Aspects of the biology and control of old man's beard (*Clematis vitalba*)

A thesis presented in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

In

Plant Science

at Massey University, Palmerston North,

New Zealand

Brenda Jarvis-Lowry

2023

Abstract

Old man's beard (*Clematis vitalba*) is an increasingly problematic liana in New Zealand, but the factors that contribute to its invasiveness are not fully understood. The work in this thesis investigated elements of old man's beard seed biology and ecology, seedling establishment, and vegetative reproduction that were unclear or unknown. The findings point to a reproductive diversification strategy that contributes to old man's beard's success as an invasive plant, due in part to dual dispersal mechanisms (by wind and water), dual seed banks (aerial and soil), dual seed dormancies (physiological and morphological), and dual reproductive modes (seeds and vegetative spread).

Summary of findings regarding the biology

- The aerial seed bank is transient: half of all achenes tagged and monitored were dispersed via anemochory during complete dormancy in autumn, and all but 5% of the remainder were gone by early spring.
- The likelihood of secondary water dispersal is high, as seeds tolerated up to 6 weeks of immersion, germinated readily in water, and produced seedlings that remained robust, if removed from water within the 6-week period.
- Pre-chilling was found to be unnecessary for germination, even for seeds that had not undergone a full winter of after-ripening: although it increased the speed at which seeds incubated at constant temperatures germinated, it did not promote total germination as successfully as a fluctuating temperature regime without pre-chilling.
- Seeds collected off the vine and tested for germination over a 2-year period were fully physiologically dormant until completely senesced. Thereafter, dormancy declined during winter, and seeds were largely non-dormant by early spring. However, morphological dormancy did not change until seeds were exposed for several days to suitable germination conditions. Fewer than 72% of seeds were ultimately viable.
- The soil seed bank was confirmed to be relatively small but persists at least for two years. Seeds in the soil experience the same cyclic physiological dormancy changes as those in the aerial seed bank, though can also enter a secondary dormancy when appropriate germination conditions are not met.
- Seedlings were not able to survive competition exerted by established perennial grass cover unless the cover was very sparse. However, seedlings that survived began producing multiple, elongating stems within six months of emergence.
- Vegetative growth produces an extensive network of creeping stems on the ground. Also, two-node woody stem fragments from both creeping and climbing stems are capable of rooting and growing vigorously as individual, clonal plants.

Current management of old man's beard infestations necessarily involves chemical control. The efficacy of two types of herbicide control was also assessed. As a precision technique for climbing vines that avoids non-target damage, the basal bark method with triclopyr in oil provides highly effective chemical control of individual stems, with >95% mortality. The cut stem method, using a 45% glyphosate gel formulation was less effective (55% mortality). For creeping stems, triclopyr alone and a triclopyr/picloram/aminopyralid mixture were effective herbicide sprays that preserved grass cover. Favouring a dense grass cover can help suppress subsequent establishment of old man's beard by seed. Management should also consider that waterways are potential conduits of propagule spread, and that mechanical fragmentation of stems serves to produce more individuals, due to regeneration.

Keywords: *Clematis vitalba*, old man's beard, liana, woody invasive, riparian, seed bank, seed dispersal, germination, dormancy, seedling competition, vegetative growth, basal bark method, cut stem method, foliar herbicide, weed control.

Acknowledgements

Thanks to my supervisory committee for their support and guidance, for their ideas, responsiveness, and helpful feedback. As my principal supervisor, Kerry Harrington was invaluable. I could always count on his insight, acute attention to detail, conscientiousness, punctuality, practical advice, and constructive criticism. He always made himself available for consultation and was quick to answer emails and texts, which is incredibly important to a stressed-out PhD student. My co-supervisor, Alastair Robertson, provided a much-needed ecology perspective and astute advice. His knowledge of R was crucial, as he was able to give me salient feedback on my analyses. My co-supervisor, Hossein Ghani Zadeh, got me started with my seed work and helped me with ideas for my glasshouse experiments. He also gave me helpful advice on analysis and on preparing for life after the PhD.

This endeavor would not have been possible without funding and input from Horizons Regional Council. Craig Davey put me on the old man's beard track and helped drive the sponsorship agreement. Craig, Jack Keast, and Kelsi Hoggard were instrumental in finding suitable field sites and giving me valuable information.

I am truly grateful for financial support from the following, which allowed me to dedicate as much time as possible to my research: Massey Doctoral Scholarship, Dan Watkins Scholarship, the New Zealand Biosecurity Institute Study Funds, George Mason Sustainable Land Use Scholarship, Taranaki Tree Crops Scholarship, and Seed Tech Services Scholarship.

Thanks to Ruth Morrison et al. at Seed Tech Services, who permitted me to use their facilities and equipment. Craig McGill at the New Zealand Indigenous Flora Seed Bank gave me advice on seed collection and seed burial, allowed me to use the x-ray machine and incubator, and introduced me to Diane Bell at AsureQuality. Diane kindly gave me one-on-one training to learn tetrazolium testing. Kay Sinclair supported me in the lab, especially when I had hours and hours of tetrazolium scoring to do. Tracy Harris spent time training me to use the fancy equipment in the Ecology lab. Gina Aubia and Cristina Sato trained me on the x-ray machine, let me borrow ISTA books, and were just generally helpful and kind.

Many thanks to Lesley Taylor, Mark Osborne, Brian Smith, Su Liu, and Sunil Sapkota at the Plant Growth Unit for their support with glasshouse and field experiments, making potting mix, fencing off plots, letting me use tools and equipment, etc.

Thanks, also, to other behind-the-scenes people, like Sharon Wright (Postgraduate Administrator), GRS (Graduate Research School), and the Massey University librarians, who provided essential services and information. Many thanks to Eloise Jillings, for style and formatting tips, and for clueing me in on the exam process. And I truly appreciate the camaraderie of my office mates, Nidhi

Shrivastav, Ling Hu, Aung Myo Thant, and Zakki. They played many roles, not least academic mentors, mental health counselors, and friends.

My parents, siblings, and children gave me very necessary support and encouragement throughout this journey. Special thanks go to my brother, Tyler, who helped me with the first couple of analyses in R. His help working through the daunting germination analysis was inestimable.

Naturally, the person I am most indebted to is my husband, John. He was my unofficial supervisor, my punching bag, my biggest champion, the best field assistant ever. The whole thing would have been impossible without him.

To all the individual people who are part of the tapestry of my life, thank you. You are in everything I accomplish. I may not have named you all here, but know I am deeply grateful for you and your influence on me.

Dedication

To my sons, Toben and Samuel, who are my most important contribution to the world.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

1WK	1-week prechilling at 5°C
2WK	2 weeks prechilling at 5°C
4WK	4 weeks prechilling at 5°C
AIC	Akaike Information Criterion
ANOVA	analysis of variance
BEE	butoxy ethyl ester
Biocontrol	biological control
CEC	cation exchange capacity
Cut (Ch.6)	grass cut to 4 cm initially, with no maintenance after sowing
Cut (Ch.8)	stems cut only, no herbicide
DAT	days after treatment
DOC	Department of Conservation
GA	gibberellin
GA_3	gibberellic acid
GPS	global positioning system
GVIF	generalised variance inflation factor
IBare	plots with initially bare soil and no maintenance after sowing
KNGA	KNO_3 + GA_3
KNO_3	potassium nitrate
KUM	site at Hopelands Reserve, near Kumeroa Road
Long	unmanipulated plots (uncut grass)
M1	Manuka Site 1
M2	Manuka Site 2
MBare	plots maintained bare by weeding
MD	morphological dormancy
me	milliequivalents
met	metsulfuron-methyl
MOG	Moginie Block
NB	negative binomial regression
OM	organic matter
ORQ	ordered quantile normalization transformation
OMB	old man's beard (*Clematis vitalba*)
Paste	stems cut and pasted with glyphosate gel
PD	physiological dormancy
PFR	Poultry Farm Road

Ring	stems chemically ringbarked with triclopyr in oil
Scar	scarification
TL	Treeline Site
tri	triclopyr
tri/pic/amino	triclopyr/picloram/aminopyralid
TTC (TZ)	triphenyl tetrazolium chloride
Tukey HSD	Tukey honestly significant difference test
WFR	Woodlands Ferry Reserve

1. Introduction, objectives, thesis structure, literature review

1.1 Introduction

Lianas are woody vines that rely on other structures—usually trees--for support, and to gain unrestricted access to photosynthesis in the canopy. Rather than devoting significant resources to self-support, lianas use those resources to produce multiple, flexible, elongated stems which use various methods to climb their hosts. Unsupported stems grow along the ground. Highly efficient water conducting vessels allow these stems to produce high leaf area to stem ratios and in turn allow lianas to efficiently photosynthesize, and successfully compete with their hosts (Prosperi et al. 2001, Allen 2015, Angylossy et al. 2015, Pasquini et al. 2015, Schnitzer et al. 2015b). As a result of competition with lianas, host tree productivity suffers both above and below ground (Schnitzer et al. 2005, Meunier et al. 2021, Newbery and Zahnd 2021). Reproduction and survival are also restrained. Consequent corresponding effects occur in the tree community structure and function (Mori et al. 2021). Because of these features, many lianas tend to be invasive (Forseth & Innis 2004; Horvitz & Koop 2004; Leicht-Young & Pavlovic 2015; Lowry et al. 2020; Yurkonis & Meiners 2004). Some lianas have food, beverage, fiber, drug, and ornamental value, but due to the above-mentioned functional traits, can also clearly have adverse effects on their environment.

Clematis vitalba is a liana species most widely known in New Zealand as old man's beard (OMB). Native to warm temperate regions in western, central and southern Europe, northern Africa and western Asia (Tutin et al. 1964, USDA-ARS 2003), OMB has been introduced as an ornamental to many parts of the world, and is now found as a naturalised species in northern and eastern Europe (Fitter 1978, Möllerová 2005, Danielewicz and Wiatrowska 2014), North America (USDA-NRCS 2006), Australia (Biosecurity Queensland 2016) and New Zealand (Allan 1940). Despite extensive ongoing efforts to control it, OMB remains one of the most problematic invasive plant species in New Zealand.

Introduced before 1922 to New Zealand, the first recorded wild specimens of OMB were collected in 1936 (West 1992), and by 1940, it was listed in government materials as having naturalised (Allan 1940). With the exception of Fiordland and Westland, it is now found throughout the country, with concentrations in the central and southern portions of the North Island, as well as the northern portion of the South Island and the Canterbury Region (Hoskins n.d., Landcare Research n.d.). Its distribution in New Zealand appears to be mainly in habitats below 750 m, where winter temperatures are mild, although there are some exceptions (Atkinson 1984).

Within its native bounds, OMB can be a nuisance in plantation forests, vineyards, and hedges (Buxton 1985, Britt 1994, Clay and Dixon 1996), but its problematic behaviour is much more pronounced in New Zealand. Here, OMB is a vigorous liana that commonly establishes in native forest gaps and edges, in scrubland, along waterways, in hedgerows, fencerows, grassland, production forest, steep cliffs, disturbed areas, roadsides and waste areas (Atkinson 1984, West 1992, Hume et al. 1995, Baars et al. 1998, Gourlay et al. 1999, Ward and Henzell 2000). Although it was recognised as problematic by the 1960s, concerted efforts to control the weed did not begin until it was a large-scale problem, in the 1980s (West 1992). OMB is now recognised as a threat to native plant diversity and abundance, as well as native forest structure (Ogle et al. 2000, Environmental Protection Authority 2018, Redmond and Stout 2018). Ogle et al. (2000) attributed a 24% loss of shrub and small tree species and a 37% loss of herbaceous plant species in Taihape, New Zealand reserves to the increasing cover of OMB. It is also becoming an increasing problem in production forestry and agricultural riparian plantings.

OMB forms multiple stems that can each grow 2-3 m/yr, with a corresponding increase in fresh weight of 6.3 kg/m^2/yr (Gourlay et al. 1999), and develop into dense, heavy tangles. Twining leaf petioles allow the stems to rapidly climb nearby vegetation like a trellis and blanket their hosts in the canopy, depriving them of sunlight and productivity, and in some cases causing tree breakage or collapse (Atkinson 1984, Gourlay et al. 1999, Ogle et al. 2000). The species produces vast quantities of seed, which are dispersed throughout the season by wind. It is also suspected of being capable of readily producing new vegetative shoots from stem nodes by layering. Biosecurity New Zealand has listed OMB and one other naturalised Clematis species, *C. flammula* (introduced in 1968), as "unwanted organisms". Under New Zealand's Biosecurity Act of 1993, it is illegal to breed, sell or release unwanted organisms (Biosecurity New Zealand 2023). Both OMB and *C. flammula*, as well as a few of the nine native Clematis species, have been used for horticultural hybridisation purposes (Lindgren 2006). Hence apart from their rapid spread and physical damage to natural ecosystems, their presence may pose a risk of biodiversity loss through hybridisation with native species.

Millions of dollars are spent annually on control of OMB in New Zealand (Gourlay et al. 1999, Environmental Protection Authority 2018), and a wide variety of methods have been used, but none so far have severely curtailed its spread (Environmental Protection Authority 2018). Due to the difficulty of distinguishing the plant when not in bloom or fruiting, the collateral environmental damage involved in chemical control and a low success rate, some regional councils have stopped attempting to control the species throughout their entire region and have been focusing solely on key native ecosystems (C. Davey, Horizons Regional Council, pers. communication, October 16, 2019).

1.2 Research aim and objectives

The purpose of this research is to identify and clarify insufficiently understood aspects of the biology and ecology of old man's beard, and to assess the efficacy of some control techniques for the species. It is anticipated that this research will improve the body of knowledge on old man's beard and will facilitate improved management of both this species and other temperate lianas in New Zealand and elsewhere. There are areas in the current literature where knowledge is lacking or unclear about the species. For example, germination requirements and dormancy issues are not well understood. Likewise, there is no definitive information about the importance of vegetative reproduction to OMB's spread, competitive vigour of seedlings, how long seeds remain viable in the soil, or how much an aerial seed bank contributes to OMB's invasiveness. It is unknown how tolerant OMB seeds are to water, and whether water is a secondary mechanism for seed dispersal. There is also a need to study effective control techniques that are less environmentally damaging than some methods in current use, as this weed often grows over desirable plants or beside waterways.

The research in this thesis has two areas of focus, with seven specific objectives:

1. Reproductive capacity of *Clematis vitalba*
    a. Describe the patterns of dormancy and viability of persistent OMB seeds on the vine over the course of a season, as well as annual seed fall patterns.
    b. Discern the dormancy and longevity of OMB seeds within the soil.
    c. Investigate the factors involved in seed germination, both to test artificial treatments and to clarify OMB's natural germination requirements.
    d. Determine the risks of new infestations of OMB occurring in riparian zones, first by evaluating the ability of seeds to be secondarily dispersed by water, and second by testing the ability of dense grass canopies to stop new seeds from establishing.
    e. Develop an understanding of OMB's reliance on vegetative growth, by assessing the phenotypic patterns of creeping stems and ascertaining how well stems can regenerate after fragmentation.
2. Control of *Clematis vitalba*
    a. Study the efficacy of two herbicide control techniques that minimise environmental impacts to treat woody OMB plants from the base: 1) basal bark treatment with triclopyr in oil; and 2) cut stump with concentrated glyphosate gel treatment.
    b. Examine the efficacy of commonly used foliar herbicide sprays to treat creeping OMB, while leaving surrounding grasses intact: 1) metsulfuron-methyl; 2) triclopyr ; and 3) a combination of picloram, aminopyralid, and triclopyr.

1.3 Thesis structure

This thesis is organised in 9 chapters. Chapter 1 is introductory, Chapters 2-8 detail the research undertaken, and Chapter 9 is an overall discussion and conclusion. A more detailed summary of the thesis structure is provided below:

Chapter 1, Introduction, objectives, thesis structure, and literature review, gives an introduction, provides a rationale for pursuing the research, and describes the aims and structure of the thesis. In addition, it summarises the current literature relevant to the research, and identifies gaps in the knowledge.

Chapter 2, Seed dispersal, describes the timing of wind driven OMB seed dispersal over the seasons and the correlation of seed release to wind strength. It also determines the likelihood of secondary water dispersal, with tests of seed buoyancy in still and moving water, and seed and seedling tolerance to short and long-term water immersion.

Chapter 3, Factors that influence seed germination, investigates the effect of various treatment combinations on conditionally dormant OMB seed germination, to better understand its natural germination requirements and to discover the most efficient artificial methods for germination.

Chapter 4, Seed dormancy and the aerial seed bank, explores the patterns of physiological dormancy and viability of vine-persistent OMB seeds (the aerial seed bank) from autumn to spring. Additionally, it examines seed embryo size before and during germination, to discern if morphological dormancy also shifts seasonally.

Chapter 5, Seed dormancy and the soil seed bank, investigates the dormancy and viability of buried OMB seeds (the soil seed bank) over a 2-year period, while considering the influence of seed depth, environmental conditions, time spent in the soil, and seed provenance.

Chapter 6, Effects of competition on seeds and seedlings, assesses the ability of OMB to invade grassy habitats by seed, and compares seedling vigour against different levels of ground cover competition.

Chapter 7, Vegetative reproduction capacity, details field observations and measurements of OMB's vegetative growth morphology and patterns. It also confirms the ability of stem fragments to regenerate and become independent individuals.

Chapter 8, Control, compares the efficacy of techniques using herbicides to control individual woody OMB stems, while minimising non-target plant damage. It also compares the efficacy of

commonly used selective herbicides for foliar sprays on creeping OMB, while minimising damage to grasses.

Chapter 9, General discussion and conclusions, provides a summary of the main findings from the experimental chapters (2 - 8). It also includes recommendations for future research related to the topic. Concluding remarks are also given, which include a short analysis of implications of the research findings and their relevance to management of old man's beard.

1.4 Literature review

1.4.1 Classification and description

Clematis vitalba (old man's beard) is a species taxonomically classified in the buttercup family, Ranunculaceae (Royal Botanic Gardens). Although most familiarly known in New Zealand as old man's beard, other English common names include traveler's joy, evergreen clematis, and virgin's bower. The genus *Clematis* comprises over 380 species, largely woody lianas or shrubs, which are native to nearly every continent of the world, including New Zealand (Royal Botanic Gardens), where five Clematis species are endemic.

Most old man's beard (OMB) seeds germinate in the spring, producing seedlings with elliptical to obovate cotyledons (Figure 1.1). Subsequent early leaves are 3-lobed and dentate (Essig 1991), or compound leaves with three 3-lobed leaflets. As the plant matures and elongates, it produces compound leaves with 5 leaflets, in opposite pairs along the 6-angled stem. Each leaflet grows 8-9 cm long and 4-5 cm across, with untoothed, toothed and/or lobed margins.

Figure 1.1: *Clematis vitalba* seedling development. Left: cotyledonous seedling, with and without true, dentate leaf. Middle: seedling with 3-lobed leaves and trifoliate leaf. Right: 5-foliate leaf.

Young stems have a higher rigidity than older stems, which allows them to grow upright in the early stages while searching for supports (Isnard et al. 2003). Leaflet petioles and rachises are prehensile, and will twine around any available small supports, up to 1 cm in diameter (Figure 1.2), allowing the liana to climb up and over tree canopies (Darwin 1865, West 1991, Leicht-Young 2014). Mature

woody stems are rough and stringy, grow to 10 cm in diameter and up to 30 m long. Each individual vine produces new stems annually, and each stem can live a few years to a few decades (West 1991, Bungard 1996). When trailing on the ground, stems will root and produce shoots at the nodes.

Figure 1.2: Left: twining leaf petiole. Middle: stringy, rough bark on mature stem. Right: nodal roots from ground-based stem.

Flower panicles are produced on stems as young as one year old from early to late summer. Perfect flowers arise in compound dichasial cymes from leaf axils and terminally (Figure 1.3). When open, flowers are 2-3cm across. Flowers lack petals, but have 4-5 lanceolate, white, hairy sepals that surround the androecium with up to 60 stamens.

Figure 1.3: Left: compound dichasial cymes with flowers and flower buds. Right: perfect flowers with hairy sepals.

The superior gynoecium consists of many separate carpels, usually not more than 36, each of which ideally produces a single-seeded, hairy achene, 2 mm x 4-5 mm. Carpel number is usually higher in terminal flowers than lateral flowers (Salisbury 1920). Green achenes ripen to lignified, dark brown or reddish-brown, almond-shaped fruits covered with dense, flattened hairs (Figure 1.4). Each achene is generally 1-2 mm wide and 2.5-4 mm long, topped with a 3-4 cm long, style (Tamura 1993). Styles curl and become plumose as the achene ripens.

Figure 1.4: Left: early development of achenes. Middle: ripe achenes with plumose styles. Right: single, ripe achene.

1.4.2 Ecological requirements

Old man's beard generally grows in soils that are moderate to well-drained, are not highly acidic, and do not have severe nutrient deficiencies. The species is associated with arbuscular mycorrhizal fungi (Maremmani et al. 2003, Renker et al. 2005), and has a positive response to calcium, phosphorous and nitrates (Hume et al. 1995, Bungard et al. 1998). OMB occurs most commonly in sites where precipitation is within a range of 800-1600 mm and is often associated with riparian areas (Atkinson 1984).

Old man's beard seedlings have been reported to be intolerant of dry conditions (Kozlovskiy et al. 2017), although adult OMB plants have been shown to be tolerant to temporary heat shock (Zhang et al. 2021). Also, due to the presence of wide, efficient water-conducting vessels in its earlywood, adult OMB is well-adapted to moderately dry conditions (Kazda 2015). However, the same wide vessels that promote water efficiency are susceptible to freezing- and drought-induced embolisms (Leicht-Young and Pavlovic 2015). This is very likely partly why OMB does not commonly occur in New Zealand above 750 m, in areas where freezing temperatures are frequent, or in Northland, where median annual average temperatures are highest (Atkinson 1984). However, at elevations below 750 m throughout its naturalised range, OMB tolerates occasional freezing temperatures in winter. In addition to the wide earlywood vessels, Clematis species produce narrower latewood vessels, which are less apt to fail from embolism. This mix of vessel sizes (heteroxylly) insures that occasional embolisms do not fully interrupt water conduction (Carlquist 1985, Angylossy et al. 2015).

There have been discrepancies in research claims on light requirements for OMB survival. According to Paliwal, Küppers and Schneider (1994), OMB makes more efficient photosynthetic use of short light flecks in forest environments than other forest species studied, enabling it to have more carbon to fuel its growth. Other researchers found that although OMB responds best to higher light levels, it can survive and keep growing on as little as 2-3.5% irradiance (Baars and Kelly 1996). Indeed, Nikoloff (2011) found seedling survival rates were higher and relative stem elongation longer in forested areas than in open sites or on forest edges. In contrast, Van Gardingen reported that shaded OMB plants had

a higher leaf area but leaves were thinner than leaves of non-shaded plants, and claimed that the species requires high light levels for establishment (1986). Furthermore, Bungard claimed that seedlings planted in undisturbed forest did not survive (1998). Regardless of how well OMB itself uses available light, as a petiole climber, it requires small supports to wrap around, which are more common in understorey and forest gaps, edges, and other places with higher light intensity (Putz 1984, Leicht-Young 2014).

1.4.3 General phenology

West (1992) identified eight phenological stages for OMB in New Zealand (Figure 1.5), the timing of which may shift slightly between microclimates, altitudes, and latitudes. First, the commencement of new vegetative growth begins in late winter or early spring, and by mid-spring, rapidly growing, green foliage is widespread. Vegetative growth continues throughout the growing season, although it slows down as soon as flowering is initiated. Flower bud production begins in late spring to early summer, followed closely by flowering. New achenes develop 2-3 weeks after flowering begins (Van Gardingen 1986), ripen on the plant in autumn, and can be found on the vine throughout the year, except in late summer. Leaves senesce and begin falling at the same time fruit begins to ripen, and seed dispersal takes place from late autumn to early summer.

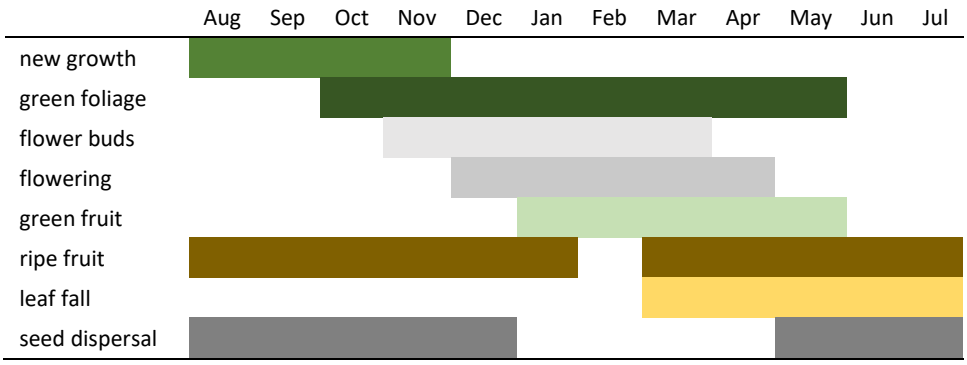

Figure 1.5: *Clematis vitalba* phenology, modified from West (1992), by combining phenological stage ranges from observations by West at Taihape (North Island) and Brown River Scenic Reserve (South Island), New Zealand, and observations by Van Gardingen (1986) at Christchurch (South Island), New Zealand.

1.4.4 Pollination and seed set

According to research carried out in Ireland (Redmond and Stout 2018), where OMB is naturalised and potentially invasive, it is pollinated by generalist insects (Figure 1.6) but is also able to self-pollinate within the same flower and between flowers on the same plant. These are both advantageous characteristics for alien plant species, as they don't need specific pollinators in their new environment, and don't necessarily need any pollinating agents at all to successfully reproduce by sexual means. However, fruit and seed set in OMB is lower in self-pollinated flowers than those that are insect-

pollinated. Also, due to inbreeding depression, genetic diversity may be lower, and offspring vigour may be weak, among other things.

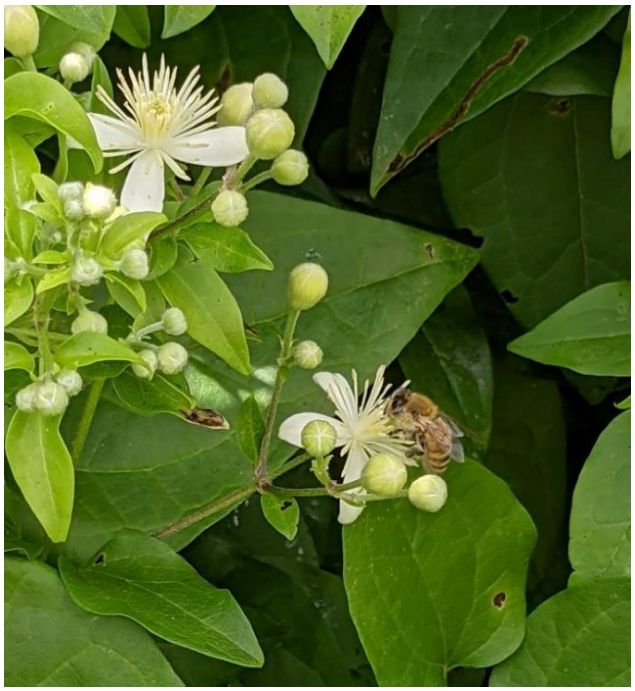

Figure 1.6: One of many generalist pollinators of *Clematis vitalba.*

The number of carpels per OMB flower is highly variable and ranges from 9-42 or more, although most flowers have between 20 and 30 carpels. Salisbury (1920) found that abortion of achenes on OMB plants in England occurs in approximately 12% of seeds produced, and that very few seed heads are 100% fertile. He determined that lack of pollination caused abortion in only 1-2% of the aborted achenes, which validates the above research in Ireland. Lindgren also confirmed that it is uncommon in the Clematis genus for every individual carpel to produce a viable seed (2006). OMB produces large quantities of seeds. Along with 12% abortion, some seeds that suffer predation or disease, or lose viability due to unfavourable temperature, moisture, or light levels. Despite those losses and the trade-offs associated with self-pollination, OMB can still produce large quantities of viable seed. Selfing confers a clear benefit on the species.

1.4.5 Seed dispersal

Seeds can be dispersed by a variety of biotic and abiotic means, including by humans, birds, insects, animals, wind, and water. OMB achenes are best adapted to wind dispersal (anemochory) when the fruit and style are mature and desiccated (Sitte 1973). It has also been hypothesised that OMB can be water dispersed. Anemochory is not usually as efficient as hydrochory (water dispersal) or biotic dispersal (van der Pijl 1982). In fact, 99% of anemochorous seeds do not travel more than 200 m from the mother plant (Tackenberg et al. 2003), travelling an average of only 8.5 m (Thomson et al. 2011).

In anemochory, dispersal distance is often associated with higher wind speed, lower seed mass, and higher surface area of the dispersal unit (Benvenuti 2007), although Thomson et al. (2011) found that plant height is more closely correlated with wind dispersal distance than seed mass. The longest recorded anemochorous distance for OMB was 500 m, but the vast majority of wind-dispersed OMB achenes have been recorded within 30 m of the source (Nikoloff 2011).

Upon ripening, OMB achenes are embryonically underdeveloped and physiologically dormant (Baskin and Baskin 2014) and require an after-ripening period to fully mature (Lhotská 1974). The achenes remain attached to the parent plant until fully desiccated, after which the fruits are dispersed gradually (bradyspory). West (1992) has claimed that those achenes exposed to more wind tend to disperse earlier in winter than sheltered fruit.

The persistent style on each OMB achene can reach 4.5 cm long, and is covered in approximately 1,300 hairs, each about 4 mm in length. When dry, style hairs open at a wide angle and essentially render the fruit an order of magnitude greater surface area than when wet, providing the achene a semi-efficient mechanism of wind-dispersal; In still air, the wet fruit has a terminal velocity of 308 cm/second, and the dry fruit velocity slows to 108 cm/second. When wind is present and the seed is sufficiently desiccated, dry style hairs increase the leverage of the wind to tear the fruit from the receptacle (Sitte 1973).

In addition to the presence of more abundant small supports for climbing, another explanation for OMB's common occurrence in forest edges, gaps and other areas of high light is that wind dispersal is favoured by the open conditions of such sites and could have much to do with this pattern of distribution (Ladwig and Meiners 2015).

1.4.6 Water dispersal

Seeds can be dislodged by rain, but hydrochory largely occurs in downstream movement of runoff, streams, rivers or other water channels (van der Pijl 1982, Benvenuti 2007, Hyslop and Trowsdale 2012). Hydrochory dispersal distances can vary considerably but are typically greater than dispersal distances by wind. In fact, hydrochory can extend dispersal distances by several times for anemochorous species (Säumel and Kowarik 2010). Mean dispersal distance of water-borne seeds is over 500 m, as opposed to 8.5 m for wind-borne seeds (Thomson et al. 2011). Hydrochorous propagules need to remain viable following extended or periodic contact with or immersion in water. Hydrochory of seeds may be obligate or facultative, that is, they may require water for dispersal, or may merely take advantage of it when available (Benvenuti 2007). Some hydrochorous seeds float by surface tension (kowhai, willow), some via specialised structures (mangrove, coconut), and some have transient floating mechanisms (water chestnut) (van der Pijl 1982).

Assumptions have been made that OMB is secondarily water dispersed, but nothing has been published on the subject. The inference is due to the fact that OMB often grows along waterways (West 1992, Bungard 1996), and also to the belief that the hairy persistent style provides enough surface tension to the achene for buoyancy to be achieved (Van Gardingen 1986).

1.4.7 Seed bank

A seed bank is a cache of viable, un-germinated seeds. The purpose of a seed bank is to ensure perpetuation of a plant species over time: seeds that can remain viable in the seed bank for a number of years guarantee a higher chance of species survival, especially when the species has unpredictable reproductive intervals (Shen, Zhao, & Liu, 2011). Seed banks can be described as transient or persistent (Thompson & Grime, 1979). Walck et al. (2005) define a transient seed bank as constituting seeds that remain viable for one germination season and germinate or die within that season. A persistent seed bank constitutes seeds that retain viability until at least the second germination season, and long-term persistent seed banks require viability until at least the sixth germination season.

Seed banks are generally associated with accumulation of seeds on or under the soil surface, following dispersal from the parent plant. However, seed reserves can also occur before dispersal, when a parent plant retains propagules after maturity for a period of time. This is referred to as an "aerial seed bank", or "serotiny" (Baskin and Baskin 2014). Bradisporic serotiny is the intermittent dispersal of seeds over time.Bradysporic serotiny results in a dual soil and aerial seed bank (Gao et al. 2014), until all aerial seeds are dispersed, or buried seeds germinate or die. One advantage to having the two different seed bank sources is that environmental conditions vary between them (moisture, gases, light availability, granivory, microorganisms), and not all seeds are subjected to the same mortality risks. Therefore, at least a portion of the propagules have the potential to survive, even when conditions are extremely unfavourable in one of the two settings. Dormancy and germination responses could also vary as a result of different conditions in the two seed banks, resulting in further staggering of germinability through time (Bhatt et al. 2016), thereby increasing the chances of species survival.

Some researchers have suggested that OMB's seeds remain on the vine at least until late spring or summer (Lhotská 1974, Van Gardingen 1986, Bungard 1996). Bungard (1996) claimed that seeds on the parent plant are viable but remain dormant, and that they serve as a bradysporic seed bank, with continuous, intermittent release over time. Indeed, seed traps set up by West (1992) collected a minimum of 10 OMB seeds/m^2 every month of the year.

Old man's beard seed longevity in the soil is disputed, and previous research has provided conflicting results. Studies in Christchurch, NZ, by Van Gardingen (1986) found no viable seeds following 7 months of burial in moist condition at 10 cm deep. Tucker (1979) claimed that OMB seed in NZ does

not remain viable for long following hydration. In Europe, studies where OMB was common aboveground have found OMB either absent in the soil seed bank or detected in low quantities (Warr et al. 1994, Dutoit and Alard 1995, Roovers et al. 2006, Chaideftou et al. 2009). Other European researchers found moderate numbers of OMB in the soil seed bank (Dölle and Schmidt 2009).

Seedling emergence research on Banks Peninsula, NZ, by Nikoloff (2011), suggested the soil seed bank is small and short term, probably between 1 and 5 years. However, In Oregon, USA, Clements and Bierzychudek (2017) discovered that, even 3 years after complete removal of all OMB plants, OMB was the most commonplace invasive species to germinate from soil samples. Also, from her seed trapping and seedling emergence work in Marlborough, NZ, West (1992) concluded OMB has an extensive soil seed bank, and seeds could persist for between 5 and 10 years. This is an indication that establishment by seed is an issue.

1.4.8 Seed dormancy and germination

Germination is the emergence of an embryonic plant from a seed and begins when a mature seed imbibes water needed for enzymatic and chemical processes and is completed when the embryo radicle breaks through the outer seed coat. Seed dormancy is the temporary inability of a viable seed to complete germination, even when exposed to suitable environmental conditions (Bewley 1997, Baskin and Baskin 2014, Davies et al. 2015). This period of inactivity makes a plant's seed bank possible, providing a source of seedling recruitment over time. Seeds that are dormant at development are said to have primary dormancy (Bewley et al. 2013). Dormancy can be caused by exogenous or endogenous mechanisms, or a combination of the two. Exogenous dormancy is usually caused by physical or chemical restrictions in the seed coat, and endogenous dormancy is caused by internal conditions associated with the seed embryo (Davies et al. 2015).

Germination usually takes place when all dormancy mechanisms are removed, environmental conditions (daylength, temperature) are optimal for survival of young seedlings, and the seed embryo reacts to the requisite stimuli (Vleeshouwers et al. 1995). The stimuli include a combination of the correct levels of moisture, light, temperature, gas, hormones and other chemicals. In the absence of the stimuli non-dormant seeds are not likely to germinate, and as a result, may even enter a secondary dormancy (Bewley 1997, Thompson and Ooi 2010, Bewley et al. 2013).

Old man's beard seeds initially exhibit two types of primary endogenous dormancy: they are both morphologically (MD) and physiologically dormant (PD) upon maturity (Nikolaeva et al. 1985). Physiological dormancy occurs as a result of some mechanism that suppresses the normal physiological process and is often associated with seasonal changes. Morphological dormancy is due to a differentiated, but temporarily undersized embryo, and is broken when the embryo reaches an adequate size for germination (Lhotská 1974, Forbis et al. 2002, Bewley et al. 2013, Baskin and

Baskin 2014, Kildisheva et al. 2020, Copete et al. 2021). Physiological dormancy exists on a continuum and can come and go (Baskin and Baskin 2004b, Finch-Savage and Leubner-Metzger 2006), but overcoming MD is a singular event (Bewley et al. 2013). Both dormancies must be broken before germination can take place.

Previous research suggested that a two- to four-month period of cold stratification at about 5°C is necessary to break OMB seed dormancy (Rudolf 1974, Grime et al. 1981, Van Gardingen 1986, Bungard et al. 1997b). This period corresponds with winter temperatures in OMB's native and much of its naturalised range. OMB seed embryos do not grow during cold stratification (Bungard 1996, Copete et al. 2021), but after a period of 8-12 weeks at 5°C in moist conditions, seeds transferred to warm temperatures germinate rapidly (Lhotská 1974, West 1992, Bungard et al. 1997b, Vinkler et al. 2004, Picciau et al. 2017). This is an indication that chilling allows embryo growth to occur as soon as suitable conditions are met, allowing seedlings to establish quickly.

Several germination experiments on OMB seed have demonstrated that various alternating temperature regimes—almost all with a low temperature above 5°C--can also break dormancy (Lhotská 1974, Tucker 1979, West 1992, Vinkler et al. 2004, Picciau et al. 2017). In fact, seeds collected in autumn before natural cold stratification has occurred have been shown to readily germinate under fluctuating temperatures after a period of time similar to the cold stratification recommendation. Furthermore, seeds in fluctuating temperature regimes alone have had higher total germination than those in constant temperatures that follow cold stratification (West 1992, Picciau et al. 2017). These results imply that OMB seeds do not require wintry temperatures to emerge from dormancy, although cold temperature prechilling increases the speed at which seeds germinate when exposed to suitably warm temperatures.

Other research has produced mixed conclusions about OMB germination requirements. For example, some researchers have reported that seeds germinate poorly both in darkness and under long day conditions (Van Gardingen 1986). However, others claim that light is unnecessary for germination, but it does increase germination under certain circumstances, such as following a chilling period, or in combination with nitrates (Bungard et al. 1997b). Still others found alternating temperatures promoted good germination for both light and dark treatments, but dark treatment promoted higher germination at constant temperatures (Vinkler et al. 2004).

1.4.9 Vegetative reproduction

Vegetative reproduction is reproduction by asexual means. Many researchers believe vegetative reproduction is an important element in liana colonization success, both in tropical and temperate environments (Prosperi et al. 2001, Yorke et al. 2013, Ladwig and Meiners 2015, Leicht-Young and Pavlovic 2015, Schnitzer et al. 2015a, Buru et al. 2016, Mori et al. 2018). For example, on a 50-ha

plot in tropical Panama, a census found 30% of nearly 70,000 individually rooted liana stems present were clones (Schnitzer et al. 2015a). Researchers in Veracruz, Mexico discovered that none of the 423 upright liana shoots found in 10 hectares of intact forest were seedlings, but rather were all connected to existing lianas (Penalosa 1984). Another study in temperate Japan used genotyping with microsatellite markers to measure the clonal proportion of the population of a single, abundant liana species, *Wisteria floribunda*, in a 6-ha plot (Mori et al. 2018). They found that 71% of the approximately 400 *W. floribunda* stems sampled were clonal.

Many liana stems creep laterally along the ground in search of a suitable host, with the help of roots that develop at the nodes and act as multiple points for resource intake, and of anchoring. Fallen and cut stems in contact with the ground will follow the same pattern (Schnitzer and Bongers 2011, Ledo and Schnitzer 2014, Ladwig and Meiners 2015, Mori et al. 2018). This is called layering. Layered stems will also branch and produce new shoots at the nodes as they elongate. This vegetative growth makes lateral expansion possible, which can be valuable when seedling colonization is limited, and/or when no suitable hosts are available for climbing (Sakai et al. 2002, Deiller et al. 2003, Leicht-Young and Pavlovic 2015). In case of subsequent detachment or injury along the stem, the rooted fragments can become independent individuals (Penalosa 1984, Jeník 1994, Prosperi et al. 2001, Ewers et al. 2015).

Old man's beard has a reputation for vigorous regrowth after cutting, and for its prostrate stems to layer easily (Bungard et al. 1997a, Vinkler et al. 2004, Williams 2009). Horticulturally, OMB is used as a rootstock for grafting other clematis scions, because of its robustness (Kreen et al. 2002). However, with the exception of two poorly documented studies, very little work has been done to determine the extent of OMB's vegetative regeneration capacity, or its importance in the reproductive strategy of the species: 1) Van Gardingen (1986) found that two-node young OMB stem fragments collected in the autumn did not produce any roots or shoots over an 8-month period; 2) An unpublished report, mentioned in proceedings from a Department of Land and Survey seminar on the invasiveness of OMB (Kennedy 1982), looked at regeneration of stem fragments with a single node and 0.5 cm on either side of the node in stems of different ages. No root or shoot growth was witnessed from young stems one and two generations old, and very poor regeneration from nodes of older stems. It is unknown what time of year this was done.

1.4.10 Control

Effective management of invasive plants and other weeds often involves a combination of several different methods of control (Simberloff 2013). In addition to prevention of spread into un-infested locations, successful management may include the use of biological, cultural, mechanical, and chemical practices.

1.4.10.1 Biological control

Biological weed control (biocontrol) is the use of a living organism to reduce a plant population. Biocontrol agents are natural enemies of the target species, such as fungi, insects, mites, or herbivores, and are generally chosen to attack only one species. The most common type of biocontrol method involves releasing the agents in an area infested by the target species. The agents are then allowed to establish self-perpetuating populations that keep the target population at a tolerable density, and ideally establish a long-term ecological balance with the weed.

An early assessment of prospects for biological control of OMB in New Zealand concluded that the species is not well controlled in its native environment in Europe, and therefore it would be difficult to find a suitably host specific agent in its introduced range (Groppe 1991, Syrett 2002). Indeed, two studies on OMB in the UK noted that it had an "invasive" habit (Clay and Dixon 1996), and is "capable of smothering hedges" (Britt 1994). More recent genetic work concluded that the New Zealand material is diverse enough to have been introduced up to three different times from different places of origin (with genetic material from the UK, Germany, France, and Italy closely matching some from New Zealand), making it difficult to find a biological agent that could control all the variants (Mitchell 2019, 2020).

Three biocontrol agents introduced previously have not had a significant impact to date (Ward and Henzell 2000, Smith 2016, Horizons Regional Council 2017). The first two agents were released in 1996: *Phytomyza vitalbae*, a leaf-mining fly, and *Phoma clematidina*, a leaf and stem fungus. Natural enemies of *P. vitalbae* seem to reduce its effectiveness, and *P. clematidina* did not sustain itself after release. *Monophadnus spinolae*, a sawfly whose larvae eat OMB leaves, was released in 1998 at 16 different sites, but with one exception, it failed to establish itself. A new attempt to release the sawfly was made in 2019 on the South Island of New Zealand, though it has not been as damaging as hoped (M. Beech, Horizons Regional Council, pers. communication, 14 December 2022). A new agent, *Aceria vitalbae,* is a leaf and bud galling mite that causes contortion of leaves and growing tips. The gall mite was reared and released in 2021 at Canterbury, NZ (J. Keast, Horizons Regional Council, pers. communication, 5 March 2021). Due to a successful 65 km unassisted spread from the original release points, several additional releases have since been made around the Manawatu Region, NZ (C.

Davey, Horizons Regional Council, pers. communication, 8 December 2022). However, it could be several years before the mites have spread widely. It also remains to be seen how much of an impact they can make on OMB's vigour and spread on a large scale. To complement the invertebrate agents, efforts are underway to find an effective pathogenic agent as well (den Breeyen 2022).

Although there were reports several decades ago of stock losses due to ingestion of OMB (Moore 1971, Connor and 1977), grazing is now considered one of the most effective ways to control the plant in New Zealand. On farmland where OMB is present, sheep and cattle will keep it under control (Gous 2003), but upon removal of the stock it becomes an almost immediate problem (A. Gordon, Rangitikei District Council, pers. communication, 18 February 2019). In conservation settings, however, the presence of stock does not allow regeneration of desirable species to occur, and thus can contribute to a loss of biodiversity in those areas (Ogle et al. 2000, Smale et al. 2008).

1.4.10.2 Cultural control

Cultural control is land management to increase a crop's competitive ability and simultaneously decrease that of undesirable species, and involves seed or stock selection, rotation, manipulation of plant density, timing of planting, nutrient and water input (Monaco et al. 2002). Indeed, previous research has shown that interspecific competition can be more important than herbivory at suppressing invasive plant growth (Vilà and Weiner 2004). Living mulches restrict light penetration to the soil surface and provide competition to potential plant invaders both above and below ground. In farm and production forests, a common cultural control technique to help prevent scrub weeds is to sow living mulches, such as grass alone or grass and legume species between forestry seedlings (West and Dean 1998, Tran et al. 2018). The ground cover grows quickly and competes with establishing weeds in new forests. However, the grass/legume ground cover must also be constrained from competing too vigorously with the young tree crop. As a rule, a ring of bare soil 1.5-2.0 m in diameter (created with the application of pre-emergence herbicides) is maintained around each seedling for the first year or two after planting, to protect it from direct competition (NZ Ministry of Forestry and NZ Forest Research Institute Ltd 1996).

In recent years there has been an emphasis on improving freshwater quality in New Zealand. One response to the emphasis has been to revegetate riverbanks near pastureland and reduce stock access to waterways, thereby reducing sediment and nutrient runoff (Reeves et al. 2006, Dairy NZ 2023). The area is usually fenced off and planted in native species and is referred to as a "riparian zone". Most of these zones have at least some grass cover. Old man's beard has become a problem in farm forestry in New Zealand and local land managers have reported that OMB occurs in the grass swards on forestry farms and in grasses of riparian zones (A. Gordon, Rangitikei District Council, pers. communication, 7 October 2019; J. Keast, Horizons Regional Council, pers. communication 17 April

2019), but it is unclear if the OMB present in these areas are shoots from existing vines, or if they are entirely new recruits.

1.4.10.3 Mechanical control

Mechanical weed control involves the physical disturbance of the target weed above or below the soil, e.g. by cutting, tilling, hand pulling, mulching with non-living materials, burning, and flooding. Studies that have evaluated mechanical management of temperate stem-twining lianas have shown that, unless repeatedly done, or done in combination with chemical control, cutting, mowing or excavating are not effective techniques for their control (Nyboer 1992, Webster et al. 2006, Lynch 2009). Researchers in the U.K. have reported that mechanical cutting of OMB stems without any additional treatment appears to be ineffective, even when done repeatedly (Britt 1994, Clay and Dixon 1996).(2000) In New Zealand, Ward and Henzell (2000) found that rooted OMB stems that were cut but untreated with herbicide resulted in 61% regrowth when pruned in late spring, and nearly 97% regrowth when pruned in winter. In the interval between tree harvest and restocking with a new crop, production foresters in New Zealand commonly fragment and clear logging debris and weeds (including OMB) mechanically with a bulldozer, leaving the debris in long windrows parallel to the rows in which the tree seedlings are planted, or planting the seedlings directly in the mulched debris (NZ Ministry of Forestry and NZ Forest Research Institute Ltd 1996). The problematic OMB in forestry sites could originate from fragmented stems that have regenerated.

1.4.10.4 Chemical control

Chemical weed control is the application of herbicide in the treatment of a plant or the soil to restrict germination or growth of the plant. Chemical control has become the most effective and economical way to manage many weedy species. For example, as New Zealand's fourth-largest export earner, the forestry industry relies heavily on herbicide to establish its crop (usually *Pinus radiata*) in the first two years, without which forest production would not be profitable (Rolando et al. 2015): the ring of bare soil around *P. radiata* seedlings mentioned above (Section 1.4.10.2) is created with the application of soil-applied residual herbicides (terbuthylazine and/or hexazinone) that kill weeds as they emerge, but are not toxic to the tree seedlings (Rolando et al. 2011). Yet, despite the routine use of soil-applied herbicides in forestry and other crops to control emerging weeds, most control of woody vines like OMB in temperate zones is done with systemic herbicides applied after plant emergence, often on mature plants (Smith 1982, Boatman and Bain 1992, Clay and Dixon 1996, 2000, Ward and Henzell 2000, 2003, Webster et al. 2006, Langeland and Meisenburg 2009, Raal and Timmins 2018, Bierzychudek 2020). Most herbicides used on woody vines selectively control broadleaf (dicotyledonous) weeds without damaging monocotyledonous plants, such as grasses.

Among the selective herbicides currently used in New Zealand for woody vines, picloram, triclopyr, and aminopyralid are related, pyridine derivative, growth-regulating herbicides that work by disrupting the plant's balance of hormones (Monaco et al. 2002). Metsulfuron-methyl, a sulfonylurea, is another commonly used selective herbicide on woody species, which works by inhibiting branch-chain amino acid biosynthesis. Non-selective glyphosate, which inhibits biosynthesis of aromatic amino acids, has also been used. As a nonselective herbicide, it is likely to damage any vegetation with which it comes in contact, although careful, directed placement can accomplish selectivity (Monaco et al. 2002).

Increased awareness of herbicide persistence and non-target effects by herbicides on the environment have contributed to a focus on the use of less persistent chemicals, lower rates of herbicide use, and the use of alternative chemical control techniques (Ward et al. 1999, Simberloff 2008, Alavanja and Bonner 2012, Rolando et al. 2015). Currently, common chemical control methods for OMB and other woody vines include: 1) the basal bark method (applying oil-based herbicide sprays to stems without cutting them) (Nelson et al. 2006, Raal and Timmins 2018); 2) the cut stem method (treating cut stems individually with systemic herbicides) (Smith 1982, Ward and Henzell 2000, 2003, Webster et al. 2006, Bierzychudek 2020); and 3) foliar spray (applying herbicide sprays to foliage) (Boatman and Bain 1992, Clay and Dixon 1996, 2000, Langeland and Meisenburg 2009).

The basal bark method has been adopted in New Zealand, largely by the Department of Conservation, as a reliable, targeted technique for treating small diameter woody weeds, including OMB (Raal and Timmins 2018). Basal bark applications involve spraying herbicide plus penetrant completely around the basal circumference of a stem. Before the mid-1980s, a high volume of herbicide was used, which often pooled at the base of the treated stem, causing potentially severe damage to non-target plants (Nelson et al. 2006). The common current technique in New Zealand uses triclopyr (120 g/L as the butoxy ethyl ester) mixed with oil and a biodegradable penetrant applied at low pressure. This has a much lower impact on the environment, as the herbicide is not allowed to pool at the base of the stem.

The cut stem method has been widely used in New Zealand on OMB since the 1970s (Smith 1982). It involves both mechanical and chemical control, by cutting each individual stem close to or at ground level and treating each cut end either with a concentrated liquid or gel herbicide. Picloram, or picloram-based herbicides, have been cited as the most consistently effective for this technique (Smith 1982, Ward and Henzell 2000, 2003, 2004). Glyphosate is strongly adsorbed by soil, and therefore has very low mobility (Duke and Powles 2008). As an alternative to picloram gel, Ward and Henzell (2000) claimed that glyphosate gel appeared to give good control of OMB in the first year after cut stem treatment, but that many stems regenerated from the roots by the end of the second season. The glyphosate concentration in the gel was not stated. In subsequent New Zealand studies on woody plants, Ward and Henzell (2004) used gel containing 10% glyphosate and found it to be highly

effective in controlling grey willow (*Salix cinerea*) following stem cutting. A New Zealand company specialising in gel formulations of herbicides currently market products they claim to be effective on some weeds, e.g. metsulfuron gel for Chinese privet (*Ligustrum sinense*), buckthorn (*Rhamnus* spp.), ginger (*Zingiber* spp.), agapanthus (*Agapanthus praecox*), and Arum lilly (*Zantedeschia aethiopica*); and picloram gel (43 g/L) and several concentrations of glyphosate gel (120 g/L, 240 g/L, 400 g/L), for shrubs, trees, and vines (Landman Limited).

The treatment of individual stems with the cut stem and basal bark methods is labour-intensive and time-consuming (Bungard 1996, Raal and Timmins 2018). However, it is often the best way to deliver targeted application with little collateral damage. Foliar spraying for control of woody vines is used when an infestation covers a large area, or when stems cannot be individually treated. When the target vegetation is inaccessible from the ground, foliar spraying is done aerially (Smith 1982, Environmental Protection Authority 2018). Old man's beard has been treated by foliar spray with triclopyr, picloram, aminopyralid, clopyralid, metsulfuron-methyl, and glyphosate, either singly or in combination with each other. Aerial herbicide application is expensive, has poor precision, and usually damages non-target vegetation, not only when using glyphosate, but also with the other listed herbicides, as they damage a wide range of broadleaf plant species (Ward and Henzell 2000, Rolando et al. 2013). Insufficient selectivity of foliar herbicides when OMB is growing amongst native vegetation or other desirable species is a major issue for its control (P. Raal, NZ Dept. of Conservation, pers. communication, March 2019).

In areas where no trees or other supports are available, OMB creeps along the ground and sprawls over grasses and other vegetation. In riparian zones where this is true, aerial spraying is not an option, as the herbicides usually used are toxic to aquatic ecosystems and not registered for use over waterbodies. In New Zealand, all chemicals except glyphosate are restricted within 10 metres of a waterbody (J. Keast, Horizons Regional Council, pers. communication 25 March 2021). In these situations, a directed foliar spray can be applied from the ground, using selective herbicides that leave grasses intact, such as those listed above for aerial spraying. This reduces spray drift and keeps the herbicide out of waterways. However, in addition to poor selectivity to the native plants often established in these zones, for some commonly used herbicides, the persistence and phytotoxicity that make them valuable against target species are simultaneously detrimental in other ways. For example, picloram and aminopyralid are effective against woody weeds but highly persistent; because of that persistence, Hickman et al. (1990) found that picloram can remain soil-active and may be absorbed by non-target species after leaching from a target plant's roots. Picloram and aminopyralid are also both known to persist in plant tissues (WSSA 1994, Kline et al. 2005), and be prone to run-off and leaching into surface and groundwater (Smith et al. 1988). As alternative selective herbicides, triclopyr and metsulfuron-methyl have been shown to be less environmentally persistent than picloram and

aminopyralid (Jotcham et al. 1989, Ismail and Lee 1995, Monaco et al. 2002, Ferrell et al. 2006, Trabue et al. 2006, Tran et al. 2015), though as a drawback to its use, metsulfuron-methyl can damage some grass species (Harrington and He 2010, Harrington et al. 2017). There is a need to evaluate the efficacy of chemicals in common use by local governments in New Zealand to control OMB without harming existing grass cover, comparing herbicides with lower environmental persistence with those of higher persistence.

1.5 Conclusion

Old man's beard is one of the most problematic and invasive plant species introduced into New Zealand, where control of the species to date has done little to slow its spread. In this chapter, key points from academic literature on relevant aspects of OMB's biology, ecology, and control were addressed, as a way of providing the reader with background about the species and informing the reader about what is known and where knowledge is lacking. The remainder of this thesis details research that helps fill in the knowledge gaps about OMB and can potentially improve the effectiveness of its management. The focus of the thesis is on OMB seed biology and ecology, vegetative reproduction, and herbicide efficacy.

2. Seed dispersal

2.1 Introduction

An overview of old man's beard phenology, pollination, seed set, and seed dispersal was provided in Sections 1.4.3-1.4.6. Some researchers have claimed that a large fraction of OMB seeds persist on the mother plant throughout the year, acting as an aerial seed bank for the species (Van Gardingen 1986, Bungard et al. 1997a): the intermittent release of seeds via wind dispersal from this seed bank presumably help to facilitate its invasion of new habitats in New Zealand. Determining the significance of this "aerial seed bank" as a potential driving factor of OMB establishment in new areas could help improve understanding of OMB's invasion success. Also important is understanding how OMB's dispersal methods influence its invasiveness.

For small propagules that are dispersed via anemochory, the most common direct method of monitoring dispersal involves the use of a trap. Two previous studies used trapping methods to monitor OMB fruit dispersal. West (1992) studied a single forested 10 x 20 m location in the Rai Valley, NZ, in which OMB took up approximately 10% of the basal area. Ten traps were positioned at random throughout the study site, to estimate seed density and duration of dispersal. Seed traps were removed and replaced monthly for one year, and viable seed was found upon each trap removal. Substantially higher seed catch occurred in June and August, but an average of at least 10 seeds/m^2 were caught each month. The second study (Nikoloff 2011) focused on dispersal distance; four seed traps were placed at regular intervals along a straight 30 m transect extending from each of three separate mature OMB plants. This was replicated in four different locations near Christchurch, NZ. Some traps were also placed 500 m away from any known mature OMB plants. The traps at 500 m caught a small handful of seeds, indicating that long distance wind dispersal of the species can occasionally occur. Transect traps were emptied only twice during the seven-month study period (March-September) but caught significantly more total seeds on average than in West's study. The disparity between the results of the two studies suggests that the methods were inadequate to get a true representation of dispersal, the density of OMB may have been quite different in the two studies, there is large variation in OMB seed output between populations, and/or that site physical differences had some effect. Very little research has been conducted to determine the timing of OMB's anemochorous seed dispersal. Despite being relatively valuable for observing dispersal, measuring wind dispersal by trapping is limited by inexact predictions about where achenes might land (Bullock et al. 2006).

Plant invaders in riparian zones have been shown to use more methods of propagule dispersal than invaders of other ecosystems (Catford and Jansson 2014). Old man's beard seeds are primarily wind dispersed, yet the species is common in Mediterranean and Central European floodplains (Deiller et

al. 2003, Guariento et al. 2020, Copete et al. 2021), and West (1992) claimed that most OMB infestations in New Zealand are found near rivers. She speculated that part of the reason for this was that stem fragments and seeds can also be dispersed by water courses, particularly during flooding events (Figure 2.1). Seeds could be blown off stems to waterways, and those that fall or are blown to the ground near waterways could get washed into the waterway by a flood. High flow events could deposit these seeds in safe, favourable locations for colonization (Truscott et al. 2006, Dołkin−Lewko and Zajączkowska 2022). However, no documentation has shown that OMB seeds are tolerant of prolonged contact with water, that they can be secondarily dispersed by water, nor that seedings can potentially establish after flooding.

Figure 2.1: Riparian area on the Manawatu River in Awapuni, Palmerston North, NZ. Left: during a short-term flooding event 14 September 2021; right: the same location, captured several weeks after the flood waters receded. This is a typical environment for *Clematis vitalba*. Due to a large infestation above the average waterline, this area was used for three separate experiments for this thesis.

The current research objectives were twofold: 1) determine how long seeds remain attached to the mother plant by measuring wind dispersal rate over time from the point of origin; and 2) determine OMB seed and seedling water tolerance with a series of small-scale studies. Knowledge from these investigations will contribute to an understanding of the plant's reproductive strategy. In addition, the outcome here augments research results on the viability of aerial seeds throughout the year (Chapter 4) in determining the extent of OMB's aerial seed bank.

2.2 Materials and methods: Anemochory

Three heavily infested river floodplain locations (Figure 2.2) in the Manawatu Region were chosen as monitoring sites, with a minimum distance of 3 km between them: 1) Site A, land under the

responsibility of Horizons Regional Council and at the junction of NZ State Highway 3 and the Oroua River, at Awahuri (-40.2765, 175.5208); 2) Site K, Horizons Regional Council land approximately 3 km east of NZ State Highway 1 at the junction of Kakariki Road and the Rangitikei River (-40.1238, 175.4458); and 3) Site M, an area defined by Land Information New Zealand (LINZ) as stop-bank land on the Oroua River approximately 1.5 km NW of NZ State Highway 54, in Aorangi (-40.2589, 175.5551).

Figure 2.2: A *Clematis vitalba* vine heavily laden with achenes before dispersal.

To avoid the imprecision of predicting landing locations, achene release was regularly monitored from the vine, by tagging individual seed heads. At each study site, five randomly selected seed heads per stem were tagged with jeweller's tags on ten separate OMB stems varying in exposure to wind and height from the ground (between 30 cm and 2 m) in March 2020. GPS coordinates of each stem were recorded, as well as the initial number of achenes per seed head. Numbers of remaining achenes were determined monthly until all had been released. A small fraction of achenes with conspicuous evidence of granivory were also counted as lost seeds, because the seeds were no longer viable.

As a measure of relative wind and moisture exposure of each stem, a tatter flag was positioned on a free-standing bamboo stake as near as possible and at the same height as each stem (Linklater 1998). Tatter flags were constructed by cutting 100% natural cotton calico into 30 x 37 cm pieces, then 7 cm of the longer end was wrapped around and glued to a length of Wetta Eco Tube plastic irrigation piping (19 mm x 30 cm). Thus, the freely moving part of the flag measured 30 x 30 cm. The tatter flags were positioned on a free-standing bamboo stake (1 cm x 2.4 m) as close as possible to the stems

at the same height without touching them. To keep the flags in place but allow unobstructed movement in the wind, a piece of Wetta reinforced vinyl tubing (12.5 mm x 1-2 cm) was positioned above and below each tatter flag and taped to the bamboo stake with duct tape (Figure 2.3). Tatter flags were replaced with new flags every two months. The intention was to oven-dry (60^{o}C) the flags and weigh them before set-up, and then repeat this process after removal, to determine weight loss over the two-month period. Tatter flag weight loss has been shown to correlate with wind run, and to increase with increased precipitation (Rutter 1965). Because OMB seeds are wind-dispersed, it has been assumed that those most exposed to wind are most likely to disperse early (West 1992). Heavy rain could also dislodge mature seeds. Therefore, a correlation between fruit loss and flag tatter would be expected.

Tagging set-up was planned for the end of March 2020 but was unexpectedly moved forward several days due to the Covid-19 pandemic response and took place in the final hours before the NZ lockdown. Due to the expedited nature of the set-up, the tatter flags could not be weighed beforehand. A sample of fresh flags was weighed 2 months later when the lockdown was over, so an approximate average initial weight and range of variation in weight could be determined. The pandemic lockdown also meant that no counts of achenes could be made 1 month after the start of observations but started at 2 months and continued every 2 months thereafter. At each 2-month removal, flags were oven-dried again and weighed. By month 8 (November), almost all seed heads had been overgrown and covered by the current year's growth and other vegetation and were thus no longer exposed to the wind. Therefore, no flags were used from months 8 to 10.

Figure 2.3: Tatter flag set up level to and as close as possible to labelled stem (white arrow) with five tagged seed heads (red dots).

2.2.1 Analysis: Anemochory

Achene loss data were fitted to a 2-parameter log-logistic time-to-event model, using the drm function in drc package in R 4.0.4 (Ritz et al. 2015, R Core Team 2021), with the following equation:

$$f(t) = \frac{d}{1 + \exp\left[b\{\log(t) - \log(t50)\}\right]}$$

where t = time elapsed in months; d = upper limit of achene loss (1); $t50$ = time to 50% seed loss relative to the initial number observed; b = the rate of change of the curve between the upper and lower limits. A one-way ANOVA was performed on the parameter estimates for $t50$ and b from the drm model, and a Tukey post hoc test was used to compare differences between the means (Larson 1992, Ghanizadeh et al. 2015) at α=0.05, using Graphpad Prism v.9.2.0. The relationship between the rate of seed loss and flag tatter was tested with a Kendall's tau-b correlation test, run in R base package, version 4.0.4 (R Core Team 2021). R code for analyses is given in Appendix G.

2.3 Results: Anemochory

As a vine, OMB exhibits indeterminate growth. Its seeds, therefore, are produced asynchronously throughout summer. As seeds develop, achenes are initially green and fleshy, and style hairs lie flat against the styles. Following achene maturity, they progressively senesce, flatten slightly, and darken to purple and then to reddish-brown or dark brown, while the style hairs become white and fluffy. At the time of tagging in March 2020, most achenes were still fleshy green or purple, and firmly attached to the seed head receptacle, though a small, more mature proportion were drier and darker. By the end of May, almost all achenes had darkened and become brittle enough that extremely gentle handling was required to avoid causing achenes to break off the receptacle. In those first two months, all but ten out of 150 seed heads experienced loss, constituting an average of 49% of all initial achenes.

Overall, by the end of June (three months since tagging), all achenes at all locations were dark brown and senescent with fluffy styles. Total seed loss averaged 77% by that stage, showing a high dispersal rate within the first few months after maturity. Time to 50% seed loss was highly significantly different between all three sites, but by six months, all three had comparable losses (Figure 2.4, Table 2.1): at the end of September, over 95% of achenes had gone. The rate of seed loss change varied slightly though significantly only between Sites A and K. This demonstrates that although seed head maturity could be somewhat correlated within a site, due to microclimate, asynchronous development of seeds can lead to variations in seed loss rate, i.e. even if they are released at the same rate when sufficiently desiccated, indeterminate seed release follows indeterminate seed production.

Dense new OMB growth was beginning to cover the previous year's stems in October, and by November, many stems were so buried under new vegetation that they were difficult to locate. At the end of December, no achenes were left at Sites K and M, and only one individual achene remained (0.03%) at Site A. That achene was gone by the end of the next month.

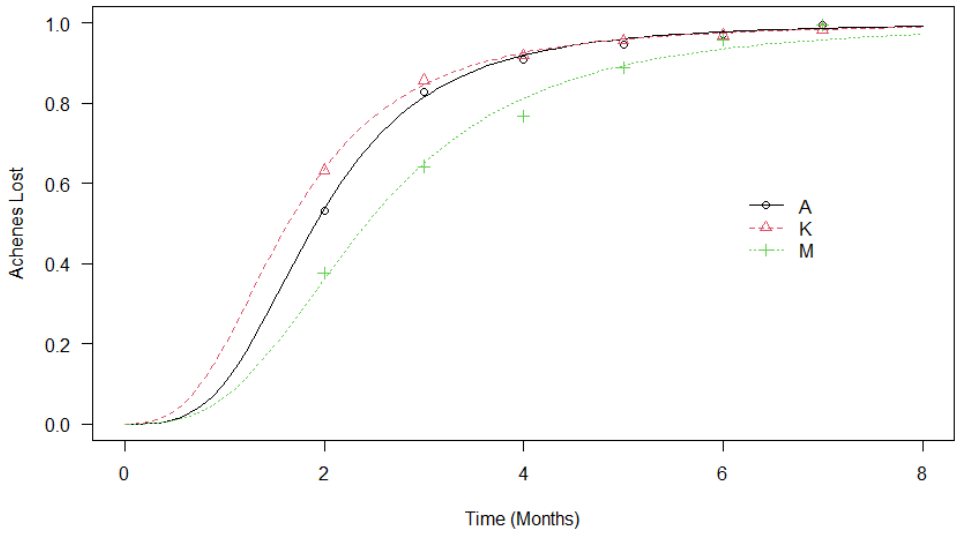

Figure 2.4: Seed loss curves (derived using 2 parameter log-logistic model, drc package R) and the site-month averages from tagged stems over time between March and November 2020 from three different sites (Site A=Junction State Highway 3 and Oroua River, Awahuri; Site K=Junction Halcombe Road and Rangitikei River, Kakariki; Site M=1119 Camerons Line, Aorangi). Time 0=March, 2=May, 4=July, 6=September, 8=November.

Table 2.1: Parameter estimates (log-logistic model, drc package R) of seed loss from three populations: Awahuri, Kakariki, and Aorangi populations. Slope (b) = rate of change of the curve between 0 and 1; T50 = time to 50% seed loss in months. Estimates with at least one common superscripted letter are not significantly different (α<0.05).

Site	*b* slope (SE)	T50 (SE)
Awahuri (A)	-3.31 (0.14)^{b}	1.92 (0.04)^{b}
Kakariki (K)	-2.83 (0.13)^{a}	1.65 (0.04)^{c}
Aorangi (M)	-2.95 (0.10)^{ab}	2.43 (0.05)^{a}
p-value	0.031	<0.001

At each tatter flag removal census, either the seed loss data or the flag weight loss data, or both, had non-normal distribution. The subsequent rank correlation test between the pairs of observations found no relationship between the two data sets at any of the two-month intervals, at α=0.1 (tau range was 0.018-0.28). Figure 2.5 shows the lack of correlation, but also displays the progression of seed desiccation and loss: each interval shows seed loss proportions nearing 100%, but fewer seeds overall.

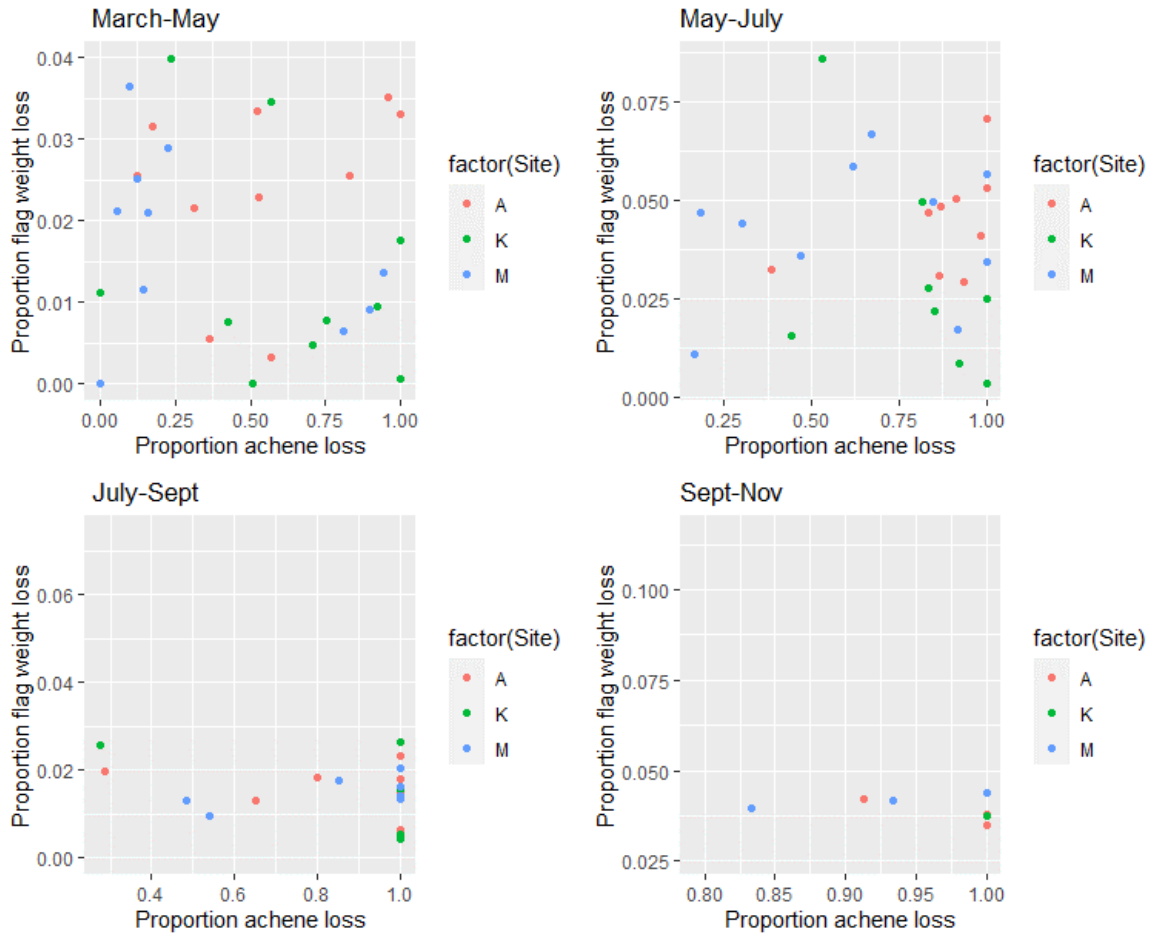

Figure 2.5: Relationship between proportions of achene loss and flag tatter for two-month intervals from March to November 2020 at three different sites (Site A=Junction State Highway 3 and Oroua River, Awahuri; Site K=Junction Halcombe Road and Rangitikei River, Kakariki; Site M=1119 Camerons Line, Aorangi).

2.4 Materials, methods, and results: Hydrochory

Achenes for all of the following studies were collected in September 2020 from the same provenances as listed in Section 4.2 and were stored stored dry in an airtight container at 5°C until required.

2.4.1 Seed buoyancy in still water (24 Oct-19 Nov 2020, 12-20 Dec 2020)

The achene with a persistent style is the natural dispersal unit of OMB. With styles intact, 100 achenes from each source were placed in still water in 15 x 15 x 8.5 cm polypropylene basins, 50 achenes per basin in an unheated glasshouse All achenes were observed to float on the water surface for 1 week, and then for an additional 2.5 weeks just below the surface. In contrast, 20 achenes with styles removed floated for a maximum of 8 days, and then sank completely. Thus, it appears that the lightweight style supports buoyancy in still water.

2.4.2 Seed buoyancy in moving water (Nov 2020, Feb 2021, Jul 2021)

Experiments with no water movement exaggerate seed buoyancy, due to surface tension (Vivian-Smith and Panetta 2005, Truscott et al. 2006), hence this follow-up buoyancy study was undertaken. After 2 months of dry storage at 5°C, a sample of 100 achenes were placed in a 450 ml jars half-way full of water, 10 achenes to a jar. The water was swirled vigorously for 30 seconds, and allowed to settle. All achenes remained at the bottom of the jar. Samples from the same collection, removed after 4 months and 10 months of storage in the same conditions, were swirled in the same way, but re-surfaced and floated. Swirling was repeated 4-5 times over the course of 24 hours, by which time no achenes re-surfaced after settling.

2.4.3 Seed viability response to short-term immersion (8 Nov 2020-2 Jan 2021)

After 2 months of dry storage at 5°C, 10 OMB achenes with intact styles were placed in each of seven 450 ml jars, which were filled halfway with water. Each jar was repeatedly swirled until all achenes sank to the bottom of the jar and remained there. The contents of one jar were removed from the water each day for 7 days, sown in a 750 ml pot in potting mix, and watered regularly. Thus, by Day 7, all seeds had been removed from immersion and planted. Pots were monitored weekly for seedlings until no new seedlings had emerged for 2 weeks; then a final assessment of seedling numbers and size was made (Day 59). Table 2.2 summarises the results and shows that seed viability was not adversely affected by up to 7 days of immersion before germination.

Table 2.2: Presence and size at 7 weeks of seedlings produced by *Clematis vitalba* seeds immersed in water from 1-7 days, followed by a transfer to potting mix.

immersion (days)	Seeds with surviving seedlings (N=10)	Seedling mean maximum height (cm)	Seedling mean number true leaves
1	7	5.0	2.9
2	8	4.6	3.1
3	10	2.9	1.9
4	5	4.5	2.6
5	6	6.7	4.0
6	7	6.0	3.3
7	10	6.2	3.0
Mean (SE)	7.57 (0.719)	5.13 (0.489)	1.1 (0.243)

2.4.4 Seed response to prolonged immersion (24 Oct-26 Dec 2020)

In October 2020, preliminary work to test OMB seed germination rate in soil was done, by sowing 20 seeds just under the surface in each of 10 1.5 L plastic planter bags filled with a potting mixture of 50% peat moss, 25% sand and 25% pine bark.. The first seedlings began emerging 20 days after sowing. Similarly, after 3.5 weeks floating on or just below the surface in a full 15 x 15 x 8.5 cm polypropylene basin of still water, an average of 90% of achenes per basin had germinated in the

water (4 basins with 20 achenes each). Half of the germinated seeds were then removed from immersion and sown in 750 ml pots filled with potting mix, while the remaining seeds were left in water. Water was replenished as needed, to maintain a full basin. Weekly monitoring for an additional 5 weeks took place to observe seedling development and survival, both in water and in soil. No more seeds germinated in water after 3.5 weeks immersion. Figure 2.6 details the timeline of seedling development of both the potted and immersed seedlings. In summary, both cohorts continued elongation and remained healthy for approximately 1.5 week after germination, after which immersed seedlings began to decline (Figure 2.7), but potted seedlings continued robust development.

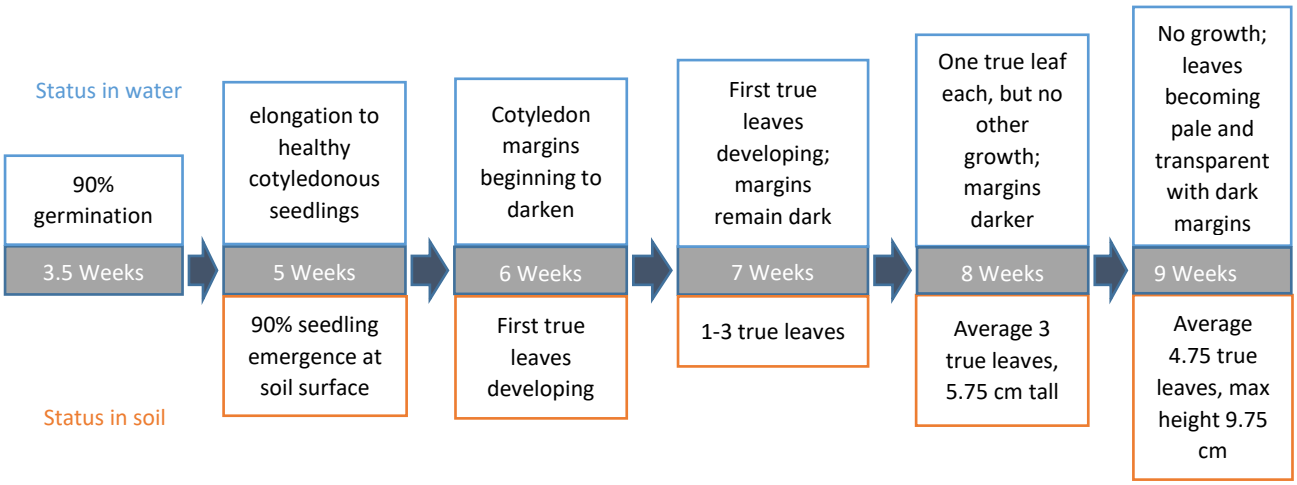

Figure 2.6: Contrast of *Clematis vitalba* seedling development in soil after removal from water following germination (lower boxes) compared to seedling decline under continuing immersion conditions (upper boxes).

Figure 2.7: Response of *Clematis vitalba* seeds to prolonged immersion in water. Left: Healthy cotyledonous seedlings 1.5 weeks after germinating in water (5 weeks total immersion). Right: Immersed true seedling decomposing 3.5 weeks after germinating in water (7 weeks total immersion).

2.4.5 Seed response to intervals of immersion after germination (12 Dec 2020-6 Feb 2021)

After 3 months in dry storage at 5°C, 52% of seeds (26/50) immersed in a 15 x 15 x 8.5 cm polypropylene basin of still water had germinated within 20 days. To test if germinated seeds can resume normal development in potting soil and produce seedlings after extended immersion, a sample of germinated seeds were removed from the water at weekly intervals after germination and sown in 750 ml pots filled with potting mix: immediately after germination by Day 20, 1 week after germination by Day 20, two weeks after germination by Day 20, three weeks after germination by Day 20, one week after germination by Day 27, and one week after germination by Day 34. After 5 weeks immersion, no more seeds germinated. Table 2.3 summarises the results, namely that 78% of seeds germinated while immersed, and by 8 weeks after the trial began, of the germinated seeds, approximately half produced seedlings after being sown in soil. The study was discontinued at 8 weeks.

Table 2.3: *Clematis vitalba* seedling development in soil following germination while immersed at 3 weeks, 4 weeks, and 5 weeks.

Week germinated	Seeds germinated (proportion of total, N=50)	Week potted	Seeds potted (proportion of germinated seeds from indicated week)	Seedling emergence through soil by 8 weeks (proportion of germinated, potted seeds)
3	26 (0.52)	3	7 (0.269)	3 (0.429)
3		4	6 (0.231)	4 (0.667)
3		5	7 (0.269)	6 (0.857)
3		6	6 (0.231)	3 (0.5)
4	5 (0.10)	5	5 (1)	3 (0.6)
5	8 (0.16)	6	8 (1)	1 (0.125)
Total	39 (0.78)		39 (1)	20 (0.513)
Mean (SE)				0.53 (0.101)

2.4.6 Summary of results: Hydrochory

Research conducted October 2020-January 2021 showed that:

1. The persistent, plumose style of OMB achenes can aid it in temporary buoyancy, perhaps by lowering the overall density of the achene (Carthey et al. 2016). However, the style does not provide enough support to allow the achene to re-emerge and float at the surface of moving water after imbibing and sinking within 24 hours;
2. Longer dry storage leads to lower seed water content, and drier achenes are more likely to float, at least temporarily. Yet ultimately, all OMB seeds that land in water with some turbulence will sink within a one-day period, even with intact styles.
3. Seeds immersed up to six weeks in still water can germinate and do so at a similar rate to seeds in soil.

4. Seedlings that remain immersed in still water after germinating there begin to decay by approximately 6 weeks total immersion.
5. Seeds that germinate while immersed and are removed from water at the cotyledon stage can continue to develop robustly when removed from water and sown in potting soil.

2.5 Discussion

Results of the wind dispersal study show that most OMB seeds persist on the stems for less than a year after ripening, and therefore provide only a transient seed bank from one reproductive cycle to the next. West's (1992) seed trapping results did show that in some situations, a small proportion of seeds can remain on the mother plant throughout the year. Yet, the outcome of the current research is in contradiction with claims by other authors, that OMB plants retain their seeds throughout the winter, and that a large proportion of seeds remain into the following summer (Lhotská 1974, Van Gardingen 1986).

Presumably some of the "dispersed" seeds were lost to granivory. An average of less than 2% of achenes were damaged by predation but persisted on the receptacle, so it is likely that other achenes suffered predation and were removed entirely. From July onwards, low-growing OMB stems appeared to have OMB achene "nests" resting in the foliage below them, comprising masses of fluffy styles with partially eaten achenes still attached (Figure 2.8). These appeared to have been removed from the receptacle by granivory, and either dropped or intentionally placed below the seed head by the predator. However, it was impossible to know what proportion of the achenes in the nests originated from the tagged seed heads.

Figure 2.8: Predated achene "nests" resting among the foliage.

All tagged seed heads needed to be easily accessible for monitoring and so were between 30 cm and 2 metres above the ground, some on stems hanging from trees, and some sprawling along the ground. Thus, no data was taken of seed dispersal from stems high in the canopy, from which it is likely there would be fewer obstructions and greater exposure to wind (Wright et al. 2008). Thus, the data collected from this study is likely to underestimate the rate at which seeds are released from OMB stems at heights greater than 2 metres.

Monthly monitoring of seed loss from tagged stems showed that the peak dispersal period (i.e., the period that included ≥ 95% of seed release) for OMB seeds is a 6-month window from autumn (March) through to early spring (September), with approximately half of all seeds dispersing before winter sets in. These results are supported by my observations during regular seed collections in the Hawke's Bay Region over a 2-year period, where some populations have few or no visible persistent achenes, or achenes that are increasingly rare past October (no data collected). West (1992) reported achenes first ripening a little later in April in the Marlborough region, so this dispersal window may vary slightly around the country.

The deciduous branches of OMB are largely bare of leaves in the late autumn and winter and pose less of an obstacle to dispersal of windblown seeds than leafy stems (Nathan and Katul 2005). Hence this period is advantageous for long-distance dispersal.

Another factor that likely decreases the chances of persistent aerial seeds contributing to a viable seed bank beyond the first year is that other weeds and the current year's new growth rapidly covers and intertwines with growth from the previous year. As early as May, some of the lower OMB stems at Site M were being overgrown by *Delairea odorata* (German ivy), and at Sites A and M, blackberry was growing into some OMB stems. By July, some of the low stems were also being overgrown by tall, vigorous grasses and other weeds. By the end of September, new shoots 1-6 cm long were observed developing on stems hanging from trees, and the new growth had largely blanketed old stems by October. This caused the remaining seeds to be less exposed, and thus less likely to be dispersed very far by the wind. Therefore, if/when the seeds are released, they are apt to drop directly below the mother vine, many getting caught on lower vegetation, with a very small chance of reaching the ground.

Thus, the end of the peak dispersal season coincides with the new flush of vegetative growth of the plant; before the new growth occurs, almost all seeds have dispersed as far as possible. Consequently, it is reasonable to assume that any seeds remaining on the mother plant past the peak dispersal period do not make an appreciable difference in OMB's survival or the expansion of its range.

The scatter plots and a Kendall rank correlation test showed no relation between seed loss proportion and flag weight loss at any of the 2-month intervals, which suggests no clear effect of degree of wind/rain exposure on seed loss rates. It should be noted that flag tatter measurement in the first 2

months was an imprecise estimate, due to the inability to weight the flags during the pandemic lockdown. In subsequent months the weight loss was recorded but showed little relation to the seed dispersal rates. One possible factor contributing to the poor correlation could be that wind strength alone does not facilitate achene release, and that seed senescence needs to reach a critical stage. Due to the indeterminate nature of OMB, new seeds develop throughout the season, and by autumn mature achenes are at different stages of senescence (Figure 2.9); fruit at earlier stages of senescence tended to remain attached to the receptacle, regardless of wind strength. To get more meaningful information about seed loss, shorter tatter flag intervals might be valuable, although if a seed head releases most of its achenes within a few days, tatter loss measured after that point still would not be useful, despite the shorter interval length. The overgrowth from weeds and new OMB stems noted above reduced wind exposure, which could also have contributed to the lack of correlation. Predation was probably also partially responsible for an inadequate relationship between seed loss and flag tatter, as seed loss due to granivory is unlikely to be associated with wind movement.

As noted in Section 1.4.8, although fully differentiated, seeds are dormant at maturity, with an undersized embryo. The results clearly indicate that OMB seeds can and do disperse early in the season, when still dormant. Under natural conditions, after-ripening occurs during the autumn and winter months, when the embryo gradually loses dormancy. This can take place both on the mother plant or on the ground. However, not only does early dispersal provide a greater chance of long-distance dispersal, it also puts seeds in a position to germinate as soon as after-ripening conditions have been met and external conditions are favourable.

Figure 2.9: Achenes at different stages of senescence

The water tolerance studies suggest that since OMB can tolerate prolonged immersion, the assumption that the seed is adapted to secondary hydrochory is reasonable. Water dispersal could extend its anemochorous dispersal distance considerably. The bulk of OMB achenes are released intermittently by parent plants over the autumn and winter seasons. Anemochory may cause some achenes from plants growing near waterways to land in water, where they could stay immersed several weeks before a flood or blow onto the waterway in a more severe event. Regardless of how they arrive, seeds in water are unlikely to get washed up onto a suitable site without a flooding event. Results from these studies indicate that OMB achenes will not remain buoyant long while immersed, but flooding can also transport non-buoyant fruit, along with sediment and other debris (Gurnell et al. 2008). Flooding creates bare, sediment-rich sites above the average waterline, favourable for seedling establishment (Vesipa et al. 2017). If, after up to 6 weeks' immersion, OMB seeds or seedlings are washed ashore by a flood at a suitable site they could conceivably colonise the riparian zone.

Six weeks seems to be the threshold duration of water tolerance for OMB during late spring and summer (average maximum temperatures 18-24°C), at least for germinated seeds and seedlings in still water. In Section 2.4.4, the breakdown of the immersed seedlings began just as their first true leaves were developing, i.e., just as a higher rate of photosynthesis was required to support seedling growth (Figures 2.6, 2.7). CO_2 movement and transpiration are both necessary for normal photosynthetic processes (Catford and Jansson 2014), but under water, CO_2 movement slows and transpiration cannot take place. Thus, they become limiting factors for immersed seedling survival.

The difference between the immersed seedlings and the potted seedlings could also have been partly due to an increasing lack of oxygen availability for the seedlings in water. Seedling respiration and small but building amounts of algae in the water likely contributed to the oxygen decline in that environment. Moving water tends to have more dissolved oxygen in it than still water, which is why other researchers have simulated movement on a water course with orbital shaking or other methods (van den Broek et al. 2005, Truscott et al. 2006, Mao et al. 2019). It is therefore possible that OMB seedlings present in moving water could survive longer than was observed here in still water.

Additionally, Mao et al. (2019) found that immersed propagules survive longer in cool temperatures (10-15°C) than those above 20°C. Hence, it is probable that OMB seeds dispersed during the peak autumn-spring dispersal window could survive longer than 6 weeks. Furthermore, flooding events that occur in springtime are more likely to result in successful seedling recruitment, as spring conditions are the most favourable for OMB germination and growth.

The un-germinated seeds from these studies were not tested for viability, and so it cannot be assumed that they were not simply dormant. However, a gradual accumulation of algae in the water and on all immersed plant material indicated that the seeds were also breaking down.

2.6 Conclusions

Data from the wind dispersal research indicate that nearly 50% of OMB seeds are released from the mother plant during dormancy, before winter temperatures occur. Old man's beard (OMB) seed production has been estimated at approximately 35,000 seeds/m^2/yr (Van Gardingen 1986). However, this estimate was made in June, at which time around half of seeds would already have dispersed, and thus could be well over 70,000 seeds/m^2/yr. All but about 5% of the remaining seeds are lost while OMB stems are bare, and before stems develop new spring growth. Granivory certainly contributes to this loss, but it is unknown what proportion of seeds are actually eaten and which are viable, wind-dispersed propagules. Further investigations into the extent of seed predation could help clarify how much seedling recruitment contributes to OMB's survival and invasiveness. In order to learn how representative the results from this study are for overall OMB seed loss, research on seed loss rates from stems above 2 m high would also be valuable, especially from the tree canopy.

Although the water tolerance studies were done on a small scale, the results point to the plausibility that OMB employs both wind and water as methods of seed dispersal. OMB produces roughly 50,000 viable seeds/m^2/year (between 65% and 72% of the total; see Section 4.4.1). Employing at least two forms of dispersal methods allows this species to disperse its multitude of propagules more widely. West (1992) suggested that stem fragments and seeds of OMB are dispersed by flooding of water courses. These studies did not look at hydrochory of vegetative propagules. However, young stems from OMB that inhabit trees along rivers often hang down over the water, which could be broken and transported downstream in a flood event. Indeed, fragments can be valuable supplementary sources of plant dispersal by flood (Truscott et al. 2006, Stella et al. 2013, Vesipa et al. 2017), particularly because stem fragments may have more food reserves than seeds, and also young seedlings are fragile and highly susceptible to environmental stress (Deiller et al. 2003). In addition, it is now known that OMB stem fragments that are kept moist and buried after fragmentation have approximately a 50% chance of regenerating (Chapter 7).

As likely vectors of propagule dispersal, waterways could promote the invasion of OMB. Thus, management strategies that focus on controlling riparian populations to prevent downstream dispersal could be highly beneficial. These results could be improved by assessing seed and seedling survival in moving water in a range of temperatures, and by testing the viability of un-germinated seeds with a tetrazolium assay. However, propagules swept into waterways by a flood event are not likely to spend more than a few days in the water before deposition, and temperature would be of minor importance in such a case. Without a flood event, most propagules in moving water would ultimately end up in the ocean and lose viability from salinity stress.

3. Factors that influence germination

3.1 Introduction

A key aspect of invasive plant success is the ability to reproduce and proliferate beyond the bounds of its original introduction. Annual seed production for old man's beard is likely over 70,000/m^2/yr (Section 2.6). Due to the sheer abundance of propagules they produce, plants like OMB often have a high potential for successful establishment in new locations, even when a portion of the seeds are not viable (Leishman and Harris 2011; Simberloff 2009). Upon arrival to new areas, successful germination in a variety of environmental conditions improves the chances of successful establishment (Baker 1974, Colautti et al. 2006, Pyšek and Richardson 2007, Baskin and Baskin 2014, Gioria and Pyšek 2017). Therefore, evaluating the germination characteristics of a species can help determine if traits associated with the embryonic plant are an important component of its invasiveness.

As mentioned in Section 1.4.8, seed germination occurs following the alleviation of dormancy as a response to the presence of suitable environmental conditions (Vleeshouwers et al. 1995), which include light, temperature, and nutrient availability. Pre-chilling at 5°C has been claimed to be necessary to break dormancy and induce germination (Rudolf 1974, Grime et al. 1981, Bungard et al. 1997b, Copete et al. 2021). Incubation of OMB seeds in constant, warm temperatures (especially ≥ 20°C) does not appear to be conducive to germination, unless seeds are prechilled (Lhotská 1974, West 1992). However, a regime of fluctuating temperatures with a low temperature of 10°C, can also break OMB dormancy, including after incubation in warm temperatures (Lhotská 1974, West 1992, Vinkler et al. 2004, Picciau et al. 2017). Some such regimes have been shown to result in a higher maximum percentage of germination than in seeds prechilled at 5°C (West 1992, Picciau et al. 2017).

Adding nitrogen or gibberellins appears to have a positive influence on OMB germination (Bungard et al. 1997b, Picciau et al. 2017). Gibberellins (GAs) are said to promote germination in seed after abscisic acid (ABA) synthesis and the expression of genes blocking germination are removed (Baskin and Baskin 2004b). ABA levels are lowest and GA levels highest in non-dormant seeds (Obroucheva 2010). GA produced by the embryo activates enzymes that break down the endosperm and allow the radicle to break through the seed (Leubner-Metzger 1996, Leubner-Metzger 2001, Linkies & Leubner-Metzger 2012). Researchers have also suggested that potassium nitrate may help regulate ABA or GA levels, and thus have a germination signalling role in dormant seeds (Alboresi et al. 2005, Hernandez et al. 2022). Higher nitrate presence is correlated with soil fertility, and it is commonly used to break seed dormancy, sometimes with the

addition of light (Alboresi et al. 2005, Bewley et al. 2013, Kołodziejek et al. 2017). Indeed, Bungard et al. (1997b) found that a combination of chilling, nitrogen (the form is unimportant) and light, or simply two of the three factors, improved germination of OMB more than any one factor alone. However, other studies have come to conflicting conclusions about the effect of light on OMB germination (Van Gardingen 1986, Vinkler et al. 2004).

Chilling is known to promote seed reactivity to applied GAs (Bewley et al. 2013). Yet, Picciau et al. (2017) reported that although chilling alone did significantly increase germination rate, OMB seeds germinate as well in response to added GA_3 without chilling as they did to chilling alone.

Studies have shown that GAs can increase nitrate absorption and metabolism, and thereby improve plant growth (Bai et al. 2016, Wang et al. 2020, Sun et al. 2021). Others have suggested that the presence of high nitrate levels in seeds can enhance GA synthesis, and thus germination (Alboresi et al. 2005). Regardless of which promotes the other, the combination of gibberellin and nitrogen has been used to improve seed germination in a variety of plant species (Chauhan et al. 2006, Ghani Zadeh 2015, Dev et al. 2020). However, no OMB research has investigated the effect of nitrogen and gibberellin combined on germination. Scarification has also been overlooked in OMB germination research. Seed coats of OMB soften considerably within 3 months of burial and are easily naturally scarified with any frictional contact (personal observation during research detailed in Chapter 5). Achenes removed directly off the vine do not have a softened seed coat, and although the OMB achene does not have an especially hard coat that prevents imbibition, scarification can make a seed coat more permeable, allowing quicker imbibition, gas exchange, and even change light or stratification requirements of seeds (Baskin and Baskin 2014).

The natural dispersal propagule of OMB is an achene with a persistent style (Figure 3.1). The hairy OMB style is quite long and curly and could potentially make it more difficult to handle the achene in germination experiments. Also, when dry, the lightweight style is lifted easily by wind, which could pose a problem in field experiments. It has been suggested that germination in the presence of a style is no different from germination without it (Van Gardingen 1986, West 1992), but this has not been documented. Removing the style would likely have made other experiments for this thesis easier, and therefore was scrutinised here for its effect.

Figure 3.1*: Clematis vitalba* achenes with persistent, hairy styles.

This experiment was designed to find the most efficient ways to artificially break dormancy and stimulate germination of seeds collected mid-winter, so that this knowledge could be applied to subsequent experiments.

The objectives for the experiments outlined in this chapter were to determine the response of OMB seeds to the following artificial treatments:

- mechanical scarification
- removal of the style
- exogenous nitrate
- exogenous gibberellic acid
- exogenous nitrate and gibberellic acid combined
- complete darkness
- various temperature/light regimes

The information gained here could potentially provide deeper insight into OMB's natural germination requirements.

3.2 Materials and methods

3.2.1 Seed material and methods: Experiment 1

Achenes from two locations in the Woodville/Dannevirke area (Woodville Ferry Reserve, -40.3369, 175.8185 and Hopelands Reserve, -40.3590, 175.9627) were collected randomly throughout the sites in mid-July 2019 and stored dry at 5°C for 5 days before incubation. Seeds were expected to be dormant at this time of year (winter). All apparently non-viable seeds were discarded, about 1/3 of the total (flat, eaten, or smaller than 50% of average size). A sample of 200 achenes was x-rayed, of which 99.5% appeared filled. A follow-up tetrazolium assay (following the International Seed Testing Association's protocol (Leist et al. 2003)) for viability made on the x-rayed sample found 88% of the achenes had viable embryos, making the overall viability of the collection approximately 59%.

Achenes from both locations were pooled together. Four replicates of 20 achenes with styles intact (S) and four replicates of 20 achenes with styles removed (NS) each were subjected to five different treatments in varying temperature regimes for 4 weeks. The Massey University Seed Technology Laboratory, where this research was done, runs germination tests using a chilling room at constant 5°C with no light; incubators at constant 15°C, 20°C, and 25°C with constant light; and incubators set with alternating temperatures (20/30°C) and corresponding alternating dark/light conditions (16h:8h). These were thus the conditions available for use. Two replicates for each treatment were placed side-by-side in a 900 ml polypropylene rectangular container with an air-tight lid on double-thick germination blotters moistened with reverse osmosis water, unless otherwise noted. Treatments were as follows:

1) untreated control [Control];

2) scarification on one side of the achene (1 mm x 1 mm sliver sliced off the exocarp) [Scar];

3) blotters moistened with 0.02% v/v potassium nitrate solution [2 mM KNO_3], (Bungard et al. (1997b) reported that optimal germination in OMB was stimulated with up to 2.5 mM nitrogen).

4) achenes soaked overnight in 250 ppm gibberellic acid [GA_3] (Picciau et al. (2017) reported that this level of GA_3 enhanced germination of OMB at all temperatures they tested);

5) achenes soaked overnight in 250 ppm GA_3 and placed on blotters moistened with 0.02% KNO_3 solution [KNGA].

Temperature regimes for each treatment were:

- 1 week, 2 weeks or 4 weeks prechilling [1WK, 2WK, 4WK] at 5°C in the dark, then a transfer to constant 20°C incubator in constant

- constant 15, 20, or 25°C in constant light, with no prechilling;
- and alternating temperatures 20/30°C in corresponding alternating dark/light conditions (16h:8h), with no cold stratification.

Blotters were kept moist throughout the experiment. Germination cabinets provided light with four 40 W fluorescent white tubes with a photosynthetic photon flux density (PPFD) of 40 µmol/m2/s. Germination, defined as radicle breakthrough of the exocarp, was monitored and recorded every second day over the course of the experiment. Incubation for all treatments was limited to 28 days.

3.2.2 Seed material and methods: Experiment 2

Achenes were collected at the end of June 2020 from the same two locations as in 2019 and stored dry at 5°C until incubation. Again, approximately 1/3 of seeds were discarded for apparent non-viability. X-rayed achenes were 94.5% filled. Of those, 94.8% were estimated viable in a tetrazolium assay. Thus, total viability was again roughly 60%.

Seeds were subjected to the same treatments and temperatures as in Experiment 1, except the 1-week prechilling followed by 20°C incubation regime was dropped. Instead, all treatments in constant 15°C with no prechilling were duplicated, but with the dishes wrapped in foil, to block out all light for complete darkness and simulate burial. For each replicate, 25 seeds were placed on double-thick blotters moistened as in Experiment 1, in 90 mm diameter x 15 mm high polystyrene petri dishes and sealed with Parafilm (PM-992). A comparison between S and NS achenes was made in all treatments at 15°C and 20/30°C, but only in the control and scarification treatments at 20°C, 25°C and both prechilling regimes (the most natural treatments). Treatments lasted 4 weeks, and one replicate was made every 2 weeks three separate times (3 replicates per treatment combination altogether). Moisture was maintained throughout the experiment, and germinated seeds were recorded and removed every 4 days. All seeds that did not germinate or decompose during the incubation period were tested for viability using a 1% 2,3,5-phenyl tetrazolium chloride solution.

3.2.3 Statistical analysis

Except as stated, all analyses were performed using R statistical software, version 4.2.0 (R Core Team 2021). Style effect on germinability was analysed for each experiment separately, with the 2-step meta-analytic event-time model proposed by Jensen et al. (2020), using the following packages: car (Fox & Weisberg, 2019), plyr (Wickham, 2011), dplyr (Wickham, Francois, Henry, & Mueller, 2022), ggplot2 (Wickham, 2016), magic (Hankin, 2005), metafor (Viechtbauer, 2010), multcomp (Hothorn, Bretz, & Westfall, 2008), devtools (Wickham, Hester,

Chang, & Bryan, 2021), drc and drcData (Ritz, Baty, Streibig, & Gerhard, 2015), and ggpubr (Kassambara 2020). Germination data were fitted to separate 3-parameter log-logistic time-to-event models, using the following equation:

$$f(t) = \frac{d}{1 + \exp\left[b\{\log(t) - \log(t50)\}\right]}$$

where $f(t)$ = the proportion of seeds that germinated between time 0 and Day 28; d = upper limit of achene loss (assumed to be 1); $t50$ = time to 50% germination relative to the maximum number observed; b = the rate of change of the curve between the upper and lower limits (lower limit= 0). Parameter estimates from the fitted models were then estimated as weighted means in a meta-analytic model, with replicates as random effects, and treatment, style, and model parameter as fixed effects:

$$\hat{b}_i = \mu_{1i} + \theta_{1i} + \varepsilon_{1i}$$
$$\hat{d}_i = \mu_{2i} + \theta_{2i} + \varepsilon_{2i}$$
$$\hat{t}_{50i} = \mu_{3i} + \theta_{3i} + \varepsilon_{3i}$$

where $\hat{b}_i$, $\hat{d}_i$, and $\hat{t}_i$ = estimates for the model parameters for each replicate i; μ_{1i} , μ_{2i} , μ_{3i} = averaged estimates of replicates for the same treatment; θ_{1i}, θ_{2i}, θ_{3i} = quantified variability between replicates; ε_{1i}, ε_{2i}, ε_{3i} = residual error.

Multiple pairwise comparisons were then made on all parameters of each treatment, between S and NS seed germination, using the Benjamini-Hochberg procedure for controlling false discovery rate.

Because the 2-step models showed inconsistency in the influence of the style, and because OMB's natural dispersal unit is an achene with an intact style, all further analyses were restricted to seeds with intact styles. Maximum germinability of seeds was predicted with binomial logistic regression analysis for Experiment 1 (packages used: tidyverse, caret, aod and arm ((Lesnoff and Lancelot 2012, Wickham et al. 2019, Gelman and Su 2021, Kuhn 2021). Goodness of model fit was tested by plotting simulated residuals with the DHARMa package (Hartig 2022), shown in Appendix A, Figures A.1 and A.2. Data on seed decay and post-incubation viability were also recorded for Experiment 2, and therefore a multinomial logistic regression was used to predict ultimate seed status in the repeat experiment. In addition to the above, R packages nnet, ggplot2, and reshape2 were employed for this analysis (Venables and Ripley 2002, Wickham 2007, 2016). The dependent variable categories were: "germinable", "decayed", or "viable but dormant". Multicollinearity was calculated using the generalised variance inflation factor (GVIF) in R, using the car package (Fox and Weisberg 2019). As this could not be done on the multinomial model, a GVIF was calculated on a separate binomial model for each of the dependent categories.

In addition, a meta-analytic random-effects event-time model (Jensen et al. 2020) was used to generate predictions of germination curves for the two temperature regimes with the highest germinability overall (4WK and 20/30°C; packages used: as listed above for the influence of the style). To simplify the model fit process, prior to meta-analysis, chi-square tests of independence indicated no significant variation in the maximum germination between the replicates of the same treatment combinations (SPSS, version 27). Therefore, the six replicates were merged into two replicates for each treatment combination.

3.3 Results

3.3.1 Patterns in effects of temperatures and treatments

Total germinability of seeds across all temperature/treatment combinations in both experiments was low (Figure 3.2), although germinability in Experiment 2 in 2020 (27%) was substantially lower than in Experiment 1 in 2019 (40%). This difference may have been due to seeds being collected about 3 weeks earlier in 2020 (end of June) than they had been in 2019 (mid-July) and being presumably more deeply dormant. TZ assays showed 83% of ungerminated seeds to be viable from Experiment 2, so the majority were either germinable or viable, but dormant.

Despite some differences between the seed collections, clear patterns were also apparent for both lots of seed: 1) seeds responded poorly to all treatments at 20°C and 25°C, except if the seeds were pre-chilled for at least 2 weeks; 2) scarification significantly improved germinability of otherwise untreated seeds, both in constant and alternating temperatures; 3) potassium nitrate and gibberellic acid together were often more effective than any other treatment, but not always significantly more than scarification, GA_3, or KNO_3 alone.

In addition to the poor germinability at 20°C and 25°C, a single week of prechilling (Experiment 1 only) did not result in substantially more germination at 20°C than no prechilling, nor did the constant 15°C regime in complete darkness (Experiment 2 only). None of these temperature regimes achieved 50% germination or more in any treatment in either experiment, but tetrazolium assays indicated most remained viable. They were thus eliminated from any statistical analysis, as these were all clearly insufficient conditions to bring seeds out of dormancy. Seeds collected mid-winter responded much more readily to 20/30°C, 15°C with light, or 2 or 4 weeks prechilling followed by 20°C incubation. Hence, the subsequent analysis focused on these four regimes.

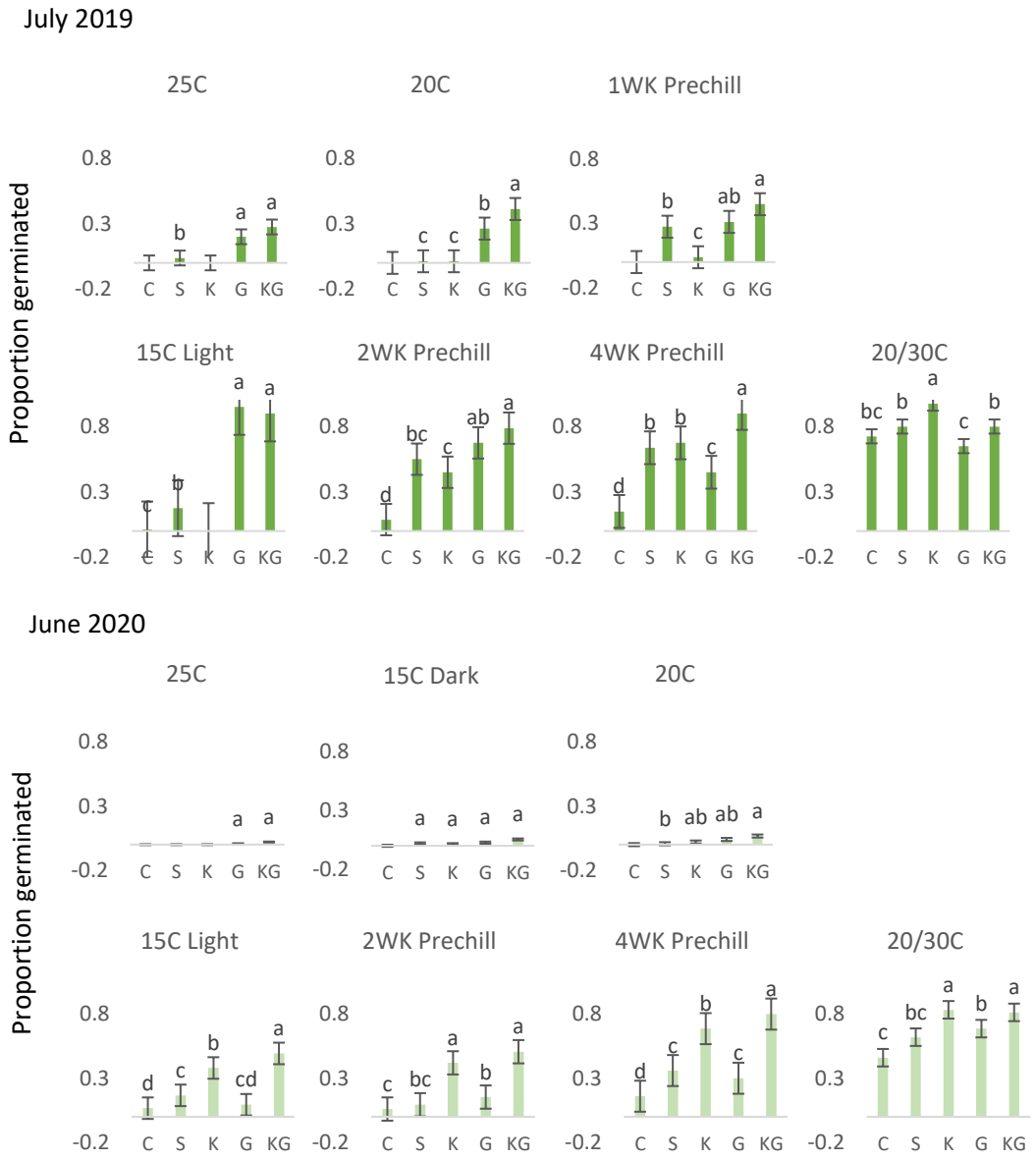

Figure 3.2: Maximum mean germination (proportion) of *Clematis vitalba* seeds at 28 days (raw data) for Experiment 1, July 2019 (top) and Experiment 2, June 2020 (bottom). Comparison between five different treatments: C = Control (blotters moistened with water); S = Scar (scarification: 1 mm sliver sliced off achene exocarp, blotters moistened in water); K = KNO_3 (blotters moistened with 0.02% KNO_3 solution); G = GA_3 (achenes soaked overnight in 250 ppm GA_3, blotters moistened with water); KG = KNGA (achenes soaked overnight in 250 ppm GA_3, blotters moistened with KNO_3)) within seven different light/temperature regimes (constant light at constant 25°C; constant light at constant 20°C (no prechilling, 1 week 2 weeks or 4 weeks prechilling (1WK 5°C, 2WK 5°C, 4 WK 5°C); constant light at constant 15°C (15°C Light), constant darkness at constant 15°C (15°C Dark); and alternating dark and light (16h:8h) with alternating temperatures (20/30°C)). Proportions with at least one common letter are not significantly different (2-sample tests for equality of proportions without continuity correction in Base-R; $p<0.05$). Error bars based on standard errors.

3.3.2 Influence of intact style

Seeds in the 2-step meta-analysis model of the 2WK, 4WK, 15°C and 20°/30°C treatments from Experiment 1 had enough germination to produce a curve, and here, the importance of an intact style on the rate and maximum germination % varied by treatment (Appendix A, Tables A.1 and

A.2). Two-thirds of the treatments indicated significant differences between S and NS seeds for maximum germination or speed, and 80% of those indicated that an intact style was beneficial. Figure 3.3 shows treatments where seeds with styles were significantly more germinable than those without.

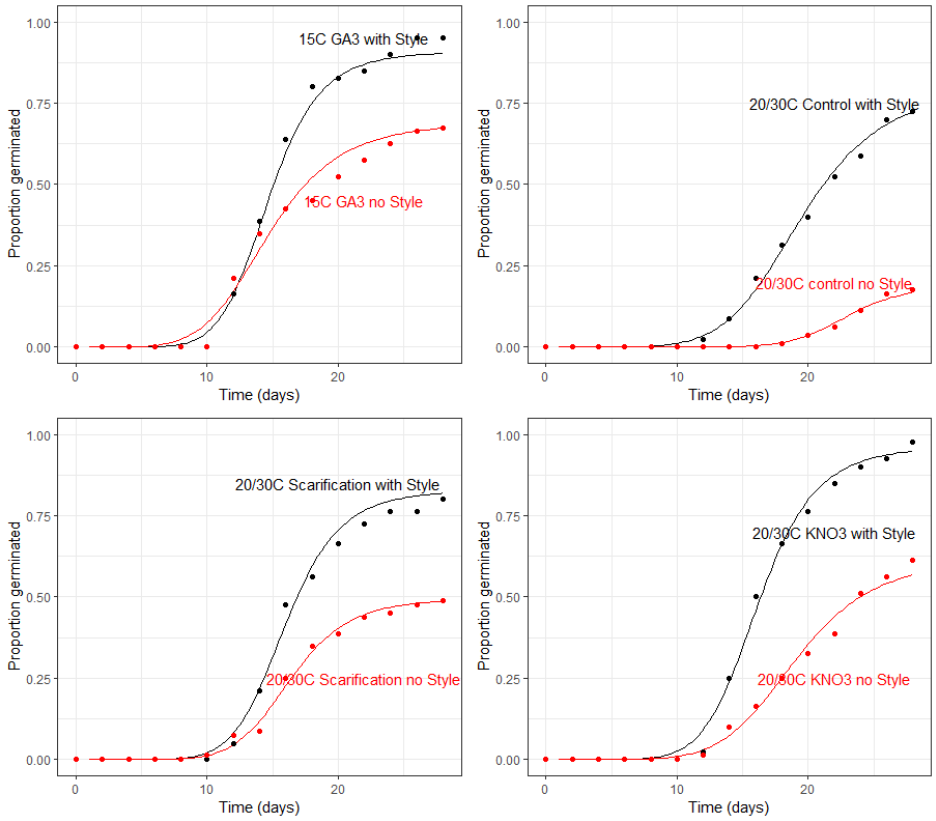

Figure 3.3: Significant effect ($p<0.05$) of an intact style on maximum germinability of *Clematis vitalba* seeds in various treatments in experiment 1 (Control = no treatment other than moisture with reverse-osmosis water, GA_3 = gibberellic acid, KNO_3 = potassium nitrate, Scarification = sliver of seed coat removed) during incubation for 28 days in July 2019 at 15°C or alternating between 20°C and 30°C (20/30C), as indicated.

However, in the event-time model for Experiment 2, none of the treatments for which significant differences occurred due to style presence or removal in 2019 were also significantly different in 2020 in the same way (Appendix A, Tables A.1 and A.2). Indeed, in all treatments that showed a significant effect, the presence of a style had the opposite effect from Experiment 1, NS seeds germinated better and/or faster. Some treatments indicated a significant effect in an entirely different parameter (maximum germination, slope, or time to 50% germination—20/30°C Scar, KNO_3 and KNGA). Also, one showed an effect in the repeat experiment where there was none in the earlier experiment (2WK Scar). Figure 3.4 shows treatments where seeds without styles were significantly more germinable than those with styles.

Because of the inconsistencies between the experiments, all further analyses for these experiments were restricted to seeds with intact styles. Also because of the inconsistencies and because the natural dispersal unit is an achene with a style, the style was left intact for all subsequent experiments.

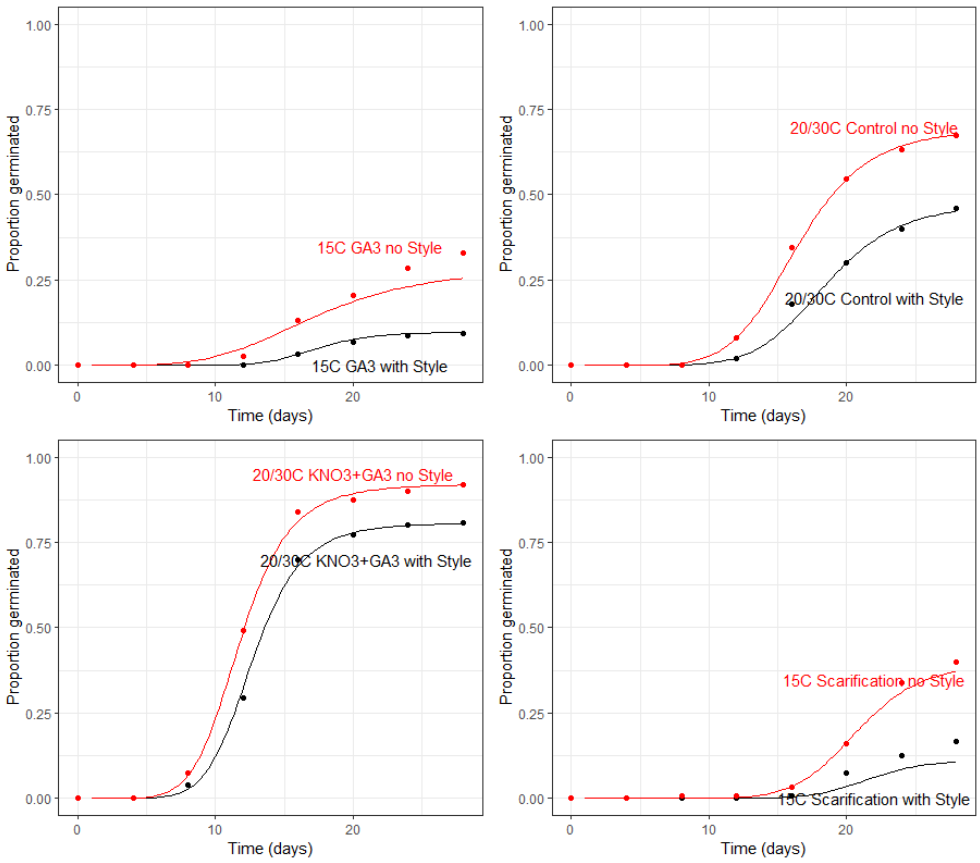

Figure 3.4: Significant effect (p<0.05) of removing the style on maximum germinability of *Clematis vitalba* seeds in various treatments (Control = no treatment other than moisture with reverse-osmosis water, GA_3 = gibberellic acid, KNGA = potassium nitrate and gibberellic acid, Scarification = sliver of seed coat removed) during incubation for 28 days in July 2020 at 15°C, alternating between 20°C and 30°C, or prechilling at 5°C for 2 weeks and incubating at 20°C (2WK Prechill), as indicated.

3.3.3 Influence of temperature/treatment combinations

In Experiment 1 (Figure 3.5), data on decay and dormancy were not collected, and therefore the reason for lack of germinability could not be ascertained. The following two results were unique to Experiment 1 in a binomial regression analysis for germinability: 1) A combination of 15°C with light appeared to be highly conducive to germination in the GA_3 and KNGA treatments (95% and 90%, respectively); and 2) although all three treatments improved germinability over control, the relative effectiveness of scarification, KNO_3 and GA_3 to encourage germination was highly variable in 2019.

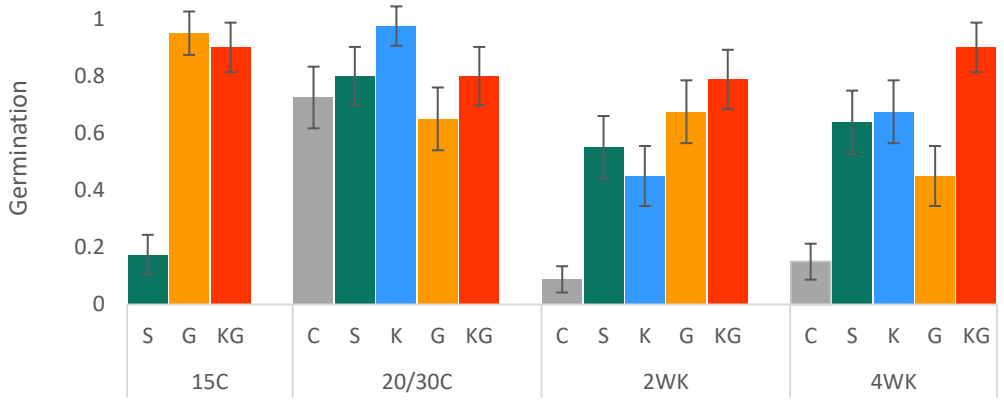

Figure 3.5: Observed treatment effect in experiment 1 (15C = 15°C, 20/30C = temperature alternating between 20° and 30°, 2WK = incubation at 20°C following 2 weeks prechilling, 4WK = incubation at 20°C following 4 weeks prechilling; C = Control, G = GA_3, KG = KNO_3 + GA_3, K = KNO_3, S = Scarification) on *Clematis vitalba* seed germination in 2019. Error bars denote 95% confidence intervals.

Figure 3.6 displays differences between treatments in Experiment 2, in which germinability, dormancy, and decay were considered. In contrast to Experiment 1, seeds in all temperatures responded more positively to KNO_3 in Experiment 2 than seeds in the scarification or GA_3 treatments. Also, 15°C with light failed to promote germination at the same high rates as in Experiment 1 for GA_3 and KNGA: respective Experiment 2 germinabilities were 9% and 49%. Control seeds in all temperature regimes other than 20/30°C stayed largely dormant, with less than 20% average final germination.

Multinomial regression analysis results showed that treatments which resulted in the most germination were also more likely to have seeds that decayed rather than stayed dormant if they did not germinate. For example, compared to the reference treatment, 2WK Control, 4WK and 20/30°C seeds treated with KNGA had similarly high odds ratios for germination over decay (21.38 and 25.55, respectively, with respective germinabilities of 79.3% and 81.3%). The odds ratios for remaining dormant rather than decaying for both were very low (4WK: 0.15; 20/30°C: 0.17), and nearly 90% of seeds that did not germinate were likely to decay. On the other hand, treatments that resulted in low germination were more likely to remain dormant (e.g. 2WK Control germinability was 6%, decay 22.7%, and dormancy 71.3%).

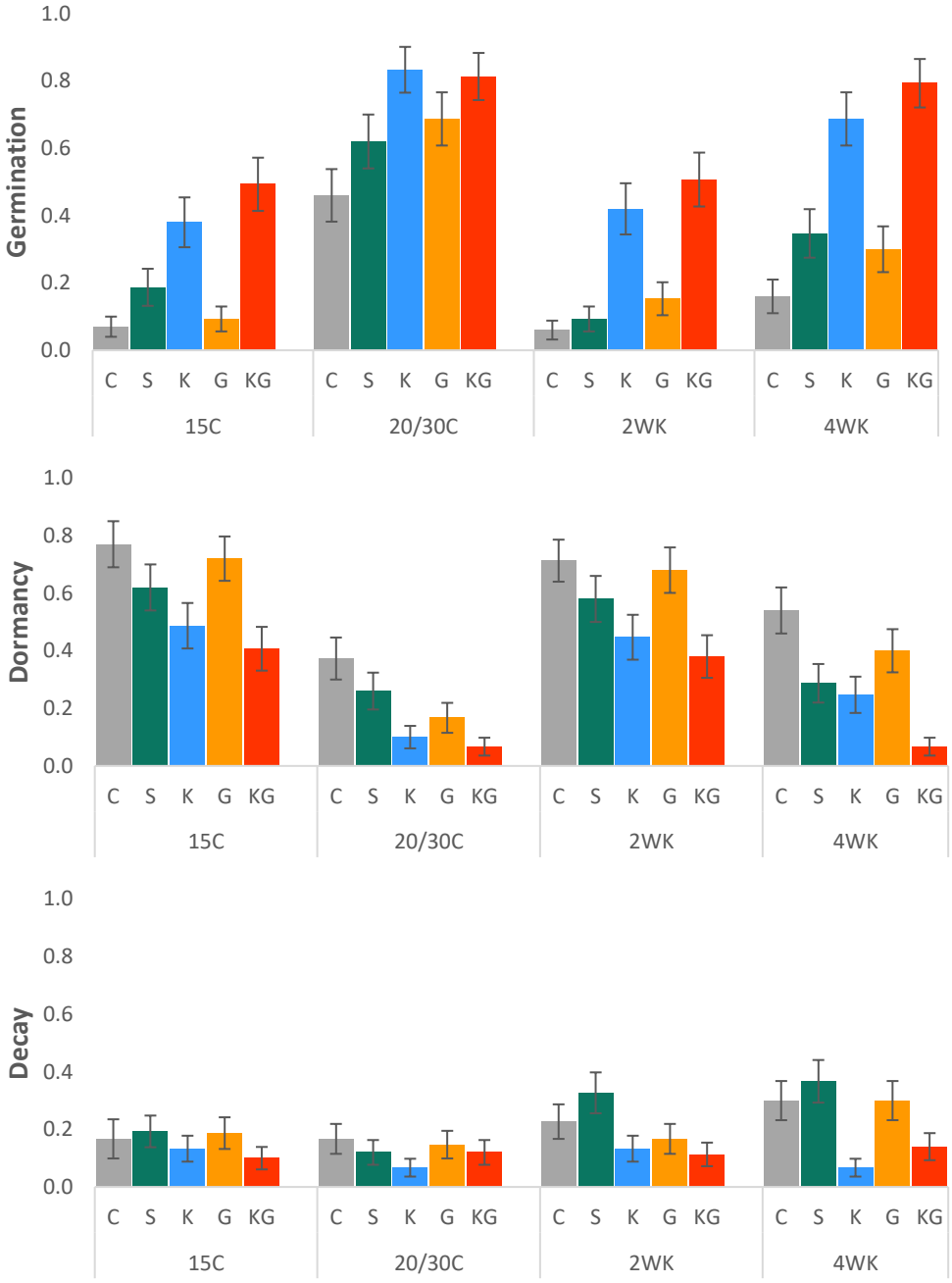

Figure 3.6: Observed effect (15C = 15°C, 20/30C = temperature alternating between 20° and 30°, 2WK = incubation at 20°C following 2 weeks prechilling, 4WK = incubation at 20°C following 4 weeks prechilling; C = Control, G = GA_3, KG = KNO_3 + GA_3, K = KNO_3, S = Scarification) on *Clematis vitalba* seed germination , dormancy , and decay following a 28-day incubation period in July 2020. Error bars denote 95% confidence intervals.

Both the binomial regression of Experiment 1 and the multinomial regression of Experiment 2 on seeds with styles indicated a highly significant overall treatment effect ($p < 0.001$; Appendix A, Table A.3). Germinability of seeds without any treatment other than the temperature/light regime (Control) was not high at 20/30°C (maximum germination 72.5% in 2019 and 46.0% in 2020), but significantly higher than that of control treatments in any other temperature regimes. Scarification,

GA_3 and KNO_3 all improved germinability of otherwise untreated seeds, both in constant and alternating temperatures.

Prechilling up to 4 weeks significantly improved overall germination for seeds incubated in most constant temperatures, and KNO_3 combined with GA_3 resulted in higher germination than any other treatments in both prechilled temperature regimes. Yet, except for similar germinability to 20/30°C in the KNGA treatment, prechilling 4 weeks (4WK) was not nearly as effective as the alternating temperature regime without prechilling for any treatment. Indeed, for both experiments, predicted probabilities for KNO_3-treated seeds in 20/30°C were the highest of any temperature/treatment combination.

The only temperature regimes that produced treatments with consistently high maximum germination in both years were 4 WK pre-chilling and 20/30°C. Meta-analysis of both experiments combined revealed that prechilling significantly reduced the time to germination onset and time to 50% germination for every treatment (Appendix A, Tables A.4 and A.5). Yet, seeds subjected to alternating temperature and light were significantly more germinable than the prechilled seeds, except in the KNGA treatment.

In both temperatures, treatment with gibberellic acid alone significantly increased germination speed over control and KNO_3 treatments, although KNO_3 alone resulted in significantly better germinability than GA_3. The combination of potassium nitrate and gibberellic acid resulted in both significantly faster rates and higher germinability than GA_3 alone, and consistently produced some of the highest maximum germination estimates. Although slower than treatments with GA_3, in the fluctuating temperature regime, seeds responded just as well or better to KNO_3 alone than to KNO_3 and GA_3 together (Figure 3.7), showing that exogenous GA was unnecessary to achieve optimal germination, given the right incubation conditions.

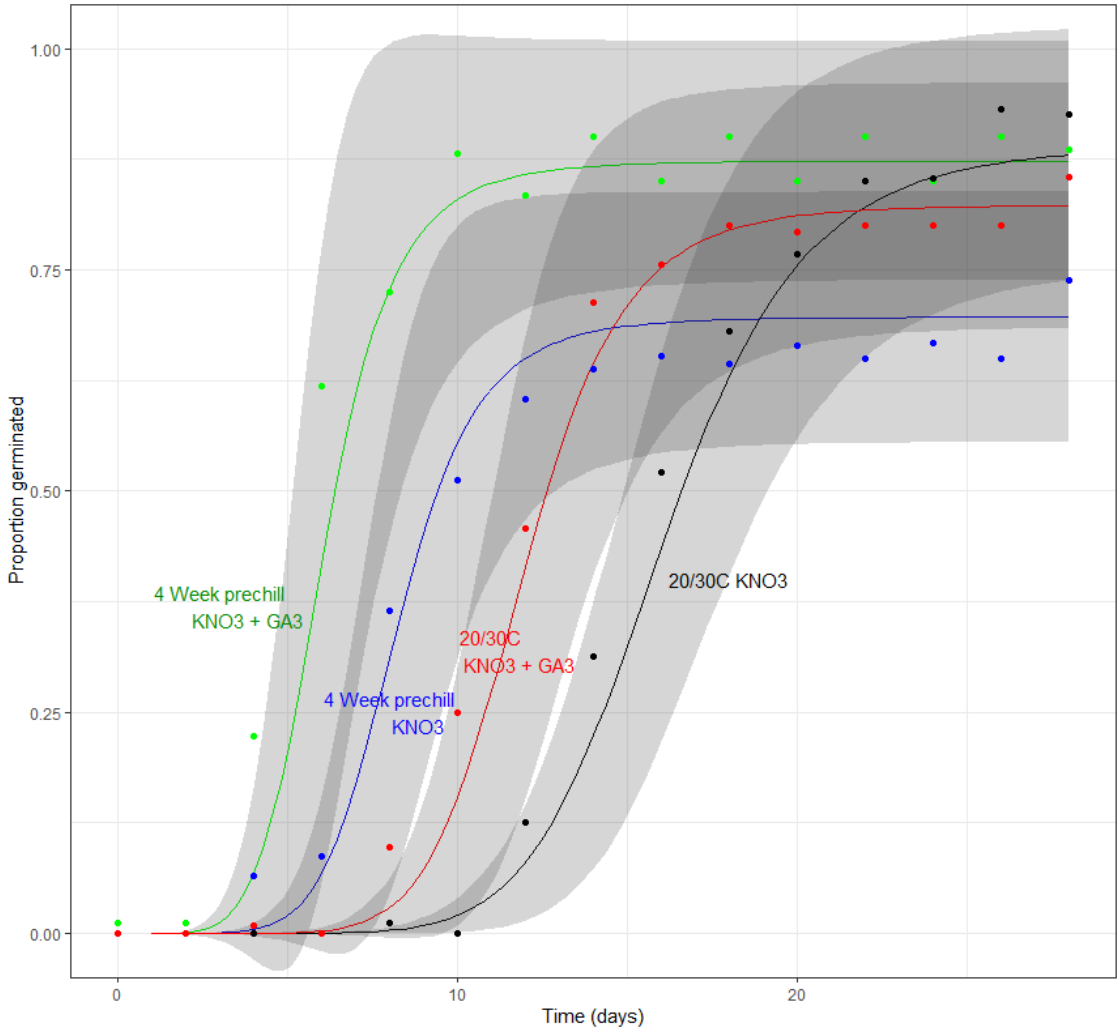

Figure 3.7: Comparison of fitted germination curves with 95% confidence bands for *Clematis vitalba* seeds treated with KNO_3 or with a combination of KNO_3 and GA_3, incubated in an alternating temperature (20/30°C) and light (16h dark:8h light) or constant temperature (20°C) and light after 4 weeks of prechilling at 5°C. Curves were predicted using a meta-analytic random-effects event-time model from data obtained in two experiments, July 2019 and July 2020.

3.4 Discussion

The present study was designed to assess the effect of a variety of treatment combinations on OMB seed germination for two reasons: a) to better understand OMB's natural germination requirements, and b) to determine which combination of factors would produce the best results for the least inputs (i.e. researcher time, money and effort), in order to apply the information in later experiments for this thesis. The results of this investigation complement those of earlier studies on OMB by substantiating some previous claims, but adding new information, and helping to clarify some issues.

Chapter 4 explains that OMB seeds come gradually out of physiological dormancy naturally between May and September: under an alternating temperature incubation regime immediately after collection, embryos of seeds produced in summer and collected in late autumn (early May), did not grow, and

remained dormant for at least four weeks. Seeds collected and immediately incubated at any time of the season beyond late autumn were increasingly more germinable. Three fewer weeks of after-ripening for the 2020 cohort compared to the 2019 cohort could certainly account for the lower overall germinability of seeds in Experiment 2 than Experiment 1. Even so, maximum germinability for seeds of all treatments in the 20/30°C regime was higher (though not all significantly so) in Experiment 2 than in Experiment 1, showing that the fluctuating temperature and light easily met the conditions to overcome the extra dormancy and stimulate germination.

Non-dormant seeds will germinate within a variety of conditions, but seeds such as those used in this research, that are progressing from complete physiological dormancy to non-dormancy are said to be partially, or "conditionally dormant" and respond to fewer stimuli (Baskin and Baskin 2004a, Kildisheva et al. 2020). Neither seeds collected in June, nor those collected in July, responded well to constant temperatures 20°C and 25°C. This finding is consistent with earlier work (Lhotská 1974, West 1992). An additional week of moist prechilling did little to improve germination.

Data regarding the importance of light for OMB germination has thus far been inconclusive. The differences may be due in part to differences in season of seed collection. For instance, Van Gardingen (1986), who reported poor germination in darkness, was working with seeds that were likely dormant, based on the findings reported in Chapter 4. On the other hand, the seeds used by Vinkler et al. (2004), which germinated well in darkness, were collected in mid-spring, and would have had very little physiological dormancy at that time. Seeds buried shallowly in the soil in the mid-central region of New Zealand's North Island would experience average temperatures as high as 15°C in September (early spring) when most OMB germination occurs naturally. Seeds collected for this study in June, incubated in total darkness at constant 15°C, had negligible germination with all treatments. Those seeds exposed to constant light at constant 15°C were significantly more germinable than those in 15°C Dark, but significantly less germinable than seeds subjected to alternating temperatures and light. Section 4.3.1 shows that control seeds of the same collection (June 2020) kept in complete darkness and incubated at 20/30°C were 17% germinable, compared to 1% at 15°C *Dark* and 10% at 15°C *Light* in this study. Along with the poor germinability of seeds in constant 20°C and 25°C, this clearly indicates that constant 15°C without prechilling is not conducive to germination of conditionally dormant seeds. Also, seeds buried too deeply to detect and respond to light will not germinate as well as those exposed to light.

In corroboration of the findings of others (Van Gardingen 1986), pre-chilling of imbibed seeds unequivocally shortened the time to germination onset and increased germination rate for seeds in all treatments: time to 50% germination was reached on average 47% faster with 4 weeks of prechilling than in the 20/30°C regime. Prechilling also improved germination at constant 20°C compared with non-chilled treatments, but the response of prechilled, untreated control seeds was poor (< 20%), so 4

weeks was inadequate to break conditional seed dormancy. This finding supports other studies that suggested maximum germination benefits were not achieved with less than two months of prechilling (Grime et al. 1981, Bungard et al. 1997b).

In contrast, although still not high, maximum germinability of control seeds with styles in 20/30°C without prechilling was near 60%. Alternating temperatures and light also resulted in relatively high final germination in all other treatments. This is consistent with previous research (Lhotská 1974, West 1992, Vinkler et al. 2004, Picciau et al. 2017). Extensive research has shown that germination stimulated by fluctuations in temperature and light are a protective adaptation to ensure seedling establishment from seeds at or just below the soil surface in areas with sufficient light, such as in forest gaps or edges (Thompson et al. 1977, Liu et al. 2013, Picciau et al. 2017). Given the current results, it is conceivable that subjecting seeds to alternating temperatures after prechilling could produce even higher germinability without increasing prechilling time.

Bungard et al. (1997b) showed that a combination of chilling, nitrogen and light, or of just two of those factors, significantly increased both speed and total germination of OMB seeds. Certainly in nature, it may be advantageous to germinate quickly after a chilling period, in order to colonise gaps, have an early advantage over other vegetation, and grow as much as possible while temperatures remain warm (Grime et al. 1981). In this study, all but one of the incubation regimes (15°C Dark) involved light. Potassium nitrate did significantly improve germination over control seeds in the prechilled regimes. Gibberellic acid also improved germination over control in prechilled regimes, as well as in non-chilled regimes, but not consistently more than KNO_3 or scarification. Indeed, in the two temperature regimes with the consistently highest germinability (20/30°C and 4WK), GA_3 improved speed of germination over KNO_3, but KNO_3 alone produced significantly higher germinability than GA_3. However, combining KNO_3 with GA_3 significantly increased germination rate. In addition, KNO_3 seems to have a synergistic relationship with GA_3. That is, the interaction generally resulted in higher germinability than with KNO_3 or GA_3 alone.

Scarification improved rate and final germination of otherwise untreated seeds, both in constant and alternating temperatures. In nature, prolonged exposure to wet soil and microorganisms promotes decay of a seed's pericarp (Baskin and Baskin 2014). As outer layers weaken and break down, the seed itself is less protected and more exposed to the immediate environment. Thus, it may respond by germinating or decaying more quickly than a seed with an intact pericarp. Scarification crudely simulates these natural processes.

Constant 15°C with light appeared to be highly conducive to germination in Experiment 1 in the GA_3 and KNGA treatments but failed to have the same results in Experiment 2. Possible explanations could be differences in seed response to that specific temperature, due to timing of seed collections, or undetected incubator differences. Differences of germinability between GA_3, KNO_3, and scarification

treatments could also be due to collection time; June seeds responded more positively to KNO_3 than the other two treatments.

An intact style does not appear to be consistently or comprehensively beneficial for germination. It is possible that a pathogen was present on the NS seeds in 2019, spread by the scalpel that was used for style removal, and causing poor germination of NS seeds (Van Gardingen (1986) reported that treatment of OMB seeds with hypochlorite reduced germination; therefore seeds were left untreated here). Dormancy differences may have also contributed to the different outcomes (collection times): perhaps removal of the style helped break dormancy in the earlier collection (2020), but not in the later collection (2019), because the later seeds were more germinable. Regardless, because of the inconsistencies, the question about style effect remains unresolved. Because of inconsistencies in style effect and the fact that the natural dispersal unit is an achene with a style, the style was left intact for all subsequent experiments.

In treatments with higher germinability, ungerminated seeds were more likely to decay by the end of incubation than remain dormant, very probably due to embryo elongation and enzymatic digestion of seed endosperm having progressed to a point where it is no longer possible for the seed to revert back to dormancy (Bewley et al. 2013); it must germinate or die. On the other hand, seeds in treatments that produced low germinability were much more likely to remain dormant than to decay, suggesting that those treatments simply were not effective enough to promote embryo growth. In nature, this tendency to remain dormant would be advantageous for OMB, allowing the species to form a seed bank, thus safeguarding its survival. It is worth noting that, after following exact protocols for cutting and staining, scoring with tetrazolium involves a subjective interpretation of the seed stain, which requires an arbitrary threshold for colour. Hence, a reproducible outcome is contingent on the technician's interpretation (França-Neto and Krzyzanowski 2019).

The conditional physiological dormancy of OMB seeds collected in mid-winter likely evolved to avoid winter germination when temperatures are not suitable for seedling survival. In this state, only a narrow range of artificial incubation conditions can break dormancy and induce satisfactory germination. This study identified three conditions which reliably fall within that range: 1) a treatment of KNO_3 incubated in an alternating temperature regime of 8 hours at 30°C with light and 16 hours at 20°C in darkness; 2) a treatment of KNO_3 combined with GA_3 (KNGA) incubated in the same alternating temperature/light regime; and 3) KNGA treatment with 4 weeks of prechilling at 5°C followed by 10-12 days incubation at 20°C in constant light. In nature, nitrogen availability in the soil changes throughout the year, with higher levels in spring, due to higher microorganism activity (Carran 1978, Taylor et al. 1982, Dawes et al. 2017). The highly positive seed response to KNO_3 in all three of these conditions simulates what might happen in spring. In the prechilling regime, adding GA_3 significantly enhanced seed response to KNO_3, although in the alternating regime this did not

occur: GA_3 seemed to have no added benefit, and was unnecessary to achieve high germinability in that setting.

Germinability differences between the three sets of conditions listed above were non-significant. Despite the accelerated germination speed following prechilling compared to the alternating regime, prechilling prolonged the total time to reach maximum germination by 10-12 days over that of the treatments incubated in 20/30°C. In the region where seeds for this experiment were collected, ambient temperature fluctuations do not consistently reach 20/30°C, even at the height of summer (February), and at no time during the year does New Zealand have fewer than 9 hours daylight. Yet, seeds still responded very favourably to 20/30°C with corresponding 16h dark/8h light. The key seems to be alternation of both temperatures and light. Therefore, considering the added length of time required to prechill seeds plus the cost of adding GA_3 to the treatment, the most efficient and efficacious treatment was KNO_3 in 20/30°C and fluctuating light. This treatment combination was used in subsequent OMB germination experiments to achieve maximum germinability.

3.5 Conclusions

These findings suggest that for any subsequent germination experiments, OMB seeds will germinate best in alternating temperatures with light/dark periods. Those treated with potassium nitrate and incubated at 20/30°C and 16h/8h light/dark should reliably achieve germinability above 80% within 4 weeks, even when seeds have residual physiological dormancy. However, this experiment did not test the performance of seeds in other alternating temperature or light regimes. It would be helpful to evaluate their effects and make comparisons with the conditions already assessed. As only constant temperatures were employed in the prechilling treatments in this study, more work could also be done to determine if partially dormant seeds without exogenous nutrients or hormones could be induced to greater germinability with alternating temperatures following a short chilling period.

Fluctuating temperatures and light can provide the requisite conditions for overcoming conditional dormancy, regardless of whether those conditions are accompanied by chilling. An implication of this is that warming temperatures due to climate change may not have a severely negative impact on the species in its current range, and could possibly allow it to expand its range, as temperature shifts occur.

A proportion of the winter-collected seeds examined in this study remained dormant in every treatment combination. Long-term seed dormancy allows a species to scatter its germination over time and helps improve its chances of successful establishment and survival. In OMB's case, long-term dormancy could contribute to its invasion success. The next chapter will look at changes in OMB seed dormancy and viability over the entire course of the after-ripening period (autumn to spring) and will

investigate whether fully after-ripened seeds can also remain dormant. Chapter 5 will look at dormancy and longevity of seeds in the soil seed bank.

4. Seed dormancy and the aerial seed bank

4.1 Introduction

Dormant seeds are inactive but viable seeds, which act as a seed bank of potential recruitment for a species over time (Section 1.4.7). Reduction of dormancy and germination of non-dormant seeds at the appropriate time and under the appropriate conditions may be pivotal features of plant establishment (Willis et al. 2014), and therefore of weedy plant invasiveness. Hence, knowledge of the seed bank of invasive species is critical for successful management of their abundance and spread. Persistent old man's beard achenes on the mother plant have been termed an aerial seed bank for the species (Bungard et al. 1997a), though Chapter 3 established that 95% of OMB seeds (70,000/m^2/yr) disperse from the mother vine by early spring, showing that the aerial seed bank has a short-term existence. Yet, the presence of such a large but temporary annual reservoir demonstrates its value for the success of the species.

OMB seeds develop asynchronously during the summer and begin to mature and dry by mid-autumn. Asynchronous dispersal from the mother plant then takes place when seeds are fully dry. As a protection against germination under circumstances that would not allow seedling maturation (winter), mature seeds are both physiologically and morphologically dormant (see Section 1.4.8), and both forms of dormancy must be overcome for germination to occur. Nikolaeva (1985) stated that OMB seeds need to undergo after-ripening with warm stratification followed by cold stratification to break dormancy. It is common for seeds to transition between physiological dormancy (PD) and activity in response to seasonal changes in shifting temperatures and daylength (Bewley et al. 2013, Baskin and Baskin 2014, Willis et al. 2014, Finch-Savage and Footitt 2017, Leuschner and Ellenberg 2017). Indeed, of OMB seeds collected over a 6-month period between winter and spring, Lhotska (1974) reported that both speed and percentage of OMB seed germination were influenced by time of collection, with highest germinability occurring from seeds collected in mid-spring. Yet, it is unclear precisely how the two types of stratification affect the development of the seed embryo, which must reach a threshold size to break morphological dormancy. In some seeds, breaking of PD happens before embryo growth, and in others, PD and MD are broken simultaneously (Baskin and Baskin 2014). Researchers have reported that OMB embryos do not grow during cold, moist stratification at constant 5°C (Bungard 1996, Copete et al. 2021). Whether or not they grow while still attached to the vine has not been established.

The objective of this experiment was to determine the extent to which aerial seed contributes to the OMB seed bank at different times of the year, by ascertaining how dormancy and viability of seeds remaining on the vine change over the course of a season. Knowing the proportion of OMB aerial seeds that are viable, and how both their germinability and their dormancy changes over time, will

give insight into the magnitude of the aerial seed contribution to OMB's invasiveness. In addition, supplemental work was carried out to discover if the MD of OMB seed embryos undergoes a similar seasonal shift like that of its PD, or if seed embryos remain underdeveloped and morphologically dormant during natural and artificial storage.

4.2 Materials and Methods

4.2.1 Seed material and population sources

To account for any population effect, achenes for this experiment were collected from two source populations. The population sites were both in riparian zones, at Woodville Ferry Reserve (WFR; -40.3369, 175.8185) and at Hopelands Reserve, near Kumeroa (KUM; -40.3590, 175.9627), Tararua District. The OMB population at WFR is on a treeless slope, where the lianas scramble over long grasses. In contrast, the KUM population is moderately dense with trees, woody shrubs, and other scrambling vines, and is less exposed to sun and wind.

Three cohorts of seeds were used in this experiment: Cohort 1 seeds began developing in December 2018, Cohort 2 in December 2019, and Cohort 3 in December 2020. At the time the new cohort of fresh seeds developed on the vines each year, only a small fraction of achenes from the previous cohort remained for collection. Indeed, achenes from each December cohort were increasingly hard to find for collection between the following November and March (11 to 15 months later), for two reasons: 1) more achenes were dispersed by the wind as time passed (see Chapter 2); and 2) new growth began covering the previous year's stems, obscuring persistent achenes (Figure 4.1). Achenes that could be found during that time were typically in very protected areas, e.g. on the leeward side of trees.

At both population sources, many of the new cohort of achenes had darkened by May, although achenes were in various stages of maturity, varying in colour from green to reddish to dark brown (Figure 4.1). This is due to differences in position on the vine (flowers produced earlier are pollinated earlier, leading to earlier seed development). Leaves had dropped from the vines, and senescence of the most mature seed heads had progressed to the point where achenes were dispersing in the wind. The darkest achenes were collected from both populations.

Figure 4.1: Left: Current year's vegetative growth covering previous year's *Clematis vitalba* achenes, January 2020. Right: *Clematis vitalba* achenes at various stages of maturity (green to reddish to dark brown and fluffy) in autumn, 2021.

By June, most OMB stems were still largely bare of leaves, and many seed heads had lost all their achenes, especially at WFR. This is consistent with monthly dispersal observations at three other OMB populations, where 60-85% of all achenes were lost between March and the end of June 2020 (see Section 2.3). By September, most stems were vigorously producing new terminal and axillary growth. Less than 5% of achenes at WFR remained intact, whereas at the less exposed KUM site, closer to 20% of achenes persisted. At WFR, no achenes were visible for collection from November through March. Greater exposure to wind at WFR likely resulted in earlier dispersal of achenes than at KUM. Therefore, only seeds from the KUM population were tested year-round, and the population comparison was restricted to the months of May, June or July and September during the two-year study period.

4.2.2 Experiment Methods

Achenes were collected every 2 months for 2 years (September 2019-September 2021) and incubated for 28 days using the most effective temperature/light regime from the germination experiments in July 2019 and 2020 (described in Section 3.5). The germination trials either began immediately following collection, or achenes were stored dry for up to five days at 5°C. For each population, three replicates of 50 seeds each were subjected to three treatments in a fluctuating temperature of 20/30°C with a corresponding fluctuation of dark/light at 16 h:8 h. Germination cabinets provided light with four 40 W fluorescent white tubes with a photosynthetic photon flux density (PPFD) of 40 μmol/m2/s. Each replicate was placed in a 900 ml rectangular polypropylene container with an air-tight lid on double-thick blotters moistened with reverse-osmosis water, unless otherwise noted. Treatments were: 1) untreated control; 2) blotters moistened with 0.02% potassium nitrate (KNO_3) solution; and 3) dark (untreated seeds in a container sealed with Parafilm and wrapped entirely with foil).

Germination was defined as radicle breakthrough of the exocarp. In all trials, germination of control and KNO_3 treatments (i.e. Treatments 1 and 2) was monitored and recorded three times per week. Any germinated or mouldy seeds were removed at each monitoring interval. Blotters were moistened as needed, usually every 4 days. Dark treatments (i.e. Treatment 3) remained sealed and wrapped during incubation, so only final germination was recorded, on Day 28. Following the 4-week incubation period, a tetrazolium assay was performed on all ungerminated seeds, to determine post-incubation viability.

4.2.3 Observations of embryo development

An average OMB seed is 2.5-4.0 mm long. Dormant embryo length is approximately 0.75 ± 0.25 mm long, between 20% and 25% as long as the seed (Figure 4.2). For a seed to germinate, the embryo needs to reach the threshold size of 1.75 ± 0.04 mm long (Copete et al. 2021), which is 50-60% as long as the seed (embryo: seed length ratio roughly 1:2).

Figure 4.2: Mature *Clematis vitalba* seed with a dormant, undersized embryo (as opposed to a non-dormant, elongated embryo shown in Figure 4.9).

4.2.3.1 Embryo size during storage

In the experiment described above, seasonal germinability patterns of freshly collected seeds were examined. As a supplement to that work, and for the purpose of discovering if seeds remaining on the mother plant lose morphological dormancy over time, seed collections stored outdoors were examined

monthly for changes in embryo size. Seeds collected in July and September 2021 were stored in synthetic mesh bags within an open-air shed, sheltered from direct precipitation, but otherwise exposed to all fluctuations of ambient temperatures and moisture. Ten to fifteen firm seeds randomly selected from each collection were imbibed and dissected each month for 10 months for embryo measurement.

4.2.3.2 Embryo growth during incubation

To investigate possible loss of seed viability during dry storage at 5°C, 200 seeds collected in September 2020 were subjected to a germination test without any exogenous nutrients or hormones added (20/30°C) when fresh and were 95% germinable. After 21 months of dry storage, 400 seeds were subjected to a second germination test in the same conditions, during which a sample of 10 randomly selected seeds were dissected and embryos measured (using Olympus CellSens Dimensions 1.6) at intervals of 2-3 days until all seeds had germinated, decayed or had been dissected.

4.2.4 Analysis

Because no samples could be collected at WFR between November and March due to complete dispersal, and because samples collected at KUM during that period were too small to be representative of the population (also due to dispersal), there were only seven collections from both sources that were sufficient to use in the statistical analysis. Still, the six additional KUM collections were subjected to incubation, to get a sense for year-round viability.

A prediction of seed non-viability at the end of the incubation period was generated with a binomial regression model, using tidyverse, caret, aod, and arm packages in R statistical software, version 4.2.0 (Lesnoff and Lancelot 2012, Wickham et al. 2019, Gelman and Su 2021, Kuhn 2021, R Core Team 2021). Another binomial regression model, using the same packages, with the same predictor interactions, was then run to predict germinability versus dormancy on the viable seeds. Multicollinearity was calculated using the generalised variance inflation factor (GVIF) with the car package (Fox and Weisberg 2019), by calculating a GVIF on separate binomial models for the main effects without interaction. Goodness of model fit was tested by plotting simulated residuals with the DHARMa package (Hartig 2022), shown in Appendix B, Figures B.1 and B.2.

4.3 Results

4.3.1 Predictor effects

At each seed collection throughout the two-year period, one-quarter to one-third of seeds from both populations were too small, flat, eaten or otherwise non-viable to be worth considering further. These were discarded. Of the rest, 94-100% were full upon x-ray examination of 100-200 achenes from each site. Therefore, approximately 72% of all seeds collected appeared to be viable during the initial assessment. An additional 15.5% of seeds either decayed during incubation or were scored non-viable after incubation (Figure 4.3). Average combined population decay during incubation of control seeds (the most natural treatment) in the three spring (the most natural season of OMB germination) collections was 3% and a further 5% scored non-viable in TZ assays.

In the binomial logistic regression analysis, of the three independent variables, neither population source nor treatment were statistically significant predictors of seed decay during or after incubation ($\alpha = 0.05$), neither as individual effects, nor in the interaction that contained both variables together (Appendix B, Table B.1). Even so, Wald tests showed the overall effect of collection date on seed viability following incubation was highly significant ($p < 0.001$), and the interaction of date and treatment was significant, as was the interaction of date and population (both $p < 0.001$). In other words, the effect of treatment or population on seed viability was unimportant, except when collection date was considered. More specifically, holding population and treatment constant, seeds in all three 2021 collections were significantly more likely to decay ($p < 0.01$) than seeds in the 2019 and 2020 cohorts. Furthermore, seeds in both control and/or KNO_3 treatments were significantly less likely to decay ($p< 0.05$) than those in the dark treatment on five of the seven collection dates: September 2019, June and September 2020, and July and September 2021. Also, seeds from the WFR population were significantly more likely to decay ($p < 0.001$) in May 2020 than KUM seeds of the same collection date: in all three treatments, the average predicted probability of decay for WFR seeds was 25.5%, and for KUM seeds 3%. Wald tests showed significantly more overall decay in May 2020 than June or September, probably because of high WFR decay.

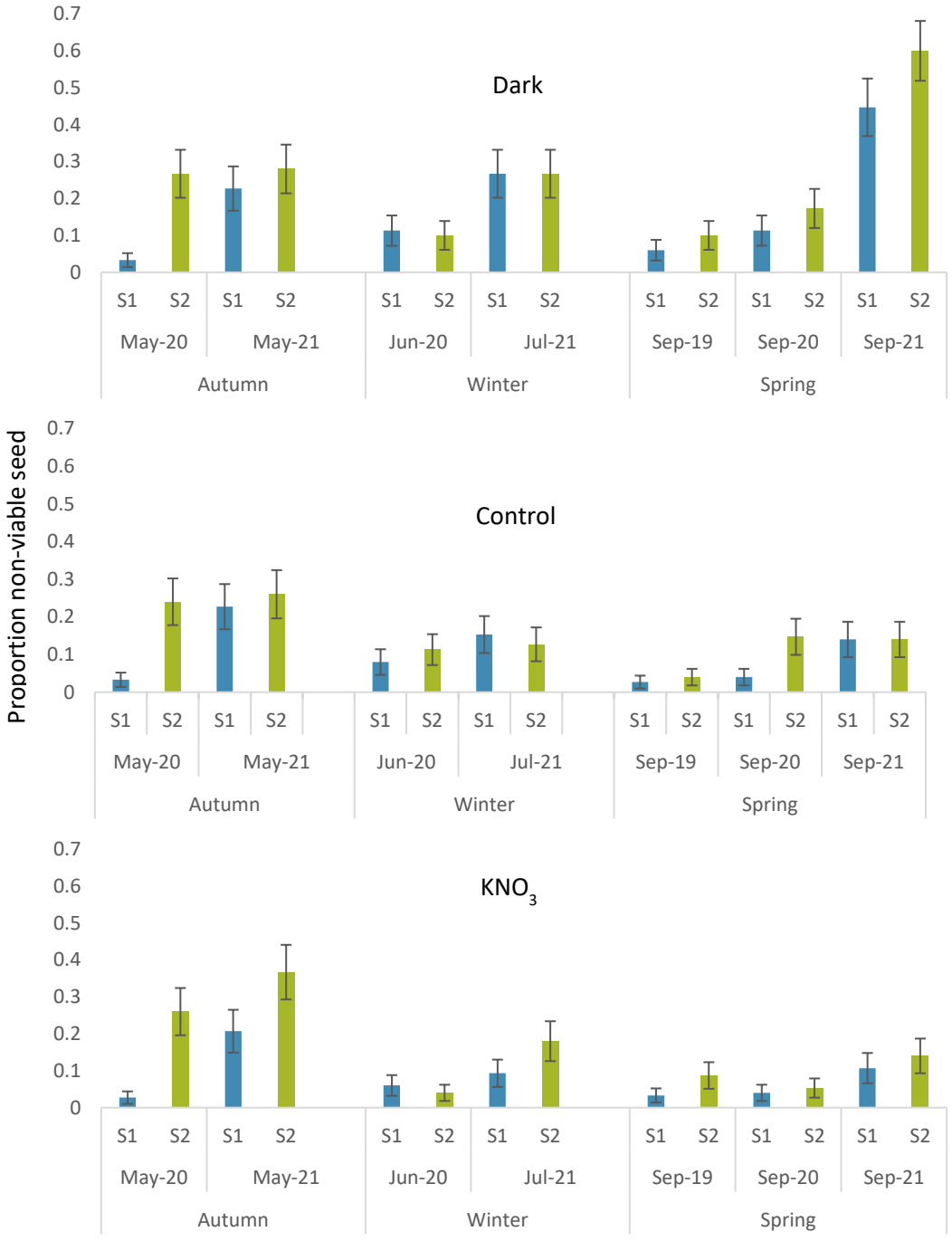

Figure 4.3: Observed proportions of *Clematis vitalba* seed that were found to be not viable after being collected from plants during autumn to spring and then subjected to one of three treatments (Dark = complete darkness; Control = blotters moistened with reverse-osmosis water; and KNO_3 = blotters moistened with KNO_3), incubated 28 days in alternating dark and light (16h:8h) with alternating temperatures (20/30°C). Seeds were collected from two separate populations (KUM, WFR) in the Tararua District, New Zealand. Error bars denote 95% confidence intervals.

Table 4.1 shows the sequential analysis of deviance table for the binomial model compared to the null model, in which deviance reduction for date was roughly triple that of treatment or the treatment by date interaction, thus demonstrating that date was the most influential term in the model.

Table 4.1: Analysis of deviance table for binomial logistic regression model of *Clematis vitalba* seed decay/non-viability following incubation, with first order interactions.

	Df	Deviance	Resid.Df	Resid.Dev	Pr(>Chi)
NULL			6299	5432.5	
Treatment	2	91.033	6297	5341.5	<0.001
Population	1	58.924		5282.5	<0.001
Date	6	298.189	6290	4984.3	<0.001
Treatment:Population	2	3.806	6288	4980.5	0.1491
Treatment:Date	12	113.58	6276	48.67.0	<0.001
Population:Date	6	67.802	6270	4799.2	<0.001

The binomial model for germinability of viable seeds showed that overall, both treatment and collection date were highly significant predictors of whether a seed germinated or remained dormant ($p < 0.001$; Appendix B, Table B.2). Seeds in both the control and KNO_3 treatments were significantly more germinable than those in the dark treatment ($p < 0.001$), and seeds treated with KNO_3 were also more germinable than control seeds ($p < 0.001$), which were exposed to light but not KNO_3.

Germinability in autumn of both cohort 2 and 3 seeds was negligible to non-existent (Figure 4.4). Wald tests between Cohorts 2 and 3 also revealed a significant increase in germinability of seeds collected in winter to those of autumn ($p<0.001$), and a significant increase in germinability of seeds collected in spring to those of winter ($p<0.001$). Even germinability of mid-winter seeds collected in July (Cohort 3) was significantly higher than that of those collected in June (Cohort 2). Significant germinability differences between all three September collections ($p<0.001$) point to cohort variation, mostly due to differences in response to the dark treatment. As it was for decay, date was the most important predictor of seed status after incubation (Table 4.2): deviance reduction for date was by far the largest, although the deviance reduction generated by treatment was also considerable.

In general, source did not contribute significantly to final seed status ($p=0.091$), although there were some conditions in which significant differences between populations did occur, e.g. September 2020 in the dark and control treatments.

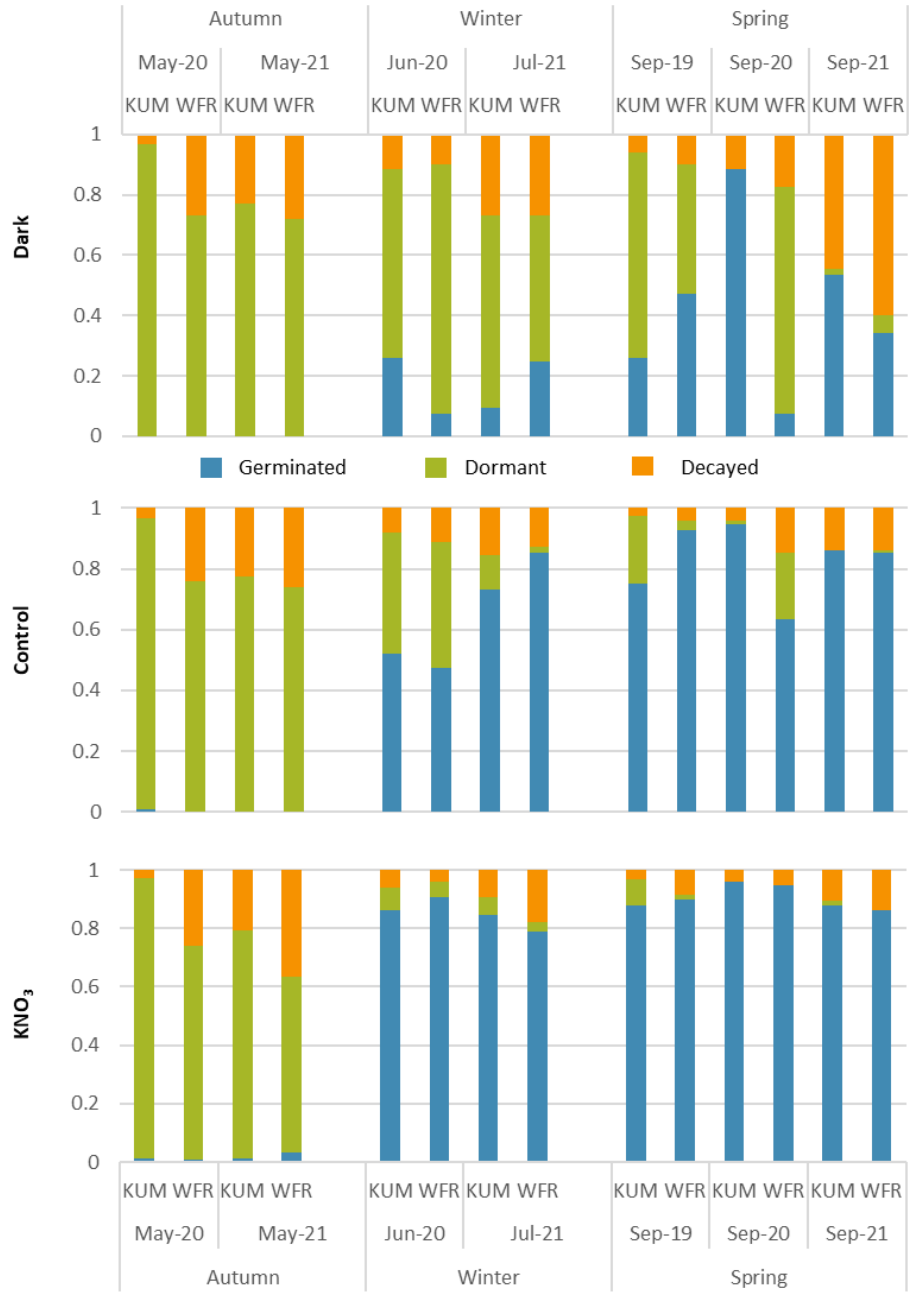

Figure 4.4: Observed proportions of *Clematis vitalba* seed status (Germinated, Dormant, Decayed) following 28-day incubation (in alternating dark and light (16h:8h) with alternating temperatures (20/30°C)) in three treatments (Dark = complete darkness; Control = blotters moistened with reverse-osmosis water; and KNO_3 = blotters moistened with KNO_3), incubated). Seeds collected autumn, winter, and spring over 2 years, from two separate populations (KUM, WFR) in Tararua District, New Zealand.

Table 4.2: Analysis of deviance table for binomial logistic regression model of *Clematis vitalba* seed germinability during incubation (multicollinearity of interaction terms made models with those terms inappropriate).

	Df	Deviance	Resid.Df	Resid.Dev	Pr(>Chi)
NULL			5323	7294.6	
Treatment	2	627.3	5321	6667.3	<0.001
Date	6	3793.8	5315	2873.5	<0.001
Population	1	2.9	5314	2870.7	0.091

Figure 4.5 demonstrates the average trend of dormancy loss in both populations combined, from autumn (May) to spring (September) when incubated directly after collection, in alternating temperature (20/30°C) and light (16h/8h). In two consecutive years, germination onset (earliest visible breakthrough of radicle) of untreated seeds (control treatment) was not observed in May until the last day of incubation (Day 28), at which point only one individual seed germinated (0.2%). Average germination onset for control seeds collected the third week of June, incubated in the same conditions, was 10 days, with 49.5% germinability. By September, average germination onset had decreased to 7 days, and average germinability had increased to 83%.

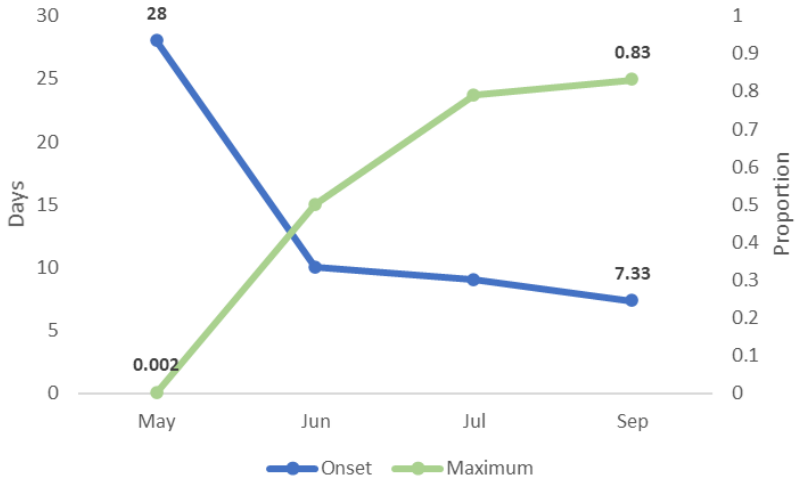

Figure 4.5: Average change in *Clematis vitalba* seed germination onset (in days) and maximum germination (proportion) during 28 days incubation in alternating temperatures (20/30°C) and light (16h/8h), between autumn and spring over two years, September 2019-September 2021. Seeds from two populations in the Manawatu Region of New Zealand.

4.3.1.1 Year-round germination and dormancy

Because the bulk of achenes had dispersed between May and September, KUM achene samples collected in November 2019 and 2020, January 2020 and 2021, and March 2020 and 2021 were too small to be representative of the population and cannot be used to support any predictions of effect

from source, treatment or date. Yet, Figure 4.6 shows that for each collection and in each treatment, germination did occur and some seeds remained dormant, as well.

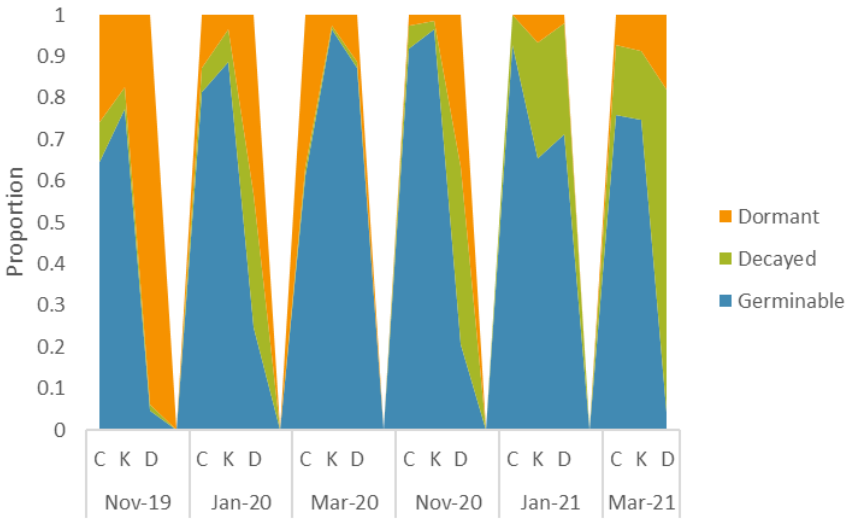

Figure 4.6: Observed proportion of *Clematis vitalba* seed dormancy, decay and germinability in three different treatments (C = control; K = KNO_3, D = complete darkness) for seeds collected from plants during two summers, 2019-2020 and 2020-2021, that had formed in the previous autumn.

4.3.2 Observations of Embryo Development

4.3.2.1 Embryo size during storage

The approximate average embryo size range of 0.5-1.0 mm was found in both batches of stored seed after storage of 9 and 11 months, respectively (Figure 4.7).

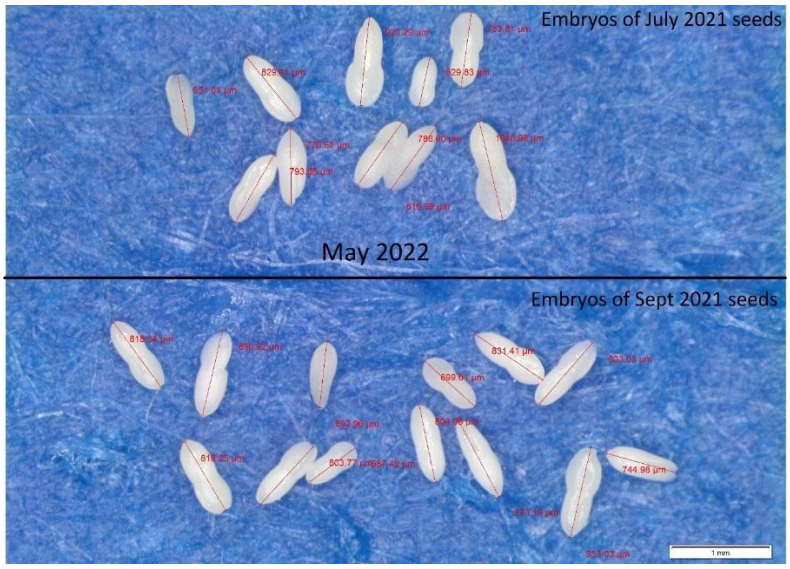

Figure 4.7: *Clematis vitalba* seed embryos measured in May 2022 after 9-11 months of ambient outdoor storage. Top: embryo length range of July 2021 seeds = 530-1041 µm (top); bottom: embryo length range of September 2021 seeds = 587-953 µm.

4.3.2.2 Embryo growth during incubation

Measurement of a sample of embryos prior to incubation established that the seeds had remained morphologically dormant during the 21 months of storage. However, once placed in conditions suitable for germination, the average embryo size which was 0.85 mm at the beginning of the study, grew to 1.37 mm at the time of germination onset (Day 7-Day 9), and peaked at 1.75 mm at 16 days, shortly after germination had peaked. Embryo length declined from thereon (Figures 4.8, 4.9), with fewer and fewer seeds being ungerminated, but a significant proportion of that small pool of remaining embryos never grew. Germinability of undissected seeds was 92%, slightly lower than when fresh, though not significantly so. The same test had been performed 2 months previously, with less precise measurement, but with similar results.

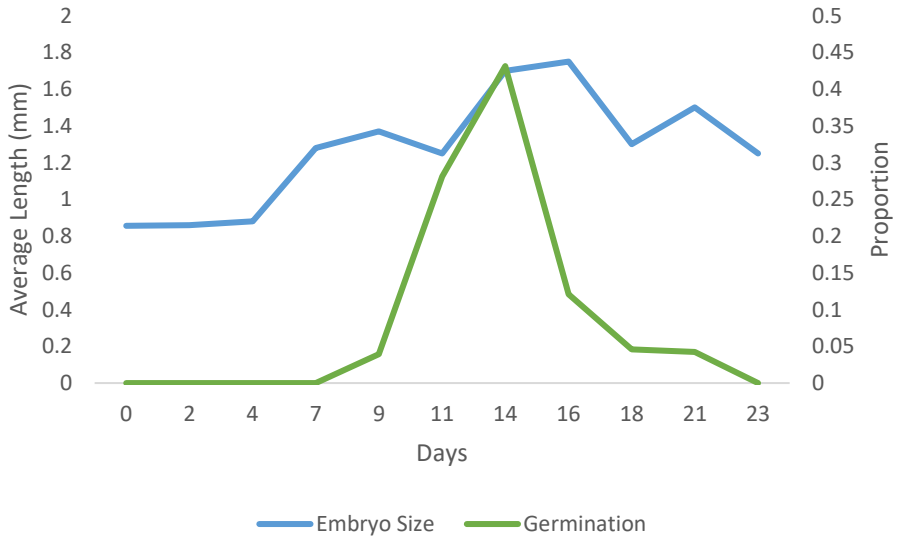

Figure 4.8: Correlation of average *Clematis vitalba* seed embryo size with proportion of seeds germinated while incubating in alternating temperatures (20/30°C) and light (16h/8h). Seeds were collected in September 2020, stored dry at 5°C, and then tested in May 2022.

Figure 4.9: *Clematis vitalba* seed at the point of radicle breakthrough (left). The same seed, showing size of non-dormant embryo relative to seed (as opposed to the dormant embryo shown in Figure 4.2)

4.4 Discussion

4.4.1 Influence of date, treatment, population

Collection time appeared to be the most important variable in predicting post-incubation viability of OMB seed. That is, season, which dictates the length of after-ripening period and stage of seed senescence, and seed cohort (year), moderated the relationship between viability and population source or treatment. For example, although KUM seeds in 2020 were an exception, there was relatively high decay in both autumn seed collections, much of which occurred during incubation. The high proportion of decay could be due to insufficient senescence of seeds in autumn, compared to those in winter or spring. Existing literature (Keenan and Richardson 2015) has established that when spring phenological processes are delayed or accelerated, autumn processes are equally affected. Indeed, in May 2020, researcher notes taken during collection indicated that seeds from the KUM population, which had low decay rates, were drier and came off the receptacle more easily than WFR seeds did, suggesting that KUM seeds had both developed and senesced earlier. By June 2020, differences between populations were non-significant, probably because WFR seeds had senesced adequately by then.

As for differences in cohort, environmental stresses on the mother plant during seed development can lead to lower seed fitness (Finch-Savage and Leubner-Metzger 2006b, Naithani et al. 2017). Overall, Cohort 3 (2021) seeds were less viable than those of Cohort 1 or 2: more decay in all treatments occurred that autumn, and substantially more decay occurred in spring 2021 in the dark treatment than in 2020. Researcher notes from September 2021 indicated that seeds were wet from rain when collected, so extra moisture could also have contributed to the higher decay in both populations for that date.

Finch-Savage and Footitt (2017) have shown that light can help activate germination in mature seeds of some species that are conditionally dormant. In general, for this study, except in autumn, seeds incubated without light were more likely to decay than those exposed to light, especially in 2021. That is, light exposure during incubation increased the chances of germination, thereby offsetting the chances of decay in otherwise susceptible seeds, except in those that had not after-ripened sufficiently and were fully dormant. This implies that buried seeds are more likely to decay than those on the soil surface, particularly those from a low-fitness cohort or population, such as occurred in 2021.

Regarding germinability and dormancy, the most critical differences in final seed status were due to collection date, i.e., stage of seasonal dormancy. Extremely low germinability occurred in May 2020 and May 2021, which supports previous researchers' claims that newly matured seeds are dormant in autumn, requiring a period of after-ripening before germination (Lhotská 1974, West 1992, Bungard et al. 1997b, Vinkler et al. 2004, Picciau et al. 2017). In contrast, in each cohort, seeds that stayed on

the vine at least until June were significantly more germinable than May seeds, and those not collected until September were significantly more germinable than winter seeds, suggesting a seasonal progression out of dormancy. Moreover, despite being from a cohort of overall lower viability, Cohort 3 winter seeds, collected the second week in July, were significantly more germinable than Cohort 2 winter seeds, which were collected the third week in June. This result implies that even 3 extra weeks of after-ripening can cause a significant reduction of physiological dormancy.

Treatment also had a sizeable influence on germinability. Autumn seeds incubated in the dark that did not decay remained dormant. Also, winter seeds in the dark treatment were much more likely to remain dormant than germinate or decay. In spring, dark seeds were more likely to germinate than winter seeds, but not as well as seeds in the other two treatments, control and KNO_3, which included fluctuating light periods. Incubation in complete darkness was chosen to simulate seed burial following dispersal. This result shows that light can promote germination in non-dormant and conditionally dormant OMB seeds, and that buried seeds are less likely to germinate than those exposed to light at the soil surface. Furthermore, exogenous nitrogen together with light (KNO_3 treatment) stimulated germination even more than light alone, which confirms the findings of others (Bungard et al. 1997b). The addition of KNO_3 simulates higher soil nitrogen availability to plants in spring. This occurs when soil fauna respond to warmer temperatures by increasing activity and decomposing organic matter at higher rates, which results in higher levels of inorganic nitrogen being released. If temperatures and other conditions mimic spring conditions, seeds then respond to the presence of the increased nitrogen levels (Taylor et al. 1982, Dawes et al. 2017).

Production of a large quantity of seeds appears to be a common life history strategy for invasive plant species: Mason et al. (2008) found that woody invasives produce >26 times more seeds than their native congeners, thus improving their chances of establishment after dispersal. An average of 72% of OMB seeds in this research did not appear viable at collection and were discarded before incubation began. Spring provides the most favourable conditions and is the most natural season of germination for OMB. Yet, September seeds in the control treatment decayed an additional 3% during incubation and another 5% tested non-viable following incubation. It is unknown what proportion of that 5% were never viable, and what proportion lost viability during the experiment, but based on these results, it is reasonable to assume that the percentage of seeds annually produced by OMB that remain germinable or dormant is less than 72%. That said, 72% of 70,000/m^2/yr (estimated total seed production) is 50,000/m^2/yr, a number which still offers formidable colonisation/invasion potential.

4.4.2 Dormancy change over time

Although too few to be indicative of the population, the six additional KUM collections incubated in November 2019 and 2020, January 2020 and 2021, and March 2020 and 2021were useful, in that they

demonstrated germinability of seeds directly off the vine throughout the summer. Germinable and/or dormant seeds were present in each collection over the 2-year period, which confirms that persistent seeds on the mother plant function as a seed bank, however small. Yet, given that new spring growth of vines blanket the previous year's achenes, there is little chance of them being widely dispersed thereafter.

According to Picciau et al. (2017), the earliest germination of un-chilled OMB seeds collected in mid-autumn and left in incubation 140 days did not occur until day 50. In the current study, limited to 28-day incubation periods, seeds collected in late autumn (May) also experienced delayed onset of germination (earliest germination of Control seeds occurred on Day 28 in Cohort 2, and no seeds germinated in Cohort 3), which is consistent with a period of dormancy at maturation of the fruit. Seeds appeared to be fully dormant through late autumn, but by mid-winter a portion could be induced to germinate. Germinability increased even more by early spring, indicating a decline in physiological dormancy over the seasons.

Peak OMB germination is expected to occur once temperatures have risen reliably in spring. Seeds collected in September would have experienced after-ripening throughout the autumn and winter, with an accompanying loss of physiological dormancy, and thus should be expected to respond more favourably to germination conditions than those collected in winter. Germination results from all three September collections are compatible with this expectation. Yet, a small proportion of seeds remained dormant in spring. According to some researchers (Finch-Savage and Leubner-Metzger 2006, Mitchell et al. 2016), along with inherent characteristics, such as genetic differences and hormone levels, position on the mother plant and asynchronous development of seeds lead to differences in senescence and dormancy levels, even as seasonal shifts occur. Accordingly, seeds produced later on the source vine would retain dormancy longer, even during the most suitable environmental conditions for germination.

No embryo growth was observed during moist (Copete et al. 2021) or dry seed storage at 5°C, nor during storage at ambient outdoor temperatures and moisture (Section 4.3.2), indicating that in any situation where the necessary combination of germination stimuli are lacking, regardless of physiological dormancy level, seeds remain morphologically dormant. Even so, the length of time between Day 0 of incubation and onset of germination in incubation shortened as physiological dormancy decreased (from 28 days in May to 7 days in September), implying a positive correlation between physiological dormancy loss and speed of embryo growth when exposed to suitable conditions. Morphologically dormant seeds never germinate immediately, because they need time for the embryo to grow to the critical size. Still, the shortening of germination onset over the course of the seasons suggests that, although there is no growth during after-ripening, longer after-ripening primes the embryo for more rapid growth when suitable conditions present themselves. As a simulation of

after-ripening during winter, artificial chilling was also observed to prompt accelerated germination onset and time to 50% germination (Section 3.3.3) (Lhotská 1974, Van Gardingen 1986, West 1992, Bungard et al. 1997b, Vinkler et al. 2004, Picciau et al. 2017).

4.5 Conclusions

The results of this study showed that mature OMB seeds on the mother plant persist in complete morphophysiological dormancy throughout autumn. Thereafter, although asynchronous seed development leads to non-uniform seed dormancy levels, OMB seeds follow a pattern of progressive physiological dormancy loss throughout winter. Morphological dormancy does not experience the same seasonal shift; seed embryos do not grow until conditions appropriate for germination present themselves, usually in the spring. Despite the lack of embryo growth during stratification, the alleviation of physiological dormancy is accompanied by a shortening of the period needed to break morphological dormancy once seeds are exposed to the right conditions. Quicker germination under optimal conditions improves the chances of seedling establishment.

This experiment found that less than 72% (< 50,000) of total OMB seeds produced are viable. That is, approximately 28% of seeds are aborted or diseased (too small or flat) or eaten while still attached to the mother plant. A further small proportion of decay occurs post-dispersal, even under very favourable conditions. Length of after-ripening (level of dormancy) appears to be critically important for determining whether OMB seeds will remain viable post-dispersal. That is, fully dormant autumn seeds decay at higher rates than non-dormant or partially dormant seeds, if exposed to moisture and warm temperatures normally adequate for germination. At the other extreme, spring seeds are highly germinable, with low levels of decay and dormancy.

The post-dispersal environment also effects seed survival. Light is not essential for OMB germination, but lack of light exposure increases chances of seed decay or dormancy, even when seeds are fully after-ripened and conditions are otherwise suitable for germination. This result suggests that seeds that become buried would also be more prone to decay or remain dormant until disturbance brings them to the soil surface and allows for enough light exposure for germination. Also, except in fully dormant seeds, the addition of nitrogen enhances OMB seed germinability, indicating that chances for successful germination improve with increased levels of nitrogen in the soil. Nonetheless, successful germination is not always followed by successful seedling establishment. Chapter 6 will address OMB seedling emergence, establishment, and survival in different levels of competition.

A small fraction of spring seeds on the vine remains dormant (up to approximately 8%). However, as mentioned in Section 2.5, due to low supply and lower chances of long-distance dispersal, seeds that persist past the peak dispersal season, regardless of their state of dormancy, do not make an important

contribution to the seed bank and are not a reliable source of recruitment for OMB. Chapter 5 will examine whether OMB's soil seed bank is a more extensive source than the aerial seed bank.

5. Seed dormancy and the soil seed bank

5.1 Introduction

Seeds that are present on the soil surface or buried in soil after dispersal become a source of potential recruitment for a plant species and are collectively called a soil seed bank (Section 1.4.7). Of course, recurrent seed accumulation bolsters the size of a soil seed bank, and other factors contribute, but the longevity of the bank is influenced to a large degree by the dormancy of its seeds: seeds with no dormancy do not survive past one season but those that experience dormancy can act as a repository from which a species can draw over time. A long-term, persistent soil seed bank can play a key role in the invasiveness of a species (Fenner and Thompson 2005, Baskin and Baskin 2014, Gioria et al. 2021).

Buried seeds experience seasonal cycles of physiological dormancy in response to their temporal and spatial environment (Schafer and Chilcote 1970, Baskin and Baskin 1985, Finch-Savage and Footitt 2017). For example, dormancy gradually declines in seeds that sense warming temperatures in spring, allowing them to respond to other germination stimuli (e.g. light, moisture, nutrients, hormones, oxygen). Without these stimuli, seeds may cycle back into dormancy. Indeed, many species go through years of dormancy cycling before germinating (Kildisheva et al. 2020). Seeds buried too deeply to be subjected to large diurnal temperature fluctuations are more likely to remain dormant until natural processes bring them closer to the soil surface during a period suitable for germination (Thompson and Ooi 2010, Bewley et al. 2013, Baskin and Baskin 2014). Not only can seeds at different soil levels display different dormancy stages, but dormancy stages can vary among seeds at the same soil level within a seed bank, as a consequence of intrinsic differences and differences in timing of seed development (Finch-Savage and Leubner-Metzger 2006, Mitchell et al. 2016). These differences allow a seed bank to retain some seeds over time, even while others germinate and are lost from the bank. This temporally syncopated germination protects a plant population from the risk of having all seeds germinate at once and potentially losing all consequent seedlings collectively to adverse circumstances (Bewley et al. 2013).

Although germination is probably the most important factor in seed loss from the soil seed bank (Roberts 1972, Bewley et al. 2013), seeds can be lost through granivory, pathogens, movement by soil fauna or erosion, and decline of viability (James and Rahman 2003, Baskin and Baskin 2014, Mašková et al. 2022). Soil seed longevity varies by species and environmental conditions and ascertaining the size and persistence of the seed bank for an invasive species can help create successful management plans (Saatkamp et al. 2009). Along with dormancy and other intrinsic characteristics of a species, soil type, moisture, temperature, depth of burial, and time in the soil have

all been shown to be important factors in buried seed survival. For example, clay soil is often less favourable than sandy soil for seed longevity (James and Rahman 1999, 2000), because the reduced microporosity of clay soils restricts movement of both water and gases, leading to more decay (Brady and Weil 1999). Fungal disease is also associated with higher soil temperatures and moisture, due in part to resultant increased microbial activity (Schafer and Chilcote 1970, Schafer and Kotanen 2003). Deeper burial is generally more favourable to seed survival than shallow burial because depth protects seeds from foragers, and reduced fluctuations of temperature and light at greater depths keep seeds dormant (James et al. 1998, Conn et al. 2006, Vandelook et al. 2008, Kołodziejek and Patykowski 2015). In addition, almost all seeds lose viability over time as they age (Conn et al. 2006, James et al. 2010, Moravcová et al. 2022), unless kept in dry, stable conditions (Liu et al. 2018).

As noted in Section 1.4.7, previous researchers have made vastly different conclusions about the longevity of old man's beard's soil seed bank. Some studies have suggested that OMB seeds do not survive in the soil and that its soil seed bank is transient (Van Gardingen 1986, Deiller et al. 2003). Most research has involved measuring seedling emergence from soil samples, and when this has resulted in very few, if any, seedlings emerging, conclusions have been that OMB's seed bank is small and short-term (Warr et al. 1994, Dutoit and Alard 1995, Roovers et al. 2006, Chaideftou et al. 2009, Nikoloff 2011). In contrast, other seedling emergence studies found that OMB presence in the soil was abundant throughout the year (West 1992), and persistent over time, even when plant removal precluded new seed accumulation for several years (Clements and Bierzychudek 2017, Bierzychudek 2020). Furthermore, West (1992) reported that seeds buried at five different depths (2, 5, 10, 15, 20 cm) in October (spring) for 3 and 6 months did not germinate after removal, and about 20% had decomposed during burial, but the remainder appeared viable. She assumed the seeds had re-entered dormancy. Although difficult to pinpoint, in addition to differences in study methods, differences in dormancy and experiment timing may have contributed to the variability of outcomes.

The objective of the current experiments was to measure the viability and dormancy of OMB seeds during a 2-yr period of burial in soil, to help determine the likely soil seed bank size and longevity for this species in the field. The influence of seed provenance, time spent in the soil, type of soil/environmental conditions during burial, and depth of burial will be considered. Determining the longevity of viable OMB seeds in the soil is important so that site reinvasion risks following clearance of the weed can be predicted.

5.2 Materials and methods

5.2.1 Seed material and burial sites

Achenes were collected in mid-September 2019 from two OMB populations in riparian zones along the Manawatu River in Tararua District, New Zealand: Woodville Ferry Reserve (WFR; -40.3369, 175.8185) and Hopelands Reserve (KUM; -40.3590, 175.9627). In the 5-day interval between collection and burial, achenes were stored in mesh bags in an unheated shade house. Achenes that were smaller than half the average size, or were obviously not filled (flat), or were damaged or decomposed were discarded (roughly 30%), and then a sample of 200 achenes from the remaining seeds of each population were tested for viability in a tetrazolium (TZ) assay. The TZ-tested seeds from both populations were 98% viable.

Two sites at the Massey University Pasture and Crop Research Unit were selected for the burial experiments, which were just over 0.5 km apart: 1) Moginie Block (MOG), on a slight slope (25°) and with a silt loam soil. 2) Poultry Farm Road (PFR) agronomy plots, on a flat surface with a fine sandy loam. The MOG site was in a large 5 x 5 m grassy block lined on both sides (west and east) with rows of young, 1.5 m-tall manuka bushes. The site experienced some shading of full sun from mānuka plants, as it was within 2 metres of the plants. The PFR site was open, with no shade. Soil samples were tested at Hill Laboratories in Hamilton, New Zealand, using their standard techniques. Characteristics of the soils are listed in Table 5.1.

Table 5.1: Soil characteristics at the two burial sites (OM = organic matter; CEC = cation exchange capacity; me/100 g = milliequivalents per 100 grams of soil).

Site	Ratio Sand:silt:clay	pH	% OM	Olsen P mg/L	K me/100g	Ca me/100g	Mg me/100g	Na me/100g	CEC me/100g	% Base Saturation
Moginie Block	8:69:23	5.9	8.94	48	1.17	7.7	1.48	0.14	17	62
Poultry Farm Rd	67:20:13	6.0	4.13	46	0.38	4.4	1.21	0.08	10	61

5.2.2 Methods: Experiment 1

In Rai Valley, New Zealand, West (1992) found that of OMB seeds naturally buried in the top 6cm of soil, the largest proportion of seedlings (60%) emerged from a 0-2 cm depth, with fewer seedlings emerging from lower depths, and only 5% of seedlings emerging from a 4-6 cm depth. In preparation

for burial, 100 achenes with styles intact, mixed with 20 g of washed, fine landscape sand were placed in 7.5 cm x 10 cm polyester gauze bags. Thirty-two bags of achenes from each of the two populations were made up for each site, 16 for burial at 2 cm and 16 for burial at 5 cm. For retrieval purposes, one end of a 30 cm piece of string was tied to the bag, and the other end to a nursery label, which had details of burial site, bag number, population source, and burial depth. Two labels (one each for 2 cm and 5 cm burial depth) were taped securely to a 15-cm galvanised nail. Bags were buried randomly in pairs, and the nails were driven into the ground between each 2 cm/5 cm pair following burial. Grazing exclusion cages were used to cover the plots, to discourage rabbit burrowing (Figure 5.1).

Figure 5.1: Buried seed experiment set-up, clockwise from top left: 100 seeds mixed with 20 g sand buried in polyester gauze bags; labels of randomly buried bag pairs; Poultry Farm Road site; Moginie Block site.

Bag numbers were randomly assigned to eight retrieval dates, 3 months apart (2 years total). At each retrieval date, four bags from each depth were removed from each site. Following retrieval, empty achenes were noted and discarded. Full achenes were rinsed and separated from any remaining sand or soil and subjected to germination tests for 28 days, using the most effective treatment for artificial stimulation (Section 4.5): KNO_3 soaked blotters in 20/30°C coinciding with 16h/8h dark/light). Any ungerminated seeds were further tested with a TZ assay.

Except during the two New Zealand COVID-19 lock downs (March-May 2020 and August-September 2021), sites were monitored every second week, seedling weeds were removed by hand, and weeds with creeping rootstocks sprayed with glyphosate, when necessary. Over the course of the experiment, weather data from the nearby AgResearch weather station were recorded (Figure 5.2). The maximum average daily temperature ranged from 24.6°C in February to 14.5°C in July.

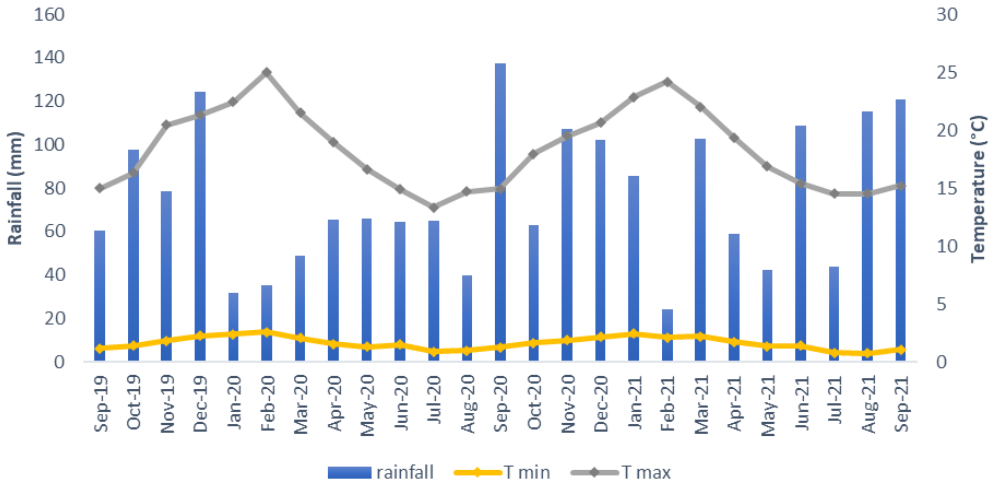

Figure 5.2: Mean total monthly rainfall (mm) and mean monthly minimum (Tmin) and maximum (Tmax) temperature (°C) over the duration of the two experiments (Experiment 1: September 2019-September 2021; Experiment 2: June 2020-June 2021).

During the 2020 New Zealand COVID-19 lock down, access to incubators was not permitted. Seeds retrieved in March 2020 were kept in an unheated conservatory at the researcher's home, in which night-time temperatures averaged approximately 8°C and day-time temperatures averaged approximately 35°C. Therefore, the temperature fluctuations were larger for the seeds of that retrieval than for any previous or subsequent retrievals kept in the incubator (20/30°C). Also, the light/dark ratio changed from 8:16 in the incubator to 11-11.5h:12.5-13h in the conservatory.

5.2.3 Methods: Experiment 2

Seeds buried in September 2019 in experiment 1 had experienced an entire winter of stratification and it is now known they would have been largely non-dormant prior to burial (see chapter 5). To

determine if seeds at different dormancy levels respond differently to burial, a second, 1-year experiment was conducted, using seeds collected and buried in mid-winter (June 2020), which would have been conditionally dormant (see Section 4.4). Seeds were collected from the same two provenances used in Experiment 1, and the bags were filled in the same way, and the same two burial sites were used. Bags were randomly assigned to spots in the original burial sites vacated when Experiment 1 bags had been retrieved. Experiment 2 bags were buried only at the 2 cm depth, each randomly assigned to one of three retrieval dates: 3 months, 6 months, 12 months. Eight bags were removed from each site per retrieval date, four from each population source. Seed germination and viability tests were again performed on the recovered seed in the same way as Experiment 1.

5.2.4 Analysis

A factorial analysis of variance (ANOVA) was performed on data from each experiment separately in R, version 4.2.0, after transforming the data with rank-based, ordered quantile normalization (ORQ), which was chosen following a comparison of alternative transformations with a Pearson P goodness of fit statistic. Akaike Information Criterion (AIC) model selection was used to compare different models: a full interaction model, a first order interaction model, and a model with no interactions. Packages used were: bestNormalize, lmtest, and AICcmodavg (Zeileis and Hothorn 2002, Mazerolle 2020, Peterson and Cavanaugh 2020, Peterson 2021, R Core Team 2021). In each case, the best-fit model, carrying 100% of the cumulative model weight, included every parameter with no interaction effects. Model diagnostic plots on the untransformed and transformed data and model selection tables based on AIC are shown in Appendix C (Figures C.1-C.4; Tables C.1, C.2). For Experiment 1, independent variables were population source, burial site and burial depth, each with two levels, and retrieval time, with eight levels. For Experiment 2, independent variables were population source and burial site, with two levels each, and retrieval time, with three levels. The response was the proportion of viable seeds remaining in each buried bag. Contrasts were then made between levels of statistically significant factors using Tukey honestly significant difference (HSD) tests.

5.3 Results

Proportions of seed viability (raw averages) for each combination of factors for Experiment 1 are shown in Figure 5.3, with clearly visible differences of survival between depths, burial sites, populations, and retrieval times. In addition, part-to-whole comparisons are shown between viable seeds that responded to incubation following retrieval by germinating and those that stayed dormant (TZ assay score). The first year's summer (December) and autumn (March) retrievals demonstrated high proportions of deep dormancy, especially in the seeds buried at 5cm, meaning they could not be induced to germinate, although they appeared to be viable. Full germinability in the winter (June) and spring (September) retrievals of the same year indicated non-dormancy, or conditional dormancy, the limited conditions of which were met by incubation. In the second year, the summer, autumn, and winter retrievals exhibited partial deep dormancy, with full germinability again in spring. These results point to a pattern of cyclical, seasonal dormancy in buried OMB seeds.

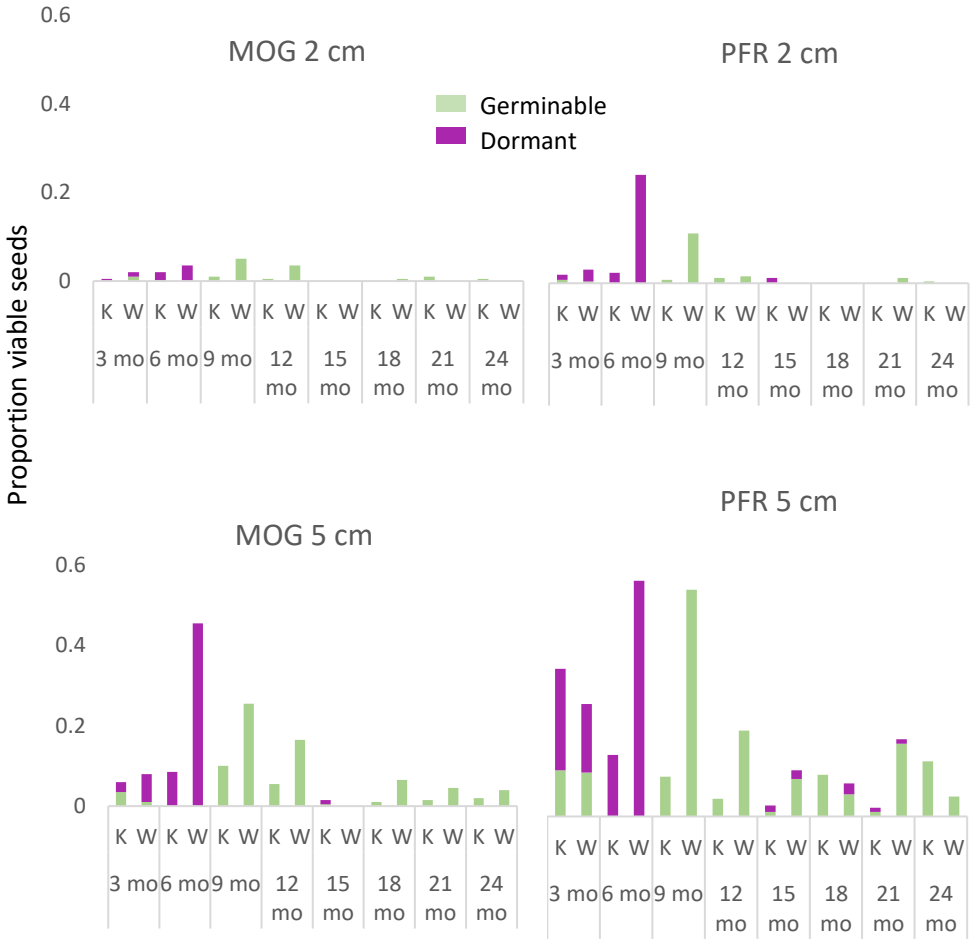

Figure 5.3: Observed proportion of viable *Clematis vitalba* seeds after burial over a 2-year period (September 2019-September 2021, Experiment 1) at two agricultural sites in Palmerston North, NZ (MOG = silt loam soil, PFR = fine, sandy loam), retrieved at 3-month intervals. Seeds were collected from two separate populations (K (KUM), W (WFR) in the Tararua District, NZ. Germinable = seeds that germinated during a 28-day incubation following retrieval (in alternating dark and light (16h:8h) with alternating temperatures (20/30°C)); Dormant = seeds that remained viable but dormant throughout the incubation period. The remaining proportion must have germinated or decayed before retrieval.

The ANOVA model for Experiment 1 (Table 5.2) revealed that there were significant differences among and between levels of all four independent variables ($p<0.01$).

Table 5.2: ANOVA summary of data transformed with ordered quantile normalization for Experiment 1, showing the effect of burial length (retrieval date), burial site (site), burial depth (depth), and provenance (population) on viability of *Clematis vitalba* seeds buried from September 2019 to September 2021. Significance codes: < .05*, < .01**, < .001***.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
retrieval date	7	27.28	3.9	12.115	<0.001***
site	1	6.24	6.24	19.406	<0.001***
depth	1	33.37	33.37	103.734	<0.001***
population	1	3.02	3.02	9.382	0.0027**
residuals	117	37.63	0.32		

Burial depth was decidedly the most influential factor in seed viability ($F_{1, 117} = 103.374$, $p < 0.001$), with significantly more seeds remaining viable at 5 cm than at 2 cm ($p < 0.001$, 95% C.I. = 1.021 [0.823, 1.220]). Of the two burial sites, seeds buried at PFR were significantly more likely to remain viable than those buried at MOG ($F_{1,117} = 19.406$, $p < 0.001$), 95% C.I. = 0.442 [0.243, 0.640]), and seeds from the WFR population were significantly more likely to remain viable than those from KUM ($F_{1,117} = 9.382$, $p =0.003$), 95% C.I. = 0.307 [0.109, 0.506]). Results from multiple pairwise comparisons (Figure 5.4) made clear that the significant effect of time spent buried on seed viability ($F_{7,117} = 12.115$, $p < 0.001$) was due to significantly lower survival from months 15-24 than from months 3-9. Seed survival did not significantly decline in the second year of burial, between months 15 and 24: during that period, 38% of bags buried at 2 cm contained at least one viable seed (an average of 0.25% of total seeds at MOG, and 0.44% of seeds at PFR), as did 89% of bags buried at 5 cm (2.6% at MOG and 8.3% at PFR).

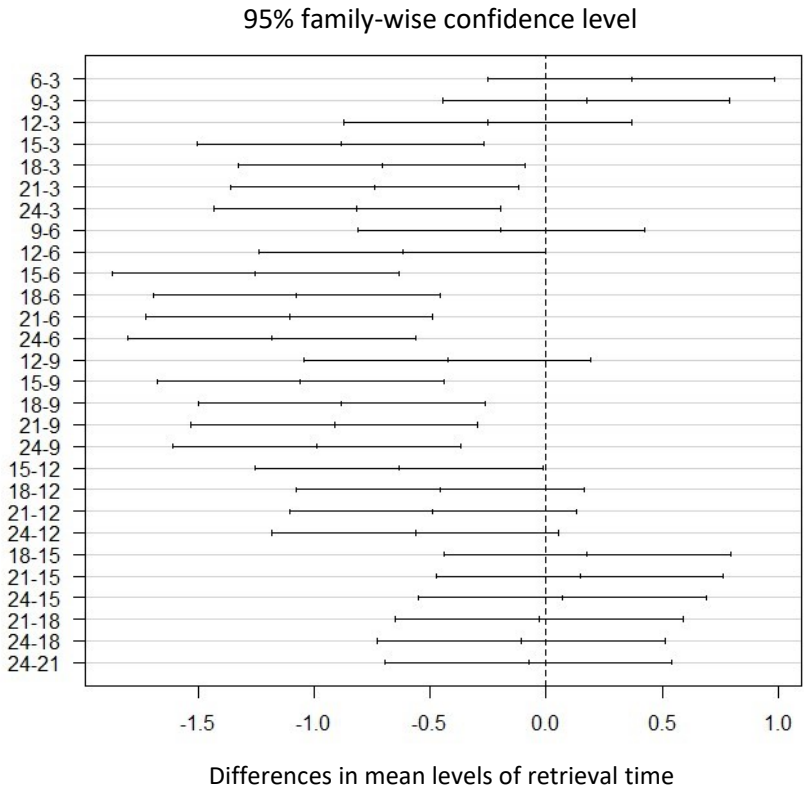

Figure 5.4: Comparisons plot of seed viability for all possible retrieval times (3, 6, 9, 12, 15, 18, 21, 24 months) in Experiment 1, in which buried *Clematis vitalba* seeds were retrieved at 3-month intervals over a 2-year period (September 2019-September 2021). Significant groupwise differences are shown where the 95% confidence interval does not include zero.

Conditionally dormant seeds buried in June 2020 (Experiment 2) seemed to follow the same cyclical pattern of dormancy that non-dormant seeds buried in September 2019 followed, i.e., little to no deep dormancy in the spring and winter retrievals, and a partial dormancy in winter (Figure 5.5). Indeed, a large majority of both sets of seeds (September 2019 and June 2020) were germinating in-situ at the time of retrieval in September (this also occurred both years in Experiment 1; Figure 5.6).

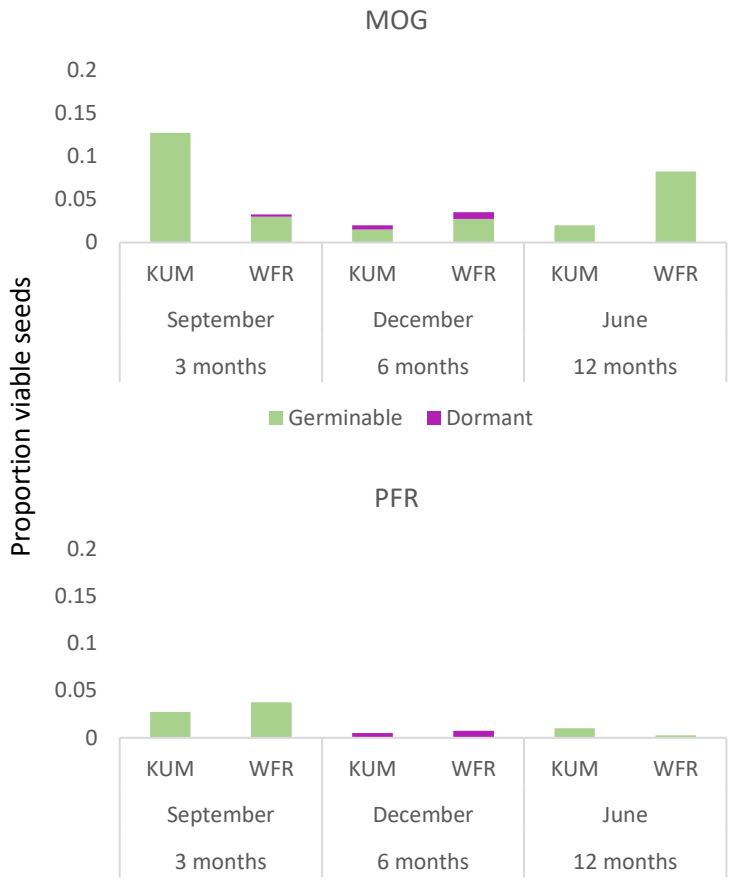

Figure 5.5: Proportion of viable *Clematis vitalba* seeds after burial for 3, 6, and 12 months (June 2020-June 2021, Experiment 2) at two agricultural sites in Palmerston North, NZ (MOG = silt loam soil, PFR = fine, sandy loam). Seeds were collected from two separate populations (KUM, WFR) in the Tararua District, NZ. Germinable = seeds that germinated during a 28-day incubation following retrieval (in alternating dark and light (16h:8h) with alternating temperatures (20/30°C)); Dormant = seeds that remained viable but dormant throughout the incubation period.

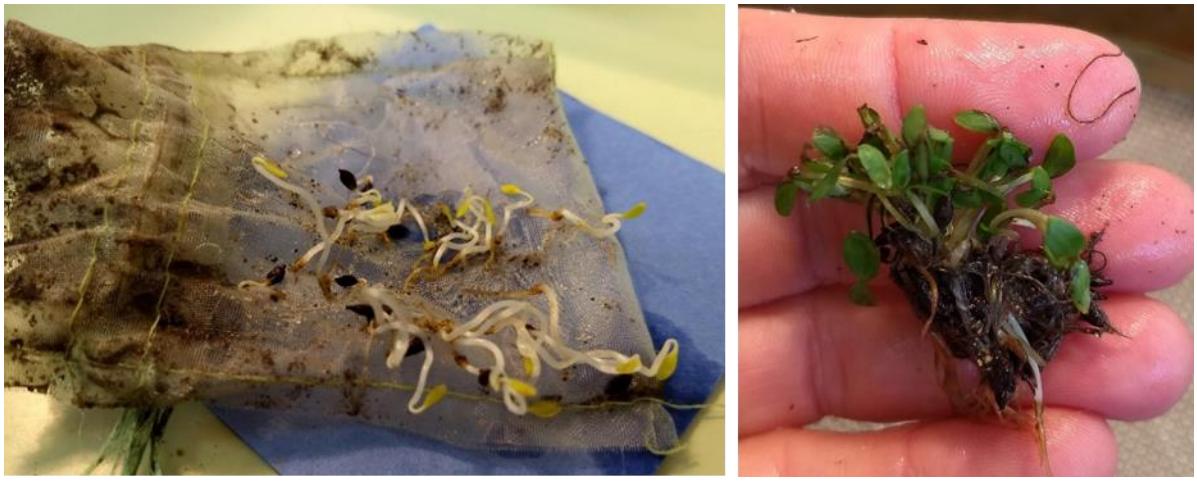

Figure 5.6: Germination of buried *Clematis vitalba* seeds at the time of retrieval, September 2020 (early spring). Left: germination of seeds buried 5 cm deep for 1 year, non-dormant at burial; right: germination of seeds buried 2 cm deep for 3 months, conditionally dormant at burial.

The ANOVA model for Experiment 2 (Table 5.3) detected a statistically significant difference in mean seed viability between the two burial sites ($F_{1,43} = 11.221$, $p = 0.002$). In contrast to Experiment 1, in which significantly more seeds remained viable at PFR, significantly more seeds survived at MOG in Experiment 2. Less influential but still significant was the difference between retrieval dates ($F_{2,43} = 3.959$, $p = 0.026$). Tukey's HSD found that this was largely due to the mean differences between months 6 and 3 ($p = 0.03$, 95% C.I. = -0.743 [-1.425, -0.060], where total viability decreased from September to December. In another contrast with Experiment 1, seed provenance did not appear to be important in seed survival.

Table 5.3: ANOVA summary of data transformed with ordered quantile normalization for Experiment 2, showing the effect of burial length (retrieval time), burial site (site), and provenance (population) on viability of *Clematis vitalba* seeds buried from June 2020 to June 2021. Significance codes: < .05*, < .01**, < .001***.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
retrieval time	2	5.005	2.502	3.959	0.026*
site	1	7.092	7.092	11.221	0.002**
population	1	0.034	0.034	0.053	0.819
residuals	43	27.180	0.632		

5.4 Discussion

5.4.1 Influence of depth, length of burial, site, population

In temperate climates, seed longevity studies commonly find that seeds buried more deeply retain viability longer than those that are superficially buried (Conn et al. 2006), as seeds at depth are not as exposed to temperature fluctuations which initiate germination, and thus are more likely to remain dormant. Indeed, the effect of burial depth was of greater consequence for OMB seed viability than any other factor considered in this research. That is, mean viability of seeds buried at 2 cm was significantly lower than that of seeds buried at 5 cm; the difference of 3 cm is not large, but apparently important, in terms of OMB seed longevity, due to the seeds germinating at 2 cm.

Also significant was the effect of time spent buried. It was particularly clear in Experiment 1 that after one year of burial, the seed bank was reduced to a fraction of its original size: an average of 5.5% at 5 cm and < 0.5% at 2 cm between months 15 and 24. However, mean viability did not decline significantly between months 15 and 24, remaining low but relatively stable. It cannot be ascertained from this study how long this minimal seed bank might persist, but Clements and Bierzychudek (2017) reported the emergence of OMB seedlings in 75% of soil samples (4 seedlings/600 ml) collected from a North American site where OMB vines had been completely removed 4 years

previously. It should be noted, however, that seedling emergence studies do not account for seeds that are present in the seed bank but remain dormant during the emergence test.

In terms of distribution in the soil, West (1992) also conducted a year-long, monthly seedling emergence experiment (mentioned in Section 5.2.1), which investigated the natural presence of OMB seeds within the top 6 cm of soil in an infested riparian zone in New Zealand. She observed that 21% of seedlings emerged from the litter layer, 60% from the top 2 cm of soil, 14% from between 2 and 4 cm deep, and only 5% from between 4 and 6 cm deep. Again, no attempt was made to determine if dormant seeds were present in the samples. Given these findings and those of the current study, it can be assumed that the vast majority of OMB seeds are buried superficially, if at all, and that the relative proportion of seeds in that range that survive past one year is minimal. Because few seeds become buried to 5 cm or more, where they have the potential for greater longevity, the seed bank remains small. In most environments where OMB has established, the land is not cultivated, and farm animals are absent. Thus, any seed burial is left to earthworms or other burrowing fauna (Willems and Huijmans (1994) found germinable OMB seeds in Dutch worm casts); to the achene's own movement (time-lapse videos by the researcher, showing hygroscopic movements of the achene in response to moisture fluctuations can be viewed at: https://youtu.be/_z1uk-xTBjY and https://youtu.be/8WdORXO78VM); to dropping into cracks in the soil formed by dryness; or possibly to flood deposition of silt over areas where OMB seeds are present. Considering the size of the total viable seed production (<50,000/m^2/yr), the seed bank at all levels is probably not inconsequential.

Burial site conditions had a significant effect on seed viability. However, the effect was different in each experiment. The sites were close enough to each other (roughly 0.5 km) that they would have received similar amounts of rainfall. During both years in Experiment 1, seeds buried at both depths in the fine, sandy loam of the flat plots at PFR survived significantly better than those in the silt loam of the slightly sloping plots at MOG. As sandy soil has lower water holding capacity than silt loam, seeds would not have as much moisture available to support germination and would be more likely to stay dormant when rainfall is limiting (Monaco et al. 2002). Yet, neither year of the experiments was particularly dry. Shading from the manuka plants at MOG would have kept it somewhat more moist, but the slight slope of the plots at MOG would have helped improve its drainage properties., Furthermore, the opposite outcome was true in Experiment 2: significantly more seeds buried in silt loam (MOG) retained viability than those buried in fine, sandy loam (PFR). The effect of burial site on seed persistence may therefore not have been a function of soil type or physical characteristics, such as sloping or shade, but rather of other factors, such as different levels of microorganism activity, or perhaps differences between the cohorts of seeds used.

Seed provenance was not consistently significant as a factor in seed survivability, which can also likely be attributed to differences in cohorts. Seeds used in Experiment 1 were collected in September 2019, and mean viability for WFR seeds was significantly higher than for KUM seeds throughout the 2 years of burial. A different cohort, collected in June 2020 was used in Experiment 2, in which there was no clear evidence that population was an influential factor in seed viability. This result reflects one finding from Chapter 4 (Section 4.4.1), that both inter- and intra-population variability of OMB seeds occurs (due to genetics and environmental factors at the time of seed maturation), although the differences were not always significant.

5.4.2 Dormancy change

The most valuable result from Experiment 2 was that the dormancy patterns of partially dormant seeds buried in June mirrored those of the non-dormant seeds buried in September for Experiment 1 (Figures 5.4 and 5.6). That is, nearly all seeds could be induced to germinate after retrieval in winter (June) and seeds were almost entirely non-dormant in spring (September), but partial deep dormancy was apparent in summer (December). In addition, partial deep dormancy continued through autumn in Experiment 1. These patterns indicate that seeds in the ground cycle seasonally in and out of physiological dormancy just as aerial seeds do (Section 4.3), regardless of when or at what dormancy level seeds become buried. It cannot be assumed that dormancy levels of OMB seeds in the ground mimic those of unburied seeds precisely, but the same general pattern of lower dormancy in winter and non-dormancy in spring does occur. Furthermore, aerial seeds develop during the summer and are naturally dormant through summer and autumn. Subterranean seeds also appear to have greater dormancy during those seasons, as an adaptation to avoid seedling emergence during hotter temperatures and at times which are too late for seedlings to achieve an adequate size before winter.

5.4.3 Limitations and other considerations

Using mesh bags to bury seeds allows for convenience of retrieval while also allowing for exposure to fluctuations in soil temperatures and moisture. Yet, there are at least two drawbacks to determining an accurate estimate of seed longevity when using bags: 1) seed density in bags may be much higher than is natural; and 2) the bags protect seeds from granivory and movement in the soil. High seed density increases seed-to-seed contact, which can lead to high rates of seed decay, which can result in seed viability being under-reported (Van Mourik et al. 2005). To mitigate the spread of pathogens from seed-to-seed contact, sand was mixed in with the seeds in each bag for both experiments. However, because of the protection afforded by the bags, seed viability may have been exaggerated.

In March 2020, seeds were removed from the incubator and moved to the researcher's conservatory, due to the Covid-19 lockdown in New Zealand. There was a larger diurnal temperature fluctuation on

sunny days in the conservatory than the incubator, because outside temperatures averaged between 9.8°C and 20.23°C, but maximum temperatures on sunny days were 30-35°C in the conservatory, due to glass trapping the heat. The buried seeds retrieved that month did not germinate at all in those circumstances, although they did appear to be mostly viable by a TZ assay. It is uncertain if the incubation difference had a major effect on the outcome. In March of the following year, seeds incubated in the growth chamber were mostly germinable, though a portion remained dormant.

Hume et al. (1995) discovered that most OMB in New Zealand is found on moderately to well-drained, alluvial or colluvial soils, which was the case at the two research sites used. Although representative of many alluvial and agricultural areas in the Manawatu Region of New Zealand (Landcare Research NZ Ltd 2019), the two soil types, silt loam and sandy loam, investigated in these experiments do not cover the full range of soils OMB may colonise. Also, the two sites used were limited to an area subject to the same climatic conditions, which likewise does not represent all possible climatic conditions where OMB is present or could potentially occupy. For example, OMB is prevalent in the Otago Region of New Zealand, which is drier and colder than the Manawatu. Also, the study sites had no leaf litter present, nor was there much shading from overhead vegetation, both of which would be common in more natural settings.

As a 2-year study, this project was not intended to discover with certainty the maximum length of time OMB seeds can persist in the soil. However, the results clearly indicate that a small soil seed bank can exist after the first year of burial. Along with previous observations (Clements and Bierzychudek 2017) of a seed bank persisting after 4 years, it is reasonable to conclude that OMB has the capacity to maintain a relatively small, long-term persistent seed bank.

5.5 Conclusion

Achenes dispersed from OMB's large but transient aerial seed bank that are buried in the soil become part of OMB's soil seed bank. The outcome of this study suggests that the proportion of viable OMB seeds after 1 year in sandy or silty loam is small relative to total viable seed production but could still be a substantial number. If, for example, nearly all seeds in the soil are incorporated within the top 5-6 cm, are distributed as West (1992) suggested, and retain viability over time as found here (Section 5.4.1), more than 1,000 viable seeds/m^2 could hypothetically remain in the soil seed bank after 1 year. Additionally, regardless of burial site, length of burial, or provenance, seeds buried at 5 cm are more apt to survive than those buried up to 2 cm, most likely because of more dormancy when buried more deeply. Cyclic changes in buried OMB seed dormancy follow a similar pattern to those in seeds retained on the mother vine, namely, a gradual decrease from mid-winter to spring, with greater dormancy in summer and autumn.

In areas with an existing OMB seed bank, machinery used to clear land after forestry harvesting or to prepare land for other long-term uses could bury some seeds at the soil surface and unearth other seeds. Evidence from this research implies that seeds near the soil surface would likely germinate *en masse* in spring, at which time seedlings could easily be controlled. On the other hand, those seeds that become buried could potentially be a source of future OMB re-establishment. However, it is still unknown how long-lived OMB's soil seed bank is. A long-term study, expanded to include more soil types and a variety of other environmental conditions, could provide useful information on how long control measures would need to focus on seedling removal after removal of existing vines.

The next chapter will address the ability of OMB seedlings to emerge successfully and survive when subjected to varying levels of competition, which will give additional insight into the importance of the soil seed bank for this species.

6. Effects of competition on seeds and seedlings

6.1 Introduction

Most research on liana competition has studied relationships between lianas and trees, or other woody plants. For example, previous research has shown that liana seedlings can outperform tree seedlings in low light situations (Pasquini et al. 2015). Also, the combined effect of underground and aboveground competition with lianas has been shown to reduce tropical tree biomass by 42% ((Toledo-Aceves and Swaine 2008).

Plants compete with each other for nutrients, water, and light (Wilson et al. 2007) and successful plant invasion is contingent on advantageous interactions with the biotic and abiotic elements in its environment (Gurevitch 2011). Although it has shown tolerance to waste areas in New Zealand, old man's beard generally grows in soils that are moderate to well-drained, are not highly acidic, and do not have severe nutrient deficiencies. The species also has a positive response to calcium, phosphorous and nitrates (Hume et al. 1995, Bungard et al. 1998). Zhang et al. (2021) demonstrated that mature OMB plants are resistant to heat shock, which may be advantageous during a particularly hot summer or when growing in open areas with direct sunlight. As described in Section 1.4.2, OMB's shade tolerance at the seedling stage has been confirmed by some researchers, but they also acknowledge that higher irradiance levels stimulate rapid growth (Paliwal et al. 1994, Baars and Kelly 1996, Bungard et al. 1998). Williams (2009) reported that, once established and mature, OMB is "highly competitive with all associated vegetation". In the Rangitikei Ecological Region of New Zealand, OMB has become increasingly abundant and problematic in local forests since its introduction as a garden ornamental in the early twentieth century, causing damage to the forest structure and a decline in indigenous biodiversity (Ogle et al. 2000). Yet, no experimental studies looking at the direct effects of competition between OMB and other species have been published, at any stage of life.

The phenological stages of germination and seedling establishment are particularly vulnerable periods in the plant life cycle (Batlla and Benech-Arnold 2014, Buru et al. 2016, Mori et al. 2020). Indeed, West (1992) has suggested that OMB seedlings in New Zealand have high mortality and are negatively density dependent. Also, in contrast to mature plants, OMB seedlings in southwestern Russia have been reported to be intolerant of dry conditions (Kozlovskiy et al. 2017). Bungard (1998) claimed that despite OMB's wide tolerance to light levels, seedling transplants in New Zealand did not survive in a deeply shaded forest. However, another New Zealand study (Nikoloff 2011) showed that, when grown in situ from seed, OMB seedling survival was proportionally higher in forested

settings than in open settings. Survival notwithstanding, the proportion of OMB seedling emergence was 20 times higher in open habitats, and in consequence, open habitats supported more seedlings.

As with many lianas, initial establishment of OMB often occurs in forest gaps or edges, such as the open habitats noted above. In such settings, competition with grasses and other ground level vegetation is a major factor in liana success. OMB is also common in riparian zones. Section 1.4.10.2 gives background on living mulches as a form of cultural weed control in production forestry, and an introduction to riparian zones in New Zealand. In terms of grass mulches, perennial grasses were highly effective at limiting the establishment of woody plants in a sub-Mediterranean ecosystem in British Columbia, Canada (MacDougall and Turkington 2005). Also, established perennial grass swards have been shown to prevent the emergence of the woody shrub species, *Cytisus scoparius*, in New Zealand (Tran et al. 2018).

As noted in Section 1.4.10.2, local land managers have found OMB in grasses of riparian zones and in production forests (A. Gordon, Rangitikei District Council, pers. communication, October 7, 2019; J. Keast, Horizons Regional Council, pers. communication April 17, 2019), but it is uncertain whether the OMB present in these areas are shoots from existing vines, or if they have established from seed. Also, in forestry settings, OMB seems to be less of a problem in grass swards than in the soil directly around tree seedlings, kept bare by herbicides to allow the seedling to grow competition-free. Research summarised in Section 2.4.6 has established that OMB seeds could survive a flooding event. How likely seeds washed up by a flooding event are to produce seedlings is unknown. In addition, germination of OMB seeds in the presence of established grasses has not been studied, and no formal research has investigated the risk of invasion of grassy habitats by OMB. OMB seed dormancy and germination were examined in Chapters 4 and 5. Here, seedling emergence and establishment will be considered. The purpose of this experiment was to assess the ability of OMB seeds to germinate and of subsequent seedlings to survive within grass swards, such as are found in riparian zones or exotic forests.

6.2 Materials and methods

6.2.1 Study sites

These experiments were conducted initially in November 2019-November 2020, and then were expanded and repeated September 2020-September 2021, with additional data collected September 2021-September 2022. The original experiment, Experiment 1, was run as a preliminary trial at one location, beginning in late spring, on a portion of the Moginie Block in the Massey University Crop and Pasture Research Unit (-40.3878, 175.6122; Site Mānuka 1 (M1)). Plots were set up on ground with slopes of 25-30° in large 5 m x 5 m grassy blocks between rows of young, 1.5 m-tall mānuka

(*Leptospermum scoparium*) bushes, fenced off and ungrazed for several years before the experiment commenced. Ground-level vegetation in the blocks was dense and vigorous. Apart from the mānuka, vegetation in the original blocks consisted of about 50% Yorkshire fog (*Holcus lanatus)*, 25% creeping buttercup (*Ranunculus repens)*, 10% rough-stalked meadow grass (*Poa trivialis)*, 5% *Trifolium repens*, 5% *Lolium perenne*, and the remaining 5% was an assortment of other grasses and broad-leaved weeds.

In anticipation of higher emergence and survival with earlier sowing, Experiment 2, conducted in the following year began 2 months earlier (September) than Experiment 1, and two locations in the Moginie Block were used: 1) Site Mānuka 2 (M2), adjacent to the original site (-40.3880, 175.6124), with nearly identical properties; and 2) Site Treeline (TL), approximately 500 m west (-40.3867, 175.6088) of Mānuka 2, located inside the dripline beneath a row of 20-30 m *Pinus radiata* trees planted on the edge of the research unit as a windbreak. The Treeline site was chosen to contrast in two important ways with the Mānuka sites: a) It had been recently grazed and was fenced off 2 weeks prior to the commencement of the experiment; and b) it was much drier and ground-level vegetation was sparser than at the other two sites, most probably due to a large number of shallow tree roots, and the shading effect of the pine trees. None of the sites had a previous history of OMB presence. Ground-level vegetation in the TL plots consisted of roughly 40% perennial ryegrass (*Lolium perenne*), 15% soft brome (*Bromus hordeaceus*), 15% daisy (*Bellis perennis*), 15% white clover (*Trifolium repens*), 10% chickweed (*Stellaria media*), and 5% was an assortment of other grasses and broad-leaved weeds.

6.2.2 Site Preparation

A randomised complete block design with four replicates was used. The following three treatments were applied in both experiments: 1) soil kept bare throughout the experiment with hand-weeding (*MBare*); 2) vegetation cut to 4 cm (*Cut*); and 3) uncut grass (*Long*). In addition, one extra treatment was added for the repeat experiment: bare soil initially, with no maintenance after sowing (*IBare*). Treatment plots measuring 60 cm x 60 cm were marked out with wooden pegs, with a 60 cm buffer between them, a 1 m buffer between mānuka trees and the plots in M1 and M2, and a 6 m buffer between the main axes of the pine trees and the plots at TL. Plots in each block were randomly assigned treatments, and plots for bare soil treatments were sprayed with glyphosate (using 10 ml/L of Roundup 360 Pro applied to run-off) 3 weeks before sowing the seeds. All above-ground vegetation and as much as possible of the root system was removed in bare soil plots 1 day prior to seed sowing. Also, vegetation in the cut grass plots was trimmed to approximately 4 cm on the day prior to sowing, and approximately 30 cm around the bare soil and cut grass plots, to ensure no extra shading occurred (Figure 6.1). On average, uncut vegetation in the *Long* plots was 60 cm tall at the time of sowing.

Figure 6.1: Experimental plots showing competition treatments at Treeline site (left) and Mānuka 2 (right) on sowing day, September 2020: L (*Long*)= uncut, long grass; C (*Cut*)= grass cut to 4 cm; B (*Bare*)= bare soil.

Vegetation samples were taken outside of the sown plots, using a 50 cm x 50 cm quadrat to estimate percent dry matter composition at the beginning of each experiment: 18 samples at M1, and 12 each at M2 and TL. Half of the samples were intended to represent the initial competition (4 cm) present in the *Cut* plots. These were cut down to 4 cm prior to sampling. The other half were samples of uncut vegetation, representing competition present in the *Long* plots (Table 6.1). Figures for samples at M1 and M2 were particularly high in some cases, due to the density of the grasses and an accumulation of dead matter in the ungrazed sites. The expectation was that this site would be similar to many other waste areas and riparian zones. In Experiment 2, to get an indication of how the dry weight might have changed between spring 2020 and autumn 2021, six additional 50 cm x 50 cm quadrat samples were taken in April 2021.

Table 6.1: Average dry weight of ground-based vegetation in kg/ha in plots cut to 4 cm (*Cut*) and uncut plots (*Long*) at three sites: Mānuka 1 (M1), Mānuka 2 (M2), Treeline (TL). Samples taken in late spring (November 2019), early spring (September 2020) and mid-autumn (April 2021).

	M1	M2	TL	M2	TL
	November 2019	September 2020	September 2020	April 2021	April 2021
Cut	5,822	5,187	3,500		
Long	21,244	13,920	4,947	10,893	3,513

Soil samples were tested at Hill Laboratories in Hamilton, New Zealand, using their standard techniques. Analysis results of soil organic matter, pH, phosphorous, calcium, magnesium, potassium, sodium, cation exchange capacity, base saturation, and texture are given in Table 6.2. Soil type at all three sites is a silt loam, and, according to S-map online (Landcare Research NZ Ltd 2019), is moderately deep and poorly drained. At TL, the ubiquitous presence of shallow tree roots made it difficult to obtain sufficiently deep soil cores and made it difficult to completely remove the grass root system. As nutrients are stratified in the soil, with more in the upper than lower parts (Brady and Weil 1999), elevated levels of base minerals, organic matter and cation exchange capacity in the shallow samples at TL reflect that. Also, because of recent grazing at the site, stock sheltering underneath the trees from adverse weather could have contributed to the higher cation levels by their excretions. At M1 and M2, which had not been grazed in several years, no excretions or fertilizer had been added.

Table 6.2: Soil characteristics at the two experiment locations: Manuka 1 & 2 are adjacent, largely open, ungrazed sites and were sampled as one location; Treeline is a recently grazed site underneath a windbreak (OM = organic matter; CEC = cation exchange capacity; me/100 g = milliequivalents per 100 grams of soil).

Site	Ratio	pH	%OM	Olsen P mg/L	K	Ca	Mg	Na	CEC	% Base Saturation
	Sand:silt:clay				me/100g	me/100g	me/100g	me/100g	me/100g	
Mānuka 1 & 2	8:69:23	5.9	8.94	48	1.17	7.7	1.48	0.14	17	62
Treeline	7:66:27	5.9	21.40	41	1.33	10.5	5.16	1.16	33	56

Achenes of OMB were collected in September from two populations both years (see Section 4.2.1). After discarding achenes that were flat, eaten or smaller than 50% of the average size, all seeds were stored dry at 5°C until the day of sowing. On the day of sowing, seeds from both populations were mixed together. Average laboratory germinability of a sample of the seeds in complete darkness was observed to be approximately 37% in September 2019 (see Figure 4.4). In anticipation of an equivalent number of seedlings emerging for this experiment, 100 achenes were sown in each plot,

just under the soil surface in five lines 20 cm long and 5 cm apart (see Figure 6.2). The second cohort of seeds from September 2020 were 48% germinable in the same laboratory conditions.

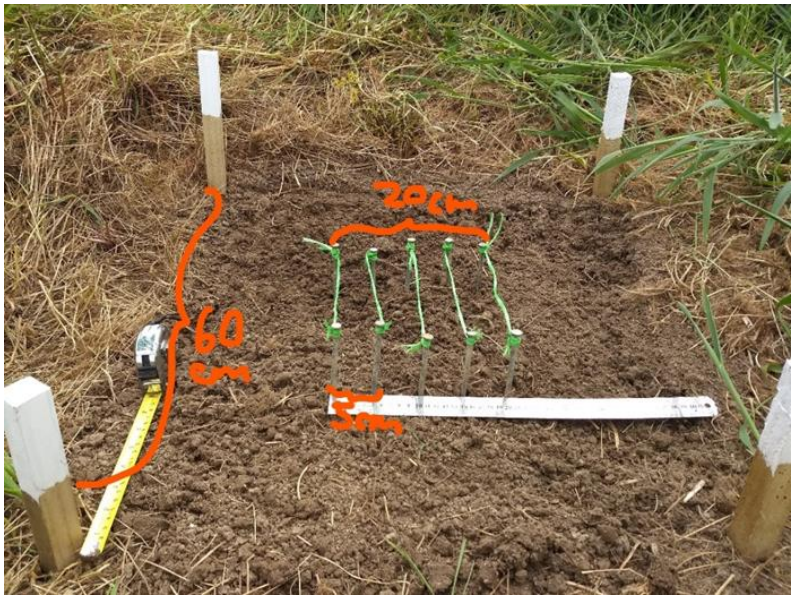

Figure 6.2: Sowing lines in the middle 20 cm of a 60 cm x 60 cm bare plot, November 2019.

Over the course of the experiment, weather data from the nearby AgResearch weather station were recorded (Figure 6.3). The maximum average daily temperature ranged from 24.6°C in February to 14.5°C in July. No supplemental watering was provided to the plots.

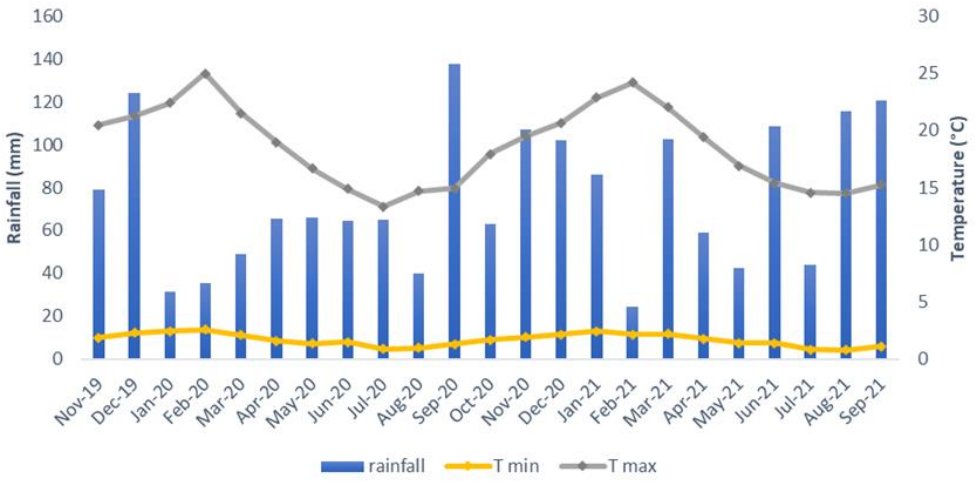

Figure 6.3: Mean total monthly rainfall (mm) and mean monthly minimum (Tmin) and maximum (Tmax) temperature (°C) over the duration of the two experiments (Experiment 1: November 2019-November 2020; Experiment 2: September 2020-September 2021).

6.2.3 Plot monitoring/data collection

Plots were monitored regularly for germination of OMB, and *MBare* plots were weeded as necessary. Numbers, size, and condition of seedlings present in each plot were recorded at 10 or 11 weeks, 15 weeks, 25 weeks (6 months), and 52 weeks (12 months) after sowing. Seedlings that survived treatment conditions over winter, between 6 and 12 months, were considered to have successfully established. At TL, new OMB seedlings began emerging in some plots after 12 months. Although the plots were not weeded after Week 52, monitoring was therefore extended at TL another year, to assess the survivability of these second-year seedlings.

The electrified fencing surrounding the plots at M2 was inadvertently left off for a short period following seed sowing in September, and lamb hoofprints were found in the *MBare* and *Cut* plots of Block 4 during the first monitoring/weeding session. Thereafter, no seedlings emerged in those plots, so no data could be obtained from them.

6.2.4 Data analysis

All experimental analyses were performed using R statistical software, version 4.2.0 (R Core Team 2021). Seedling survival at each site was analysed independently, with eight different generalised linear mixed-effects regression models for counts using the lme4 package (Bates et al. 2015). Block was included as a random factor, with treatment as a fixed factor. For each of the three sites, one model was fitted to the data from Week 10 (initial seedling establishment), and a separate model fit to the data at the last monitoring event, Week 52 (survival). For TL, an additional two models were fitted from Week 10 (61) and 52 (104) in the 2nd year to estimate treatment effects on the year 2 seedling establishment and subsequent survival. Due to overdispersion, a negative binomial analysis was used for TL's Weeks 10 and 61 models, but a Poisson was used for all other models. Because no seedlings ever established in the unmanipulated, *Long* plots at either M1 or M2, only the *Bare* and *Cut* plots were used in those analyses. In addition, failure of model convergence for Week 52 at TL was resolved by removing *Long* plot data (all 0). Goodness of fit for each model was tested by plotting simulated residuals with the DHARMa package (Hartig 2022), shown in Appendix D, Figures D.1-D.8. Post-hoc multiple pairwise comparisons were made using the multcomp package (Hothorn et al. 2008). However, low seedling emergence and even lower survivorship made robust model estimates of block or treatment effect difficult to obtain, especially for analyses at 52 and 104 weeks, in which large standard errors were produced.

6.3 Results

More seedlings established at the early spring-sown M2 than the late spring-sown M1 (Figure 6.4). In both cases, by Week 15, seedlings in *MBare* plots had begun forming multiple stems. By Week 25, the seedlings had developed into vigorous, bushy, multi-stemmed masses, regardless of seedling number. In all other treatments, seedlings remained small and single-stemmed. As mentioned above, no seedlings were detectable in the dense, uncut grass of the *Long* plots at any time during the year of the preliminary experiment at M1, nor were any found in the year of the repeated experiment at M2. Also, no seedlings in either Mānuka site survived the winter in any treatment other than *MBare* (no competition).

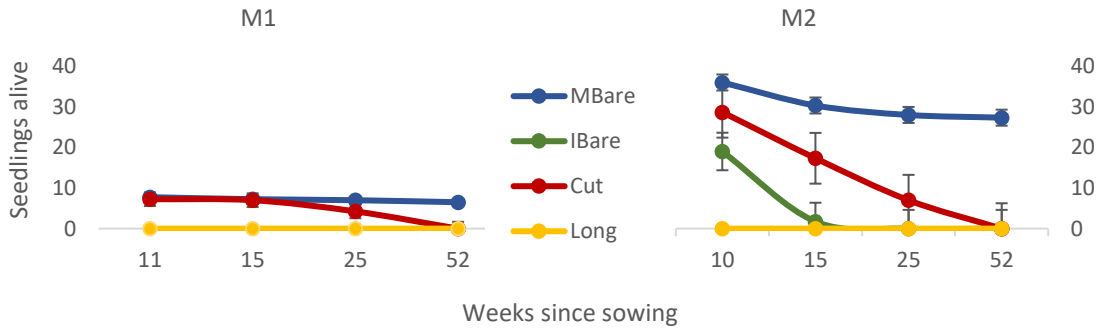

Figure 6.4: Average *Clematis vitalba* seedling survival (raw data) per plot over a 1-year period after sowing (100 seeds/plot) at a densely vegetated, site interspersed with mānuka plants; from November 2019-November 2020 (M1, left) and from September 2020-September 2021 (M2, right). MBare = plots maintained bare (bare at the time of sowing, kept weeded and trimmed throughout the experiment); IBare = plots initially bare (bare at the time of sowing, but not weeded or trimmed thereafter); Cut = vegetation in plots cut to ~4cm at the time of sowing, but not trimmed again; Long= seeds sown in plots with unmanipulated vegetation. Error bars based on standard errors of the arithmetic mean of raw data.

At Week 52, some seedling stems at M1 and M2 had senesced and dropped some or all their leaves (Figure 6.5). The smallest of those had been reduced to a single-stemmed shoot and appeared to have died. However, upon removal, all seedlings had healthy root systems, showing that they were simply dormant, and had not yet begun their spring growth.

Unexpectedly, more seedlings emerged in *Cut* plots than in *IBare* plots at M2. Between Weeks 10 and 15, average seedling survival in *Cut* plots declined 30%, whereas survival in *IBare* plots declined 90%. Further inspection of plot vegetative makeup revealed that all *Cut* plots were almost entirely buttercup (33%) and perennial grasses (65%), with a small amount of clover (2%). In *IBare* plots, buttercup and clover density was similar to *Cut* plots, but grass density was slightly lower (55%) and species diversity was also much higher, with broad-leaved fleabane (*Conyza sumatrensis*), Scotch

thistle (*Cirsium vulgare*), broad-leaved dock (*Rumex obtusifolius*), and sow thistle (*Sonchus oleraceus*) having colonised the *IBare* plots in small numbers.

Figure 6.5: Surviving *Clematis vitalba* seedlings with senescent stems in a plot kept bare by hand weeding during the first year of growth. Left: new, early spring growth, right: early spring dormant seedling shoot with a healthy root system.

Because of poor emergence, Poisson models showed very little variation among blocks and no treatment effect in M1, at either Week 10 or 52 (Figure 6.4; Appendix D, Table D.1). At M2 (Appendix D, Table D.2), where more seedlings emerged than at the other two sites, initial seedling establishment (Week 10) was significantly different among all treatments (<0.001), with *MBare* plots having the highest emergence (average 36%), *Cut* plots the next highest (average 29%), followed by *IBare* plots (average 19%), and *Long* plots (0%). Survivability at the *MBare* treatment (average 27%) remained significantly higher (<0.001) than all other treatments (0%) at Week 52.

At TL, initial seedling establishment occurred in every treatment (Figure 6.6). Grass and other weeds were slow to regrow in *IBare* plots, which remained largely bare through to Week 10. Thus, there was no significant difference between seedling numbers in the *MBare* and *IBare* treatments at that time. A Poisson model for Week 10 at TL showed a treatment effect and a very small block effect, with both the *Long* and *Cut* treatments resulting in significantly less seedling establishment (<0.05) than the *IBare* treatment, reflecting what seems to be apparent from the observed data. However, due to overdispersion, a more conservative, negative binomial was fitted, which resolved the overdispersion, but did not detect a block or treatment effect (Appendix D, Table D.3).

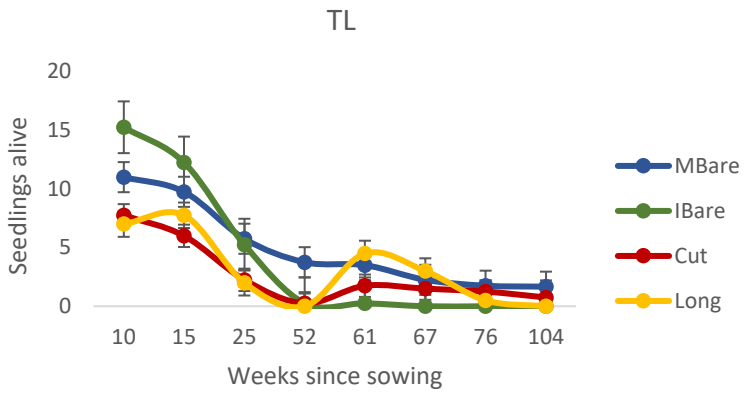

Figure 6.6: Average *Clematis vitalba* seedling survival (raw data) per plot over a 2-year period (September 2020-September 2022) after sowing (100 seeds/plot) at a recently grazed, sparsely vegetated, dry site, beneath a row of pine trees. MBare = plots maintained bare (bare at the time of sowing, kept weeded and trimmed throughout the 1st year of the experiment, but not the 2nd year); IBare = plots initially bare (bare at the time of sowing, but not weeded or trimmed thereafter); Cut = vegetation in plots cut to ~4cm at the time of sowing, but not trimmed again; Long= seeds sown in plots with unmanipulated vegetation. Error bars based on arithmetic mean of raw data.

Although not the only treatment with seedlings that survived to Week 52 at TL, the largest proportion of seedlings persisted in plots kept bare by weeding (*MBare*): significantly more seedlings survived in the *MBare* treatment (<0.05) than in any other treatments. Only a small fraction of seedlings in the *IBare* and *Cut* treatments survived the winter, with no seedlings in the *Long* plots surviving. By Week 61, new seedlings had emerged in the *Cut* plots (average 0.25 seedlings/plot at Week 52; 1.75 seedlings/plot at Week 61) and the *Long* plots (0 seedlings/plot at Week 52; 4.5/plot at Week 61). Significantly more seedlings were found in the *Long* plots ($p < 0.05$) than in the *IBare* plots at Week 61, where all first-year seedlings had died and no new seedlings had emerged. By the end of the second year (Week 104), there was again a higher proportion of seedlings in the *MBare* treatment than any other, but so few seedlings persisted that there was no significant difference among any treatments. At the end of the 2nd year (Week 104), *Cut* plots averaged 0.75 seedlings/plot and *Long* plots were again at 0.

Apart from the critical decline in survival over winter, in *IBare* plots at M2 and TL, as well as in *Cut* plots at all three sites, seedling numbers declined over the summer. In addition to competition, water stress, heat stress and herbivory likely contributed to summer seedling loss. Summer leaves on most of the seedlings at TL, as well as on some seedlings at M1 and M2 in *MBare* plots, turned reddish-purple, caused by production of anthocyanins, a sign of environmental stress (Kovinich et al. 2015) (Figure 6.7). Also, some seedlings were chewed down to the ground, most probably by rabbits or ducks.

Figure 6.7: Stressed *Clematis vitalba* seedling leaves with anthocyanin pigmentation at the Treeline site.

Of the two sites sown in 2020 for Experiment 2, fewer total seedlings emerged at TL (164) than at M2 (270). By 6 months, all seedlings at TL remained single-stemmed, and average TL seedling height was 2.4 cm (Figures 6.8, 6.9).

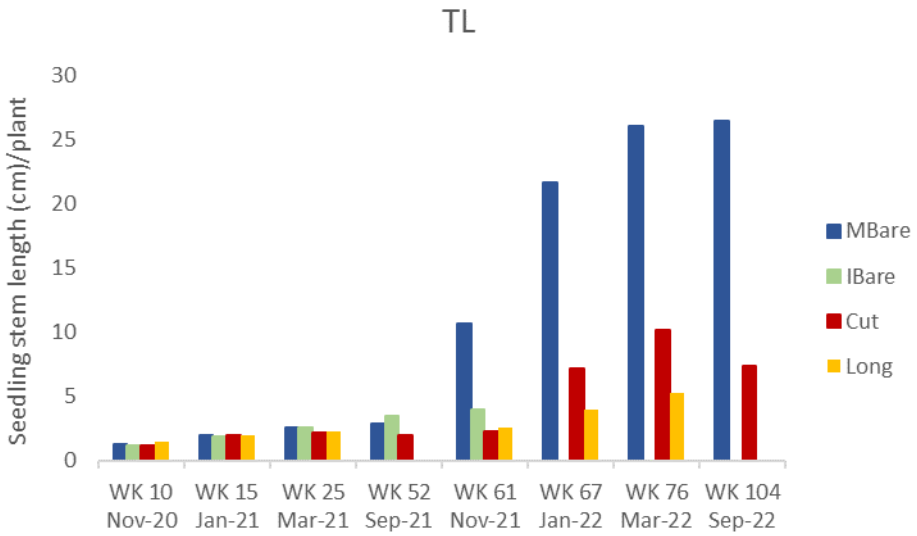

Figure 6.8: Average stem length of *Clematis vitalba* seedlings after sowing in four levels of competition (MBare = plots maintained bare (bare at the time of sowing, kept weeded and trimmed throughout the 1st year of the experiment, but not the 2nd year); IBare = plots initially bare (bare at the time of sowing, but not weeded or trimmed thereafter); Cut = vegetation in plots cut to ~4cm at the time of sowing, but not trimmed again; Long= seeds sown in plots with unmanipulated vegetation) at Treeline (TL) site sparsely vegetated, dry site beneath a row of pine trees. All seedlings between Weeks (WK) 10-61 remained single-stemmed. At Week 67 (WK 67), seedlings in treatment A and C were multi-stemmed and seedling average stem length was multiplied by the average number of stems to reflect average total stem length/plant.

Figure 6.9: Size and vigour of *Clematis vitalba* seedlings 25 weeks after sowing in plots kept bare by weeding at two different sites: Treeline (left), a sparsely vegetated, dry site, beneath a row of pine trees; and Mānuka 2 (right), a densely vegetated site interspersed with mānuka plants. Distance between pegs, both left to right and top to bottom is 60 cm.

In contrast, M2 seedlings had an average of 2.5 stems each by 6 months, and average stem length was 36 cm (Figures 6.9, 6.10).

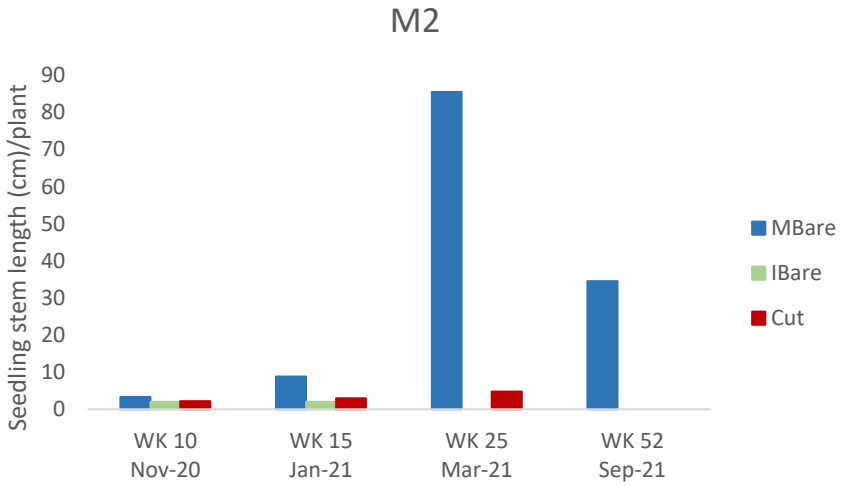

Figure 6.10: Average stem length of *Clematis vitalba* seedlings after sowing in three levels of competition (MBareA = plots maintained bare (bare at the time of sowing, kept weeded and trimmed throughout the 1st year of the experiment, but not the 2nd year); IBare = plots initially bare (bare at the time of sowing, but not weeded or trimmed thereafter); Cut = vegetation in plots cut to ~4cm at the time of sowing, but not trimmed again; at Manuka 2 (M2). All seedlings in IBare and Cut treatments remained single-stemmed. By 15 weeks (WK 15), seedlings in treatment MBare had developed multiple stems and average seedling height was multiplied by the average number of stems to reflect average total stem length/plant. A fourth treatment, where seeds were sown in plots with unmanipulated vegetation, prevented all seedling emergence.

Only 27% of all seedlings in all treatments present at Week 25 persisted through to Week 52 at TL, while 78% persisted at M2. Yet, at TL, small, single-stemmed seedlings no longer than 3 cm survived to 52 weeks in both the *IBare* and *Cut* plots, whereas at M1 and M2, seedlings up to 9 cm tall did not survive winter in those same treatments. In the second year, although no weeding was done, TL seedlings that had survived the first year in the *MBare* plots elongated considerably more than they had in the previous year, and by Week 67, had developed multiple stems, as had one seedling in the *Cut* treatment.

6.4 Discussion

Higher invasion potential has been attributed to plants whose seedlings germinate and emerge earlier in the growing season, because early access to finite resources can enhance their prospects for reaching maturity (Bakker et al. 1980, Guido et al. 2017). In this research, sowing time was an important factor in determining OMB seedling emergence. Under optimal laboratory circumstances, approximately 80% of OMB seeds collected and incubated in September will germinate when exposed to light, but without light only about 40% of seeds will germinate (Figure 4.4). For seeds sown in late spring (M1, November 2019) in the plots kept bare by weeding (*MBare*), fewer than 10% produced a seedling that emerged through the soil. In contrast, of seeds sown in 2020 earlier in the spring (M2, September 2020) in bare plots, 36% produced a seedling that emerged, slightly lower than the laboratory germination results for seeds kept in darkness. It should be noted that, as one *MBare* M2 plot was trampled by lambs and did not support any OMB seedling growth, M2 results were averaged across only three of the four plots for the *MBare* treatment. In addition to the advantage of an early start, the September 2020-sown seedlings benefitted from more rainfall in the first 4 months of the early establishment phase than did those of the November 2019-sown seedlings (Figure 6.3). Hotter temperatures in November may also have been less hospitable than September temperatures for emerging seedlings. Differences between seed cohorts could also have had a small effect on the differences in outcome between M1 and M2 (see Section 4.3.1). Lower emergence in the field plots than in the incubator could be explained by: a) seedling emergence through the soil is an additional process after germination; and b) more factors are involved in the successful germination of seeds and subsequent seedling emergence in a natural setting than in the laboratory. For example, outside of the laboratory setting, moisture, temperature, and other resources are not controlled; soil properties can restrict gas exchange, availability of nutrients or minerals (Brady and Weil 1999). In addition, seed predators, soil microorganisms, intra- and interspecific competitors can all slow down, damage or destroy seeds and emerging seedlings (Van Mourik et al. 2005, Gurevitch 2011, Gioria and Pyšek 2017).

At the Mānuka sites (M1 and M2), plots experienced some shading of full sun from mānuka plants, as some plots were within 2 metres of the mānuka. However, the main source of competition came from

crowding by the grasses and weeds at ground level, which restricted OMB seedling access to limiting resources (light, water, nutrients). Previous researchers have shown that availability of light, soil moisture and soil nitrogen to plants grown without competitors is two to five times greater than for plants grown in the presence of competitors (Peltzer and Köchy 2001). In this study, of those seedlings at M1 and M2 that emerged in the plots without competitors (*MBare* plots) both years, within 6 months, almost all seedlings had produced vigorous, multiple stems, averaging more than 30 cm long. More than 75% of the *MBare* seedlings at the Mānuka sites persisted at Week 52. By comparison, no recognisable seedlings ever emerged in the plots with dense, grassy vegetation (*Long* plots) at either of the two sites. Also, seedlings that emerged in all other treatments remained single-stemmed and small, with numbers that declined as competition from regrowth of the grass and other weeds increased. None of those seedlings survived a full year. The differences between the treatments are evidence that OMB seedlings are negatively density dependent, as suggested by West (1992).

Regarding the multiple stems of MBare seedlings at M1 and M2, each new stem represents a potential searching climber for lianas. In addition, according to Buru et al. (2016), development of multiple stems allows vines to maximise photosynthesis and improve competitive ability. Tanentzap et al. (2012) have also reported that forest understorey trees with multiple stems have a greater chance of survival, due to the additive growth of each stem and the buffering effect of allocating resources among several stems, in case of individual stem loss. Thus, reaching the stage where ancillary stems are produced may be critical for OMB seedling survival to maturity, especially in highly competitive habitats in combination with other stresses. In fact, only the smallest seedlings in the *MBare* plots at the Mānuka sites, all single-stemmed, died over winter (7% at M1 and 2% at M2). Despite complete winter senescence of the smallest *MBare* seedlings (Figure 6.5), and dieback of some stems on most seedlings, all *MBare* seedlings that did persist through winter had developed vigorous root systems when removed after Week 52. These appeared to be well-positioned to continue into maturity.

Zhang et al. (2021) have claimed that OMB leaves are heat tolerant. Indeed, as the summer progressed, seedlings in *MBare* plots at the Mānuka sites and most TL seedlings began producing anthocyanins in their leaves, which are recognised to help protect against extreme temperatures, UV radiation, free radicals, drought, injury, and soil contamination (Chalker-Scott 2002, Gould 2004, Kovinich et al. 2015, Laxa et al. 2019). At TL, overhanging pine branches intercepted much of the direct rainfall TL seedlings would have received, and large pine tree roots competed with seedlings for access to soil resources. At M1 and M2, *MBare* seedlings were more exposed to direct, harsh sunlight than any other treatments. Anthocyanin production was likely a response to the accumulating stresses of limited water supply with high light intensity. It is possible that the anthocyanin production helped mitigate seedling loss during the summer months, but it could not protect seedlings from the effects of competition with other plants.

The purpose of adding the *IBare* treatment at M2 and TL was to simulate what might happen in a situation where no intervention occurs after initially clearing the land, or after a treefall occurs in a forest, or after a flooding event leaves silt deposits behind. It was also intended to be an intermediate level of competition between the *MBare* and *Cut* treatments (grass initially cut down to 4 cm). At TL, the *IBare* plots supported significantly more seedlings than *Cut* plots at first, as expected. However, at M2, not only did *Cut* plots support significantly more seedlings than *IBare* plots, but seedling numbers in the *IBare* plots also declined much more rapidly than those in the *Cut* plots, suggesting that after the temporary bare phase, the competition in the *IBare* plots was higher. More diverse types of weeds, and more large-leaved individuals (dock, Scotch thistle, sow thistle, broad-leaved fleabane) were found to have populated M2's *IBare* plots after the first few weeks, whereas, aside from the height of the plants, the vegetative makeup of the *Cut* plots did not change over time. It may be that the difference between the two treatments was a result of the large broad-leaved weeds in the *IBare* plots reducing the amount of light available to the OMB seedlings in the plots. Regardless of the abundance of seedlings present within the first few months of life, no seedlings progressed past the single-stem stage, nor did any survive the winter in either treatment. These results demonstrate that increasing competition during the vulnerable young seedling stage prevents OMB from establishing in a grassy habitat. It is worth mentioning here that the *Cut* treatment was not intended as a simulation of a grazed pasture, but as a form of intermediate competition. Livestock readily graze OMB. Therefore, seedlings that emerge in a grazed pasture would be very unlikely to reach maturity, due to grazing.

Biomass of ground-based vegetation at the Treeline site (TL) was considerably lower than at either Mānuka site. Initial establishment of seedlings in every treatment at TL is evidence that the reduced direct ground cover competition was advantageous in the emergence to early seedling stage. However, in addition to the ground cover, seedlings at TL were subjected to drier soil due to the prevalence of large radiata pine tree roots, shading and obstruction of rainfall from overhanging tree branches, shading from pine needle litter, and possibly an allelopathic effect of the needles (Kimura et al. 2015). Thus, fewer seedlings emerged at TL than M2. Also, after the initial emergence, TL seedlings failed to grow taller than an average of 2.4 cm, to elongate, or produce multiple stems in the first year, even in the *MBare* treatment. Yet, survival to one year at TL was almost entirely limited to seedlings in the *MBare* treatment, which suggests that direct competition from grasses and weeds did still have a decisively negative effect on the seedlings in the other treatments, despite the lower density.

That a smaller proportion of initially established seedlings persisted between 6 months and 1 year at TL (27%) than at M2 (76%) may have been due to a failure to achieve a critical size to withstand the winter. Notwithstanding their small size, likely because of low direct competition from ground-level vegetation, a few seedlings did persist through the first winter (1% of all seeds sown). A small flush of emergence in the second year produced new seedlings, but 40% fewer seedlings persisted at TL at the end of that year (0.5% of all seeds sown) than at the end of the first year, demonstrating the

difficulty of OMB establishment in such an environment. However, even though no weeding was done in the second year at TL, seedlings began developing multiple stems, indicating that establishment in restrictive habitats can occur on a limited scale. Thus, if a seed germinates at a site that is open for a window of time that allows a seedling to reach the multi-stemmed stage, it will likely survive to maturity, even if other vegetation eventually re-populates the site. As an aside, emergence of new seedlings a year after sowing confirms the findings detailed in Chapter 5, that buried OMB seeds, even those buried superficially, can constitute a dormant (temporary at least) soil seed bank.

6.4.1 Limitations

Low numbers of seedlings at M1 during the year led to poor statistical power to detect treatment effects. Also, the models for Week 52 at M1 and M2 and for Weeks 10 and 61 at TL, returned matrices with a singular fit. According to Bates, et al. (2015), singular models are statistically sensible, in theory, but can sometimes signal overfitting, and result in poor predictive power. Poor prediction seemed to be especially evident for TL's Week 10, where no treatment effect was detected by the model, although the data points appeared to indicate one (see Section 6.3, Figure 6.6). Removing the random block variable in the models did not change the singularity, so overfitting was likely not the problem. Some researchers have recommended using a Bayesian approach to overcome the singularity issue, by incorporating prior information into the model (Gelman and Hill 2006, McElreath 2015). This study offers insight into the unexceptional competitive ability of OMB seedlings, although, to achieve more meaningful statistical information, it may be useful to apply Bayesian methods and fit a Bayesian model assuming that sensible prior data is available.

6.5 Conclusions

Plant establishment from seed in a new location requires successful seed germination, seedling survival and growth to maturity (Richardson et al. 2007). As demonstrated in Chapter 2, nearly all OMB seeds are dispersed by the end of September (early spring). Viable OMB seeds have a relatively high germinability rate in spring (Chapter 4). However, despite the potential for an early start, even when OMB seeds land in suitable sites for germination, obstacles to seedling emergence and unremarkable competitiveness at the young seedling stage severely limit OMB's chances for successful establishment from seed. Indeed, even when competition was removed, seedling emergence was lower than 40% of sown seeds. Therefore, risk of invasion by OMB seed of dense grassy habitats or otherwise competitive habitats, is not high.

Avoiding competition during vulnerable seedling stages by colonising bare or low competition habitats is a strategy employed by many invasive plants (Gioria and Pyšek 2017), as seems to be the

case for OMB. A suitably vacant site may occur along rivers when silt is deposited during a flooding event, or when gaps in the forest floor open as a result of treefalls or other disturbances. Clearing by machinery can also open sites for colonisation. Yet, if gaps or cleared land are allowed to be quickly recolonised with dense vegetation, OMB seedlings that do emerge are unlikely to reach maturity. However, dense vegetation is not always achievable, and seedlings are capable of colonising habitats with lower density ground cover, even where the presence of large tree roots and shading make establishment even more problematic. The prospect of successful seedling establishment increases with increasing numbers of seeds (Clarke et al. 2001) and copious seed production is a recognised trait of many invasive plant species (Mason et al. 2008). With between 40,000 and 50,000 viable OMB seeds produced/m^2/yr (Section 4.4.1), if even 0.5-1% land on soil that remains bare long enough for seedlings to reach a critical size, several hundred successful new recruits could result. Also, dormant seeds in the soil could take advantage of newly bare habitats. Thus, the magnitude of its sexual propagule pressure helps OMB compensate for its low investment in quality seed and establish itself in new locations. Importantly, as a consequence of climate change, more extreme and frequent storms and flooding events that generate more vacant sites may improve OMB's chances of establishing by seed.

Researchers agree that invasive plants often employ effective long-distance dispersal in combination with effective, rapid expansion over shorter distances (Schupp 2011). As an invasive species, OMB seeds have the potential for long-distance dispersal via wind and water. However, this study has established that few OMB seedlings are expected to survive to maturity. For those few that do successfully colonise new habitats, it is probable that the primary means of rapidly expanding their range is by vegetative reproduction. Some lianas are well known for spreading by clonal stems along the ground (Prosperi et al. 2001, Schnitzer et al. 2004, Yorke et al. 2013, Ledo and Schnitzer 2014, Ladwig and Meiners 2015, Buru et al. 2016, Mori et al. 2018). The next chapter will explore OMB's use of vegetative reproduction as a means of local dispersal and population growth.

7. Asexual reproduction

7.1 Introduction

Successful dispersal, recruitment of new plants, and maintenance of a reproducing population are all elements of a successful plant invasion (Bufford and Daehler 2011). Employing multiple mechanisms to those ends improves the chances of a favourable outcome. Indeed, as mentioned in Section 1.4.9, in addition to reproduction by seed, many lianas, including invasive species, commonly rely on vegetative growth (growth that involves non-flowering plant parts) for dispersal, recruitment, and reproduction (Penalosa 1984, Putz 1984, Leicht-Young and Pavlovic 2015, Buru et al. 2016, Mori et al. 2020). The main benefit of sexual reproduction is that it ensures genetic diversity in a population, which leads to adaptability in changing environments. As a complementary system, vegetative reproduction generally supports more rapid growth and range expansion with fewer resource inputs than sexual reproduction (Schurko et al. 2009). Lianas produce multiple stems, which elongate and branch, root and develop shoots, both on the ground and as climbers. This is a form of short-distance dispersal. Pollinators and mates for cross-fertilisation are unnecessary in asexual reproduction. Thus, it is possible for a single successful, genetically fit seedling to arrive and populate a new habitat by vegetative expansion (Barrett 2011). When vegetative plant parts form adventitious roots and are subsequently separated from the mother plant (genet), they become independent clones (ramets) of the genet (Jeník 1994, Oborny and Bartha 1995, Munné-Bosch 2015). Another form of clonal reproduction is through the regeneration of plant parts into individual ramets after fragmentation (Stuefer et al. 2002). Ramets represent new recruits, which increase propagule pressure and a plant's odds of surviving in a new environment, because the risk of destruction is reduced with the presence of more individuals (Richardson et al. 2007, Duncan 2011).

For plant species that are incapable of growing upright on its own, the optimal circumstance is to climb a tree or other support, which allows it to maximise its photosynthetic capacity. Ground-based vegetative spread is a way for an established liana to expand its range temporarily, albeit incrementally, while searching for trees to support its stems (Wyka et al. 2019). Researchers have shown that creeping lianas can produce dense networks of branching stems, both above and below ground (Putz 1984, Schnitzer et al. 2000, Sakai et al. 2002, Buru et al. 2016). The lateral stems root at the nodes, which physically anchor them to the ground and provide them with an increased uptake of water and soil nutrients. As discussed above, rooted fragments that become severed from the rest of the stem can become self-sufficient plants, yet any connections between the rooted nodes allow them to share resources and enhance their resiliency in less-than-ideal environments (Marshall 1990, Roiloa et al. 2014, Mori et al. 2021). The connections also make it possible for any shoots and leaves along the continuum to provide photosynthates to other sections of the stem. Furthermore, the active and

dormant buds on vegetative growth serve as a “bud bank” (Ott et al. 2019), complementary to the seed bank a liana may develop, to allow the species to persist over time.

As stated in Section 1.4.10.2, old man’s beard has become a problem in farm forestry settings in New Zealand (A. Gordon, Rangitikei District Council, pers. communication, November 2019), where site preparation between rotations and before planting tree seedlings involves mechanically breaking and clearing debris with bulldozers and stacking it into windrows, including any OMB that may be present. Rudimentary investigations of small OMB individuals at a 6-year-old forest in Taihape suggested that nearly all shoots arose from creeping stems that appeared to originate in the windrows between the trees, indicating that broken stem fragments had regenerated (personal observations, December 2019; Figure 7.1).

Figure 7.1: Six-year-old farm forestry *Pinus radiata* block near Taihape, New Zealand, December 2019. Left: *Clematis vitalba* climbing the trees; right: new *Clematis vitalba* growth from a windrow between the trees (current year’s growth displayed, between 2 and 3 metres).

OMB may rely heavily on vegetative reproduction, yet there has been no peer-reviewed research published on the ability of OMB to asexually reproduce. Two New Zealand studies (Kennedy 1982, Van Gardingen 1986) have looked at the capacity of OMB stem fragments to regenerate. Van Gardingen (Master’s thesis) collected fragments in April, while the plant was going dormant. She collected two-node fragments from a mature vine, placed them upright in 1:1 mix of sand and loam, with one node covered, and watered overhead. Monitored over the course of 8 months, there was no growth of any kind. McClelland (unpublished report mentioned in Dept of Lands and Survey seminar proceedings by Kennedy (1982)) claimed poor regeneration from one-node sections but found that

20% of fragments from 3-year-old and older stems produced shoots. Only 12.5% of 2-year-old fragments produced regrowth, and no younger material produced any regrowth. Few other details are known about either one of these studies (Section 1.4.9).

The investigations in this chapter were carried out to gain a better understanding of the role vegetative growth plays in OMB's invasion success. Initially, an observational study was carried out to investigate *in situ* the frequency of natural layering and branching of OMB lateral stems as potentially important contributors to dispersal, recruitment, and survival of the species. Based on the patterns observed, the question of whether injured stems were capable of re-rooting and regenerating was raised. An experimental study was then designed to test *ex situ* the ability of OMB stem fragments to act as vegetative propagules after injury. As the first steps in characterising and defining the magnitude of OMB's vegetative reproduction, these results may help predict its spread and inform choices for its control.

7.2 Methods

7.2.1 Methods: Observational study

In February 2021, three river flood plain sites in the Manawatu Region: 1) Manawatu River at Awapuni (-40.3861, 175.5858), 2) Oroua River at Awahuri (-40.2763, 175.5208), and 3) Rangitikei River near Halcombe (-40.1238, 175.4458), each separated by at least 17 km from each other, were selected as representative of riparian zones highly infested with OMB. Across the three sites, 10 random 1m x 1m plots on the ground were excavated by hand to determine the characteristics, abundance, and frequency of rooting, branching and shoot development of all OMB stems present, both above and below the soil surface. Plots represented a range of shade levels, from 10 to 75% of full sunlight; the canopy cover index (immediately above the ground level vegetation) of each plot was calculated using the Gap Light Analysis Mobile Application (GLAMA) for Android phones (Tichý 2019). All non-OMB vegetation within each plot was removed, and all OMB stems and associated parts were then examined, measured, and traced to their lowest point underground, between 20 and 40 cm deep (Figure 7.2). In each plot, the following was recorded:

- whether stems were above or below the soil surface
- total length of stems
- any stem branching
- number of stem nodes and the distance between nodes
- number of nodes with roots

- number of nodes with shoots (current year's new stems)
- total length of shoots
- any flower or achene production from lateral shoots

Figure 7.2: L: Plot (1 m^2) staked and edges cut; R: All above and below ground *Clematis vitalba* stems removed (30 cm deep).

7.2.2 Methods: Fragment regeneration experiment

In mid-September 2021, woody stem fragments of both climbing (vertical) and creeping (lateral) stems were collected from the same three sites where the observational study was conducted. Due to the time required to dig up underground stems, 90% of the lateral stems were obtained from above ground. Approximately 80 fragments of each stem type were obtained and cut to 30 cm in length, with two nodes. To avoid using material with dormant buds, stem fragments for this experiment were collected just as OMB's first spring flush of growth began. Bud activity at the nodes (leaves/shoots or roots) and fragment diameter (which ranged from 2 cm to 25 cm) were recorded and each fragment was labelled with a unique identifier. Water stress of fragments was minimised by keeping them in a wet burlap (hessian) bag until they could be buried. Immediately following collection, five fragments were placed horizontally at random in a polypropylene punnet tray (26 cm x 30 cm x 5 cm) lined with paper and a 1 cm layer of potting mix (50% fine bark, 30% coconut fibre, 20% Pacific pumice (7 mm), with 500 g Osmocote Pro 5-6 month controlled-release fertiliser, 50 g Osmoform, and 150 g dolomite per 100 L of potting mix), then covered completely with 2-3 cm of potting mix. Trays were

kept in a shade house, with no supplemental light, but with regular hand weeding. Overhead watering was provided once daily for 20 minutes (flow rate of 0.87 ml/minute) from September to 23 December and increased to twice daily from 23 December until the end of the experiment. Temperature in the shade house was monitored with a HortPlus MicroLogger. The maximum daily temperature ranged from 19.29 °C in September to 27.27 °C in January (Figure 7.3). Four months after burial, fragments were removed from the trays. As a measure of regeneration, the presence of any nodal growth and the number and length of shoots at each node were recorded.

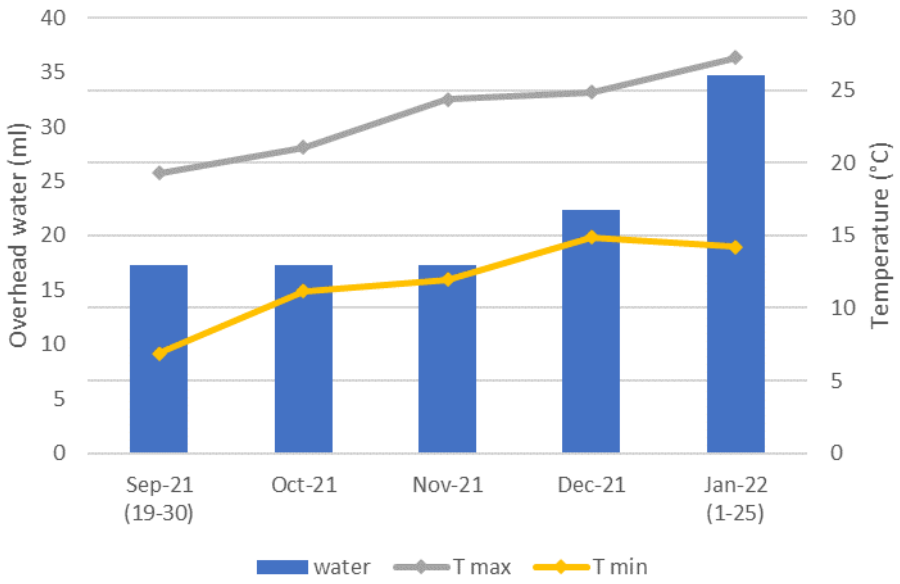

Figure 7.3: Mean monthly overhead water volume and mean monthly minimum (Tmin) and maximum (Tmax) temperature (°C) over the duration of the experiment, 19 September 2021-25 January 2022.

7.2.2.1 Experiment analysis

A two-stage analysis was carried out using R statistical software, version 4.2.0 (R Core Team 2021). First, analysis of stem regeneration (presence of shoots 4 months after cutting; yes/no) was made by fitting a binomial logistic mixed effects regression model, with the lme4 package (Bates et al. 2015). Stem type (creeping or climbing), initial stem diameter, and initial presence of an active bud (whether root or shoot) were included as fixed effects; tray was included as a random effect. As only 10% of creeping stem fragments were from below ground, no differentiation between above and belowground was made.

Second, an analysis of regeneration yield was made by 1) fitting a Poisson mixed effects model to predict the number of shoots produced from fragments that regrew, using the lme4 package (Bates et al. 2015); and 2) fitting a linear regression mixed model to predict the total length of new shoots produced, with the lmerTest package (Kuznetsova et al. 2017). Stem type and initial stem diameter

were included as fixed variables, with tray as a random variable. For the linear model, assumptions of normality and heterogeneity of variance were violated, so a standardised square root transformation was performed on the data before fitting the model (the most appropriate transformation was calculated using the bestNormalize package (Peterson 2021)).

For each analysis, stem provenance (origin) was also initially included as a random effect. However, all models including both random effects returned singular matrices, which generally is an indication of overfitting, with an increased chance of a false positive outcome (Bates et al. 2015). To achieve a non-singular fit, stem provenance was removed as a predictor for all final models. All models were selected based on AIC and goodness of fit calculated with the DHARMa package (Hartig 2022); plots of simulated residuals are shown in Appendix E, Figures E.1-E.4. Multicollinearity of fixed predictors was calculated using the variance inflation factor (VIF) with the car package (Fox and Weisberg 2019), and visualization of the Poisson and binomial models was accomplished with the effects (Fox 2003) and sjPlot packages (Lüdecke 2022).

7.3 Results

7.3.1 Results: Observational study

In the ten plots (excavated in mid-summer), OMB formed a complex lateral network of stems, roots, and shoots (Figure 7.4).

Figure 7.4: Typical *Clematis vitalba* lateral stem network. About half of this network was above ground, the other half buried within 30 cm of the soil surface.

Table 7.1 gives a combined summary of the findings. Most stems were above ground, but about 18% were below ground, found up to 40 cm deep, but usually less than 30 cm deep. On average, a 1 m^2 plot yielded 21 m of older OMB stems (5 m underground, 15.6 m aboveground) and 42 m of young, leafy OMB shoots (the current year's stems; 11 m emerging from underground stems, 30.8 m from aboveground). Average stem diameter in the plots was 3.6 mm (3.3 mm belowground, 3.8 above). Aboveground stems branched approximately once per metre, and belowground stems every 0.3 m. Dieback of growing tips occurred occasionally, which probably influenced the branching patterns. Branching also occurred sometimes in multiple directions, meaning more than one shoot per node survived to become a stem. Nodes were produced approximately every 9 cm (10.4 cm on underground stems, 7.7 cm on aboveground stems), with roots present at 25% of all nodes (45% of underground nodes, 5.5% of aboveground nodes), and shoots at approximately 17% of all nodes (7% underground, 26% above). Most shoots were produced at nodes with roots present, and often multiple shoots were present at a single node (Figure 7.5). No seedlings were found in any of the plots investigated; all small individuals were shoots originating from a creeping stem (which could have been mistaken as seedlings if not excavated). Nearly 6% of all shoots produced by aboveground stems had also produced flowers or flower buds.

Table 7.1: Summary of findings, average of 10 plots (1 m^2).

	Underground (SE)	Aboveground (SE)
% stem material	18.33 (7.2)	81.67 (10.73)
depth of stems (cm)	26.70 (1.45)	
length of stems (m)	5.10 (3.0)	15.60 (4.45)
stem diameter (mm)	3.29 (0.98)	3.81 (0.57)
branching frequency/m	0.29 (0.14)	1.04 (0.2)
distance between stem nodes (cm)	10.39 (0.46)	7.68 (0.02)
% nodes with roots	44.80 (13.14)	5.51 (5.17)
% nodes with shoots	6.98 (2.71)	26.33 (4.27)
length of shoots (m)	11.12 (4.64)	30.83 (12.9)
% shoots producing flowers	0.00	5.60 (0.03)

Figure 7.5: Multiple shoots and roots from one *Clematis vitalba* stem node.

7.3.2 Results: Fragment regeneration experiment

At the end of January, 4 months after collection, 48.7% of all stems (48.0% of lateral stems and 49.4% of vertical stems) had produced new shoots and roots and were growing as individual ramets. All new growth originated from the nodes (32% of all nodes: 30% of lateral stem nodes and 34% of vertical stem nodes; Figure 7.6).

Figure 7.6: Regrowth on a 2-node, 30 cm *Clematis vitalba* stem fragment 4 months after burial.

Of those stems that had had at least one visibly active bud (roots or shoots present) at the time of fragmentation and burial, 50.7% regenerated, whereas 47.7% of stems with no active initial bud regenerated. Logistic regression model results indicated that neither stem type nor the presence of an active bud at the time of fragment collection were significant predictors of fragment regeneration (stem type $p = 0.78$; active bud $p = 0.66$). On the other hand, initial diameter of stem fragments was significant at $p = 0.04$: for every one mm unit increase in initial diameter, the odds ratio of stem regeneration increased by a factor of 2.16 (Figures 7.7 and 7.8, Table E.1 in Appendix E).

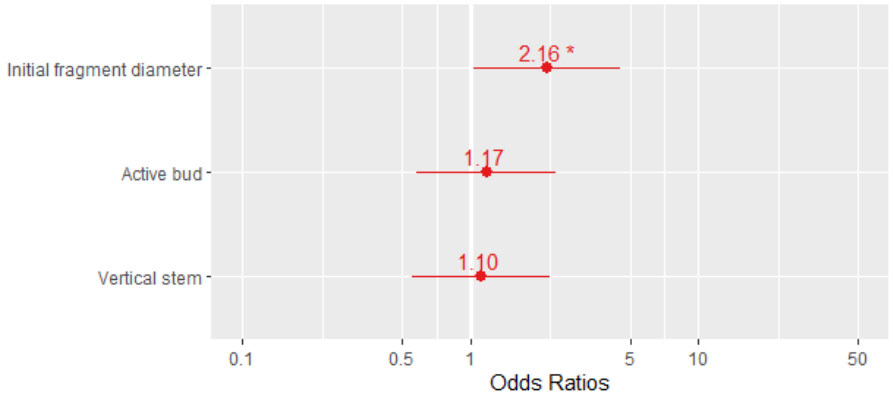

Figure 7.7: Logistic regression modelled effects of factors on *Clematis vitalba* fragment regeneration: initial fragment diameter (* indicates significance at $\alpha < 0.05$); initial presence of an active bud (as opposed to no active bud); vertical (climbing) stems (as opposed to lateral (creeping) stems.

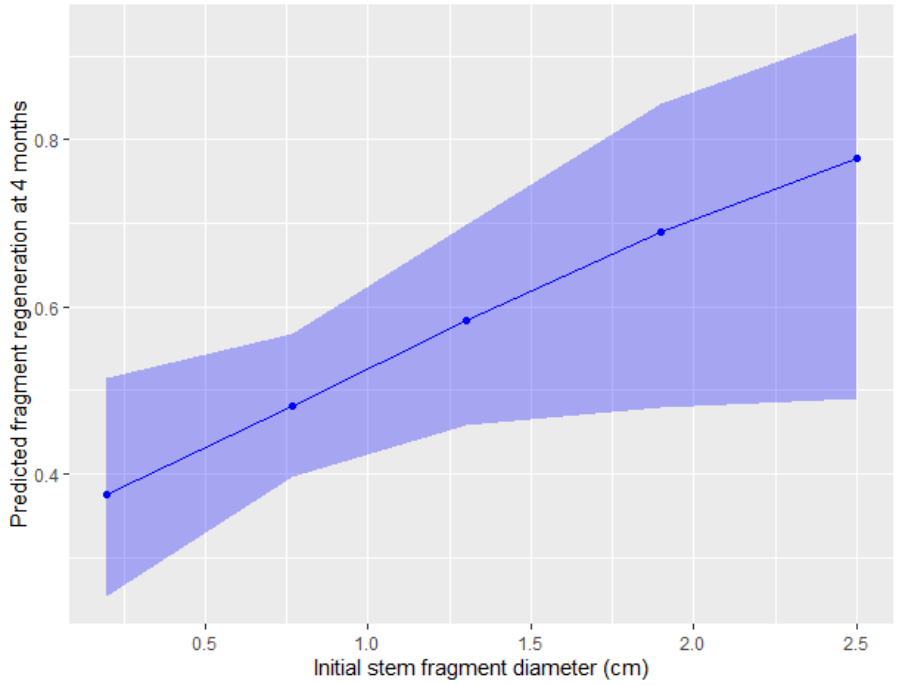

Figure 7.8: Logistic regression modelled probability of *Clematis vitalba* stem regeneration as a function of initial stem diameter, with 95% confidence band.

Stems that did regenerate produced an average of three shoots each; multiple shoots on each side of one node was not uncommon (Figure 7.9). In the Poisson model, the number of new shoots a fragment was likely to produce was significantly (p= 0.006) associated with initial stem diameter (Figures 7.10 and 7.11, Table E.2 in Appendix E): for every one mm unit increase in initial diameter, the incidence of multiple shoot production increased by 44%. Total new shoot length per 30 cm stem fragment averaged 61 cm, but according to the LMM, neither initial diameter nor stem type were important predictors of shoot length at $\alpha = 0.1$ (Table E.3 in Appendix E).

Figure 7.9: *Clematis vitalba* stem fragment with multiple shoots on each side of one node.

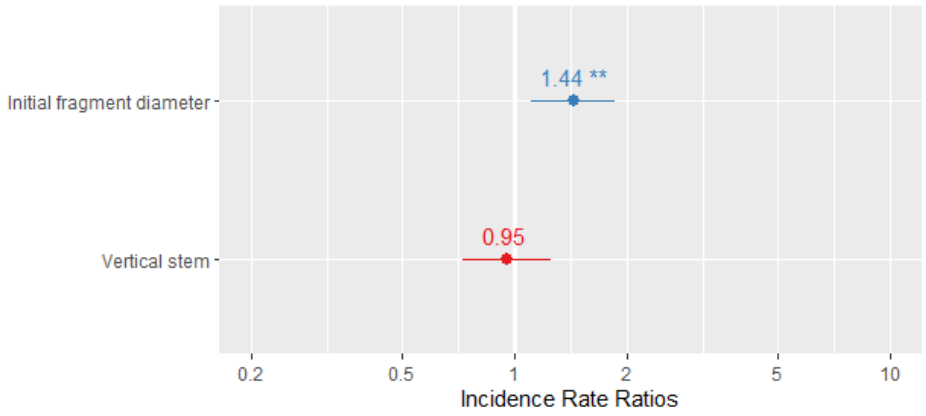

Figure 7.10: Poisson regression modelled effects of factors on *Clematis vitalba* fragment regeneration: initial fragment diameter (** indicates significance at $\alpha = 0.01$); and vertical (climbing) stems (as opposed to lateral (creeping) stems.

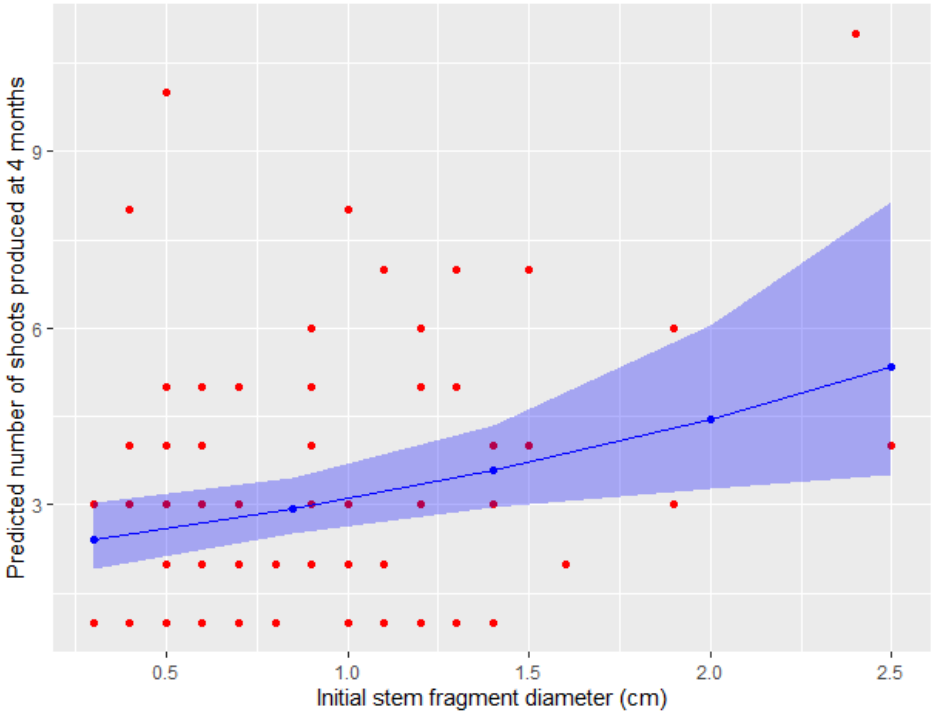

Figure 7.11: Poisson regression modelled probability of fragmented *Clematis vitalba* stems producing multiple shoots when regenerating, as a function of initial stem diameter. Red data points are observed values, blue line and blue 95% confidence band are model estimates.

An additional observation of note was the development of flower buds on shoots from three stem fragments, two vertical and one lateral (Figure 7.12). All three fragments measured between 3 and 5 mm in diameter at the time of collection.

Figure 7.12: Regrowth of *Clematis vitalba* stem fragments 4 months after burial, including flower buds.

7.4 Discussion

7.4.1 Discussion: Observational study

The observational study was an exploratory investigation of OMB's horizontal stem morphology and growth patterns, and due to the limited number of plots assessed, no statistical analysis was undertaken. However, the measurements and observations made from the plots give a good indication that OMB may rely quite heavily on lateral vegetative growth: consistent with research on other liana species (Penalosa 1984, Sakai et al. 2002, Leicht-Young and Pavlovic 2015, Buru et al. 2016, Mori et al. 2018), creeping OMB stems were pervasive in highly infested sites, forming an intricate, branching web of vegetative growth at the ground level. Aboveground stems comprised the majority of the lateral network (81.7%), and, in all the plots examined, no true seedlings were found; all small individuals were connected to lateral stems. It is worth noting, though, that the ground in these sites had a heavy vegetation cover with no bare soil, and therefore true seedlings would not likely have survived there, due to their poor ability to compete (Chapter 6).

West (1992) documented vigorous, rapid elongation of OMB shoots in New Zealand, and found average shoot elongation in one season to be just over 2 m. As a validation of that vigour, in the plots of the current observational study, fewer than 20% of all stem nodes developed new, leafy shoots (7% of underground stem nodes, 26% of aboveground stem nodes), but the length of shoots generated by the stems was double that of the stems themselves (42 m and 21 m, respectively). In addition, a single node often produced multiple shoots, which could be considered a "branch bank", in case of dieback. The association of abundant leafy shoots with the prostrate stems suggests that the stems have a direct source of photosynthates and are not reliant on energy harvested up in the canopy.

Roots had developed on 25% of all stem nodes in the observation plots (5.5% of aboveground stem nodes, 44.8% of underground stem nodes). In Germany, Plavcová et al. (2016) found that OMB has a much higher proportion of living fibres in the storage tissue of its stems and roots than most temperate woody species, which have more ray and axial parenchyma. The living fibres have a lower storage capacity than ray and axial parenchyma. Assuming that the structure of OMB storage tissue in New Zealand is similar to OMB in Germany, it may be that, at least for creeping stems, food reserves are distributed along the length of the stem with multiple rooted nodes, reducing the need for a large storage capacity in any one place. The well-established concept of resource sharing in clonal plants (Marshall 1990, Alpert 1996, Saitoh et al. 2002, Saitoh et al. 2006, Roiloa et al. 2014, Mori et al. 2021) would support this.

A small fraction of shoots (<3%) from the creeping stems bore buds or flowers at the time of the observational study (January/February), which was during the middle of the phenological flowering period for OMB (West 1992). Hence, the prostrate stems can support sexual reproduction as well as

vegetative growth. However, the primary function of aboveground stems appears to be to produce photosynthetically active growth (26.33% of nodes) while elongating, with only a small investment in rooting (5.5% of nodes). On the other hand, underground stems seem to primarily function as conduits for water and nutrients: nearly half of underground stem nodes developed roots (44.8%), whereas only 7% produced shoots.

OMB seedlings are negatively density dependent and are severely limited by crowding and competition (Chapter 6). However, researchers studying mature vines have shown that strongly clonal liana species are often positively density dependent (Ledo and Schnitzer 2014). Considering the dense network of lateral OMB stems and shoots within a single square meter observed in this study, OMB also appears to be positively density dependent once it matures enough to produce rooting, vegetative stems, as a vegetative stem's enhanced ability to extract resources from the surrounding environment makes it less vulnerable to competition than a seedling. Positive density dependence permits heavy propagule pressure on a habitat and may help explain OMB's invasion success.

7.4.2 Discussion: Fragment regeneration experiment

Regeneration begins with adventitious rooting, which is contingent on numerous factors, including appropriate environmental conditions, an appropriate balance of endogenous compounds, and an inherited ability to allow differentiated cells to dedifferentiate and form new tissue (Geiss et al. 2009). No attempt was made in this experiment to manipulate any endogenous factors, although environmental conditions were optimized for rooting of OMB stem fragments, by: 1) timing the experiment to use hardwood stems with active buds; 2) using soilless potting mix to minimize disease; 3) providing essential nutrients in the potting mix; 4) covering the fragments, in case of light-induced inhibition of rooting; and 5) watering and weeding regularly. No published descriptions of climbing OMB stems refer to adventitious rooting, nor have inspections of vertical stems by the researcher produced any evidence of rooting (personal observations, 2019-2022). Accordingly, any active buds on climbing stems would have been forming shoots and leaves at the time of collection, not roots. Based on the observation study, between 5.5% (aboveground stems) and 45% (underground stems) of nodes on creeping stems developed adventitious roots under normal circumstances. Therefore, lateral stem fragments should have been more likely than vertical stems to recover from fragmentation by rooting, especially if active buds were present on the fragment nodes. Yet, according to the model analyses, whether woody OMB fragments originated from creeping or climbing stems, they had the same propensity in the optimal conditions to regenerate (48.7% for a 30 cm fragment; $p = 0.782$). Ward and Henzell (2000) found that OMB stems that were cut but not treated with herbicide resulted in 61% regrowth when pruned in late spring. The current experiment shows that spring-fragmented

stems regenerate at a slightly lower rate than spring-cut stumps that are still rooted in the ground. In addition, the presence of a visibly active bud was not a significant predictor of regeneration ($p = 0.656$). This demonstrates the phenotypic plasticity of the species. That is, injury or separation from the mother plant can prime OMB stems to produce adventitious roots for regeneration, regardless of their previous phenotype. Given that OMB in the canopy can cause the death and collapse of host trees, this plasticity should permit OMB stems that fall with the host to develop roots and search for a new host, as is the case with many tropical lianas (Putz 1984, Schnitzer et al. 2000, Schnitzer et al. 2004, Yorke et al. 2013).

Though vertical and lateral stems are equally capable of regrowth, the results suggest larger diameter OMB fragments have a significantly better chance of successfully forming independent ramets than their smaller counterparts: the ability to regenerate and the number of shoots produced by regenerating fragments increased significantly with an increase in initial fragment diameter ($p = 0.04$ for regrowth; $p = 0.006$ for shoot number). Researchers have typically documented a *loss* of ability to form adventitious roots as a plant ages, though the decline varies by species (Greenwood et al. 1989, Diaz-Sala et al. 1996, Díaz-Sala et al. 2002). Fragment diameters ranged from 2.5 mm to 2.5 cm in the current experiment. Certainly, annual secondary growth depends on environmental conditions, and stem age cannot be precisely determined based solely on diameter. Nonetheless, several 2 cm OMB stem cross sections observed by the author had 8-10 annual rings (personal observations, 14 January 2021; Figure E.4 in Appendix E). Using that measure as a loose standard, a 2.5 mm stem can be considered 1 year old, and a 2.5 cm stem 10-12 years old. Thus, adopting diameter as a surrogate for age, the results of the experiment suggest that OMB's rooting capability *increases* with age, at least up to 10-12 years, and that age-related decline does not take place in the short term for this species.

Employing a variety of reproductive strategies allows a species to enter new habitats and reduce its risk of destruction in volatile environments (Zhang and Zhang 2007, Barrett 2011, Buru et al. 2016). Although initial seedling colonisation may be important, Chapter 6 showed that seedling establishment is difficult for OMB. Yet, when followed by vigorous asexual reproduction, an abundance of successful seedlings is not necessary for establishment of a population. Other researchers have shown that vegetative propagules are more resilient than seedlings and can tolerate more environmental stress (Oborny and Bartha 1995, Deiller et al. 2003, Richardson et al. 2007). For OMB, vegetative stems, whether connected or fragmented, may be more likely to weather extreme temperatures, storms, or herbivory than seedlings. Vegetative propagules transported by flood waters may also have a better chance of colonising new downstream habitats than seeds. In addition, vegetative reproduction is likely a quicker way for OMB to return to the tree canopy than growth from a seedling, as lateral stems can rely on a well-developed root system as they lengthen, which allows them to exploit available soil resources more readily than seedlings. The balance of a) genetic diversity, b) long-distance seed dispersal, and c) a seed bank of sexual reproduction on one hand, and

a) resilience, b) heavy propagule pressure, c) short-distance dispersal, and d) a bud bank of vegetative reproduction on the other, confers clear advantages on OMB for successful invasion of new territory.

7.5 Conclusions

As noted in Chapter 6, OMB seedlings are not highly competitive. The existence of extensive, branching networks of creeping OMB stems in infested river flood plains points to a reliance on vegetative reproduction as a prominent mechanism for persistence and spread through propagule pressure. Indeed, being multi-stemmed and positively density dependent may be central to OMB's success as an invasive species. When conditions permit, OMB seedlings produce multiple stems within the first few months of emergence (Section 6.3). Furthermore, this research has shown that creeping OMB stems not only root adventitiously, but consistently generate multiple shoots from one node. If they survive, each shoot will become a woody stem. This experiment also demonstrated that nodes on buried woody OMB stem fragments exhibit morphological plasticity, allowing both creeping and climbing fragments to develop roots and regenerate as self-sufficient clonal ramets when buried. Thus, multiple stems represent multiple vegetative propagules. Regenerating stem fragments also produce multiple shoots, thus reiterating the plant morphology. As with other lianas, it appears that once an OMB seedling succeeds to maturity, consequent vigorous vegetative reproduction from its multiple stems assures its establishment and spread, despite low initial colonization rates (Sakai et al. 2002, Deiller et al. 2003, Schnitzer et al. 2012, Ladwig and Meiners 2015).

In a forestry plantation or other environments where land is cleared mechanically, each metre of OMB stem broken up by machinery in spring could yield several new, independent plants, each with multiple shoots. Flower production can occur in the first year from a regenerated stem fragment, and hence both asexual and sexual propagation are possible in the same year as the clearing. Thus, mechanical clearing into windrows could exacerbate an infestation, unless OMB growth from the windrows is repeatedly controlled. Removal of all stems would be a preferable alternative, although considering the extensive nature of lateral OMB networks, full removal may be impractical where infestations are severe. That Van Gardingen (1986) observed no regrowth (over 8 months of monitoring) from stem fragments taken in autumn suggests that regrowth from broken OMB stems may be less likely if bulldozing is done in autumn.

Clones are genetically identical other than somatic mutations that accumulate in ramets through time (Schoen and Schultz 2019). An over-reliance on asexual reproduction in a species can lead to a lower ability to adapt to change over time, as well as an accumulation of deleterious mutations in clones (Schurko et al. 2009, Barrett 2011), although in some cases beneficial mutations accumulate (Schoen and Schultz 2019, Vondras et al. 2019, Cruzan et al. 2022). Because OMB makes use of both sexual

and asexual means of reproduction, variation and adaptation to change can still take place, along with rapid local expansion. However, if its populations are largely clonal, that genetic uniformity may leave it more susceptible to disease and other natural enemies. So far, two biological control agents, a gall mite (*Aceria vitalba*) and a sawfly (*Monophadnus spinolae*), introduced into New Zealand have shown some promise in reducing OMB vigour and spread, but have not yet been very damaging to the species. To augment the damage of the other agents, a search for an effective biocontrol pathogen is also ongoing (den Breeyen 2022). This research has laid the groundwork for future investigations into OMB's reliance on clonal reproduction, possibly with the use of microsatellite markers or other genetic analyses. A more comprehensive, genetic study of the contribution of OMB's clonality could also help in understanding its distribution patterns.

8. Herbicide Efficacy

8.1 Introduction

Section 1.4.10.4 gives an overview of the herbicides and techniques currently being used in New Zealand against old man's beard. Herbicides are indispensable for the control of many problematic weeds (Simberloff 2013), although increasingly, techniques that improve precision and reduce non-target damage are preferred. This is especially true when invasive species, like old man's beard, are associated with valuable natural ecosystems, aquatic environments, and production forestry, where methods for control may be limited due to environmental sensitivity and government regulations (Hamilton et al. 2003, Baillie et al. 2015, Rolando et al. 2015, Raal and Timmins 2018). Some effective herbicides are problematic as they persist in the environment, and alternative herbicides and techniques are being sought to reduce the burden of environmental contamination from their use (Ward et al. 1999, Tu et al. 2001, Simberloff 2008, Alavanja and Bonner 2012).

As a pyridine herbicide, picloram acts as a synthetic auxin which is quite persistent in soil. MacDiarmid (1975) found that sensitive species white clover (*Trifolium repens*) was unable to establish in soil treated with 1.1 kg ai/ha of picloram until up to 15 months after treatment. Picloram does not break down quickly in plant tissues or in the soil and is characterised as highly mobile to very highly mobile in soil (Rao et al. 1985, Liu et al. 1997, Chu and Chan 2000). Due to this mobility, it can easily leach into groundwater or runoff into water bodies (Tu et al. 2001, Vencill 2002). Since the early 2000s, picloram in a concentrated gel form has been increasingly used in New Zealand to treat woody weeds with the cut stem method, in which herbicide is applied directly to both sides of a cut stem (Ward and Henzell 2000, 2003, 2004). Gels are valued as easy and ready-to-use, and the technique is preferred, because its direct application on individual stems reduces damage to nearby non-target species, allows complete avoidance of spray drift, and reduces chances of herbicide runoff at the time of application (Ward et al. 1999). Active ingredient concentrations are usually higher for herbicides applied directly to stems, as opposed to a foliar spray, but the overall amount of herbicide product used is often smaller for the cut stem method (Miller 2016). Still, due to the persistence of its residues that could affect nearby desired species through root uptake, alternatives to picloram gel have been sought by New Zealand's Department of Conservation (DOC) (Raal and Timmins 2018).

Triclopyr's targeted use in basal bark applications is recognised by researchers as highly effective against a wide variety of woody plants (Nelson et al. 2006, DiTomaso and Kyser 2007), and likewise cut stem applications of triclopyr on OMB (Bierzychudek 2020). A synthetic auxin in the pyridine group, triclopyr is closely related to picloram but is somewhat less toxic and breaks down more quickly in soil (Jotcham et al. 1989, Monaco et al. 2002, Ferrell et al. 2006); its soil residual activity is

between 10 and 100 days after treatment (DAT) (Cox 2000). However, researchers in Oregon, USA (Newton et al. 1990), have reported that the half-life of triclopyr (sprayed as a butoxy ethyl ester at 1.68 kg ai/ha) in Tanoak (*Lithocarpus densiflorus*) plant tissues can be up to 291 days, and that the herbicide residues do not break down in the plant matter until it becomes part of the soil litter layer. On the other hand, triclopyr is increasingly bound to soil over time, and adsorption increases with increasing levels of organic matter (Ghassemi et al. 1981, Buttler et al. 1993). DOC uses a basal bark method, with an ester formulation of triclopyr (butoxy ethyl ester (BEE); 120 g/L) in an oil carrier and a non-toxic biodiesel as a penetrant, in which a low-pressure spray is applied to saturation to the entire circumference of intact individual woody stems to a height of 30-50 cm (Raal and Timmins 2018). As with the gel technique, spray drift is avoided with the basal bark method. However, the basal bark method is more expeditious, in that it does not require the extra step of cutting the stem before treating it.

Glyphosate is adsorbed rapidly to soil and is also quickly degraded microbially, and therefore is nearly immobile (Duke and Powles 2008). As a broad-spectrum herbicide, glyphosate can potentially harm any vegetation type, but by applying it directly to target plants, such as in the cut stem method, it can be used in a selective way. Early work in New Zealand comparing various gel herbicides for control of OMB (Ward and Henzell 2000) showed that picloram (ai 5%) was highly effective, while glyphosate (no % ai given) only temporarily set back treated stems in the first year. A similar outcome was described by Di Tomaso and Kyser (2007), where stumps of vigorous invasive, *Ailanthus altissima*, were treated with an aqueous solution containing 479 g/ L glyphosate, diluted 50%, but after two years, 41% of the treated stumps had resprouted as well as untreated control stumps. Cut stem treatment on grey willow (*Salix cinerea*) with 10% glyphosate gel appeared to be 95% effective 24 months after treatment when applied at 0.3 m aboveground, and 75% effective at 1 m aboveground (Ward and Henzell 2004). Campbell et al. (2021) reported 100% control of *Cereus uruguayanus* cacti 42 months after treatment with undiluted 360 g/ L glyphosate in water plus a penetrant. No literature is available on cut stem treatment with glyphosate gel at concentrations higher than 10%.

Directed techniques like cut stem and basal bark methods are inappropriate when a target weed is spread extensively over an area, as is the case with OMB in many New Zealand riparian zones which are often fenced off to cattle and remain relatively unmaintained. These areas are often populated by grasses and weedy species. When OMB can establish itself in such areas, it tends to spread rapidly via vegetative growth of its branching stems, which can develop into web-like networks of both creeping and climbing stems (Chapter 7). In these situations, broadcast spraying of herbicides from the ground can be the best method for control of OMB, using as much precision as possible to avoid contamination of waterways and damage to desirable vegetation which may be present. In these situations, it is important to leave grasses intact, which can help protect against re-colonisation by

OMB or other weedy species. The herbicides most widely used by regional councils around New Zealand for such cases include triclopyr, metsulfuron-methyl, and a combination of picloram, triclopyr, and aminopyralid (J. Keast, Horizons Regional Council, pers.communication, 25 March 2021).

Aminopyralid, which is structurally like picloram is likewise persistent in plant tissue and soil, with high soil mobility (Kline et al. 2005, MacBean 2010): Ferrell et al (2006) reported that aminopyralid (0.12 kg ai/ ha) provided > 97% control of tropical soda apple (*Solanum viarum*) for up to 335 DAT.

With the principal purpose of enhancing efficacy, combining two or more herbicides is common in weed control. Related herbicides are more likely to work synergistically than those with different chemical structures (Zhang et al. 1995). Indeed, the pyridine herbicides triclopyr and picloram have often been mixed together, recently also with aminopyralid, to achieve improved control of brush weeds (Webb and Harrington 2005, Moore et al. 2010, Gawn et al. 2013, Tran et al. 2015, Campbell et al. 2021).

Although metsulfuron-methyl can be highly mobile in some soils (USDA-ARS , Thompson et al. 1992), studies have shown it to have relatively low residual soil activity, from 5-63 DAT (Ismail and Lee 1995, Trabue et al. 2006, Harrington et al. 2017). Indeed, Tran et al. (2015) compared the relative persistence of several residual herbicides applied in summer in a New Zealand silt loam soil, metsulfuron, triclopyr, and a combination of triclopyr and picloram among them, and found that metsulfuron was the least persistent of all herbicides evaluated (although it was noted that if applied in cooler conditions, its persistence was likely to increase). Triclopyr on its own was only slightly, but not significantly, more persistent than metsulfuron, but the triclopyr/picloram mixture had the most persistent soil residual effect. Notwithstanding its lower persistence overall, metsulfuron has also been shown to damage some grass species, such as perennial ryegrass (*Lolium perenne*) and Yorkshire fog (*Holcus lanatus* L.) (Harrington and He 2010, Harrington et al. 2017), despite being labelled for selective broadleaf weed control.

The first objective of the current research was to compare the efficacy of different targeted basal stem herbicide techniques on woody OMB stems, using two different herbicides, i.e. the basal bark method using triclopyr plus oil mixture, and the cut stem technique using concentrated glyphosate gel. The second objective was to determine the efficacy of several selective herbicides used widely in New Zealand as foliar sprays on sprawling OMB, while also taking the effect on grass cover into account. The results from these experiments may be helpful for informing OMB management decisions.

8.2 Methods

8.2.1 Study sites: Basal herbicide experiment

Three rural sites along the Manawatu River in the Tararua District, New Zealand were chosen for the study, with a minimum of 4 km between them: 1) Kaitoki (-40.2401, 176.1145), 5,000 m^2 of private farmland between Weber Road and Kaitoki Road; 2) Tamaki (-40.2527, 176.0672), 6,000 m^2 of private farmland on the Tamaki River, a tributary of the Manawatu, between Wi Duncan Road and Totaramahonga Road; and 3) Hopelands (-40.3613, 175.9604), a 1.5 ha section of Hopelands Reserve, public land near the junction of River Road and Hopelands Road. All three sites were largely unmanaged riparian zones infested by OMB, both on the ground and in the tree canopy. Kaitoki was an open, grassy site, dominated by tall fescue (*Schedonorus arundinaceus*), perennial ryegrass (*Lolium perenne*), tall oat grass (*Arrhenatherum elatius*), and cocksfoot (*Dactylis glomerata*), with tradescantia (*Tradescantia fluminensis*), blackberry (*Rubus fruticosus*), hemlock (*Conium maculatum*), and various other weeds scattered throughout the site, as well as occasional willow (*Salix spp.*), tōtara (*Podocarpus totara*), and mataī (*Prumnopitys taxifolia*) trees. Horses were occasionally allowed to graze at Kaitoki. Sites Tamaki and Hopelands were much more densely inhabited by trees, and neither one was used for grazing. At Tamaki, 95% of the trees were willow (*Salix* spp.), with an occasional radiata pine (*Pinus radiata)*, or tree lucerne (*Chamaecytisus palmensis*) in the understory. Groundcover/vine species at Tamaki consisted mainly of high densities of tradescantia, grasses (mostly cocksfoot and perennial ryegrass), three-cornered garlic (*Allium triquetrum*), and great bindweed (*Calystegia sylvatica*), along with lower densities of periwinkle (*Vinca major*), blackberry, and montbretia (*Crocosmia x crocosmiiflora*). At Hopelands, crack willow also dominated the canopy, with some large, impenetrable clumps of Oldham's bamboo (*Bambusa oldhamii*); occasional māhoe (*Melicytus ramiflorus*) and tree lucerne were found in the understory. Tradescantia, periwinkle, and cocksfoot were the most abundant ground cover/vine species at Hopelands, with blackberry, great bindweed, ivy (*Hedera helix*), and montbretia somewhat less abundant.

8.2.2 Site preparation: Basal herbicide experiment

Across the three sites, 48 circular plots, each in a radius around a central cluster of climbing OMB stems, with a minimum buffer of 3 m separating them, were classified in one of three levels of infestation (low, medium, high). The infestation levels were based on the approximate number of OMB stems in the plot given its size (minimum 3 m, maximum 8 m diameter). A group of four plots at each classification level comprised a single experimental block (12 blocks total). With infestation levels distributed as equally as possible between them, half of the blocks were assigned for treatment in autumn 2020, and the other half in autumn 2021.

Prior to treatment, as well as at each post-treatment assessment, the following were recorded for each plot: a) major tree canopy species and major ground cover species (as listed in Section 8.2.1); b) tree canopy cover, estimated using hemispherical photography with 'Gap Light Analysis Mobile App' (GLAMA); c) percentage live groundcover density around the central OMB cluster (fractional green canopy cover (FGCC)) measured at 1 m above ground, using the Canopeo App for Android phones, Oklahoma State University; and d) OMB as an estimated proportion of the ground cover.

In addition to taking GPS coordinates, each plot was marked in the centre with a bamboo pole and identification label. To simplify cut stem relocation for post-treatment assessment, all treatments were made at 1 m above the ground (following Ward and Henzell (2000)). Four treatments were assigned randomly to each stratified block (herbicide details listed in Table 8.1): 1) an untreated control (*Control*); 2) basal bark application of triclopyr butoxyethyl ester (BEE), by chemically ring-barking stems (*Ring*; 120 g/ L plus 620 g/L methyl and ethyl esters of fatty acids derived from canola oil and hydrocarbon liquids in a ready-to-use formula); 3) cut stump with application of concentrated glyphosate isopropylamine salt (IPA) gel (*Paste*; 450g/L); and 4) cut stump with no herbicide application (Cut); a control treatment for *Paste*. For the *Paste* treatment, both upper and lower cut surfaces of the stem were immediately covered with a 3-5 mm layer of glyphosate gel (untreated cut stems can re-root if they fall to the ground), using the bottle brush top supplied with the herbicide. The gel has a non-toxic blue dye added, for easy temporary identification of treated stems (Figure 8.1). For the *Ring* treatment, a 20 ml Forestry Spotgun (modified drench gun, similar to that described by Porter (1979); ChemAgro, Auckland, NZ) with a solid cone nozzle (GG 4.3W; Spraying Systems, Auckland, NZ), calibrated to release 2.5 ml of solution with each pull of the trigger, was used at low pressure to thoroughly wet stems (volume 5 ml per stem up to 2 cm diameter, with another 2.5 ml for each additional cm increase in diameter) with the triclopyr + oil product (Figure 8.1). The technique allowed the herbicide to encircle the whole stem when applied from two sides, essentially chemically ringbarking the stem, but without run-off. An oil-based white enamel paint was added to the herbicide mixture in 2020, to allow identification of treated stems (250 ml/5 L) but did not prove effective in improving visibility of treated areas. In 2021, no paint was added to the triclopyr mixture. Treated stems varied in size from approximately 5 mm to 10 cm in diameter.

Table 8.1: Ready-to-use herbicides in the basal herbicide experiment.

Herbicide	Trade Name*	Commercial formulation/rates of active ingredient	Application dosage
Glyphosate gel	Cut'n'Paste Glimax Professional	450 g ai/L glyphosate isopropylamine salt with 0.5% alkyl polyglucoside surfactants	3-5 mm layer/cut stem end
Triclopyr	X-Tree Wet & Dry	120 g ai/L triclopyr butoxyethyl ester and 620 g/L methyl and ethyl esters of fatty acids	2.5 ml x 2 per stem up to 2 cm diameter + 2.5 ml for each additional cm increase in diameter

*Manufacturers: Landman, Waiheke Island, NZ (Glimax); UPL, Auckland, NZ (X-Tree)

Figure 8.1: Left: Climbing *Clematis vitalba* cut stems treated with 45% glyphosate gel (Cut'n'Paste Glimax Professional). Right: A modified drench gun and backpack with triclopyr in oil, used to chemically ringbark the climbing *Clematis vitalba* stems (by dispensing 2.5 ml with each trigger pull). March 2021.

At treatment in late February 2020, weather conditions were dry, partly cloudy, with light winds (up to 20 kph), and a high temperature of 26°C. On treatment day in early March 2021, conditions were dry, mostly cloudy, with winds up to 29 km/h and a high temperature of 26°C. As all treated stems were sheltered under tree canopy cover, and herbicide applications were either directly applied as a gel or applied at low pressure about 2.5 cm away from the stem, potential drift due to wind was unlikely. Shelter from the canopy also reduced temperatures and potential volatilization of triclopyr during application. Light rain occurred approximately 24 hours after treatment in 2020 and 18 hours after treatment in 2021.

8.2.3 Plot assessments/data collection: Basal herbicide experiment

Treatment effects were assessed at 1 year and 2 years after the 2020 treatment (2020.1yr and 2020.2yr), and at 1 year after the 2021 treatment (2021.1yr), on six stems per plot. Stems were

selected for assessments using a systematic approach, in which the plot was divided into six equal sectors, and a stem in each sector was randomly chosen. In addition to the items listed in Section 8.2.2, the following data were collected at the post-treatment assessments:

- average number of nodes on the stem up to 1 m;
- proportion of nodes producing active growth;
- total number of live shoots produced on the stem up to 1 m;
- length of live shoots, up to 3 m;
- at the Tamaki site, the final assessment included a check of stem brittleness (dead wood that breaks).

8.2.4 Analysis: Basal herbicide experiment

A two-stage analysis was carried out using R statistical software, version 4.2.2 (R Core Team 2021). First, a comparison of live stems across all treatments was made by using the data for the proportion of active nodes per stem. These data did not meet the assumptions for linear regression, nor could they be satisfactorily transformed, as determined by the following packages: broom, rstatix, tidyverse, and bestNormalize (Wickham et al. 2019, Kassambara 2021, Peterson 2021, Robinson et al. 2022). Hence, a separate Kruskal-Wallis test was performed on ranked data for each of the three efficacy assessments (2020.1yr, 2020.2yr, 2021.1yr), followed by pairwise comparisons of the treatments using Wilcoxon rank sum tests. To control the false discovery rate, the Benjamini-Hochberg p-value adjustment method was employed.

Second, an analysis of the vigour of surviving stems (number of shoots produced by each stem) was made by fitting a Poisson to the treatment pairs, i.e., the herbicide treatments and their controls (*Control/Ring; Cut/Paste*), as well as a separate model for the pair of herbicide treatments alone (*Ring/Paste*). Negative binomial (NB) models were used when data were over dispersed, and treatment block was used as a random variable in each model, except when its inclusion resulted in a singular matrix. Packages used for Poisson model fitting, diagnostics, and data sub-setting were: lme4, DHARMa, dplyr (Bates et al. 2015, Hartig 2022, Wickham et al. 2022). Model diagnostic plots are shown in Appendix F, Figures F.1-F.9. Plots for all analyses were made using the following packages: magrittr, iNZightPlots, ggplot2, ggpubr, and patchwork (Wickham 2016, Kassambara 2020, Bache and Wickham 2022, Elliot et al. 2022, Pedersen 2022).

8.2.5 Study sites: Foliar spray experiment

Two unmanaged riparian sites along the Manawatu River, New Zealand, were selected for this experiment: 1) Kaitoki (-40.2401, 176.1145), a 2,000 m^2 section of farmland in the Tararua District,

adjacent to a site used in the basal herbicide experiment (Section 8.2.1), with an approximately 10-15° slope toward the river; and 2) Awapuni (-40.3861, 175.5858), a 3,000 m^2 section of relatively flat private land 300 m southwest of Paneiri Park in Awapuni, Palmerston North. S-map online (Landcare Research NZ Ltd 2019) characterizes the soil at Awapuni as moderately deep, well-drained alluvial sand, and while no detailed soil information was available for Kaitoki, it is estimated as having a recent fluvial, well-drained soil. OMB was prevalent in both locations, with extensive networks of creeping stems sprawling over the grass, other low-lying vegetation, and fallen trees. OMB was also present in the tree canopy, although plots in this study did not include any trees, and only creeping stems were treated. As stated in Section 8.2.1, Kaitoki was an open, grassy site, dominated by tall fescue, perennial ryegrass, tall oat grass, and cocksfoot, with tradescantia, blackberry, hemlock, and various other weeds scattered throughout the site, as well as occasional willow, tōtara, and mataī trees. Horses were occasionally allowed to graze at Kaitoki. The Awapuni site was slightly more densely populated with willow trees, but also had large open, areas, in which the ground cover/vines consisted mainly of the grass species tall fescue, tall oat grass, and cocksfoot, with great bindweed (*Calystegia sylvatica*), German ivy (*Delairea odorata*); occasional cleavers (*Galium aparine*) and blackberry shrubs also present. No grazing animals were present at Awapuni.

8.2.6 Site preparation and herbicide treatments: Foliar spray experiment

The heterogeneity of OMB infestation at each site was accounted for by creating eight circular plots at each site, with a 5 m radius (78.5 m^2) and a minimum buffer of 5 m between them and classifying them in one of two levels of infestation (by dividing plots into four sections, estimating the density of OMB cover in each section, and taking the average of the four densities): Level 1 OMB density was 40-60%, Level 2 was >60-80%. A group of four plots at each level comprised a single experimental block, two at each site. In late March (autumn) 2021, along with the estimated OMB cover, GPS coordinates of each plot were recorded, other groundcover species were identified (as noted in Section 8.2.5), and percentage live groundcover density (FGCC) was recorded in each plot section measured at 1 m above ground, using the Canopeo App for Android phones, Oklahoma State University (area approx. 1.73 m^2/Canopeo image). Along with one control treatment, three herbicide treatments commonly used by New Zealand regional councils for control of OMB were assigned randomly to each block: metsulfuron-methyl ester (*met*; 600 g/kg of water dispersible methyl ester granules); a triclopyr/picloram/aminopyralid mixture (*tri/pic/amino*; 300 g/litre triclopyr as an emulsifiable concentrate as butoxyethyl ester (BEE), 100 g/litre picloram, 8 g/litre aminopyralid as amine salts); and triclopyr (*tri*; 600 g/litre BEE as an emulsifiable concentrate).

In late March 2021 (autumn), plots were staked in the middle with 1.8 m bamboo poles with identification labels attached, and plot borders were temporarily marked with fluorescent aerosol marking paint (GloKote, Damar, Rotorua, NZ) for herbicide application accuracy. OMB plants had

not dropped any leaves at that time. Treatments at the recommended rates for woody plants plus an organosilicone surfactant (Boost Penetrant, Dow Agrosciences, New Plymouth, NZ), and a blue marker dye (FIL Done That, GEA Farm Technologies, Mt Maunganui, NZ) were then applied with 15 L knapsack sprayers (Solo 425, or Swissmex SW503), using a fan nozzle (TeeJet XR 11004-VP). Application rates of the herbicides are given in Table 8.2. The surfactant and dye were each added at the recommended rate (100ml/100L spray mix). As detailed in Chapter 7, creeping OMB stems form dense networks. To ensure that all OMB stems in the plots were covered, the spray mix (a total of 10 L/plot) was broadcast uniformly over each 78.5 m^2 plot to the point of run-off. Control plots were left untreated. Weather conditions at both sites at the time of treatment were dry, sunny, with light winds (Awapuni: up to 17 kph; Kaitoki <10 kph), and a high temperature of 25°C. No precipitation occurred at either site for at least 48 hours after treatment.

Table 8.2: Herbicides used in the foliar spray experiment ; RR = label recommended rate; ai = active ingredient.

Herbicide	Trade Name	Commercial formulation	Knapsack sprayer product RR	Rates of active ingredient	Treatment abbreviation
metsulfuron	Agpro Meturon	600 g/kg metsulfuron-methyl	5 g/10 L	30 g ai/100 L	met
triclopyr	Grazon	600 g/L triclopyr butoxyethyl ester	6 ml/ L	0.36 kg ai/100 L	tri
Triclopyr/picloram/ aminopyralid	Tordon Brushkiller XT	100 g/L picloram, 8 g/L aminopyralid, 300 g/L triclopyr butoxyethyl ester	6 ml/ L	60 g ai/100 L picloram + 48 g ai/100 L aminopyralid + 0.18 kg ai/100 L triclopyr	tri/pic/amino

*Manufacturers: AgPro, Auckland, NZ (Meturon); Dow AgroSciences, New Plymouth, NZ (Tordon, Grazon)

8.2.7 Plot assessments/data collection: Foliar spray experiment

Eight months following treatment (late November 2021), plots were assessed for herbicide efficacy. Plot borders were marked again with fluorescent paint before assessments began. However, to mitigate interference with results by potential spread of OMB from outside the treated area into the plots, measurements were limited to the central-most 2.5 m radius area. All measurements were made in each of four equal sections of the plot. To compare with density before treatment, percentage ground cover density was again documented (Canopeo App). Major grass species were identified, and as a measure of relative grass health, average height of grasses in the plots was recorded. Additionally, OMB biomass in the plot was measured by removing all aboveground OMB plant parts

within four random 50 cm x 50 cm quadrat samples and taking the weight of the samples after drying them over a 3-day period in a 60°C oven.

8.2.8 Analysis: Foliar spray experiment

All analyses were made using R statistical software, version 4.2.2 (R Core Team 2021). Ground cover density change and grass height were each analysed with separate two-way Analysis of Variance (ANOVA) and Tukey multiple comparisons of means post hoc tests, using the car, broom, and tidyverse packages (Fox and Weisberg 2019, Wickham et al. 2019, Robinson et al. 2022). Independent variables (IVs) for both ANOVAs were treatment and site. The OMB dry weight data did not meet assumptions for a linear model, nor could they be satisfactorily transformed, given that the median was zero. These were analysed with a Scheirer-Ray-Hare (SRH) test, an extension of the Kruskal-Wallis test, with treatment and site again used as IVs. Variance and distribution of dry weight data for *Control* treatment plots was clearly substantially different from that of all other treatments. Because treatment was the sole statistically significant predictor from the SRH test, and also to avoid drawing invalid inferences from post-hoc tests on the complete dataset, the *Control* data were removed, and an additional Kruskal Wallis test was run on the remaining treatments. In addition to the aforementioned packages, the rcompanion, FSA, and dplyr packages (Mangiafico 2022, Ogle et al. 2022, Wickham et al. 2022) were used in the dry weight analysis. Plots for all analyses were made using the following packages: magrittr, iNZightPlots, ggplot2, and patchwork (Wickham 2016, Bache and Wickham 2022, Elliot et al. 2022, Pedersen 2022).

8.3 Results

8.3.1 Results: Basal herbicide experiment

Control of OMB was quantified by 1) using the proportion of active nodes per stem as a measure of living stems, and 2) using the total number of shoots produced per stem as a measure of living stem vigour. Of the stems treated with triclopyr (*Ring*), no more than 4.8% were producing active growth from any nodes at any of the three assessments (> 95% mortality). In comparison, 58.3% (2020.1yr), 66.7% (2021.1yr), and 44.4% (2020.2yr) of stems treated with the cut and paste method (*Paste*) had active nodes (42%, 33.3%, and 55.6% mortality, respectively). Figure 8.2 shows the median (with bootstrapped confidence intervals), quartiles, and outliers for the proportion of active nodes per stem for each treatment, at each assessment. Kruskal-Wallis rank sum tests consistently indicated there were statistically significant different distributions between treatment groups ($F(3) = 62.44$

(2020.1yr); 51.443 (2020.2yr); 62.072 (2021.1yr); $p < 0.001$ at each assessment; Appendix F, Table F.1).

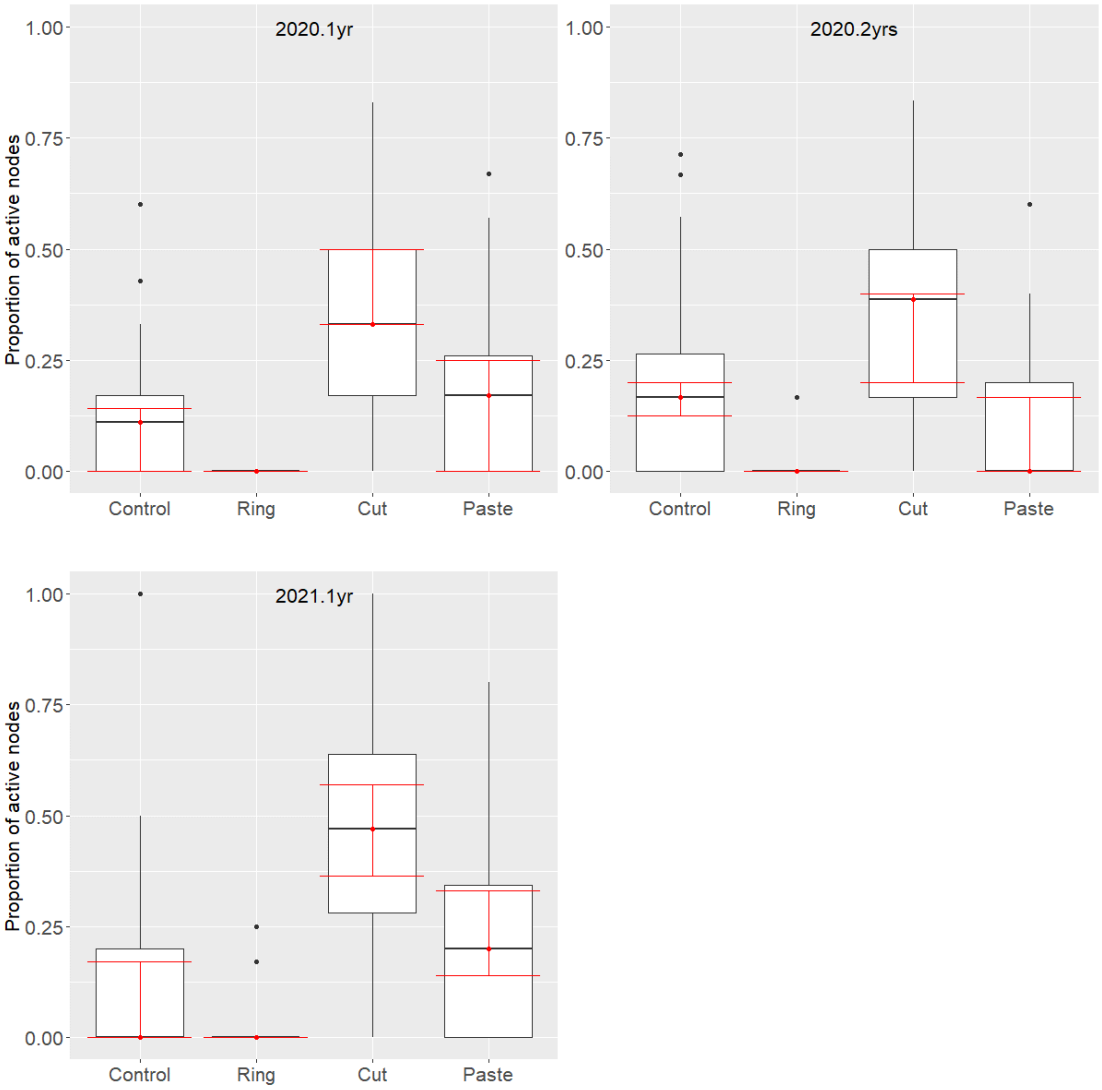

Figure 8.2: Median observed proportion of active nodes per *Clematis vitalba* stem by treatment (Control = untreated stems; Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)) in three different assessments (2020.1yr = 1 year after treatment in autumn 2020; 2020.2yrs = 2 years after treatment in autumn 2020; 2022.1yr = 1 year after treatment in autumn 2021). Bootstrapped confidence intervals around median in red.

Post-hoc pairwise Wilcoxon tests showed that there were significant differences ($p < 0.01$) between treatments in the three main treatment pairs of interest for all three assessments: *Control/Ring*, *Cut/Paste*, and *Paste/Ring* (Table 8.3). Also, stems in the *Cut* treatment had a significantly larger proportion of active nodes than any other treatment, while stems in the *Ring* treatment had a significantly smaller proportion of active nodes than any other treatment. Figure 8.3 demonstrates the

contrast in node activity 1 year after treatment, between a stem given the *Cut* treatment and stems given the basal bark treatment (*Ring*).

Table 8.3: P-value results from three assessments (2020.1yr = 1 year after treatment in autumn 2020; 2020.2yr = 2 years after treatment in autumn 2020; 2022.1yr = 1 year after treatment in autumn 2021) of pairwise Wilcoxon rank sum tests with continuity correction, showing differences between treatments (Control = untreated stems; Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)) in proportion of active nodes per *Clematis vitalba* stem. Benjamini-Hochberg p-value adjustment method used.

	2020.1yr			2020.2yr			2021.1yr		
	Control	Cut	Paste	Control	Cut	Paste	Control	Cut	Paste
Cut	<0.001			0.005			<0.001		
Paste	0.141	<0.001		0.040	<0.001		0.014	0.005	
Ring	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figure 8.3: Activity of stem nodes on *Clematis vitalba* stems treated by cutting only (left), and by chemically ringbarking with triclopyr in oil (basal bark method; right).

Active nodes commonly produced multiple shoots; indeed, stems cut with no herbicide applied commonly produced especially vigorous multiple shoots per node (Figure 8.4).

Figure 8.4: Multiple, vigorous shoots produced from one node on *Clematis vitalba* stem cut at 1 m aboveground. One year after treatment.

Poisson and negative binomial models for all three assessments indicated that *Cut* stems produced significantly more shoots per live stem than those pasted with glyphosate after being cut ($p < 0.001$; Figure 8.5 and Appendix F, Table F.2). Likewise, results showed *Control* and *Paste* stems each produced significantly more shoots than *Ring* stems ($p < 0.001$), except for models fit for the first-year assessment of plots treated in 2020. These two models produced very large standard errors, demonstrating that the model estimates were not robust, almost certainly because no shoots were found on any *Ring* stems in that assessment (as compared to at least one shoot on *Ring* stems in the 2020.2yr and 2021.1yr assessments).

Of the nine models (Poisson and NB) used to analyse shoot number per stem, four could be fitted as mixed-effects models, with block as a random variable (all 2021 models and the *Paste/Ring* model for 2020, second year). The block effect varied widely between the models: from a minimal variance for the 2020, second year *Paste/Ring* model (0.016), to a small variance in the 2021 *Cut/Paste* (0.228) and *Paste/Ring* (0.196) models, to a more substantial variance in the 2021 *Control/Ring* (0.428) model.

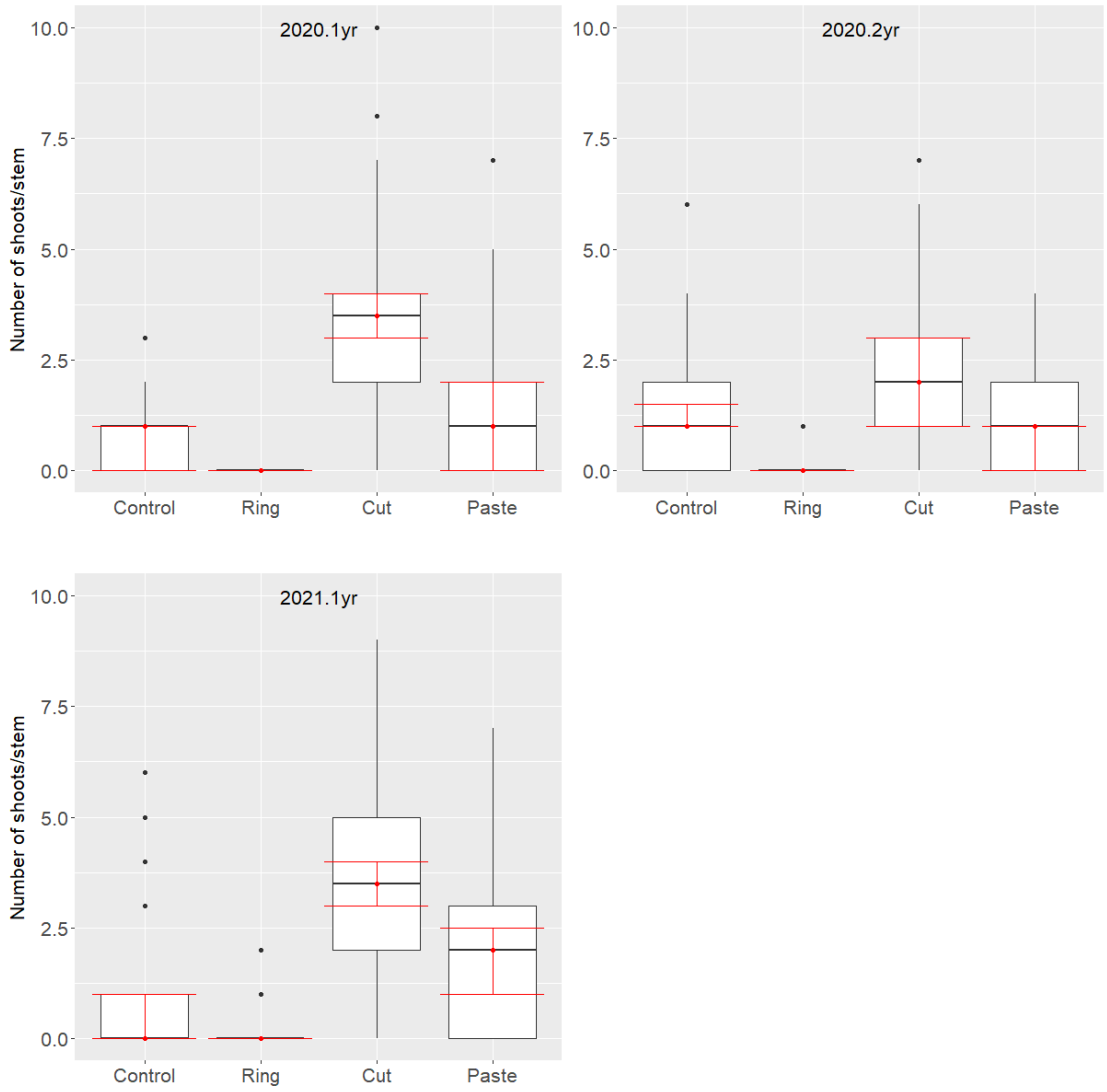

Figure 8.5: Median observed number of shoots per *Clematis vitalba* stem by treatment (Control = untreated stems; Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)) in three different assessments (2020.1yr = 1 year after treatment in autumn 2020; 2020.2yrs = 2 years after treatment in autumn 2020; 2022.1yr = 1 year after treatment in autumn 2021). Bootstrapped confidence intervals around median in red.

As supplementary, anecdotal evidence of stem survival, the final post-treatment assessment at Tamaki (both 2020.2yr and 2021.1yr) included an audit of above-ground stem brittleness (tested by breaking stems or checking cambium) on *Cut*, *Paste*, and *Ring*-treated stems up to 1 m (Figure 8.6). Some dieback occurred in all three treatments, yet *Ring*-treated stems (basal bark treatment with triclopyr) were nearly always completely brittle (91.7%). On the other extreme, no cut-only stems were completely brittle; brittleness usually appeared to be limited to the top two nodes. At Tamaki, 1-year post-treatment, 44% of cut stems treated with glyphosate (*Paste*) were completely brittle, while at 2 years post-treatment, 67% were completely brittle. The remaining stems were partially brittle (up to at least 2 nodes).

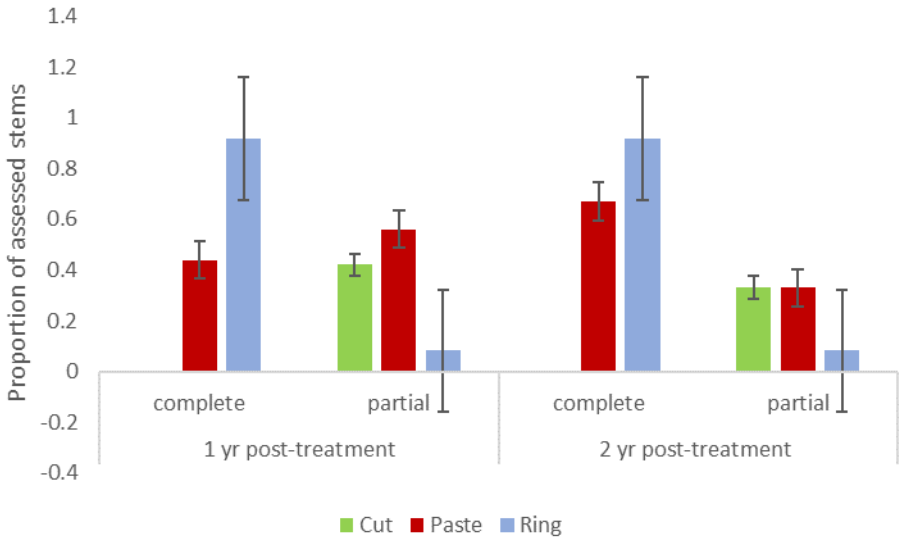

Figure 8.6: Brittleness of stems (aboveground, up to 1 m) at one site, 1 year and 2 years post-treatment. Complete = 100% brittleness; partial = <100% brittleness; Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method). Error bars based on standard error of the mean.

Notably, in nearly all plots, regardless of treatment efficacy, within 1 year of treatment, healthy OMB had recolonised the tree canopy. For example, in some *Ring* plots, all visible stems in the plot were completely brittle, though the canopy was full of OMB (Figure 8.7). Thus, OMB in the canopy must have come from climbing stems that were missed, from below the brittle part (belowground), or from untreated creeping stems. Also, the estimated proportion OMB of the groundcover, did not change between pre-treatment assessment and post-treatment assessments, demonstrating that treatment of the climbing stems had no noticeable effect on the density of ground-based OMB stems.

Figure 8.7: Demonstration of *Clematis vitalba* re-colonisation of dead tree after treatment of climbing stems with triclopyr in oil, using the basal bark method: top, before treatment; middle, one season after treatment (at which time all treated stems appeared to be dead); bottom, vigorous growth two seasons after treatment, likely from missed stems or creeping stems entering the plot.

8.3.2 Results: Foliar spray experiment

Although the ground cover at Kaitoki appeared to be somewhat less dense than Awapuni, both before treatment and at 8-months post-treatment, a two-way ANOVA of the ground cover density change indicated that there were no statistically significant differences among means for site, treatment, or their interaction in ground cover density change ($p = 0.711, 0.195, 0.128$, respectively; Figure 8.8; Appendix F, Table F.3). However, grass height measurements at assessment (Figure 8.9) suggest that metsulfuron did more lasting damage to the grasses than all other treatments, including *Control*, in which OMB would have been suppressing the grasses somewhat.

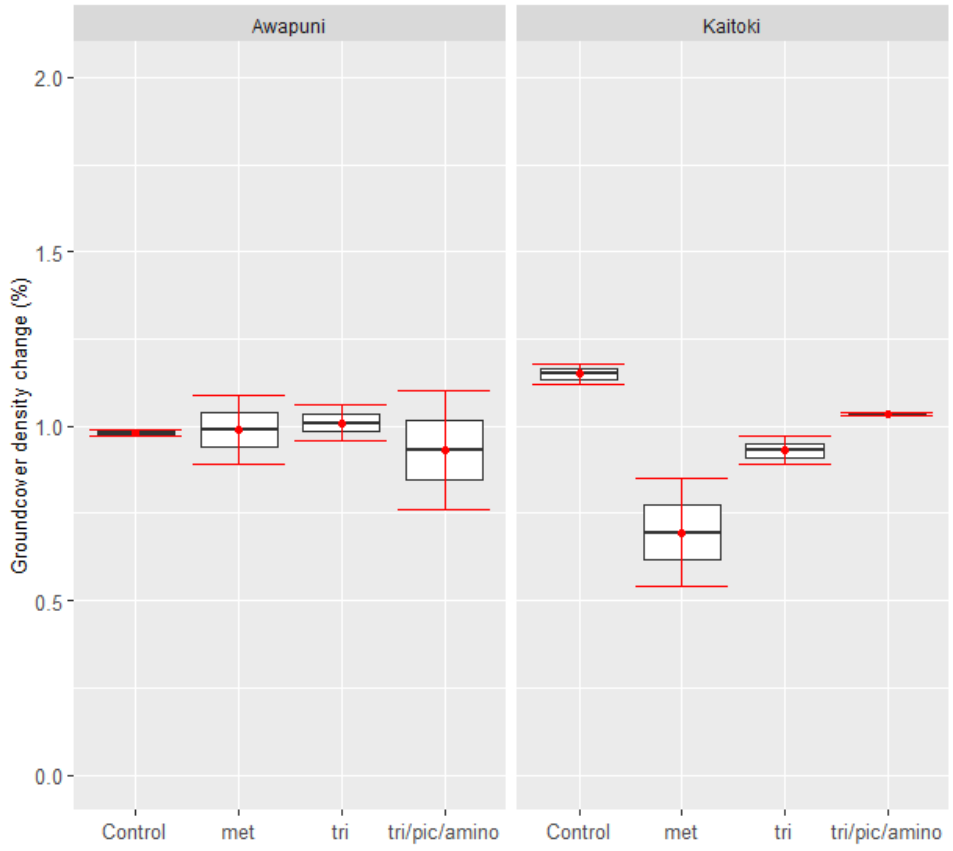

Figure 8.8*:* Ground cover density change of plots by foliar spray herbicide treatment (Control = untreated; Met= metsulfuron; tri = triclopyr; tri/pic/amino = a combination of triclopyr, picloram, and aminopyralid) and site (Awapuni, Kaitoki), 8 months after treatment. Estimations calculated by taking percentage live groundcover density (FGCC), using the Canopeo App for Android phones (one image in each of four equal plot sections; area approx. 1.73 m2/Canopeo image). Range of measurements in red.

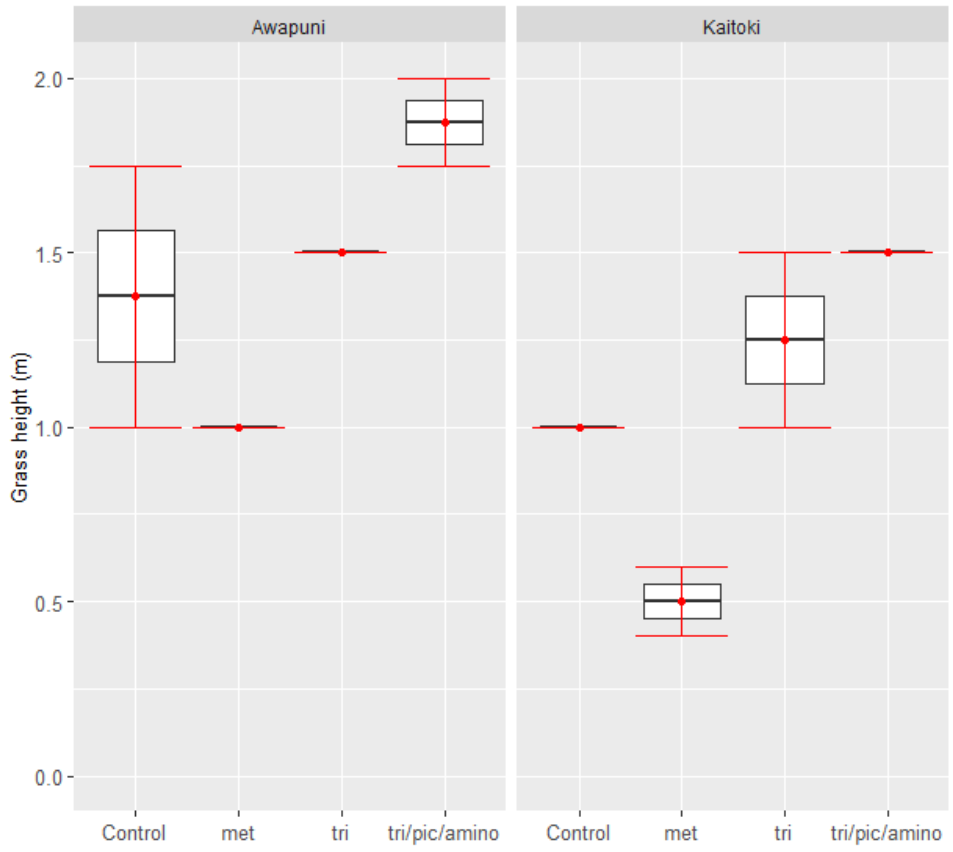

Figure 8.9: Measured height of plot grasses by foliar spray herbicide treatment (Control = untreated; met= metsulfuron; tri = triclopyr; tri/pic/amino = a combination of triclopyr, picloram, and aminopyralid) and site (Awapuni, Kaitoki) 8 months after treatment. Range of measurements in red.

Figures 8.10 and 8.11 demonstrate the relative effects of *met* and *tri/pic/amino* in adjacent plots at Kaitoki: in both plots, OMB was well-controlled, yet whole plot recovery and grass height was clearly not as strong in the *met* plots as in the *tri/pic/amino* plots. Indeed, great bindweed had begun moving into the *met* plot, covering the mounds of dead OMB stems. ANOVA results for average plot grass height at 8 months showed both a treatment and site effect ($F(3) = 10.747$, $p = 0.004$; $F(1) = 9.836$, $p = 0.014$, respectively), but no interaction effect. Adjusted p-values from Tukey post hoc tests indicated significant differences between *met* and *tri/pic/amino* ($p = 0.002$), and between *met* and *tri* ($p = 0.025$; Appendix F, Table F.4).

Figure 8.10: Plot on riparian farmland near Dannevirke, NZ, infested with *Clematis vitalba* before treatment with foliar spray of metsulfuron in late February 2021 (left), and 8 months after treatment in late November 2021 (right), with short grasses and *Calystegia sylvatica* colonising dead *Clematis* mounds.

Figure 8.11: Plot on riparian farmland near Dannevirke, NZ, infested with *Clematis vitalba* before treatment with foliar spray of a mixture of triclopyr, picloram, and aminopyralid) in late February 2021 (left), and 8 months after treatment in late November 2021 (right), with tall grasses dominating.

At the 8-month assessment, OMB remained healthy and vigorous in all *Control* plots, at both sites; a random square metre of OMB plant parts in a *Control* plot yielded between 115-360 g of dry matter (median at Awapuni 238 g/m^2, at Kaitoki 269 g/m^2; Figure 8.12). At Awapuni, no OMB was visible within the 2.5 m diameter inner circle of any plots other than *Control* plots. However, at Kaitoki, one *met* and both *tri* plots did have a small amount of OMB remaining (< 35 g/ m^2). The Scheirer-Ray-Hare analysis showed there was a statistically significant difference in dry weight among treatments ($H(3) = 11.25$, $p = 0.01$; Appendix F, Table F.5), but not between sites or the interaction of site and treatment. A Kruskal-Wallis test on the OMB dry weight of treatments with *Control* removed indicated no significant difference between the three remaining groups ($H(2) = 2.386$; $p = 0.303$).

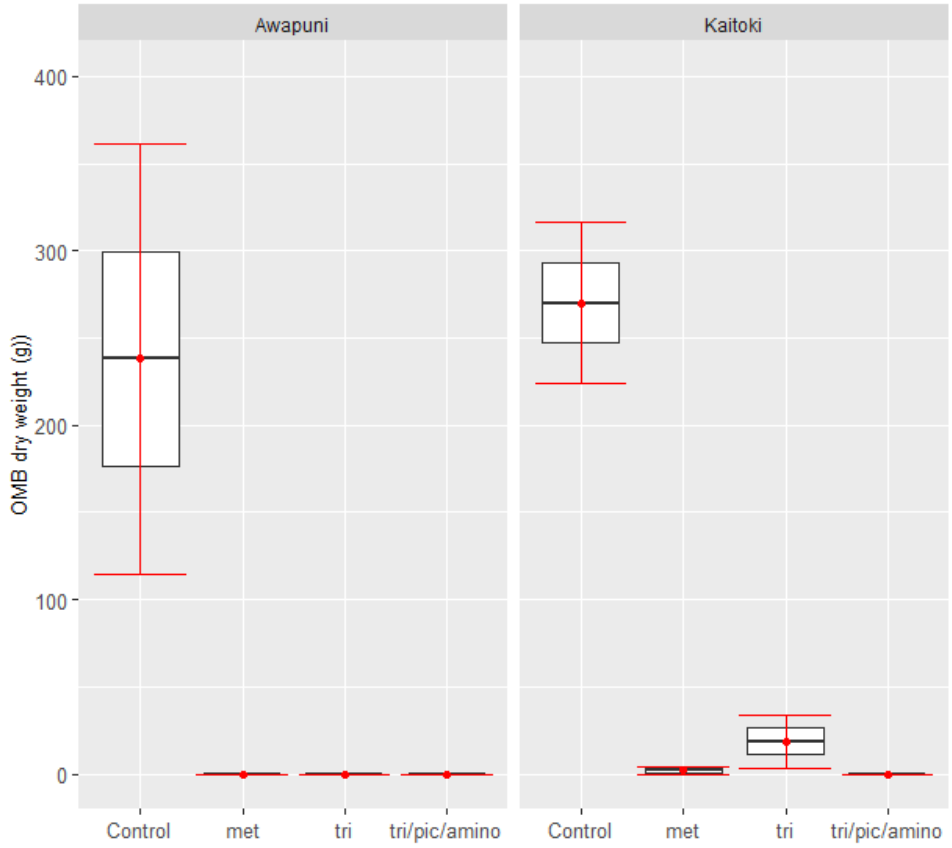

Figure 8.12: Measured dry weight of *Clematis vitalba* remaining in plots 8 months after treatment. Foliar spray herbicide treatments: Control = untreated; met= metsulfuron; tri = triclopyr; tri/pic/amino = a combination of triclopyr, picloram, and aminopyralid. Range of measurements in red.

8.4 Discussion

8.4.1 Discussion: Basal herbicide experiment

Manufacturer labels for both the basal bark technique using X-Tree Wet & Dry (triclopyr ester) and the cut and paste technique using Glimax Professional (glyphosate gel) recommend treating stems at the base, including the root collar, or very close to the base. However, for this study, all stems were treated at 1 m aboveground (after Ward and Henzell (2000)), to ensure that cut stems could be relocated for efficacy assessments 1 and 2 years later. In this study, the basal bark method with triclopyr in oil was unquestionably the most effective treatment among those tested for control of OMB. The mortality of stems chemically ring-barked with triclopyr (*Ring*), based on stems with active nodes, was > 95%, both 1 and 2 years after treatment. *Ring* stems had significantly fewer active nodes per stem than stems in any other treatment ($p < 0.001$). This outcome was roughly supported by anecdotal evidence of brittle stems at Tamaki: Ring stems were 91.7% completely brittle, from the ground up. These results are consistent with previous research reporting successful woody plant

control using the basal bark method with triclopyr ester on other species (Nelson et al. 2006, DiTomaso and Kyser 2007, Langeland and Meisenburg 2009), notwithstanding the lack of adherence to label guidelines in the current study.

The average mortality of *Paste* stems at 1-year post-treatment was 37.5%, and at 2 years 55.6% (complete brittleness of *Paste* stems at Tamaki was 44% at 1 year and 67% at 2 years post-treatment). When cutting and pasting with 10% glyphosate gel, Ward and Henzell (2004) indicated that grey willow was controlled better when applying the treatment at 0.3 m as opposed to 1 m. Certainly, given the label recommendations, treatment lower on the stem with 45% glyphosate may likewise improve effectiveness of control for OMB. Yet, Whitwell et al. (2016) reported that the invasive beach vitex (*Vitex rotundifolia*), also a plant that spreads via creeping stems, cut at ground level and treated with 50% v/v glyphosate (approximately 2.4 g ai/cm) and with 50% v/v triclopyr as a triethylamine salt (1.8 g ai/cm) five times in 3 years, was knocked back but not eradicated by either herbicide. They suggested the inadequate control may have been due to poor mobility of the herbicides in lateral stems.

Although there was still significantly more activity than on *Ring* stems, relatively few nodes on *Control* stems were active. This can be attributed to the suppression of lower nodes by the apical meristem's dominance (Monaco et al. 2002, Kebrom 2017): the untreated stems had significantly fewer active nodes at 1 m and below than cut stems (but no brittleness). Thus, while stem node activity was used as an indicator of stem survival, in the case of *Control* stems, non-active nodes were simply suppressed by other dominant nodes.

In stark contrast to stems in the *Ring* treatment, stems in the cut-only treatment (*Cut;* included as a control for the cut and *Paste* treatment) consistently had significantly more active nodes per stem than any other treatment. This may be explained by the removal of apical dominance brought on by the cut, and the simultaneous shift of translocating sugars to new sinks below the cut (Kebrom 2017). Research in North America (Leicht-Young and Pavlovic 2015) suggested that the invasive liana, climbing spindleberry (*Celastrus orbiculatus*), uses up roughly half of its stored nonstructural carbohydrates to support its spring growth, and that subsequent photosynthesis during the growing season restores the reserves. If cut during the growing season, however, resprouting causes sugars to be reduced a further 25%, indicating that cutting can reduce a liana's vigour. The cut-only treatment was certainly beneficial to the host trees, in that it immediately caused OMB stems in the canopy to die back and deprived the remaining parts of new photosynthates from above, weakening OMB stems overall. Cut stems treated with glyphosate, however, had significantly fewer live stems and produced significantly fewer shoots per live stem than those that were cut-only, which undoubtedly indicates that the gel treatment substantially undermined the growth that would have been stimulated by the cut.

The Poisson models showed some variance between blocks, demonstrating that block conditions did have some impact on the outcome. However, given the consistent differences between the treatments, it is clear the random variable did not influence the outcome to a large degree.

To ensure their root reserves are adequate for winter dormancy, at the end of the growing season, translocation of carbohydrates in woody plants is increasingly directed toward the roots (Loescher et al. 1990). All treatments in this experiment were applied in autumn, in anticipation that the systemic herbicides would predominantly move toward the roots, accumulate there, and irreparably damage the treated plants. Although the autumn application of the triclopyr ester was highly effective, the glyphosate gel was less so. In a previous cut stem study on OMB in New Zealand (Ward and Henzell 2000), late spring applications of picloram gel herbicide on cut stems were highly effective, and more effective than winter applications, and in lower concentrations. This result is likely due to high phloem movement toward growing tips and reproductive tissues in spring, which facilitates active elongation and photosynthesis (Loescher et al. 1990). Herbicides that are applied in spring would thus move toward the same sinks, doing considerable damage to the upper plant body. Little or no phloem movement occurs in plant tissues during winter dormancy (Ray and Savage 2021). It may be worth testing the efficacy of the cut stem method with glyphosate earlier in the season.

As discussed in Chapter 7, OMB infestations appear, at least in part, to be driven by extensive, branching lateral networks of stems. Measuring the effect of different herbicide techniques on individually treated climbing stems was the focus of this experiment. Yet, during efficacy assessments, both 1 and 2 years after treatment, there appeared to be no good correlation between treatment efficacy and the presence of healthy OMB stems in a plot's tree canopy (Figure 8.7). That is, although the basal bark method was more than 95% effective on treated stems, the effect was not necessarily reflected in the canopy, particularly at 2 years post-treatment. Likewise, OMB density in the ground cover remained largely unchanged. This suggests that a) untreated, smaller diameter climbing stems; b) missed climbing stems; c) stems regenerating from below the treated area; and d) untreated creeping stems, both in the plot understorey and outside of it, had been responsible for the quick re-colonization of the canopy. Also, dead climbing stems remained intact, acting as convenient, expeditious trellises for living stems to climb. Schnitzer et al. (2000) found that lianas recruited so vigorously into natural treefall gaps and abandoned pastures in Cameroon that tree regeneration was completely overwhelmed within 5 years. Ward and Henzell (2000) noted the difficulty of finding and individually treating 100% of OMB stems without follow-up work, observing that 10-40% of stems in research plots were missed. Furthermore, Clay and Dixon, (2000) reported that following effective control of OMB in the centre of small, infested areas (with a foliar spray of imazapyr), peripheral stems had thoroughly re-colonised them within a year. Their results corroborate the findings of this research and suggest that the vigour of OMB stems and the web-like lateral OMB networks prevent

anything more than a temporary setback to infestations if all climbing and creeping stems are not also comprehensively treated.

8.4.2 Discussion: Foliar spray experiment

All herbicide treatments gave effective control of OMB at both research sites. Average OMB dry weight per m^2 was nil, or nearly so, for all three herbicide treatments at both sites, except triclopyr at Kaitoki (Figure 8.12), but statistically, no differences between the treatments were detectable ($p = 0.01$). Furthermore, as median dry weight of OMB in *tri* plots 19 g/m^2 as opposed to >260 g/ m^2 in *Control* plots at Kaitoki, biomass in the *tri* plots was approximately 7% that in the *Control* plots, an incontestably consequential reduction. Still, any remaining OMB is a source from which the infestation can re-establish itself. Thus, in some situations, spot-spraying may need to be done following broadcast spraying, to get more complete control.

Healthy grass cover can help prevent OMB and other weeds from establishing by seed (Chapter 6). An intact ground cover layer is also beneficial to prevent erosion. The effect of herbicide treatments on the ground cover layer other than OMB was quantified by determining the change in ground cover density from pre- to post-treatment, as well as the relative height of the grasses in the plot post-treatment. The ground cover density at Kaitoki was more irregular than at Awapuni, most probably due to occasional horse grazing. Statistically, regarding the change in ground cover density, no treatment appeared to have a more detrimental or beneficial effect than any other, nor did site have a significant effect. Yet, Figure 8.8 shows larger variability of density change between treatments at Kaitoki than at Awapuni, which belie the statistical result, somewhat, at least for the *Control* (slight increase) and *met* treatments (marked decrease). The drop in ground cover density in *met* plots at Awapuni, compared to all other treatments and compared to Kaitoki, points to a larger adverse effect on the grasses by metsulfuron at that site. This was also supported both by the statistical analysis and graphical representation (Figure 8.9) of the differences in final grass height between sites and treatments: metsulfuron clearly reduced grass vigour at both sites, more than triclopyr or the triclopyr/picloram/aminopyralid mix, but more so at Kaitoki. This result is consistent with that of other studies that have reported sensitivity of some grass species to metsulfuron (Harrington and He 2010, Harrington et al. 2017). Harrington et al. (2017) reported severe damage to perennial ryegrass by metsulfuron, while clopyralid and a triclopyr/picloram mixture had no effect on the grass. As mentioned in Section 8.2.1, the main grass species present at Kaitoki included perennial ryegrass, tall fescue, tall oat grass and cocksfoot. Tall fescue, tall oat grass and cocksfoot were also present at Awapuni, but perennial ryegrass was not among the dominant species there. Although no attempt was made in this study to determine the proportions of each grass species present in the research plots, metsulfuron's stronger effect at Kaitoki could be partly attributed to its impact on the perennial

ryegrass there. It is also worth noting that in the met plots at Kaitoki, OMB had been controlled, but was being replaced by great bindweed, indicating a shift in dominance from one invasive species to another. In fact, Gawn et al. (2013) have shown that metsulfuron is not effective against great bindweed.

8.4.2.1 Limitations: foliar spray experiment

Restricted to two research sites with only two replicates per treatment per site, the statistical power of this study to detect a true treatment effect was rather low. In addition, the need to use non-parametric methods reduced the sensitivity further. To improve statistical accuracy, large plots were used, but due to possible interference from peripheral stems moving into the plots, only the plot inner half was assessed. The results of this study were generally clear, but could be strengthened by repeating it, using multiple sites, and by assessing herbicide effects over a longer period.

8.5 Conclusions

Picloram gels are widely used in New Zealand to control OMB but are likely to have undesirable effects in some sites. The aim of the basal herbicide experiment was to identify the least environmentally persistent herbicide that can effectively control individually treated OMB stems, using selective techniques that protect tree hosts and other non-target vegetation. For stems treated 1 m aboveground in autumn, the basal bark method, using triclopyr ester in oil plus biodiesel (X-Tree Wet & Dry) was highly effective (> 95% mortality), even though stems were treated higher up than recommended by the manufacturer label. The cut stem method, using a 45% glyphosate gel formulation (Glimax Professional) was less effective (55% mortality 2 years after treatment) at the same stem height. However, glyphosate's rapid adsorption and degradation in soil greatly limits its mobility, making it much safer, environmentally, than most other herbicides (Duke and Powles 2008), including triclopyr.

Both basal herbicide stem treatments tested in this research are well-suited for situations where OMB density is not high and can be implemented without damaging high priority trees and shrubs (Raal and Timmins 2018). However, due to the lateral stem network OMB develops, an infestation cannot be controlled by solely focusing on climbing stems, nor would it be practical to treat individual stems where OMB is widespread, as basal herbicide methods are labour-intensive and time-consuming. To effectively manage both climbing and creeping stems while at the same time minimising non-target damage, separate approaches are necessary for each. Directed foliar herbicide spraying can be used to supplement basal methods.

The results of the foliar herbicide study support other work demonstrating that mixtures with triclopyr and picloram or with triclopyr, picloram and aminopyralid, are highly effective as foliar applications against other woody species (Webb and Harrington 2005, Gawn et al. 2013, Campbell et al. 2021). Regional councils in New Zealand and herbicide manufacturers of the compounds tested in this research recommend their use against OMB, but no other publication has evaluated the efficacy of the herbicides side-by-side on OMB, as was done here. However, the evidence in this study was not strong that the mixture was much more effective against OMB than triclopyr alone, or metsulfuron, which are less persistent in the environment. Despite its effectiveness against OMB, the drawback to using metsulfuron is that it can severely damage some grass species, such as perennial ryegrass, increasing the chances of re-colonisation from OMB or colonisation from other weeds through the weakened grass cover (Tran 2013). Also, because metsulfuron is not effective against great bindweed, it is not a suitable choice for control of OMB when great bindweed is also present, as it could release great bindweed from OMB competition and encourage its growth. The outcome of this research may be helpful for informing management of OMB.

9. General discussion and final conclusions

9.1 Introduction

Old man's beard is an introduced liana that is increasingly problematic and widespread in NZ, and so difficult to control that in many regions, it is only actively managed in high priority areas (C. Davey, Horizons Regional Council, pers. communication, October 16, 2019). Research that identifies characteristics of a species that facilitate its invasiveness is critical for its control (Harris and Gallagher 2011, Simberloff 2013). The bulk of previous research on OMB reproduction has focused largely on aspects of seed ecology, seed germination, and seedling establishment (Lhotská 1974, Nikolaeva et al. 1985, Van Gardingen 1986, West 1992, Baars and Kelly 1996, Bungard 1996, Vinkler et al. 2004, Nikoloff 2011, Picciau et al. 2017, Redmond and Stout 2018, Copete et al. 2021). Research reported in this thesis has taken new approaches and built on earlier work to clear up uncertainties, and has also broadened the scope of inquiry, looking beyond seed establishment to vegetative growth as a major determinant of OMB invasiveness. The current study revealed new information on OMB's aerial seed bank, which also lead to a re-estimation of its annual seed production. It confirmed that OMB seeds can be water-dispersed, discovered that chilling is not a requirement for seed germination, and helped confirm that OMB relies on a limited, short-term soil seed bank. It explained how morphological dormancy works in this species and explained how seeds cycle through physiological dormancy, both on the vine and in the soil. It described the unremarkable competitive ability of OMB seedlings and demonstrated the vigour of its vegetative spread. In addition, it tested the efficacy of chemical control techniques for climbing stems and herbicides to control creeping stems, at lower environmental cost. In this chapter, a summary of the research, major research findings, and implications of those findings will be discussed. The conclusion will bring together the research findings, by summarising the traits of OMB that contribute to its success as an invasive species, suggesting management strategies and future research.

9.2 Summary of work and findings

9.2.1 Dispersal

No previous studies have attempted to investigate the length of time achenes remain attached to the mother plant after development. In Chapter 2, the duration of OMB's aerial seed bank was determined, by tagging individual seed heads and monitoring seed presence over time. This was done to establish how intermittent release of seeds from the aerial seed bank via wind dispersal help facilitate OMB invasion of new habitats. Also, although it has long been assumed OMB can be secondarily water-dispersed, no research literature has shown that it can. Therefore, the likelihood of

water dispersal being a secondary dispersal mechanism was investigated, as employing dual dispersal methods would allow OMB to disperse its propagules more widely.

Nearly 50% of achenes were released from the vine during autumn, while fully dormant, and a further 45% were lost during winter and into early spring. Achenes remaining past mid-summer were extremely rare. A previous estimate of annual seed production (35,000/m^2/yr), based on counts taken in June (Van Gardingen 1986), assumed that OMB plants retained most of their aerial seed bank throughout the winter. Based on observations from this study, an adjusted estimate of annual seed production was calculated to be >70,000/m^2/yr. Thus, the aerial seed bank is large and ephemeral, but restored yearly.

OMB seeds germinated while immersed in water and seedlings tolerated immersion in water for up to 6 weeks. Seeds removed from water at the cotyledon stage developed robustly after being sown in potting soil. These results clearly indicate tolerance of the seed to water and point to waterways as likely conduits of dispersal.

9.2.2 Germination

Evaluating the germination characteristics of a species can help determine if traits associated with the embryonic plant are an important component of its invasiveness. A systematic understanding of OMB seed germination requirements is still lacking. In Chapter 3, to gain a better understanding of OMB germination requirements, conditionally dormant seeds collected mid-winter were tested for their response to various treatments to artificially break dormancy and stimulate germination. The treatments included mechanical scarification, removal of the achene style, exogenous nitrate, exogenous gibberellic acid, exogenous nitrate and gibberellic acid combined, complete darkness, and various temperature and light regimes.

The study found that OMB seeds germinated best in alternating temperatures with alternating dark/light periods, which is consistent with other research which noted similar outcomes (Picciau et al. 2017). The most effective treatment was exogenous nitrate: > 80% of conditionally dormant seeds treated with potassium nitrate, could be induced to germinate within 4 weeks when incubated in temperatures alternating between 20°C and 30°C, with corresponding dark/light periods of 16h/8h. Also, although prechilling increased the speed at which seeds incubated at constant temperatures germinated, it did not promote total germination as well as the fluctuating temperature regime without prechilling, regardless of treatment. Thus, despite claims by previous researchers (Rudolf 1974, Grime et al. 1981, Bungard et al. 1997b, Copete et al. 2021), beyond the natural chilling seeds experienced before collection in mid-winter, chilling was unnecessary for overcoming dormancy. This shows that OMB seeds will germinate in response to simulated spring conditions (fluctuating temperatures that are appropriately warm and higher soil nitrogen availability), even in mid-winter,

which could have important ramifications for its invasiveness climate as temperature shifts occur in a warming climate.

9.2.3 Aerial seed bank and dormancy

Mature OMB seeds are both physiologically and morphologically dormant (Nikolaeva et al. 1985), but no prior studies have attempted to demonstrate how the two dormancies may change between seed development and germination. To better understand the dormancy process and viability of seeds in the aerial seed bank, further germination experiments in Chapter 4 tracked dormancy and viability changes in OMB seed from the aerial seed bank throughout the entire after-ripening period (autumn to spring). Seeds collected every second month for 2 years were incubated for 28 days in alternating temperatures (20°C/30°C), with either no treatment or the addition of potassium nitrate, in complete darkness or in an alternating light regime (16h dark/8h light).

Less than 72% of total OMB seeds produced were viable (50,000/m^2/yr). That is, approximately 28% of seeds were aborted or diseased (too small or flat) or eaten while still attached to the mother plant. OMB seeds on the mother plant remained completely morphophysiologically dormant throughout autumn, even when treated with exogenous nitrogen. Physiological dormancy was progressively lost over winter, in response to seasonal changes, yet morphological dormancy did not change until seeds had been exposed to appropriate germination conditions for several days. Fully dormant autumn seeds decayed at higher rates than non-dormant or partially dormant seeds, when exposed to moisture and warm temperatures normally adequate for germination. At the other extreme, non-dormant spring seeds were highly germinable, with low levels of decay and dormancy. Also, seeds incubated in complete darkness were more likely to decay or remain dormant than those exposed to some light during incubation.

This study demonstrated that total fertility is very high, even though fewer than three-quarters of seeds produced are viable and further decay occurs after dispersal. Thus, from seeds alone, propagule pressure from this species is enormous. In addition, viable seeds are protected with two forms of dormancy, one which changes with seasonal shifts and one which requires additional cues to break.

9.2.4 Soil seed bank and dormancy

Dormant seeds in a long-term seed bank can help ensure the success of an invasive species (Fenner and Thompson 2005, Baskin and Baskin 2014, Gioria et al. 2021). Other researchers have not been in agreement about whether or not OMB has a long-term persistent seed bank (Van Gardingen 1986, West 1992, Warr et al. 1994, Dutoit and Alard 1995, Deiller et al. 2003, Roovers et al. 2006, Nikoloff 2011, Clements and Bierzychudek 2017). In Chapter 5, to evaluate OMB seed dormancy and longevity in the soil, buried seeds were retrieved at 3-month intervals over a 2-year period and

subjected to germination tests. Seed provenance, time spent in the soil, type of soil/environmental conditions during burial, and depth of burial (2 or 5 cm) were all factors considered.

As did aerial seeds, buried seeds experienced cyclic changes to physiological dormancy, with greater dormancy in summer and autumn and a gradual decrease from winter to spring. Being less exposed to temperature fluctuations, seeds buried at 5 cm were less likely to decay and more likely to remain dormant than those buried at 2 cm, regardless of time spent in the soil. Between 15 and 24 months after burial, an average of < 0.5% of the total number of seeds buried at 2 cm remained viable, and of those buried at 5 cm, approximately 5.5% remained viable. The outcome of this study suggests that the proportion of viable buried OMB seeds after 1 year is relatively small but could still be a substantial number, given total viable seed production (< 50,000/m^2/yr). Thus, OMB seedlings could potentially be recruited from the soil seed bank to re-colonise an area following removal of mature plants. This finding validates observations of a persistent OMB seed bank made by others (Clements and Bierzychudek 2017).

9.2.5 Seedling competition

Superior competitive ability is often attributed to invasive species (Gurevitch 2011), and as a mature vine old man's beard is known to have devastating negative impacts on the trees it colonises (Atkinson 1984, Gourlay et al. 1999, Ogle et al. 2000). No other experimental studies looking at the direct effects of competition between OMB and other species have been published, at any stage of life. OMB has had an increasing presence in forestry blocks and riparian zones, both of which usually maintain some grass cover. To ascertain the competitive ability of OMB at the seedling stage, Chapter 6 examined OMB seedling emergence, establishment, and survival in different levels of competition with grass at three different sites. Competition was represented by four different grass heights: 1) no grass; 2) grass allowed to recolonise after initial clearing; 3) grass cut to 4 cm then allowed to regrow; and 4) unmanipulated grass.

In plots at the highest level of grass competition, no seedlings were ever detected, throughout a 1-year monitoring period. At all other levels, poor seedling emergence was observed, with a maximum of 36% of seeds sown in competition-free plots producing a seedling. Also, seedlings did not survive past 1 year, except in plots without competition in the first few months of growth, or in plots where grass grew more sparsely. However, seedlings that did survive began producing multiple stems within 6 months of emergence. These results indicate that obstacles to seedling emergence and mediocre competitiveness at the young seedling stage severely limit OMB's chances for establishment from seed. Yet, successful seedling recruitment can occur, and may not be insubstantial, due to the magnitude of the propagule pressure. Also, increasing frequency of storms and flooding events due to climate change may generate more suitably bare sites for OMB seed establishment.

9.2.6 Vegetative reproduction

Lianas in both tropical and temperate climates are well known for spreading by clonal stems along the ground (Prosperi et al. 2001, Schnitzer et al. 2004, Yorke et al. 2013, Ledo and Schnitzer 2014, Ladwig and Meiners 2015, Buru et al. 2016, Mori et al. 2018), yet no investigative work had been done on OMB's vegetative spread. Considering the findings in Chapter 6, Chapter 7 investigated OMB's use of vegetative reproduction as a means of local dispersal and population growth. By excavating 1 m^2 plots, an observational study documented and characterised the patterns of lateral stem growth in infested riparian zones. The measurements and observations made from the plots revealed the existence of an extensive, branching network of creeping stems, both above and belowground, with single nodes consistently generating multiple new shoots.

In addition, an experimental study tested the ability of OMB stem fragments, both creeping and climbing, to act as vegetative propagules. Two-node woody stem fragments in a range of diameters were buried in trays filled with potting mix and watered overhead for 18 weeks. After 4 months, approximately 50% of fragments, whether creeping or climbing, had regenerated, and were growing as individual plants. Larger fragment diameter improved the odds of fragment regrowth, as well as regrowth vigour, likely due to larger carbohydrate reserves. These studies provided good evidence that OMB may rely quite heavily on vegetative growth, and that once an OMB seedling succeeds to maturity, consequent vigorous vegetative reproduction from its multiple stems assures its establishment and spread, despite low initial colonization rates. This has implications for management, as creeping stems are apt to recolonise areas where only climbing stems have been controlled. Also, any plant material left behind after land clearance by machinery has the potential to recover. Furthermore, OMB stems fragmented during storms and flooding events that get washed downstream in waterways and lodged in silt deposits could conceivably establish, as well. Indeed, along with seedling establishment, this may partly explain OMB's common presence along riverbanks.

9.2.7 Chemical Control

As part of effective management plans, herbicides are indispensable for the control of many problematic weeds (Simberloff 2013). Increasingly, techniques that improve precision and reduce non-target damage are preferred, yet these techniques have not been compared in published research for their use against OMB. Chemical methods for controlling climbing and creeping stems separately were evaluated in Chapter 8. Using two different herbicides with lower relative environmental persistence than commonly used picloram, the efficacy of two techniques for controlling climbing stems was tested: 1) chemical ringbarking (basal bark method) with triclopyr ester in oil plus biodiesel, without any cutting of stems; and 2) application of 45% concentrated glyphosate gel on the

surfaces of freshly cut stems (cut stem method). Herbicides were applied directly to individual OMB stems at the base, thereby protecting tree hosts and other non-target vegetation. The basal bark method was highly effective (> 95% mortality), even though stems were treated at 1m aboveground, higher up than recommended by the manufacturer label. The cut stem method, using a 45% glyphosate gel formulation (Glimax Professional) was less effective (56% mortality 2 years after treatment) at the same stem height. However, quick recolonisation of the canopy by untreated stems and creeping stems from outside plots demonstrated the need to treat the entire infestation for successful control.

Herbicide efficacy of three selective herbicide sprays on creeping stems that do not damage existing grass cover was also assessed: 1) metsulfuron-methyl ester; 2) triclopyr butoxyethyl ester; and 3) a mixture of triclopyr, picloram, and aminopyralid. All herbicide treatments gave effective control of OMB, although metsulfuron had a negative effect on grass vigour. Preserving the grass cover is valuable when clearing infestations from waste areas or riparian zones being prepared for native plantings, as it can help suppress re-colonisation by OMB and colonisation by other weeds.

9.3 Final Conclusions

9.3.1 Invasive success through a diversified reproductive strategy

The combination of findings from this research help delineate the traits that allow old man's beard to be an invasive species. In addition to factors such as efficient use of light in seedlings (Baars and Kelly 1996, Bungard et al. 1997c), tolerance to heat (Zhang et al. 2021), and advantageous stem anatomical features (Carlquist 1995, Isnard et al. 2003, Plavcová et al. 2019), a considerable component of its success appears to be a reproductive diversification strategy. That is, investing in a variety of reproductive methods that result in significant propagule pressure and improve its chances of survival. Those methods include dual pollination mechanisms, dual dispersal mechanisms, dual seed banks, dual seed dormancies, and dual reproductive forms.

Fertilisation of OMB is accomplished by generalist pollinators, but in the absence of pollinators, self-pollination can take place (Redmond and Stout 2018). Due to lower genetic diversity of self-pollinated seeds, self-pollination is less optimal than out-crossing, but it nevertheless allows fertilisation to occur in the absence of pollinators. Seeds are mass produced annually: >70,000/m^2/yr. Approximately 12% of seeds are aborted (Salisbury 1920), and others suffer predation, disease, decay, or do not develop properly, due to unfavourable conditions. Thus, the net fertility is reduced by more than one-quarter, to approximately 50,000 viable seeds/m^2/yr, which is nevertheless still considerably large.

Seeds are valuable for long distance dispersal and initial establishment in new locations. Also, due to genetic recombination, pollinated seeds by out-crossing are genetically diverse, conferring adaptive fitness on seedling recruits (Busch et al. 2022). Seeds that develop on OMB vines each summer are completely morphologically and physiologically dormant until fully mature, and constitute a temporary aerial seed bank, from which seeds are wind-dispersed when completely senesced, beginning in late autumn. Because seeds develop asynchronously, they likewise senesce and disperse asynchronously, throughout the winter and into spring, but nearly all seeds are lost to the aerial seed bank by early spring. Secondary long distance seed dispersal can be facilitated by hydrochory, and thus water channels become corridors of OMB spread.

Physiological dormancy of seeds gradually declines while dispersal takes place, largely in response to seasonal shifts in temperature, but also to daylength (Finch-Savage and Footitt 2017). This occurs in OMB seeds still attached to the mother plant, as well as those that have become buried in the soil. Seeds in the soil constitute a second seed bank. Most seeds on the ground germinate within the first season after dispersal, and others lose viability, but a nominal proportion either remain dormant or re-enter physiological dormancy. Seeds buried more deeply are more likely to remain dormant, contributing to a small, persistent soil seed bank that endures for at least two seasons after dispersal.

After physiological dormancy is broken, seeds must be exposed to suitable germination conditions for several days before morphological dormancy can be broken. Hence, morphological dormancy acts as an extra protective mechanism against responding too quickly to ephemeral warm weather. Still, OMB seeds can and will germinate in response to extended simulated spring conditions any time after initial autumn dormancy.

OMB seedlings are not vigorous competitors with dense ground cover and are unlikely to survive to maturity unless seeds germinate and seedlings grow, at least for the early stages, in a very low competition setting. Thus, despite heavy propagule pressure presented by the masses of seeds, relatively few seedlings can establish. Suitably vacant sites may occur along rivers when silt is deposited during a flooding event, when gaps in the forest floor open because of treefalls or other disturbances.

Successful seedlings begin producing multiple stems within the first few months of existence. OMB invests heavily in vegetative growth, eventually producing extensive networks of creeping and climbing stems, which, unlike young seedlings, are able to thrive in densely vegetated areas. Any stems in contact with soil can produce roots at the nodes, and both creeping and climbing stems are capable of regenerating after fragmentation. The vegetative growth is a second form of reproduction, which allows OMB to spread locally from the initial point of establishment, and to intensify its propagule pressure. In addition, as found for other species (Ott et al. 2019), dormant nodes on stems function as a “bud bank”, a cache from which the plant can regenerate.

9.3.2 Recommendations for management and further research

Most of the riparian areas encountered in this research were fenced off to exclude cattle, but had not been revegetated or managed, and were essentially waste areas, in which weedy species, such as OMB, are prevalent. As potential sources of propagule dispersal, waterways can act as conduits for OMB spread. Management strategies that focus on controlling riparian populations to prevent downstream dispersal could therefore be highly beneficial.

Seedling recruitment can be reduced by limiting bare soil and encouraging a dense, desirable ground cover, such as a healthy grass cover beneath planted natives in riparian zones and beneath pines in forestry blocks. In circumstances where seedlings establish in bare sites generated by natural or man-made disturbances, seedlings must be removed completely, so they cannot begin spreading vegetatively. Breaking up mature OMB stems, by bulldozing or other mechanical means, effectively produces fragments capable of regenerating and growing as independent individuals. Forestry windrows with OMB fragments should be monitored for regrowth. Infestations of creeping stems (including windrows) can be treated by broadcasting a foliar spray, ideally one that preserves existing grasses, such as triclopyr or a combination of triclopyr, picloram, and aminopyralid. Native or other broadleaf vegetation must be shielded from the spray, although other research has shown that *Pinus radiata* (the species most commonly grown in production forests) is tolerant to these pyridine herbicides (Tran et al. 2016). Once the infestation has been reduced, following up with spot spraying may be necessary. Individual stems can be effectively treated with triclopyr in oil, using the basal bark method, which is especially valuable as a precision technique that avoids damage to nearby non-target vegetation, such as trees hosting climbing OMB.

This research found that vegetative growth helps drive OMB infestations, but just how much its populations rely on clonality for colonisation is unknown. Future investigations, using microsatellite markers or other genetic analyses, could reveal the extent of OMB population genetic uniformity, its distribution patterns, and its potential vulnerability to disease and other natural enemies. Currently, two biocontrol agents in New Zealand show promise in reducing OMB vigour and spread in the early stages of their establishment, and a third, pathogenic agent, is being sought, in anticipation that it may also effectively antagonise the species (den Breeyen 2022). Research in new directions will continue to be necessary, to mitigate OMB's destructive spread and reduce the environmental and financial costs associated with it. Nonetheless, this project has contributed important information for understanding and managing this harmful species.

References

Alavanja, M. C., and M. R. Bonner. 2012. Occupational pesticide exposures and cancer risk: A review. Journal of Toxicology and Environmental Health, Part B **15**:238-263.

Alboresi, A., C. Gestin, M. T. Leydecker, M. Bedu, C. Meyer, and H. N. Truong. 2005. Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant, Cell & Environment **28**:500-512.

Allan, H. H. 1940. A handbook of the naturalised flora of New Zealand. N.Z. Department of Scientific and Industrial Research (DSIR). Government Printer, Wellington, NZ.

Allen, B. P. 2015. Patterns of liana abundance, diversity and distribution in temperate forests. Pages 7-15 *in* N. Parthasarathy, editor. Biodiversity of Lianas. Springer International, Switzerland.

Alpert, P. 1996. Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*. Journal of Ecology **84**:395-406.

Angylossy, V., M. R. Pace, and A. C. Lima. 2015. Liana anatomy, physiology, and biomechanics: a broad perspective on structural evolution of the vascular system. Pages 253-288 *in* S. A. Schnitzer, F. Bongers, R. J. Burnham, and F. E. Putz, editors. Ecology of Lianas. Wiley Blackwell.

Atkinson, I. A. E. 1984. Distribution and potential range of old man's beard, *Clematis vitalba*, in New Zealand. Pages 6-25 *in* The Clematis vitalba Threat. Department of Public Lands and Survey, New Zealand, Wellington, NZ.

Baars, R., and D. Kelly. 1996. Survival and growth responses of native and introduced vines in New Zealand to light availability. New Zealand Journal of Botany **34**:389-400.

Baars, R., D. Kelly, and A. D. Sparrow. 1998. Liane distribution within native forest remnants in two regions of the South Island, New Zealand. New Zealand Journal of Ecology **22**:71-85.

Bache, S. M., and H. Wickham. 2022. magrittr: A forward-pipe operator for R. https://CRAN.R-project.org/package=magrittr.

Bai, L., H. Deng, X. Zhang, X. Yu, and Y. Li. 2016. Gibberellin is involved in inhibition of cucumber growth and nitrogen uptake at suboptimal root-zone temperatures. PLOS ONE **11**:e0156188.

Baillie, B. R., D. G. Neary, S. Gous, and C. Rolando. 2015. Aquatic fate of aerially applied hexazinone and terbuthylazine in a New Zealand planted forest. Journal of Sustainable Watershed Science & Management **2**:118-129.

Baker, H. G. 1974. The Evolution of Weeds. Annual Review of Ecology and Systematics **5**:1-24.

Bakker, J. P., M. Dekker, and Y. De Vries. 1980. The effect of different management practices on a grassland community and the resulting fate of seedlings. Acta Botanica Neerlandica **29**:469-482.

Barrett, S. C. H. 2011. Reproductive systems, plant *in* D. Simberloff and M. Rejmánek, editors. Encyclopedia of Biological Invasions. University of California Press, London, England.

Baskin, C. C., and J. M. Baskin. 2004a. Germinating seeds of wildflowers, an ecological perspective. HortTechnology **14**:467-473.

Baskin, C. C., and J. M. Baskin. 2014. Seeds: Ecology, biogeography, and evolution of dormancy and germination. 2nd edition. Academic Press.

Baskin, J. M., and C. C. Baskin. 1985. The annual dormancy cycle in buried weed seeds: A continuum. Bioscience **35**:492-498.

Baskin, J. M., and C. C. Baskin. 2004b. A classification system for seed dormancy. Seed Science Research **14**:1-16.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1-48.

Batlla, D., and R. L. Benech-Arnold. 2014. Weed seed germination and the light environment: Implications for weed management. Weed Biology and Management **14**:77-87.

Benvenuti, S. 2007. Weed seed movement and dispersal strategies in the agricultural environment. Weed Biology and Management **7**:141-157.

Bewley, J. D. 1997. Seed germination and dormancy. The Plant Cell **9**:1055-1066.

Bewley, J. D., K. J. Bradford, H. W. M. Hilhorst, and H. Nanogaki. 2013. Seeds: Physiology of development, germination and dormancy. 3rd edition. Springer, New York Heidelberg Dordrecht London.

Bhatt, A., P. C. Phondani, S. S. Phartyal, A. Santo, and D. Gallacher. 2016. Influence of aerial seed banks on germination response in three desert plant species. Journal of Plant Ecology **10**:994-1000.

Bierzychudek, P. 2020. Passive recovery of an urban forest in the Pacific Northwest after removal of invasive plants. Urban Ecosystems **23**:1023-1038.

Biosecurity New Zealand. 2023. Pests of concern to New Zealand. https://pierpestregister.mpi.govt.nz/pests-of-concern/?scientificName=&organismType=&freeSearch=Clematis.

Biosecurity Queensland. 2016. Weeds of Australia. https://keyserver.lucidcentral.org/weeds/data/media/Html/clematis_vitalba.htm.

Boatman, N. D., and A. B. Bain. 1992. Evaluation of quinmerac and fluroxypyr against hedgerow flora and uncommon arable weeds. Tests of Agrochemicals and Cultivars **13**:42-43.

Brady, N. C., and R. R. Weil. 1999. The nature and properties of soils. 12th edition. Prentice Hall, Upper Saddle River, N.J.

Britt, C. P. 1994. Clematis vitalba (old man's beard) as a competitive "weed" in hedgerows and the effects of hedge cutting regimes on its development. Pages 241-246 *in* N. Boatman, editor. Field margins: Integrating agriculture and conservation. British Crop Protection Council (BCPC), Farnham, UK.

Bufford, J. L., and C. Daehler. 2011. Life history strategies *in* D. Simberloff and M. Rejmánek, editors. Encyclopedia of Biological Invasions. University of California Press, London, England.

Bullock, J. M., K. Shea, and O. Skarpaas. 2006. Measuring plant dispersal: An introduction to field methods and experimental design. Plant Ecology **186**:217-234.

Bungard, R. A. 1996. Ecological and physiological studies of *Clematis vitalba* L. PhD, Lincoln University, Canterbury, New Zealand.

Bungard, R. A., G. T. Daly, D. L. McNeil, A. V. Jones, and J. D. Morton. 1997a. *Clematis vitalba* in a New Zealand native forest remnant: Does seed germination explain distribution? New Zealand Journal of Botany **35**:525-534.

Bungard, R. A., D. McNeil, and J. D. Morton. 1997b. Effects of chilling, light and nitrogen-containing compounds on germination, rate of germination and seed imbibition of *Clematis vitalba* L. Annals of Botany **79**:643-650.

Bungard, R. A., D. McNeil, and J. D. Morton. 1997c. Effects of nitrogen on the photosynthetic apparatus of *Clematis vitalba* grown at several irradiances Functional Plant Biology **24**:205-214.

Bungard, R. A., J. D. Morton, D. L. McNeil, and G. T. Daly. 1998. Effects of irradiance and nitrogen on Clematis vitalba establishment in a New Zealand lowland podocarp forest remnant. New Zealand Journal of Botany **36**:661-670.

Buru, J. C., K. Dhileepan, O. Osunkoya, and J. Firn. 2016. Comparison of growth traits between abundant and uncommon forms of a non-native vine, *Dolichandra unguis-cati* (Bignoniaceae) in Australia. NeoBiota **30**:91-109.

Busch, J. W., S. Bodbyl-Roels, S. Tusuubira, and J. K. Kelly. 2022. Pollinator loss causes rapid adaptive evolution of selfing and dramatically reduces genome-wide genetic variability. Evolution **76**:2130-2144.

Buttler, I. W., D. W. Roberts, L. E. Siders, and R. C. Gardner. 1993. Non-crop right-of-way terrestrial dissipation of triclopyr in California. DowElanco. Report No. ABM-140265-E. DPR# 51566-017.

Buxton, J. M. 1985. The potential for the biological control of *Clematis vitalba* L. M.Sc., University of London, UK.

Campbell, S., A. Bajwa, K. Hosking, D. Brazier, V. Mellor, and M. Perkins. 2021. Herbicide Options to Control Naturalised Infestations of *Cereus uruguayanus* in Rangeland Environments of Australia. Plants **10**:2227.

Carlquist, S. 1985. Observations on functional wood histology of vines and lianas. Aliso: A Journal of Systematic and Floristic Botany **11**:139-157.

Carlquist, S. 1995. Wood and bark anatomy of Ranunculaceae (including Hydrastis) and Glaucidiaceae. Aliso: A Journal of Systematic and Floristic Botany **14**:65-84.

Carran, R. A. 1978. Soil nitrogen and pasture management. Pages 44-50 *in* New Zealand Grassland Association **40**:44-50.

Carthey, A. J. R., K. A. Fryirs, T. J. Ralph, H. Bu, and M. R. Leishman. 2016. How seed traits predict floating times: a biophysical process model for hydrochorous seed transport behaviour in fluvial systems. Freshwater Biology **61**:19-31.

Catford, J. A., and R. Jansson. 2014. Drowned, buried and carried away: Effects of plant traits on the distribution of native and alien species in riparian ecosystems. New Phytologist **204**:19-36.

Chaideftou, E., C. A. Thanos, E. Bergmeier, A. Kallimanis, and P. Dimopoulos. 2009. Seed bank composition and above-ground vegetation in response to grazing in sub-Mediterranean oak forests (NW Greece). Pages 255-265 *in* A. G. Van der Valk, editor. Forest Ecology: Recent Advances in Plant Ecology. Springer Netherlands, Dordrecht.

Chalker-Scott, L. 2002. Do anthocyanins function as osmoregulators in leaf tissues? Pages 103-127 *in* K.S. Gould and D.W. Lee, editors. Anthocyanins in Leaves. Academic Press, Amsterdam.

Chauhan, B. S., G. Gill, and C. Preston. 2006. Factors affecting seed germination of threehorn bedstraw (*Galium tricornutum)* in Australia. Weed Science **54**:471-477.

Chu, W., and K.-H. Chan. 2000. The prediction of partitioning coefficients for chemicals causing environmental concern. Science of the Total Environment **248**:1-10.

Clarke, J. S., M. Lewis, and L. Horvath. 2001. Invasion by extremes: Population spread with variation in dispersal and reproduction. The American Naturalist **157**:537-554.

Clay, D. V., and F. L. Dixon. 1996. Investigations of control methods for *Clematis vitalba* (old man's beard). Aspects of Applied Biology **44**:313-318.

Clay, D. V., and F. L. Dixon. 2000. Further investigations on the control of *Clematis vitalba* (Old Man's Beard). Aspects of Applied Biology **58**:71-76.

Clements, H., and P. Bierzychudek. 2017. Can the persistent seed bank contribute to the passive restoration of urban forest fragments after invasive species removal? Ecological Restoration **35**:156-166.

Colautti, R. I., I. A. Grigorovich, and H. J. MacIsaac. 2006. Propagule pressure: A null model for biological invasions. Biological Invasions **8**:1023-1037.

Conn, J. S., K. L. Beattie, and A. Blanchard. 2006. Seed viability and dormancy of 17 weed species after 19.7 years of burial in Alaska. Weed Science **54**:464-470.

Connor, H. E., and 1977. The poisonous plants in New Zealand. Page 247 *in* N. Z. Department of Science and Industrial Research (DSIR), editor. Bulletin 99. Government Printer, Wellington, NZ.

Copete, M. A., J. M. Herranz, R. Herranz, E. Copete, and P. Ferrandis. 2021. Effects of desiccation of seeds in nine species with morphophysiological dormancy on germination and embryo growth. Journal of Plant Ecology **14**:132-146.

Cox, C. 2000. Herbicide factsheet: Triclopyr. Journal of Pesticide Reform **20**:12-19.

Cruzan, M. B., M. A. Streisfeld, and J. A. Schwoch. 2022. Fitness effects of somatic mutations accumulating during vegetative growth. Evolutionary Ecology **36**:767-785.

Dairy NZ. 2023. Sustainable dairying: Water accord. DairyNZ. https://www.dairynz.co.nz/environment/environmental-leadership/sustainable-dairying-water-accord/.

Danielewicz, W., and B. Wiatrowska. 2014. The most invasive tree and shrub species in Polish forests. Peckiana **9**:59-67.

Darwin, C. R. 1865. On the movements and habits of climbing plants. Journal of the Linnean Society of London (Botany) **9**:1-118.

Davies, R., A. Di Sacco, and R. Newton. 2015. Germination testing: Environmental factors and dormancy-breaking treatments. Millenium Seed Bank Partnership. http://brahmsonline.kew.org/msbp/Training/Resources.

Dawes, M. A., P. Schleppi, S. Hättenschwiler, C. Rixen, and F. Hagedorn. 2017. Soil warming opens the nitrogen cycle at the alpine treeline. Global Change Biology **23**:421-434.

Deiller, A.-F., J.-M. N. Walter, and M. Trémolières. 2003. Regeneration strategies in a temperate hardwood floodplain forest of the Upper Rhine: Sexual versus vegetative reproduction of woody species. Forest Ecology and Management **180**:215-225.

den Breeyen, A. 2022. A fresh search for old man's beard pathogens. Manaaki Whenua Landcare Research, Auckland, New Zealand. https://www.landcareresearch.co.nz/publications/weed-biocontrol/weed-biocontrol-articles/a-fresh-search-for-old-mans-beard-pathogens/

Dev, R., D. Dayal, and M. Sureshkumar. 2020. Gibberellic acid and potassium nitrate promote seed germination and growth of grey-leaved saucer-berry (*Cordia sinensis* lam.) seedlings. International Journal of Fruit Science **20**:S937-S954.

Díaz-Sala, C., G. Garrido, and B. Sabater. 2002. Age-related loss of rooting capability in Arabidopsis thaliana and its reversal by peptides containing the Arg-Gly-Asp (RGD) motif. Physiologia Plantarum **114**:601-607.

Diaz-Sala, C., K. W. Hutchison, B. Goldfarb, and M. S. Greenwood. 1996. Maturation-related loss in rooting competence by loblolly pine stem cuttings: The role of auxin transport, metabolism and tissue sensitivity. Physiologia Plantarum **97**:481-490.

DiTomaso, J. M., and G. B. Kyser. 2007. Control of *Ailanthus altissima* using stem herbicide application techniques. Arboriculture & Urban Forestry **33**:55-63.

Dołkin−Lewko, A., and U. Zajączkowska. 2022. Threats to ecosystems and methods for controlling invasive vines in temperate forests. Sylwan **166**:267-282.

Dölle, M., and W. Schmidt. 2009. The relationship between soil seed bank, above-ground vegetation and disturbance intensity on old-field successional permanent plots. Applied Vegetation Science **12**:415-428.

Duke, S. O., and S. B. Powles. 2008. Glyphosate: A once-in-a-century herbicide. Pest Management Science **64**:319-325.

Duncan, R. P. 2011. Propagule pressure *in* D. Simberloff and M. Rejmánek, editors. Encyclopedia of Biological Invasions. University of California Press, London, England.

Dutoit, T., and D. Alard. 1995. Permanent seed banks in chalk grassland under various management regimes: Their role in the restoration of species-rich plant communities. Biodiversity & Conservation **4**:939-950.

Elliot, T., Y. Soh, and D. Barnett. 2022. iNZightPlots: Graphical tools for exploring data with 'iNZight'. https://CRAN.R-project.org/package=iNZightPlots.

Environmental Protection Authority. 2018. Decision--Application to release a leaf-galling mite, Aceria vitalbae, as a biological control agent for old man's beard (*Clematis vitalba*), APP203313*in* Application to import for release and/or release from containment any new organism under section 34 of the Hazardous Substances and New Organisms Act 1996. N. Z. EPA, editor.

Essig, F. B. 1991. Seedling morphology in clematis (Ranunculaceae) and its taxonomic implications. SIDA, Contributions to Botany **14**:377-390.

Ewers, F. W., J. A. Rosell, and M. E. Olson. 2015. Lianas as structural parasites. Pages 163-188 *in* U. Hacke, editor. Functional and Ecological Xylem Anatomy. Springer International Publishing, Cham.

Fenner, M., and K. Thompson. 2005. The ecology of seeds. Cambridge University Press, Cambridge.

Ferrell, J. A., J. J. Mullahey, K. A. Langeland, and W. N. Kline. 2006. Control of Tropical Soda Apple (Solanum viarum) with Aminopyralid. Weed Technology **20**:453-457.

Finch-Savage, W. E., and S. Footitt. 2017. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. Journal of Experimental Botany **68**:843-856.

Finch-Savage, W. E., and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. New Phytologist **171**:501-523.

Fitter, A. 1978. An atlas of the wild flowers of Britain and Northern Europe. Collins, London, UK.

Forbis, T. A., S. K. Floyd, and A. de Queiroz. 2002. The evolution of embryo size in angiosperms and other seed plants: Implications for the evolution of seed dormancy. Evolution **56**:2112-2125.

Forseth, I. N., and A. F. Innis. 2004. Kudzu (*Pueraria montana*): History, physiology, and ecology combine to make a major ecosystem threat. Critical Reviews in Plant Sciences **23**:401-413.

Fox, J. 2003. Effect displays in R for generalised linear models. Journal of Statistical Software **8**:1 - 27.

Fox, J., and S. Weisberg. 2019. An R companion to applied regression. 3rd edition. Sage, Thousand Oaks, CA.

França-Neto, J. d. B., and F. C. Krzyzanowski. 2019. Tetrazolium: An important test for physiological seed quality evaluation. Journal of Seed Science **41**:359-366.

Gao, R., X. Yang, F. Yang, L. Wei, Z. Huang, and J. L. Walck. 2014. Aerial and soil seed banks enable populations of an annual species to cope with an unpredictable dune ecosystem. Annals of Botany **114**:279-287.

Gawn, T. L., K. C. Harrington, and C. Matthew. 2013. Susceptibility of great bindweed (*Calystegia silvatica*) to herbicides. New Zealand Plant Protection **66**:94-98.

Geiss, G., L. Gutierrez, and C. Bellini. 2009. Adventitious root formation: New insights and perspectives. Annual Plant Reviews **37**:127-156

Gelman, A., and J. Hill. 2006. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press.

Gelman, A., and Y.-S. Su. 2021. arm: Data analysis using regression and multilevel/hierarchical models. https://CRAN.R-project.org/package=arm.

Ghani Zadeh, H. 2015. Aspects of herbicide resistance in three New Zealand weed species. PhD, Massey University, Manawatu, New Zealand.

Ghanizadeh, H., K. C. Harrington, and T. K. James. 2015. Glyphosate-resistant population of *Lolium perenne* loses resistance at winter temperatures. New Zealand Journal of Agricultural Research **58**:423-431.

Ghassemi, M., L. Fargo, P. Painter, P. Painter, S. Quinlivan, R. Scofield, and A. Takata. 1981. Picloram. Pages A221-267 *in* Environmental fates and impacts of major forest use pesticides. Office of Pesticides and Toxic Substances. Washington D.C. U.S. EPA.

Gioria, M., A. Carta, C. C. Baskin, W. Dawson, F. Essl, H. Kreft, J. Pergl, M. van Kleunen, P. Weigelt, M. Winter, and P. Pyšek. 2021. Persistent soil seed banks promote naturalisation and invasiveness in flowering plants. Ecology Letters **24**:1655-1667.

Gioria, M., and P. Pyšek. 2017. Early bird catches the worm: germination as a critical step in plant invasion. Biological Invasions **19**:1055-1080.

Gould, K. S. 2004. Nature's swiss army knife: The diverse protective roles of anthocyanins in leaves. Journal of Biomedicine and Biotechnology **2004**:314-320.

Gourlay, A. H., R. Wittenberg, R. L. Hill, A. G. Spiers, and S. V. Fowler. 1999. The biological control programme against *Clematis vitalba* in New Zealand. Pages 709-718 *in* N.R. Spencer, editor. 10th International Symposium on Biological Control of Weeds, Montana State University, Bozeman, Montana.

Gous, S. 2003. Overview of the use of agri-chemicals and alternatives to control competing vegetation in forestry. Scion. https://fgr.nz/documents/download/5954

Greenwood, M. S., C. A. Hopper, and K. W. Hutchison. 1989. Maturation in larch: Effect of age on shoot growth, foliar characteristics, and DNA methylation. Plant Physiology **90**:406-412.

Grime, J. P., G. Mason, A. V. Curtis, J. Rodman, and S. R. Band. 1981. A comparative study of germination characteristics in a local flora. Journal of Ecology **69**:1017-1059.

Groppe, K. 1991. Literature review and preliminary survey of biotic agents associated with old man's beard, Clematis vitalba L., in Europe. Unpublished., International Institute of Biological Control, Delémont, Switzerland.

Guariento, E., P. Strutzenberger, C. Truxa, and K. Fiedler. 2020. The trinity of ecological contrasts: A case study on rich insect assemblages by means of species, functional and phylogenetic diversity measures. BMC Ecology **20**:14.

Guido, A., D. Hoss, and V. D. Pillar. 2017. Exploring seed to seed effects for understanding invasive species success. Perspectives in Ecology and Conservation **15**:234-238.

Gurevitch, J. 2011. Competition, Plant *in* D. Simberloff and M. Rejmánek, editors. Encyclopedia of biological invasions. University of California Press, London.

Gurnell, A., K. Thompson, J. Goodson, and H. Moggridge. 2008. Propagule deposition along river margins: linking hydrology and ecology. Journal of Ecology **96**:553-565.

Hamilton, D. J., Á. Ambrus, R. M. Dieterle, A. S. Felsot, C. A. Harris, P. T. Holland, A. Katayama, N. Kurihara, J. Linders, J. Unsworth, and S.-S. Wong. 2003. Regulatory limits for pesticide residues in water (IUPAC Technical Report). Pure and Applied Chemistry **75**:1123-1155.

Harrington, K. C., H. Ghanizadeh, C. Moyo, P. D. Kemp, and E. J.P.J. 2017. Pasture damage from spot-sprayed herbicides. New Zealand Plant Protection **70**:179-185.

Harrington, K. C., and X. Z. He. 2010. Determining how soon ground covers can be planted in forests after applying metsulfuron. Pages 340-343 *in* 17th Australasian Weeds Conference, Christchurch, New Zealand.

Harris, C. J., and R. Gallagher. 2011. Vines and lianas. Pages 680-684 *in* D. Simberloff and M. Rejmánek, editors. Encylopedia of biological invasions. University of California Press, Berkeley and Los Angeles, California.

Hartig, F. 2022. DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models. https://CRAN.R-project.org/package=DHARMa.

Hernández, J. A., P. Díaz-Vivancos, J. R. Acosta-Motos, and G. Barba-Espín. 2022. Potassium nitrate treatment is associated with modulation of seed water uptake, antioxidative metabolism and phytohormone levels of pea seedlings. Seeds **1**:5-15.

Hickman, M. V., C. G. Messersmith, and R. G. Lym. 1990. Picloram release from leafy spurge roots. Journal of Range Management **43**:442-445.

Horizons Regional Council. 2017. What's happening with old man's beard biological control? Horizons Regional Council Environment Committee, New Zealand. http://www.horizons.govt.nz/HRC/media/Media/Agenda-Reports/Environment-Committee-2017-12-12/17249%20Annex%20A%20Old%20Mans%20Beard%20Biological%20Control.pdf

Horvitz, C. C., and A. Koop. 2004. Removal of nonnative vines and post-hurricane recruitment in tropical hardwood forests of Florida. Biotropica **33**:268–281.

Hoskins, G. n.d. Plant pests. New Zealand Biosecurity Institute. https://www.biosecurity.org.nz/about-us/what-is-biosecurity/plant-pests/.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal **50**:346-363.

Hume, L. J., C. J. West, and H. M. Watts. 1995. Nutritional requirements of *Clematis vitalba* L. (old man's beard). New Zealand Journal of Botany **33**:301-313.

Hyslop, J., and S. Trowsdale. 2012. A review of hydrochory (seed dispersal by water) with implications for riparian rehabilitation. Journal of Hydrology (NZ) **51**:137-152.

Ismail, B. S., and H. J. Lee. 1995. Persistence of metsulfuron‐methyl in two soils. Journal of Environmental Science and Health, Part B **30**:485-497.

Isnard, S., T. Speck, and N. P. Rowe. 2003. Mechanical architecture and development in Clematis: Implications for canalised evolution of growth forms. New Phytologist **158**:543-559.

James, T. K., and A. Rahman. 1999. Survival of giant buttercup seeds buried at different depths in four soils. Proceedings of the NZ Plant Protection Conference **52**:234-239.

James, T. K., and A. Rahman. 2000. Longevity of buried ragwort seed in four soils. New Zealand Plant Protection **53**:253-257.

James, T. K., and A. Rahman. 2003. Survival of Scotch thistle seed buried at three depths in four New Zealand soils. New Zealand Plant Protection **56**:113-117.

James, T. K., A. Rahman, and P. Trivedi. 2010. Germination of seed from five broadleaf weeds after burial for up to 28 years in two soils. New Zealand Plant Protection **63**:84-89.

James, T. K., A. Rahman, D. A. Wardle, and K. I. Bonner. 1998. Survival of nodding thistle (*Carduus nutans*) seed buried at different soil depths in four soils. Proceedings of the NZ Plant Protection Conference **51**:33-37.

Jeník, J. 1994. Clonal growth in woody plants: A review. Folia Geobotanica **29**:291-306.

Jensen, S. M., D. Wolkis, E. Keshtkar, J. C. Streibig, and C. Ritz. 2020. Improved two-step analysis of germination data from complex experimental designs. Seed Science Research **30**:194-198.

Jotcham, J. R., D. W. Smith, and G. R. Stephenson. 1989. Comparative Persistence and Mobility of Pyridine and Phenoxy Herbicides in Soil. Weed Technology **3**:155-161.

Kassambara, A. 2020. ggpubr: 'ggplot2' based publication ready plots. https://CRAN.R-project.org/package=ggpubr.

Kassambara, A. 2021. rstatix: Pipe-friendly framework for basic statistical tests. https://CRAN.R-project.org/package=rstatix.

Kazda, M. 2015. Liana-nutrient relations. Pages 309-322 *in* S. A. Schnitzer, F. Bongers, R. J. Burnham, and F. E. Putz, editors. Ecology of Lianas. John Wiley & Sons, Ltd., West Sussex, U.K.

Kebrom, T. H. 2017. A growing stem inhibits bud outgrowth – The overlooked theory of apical dominance. Frontiers in Plant Science **8**:1874.

Keenan, T. F., and A. D. Richardson. 2015. The timing of autumn senescence is affected by the timing of spring phenology: implications for predictive models. Global Change Biology **21**:2634-2641.

Kennedy, P. C. 1982. The general morphology and ecology of *Clematis vitalba*. Pages 26-35 *in* The *Clematis vitalba* threat: Distribution and control of the introduced weed old man's beard (*Clematis vitalba*). Department of Public Lands and Survey, Wellington, NZ.

Kildisheva, O. A., K. W. Dixon, F. A. O. Silveira, T. Chapman, A. Di Sacco, A. Mondoni, S. R. Turner, and A. T. Cross. 2020. Dormancy and germination: Making every seed count in restoration. Restoration Ecology **28**:S256-S265.

Kimura, F., M. Sato, and H. Kato-Noguchi. 2015. Allelopathy of pine litter: Delivery of allelopathic substances into forest floor. Journal of Plant Biology **58**:61-67.

Kline, W. N., A. A. Chemello, J. L. Troth, and J. M. Breuninger. 2005. Aminopyralid: global opportunities.*in* Southern Weed Science Society **58**:140.

Kołodziejek, J., and J. Patykowski. 2015. Effect of environmental factors on germination and emergence of invasive *Rumex confertus* in Central Europe. Scientific World Journal **2015**:170176.

Kołodziejek, J., J. Patykowski, and M. Wala. 2017. Effect of light, gibberellic acid and nitrogen source on germination of eight taxa from dissapearing European temperate forest, *Potentillo albae-Quercetum*. Scientific Reports **7**:13924.

Kovinich, N., G. Kayanja, A. Chanoca, M. S. Otegui, and E. Grotewold. 2015. Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. Plant Signaling & Behavior **10**:e1027850-e1027850.

Kozlovskiy, B. L., O. I. Fedorinova, M. V. Kuropyatnikov, and P. A. Dmitriev. 2017. *Clematis vitalba* L. as potentially invasive species of Rostov region. Ukrainian Journal of Ecology **7**:116-124.

Kreen, S., M. Svensson, and K. Rumpunen. 2002. Rooting of clematis microshoots and stem cuttings in different substrates. Scientia Horticulturae **96**:351-357.

Kuhn, M. 2021. caret: Classification and regression training. R package 6.0-9.0. https://CRAN.R-project.org/package=caret.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: Tests in linear mixed effects models. Journal of Statistical Software **82**:1-26.

Ladwig, L. M., and S. J. Meiners. 2015. The role of lianas in temperate tree communities. Pages 188-202 *in* S. A. Schnitzer, F. Bongers, R. J. Burnham, and F. E. Putz, editors. Ecology of Lianas. Wiley Blackwell, West Sussex, UK.

Landcare Research. n.d. *Clematis vitalba* L. http://www.nzflora.info/factsheet/Taxon/Clematis-vitalba.html. http://www.nzflora.info/factsheet/Taxon/Clematis-vitalba.html.

Landcare Research NZ Ltd. 2019. S-map - New Zealand's national digital soil map. Manaaki Whenua - Landcare Research. https://doi.org/10.7931/L1WC7.

Landman Limited. Cut'N'Paste: Easy to use, safe weed killer gel. https://www.cutnpaste.co.nz/.

Langeland, K., and M. Meisenburg. 2009. Herbicide evaluation to control *Clematis terniflora* invading natural areas in Gainesville, Florida. Invasive Plant Science and Management **2**:70-73.

Larson, D. A. 1992. Analysis of variance with just summary statistics as input. The American Statistician **46**:151-152.

Laxa, M., M. Liebthal, W. Telman, K. Chibani, and K.-J. Dietz. 2019. The role of the plant antioxidant system in drought tolerance. Antioxidants (Basel, Switzerland) **8**:94.

Ledo, A., and S. A. Schnitzer. 2014. Disturbance and clonal reproduction determine liana distribution and maintain liana diversity in a tropical forest. Ecology **95**:2169-2178.

Leicht-Young, S. 2014. Seeing the lianas in the trees: Woody vines of the temperate zone. Arnoldia **72**:1.

Leicht-Young, S., and N. B. Pavlovic. 2015. Lianas as invasive species in North America *in* S. A. Schnitzer, F. Bongers, R. J. Burnham, and F. E. Putz, editors. Ecology of Lianas. Wiley Blackwell.

Leishman, M. R., and C. J. Harris. 2011. Seed Ecology *in* D. Simberloff and M. Rejmánek, editors. Encyclopedia of biological invasions. University of California Press, Berkeley, Los Angeles, London.

Leist, N., S. Krämer, and A. Jonitz. 2003. ISTA Working Sheets on Tetrazolium Testing: Tree and shrub species. International Seed Testing Association, Zurich, Switzerland.

Lesnoff, M., and R. Lancelot. 2012. Analysis of overdispersed data. https://cran.r-project.org/package=aod.

Leuschner, C., and H. Ellenberg. 2017. Life forms and growth types of Central European plant species. Pages 23-28 *in* C. Leuschner and H. Ellenberg, editors. Ecology of Central European forests: Vegetation ecology of Central Europe. Springer International Publishing.

Lhotská, M. 1974. The after-ripening of embryos on the mother plant. Folia Geobotanica et Phytotaxonomica **9**:231-240.

Lindgren, D. T. 2006. Clematis. Pages 781-799 *in* N. O. Anderson, editor. Flower Breeding and Genetics: Issues, Challenges and Opportunities for the 21st Century. Springer Netherlands, Dordrecht.

Linklater, W. L. 1998. The social and spatial organisation of horses. PhD, Massey University, Palmerston North, New Zealand.

Liu, K., J. M. Baskin, C. C. Baskin, H. Bu, G. Du, and M. Ma. 2013. Effect of diurnal fluctuating versus constant temperatures on germination of 445 species from the Eastern Tibet Plateau. PLOS ONE **8**:e69364.

Liu, L.-c., J. A. Dumas, and C. L. Cacho. 1997. Picloram groundwater contamination from pasture use. The Journal of Agriculture of the University of Puerto Rico **81**:211-218.

Liu, U., E. Breman, T. A. Cossu, and S. Kenney. 2018. The conservation value of germplasm stored at the Millennium Seed Bank, Royal Botanic Gardens, Kew, UK. Biodiversity and Conservation **27**:1347-1386.

Loescher, W. H., T. McCamant, and J. D. Keller. 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience **25**:274-281.

Lowry, B. J., J. H. Lowry, K. J. Jarvis, G. Keppel, R. R. Thaman, and H. J. Boehmer. 2020. Spatial patterns of presence, abundance, and richness of invasive woody plants in relation to urbanization in a tropical island setting. Urban Forestry & Urban Greening **48**:126516.

Lüdecke, D. 2022. sjPlot: Data visualization for statistics in social science. https://CRAN.R-project.org/package=sjPlot.

Lynch, A. L. 2009. Investigating distribution and treatments for effective mechanical and herbicide application for controlling oriental bittersweet (*Celastrus orbiculatus* Thunb.) vines in an Appalachian hardwood forest. M.S., West Virginia University, Ann Arbor.

MacBean, C. 2010. Aminopyralid (150114-71-9) The e-Pesticide Manual. 15th edition. British Crop Protection Council, Surrey UK.

MacDiarmid, B. N. 1975. Soil residues of picloram applied aerially to New Zealand brushweeds. New Zealand Plant Protection **28**:109-114.

MacDougall, A. S., and R. Turkington. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology **86**:42-55.

Mangiafico, S. 2022. rcompanion: Functions to support extension education program evaluation. https://CRAN.R-project.org/package=rcompanion.

Mao, R., T. L. T. Nguyen, O. O. Osunkoya, and S. W. Adkins. 2019. Spread pathways of the invasive weed *Parthenium hysterophorus* L.: The potential for water dispersal. Austral Ecology **44**:1111-1122.

Maremmani, A., S. Bedini, I. Matoševic, P. E. Tomei, and M. Giovannetti. 2003. Type of mycorrhizal associations in two coastal nature reserves of the Mediterranean basin. Mycorrhiza **13**:33-40.

Marshall, C. 1990. Source-sink relations of interconnected ramets. Pages 23-41 *in* J. Groenendael and H. de Kroon, editors. Clonal growth in plants: Regulation and function. SPB Academic Publishing, Hague.

Mašková, T., S. S. Phartyal, M. Abedi, M. Bartelheimer, and P. Poschlod. 2022. Soil moisture level and substrate type determine long-term seed lifespan in a soil seed bank. Plant and Soil **477**:475-485.

Mason, R. A. B., J. Cooke, A. T. Moles, and M. R. Leishman. 2008. Reproductive output of invasive versus native plants. Global Ecology and Biogeography **17**:633-640.

Mazerolle, M. J. 2020. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). https://cran.r-project.org/package=AICcmodavg.

McElreath, R. 2015. Statistical rethinking: A Bayesian course with examples in R and Stan. Chapman and Hall/CRC.

Meunier, F., G. M. F. van der Heijden, S. A. Schnitzer, H. P. T. De Deurwaerder, and H. Verbeeck. 2021. Lianas significantly reduce aboveground and belowground carbon storage: A virtual removal experiment. Frontiers in Forests and Global Change **4**:75.

Miller, T. W. 2016. Integrated strategies for management of perennial weeds. Invasive Plant Science and Management **9**:148-158.

Mitchell, C. 2019. Genetic patterns of the invasive vine *Clematis vitalba* (old man's beard) in New Zealand. LC3498, Manaaki Whenua Landcare Research, Horizons Regional Council.

Mitchell, C. 2020. Potential origins of the invasive vine *Clematis vitalba* (old man's beard) in New Zealand. LC3674, Manaaki Whenua Landcare Research, Horizons Regional Council.

Mitchell, J., I. G. Johnston, and G. W. Bassel. 2016. Variability in seeds: biological, ecological, and agricultural implications. Journal of Experimental Botany **68**:809-817.

Möllerová, J. 2005. Notes on invasive and expansive trees and shrubs. Journal of Forest Science **51**:19-23.

Monaco, T. J., S. C. Weller, and F. M. Ashton. 2002. Weed Science: Principles and Practices. 4th edition. John Wiley & Sons, Incorporated, New York.

Moore, J. H., B. Rayner, and B. Vukelik. 2010. Movement of triclopyr, picloram and aminopyralid down a river after blackberry spraying. Pages 315-317 *in* 17th Australasian weeds conference. New Zealand Plant Protection Society, Christchurch, New Zealand.

Moore, R. H. S. 1971. Poisoning by old man's beard (*Clematis vitalba*) Veterinary Record **89**:569-570.

Moravcová, L., A. Carta, P. Pyšek, H. Skálová, and M. Gioria. 2022. Long-term seed burial reveals differences in the seed-banking strategies of naturalized and invasive alien herbs. Scientific Reports **12**:8859.

Mori, H., T. Masaki, Y. Tsunamoto, and S. Naoe. 2020. Survival rate and environmental response of current-year seedlings of the temperate liana *Wisteria floribunda* across a heterogeneous environment. Journal of Plant Research **133**:193-203.

Mori, H., S. Ueno, T. Kamijo, Y. Tsumura, and T. Masaki. 2021. Interspecific variation in clonality in temperate lianas revealed by genetic analysis: Do clonal proliferation processes differ among lianas? Plant Species Biology **36**:4.

Mori, H., S. Ueno, A. Matsumoto, T. Kamijo, Y. Tsumura, and T. Masaki. 2018. Large contribution of clonal reproduction to the distribution of deciduous liana species (*Wisteria floribunda*) in

an old-growth cool temperate forest: Evidence from genetic analysis. Annals of Botany **121**:359-365.

Munné-Bosch, S. 2015. Senescence: Is it universal or not? Trends in Plant Science **20**:713-720.

Naithani, S., H. Nonogaki, and P. Jaiswal. 2017. Exploring crossroads between seed development and stress response. Pages 415-454 *in* G. K. Pandey, editor. Mechanism of Plant Hormone Signaling under Stress. John Wiley & Sons, Hoboken, N.J.

Nathan, R., and G. G. Katul. 2005. Foliage shedding in deciduous forests lifts up long-distance seed dispersal by wind. PNAS **102**:8251.

Nelson, L. R., A. W. Ezell, and J. L. Yeiser. 2006. Imazapyr and triclopyr tank mixtures for basal bark control of woody brush in the southeastern United States. New Forests **31**:173-183.

Newbery, D. M., and C. Zahnd. 2021. Change in liana density over 30 years in a Bornean rain forest supports the escape hypothesis. Ecosphere **12**:e03537.

Newton, M., F. Roberts, A. Allen, B. Kelpsas, D. White, P. Boyd, and 1990. Deposition and dissipation of three herbicides in foliage, litter and soil of brushfields of Southwest Oregon. Journal of Agriculture and Food Chemistry **38**: 574-583.

Nikolaeva, M. G., M. V. Razumova, and V. N. Gladkova. 1985. Reference book on dormant seed germination. Nauka, Leningrad, USSR.

Nikoloff, M. H. 2011. Seed and microsite limitation in *Clematis vitalba* on Banks Peninsula. M.Sc., University of Canterbury, Christchurch, New Zealand.

Nyboer, R. 1992. Vegetation management guideline: Japenese honeysuckle (*Lonicera japonica* Thunb.). Natural Areas Journal **12**:217-218.

NZ Ministry of Forestry, and NZ Forest Research Institute. 1996. Establishing a small forest. Small Forest Management Series Vol.5. N.Z. Ministry of Forestry and the N.Z. Forestry Research Institute Ltd.

Oborny, B., and S. Bartha. 1995. Clonality in plant communities - an overview. Abstracta Botanica **19**:115-127.

Ogle, C. C., G. D. L. Cock, G. Arnold, and N. Mickleson. 2000. Impact of an exotic vine *Clematis vitalba* (F. Ranunculaceae) and of control measures on plant biodiversity in indigenous forest, Taihape, New Zealand. Austral Ecology **25**:539-551.

Ogle, D. H., J. C. Doll, P. Wheeler, and A. Dinno. 2022. FSA: Fisheries stock analysis. https://github.com/fishR-Core-Team/FSA.

Ott, J. P., J. Klimešová, and D. C. Hartnett. 2019. The ecology and significance of below-ground bud banks in plants. Annals of Botany **123**:1099-1118.

Paliwal, K., M. Kuppers, and H. Schneider. 1994. Leaf gas-exchange in lightflecks of plants of different successional range in the under-story of a central-european beech forest. Current Science **67**:29-34.

Pasquini, S. C., S. J. Wright, and L. S. Santiago. 2015. Lianas always outperform tree seedlings regardless of soil nutrients: results from a long-term fertilization experiment. Ecology **96**:1866-1876.

Pedersen, T. L. 2022. patchwork: The composer of plots. https://CRAN.R-project.org/package=patchwork.

Peltzer, D. A., and M. Köchy. 2001. Competitive effects of grasses and woody plants in mixed-grass prairie. Journal of Ecology **89**:519-527.

Penalosa, J. 1984. Basal branching and vegetative spread in two tropical rain forest lianas. Biotropica **16**:1-9.

Peterson, R. A. 2021. Finding optimal normalizing transformations via bestNormalize. The R Journal **13**:310-329.

Peterson, R. A., and J. E. Cavanaugh. 2020. Ordered quantile normalization: A semiparametric transformation built for the cross-validation era. Journal of Applied Statistics **47**:2312-2327.

Picciau, R., M. Porceddu, and G. Bacchetta. 2017. Can alternating temperature, moist chilling, and gibberellin interchangeably promote the completion of germination in *Clematis vitalba* seeds? Botany **95**:847-852.

Plavcová, L., F. Gallenmüller, H. Morris, M. Khatamirad, S. Jansen, and T. Speck. 2019. Mechanical properties and structure–function trade-offs in secondary xylem of young roots and stems. Journal of Experimental Botany **70**:3679-3691.

Plavcová, L., G. Hoch, H. Morris, S. Ghiasi, and S. Jansen. 2016. The amount of parenchyma and living fibers affects storage of nonstructural carbohydrates in young stems and roots of temperate trees. American Journal of Botany **103**:603-612.

Porter, J. F. 1979. Development and use of herbicide spot-gun applicator. Pages 211-214 *in* 32nd NZ Weed and Pest Control Conference.

Prosperi, J., G. Caballe, and Y. Caraglio. 2001. Lianas and Hemiepiphytes: Distribution, Development, and Adaptations. Selbyana **22**:197-212.

Putz, F. E. 1984. The natural history of lianas on Barro Colorado Island, Panama. Ecology **65**:1713-1724.

Pyšek, P., and D. M. Richardson. 2007. Traits associated with invasiveness in alien plants: Where do we stand? Pages 97-125 *in* W. Nentwig, editor. Biological Invasions. Springer Berlin Heidelberg, Berlin, Heidelberg.

R Core Team. 2021. R: A language and environment for statistical computing. http://www.R-project.org/.

Raal, P., and S. Timmins. 2018. Woody weed control using bark application methods--Guideline. DOCDM-1364016. New Zealand Department of Conservation.

Rao, P.S.C., A. G. Hornsby, and R. E. Jessup. 1985. Indices for ranking the potential for pesticide contamination of groundwater. Pages 1-8 in Proceedings of the Soil and Crop Science Society of Florida, University of Florida, Gainesville, FL.

Ray, D. M., and J. A. Savage. 2021. Seasonal changes in temperate woody plant phloem anatomy and physiology: implications for long-distance transport. AoB PLANTS **13**:plab028.

Redmond, C. M., and J. C. Stout. 2018. Breeding system and pollination ecology of a potentially invasive alien Clematis vitalba L. in Ireland. Journal of Plant Ecology **11**:56-63.

Reeves, P., M. Meleason, and F. Matheson. 2006. Sustainable riparian plantings in urban and rural landscapes. National Institute of Water and Atmospheric Research (NIWA). https://niwa.co.nz/publications/water-and-atmosphere/vol14-no1-march-2006/sustainable-riparian-plantings-in-urban-and-rural-landscapes.

Renker, C., V. Blanke, and F. Buscot. 2005. Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. Environmental Pollution **135**:255-266.

Richardson, D. M., P. M. Holmes, K. J. Esler, S. M. Galatowitsch, J. C. Stromberg, S. P. Kirkman, P. Pyšek, and R. J. Hobbs. 2007. Riparian vegetation: Degradation, alien plant invasions, and restoration prospects. Diversity and Distributions **13**:126-139.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. Dose-Response Analysis Using R. PLOS ONE **10**:e0146021.

Roberts, E. H. 1972. Dormancy: A factor affecting seed survival in the soil. Pages 321-359 *in* E. H. Roberts, editor. Viability of Seeds. Springer Netherlands, Dordrecht.

Robinson, D., A. Hayes, and S. Couch. 2022. broom: Convert statistical objects into tidy tibbles. https://CRAN.R-project.org/package=broom.

Roiloa, S. R., P. Sánchez-Rodríguez, and R. Retuerto. 2014. Heterogeneous distribution of soil nutrients increase intraspecific competition in the clonal plant *Glechoma hederacea*. Plant Ecology **215**:863-873.

Rolando, C., S. Gous, and M. Watt. 2011. Preliminary screening of herbicide mixes for the control of five major weed species on certified Pinus radiata plantations in New Zealand. New Zealand Journal of Forestry Science **41**:165-175.

Rolando, C., C. Todoroki, and M. Watt. 2015. Minimising the environmental impact of forest weed management in New Zealand. S0015, New Zealand Forest Research Institute Ltd.

Roovers, P., B. Bossuyt, B. Igodt, and M. J. P. E. Hermy. 2006. May seed banks contribute to vegetation restoration on paths in temperate deciduous forest? Plant Ecology **187**:25-38.

Royal Botanic Gardens, Kew. *Clematis* L. Plants of the World Online. https://powo.science.kew.org/results?q=Clematis%20L.

Rudolf, P. O. 1974. *Clematis* L. Pages 331-334 *in* C. S. Schopmeyer, editor. Seeds of Woody Plants in the United States. United States Department of Agriculture.

Rutter, N. 1965. Tattering of Flags under Controlled Conditions. Nature **205**:168-169.

Saatkamp, A., L. Affre, T. Dutoit, and P. Poschlod. 2009. The seed bank longevity index revisited: limited reliability evident from a burial experiment and database analyses. Annals of Botany **104**:715-724.

Saitoh, T., K. Seiwa, and A. Nishiwaki. 2002. Importance of physiological integration of dwarf bamboo to persistence in forest understorey: A field experiment. Journal of Ecology **90**:78-85.

Saitoh, T., K. Seiwa, and A. Y. A. Nishiwaki. 2006. Effects of resource heterogeneity on nitrogen translocation within clonal fragments of *Sasa palmata*: An isotopic (15n) assessment. Annals of Botany **98**:657-663.

Sakai, A., H. Nomiya, and W. Suzuki. 2002. Horizontal distribution of stolons of a temperate liana *Wisteria floribunda* DC. and its ecological significance. Journal of Forest Research **7**:125-130.

Salisbury, E. J. 1920. Variation in *Anemone apennina* L., and *Clematis vitalba* L., with special reference to trimery and abortion. Annals of Botany **os-34**:107-116.

Säumel, I., and I. Kowarik. 2010. Urban rivers as dispersal corridors for primarily wind-dispersed invasive tree species. Landscape and Urban Planning **94**:244-249.

Schafer, D. E., and D. O. Chilcote. 1970. Factors Influencing Persistence and Depletion in Buried Seed Populations. II. The Effects of Soil Temperature and Moisture. Crop Science **10**:342-345.

Schafer, M., and P. M. Kotanen. 2003. The influence of soil moisture on losses of buried seeds to fungi. Acta Oecologica **24**:255-263.

Schnitzer, S. A., and F. Bongers. 2011. Increasing liana abundance and biomass in tropical forests: Emerging patterns and putative mechanisms. Ecology Letters **14**:397-406.

Schnitzer, S. A., J. W. Dalling, and W. P. Carson. 2000. The impact of lianas on tree regeneration in tropical forest canopy gaps: Evidence for an alternative pathway of gap-phase regeneration. Journal of Ecology **88**:655-666.

Schnitzer, S. A., M. E. Kuzee, and F. Bongers. 2005. Disentangling above- and below-ground competition between lianas and trees in a tropical forest. Journal of Ecology **93**:1115-1125.

Schnitzer, S. A., S. A. Mangan, J. W. Dalling, C. A. Baldeck, S. P. Hubbell, A. Ledo, H. Muller-Landau, M. F. Tobin, S. Aguilar, D. Brassfield, A. Hernandez, S. Lao, R. Perez, O. Valdes, and S. R. Yorke. 2012. Liana abundance, diversity, and distribution on Barro Colorado Island, Panama. PLOS ONE **7**:e52114.

Schnitzer, S. A., S. A. Mangan, and S. P. Hubbell. 2015a. The lianas of Barro Colorado Island, Panama. Pages 76-90 *in* S. A. Schnitzer, F. Bongers, R. J. Burnham, and F. E. Putz, editors. Ecology of Lianas. Wiley Blackwell, West Sussex, UK.

Schnitzer, S. A., M. P. E. Parren, and F. Bongers. 2004. Recruitment of lianas into logging gaps and the effects of pre-harvest climber cutting in a lowland forest in Cameroon. Forest Ecology and Management **190**:87-98.

Schnitzer, S. A., F. E. Putz, F. Bongers, and K. Kroening. 2015b. The past, present, and potential future of liana ecology. Pages 3-12 *in* S. A. Schnitzer, F. Bongers, R. J. Burnham, and F. E. Putz, editors. Ecology of Lianas. John Wiley & Sons, Ltd., West Sussex, U.K.

Schoen, D. J., and S. T. Schultz. 2019. Somatic Mutation and Evolution in Plants. Annual Review of Ecology, Evolution, and Systematics **50**:49-73.

Schupp, E. W. 2011. Dispersal ability, plant *in* D. Simberloff and M. Rejmánek, editors. Encyclopedia of biological invasions. University of California Press, Ltd., London.

Schurko, A. M., M. Neiman, and J. M. Logsdon. 2009. Signs of sex: What we know and how we know it. Trends in Ecology & Evolution **24**:208-217.

Simberloff, D. 2008. We can eliminate invasions or live with them. Successful management projects. Pages 149-157 Ecological impacts of non-native invertebrates and fungi on terrestrial ecosystems. Springer, Dordrecht.

Simberloff, D. 2009. The role of propagule pressure in biological invasions. Annual Review of Ecology, Evolution, and Systematics **40**:81-102.

Simberloff, D. 2013. Invasive species: What everyone needs to know. Oxford University Press.

Sitte, P. 1973. Zellwand-Feinbau bei Flughaaren der Clematis - Frucht. Berichte der Deutschen Botanischen Gesellschaft **86**:551-561.

Smale, M. C., M. B. Dodd, B. R. Burns, and I. L. Power. 2008. Long-term impacts of grazing on indigenous forest remnants on North Island hill country, New Zealand. New Zealand Journal of Ecology **32**:57-66.

Smith, A. E., D. Aite, R. Grover, L. A. Kerr, L. J. Milward, and H. Sommerstad. 1988. Persistence and movement of picloram in a northern Saskatchewan watershed. Journal of Environmental Quality **17**: 262-268.

Smith, D. 1982. *Clematis vitalba*--a paper on past control. Pages 47-49 *in* The Clematis vitalba threat: Distribution and control of the introduced weed old man's beard (*Clematis vitalba*). Department of Lands and Survey, Wellington, NZ.

Smith, L. 2016. What's happening with old man's beard? Manaaki Whenua Weed Biocontrol Newsletter, Vol. 76. Landcare Research, Lincoln, NZ.

Stella, J. C., P. M. Rodríguez-González, S. Dufour, and J. Bendix. 2013. Riparian vegetation research in Mediterranean-climate regions: common patterns, ecological processes, and considerations for management. Hydrobiologia **719**:291-315.

Stuefer, J. F., B. Erschbamer, H. Huber, and J.-I. Suzuki. 2002. The ecology and evolutionary biology of clonal plants: An introduction to the proceedings of Clone-2000. Pages 1-8 *in* J. F. Stuefer,

B. Erschbamer, H. Huber, and J. I. Suzuki, editors. Proceedings of Clone-2000, an International Workshop held in Obergurgl, Austria, 20–25 August 2000. Springer Netherlands, Dordrecht.

Sun, H., H. Cui, J. Zhang, J. Kang, Z. Wang, M. Li, F. Yi, Q. Yang, and R. Long. 2021. Gibberellins inhibit flavonoid biosynthesis and promote nitrogen metabolism in *Medicago truncatula*. International Journal of Molecular Sciences **22**:9291.

Syrett, P. 2002. Biological control of weeds on conservation land: Priorities for the Department of Conservation. DOC Science Internal Series, Vol. 82. New Zealand Department of Conservation, Wellington.

Tackenberg, O., P. Poschlod, and S. Bonn. 2003. Assessment of wind dispersal potential in plant species. Ecological Monographs **73**:191-205.

Tamura, M. 1993. Ranunculaceae. Pages 563-583 *in* K. Kubitzki, J. G. Rohwer, and V. Bittrich, editors. Flowering Plants · Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families. Springer Berlin Heidelberg, Berlin, Heidelberg.

Taylor, A. A., J. De-Felice, and D. C. Havill. 1982. Seasonal variation in nitrogen availability and utilization in an acidic and calcareous soil. New Phytologist **92**:141-152.

Thompson, D. G., L. M. MacDonald, and B. Staznik. 1992. Persistence of hexazinone and metsulfuron-methyl in a mixed-wood/boreal forest lake. Journal of Agricultural and Food Chemistry **40**:1444-1449.

Thompson, K., J. P. Grime, and G. Mason. 1977. Seed germination in response to diurnal fluctuations of temperature. Nature **267**:147-149.

Thompson, K., and M. K. J. Ooi. 2010. To germinate or not to germinate: More than just a question of dormancy. Seed Science Research **20**:209-211.

Thomson, F. J., A. T. Moles, T. D. Auld, and R. T. Kingsford. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. Journal of Ecology **99**:1299-1307.

Tichý, L. 2019. GLAMA – gap light analysis mobile application. https://play.google.com/store/apps/details?id=new4.glama.glamanew4.

Toledo-Aceves, T., and M. D. Swaine. 2008. Above- and below-ground competition between the liana *Acacia kamerunensis* and tree seedlings in contrasting light environments. Plant Ecology **196**:233-244.

Trabue, S. L., D. E. Palmquist, T. M. Lydick, and S. K. Singles. 2006. Effects of soil storage on the microbial community and degradation of metsulfuron-methyl. Journal of Agricultural and Food Chemistry **54**:142-151.

Tran, H. 2013. The ecology and integrated management of broom (*Cytisus scoparius*) in New Zealand plantation forests. PhD, Massey University, Manawatu, New Zealand.

Tran, H., K. C. Harrington, H. Ghanizadeh, A. W. Robertson, and M. S. Watt. 2018. Suppression by three grass species of broom seedling emergence and survival. New Zealand Plant Protection **71**:57-65.

Tran, H., K. C. Harrington, A. W. Robertson, and M. S. Watt. 2015. Relative persistence of commonly used forestry herbicides for preventing the establishment of broom (*Cytisus scoparius*) seedlings in New Zealand plantations. New Zealand Journal of Forestry Science **45**:6.

Tran, H., K. C. Harrington, A. W. Robertson, and M. S. Watt. 2016. Assessment of herbicides for selectively controlling broom (*Cytisus scoparius*) growing with radiata pine (*Pinus radiata*) in New Zealand. New Zealand Journal of Forestry Science **46**:13.

Truscott, A.-M., C. Soulsby, S. C. F. Palmer, l. Newell, and P. E. Hulme. 2006. The dispersal characteristics of the invasive plant *Mimulus guttatus* and the ecological significance of increased occurrence of high-flow events. Journal of Ecology **94**:1080-1091.

Tu, M., C. Hurd, and J. M. Randall. 2001. Weed control methods handbook: Tools & techniques for use in natural areas. The Nature Conservancy. http://tncweeds.ucdavis.edu.

Tucker, A. R. 1979. The ecology of *Clematis vitalba*. Investigation in consequence of a research grant for the Department of Lands and Survey. File 22/260/14. N. Z. Dept. Land and Survey.

Tutin, T. G., V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters, and D. A. Webb. 1964. Flora Europaea. Cambridge University Press, Cambridge, UK.

USDA-ARS. Metsulfuron ME (74223-64-6). Agricultural Research Service. https://www.ars.usda.gov/ARSUserFiles/00000000/DatabaseFiles/PesticidePropertiesDatabase/IndividualPesticideFiles/METSULFURON_ME.TXT.

USDA-ARS. 2003. National Plant Germplasm System. Agricultural Research Service, Germplasm Resources Information Network (GRIN), Beltsville, Maryland, USA.

USDA-NRCS. 2006. The PLANTS Database. National Plant Data Center, Baton Rouge, LA, 70874-4490 USA.

van den Broek, T., R. van Diggelen, and R. Bobbink. 2005. Variation in seed buoyancy of species in wetland ecosystems with different flooding dynamics. Journal of Vegetation Science **16**:579-586.

van der Pijl, L. 1982. Principles of dispersal in higher plants. 3rd edition. Springer-Verlag, Berlin Heidelberg New York.

Van Gardingen, J. R. 1986. The physiological ecology of *Clematis vitalba* L. M.Sc., University of Canterbury, Christchurch, New Zealand.

Van Mourik, T. A., T. J. Stomph, and A. J. Murdoch. 2005. Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks. Journal of Applied Ecology **42**:299-305.

Vandelook, F., N. Bolle, and J. A. Van Assche. 2008. Seasonal dormancy cycles in the biennial *Torilis japonica* (Apiaceae), a species with morphophysiological dormancy. Seed Science Research **18**:161-171.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Fourth edition. Springer.

Vencill, W. K., ed. . 2002. Herbicide Handbook. 8th edition. Weed Science Society of America, Lawrence, KS.

Vesipa, R., C. Camporeale, and L. Ridolfi. 2017. Effect of river flow fluctuations on riparian vegetation dynamics: Processes and models. Advances in Water Resources **110**:29-50.

Vilà, M., and J. Weiner. 2004. Are invasive plant species better competitors than native plant species? – Evidence from pair-wise experiments. Oikos **105**:229-238.

Vinkler, I., C. Muller, and A. Gama. 2004. Evergreen clematis (*Clematis vitalba* L.) germination and potential for preventive control in forests. Revue Forestiere Francaise **56**:275-286.

Vivian-Smith, G., and F. D. Panetta. 2005. Seedling recruitment, seed persistence and aspects of dispersal ecology of the invasive moth vine, *Araujia sericifera* (Asclepiadaceae). Australian Journal of Botany **53**:225-230.

Vleeshouwers, L. M., H. J. Bouwmeester, and C. M. Karssen. 1995. Redefining seed dormancy: An attempt to integrate physiology and ecology. Journal of Ecology **83**:1031-1037.

Vondras, A. M., A. Minio, B. Blanco-Ulate, R. Figueroa-Balderas, M. A. Penn, Y. Zhou, D. Seymour, Y. Zhou, D. Liang, L. K. Espinoza, M. M. Anderson, M. A. Walker, B. Gaut, and D. Cantu. 2019. The genomic diversification of clonally propagated grapevines. bioRxiv:585869.

Wang, Y., Q. Yao, Y. Zhang, Y. Zhang, J. Xing, B. Yang, G. Mi, Z. Li, and M. Zhang. 2020. The role of gibberellins in regulation of nitrogen uptake and physiological traits in maize responding to nitrogen availability. International Journal of Molecular Sciences **21**:1824.

Ward, B. G., and R. F. Henzell. 2000. Herbicide gels for controlling old man's beard (*Clematis vitalba*) in ecologically sensitive areas. Pages 284-288 *in* S. M. Zydenbos, editor. New Zealand Plant Protection Volume 53. Proceedings of a conference, Christchurch, New Zealand. New Zealand Plant Protection Society, Rotorua, New Zealand.

Ward, B. G., and R. F. Henzell. 2003. A novel herbicidal gel technique for controlling the vine *Celastrus orbiculatus* (climbing spindleberry). Pages 331-336 *in* L. Child, J. H. Brock, G. Brundu, K. Prach, K. Pysek, P. M. Wade, and M. Williamson, editors. Plant invasions: ecological threats and management solutions. Backhuys Publishers, Leiden, Netherlands.

Ward, B. G., and R. F. Henzell. 2004. Use of herbicidal gels on woody weeds. DOC Science Internal Series Vol. 162. Science & Research Unit, Department of Conservation Science Publishing, Wellington.

Ward, B. G., R. F. Henzell, P. T. Holland, and A. G. Spiers. 1999. Non-spray methods to control invasive weeds in urban areas. Pages 1-5 *in* Proceedings 52nd New Zealand Plant Protection Conference.

Warr, S. J., M. Kent, and K. Thompson. 1994. Seed bank composition and variability in five woodlands in South-West England. Journal of Biogeography **21**:151-168.

Webb, H. J., and K. C. Harrington. 2005. Control strategies for Madeira vine (*Anredera cordifolia*). New Zealand Plant Protection **58**:169-173.

Webster, C. R., M. A. Jenkins, and S. Jose. 2006. Woody invaders and the challenges they pose to forest ecosystems in the Eastern United States. Journal of Forestry **104**:366-374.

West, C. J. 1991. Literature review of the biology of *Clematis vitalba* (old man's beard). New Zealand Department of Scientific and Industrial Research (DSIR) Land Resources Vegetation Report **725**:1-37.

West, C. J. 1992. Ecological studies of *Clematis vitalba* (old man's beard) in New Zealand. Pages 1-151 Land Resources Vegetation Report. New Zealand Department of Science and Industrial Research (DSIR). Land Resources Vegetation Report **736**:1-151.

West, G. G., and M. G. Dean. 1998. Forest oversowing and grazing trial--Progress to June 1997. Report 93. New Zealand Forestry Research Institute Ltd.

Whitwell, T., J. A. Briggs, and M. M. Cousins. 2016. Control of beach vitex (*Vitex rotundifolia)* with foliage and cut stem herbicide applications. Journal of Environmental Horticulture **34**:1-6.

Wickham et al. 2019. Welcome to the tidyverse. Journal of Open Source Software **4**:1686.

Wickham, H. 2007. Reshaping data with the reshape package. Journal of Statistical Software **21**:1-20.

Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Wickham, H., R. Francois, L. Henry, and K. Mueller. 2022. dplyr: A Grammar of Data Manipulation. https://dplyr.tidyverse.org.

Willems, J. H., and K. G. A. Huijsmans. 1994. Vertical seed dispersal by earthworms: A quantitative approach. Ecography **17**:124-130.

Williams, P. A. 2009. Biological summaries of bindweeds (Calystegia) and other climbers. Contract Report LC0809/089. Landcare Research New Zealand Limited, Nelson, NZ.

Willis, C. G., C. C. Baskin, J. M. Baskin, J. R. Auld, D. L. Venable, J. Cavender-Bares, K. Donohue, and R. Rubio de Casas. 2014. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytologist **203**:300-309.

Wilson, J. B., J. B. Steel, and S.-L. K. Steel. 2007. Do plants ever compete for space? Folia Geobotanica **42**:431-436.

Wright, S. J., A. Trakhtenbrot, G. Bohrer, M. Detto, G. G. Katul, N. Horvitz, H. C. Muller-Landau, F. A. Jones, and R. Nathan. 2008. Understanding strategies for seed dispersal by wind under contrasting atmospheric conditions. PNAS **105**:19084.

WSSA. 1994. Herbicide handbook. Weed Science Society of America, Champaign, Illinois.

Wyka, T. P., M. Zadworny, J. Mucha, R. Żytkowiak, K. Nowak, and J. Oleksyn. 2019. Species-specific responses of growth and biomass distribution to trellis availability in three temperate lianas. Trees **33**:921-932.

Yorke, S. R., S. A. Schnitzer, J. Mascaro, S. G. Letcher, and W. P. Carson. 2013. Increasing liana abundance and basal area in a tropical forest: The contribution of long-distance clonal colonization. Biotropica **45**:317-324.

Yurkonis, K. A., and S. J. Meiners. 2004. Invasion impacts local species turnover in a successional system. Ecology Letters 7:764-769.

Zeileis, A., and T. Hothorn. 2002. Diagnostic checking in regression relationships. R News **2**:7-10.

Zhang, H., C. Jiang, R. Wang, L. Zhang, R. Gai, S. Peng, Y. Zhang, C. Mao, Y. Lou, J. Mo, S. Feng, and F. Ming. 2021. Insights into heat response mechanisms in clematis species: physiological analysis, expression profiles and function verification. Plant Molecular Biology **106**:569-587.

Zhang, J., S. H. Allan, and E. W. Susan. 1995. Antagonism and synergism between herbicides: Trends from previous studies. Weed Technology **9**:86-90.

Zhang, Y., and D. Zhang. 2007. Asexual and sexual reproductive strategies in clonal plants. Frontiers of Biology in China **2**:256-262.

Appendices

Appendix A Chapter 3 supplementary material

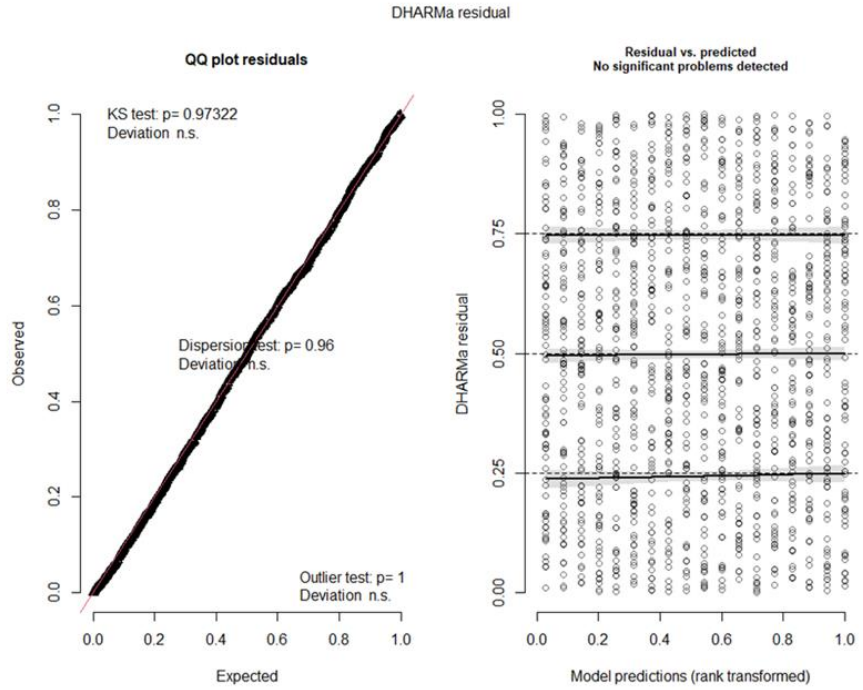

Figure A.1: Binomial logistic regression goodness of fit plots for *Clematis vitalba* seed germinability following incubation in various treatment combinations.

Table A.1: Meta-analysis model summary of parameter estimates (b = steepness of germination curve (log odds); d = maximum germination (proportion); t50 = time to 50% maximum germination (days) for temperature/treatment combinations of interest (15C = constant light at constant 15°C; 2WK = constant light at constant 20°C after 2 weeks prechilling at 5°C; 4WK = constant light at constant 20°C after 4 weeks prechilling at 5°C; 20/30C = alternating dark (16h) and light (8h) with temperatures alternating between 20°C and 30°C; Control = no treatment other than moisture; GA3 = gibberellic acid; KNO3 = potassium nitrate; KNGA = gibberellic acid and potassium nitrate; Scar = scarification; S = intact style; NS = style removed) with a significant difference between germination of *Clematis vitalba* seeds with an intact style and those without in July 2019 and June 2020.

	2019						2020					
	b est (SE)	p-val	d est (SE)	p-val	t50 est (SE)	p-val	b est (SE)	p-val	d est (SE)	p-val	t50 est (SE)	p-val
15C.Scar.S	NA	NA	NA	NA	NA	NA	-10.69 (2.33)	<.001	0.11 (0.67)	.865	21.64 (1.55)	<.001
15C.Scar.NS	NA	NA	NA	NA	NA	NA	-8.93 (1.44)	<.001	0.40 (0.67)	.551	20.81 (1.28)	<.001
15C.KNO3.S	NA	NA	NA	NA	NA	NA	-11.50 (1.85)	<.001	0.38 (0.67)	.57	20.86 (1.26)	<.001
15C.KNO3.NS	NA	NA	NA	NA	NA	NA	-8.67 (1.37)	<.001	0.49 (0.67)	.465	19.12 (1.28)	<.001
15C.GA3.S	-7.62 (0.88)	<.001	0.91 (0.37)	.012	14.70 (0.50)	<.001	-8.73 (2.46)	<.001	0.10 (0.67)	.882	17.41 (1.59)	<.001
15C.GA3.NS	-5.47 (0.84)	<.001	0.69 (0.37)	.058	14.71 (0.79)	<.001	-4.21 (1.00)	<.001	0.29 (0.67)	.664	17.34 (1.36)	<.001
15C.KNGA.S	-8.60 (1.03)	<.001	0.90 (0.36)	.013	15.56 (0.50)	<.001	-6.20 (1.12)	<.001	0.54 (0.67)	.418	19.98 (1.53)	<.001
15C.KNGA.NS	-4.99 (0.76)	<.001	0.69 (0.37)	.059	16.69 (0.92)	<.001	-5.10 (1.04)	<.001	0.57 (0.67)	.391	16.91 (1.33)	<.001
20/30C.Control.S	-6.33 (1.00)	<.001	0.80 (0.37)	.031	19.50 (0.98)	<.001	-6.91 (1.23)	<.001	0.48 (0.67)	.474	18.53 (1.31)	<.001
20/30C.Control.NS	-10.28 (2.79)	<.001	0.19 (0.37)	.601	23.01 (0.88)	<.001	-6.47 (1.09)	<.001	0.70 (0.67)	.295	16.43 (1.27)	<.001
20/30C.Scar.S	-7.77 (1.01)	<.001	0.83 (0.36)	.023	15.90 (0.57)	<.001	-8.126 (1.08)	<.001	0.69 (0.67)	.304	16.99 (1.28)	<.001
20/30C.Scar.NS	-7.37 (1.22)	<.001	0.50 (0.37)	.175	16.39 (0.71)	<.001	-4.70 (0.97)	<.001	0.76 (0.67)	.251	13.08 (1.24)	<.001
20/30C.KNO3.S	-7.48 (0.85)	<.001	0.97 (0.36)	.008	16.22 (0.52)	<.001	-8.44 (1.14)	<.001	0.84 (0.67)	.207	16.02 (1.23)	<.001
20/30C.KNO3.NS	-6.38 (0.91)	<.001	0.62 (0.37)	.095	19.10 (1.10)	<.001	-6.64 (1.04)	<.001	0.86 (0.67)	.198	13.06 (1.23)	<.001
20/30C.GA3.S	-9.39 (1.23)	<.001	0.69 (0.37)	.060	11.33 (0.45)	<.001	-6.52 (1.08)	<.001	0.70 (0.67)	.291	15.82 (1.27)	<.001
20/30C.GA3.NS	-5.53 (0.88)	<.001	0.63 (0.37)	.087	13.43 (0.69)	<.001	-4.94 (0.97)	<.001	0.72 (0.67)	.279	12.88 (1.28)	<.001
20/30C.KNGA.S	-14.68 1.90)	<.001	0.80 (0.36)	.028	11.14 (0.39)	<.001	-7.22 (1.07)	<.001	0.81 (0.67)	.226	12.69 (1.22)	<.001
20/30C KNGA.NS	-6.23 (0.89)	<.001	0.70 (0.37)	.057	12.05 (0.55)	<.001	-6.55 (1.01)	<.001	0.92 (0.67)	.167	11.78 (1.22)	<.001
2WK.Scar.S	-6.67 (1.01)	<.001	0.55 (0.37)	.131	8.45 (0.49)	<.001	-6.30 (3.30)	0.014	0.04 (0.67)	.956	12.49 (1.68)	<.001
2WK.Scar.NS	-5.01 (0.76)	<.001	0.64 (0.37)	.080	8.88 (0.57)	<.001	-3.82 (1.06)	<.001	0.21 (0.67	.758	10.65 (1.49)	<.001
4WK.Scar.S	-4.46 (0.70)	<.001	0.65 (0.37)	.076	6.55 (0.50)	<.001	-3.91 (0.97)	<.001	0.46 (0.67)	.469	6.58 (1.24)	<.001
4WK.Scar.NS	-3.65 (0.61)	<.001	0.68 (0.37)	.062	6.10 (0.54)	<.001	-5.33 (1.08)	<.001	0.36 (0.67)	.591	7.92 (1.25)	<.001

Table A.2: Summary for simultaneous targeted pairwise comparisons (general linear hypothesis S - NS = 0) of fitted parameter estimates (b = steepness of germination curve; d = maximum germination; t50 = time to 50% maximum germination) for temperature/treatment combinations of interest (15C = constant light at constant 15°C; 2WK = constant light at constant 20°C after 2 weeks prechilling at 5°C; 20/30C = alternating dark (16h) and light (8h) with temperatures alternating between 20°C and 30°C; Control = no treatment other than moisture; GA3 = gibberellic acid; KNO3 = potassium nitrate; KNGA = gibberellic acid and potassium nitrate; Scar = scarification; S = intact style; NS = style removed) with a significant difference between germination of *Clematis vitalba* seeds with an intact style and those without (glht function in multcomp pkg, R; Hochberg correction used to reduce false discovery rate) in July 2019 and June 2020. Significance codes: < .05*, < .01**, < .001***.

	2019						2020					
	b est (SE)	Pr(>|z|)	d est (SE)	Pr(>|z|)	t50 est (SE)	Pr(>|z|)	b est (SE)	Pr(>|z|)	d est (SE)	Pr(>|z|)	t50 est (SE)	Pr(>|z|)
15C.Scar	NA	NA	NA	NA	NA	NA	-1.76 (2.47)	.699	-0.28 (0.05)	<.001***	0.83 (1.12)	.922
15C.KNO3	NA	NA	NA	NA	NA	NA	-2.83 (1.98)	.699	-0.11, 0.06)	.341	1.73 (0.66)	.0498*
15C.GA3	-2.15 (1.06)	.460	0.22 (0.07)	.036*	-0.01 (0.80)	.987	-4.52 (2.37)	.522	-0.19 (0.06)	.004**	0.07 (1.25)	.958
15C.KNGA	-3.61 (1.14)	.021*	0.21 (0.07)	.046*	-1.13 (0.92)	.219	-1.10 (0.97)	.699	-0.03 (0.07)	.755	3.07 (1.15)	.0498*
20/30C.Control	3.95 (2.91)	.636	0.60 (0.09)	<.001***	-3.52 (1.22)	.004*	-0.44 (1.14)	.699	-0.22 (0.06)	.001 **	2.10 (0.73)	.031*
20/30C.Scar	-0.40 (1.47)	.786	0.33 (0.07)	<.001***	-0.40 (0.77)	.603	-1.60 (0.84)	.522	-0.08 (0.06)	.586	3.91 (0.60)	<.001***
20/30C.KNO3	-1.10 (1.10)	.636	0.35 (0.09)	<.001***	-2.89 (1.11)	.009**	-1.80 (0.98)	.525	-0.02 (0.04)	.754	2.96 (0.46)	<.001***
20/30C.GA3	-3.86 (1.39)	.073	0.06 (0.07)	.892	-2.11 (0.66)	.001**	-1.58 (0.84)	.522	-0.02 (0.06)	.754	2.93 (0.65)	<.001***
20/30C.KNGA	-8.45 (2.01)	<.001***	0.11 (0.07)	.892	-0.91 (0.46)	.051	-0.67 (0.87)	.699	-0.11 (0.04)	.018 *	0.91 (0.39)	.086
2WK.Scar	-1.66 (1.12)	.636	-0.09 (0.08)	.892	-0.43 (0.56)	.448	-4.31 (3.26)	.699	-0.17 (0.05)	.006 **	1.84 (1.49)	.655
4WK.Scar	-0.81 (0.72)	.636	-0.04 (0.07)	.892	0.45 (0.54)	.407	-1.42 (0.84)	.638	-0.11 (0.06)	.341	1.35 (0.54)	.061

Table A.3: Binomial (2019) and multinomial (2020) regression coefficients (G est = estimate for germination, V est = estimate for viable but dormant), odds ratios (OR), confidence intervals (CI lb = lower bound, CI ub = upper bound), and predicted probabilities (prob germ = probability of germination, prob dormant= probability of remaining dormant) for *Clematis vitalba* seed status after 28 days incubation at 15°C (15), alternating temperatures 20/30°C (2030), or 20°C following 2 or 4 weeks prechilling (2WK, 4WK) in five different treatments (C = Control, G = GA_3, K = KNO_3, KG = KNO_3 + GA_3, S = Scarification) . In both cases, the 2WK Control treatment was used as the base reference.

	2019				2020							
	G est (SE)	Pr (>|z|)	OR	CI lb, ub	G est (SE)	Pr (>|z|)	OR	CI lb, ub	V est (SE)	Pr (>|z|)	OR	CI lb, ub
5.Control	NA	NA	NA	NA	0.41 (0.53)	NS	1.51	-0.63, 1.45	0.38 (0.30)	NS	1.46	-0.20, 0.96
15.GA3	5.29 (0.65)	<0.001	198.14	62.16, 817.00	0.63 (0.50)	NS	1.88	-0.34, 1.61	0.20 (0.29)	NS	1.23	-0.36, 0.77
15.KNO3	NA	NA	NA	NA	2.38 (0.46)	<0.001	10.75	1.48, 3.27	0.15 (0.32)	NS	1.16	-0.48, 0.78
15.KNGA	4.54 (0.54)	<0.001	93.86	34.69, 296.75	2.92 (0.47)	<0.001	18.60	2.00, 3.84	0.26 (0.35)	NS	1.29	-0.43, 0.94
15.Scar	0.79 (0.49)	NS	2.21	0.87, 6.15	1.29 (0.46)	<0.001	3.64	0.39, 2.19	0.02 (0.29)	NS	1.02	-0.55, 0.59
2030.Control	3.31 (0.47)	<0.001	27.49	11.61, 74.21	2.34 (0.44)	<0.001	10.40	1.48, 3.21	-0.34 (0.31)	NS	0.71	-0.95, 0.27
2030.GA3	2.96 (0.46)	<0.001	19.37	8.31, 51.45	2.87 (0.44)	<0.001	17.65	2.00, 3.74	-1.02 (0.35)	<0.001	0.36	-1.71, -0.33
2030.KNO3	6.01 (0.82)	<0.001	406.71	100.73, 2839.74	3.85 (0.50)	<0.001	47.14	2.88, 4.83	-0.74 (0.45)	NS	0.48	-1.63, 0.15
2030.KNGA	3.73 (0.48)	<0.001	41.71	17.10, 116.20	3.24 (0.45)	<0.001	25.55	2.36, 4.13	-1.73 (0.44)	<0.001	0.18	-2.60, -0.87
2030.Scar	3.73 (0.48)	<.001	41.71	17.10, 116.20	2.97 (0.46)	<0.001	19.49	2.08, 3.86	-0.37 (0.35)	NS	0.69	-1.05, 0.31
2WK.Control	*reference*											
2WK.GA3	3.08 (0.46)	<0.001	21.66	9.25, 57.78	1.24 (0.47)	<0.001	3.47	0.32, 2.17	0.26 (0.30)	NS	1.30	-0.32, 0.84
2WK.KNO3	2.14 (0.46)	<0.001	8.53	3.68, 22.43	2.48 (0.45)	<0.001	11.88	1.59, 3.37	0.06 (0.32)	NS	1.06	-0.57, 0.69
2WK.KNGA	3.65 (0.48)	<0.001	38.65	15.95, 106.90	2.83 (0.46)	<0.001	16.85	1.92, 3.73	0.06 (0.34)	NS	1.07	-0.60, 0.73
2WK.Scar	2.55 (0.46)	<0.001	12.75	5.51, 33.54	0.08 (0.48)	NS	1.08	-.87, 1.02	-0.57 (0.27)	<0.01	0.56	-1.09, -0.05
4WK.Control	0.61 (0.51)	NS	1.84	0.70, 5.20	0.70 (0.45)	NS	2.01	-0.19, 1.58	-0.56 (0.27)	<0.01	0.57	-1.09, -0.03
4WK.GA3	2.14 (0.46)	<0.001	8.53	3.68, 22.43	1.33 (0.43)	<0.001	3.77	0.48, 2.17	-0.86 (0.28)	<.001	0.42	-1.41, -0.31
4WK.KNO3	3.08 (0.46)	<0.001	21.66	9.25, 57.78	3.66 (0.50)	<0.001	38.85	2.68, 4.64	0.16 (0.41)	NS	1.18	-0.64, 0.96
4WK.KNGA	4.54 (0.54)	<0.001	93.86	34.69, 296.75	3.06 (0.44)	<0.001	21.38	2.19, 3.93	-1.89 (0.43)	<0.001	0.15	-2.73, -1.04
4WK.Scar	2.91 (0.46)	<0.001	18.34	7.88, 48.63	1.27 (0.42)	<0.001	3.57	0.45, 2.10	-1.39 (0.28)	<0.001	0.25	-1.95, -0.84

Table A.4: Meta-analysis model summary of parameter estimates (b = steepness of germination curve (log odds); d = maximum germination (proportion); t50 = time to 50% maximum germination (days) for germination of *Clematis vitalba* seeds in temperature/treatment combinations of interest (4WK = constant light at constant 20°C after 4 weeks prechilling at 5°C; 20/30C = alternating dark (16h) and light (8h) with temperatures alternating between 20°C and 30°C; Control = no treatment other than moisture; GA3 = gibberellic acid; KNO3 = potassium nitrate; KNGA = gibberellic acid and potassium nitrate) in July 2019 and June 2020.

	b est (SE)	p-val	CI lwr, upr	d est (SE)	p-val	CI lwr, upr	t50 est (SE)	p-val	CI lwr, upr
20/30C.Control	-6.98 (0.66)	<.001	-8.27, -5.69	0.59 (0.07)	<.001	0.44, 0.73	18.38 (0.85)	<.001	16.72, 20.05
20/30C.GA3	-7.73 (0.62)	<.001	-8.95, -6.51	0.71 (0.07)	<.001	0.56, 0.85	12.71 (0.75)	<.001	11.25, 14.17
20/30C.KNGA	-8.16 (0.66)	<.001	-9.46, -6.86	0.82 (0.07)	<.001	0.69, 0.96	11.97 (0.72)	<.001	10.56, 13.38
20/30C.KNO3	-7.87 (0.58)	<.001	-9.00, -6.73	0.89 (0.07)	<.001	0.76, 1.03	16.09 (0.75)	<.001	14.63, 17.55
20/30C.Scar	-6.77(0.60)	<.001	-7.94, -5.59	0.74 (0.07)	<.001	0.60, 0.88	16.39 (0.78)	<.001	14.87, 17.91
4WK..GA3	-3.54 (0.49)	<.001	-4.50, -2.58	0.37 (0.07)	<.001	0.22, 0.51	7.10 (0.78)	<.001	5.58, 8.62
4WK.KNGA	-5.93 (0.49)	<.001	-6.89, -4.97	0.87 (0.07)	<.001	0.74, 1.01	6.08 (0.72)	<.001	4.67, 7.49
4WK.KNO3	-7.02 (0.58)	<.001	-8.15, -5.89	0.70 (0.07)	<.001	0.56, 0.84	8.23 (0.73)	<.001	6.80, 9.66
4WK.Scar	-4.80 (0.49)	<.001	-5.76, -3.84	0.50 (0.07)	<.001	0.36, 0.64	7.09 (0.75)	<.001	5.62, 8.56

Table A.5: Summary for simultaneous targeted pairwise comparisons (general linear hypothesis Treatment a – Treatment b = 0) of fitted parameter estimates (b = steepness of germination curve; d = maximum germination; t50 = time to 50% maximum germination) for germination of *Clematis vitalba* seeds in temperature/treatment combinations of interest (20/30C = alternating dark (16h) and light (8h) with temperatures alternating between 20°C and 30°C; 4WK = constant light at constant 20°C after 4 weeks prechilling at 5°C; GA3 = gibberellic acid; KNO3 = potassium nitrate; KNGA = gibberellic acid and potassium nitrate; Scar = scarification; glht function in multcomp pkg, R; Hochberg correction used to reduce false discovery rate) in July 2019 and June 2020. Significance codes: < .05*, < .01**, < .001***.

	b est (SE)	Pr(>|z|)	CI lwr, upr	d est (SE)	Pr(>|z|)	CI lwr, upr	t50 est (SE)	Pr(>|z|)	CI lwr, upr
20/30.KNO3 - 4WK.KNO3	-0.85 (0.75)	.257	-2.32, 0.62	0.20 (0.04)	<.001***	0.12, 0.27	7.86 (0.31)	<.001***	7.25, 8.47
20/30.KNGA - 4WK.KNGA	-2.23 (0.76)	.003**	-3.71, -0.75	-0.05 (0.03)	.139	-0.12, 0.02	5.89 (0.21)	<.001***	5.48, 6.30
20/30.GA3 - 4WK.GA3	-4.19 (0.72)	<.001***	-5.61, -2.78	0.34 (0.04)	<.001***	0.25, 0.43	5.61 (0.41)	<.001***	4.81, 6.41
20/30.Scar - 4WK.Scar	-1.97 (0.70)	.005 **	-3.34, -0.59	0.24 (0.04)	<.001***	0.15, 0.32	9.30 (0.42)	<.001***	8.48, 10.12
4WK.KNO3 - 4WK.KNGA	1.09 (0.68)	.109	-0.24, 2.42	0.18 (0.04)	<.001***	0.10, 0.25	-2.16 (0.24)	<.001***	-2.63, -1.68
4WK.KNGA - 20/30C.KNO3	1.94 (0.68)	.005**	0.60, 3.28	-0.02 (0.03)	.565	-0.08, 0.04	-10.01 (0.29)	<.001***	-10.58, -9.45
4WK.KNO3 - 20/30.KNGA	1.14 (0.81)	.160	-0.45, 2.74	-0.13 (0.04)	.002**	-0.21, -0.05	-3.74 (0.24)	<.001***	-4.22, -3.26

Appendix B Chapter 4 supplementary material

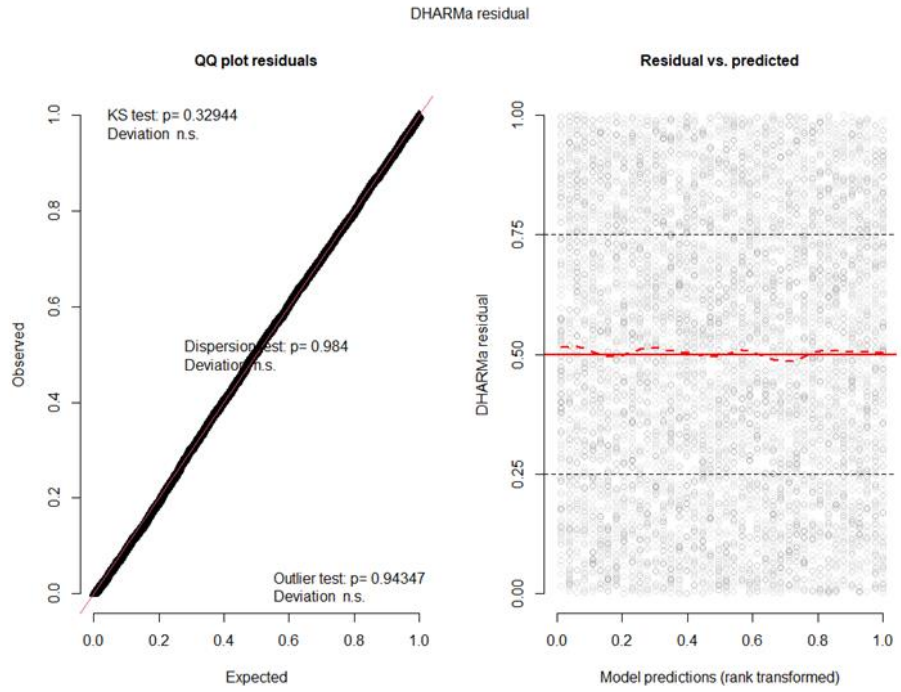

Figure B.1: Binomial logistic regression goodness of fit plots for *Clematis vitalba* seed non-viability following incubation.

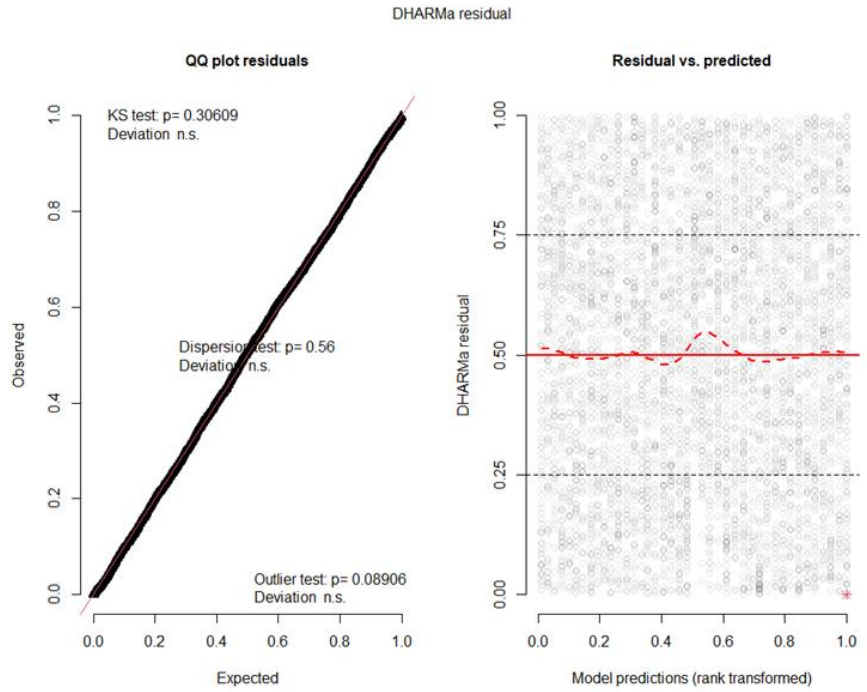

Figure B.2: Binomial logistic regression goodness of fit plots for *Clematis vitalba* seed germinability versus dormancy following incubation.

Table B.1: Binomial logistic regression model summary for *Clematis vitalba* seed viability following collection and incubation on seven separate dates (May.21 reference category) in alternating dark and light (16h:8h) with alternating temperatures (20/30°C). D est = coefficient estimate for seed decay or non-viability; fitted ave = fitted model average proportion decay; CI lb, ub = lower and upper bounds of 95% confidence interval. Three treatments: Dark (reference); Control = blotters moistened with reverse-osmosis water; and KNO3 = blotters moistened with KNO3). Seeds from two separate populations (Hopeland Reserve at Kumeroa Rd, KUM (reference), and Woodville Ferry Reserve, WFR). P-values in bold are significant at α=0.05.

	D est	SE	Pr (>|z|)	fitted	CI lb	CI ub
Intercept	-1.267	0.167	**<0.001**	0.22	0.168	0.28
Treatment						
Control	-0.024	0.214	0.911	0.494	0.391	0.598
KNO3	0.027	0.214	0.897	0.507	0.403	0.61
Population						
WFR	0.357	0.187	0.056	0.588	0.498	0.673
Date						
May.20	-2.059	0.342	**<0.001**	0.113	0.059	0.195
Jun.20	-0.842	0.272	**<0.01**	0.301	0.2	0.421
Jul.21	0.213	0.224	0.341	0.553	0.444	0.658
Sep.19	-1.514	0.325	**<0.001**	0.18	0.102	0.289
Sep.20	-0.923	0.274	**<0.001**	0.284	0.186	0.403
Sep.21	1.186	0.214	**<0.001**	0.766	0.684	0.833
Treatment:Population						
Control:WFR	-0.055	0.185	0.766	0.486	0.397	0.576
KNO3:WFR	0.258	0.193	0.181	0.564	0.47	0.654
Treatment:Date						
Control:May.20	-0.049	0.316	0.875	0.488	0.339	0.639
KNO3:May.20	-0.312	0.313	0.319	0.423	0.284	0.575
Control:Jun.20	-0.059	0.33	0.859	0.485	0.33	0.643
KNO3:Jun.20	-0.982	0.374	**<0.01**	0.273	0.15	0.435
Control:Jul.21	-0.752	0.284	**<0.01**	0.32	0.212	0.451
KNO3:Jul.21	-0.999	0.283	**<0.001**	0.269	0.174	0.39
Control:Sep.19	-0.87	0.431	**<0.05**	0.295	0.148	0.488
KNO3:Sep.19	-0.508	0.375	0.175	0.376	0.222	0.555
Control:Sep.20	-0.433	0.322	0.179	0.393	0.256	0.548
KNO3:Sep.20	-1.445	0.372	**<0.001**	0.191	0.1	0.324
Control:Sep.21	-1.866	0.278	**<0.001**	0.134	0.082	0.21
KNO3:Sep.21	-2.247	0.282	**<0.001**	0.096	0.057	0.154
Population:Date						
WFR:May.20	1.951	0.331	**<0.001**	0.876	0.791	0.933
WFR:Jun.20	-0.389	0.287	0.175	0.404	0.278	0.543
WFR:Jul.21	-0.273	0.234	0.244	0.432	0.325	0.547
WFR:Sep.19	0.245	0.339	0.469	0.561	0.399	0.716
WFR: Sep.20	0.357	0.289	0.217	0.588	0.449	0.717
WFR: Sep.21	-0.007	0.23	0.976	0.498	0.387	0.61

Table B.2: Binomial logistic regression model summary for *Clematis vitalba* seed germinability during collection and incubation on seven separate dates (May.20 reference category) in alternating dark and light (16h:8h) with alternating temperatures (20/30°C). G est = coefficient estimate for seed germination; fitted ave = fitted model average proportion germination; CI lb, ub = lower and upper bounds of 95% confidence interval. Three treatments: Dark (reference); Control = blotters moistened with reverse-osmosis water; and KNO3 = blotters moistened with KNO3). Seeds from two separate populations (Hopeland Reserve at Kumeroa Rd, KUM (reference), and Woodville Ferry Reserve, WFR). P-values in bold are significant at α=0.05.

	G est	SE	Pr (>|z|)	fitted ave	LL ci	UL ci
Intercept	-7.053	0.329	**<0.001**	0.0009	0.0004	0.0016
Treatment						
Control	2.116	0.109	**<0.001**	0.892	0.87	0.911
KNO3	3.92	0.166	**<0.001**	0.981	0.974	0.986
Population						
WFR	-0.161	0.095	0.091	0.46	0.414	0.506
Date						
May.21	-0.372	0.482	0.44	0.408	0.202	0.635
Jun.20	5.451	0.323	**<0.001**	0.996	0.992	0.997
Jul.21	6.478	0.332	**<0.001**	0.998	0.997	0.999
Sep.19	6.702	0.331	**<0.001**	0.999	0.998	0.999
Sep.20	7.771	0.341	**<0.001**	0.9996	0.999	0.9998
Sep.21	9.537	0.418	**<0.001**	0.9999	0.9998	0.99997

Appendix C Chapter 5 supplementary material

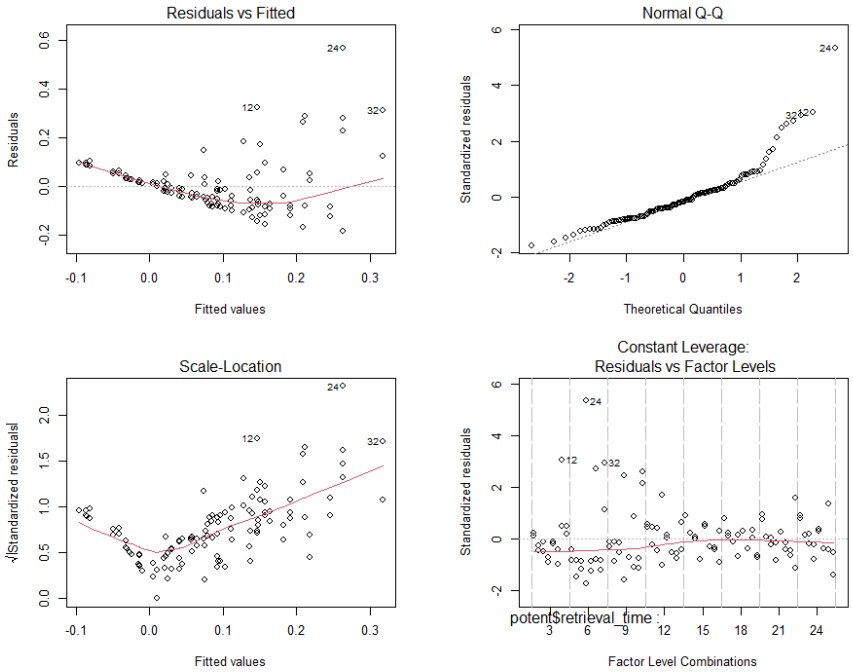

Figure C.1: Model diagnostic plots for Experiment 1 ANOVA of main effects (burial site, depth, provenance, retrieval time) on untransformed data of *Clematis vitalba* seed viability following burial beginning September 2019 and retrieved at 3-month intervals over a 2-year period.

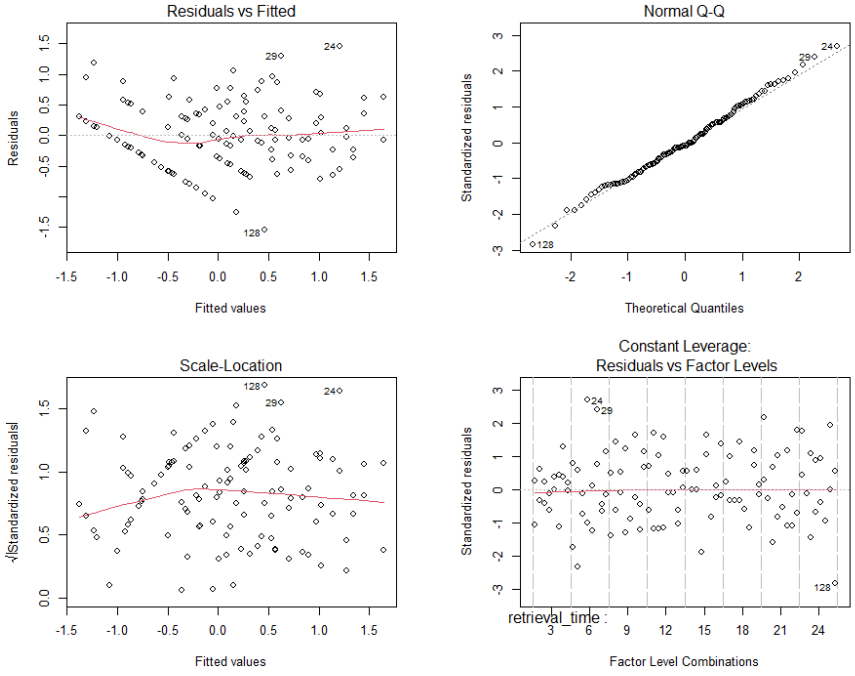

Figure C.2: Model diagnostic plots for Experiment 1 ANOVA of main effects (burial site, depth, provenance, retrieval time) on data transformed using ordered quantile normalization (ORQ) of *Clematis vitalba* seed viability following burial beginning September 2019 and retrieved at 3-month intervals over a 2-year period.

Table C.1: Experiment 1 ANOVA model comparison output (K= number of parameters; AIC_c = information score of the model, with lower-case 'c' indicating a correction for small sample sizes; Delta AIC_c = difference in AIC score between best model and model being assess; AIC_c Wt = AIC_c weight, the proportion of total amount of predictive power from the full set of models in the model being assessed; Cum.Wt = sum of AIC_c weights; LL = log-likelihood).

	K	AIC_c	Delta_AIC_c	AIC_c Wt	Cum.Wt	LL
Main effects	12	233.27	0.00	1	1	-103.28
First order interactions	36	275.91	42.63	0	1	-87.32
Full interactions	65	401.99	168.72	0	1	-66.80

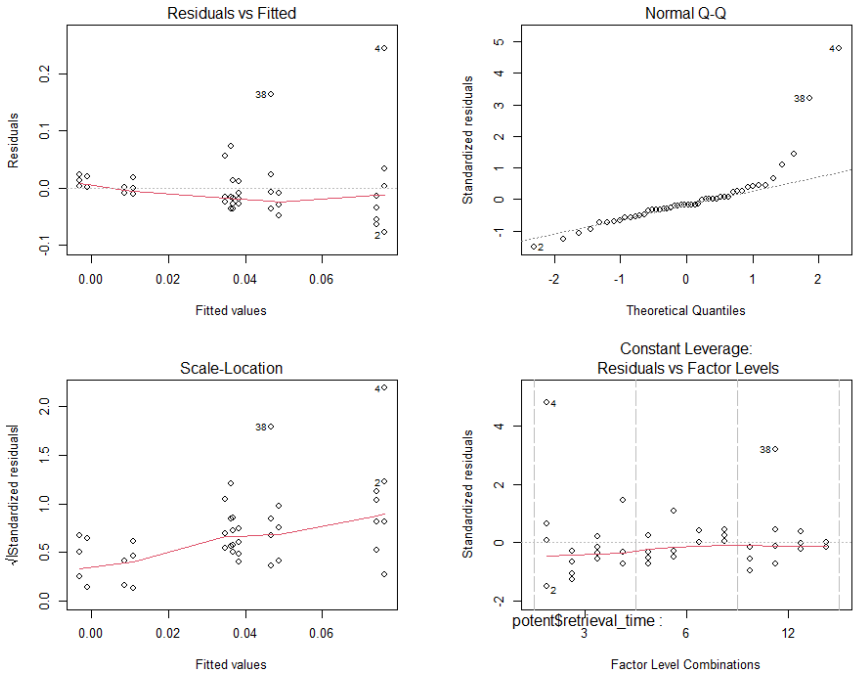

Figure C.3: Model diagnostic plots for ANOVA of main effects (population source, burial site, retrieval time) on untransformed data of *Clematis vitalba* seed viability following burial beginning June 2020 for 3, 6, and 12 months.

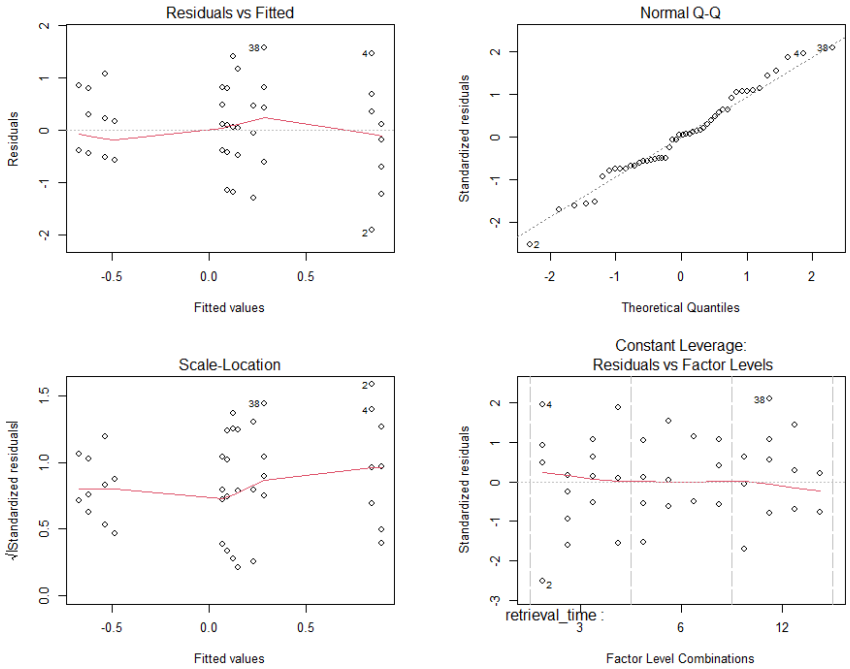

Figure C.4: Model diagnostic plots for ANOVA of main effects (population source, burial site, retrieval time) on data transformed using ordered quantile normalization (ORQ) of *Clematis vitalba* seed viability following burial beginning June 2020 for 3, 6, and 12 months.

Table C.2: Experiment 2 ANOVA model comparison output (K= number of parameters; AICc = information score of the model, with lower-case 'c' indicating a correction for small sample sizes; Delta AICc = difference in AIC score between best model and model being assess; AICc Wt = AICc weight, the proportion of total amount of predictive power from the full set of models in the model being assessed; Cum.Wt = sum of AICc weights; LL = log-likelihood).

	K	AIC_c	Delta_AIC_c	AIC_c Wt	Cum.Wt	LL
Main effects	6	132.43	0.00	1	1	-59.20
First order interactions	11	143.67	11.22	0	1	-57.17
Full interactions	13	146.46	14.00	0	1	-54.88

Appendix D Chapter 6 supplementary material

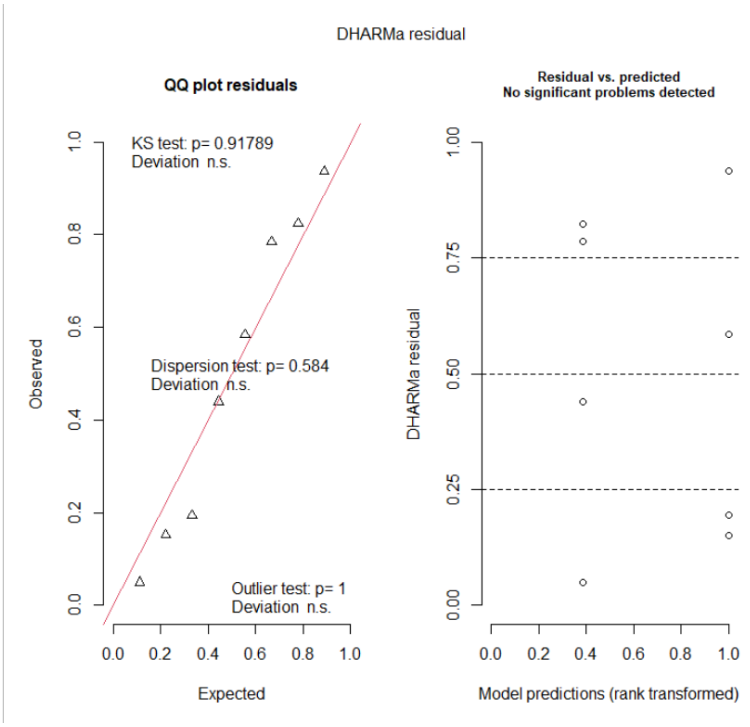

Figure D.1: Poisson regression goodness of fit plots for initial establishment (10 weeks) of *Clematis vitalba* seedlings at Manuka 1 site. Too few data points were generated at this site for the model to calculate quantile regression.

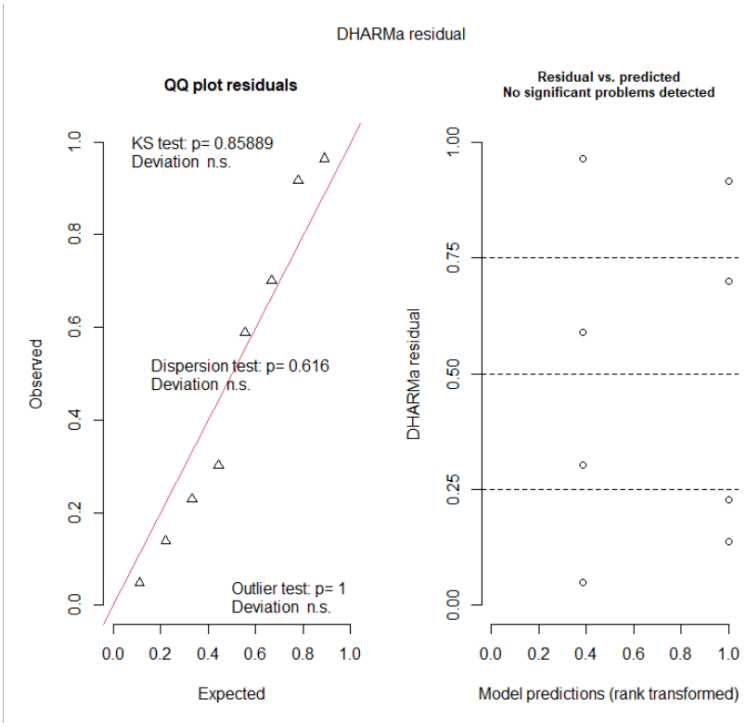

Figure D.2: Poisson regression goodness of fit plots for final assessment (52 weeks) of *Clematis vitalba* seedling survival at Manuka 1 site. Too few data points were generated at this site for the model to calculate quantile regression.

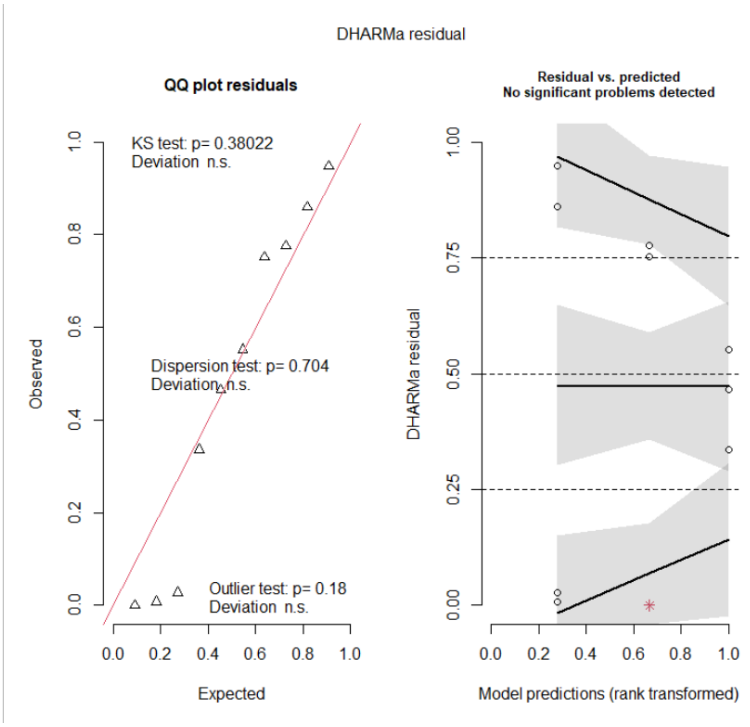

Figure D.3: Poisson regression goodness of fit plots for initial establishment (10 weeks) of *Clematis vitalba* seedlings at Manuka 2 site.

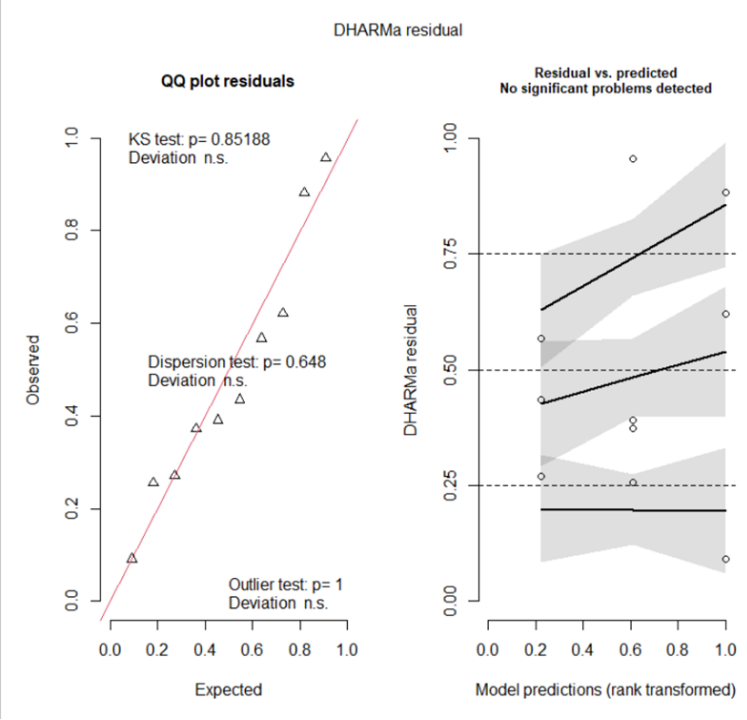

Figure D.4: Poisson regression goodness of fit plots for final assessment (52 weeks) of *Clematis vitalba* seedling survival at Manuka 2 site.

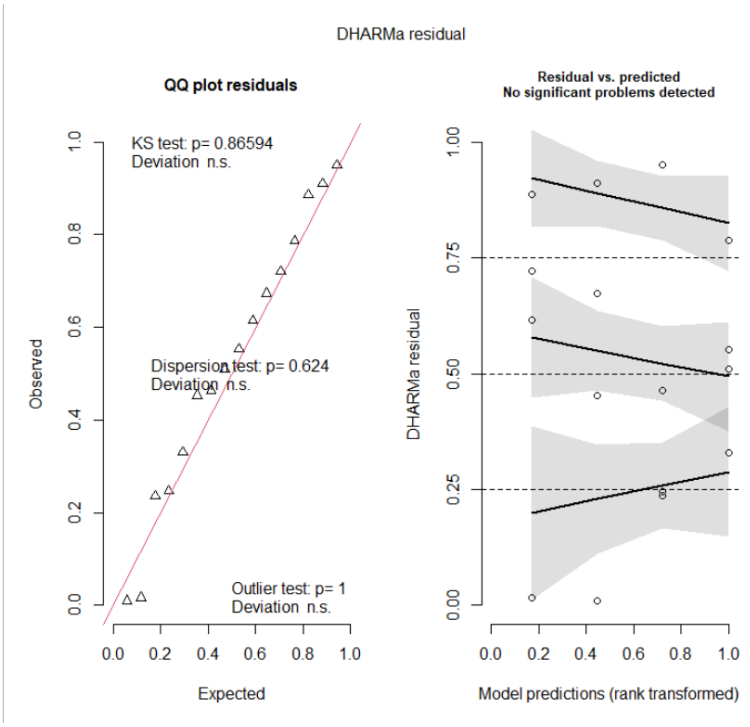

Figure D.5: Negative binomial regression goodness of fit plots for initial establishment (10 weeks) of *Clematis vitalba* seedlings at Treeline site.

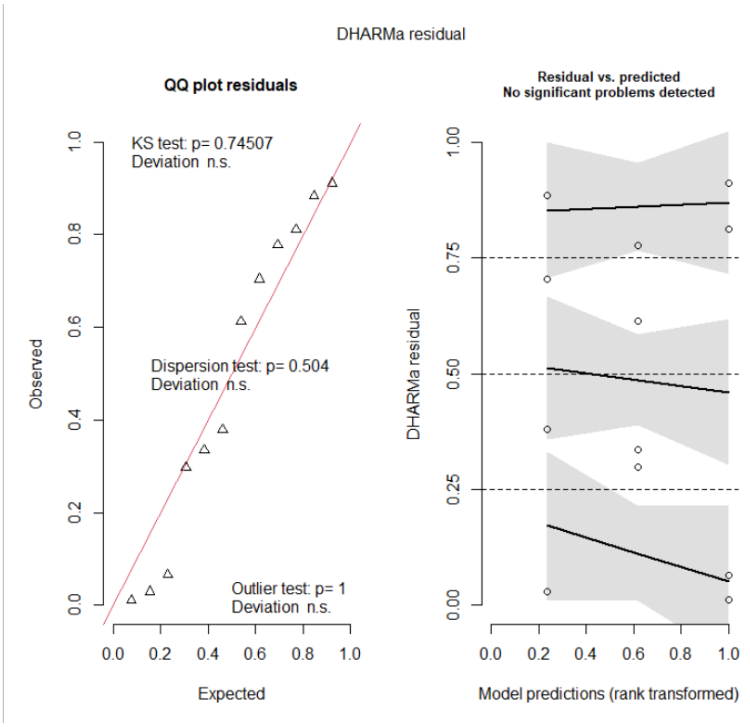

Figure D.6: Poisson regression goodness of fit plots for 1-year assessment of *Clematis vitalba* seedling survival at Treeline site.

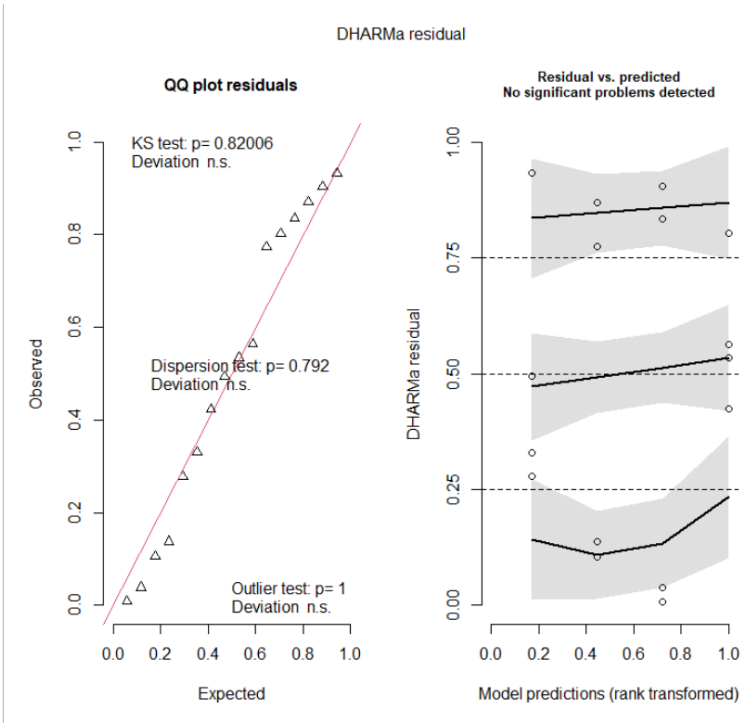

Figure D.7: Negative binomial regression goodness of fit plots for *Clematis vitalba* seedling survival after 61 weeks at Treeline site.

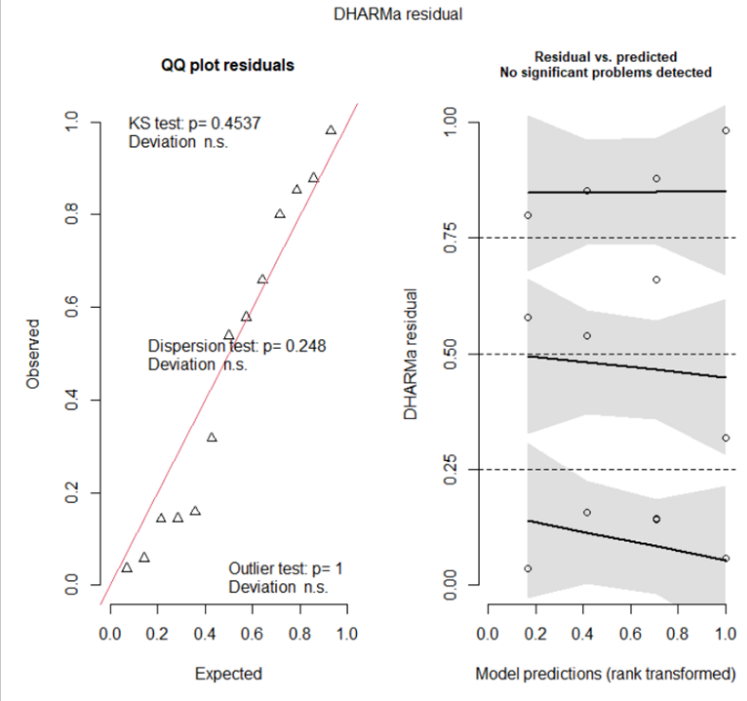

Figure D.8: Poisson regression goodness of fit plots for final assessment (104 weeks) of *Clematis vitalba* seedling survival at Treeline site.

Table D.1: Poisson generalised linear mixed model summary for *Clematis vitalba* seedling survivability at Manuka 1 site 10 and 52 weeks (WK) after sowing. P-values in bold are significant at α=0.001.

WK 10				**WK 52**		
Random effects	VAR	SD		VAR	SD	
block(intercept)	0.105	0.324		0.092	0.304	
Fixed effects	Est	SE	Pr (>|z|)	Est	SE	Pr (>|z|)
intercept	2.145	0.205	**<0.001**	1.826	0.26	**<0.001**
Treatment C	0.28	0.237	0.237	-29.603	2048	0.988

Table D.2: Poisson generalised linear mixed model summary for *Clematis vitalba* seedling survivability, M2; significant pairwise comparisons from simultaneous tests for general linear hypotheses for WK 10. P-values in bold are significant at α=0.001.

WK 10				**WK 52**		
Random effects	VAR	SD		VAR	SD	
block(intercept)	0.223	0.472		0.016	0.127	
Fixed effects	Est	SE	Pr (>|z|)	Est	SE	Pr (>|z|)
intercept	7.263	0.237	**<0.001**	3.3	0.133	**<0.001**
treatment B	-1.258	0.034	**<0.001**	-27.46	87940	1
Treatment C	-0.227	0.024	**<0.001**	-28.44	165700	1

Multiple Comparisons of Means: Tukey Contrasts			
	Est	SE	Pr(>|z|)
B-A==0	-1.258	0.034	**<0.001**
C-A==0	-0.227	0.024	**<0.001**
C-B==0	1.031	0.035	**<0.001**

Table D.3: Generalised linear mixed model summary (negative binomial (NB) and Poisson) for *Clematis vitalba* seedling survivability at Treeline site 10, 52, 61 and 104 weeks (WK) after sowing, with significant pairwise comparisons from simultaneous tests for general linear hypotheses for WK 52 and 61. P-values in bold are significant at α=0.05.

WK 10 NB model						
Random effects	VAR	SD				
block(intercept)	3.01E-11	5.49E-06				
Fixed effects	Est	SE	Pr (>|z|)			
intercept	2.398	0.341	**<0.001**			
treatment B	0.327	0.475	0.492			
Treatment C	-0.35	0.492	0.476			
Treatment D	-0.452	0.495	0.361			
WK 52 Poisson model				**WK 61 NB model**		
Random effects	VAR	SD		VAR	SD	
block(intercept)	0.75	0.866		0	0	
Fixed effects	Est	SE	Pr (>|z|)	Est	SE	Pr (>|z|)
intercept	0.926	0.588	0.1153	1.253	0.472	**0.008**
treatment B	-2.639	1.035	**0.011**	-2.639	1.173	**0.024**
Treatment C	-2.639	1.035	**0.011**	-0.693	0.72	0.335
Treatment D	NA	NA	NA	0.305	0.654	0.64
	Multiple Comparisons of Means: Tukey Contrasts					
	Est	SE	Pr(>|z|)	Est	SE	Pr(>|z|)
B-A==0	-2.639	1.035	**0.0276**	NA	NA	NA
C-A==0	-2.639	1.035	**0.0276**	NA	NA	NA
D-B==0	NA	NA	NA	2.94	1.165	0.053
WK 104 Poisson model						
Random effects	VAR	SD				
block(intercept)	0.008	0.092				
Fixed effects	Est	SE	Pr(>|z|)			
intercept	0.51	0.451	0.258			
treatment B	-29.8	1.33e+06	1			
Treatment C	-0.802	0.732	0.273			
Treatment D	-49.65	3.88e+07	1			

Appendix E Chapter 7 supplementary material

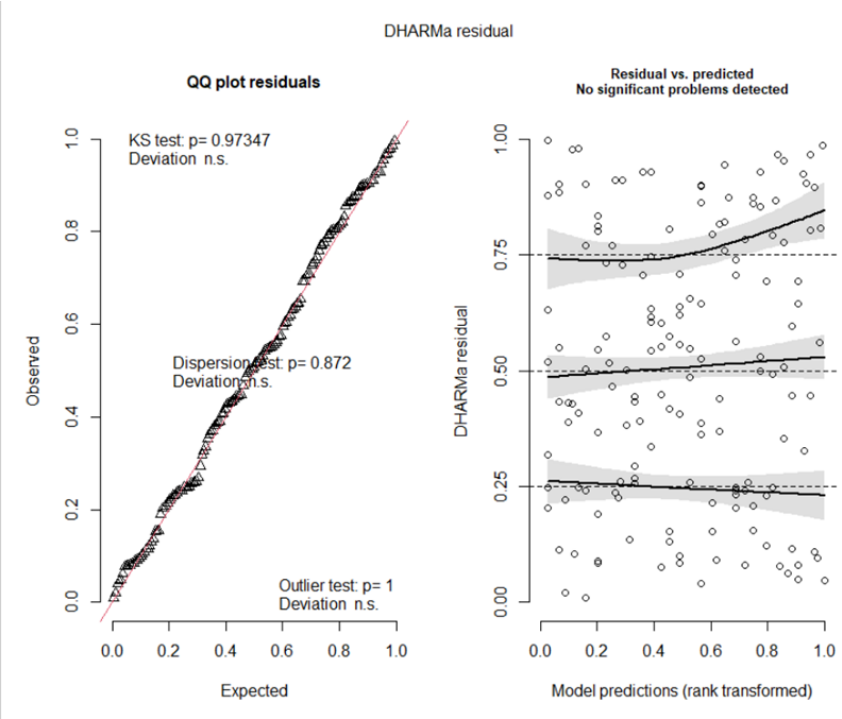

Figure E.1: Binomial logistic regression goodness of fit plots for regeneration of *Clematis vitalba* stem fragments, as a function of initial stem diameter, active initial bud, and stem type (climbing or creeping).

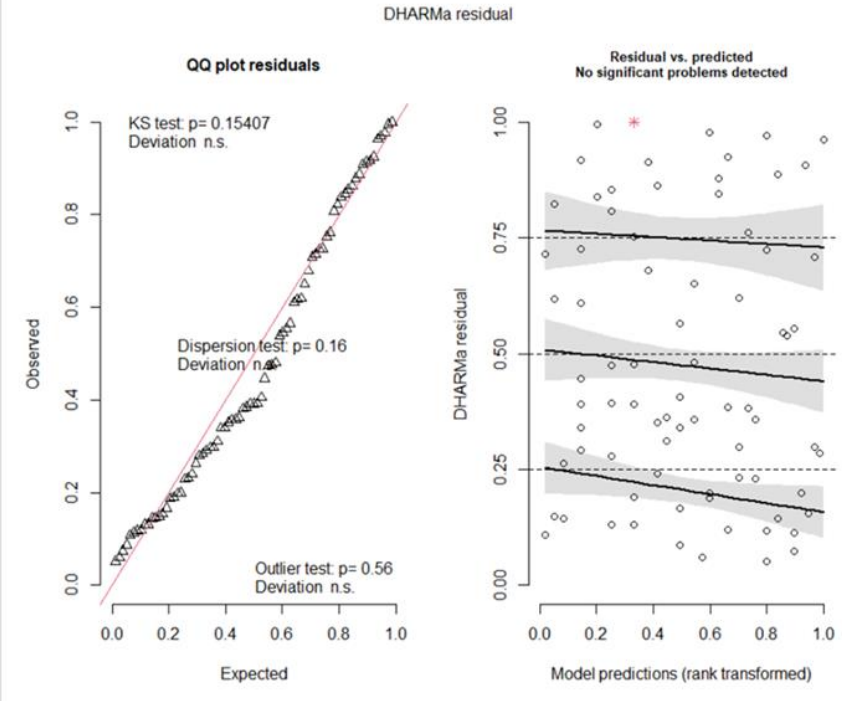

Figure E.2: Poisson regression goodness of fit plots for number of shoots produced by *Clematis vitalba* stem fragments, as a function of initial stem diameter and stem type (climbing or creeping).

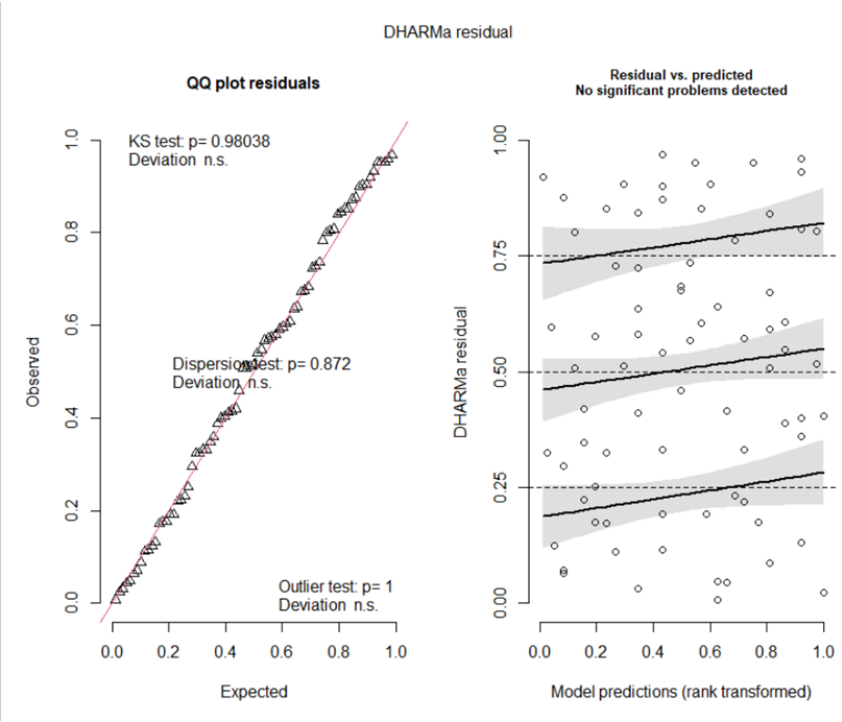

Figure E.3: Linear mixed regression goodness of fit plots for regenerative shoot yield (total length of shoots) of *Clematis vitalba* stem fragments, as a function of initial stem diameter and stem type (climbing or creeping).

Table E.1: Generalised linear mixed model summary (binomial logistic regression) for *Clematis vitalba* stem fragment regeneration, as a function of initial stem diameter (Initial diam), initial root or shoot presence (active bud Y; inactive bud is reference category), and stem type (vertical (Type V; lateral is reference category). P-values in bold are significant at α=0.05. OR = odds ratio.

Random effects	VAR	SD						
Tray (intercept)	0.113	0.336						
Fixed effects	Est	SE	Pr (>|z|)	Est ci lb	Est ci ub	OR	OR ci lb	OR ci ub
intercept	0.782	0.456	0.086	1.189	0.375	0.457	0.304	0.687
Initial diam	0.765	0.375	**0.042**	0.489	1.04	2.148	1.63	2.83
Active bud Y	0.158	0.355	0.655	0.088	0.405	1.172	0.915	1.5
Type V	0.095	0.353	0.789	-0.15	0.339	1.099	0.86	1.404

Table E.2: Poisson mixed regression model summary for number of shoots produced by *Clematis vitalba* stem fragments 4 months after fragmentation and burial, as a function of initial stem diameter (Initial diam) and stem type (vertical (Type V) or lateral (reference category)). P-values in bold are significant at α = 0.05. IRR = incidence rate ratio.

Random effects	VAR	SD						
Tray (intercept)	0.048	0.219						
Fixed effects	Est	SE	Pr (>|z|)	Est ci lb	Est ci ub	IRR	IRR ci lb	IRR ci ub
intercept	0.798	0.173	**<0.001**	0.74	0.857	2.222	1.58	3.12
Initial diam	0.361	0.131	**0.006**	0.328	0.395	1.435	1.11	1.86
Type V	-0.05	0.137	0.716	-0.086	-0.013	0.951	0.73	1.24

Table E.3: Linear mixed regression model summary for regenerative shoot yield (total length of shoots) of *Clematis vitalba* stem fragments 4 months after fragmentation and burial, as a function of initial stem diameter (Initial diam) and stem type (vertical (Type V) or lateral (reference category)).

Random effects	VAR	SD					
Tray (intercept)	0.006	0.08					
Residual	0.993	0.997					
Fixed effects	Est	SE	df	t value	Pr (>|z|)	Est ci lb	Est ci ub
intercept	0.286	0.283	68.5	1.014	0.314	0.13	0.442
Initial diam	-0.131	0.246	73.991	-0.533	0.595	-0.249	-0.013
Type V	-0.317	0.229	73.845	-1.385	0.17	-0.419	-0.214

Figure E.4: Cross section of a 2 cm diameter woody *Clematis vitalba* stem with 8 annual rings. Each ring is made up of larger vessels that developed during the beginning of the growing season (earlywood), and smaller vessels that developed later in the season (latewood).

Appendix F Chapter 8 supplementary material

Table F.1: Basal herbicide experiment summary of Kruskal-Wallis rank sum tests comparing proportion of active nodes per *Clematis vitalba* stem across four treatment groups, at three separate assessments (2020.1yr = 1 year after treatment in autumn 2020; 2020.2yr = 2 years after treatment in autumn 2020; 2022.1yr = 1 year after treatment in autumn 2021).

treatment yr.assessment	χ^2	df	p-val
2020.1yr	62.44	3	<0.001
2020.2yr	51.44	3	<0.001
2021.1yr	62.07	3	<0.001

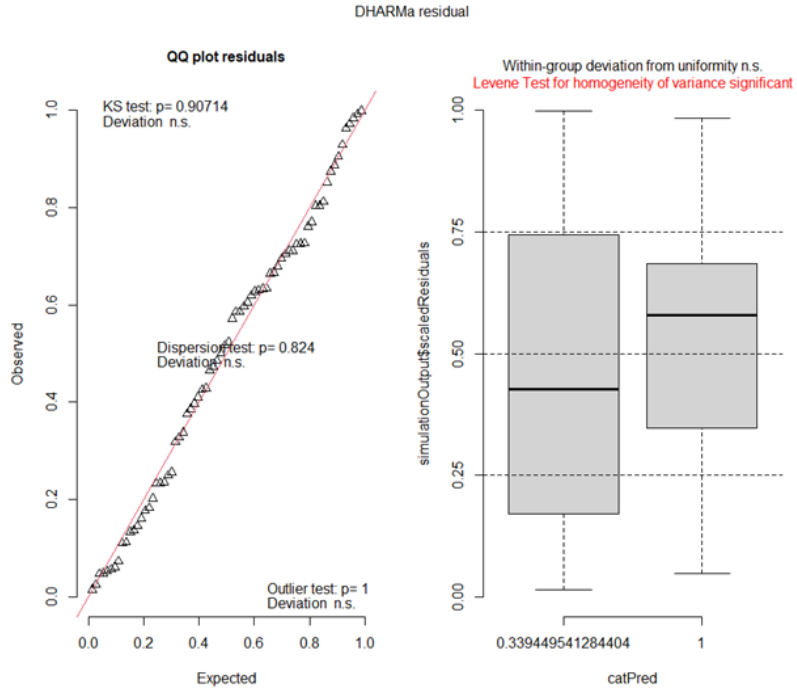

Figure F.1: Negative binomial regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Cut and Paste treatments (Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel), 1 year after treatment in 2020.

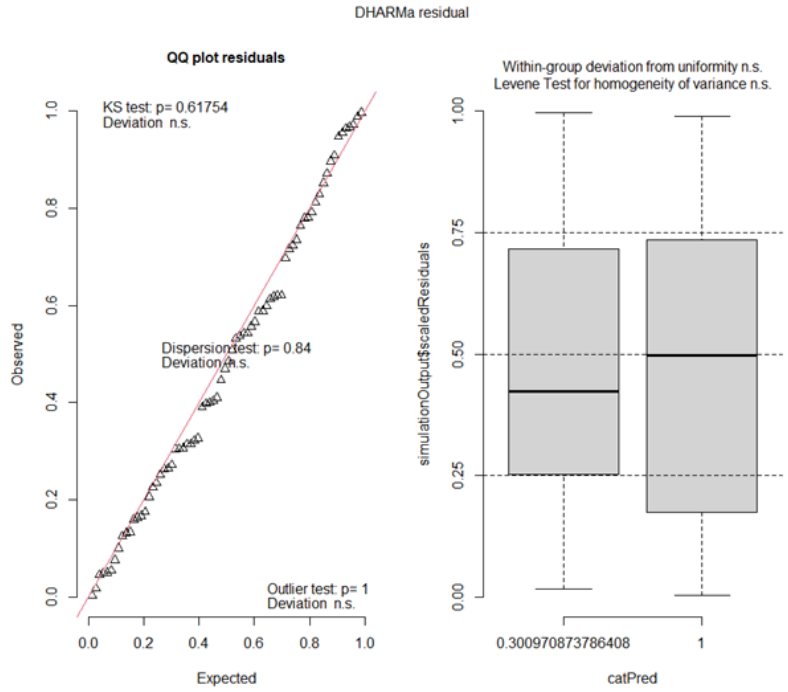

Figure F.2: Poisson regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Control and Ring treatment (Control = untreated stems; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)), 1 year after treatment in 2020.

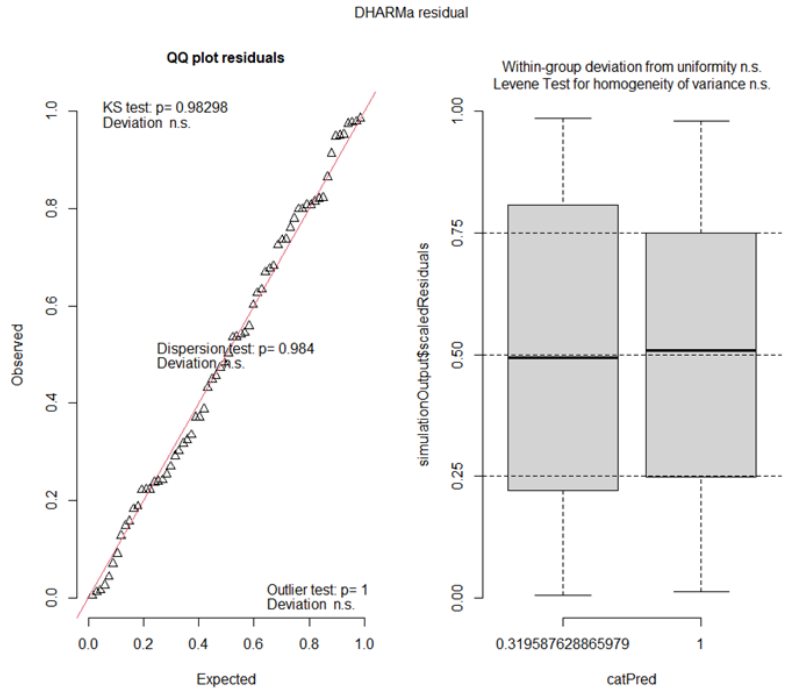

Figure F.3: Negative binomial regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Ring and Paste treatments (Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)), 1 year after treatment in 2020.

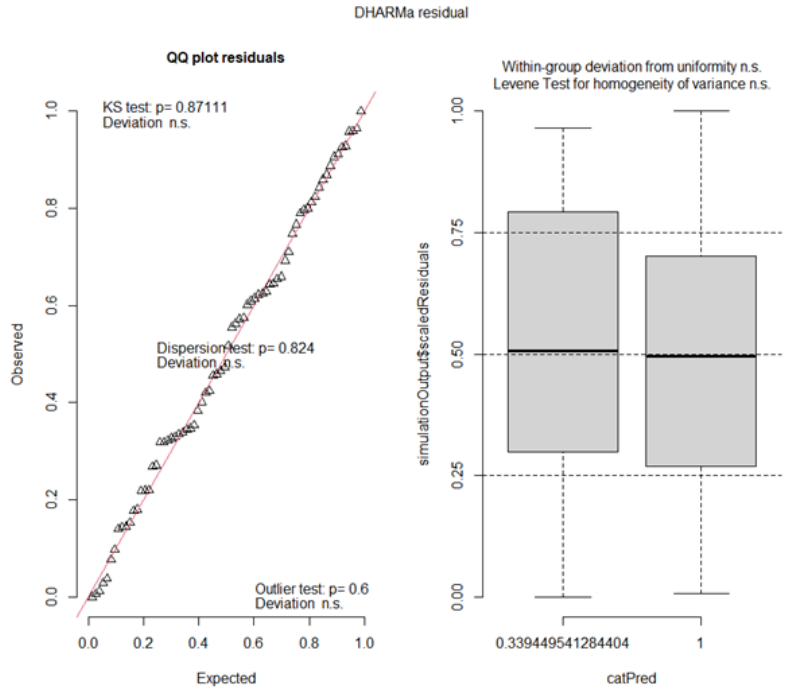

Figure F.4: Negative binomial regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Cut and Paste treatments (Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel), 2 years after treatment in 2020.

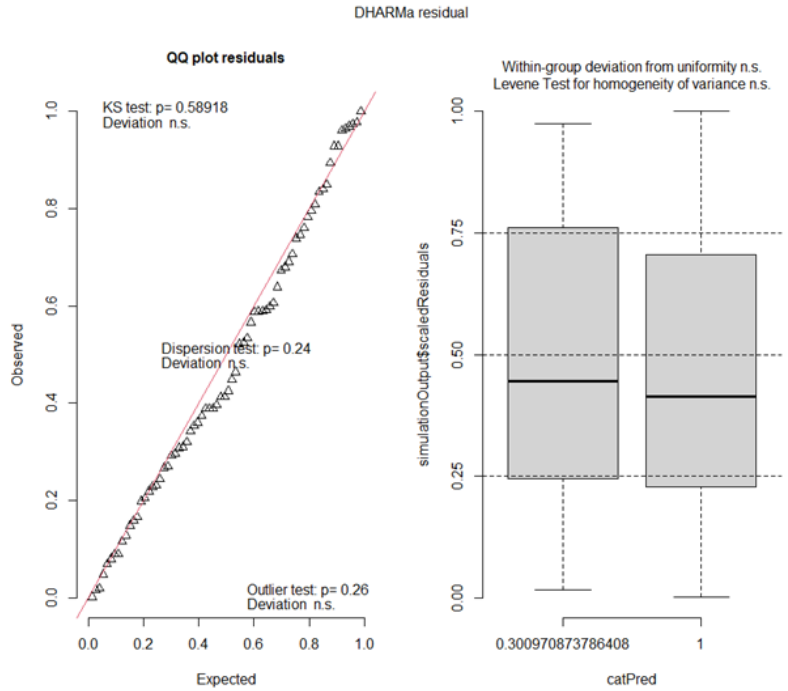

Figure F.5: Poisson regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Control and Ring treatment (Control = untreated stems; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)), 2 years after treatment in 2020.

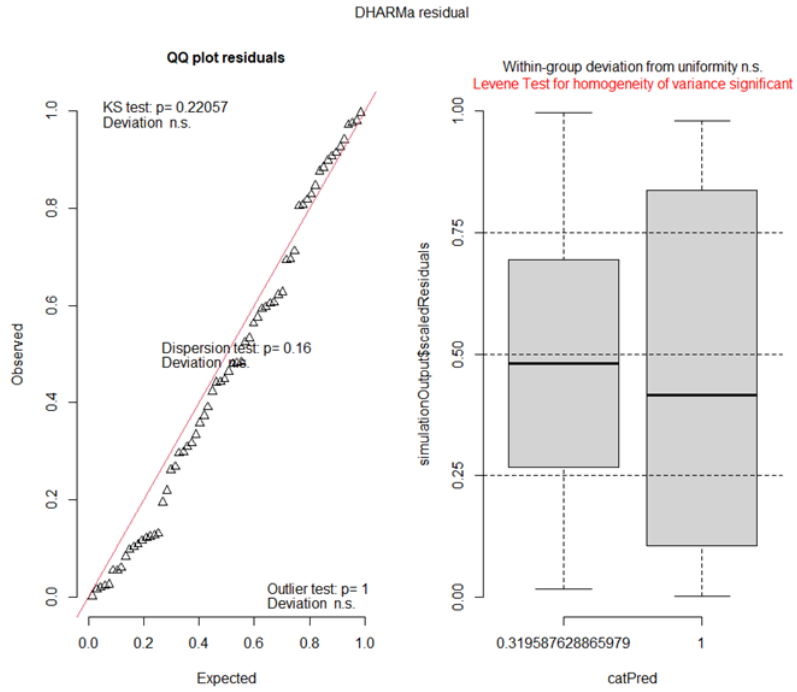

Figure F.6: Generalised linear mixed Poisson regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Ring and Paste treatments (Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)), 2 years after treatment in 2020.

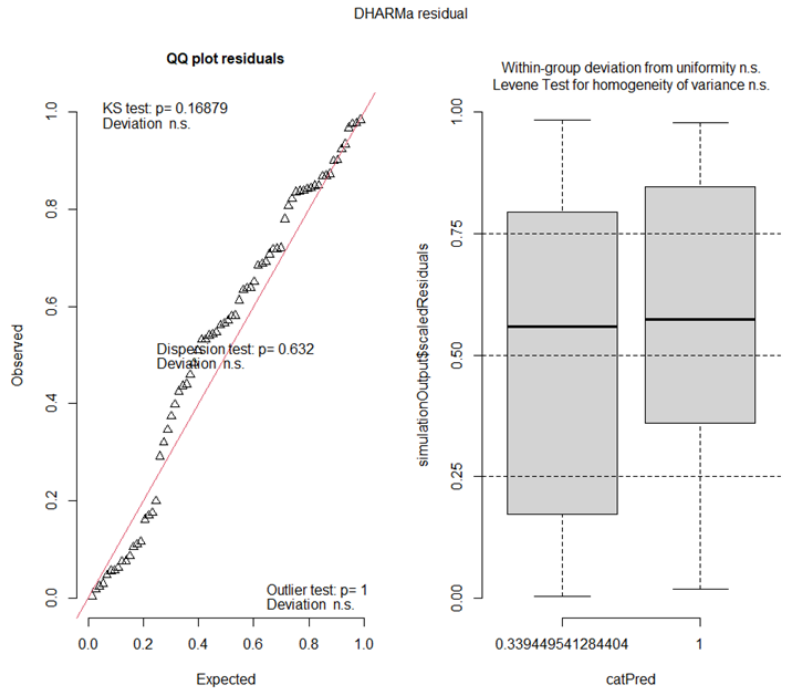

Figure F.7: Generalised linear mixed Poisson regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Cut and Paste treatments (Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel), 1 year after treatment in 2021.

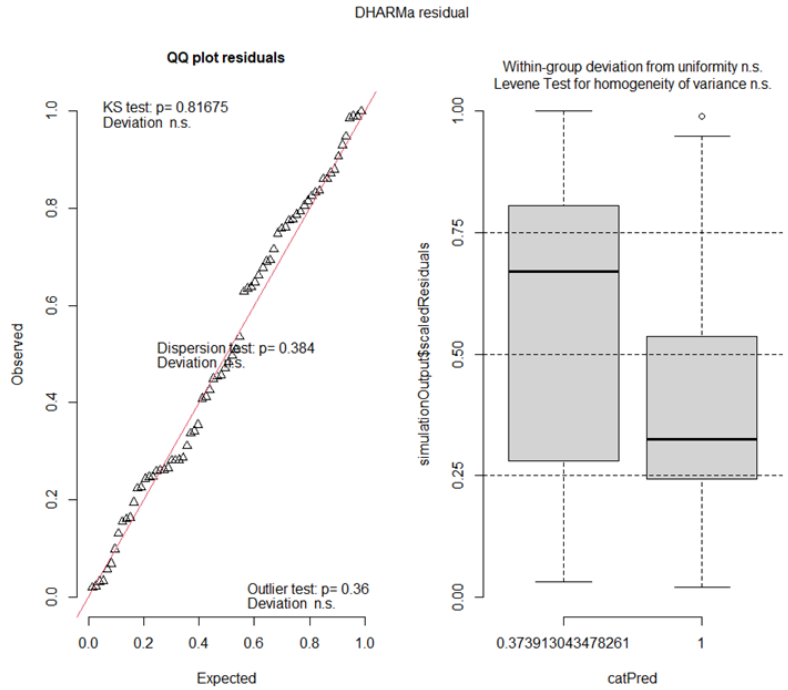

Figure F.8: 2021 control/ring, GLMM Poisson Generalised linear mixed Poisson regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Control and Ring treatment (Control = untreated stems; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)), 1 year after treatment in 2021.

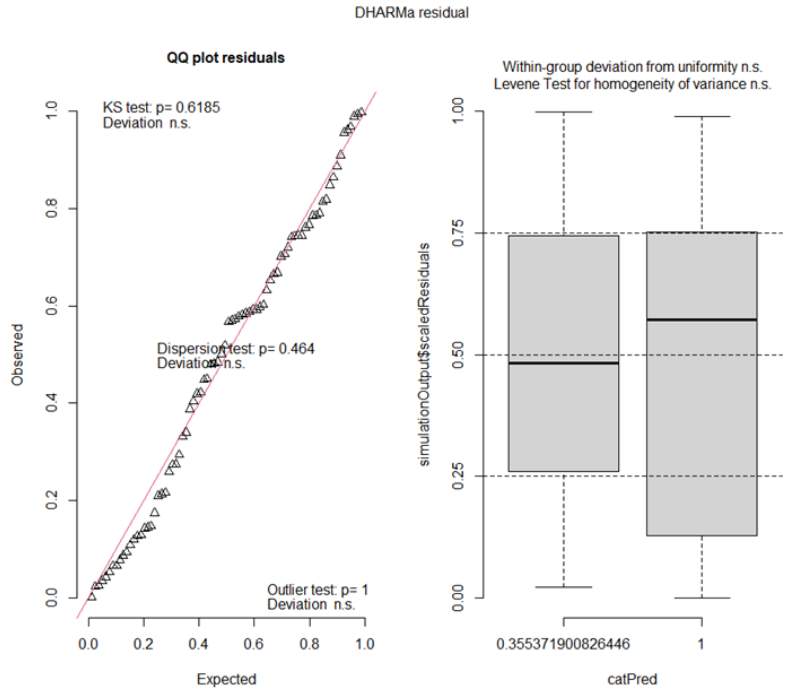

Figure F.9: Generalised linear mixed Poisson regression goodness of fit plots for number of shoots produced per *Clematis vitalba* stem, comparison of Ring and Paste treatments (Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method)), 1 year after treatment in 2021.

Table F.2: Basal herbicide experiment generalised linear model summaries (negative binomial (NB) and Poisson) for *Clematis vitalba* stem vigour between treatment pairs (Control = untreated stems; Cut = stems severed at 1 metre above ground; Paste = stems severed at 1 metre above ground and treated with 45% glyphosate gel; Ring = stems chemically ringbarked with triclopyr in oil (basal bark method))) at three separate assessments: 1 year after 2020 treatment (2020.1yr), 2 years after 2020 treatment (2020.2yr), and 1 year after 2021 treatment (2021.1yr). Treatment p-values in bold are significant at α=0.001.

Cut vs Paste				Control vs Ring				Paste vs Ring			
2020.1yr NB model				2020.1yr Poisson model				2020.1yr NB model			
	EST	SE	Pr (>|z|)		EST	SE	Pr (>|z|)		EST	SE	Pr (>|z|)
Intercept	1.27	0.14	<0.001	Intercept	-0.241	0.17	0.166	Intercept	0.42	0.21	0.048
Paste	-0.85	0.22	<0.001	Ring	-19.06	1721.06	0.991	Ring	-20.73	2837.54	0.994
2020.2yr NB model				2020.2yr Poisson model				2020.2yr Poisson model			
	EST	SE	Pr (>|z|)		EST	SE	Pr (>|z|)	Random effects	VAR	SD	
Intercept	0.96	0.15	<0.001	Intercept	0.25	0.14	0.065	Block (intercept)	0.02	0.13	
Paste	-0.91	0.24	<0.001	Ring	-3.65	1.01	<0.001	Fixed effects	EST	SE	Pr (>|z|)
								Intercept	0.05	0.18	0.795
								Ring	-3.45	1.01	<0.001
2021.1yr Poisson model				2021.1yr Poisson model				2021.1yr Poisson model			
Random effects	VAR	SD		Random effects	VAR	SD		Random effects	VAR	SD	
Block (intercept)	0.23	0.48		Block (intercept)	0.43	0.65		Block (intercept)	0.20	0.44	
Fixed effects	EST	SE	Pr (>|z|)	Fixed effects	EST	SE	Pr (>|z|)	Fixed effects	EST	SE	Pr (>|z|)
Intercept	1.12	0.24	<0.001	Intercept	0.02	0.38	0.957	Intercept	0.68	0.23	0.004
Paste	-0.54	0.15	<0.001	Ring	-2.82	0.68	<0.001	Ring	-3.40	0.60	<0.001

Table F.3: Foliar herbicide experiment two-way ANOVA table for ground cover density change following treatment of *Clematis vitalba* within an 8-month period, between two sites and four treatments.

Ground cover density change

	Df	Sum sq	Mean sq	F value	Pr(>F)
treatment	3	0.101	0.034	1.986	0.195
site	1	0.003	0.003	0.147	0.711
treatment:site	3	0.131	0.044	2.564	0.128
residuals	8	0.136	0.017		

Table F.4: Foliar herbicide experiment two-way ANOVA table, with Tukey multiple comparisons of means, for average grass height at 8 months post-treatment to control *Clematis vitalba*, between two sites (MM, AP) and four treatments (Control = untreated; Grazon = triclopyr in oil; Meturon = metsulfuron-methyl; Tordon = combination of picloram, aminopyralid, triclopyr). P-values in bold are significant at α=0.05.

Grass height

	Df	Sum sq	Mean sq	F value	Pr(>F)
treatment	3	1.844	0.615	10.747	**0.004**
site	1	0.563	0.563	9.836	**0.014**
treatment:site	3	0.031	0.01	0.182	0.906
residuals	8	0.458	0.057		

Tukey multiple comparisons of means

treatment	diff	lwr	upr	p adj
Grazon-Control	0.188	-0.354	0.729	0.695
Meturon-Control	-0.438	-0.979	0.104	0.119
Tordon-Control	0.5	-0.042	1.042	0.071
Meturon-Grazon	-0.625	-1.167	-0.083	**0.025**
Tordon-Grazon	0.313	-0.229	0.854	0.320
Tordon-Meturon	0.938	0.396	1.479	**0.002**

site	diff	lwr	upr	p adj
MM-AP	-.375	-.651	-.099	**.014**

Table F.5: Foliar herbicide experiment two-way Scheirer-Ray-Hare table for dry weight of *Clematis vitalba* remaining in plots 8 months post-treatment, between two sites and four treatments. Also, a summary of Kruskal-Wallis rank sum test comparing three treatments (no control treatment). P-values in bold are significant at α=0.05.

OMB dry weight

	Df	Sum Sq	H	p-value
treatment	3	210	11.25	**0.01045**
site	1	20.25	1.0848	0.29762
treat:site	3	24.75	1.3259	0.72299
residuals	8	25		

Kruskal-Wallis rank sum test

χ^2	df	p-val
2.386	2	0.303

Appendix G R code

R code for time-to-event model, Chapter 2

```
# seed loss over time

library(drc)

interval.data<-read.csv("interval.csv")

interval.data

interval.data <- na.omit(interval.data) # drop lines with start time = na (end time
0)


interval.basic <- drm(Count_Delta ~ Start_Time + End_Time,

                      Location,

                      data = interval.data,

                      fct = LL.2(names=c("Slope","T50")),

                      type = "event")

summary(interval.basic)

plot(interval.basic, ylim = c(0, 1), xlim = c(0,8),

     main="OMB Seed Loss Rate--2-parameter log-logistic event model", ylab= "Seeds
Lost", xlab = "Time (Months)",

     legendPos = c(7,0.6),

     log="",col=1:3)


interval.LN <- drm( Count_Delta ~ Start_Time + End_Time,

                    Location,

                    data = interval.data,

                    fct = LN.2(names=c("Slope","T50")),

                    type = "event")

interval.W1 <- drm( Count_Delta ~ Start_Time + End_Time,

                    Location,

                    data = interval.data,

                    fct = W1.2(names=c("Slope","T50")),

                    type = "event")

interval.W2 <- drm( Count_Delta ~ Start_Time + End_Time,

                    Location,

                    data = interval.data,

                    fct = W2.2(names=c("Slope","T50")),

                    type = "event")

c(AIC(interval.basic), BIC(interval.basic))

c(AIC(interval.LN), BIC(interval.LN))
```

```
c(AIC(interval.W1), BIC(interval.W1))
c(AIC(interval.W2), BIC(interval.W2))

#plot residuals for LL model
plot(fitted(interval.basic), residuals(interval.basic),
     main="Residuals vs Fitted")
abline(h = 0, lty = 2, col = "darkorange", lwd = 2)

#plot residuals of LN.2 model
plot(fitted(interval.LN), residuals(interval.LN),
     main="Residuals vs Fitted")
abline(h = 0, lty = 2, col = "darkorange", lwd = 2)

library(moments)
skewness(residuals(interval.basic))
skewness(residuals(interval.LN))

kurtosis(residuals(interval.basic))
kurtosis(residuals(interval.LN))

shapiro.test(residuals(interval.basic))
shapiro.test(residuals(interval.LN))

library(car)
qqPlot(residuals(interval.LN), "norm")
qqPlot(residuals(interval.basic),"logis")
summary(interval.basic)
#-------------------------------------
```

R code for 2-step analysis, Chapter 3

```
#style effect on germinability, expt 1
library(car)
library(plyr)
library(dplyr)
library(ggplot2)
library(magic)
library(metafor)
library(multcomp)
```

```
library(devtools)
install_github("DoseResponse/drcData")
install_github("DoseResponse/drc")
library(drc)
library (drcData)
library(ggpubr)

#defining helper functions for step 1
paramToWide <- function(allFits, tVal = NULL)
{
  mpNames <- c("b", "d", "e")
  numPar <- length(mpNames)
  numTval <- length(tVal)
  perFit <- function(fitObj)
  {
    if (is.null(fitObj)) {return(c(rep(NA, 2 * (numPar + numTval)), 0))}
    tempNames <- fitObj[["fct"]][["names"]]
    numPar2 <- length(tempNames)
    tempNames2 <- paste(tempNames, ":(Intercept)", sep = "")
    returnMat <- matrix(NA, numPar + numTval, 2)
    rmNames <- mpNames
    if (!is.null(tVal)) {rmNames <- c(rmNames, paste("t:", tVal, sep = ""))}
zh
    rownames(returnMat) <- rmNames
    coefSum <- coef(summary(fitObj))
    for (i in 1:numPar2)
    {
      returnMat[tempNames[i], 1:2] <- coefSum[tempNames2[i], 1:2]
    }
    if (numTval > 0) {for (i in 1:numTval)
    {
      returnMat[i + numPar, 1:2] <- ED(fitObj, tVal[i], display = FALSE)[1:2]
    }}
    returnVec <- c(as.vector(t(returnMat)), numPar2)
    names0 <- paste(rep(mpNames, rep(2, numPar)), rep(c(".est", ".se"), numPar),
sep = "")
    tvNames <- paste("t", tVal, sep = "")
    if (numTval > 0) {names0 <- c(names0,
                                  paste(rep(tvNames, rep(2, numTval)),
```

```
                                        rep(c(".est", ".se"), numTval), sep = ""))}
    names(returnVec) <- c(names0, "npar")
    returnVec
  }
  ldply(allFits, perFit)
}
paramToLong <- function(allFits, groupVars, parmsWide)
{
  mpNames <- c("b", "d", "e")
  longData0 <- parmsWide
  longData <- longData0[rep(row.names(longData0), longData0[["npar"]]), ]
  coefListN <- lapply(allFits, function(modelFit) {if (!is.null(modelFit))
  {names(coef(modelFit))} else {NULL}})
  coefListN2 <- compact(coefListN) # removing NULL elements
  coefVecN <- do.call("c", coefListN2)
  longData[["Parm"]] <- as.factor(coefVecN)
  levels(longData[["Parm"]]) <- mpNames
  coefList <- lapply(allFits, function(modelFit) {if (!is.null(modelFit))
  {coef(modelFit)} else })
  coefList2 <- compact(coefList) # removing NULL elements
  coefVec <- as.numeric(do.call("c", coefList2))
  vcovList <- lapply(allFits, function(modelFit) {if (!is.null(modelFit))
  {vcov(modelFit)} else {NULL}})
  vcovList2 <- compact(vcovList) # removing NULL elements
  vcMat <- do.call("adiag", vcovList2)
  return(list(data = data.frame(longData, Resp = coefVec),
              vcovMat = vcMat))
}
updateStep1Data <- function(allFits, groupVars, tVal = NULL)
{
  parmsWide <- paramToWide(allFits, tVal)
  parmsLong <- paramToLong(allFits, groupVars, parmsWide)
  return(list(fits = allFits, wide = parmsWide, long = parmsLong))
}
makeStep1Data <- function(GerVar, StartVar, EndVar, drmFct, fullData, groupVars,
                          tVal = NULL, plotFit = FALSE)
{
  fitFct <- function(dataSet)
```

```
  {
    modelFit <- try(drm(as.formula(paste(GerVar, "~", StartVar, "+", EndVar)),
                        data = dataSet,
                        fct = drmFct,
                        type = "event"), silent = TRUE)
    if (inherits(modelFit, "try-error")) {modelFit <- NULL}
    if (plotFit & !is.null(modelFit)) {plot(modelFit)}
    return(modelFit)
  }
  allFits <- dlply(fullData, groupVars, fitFct)
  updateStep1Data(allFits)
}
#Defining helper functions for plotting after Step 2
makeRawData <- function(subSet, subexVar, startVar, gerVar)
{
  #tempD <- subset(dataSet, Treat == "GA3.0")

  obsMat <- ddply(subSet, subexVar,
                  function(x) data.frame(Time = x[[startVar]],
                                         Ger = head(c(0,
cumsum(x[[gerVar]])/sum(x[[gerVar]]) ), -1)))
               retMat <- ddply(obsMat[order(obsMat[, 2]), ], "Time",
                           function(x){mean(x[["Ger"]])})
  colnames(retMat) <- c("Time", "Germinated")
  return(retMat)
}
makePlotData <- function(modelFit, groupLevel, timeRan) # works only for LL.2() and
LL.3() models
{
  coefVec <- coef(summary(modelFit))[, 1]
  selInd <- regexpr(groupLevel, row.names(coef(summary(modelFit)))) > 0
  coef1 <- coefVec[selInd]
  lenCoef <- length(coef1)
  parNames <- c("b", "d", "e")
  names(coef1) <- parNames[1:lenCoef]
  if (lenCoef<3) {names(coef1) <- parNames[c(1, 3)]} else {names(coef1) <-
parNames}
  vcMat1 <- vcov(modelFit)[selInd, selInd]

  # Generating fitted values and corresponding 95% confidence intervals
```

```
  # (based on a grid on size 100)
  timeVec <- seq(timeRan[1], timeRan[2], length.out = 100)
  resultsMat <- matrix(NA, 100, 5)
  if (lenCoef<3) {denomPart <- "1/(1+("} else {denomPart <- "d/(1+("}
  for (i in 1:100)
  {
    resultsMat[i, ] <-
      c(timeVec[i], as.vector(unlist(deltaMethod(coef1, paste(denomPart,
                                                              timeVec[i],
                                                              "/e)^b)"), vcMat1))))
  }
  colnames(resultsMat) <- c("Time", "Fitted", "SE", "LCI", "UCI")
  resultsMat <- as.data.frame(resultsMat)
  return(resultsMat)
}
#load data
mydata<-read.csv("2019hypothesisTest_prelim.full.csv", header =TRUE)
head(mydata)
tail(mydata)
#-----------------------------------------------
#step 1
#Fitting event-time models in a loop:
  mydata.step1 <- makeStep1Data("Germinated", "Start", "End",
                                LL.3(), mydata,
                                c("Treat", "Style", "Replicate"))

  #Looking at the output: The parameter estimates for the top 6 rows:
  dim(mydata.step1[["wide"]])
  head(mydata.step1[["wide"]])

 # Finding the treatment combinations not resulting in a model fit:
  options(max.print=1000000)
  mydata.step1[["wide"]][is.na(mydata.step1[["wide"]][, "b.est"]), ]

  (mydata.step1[["wide"]])
 #  Looking at the data for the the combinations where no fits were obtained.
  # all seeds germinated, need LL.2 :
  subset(mydata,Treat == "15GA" & Style == "S" & Replicate == 2)
```

```
  subset(mydata,Treat == "2030KN" & Style == "S" & Replicate == 2)


     # Manual fitting of the above using a two-parameter log-logistic model:
      mydata.step1.15GA.S.2 <- drm(Germinated ~ Start + End,
    data = subset(mydata, Treat == "15GA" & Style == "S" & Replicate == 2),
          fct = LL.2(),
          type = "event")
    plot(mydata.step1.15GA.S.2)
    summary(mydata.step1.15GA.S.2)


    mydata.step1.2030KN.S.2 <- drm(Germinated ~ Start + End,
                                   data = subset(mydata, Treat == "2030KN" & Style ==
"S" & Replicate == 2),
                                   fct = LL.2(),
                                   type = "event")
    plot(mydata.step1.2030KN.S.2)
    summary(mydata.step1.2030KN.S.2)


    #Adding the model fits from the manual fittings to the list of model fits from
the automated run:
    mydata.step1[["fits"]][["15GA.S.2"]] <- mydata.step1.15GA.S.2
    mydata.step1[["fits"]][["2030KN.S.2"]] <- mydata.step1.2030KN.S.2


    mydata.step2 <- updateStep1Data(mydata.step1[["fits"]],
                                        c("Treat", "Style", "Replicate"))
    #extracting data for step 2
    mydata.long <- mydata.step2[["long"]]
    names(mydata.long)


 # Showing the top lines of the data in the long format:
    mydata.long.data <- mydata.long[["data"]]
    dim(mydata.long.data)
    head(mydata.long.data, 20)
#-----------------------------------------
   #step 2
    #Defining an explicit three-way interaction term:
      mydata.long.data[["TreatStyleParm"]] <-
      with(mydata.long.data, interaction(Treat, Style, Parm))
```

```
     # Fitting the model:
        mydata.meta1 <- rma.mv(Resp, V = mydata.long[["vcovMat"]],
                               mods = ~ TreatStyleParm-1,
                                random = list(~Parm|Treat, ~ Parm|Replicate),
                                 data = mydata.long.data,
                                 struct = "UN")
             summary(mydata.meta1)
plot(fitted(mydata.meta1), residuals(mydata.meta1, type = "rstandard")) # residual
plot
        qqnorm(residuals(mydata.meta1, type = "rstandard")) # QQ plot
# ------------------------------------------------------------
#Targeted pairwise comparisons
#Comparisons for the parameter b:
        targetedPairWiseComp.b1 <-
          c("
            TreatStyleParm15GA.S.b - TreatStyleParm15GA.NS.b = 0",
            "TreatStyleParm15KG.S.b - TreatStyleParm15KG.NS.b = 0",
            "TreatStyleParm2WKS.S.b - TreatStyleParm2WKS.NS.b = 0",
            "TreatStyleParm2WKKN.S.b - TreatStyleParm2WKKN.NS.b = 0",
            "TreatStyleParm2WKGA.S.b - TreatStyleParm2WKGA.NS.b = 0",
            "TreatStyleParm2WKKG.S.b - TreatStyleParm2WKKG.NS.b = 0",
            "TreatStyleParm4WKS.S.b - TreatStyleParm4WKS.NS.b = 0",
            "TreatStyleParm4WKKN.S.b - TreatStyleParm4WKKN.NS.b = 0",
            "TreatStyleParm4WKGA.S.b - TreatStyleParm4WKGA.NS.b = 0",
            "TreatStyleParm4WKKG.S.b - TreatStyleParm4WKKG.NS.b = 0",
            "TreatStyleParm2030C.S.b  - TreatStyleParm2030C.NS.b = 0",
            "TreatStyleParm2030S.S.b  - TreatStyleParm2030S.NS.b = 0",
            "TreatStyleParm2030KN.S.b  - TreatStyleParm2030KN.NS.b = 0",
            "TreatStyleParm2030GA.S.b  - TreatStyleParm2030GA.NS.b = 0",
            "TreatStyleParm2030KG.S.b - TreatStyleParm2030KG.NS.b = 0")

        mydata.targeted.pairwise.b1 <- glht(mydata.meta1,
                                            linfct = targetedPairWiseComp.b1)
        summary(mydata.targeted.pairwise.b1, test = adjusted("hochberg"))
        confint(mydata.targeted.pairwise.b1, calpha = 1.96)

        #Comparisons for the parameter d:
        targetedPairWiseComp.d1 <-
          c("
```

```
    TreatStyleParm15GA.S.d - TreatStyleParm15GA.NS.d = 0",
    "TreatStyleParm15KG.S.d - TreatStyleParm15KG.NS.d = 0",
    "TreatStyleParm2WKS.S.d - TreatStyleParm2WKS.NS.d = 0",
    "TreatStyleParm2WKKN.S.d - TreatStyleParm2WKKN.NS.d = 0",
    "TreatStyleParm2WKGA.S.d - TreatStyleParm2WKGA.NS.d = 0",
    "TreatStyleParm2WKKG.S.d - TreatStyleParm2WKKG.NS.d = 0",
    "TreatStyleParm4WKS.S.d - TreatStyleParm4WKS.NS.d = 0",
    "TreatStyleParm4WKKN.S.d - TreatStyleParm4WKKN.NS.d = 0",
    "TreatStyleParm4WKGA.S.d - TreatStyleParm4WKGA.NS.d = 0",
    "TreatStyleParm4WKKG.S.d - TreatStyleParm4WKKG.NS.d = 0",
    "TreatStyleParm2030C.S.d  - TreatStyleParm2030C.NS.d = 0",
    "TreatStyleParm2030S.S.d  - TreatStyleParm2030S.NS.d = 0",
    "TreatStyleParm2030KN.S.d  - TreatStyleParm2030KN.NS.d = 0",
    "TreatStyleParm2030GA.S.d  - TreatStyleParm2030GA.NS.d = 0",
    "TreatStyleParm2030KG.S.d - TreatStyleParm2030KG.NS.d = 0")

mydata.targeted.pairwise.d1 <- glht(mydata.meta1,
                                    linfct = targetedPairWiseComp.d1)
summary(mydata.targeted.pairwise.d1, test = adjusted("hochberg"))
confint(mydata.targeted.pairwise.d1, calpha = 1.96)

#Comparisons for the parameter t50:
targetedPairWiseComp.e1 <-
  c("
    TreatStyleParm15GA.S.e - TreatStyleParm15GA.NS.e = 0",
    "TreatStyleParm15KG.S.e - TreatStyleParm15KG.NS.e = 0",
    "TreatStyleParm2WKS.S.e - TreatStyleParm2WKS.NS.e = 0",
    "TreatStyleParm2WKKN.S.e - TreatStyleParm2WKKN.NS.e = 0",
    "TreatStyleParm2WKGA.S.e - TreatStyleParm2WKGA.NS.e = 0",
    "TreatStyleParm2WKKG.S.e - TreatStyleParm2WKKG.NS.e = 0",
    "TreatStyleParm4WKS.S.e - TreatStyleParm4WKS.NS.e = 0",
    "TreatStyleParm4WKKN.S.e - TreatStyleParm4WKKN.NS.e = 0",
    "TreatStyleParm4WKGA.S.e - TreatStyleParm4WKGA.NS.e = 0",
    "TreatStyleParm4WKKG.S.e - TreatStyleParm4WKKG.NS.e = 0",
    "TreatStyleParm2030C.S.e  - TreatStyleParm2030C.NS.e = 0",
    "TreatStyleParm2030S.S.e  - TreatStyleParm2030S.NS.e = 0",
    "TreatStyleParm2030KN.S.e  - TreatStyleParm2030KN.NS.e = 0",
    "TreatStyleParm2030GA.S.e  - TreatStyleParm2030GA.NS.e = 0",
```

```
            "TreatStyleParm2030KG.S.e - TreatStyleParm2030KG.NS.e = 0")

        mydata.targeted.pairwise.e1 <- glht(mydata.meta1,
                                            linfct = targetedPairWiseComp.e1)
        summary(mydata.targeted.pairwise.e1, test = adjusted("none"))
        confint(mydata.targeted.pairwise.e1, calpha = 1.96)

        # ------------------------------------------------------------
#Extracting the relevant information for plotting accumulated observed data
                mydata.rawdata.2030C.S <-
          makeRawData(subset(mydata, Treat == "2030C"& Style== "S"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.2030C.S)
        mydata.rawdata.2030C.NS <-
          makeRawData(subset(mydata, Treat == "2030C" & Style=="NS"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.2030C.NS)
        #Extracting the relevant information for plotting
 mydata.plotdata.2030C.S <- makePlotData(mydata.meta1, "2030C.S", c(1, 28))
        head(mydata.plotdata.2030C.S)
mydata.plotdata.2030C.NS <- makePlotData(mydata.meta1, "2030C.NS", c(1, 28))
        head(mydata.plotdata.2030C.NS)
#Extracting the relevant information for plotting accumulated observed data
         mydata.rawdata.2030S.S <-
          makeRawData(subset(mydata, Treat == "2030S"& Style== "S"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.2030S.S)
        mydata.rawdata.2030S.NS <-
          makeRawData(subset(mydata, Treat == "2030S" & Style=="NS"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.2030S.NS)
        #Extracting the relevant information for plotting
 mydata.plotdata.2030S.S <- makePlotData(mydata.meta1, "2030S.S", c(1, 28))
        head(mydata.plotdata.2030S.S)
mydata.plotdata.2030S.NS <- makePlotData(mydata.meta1, "2030S.NS", c(1, 28))
        head(mydata.plotdata.2030S.NS)
        #Extracting the relevant information for plotting accumulated observed data
         mydata.rawdata.2030KN.S <-
```

```
  makeRawData(subset(mydata, Treat == "2030KN"& Style== "S"),
              "Replicate", "Start", "Germinated")
head(mydata.rawdata.2030KN.S)
mydata.rawdata.2030KN.NS <-
  makeRawData(subset(mydata, Treat == "2030KN" & Style=="NS"),
              "Replicate", "Start", "Germinated")
head(mydata.rawdata.2030KN.NS)
#Extracting the relevant information for plotting
mydata.plotdata.2030KN.S <- makePlotData(mydata.meta1, "2030KN.S", c(1, 28))
head(mydata.plotdata.2030KN.S)
mydata.plotdata.2030KN.NS <- makePlotData(mydata.meta1, "2030KN.NS", c(1,
28))
head(mydata.plotdata.2030KN.NS)
#Extracting the relevant information for plotting accumulated observed data
  mydata.rawdata.2030GA.S <-
  makeRawData(subset(mydata, Treat == "2030GA"& Style== "S"),
              "Replicate", "Start", "Germinated")
head(mydata.rawdata.2030GA.S)
mydata.rawdata.2030GA.NS <-
  makeRawData(subset(mydata, Treat == "2030GA" & Style=="NS"),
              "Replicate", "Start", "Germinated")
head(mydata.rawdata.2030GA.NS)
#Extracting the relevant information for plotting         mydata.plotdata.2030GA.S
<- makePlotData(mydata.meta1, "2030GA.S", c(1, 28))
head(mydata.plotdata.2030GA.S)
mydata.plotdata.2030GA.NS <- makePlotData(mydata.meta1, "2030GA.NS", c(1,
28))
head(mydata.plotdata.2030GA.NS)
#Extracting the relevant information for plotting accumulated observed data
 mydata.rawdata.2030KG.S <-
  makeRawData(subset(mydata, Treat == "2030KG"& Style== "S"),
              "Replicate", "Start", "Germinated")
head(mydata.rawdata.2030KG.S)
mydata.rawdata.2030KG.NS <-
  makeRawData(subset(mydata, Treat == "2030KG" & Style=="NS"),
              "Replicate", "Start", "Germinated")
head(mydata.rawdata.2030KG.NS)
#Extracting the relevant information for plotting
mydata.plotdata.2030KG.S <- makePlotData(mydata.meta1, "2030KG.S", c(1, 28))
head(mydata.plotdata.2030KG.S)
```

```
        mydata.plotdata.2030KG.NS <- makePlotData(mydata.meta1, "2030KG.NS", c(1,
28))
        head(mydata.plotdata.2030KG.NS)
#Extracting the relevant information for plotting accumulated observed data
        mydata.rawdata.15GA.S <-
          makeRawData(subset(mydata, Treat == "15GA"& Style== "S"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.15GA.S)
        mydata.rawdata.15GA.NS <-
          makeRawData(subset(mydata, Treat == "15GA" & Style=="NS"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.15GA.NS)
        #Extracting the relevant information for plotting
  mydata.plotdata.15GA.S <- makePlotData(mydata.meta1, "15GA.S", c(1, 28))
        head(mydata.plotdata.15GA.S)
 mydata.plotdata.15GA.NS <- makePlotData(mydata.meta1, "15GA.NS", c(1, 28))
        head(mydata.plotdata.15GA.NS)
#Extracting the relevant information for plotting accumulated observed data
        mydata.rawdata.15KG.S <-
          makeRawData(subset(mydata, Treat == "15KG"& Style== "S"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.15KG.S)
        mydata.rawdata.15KG.NS <-
          makeRawData(subset(mydata, Treat == "15KG" & Style=="NS"),
                      "Replicate", "Start", "Germinated")
        head(mydata.rawdata.15KG.NS)
        #Extracting the relevant information for plotting
   mydata.plotdata.15KG.S <- makePlotData(mydata.meta1, "15KG.S", c(1, 28))
        head(mydata.plotdata.15KG.S)
 mydata.plotdata.15KG.NS <- makePlotData(mydata.meta1, "15KG.NS", c(1, 28))
        head(mydata.plotdata.15KG.NS)
     #---------------------------------------
        #Visualization
        #Rendering the plot with fitted curves using "ggplot2":
        mydata.plot1 <- ggplot(mydata, aes(x = End, y = Germinated)) +
          coord_cartesian(xlim = c(0, 28), ylim = c(0, 1)) +
          xlab("Time (days)") + ylab("Proportion germinated") +
          theme_bw() + ## white background
 #20/30 KNO3 S
```

```
          geom_line(data = mydata.plotdata.2030KN.S, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid") +
          #geom_ribbon(data = mydata.plotdata.2030KN.S, # confidence band
                     # aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
                      #alpha = 0.2)+
 # 20/30C.KNO3 NS
          geom_line(data = mydata.plotdata.2030KN.NS, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid", colour = "red")# +
          #geom_ribbon(data = mydata.plotdata.2030KN.NS, # confidence band
                     # aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
           #alpha = 0.2)
        mydata.plot1
        #Adding the cumulated observed data:
      mydata.plot2 <- mydata.plot1 +
          geom_point(data = mydata.rawdata.2030KN.S,
                     aes(x = Time, y = Germinated)) +
          geom_point(data = mydata.rawdata.2030KN.NS,
                     aes(x = Time, y = Germinated), colour="red")+
          theme(legend.position = "none")
        mydata.plot2
   #Adding text to the plot using the plotmath functionality in R:
        mydata.plot3 <- mydata.plot2 +
          annotate("text", x = 22, y = 0.70, label = "20/30C KNO3 with Style") +
          annotate("text", x = 22, y = 0.25,  label = "20/30C KNO3 no Style",
colour = "red")
          mydata.plot3
    #Rendering the plot with fitted curves using ""ggplot2":
        mydata.plot4 <- ggplot(mydata, aes(x = End, y = Germinated)) +
          coord_cartesian(xlim = c(0, 28), ylim = c(0, 1)) +
          xlab("Time (days)") + ylab("Proportion germinated") +
          theme_bw() + ## white background


#15 GA S
          geom_line(data = mydata.plotdata.15GA.S, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid") +
          #geom_ribbon(data = mydata.plotdata.15GA.S, # confidence band
                     # aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
```

```
                    # alpha = 0.2)+
# 15C.GA NS
          geom_line(data = mydata.plotdata.15GA.NS, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid", colour = "red")# +
          #geom_ribbon(data = mydata.plotdata.15GA.NS, # confidence band
                     # aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
                      #alpha = 0.2)
        mydata.plot10
        #Adding the cumulated observed data:
        mydata.plot11 <- mydata.plot10 +
          geom_point(data = mydata.rawdata.15GA.S,
                     aes(x = Time, y = Germinated)) +
          geom_point(data = mydata.rawdata.15GA.NS,
                     aes(x = Time, y = Germinated), colour="red")+
          theme(legend.position = "none")
        mydata.plot11
        #Adding text to the plot using the plotmath functionality in R :
        mydata.plot12 <- mydata.plot11 +
          annotate("text", x = 22, y = 0.95, label = "15C GA3 with Style") +
          annotate("text", x = 22, y = 0.45,  label = "15C GA3 no Style", colour =
"red")
        mydata.plot12
        #Rendering the plot with fitted curves using ""ggplot2":
        mydata.plot13 <- ggplot(mydata, aes(x = End, y = Germinated)) +
          coord_cartesian(xlim = c(0, 28), ylim = c(0, 1)) +
          xlab("Time (days)") + ylab("Proportion germinated") +
          theme_bw() + ## white background


 #20/30 C S
          geom_line(data = mydata.plotdata.2030C.S, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid") +
          #geom_ribbon(data = mydata.plotdata.2030C.S, # confidence band
                      #aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
                      #alpha = 0.2)+
          # 20/30C.C NS
          geom_line(data = mydata.plotdata.2030C.NS, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid", colour = "red") #+
```

```
          #geom_ribbon(data = mydata.plotdata.2030C.NS, # confidence band
                       #aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
                       #alpha = 0.2)
        mydata.plot16
      #Adding the cumulated observed data:
        mydata.plot17 <- mydata.plot16 +
          geom_point(data = mydata.rawdata.2030C.S,
                     aes(x = Time, y = Germinated)) +
          geom_point(data = mydata.rawdata.2030C.NS,
                     aes(x = Time, y = Germinated), colour="red")+
          theme(legend.position = "none")
        mydata.plot17
        #Adding text to the plot using the plotmath functionality in R :
        mydata.plot18 <- mydata.plot17 +
          annotate("text", x = 22, y = 0.75, label = "20/30C Control with Style") +
          annotate("text", x = 22, y = 0.20,  label = "20/30C control no Style",
colour = "red")
        mydata.plot18
        #Rendering the plot with fitted curves using ""ggplot2":
        mydata.plot19 <- ggplot(mydata, aes(x = End, y = Germinated)) +
          coord_cartesian(xlim = c(0, 28), ylim = c(0, 1)) +
          xlab("Time (days)") + ylab("Proportion germinated") +
          theme_bw() + ## white background
 #20/30 S. S
          geom_line(data = mydata.plotdata.2030S.S, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid") +
       # confidence band  geom_ribbon(data = mydata.plotdata.2030S.S,
                     # aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
                     # alpha = 0.2)+
 # 20/30C.S.NS
          geom_line(data = mydata.plotdata.2030S.NS, aes(x = Time, y = Fitted), #
fitted curve
                    linetype = "solid", colour = "red") #+
         # geom_ribbon(data = mydata.plotdata.2030S.NS, # confidence band
          #            aes(x = Time, y = Fitted, ymin = LCI, ymax = UCI),
           #           alpha = 0.2)
        mydata.plot19
        #Adding the cumulated observed data:
       mydata.plot20 <- mydata.plot19 +
```

```
          geom_point(data = mydata.rawdata.2030S.S,
                     aes(x = Time, y = Germinated)) +
          geom_point(data = mydata.rawdata.2030S.NS,
                     aes(x = Time, y = Germinated), colour="red")+
          theme(legend.position = "none")
        mydata.plot20
        #Adding text to the plot using the plotmath functionality in R :
        mydata.plot21 <- mydata.plot20 +
          annotate("text", x = 20, y = 0.85, label = "20/30C Scarification with
Style") +
          annotate("text", x = 22, y = 0.25,  label = "20/30C Scarification no
Style", colour = "red")
        mydata.plot21
#plots with significant d parameter differences
ggarrange(mydata.plot12,mydata.plot18,mydata.plot21,mydata.plot3,
           ncol=2, nrow=2)
#-----------------------------------------------------
```

R code for binomial regression, Chapter 3

```
#experiment 1, germinability
library(tidyverse)
library(caret)
library(aod)#
library(effects)#visualization
library(ggplot2)
library(stargazer)
# load my data
mydata<-read.csv("binomial_prelim_germ_only_S3.csv", header =TRUE)
head(mydata)
tail(mydata)
#descriptive stats
xtabs(~Germination + Treatment, data = mydata)
str(mydata)
# Converting to factor variables
mydata$Treatment <- as.factor(mydata$Treatment)
mydata$Germination <- as.factor(mydata$Germination)
str(mydata)
#set reference level for treatment
```

```
mydata$Treatment=relevel(mydata$Treatment, ref="2WK.C")

#fit model
model<-glm(Germination~ Treatment,family=binomial(link='logit'), data=mydata)
summary(model)
stargazer(model, type="text", out="logit.htm")

wald.test(b=coef(model), Sigma = vcov(model),Terms=2:18)
#P-value=0, Treatment effect is significant

# To test differences in coefficients for different levels
Prepare the comparison code between the 2nd and 3rd terms of the model
l <- cbind(0, 1, -1, 0,0,0,0,0, 0, 0,0,0,0,0,0,0,0)
# Run wald test to see whether 15G is equal to 15KG
wald.test(b = coef(model), Sigma = vcov(model), L = l)

# odds ratios
exp(coef(model))
#odds ratios and 95% CI
exp(cbind(OR = coef(model), confint(model)))

# Make predictions
probabilities <- model %>% predict(mydata, type = "response")
probabilities

#model fit
with(model, null.deviance - deviance)
# The degrees of freedom for the difference between the two models can be obtained
using:
with(model, df.null - df.residual)
# p-value can be obtained using:
with(model, pchisq(null.deviance - deviance, df.null - df.residual, lower.tail =
FALSE))

#test for multicollinearity
car::vif(model)

#plot
plot(allEffects(model), main='',
```

```
     ylab="Germination (probability)")
#-----------------------------------
```

R code for multinomial regression Chapter 3

```
# for germinability, decay and dormancy, Experiment 2, (ultimate seed status)
require(nnet)
require(ggplot2)
require(reshape2)
library(stargazer)
library(effects)
# load my data
mydata<-read.csv("multinomial_repeat_germ_StyleOnly_LowOut.csv", header =TRUE)
head(mydata)
tail(mydata)

#descriptive stats
with(mydata, table(Treatment, Status))

# Converting to factor variables
mydata$Treatment <- as.factor(mydata$Treatment)
mydata$Status <- as.factor(mydata$Status)
str(mydata)

#set reference level for treatment
mydata$Treatment=relevel(mydata$Treatment, ref="2WK.C")
#run only intercept model
OIM<-multinom(Status~1, data=mydata)
summary(OIM)

#run model
test <- multinom(Status ~ Treatment, data = mydata)
summary(test)
#lower AIC than OIM
#D is baseline outcome
#The multinom function does not include p-value calculation for the regression
coefficients;
#you can get significance of the coefficients using the stargazer() function from
the package stargazer.
stargazer(test, type="text", out="test.htm")
```

```
## extract the coefficients from the model and exponentiate; log odds relative to
baseline

test.rrr = exp(coef(test))

test.rrr

stargazer(test, type="text", coef=list(test.rrr), p.auto=FALSE, out="test.htm")


#Compare test with OIM

anova(OIM,test)

# predicted probabilities

head(pp <- fitted(test), 3000)

pp

#plot

plot(allEffects(test), main='')

#----------------------------------------
```

R code for binomial logistic regression, Chapter 4

```
#aerial seed bank germination/dormancy binomial

library(tidyverse)

library(caret)

library(aod)

library(arm)

library(DHARMa)


#load my data

mydata<-read.csv("binomial_aerial_germdorm7.csv", header =TRUE)

head(mydata)

tail(mydata)

# Converting to factor variables

mydata$Date <- as.factor(mydata$Date)

mydata$Treatment <- as.factor(mydata$Treatment)

mydata$Population <- as.factor(mydata$Population)

mydata$Germ <- as.factor(mydata$Germ)


#set reference level for treat

mydata$Treatment=relevel(mydata$Treatment, ref="Dark")

#set reference level for date

mydata$Date=relevel(mydata$Date, ref="5.20.")
```

```
#null model
logit.nullG <- glm(Germ~ 1, data=mydata, family=binomial(link='logit'))
summary(logit.nullG)

#fit model
modelG<-glm(Germ~ Treatment + Date +Population ,family=binomial(link='logit'),
data=mydata)
summary(modelG)
#all interactions--lower AIC, but with giant SEs and no significant terms
model2G<-glm(Germ~ Treatment *Population * Date ,family=binomial(link='logit'),
data=mydata)
summary(model2G)

####only 2-way interactions###### also lower AIC, but giant SEs
model2aG<-glm(Germ~ Treatment +Population + Date + Treatment:Population +
Treatment:Date + Population: Date ,family=binomial(link='logit'), data=mydata)
summary(model2aG)
#compare models--
anova(logit.nullG,modelG,model2G, model2aG, test="Chisq")

#test for multicollinearity
car::vif(modelG)
#goodness of fit main effects model
ouputmodelG<-simulateResiduals(modelG,plot = T)

#analysis of deviance
anova(modelG, test="Chisq")

#fitted averages
avefit.modelG <-invlogit(coef(modelG))#average fitted values for terms (proportion
chance of occurrence)
avefit.modelG
confint.modelG <- invlogit(confint(modelG))# 95 ci
confint.modelG

#Overall effect of treatment, pkg AOD
wald.test(b=coef(modelG), Sigma = vcov(modelG),Terms=2:3)
#P-value=0.0; Treatment effect is significant
```

```
#overall effect of date
wald.test(b=coef(modelG), Sigma = vcov(modelG),Terms=4:9)
#P-value=0***, Treatment effect is significant
# To test differences in coefficients for different levels of Date
#Prepare the comparison code between the xth and yth terms of the model
l <- cbind(0, 0, 0, 1,-1,0,0,0, 0, 0)
# Run wald test to see whether 6.20 is equal to 2021.05.
wald.test(b = coef(modelG), Sigma = vcov(modelG), L = l)
#-----------------------------------------
```

R code for ANOVA, Chapter 5

```
# burial, experiment 1
potent <-read.csv("AllBurial_Viability_empty_clean.csv")
head(potent)
#convert numbers to factors
potent$depth<-as.factor(potent$depth)
potent$site<-as.factor(potent$site)
potent$population<-as.factor(potent$population)
potent$retrieval_time<-as.factor(potent$retrieval_time)
str(potent)

#full interaction model
model<-aov(potent$prop_alive ~
potent$retrieval_time*potent$site*potent$depth*potent$population, data = potent)
summary(model)
#residuals
par(mfrow=c(2,2))
plot(model)
#no interactions
modela<-aov(potent$prop_alive ~
potent$retrieval_time+potent$site+potent$depth+potent$population, data = potent)
summary(modela)
#residuals
par(mfrow=c(2,2))
plot(modela)
vis=residuals(model)
hist(vis, main="Histogram of residuals")
plot(density(vis),main="Density plot of residuals",ylab="Density",xlab="Residuals")
# see if the assumption of homoscedasticity is met#Breusch-Pagan Test.
```

```
library(lmtest)

bptest(model)#no

bptest(modela)

library(bestNormalize)

(BNpotent <- bestNormalize(potent$prop_alive))

plot(BNpotent)

potent$prop_alive.t <-BNpotent$x.t

#interaction

#normal quantile transformation

model4<-aov(prop_alive.t ~
potent$retrieval_time*potent$site*potent$depth*potent$population, data = potent)

summary(model4)

#resids

par(mfrow=c(2,2))

plot(model4)

#no interactions

model5<-aov(prop_alive.t ~ retrieval_time+site+depth+population, data = potent)

summary(model5)

#resids

par(mfrow=c(2,2))

plot(model5)

#lower order interactions

model6<-aov(prop_alive.t ~
potent$retrieval_time+potent$site+potent$depth+potent$population+


potent$retrieval_time:potent$site+potent$site:potent$depth+potent$depth:potent$popu
lation+


potent$retrieval_time:potent$depth+potent$retrieval_time:potent$population+potent$s
ite:potent$population,

            data = potent)

summary(model6)

#resids

par(mfrow=c(2,2))

plot(model6)


#best fit

library(AICcmodavg)

model.set <- list(model4, model5, model6)#no interaction (combination model) best

model.names <- c( "model4", "model5", "model6")

aictab(model.set, modnames = model.names)
```

```
options(max.print=100000)

#post hoc

tukey.model5<-TukeyHSD(model5)

tukey.model5

par(mfrow=c(1,1))

par(mar = c(8, 8, 8, 8)) # Set the margin on all sides

plot(tukey.model5, las=1)

#-----------------------------------------------
```

R code for negative binomial and Poisson regression, Chapter 6

```
#TL, Experiment 2

library(lme4)

mydata <-read.csv("TreelineRepeat_grass_comp_nd.csv")

head(mydata)

#Time1

T1<-subset(mydata, time==1)

T1

hist(T1$survival)


m<- glmer(survival ~ treatment+ (1|block), na.action = na.omit,family = poisson,
data = T1)

summary(m)#no singularity,but overdispersion--model overfitted

#block removed from model

mf<- glm(survival ~ treatment, na.action = na.omit,family = poisson, data = T1)

summary(mf)#even more overdispersed

m_0 <- update(m, .~1+(1|block))

summary(m_0)


#comparison intercept only and predictor

AIC(m, m_0)#predictor best


m.nb <- glmer.nb(survival ~ treatment + (1|block),  na.action = na.omit,data=T1,
verbose=TRUE)

summary(m.nb)

m.nb_0 <- update(m.nb, .~1+(1|block))

summary(m.nb_0)#singular

#comparison intercept only and predictor

AIC(m, m.nb)#nb best

AIC(m.nb, m.nb_0)#int best
```

```
library(DHARMa)
output<-simulateResiduals(m, plot=T)
testDispersion(output)#overdispersion and outliers
output<-simulateResiduals(m.nb, plot=T)
testDispersion(output)#good, but singular

#posthoc
library(multcomp)
summary(glht(m.nb, mcp(treatment="Tukey")))

#Time4
T4<-subset(mydata, time==4)
T4
hist(T4$survival)
survivalT4<- T4$survival

m4 <- glmer(survival ~ treatment + (1|block), na.action = na.omit, family =
poisson, data = T4)
summary(m4)
m4_0 <- update(m4, .~1+(1|block))
summary(m4_0)#failed to converge
#comparison intercept only and predictor
AIC(m4, m4_0)#predictor better
m4.nb <- glmer.nb(survival ~ treatment + (1|block), na.action = na.omit, data=T4,
verbose=TRUE)
m4.nb#iteration limit reached
#comparison intercept only and predictor
AIC(m4, m4.nb)#m4 better

output<-simulateResiduals(m4, plot=T)
testDispersion(output)
#posthoc
summary(glht(m4, mcp(treatment="Tukey")))

#Time5
T5<-subset(mydata, time==5)
T5
hist(T5$survival)
```

```
m5<- glmer(survival ~ treatment + (1|block), na.action = na.omit, family = poisson,
data = T5)

summary(m5)#isSingular

m5_0 <- update(m5, .~1+(1|block))

summary(m5_0)

#comparison intercept only and predictor

AIC(m5, m5_0)#predictor best


m5.nb <- glmer.nb(survival ~ treatment + (1|block), data=T5, verbose=TRUE)

summary(m5.nb)#isSingular

#comparison nb and poisson

AIC(m5, m5.nb)#nb better


output5.nb<-simulateResiduals(m5.nb, plot=T)

testDispersion(output5.nb)

output5<-simulateResiduals(m5, plot=T)

testDispersion(output5)#overdispersed


#posthoc

summary(glht(m5.nb, mcp(treatment="Tukey")))


#Time8

T8<-subset(mydata, time==8)

T8

hist(T8$survival)


m8<- glmer(survival ~ treatment + (1|block), na.action = na.omit, family = poisson,
data = T8)

summary(m8)#isSingular

m8_0 <- update(m8, .~1+(1|block))

summary(m8_0)#isSingular

#comparison intercept only and predictor

AIC(m8, m8_0)#predictor best


m8.nb <- glmer.nb(survival ~ treatment + (1|block), data=T8, verbose=TRUE)

m8.nb#singularity

#comparison nb and poisson

AIC(m8, m8.nb)#Poisson better
```

```
output8<-simulateResiduals(m8, plot=T)
testDispersion(output8)#
#----------------------------------------
```

R code for mixed effects binomial logistic regression, Chapter 7

```
#stem regeneration at 4 months
mydata<-read.csv("frag_regen_final.inigrowth.csv")
head(mydata)
summary(mydata)
str(mydata)

# Converting to factor variables
mydata$origin <- as.factor(mydata$origin)
mydata$tray <- as.factor(mydata$tray)
mydata$type <- as.factor(mydata$type)
mydata$active.bud <- as.factor(mydata$active.bud)
#mydata$regen <- as.factor(mydata$regen)

str(mydata)
#descriptive stats
#did stem regenerate?
with(mydata, table(active.bud, regen))#almost equal
with(mydata, table(origin, regen))#

library(lme4)
#set reference level for origin
mydata$origin=relevel(mydata$origin, ref="A")

#interaction
binom1= glmer(regen ~ initial.diam * active.bud *type + (1 | origin) + (1 | tray),
data=mydata,
              na.action = na.omit,family=binomial(link='logit'))
summary(binom1)#no warnings, but when ci's are calculated, lots of warnings

binom2= glmer(regen ~ initial.diam * active.bud + type + (1 | origin) + (1 | tray),
data=mydata,
              na.action = na.omit,family=binomial(link='logit'))
summary(binom2)#isSingular, but lower AIC and better SEs
```

```
#main effects

binom2a= glmer(regen ~ initial.diam + active.bud + type + (1 | origin) + (1 |
tray), data=mydata,

              na.action = na.omit,family=binomial(link='logit'))

summary(binom2a)


#removing terms to get non singular fit--origin

binom3= glmer(regen ~ initial.diam + active.bud + type + (1|tray), data=mydata,

              na.action = na.omit,family=binomial(link='logit'), control =
glmerControl(optimizer = "bobyqa"),

              nAGQ = 100)

summary(binom3)#no singularity


#interaction with no origin

binom4= glmer(regen ~ initial.diam * active.bud *type + (1 | tray), data=mydata,

              na.action = na.omit,family=binomial(link='logit'))

summary(binom4)# no singularity, no significant terms

anova(binom3, binom4, test="Chisq")#binom3


#goodness of fit

library(DHARMa)

#binom3

outputbinom3<-simulateResiduals(binom3,plot = T)

residuals(outputbinom3)

testZeroInflation(outputbinom3)#fine

se <- diag(vcov(binom3))


# table of estimates with 95% CI

(tab3 <- cbind(Est = fixef(binom3), LL = fixef(binom3) - 1.96 * se, UL =
fixef(binom3) + 1.96 *

                 se))

#Odds ratios

exp(tab3)

#test for multicollinearity of fixed factors with car pkg

car::vif(binom3)#good


#visualizations https://lmudge13.github.io/sample_code/mixed_effects.html

library(effects)

plot(allEffects(binom3))
```

```
library(sjPlot)

# To see the values of the effect size and p-value, set show.values and show.p=
TRUE.

plot_model(binom3,

           axis.labels=c("Vertical stem", "Active bud","Initial fragment
diameter"),

           show.values=TRUE, show.p=TRUE,

           title="")


#table of output

tab_model(binom3,

          show.re.var= TRUE,

          pred.labels =c("(Intercept)", "Initial fragment diameter", "Active
initial bud",

                         "Vertical stem"),

          dv.labels= "Effects of factors on fragment regeneration")


#plot model estimates with data

#stem diameter effect

effects_diam <- effect(term= "initial.diam", mod= binom3)

summary(effects_diam)


# Save the effects values as a df:

x_diam <- as.data.frame(effects_diam)


#1

diam_plot <- ggplot() +

  #2: observed values

  #geom_point(data=mydata, aes(initial.diam, regen), color= "red") +

  #3: model estimates

  geom_point(data=x_diam, aes(x=initial.diam, y=fit), color="blue") +

  #4

  geom_line(data=x_diam, aes(x=initial.diam, y=fit), color="blue") +

  #5: CI limits

  geom_ribbon(data= x_diam, aes(x=initial.diam, ymin=lower, ymax=upper), alpha=
0.3, fill="blue") +

  #6

  labs(x="Initial stem fragment diameter (cm)", y="Predicted fragment regeneration
at 4 months")

diam_plot
```

```
#-----------------------------------
```

R code for mixed effects Poisson regression, Chapter 7

```
#prediction of shoot number

library(lme4)

mydata<-read.csv("frag_regen_final.5.csv")

head(mydata)

summary(mydata)

str(mydata)


# Converting to factor variables

#mydata$tray <- as.factor(mydata$tray)

mydata$stem <- as.factor(mydata$stem)

mydata$origin <- as.factor(mydata$origin)

mydata$type <- as.factor(mydata$type)

str(mydata)

hist(mydata$shoot.num)


#model fit both random effects

modelP<- glmer(shoot.num ~ initial.diam *type + (1|origin) + (1|tray), na.action =
na.omit,family = poisson, data = mydata)

summary(modelP)

modelPa<- glmer(shoot.num ~ initial.diam *type  + (1|tray), na.action =
na.omit,family = poisson, data = mydata, nAGQ = 100)

summary(modelPa)

#intercept model

Pa_0 <- glmer(shoot.num ~ 1+(1|tray), data=mydata, family= poisson,nAGQ = 100)

summary(Pa_0)

AIC(modelPa, Pa_0)#Pa


#no interaction

modelPb<- glmer(shoot.num ~ initial.diam + type  + (1|tray), na.action =
na.omit,family = poisson, data = mydata, nAGQ = 100)

summary(modelPb)


AIC(modelPa, modelPb)#Pb


library(DHARMa)
```

```
outputPb<-simulateResiduals(modelPb, plot=T)#qq left, resids right; plotted against
predicted values

residuals(outputPb)#

testDispersion(modelPb)

se <- diag(vcov(modelPb))


# table of estimates with 95% CI

(tabPb <- cbind(Est = fixef(modelPb), LL = fixef(modelPb) - 1.96 * se,

                UL = fixef(modelPb) + 1.96 * se))

#Odds ratios

exp(tabPb)

predict(modelPb, data= mydata, type = "response")


library(sjPlot)

plot_model(modelPb)

# Notes: axis labels should be in order from bottom to top.

# To see the values of the effect size and p-value, set show.values and show.p=
TRUE. Pvalues will only be shown if the effect size values are too


plot_model(modelPb,

                   axis.labels=c("Vertical stem", "Initial fragment diameter"),

                   show.values=TRUE, show.p=TRUE,

                   title="")

#table of output

tab_model(modelPb)


# Notes: predictor labels (pred.labels) should be listed from top to bottom;
dv.labels= the name of the response variable that will be at the top of the table.

tab_model(modelPb,

                  show.re.var= TRUE,

                  pred.labels =c("(Intercept)", "Initial fragment diameter",
"Vertical stem"),

                  dv.labels= "Effects of factors on shoots produced")


lmmod <-  lm(stem.num ~ 1 + as.factor(tray), data=mydata)

summary(lmmod)#tray 16 is only tray sig diff from others


#plot model estimates with data

library(effects)

#stem diameter effect
```

```
effects_diam <- effect(term= "initial.diam", mod= modelPb)
summary(effects_diam) #output of what the values are

# Save the effects values as a df:
x_diam <- as.data.frame(effects_diam)

#1
diam_plot <- ggplot() +
  #2: observed values
  geom_point(data=mydata, aes(initial.diam, shoot.num), color= "red") +
  #3: model estimates
  geom_point(data=x_diam, aes(x=initial.diam, y=fit), color="blue") +
  #4
  geom_line(data=x_diam, aes(x=initial.diam, y=fit), color="blue") +
  #5: CI limits
  geom_ribbon(data= x_diam, aes(x=initial.diam, ymin=lower, ymax=upper), alpha=
0.3, fill="blue") +
  #6
  labs(x="Initial stem fragment diameter (cm)", y="Predicted number of shoots
produced at 4 months")
diam_plot
#-----------------------------------
```

R code for mixed effects linear regression, Chapter 7

```
#total length of new shoots
mydata<-read.csv("frag_regen_final.4.csv")
head(mydata)
summary(mydata)
str(mydata)

# Converting to factor variables
#mydata$tray <- as.factor(mydata$tray)
mydata$stem <- as.factor(mydata$stem)
mydata$origin <- as.factor(mydata$origin)
mydata$type <- as.factor(mydata$type)
str(mydata)

#check ANOVA assumptions
hist(mydata$yield.cm)#long right tail
```

```
#transform data

library(bestNormalize)

(BNyield <- bestNormalize(mydata$yield.cm))

plot(BNyield)

mydata$yield.cm.t <-BNyield$x.t

mydata$yield.cm.t

hist(mydata$yield.cm.t)


library(lmerTest)

#fit model

#all models with interaction (with random vars) between type and diam are singular,
so not shown


frag0<-lmer(yield.cm.t ~1 +(1|origin)+ (1|tray), data=mydata)

summary(frag0)#is singular

frag1<- lmer(yield.cm.t ~ initial.diam *  type + (1|origin)+ (1|tray), data =
mydata)

summary(frag1)#is singular

frag2<-lmer(yield.cm.t ~ initial.diam +  type + (1|origin)+ (1|tray), data =
mydata)

summary(frag1)#is singular

frag3<- lmer(yield.cm.t ~ initial.diam *  type + (1|tray), data = mydata)

summary(frag3)#is singular

frag4<- lmer(yield.cm.t ~ initial.diam +  type + (1|tray), data = mydata)

summary(frag4)

#test for multicollinearity of fixed factors

library(car)

vif(frag4)#good


library(DHARMa)

output4<-simulateResiduals(frag4, plot=T)#qq left, resids right; plotted against
predicted values

residuals(output4)

testDispersion(output4)#fine


# visualize residuals and fitted values.

plot(frag4, pch=16, which=1)

se <- diag(vcov(frag4))

# table of estimates with 95% CI
```

```
(tab4 <- cbind(Est = fixef(frag4), LL = fixef(frag4) - 1.96 * se, UL = fixef(frag4)
+ 1.96 *
                  se))
#diam*type models all give singular matrices
#frag4 gives output with no singularity;
#stepwise backward elimination of terms to get best model results in all terms
#being eliminated, and intercept-only model as best model
#must mean that no model really fits data; predictors don't predict outcome well
#-----------------------------------
```

R code for Kruskal Wallis/Wilcoxon tests, Chapter 8

```
#basal herbicide expt
library(bestNormalize)
library(rstatix)
library(ggpubr)
library(broom)
library(tidyverse)

#2020.1yr
#load data
   mydata<-read.csv("2020_basalHerb.1year.2.csv", header =TRUE)
   mydata

   #compute summary stats by treatment for each outcome variable
   mydata %>%
     group_by(treat) %>%
     get_summary_stats(activenode.prop, numshoot, type = "mean_sd")
   mydata %>%
     group_by(treat) %>%
     get_summary_stats(activenode.prop, numshoot, type = "median_iqr")
   # Group the data by Treatment and then identify outliers in the Livenode.prop
variable:
   mydata %>%
     group_by(treat) %>%
     identify_outliers(activenode.prop)
   #3 outliers
     hist(mydata$activenode.prop)

   # QQ plot of Livenode.prop
```

```
  ggqqplot(mydata, "activenode.prop", facet.by = "treat",
           ylab = "Proportion live nodes", ggtheme = theme_bw())

  #check homogeneity of variances
  mydata %>%
    gather(key = "variable", value = "value", activenode.prop) %>%
    group_by(variable) %>%
    levene_test(value ~ treat) #violated

  #transform data
  (BNactivenode <- bestNormalize(mydata$activenode.prop))
  plot(BNactivenode)
  mydata$activenode.prop.t <-BNactivenode$x.t
  mydata$activenode.prop.t
  hist(mydata$activenode.prop.t)#Didn't change much

  #Kruskal-Wallis test by rank
  kruskal.test(activenode.prop ~ treat, data = mydata)

  #median
  pairwise.wilcox.test(mydata$activenode.prop, mydata$treat,
                       p.adjust.method = "BH",  exact=FALSE)
 #following has confidence intervals:
 mydata %>% wilcox_test(activenode.prop~treat,detailed=TRUE)

#plot with ci
 library(magrittr)  # enables the pipe (%>%) operator
  library(iNZightPlots)
  library(ggplot2)
  library(patchwork)
 # function to create confidence intervals
 median_cl_boot <- function(x, conf = 0.95) {
   lconf <- (1 - conf)/2
   uconf <- 1 - lconf
   require(boot)
   bmedian <- function(x, ind) median(x[ind])
   bt <- boot(x, bmedian, 1000)
   bb <- boot.ci(bt, type = "perc")
```

```
    data.frame(y = median(x), ymin = quantile(bt$t, lconf), ymax = quantile(bt$t,
                                                                            uconf))
  }
  # create the plot
  windows(6.5,6)
  basal2020.1 <- ggplot(mydata, aes(x=factor(treat,
level=c('Control','Ring','Cut','Paste')),y=activenode.prop,))+
    geom_boxplot()+
    stat_summary(fun.data = median_cl_boot, geom = "errorbar", colour = "red") +
    stat_summary(fun = median, geom = "point", colour = "red")+
    labs(x="")+ ylim(0,1)+ylab("Proportion of active nodes")+
    ggtitle("2020.1yr")+
    theme(plot.title = element_text(hjust= 0.5,vjust=-10, size = 12),
          axis.text.x = element_text(size=12), axis.text.y=element_text(size = 12),
          axis.title.y = element_text(size=12))
  basal2020.1
#-------------------------------------------
```

R code for Poisson and negative binomial regression, Chapter 8

```
#2020.1year, basal herbicide expt
library(lme4)
#load data
mydata <-read.csv("2020_basalHerb.1year.2.csv")
head(mydata)
hist(mydata$numshoot)
numshootmydata<- mydata$numshoot
numshootmydata
#boxplot with ci
library(magrittr)  # enables the pipe (%>%) operator
library(iNZightPlots)
library(ggplot2)
library(patchwork)

# function to create confidence intervals
median_cl_boot <- function(x, conf = 0.95) {
  lconf <- (1 - conf)/2
  uconf <- 1 - lconf
  require(boot)
```

```
  bmedian <- function(x, ind) median(x[ind])
  bt <- boot(x, bmedian, 1000)
  bb <- boot.ci(bt, type = "perc")
  data.frame(y = median(x), ymin = quantile(bt$t, lconf), ymax = quantile(bt$t,
                                                                          uconf))
}
# create the plot
basal2020.1 <- ggplot(mydata, aes(x=factor(treat,
level=c('Control','Ring','Cut','Paste')),y=numshoot,))+
  geom_boxplot()+
  stat_summary(fun.data = median_cl_boot, geom = "errorbar", colour = "red") +
  stat_summary(fun = median, geom = "point", colour = "red")+
  labs(x="")+ ylim(0,10)
basal2020.1


#-------------------------
library (dplyr)
#subset
#cut vs cut/paste 2020.1year
cutdata<-mydata %>% filter(treat == "Cut" | treat == "Paste")
cutdata


m1<- glmer(numshoot ~ treat + (1|block), family = poisson, data = cutdata)
summary(m1)#no warnings
m1_0 <- update(m1, .~1+(1|block))
summary(m1_0)
#comparison intercept only and predictor
AIC(m1, m1_0)#predictor


library(DHARMa)
outputcut<-simulateResiduals(m1, plot=T)#dispersion sig, outliers
testZeroInflation(outputcut)#p< 2.2e-16
#negative binomial
m1.nb <- glmer.nb(numshoot ~ treat + (1|block), data=cutdata, verbose=TRUE)
m1.nb#issingular
#removing block
glm1.nb <- glm.nb(numshoot ~ treat , data=cutdata)
summary(glm1.nb)#no warnings
```

```
outputcut.nb<-simulateResiduals(glm1.nb, plot=T)#levene test significant
(heterogeneity)

testZeroInflation(outputcut.nb)#p=0.464

#---------------------------------------

#control vs ring 2020.1 year

ringdata<-mydata %>% filter(treat == "Control" | treat == "Ring")

ringdata


ma<- glmer(numshoot ~ treat + (1|block), family = poisson, data = ringdata)

summary(ma)#In vcov.merMod(object, use.hessian = use.hessian) :

#variance-covariance matrix computed from finite-difference Hessian is

#not positive definite or contains NA values: falling back to var-cov estimated
from RX

m_0a <- update(ma, .~1+(1|block))

summary(m_0a)#issingular

#removing block

ma<- glm(numshoot ~ treat , family = poisson, data = ringdata)

summary(ma)

m_0a <- update(ma, .~1)

summary(m_0a)

AIC(ma, m_0a)#predictor


outputring<-simulateResiduals(ma, plot=T)#all good

testZeroInflation(outputring)#p=1


ma.nb <- glmer.nb(numshoot ~ treat + (1|block), data=ringdata, verbose=TRUE)

ma.nb#issingular

#removing block

ma.nb <- glm.nb(numshoot ~ treat, data=ringdata)

summary(ma.nb)#no warnings

outputring.nb<-simulateResiduals(ma.nb, plot=T)#all good

testZeroInflation(outputring.nb)#p=1

#-------------------------------------------

#subset

#ring vs vs cut/paste 2020 yr1

ringpastedata<-mydata %>% filter(treat == "Ring" | treat == "Paste")

ringpastedata

m2a<- glm(numshoot ~ treat , family = poisson, data = ringpastedata)

summary(m2a)
```

```
m2a_0 <- update(m2a, .~1)
summary(m2a_0)
#comparison intercept only and predictor
AIC(m2a, m2a_0)#predictor

outputringpaste<-simulateResiduals(m2a, plot=T)# dispersion, outliers
testZeroInflation(outputringpaste)#p=0.008
library(MASS)
#neg binom
m2a.nb <- glm.nb(numshoot ~ treat, data=ringpastedata)
summary(m2a.nb)#no warnings
outputringpaste.nb<-simulateResiduals(m2a.nb, plot=T)#all good
testZeroInflation(outputringpaste.nb)#p=0.992
#-------------------------------
```

R code for ANOVA and Kruskal Wallis analysis, Chapter 8

```
#foliar herbicide experiment
#load data
mydata<-read.csv("foliarherb.3.csv", header =TRUE)
mydata

#check ANOVA assumptions
hist(mydata$grounddens.delta)
hist(mydata$grassheight)
hist(mydata$OMB.dWt)

shapiro.test(mydata$grounddens.delta)#0.11
shapiro.test(mydata$grassheight)#0.18
shapiro.test(mydata$OMB.dWt)#1.55e-05

library(car)#for ANOVA analyses
library(ggpubr)#for creating easily publication ready plots
#visualization
ggboxplot(
  mydata, x = "treatment", y = c("grounddens.delta"),
  merge = TRUE, palette = "jco"
)
ggboxplot(
```

```
  mydata, x = "treatment", y = c("grassheight"),
  merge = TRUE, palette = "jco"
)
ggboxplot(
  mydata, x = "treatment", y = c("OMB.dWt"),
  merge = TRUE, palette = "jco"
)
library(broom)#for printing a nice summary of statistical tests as data frames
#compute summary stats by treatment for each outcome variable

mydata %>%
  group_by(treatment) %>%
  get_summary_stats(grounddens.delta,grassheight, OMB.dWt,type = "mean_sd")
#check sample size assumption: the n in each cell > the number of outcome
variables.
library(tidyverse)
mydata %>%
  group_by(treatment) %>%
  summarise(N = n())
#all 4
#-------------------------------------------------------------------------
#separate response variables
#grassheight
grassheight.anova<-aov(grassheight~treatment*site, data=mydata)
summary(grassheight.anova)
TukeyHSD(grassheight.anova)

#plot with conf intervals
library(magrittr)  # enables the pipe (%>%) operator
library(iNZightPlots)
library(ggplot2)
library(patchwork)

# function to create confidence intervals
median_cl_boot <- function(x, conf = 0.95) {
  lconf <- (1 - conf)/2
  uconf <- 1 - lconf
  require(boot)
  bmedian <- function(x, ind) median(x[ind])
```

```
  bt <- boot(x, bmedian, 1000)
  bb <- boot.ci(bt, type = "perc")
  data.frame(y = median(x), ymin = quantile(bt$t, lconf), ymax = quantile(bt$t,
                                                                          uconf))
}

# create the plot; to look at just treatments, no site effect, remove facet_grid(.~
site)+
windows(6.5,6)
foliar <- ggplot(mydata, aes(x=factor(treatment,
level=c('Control','met','tri','tri/pic/amino')),y=grassheight,))+
  geom_boxplot()+  facet_grid(.~ site)+
  stat_summary(fun.data = median_cl_boot, geom = "errorbar", colour = "red") +
  stat_summary(fun = median, geom = "point", colour = "red")+
  labs(x="")+ ylim(0,2 )+ ylab("Grass height (m)")+
  theme(axis.text.x = element_text(size=10), axis.text.y=element_text(size = 10),
        axis.title.y = element_text(size=10))
foliar
#-------------------------------------
#groundcover density change
grounddens.deltagrounddens.anova<-aov(grounddens.delta~treatment*site, data=mydata)
summary(grounddens.anova)
#---------------------------------------------
#OMB dry weight
# KW test is significant, but in pairwise comparisons, it isn't;
#must be because the distribution and variance of Control is very different from
the rest
#dry weight--site not used as IV
kruskal.test(OMB.dWt ~ treatment, data = mydata)
#median
#pairwise.wilcox.test(drywt$OMB.dWt, mydata$treatment,
 #p.adjust.method = "BH",  exact=FALSE)# no sig diffs???

#Scheirer-Ray-Hare test
library(rcompanion)
library(FSA)
scheirerRayHare(OMB.dWt~ treatment*site, data=mydata)
library(rstatix)
pwc.srh<-mydata%>%
  dunn_test(OMB.dWt~treatment, p.adjust.method="bonferroni")
```

```
pwc.srh#only sig diff is between control and tordon???

#-------------------------

#Dunnett's test to compare 3 treatments to a control was not possible, due to
assumptions of normality and homogeneity of variance

#removed control from analysis, and none of treatments are sig diff from each other

library (dplyr)

#subset

#all treats but control

drywt<-mydata %>% filter(treatment == "Meturon" | treatment ==
"Tordon"|treatment=="Grazon")

drywt

kruskal.test(OMB.dWt ~ treatment, data = drywt)
```