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RESEARCH ARTICLE

Viability and dormancy of the *Clematis vitalba* aerial seed bank

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ABSTRACT

- Old man's beard (*Clematis vitalba* L.) is a liana species that has become invasive in many areas of its introduced range. Seeds are produced in abundance and are both physiologically and morphologically dormant upon maturity. To understand the importance of seeds to its invasiveness, changes in viability and dormancy of the aerial seed bank were tracked throughout the after-ripening period and during storage.
- Seeds collected every second month for 2 years were subjected to germination tests. Other seeds stored in outdoor ambient conditions or in a dry, chilled state were dissected before, during, and after imbibition, as well as during incubation, to measure embryo size.
- Less than 72% of seeds on the mother plant were viable. Viable seeds remained completely morpho-physiologically dormant throughout autumn, even when treated with nitrate. Physiological dormancy declined 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 during incubation than partially or fully after-ripened seeds, which were also more germinable and less dormant. Furthermore, seeds incubated in complete darkness were more likely to decay or remain dormant than those exposed to light.
- This study demonstrates that fewer than three-quarters of seeds produced are viable and further decay occurs after dispersal, yet total fertility is still very high, with enormous propagule pressure from seeds alone. Viable seeds are protected with two forms of dormancy; morphological dormancy requires additional germination cues in order to break after seasonal changes break physiological dormancy.

INTRODUCTION

Dormant seeds are inactive but viable seeds, which act as a seed bank for potential recruitment of a species over time. 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, understanding the seed banks of an invasive species is necessary for controlling their abundance and spread. The diaspore of the invasive temperate liana, old man's beard (*Clematis vitalba* L.), is a wind-dispersed achene, which is retained on the mother plant in an aerial seed bank until release (Bungard *et al.* 1997a). In early winter, Van Gardingen (1986) estimated mean seed production of *C. vitalba* as 35,300 m^{-2}·$year^{-1}$. It is now known that each year, 50% of *C. vitalba* seeds disperse from the mother plant during autumn dormancy (Jarvis-Lowry 2023), and thus that estimate may have been only half what it should have been (70,600 m^{-2}·$year^{-1}$) because of the seeds missed due to early dispersal. A further 45% of seeds disperse 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 potential value for the invasiveness of the species.

Seeds of *C. vitalba* develop asynchronously during the summer and begin to mature and dry by mid-autumn. Asynchronous dispersal from the mother plant then takes place once seeds are fully dry. Presumably as a protection against germination under circumstances that would not allow seedling maturation (winter), mature seeds are both physiologically and morphologically dormant (Nikolaeva *et al.* 1985), and both forms of dormancy must be overcome for germination to occur. In general, physiological dormancy (PD) is often associated with seasonal conditions that suppress normal physiological processes (Bewley *et al.* 2013; Baskin & Baskin 2014; Willis *et al.* 2014; Leuschner & Ellenberg 2017). Morphological dormancy (MD) 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 & Baskin 2014; Kildisheva *et al.* 2020; Copete *et al.* 2021; Walker *et al.* 2021).

It is common for seeds to break physiological dormancy as a response to seasonal changes in shifting temperatures and daylength (Baskin & Baskin 2004; Finch-Savage & Leubner-Metzger 2006; Finch-Savage & Footitt 2017). Indeed, of *C. vitalba* seeds collected over a 6-month period between winter and spring, Lhotská (1974) reported that both speed and percentage seed germination were influenced by time of collection, with highest germinability occurring in seeds collected in mid-spring. However, it is unclear precisely how seasonal changes and germination conditions affect the development of the *C. vitalba* seed embryo, which must reach a threshold size

to break morphological dormancy. In some seeds, breaking of PD occurs before embryo growth, and in others, PD and MD are broken simultaneously (Baskin & Baskin 2014). Researchers have reported that *C. vitalba* 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 study was to ascertain how viability and physiological dormancy of seeds remaining on the vine of *C. vitalba* change between seed development and germination. Knowing the proportion of *C. vitalba* seeds in the seed bank that are viable, and how their germinability changes over time, will provide insight into the magnitude of *C. vitalba* invasiveness. In addition, supplemental work was carried out to discover if the MD of *C. vitalba* seed embryos undergoes a similar seasonal shift to that of its PD, or if seed embryos remain underdeveloped and morphologically dormant during natural and artificial storage.

MATERIAL AND METHODS

Seed material and population sources

To account for any population effect, *C. vitalba* achenes for this experiment were collected from two provenances approximately 12 km apart. The population sites were both in riparian zones, at Hopelands Reserve, near Kumeroa (S1; 40.3590° S, 175.9627° E), and at Woodville Ferry Reserve (S2; 40.3369° S, 175.8185° E) Tararua District, New Zealand. Average rainfall and growing degree-day totals above 5°C and 10°C are provided in Table 1. The population at S2 was on a treeless slope, where the lianas scrambled over long grasses. In contrast, the S1 population was moderately dense within trees, woody shrubs, and other scrambling vines, and was less exposed to sun and wind.

In the Southern Hemisphere, where this research was conducted, the spring season occurs from September to November, summer from December to February, autumn from March to May, and winter from June to August. Phenological stage ranges for *C. vitalba* in New Zealand are given in Fig. 1. 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 when 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 and collect 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; and (2) new growth began that covered the previous year's stems, obscuring persistent achenes (Figs 1 and 2A). 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 achenes of the new cohort had darkened by May, although achenes were in various stages of maturity, varying in colour from green to reddish, to dark brown (Figs 1 and 2B). This is related to differences in position on the vine (flowers produced earlier are pollinated earlier, leading to earlier seed development). By this stage, leaves had dropped from the vines, and senescence of the most mature seed heads had progressed to the point where achenes were dispersed by the wind. The darkest achenes were collected from both populations for our experiments.

By June, most *C. vitalba* stems were still largely bare of leaves, and many seed heads had lost all their achenes, especially at S2. This is consistent with monthly dispersal

Table 1. Average rainfall and growing degree-day (GDD) totals above 5°C and 10°C for Dannevirke, Tararua District, New Zealand (Chappell 2015).

	sep	oct	nov	dec	jan	feb	mar	apr	may	jun	jul	aug
Rainfall (cm)	89	101	88	82	76	82	75	75	84	99	102	82
GDD >5°C	160	219	258	339	386	351	328	234	171	107	93	111
GDD >10°C	37	75	111	185	231	210	174	90	42	17	10	13

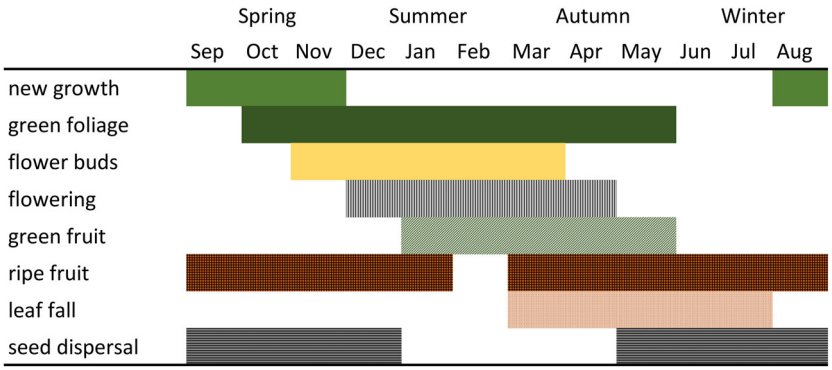

Fig. 1. *Clematis vitalba* phenology, modified from West (1992). Obtained by combining phenological stage ranges from observations of West at Taihape (North Island) and Brown River Scenic Reserve (South Island), New Zealand, observations of Van Gardingen (1986) at Christchurch (South Island), New Zealand, and observations of the current authors in the Tararua District and Manawatu Region (North Island).

Fig. 2. (A) Current year's vegetative growth covering previous year's *Clematis vitalba* achenes, January 2020. (B) *Clematis vitalba* achenes at various stages of maturity (green to reddish to dark brown and fluffy) in autumn 2021.

observations of three other *C. vitalba* populations at different sites, where 60–85% of all achenes were lost between March and the end of June 2020 (Jarvis-Lowry 2023). By September, most stems were vigorously producing new terminal and axillary growth. Less than 5% of achenes at S2 remained intact, whereas at the less exposed S1 site, closer to 20% of achenes persisted. At S2, no achenes were visible for collection from November through March. Increased exposure to wind at S2 likely resulted in earlier dispersal of achenes than at S1. Therefore, only seeds from the S1 population were tested for the full year, and the population comparison was restricted to May, June/July and September (late autumn to early spring) during the 2-year study period.

Experimental 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 found in preliminary germination experiments carried out in 2019 (Jarvis-Lowry 2023): day/night temperatures of 20°C/30°C with a corresponding 16-h dark:8-h light photoperiod. The germination trials either began immediately following collection, or after the achenes were stored dry for up to 5 days at 5°C. For each population, three replicates of 50 seeds each were subjected to three treatments. Treatments were: (1) untreated on blotting paper *Control*; (2) blotting paper moistened with 0.2% potassium nitrate (KNO_3) solution; and (3) *Dark* (untreated seeds on blotting paper in a container sealed with Parafilm and wrapped entirely in foil). Each replicate was placed in a 900-ml rectangular polypropylene container with an air-tight lid on double-thickness blotting paper moistened with reverse-osmosis water, unless otherwise stated.

Germination was defined as radicle breakthrough from the exocarp. In all trials, germination of *Control* and KNO_3 treatments was monitored and recorded three times per week. Any germinated or mouldy seeds were removed at each census. Blotting paper was moistened as needed, usually every 4 days. *Dark* treatments (Treatment 3) remained sealed and wrapped during incubation to ensure seeds were unaffected by any light source, so only final germination was recorded, on day 28. Following the 4-week incubation period, a tetrazolium (TZ) assay (Leist *et al.* 2003) was performed on all ungerminated seeds to determine post-incubation viability.

Observations of embryo development

Embryo size during storage

An average *C. vitalba* seed is 2.5–4.0-mm long. Dormant embryo length is approximately 0.75 ± 0.25 mm, between 20% and 25% as long as the seed (Fig. 3A). For a seed to germinate, the embryo needs to reach the threshold length of 1.75 ± 0.04 mm (Copete *et al.* 2021), which is 50–60% as long as the seed (embryo:seed length is roughly 1:2; Fig. 3B,C).

In the experiment described above, seasonal germinability patterns of freshly collected seed were examined. As a supplement to that work, and to assess whether seeds remaining on the mother plant lose MD over time, seed collections stored outdoors were examined each month for changes in embryo size. Seeds collected in July and September 2021 from S1 and S2 were stored in synthetic mesh bags within an open-air shed, sheltered from direct precipitation, but otherwise exposed to all fluctuations in ambient temperatures and moisture. Ten to 15 firm seeds randomly selected from each collection were imbibed and dissected each month for 10 months for embryo measurement.

Embryo growth during incubation

To investigate possible loss of seed viability during dry storage at 5°C, seeds collected in September 2020 from S1 and S2 were subjected to a germination test without any added exogenous nutrients or hormones (20°C/30°C) when fresh and were found to be 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

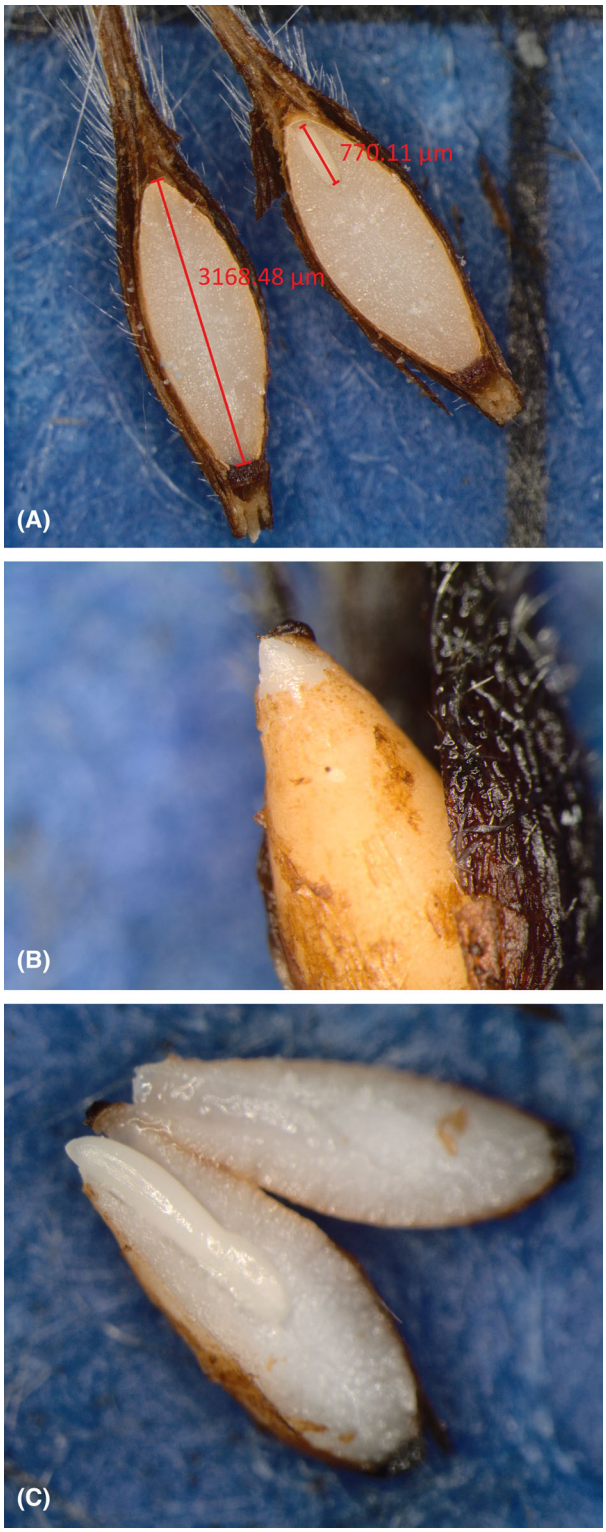

Fig. 3. (A) Mature *Clematis vitalba* seed with a dormant, undersized embryo. (B) Non-dormant *Clematis vitalba* seed at the point of radicle breakthrough. (C) The same seed, showing size of fully elongated embryo relative to seed (as opposed to the dormant embryo in A).

selected seeds was dissected and the embryos measured (using Olympus CellSens Dimensions 1.6) at intervals of 2–3 days until all seeds had germinated, decayed or been dissected.

Statistical analysis

Because no samples could be collected at S2 between November and March due to their complete dispersal, and because sample sizes collected at S1 during that period were too small to be representative of the population (also related to dispersal), there were only seven collections from these two sources that were sufficient to use for the statistical analysis.

A prediction of seed non-viability at the end of the incubation period was generated using a binomial regression model and provenance, date, and germination treatment as factors, with *tidyverse*, *caret*, *aod*, and *arm* packages in R statistical software, version 4.2.0 (Lesnoff & Lancelot 2012; Wickham *et al.* 2019; Gelman & 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 generalized variance inflation factor (GVIF) with the *car* package (Fox & 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 using the *DHARMa* package (Hartig 2022).

RESULTS

Seed germinability and dormancy rates

The collection date and incubation treatments for *C. vitalba* seeds both had significant effects on the likelihood of seed germination, decay, or remaining dormant but viable after the 28-day germination trial period (Fig. 4). 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 ($P < 0.001$; Table 2; for regression coefficients see Table S1). 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. In general, the seed 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.

Germinability or *C. vitalba* seeds in autumn was negligible to non-existent (Fig. 4). Wald tests between cohorts 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 seeds collected in June (Cohort 2). Germinability differences between all three September collections ($P < 0.001$) point to cohort variation, mostly related to differences in response to the *Dark* treatment. Date was the most important predictor of seed status after incubation (Table 2): deviance reduction for date was by far the largest, although the deviance reduction generated by treatment was also considerable.

Figure 5 demonstrates the average trend of dormancy loss in *C. vitalba Control* seeds in both populations combined, from

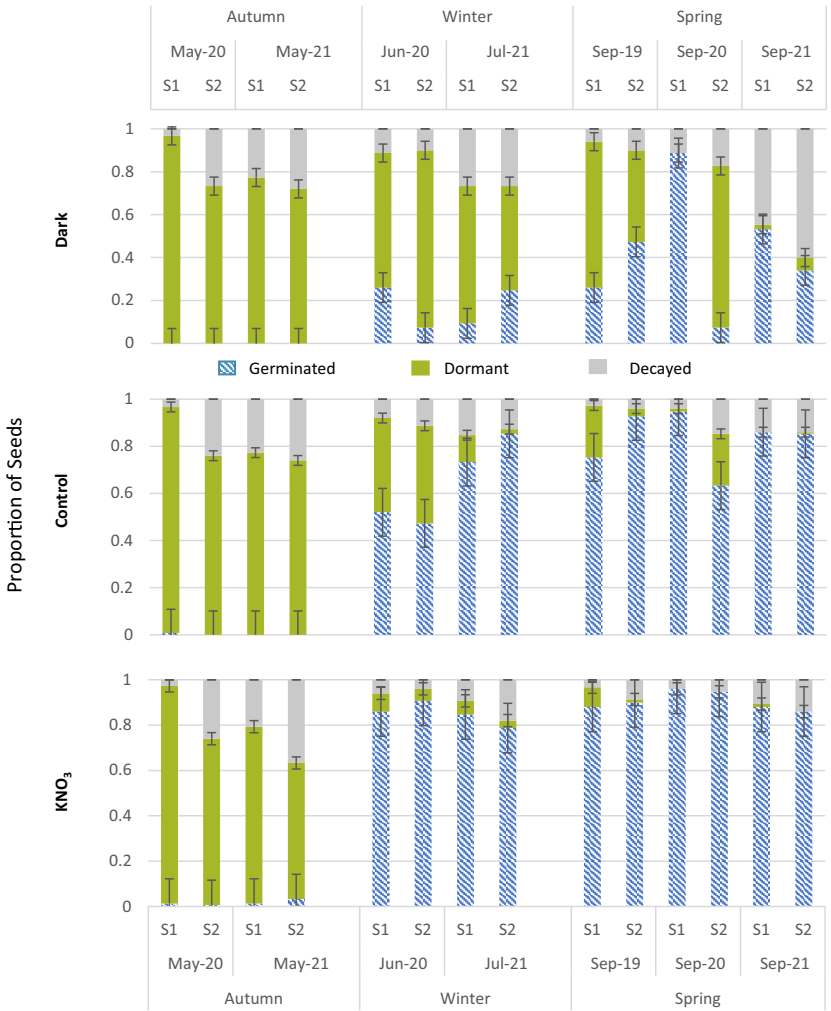

Fig. 4. Proportions of *Clematis vitalba* seed status (Germinated, Dormant, Non-viable) following 28-day incubation (16-h dark/8-h light, 20°C/30°C photoperiod) and three treatments (Dark = complete darkness; Control = blotting paper moistened with reverse-osmosis water; and KNO_3 = blotting paper moistened with KNO_3) ± SE. Seeds collected in autumn, winter, and spring over 2 years from two separate populations (S1, S2) in Tararua District, New Zealand.

Table 2. Analysis of deviance table for binomial logistic regression model of *Clematis vitalba* seed germinability during incubation (multicollinearity of interaction terms rendered 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

autumn (May) to spring (September), when incubated directly after collection, at night/day temperature (20°C/30°C) and dark/light (16 h/8 h) photoperiod. In two consecutive years, germination onset (earliest visible breakthrough of radicle) of untreated seeds (*Control* treatment) was not observed in autumn-collected seed (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 early spring (September), average germination onset had decreased to 7 days, and average germinability had increased to 83%.

Seed viability

At each *C. vitalba* seed collection throughout the 2-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 seeds 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 (Fig. 4). The average combined population decay percentage during incubation of *Control* seeds (the most natural treatment) in the three spring (the most natural season for *C. vitalba* germination) collections was 3%, and a further 5% were scored non-viable in tetrazolium assays.

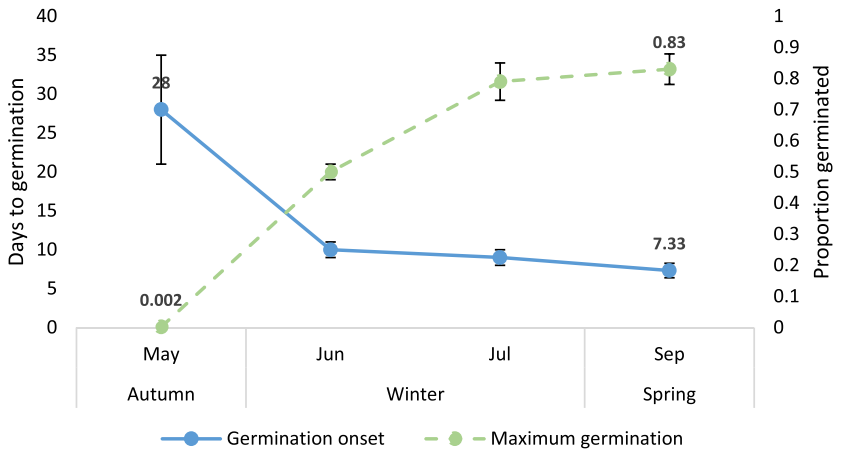

Fig. 5. Average change in *Clematis vitalba* seed germination onset (in days) and maximum germination (proportion) ± SE, during 28 days of incubation (16-h dark/8-h light, 20°C/30°C photoperiod), between autumn and spring over 2 years, September 2019–September 2021.

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, either as individual effects or in the interaction that contained both variables together (Table 3; see Table S2 for regression coefficients). The date effect was roughly triple that of treatment or the treatment x date interaction, thus demonstrating that date was the most influential term in the model. 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 × treatment was significant, as was the interaction of date x population (both $P < 0.001$). In other words, the effect of treatment or population on *C. vitalba* 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 S2 population were significantly more likely to decay ($P < 0.001$) in May 2020 than S1 seeds of the same collection date: in all three treatments, the average predicted probability of decay for S2 and S1 seeds was 25.5% and 3.0%, respectively. Wald tests showed significantly more overall decay in May 2020 than June or September, probably because of high S2 decay.

Table 3. 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	6296	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	113.580	6276	4867.0	<0.001
Population:Date	6	67.802	6270	4799.2	<0.001

Observations of embryo development

Embryo size during storage

An approximate average embryo size range of 0.5–1.0 mm was found in both batches of stored seed after ambient outdoor storage for 9 and 11 months: embryo length of July 2021 seeds ranged from 530 to 1041 μm, and September 2021 embryo length ranged from 587 to 953 μm (Fig. 6), with no consistent change in size with storage time.

Embryo growth during incubation

Measurement of a sample of embryos stored chilled prior to incubation established that *C. vitalba* seeds had remained morphologically dormant during the 21 months of dry 5°C storage and were in the same size range as those stored at ambient temperatures. However, once placed in conditions suitable for germination, the average embryo size, which was 0.85 mm at the beginning of the study, increased to 1.37 mm at the time of germination onset (Day 7–Day 9; Fig. 3A), and peaked at

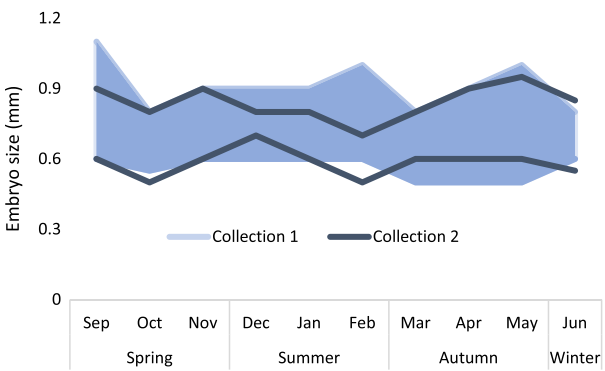

Fig. 6. Monthly embryo size range (maximum and minimum) over 10 months of *Clematis vitalba* seed collections taken in mid-winter (Collection 1, July) and early spring (Collection 2, September) 2021, stored outdoors at ambient temperatures, sheltered from direct precipitation.

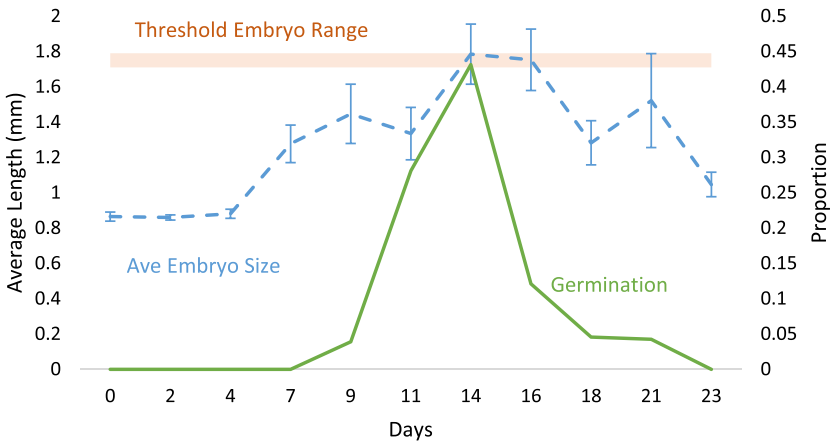

Fig. 7. Correlation of average *Clematis vitalba* seed embryo size with proportion of seeds germinated at 2-day increments after incubation (16-h dark/8-h light 20°C/30°C photoperiod) ± SE. Orange line = range of threshold embryo length to break morphological dormancy (1.75 ± 0.04 mm); blue dashed line = average embryo length during the test period; green line = proportion of seeds that germinated in each increment. Seeds were collected from two populations (S1 and S2 in Tararua District, New Zealand) in September 2020, stored dry at 5°C, and then tested in May 2022.

1.75 mm at day 16, shortly after germination had peaked (Figs 3B,C and 7). Embryo length declined from thereon, with fewer and fewer seeds being ungerminated, but a significant proportion of that small pool of remaining embryos did not grow. Germinability of undissected seeds was 92%, slightly lower than when fresh, although not significantly so.

DISCUSSION

Influence of date, treatment and population

Regarding germinability and dormancy of *C. vitalba* seed, the most critical difference in final seed status was related to collection date, i.e., stage of seasonal dormancy. Extremely low germinability occurred in May 2020 and May 2021, which supports previous claims that newly mature 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 seeds from May, 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 in the second week of July, were significantly more germinable than Cohort 2 winter seeds, which were collected in the third week of June. This result implies that, even three extra weeks of after-ripening, can lead to a significant reduction of PD.

Treatment also had a sizeable influence on germinability, demonstrating that the post-dispersal environment affects seed survival. 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 to 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 photoperiods. Finch-Savage & Footitt (2017) have shown that light activates germination in mature seeds that are provisionally dormant. Results from the current study support this: light promoted germination in non-dormant (spring-collected) and conditionally dormant (winter-collected) *C. vitalba* seeds. Also, 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, indicating that chances for successful germination improve with increased levels of nitrogen in the soil. This 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 release. If temperatures and other conditions mimic spring conditions, seeds then respond to the presence of the increased nitrogen availability (Taylor *et al.* 1982; Dawes *et al.* 2017).

Nitrate has been found to be a suitable proxy for light in some species incubated in complete darkness (Koutsovoulou *et al.* 2013). It could be useful to repeat the present study with an additional nitrate treatment in the dark, to help determine if this holds true for *C. vitalba*. Indeed, at least for spring-collected seeds, the propensity to die of *C. vitalba* seeds in the *Dark* treatment may be partly related to the absence of appropriate levels of nitrate that are normally available to buried seeds in spring.

Less than 72% of total *C. vitalba* seeds collected were viable. An average of 28% of seeds were aborted or diseased (too small or flat) or eaten while still attached to the mother plant, and were discarded before incubation began. Spring provides the most favourable conditions and is the most natural season of germination for *C. vitalba*. Yet, September seeds in the *Control* treatment decayed an additional 3% during incubation, and a further 5% were 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 *C. vitalba* (ca. 70,000 $m^{-2}{\cdot}year^{-1}$) that remain germinable or dormant is <72% (50,000 viable seeds $m^{-2}{\cdot}year^{-1}$).

Collection time appeared to be the most important variable in predicting post-incubation viability of *C. vitalba* seed. Namely, the length of the after-ripening period and stage of seed ripening, as well as seed cohort (year), moderated the relationship between viability and population source or treatment. For example, there was generally relatively high decay in both autumn seed collections, much of which occurred during incubation (S1 seeds in 2020 were an exception to this general pattern). The high proportion of decay could be partly related to insufficient after-ripening of seeds in autumn, compared to that in winter or spring. Existing literature has established that when spring phenological processes are delayed or accelerated, autumn processes are equally affected (Keenan & Richardson 2015). Indeed, in May 2020, notes taken during collection indicated that seeds from the S1 population, which had low decay rates, were drier and more easily removed from the receptacle than S2 seeds, suggesting that S1 seeds had both developed and ripened earlier. By June 2020, differences between populations were not significant, probably because S2 seeds had ripened adequately by that time.

As for differences in cohort, environmental stresses on the mother plant during seed development can lead to lower seed fitness (Finch-Savage & Leubner-Metzger 2006; Naithani *et al.* 2017). Overall, Cohort 3 (2021) seeds were less viable than those of Cohorts 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. Our notes from September 2021 indicated that seeds were rain wetted when collected, so this extra moisture could also have contributed to the higher decay in both populations for that date.

Although treatment was not a significant predictor of seed decay, for this study, except in autumn, *C. vitalba* seeds incubated without light were more likely to decay than those exposed to light, especially in 2021. Thus, light exposure during incubation seemed to increase chances of germination, offsetting chances of decay in otherwise susceptible seeds, except in those that had not after-ripened sufficiently and remained 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. It is also possible that decay occurred after germination in the *Dark* seeds. That is, some seeds germinated but, like buried seeds that germinate and use up their seed reserves before reaching the surface where they could begin photosynthesis (Batlla & Benech-Arnold 2014), subsequently succumbed to decay from lack of light.

Because the *Dark* treatment containers were not opened and mouldy seeds were not removed during incubation, as they were in the other two treatments, some seed mortality may have occurred in the dark due to contamination from infected seeds. Surface sterilization of seeds before incubation could have minimized such contamination, although mouldy seeds in the natural soil seed bank are not removed, and buried seeds would similarly experience more exposure to pathogens, both soil- and seed-borne. Thus, the *Dark* treatment may have been a simulation of conditions in the soil seed bank for that reason, as well as a lack of light. Indeed, Jarvis-Lowry (2023) reported that fewer than 40% of *C. vitalba* seeds sown in field plots emerged as seedlings, which is similar to germination results in the *Dark* treatment of the current experiment.

Dormancy changes over time

The six S1 *C. vitalba* collections from November 2019 and 2020, January 2020 and 2021, and March 2020 and 2021 were not representative of the population, but were useful in that they demonstrated germinability of seeds taken directly off the vine throughout the summer. Germinable and/or dormant seeds were present in each collection over the 2-year period (Jarvis-Lowry 2023), which confirms that persistent seeds on the mother plant function as a seed bank, however small.

According to Picciau *et al.* (2017), the earliest germination of non-chilled *C. vitalba* seeds collected in mid-autumn and incubated for 140 days did not start until Day 50. In the current study, which was 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, no seeds germinated in Cohort 3), which is consistent with a period of complete dormancy at maturation of fruits. Seeds appeared to be fully morpho-physiologically dormant through late autumn, but by mid-winter a portion of these seeds could be induced to germinate. Germinability increased even more by early spring, indicating a decline in PD over the seasons.

Peak *C. vitalba* seed germination is expected to occur once temperatures have risen reliably in spring. Seeds collected in September would have experienced after-ripening throughout autumn and winter, with an accompanying loss of PD, and thus would be expected to respond more favourably to germination conditions than seeds collected in winter. Germination results from all three September collections were compatible with this expectation. Yet, a small proportion of the seeds (up to approximately 8%) remained dormant in spring. According to some researchers (Finch-Savage & Leubner-Metzger 2006; Mitchell *et al.* 2016), along with inherent characteristics, genetic differences and hormone levels, position on the mother plant and asynchronous development of seeds will lead to differences in senescence and dormancy levels, even as seasonal shifts occur. Accordingly, seeds produced later would retain dormancy for longer, even during the most suitable environmental conditions for germination.

Morphological dormancy does not lead to the same seasonal shift. Copete *et al.* (2021) did not observe any embryo growth during moist seed storage of *C. vitalba*. Similarly, no embryo growth occurred in the present study during dry seed storage at 5°C, or during storage at ambient outdoor temperature and moisture conditions. This indicates that, in any situation where the necessary combination of germination stimuli is lacking, regardless of PD level, seeds will remain morphologically dormant. Even so, the length of time between Day 0 of incubation and the onset of germination during incubation shortened as PD decreased (from 28 days in May to 7 days in September), implying a positive correlation between PD 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 reach a 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 conditions are suitable (accumulated thermal time may be involved in this process), improving chances

of early seedling establishment. As a simulation of after-ripening during winter, artificial chilling has also been found to prompt accelerated germination onset and time to 50% germination (Lhotská 1974; Van Gardingen 1986; West 1992; Bungard *et al.* 1997b; Vinkler *et al.* 2004; Picciau *et al.* 2017; Jarvis-Lowry 2023).

Fewer than 5% of *C. vitalba* seeds persist on the mother plant beyond early spring, a fraction of which may be dormant. Seeds that do remain become covered by new vegetative growth as spring progresses, and thus have much reduced chances of long-distance dispersal. Therefore, seeds that persist past the peak dispersal season, regardless of their dormancy state, do not make an important contribution to the seed bank. On the other hand, the small percentage of dormant seeds that have dispersed and become part of the soil seed bank may become a longer-term source of recruitment for *C. vitalba*.

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AUTHOR CONTRIBUTIONS

BJ-L helped design the study, collected the data, analysed the results, wrote the manuscript. KCW helped design the study, and helped writing the manuscript. HG helped design the study, and helped writing the manuscript. AWR helped design the study, helped with the statistical analysis, and helped writing the manuscript.

DATA AVAILABILITY STATEMENT

Data are available from the Open Science Framework Repository (Jarvis-Lowry *et al.* 2024; osf.io/pbvk8).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Binomial logistic regression model summary for *Clematis vitalba* seed germinability during collection and incubation on seven separate dates.

Table S2. Binomial logistic regression model summary for *Clematis vitalba* seed viability following collection and incubation on seven separate dates.

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