OPTIMISING THE SEED PRODUCTION CYCLE OF *Delphinium elatum* L.

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ABSTRACT

Delphinium elatum L. (Ranunculaceae) hybrids are developed by Dowdeswell's Delphiniums located at 692 Brunswick Road, Wanganui (www.delphinium.co.nz), who grow delphiniums for seed export. Development of new cultivars is important for commercial purposes, and requires development of high quality breeds. The main objectives of this study were to determine reasons for variable seed set and low germination in commercial *D. elatum* hybrids and determine optimal parameters for seed germination and storage.

Seed set was investigated by examining pollen viability, carpel structure, and pollen tube growth *in vitro* and *in vivo*. Seed structure was determined by paraffin embedding and sectioning of seeds. Results investigating variable seed set were inconclusive. Seed set is limited by pollen viability and age. Pollen viability is low (below 50%) in most cultivars, and pollen tubes growth rate is variable between cultivars and individual pollen grains. Ovule development showed no abnormalities.

Germination-promoting treatments were used to improve germination in *D. elatum*. Stratification (5°C) and application of gibberellic acid both improved germination in *D. elatum* cultivars. Chilling at 5°C reduced germination time in all cultivars, and increased total germination in cv. LB01130x. Storage trials were carried out for 12 months, with seeds stored at room temperature or 5°C in open and closed, water impermeable, aluminium bags. Storage trials determined that at low temperature storage (5°C) seed germinability is retained for up to 12 month. Storage at room temperature leads to seed deterioration and viability loss. Application of GA_3 improved germination in seed stored at 5°C.

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1.0 GENERAL INTRODUCTION

The *Delphinium* genus (Ranunculaceae) consists of over 300 species of annual and perennial herbs (Edwards, 1981; Hosokawa et al, 2001). Wild species of *Delphinium* are distributed within the North Temperate Zone and the North Frigid Zone (Honda and Tsutsui, 1997; Honda et al, 2003). *Delphinium* species have been in cultivation for over 300 years although only three species are commonly grown today; *Delphinium elatum* L., *D. ajacis,* and *D. naudicaule* (Legro, 1963). Of these three species, *D. elatum* is by far the most widely grown, with over 4000 named cultivars of *D. elatum* in existence by 1960 (Legro, 1961).

D. elatum is a clump-forming perennial usually flowering from early to late summer. Cultivars comprise of many flower shades and colour combinations formed from whites, blues and pinks. Yellow, orange and red flower colours are not found in this species (Legro, 1961). The leaves of *D. elatum* are deeply pinnatisect and flowers are zygomorphic (Tutin et al, 1964). Flowers are showy (Plate 1.1; Plate 1.2), occurring on tall spikes with numerous flowers per spike. The wild-type flower typically has a calyx of 5 showy sepals and a corolla of 4-5 petals (small or absent) known as the 'bee'. Hybrids may be double, semi-double, or single (Tutin et al, 1964). *Delphinium elatum* hybrids produce around 75-100 florets per floral spike, with each floret producing 3-5 pods. Seeds are harvested January-May in New Zealand.

This research focuses on aspects of pollination, seed set, and storage and germination in commercial *Delphinium* cultivars. *Delphinium* cultivars examined are those grown by Dowdeswell's Delphiniums, a business located in Wanganui, New Zealand, which produces delphinium seed for export. *Delphinium* cultivars are developed through hand-pollination, with new varieties produced on a regular basis. Development of new cultivars is important for commercial purposes, and requires development of high quality breeds. Several key problems limiting seed production have been identified by Dowdeswell's Delphiniums. These problems range from seed set through to seed storage. At present

seed set in *Delphinium* hybrids tends to be variable between cultivars. Many hybrids show poor germination, and seeds deteriorate in storage. The aims of this research are:

- To determine reasons for variable seed set in commercial *Delphinium* hybrids following controlled pollinations.
- To determine reasons for low germination of *Delphinium* seed and define optimal conditions for germination.
- To determine optimal storage conditions for *Delphinium* seed. Delphinium seed is known to deteriorate in storage. Seed exported from New Zealand has shown losses in germinability compared with pre-export performance.

Determining the most advantageous conditions for seed set, germination and growth will allow the business to optimise seed production and increase economic output.

Plate1.1 Blue and pink flowering *D. elatum* hybrids

Plate 1.2 Dark blue *D. elatum* cultivar

2.0 LITERATURE REVIEW

2.1 *Delphinium* species

Delphinium (Ranunculaceae) is a large genus of annual and perennial erect herbs native to northern temperate zones (Tutin et al, 1964; Edwards, 1981). The Ranunculaceae (buttercup) family characteristically exhibits hypogyny (superior ovaries) and floral whorls in sets of five (Raven et al, 1999). *Delphinium* flowers are double, semi-double, or single. Doubles have 2 or more whorls of 5 sepals. Stems are 40-200cm long. There are usually several to numerous carpels produced from each floret. Ovules are numerous in each carpel. Carpels are glabrous or patent-pilose and seeds are winged at angles (Zomlefer, 1994). The Ranunculaceae family belongs to the basal section of the tricolpate pollen group of dicots (Zomlefer, 1994), and *Delphinium* pollen is binucleate (Brewbaker, 1967).

D. elatum is also commonly known as delphinium, larkspur, alpine larkspur, or candle larkspur.

2.2 Pollination structures and events

2.2.1 Stigma-style complex

The angiosperm carpel is comprised of the stigma, style and ovary. The stigma is the receptive surface for pollen, which germinates on the stigmatic surface and migrates down the style to fertilise the ovule inside the ovary. Maximum receptivity is usually reached soon after anthesis (Bhattacharya and Mandal, 2004), although length of the receptivity period varies between species. Stigmas are of types 'dry' or 'wet'. Receptive surface cells of 'dry' stigma bear a thin proteinaceous surface layer, often with lipid components (Heslop-Harrison, 1987). With stigma of the 'wet' type, pollen immediately enters a liquid matrix. Pollen of this type generally germinates more readily *in vitro* in liquid medium whereas pollen from 'dry' types is more likely to require specialist conditions (Heslop-Harrison, 1987). Stylar tissues provide physical and chemical directional support to the

pollen tube growth process (Cheung, 1995). The stigma-style complex may be solid (e.g. *Arabidopsis*) or hollow (e.g. lily) (Lord, 2000). In both types the pollen tube travels through an extra-cellular matrix that either lines a hollow channel in the centre of the style, or is secreted between style cells which form a solid transmitting tract (Wilhelmi and Preuss, 1997). Transmitting tract is the stylar tissue through which pollen tubes grow (Mascarenhas, 1993). In 'dry' stigma, adhesion to a stigmatic papilla usually determines point of entry of pollen tube into the transmitting tract, whereas in 'wet' stigma the extra-cellular matrices cover the stigma surface, and tubes can grow to some length before penetrating the style (Lord, 2003).

2.2.2 Ovary complex

The angiosperm ovary contains one to numerous ovules which develop into seeds. Ovules are attached to the ovary via the placental strand which may be located on the ovary wall (placietal), or form a column down the centre of the ovary (central or axial) (Raven et al, 1999).

2.2.3 The ovule structure

The angiosperm ovule consists primarily of a stalk (the funiculus) bearing a nucellus (which is enclosed by one-two integuments) (Reiser and Fischer, 1993; Raven et al, 1999). The embryo sac develops within the nucellus, with most of the nucellus degenerating before the embryo sac matures (Willemse and Went, 1984; Reiser and Fischer, 1993). The integuments completely enclose the nucellus and embryo sac except for a small opening, the micropyle, through which the pollen tube enters (Raven et al, 1999). The opposing end of the ovule from the micropyle is termed the chalazal (Willemse and Went, 1984). The ovule is connected to the ovary wall via the funiculus, which extends from the chalazal end of the ovule to the ovary (Reiser and Fischer, 1993). In anatropous ovules, the ovule undergoes curvature, so the micropylar end comes to lie next to the funiculus (Babis, 1973). A single vascular strand usually runs through the funiculus from the placenta

(ending at the base of the embryo sac) which is used to supply the ovule with nutrients (Johri and Ambegaokar, 1984; Reiser and Fischer, 1993).

2.2.3.1 Embryo sac

The embryo sac of angiosperms is usually composed of: an egg cell; two synergids; three antipodal cells (all with one nucleus); and the central cell (two nuclei) (Willemse and Went, 1984; Raven et al, 1999).

2.2.3.2 Egg cell

The egg cell is located at the micropylar end of the embryo sac. During fertilisation the egg cell fuses with a sperm cell to produce the zygote (2n). The egg cell is adjacent to the synergids, separated by partial cell walls or plasmalemma alone (Reiser and Fischer, 1993). It contains a large vacuole which restricts the nucleus and majority of the cytoplasm to the chalazal end of the embryo sac (Willemse and Went, 1984).

2.2.3.3 Synergids

The synergids are located on either side of the egg cell at the micropylar end of the embryo sac. These play a role in pollen tube reception, with the tube depositing its contents into one of the synergids prior to fertilisation of the egg and central cell by a male nucleus (Faure et al, 2002). Synergids are also thought to play a possible role in pollen tube guidance (Lush, 1999; Lord, 2003).

2.2.3.4 Central cell

The central cell is positioned in the centre of the embryo sac and contains two nuclei. During fertilisation these nuclei fuse with a male nucleus to form the endosperm (3n). The mature endosperm provides nutrients for the developing embryo and seedling (Reiser and Fischer, 1993).

2.2.3.5 Antipodal cells

The three antipodal cells are located opposite the egg at the chalazal end. No specific function during fertilisation has been assigned to these cells and they are generally thought to be relic cells of ancestral larger megagametophytes. They may play a role in the import of nutrients (Reiser and Fischer, 1993).

2.2.4 *Delphinium* ovule structure

The Ranunculaceae family shows heterogeneity in respect to embryogenesis (Babis, 1980a). In *D. elatum* the zygote divides transversely (Asterad type) and develops into a two-tiered, binuclear embryo (Babis, 1980b). The two-tiered embryo consists of a smaller apical cell and a larger basal cell (Babis, 1979). The apical tier later gives rise to the cotyledons; the shoot-growing tip; and part of the hypocotyl (Babis, 1979). The *Delphinium* L. (Ranunculaceae) genus is anatropous, and bitegmic (two integuments) (Babis, 1973). In anatropous ovules the ovule undergoes curvature of approximately 180° during development. This causes the nucellus and integuments to turn over, and the micropyle comes to lie next to the future hilum (scar left on seed after separation from the funiculus) (Boeswinkel and Kigel, 1995). Inside the mature embryo sac lie three large antipodal cells with large nuclei (Babis, 1973). The nucleus of the central cell is situated directly below the antipodal cells at ¾ the height of the embryo in a mass of cytoplasm (Babis, 1973). In *Delphinium elatum* the egg cell is separated from the micropyle by three layers of cells (Babis, 1973) and localised at one side of the micropyle. The synergids are of a secretory type and filled with dense cytoplasm (devoid of vacuoles). Endosperm development is nuclear, with several free nuclear divisions prior to cell-wall formation (Babis, 1973; Bewley and Black, 1994). In *D. elatum* the endosperm later becomes cellular (Babis, 1973).

2.2.5 Pollination events

The first step in pollination is adhesion of the pollen grain to the stigmatic surface, which is then followed by pollen hydration (Johri and Ambegaokar, 1984; Lord, 2000; Wheeler et al, 2001). After hydration the pollen grain germinates, producing a pollen tube that penetrates the stigma and enters the style. The function of the pollen grain tube is to deliver two male gametes to the embryo sac (Heslop-Harrison, 1987; Franklin-Tong, 1999). Plants can either reject or accept pollen depending on compatibility. Compatibility mechanisms vary between species and barriers to fertilisation may occur during pollen adhesion, or at any point in pollen tube growth (Franklin-Tong and Franklin, 2003).

2.2.5.1 Pollen adhesion and rehydration

Initiation of pollination is dependent on the pollen grain adhering to the stigmatic surface (Wheeler et al, 2001). Compatible pollen grains and stigma 'recognise' one other, allowing initiation of germination. Pollen reaching a receptive stigma usually will have equilibrated with the surrounding atmosphere, and be near its minimal water content (Heslop-Harrison, 1987). The stigma provides the appropriate chemical and physical conditions for hydration and germination of the pollen grain. Rate of hydration depends on compatibility of the pollen and the receptive style (Heslop-Harrison, 1987). In 'wet' stigmas adhesion is thought to be on the stigmatic side. Specific molecular interactions between stigma and pollen grains are unknown (Lord, 2000). The pollen coat proteins, lipids and waxes play a role in initiation of signals required for adhesion and germination (Franklin-Tong, 1999; Lord, 2000).

2.2.5.2 Pollen germination and tube growth

Once pollen has hydrated, it germinates and grows through the stigma and style until it reaches the ovary (Wheeler et al, 2001). Failure to germinate can be due to uncontrolled or insufficient pollen rehydration (Onus, 2000). Mutations in the pollen coat can also prevent hydration, preventing pollen from germinating (Lord, 2000). Pollen grains require suitable

conditions on the stigmatic surface for germination to occur. For example, many species require the presence of boron and calcium for effective germination (Onus, 2000). Rates of pollen-tube extension after germination vary extensively among species, possibly due to differences in speed of tube wall synthesis (Heslop-Harrison, 1987). In the majority of plants, pollen germination is very rapid, and rate of tube growth is high. For example, maize pollen germination occurs within five minutes of pollen reaching the stigma (Mascarenhas, 1993). Initial penetration of the stigmatic surface appears to be variable, depending on species and stigma type (Wheeler et al, 2001). The rate of pollen tube growth is usually significantly slower when germinated *in vitro* (Lush, 1999; Wheeler et al, 2001). Actual mechanism of guidance through the stylar tissue is unknown, but is generally thought to be due either to mechanical guidance or a chemical gradient (Heslop-Harrison, 1987; Lush, 1999; Wheeler et al, 2001; Lord, 2003). Components of the style are thought to have a number of functions in respect to pollen tube growth including adhesion, nutrition and directional guidance (Franklin-Tong, 1999).

The pollen tube cell is a moving cell independent of the pollen grain and the spent tube (Lord, 2000). The cytoplasm at the growing tip of the cell is separated from the spent tube by callose plugs which are laid down at regular intervals as the pollen tube grows, concentrating cytoplasm in the tube tip (Franklin-Tong, 1999). The walls of the pollen tube consist primarily of pectin and callose. Extent of thickness depends on species. For example, tobacco has a thick callose wall, whereas it is negligible in lily (Lord, 2000).

There are two stages to pollen tube guidance: the sporophytic stage (through the stigma and style), and the gametophytic stage, which depends on guidance from the female gametophyte (embryo sac) (Higashiyama et al, 2003). Gametophytic guidance is required to direct the pollen tube into the micropyle of the ovule in order for fertilisation to occur (Heslop-Harrison, 1987). Studies of the female gametophytic *Arabidopsis* mutant *maa* show normal growth of pollen tubes along the funiculus of the ovule, but inability to locate and enter the micropyle, indicating gametophytic guidance is necessary (Higashiyama et al, 2003). A feature of pollen tube guidance is that usually only one pollen tube is guided to each ovule, even when an ovary contains multiple ovules. This indicates some sort of

signal is sent from the ovule which prevents further fertilisation attempts (Wheeler et al, 2001). This signalling is critical to ensure pollen tubes are distributed amongst ovules, especially in species with high numbers of ovules (Higashiyama et al, 2003).

One of the most common fertilisation barriers is failure of the pollen tube to reach the ovary. This may be due to a pollen tubes' inability to utilise style nutrients, due either to a lack of suitable nutrients or inability of the pollen tube to receive them (Onus, 2000).

2.2.5.3 Incompatibility

Most angiosperm species show compatible pollination (Lord, 2003). Self-incompatibility is a mechanism developed to prevent self-fertilisation, and thus inbreeding (Franklin-Tong and Franklin, 2003). Incompatibility mechanisms provide barriers to fertilisation in some species (Wheeler et al, 2001). Self-incompatibility is genetically controlled by the S-locus, allowing the recognition of phenotypically identical pollen (Franklin-Tong and Franklin, 2003). Self-incompatibility involves cell-cell recognition that allows incompatible pollen to be recognised upon adhesion and inhibited at a specific stage (species-dependent) during pollination (Wheeler, 2001). Two main types of self-incompatibility (SI) exist; gametophytic SI (GSI), and sporophytic SI (SSI) (Franklin-Tong and Franklin, 2003). GSI is thought to be the more widespread of the two. In this type of self-incompatibility, the incompatibility phenotype of the pollen is determined by its haploid (gametophytic) genome. In sporophytic self-incompatibility, the pollen exhibits the incompatibility of its diploid (sporophytic) parent. Gametophytic self-incompatibility is usually controlled by a multi-allelic S-locus that encodes both the pollen and carpel S-specificity. Phenotype of the pollen will match either haplotype of the parent. Thus, there are three possible outcomes; (1) total incompatibility (all pollen recognised), (2) 50% incompatibility (half the pollen recognised), (3) total compatibility (all pollen unrecognised (Franklin-Tong and Franklin, 2003). Incompatible pollen may be stopped at the stigmatic surface or at any point during pollen tube growth (Cheung, 1995). For example, in the *Brassica* genus self-incompatibility is sporophytic. The self-incompatibility reaction is mediated by the pollen parent and incompatible pollen is inhibited at or near the stigmatic surface (Heslop-

Harrison, 1987). Self-incompatibility mechanisms have not yet been studied in the Ranunculaceae (Franklin-Tong and Franklin, 2003).

2.2.5.4 Pollen viability

One of the most widely used methods of testing pollen viability is the fluorochromatic reaction (FCR) test (Heslop-Harrison and Heslop-Harrison, 1970). Pollen grains are treated with fluorescein diacetate (FDA) and examined under a fluorescent microscope. Fatty acid esters of fluorescein enter cells and are hydrolysed to fluorescein (Aronne et al, 2001). If cell membranes are intact, fluorescein accumulates within the cell and is detectable as fluorescence under a microscope. This is primarily testing the intactness of the plasmalemma of the vegetative cell, which may be closely correlated with viability of the pollen grain. However, Sato et al (1998) found that in tests on *Brassica* pollen, the FCR test did not always coincide with *in vitro* germination tests, and pollen samples with high germination rates were often poorly stained by FDA.

2,3,5-triphenyl tetrazolium chloride (TTC) is also used to test pollen viability (Shivanna and Rangaswamy, 1992). Pollen is soaked in 2,3,5-triphenyl tetrazolium chloride (TTC) which stains living cells red. The test is based on the presence of dehydrogenase enzymes and indicates the presence of respiratory activity (Heslop-Harrison and Heslop-Harrison, 1970).

The clearest method of assessing pollen viability is to germinate pollen *in vitro*, and score percentages of germinating pollen (Shivanna and Rangaswamy, 1992; Sato et al, 1998). However, some pollen does not germinate well *in vitro* and will return a false viability reading. Using two or more methods for viability testing helps eliminate false results (Heslop-Harrison and Heslop-Harrison, 1970).

Honda et al (2002) examined cryopreservation of pollen in five perennial species of *Delphinium*. Viability was retained in pollen cryopreserved at -30°C even after 180 days storage. Pollen stored at 25°C showed reduced viability after only 10 days. Field

pollination with cryopreserved pollen also achieved higher rates of seed set than pollination using 25°C stored pollen and showed similar seed set levels to fresh pollen.

2.2.6 Pollination in *Delphinium*

The chromosome number of *D. elatum* is 2n = 32 (tetraploid). Wild-type species are 2n = 16 (Legro, 1961). Honda et al (2003) crossed *Delphinium* species of different ploidy and observed fruit set in reciprocal crosses. Observation revealed that although fertilisation itself might occur naturally, a post-fertilisation barrier arose. Attempts at crossing *D. elatum* with *D. ajacis* by Legro (1961) led to the conclusion that there is a cross barrier between diploid and tetraploid cultivars based on differences in chromosome number. Differences in fertilisation were encountered when using one species as the male or female. For example, a species might work as a female plant but not as a male plant, indicating there may be a disruption in the pollination process, such as a failure to recognise certain pollens (Legro, 1961).

Pollination has not been examined in detail within *Delphinium elatum*. Examination of *Delphinium* species *D. nelsonii* (Waser and Price, 1991) and *D. nuttalianum* (Williams et al, 2001) reveal reduced seed set in selfed plants when compared to outcrossed plants. Outcrossing distance also appeared to have an effect with optimal seed set in *D. nelsonii* occurring at a 10m interval (Waser and Price, 1991). However, studies of *D. barbeyi* showed no reduction in seed set between selfed (hand-pollinated) plants and outcrossed plants (Williams et al, 2001), indicating effects of parental genetic similarity is not fixed for *Delphinium* species.

Waser and Price (1991) observed that in *Delphinium nelsonii*, pollen tubes had reached the ovary 26 hours after pollination whereas Xuhan and van Lammeren (1997) observed that in *Ranunculus scleratus* (Ranunculaceae), pollen tubes enter embryo sacs within 8 hours of pollination. Rates of pollen tube growth in *D. elatum* are unknown.

2.2.7 Reasons for low seed set

Factors influencing seed set include pollen viability and quantity (Waser &Price, 1991), pollen tube growth, initial ovule number, fertilisation, and seed development. Any disruption in these processes may affect seed production. For example, mutations within pollen coat proteins, waxes and lipids result in defective pollen, impairing fertility (Franklin-Tong, 1999; Lush, 1999; Lord, 2000). Waser &Price (1991) and Lush (1999) have matched low seed set in some species to pre-zygotic pollen tube performance and failure to adhere to surfaces within the locule. The amount of pollen deposited on the stigma will determine the number of ovules that are fertilised (Vaughton and Ramsey, 1995). Reproduction is considered to be pollen limited if hand-pollination increases seed set (Vaughton and Ramsey, 1995).

In self-compatible species, inbreeding depression may lower seed set (and seed quality) after self-fertilisation (Vaughton and Ramsey, 1995). Seed set in some species may also be affected by resource limitation, where resources are not sufficient to allow development of all fertilised ovules. Lack of sufficient nutrients may also play a role (Vaughton and Ramsey, 1995; Niesenbaum, 1996). For example, application of pollen and nutrients to *Banksia spinulosa* (Proteaceae) showed that both factors limited fruit set (Vaughton and Ramsey, 1995). Fruit set increased when each factor was added separately, but increased further when both were added in conjunction. *Arabidopsis* with low levels of gibberellins also showed abortion of seeds at various stages of development and impaired pollen-tube elongation (Singh et al, 2002).

Delphinium pollen is binucleate (pollen shed at binuclear stage) (Brewbaker, 1967). Pollen of this type usually retains viability *in vitro* and has greater storage longevity than trinucleate pollen. Self-incompatibility in binucleate pollen usually occurs in the style and ovary and is of the gametophytic type (Brewbaker, 1967).

2.3 Seed germination

A seed is a mature, fertilised ovule containing an embryo (Raven et al, 1999). Bewley and Black (1994) describe the seed as typically consisting of (1) the embryo (result of fertilisation of the egg cell with a male pollen nuclei), (2) the endosperm (result of fusion of two polar nuclei in the embryo sac with a male pollen nuclei), (3) the perisperm (development of the nucellus), and (4) the testa or seed coat (formed from the integument(s)). The endosperm is storage tissue, creating a food reserve for the embryo (Bewley and Black, 1994), and endosperm development almost always precedes embryo development (Bewley and Black, 1994; Boeswinkel and Kigel, 1995). The embryo consists of a central axis bearing 1-2 cotyledons which also store food (Bewley and Black, 1982; Raven et al, 1999).

2.3.1 Germination requirements

Seeds have certain basic requirements which need to be met for germination to occur. These are the presence of water; a favourable temperature; and oxygen (Bradbeer, 1988). Specific requirements vary from species to species. Sufficient moisture is required for imbibition and hydration of the embryo to occur (Bewley and Black, 1994). Most species will germinate even with an excess of water, but in some species, such as beetroot, oxygen will be reduced and germination inhibited (Bradbeer, 1988). The survival and growth of a seed will be affected by surrounding physical, chemical, and biotic factors (Staub et al, 1989).

2.3.2 Dormancy

Dormancy is an adaptive mechanism allowing a plant to survive unfavourable growth conditions (Willemsen and Rice, 1972; Raven et al, 1999). In temperate zones this may prevent germination until spring, when conditions are favourable for seedling growth (Willemsen and Rice, 1972). Dormancy is described as failure to germinate when the environment is adequate and the seed fails to respond to favourable conditions (Bewley and

Black, 1982; Bradbeer, 1988). This is caused by blocks within the seed, which are influenced by the surrounding environmental conditions (Raven et al, 1999). Dormancy is terminated by a trigger which is not required for germination itself, but rather primes the seed to respond to conditions which promote germination (Bewley and Black, 1982, Bradbeer, 1988). For example, chilling may be required to break dormancy in some species, but seeds need to be transferred to a more favourable temperature to allow germination itself. Non-dormant species usually germinate when provided with water, whereas dormant seeds require additional treatment (Raven et al, 1999).

Seed dormancy may be of a primary or secondary type. Primary dormancy (also known as innate dormancy) occurs when seeds are released from the plant in a dormant state. In secondary dormancy seeds are initially non-dormant, but develop dormancy after dispersal if germination conditions are unfavourable (also known as induced dormancy) (Bewley and Black, 1994; Taiz and Zeiger, 1998).

Two main types of dormancy have been recognised; coat-imposed dormancy and embryo dormancy (Choate, 1940; Taiz and Zeiger, 1998). In some species these may exist simultaneously or consecutively (Bewley and Black, 1982).

2.3.2.1 Embryo dormancy

Embryo dormancy refers to dormancy imposed by the embryo itself (Bradbeer, 1988). Embryo dormancy is thought to be caused by the presence of inhibitors such as abscisic acid (ABA) and the absence of growth promoters such as gibberellic acid (GA). Release from embryo imposed dormancy is often associated with a drop in the ratio of ABA to GA (Taiz and Zeiger, 1998). Embryo dormancy is commonly recognised by the failure of viable embryos to germinate even when isolated from the seed (Bewley and Black, 1982). It includes (a) metabolic blocks within the cotyledons, and (b) germination inhibitors (Bradbeer, 1988), and may be limited to part of the embryonic axis or entire embryo.

Embryo dormancy can also include embryo immaturity (Choate, 1940). Embryos of some species are morphologically immature when released from the parent plant and require further development before maturing. Embryos of this type are usually small and often poorly developed. Further growth is required before germination (Bewley and Black, 1982). For example, *Panax* embryos are poorly differentiated when dispersed and need several months imbibed for differentiation to proceed. The developmental period is then followed by embryo dormancy, which is broken by chilling (Bewley and Black, 1982).

2.3.2.2 Coat-imposed dormancy

Seed-coat dormancy is found in the majority of species exhibiting dormancy (Bewley and Black, 1982). Structures included in this type of dormancy are the pericarp, testa, perisperm, and endosperm (Bunker, 1994). Structures responsible for imposing dormancy vary between species. Recognition of this type of dormancy is achieved when the embryo in the intact seed is dormant, but will germinate when isolated from surrounding structures (Bradbeer, 1988). Complete removal of the seed coat is not always necessary. Various physical or chemical treatments on the seed coat are sufficient to allow germination (Bunker, 1994). This includes perforation or abrasion, commonly known as scarification (Bewley and Black, 1994)

There are five common types of coat-imposed dormancy (Bewley and Black, 1982):

1) Prevention of water-uptake. This is common in species from arid or semi-arid regions, from structures such as waxy layers.
2) Mechanical constraint. The seed coat may be too rigid for the radicle to penetrate. This must be broken in order for seeds to germinate. In some species other tissues, such as the endosperm, may prevent embryo expansion.
3) Interference with gas exchange. The seed coat may limit oxygen reaching the embryo.
4) Retention of inhibitors. The seed coat may prevent leaching of growth inhibitors from the seed.

5) Inhibitor production. Seed coats may contain growth inhibitors that can suppress embryo germination.

Only one of these factors is necessary to cause dormancy, but one or more may operate in conjunction.

2.3.3 Breaking dormancy/inducing germination

Release from dormancy is controlled by various environmental factors. Many dormant seeds respond to more than one condition. In natural environments seed dormancy is broken by (a) light, (b) temperature, (c) ageing, and (d) changes to covering structures. One or more of these factors will operate to convert the seeds to a germinable state (Bewley and Black, 1982; Bradbeer, 1988).

2.3.3.1 After-ripening

The most widespread change exhibited by seeds post-dispersal is a gradual reduction in dormancy level, known as after-ripening (Bewley and Black, 1982). This takes place in seeds with low water contents and hence is common in storage. Some plant species which exhibit a requirement for light or chilling to break dormancy after harvest slowly become partially or completely independent of such requirements, and gain ability to germinate under previously unfavourable conditions (Hidayati et al, 2002). The time required for after-ripening is affected by moisture content, temperature, and oxygen (Bewley and Black, 1982).

2.3.3.2 Scarification

Scarification is the rupturing or softening of a seed coat to allow germination to occur (Raven et al, 1999; Gebre and Karam, 2004). This is required in families such as the Leguminaceae which have hard, impermeable seed coats. In a natural environment scarification may occur through a number of processes, such as freezing, heating, or

abrasion from natural forces. There are a range of artificial means available to release seed dormancy. These include concentrated acid, mechanical scarification, freezing, heating, radiation, percussion, and pressure (Bewley and Black, 1982).

2.3.3.3 Temperature

Low temperature treatment may be described as chilling, cold after-ripening, or stratification. Temperature-controlled termination of dormancy occurs in both fully imbibed seeds and dry seeds (Bewley and Black, 1982). Dry seeds undergo temperature-sensitive changes (dry after-ripening) resulting in dormancy loss. Imbibed seeds may require alternating, low, or (rarely) high temperatures in order to break dormancy. Seeds of many woody and herbaceous species are released from dormancy when they experience low temperature (Taiz and Zeiger, 1998). Although dormancy is terminated at low temperature, germination usually (but not invariably) awaits higher temperatures which are more favourable for the biochemical and physiological processes promoting emergence of the radicle (Bradbeer, 1988). An absolute dependence upon chilling to remove dormancy is exhibited by a number of species, but more often low temperature is just one of several environmental factors which can release a seed from dormancy (Bewley and Black, 1994; Bungard et al, 1997). Others factors include light, alternating temperature, various chemicals (such as nitrate), and hormones (e.g. gibberellins). Chilling can interact with any of these (Bewley and Black, 1994).

Germination is often defined as radicle emergence; the actual physiological initiation of germination occurs with the onset of cell elongation in the radicle (Bewley and Black, 1994; Bungard et al, 1997). Factors influencing the rate of elongation could therefore be expected to affect radicle emergence and therefore the rate of germination when measured as radicle emergence. It is possible that increased rates of germination may be a result of chilling leading to an increase in cell wall extensibility, affecting cell expansion and eventually radicle emergence (Bungard et al, 1997).

Many seeds require a chilling period (0 to 10°C) (Baron, 1978; Taiz and Zeiger, 1998) while imbibed (hydrated) in order to germinate. The level and depth of chilling required to break dormancy varies between species and individual seeds within a population (Bungard et al, 1997). Chilling of seed may also promote germination over a wider range of temperatures (Bewley and Black, 1982).

Low temperature is especially important for species living in climates where winters are unsuitable for seedling growth, and which might prove fatal to an established seedling (Bewley and Black, 1994). A seed that remains dormant until several weeks or months of chilling have been experienced will usually not germinate until the winter is over and conditions are favourable for seedling establishment. There are two possible controls behind this; (1) a winter's duration of cold is required to break dormancy; (2) germination cannot begin until warmer temperatures arrive (there are exceptions to this rule). Chilling is commonly required by seeds of woody species exhibiting embryo dormancy (e.g. *Acer saccharum*) (Bewley and Black, 1982; Bungard et al, 1997). Herbaceous and woody species with coat-imposed dormancy also respond to chilling, as do seeds with secondary dormancy. Chilling is therefore effective in a range of dormancy types. Differences in depth of dormancy are reflected by the duration of chilling required to produce germinable seeds. In sugar pine, speed and percentage of germination are related to length of stratification (Baron, 1978).

There is no absolute in temperature requirements for any species (Taiz and Zeiger, 1998). The period of chilling necessary for dormancy breaking varies. For example, seed provenance may exert an influence. *Fraxinus excelsior* seed from latitudes with long, cold winters requires an extended period of cold to break dormancy compared with less rigorous winters (Bewley and Black, 1982). Germination responses in introduced plant species are usually shaped under different climatic conditions (Wagner, 1988). After-ripening and therefore breaking of dormancy in *Echinocystis lobata* can be induced by stratification at 5-10°C (Choate, 1940), and dormancy in common ragweed is broken by stratification at 4°C (Willemsen, 1975). While the natural mechanism for breaking dormancy in seeds with

stratification requirements is moist winter chilling (Willemsen, 1975), dormancy in seeds of many plant species can be broken by stratification in the laboratory (Willemsen, 1975).

2.3.3.4 Light

Many seeds exhibit a requirement for light in order to break dormancy. This may be limited to a few moments, intermittent treatment, or specific photoperiods (Naidu and Amritphale, 1994). Phytochrome is the main sensor that regulates germination in most light-requiring species. The majority of light-requiring seeds exhibit coat-imposed dormancy. Few (e.g. *Nigella arvensis*) possess embryo dormancy (Bewley and Black, 1982; Taiz and Zeiger, 1998). Release from light requirements can be modified by temperature. Light allows the radicle to penetrate the seed coat and usually involves enzymatic weakening of surrounding tissues. The effect of light on seed germination is mediated by red (R) and far-red (FR) light (Yamauchi et al, 2004). The physiology of photo-control is varied and complex. Some species may also exhibit sensitivity to light, and secondary dormancy may be imposed on these species by exposure to light (Bewley and Black, 1982).

2.3.3.5 Hormones

Gibberellins are a family of plant hormones which play a role in seed germination (Taiz and Zeiger, 1998). These are thought to be required for several steps in seed germination; (1) activation of vegetative growth of the embryo, (2) weakening of a growth-constraining endosperm layer surrounding the embryo, (3) mobilisation of food reserves in the endosperm (Taiz and Zeiger, 1998). Seed dormancy is controlled by the ratios of abscisic acid (ABA) to gibberellic acid (GA) in the seed (Taiz and Zeiger, 1998), with GA promoting germination in many species (Yamauchi et al, 2004).

Some species of seed require light or cold to break dormancy and induce germination (Bunker, 1994; Taiz and Zeiger, 1998). This type of dormancy can often be overcome by gibberellin. Chilling often results in changes to endogenous levels of gibberellin in seed

(Karssen, 1995; Taiz and Zeiger, 1998). Gibberellins stimulate cell elongation and division by increasing cell wall extensibility, which influences cell elongation rate (Taiz and Zeiger, 1998). Light and temperature regulation of GA biosynthesis may be important in ensuring germination occurs under conditions beneficial to germination (Yamauchi et al, 2004). The most effective treatment for breaking dormancy in *Cercis siliquastrum* is stratification of imbibed seeds (Gebre and Karam, 2004). However, GA also promotes germination, but to a lesser degree. Imbibed seeds of *Cercis siliquastrum* will germinate if pre-treated with chilling or GA (Gebre and Karam, 2004). Edelstein et al (1995) suggests that higher germination in certain melon cultivars may be due to higher concentrations of endogenous gibberellins in those cultivars.

Gibberellins may represent a natural regulator of some germination processes (Taiz and Zeiger, 1998). Cold treatment has been discovered to increase sensitivity to exogenous GA in imbibed *Arabidopsis* seeds (Derkx and Karrsen, 1993). Studies of *Arabidopsis* mutants have indicated that a subset of gibberellin-inducible genes is upregulated at 4°C in imbibed seeds (Yamauchi et al, 2004). Concentration of GA was also examined in *Onopordum nervosum* seeds. Concentrations of GA_3 and GA_{20} in seeds with high germination rates were much higher than in seeds with low rates of germination, indicating a possible role in release from dormancy (Fernández et al, 2002). Results suggest that GA plays a substantial role in stimulating seed germination during cold treatment (Yamauchi et al, 2004).

2.3.3.6 Priming

Time to germination may be reduced in some plant species by hydrating seeds then re-dehydrating. When seeds are re-hydrated germination will advance more rapidly, due to the previous hydration initiating the germination process (Bewley and Black, 1982). Another method involves initial imbibition in an osmoticum such as mannitol before imbibing in water. Conditions and osmoticum required for optimal priming vary from species to species. In some species, such as carrot, the advantages of pre-treatment are lost if the seed is re-dried before germinating (Bewley and Black, 1982).

2.3.3.7 Viability

A viable seed is classified as being able to germinate under suitable conditions. This includes dormant seeds which require a trigger to induce germination (Bradbeer, 1988). A non-viable seed is one which fails to germinate under favourable conditions once dormancy-breaking requirements have been met (Bewley and Black, 1982). The tetrazolium test can be used to assess viability of a population which may exhibit dormancy. Seeds are soaked in 2,3,5-triphenyl tetrazolium chloride (TTC) which stains living cells red and leaves dead cells colourless (Shivanna and Rangaswamy, 1992). The test is based on the presence of dehydrogenase enzymes and indicates the presence of respiratory activity. A positive result will, however, also be given for micro-organisms which possess dehydrogenase activity (Bradbeer, 1988). The most accurate measure of viability is the germination test, where seed viability is expressed as percent of seeds germinated (Bewley and Black, 1982; Bradbeer, 1988).

2.3.3.8 Seed deterioration

Symptoms of seed deterioration include (a) changes in colour to the seed coat or embryo, (b) delayed radicle emergence and seedling growth, (c) decreases in total germination, (d) decline in tolerance to sub-optimal germination conditions, (e) increase in number of abnormal seedlings (Bradbeer, 1988). The tetrazolium test for seed viability generally shows a correlation between reduction in intensity of staining, respiration and seedling vigour. However, after some ageing treatments loss of viability can occur without subsequent loss of dehydrogenase enzymes, which leads to an overestimation of seed viability (Bewley and Black, 1988).

Nigella is an annual bedding plant that shows poor germination when seeds are sown directly into soil. Low percentages of germination are seen in the laboratory when seeds are grown under optimal temperature (Rudnicki and Kaukovirta, 1991). Seeds were subjected to various germination-promoting treatments to see if this is due to poor seed quality or seed dormancy. Treatment with red light or GA stimulated germination but still

did not explain why 20-30% of seed would not germinate under these conditions. Seeds either remained un-germinated or rotted (Rudnicki and Kaukovirta, 1991). Seed size was found to play a role in germination, with larger seeds less likely to be affected by fungi, and exhibiting higher germination.

2.4 *Delphinium* seed germination

Carpenter & Boucher (1992) examined the temperature requirements for germination of *Delphinium x cultorum* seed by storing seeds at various temperatures and humidities. Moisture content for each storage condition was examined. Carpenter and Boucher (1992) found that germination of *delphinium* x *cultorum* seed was unaffected by light or darkness, and germination was optimal at temperatures of a constant 15-20°C. Dry storage of seed at 2°C for three weeks before germinating reduced time to 50% of germination but did not increase overall germination. Hot oven drying was performed on fresh seed. Reducing seed moisture below a critical level significantly lowered the total germination rate. Relative humidity and temperature during storage appeared to determine the germination rate at the end of storage. Total germination percentage declined when relative humidity was below 30% or above 50%. Storage at 15 °C also led to lower germination than storage at 5 °C. However responses were found to differ between cultivars. Seeds also did not appear to have any discernable need for light or dark conditions during storage.

Overall the *Delphinium* seed was found tolerant of low, non-freezing and below zero temperatures at moisture levels of 6.8-7.0%. A significant decline in germination was noted when seed moisture content declined below 6.7%. Similar results were noted by Carpenter et al (1995), who examined temperature and moisture effects on storage of annual seed. *Delphinium* germination percentage was generally found to decline as seed moisture content increased during storage. Highest germination was seen after storage at conditions of 5 °C and 32% relative humidity. In these conditions seed moisture content was ~6.8%. Highest seed germination for *Delphinium* coincided with moisture content of 6.8-8.3%. Germination declined above or below this range, or if storage temperature increased. The results from these two papers suggest that temperature and humidity may

play a large role in the decline of viability of stored seeds due to their influence on moisture content. Little research appears to have been carried out on horticultural *Delphinium* species since these two papers were published. However the trials undertaken are still viable today, and remain applicable to current *Delphinium* cultivars.

Legro (1961) achieved optimal germination in *Delphinium elatum* by growing seeds on wet blotting paper at 11°C in a controlled dark room. Seedlings were then transferred to 18°C for seedling growth. Deno (1993) preferred germinating *D. elatum* at 21°C over an eight week period (however, only low levels of germination were achieved in this study).

Many species whose dormancy is only expressed above a certain temperature (relative dormancy) also respond to chilling. *Delphinium* and *Latuca sativa* are good examples of this (Bewley and Black, 1982), where the temperature range over which germination eventually occurs is considerably widened by previous exposure to low temperature. *Delphinium ambiguum* (Bewley and Black, 1982) was found to have a chilling range of 2-6°C with optimum germination after 14 days at 6°C. The recorded optimum for most species is close to 5°C (Bewley and Black, 1982).

2.5 Seed storage

As seeds age, they begin deteriorating, and becoming more sensitive to environmental stress (Walters, 1998). Eventually the ability to germinate may be lost. The main parameters of a storage environment determining seed survival are seed moisture content and temperature (Oladrian and Agunbade, 2000). In the majority of cases viability declines more rapidly at higher temperatures and is prolonged at low temperatures. Dry storage has been shown to deepen dormancy in some species, and cause other species to re-enter dormancy (Hidayati et al, 2002).

Speed with which seed loses viability and vigour depends on initial seed quality, as well as storage condition and genetic constitution of the cultivar (Freitas et al, 2002). Once seeds have developed, storage conditions are important for maintaining optimum germinability.

Most horticultural seed spends at least part of their lifespan in storage. There are two main types of seed: recalcitrant and orthodox (Santana-Buzzy et al, 2002). Recalcitrant seed is shed moist from the parent (Greggains et al, 2000) and needs to retain a high moisture content to stay viable (Varghese et al, 2002). Generally these seeds do not store well. Reduction in the germination rate of stored seed is often attributed to loss of viability, especially in seeds with some degree of recalcitrance (Santana-Buzzy et al, 2002).

The capacity of seeds to maintain physiological integrity during dry storage, and retain metabolic activity upon rehydration is a determining factor in seed vigour and viability (Carpenter & Boucher, 1992). Orthodox seed usually undergoes a period of drying on the plant, and prove able to retain viability during dry storage (Greggains et al, 2000). The majority of seeds are orthodox, including *Delphinium* (Kwong et al, 2001). The onset of desiccation tolerance in orthodox seeds varies according to species. For example, a study by Corbineau et al (2000) discovered that in peas (*Pisum sativum*) seeds do not become tolerant of fast drying until moisture content has dropped naturally to ~50%, and reserve accumulation is complete. Incomplete embryos which were fast dried did not complete desiccation tolerance, and showed decreased viability. However, early removal of seeds from plants proved non-detrimental provided drying was slow and humidity high (Corbineau et al, 2000). Achieving the optimal seed moisture content is an important goal in horticulture, as it allows optimal storage conditions to be found (Fang et al, 1998).

2.5.1 Moisture content

The moisture content of a seed in storage is established by the surrounding atmosphere, and is equilibrated with ambient relative humidity (RH). Water contents of dry seed are usually between 5-15% and vary according to species and climatic conditions (Bewley and Black, 1982). At higher than normal moisture contents, complications may occur and viability may be lost (Carpenter el al, 1995). Effective after-ripening can only occur at moisture contents within the optimal range determined by retention of viability and onset of secondary dormancy. The rate of after-ripening is temperature dependant, with higher temperatures usually enhancing after-ripening (Bradbeer, 1988).

Storage is affected by surrounding environmental conditions, especially temperature, humidity, and light. The rate of ageing, or deterioration, in seeds is known to be determined by the temperature and moisture content at which seeds are stored (Walters & Engels, 1998). As storage temperature increases at constant relative humidity levels, moisture contents also increase (Carpenter et al, 1995). The effects of temperature, humidity and moisture content vary according to species and seed age (Walters, 1998). Varghese et al (2002) noted a decrease in seed viability in *Madhuca indica* when seed was desiccated below a critical moisture level, with similar results shown for other species by Corbineau et al (2000), Eeswara et al (1998), and Olidiran & Agunbiade (2000). Carpenter & Boucher (1992) and Carpenter et al (1995) discovered similar results when examining *Delphinium* and other horticultural flower species.

2.5.2 Seed longevity

The length of time a seed can stay dormant but still retain viability varies greatly between species. Older seeds are known to produce higher levels of genetic variability than younger seed (Bradbeer, 1988). A loss of enzyme activity may also occur. Seed quality is determined by the viability and vigour of a seed lot (Bradbeer, 1988). At a cellular level, viability and vigour depend on the integrity of cellular organelles and processes which influence the length of time a seed can be stored and retain viability (Bewley and Black, 1982).

2.5.3 Pathogens

Seeds are usually resistant to microbial contamination. Nevertheless, seeds can be contaminated with pathogens, and it is common for mould to invade dead seeds and dead outer coverings of viable seeds (Bradbeer, 1988). This sort of contamination is favoured by high humidities encountered within germination chambers. Surface sterilisation can be used to eradicate most contamination, but not necessarily, as it may be incorporated deeply into the tissues (Bradbeer, 1988). Fungal contamination may relate to seed death during storage.

3.0 SEED SET IN DELPHINIUM

3.1 INTRODUCTION

Hybrids of *Delphinium elatum* are developed through selective hand-pollination. Ripe pollen is brushed directly from anthers of selected cultivars onto the receptive stigma of flowers to be pollinated. Stigmas unfurl as they become receptive, and pollen is generally visible once brushed onto the stigmas. Cultivars differ in time to anther dehiscence, stigma receptivity, and pollen quality. Flowers are bisexual, and developing flowers are emasculated before anther dehiscence to prevent self-pollination.

The pollination processes include pollen adhesion to stigma, pollen tube growth, pollen tube entry into the ovary, and ovule penetration. The success of the pollination process will depend firstly on the quantity and quality of pollen reaching the stigma. It is determined by pollen's ability to recognise the stigma, and locate and fertilise the ovule successfully (Waser & Price, 1991). Infertile hybrids are at times able to develop pollen, but the pollen is dysfunctional, or not given correct signals for tube growth. Incompatibility may also prevent seed set, with incompatible pollen stopped at the stigmatic surface or at any point during pollen tube growth (Cheung, 1995). Seed set in some species may also be affected by resource limitation, where resources are not sufficient to allow development of all fertilised ovules.

The objective of this research is to determine reasons behind variable seed set in commercial *Delphinium elatum* hybrids. Poor seed set occurs particularly in pink and white cultivars. Since Dowdeswell's Delphiniums develops new varieties of delphiniums, discovery of reasons behind low seed set is important as the commercial viability of cultivars is affected. Experiments described here have the following aims: 1) discover reasons for low seed set in *D. elatum* cultivars; and 2) examine pollen viability levels of selected *D. elatum* hybrids.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials

Delphinium elatum L. (Ranunculaceae) plants used for studying pollination events were grown at Dowdeswell's Delphiniums located at 692 Brunswick Road, Wanganui (www.delphinium.co.nz). Mature plants were also provided by Dowdeswell's Delphiniums and grown in a glasshouse at the Centre for Plant Reproduction and Seed Technology, Massey University, Palmerston North. Plants grown at the university were used for pollen viability tests. The temperature in the glasshouse varied between 25-30°C daily. Plants were watered 4 times a day for 5 minutes. Plants used were the following cultivars:

Pollen viability:

- Pu5
- P35 (Celia)
- P01370
- P9908
- White DB sport

Pollen from cultivars Pu5 and P35 usually results in high seed set when used in crosses. Pollen from cultivar White DB Sport gives variable results depending on the female cultivar. Pollen results for cultivars P01370 and P9908 are unknown (Terry Dowdeswell[1], pers. comm.).

Pollen tube growth *in vivo*:

- P9827 (f) x PO1365 (m) (poor set)
- P9827 (f) x P35 (m) (good set)
- Pu5 (f) x white DB sport (m) (poor set)
- Pu5 (f) x WO208 (m) (good set)

[1] Owner of Dowdeswell's Delphiniums

3.2.1.1 Pollination and pollen collection

Pollination was carried out using procedures developed by Dowdeswell's Delphiniums. Pollination was carried out in the morning between 6.00 and 11.00, except for the time trial of 18 hours where pollination was carried out in the evening. Flowers were pollinated by brushing the anthers from one flower directly over the receptive stigma of the flower to be pollinated. Flowers to be pollinated were emasculated before anther dehiscence to prevent self-pollination. Stigmas were brushed with pollen several times to ensure a full load of pollen was received.

Pollen used in pollen viability testing was collected in the morning between 9.00 and 11.00am. Only freshly dehisced anthers were collected, except when testing viability of aged pollen. A small brush was used to dislodge pollen from anthers. Pollen was suspended in a liquid medium (containing nutrients listed for the medium in section 3.2.2.3 below) before being applied to slides.

3.2.2 Pollen viability

3.2.2.1 Tetrazolium test

Fresh pollen of cultivars Pu5, P35 and PO1370 was collected between 9am-11am from glasshouse plants. Pollen was distributed uniformly in drops of 0.2%, 0.5%, and 1.0% 2,3,5-triphenyl tetrazolium chloride (TTC) in 10% sucrose solution on slides (Shivanna & Rangaswamy, 1992). Slides were then incubated in the dark at 30°C for 60 minutes before being examined under an Olympus compound light microscope for red staining.

3.2.2.2 Fluorochromatic test

Pollen samples were collected between 9am-11am on the morning of testing. Four replicates each of pollen types Pu5, P35 (Celia), PO1370, P9908 and White DB sport were collected. Pollen of type P9908 was examined at three ages within the floret; (1) newly

dehisced (majority of anthers still green); (2) ½ - ¾ anthers dehiscing; (3) anthers dying, to determine when pollen was most viable (usually pollination is carried out using anthers between ages (1) and (2)). Anthers were brushed directly over microslides. Slides were then tapped gently at an angle to obtain an even coating of pollen. A stock solution of 2mg/ml fluorescein diacetate (FDA) in acetone was prepared (Heslop-Harrison and Heslop-Harrison, 1970). A few drops of FDA solution was added to vials containing 5ml of 0.1M, 0.2M, 0.3M, 0.4M, and 0.5M sucrose solution until solutions turned milky. Sucrose prevents pollen grains from bursting (Heslop-Harrison and Heslop-Harrison, 1970). Several drops of each solution were then added to slides containing pollen. Non-viable pollen was used as a control. Non-viable pollen was created by baking pollen at 100°C for 2-3 hours. A further control of H_2O with no FDA was also used. Slides were examined immediately at 100x and 200x using an Olympus compound fluorescence microscope equipped with a UV source. Excitation was at 300-400nm. Fluorescence attributable to the fluorescein moiety was observed as white colouration.

3.2.2.3 *In vitro* germination

A solidified medium was found to be most efficient in germinating pollen grains. Four replicates each of pollen types Pu5, P35 (Celia), PO1370, P9908 and White DB sport were germinated on solidified agar (2%). The effects of various concentrations of sucrose (5, 7.5, 10, 12.5%), boric acid (50mg/l, 100mg/l, 500mg/l) and calcium nitrate (50mg/l, 100mg/l, 300mg/l, 500mg/l) were trialled in preliminary work to determine optimal concentrations for both pollen germination and tube growth. The medium found most suitable was a modified version of Brewbaker and Kwack's medium listed in Shivanna and Rangaswamy (1992) (from Brewbaker and Kwack (1963)), which consisted of:

Agar	2%
Sucrose	10%
Boric acid	100mg/l
Calcium nitrate	300mg/l

(Brewbaker and Kwack's medium also contains 100mg/l potassium nitrate which was found unnecessary for this species in preliminary work).

This medium was used for all *in vitro* germination tests. To achieve separation of pollen grains for easier viewing, pollen was suspended in a solution of liquid medium which contained all nutrients listed excepting agar. A few drops of this solution were then pipetted onto the surface of a thin layer of solidified agar, and a coverslip applied. Liquid medium was also trialled but showed lower levels of pollen germination than solid medium. Slides were incubated in the dark at 25°C +/- 2°C. Tube growth was measured with a calibrated eyepiece micrometer at 100x under an Olympus compound light microscope at intervals of 0.5, 1, 2, 4, 8, 18 and 24 hours after incubation. Each slide was scored for pollen germination and pollen tube length. Germination percentage was calculated by counting all pollen grains in the field of view and scoring for germination (five replicates per time interval). Average tube length was calculated by measuring ten separate tube lengths in each field of view. Pollen was considered to be germinated when pollen tube length exceeded the diameter of the pollen grain (Wang et al, 2003).

3.2.3. Pollen adherence

Pollinated stigmas were excised from plants 1-2 hours after pollination to examine pollen adherence and size of pollen loads. Because pollen adheres to the inner stigmatic surface in *Delphinium elatum*, stigma were cleared in 6M NaOH and stained with water soluble aniline blue (see method 3.2.4.1 below) to allow pollen to be seen. Slides were mounted in glycerol and examined under an Olympus compound bright field light microscope at 200x for pollen adherence.

3.2.4 Examination of pollen germination *in vivo*

Four crosses were used to examine pollen tube growth within the style. These were:

1) P9827 (f) x P35 (m) and Pu5 (f) x WO208 (m) (showing high seed set)
2) P9827 (f) x PO1365 (m) and Pu5 (f) x white DB sport (m) (poor seed set)

Carpels were excised at intervals of 1, 3, 5, 6, 8, 10, 12, 18, 24, and 48 hours after hand pollination. Samples were immediately fixed in FAA (formaldehyde, glacial acetic acid, and ethanol).

3.2.4.1 Aniline blue fluorescence method

A modified aniline blue squash method (Shivanna and Rangaswamy, 1992) was used to observe pollen tube growth *in vivo*. Carpels fixed in FAA were transferred to 8M NaOH for 16 hours to clear tissue. Specimens were then rinsed twice in distilled H_2O, before being soaked in distilled H_2O for 1-2 hours to remove NaOH from the tissue. After clearing, material was stained in decolourised aniline blue solution (0.15% water soluble aniline blue dissolved in 0.05M Na_2HPO_4) for 12 hours before being mounted in a 1:1 ratio of decolourised aniline blue solution and glycerine. Pressure was then applied to coverslips to spread the material for observation. Slides were examined under an Olympus fluorescence compound microscope equipped with a UV source (excitation at 350-425 nm) for presence of pollen tubes in the style and ovary. Pollen tubes fluoresce when treated with aniline blue due to the presence of callose in the tube walls.

3.2.5 Structural analysis

Carpels were excised at intervals of 1, 3, 5, 6, 8, 10, 12, 18, and 24 hours after hand pollination, and placed in fixative. Samples were fixed in a mixture of 40% formaldehyde, glacial acetic acid, and 70% ethanol (FAA) in a ratio of 5:5:90 for 24-48 hours. The samples were rinsed in 70% ethanol before dehydration. Eosin dye was added to help locate tissue in blocks. Tissue was then infiltrated with roticlear (Carl-Roth, Germany) using an increasing ratio of roticlear/ethanol. After 2 changes with pure roticlear, a combination of roticlear/paraffin wax (50:50) was used to link roticlear with pure paraffin wax. After 6 repeats of melted wax at 60°C, samples were embedded into aluminium moulds.

Sectioning was performed using a LEICA RM 2145 microtome. Sections were cut in layers 10μm thick, mounted on poly-lysine coated slides, and dried at 42°C overnight. Wax was then removed by roticlear and roticlear by ethanol. Sections were stained in 1% safranin for 48 hours before staining in 0.5% fast green for 1-5 seconds before de-staining with ethanol and roticlear. Slide mounting was carried out using DPX. Samples were examined and photographed under an Olympus compound bright field light microscope at 40x, 100x and 200x.

3.2.6 Statistical analysis

Measurements of pollen tube growth were evaluated using the SAS system for Windows V8 program. The DUNCAN mean separation test was used to determine significant differences ($p<0.05$) in germination and pollen tube lengths amongst cultivars.

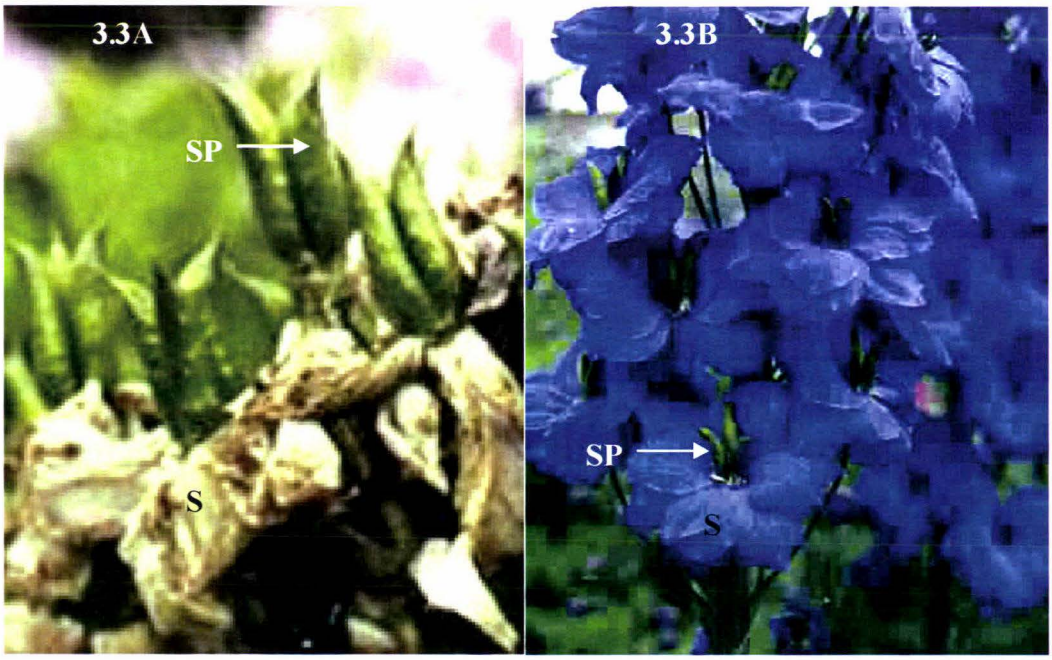
3.3 RESULTS

3.3.1 Floral description

Delphinium elatum hybrid flowers consist of a calyx of five or more showy sepals (Plate 3.1) and a corolla of four-five small petals (Plate 3.2). Flowers are bisexual and zygomorphic (one plane of symmetry). Hybrids differ in the colour of sepals, petals, or both (Plate 3.2; Plate 3.5A-B), and may be single (5 sepals), semi-double, or double. Sepal and petal shapes and colour combinations differ between hybrids (Plate 3.2; Plate 3.4). Stamens occur in whorls of eight or more. The actual number of stamens varies between cultivars. Flowers are hypogynous (sepals, petals and stamens attached below the ovary; Plate 3.1A) and apocarpous (carpels formed separately; Plate 3.3A). Carpels commonly occur in threes (Plate 3.3), but double flowers may have five to eight carpels per floret and carpel number can vary between florets on the same stem (data not shown). Style length is approximately 4-8mm and carpel length approximately 6-10mm. Anther dehiscence and stigma receptivity usually occur at the same time but timing varies between cultivars. Anthers begin dehiscing in the outer whorl first and progress inwards.

Plate 3.1 *D. elatum* flower (cultivar P9908): S, sepals; P, petals; St, stigma; Ca, carpel.

Plate 3.2 *D. elatum* flower spikes. (A) Cultivar 'Pern' (pink double cultivar with bi-coloured sepals and petals). (B) Cultivar 'Emma' (dark blue double with single-coloured sepals and bi-coloured petals).



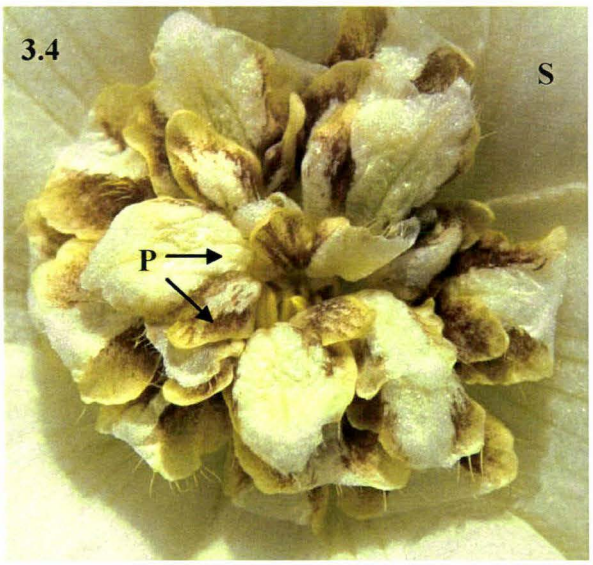


Plate 3.3 Green seed-pods of *D. elatum*. Carpels are occurring in threes and fours. (A) Pink flowering cultivar. SP, seed pod (carpel); S, sepals. (B) Blue-flowering cultivar. Seed-pods are well-developed before flowers lose their sepals.

Plate 3.4 Petals ('bee') of a double flower. Petals are small and numerous. Stamens and stigma are not visible but are located within the petal whorl. P, Petals; S, sepals

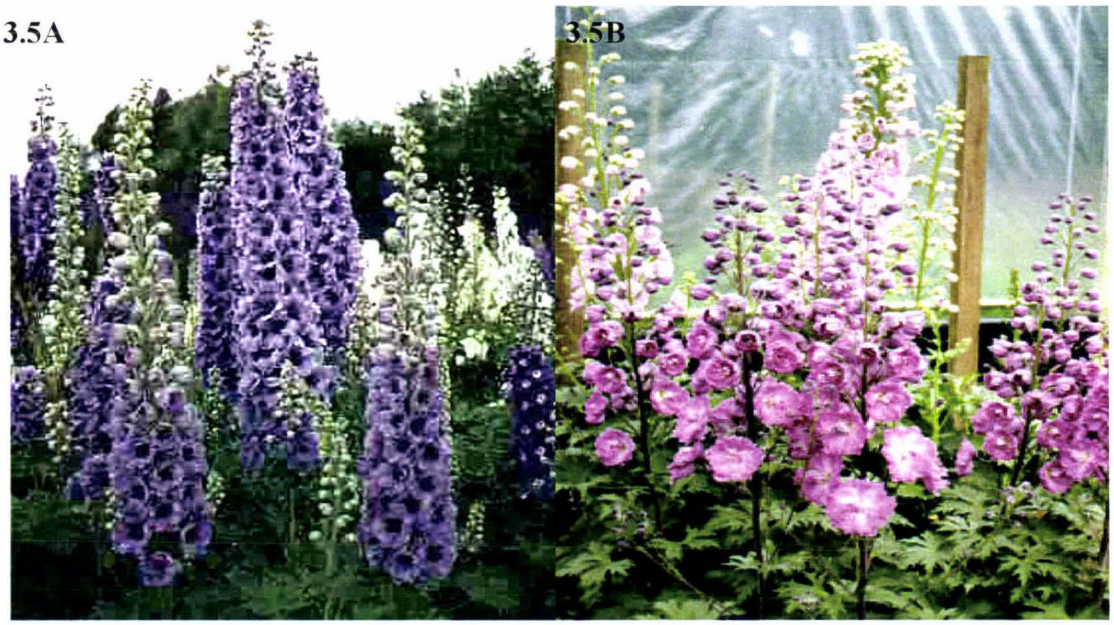


Plate 3.5 Delphinium flower spikes. Flowers open at the bottom of the spike first. (A) Blue sepals with dark petals. (B) Pink sepals with white petals.

3.3.2 Pollen viability

Highest viability was seen in pollen types White DB Sport (62.67%) and P35 (52.94%) using the *in vitro* germination test. Viability results were similar for germination *in vitro* and the fluorochromatic (FCR) test. Pollen fluoresced to differing degrees with the FCR test (Plate 3.6). Only pollen grains fluorescing completely were categorised as viable. The majority of pollen showed less than 50% viability. Lowest germination was seen in P9908 at 35.26% (Figure 3.1). Using the FCR test, highest germination was seen in newly dehisced pollen (Plate 3.6A) out of the three ages (newly dehisced, half-dehisced, and dying) tested.

The tetrazolium assay for examining viability of pollen showed no results for *Delphinium elatum*. No staining was visible in any pollen tested for viability using the TTC assay. Viability was therefore assessed using the *in vitro* germination test and the fluorochromatic test.

3.3.3 Pollen growth rate

Pollen germinated within 30 minutes of incubation on solid medium (Figure 3.1). The majority of pollen germinated within the first 2 hours but pollen continued germinating for at least 18 hours (Figure 3.1). Germination increased rapidly up to 4 hours after initial incubation for cultivars White DB Sport, Pu5, and P9908. P35 and PO1370 also increased rapidly up to 4 hours after incubation, but showed a higher levels of germination after 4 hours compared with White DB Sport, Pu5 and P9908, increasing steadily up until 18 hours. White DB Sport showed both the fastest and highest germination (Figure 3.1).

The longest pollen tube grown *in vitro* was 0.195mm (cultivar P9908; Figure 3.2). Pollen tube growth rates were similar to rates of germination. However, germination rate did not match tube lengths for individual cultivars. P9908 had the longest average pollen tube length (0.195mm) but showed the lowest germination rate (26.77%). P35 showed optimal growth, with higher germination (52.94%) and a higher average tube length (0.164mm). White DB Sport had the highest germination (62.67%) but its average tube length was only 0.137mm (3rd highest). Lowest average tube length was Pu5 at 0.077mm (Figure 3.2). P01370 showed the longest individual tube length at 0.968mm (after 4 hours) and P9908 the second highest at 0.768mm (data not shown).

Pollen tube growth *in vitro* showed no particular directionality (Plate 3.7A). Pollen grains tended to aggregate in clumps (Plate 3.7B) with pollen tubes protruding in any direction. Tubes tended to meander, with few tubes traversing a straight line (Plate 3.7A). Pollen grains germinated *in vitro* show an affinity for style material embedded in the same medium (data not shown). Germination was highest around the edge of the cover slip, suggesting oxygen may be important in pollen germination.

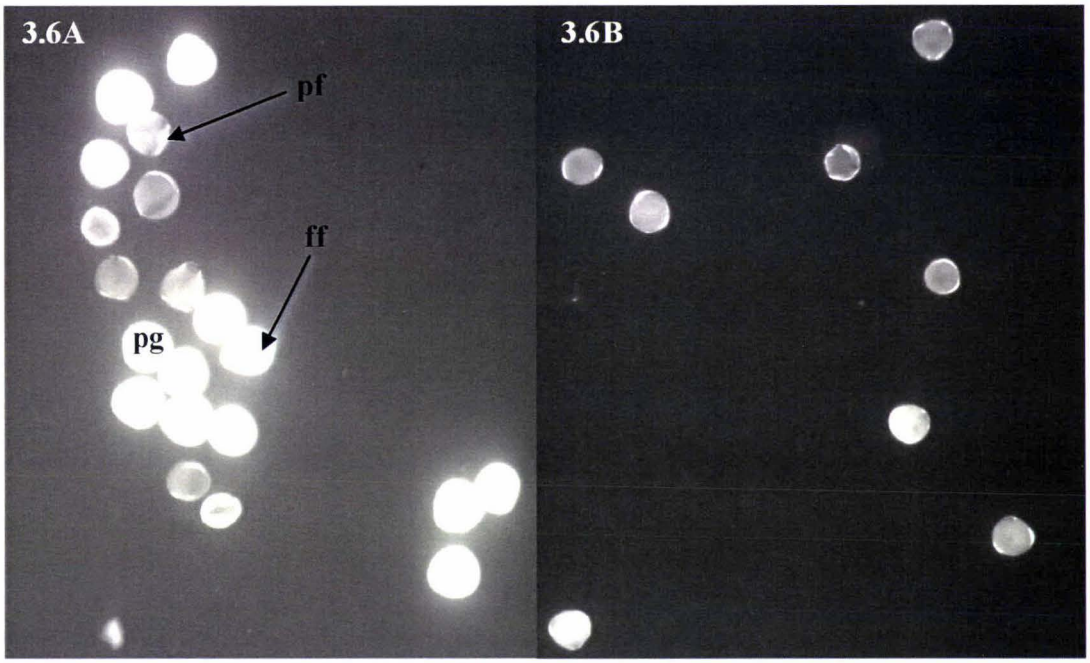


Plate 3.6 *Delphinium elatum* pollen (cultivar P9908) fluorescing after staining with fluorescein diacetate (100x): pg, pollen grain; pf, partially fluorescing (not viable); ff, fully fluorescing (viable). (A) Pollen from newly dehisced anthers. (B) Pollen from old (dying) anthers.

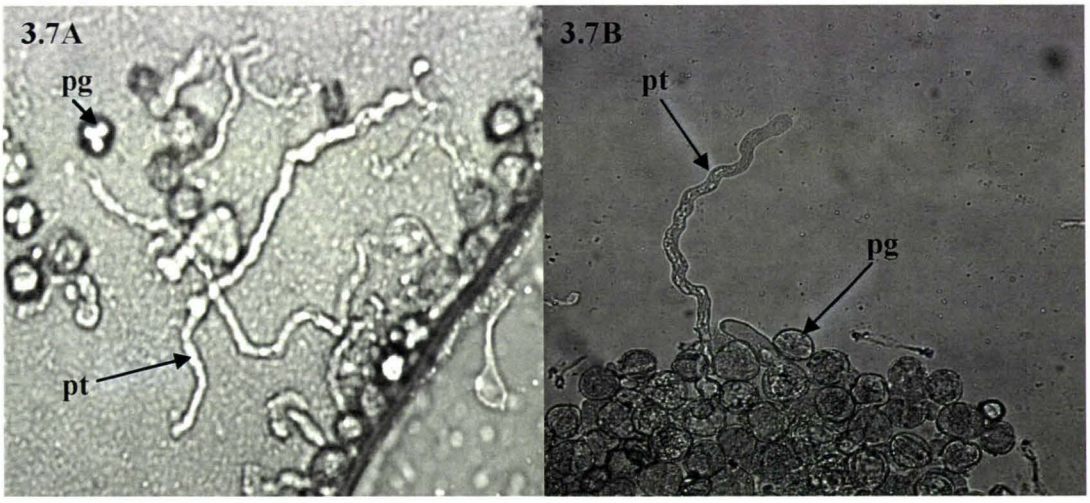


Plate 3.7 *D. elatum* pollen grown on a solid medium *in vitro* (100x): pt, pollen tube; pg, pollen grain.

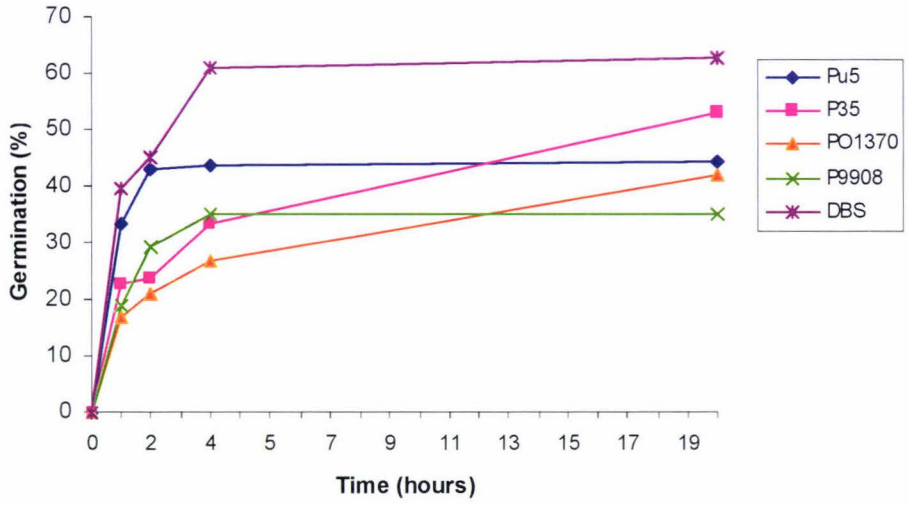


Figure 3.1 Germination of *D. elatum* pollen tubes *in vitro* over a 21 hour period. Five replicates of 10 pollen tubes were measured at each time interval.

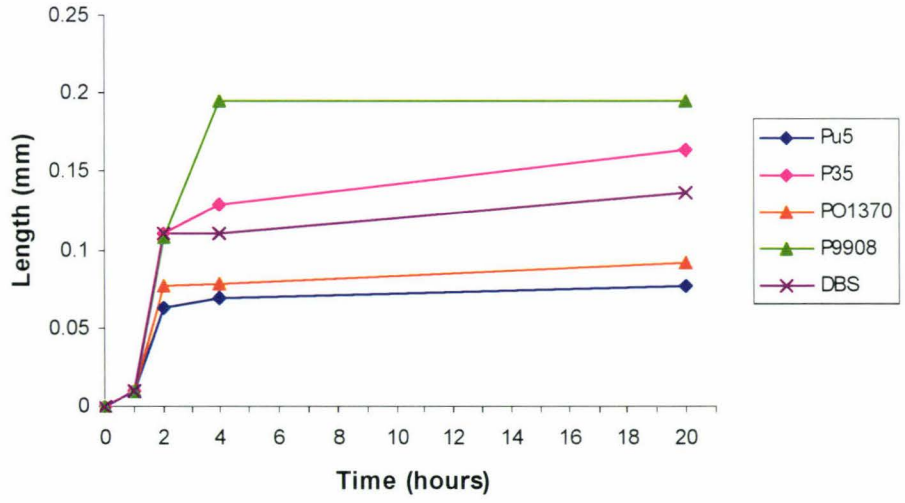


Figure 3.2 Average pollen tube lengths of 5 *D. elatum* cultivars grown *in vitro* over a 21 hour period.

3.3.4 Pollen adherence

Pollen from cultivars P01365, P35, White Db Sport, and W0208 adhered equally well to stigmas of Pu5 and P9827 (Plate 3.8). Amount of pollen adhering to individual stigmas varied within cultivars. Plate 3.8D shows an unopened stigma of cultivar Pu5 with no pollen adherence. The stigma is closed, preventing pollen from reaching the inner stigmatic surface to which pollen adheres during pollination. Once stigmas are open, pollen adherence can occur as the stigma is receptive. Individual numbers of adhering pollen grains were not counted as they generally occurred in large quantities (Plate 3.8A).

3.3.5 Pollen germination *in vivo*

Microscopic examination of pollen tube growth *in vivo* revealed pollen germinating in the stigma and multiple pollen tube growth occurring through the style in crosses P9827 x P35 (Plate 3.9A-B), Pu5 x White DB Sport (Plate3.9D; Plate 3.10A), and Pu5 x W0208 (Plate 3.10C). No pollen germination was visible in crosses of P9827 x P01365 at 3 hours after pollination (Plate 3.9C) but pollen tubes were visible on the stigma at 12 hours after pollination (Plate 3.10B). Pollen tubes were visible in the stigma, style and ovary of cultivar P9827, 48 hours after pollination with P01365 (Plate 3.11).

Some difficulty was encountered in detecting pollen tubes due to high fluorescence of vascular tissue which is prevalent in the style and ovary.

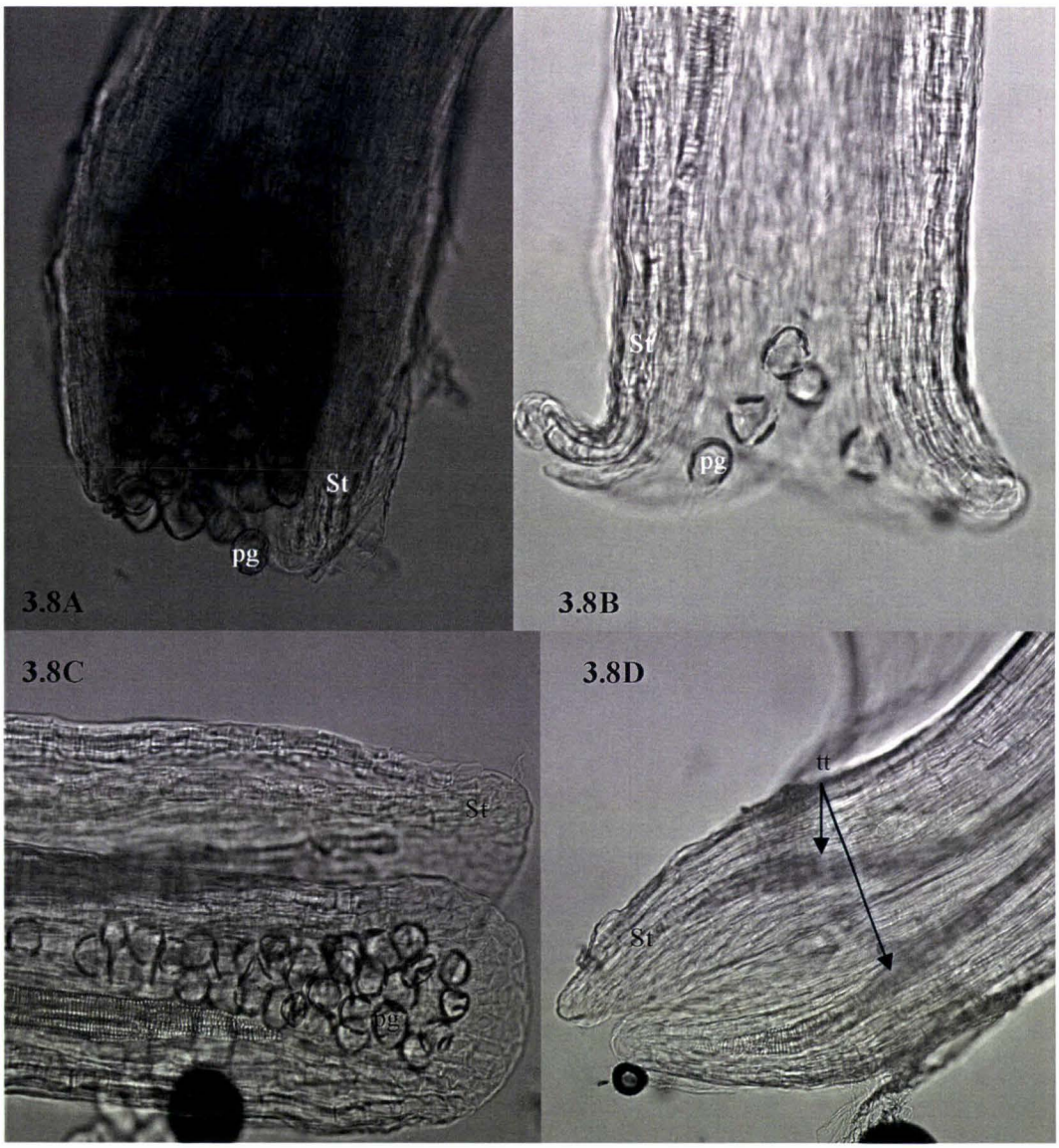


Plate 3.8 Pollen grain adherence to partially cleared delphinium stigma (100x): St, stigma; pg, pollen grain; tt, transmitting tract tissue. (A) White DB Sport pollen adhering to a stigma of cultivar Pu5. (B) P35 pollen adhering to a stigma of P9827. (C) P01365 pollen within a P9827 stigma. (D) Closed Pu5 stigma (no pollen adherence).

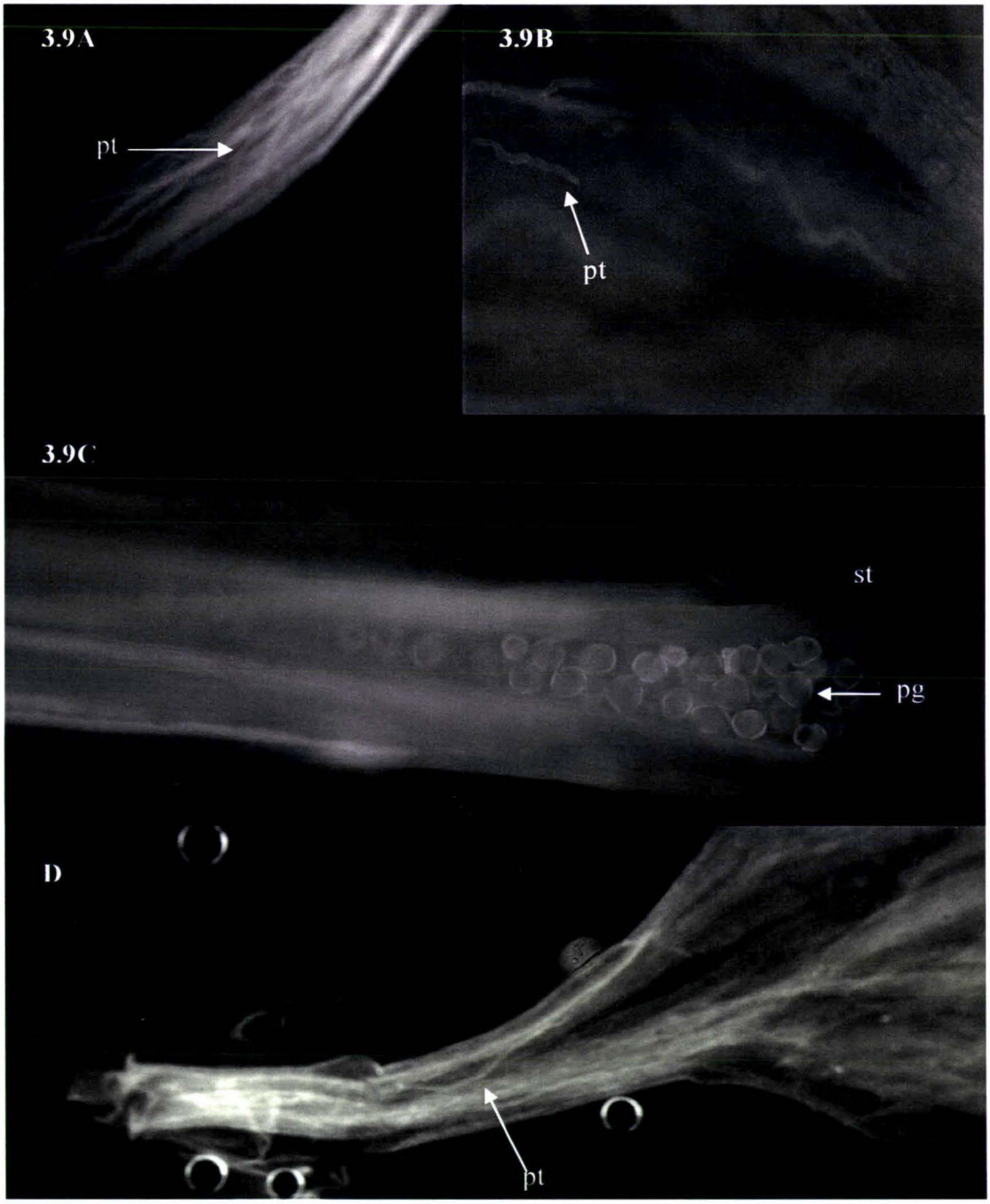


Plate 3.9 Pollen tube growth *in vivo*: pt, pollen tube; st, stigma; pg, pollen grain. (A) Pollen tubes growing in the style of P9827 12 hours after pollination with P35 (40x). (B) Pollen tubes in the basal section of the style of P9827 12 hours after pollination with P35 (100x). (C) Stigma of P9827 3 hours after pollination with P01365 (100x). No pollen tubes are visible. (D) Stigma of Pu5 3 hours after pollination with White DB Sport (40x). Pollen tubes are visible as white fluorescence.

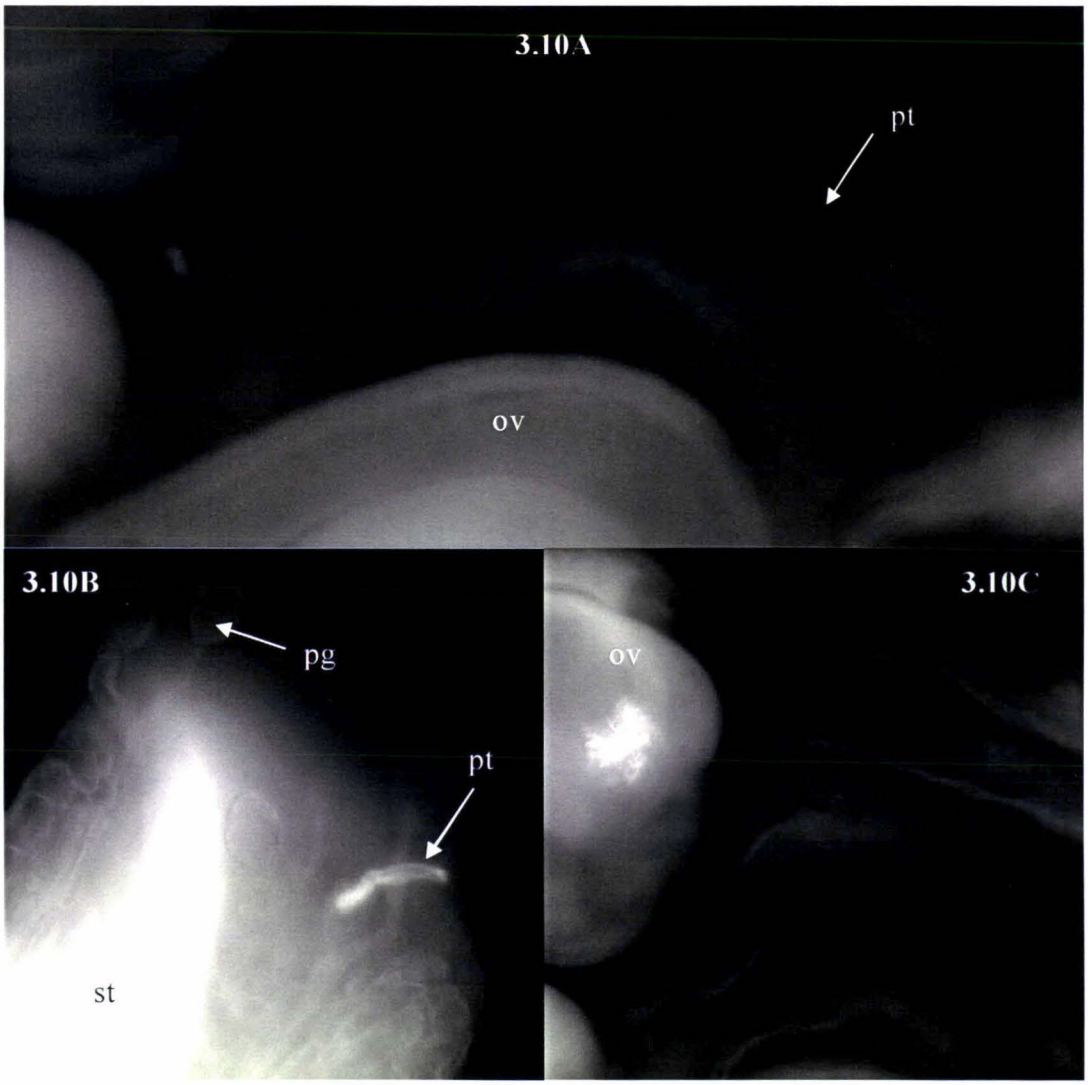


Plate 3.10 Pollen tubes growing *in vivo*: pt, pollen tube; ov, ovule; pg, pollen grain. (A) Pollen tubes growing towards an ovule of cultivar Pu5 24 hours after pollination with cultivar White DB Sport (200x). (B) Pollen grains germinated on the stigmatic surface of cultivar P9827 12 hours after pollination with cultivar P01365 (100x). (C) Pollen tubes in the ovary of cultivar Pu5 24 hours after pollination with cultivar W0208 (100x).

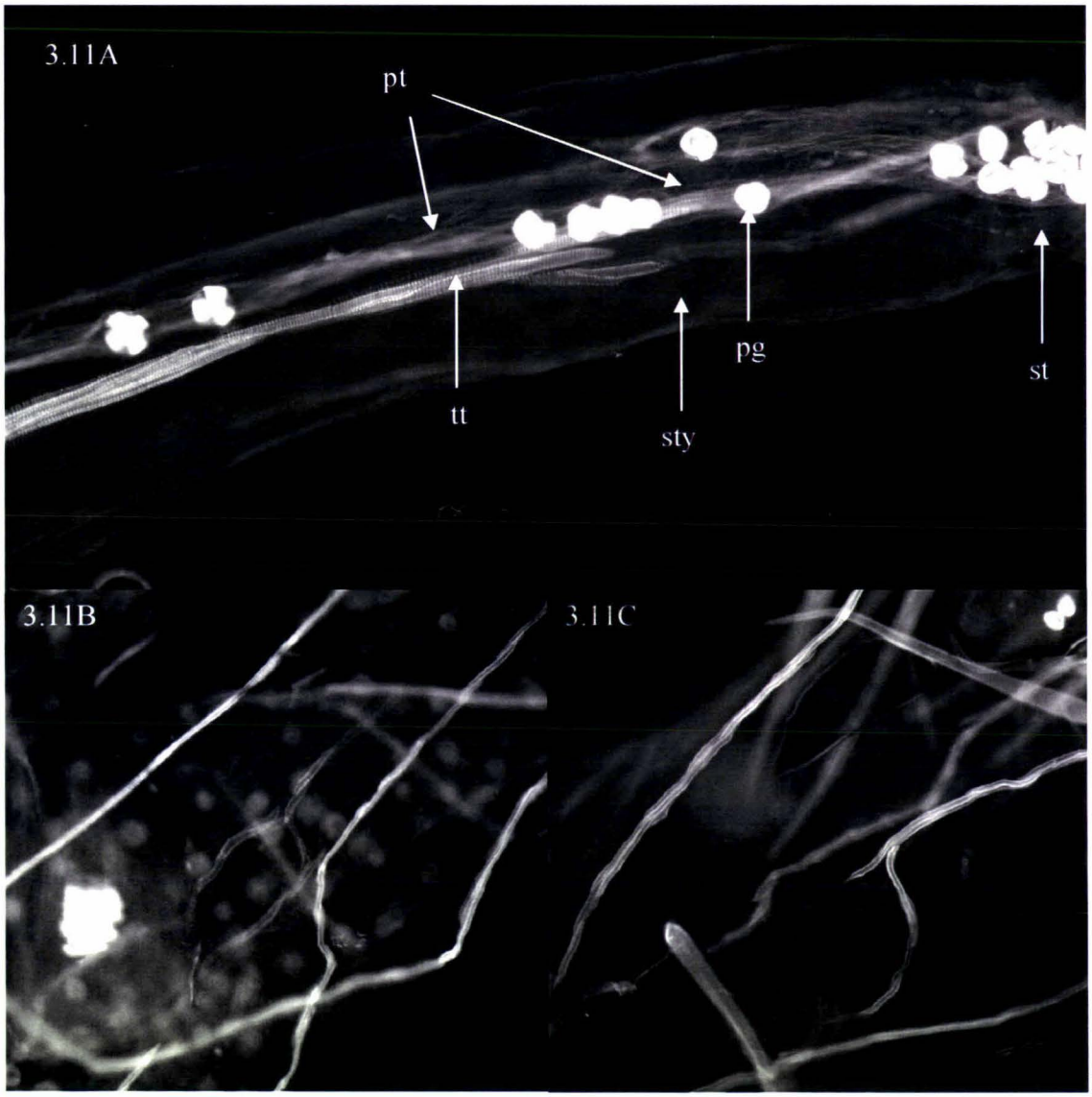


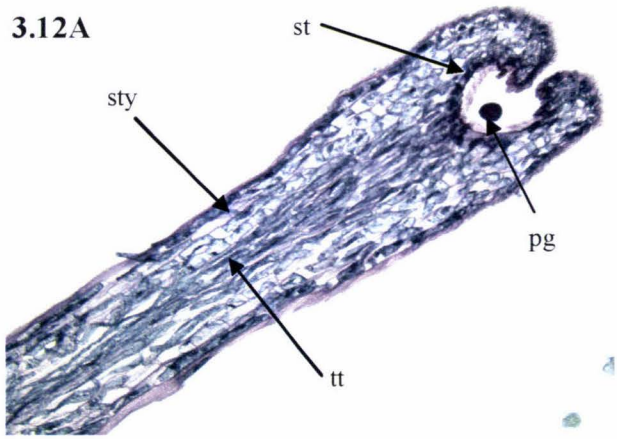
Plate 3.11 Pollen tubes growing *in vivo*. *D. elatum* cultivar P9827 48 hours after pollination with P01365: pt, pollen tube; pg, pollen grain; tt, transmitting tract tissue; st, stigma; sty, style. Pollen grains and tubes are fluorescing due to the presence of aniline blue. (A) Pollen tube growth in stigma and style (100x). (B) and (C) Pollen tube growth in the ovary (200x).

3.3.6 Structural examination

Stigma-style morphology and carpel structures were examined using microscopy to determine structure and to compare it with descriptions given in the literature by Babis (1973) for *Delphinium*.

3.3.6.1 Stigma-style complex

Microscopic examination of sections of *D. elatum* stigmas revealed open stigmas with pollen grain adherence on the inner surface. The inner surface of the open stigma is lined with transmitting tract epidermis (Plate 3.8A). Young stigmas (not yet receptive) have a closed structure, visible in cleared stigma (Plate 3.8D), which physically prevents pollen adhering to the non-receptive stigmatic surface. Later the stigma unfolds, allowing pollen to be deposited on the inner stigmatic surface (Plate 3.8). The inner surfaces of the open stigmas are lined with transmitting tract tissue which traverses the style and continues down into the ovary (Plate 3.9). The style is open, consisting of an open central canal surrounded by transmitting tract tissue (Plate 3.12B). This tissue branches at the base of the style to line the sides of the ovary (Plate 3.10). Close examination of the style reveals this tissue to be composed, in part, of vascular tissue (Plate 3. 13) which lines the sides of the open stigma (Plate 3.12A), and gradually narrows below the stigma (Plate 3.14A) before travelling the length of the style (Plate 3.14B) and entering the outer integuments of individual ovules (Plate 3.14C). Plate 3.15 shows fluorescing vascular tissue leaving the style (Plate 3.15A) and branching to enter individual ovules (Plate 3.15B) within the ovary.



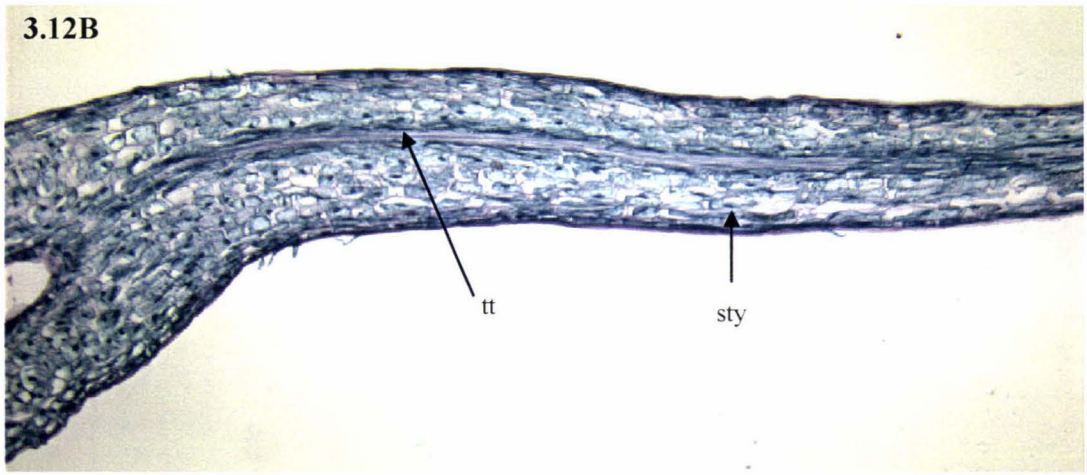


Plate 3.12 10µm sections of delphinium stigma and style (100x): st, stigma; sty, style; tt, transmitting tract tissue; pg, pollen grain. (**A**) A longitudinal section of a cultivar Pu5 stigma and style 3 hours after pollination with cultivar White DB Sport. The hollow stigma is clearly visible. (B) A Longitudinal section of the style of cultivar P9827 12 hours after pollination with P01365.

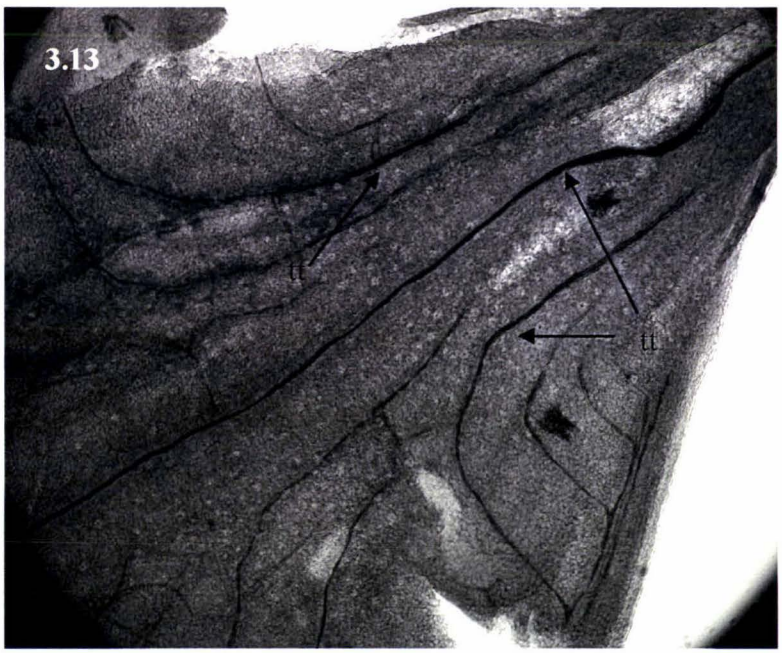
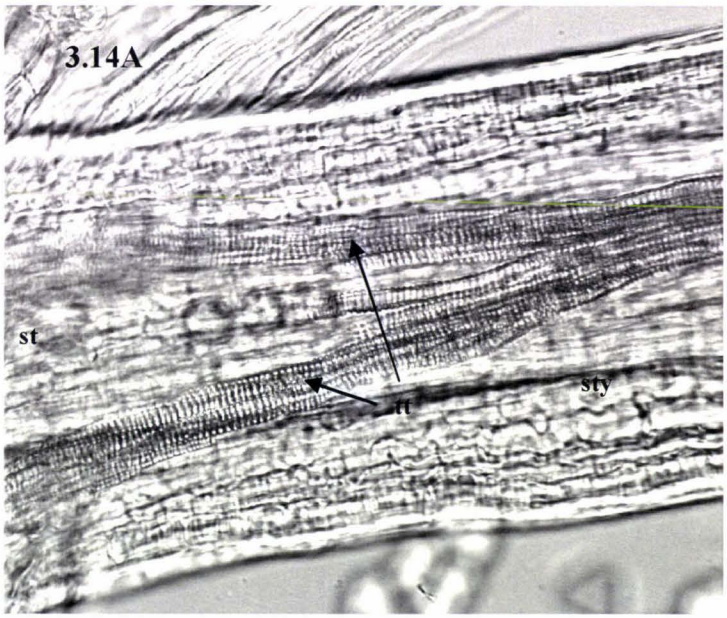


Plate 3.13 The spread tissue of a delphinium style base and ovary (100x): tt, transmitting tract tissue. Transmitting tract tissue is clearly visible. This tissue traverses the length of the style before branching to follow the placental tissue in the ovary.



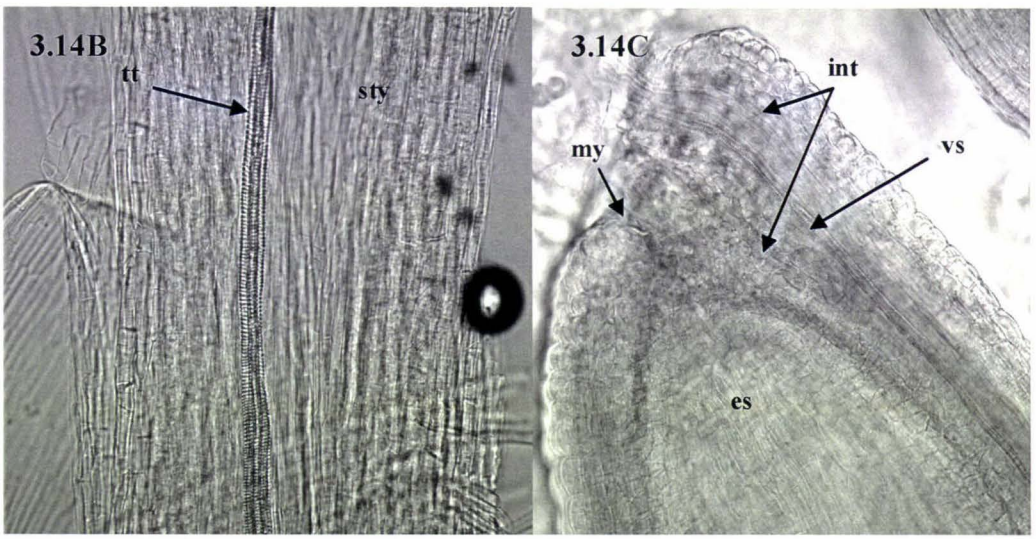
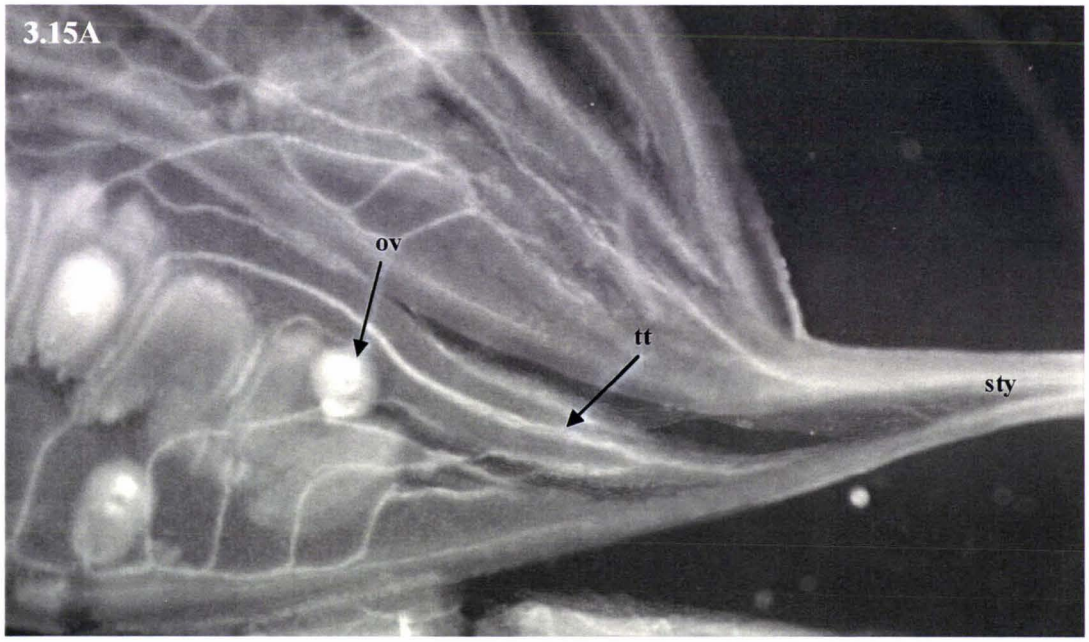


Plate 3.14 Partially cleared tissue of cultivar P9827 3 hours after pollination with P35 pollen: st, stigma; tt, transmitting tract tissue; sty, style; int, integument; es, embryo sac; vs, vascular tissue; my, micropyle. (A) Vascular tissue (transmitting tract tissue) lining the sides of the stigma and style (100x). (B) Vascular tissue running the length of a style (100x). (C) Vascular tissue entering the outer integument of an ovule at the micropylar end (200x).



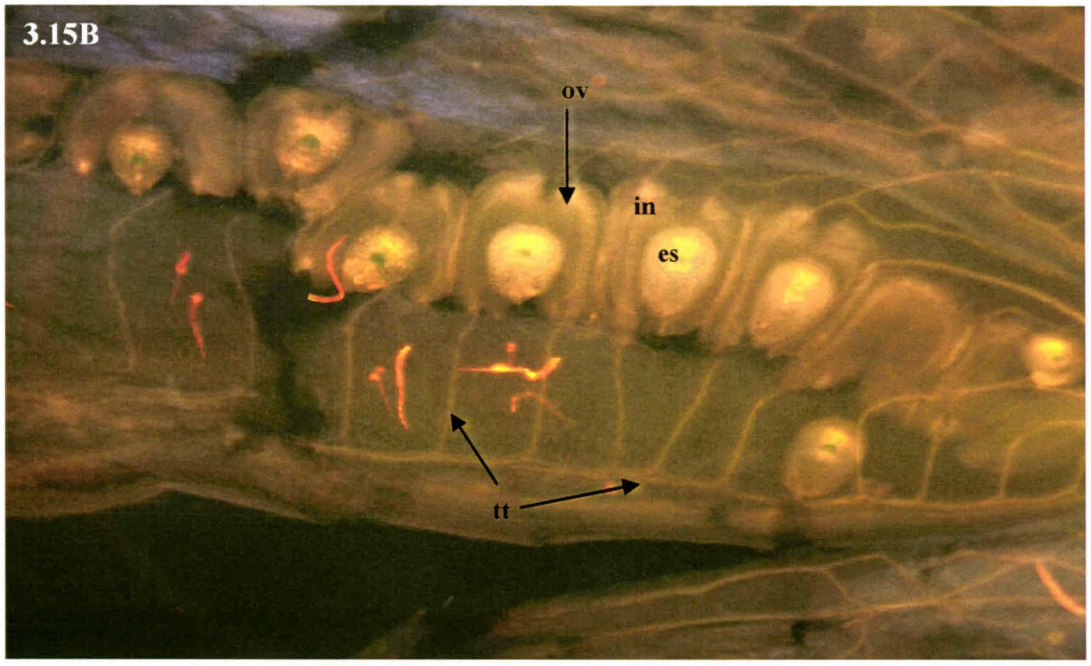
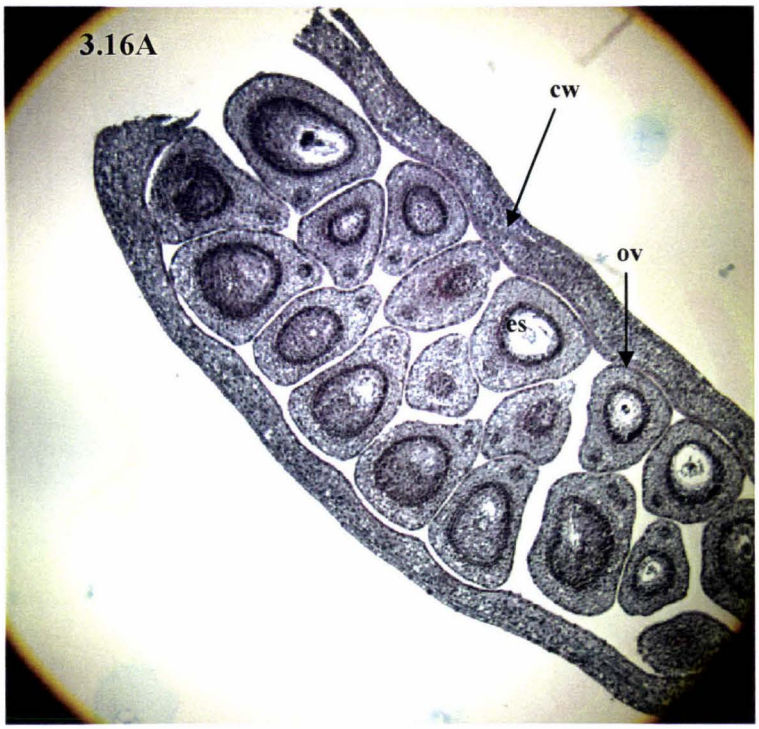


Plate 3.15 Transmitting tract tissue fluorescing after staining with aniline blue (40x): ov, ovule; sty, style; tt, transmitting tract tissue; in, integument; es, embryo sac. (A) Vascular tissue leaving the style and entering the ovary. (B) Vascular tissue entering the outer integuments of individual ovules.



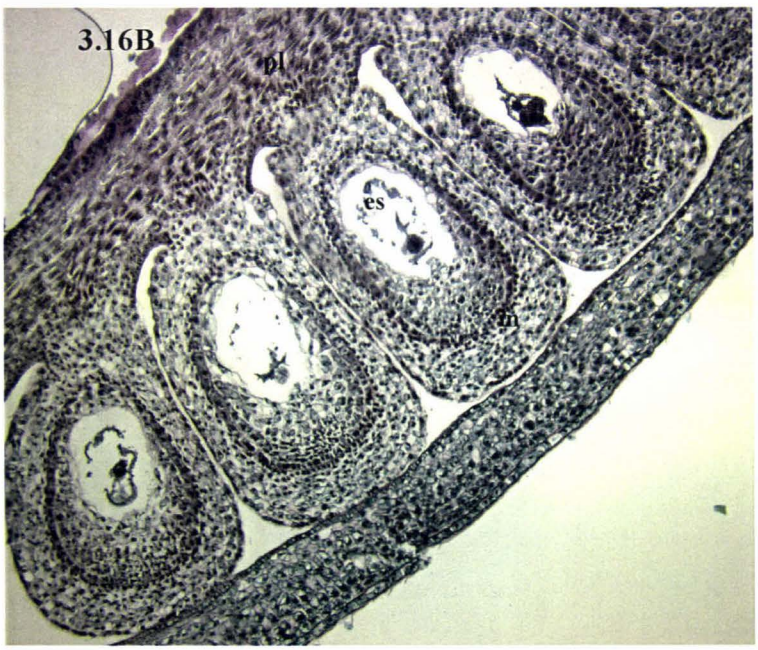


Plate 3.16 A section of 10µm thick cultivar Pu5 carpel: cw, carpel wall; ov, ovule; es, embryo sac; pl, placenta. (A) Ovary of cultivar Pu5 containing numerous ovules (40x). (B) Ovary of *D. elatum* cultivar Pu5 8 hours after pollination (100x).

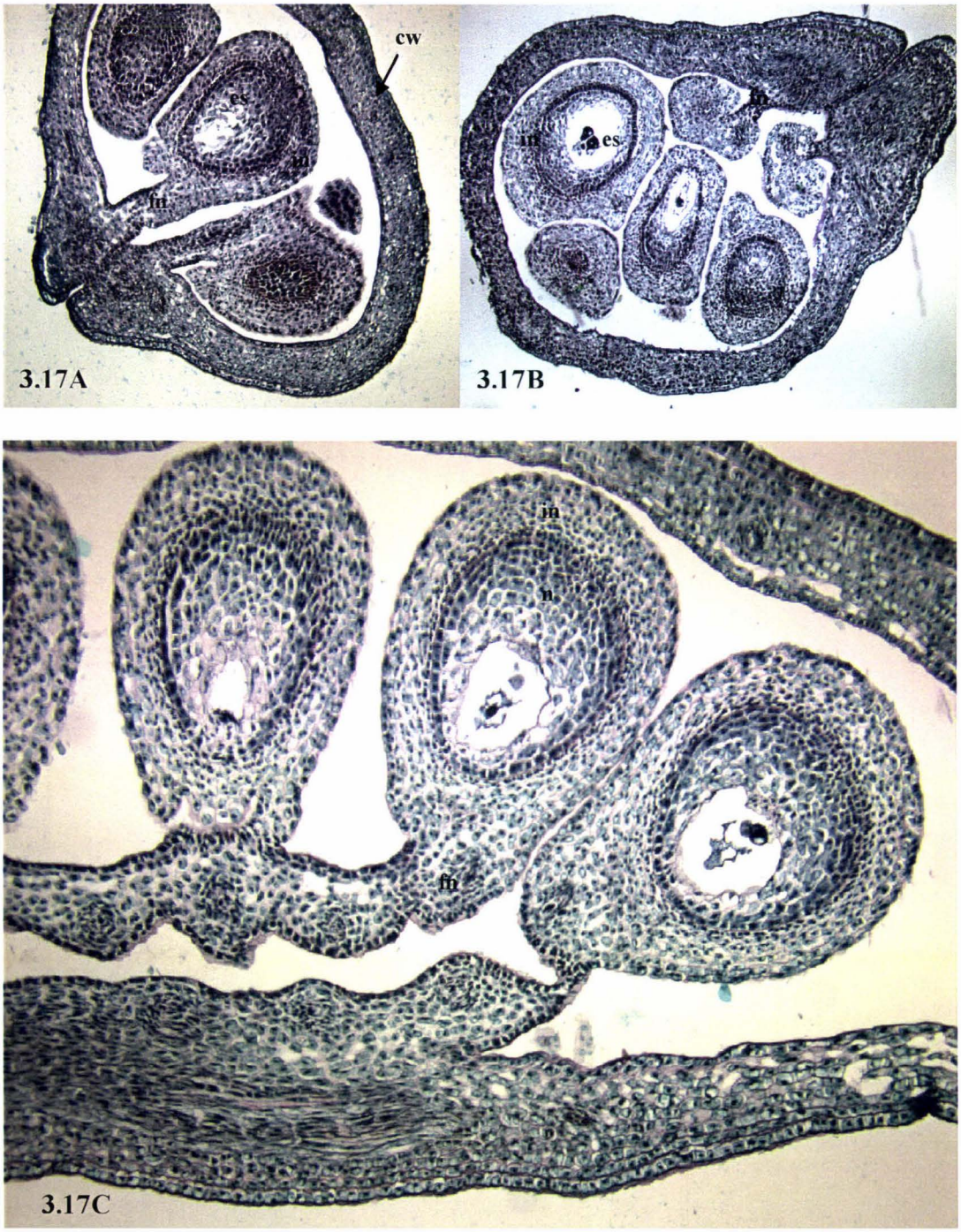


Plate 3.17 10µm thick sections of a delphinium carpel: cw, carpel wall; es, embryo sac; in, integuments; fn, funiculus; n, nucellus. (A) Transverse section of a cultivar P9827 carpel (40x). (B) Transverse section of cultivar Pu5 ovary (40x). (C) Longitudinal section of ovules of cultivar P9827. Developing endosperm is visible at the chalazal end of the ovule (200x).

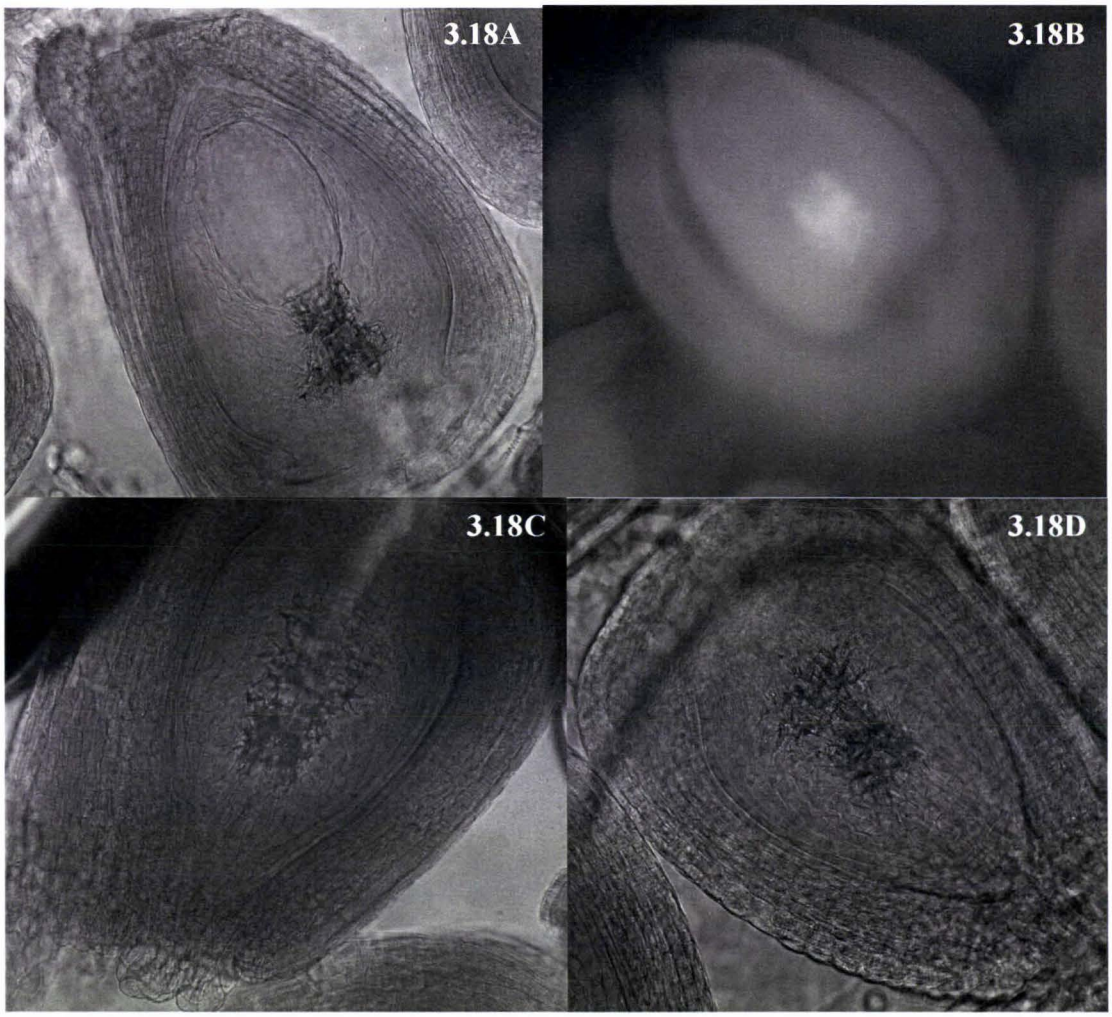
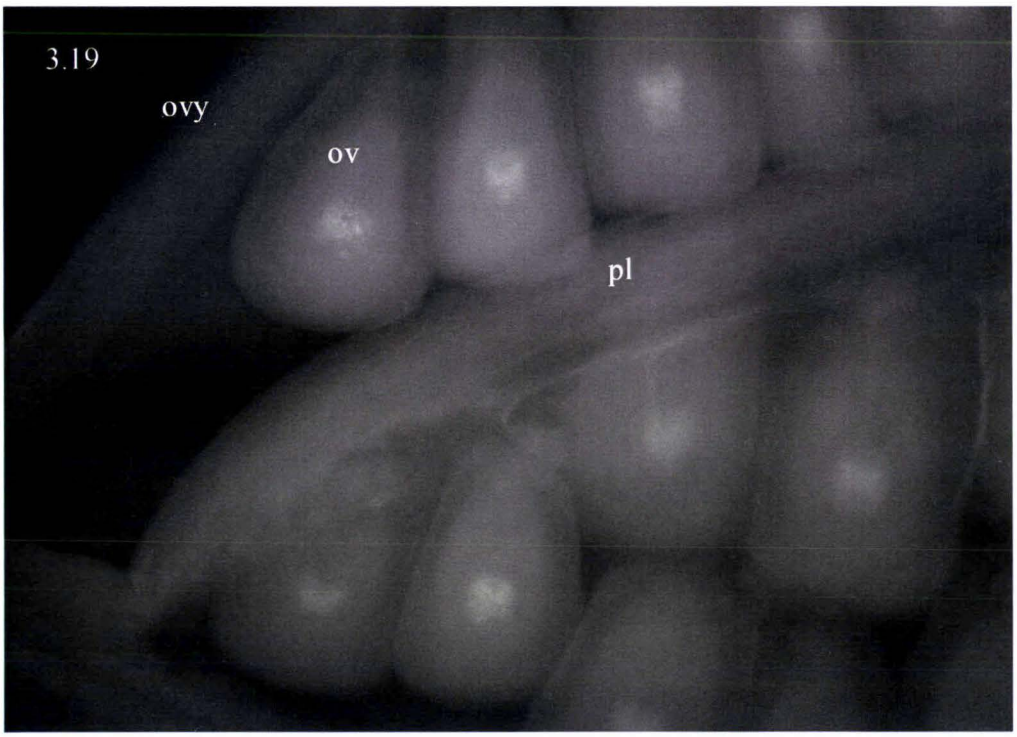


Plate 3.18 Partially cleared ovules of delphinium cultivars (200x). (A) P9827 ovule 3 hours after pollination with P35. (B) A fluorescing ovule of cultivar Pu5 24 hours after pollination with White DB Sport. (C) A Pu5 ovule 24 hours after being pollination with W0208. (D) P9827 ovule 3 hours after pollination with P01365.



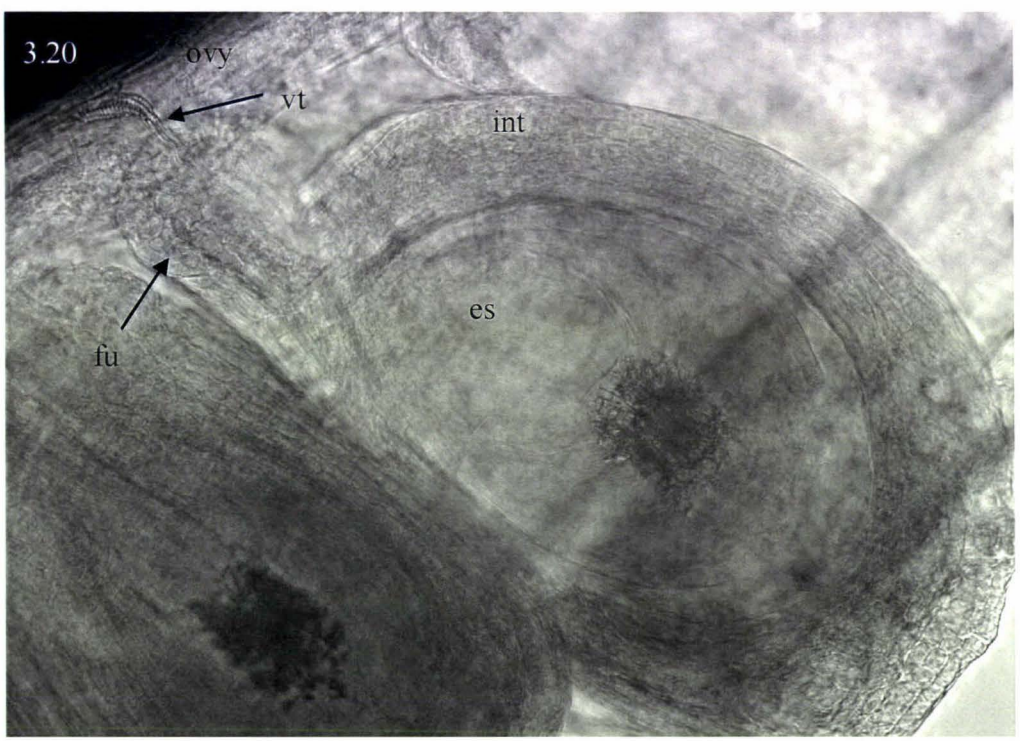


Plate 3.19 Ovules of cultivar Pu5 24 hours after pollination (100x): ovy, ovary wall; ov, ovule; pl, placenta. Ovules are fluorescing due to treatment with aniline blue.

Plate 3.20 Partially cleared ovule of cultivar Pu5 24 hours after pollination (200x): ovy, ovary wall; vt, vascular tissue; int, integuments; fu, funiculus; es, embryo sac.

3.3.6.2 Ovary and ovules

The ovaries contain multiple ovules (Plate 3.16), each attached to the ovary wall via the placental membrane (Plate 3.17; Plate 3.19). There appears to be two placental strands per carpel, and placenta is parietal (ovules are borne on extensions of the ovary wall) (Plate 3.17C). Transmitting tract tissue forms a continuous surface down the centre of the style and into the ovary. Spread carpel tissue reveals the transmitting tract tissue branches once it reaches the ovary, with individual strands corresponding to individual ovules (Plate 3.14; Plate 3.15). Ovule structure is the same for all cultivars (Plate 3.18). Ovules consist of two outer integuments enclosing the nucellus which contains the embryo sac (Plate 3.18). Plate 3.14C shows the micropylar region, and Plate 3.20 the funiculus. Vascular tissue runs through the funiculus and into the outer integument (Plate 3.20). Fluorescing tissue was visible in the centre of all ovules treated with aniline blue (Plate 3.15; 3.18B; 3.19). The position of this fluorescence corresponds with the nucleus of the central cell, as described by Babis (1973) and is the same as the dark, dense region of cell seen in the ovule centre in Plate 3.20.

3.4 DISCUSSION

3.4.1 Pollen viability

Overall, *D. elatum* pollen showed low viability. Similar rates of viability were seen when germinating pollen *in vitro* and using the FCR (fluorochromatic test). Usually it is assumed that pollen germination *in vitro* will be lower than viability assessed using stain or fluorescence (Heslop-Harrison, 1987). Pollen had no difficulty germinating *in vitro*. In most cases germination began within 30 minutes of pollen reaching the medium, which suggests germination *in vivo* occurs at a very fast rate. Generally pollen germinating on the stigma takes less time to hydrate and germinate than *in vitro* germination. A solid medium was used for *in vitro* tests, although pollen also germinated in liquid medium.

3.4.2 Pollen tube growth

First measurements of *in vivo* pollen tube growth were taken 3 hours after pollination. In all cultivars pollen tubes had reached ovaries within 48 hours of pollination, suggesting tube growth is faster *in vivo*. The longest pollen tube *in vitro* was 0.195mm over 24 hours. Style lengths are on average 5-10mm long, so although germination is fast, pollen tube growth must be slower *in vitro*. Individual pollen tubes within the style and ovary were not countable due to the difficulty in distinguishing pollen tubes from vascular bundles, which also fluoresced under aniline blue treatment. This may also be due to pollen tubes growing within the transmitting tract (which fluoresced under aniline blue) surrounding the style canal, as well as in the canal. A similar problem was observed by Rugkhld et al (1997) when examining seed set *Santalum spicatum*. Observations of pollen tube growth in the style showed the majority of pollen tubes to grow within extra-cellular matrices located in the transmitting tract tissue surrounding the hollow canal in the centre of the style, with few tubes growing within the canal itself. Rates of pollen-tube extensibility after germination varied not only between cultivars, but also between individual pollen grains when grown *in vitro*. This suggests individual pollen grains may differ in their ability to grow, perhaps due to an inability to utilise nutrients provided by the medium, which may correspond with a similar inability to utilise style nutrients when germinating *in vivo*.

Pollen from cultivars Pu5 and P35 usually results in high seed set when used in crosses which does not correlate with results from pollen grain viability and tube growth. These cultivars have the 2nd and 3rd highest levels of pollen germination, but Pu5 had the lowest rate of pollen tube growth. Pollen from cultivar White DB Sport gives variable results in actual crosses, but showed highest pollen viability in these trials and the 3rd highest level of pollen tube growth rate, out-performing cultivar Pu5 which usually results in high seed set when used in tests.

3.4.3 Pollen adherence

D. elatum pollen had no trouble adhering to the stigmatic surface in any cultivar. The amount of pollen adhering to individual stigma varied within cultivars. This is possibly due either to different amounts of pollen reaching individual stigmas or to stigmas varying in their ability to hold pollen. The cause is unlikely to be due to the pollen itself, or similar rates of adherence would have been seen in all stigmas from each cultivar. This suggests that either individual stigma vary in ability to accept pollen, or these stigmas received less pollen. Flowers were pollinated twice to ensure full pollen coverage, making the latter scenario less likely. The more likely explanation is that these stigmas may not have been fully receptive at the time of pollination, and that not all stigmas within a floret initiate receptivity at the same point in time. As seen in Plate 3.8D, a stigma needs to be open to receive pollen. Immature stigmas are physically incapable of receiving pollen on the inner stigmatic surface where adherence occurs. Lack of pollen adherence is most likely due to initial pollen amounts reaching the stigma, or receptivity of stigmas at time of pollen reception.

In at least several of the cultivars (such as P9908), low seed set after selfing (without hand-pollinating) is due to lack of pollen reaching the stigma. Flowers of P9908 could be selfed with human intervention, but usually did not self naturally, although stigma receptivity occurred at the same time as anther dehiscence. Differences in number of pollen tubes growing through the style and entering ovules occurred between individual carpels but no pattern was seen linking pollen tube growth to cultivar type.

3.4.4 Structural examination

The structural examination revealed *D. elatum* stigmas to be open-ended. Receptivity occurs when stigma unfurl to expose the inner surface of the stigma. The surface of the stigma is line with a callose containing tissue which tapers at the base of the stigma to form a column of transmitting tract around the hollow canal of the style. Pollen grains landed on

the inner stigmatic surface and grew down the transmitting tract lining the inner wall of the style.

Transmitting tract tissue (or vascular tissue) is prevalent in the style and ovary. It forms a column down the centre of the style before branching at the base of the ovary to form 2-3 columns traversing the placental tissue on the sides of the ovary. This tissue then branches further to form individual strands penetrating the outer integument of individual ovules. The vascular strand is used to supply nutrients to the ovule (Johri and Ambegaokar, 1984). However, this tissue may play an additional role in guidance of pollen tubes to the micropylar entrance of ovules. Rugkhld et al (1997) discovered that in *Santalum spicatum*, pollen tubes grow in transmitting tract tissue around the sides of the ovary wall, and emerged next to the embryo sac of individual ovules.

Sectioning revealed the amount of ovules per carpel to be numerous. Vascular tissue is prevalent throughout the style and carpel. Ovule structure correlated with descriptions given by Babis (1973) for *Delphinium elatum*. No differences in ovule structures were observed between cultivars, and examined ovules displayed no abnormalities. Ovule shape tended to vary from oblong to oval due to the number of ovules contained within each ovary. Rugkhld et al (1997) suggest the fluorescence seen in the egg apparatus after treatment with aniline blue may be an indicator of embryo sac receptivity.

Babis (1973) states that the nucleus of the central cell is situated directly below the antipodal cells at ¾ the height of the embryo in a mass of cytoplasm. This corresponds to the dark mass seen off-centre in partially cleared ovules, and may be the region of ovule tissue fluorescing under treatment with aniline blue.

4.0 SEED GERMINATION IN DELPHINIUM

4.1 INTRODUCTION

Delphinium elatum hybrids produce around 75-100 florets per flower spike. Each floret produces 3-5 pods, containing approximately 25 seeds each. Seeds are harvested January-May when pods turn brown. Seeds are not left on plants until fully dried, as pods tend to shatter and seed is lost. Once removed from the plant, seed is air dried before being packaged. Many *D. elatum* hybrids appear to have low rates of germination, limiting commercial viability.

Seeds have certain requirements for water, temperature, and oxygen in order to germinate (Bradbeer, 1988). Specific requirements vary from species to species. Many species also require breaking of dormancy before germination can occur. In natural environments seed dormancy is broken by light, temperature, ageing, and changes to covering structures. Determining the best levels of each factor required for a particular species or cultivar allows germination to be optimised. For example, chilling may be required to break dormancy in some species, but seeds need to be transferred to a more favourable temperature to allow germination itself.

The aim of this research is to determine reasons for low germination of *Delphinium elatum* seed and define optimal conditions for germination.

4.2 MATERIALS AND METHODS

4.2.1 Seed Source

Delphinium elatum seeds were supplied by Dowdeswell's Delphiniums and produced from plants growing in glasshouses at the Centre for Seed Production and Technology, Massey University. Seeds were harvested in December 2002 – January 2004 at Dowdeswell's Delphiniums and in May 2004 at Massey University. Seeds provided by the business were

used in all experiments. Seeds harvested May 2004 at Massey University were used in examination of seed structure and in germination experiments. All seeds were stored in plastic bags in a sealed plastic container at 5°C until required.
Seeds from the following cultivars were supplied by the business:

- Open-pollinated mix (O/P mix)
- 63 x bed 2
- P9827 x 35
- LB01130x
- W0204x

Crosses made at the University were:

- P9908 selfed
- P9827 selfed
- P01370 selfed
- P01370 (f) x P01433 (m)
- P35 (Celia) selfed

4.2.2 Embedding and sectioning of seeds

All seed types listed above were fixed for structural examination. Five replicates of each type were used. Seed samples were fixed in a mixture of 40% formaldehyde, glacial acetic acid, and 70% ethanol (FAA) in a ratio of 5:5:90 for 2-4 days. The samples were rinsed in 70% ethanol before dehydration. Dehydration was carried out using an ascending ethanol/water ratio over 48 hours. Tissue was infiltrated with roticlear (Carl Roth, Germany) using an increasing ratio of roticlear/ethanol. After 2 changes with pure roticlear, a combination of roticlear/paraffin wax (50:50) was used to link roticlear with pure paraffin wax. After 6 repeats of melted wax at 60°C, samples were embedded into aluminium moulds. Some difficulty was encountered in manoeuvring seeds to gain a clear transverse or longitudinal view as seed coats obscure orientation of seed.

Sectioning was performed using a LEICA RM 2145 Microtome. Sections were cut in layers 12μm thick, mounted on poly-lysine coated slides, and dried at 42°C overnight. Attempts were made to cut material at 10μm but material tended to fray and sections were lost. Wax was removed by roticlear and roticlear by ethanol. Sections were stained in 1% safranin for 48 hours before staining in 0.5% fast green for 1-5 seconds before de-staining in ethanol and roticlear. Slide mounting was carried out using DPX. Samples were then examined and photographed under an Olympus compound light microscope. Comparisons were made between cultivars.

4.2.3 Seed viability

50 seeds from each seed type were soaked in water for 20 hours at 20°C. Each seed was then cut longitudinally approximately ⅓ of the way through the distal end of the endosperm. Seeds were then immersed in a 1.0% solution of 2,3,5-triphenyl tetrazolium chloride for 18 hours at 30°C (ISTA working sheets for tetrazolium testing, 2003). Stained seeds were cut longitudinally and examined under a light microscope for red staining. Seeds were classified as viable (total staining), or non-viable (sections of embryo or endosperm left unstained) (Classification according to ISTA working sheets for tetrazolium testing, 2003). Hard seed left at the end of germination experiments was also stained with TTC for assessment of viability following sectioning as above.

4.2.4 Optimising germination

Seeds were germinated on two layers of moist blue germination paper in 11x9cm clear plastic containers. Germination paper was soaked for 30-60 seconds in distilled water (H_2O) before spreading out of seeds. Containers were sealed to retain moisture. Four replicates of 50 seed were used in each experiment. All seed germinations (excepting those examining the effects of temperature) were performed at 15°C. Table 4.1 shows a summary of germination treatments trialled. Germination was recorded every 2-4 days for 20-30 days, except in experiments examining germination rates over time, where germination was recorded until the majority of viable seeds had germinated or deteriorated.

Percent of germinated seeds showing fungal growth was also recorded. Germination was defined as the visible emergence of the radicle from the seed coat (Bewley and Black, 1994). At the end of each germination period remaining seeds were classified as 'hard' (water impermeable seed coat) or dead (soft seeds that burst under gentle pressure). Any remaining hard seeds were tested for viability using the TTC assay.

The following sections give details relating to two factors: firstly, optimising of overall germination percentage, and secondly, decreasing of germination time.

4.2.4.1 Temperature

Cultivars Open-pollinated mix (O/P mix), LB01130x, P9827x35, and 63xbed2 were used in this experiment. Seeds were germinated at temperatures of 15°C, 20°C, 25°C, and room temperature, with germination percentage scored every 2-4 days. Individual seeds were removed once germinated to limit spread of mould.

4.2.4.2 Chilling

Cultivars O/P mix and LB01130x (2 weeks only) were trialled. Seeds were imbibed on moist germination paper and set to germinate at 5°C for periods of 2, 6 and 30 weeks. Four replicates of each seed type were used in both time trials. Germination percentages were recorded every 1-2 weeks for the 30 week trial and every 2-6 days for the 2 and 6 week trials. After 2, 6 and 30 weeks, the remaining seed was transferred to 15°C for 2-3 weeks. Seeds were scored for presence or absence of fungal growth.

4.2.4.3 Light

Cultivars O/P mix, LB01130x, and P9827x35 were used in this experiment. Seeds were germinated in germination containers placed in sealed black polythene bags (photographic film light-excluding grade) at 15°C to compare with germination in daylight at 15°C. A

weak green safe light was used to score for germination (Photosynthetically active radiation (PAR) was up to 0.17 μmol $m^{-2}s^{-1}$level, as measured by Mackay (2004)).

4.2.4.4 Scarification

Cultivars O/P mix and 63xbed2 were used in this experiment. Seed coats were nicked with a razor blade near the distal end of the endosperm before being imbibed in distilled H_2O. Seed were soaked for 10 minutes first to make cutting easier. Germination was then compared to a control (soaked for 10 minutes, unscarified).

4.2.4.5 Chemical

O/P mix seeds were used in this experiment. Seeds were placed on blue germination paper which had been soaked in potassium nitrate (KNO_3) solution for 30 seconds. Concentrations of 10mg/l, 100mg/l, 500mg/l, and 1000mg/l KNO_3 were used to test the effects of concentration on germination. Distilled water was used as the control.

4.2.4.6 Hormone treatment

Germination of seeds of O/P mix, LB01130x, P9827x35, and 63xbed2 was examined. Seeds were germinated on paper soaked in gibberellic acid (GA_3). Concentrations of 10mg/l, 100mg/l, 500mg/l GA_3 were used to test the effects of concentration on open-pollinated seed. Distilled water solvent was used as a control.

4.2.4.7 Combined treatments

Several treatments were combined to test their joint effect on germination of seed type O/P mix. Four replicates of 50 seeds of O/P mix were germinated on blue germination paper under each treatment. Treatments consisted of: (1) 50:50 solution of 100mg/l GA_3 and 100mg/l KNO_3 applied to germination paper; (2) 100mg/l GA_3 applied to seed replicates germinated at 5°C; (3) Scarified seeds germinated in sealed black polythene bags

(photographic film light-excluding grade; no light); and (4) Seeds surface sterilised and imbibed at 5°C.

4.2.5 Effects of fungicide and surface sterilisation on germination

Four replicates of 50 seeds of O/P mix were used. For surface sterilisation treatment seeds were soaked for 10 minutes in 21% household bleach (Janola) containing 4.2% sodium hypochlorite before being rinsed 3x with distilled H_2O (control was rinsed 3x in distilled H_2O). Seeds were then separated out and germinated on blue germination paper soaked in distilled H_2O.

Effects of fungicide (thiram) on seed germination were also tested. Seeds were placed in a sieve and dusted with thiram before being transferred to germination paper soaked in distilled H_2O. Excess thiram was removed by shaking the sieve for 30-60 seconds after application. Seeds were transferred to the germination paper with forceps. A control (no fungicide) was used.

4.2.6. Seed imbibition

100 seeds of open-pollinated (O/P) mix were measured individually for water uptake over 48 hours. Seeds were weighed before placing on moistened filter paper. At intervals of 1, 2, 4, 8, 24, and 48 hours seeds were removed and weighed. Each seed was rolled gently between 2 layers of tissue paper for 2-4 seconds to remove excess water before weighing. At the end of 48 hours seeds were transferred to blue germination paper. Days to germination were recorded and compared to seed water uptake and dry weight. Appearance and rates of fungal growth were also recorded.

4.2.7 Seed weight distribution

To evaluate seed weight, 50 dry seeds of O/P mix, LB01130x, P9827x35, 63xbed2, and W0204x were individually weighed (4 decimal places). Weights were then ranked from

lightest to heaviest and compared between cultivars. Average seed weights for each cultivar were also calculated. Average weight was then compared to cultivar viability to look for correlations between weight and viability.

4.2.8 Seed moisture

Cultivars O/P mix, 63xbed2, P9827x35, LB01130x, and W0204x were all moisture tested. Four replicates of ten seeds were used for each test. Seeds were dried in a hot air oven at 103°C for 17 hours (in accordance with International Standards For Seed Testing) before weighing. Moisture percent was then calculated for each cultivar.

Table 4.1 Summary of treatments used in germination experiments

Treatment type	Description	Seed type
Temperature		
	Room temperature	O/P mix, LB01130x, P9827 x 35, 63 x bed 2
	15°C	O/P mix, LB01130x, P9827 x 35, 63 x bed 2, W0204x
	20°C	O/P mix, LB01130x, P9827 x 35, 63 x bed 2
Stratification		
(Imbibed, 5°C)	30 weeks	O/P mix,
	6 weeks	O/P mix,
	2 weeks	O/P mix, 63 x bed 2, LB01130x
Scarification		
	Nicking with razor blade	O/P mix, 63 x bed 2
Fungal control		
	Thiram	O/P mix
	Surface sterilisation	O/P mix
Light/dark		
	Black impermeable bags	O/P mix, 63 x bed 2, LB01130x, P9827 x 35
Chemical		
(KNO_3)	10mg/l, 100mg/l,	O/P mix
	500mg/l, 1000mg/l	
Hormone		
(GA_3)	10mg/l	O/P mix
	100mg/l	O/P mix, LB01130x, P9827 x 35, 63 x bed 2
	500mg/l	O/P mix
Priming		
	Wet, dry, wet	O/P mix, 63 x bed 2
Combined treatments		
	100 mg/l KNO_3	O/P mix
	/100mg/l GA_3	
	Dark, scarification	O/P mix
	5°C and GA_3	O/P mix
	5°C, surface sterilisation	O/P mix

4.2.9 Statistical analysis

Measurements of germination made in this trial were analysed using the SAS system for Windows V8 program. DUNCAN and LSD mean separation tests were used to determine significance differences at the 95% confidence level ($p<0.05$) between germination treatments. Statistical analysis was carried out on all experiments testing the effects of different treatments on seed germination within cultivars.

4.3 RESULTS

4.3.1 Seed structure

Delphinium elatum seed is an irregular triangular to oval shape, 2-4mm in length. Size varies between cultivars and individual seeds. Removal of the seed coat exposes a smooth triangular seed with a pointed end. Endosperm tissue within the seed is white. The embryo is clearly visible in the mature seed (Plate 4.1-4.6).

4.3.1.1 Embryo and endosperm

The embryo of the mature seed is torpedo-shaped, extending longitudinally through the seed (Plate 4.1-4.6). Some differences were seen in embryo size and shape between cultivars. Cultivar LB01330x has a well developed embryo with short cotyledons and a thick embryonic axis (Plate 4.1). The cotyledons make up approximately ⅓ of the embryo with the embryo itself is just under ½ the length of the seed. 63xbed2 has cotyledons curving around the central axis in the transverse section (Plate 4.2). This was not visible in other cultivars. A column of darkly stained intense cells are visible down the central axis of the seed. Open-pollinated mix seed (O/P mix) also has a well developed embryo (Plate 4.3) with cotyledons over ½ the length of the embryo. The embryo has long, thin cotyledons with a thick embryonic axis. The seed is elongate and oval. Plate 4.3C shows the embryo from the side. Cotyledons are thicker than the embryonic axis in this view.

W01120X had cotyledons shaped differently from all others examined (Plate 4.4). The cotyledons appear widely separated and curved. The seed is almost round rather than oval. The embryo is over half the length of the seed. P9827x35 cotyledons are straight and densely stained (Plate 4.5). The embryo extends about half the length of the seed. The seed is oval-triangular. The fresh seed more is elongate than dried seed. P9908 (selfed) had an embryo extending over half the length of the seed with short and thick cotyledons (approximately ⅓ the length of the embryo) (Plate 4.6). The seed is triangular shaped. Cotyledons are straight with a typical torpedo shape. No differences were seen between selfed P9908 and out-crossed P9908 seeds.

Plate 4.7 shows stages of development in cultivar P01370x; the ovule before fertilisation; seed 11 days after pollination; seed coat of immature seed; and the mature seed. At 11 days after pollination, the seed is already showing developing endosperm, but embryo is not visible. Seed coat is highly visible. The mature seed was allowed to dry on the plant (Plate4.7D). Embryos are very darkly stained.

Endosperm is of the nuclear type, but becomes cellular later in development. The endosperm is well developed in the mature seed (Plate 4.6) and occupies most of the seed body (Plate 4.6). Cells are thick and dense near the axis of the seed (Plate 4.1B). Endosperm develops early during seed development (Plate 4.7).

4.3.1.2 Seed coat

Delphinium elatum seed coats are wrinkled and dark brown to black in colour. As seeds imbibe water, seed coats swell. Seed coat size in relation to seed volume depends upon individual seed and varies within cultivars. Cultivars such as 63xbed2 and P9827x35 which have proportionately higher seed weight tend to have a lower coat to volume ratio. Seed coats are brittle in the dry seed (easily pierced with forceps or broken by rubbing between fingers), and remove easily from the seed once imbibed. Sectioning of embedded seed generally left the seed coat behind, or tore it. The layers of the developing seed coat are easily visible in the immature seed (Plate 4.7B-C) but harder to distinguish in the

mature, desiccated seed (Plate 4.7D). At 11 days after pollination (Plate 4.7C) the seed coat layers comprised of the two integuments are evident. The outer integument is already one-layered, forming a thin epidermis of intense cells. The inner integument in comprised of two cell layers: a thin inner thick-walled layer, and a surrounding outer layer of large thin-walled cells. This becomes a single layer coating the endosperm in the mature seed. Safranin stains both layers of seed coat red, indicating presence of lignin.

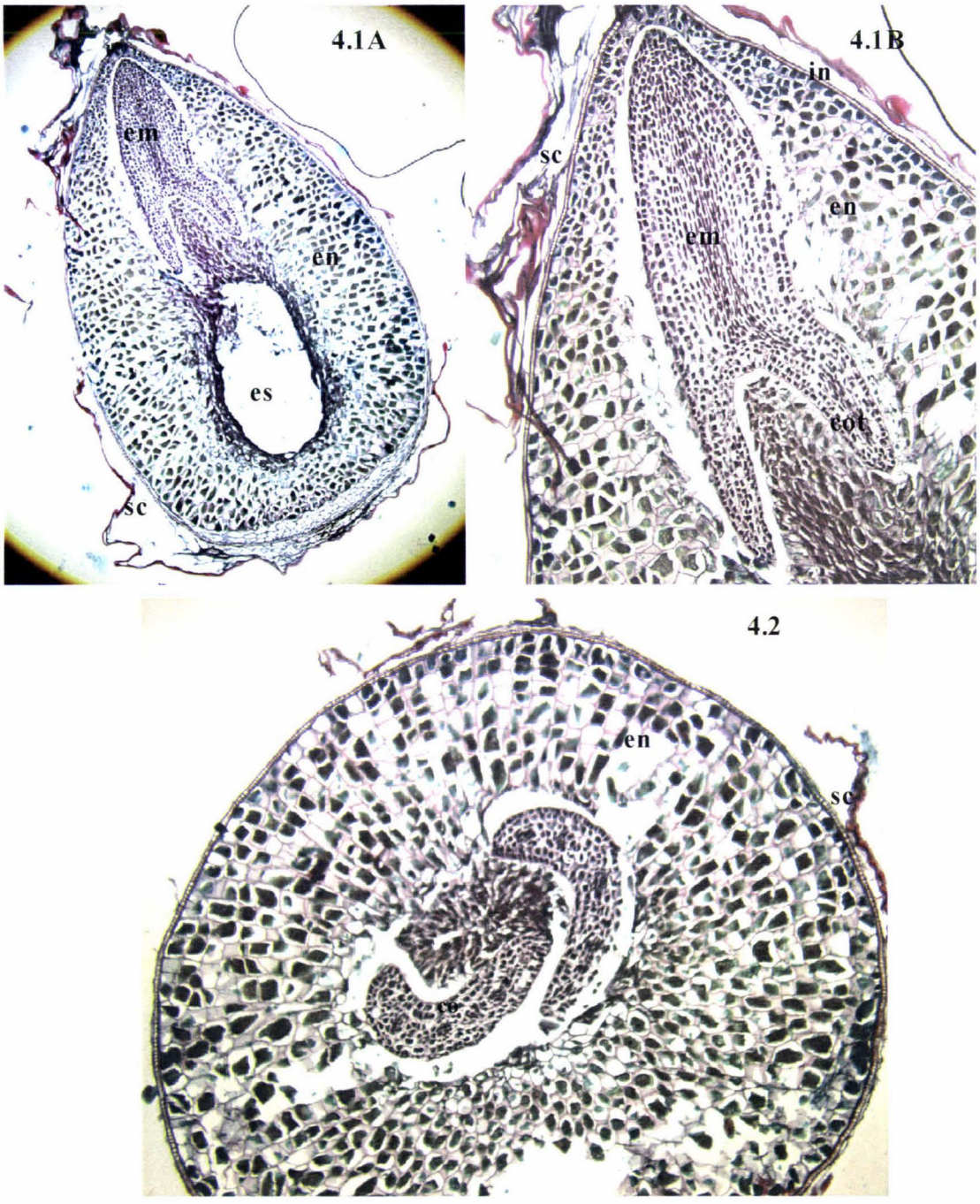


Plate 4.1 Longitudinal section of mature LB01330x seed: en, endosperm; em, embryo; es, empty space; co, cotyledons; sc, seed coat; in, inner seed coat. (A) Full seed (40x). (B) Embryo (100x).

Plate 4.2 Transverse section of a mature seed of type 63xbed2 (100x): en, endosperm; co, cotyledons; sc, seed coat.

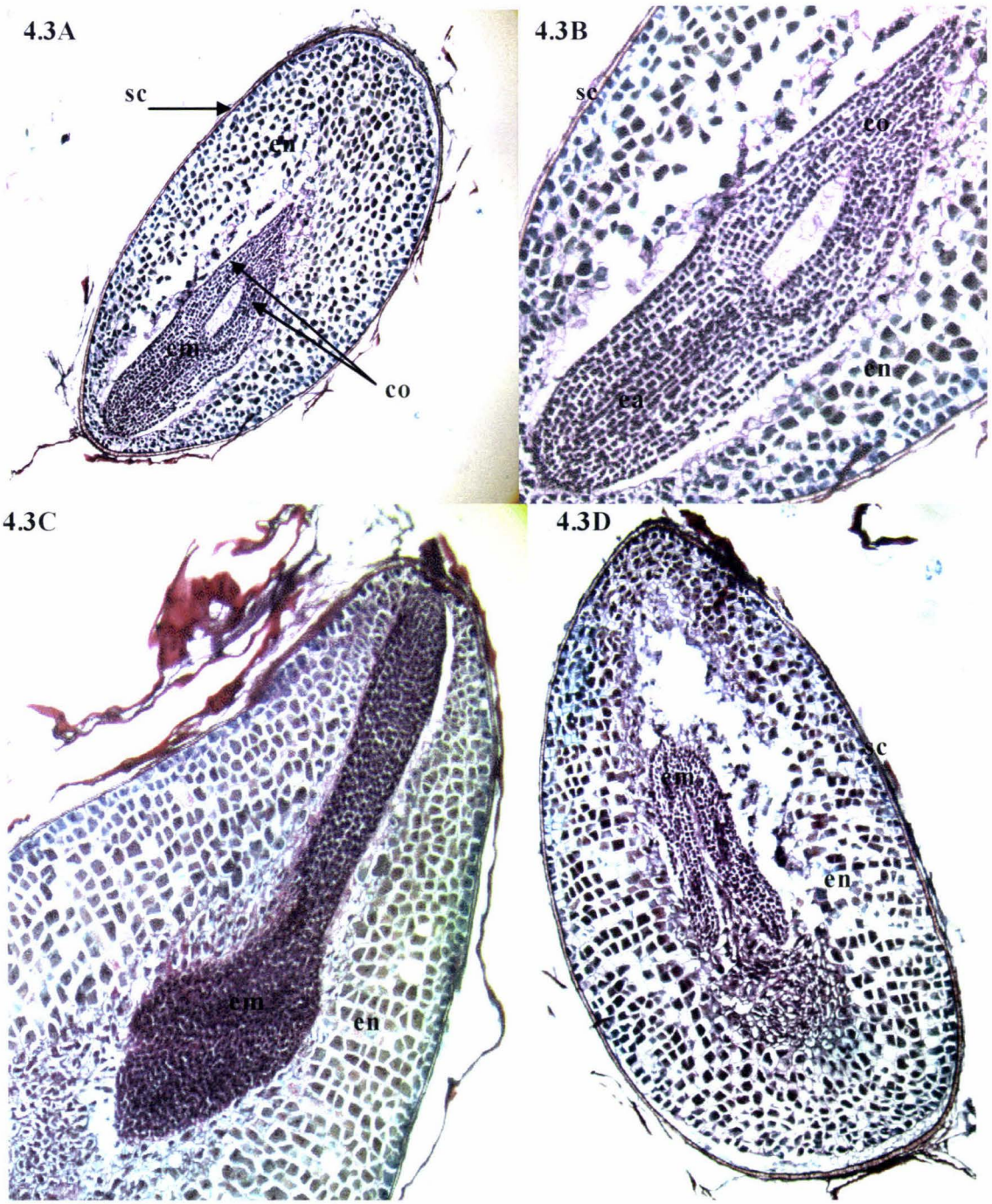
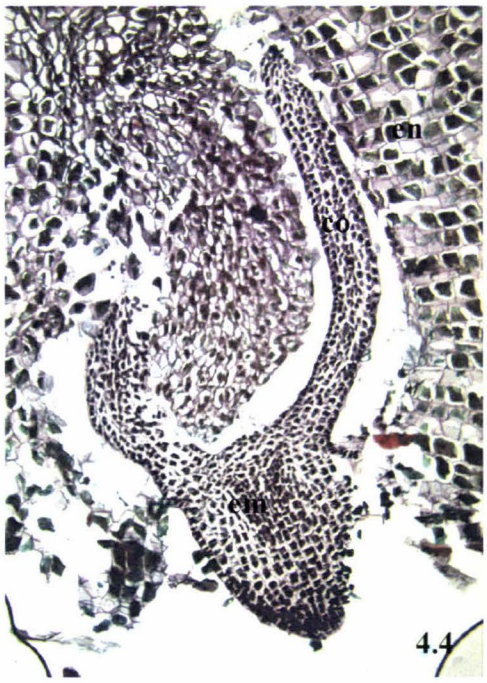


Plate 4.3 Longitudinal sections of mature seed of type open-pollinated mix: sc, seed coat; en, endosperm; em, embryo; co, cotyledons; ea, embryonic axis. (A) Full seed showing embryo and endosperm (40x). (B) The embryonic axis and cotyledons (100x). (C) Intensely stained embryo showing an elongated axis and short cotyledons (100x). (D) Full seed showing cotyledons in centre (40x).



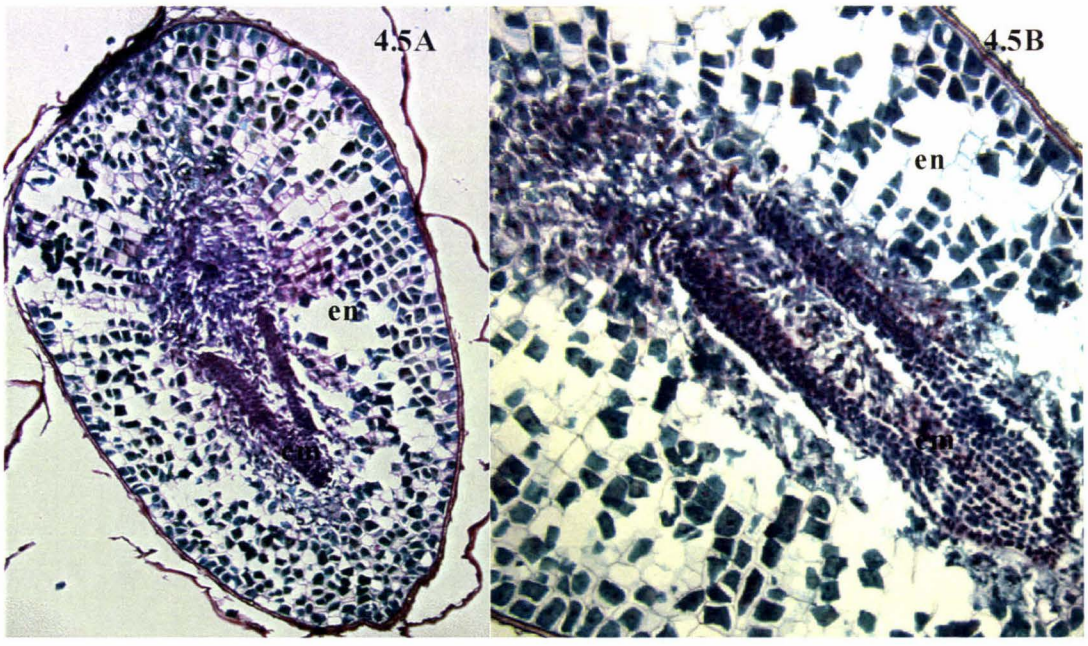
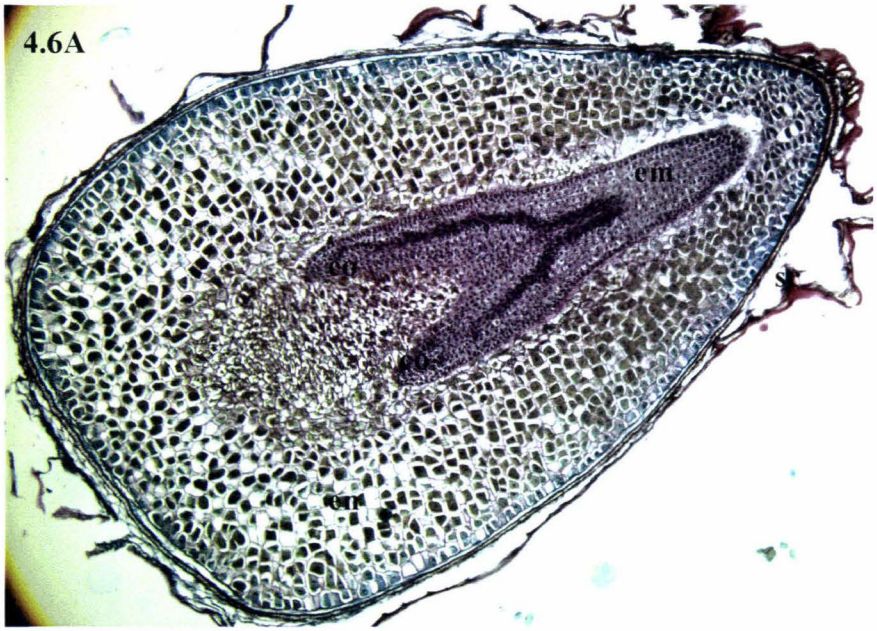


Plate 4.4 Longitudinal section of W01220x (100x): en, endosperm; co, cotyledons; em, embryo.

Plate 4.5 Longitudinal section of a mature seed of cultivar P9827x35: en, endosperm; em, embryo. (A) Entire seed (40x) showing embryo in centre of seed. (B) Close up of embryo (100x) showing red staining of the embryo.



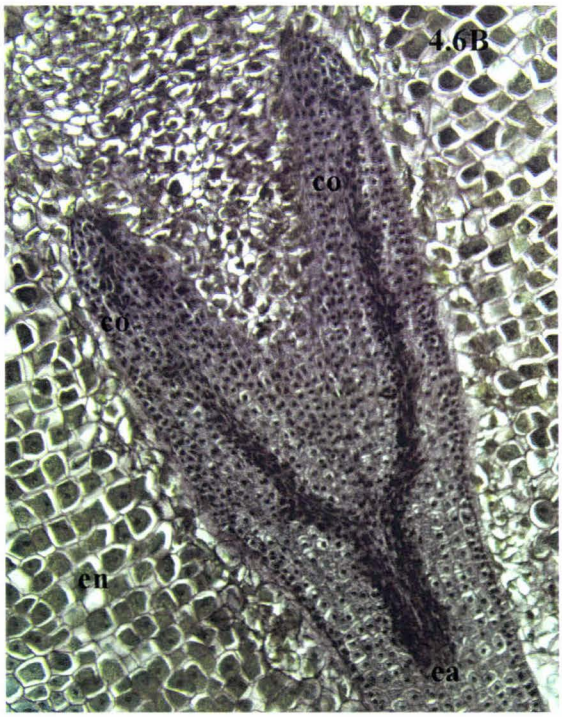


Plate 4.6 Longitudinal sectioning of a mature seed of P9908 (selfed): sc, seed coat; en, endosperm; co, cotyledons; ea, embryonic axis. (A) Entire seed (40x) with the outer seed coat mostly intact. (B) Close up of the embryo showing dense staining down the centre of the cotyledons and embryonic axis (100x).

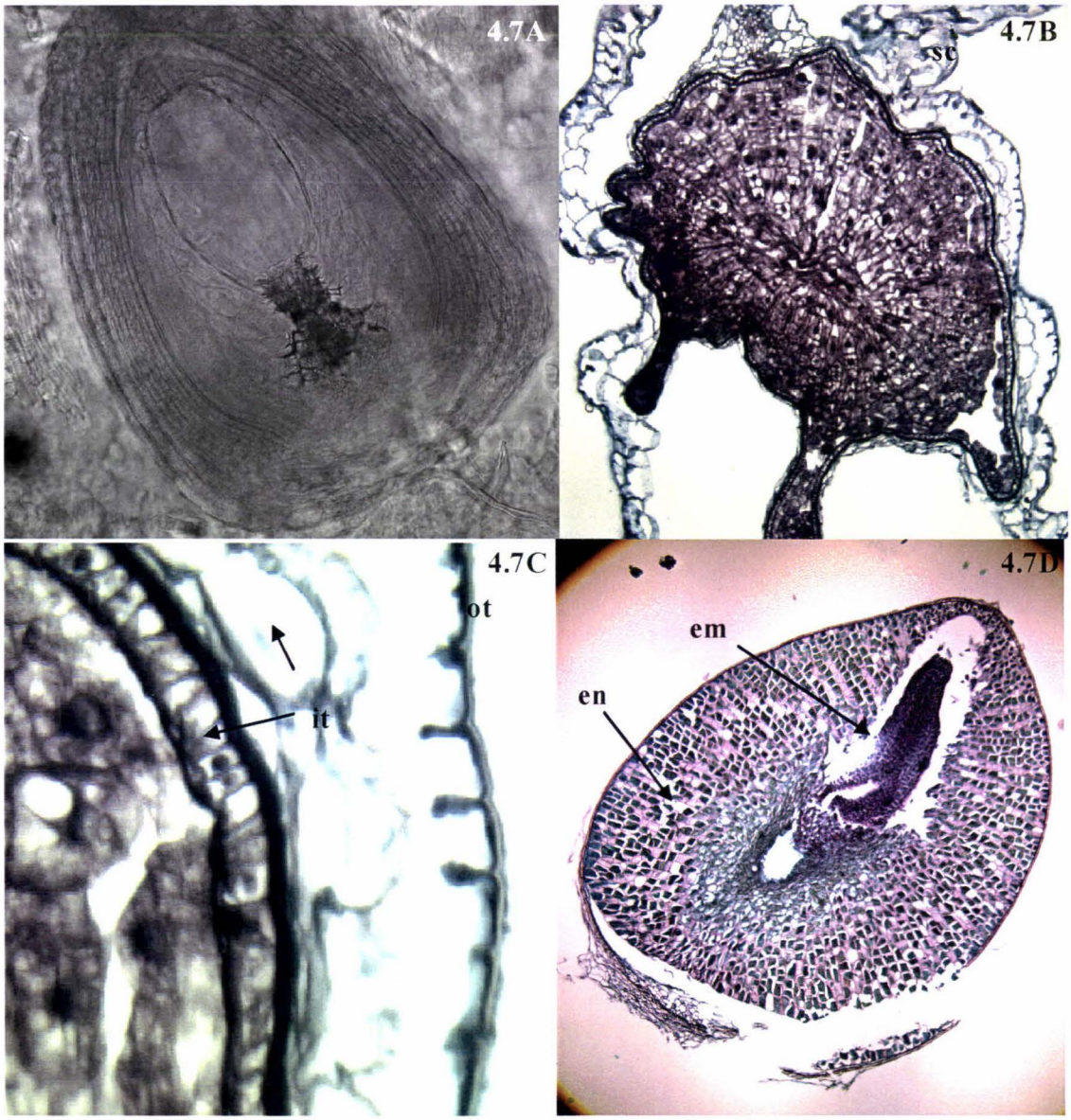


Plate 4.7 Ovule and seed of cultivar P01370: ot, outer integument; it, inner integument; sc, seed coat; en, endosperm; em, embryo. (A) Unfertilised ovule (200x). (B) Immature seed 11 days after pollination (100x). (C) Close up of seed coat of 11 day old seed (200x). (D) Mature seed (dried on plant) (40x).

4.3.2 Seed viability

50 seeds of each *Delphinium elatum* cultivar used in germination tests were examined for viability using the Tetrazolium (TTC) test. Viability results for each cultivar are shown in Table 4.1. No seed type had more than 10% of its seeds classified as non-viable using the TTC test. According to the ISTA Worksheet on Tetrazolium Testing (2003) for *Delphinium*, only seeds showing full staining are classified as viable. Cultivar W0204x showed total viability in all 50 seeds tested. O/P mix was the only variety with any seeds left totally unstained. Seed samples for LB01130x, P9827x35, and 63xbed2 showed some seeds with sections of endosperm or embryo left unstained. Lowest viability (92%) was seen in types O/P mix and LB01130x. Comparing viability estimates with actual germinability under standard conditions at 15°C showed a correlation between seed viability and seed germination (Table 4.2). Highest germination (87.5%) was found in seeds of P9827x35 and W0204x, which also had the highest estimated viabilities (96% and 100% respectively).

Table 4.2 Numbers of viable (totally stained) *D. elatum* cultivar seeds and descriptions of staining patterns for non-viable seeds after using the tetrazolium test. Mean germination rates for each cultivar grown at a constant 15°C are also given.

Seed type	Description of non-viable seed	Viable seed	Mean Germination at 15°C (%)
O/P mix	Totally unstained, endosperm partially unstained or embryo unstained	46/50 (92%)	78.5
LB01130x	Embryo or endosperm partially unstained	46/50 (92%)	61.5
P9827x 35	Embryo partially unstained	48/50 (96%)	87.5
63x bed 2	Embryo partially unstained	47/50 (94%)	85.5
W0204x	N/A	50/50 (100%)	87.5

4.3.3 Rates of germination

A sigmoidal rate of germination was seen in all cultivars with most attaining at least 75% germination within 20 days of sowing (Figure 4.1). LB01130x was anomalous in exhibiting delay in starting germination and attaining only 61.5% germination. Lowest rates of germination were seen in LB01130x and O/P mix, which also showed the lowest estimated viability rates (Table 4.2). Germination was less than 25% for any cultivar at day 5. By day 10, germination was almost complete in P9827x35, W0204x and 63xbed2. All cultivars showed two germination peaks (Figure 4.2), except for cultivars P9827x5 and W0204x, which showed a single peak and highest overall germination (87.5%).

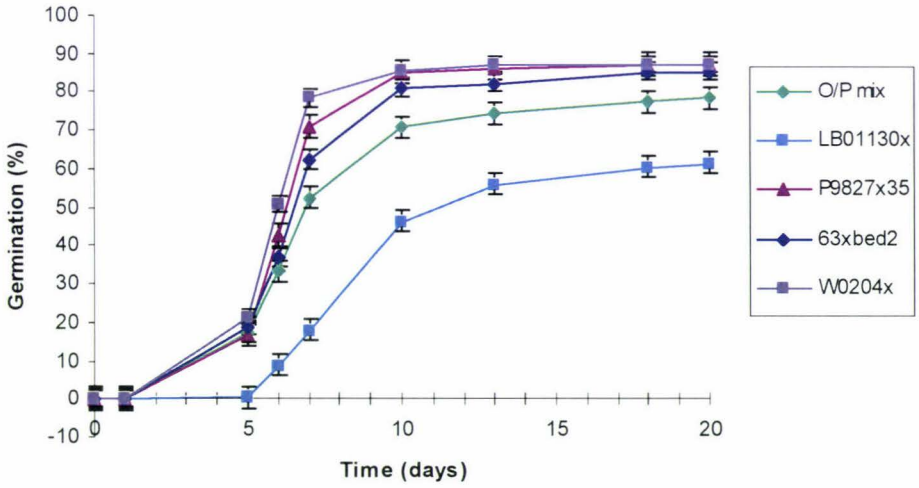


Figure 4.1 Germination percentages of *D. elatum* cultivars germinated at 15°C. Germination was measured 5, 6, 7, 10, 13, 18 and 20 days after imbibition. Percentages given are means +/- standard error (SE)

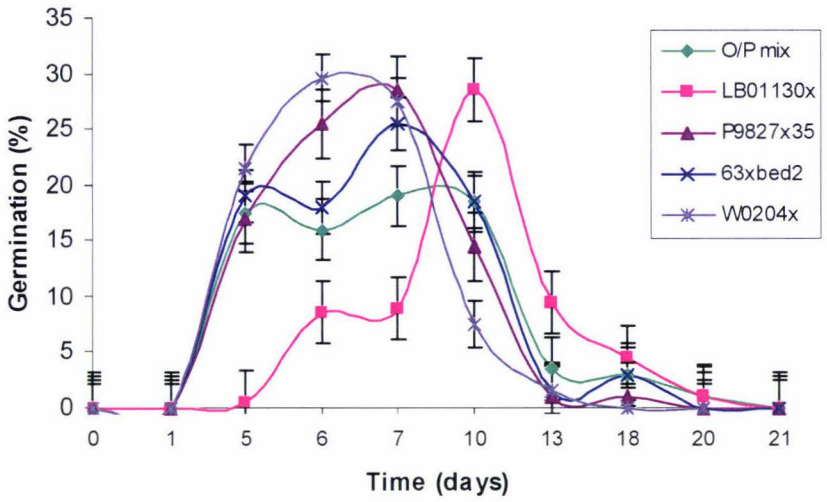


Figure 4.2 Percentage of seed germinating at each time period (days). Germination was measured on days 5, 6, 7, 10, 13, 18 and 20. Percentages given are means +/- SE.

4.3.4 Germination treatments

4.3.4.1 Temperature

Delphinium elatum cultivars O/P mix and P9827x35 showed significant differences in germination at temperatures of 15°C, 20°C and room temperature (Table 4.3). Nearly 80% germination was achieved for O/P mix at 15°C and 20°C while P9827x35 showed significantly lower germination at constant 20°C (80.5%). No other cultivars showed significant differences in overall germination at the three temperatures. However, temperature did have an effect on germination rate, with time to 50% of total germination (t_{50}) reduced in all cultivars when germinated at 20°C. This reduction was significantly different in LB01130x when compared to germination at 15°C (7.40 days c.f. 8.37 days) and in P9827x35 when compared to room temperature (5.50 days c.f. 6.62 days). Mould growth was also consistently increased at 20°C and lowest at 15°C for all cultivars.

4.3.4.2 Germination promoting treatments

Responses of seed to germination promoting treatments varied between *D. elatum* cultivars (Table 4.4). In O/P mix seed no treatments showed a significant difference in mean germination percent from the control (germination at 15°C, no treatment). Scarified seeds (72%) showed a significant difference in mean germination compared to seed treated with 100mg/l gibberellic acid (GA_3) (84.5%). Cultivars P9827x35 and 63xbed2 showed no significant difference in mean germination for any treatment. Cultivar LB01130x saw a significant increase in mean germination for treatments 100mg/l GA_3 (77.5%) and cold imbibed (79%) compared with the control (61.5%). All cultivars displayed a significant increase in time to 50% total germination (t_{50}) for seed imbibed at 5°C for two weeks (O/P mix 2.63 days; 63x bed2 2.45 days; LB01130x 3.51 days). Germination time was recorded from when seeds were transferred to 15°C, and did not include the two weeks imbibed at 5°C. O/P mix saw a significant increase in t_{50} for scarified seeds (8.06 days) compared to the control (6.23 days). Cultivar 63xbed2 saw no significant differences in t_{50} from the control, except for the cold imbibed treatment. P9827x35 showed no significant differences in t_{50} under any treatments (no cold, imbibed treatment for this cultivar). LB01130x saw a significant decrease in t_{50} with treatment of GA_3 (7.30 days c.f. 8.37) but not with dark-grown seeds.

Table 4.3 Effects of temperature on mean germination and time to 50% (t_{50}) total germination in *Delphinium elatum* cultivars. Four replicates of 50 seeds were examined. Means that are followed by the same letters are not significantly different at the $p<0.05$ level.

Seed type	Temperature	Seed germinated/50 (replicates)				Mean germination	Mean t_{50}*	Mean mould
		1	2	3	4	%	days	%
O/P mix	room temp	31	30	37	32	65.0 **b**[1]	6.85 **a**	19.5
	20°C	36	40	39	42	78.5 **a**	5.98 **a**	31.5
	15°C (control)	38	38	37	44	78.5 **a**	6.23 **a**	11.0
LB01130x	room temp	31	22	32	27	56.0 **a**	8.17 **ab**	23.0
	20°C	27	30	33	26	58.0 **a**	7.40 **a**	36.0
	15°C (control)	31	30	32	30	61.5 **a**	8.37 **b**	15.0
P9827x 35	room temp	48	47	45	45	92.5 **a**	6.62 **b**	19.5
	20°C	41	37	41	42	80.5 **b**	5.50 **a**	48.5
	15°C	41	48	40	46	87.5 **ab**	6.04 **ab**	13.0
63x bed 2	room temp	40	39	45	42	83.0 **a**	6.25 **a**	22.0
	20°C	42	47	45	40	87.0 **a**	5.90 **a**	33.5
	15°C (control)	42	47	37	45	85.5 **a**	6.15 **a**	17.5
W0204x	15°C (control)	45	42	46	42	87.5 **a**	5.00 **a**	15.0

* Time to 50% of total germination

[1]Letters represent differences in mean values within a cultivar according to Duncan's Multiple Range Test.

Table 4.4 Effects of various germination treatments on mean germination and time to 50% germination (t_{50}) in four seed types of *Delphinium elatum*

Seed type	Treatment	Seed germinated/50				Mean germination	t_{50}*
		1	2	3	4	%	days
O/P mix	Control (15°C, no treatment)	38	38	37	44	78.5 **ab**[1]	6.23 **bc**
	Dark	36	39	35	38	74.0 **ab**	6.75 **bcd**
	Scarification	32	40	42	30	72.0 **b**	8.06 **d**
	GA3 100mg/l	40	45	45	39	84.5 **a**	7.58 **cd**
	Imbibed, 5°C (2 wks)	42	36	42	40	80.0 **ab**	2.63 **a**
	Priming	43	35	44	41	81.5 **ab**	6.56 **bcd**
	KNO3 100mg/l	38	37	41	36	76.0 **ab**	6.23 **bc**
	Dark, scarification	35	39	32	38	72.0 **b**	7.89 **cd**
	100mg/lGA3 and 100mg/lKNO3	38	43	41	31	76.5 **ab**	6.64 **bcd**
63x bed 2	Control (15°C)	42	47	37	45	85.5 **a**	6.15 **b**
	Dark	44	41	37	46	84.0 **a**	5.87 **b**
	Scarification	43	38	27	45	76.5 **a**	6.97 **b**
	GA3 100mg/l	44	43	48	36	86.5 **a**	6.81 **b**
	imbibed, 5°C (2 wks)	45	46	36	43	85.0 **a**	2.45 **a**
	Priming	41	43	37	44	83.0 **a**	5.98 **b**
LB01130x	Control (15°C)	31	30	32	30	61.5 **b**	8.37 **c**
	Dark	31	29	32	29	60.5 **b**	8.12 **bc**
	GA3 100mg/l	39	43	37	36	77.5 **a**	7.30 **b**
	imbibed, 5°C (2 wks)[2]	41	37	39	41	79.0 **a**	3.51 **a**
P9827x 35	Control (15°C)	41	48	40	46	87.5 (+/-4.64) **a**	6.04 **a**
	Dark	41	45	43	47	88.0 **a**	5.71 **a**
	GA3 100mg/l	42	45	45	43	87.5 **a**	6.05 **a**

* Time to 50% of total germination

[1]Letters represent significant differences in means within cultivars (Duncan's Multiple Range Test)

[2]Transferred to 20°C after 2 weeks stratified at 5°C

4.3.4.3 Stratification time

Delphinium elatum O/P mix type seeds were imbibed at 5°C for periods of two weeks, six weeks, and 30 weeks (Table 4.5). Seeds in all trials began germinating within 1-2 weeks of being imbibed at 5°C. There was no significance difference in mean germination for any of the three treatments or the control (no chilling) (Table 4.5). A large difference was seen in the rate of germination between treatments. T_{50} was greatly reduced (2.63 c.f. 6.48 days) in seeds that were imbibed at 5°C for two weeks. In seed imbibed at 5°C for periods of 6 and 30 weeks t_{50} was greatly increased. Time was recorded from when seeds were transferred to 15°C except for the 6 week and 30 week trials. This was due to seed initiating germination at 5°C. By the end of 6 weeks stratification, 82.5% of seed had germinated (79% for surface sterilised seed). After 30 weeks stratification, 83.5% of seed had germinated (data not shown). The mean total germination (80%) for seed imbibed at 5°C for two weeks was reached 7-8 days after transferral to 15°C (Figure 4.3), giving a much more uniform germination rate than the control (16-18 days). Surface sterilisation of seed imbibed at 5°C had no significant impact on mean seed germination. Treating cold imbibed seed with 100mg/l GA_3 also had no significant impact on total germination (82% germination compared with 75.5% for the control and 80% for 2 weeks cold imbibed, no GA_3). Rate of germination was not significantly affected by addition of GA_3 to seed stratified at 5°C, when compared to seeds stratified at 5°C with no GA_3 (Table 4.5).

Table 4.5 Effect of stratification (5°C) time on germination of *D. elatum* type O/P mix. Values are recorded from when seeds were first imbibed at initiation of stratification except for seeds stratified for two weeks where recordings were initiated upon transferral to 15°C.

Treatment	seed germinated/50				Mean germination	t50
	1	2	3	4	%	
Control (imbibed, 15°C)	40	40	33	38	75.5 **a**[1]	6.48**b**
2 weeks*	42	36	42	40	80.0 **a**	2.63**a**
2 weeks* 100mg/l GA_3	43	35	44	42	82.0 **a**	3.76 **a**
6 weeks	41	43	42	44	85.0 **a**	22.59 **e**
6 weeks, surface sterilisation	41	36	42	41	80.0 **a**	17.89 **c**
30 weeks	45	37	45	45	86.0 **a**	20.53 **d**

*Time to germinate began when seeds were transferred to 15°C (does not include the 2 weeks at 5°C).
[1]Letters represent significant differences at the $p<0.05$ level according to Duncan's Multiple Range Test

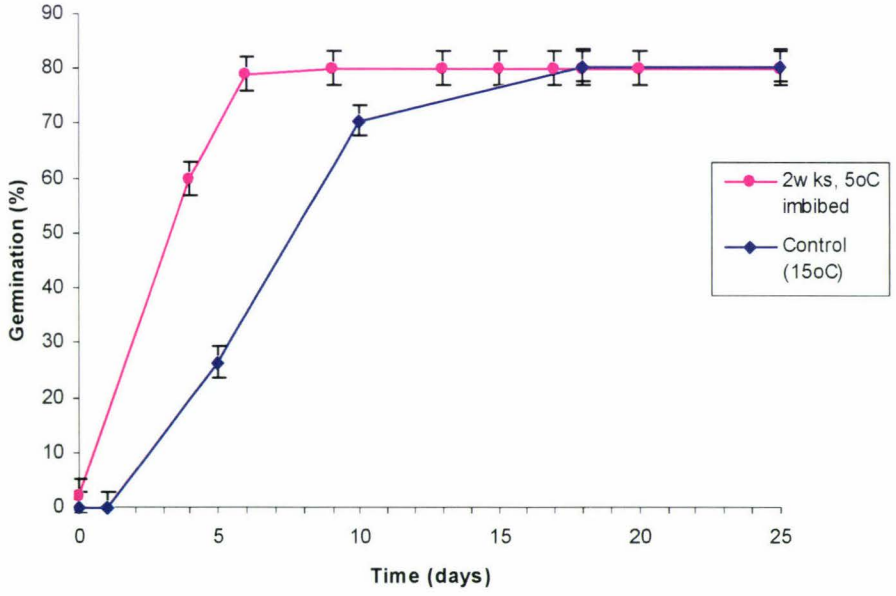


Figure 4.3 Rate of germination of *Delphinium elatum* O/P type seeds imbibed at 5°C for two weeks. Time to germination for cold imbibed seeds was begun upon transferral to 15°C after two weeks at 5°C. Germination percent is mean total germination +/- the standard error of the mean.

4.3.4.4 Potassium nitrate concentration

The effect of potassium nitrate (KNO_3) concentration on seed germination was mostly insignificant (Table 4.6). No significant differences in mean germination were seen, except for 500mg/l KNO_3 where germination was reduced to 66% (c.f. 76.5%). Germination at 1000mg/l KNO_3 was the same as for the control (76.5%). At no concentration of KNO_3 was there any increase in germination above the control.

4.3.4.5 Gibberellic acid concentration

The effect of different concentrations of gibberellic acid (GA_3) on germination was tested on O/P mix seed (Table 4.7). GA_3 had no significant effect on germination of O/P mix seed, except at levels of 500mg/l, where mean germination was significantly lower than germination at levels of 10mg/l and 100mg/l (Table 4.7). It was not significantly different from the control, except in time to 50% total germination (t_{50}), which was increased to 8.05 days at 500mg/l GA_3 (control took 6.48 days).

Table 4.6 Effect of concentration of KNO_3 on germination percentages of *D. elatum* O/P mix seed type

Treatment	seed germinated/50				Mean germination
	1	2	3	4	%
Control (no KNO3)	38	40	37	38	76.5 (+/-9.20)[1] **a**[2]
10mg/l	33	40	34	33	70.0 **ab**
100mg/l	38	37	41	36	76.0 **a**
500mg/l	34	32	33	33	66.0 **b**
1000mg/l	36	38	39	40	76.5 **a**

[1]+/- standard error of the mean
[2]Letters represent significant differences between means at $p<0.05$ according to Duncan's Multiple Range Test

Table 4.7 Effects of concentrations of GA_3 on germination of *D. elatum* O/P seed mix

Treatment	seed germinated/50				Mean germination	t50*
	1	2	3	4	%	
control (no GA_3)	38	40	37	38	76.5 (+/-8.45)[1] **ab**[2]	6.48 **a**
10mg/l	47	39	40	37	81.5 **a**	7.34 **ab**
100mg/l	40	45	45	39	85.0 **a**	7.57 **b**
500mg/l	38	34	35	35	71.0 **b**	8.05 **b**

* Time to 50% of total germination
[1]+/- Standard error of the mean
[2]Letters represent significant differences at $p<0.05$ according to Duncan's Multiple Range Test

4.3.5 Fungicide and surface sterilisation

Delphinium elatum O/P mix seeds treated with thiram showed significantly reduced germination compared with un-treated seeds (Table 4.8). Mean germination for thiram treated seed was only 62% compared with 78% for the control ($p<0.05$). Thiram treated seeds also took a significantly longer time to reach 50% of total germination (Table 4.8). Seeds surface sterilised with 21% bleach showed no significant difference to the control in

either mean germination or time to 50% of total germination. Seeds treated with thiram showed no fungal growth at all, while 1-2% of surface sterilised seed showed fungal growth 1-2 weeks after seed imbibition (data not shown).

Table 4.8 Treatment of *D. elatum* O/P mix seed type with thiram (fungicide) and bleach (surface sterilisation) for control of seed fungi.

Treatment	seed germinated/50				Mean germination	t50*
	1	2	3	4	%	
control (15°C)	37	39	37	44	78.0 **a**[1]	6.23 **a**
Thiram (dusted over)	30	32	34	28	62.0 **b**	8.34 **b**
surface sterilisation (21% bleach)	38	41	36	37	76.0 **a**	7.66 **ab**

*Time to 50% of total germination
[1]Letters represent significant differences between means at the $p<0.05$ level according to Duncan's Multiple Range Test

4.3.6 Seed weights

Mean seed weights were recorded for all seed cultivars that were germination tested (Table 4.9). All mean seed weights are significantly different from one another, except for O/P mix (mean weight 0.002044g) and LB01130x (0.002104g). These cultivars have the lowest mean weights and also show the lowest germination per seed lot (Table 4.2). Mean moisture content is lowest for cultivar LB01130x at 9.25% (Table 4.9). This is significantly lower than moisture contents for P9827x35 and 63xbed2 but not significantly different from O/P mix and W0204x (Table 4.9).

Table 4.9 Mean seed weights for five *Delphinium elatum* cultivars. Mean weights are taken from the individual weights of 50 seed of each cultivar. Weight range and median for each cultivar are also given

Seed type	Mean wt (g) of seed	Range (g)	Median (g)	Mean moisture content (%)
O/P mix	0.002044 **d**[1]	0.0026	0.0020	10.63 **ab**[2]
P9827x 35	0.002462 **c**	0.0028	0.0025	10.77 **a**
LB01130x	0.002104 **d**	0.0034	0.0024	09.25 **b**
63x bed 2	0.002956 **a**	0.0034	0.0031	11.58 **a**
W0204x	0.002696 **b**	0.0035	0.0028	10.61 **ab**

[1]Letters represent significant differences between means at $p<0.05$ according to Duncan's Multiple Range Test
[2]Letters represent significant differences in moisture content between seed types

4.3.7 Imbibition rate

Water content of imbibed seeds increased rapidly for the first 7-8 hours after imbibition, until an approximate 70% increase over the dry weight was reached (Figure 4.4). This resulted in a plateau, with water uptake increasing only gradually up until 72 hours after initial imbibition. No correlations were seen between initial seed weight, imbibition rate and seed germination for individual seeds (data not shown). Both the heaviest and lightest seed weighed had germinated by day 6 after imbibition. Forty-six percent of seeds weighed did not germinate (perhaps due to structural damage during weighing) and were proved dead at the end of the germination period (data not shown). Individual water uptake patterns were very similar for all seeds tested (Figure 4.5).

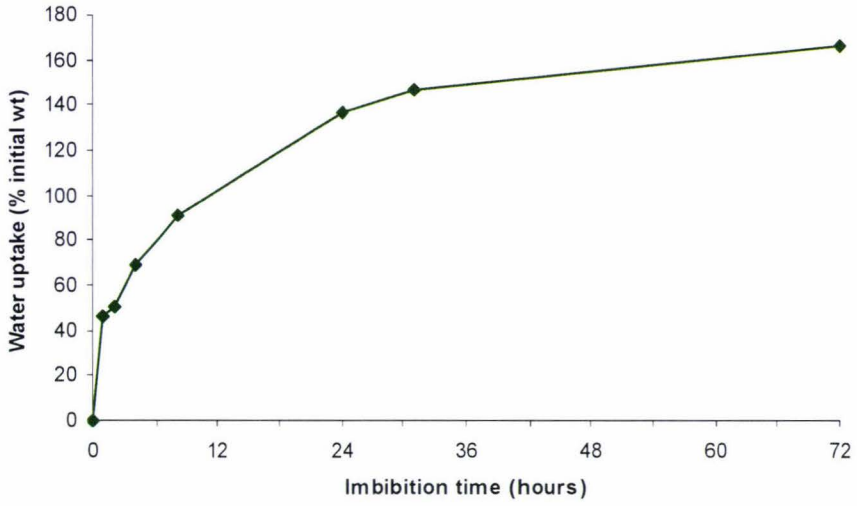


Figure 4.4 Water uptake by O/P mix seeds (as a percent of dry weight) for 72 hours of imbibition. Seed were weighed at intervals of 0, 1, 2, 4, 8, 24, 31, and 72 hours after imbibition. Values are mean percentages of 100 seeds weighed individually.

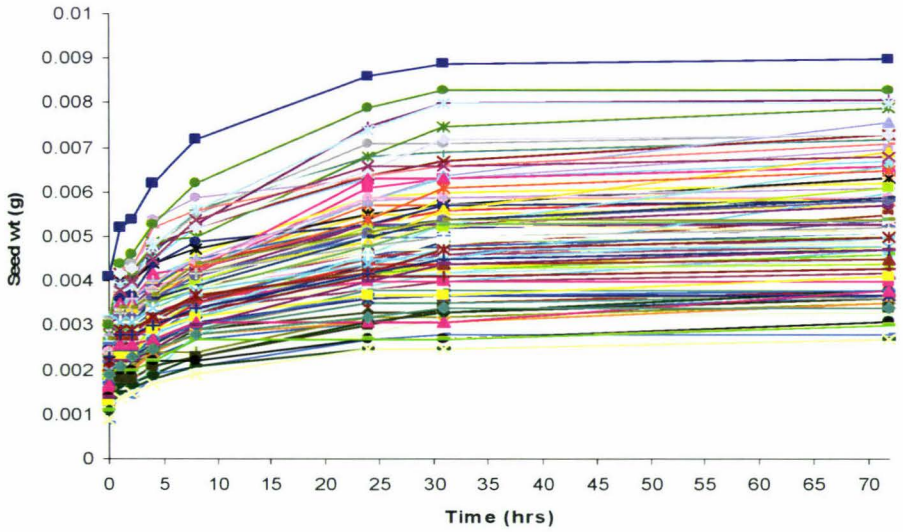


Figure 4.5 Increases in individual seed weight after imbibition for 100 seed of type O/P mix. Seeds were weighed individually at intervals of 0, 1, 2, 4, 8, 24, 31, and 72 hours after imbibition.

4.4 DISCUSSION

4.4.1 Seed structure

Differences between seeds occurred mainly in overall seed shape, embryo length and cotyledon position. Seed structure is mostly uniform once seed coats are removed. Cotyledons match Babis' (1973) description of being torpedo-shaped and linear. Cotyledons are straight in the seed but most experience a small degree of curvature. Embryos were well defined in all cultivars. Endosperm comprises the majority of the seed volume and consists of numerous thick-walled cells. Seeds are pointed at the micropylar end, although orientation is difficult to establish in some cultivars unless the seed coat is removed.

The seed coating is comprised from the two integuments. The outer integument forms the seed coat while the inner integument forms a thin, lignified (stained red) layer around the endosperm. At 11 days after pollination, the seed coat was already visible in the developing seed. The hilum (scar left by funiculus) is not apparent on the seed coat, due to the intensive creasing of the coat in the desiccated seed.

Structural examination revealed normal development of embryo and endosperm in all seeds. Seed coat varied in shape and size between cultivars and individuals but was intact in all cases. Cultivars did not exhibit any signs of deterioration and no anomalies in seed structure were observed. Inability to germinate is not due to embryo immaturity as all cultivars showed large, well-developed embryos.

4.4.2 Seed viability

The tetrazolium test shows a relationship between reduction in dye intensity, respiration and seedling vigour (Bewley and Black, 1982). Depth and degree of staining are indicators of seed viability. The *D. elatum* cultivars examined showed high levels of estimated viability (over 90% in all cultivars). Highest estimated viability was seen in W01220x

which showed 100% viability. Cultivars with the lowest viability also showed the lowest germination rates. Germination rates were still lower than viability, indicating a possible degree of dormancy in *D. elatum* seeds.

4.4.3 Germination rates

All *D. elatum* cultivars tested showed similar patterns of germination. Most seeds germinated within a short time frame, with a few seed germinating either early or late. Cultivar LB01130x showed the same pattern of germination as cultivars O/P mix, P9827x35, 63xbed2, and W0204x, but took longer to initiate germination and showed lower germination overall. Germination was highest in cultivars displaying uniformity of germination. LB01130x showed two germination peaks and took significantly longer to initiate germination than other cultivars. Results suggest that LB01130x requires further treatment to initiate germination. Mechanisms preventing germination appear only to be present in some cultivars.

4.4.4 Optimising germination

A range of treatments were selected to determine how to optimise seed germination in *Delphinium elatum* cultivars. Of the three temperatures trialled (room temperature, 20°C, 15°C), different responses were seen in all cultivars. The 15°C to 20°C temperature range was regarded as optimal. Germination was highest at 15°C to 20°C for all cultivars (excepting P9827x35). Germination rate measured as t_{50} (time to 50% total germination) was shorter in seed germination at 20°C for all cultivars. However, germination at 15°C temperatures reduced amounts of mould growth, allowing for cleaner germination and a higher chance of seedling survival. Highest germination was at a uniform temperature (i.e. 15°C or 20°C). Highest rates of mould growth and lowest rates of germination were recorded at room temperature for cultivars O/P mix, 63xbed2 and LB01130x.

4.4.4.1 Germination-promoting treatments

The majority of germinating-promoting tests were carried out on O/P seed mix (due to seed availability). No treatment saw significant differences in germination percentages for O/P mix seed. LB01130x showed significantly improved germination with treatments of GA_3 and stratification. Seeds exhibited no requirement for light or dark treatment and germinated equally well under either condition. Seeds showed no inhibition of water-uptake, and scarification treatment reduced germination, suggesting *Delphinium elatum* seeds are subject to embryo dormancy rather than coat-imposed dormancy. P9827x35 and W0204x cultivars did not require germination promoting treatments due to the high level of germination already present.

4.4.4.2 Stratification

Chilling of imbibed seed at 5°C produced varying results. Germination was unaffected in 63xbed2 seed, but increased in O/P mix and LB01330x cultivars. In all cultivars, t_{50} was significantly reduced after 2 weeks chilling at 5°C (germination was recorded from time of transferral to 15°C). Increasing stratification time above two weeks did not significantly increase germination.

D. elatum seed were able to germinate at 5°C, which is uncommon (Bewley and Black, 1982) as seeds usually require transferral to a higher temperature after stratification for germination to occur. Seedling growth was affected by chilling, however, and germinated seeds needed to be removed from the 5°C area 2-3 days after germination, or became stunted. Difficulties were found in assessing t_{50} in stratified seeds due to germination initiating at 5°C. For seeds chilled for two weeks, germination rate was recorded from when seeds were transferred to 15°C (time 0). For all other germination, germination rate was recorded from time of initial imbibition. Stratification not only increased germination in *D. elatum* seed, but also reduced fungal growth. Fungal growth on ungerminated seeds

was greatly reduced at 5°C but increased upon transferral to 15°C. Germination at 5°C also saw reduced rotting of seeds earlier on in germination.

Stratification of imbibed seeds for a short period of time (2 weeks) appears to have a significant impact on rate of germination, and increases overall germination. Rate of germination is greatly enhanced upon transferral to 15°C or 20°C, suggesting that although seeds are able to germinate at 5°C, increase in temperature is required to stimulate germination and promote seedling survival. Increasing the chilling period over two weeks did not significantly affect germination, signifying a short chilling period is sufficient to promote germination in *D. elatum* seed. Stratification appears to play an important role in reducing germination time and increasing overall germination in *D. elatum* cultivars exhibiting dormancy.

In *D. elatum* cultivars, germination was linked to length of stratification. However due to the fact that within 1-2 weeks of imbibition at 5°C seeds begin germinating, a shorter stratification period is more efficient as seedlings need to be removed to higher temperature once germination has occurred to ensure correct growth.

Positive effects of stratification on commercial *D. elatum* seed may be related to the province of origin of the wild-type ancestor. Wild-type *D. elatum* originates from Northern Temperate Zones (Honda et al, 2003) where dormancy imposed by chilling requirements may be a survival mechanism against harsh winter conditions detrimental to seedling survival.

4.4.4.3 Gibberellic acid

Chilling requirements in some seed are known to be overcome by application of exogenous gibberellins (Bewley and Black, 1982; Taiz and Zeiger, 1998). Application of GA_3 to seeds saw increased germination in all cultivars (except P9827x35 which remained unchanged). Increases in germination under gibberellin treatment were significant in LB01330x, with t_{50} also reduced. These results suggest that gibberellic acid application

may play a role in increasing germination of *D. elatum* seed. Extent of this role is influenced by gibberellic acid concentration. Rate of germination after GA_3 application, and response to GA_3, depends on the cultivar under investigation. Responses of *D. elatum* seed to both chilling and exogenous gibberellin application suggest that germination of this species may be regulated to some extent by gibberellic acid levels in the seed at time of germination. Edelstein et al (1995) suggest that higher germination in certain melon cultivars may be due to higher concentrations of endogenous gibberellins in those cultivars.

Due to the permeability of the seed coat, and reduced germination seen in scarified seed, GA_3 was not applied to scarified seed.

4.4.4.4 Scarification

Scarification treatment (seeds nicked with scalpel) was performed on two *D. elatum* seed types: O/P mix and 63xbed2. In type O/P mix, scarification was used in conjunction with dark treatment. In both cultivars scarification decreased germination. Scarification also produced the longest time to t_{50} in both cultivars (significantly different to the control: 8.06 days c.f. 6.23 days in O/P mix). *D. elatum* seed have wrinkled, brittle coats (seed can easily be squashed or damaged with forceps). It is extremely unlikely that seeds are impermeable to water, and scarification is unlikely to impact water uptake. Results instead suggest scarification may have an adverse effect on germination. This may be due to the embryo being damaged during nicking, or rupturing of the seed coat allowing pathogens to invade the seed.

4.4.5 Thiram and surface sterilisation

D. elatum seeds show very high rates of mould growth. To examine the effects of fungal growth on germination, seeds were: (1) dusted with thiram (fungicide); and (2) surface sterilised in 4.2% sodium hypochlorite for 10 minutes. Although no previous data was available on the effects of thiram on *Delphinium* species, it significantly reduced

germination of *D. elatum* cultivars in these trials. Terry Dowdeswell[2] (pers. comm.) noted it also appears to affect seedling growth after germination. Surface sterilisation had no significant impact on seed germination, and only 1-2 seed showed mould growth under this treatment. However, application of surface sterilisation did not promote germination, which leads to the supposition that mould growth does not adversely affect germination (although it may affect young seedlings). During the course of germination most non-germinating seeds rot before the end of the germination period. These seeds also show much higher rates of mould growth than germinated seed. Very few seed still showed viability at the end of germination. Mould only appears on the seed coat in germinating seed, while it usually envelops rotting seed. This suggests fungal growth may be a symptom rather than a cause of seed deterioration.

4.4.6 Seed weights and imbibition

Studies have indicated that seed weights may vary greatly within a species and larger seeds frequently exhibit greater germination and radicle emergence than small seeds (Hendrix, 1984). A correlation exists between dry seed weight and germination percentage in *D. elatum* cultivars. Lowest mean weights were seen in O/P mix and LB01330x which also show lowest viability and germination. 63xbed2 seed had highest mean seed weight but was not the highest germinator, although there was no significant difference in germination between P9827x35, 63xbed2, and W0204x cultivars. Moisture content was lowest for LB01130x and highest for 63xbed2, which may partially account for the high mean seed weight in 63xbed2.

One hundred seeds of O/P mix were also individually weighed and imbibed. Water uptake patterns were very similar for all seed regardless of initial dry weight. Water uptake rate increased rapidly (phase I), reached a plateau (phase II), then increased again (phase III). Seeds showed no impermeability to water. A high percentage (46%) of imbibed seed did not germinate. This is likely due to damage imposed while handling seeds.

[2] Owner of Dowdeswell's Delphiniums

4.4.7 Seed deterioration

The majority of non-germinating seeds had rotted by the end of the germination period. Viability tests indicate these seeds were viable at time of imbibition. Some factor is therefore causing seed deterioration of imbibed seeds before germination can occur. High levels of fungal growth on seeds would indicate this was the cause of deterioration, except that surface sterilised seeds show no improvement in germination over the control. A possibility is that pathogens have already attacked the seed, affecting germination ability but not respiratory activity.

5.0 SEED STORAGE IN DELPHINIUM

5.1 INTRODUCTION

Retention of seed viability during storage is determined by the storage environment; namely humidity and temperature (Bewley and Black, 1982) which impacts directly on the moisture content of a seed lot. Usually viability declines more rapidly at higher temperatures and is prolonged at low temperatures. However, optimal moisture contents and storage temperatures depend very much on the seed itself. At unfavourable seed moisture contents, storage temperature is known to modify rates of physiological deterioration in seed (Carpenter et al, 1995). Seed stored at high moisture levels commonly has increased enzymatic activity and respiration (Bewley and Black, 1982). However, if seeds are stored in conditions that are too dry, they may also have accelerated deterioration rates due to cellular degeneration. Detection of the optimal storage temperature range for a species is therefore important if viability is to be retained during long term storage.

Delphinium elatum seeds exhibit orthodox storage behaviour, with deterioration rate increasing as temperature or moisture content increases (Kwong et al, 2001). However, little research has been carried out on storage requirements of *Delphinium* seeds.
The aim of this research is to discover optimal conditions for storage of commercial *Delphinium elatum* seeds.

5.2 MATERIALS AND METHODS

5.2.1 Seed source

Delphinium elatum seeds were supplied by Dowdeswell's Delphiniums. Seeds were harvested in December 2002 – January 2004. All seeds were stored in plastic bags in a sealed plastic container at 5°C until use. The following seed types were used

- Open-pollinated mix (O/P)
- P9827 x 35
- LB01130x

5.2.2 Storage conditions

All seeds used in storage experiments were stored in glass vials inside water impermeable aluminium bags (Corson Seeds International Ltd). Four replicates of 50 seeds of each cultivar were used. Each replicate was stored in a separate bag. Replicates for each seed type were stored in the same bag as other seed types. Each vial contained an additional 10 seeds for moisture testing at time of germination. Seeds were stored for time periods of 0, 3, 6 12 months. At the end of each time period, seeds were removed and germinated. Vials stored in bags for 12 months contained an additional 200 seeds for structural analysis and dormancy-breaking treatment. Three storage conditions were tested. Treatment 1 seeds were stored at room temperature in open bags. Treatment 2 seeds were stored at low temperature (5°C) and reduced humidity (sealed bags with air removed) (the measured humidity in the 5°C storage facility ranged from 85-95% as recorded by McGill, 2003). Treatment 3 seeds were stored at room temperature at reduced humidity (sealed bags with air removed). Humidity at room temperature was not measured, but would have fluctuated depending on outside weather conditions. Seeds were measured at room temperature due to the likelihood that seeds exported by the business (Dowdeswell's Delphiniums) are subject to such a temperature range and thus storage at room temperature is a likely indicator of usual conditions undergone by *D. elatum* seeds.

The effect of short term dry storage at different temperatures on seed type O/P mix was also tested. Four replicates of 50 seeds were stored in plastic-lidded glass vials for 6 weeks at 5°C, 15°C, 20°C, and 25°C before being germinated. Moisture content was also tested before and after storage.

5.2.3 Seed germination

Germination of all stored seeds took place at 15°C except when testing the effects of temperature on germination of stored seeds. Germination took place on 2 layers of blue germination paper soaked for 30-60 seconds in distilled water. Seeds were germinated in sealed plastic containers to retain moisture. Seeds were germinated for 30-40 days with germination percentage and fungal growth being recorded every 3-4 days. Seeds remaining after 30-40 days were tested for viability using a TTC assay, except in cases where treatment was applied to promote further germination. Ungerminated seeds left after 40 days were judged to be dormant or non-viable. Germination percentages and time to 50% germination (t_{50}) were compared for each storage treatment at each time interval, and also between time intervals. From this, an estimated allowable storage period before germination decreases was calculated.

5.2.4 Viability testing

All seeds remaining after germination at the end of storage were tested for viability using the TTC assay (see section 4.2.3 for method).

5.2.5 Structural analysis

Ten seeds of each seed type for each treatment were imbibed for 24 hours, had their seed coats removed, and were longitudinally dissected with a scalpel. Each seed was then examined under the dissecting microscope for any marked deterioration to the embryo or endosperm.

5.2.6 Moisture tests

After storage, ten seeds from each replicate were dried in a hot oven at 103°C for 17 hours before weighing and calculation of moisture loss. Moisture testing of seed samples took place in accordance with ISTA standards.

5.2.7 Dormancy breaking treatments (after 12 months storage)

Three germination treatments were tested on stored seeds (temperature, chilling and hormonal). These were the only treatments known to have a positive effect on seed germination based on survey results of treatments shown in Table 4.1.

5.2.7.1 Temperature

Hard seeds remaining at the end of germination of 12 month stored seed at 15°C were removed to 5°C and 20°C to examine effects of temperature on ungerminated seeds. After 2 weeks at these temperatures, remaining seeds were tested for viability.

5.2.7.2 Chilling

Seeds of types open-pollinated (O/P) and LB01130x from storage treatments 1 (room temperature, open bags), and 2 (5°C, closed bags) were imbibed at 5°C to examine the effects of chilling on germination of stored seeds (see section 4.2.4.2 for method). Results were then compared between storage conditions, and also to germination of fresh seeds at 5°C, imbibed.

5.2.7.4 Hormonal

Seed of type open-pollinated (O/P) mix, LB01130x, and P9827 x 35 from storage treatment 2 (5 °C, closed bag) were germinated on blue germination paper soaked in 100mg/l GA_3

(see section 4.2.4.6 for method). Seed types O/P mix and LB01130x from treatment 1 (room temperature, open bags) were also treated with 100mg/l GA_3.

5.2.8 Statistical analysis

Measurements of germination were analysed using the SAS system for Windows V8 program. DUNCAN and LSD mean separation tests were used to determine significant differences at the 95% confidence level ($p<0.05$) between the three storage conditions and between length of storage time. Statistical analysis was carried out on all experiments testing the effects of different treatments on seed germination within cultivars.

5.3 RESULTS

5.3.1 Seed viability

Twenty seeds each of *Delphinium elatum* cultivars O/P mix, LB01130x, and P9827x35 were tested for viability after 12 months storage, using the tetrazolium test. Seeds were stored under conditions of (1) room temperature, open bags, (2) 5°C, sealed bags, and (3) room temperature, sealed bags. Seed viability as assessed by the tetrazolium test was considerably reduced by storage at room temperature (Table 5.1). No differences in viability were found between seeds stored for 12 months at 5°C in sealed bags, and fresh seeds. Viability of seeds stored at room temperature in open or sealed bags was reduced to 15% or less for all three seed types when seeds were stored in sealed bags at room temperature (Table 5.1). Viability of seeds stored in open bags at room temperature was slightly higher (at 25% for O/P mix, 20% for LB01130x, and 40% for P9827x35) than viability of seeds stored in sealed bags (Table 5.1).

5.3.2 Structural examination

Seeds of *D. elatum* cultivars examined after 12 months storage showed no discernable differences in structure between storage treatments of room temperature and 5°C storage.

Seed coat and embryo appeared intact in all 10 seeds of each cultivar examined. Approximately half of the seeds of types O/P mix and LB01330x under treatments (1) room temperature, open bags, and (3) room temperature, closed bags were soft to touch after 24 hours soaking. Endosperm of these seed appeared to be soft and waterlogged when touched with forceps, suggesting some deterioration had occurred. Seed from all three cultivars showed no softness or deterioration under 5°C storage.

Table 5.1 Viability of *Delphinium elatum* seed after 12 months storage. Seeds were stored under treatments (1) room temperature, open bags, (2) 5°C, sealed bags, and (3) room temperature, sealed bags

Seed type	Treatment	Description of non-viable seed	Viability*	Mean Germination (%)[1]
O/P mix	(1) room temp, open bag	Totally unstained, embryo partially unstained	5/20 (25%)	12.5**b**[2]
	(2) 5°C, sealed bag	Totally unstained, embryo/endosperm partially unstained	17/20 (85%)	76.5**a**
	(3) room temp, sealed bag	Most seeds totally unstained	2/20 (10%)	4.0**b**
LB01130x	(1) room temp, open bag	Half seed unstained, embryo unstained	4/20 (20%)	7.0**b**
	(2) 5°C, sealed bag	Embryo/endosperm partially unstained	18/20 (90%)	48.0**a**
	(3) room temp, sealed bag	Totally unstained, embryo unstained	2/20 (10%)	4.0**b**
P9827x35	(1) room temp, open bag	Embryo/endosperm partially unstained	8/20 (40%)	29.5**b**
	(2) 5°C, sealed bag	Embryo/endosperm partially unstained	19/20 (95%)	90.0**a**
	(3) room temp, sealed bag	Totally unstained, embryo unstained	3/20 (15%)	7.5**c**

*Viability as assessed by the TTC assay
[1]Germination after 12 months storage
[2]Letters represent significant differences between means at the $p<0.05$ level according to Duncan's Multiple Range Testing

5.3.3 Effect of storage conditions

In all *Delphinium elatum* cultivars stored for three or more months at room temperature, mean germination decreased as storage time increased (Figures 5.1-5.3). Germination decreased from 78.5% to 12.5% and 4.0% respectively, for O/P mix seed stored at room temperature in open and closed bags (Figure 5.1). Germination of seed stored at 5°C was not significantly different (76.5%) from fresh seed after 12 months of storage. LB01130x seed was similarly affected, with germination reduced to 7.0 and 4.0% in seed stored at room temperature for 12 months (Figure 5.2). P9827x35 seeds showed higher germination (29.5%) when stored in open bags at room temperature for 12 months than the other two cultivars, but seed stored at room temperature in closed bags was reduced to 6.5% (Figure 5.3). Storage for 3 months at room temperature did not greatly affect germination in O/P mix and P9827x35. Germination in LB01330x after 3 months at room temperature was significantly different from seed stored at 5°C for 3 months (57.0 and 54.5% c.f. 73.5%). The biggest drops in germination were seen in the time period of 6-12 months, for all cultivars. Seed stored at 5°C saw an increase in germination for all cultivars after 3 months storage. However, this was only significant in LB01330x which increased from 61.5% to 73.5 % (Figure 5.2).

5.3.3.1 Seed moisture content

Moisture contents of *D. elatum* seeds were variable between storage treatments and storage times. Some variability may be due to the limited amounts of seed being available for testing. Moisture content decreased over time for all seed types stored in sealed bags at room temperature (Table 5.2). However, the moisture content of seed stored in open bags at room temperature was variable for all seed types. *D. elatum* O/P mix showed significant differences in moisture content at 6 and 12 months storage for treatment 3, with moisture contents of 8.03% and 7.45% respectively. Treatment 1 (6 months) was also significantly different (7.26%). LB01130x showed significant difference from the control (no storage, 9.25%) at 12 month storage for treatments 1 and 3, with moisture contents of 13.09% and 7.88%. P9827x35 was much more variable, with treatment 1 significantly different from

the control (10.77%) at 3 months (9.24%), 6 months (8.49%), and 12 months (8.85%). Treatments 2 and 3 were both significantly different at 12 months (8.22% and 7.55% respectively). A positive correlation is visible between mean germination (Table 5.1) and moisture content (Table 5.2) at 5°C storage. After 12 months storage, germination percent fell to 48.0% with a moisture content of 8.72%. This moisture content was not significantly different from fresh seed (9.25%) but was significantly different from moisture contents at 3 and 6 months storage (9.98% and 10.06%).

Table 5.2 Mean moisture content (%) of *D. elatum* types O/P mix, LB01130x, and P9827x35 after 0, 3, 6, and 12 months of storage. Storage took place under treatments (1) room temperature, open bags, (2) 5°C, sealed bags, and (3) room temperature, sealed bags. Letters represent significant differences between means at the $p<0.05$ level according to Duncan's Multiple Range Testing

		Mean moisture content (%)			
		Time (months)			
Seed type	Treatment	0 (control)	3	6	12
O/P mix	tmt1	10.63 **a**	10.20 **a**	7.26 **b**	10.45 **a**
	tmt2	10.63 **a**	10.31 **a**	10.21 **a**	9.77 **a**
	tmt3	10.63 **a**	10.23 **a**	8.03 **b**	7.45 **b**
LB01130x	tmt1	9.25 **bc**	8.30 **cd**	9.04 **bcd**	13.09 **a**
	tmt2	9.25 **bc**	9.98 **b**	10.06 **b**	8.72 **cd**
	tmt3	9.25 **bc**	9.47 **bc**	8.68 **cd**	7.88 **d**
P9827x35	tmt1	10.77 **a**	9.24 **bcd**	8.49 **cde**	8.85 **bcde**
	tmt2	10.77 **a**	10.22 **ab**	9.81 **abc**	8.22 **de**
	tmt3	10.77 **a**	10.24 **ab**	8.63 **cde**	7.55 **e**

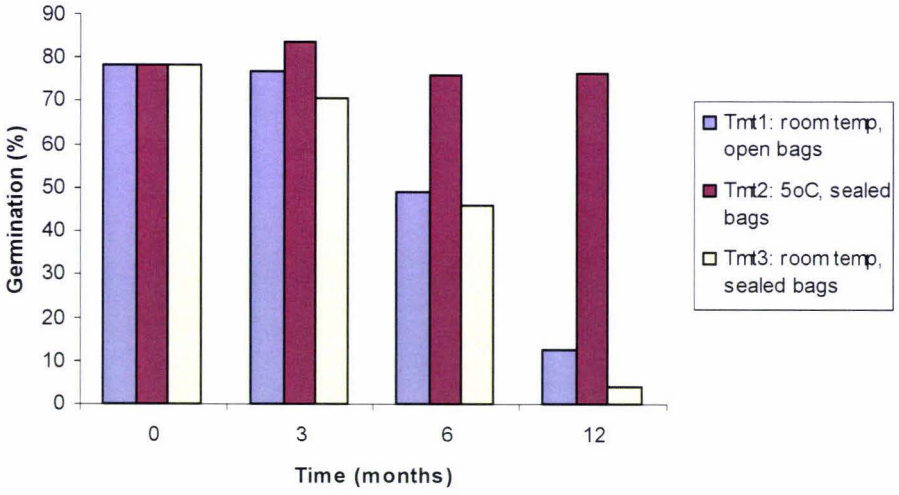


Figure 5.1 Germination of *Delphinium elatum* O/P mix seed after 0, 3, 6, and 12 months storage. At time 0 seed is fresh (control).

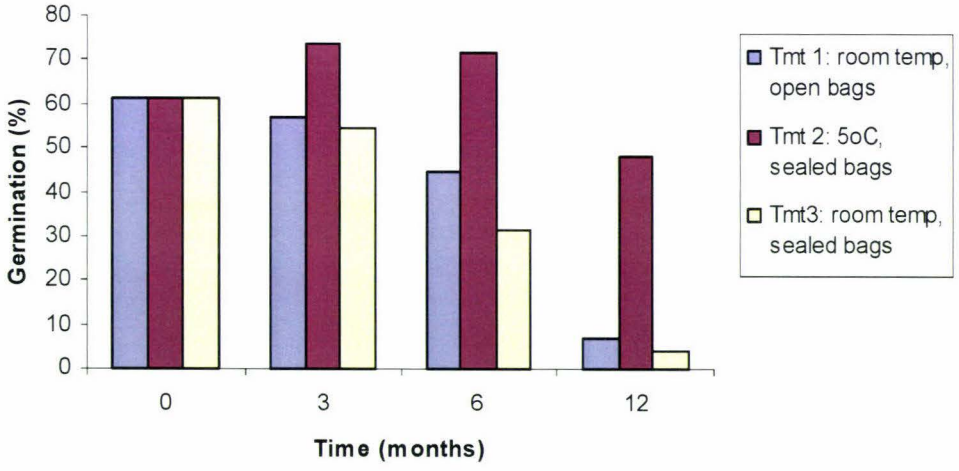


Figure 5.2 Germination of *D. elatum* LB01130x cultivar seed. Germination took place after 0, 3, 6, and 12 months storage. The control is time 0.

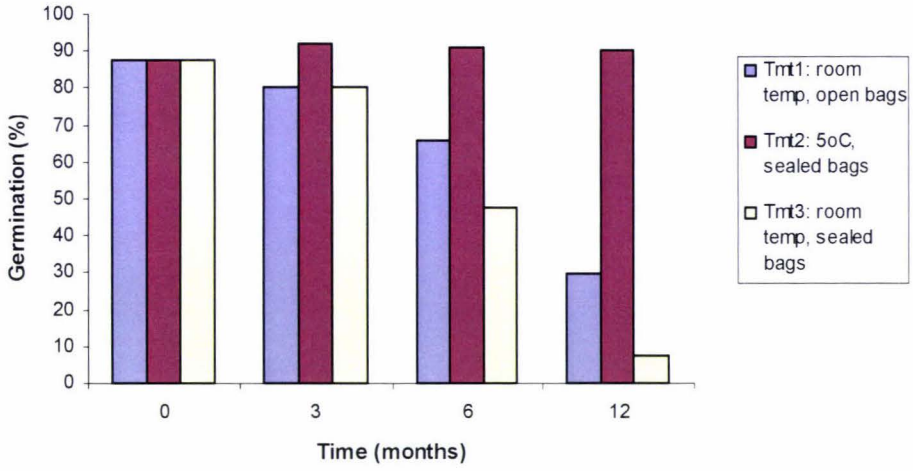


Figure 5.3 Germination of *D. elatum* P9827x35 seed. Germination took place after 0, 3, 6, and 12 months storage. The control is time 0.

5.3.4 Short term storage

D. elatum O/P mix seed showed significant differences in mean germination percent after storage at 15°C, 20°C, and 25°C for 6 weeks (Table 5.3). Germination after storage at 15°C was significantly different (77.5%) from germination at 20°C and 25°C (67.5% and 68.5%). Time to 50% of total germination (t_{50}) was significantly increased in seed stored at 25°C (12.74 days) compared with germination at 15°C and 20°C (10.28 and 10.43 days). Moisture content decreased as temperature increased and was significantly higher at 15°C (9.11% c.f. 8.21% and 8.06%). A correlation is seen between higher moisture content and higher germination percent in seed stored at 15°C (Table 5.3), with highest moisture content (9.11%) corresponding to highest germination (77.5%). Germination over time showed similar patterns for seed stored at all 3 temperatures (Figure 5.4). Rate of germination was slowest for seed stored at 25°C (Figure 5.4).

Table 5.3 Germination percent, t_{50}, and seed moisture content (%) of *D. elatum* O/P mix seed after 6 weeks storage at temperatures of 15°C, 20°C, and 25°C

Treatment	Mean germination (%)	t_{50}	Moisture %
15°C	77.5 **a**	10.28 **a**	9.11 **a**
20°C	67.5 **b**	10.43 **a**	8.21 **b**
25°C	68.5 **b**	12.74 **b**	8.06 **b**

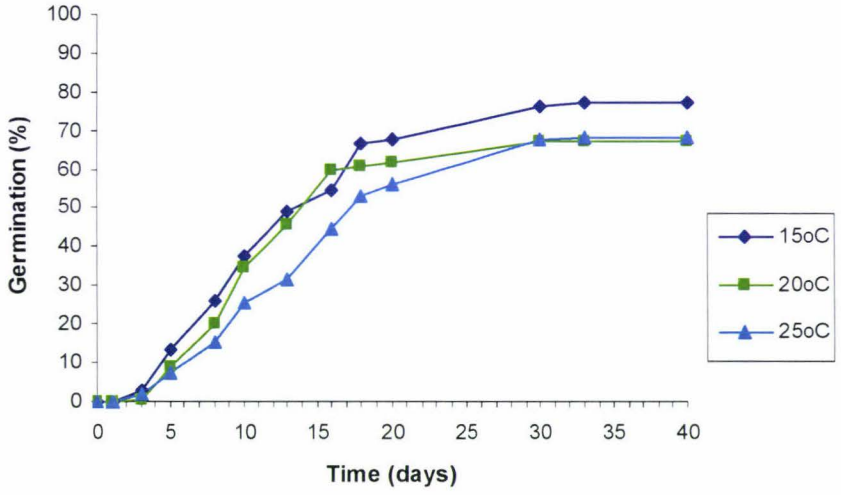


Figure 5.4 Mean germination rates for *D. elatum* O/P mix seed stored at 15°C, 20°C, and 25°C for 6 weeks. Seed was germinated in standard conditions at 15°C.

5.3.5 Dormancy-breaking treatments

5.3.5.1 Temperature

D. elatum seed stored for 12 months under treatments (1) room temperature, open bags, (2) 5°C, sealed bags, (3) room temperature, sealed bags was germinated at 15°C for 30 days (germination results in Figures 5.1-5.3). Remaining hard seed was then transferred to 5°C

and 20°C for a further 2 weeks germination to assess the effects of temperature on germination of stored seed (Table 5.4). Higher germination occurred at 20°C than 5°C (Table 5.4) for O/P mix and LB01130x. Most significant germination was seen at 20°C, under treatment 2 (5°C storage, sealed bags) where a further 37% of seed germinated. Further germination was seen for all cultivars at 20°C.

Table 5.4 Germination of *D. elatum* seed at 20°C and 5°C after germination at 15°C for 30 days. Germination took place over 2 weeks.

Seed type	Treatment	Germination (%) at 20°C	Germination (%) at 5°C	Total Germination (%)
O/P mix	1	6	6	18.5
	2	12	4	84.5
	3	6	0	7.0
LB01130x	1	2	2	9.0
	2	37	13	73.0
	3	5	0	6.5
P9827x35	1	1	5	32.5
	2	0	0	90.0
	3	4	0	7.5

5.3.5.2 Chilling

D. elatum seed of types O/P mix and LB01130x stored for 12 months was imbibed at 5°C for 2 weeks before transferral to 15°C to germinate. No significant differences were seen for seed under treatments (1) and (3) at room temperature. Treatment (2) 5°C showed similar results to fresh seed (data not shown) for both seed types.

5.3.5.3 Hormone treatment

100mg/l GA_3 was applied to *Delphinium elatum* seed stored for 12 months at (1) room temperature, open bags, (2) 5°C, sealed bags, (3) room temperature, sealed bags. No significant difference was seen in seed under treatments 1 and 3 (data not shown). Under treatment 2 (5°C, sealed bags) germination was increased significantly for LB01130x

(Figure 5.5) with application of GA_3 (77.5 c.f. 48%). Germination was also increased for O/P mix, but not to a significant degree. No significant difference was seen in germination of P9827x35 seed under the two treatments.

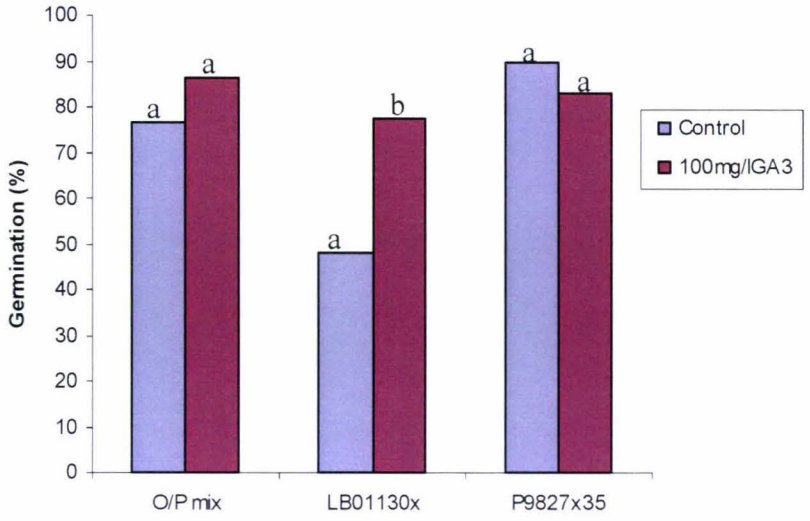


Figure 5.5 *Delphinium elatum* cultivars germinated at 15°C after 12 months storage at 5°C. Letters represent significant differences at $p<0.05$ according to Duncan's Multiple Range Test. Control seed has no GA_3 added.

5.4 DISCUSSION

5.4.1 Viability

Stored seeds in this trial were assessed for viability using the tetrazolium test. The main factors of storage environment affecting viability are seed moisture content, temperature and atmospheric oxygen concentration (Bradbeer, 1988). Roberts (1972) stated that in the majority of cases it has been shown that the lower the seed storage temperature and moisture content, the longer the period of viability. Seeds stored at 5°C in sealed bags showed no significant difference in viability from fresh seeds. Viability was significantly reduced in seeds stored at room temperature, although seeds stored in open bags tended to

have slightly higher estimated viability than seeds in sealed bags. Tetrazolium staining patterns were also affected by storage temperature. The majority of non-viable seeds after room temperature storage showed no staining at all, i.e. no respiratory activity. These findings indicate storage temperatures play an important role in seed deterioration. Seeds stored at 5°C showed no reduction in viability and retained their ability to germinate. Carpenter and Boucher (1995) discovered similar results with *Delphinium x cultorum* seeds, where storing delphinium seeds dry at 2°C improved germination after storage.

5.4.2 Effect of storage condition on germination

All seeds stored at 5°C for three months displayed increased germination. After three months storage germination decreased in all seeds stored at room temperature for each cultivar. Germination continued to decrease in seeds stored at room temperature after six months, but remained unchanged or higher for seeds stored at 5°C. The exceptions to this were LB01330x seeds stored at 5°C for 12 months in which germination was only 48%. However when seeds were transferred to 20°C for a further two weeks after the initial 30 days at 15°C, germination increased. It appears that 12 months storage at 5°C does not affect the ability of seeds to germinate, but does influence rate of germination. Results indicate that short-term storage at low temperatures promotes germination in delphinium seeds. Long-term storage may not be as beneficial.

5.4.3 Moisture content of seed

Moisture contents varied within and between cultivars. Lowest initial moisture content was seen in LB01330x, at 9.25%. O/P mix and P9827x35 had initial moisture contents of 10.63% and 10.77% respectively. LB01330x also had the lowest germination (61.5% c.f. 78.5% and 87.5%). There is no obvious correlation between the seed moisture contents and the ability of seeds to germinate. A correlation does appear between cultivars at time 0 with increasing moisture content and higher germination. This is also seen in LB01330x in storage at 5°C. Moisture contents and germination percents increase at three and six months, but fall after six months. This is not echoed in O/P mix and P9827x35, with these

cultivars showing no significant difference in germination at different moisture contents. Moisture content is likely to play a role in seed germination. From data received it would appear that responses to moisture content differ between cultivars. If moisture content plays a role in seed germination, optimum moisture contents in these trials for O/P and LB01330x falls between 9-11% and between 8-11% for P9827x35. Carpenter and Boucher (1995) saw a significant decrease in germination of *Delphinium x cultorum* when seed moisture content fell below 6.7-7.0%. Their t_{50} also increased.

5.4.4 Short-term storage

Seeds were stored for six weeks at 15°C, 20°C and 25°C to assess the effects of short-term storage temperatures on germination. Seeds stored for six weeks at 15°C showed significantly higher germination (77.5%) to seeds stored at 20°C (67.5%) and 25°C (68.5%) but was not significantly different from fresh seeds (78.5%). T_{50} was also significantly increased at 25°C storage. A correlation with moisture content appears to be present, as this decreased as temperature rose. These findings suggest that storage temperature and moisture content play an important role in determining germination after storage. Combined with results from long-term storage (12 months) it appears that optimal moisture content may be above 9% (for LB01330x and O/P mix at least). Storage temperatures above 15°C lead to decreases in moisture content and germinability. Seed germination after short-term storage is affected by moisture content. However, results from the 12 months storage trials indicate that seeds stored at room temperature deteriorate at a faster rate than seeds stored at low temperature, regardless of moisture content.

5.4.5 Dormancy-breaking treatments

As stratification and application of gibberellic acid were the only treatments that significantly influenced germination of fresh *Delphinium elatum* seeds, these appeared the best choice for trialling on stored seeds (12 months). Firstly, seeds that remained hard after 30 days germination at 15°C were transferred to (a) 20°C or (b) 5°C for two weeks to see if changes in temperature promoted further germination. Further germination took place in

all three cultivars placed under this treatment. Highest germination was seen at 20°C, although seeds also germinated at 5°C. Most remarkable was cultivar LB01330x, which saw 37% further germination in seeds transferred to 20°C, increasing total germination from 48% to 73% for seeds stored at 5°C for 12 months. Further germination was also seen in seeds stored at room temperature for 12 months. This germination seems to be affected by temperature (highest at 20°C), but is also probably due to increased germination time. A temperature of 20°C does promote shorter t_{50} in fresh seed. Germination was improved slightly in seeds stored at room temperature for 12 months. However, according to the tetrazolium test, the majority of seeds stored at room temperature had lost viability and were unable to germinate regardless of treatment.

One aim of the project is to decrease germination time of delphinium seeds and thus increase rate of germination. To achieve this, GA_3 was applied to seeds stored for 12 months under the three conditions, and germinated at 15°C. This increased germination in O/P mix and LB01330x (significantly higher in LB01330x). Germination under this treatment was also higher than in fresh LB01330x seed. No significant differences were seen in germination after room temperature storage. Chilling (two weeks imbibed at 5°C) was also trialled on O/P mix and LB01330x. Again, no difference was seen in the germination of room temperature stored seeds (although t_{50} was shorter), but similar results were seen for LB01330x and O/P mix stored for 12 months at 5°C compared with fresh stratified seeds.

6.0 CONCLUSIONS

Delphinium elatum is an important seed crop for Dowdeswell's Delphiniums. Information gathered in this thesis is significant to the production of *D. elatum* seed, as previous information is limited. From the results obtained in this thesis the subsequent conclusions can be drawn:

6.1 Seed set

Delphinium elatum stigmas have an open structure, and pollen adherence occurs on the inner stigmatic surface. Stigmas open once receptive and adherence can occur. Young stigmas present a closed appearance, and pollen is physically prevented from adhering to the stigmatic surface. Styles are hollow, with a thick column of transmitting tract tissue lining the inner tract. This transmitting tract tissue branches once the ovary is reached, and may provide physical support for growth of pollen tubes to ovules.

Carpels are hypogynous and (usually) occur in threes. There appears to be two-three placental strands per carpel and placenta is parietal (ovules are borne on extensions of the ovary wall). Ovules are anatropous, bitegmic and crassinucleate. Ovules fluoresce when treated with aniline blue, which may be an indicator of receptivity (Rugkhld et al, 1997).

Seed set in commercial *Delphinium elatum* cultivars is limited by pollen type and age. Pollen viability is low (below 50%) in most cultivars, and pollen tubes grow at different rates depending on cultivar. Pollen age also plays a role, as older anthers show lower viability than those newly dehisced. No signs of self-incompatibility were observed, although it may occur in other (untested) varieties. Pollen tube growth *in vivo* proved inconclusive in determining if pollen tubes encounter difficulty in reaching ovules. Ovules observed for development showed normal developmental patterns after pollination. Selfing did not occur naturally in cultivars tested (without the aid of a pollinator) due to inability of pollen to reach the stigma.

6.2 Seed germination

The mature *Delphinium elatum* seed is composed of an embryo, endosperm and a seed coat. Endosperm occupies most of the seed volume. Embryos are torpedo-shaped, lobose, and straight. Cultivars differ to a small extent in seed shape, embryo size, and cotyledon shape. *D. elatum* endosperm development is nuclear (but later becomes cellular). Endosperm is well developed at 11 days after pollination, but the embryo is not visible. Inability to germinate is not related to embryo immaturity as embryos were fully developed in all cultivars examined.

D. elatum seeds do not exhibit a uniform germination response across cultivars. Germination is low in LB01130x, but high in cultivars P9827x35 and W0204x. Optimal conditions for germination of *D. elatum* seed are at a uniform 15-20°C (although seed will germinate at 5°C). Compared to all treatments, chilling had most impact on germination rates, reducing germination time and promoting higher germination. GA_3 also promoted germination in some cultivars of *D. elatum* (i.e. LB01130x). Results indicate dormancy mechanisms influenced by stratification and GA_3 are present in some *D. elatum* cultivars. This may be related to differences in endogenous gibberellin levels between cultivars.

Mould growth does not affect germination but does affect seedling survival after germination if seeds are not removed following germination. Germination is also adversely affected by high humidity/high temperature which promotes rapid fungal growth and rotting of seed (i.e. above 20°C). Seeds do not respond well to treatment with the fungicide thiram (germination is reduced) but are unaffected by surface sterilisation.

6.3 Seed storage

Low temperature storage has no significant impact on germination (compared with no storage), although it may impose a degree of dormancy in some cultivars (i.e. LB01330x). This dormancy can be broken by stratification or application of GA_3. Storage at room

temperature (temperatures exceeding 15°C) reduces seed viability considerably. A negative correlation exists between seed viability and length of storage period at 15°C or above. Seeds respond best to storage at 5°C or lower. Storage appears to be optimal between seed moisture contents of 9-11% although this may depend on cultivar type. Storage above or below this may reduce viability of the seed.

7.0 RECOMMENDATIONS FOR FURTHER RESEARCH

Embryogenesis and seed development have not been examined in detail in this study. Babis (1973; 1979) has examined *Delphinium* ovule development and structure in detail, but little is available on development of the seed itself. Further work is suggested in this area to complement previous research and help understand mechanics of seed development in *D. elatum*.

A recommendation for further work on *Delphinium elatum* cultivars developed by Dowdeswell's Delphiniums is to study the known history of individual cultivars to determine degrees of relatedness, as this may be a cause of self-incompatibility in some hybrids. Communication with Terry Dowdeswell[3] has revealed that seasonal changes in seed set are sometimes observed, with variation in seed set occurring within the same cultivar. Resource allocation could be examined in these cases to determine if insufficient nutrients are a cause of variable seed set in some cultivars. Other areas to be examined are links between physical characteristics, such as doubling, and seed set performance. Examination of pollen viability and rates of pollen tube growth over a much wider range of cultivars may also prove beneficial to the business in developing crosses which will provide high levels of seed set.

Seed germination was not studied past radicle emergence and initial seedling growth in delphinium. Seedling growth is known to be affected by seed aging (Oladrian and Agunbiade, 2000). Further work in this area is of importance to determine influences of seed pre-treatment and storage on delphinium, and to establish optimal parameters for commercial growth of delphinium seed.

[3] Owner of Dowdeswell's Delphiniums

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