

1 **Reproductive biology of the invasive exotic shrub, *Rhododendron ponticum* L.**

2 **(Ericaceae)**

3

4 **Dr Jane C. Stout**

5

6 School of Natural Sciences

7 Trinity College Dublin

8 Dublin 2, Ireland

9

10 Tel +353-1-6083761

11 Fax +353-1-6081147

12 E-mail stoutj@tcd.ie

13

14 Running Title: Reproductive biology of *Rhododendron*

1 **Abstract**

2 The reproductive biology of an exotic species will affect its ability to become naturalised and
3 invasive in non-native habitats. *Rhododendron ponticum* is an ecologically damaging exotic
4 weed in the British Isles, which spreads predominantly by seed. I investigated how inbreeding
5 and out-crossing affect seed production and germination in a wild population of this species in
6 Ireland. Experimental manipulations revealed low fruit and seed set when insects were
7 excluded from flowers, suggesting that this species has limited capability for spontaneous
8 autogamy. Hand-pollination treatments showed that although flowers are self-compatible
9 (with self and same plant pollen), higher levels of seed set occur following outcrossing
10 (xenogamy). There was no significant difference in rate of germination of seeds from inbred
11 or outcrossed treatments. The addition of xenogamous pollen to open flowers did not increase
12 fruit or seed set, suggesting that flowers in this population are not pollen limited: native
13 generalist pollinators, mainly bumblebees (*Bombus* spp.), are providing an adequate pollinator
14 service. This work demonstrates that outcrossing increases seed set and pollinators are
15 required to facilitate this. Hence generalist native pollinators can promote invasion by exotic
16 plants. Native pollinators can clearly play an important part in alien species invasion.

17

18 **Keywords**

19 fruit set – hand-pollination – inbreeding – invasion – out-crossing – pollination – seed
20 production

21

1 **Introduction**

2 Although many exotic species are introduced into non-native habitats, only a fraction become
3 invasive (Williamson, 1994; Mack *et al.*, 2000; Mooney & Cleland, 2001). Successful
4 invasion involves a number of stages including introduction, establishment and range
5 expansion (Andow *et al.*, 1990). A significant factor affecting invasion is the mode of
6 reproduction of a species. Plants capable of vegetative spread may easily expand their ranges
7 in suitable habitats, and some clonal plants have been notoriously successful, for example
8 *Fallopia japonica* (Houtt.) Ronse Decraene (Japanese knotweed) (Hollingsworth & Bailey,
9 2000). In sexually reproducing species, breeding system characteristics, such as self-
10 compatibility, outcrossing rate and method of pollen and seed dispersal can dramatically
11 influence population dynamics and invasion (Kittelson & Maron, 2000).

12
13 Entomophilous species, artificially introduced into habitats that lack their mutualistic insect
14 pollinators, may set seed as a result of apomixis (asexual seed production), autogamy
15 (spontaneous self pollination in open flowers) or cleistogamy (the production of specialised
16 closed flowers) (Spencer, Kennedy & Gray, 1996). Indeed, in species with a mixed mating
17 system, a lack of pollinators can promote increased self-pollination (e.g. Redbotorstensson &
18 Berg, 1995; Kalisz & Vogler, 2003). Invasive exotic plants are often capable of self-
19 fertilisation, and this is frequently cited as a mechanism for successful invasion (Baker, 1965;
20 Williamson & Brown, 1986; Rambuda & Johnson, 2004; but see Williamson & Fitter, 1996).
21 On the other hand, obligate out-crossers, or those that require facilitation by pollinators, may
22 be reproductively constrained by a lack of pollinator visitation (Burd, 1994; Parker, 1997;
23 Larson & Barrett, 1999). Species which rely on specialised mutualistic interactions with
24 pollinators may completely fail to set seed in exotic habitats (Richardson *et al.*, 2000). Most
25 plants do not rely on specialist pollinators, however, and generalist native pollinators can

1 facilitate adequate xenogamy (Stary & Tkalcu, 1998; Richardson *et al.*, 2000; Mooney &
2 Cleeland, 2001; Chittka & Schurkens, 2001).
3
4 Generalist pollinators often visit exotic plants, and many successful aliens produce large floral
5 displays (Ghazoul, 2002). The size of the floral display can affect the behaviour of pollinators
6 (Klinkhamer *et al.*, 1989; Goulson *et al.*, 1998). This influences not only the quantity of
7 pollinating visits, but also the quality of the pollination service received. The production of
8 many flowers can increase pollinator visitation (Augsburger, 1980; Geber, 1985; Klinkhamer,
9 de Jong & de Bruyn, 1989; Klinkhamer & de Jong, 1990; Stout, 2000), which may result in
10 increased reproductive success. Alternatively, plants with large floral displays may suffer
11 from inbreeding as a result of increased geitonogamy (within-plant pollen transfer) (Geber,
12 1985; de Jong *et al.*, 1992; de Jong, Waser & Klinkhamer, 1993; Klinkhamer & de Jong,
13 1993). Inbreeding can reduce individual fitness through decreased fruit and seed production,
14 seed germination, and growth and fertility of inbred offspring (Keller & Waller, 2002;
15 Wallace, 2003). In addition, at the population level, inbreeding can result changes in genetic
16 diversity and affect the ability of a population to cope with environmental change (Lande,
17 1995). Inbreeding rates may already be relatively high in exotic plant populations since many
18 exotic plants form small isolated populations of genetically similar individuals, or are
19 descended from small founder populations (Lee *et al.*, 2004). Having said that, low genetic
20 diversity does not necessarily restrict plant invasion and some invasive plants display little
21 genetic variation within populations (Poulin, Weller & Sakai, 2005).

22

23 *Study species:*

24 *Rhododendron ponticum* L. (Ericaceae) is a highly invasive shrub in several regions in north-
25 western Europe (Cronk & Fuller, 1995). Its spread is facilitated by successful seedling

1 recruitment, which results from the production of prolific numbers of small, viable, wind
2 dispersed seeds each year (Brown, 1953; Cronk & Fuller, 1995). Seeds are produced sexually
3 rather than as a result of apomixis, and cleistogamy is not known to occur (Cross, 1975).
4 Naturalised populations in Britain and Ireland are thought to have descended from Spanish
5 ancestors, but introgression with North American *Rhododendron* species has occurred in
6 many populations (Milne & Abbott, 2000). This could have affected the breeding system of
7 *R. ponticum* in the British Isles compared with ancestral populations in Spain (Mejías, Arroyo
8 & Ojeda, 2002), but no previous studies have determined that this is the case.

9
10 Naturalised *R. ponticum* has been presumed to be insect pollinated because it produces a
11 massive floral display of large (>60 mm corolla diameter), bright pink/purple flowers that
12 secrete profuse volumes of nectar, flowers are protandrous and anthers are poricidal (although
13 natural vibrations can release pollen; King & Buchmann, 1995). Various Hymenoptera,
14 Diptera and Lepidoptera have been cited as pollinators (Cross, 1975; Cronk & Fuller, 1995).
15 Recently, it has been revealed that a range of insects visit flowers in native (Spanish) and
16 exotic (Irish) habitats, many of which pick up and transport pollen (Mejías *et al.*, 2002, Stout
17 *et al.*, 2006). Observations of pollinator behaviour suggest that levels of geitonogamy (within-
18 plant pollen transfer) are high in Irish populations (Stout, 2007). The impacts of inbreeding on
19 fruit production in native populations has been shown to be negligible (Mejías *et al.*, 2002),
20 but nothing is known about the reproductive biology or breeding system of *R. ponticum* in its
21 exotic range (Rotherham, 2001).

22
23 In this paper, I investigate the breeding system of exotic *R. ponticum*. Specifically, I test the
24 following hypotheses:

- 25 1. Exotic *R. ponticum* is capable of autogamous self-pollination

2. Pollination limitation reduces natural levels of seed production
3. Inbreeding affects both the quantity and quality of seed produced (in terms of proportion of fruit set, number of seeds produced and germination success)

Materials and Methods

Breeding experiments were performed on a wild population of *Rhododendron ponticum* plants at Howth Head (Co. Dublin) in 2003. Twelve treatments were set up on each of five plants (a randomised block design) (see Table 1). Individual plants were at least 5m apart to avoid treating clones (which arise as a result of short-distance vegetative spread via layering). On each plant, each treatment was performed on all flowers on a single inflorescence; on average, 12.97 flowers were treated per inflorescence (SE 0.57). Insects were excluded from appropriate treatments by bagging inflorescences with bridal veil material (<1 mm mesh) on 1st May 2003, before flower buds opened. Plants were visited every 3-7 days during May and June 2003 to perform hand pollinations or to emasculate flowers as necessary. When appropriate, individual flowers were hand pollinated at least twice during the period they were open. To test for spontaneous autogamy (treatment 1), inflorescences were bagged and no further manipulation was carried out. To test for facilitated autogamy (treatment 2), inflorescences were bagged, and flowers self-pollinated by bending anthers to apply pollen directly onto stigmas of the same flowers. For geitonogamy and xenogamy treatments (3 and 4 respectively), inflorescences were bagged, and pollen was applied by hand by picking dehiscing stamens from randomly selected donor flowers (from the same or different plants respectively) and applying pollen directly from these anthers onto the stigmas of recipient flowers. To test whether same-flower pollen contributes to pollination success, I used additional treatments, similar to 3 and 4, but flowers were emasculated prior to opening (by

1 carefully prising apart petals and removing immature anthers from flowers with tweezers)
2 (treatments 5 and 6). To test whether the emasculation technique was reliable, and to test for
3 apomixis, additional bagged, emasculated flowers received no further manipulation (treatment
4 7). Treatment 8 consisted of open untreated flowers, representing seed set under natural, non-
5 manipulated conditions. Treatments 9, 10 and 11 were equivalent to 2, 3 and 4 but
6 inflorescences were not bagged. To examine levels of pollen receipt from different flowers
7 (geitonogamous and/or xenogamous), treatment 12 consisted of open, emasculated flowers.
8

9 At the end of the flowering period (29th June 2003) bags were removed to prevent any damage
10 to developing fruits. Although fruits do not usually dehisce until January/February, seeds are
11 ripe by December (Cross, 1975). Fruits from all treatments were collected at the end of
12 November 2003 to ensure no seeds were lost during dehiscence. Fruit lengths were measured
13 and fruits were stored at room temperature (approximately 20-23°C) in individual paper cases
14 to allow them to dry out. Once seeds were released, they were counted from a sample of 111
15 fruits. Seeds are small (~2mm in length), but mature seeds are easily distinguished from much
16 smaller (<0.5mm in length) undeveloped ovules. Only mature seeds were counted.
17

18 In addition, once seeds were released, germination trials were conducted. Twenty mature
19 seeds were taken from a randomly selected fruit from each plant for each treatment, except
20 treatment 7 (emasculated and bagged) where no seeds were produced. Some fruits from
21 treatment 1 (spontaneous autogamy) contained less than 20 seeds, and so as many seeds as
22 were available were selected (plants 1, 3 and 4 produced 9, 6 and 0 seeds respectively). Seeds
23 were placed in Petri dishes lined with filter paper (Whatman Type 1), watered daily with 0.5-
24 1 ml of deionised water as necessary. Light is essential for germination of *R. ponticum* seeds
25 (Cross, 1981) and so Petri dishes were covered with transparent lids. Petri dishes were

1 randomly arranged to reduce position and edge effects and kept at room temperature. The
2 number of germinations (determined by the appearance of both cotyledons) were measured
3 every 1-5 days for 50 days.

4

5 *Data analysis*

6 The relationship between fruit length and the number of seeds per fruit was not linear and so
7 was analysed using quadratic regression analysis. Subsequently, the number of seeds
8 produced per fruit from each treatment was estimated from this equation. The proportion of
9 fruit set was calculated as the number of fruits produced per inflorescence divided by the
10 number of flowers on that inflorescence at the time of flowering. Comparisons of proportion
11 of fruit set and mean fruit length were made using ANOVA for Randomised Complete Blocks
12 (RCB) with each plant representing a fixed block (Sokal & Rohlf, 1995). To determine
13 whether flowers are capable of autogamous self-pollination in the absence of insects, fruits
14 from treatment 1 (bagged flowers) were compared with fruits from treatment 8 (open
15 flowers). Similarly, to establish the effect of self pollination versus outbreeding on seed
16 production, fruits from treatments 2, 3 and 4, and, separately, 9, 10 and 11, and, separately, 5
17 and 6 were compared; and when significant differences were found, post-hoc Student-
18 Newman-Keuls (SNK) tests were used to determine groupings. To establish the occurrence
19 and impact of self pollination, non-emasculated and equivalent emasculated treatments were
20 compared. To determine whether flowers were pollen limited, treatment 11 was compared
21 with treatment 8, assuming that maximum fruit set occurred in open flowers with additional
22 outcross pollen added by hand.

23

1 Self compatibility indices (SCI) and an auto-fertility index (AFI) were also calculated for the
2 population, by calculating values for each plant and then obtaining a mean for the population,
3 using the following according to Lloyd & Schoen (1992):

4

5 SCI (within flower) = mean seed set after facilitated selfing (treatment 2) / mean seed set after
6 xenogamy (treatment 6)

7

8 SCI (between flowers) = mean seed set after geitonogamy (treatment 3) / mean seed set after
9 xenogamy (treatment 6)

10

11 AFI = mean seed set after spontaneous autogamy (treatment 1) / mean seed set after
12 xenogamy (treatment 6)

13

14 The SCI ranges from 0 to greater than 1, with 1 representing full self-compatibility, and
15 values <0.75 interpreted as representing self-incompatibility (Lloyd & Schoen 1992). The

16 AFI represents the ability of flowers to self-fertilise in the absence of pollinators (Escaravage
17 *et al.*, 1997).

18

19 The proportion of seeds germinated (i.e. developing both cotyledon leaves) for each plant in
20 each treatment was calculated per day. The proportion germinated on five days during the
21 germination period (day 20, 31, 40 and 50) were compared among treatments using RCB
22 ANOVA, again with each plant representing a fixed block (Sokal & Rohlf, 1981). Open
23 treatments 8 – 12 may have received self/geitonogamous pollen in addition to outcross pollen,
24 and so these treatments were analysed separately.

25

1 **Results**

2 There was a positive relationship between fruit length and the number of seeds per fruit (Fig.
3 1). Quadratic regression analysis revealed this relationship to be highly significant ($F_{2,108} =$
4 $265.5, p < 0.0001$, the model explaining 83% of the variance). Fruits < 6.5 mm in length
5 contained no seeds. There were no significant effects of plants (blocks) in any of the RCB
6 analyses below.

7

8 *Autogamous self-pollination*

9 When insects were excluded from flowers, but no additional pollen was added (treatment 1),
10 just under half of all flowers produced a fruit (mean fruit set \pm SD: 0.45 ± 0.27), but these
11 fruits were very small and most did not contain seeds (mean number of seeds \pm SD: $29.81 \pm$
12 43.81). When insects were not excluded, but no additional pollen was added (treatment 8),
13 fruit and seed production was higher (mean \pm SD fruit set: 0.91 ± 0.08 ; number of seeds:
14 405.99 ± 294.28). The differences between treatments 1 and 8 were significant (fruit set: $F_{1,4}$
15 $= 9.41, p = 0.04$; fruit length: $F_{1,4} = 21.77, p = 0.010$).

16

17 *Facilitated self pollination vs. geitonogamy vs. xenogamy*

18 Seed set increased when geitonogamous or out-cross pollen was added, both when insects
19 were excluded from flowers (treatments 2, 3 and 4: fruit set: $F_{2,8} = 5.11, p = 0.04$; fruit length:
20 $F_{2,8} = 22.33, p = 0.001$) and when they were not excluded (treatments 9, 10 and 11: fruit set:
21 $F_{2,8} = 4.97, p = 0.04$; fruit length: $F_{2,8} = 10.15, p = 0.006$) (Fig. 2). Post-hoc tests revealed
22 significant differences in fruit length between all treatments when insects were excluded ($p <$
23 0.05), but failed to resolve differences between other groups.

24

25 *Effect of emasculation*

1 Although emasculated bagged flowers (treatment 7) occasionally produced small fruits, these
2 fruits contained no seeds. Hence both emasculation and bagging appear to be reliable methods
3 for excluding pollen and flowers do not appear to be capable of seed production via apomixis.
4 There were no significant differences in fruit set or fruit length between emasculated and non-
5 emasculated treatments (Fig. 3). There was no significant difference between emasculated
6 flowers hand-pollinated with geitonogamous or xenogamous pollen (treatments 5 and 6: fruit
7 set: $F_{1,4} = 1, p = 0.37$; fruit length: $F_{1,4} = 0.92, p = 0.39$).

8

9 *Pollen limitation*

10 When additional out-cross pollen was added to open flowers (treatment 11), 100% fruit set
11 was achieved, with a mean of 594.79 seeds per fruit (SD = 204.74). Although fruit set and
12 seed number were higher than open pollinated flowers (treatment 8, mean \pm SD, fruit set: 0.91
13 ± 0.08 ; number of seeds: 405.99 ± 294.28), these differences were not significant (fruit set:
14 $F_{1,4} = 5.94, p = 0.07$; fruit length: $F_{1,4} = 0.86, p = 0.41$).

15

16 *Self compatibility and auto-fertility*

17 The mean self compatibility index (SCI) was 0.24 (within flowers) and 0.69 (between flowers
18 on the same plant) (standard deviation = 0.24 and 0.46 respectively). In addition, the auto-
19 fertility index (AFI) was 0.044 (SD=0.06).

20

21 *Germination success*

22 There were significant differences among treatments 1-6 on day 31 ($F_{5,20} = 3.307, p = 0.024$)
23 but this difference had disappeared by day 40 and 50. Throughout the trial period, the
24 proportion of seeds germinated from the out-crossed treatments (4 and 6) was higher than
25 seeds produced by geitonogamy (3 and 5), which was higher than seeds produced by selfing

1 (1 and 2, Fig. 4). There were no significant differences in germination success among open
2 treatments (8-12) at any time point.

3

4 **Discussion**

5 Although it has long been recognised that the prolific seed production of *Rhododendron*
6 *ponticum* has facilitated its invasion in many parts of Britain and Ireland (Brown, 1953;
7 Cross, 1981), the breeding system has been unclear. I found that insect exclusion severely
8 reduces fruit and seed set. In addition, the calculated AFI was extremely low, indicating a
9 minimal ability to spontaneously self-fertilise in naturalised exotic populations. This can be
10 explained by 1) protandry within flowers and 2) poricidal anthers which require vibration for
11 pollen release. This is also the case in ancestral Spanish populations (Mejías *et al.*, 2002). It
12 has been predicted that reliance on insect visitation for seed production should be unusual
13 among highly invasive taxa (Baker, 1965) and there are some famous examples of invading
14 exotics that are chiefly autogamous (e.g. cheatgrass, *Bromus tectorum*, and Hottentot fig,
15 *Carpobrotus edulis*, in North America, Richardson *et al.*, 2000). However, there are other
16 notable alien invaders that are outcrossers and require pollinator visitation (e.g. scotch broom,
17 *Cytisus scoparius*, Parker, 1997, *Lantana camara*, Goulson & Derwent, 2004). *R. ponticum*
18 appears to fall into the latter category.

19

20 Long-lived woody plants, with multiple flowering episodes throughout their life, tend not to
21 depend upon spontaneous autogamy and generally show low levels of self-compatibility
22 (Stebbins, 1965; Rambuda & Johnson, 2004). Self-sterility has been demonstrated in six
23 *Rhododendron* species from Hong Kong (Ng & Corlett, 2000), but *R. ferrugineum*, a distantly
24 related European species, shows high levels of self-compatibility (Escaravage *et al.*, 1997). *R.*
25 *ponticum* also appears to be partially self compatible: I found that hand-selfing, via facilitated

1 autogamy and particularly by geitonogamy, resulted in fruit and seed set. Geitonogamous
2 pollination probably increased fruit and seed set in relation to facilitated selfing due to
3 protandry limiting within-flower fertilisation. The facilitated selfing carried out in this study
4 may simply have transferred pollen onto immature stigmas. It is unlikely that physical or
5 chemical self incompatibility (SI) mechanisms, common in other taxa, exist in *R. ponticum* to
6 prevent self-fertilisation (Silva & Goring, 2001; Hiscock & McInnis, 2003). When compared
7 with non-emasculated treatments, emasculation had no significant effect on fruit and seed set.
8 This supports the notion that within-flower selfing is not an important component of the
9 breeding system, and that emasculation does not affect stigma or style receptivity or
10 attractiveness of flowers to pollinators (Dafni, 1992 and references therein).

11
12 In Spain, Mejías *et al.* (2002) demonstrated that reproductive success from facilitated self
13 pollination was not significantly different to out-crossing. However, I found that the addition
14 of xenogamous pollen to bagged flowers significantly increased seed set when compared with
15 any of the inbred treatments. Therefore it may be concluded that seed quantity is adversely
16 affected by inbreeding. However, these seeds displayed no significant increase in germination
17 success, indicating that seed quality is not affected by inbreeding. Of course, other aspects of
18 seed quality, for example, seed size and progeny fitness beyond the germination stage were
19 not measured, and may be adversely affected by inbreeding. Outcrossing plants often express
20 inbreeding depression early in their life cycle, particularly at the seed production stage
21 (Barrett & Harder, 1996; Husband & Schemske, 1996). Mejías *et al.* (2002) found that
22 xenogamy did not improve fruit set in native Spanish populations of *R. ponticum*. It is
23 possible that introgression with North American *Rhododendron* species in exotic populations
24 has caused this change in the breeding system of this species. A recent study has also shown
25 differences in the biology of native and exotic *R. ponticum* plants (Erfmeier & Bruelheide,

1 2004) where exotic populations of *R. ponticum* displayed higher vegetative growth rates than
2 native Spanish ones and this difference was shown to be genetically determined (Erfmeier &
3 Bruelheide, 2005).

4
5 No pollen limitation was detected in these experiments, and this suggests that flowers are
6 receiving adequate pollinator service in this population. However, since only a small
7 proportion of the inflorescences were manipulated per plant, and this study was only carried
8 out in one flowering season, I cannot rule out the possibility that pollen limitation occurs in
9 this exotic species (Zimmerman & Pyke, 1988). Indeed, substantially lower levels of fruit set
10 have been recorded in other populations in Ireland, and a positive correlation between
11 pollinator visitation rate and fruit set has been determined (Stout *et al.*, 2006). Hence, pollen
12 limitation may occur in populations other than the one studied, although resource limitation
13 may also be responsible for lower fruit set.

14

15 *Implications for invasion*

16 Exotic *R. ponticum* in the British Isles benefits from pollination by native insects and
17 produces vast numbers of seeds which provide the primary mechanism for its invasion. In the
18 absence of pollinator visitation, this species would set few seeds, because of the inability of
19 flowers to spontaneously self-pollinate. There was no pollination limitation in the population
20 studied because of adequate service from generalist pollinators. These insects are attracted by
21 the prolific nectar and pollen production (Stout *et al.*, 2006) and seem unaffected by toxins in
22 the nectar (von Malottki & Wiechmann, 1996). Although a range of insects pick up pollen
23 from *R. ponticum* flowers (Stout *et al.*, 2006), only bumblebees appear to facilitate pollen
24 deposition (Stout, 2007). Bumblebees forage in an efficient manner, often moving between
25 adjacent flowers and can promote high levels of geitonogamy (Stout, 2000; Utelli & Roy,

1 2000). On the other hand, studies have shown that insects visit a reduced proportion of
2 flowers on large plants, and so mass flowering is not necessarily associated with low
3 outcrossing rates (Augspurger, 1980; Andersson, 1988; Parker, 1997). Individual *R. ponticum*
4 plants produce a large number of flowers and movements between plants are rare
5 (approximately 2% of bumblebee moves, Stout, 2007). Therefore, it may be that a large
6 proportion of seeds set by *R. ponticum* are as a result of geitonogamy. However, until we
7 know more about pollen carryover in *R. ponticum*, we can not speculate on this further.
8 Carryover was estimated to be negligible after 5 flowers and pollen dispersal distances were
9 estimated between 2-45m in *R. ferrugineum* (Escaravage & Wagner, 2004).

10

11 The breeding system is obviously not the only factor affecting invasion of *R. ponticum* in the
12 British Isles. The availability of safe sites for germination (Cross, 1981; Rotherham, 2001;
13 Mejías *et al.*, 2002; Erfmeier & Bruelheide, 2004), the removal of competing plants by
14 vertebrate grazers (Higgins *et al.*, 2001), disturbance (Rotherham, 2001) and climate all play a
15 role. However, the fact that this species is not pollinator limited and produces abundant seed
16 suggests that it has the potential to become more invasive, particularly in suitable habitats.
17 Given the effort and costs involved with *Rhododendron* control (Dehnen-Schmutz, Perrings &
18 Williamson, 2004), land managers should take care to prevent the establishment and
19 flowering of *R. ponticum*, as seed production and further spread is inevitable.

20

21

22 **References**

23 **Andersson S. 1988.** Size dependent pollination efficiency in *Anchusa officinalis*
24 (Boraginaceae) - causes and consequences. *Oecologia* **76**: 125-130.

25

- 1 **Andow DA, Kareiva PM, Levin SA, Okubo A. 1990.** Spread of invading organisms.
2 *Landscape Ecology* **4**: 177-188.
3
- 4 **Augsburger CK. 1980.** Mass-flowering of a tropical shrub (*Hybanthus prunifolius*):
5 influence on pollinator attraction and movement. *Evolution* **34**: 475-488.
6
- 7 **Baker HG. 1965.** Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL,
8 eds. *The genetics of colonizing species*. Academic Press, New York, 147-168.
9
- 10 **Barrett SCH, Harder LD. 1996.** Ecology and evolution of plant mating. *Trends in Ecology*
11 *& Evolution* **11**: 73-79
12
- 13 **Brown JMB. 1953.** The Rhododendron problem in the woodlands of southern England.
14 *Quarterly Journal of Forestry* **47**: 239-253.
15
- 16 **Burd M. 1994.** Bateman principle and plant reproduction - the role of pollen limitation in
17 fruit and seed set. *Botanical Review* **60**: 83-139.
18
- 19 **Chittka L, Schurkens S. 2001.** Successful invasion of a floral market. *Nature* **411**: 653.
20
- 21 **Cronk QCB, Fuller JL. 1995.** *Plant invaders: the threat to natural ecosystems*. London:
22 Chapman and Hall.
23
- 24 **Cross JR. 1975.** Biological flora of the British Isles *Rhododendron ponticum*. *Journal of*
25 *Ecology* **63**: 345-365.

1

2 **Cross JR. 1981.** The establishment of *Rhododendron ponticum* in the Killarney oakwoods,
3 S.W. Ireland. *Journal of Ecology* **69**: 807-824.

4

5 **Dafni A. 1992.** *Pollination Ecology: A practical approach*. Oxford: Oxford University Press.

6

7 **Dehnen-Schmutz K, Perrings C, Williamson M. 2004.** Controlling *Rhododendron*
8 *ponticum* in the British Isles: an economic analysis. *Journal of Environmental*
9 *Management* **70**: 323-332.

10

11 **Erfmeier A, Bruelheide H. 2004.** Comparison of native and invasive *Rhododendron*
12 *ponticum* populations: Growth, reproduction and morphology under field conditions.
13 *Flora* **199**: 120-133.

14

15 **Erfmeier A, Bruelheide H. 2005.** Invasive and native *Rhododendron ponticum* populations:
16 is there evidence for genotypic differences in germination and growth? *Ecography* **28**:
17 417-428.

18

19 **Escaravage N, Pornon A, Doche B, Till-Bottraud I. 1997.** Breeding system in an alpine
20 species: *Rhododendron ferrugineum* L. (Ericaceae) in the French northern Alps.
21 *Canadian Journal of Botany* **75**: 736-743.

22

23 **Escaravage N, Wagner J. 2004.** Pollination effectiveness and pollen dispersal in a
24 *Rhododendron ferrugineum* (Ericaceae) population. *Plant Biology* **6**: 606-615.

25

- 1 **Geber MA. 1985.** The relationship of plant size to self-pollination in *Mertensia ciliata*.
2 *Ecology* **66**: 762-772.
3
- 4 **Ghazoul J. 2002.** Flowers at the front line of invasion? *Ecological Entomology* **27**: 639-640.
5
- 6 **Goulson D, Stout JC, Hawson SA, Allen JA. 1998.** Floral display size in comfrey,
7 *Symphytum officinale* L. (Boraginaceae): relationships with visitation by three
8 bumblebee species and subsequent seed set. *Oecologia* **113**: 502-508.
9
- 10 **Goulson D, Derwent LC. 2004.** Synergistic interactions between an exotic honeybee and an
11 exotic weed: pollination of *Lantana camara* in Australia. *Weed Research* **44**: 195-202.
12
- 13 **Higgins GT, Larkin R, Dower P, Mitchell FJG, Kelly DL. 2001.** *Permanent plots for the*
14 *monitoring of woodland vegetation and regeneration in Killarney National Park:*
15 *1991-2001.* Ireland: Report submitted to Dúchas, The Heritage Service, National
16 Parks and Wildlife Service.
17
- 18 **Hiscock SJ, McInnis SM. 2003.** The diversity of self-incompatibility systems in flowering
19 plants. *Plant Biology* **5**: 23-32.
20
- 21 **Hollingsworth MI, Bailey JP. 2000.** Evidence for massive clonal growth in the invasive
22 weed *Fallopia japonica* (Japanese Knotweed). *Botanical Journal of the Linnean*
23 *Society* **133**: 463-472.
24

- 1 **Husband BC, Schemske DW. 1996.** Evolution of the magnitude and timing of inbreeding
2 depression in plants. *Evolution* **50**: 54-70.
- 3
- 4 **Jong de TJ, Waser NM, Price MV, Ring RM. 1992.** Plant size, geitonogamy and seed set in
5 *Ipomopsis aggregata*. *Oecologia* **89**: 310-315.
- 6
- 7 **Jong de TJ, Waser NM, Klinkhamer PGL. 1993.** Geitonogamy - the neglected side of
8 selfing. *Trends in Ecology and Evolution* **8**: 321-325.
- 9
- 10 **Kalisz S, Vogler DW. 2003.** Benefits of autonomous selfing under unpredictable pollinator
11 environments. *Ecology* **84**: 2928-2942.
- 12
- 13 **Keller LF, Waller DM. 2002.** Inbreeding effects in wild populations. *Trends in Ecology and*
14 *Evolution* **17**: 230-241.
- 15
- 16 **King MJ, Buchmann SL. 1995.** Bumblebee initiated vibration release mechanism of
17 *Rhododendron* pollen. *American Journal of Botany* **82**: 1407-1411.
- 18
- 19 **Kittelson PM, Maron JL. 2000.** Outcrossing rate and inbreeding depression in the perennial
20 yellow bush lupine, *Lupinus arboreus* (Fabaceae). *American Journal of Botany* **87**:
21 652-660.
- 22
- 23 **Klinkhamer PGL, de Jong TJ, de Bruyn GJ. 1989.** Plant size and pollinator visitation in
24 *Cynoglossum officinale*. *Oikos* **54**: 201-204.
- 25

- 1 **Klinkhamer PGL, de Jong TJ. 1990.** Effects of plant size, plant density and sex differential
2 nectar reward on pollinator visitation in the protandrous *Echium vulgare*
3 (Boraginaceae). *Oikos* **57**: 399-405.
4
- 5 **Klinkhamer PGL, de Jong TJ. 1993.** Attractiveness to pollinators - a plants dilemma. *Oikos*
6 **66**: 180-184.
7
- 8 **Lande R. 1995.** Mutation and conservation. *Conservation Biology* **9**: 782-791.
9
- 10 **Larson BMH, Barrett SCH. 1999.** The ecology of pollen limitation in buzz-pollinated
11 *Rhexia virginica* (Melastomataceae). *Journal of Ecology* **87**: 371-381.
12
- 13 **Lee PLM, Patel RM, Conlan RS, Wainwright SJ, Hipkin CR. 2004.** Comparison of
14 genetic diversities in native and alien populations of hoary mustard (*Hirschfeldia*
15 *incana* (L.) Lagreze-Fossat). *International Journal of Plant Sciences* **165**: 833-843.
16
- 17 **Lloyd DG, Schoen DJ. 1992.** Self- and cross-fertilization in plants. I. Functional dimensions.
18 *International Journal of Plant Sciences* **153**: 358-369.
19
- 20 **Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA. 2000.** Biotic
21 invasions: causes, epidemiology, global consequences and control. *Ecological*
22 *Applications* **10**: 689-710.
23

- 1 **Malottki Von K, Weichmann HW. 1996.** Acute life-threatening bradycardia due to
2 poisoning with Turkish wild honey. *Deutsche Medizinische Wochenschrift* **121**, 936-
3 938.
- 4
- 5 **Mejías JA, Arroyo J, Ojeda F. 2002.** Reproductive ecology of *Rhododendron ponticum*
6 (Ericaceae) in relict Mediterranean populations. *Botanical Journal of the Linnean*
7 *Society* **140**: 297-311.
- 8
- 9 **Milne RI, Abbott RJ. 2000.** Origin and evolution of invasive naturalized material of
10 *Rhododendron ponticum* L. in the British Isles. *Molecular Ecology* **9**: 541-556.
- 11
- 12 **Mooney HA, Cleland EE. 2001.** The evolutionary impact of invasive species. *Proceedings of*
13 *the National Academy of Sciences of the United States of America* **98**: 5446-5451.
- 14
- 15 **Ng SC, Corlett RT. 2000.** Comparative reproductive biology of six species of *Rhododendron*
16 (Ericaceae) in Hong Kong, South China. *Canadian Journal of Botany* **78**: 221-229.
- 17
- 18 **Parker IM. 1997.** Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive
19 exotic shrub. *Ecology* **78**: 1457-1470.
- 20
- 21 **Poulin J, Weller SG, Sakai AK. 2005.** Genetic diversity does not affect the invasiveness of
22 fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii. *Diversity*
23 *and Distributions* **11**: 241-247.
- 24

- 1 **Rambuda TD, Johnson SD. 2004.** Breeding systems of invasive alien plants in South Africa:
2 does Baker's rule apply? *Diversity and Distributions* **10**: 409-416.
3
- 4 **Redbotorstensson P, Berg H. 1995.** Seasonal cleistogamy - a conditional strategy to provide
5 reproductive assurance. *Acta Botanica Neerlandica* **44**: 247-256.
6
- 7 **Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmanek M. 2000.** Plant
8 invasions - the role of mutualisms. *Biological Reviews of the Cambridge*
9 *Philosophical Society* **75**: 65-93.
10
- 11 **Rotherham ID. 2001.** Rhododendron gone wild: conservation implications of *Rhododendron*
12 *ponticum* in Britain. *Biologist* **48**: 7-11.
13
- 14 **Silva NF, Goring DR. 2001.** Mechanisms of self-incompatibility in flowering plants.
15 *Cellular and Molecular Life Sciences* **58**: 1988-2007.
16
- 17 **Sokal RR, Rohlf FJ. 1995.** *Biometry - the principles and practice of statistics in biological*
18 *research (Third Edition)*. New York: W. H. Freeman and Co.
19
- 20 **Spencer HG, Kennedy M, Gray RD. 1996.** Perceptual constraints on optimal foraging - the
21 effects of variation among foragers. *Evolutionary Ecology* **10**: 331-339.
22
- 23 **Stary P, Tkalcu B. 1998.** Bumble-bees (Hym., Bombidae) associated with the expansive
24 touch-me-not *Impatiens glandulifera* in wetland biocorridors. *Anzeiger fur*
25 *Schadlingskunde Pflanzenschutz Umweltschutz* **71**: 85-87.

- 1
- 2 **Stebbins GL. 1965.** Colonizing species of the native California flora. In: Baker HG, Stebbins
3 GL, eds. *The genetics of colonizing species*. New York: Academic Press, 173-195.
4
- 5 **Stout JC. 2000.** Does size matter? Bumblebee behaviour and the pollination of *Cytisus*
6 *scoparius* L. (Fabaceae). *Apidologie* **31**: 129-139.
7
- 8 Stout JC. 2007. Pollination of invasive *Rhododendron ponticum* (Ericaceae) in Ireland.
9 *Apidologie* **38**: 198–206.
10
- 11 **Stout JC, Parnell JAN, Arroyo J, Crowe TP. 2006.** Pollination ecology and seed
12 production of *Rhododendron ponticum* in native and exotic habitats. *Biodiversity and*
13 *Conservation* **15**: 755-777.
14
- 15 **Utelli AB, Roy BA. 2000.** Pollinator abundance and behavior on *Aconitum lycoctonum*
16 (Ranunculaceae): an analysis of the quantity and quality components of pollination.
17 *Oikos* **89**: 461-470.
18
- 19 **Wallace LE. 2003.** The cost of inbreeding in *Platanthera leucophaea* (Orchidaceae).
20 *American Journal of Botany* **90**: 235-242.
21
- 22 **Williamson M. 1994.** Community response to transgenic plant release: predictions from
23 British experience of invasive plants and feral crops. *Molecular Ecology* **3**: 75-79.
24

1 **Williamson MH, Brown KC. 1986.** The analysis and modelling of British invasions.
2 *Philosophical Transactions of the Royal Society of London Series B Biology* **314**: 505-
3 522.

4
5 **Williamson MH, Fitter A. 1996.** The characters of successful invaders. *Biological*
6 *Conservation* **78**: 163-170.

7
8 **Zimmerman M, Pyke GH. 1988.** Reproduction in *Polemonium* - assessing the factors
9 limiting seed set. *American Naturalist* **131**: 723-738.

10

11 **Acknowledgements**

12 This work was funded by an Enterprise Ireland Post-doctoral Fellowship awarded to JCS
13 (PD/2001/050). I am grateful to Dr Tasman Crowe for statistical advice and Dr Juan Arroyo
14 for helpful comments on the manuscript.

Figure Legends

Figure 1: Relationship between fruit length and number of seeds per fruit (N = 111).

Equation of fitted line: $y = 3.33x^2 - 40.36x + 132.49$, $R^2 = 0.831$.

Figure 2: (a) proportion of fruit set and (b) fruit length (mean \pm SE) of bagged (shaded bars) and open (unshaded bars) flowers hand-pollinated with self (same flower), geitonogamous (same plant) and xenogamous (different plant) pollen.

Figure 3: (a) proportion of fruit set and (b) fruit length (mean \pm SE) of non-emasculated (shaded bars) and emasculated (unshaded bars) treatments. No significant differences were found between emasculated and non-emasculated treatments.

Figure 4: mean proportion of seeds germinated from inbred (1, 2, 3, 5, solid lines) and outcrossed (4, 6, broken lines) treatments over 50 days from the start of the germination experiment. Numbers at ends of lines refer to treatments. Significant differences among treatments were only detected at day 31.

Table 1: Treatments used in this experiment: treatment numbers 1-7 were bagged with bridal veil material to prevent insect visitation.

Number	Treatment	Pollen added from:
1	Spontaneous autogamy	No pollen added
2	Facilitated autogamy	Same flower
3	Geitonogamy	Different inflorescence, same plant
4	Xenogamy	Different plant
5	Emasculation + Geitonogamy	Different inflorescence, same plant
6	Emasculation + Xenogamy	Different plant
7	Emasculation control/Apomixis	No pollen added
8	Open control	No pollen added
9	Open + Facilitation	Same flower
10	Open + Geitonogamy	Different inflorescence, same plant
11	Open + Xenogamy	Different plant
12	Emasculation + Open	No pollen added











