Breeding system and pollination ecology of a potentially invasive alien *Clematis vitalba* L. in Ireland

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Abstract

Aims

Invasive alien plants can greatly affect native communities and ecosystem processes but only a small fraction of alien plant species become invasive. Barriers to establishment and invasion include reproductive limitations. *Clematis vitalba* L. has been a popular horticultural species for the past century and is widely distributed and can be highly invasive. In Ireland, it is considered naturalized and potentially invasive. Despite this, little is known about its reproductive biology.

Methods

We carried out manipulative field experiments in Ireland and compared fruit and seed set from a number of pollination treatments, namely cross-pollination, geitonogamy, autogamy and natural pollination. We also recorded floral visitation to *C. vitalba* through a series of timed observations.

Important Findings

We found that *C. vitalba* is capable of uniparental reproduction via geitonogamy and autonomous selfing, albeit at a reduced rate compared with outcrossing treatments. *Clematis vitalba* was visited by at least 10 native pollinator taxa, with hoverflies dominating visitation. Neither fruit set nor seed set in our study population was pollen limited. Given the lack of reproductive constraint, *C. vitalba* may easily spread in suitable habitats. This is of concern in Ireland, given its prevalence in some of the country's most floristically diverse regions.

Keywords: *Clematis vitalba*, plant invasion, invasive traits, mutualism

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INTRODUCTION

Alien (introduced, exotic, non-native) plants can invade natural and managed ecosystems, influencing economies, agricultural production and ecological structure and functioning (Vitousek *et al.* 1997). Alien plants may compete with native and/or crop plants, exacerbate the spread of pathogens, alter habitat or ecosystem function, pollute gene pools and facilitate invasion by other alien species (Grosholz and Tilman 2005; Parker *et al.* 1999; Simberloff and von Holle 1999). Given the range of potential impacts, it is important to understand both the mechanisms that allow alien plant species to successfully establish populations and the dynamics of those populations, both of which are influenced by the quantity and quality of propagule supply (Ward *et al.* 2012). In the absence of seed production, propagules are supplied via vegetative or clonal means, which, despite providing little possibility for local adaptation, can be very successful in some cases (e.g. for Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decraene) (Hollingsworth and Bailey 2000). For plants that reproduce sexually, seed production depends on a number of factors, including the ability of the plant to produce seeds in the absence of pollinators (through autonomous self-fertilization or abiotic pollen transfer) or the ability to attract resident pollinators and have sufficient pollen transferred to maximize seed set, thus preventing pollen limitation (Goodell *et al.* 2010; Harmon-Threatt *et al.* 2009).

Here, we explore two prominent hypotheses on reproductive success in invasive alien plants. Firstly, that a successful alien plant species tends to have a generalized pollination system, as this effectively increases the probability that the plant will find a suitable pollinator outside its native range,

compared with a more specialist pollinator system (Richardson *et al.* 2000; Rodger *et al.* 2010). Although there are few studies of flower visitors in both the native and the invasive range of plants (but see Montero-Castaño *et al.* 2014; Stout *et al.* 2006), several studies have shown that alien plants are visited by a range of native generalist insects in their invasive range (e.g. Chittka and Shurkens 2001; Lopezaraiza-Mikel *et al.* 2007; Nienhuis and Stout 2009; Vila *et al.* 2009). However, the degree to which pollinator generalization is important in determining invasion success varies according to the degree of self-compatibility exhibited by a species (Chrobock *et al.* 2013; Ollerton *et al.* 2012).

Secondly, Baker's Law (1955, 1967, 1974) (also referred to as Baker's Rule, Stebbins 1957) proposes that plant species that are capable of uniparental reproduction are more likely to become successful colonists than species that rely on outcrossing, because they can establish a population following a single dispersal event. There has been strong support for Baker's Law across a range of contexts (e.g. Bernerdello *et al.* 2001; Busch 2005; Herlihy and Eckert 2005; Randle *et al.* 2009, van Kluenen and Johnson 2007). For alien plants, uniparental reproduction will reduce constraints associated with mate shortages that are typically experienced in small populations (Ward *et al.* 2012). Furthermore, uniparental reproduction would be beneficial in the spread of populations along the leading edge of an invasion front (Rambuda and Johnson 2004). Studies of invasive plants have shown self-compatibility in species as predicted (Hao *et al.* 2011; Rambuda and Johnson 2004; Stout 2007; Ward *et al.* 2012). However, other studies have reported self-incompatible breeding systems in highly successful invasive alien plant species, indicating that uniparental reproduction is not a prerequisite of successful invasion (Jesse *et al.* 2006; Liu *et al.* 2006).

Although uniparental reproduction may help alien species overcome initial barriers, there will often be the intrinsic genetic issue of founder effects within a newly established population (Kliber and Eckert 2005; Mullarkey *et al.* 2013). These, typically small, populations experience genetic bottlenecks, genetic drift and inbreeding, all of which may significantly impact invasion trajectory (Novak and Mack 2005; Sakai *et al.* 2001). The reduction in genetic variation may not only reduce evolutionary potential but also cause reductions in reproductive fitness (Ellstrand and Elam 1993). Thus, understanding potential deleterious genetic effects could influence how best to address management of invasive species. We make a simple assessment of this aspect of invasion dynamics by assessing inbreeding depression through seed output.

Here, we investigate the pollination ecology of *Clematis vitalba* L. (Ranunculaceae), a species native to Britain, Central and Southern Europe, and the Caucasus (Hill *et al.* 2001). It is a woody, perennial climber and has been introduced as an ornamental species in gardens worldwide, from Australia to America. It produces cream-coloured, fragrant flowers (15–20 mm diameter) with four-petal-like sepals and prominent, widely spread stamens. Flowers are

produced in inflorescences of ~12–20 flowers, between July and September. Its seeds are dispersed by wind, thus it is capable of escaping gardens and establishing populations in the wild. It has become naturalized in many countries and even extremely invasive in some. In New Zealand it is one of the most well-known invasive plants, responsible for causing structural changes to forest canopies and facilitating the invasion of other species (Hill *et al.* 2001; Ogle *et al.* 2000). In Ireland, *C. vitalba* is relatively widespread and is particularly prevalent in the southeast of the country. It is currently classified in Ireland as a top-ranking 'amber list' species: a well-established and potentially invasive species of some concern (Invasive Species Ireland 2016). The species was first recorded in Ireland in 1866 (Reynolds 2002) and may be in a lag phase prior to being recognized as invasive (Crooks and Soulé 1997) or invasion may be prevented by intrinsic and/or extrinsic barriers (Milbau and Stout 2008; Milbau *et al.* 2009), including breeding system and pollination ecology. In this study, we investigate a number of aspects of the reproductive ecology of *C. vitalba* in Ireland. Specifically, we test whether *C. vitalba* is (i) capable of uniparental reproduction, (ii) being visited by native pollinators in Ireland, (iii) suffering from inbreeding depression and (iv) pollen limited. To do this, we investigated the breeding system, visitation rates to flowers, seed output and pollen limitation in populations in the west of Ireland. Understanding its reproductive biology will allow a better understanding of the potential threat of invasion posed by this species.

METHODS

Study site

The study was conducted near Caherlough in the Burren region of Co. Clare in the West of Ireland (52°57′7″N; 8°59′53″W, 31 m elevation) from July to September 2012. The Burren is well known for its carboniferous limestone landscape, featuring a wide range of habitat types including limestone pavements, woodlands and Turloughs. It is a region of great floristic interest and exceptional phytogeographical diversity (Ivimey-Cook and Proctor 1964). The population selected for study contained >1500 individuals among hedgerow, hazel scrub and limestone pavement.

Breeding system

Controlled hand pollinations were carried out at the beginning of the flowering period (late July to early August 2012). Thirty-one plants were selected prior to flowering, while flowers were still in the bud stage. On each plant, a group of 4 inflorescences was selected at random, of which 3 were bagged with fine meshing (bridal veil material, which excludes flower visitors while maintaining a relatively natural microclimate around the inflorescence), with each bag containing 12 flowers, with the exception of 3 that contained 9 flowers. Inflorescences were assigned to one of the four treatments: (i) cross-pollination: hand pollination using pollen removed from a donor plant, which was located a

minimum distance of 10 m from the recipient plant; (2) self-pollination: hand pollination using pollen from a different flower on the same plant (geitonogamy); (3) exclusion: bagged for the entire duration of the experiment preventing pollinators from visiting the inflorescence, allowing for an investigation into the potential for autonomous self-fertilization (autogamy); and (4) open pollination: whereby natural pollination was allowed to take place (no bag).

The bagged inflorescences were examined daily, and each open flower on an inflorescence was pollinated according to the assigned treatment on the first day of anthesis and each day thereafter until the point where the stamens began falling from the flower. This method was employed as there was no previous information regarding the stigma receptivity of *C. vitalba*, and thus multiple pollinations on multiple days were required to ensure pollination success and maximize pollen transfer. The hand pollination technique involved removing a whole flower, whose anthers had recently dehisced, from a donor plant (or same plant depending on treatment), and gently brushing the pollen from the donor flowers anthers onto the stigmatic surface of the receptive flower, being careful not to disturb recipient flower stamens. This technique was repeated on all flowers on each inflorescence, after which the inflorescence was securely bagged in order to prevent subsequent pollinator visits and to prevent insect damage to developing fruits.

Each of the 31 experimental individuals was revisited 3 to 4 weeks after hand pollination, when all experimental flowers had either developed into an infructescence, or abscised. The infructescences were collected, and for each infructescence, the proportion of flowers that had developed fruits was calculated, and the number of seeds per individual seed cluster was counted.

Pollinator observations

In order to determine which insects were visiting flowers and whether visitation rate was related to seed set, a series of observations were carried out. Twelve randomly chosen locations throughout the population were selected, at which pollinator observations were undertaken. At each of these locations, a total of seven observations were carried out, each of which was 15 min in duration, totalling 21 h of observations. Each location contained multiple flowers, and the total number of flowers under observation during each recording session was noted for subsequent calculation of visitation rate per flower per observation period. Locations were observed at different times of the day on each day over a period of 3 weeks, with no more than one observation per location per day in order to reduce temporal bias. Observations were not carried out during rainy conditions. For flower visitors, the species, the total number of flowers visited and the duration of visit were recorded for each visitor. A visit was only counted if the visitor was clearly observed to come into contact with the flower's sexual organs. After the observed plants set seed, 10 inflorescences were collected at random from each of the 12 observation locations. From these samples, the mean number of seeds per fruit was determined.

DATA ANALYSIS

Breeding system

Mean percentage fruit set (percentage of flowers that produced fruits; henceforth referred to as simply fruit set) per inflorescence and mean seed set per individual seed cluster were determined for all treatments on all 31 individual plants. Differences in fruit set data (likelihood of successful fruiting) were analysed using binary logistic regression, whereas for seed set, we used one-way analysis of variance with a randomized block design, where individual plants were treated as blocks *C. vitalba* was considered completely self-compatible if there was no significant difference between the fruit and seed set of selfed and outcrossed treatments. Also *C. vitalba* was considered to be fully capable of autonomous self-fertilization if there was no significant difference in fruit and seed set between the pollinator exclusion treatment and the selfed treatment.

Inbreeding depression, autonomous selfing and pollen limitation

Inbreeding depression indices were calculated using the equation: $\delta = 1 - (w_s/w_c)$, where w_s = fitness of progeny from selfing and w_c = fitness of progeny from outcrossing. Inbreeding depression indices were calculated for both fruit and seed set. A cumulative inbreeding depression index was calculated as the fitness ratio for fruit set (f) and seed set (z), given as $\delta = 1 - (w_{sf}/w_{cf})(w_{sz}/w_{cz})$. The level of inbreeding depression will commonly fall between 0 and 1, where 0 is indicative of no inbreeding depression, and on occasion, may even be negative. Positive values for this index suggest that outcrossed progenies are more advantageous than selfed ones, whereas negative values indicate that the performance of selfed individuals exceeds that of outcrossed individuals (outbreeding depression) (Mustajarvi *et al.* 2005), which may either be a real effect or due to experimental error (Young and Young 1992).

Clematis vitalba was considered to be pollen limited if the fruit/seed set of the open pollination treatment was significantly lower than that of the outcrossed treatment. Pollen limitation indices were calculated using the equation: $L = 1 - (P_o/P_c)$, where P_o is the reproductive output for open-pollinated treatments and P_c is the reproductive output for crossed treatments (Larson and Barrett, 2000). High values indicate strong pollen limitation, whereas low values suggest weak pollen limitation. Negative values may occur, reflecting either natural variance in non-pollen limited species or deleterious effects of the hand pollination experimental approach (Young and Young, 1992). Indices were determined for each plant, from which a mean value was calculated.

Autonomous selfing indices for fruit and seed set were calculated as follows: (mean autonomous fruit or seed set)/(mean fruit or seed set of selfed hand pollination treatment).

Visitation rates

We constructed a linear model of mean seed output per location as the dependent variable, with mean visitation rate per flower per observation period as the independent variable.

The primary flower visitors to *C. vitalba* were determined by calculating overall contribution to floral visitation for that species. It is important to note that these species may not reflect the most important species for pollination, as this must be established using pollinator exclusion and emasculation experiments; however, it provides some indication as to the potential suite of pollinators of *C. vitalba*.

RESULTS

Breeding system

Fruit set was highest in flowers that were naturally pollinated, i.e. open pollination treatment, however this was not significantly different from those that were pollinated by artificial outcrossing ($Z_{120,3}$ = 1.625, P = 0.104) (Fig. 1), suggesting no pollen limitation in terms of fruit set. Flowers that had been pollinated with pollen from a different flower on the same individual (selfed treatment) had significantly lower fruit set than open-pollinated flowers ($Z_{120,3}$ = 4.246, P ≤ 0.001). Fruit set was lowest when floral visitors were prevented from accessing flowers and no supplemental hand pollination was carried out (exclusion treatment), this was significantly lower than selfed fruit set ($Z_{120,3}$ = 3.209, P = 0.001).

Similar trends were found for seed set (Fig. 2). Seed set varied significantly on both the level of individual and treatment (Table 1). Seed set was highest in outcrossed and naturally pollinated flowers, and these again did not differ significantly, indicating that seed set per fruit in *C. vitalba* is not pollen limited. Selfed flowers and flowers from which floral visitors were excluded had lower seed set, although not significantly lower than the open pollination treatment. Autonomous selfing indices were calculated as 0.79 and 0.95 for fruit set and seed set, respectively.
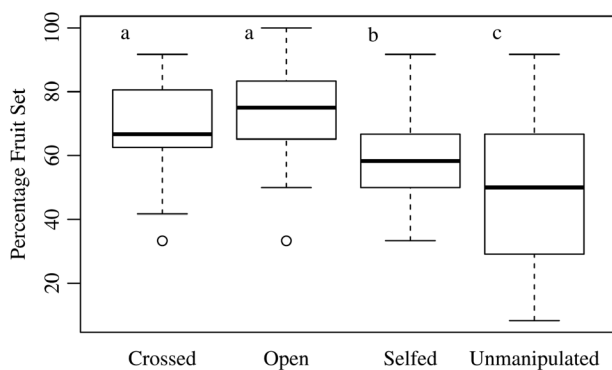
The levels of inbreeding depression in *C. vitalba*, as calculated by inbreeding depression indices, were 0.141 and 0.140 for fruit set and seed set, respectively. The level of cumulative inbreeding depression was calculated at 0.27. Given there was no significant difference between crossed and open pollination treatments with regard to fruit and seed set, it is unsurprising that pollen limitation indices were low at −0.14 and 0.03 for fruit and seed set, respectively.

Floral visitation

Ten taxa of insects were identified as visitors to *C. vitalba* (Table 2). Hoverflies (Syrphidae) dominated in terms of visitation rates, with the most frequent visitors *Metasurphus latifasciafus* and *Helophilous pendulous*. Due to the difficulty of capturing and identifying other, non-Syrphidae Diptera, they were pooled into one group. Together, these Diptera species were also very frequent visitors to *C. vitalba* flowers. Three *Bombus taxa* were recorded visiting the flowers, but far less frequently than the hoverflies. Mean visitation rate at each of the 12 observation locations was not associated with mean seed set (R^2 = 0.303, $F_{1,10}$ = 4.35, P = 0.064).
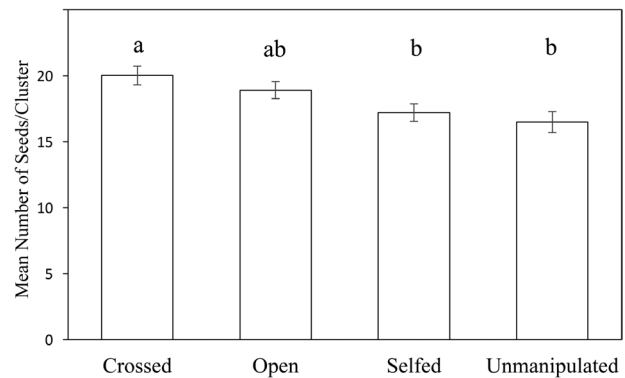

Figure 2: mean seed set/seed cluster (±S.E.) for *Clematis vitalba* following four different pollination treatments (crossed: pollinated using pollen from a conspecific plant; open: natural pollination process; selfed: pollinated using pollen from the same plant; exclusion: floral visitors excluded from flowers). Different letters above each treatment type highlight significant differences as indicated by Tukey's *post hoc* analysis of one-way analysis of variance.

Figure 1: boxplot (with the median shown as a solid line, 25th and 75th percentiles in the lower and upper boxes, respectively, and data range represented by the whiskers) of percentage fruit set for *Clematis vitalba* following four different pollination treatments (crossed: pollinated using pollen from a conspecific plant; open: natural pollination process; selfed: pollinated using pollen from the same plant; exclusion: floral visitors excluded from flowers). Different letters above each treatment type highlight significant differences.

Table 1: summary results of one-way analysis of variance examining differences in seed per seed cluster among four treatment types where individual plants are taken as the blocking factor

Variable	Factor	df	*F* value	*P*
Seed per seed cluster	Treatment	3119	5.256	0.002
	Plant	1119	4.973	0.028

Table 2: insects identified visiting flowers of *Clematis vitalba* and their contribution to the total floral visitation

Floral visitors	Contribution to total visitation (% of total flower visits)
Metasurphus latifasciafus	22.5
Helophilous pendulous	20.5
Other *Diptera* sp (largely *Drosophilidae*)	19.4
Syritta pipiens	9.6
Bombus pratorum	6.1
Platycheirus albimanus	6.5
Platycheirus Platycheirus scambus	5.0
Bombus hortorum	4.6
Bombus terrestris/lucorum agg.[a]	3.4
Helophilous trivittatus	2.4

[a]Workers of *Bombus terrestris*, *Bombus lucorum*, *Bombus cryptarum* and *Bombus magnus* are indistinguishable morphologically (Carolan *et al.* 2012) and so grouped.

DISCUSSION

Breeding system

We found that *C. vitalba* is capable of uniparental reproduction, via both geitonogamy and autonomous selfing, albeit at a reduced rate compared with natural pollination and artificial outcrossing. Within the genus *Clematis*, this ability to self-fertilize is not uncommon. A study of the breeding systems of three *Clematis* species *C. chrysocoma*, *C. akebioides* and *C. rehderiana* in their native range of southwestern China revealed similar findings for the two latter species, although neither currently pose invasive threats (Jiang *et al.* 2010). This uniparental reproduction provides *C. vitalba* with reproductive assurance, a driving force behind the evolution of self-compatibility (Darwin 1876; Busch 2005). Self-compatibility is common among invasive plant species, as it alleviates some of the reproductive constraints associated with establishing a new population, such as mate shortages and pollinator availability (Ward *et al.* 2012; Ollerton *et al.* 2012). Some species, even though they are self-compatible, are not capable of autonomous self-fertilization, either due to the morphological structure of flowers separating male and female structures (herkogamy) or due to temporal separation of male and female function (dichogamy). In southeast Queensland Australia, self-compatible invasive milkweeds, *Asclepias curassavica*, *Gomphocarpus fruticosus* and *Gomphocarpus physocarpus*, did not demonstrate a capacity for autonomous self-fertilization, as pollinators are still needed to trip the pollination mechanism (Ward *et al.* 2012). In *C. vitalba*, autonomous self-pollination may occur if inner stamens dehisce directly onto receptive stigmas or as pollen is transferred between neighbouring flowers on an inflorescence as it is buffeted by wind.

Inbreeding depression

Although *C. vitalba* was capable of uniparental reproduction, this reproductive assurance appears to come at a cost in terms of fruit and seed set. Open pollination and outcrossed hand pollination resulted in significantly greater fruit and seed set respectively compared with geitonogamy, with even greater reductions in fitness for autonomous self-fertilization. These results are indicative of the occurrence of inbreeding depression, i.e. a reduced fitness in inbred progeny in comparison with outbred progeny (Wright *et al.* 2007).

Inbreeding depression indices for *C. vitalba* were 0.141 and 0.140 for fruit and seed set, respectively. Similar levels have been recorded for other *Clematis* species, with *C. akebioides* and *C. rehderiana* found to have a lower level of inbreeding depression in fruit set but higher in seed set (Jiang *et al.* 2010). Inbreeding depression has also been associated with a number of other invasive plant species, such as *Allaria petilota*, *Brachypodium sylvaticum* and *Spartina alterniflora* (Daehler 1999; Marchini *et al.* 2016; Mullarkey *et al.* 2013), however, inbreeding depression is generally quite common in plant species (Charlesworth and Charlesworth 1987; Daehler 1999). Despite the prevalence of inbreeding depression in the plant kingdom, it is often not factored into models of the evolution and spread of invasive plants (Blossey and Notzold 1995; Daehler 1999). Levels of inbreeding depression are likely to be greatest when population density is low or where there is a lack of pollinators to transfer pollen between individuals. As such, its impact on a newly establishing population along the leading edge of an alien plant's range may be considerable, given these populations are often founded by very few individuals and have a diminished pollinator assemblage (Daehler 1999). If inbreeding leads to strong inbreeding depression, then the dynamics of the populations may be greatly affected, and population growth and spread will occur at a reduced rate, as is thought to be the case for *B. sylvaticum* (Marchini *et al.* 2016).

Floral visitation and pollen limitation

Nine species were identified visiting *C. vitalba*, however a number of other species, primarily *Drosophilidae* sp., were observed visiting the flowers but escaped before species identification could be made. Of the obligate floral visitors, there were three bumblebee (*Bombus*) and six hoverfly (Syrphidae) species. Although we cannot say for certain that the species recorded visiting the flowers of *C. vitalba* were in fact its pollinators as we did not carry out pollinator effectiveness experiments, we can be relatively confident as only visitors that clearly came into contact with the sexual organs of the flower were recorded. Most hoverflies are typically generalist pollinators, with broader feeding habits than many other pollinator taxa, having been observed feeding on nectar and pollen of a wide range of plant species (Biesmeijer *et al.* 2006; Moron 2009). Also, bumblebees in the *Bombus terrestris/lucorum agg.* are notoriously generalist foragers, with more than 300 species recorded as the host plant of *B. terrestris* in France and Belgium (Walther-Hellwig and Frankl 2000). Given the composition, diversity and frequency of floral visitors, *C. vitalba* could be considered

to have a generalist pollination system. Most alien species tend to have a more than adequate pollination service from native, generalist pollinators, especially if the invasive plant originates from the same continent, as is the case for *C. vitalba* which is native to the neighbouring island of Britain (Valentine 1978). Indeed, *C. vitalba*, alongside other nectarless congeneric species such as *C. chrysocoma*, *C. recta* and *C. ligustcifolia*, with white, dish-like flowers are already highly associated with generalist pollinators as an established pollination syndrome (Jiang *et al.* 2010).

Such generalized pollination syndromes have been recorded in other invasive plant species including a prominent invader in Ireland, *Rhododendron ponticum* (Stout *et al.* 2006). Often invasive species with entomophilous flowers, such as *C. vitalba* and *R. ponticum*, become well integrated into plant–pollinator networks in their invaded range, visited by a diverse pollinator assemblage, with more interactions accumulating over time (Pysek *et al.* 2011; Vila *et al.* 2009).

Our results show that this study population is not pollen limited. In fact, average fruit set following supplemental hand pollination was lower than openly pollinated flowers (perhaps due to stigma clogging, outbreeding depression and/or damage to floral structures during experimental manipulation; Young and Young 1992), but not significantly so. Thus, it appears that *C. vitalba* is currently adequately serviced by a native pollinator assemblage. This is likely a result of the close geographical proximity of Ireland to its native range, and the fact Ireland contains a subset of the pollinators found there. This lack of pollen limitation is also a feature of other invasive species. No pollen limitation was detected for *R. ponticum* in some of its invaded Irish range (Stout 2007). However, it is not possible to definitively say that either *C. vitalba* or *R. ponticum* are not pollen limited, as both of these studies were carried out over just one flowering season and in a small number of populations, thus pollen limitation may indeed occur in populations other than those studied, where fewer pollinators are present (Stout 2007).

CONCLUSION

C.vitalba is not reproductively limited in its novel range in Ireland. It is capable of producing seed autonomously in the absence of pollinators, although this does come at a cost of reproductive fitness. In the presence of pollinators, this seed output is greater. When we consider that *C. vitalba* spreads via seed production, it would appear that *C. vitalba* may continue to spread to wherever suitable conditions prevail. Given it has shown preference for a floristically important area of Ireland, among others, close monitoring of its population size and distribution, particularly the appearance of new populations, and appropriate management (ensuring removal of root material), is recommended in an attempt to avoid potentially costly invasions of *C. vitalba*, as seen elsewhere in the world (Global Invasive Species Database, 2016).

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