

Seed germination tests of the parasitic perennial *Viscum album* (Viscaceae) from fragmented habitats at the northern edge of its range

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Background and aims – Despite being a rather common species throughout Europe, little is known about the effects of habitat change on the fitness of *Viscum album*. We expected to find a reduction in fitness in *V. album* populations growing in fragmented habitats resulting from a loss of genetic diversity through increased inbreeding.

Methods – We studied seed germination as a measure of fitness among sixteen Belgian *V. album* populations varying in size and degree of isolation to investigate the fitness consequences of habitat fragmentation. Populations were sampled from two landscapes differing in their degree of habitat fragmentation and *V. album* population characteristics. We also compared germination percentages of three populations at three different temperatures (5, 20, 30°C) to examine the potential effects of climate change on *V. album* regeneration in northern Europe.

Key results – Germination percentages (at 20°C) were high (69–100%) and we found no evidence of relationships between germination and population size, density, or area. There was no direct relationship between germination percentage and population isolation within study regions, but connectivity among populations appears to be important. Samples from the more fragmented habitat showed a negative correlation between germination percentage and the proportion of females, suggesting reduced mate availability and pollination resulting from increased isolation of populations. There was no significant difference in mean germination percentages among the three temperature treatments, but the high temperature samples (30°C) exhibited the highest variation in germination success.

Conclusion – Our results suggest that *V. album* has evolved high germination success to compensate for limited success in establishing on a host plant. Successful germination under a wide range of environmental conditions is expected to increase the likelihood of establishing on host plants, possibly helping *V. album* respond to changes in climate.

Keywords – mistletoe, habitat fragmentation, inbreeding, germination percentage.

INTRODUCTION

The effects of habitat degradation and disturbance on parasitic plant populations are poorly understood. Parasitic plants are often involved in complex community-level interactions with host plants, pollinators and seeds dispersers, and therefore are considered keystone species in a variety of ecosystems (Watson 2001, Phoenix & Press 2005, Press & Phoenix 2005, Mathiesen et al. 2008). Despite increasing recognition of the role of parasitic plants in community ecology, little is known about how they will respond to local or regional changes to habitat or climate.

The European mistletoe, *Viscum album* L. (Viscaceae), is a dioecious, evergreen, shoot hemiparasite. It is widely dis-

tributed throughout temperate Europe, with Belgium marking the northern edge of its range (Zuber 2004). Barney et al. (1998) noted 452 host tree taxa for *V. album* in Europe, including 190 introduced species. The vast majority of hosts for *V. album* subsp. *album* in Belgium are *Populus nigra*, *P. × canadensis* and *Malus domestica* varieties in abandoned orchards. The area of poplar woodlands and apple orchards in Belgium has decreased over the last forty years through shifts in agricultural practices and the logging of poplar trees for meadow restoration projects. In some areas, a decrease in active management of poplar woodlands has resulted in a more stable habitat for *V. album*, allowing parasite populations to persist and sometimes expand.

Populations of *V. album* in Belgium tend to be rather

small because of the high degree of forest fragmentation and their location at the northern range margin for the species. Parasitic plants are useful model species for investigating the effects of habitat change. Their dependence on host plants that may themselves be vulnerable to decreased fitness related to habitat loss may result in increased susceptibility of parasites to the negative consequences of habitat fragmentation (Davies & Graves 1998, Salonen et al. 2000, Bickford et al. 2005). We expect recent changes to *V. album* habitat in Belgium to have pronounced negative influences on population genetics and dynamics, and reproductive success.

Ecological genetic theory suggests that small and isolated plant populations will suffer from genetic erosion and differentiation through increased genetic drift and elevated inbreeding (Ellstrand & Elam 1993, Young et al. 1996). These effects may be reinforced through a lack of seed and pollen flow between isolated populations in a matrix of hostile urban and agricultural land use. Increased inbreeding is expected to have a deleterious effect on short term population fitness (Keller & Waller 2002). Previous studies have demonstrated positive correlations between population size and plant fitness (Menges 1991, Vergeer et al. 2003, Reed & Frankham 2003), yet the relationships between germination success and population size and genetic diversity remain unclear (Ouborg & Van Treuren 1995, Luijten et al. 2000, Vergeer et al. 2003, Hensen & Wensche 2006). Such information is especially lacking for parasitic or epiphytic species. The majority of research investigating the effects of population size on reproductive success focuses on short-lived, herbaceous, nonparasitic plant species. There is currently a lack of information concerning population dynamics and fitness of parasitic plants in fragmented populations.

A loss of genetic diversity may affect a species' ability to respond to changing selection pressures over the long term (Young et al. 1996). If fragmented *V. album* populations in Belgium are experiencing genetic erosion and reduced reproductive fitness, they may also be losing the ability to adjust to climate variation. Previous research noted that *V. album* can germinate at a wide range of temperatures, from 8 to 30°C with optimal results between 15 and 20°C (Wangerin 1937, Lamont 1983). It is unclear whether seeds from populations at the northern range edge would respond to a similar range of temperatures and have the ability to adjust to changing climatic conditions. *Viscum album* is an ideal species for experimental investigation of the role of temperature in seed germination because, unlike many parasitic plant species, it does not require chemical signals from the host plant for germination (Lamont 1983; Musselman & Press 1995). Birds are the primary dispersers of *V. album* seeds, but passage through the gut is not necessary for successful germination or establishment.

This study investigates the interactions between habitat fragmentation and germination success of *V. album* populations. We conducted germination tests with seeds collected from *V. album* populations ranging in size and degree of isolation and from two landscapes differing in the degree of habitat fragmentation, allowing us to investigate relationships between population variables (size, density, proportion of females) and seed germination success. We also experimentally monitored seed germination at temperatures rang-

ing between 5 and 30°C to assess the long-term ability of *V. album* to respond to changing environmental conditions.

METHODS

Study area

We sampled *V. album* populations from two 800 ha regions in central Belgium (fig. 1) representing different degrees of forest fragmentation and connectivity. Both regions are characterized by agricultural landscapes interspersed with forest patches and urban development. The Voeren region in east-central Belgium includes relatively large areas of forest with poplar stands of varying age and size (a 179 ha forested area composed of 165 fragments) supporting large *V. album* populations (ranging from 124 to 1539 individuals, table 2). The Leuven region in central Belgium is more strongly fragmented (156 ha forested area of 124 fragments) with a higher density of roads and urban development, therefore the forests support smaller and fewer *V. album* populations (ranging from 93 to 386 individuals, table 2). Stanton et al. (2009) found that the Voeren study region had more forested area and fragments than Leuven, but the average fragment area was similar between the two regions (table 1). Populations in both regions had a significant female bias, and inbreeding levels were significantly higher at Leuven (Stanton et al. 2009).

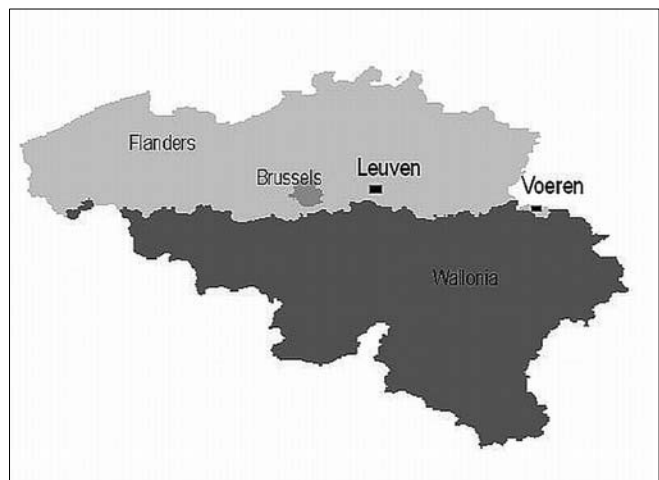


Figure 1 – Map of Belgium showing the location of the two study regions, Leuven and Voeren. See Stanton et al. (2009) for more detailed maps of study populations.

Sample collection

We randomly selected eight populations growing in poplar stands in each region. We counted the number of males, females, non-flowering juveniles, and host trees to provide an estimate of *V. album* population size and composition, and plant density. We obtained geographic coordinates of all sampled populations and their nearest neighbour populations using a GPS, and then used digital maps to calculate isolation as the linear distance to the nearest neighbouring *V. album* population. We also calculated pair wise geographic distances among all the sampled populations within each region

Table 1 – Comparison of characteristics of sixteen *Viscum album* populations from two regions in Belgium.

Descriptive statistics and Mann-Whitney U-test results (from Stanton et al. 2009); ¹average distance (m) to nearest neighbouring *V. album* population, regardless of sampling status; ²average number of *V. album* individuals per host tree.

	Mean (std. error)		Median		U-value	P-value
	Leuven	Voeren	Leuven	Voeren		
Population size	220 (43)	624 (154)	187	530	8.0	0.01
Population area (ha)	0.51 (0.17)	0.47 (0.12)	0.38	0.47	31.5	ns
Isolation¹	219.4 (56.3)	96.25 (15.2)	187.5	85	6.5	0.005
% female	57 (3)	61 (3)	59	63	22.0	ns
# juveniles	18 (3.7)	84 (20.4)	16	79	8.0	0.01
Pop. density²	10 (2)	17 (3)	7.76	15.94	13.0	0.05
Fragment area (ha)	0.004 (0.007)	0.005 (0.009)	0.001	0.002	8899	ns

and estimated the area of total forest (fragment area) and the area of forest that includes the sampled *V. album* populations (population area).

Germination tests

We collected ~100–200 fruits (i.e. 100–200 seeds) from 6–7 large, healthy *V. album* individuals within each sample population and stored the fruits in a well-lit and ventilated room at 10°C. Within one week of collection, we extracted the seeds from the fruit and pulp layer (to reduce fungal colonization and encourage germination) and placed them on in petri plates lined with filter paper. Seeds were moistened with a dilute solution (0.0001%) of merthiolate (fungicide), then

incubated in a growth chamber with constant light at 20°C and watered as needed. Germination was considered a success when the radicle emerged to a length of 2.0 mm. *Viscum album* seeds can contain more than one embryo, therefore we also tallied the number of radicles emerging from each seed. To investigate differential responses to temperature, we also tested 270 seeds from each of three populations (V7, V8, L6) as described above, whereby one third of each sample was incubated at 5°C, 20°C, and 30°C.

Data analysis

We performed analyses on the entire data set (sixteen populations), as well as among populations from each region (Leuven and Voeren) separately. We used Mann-Whitney U-tests to identify differences in population variables

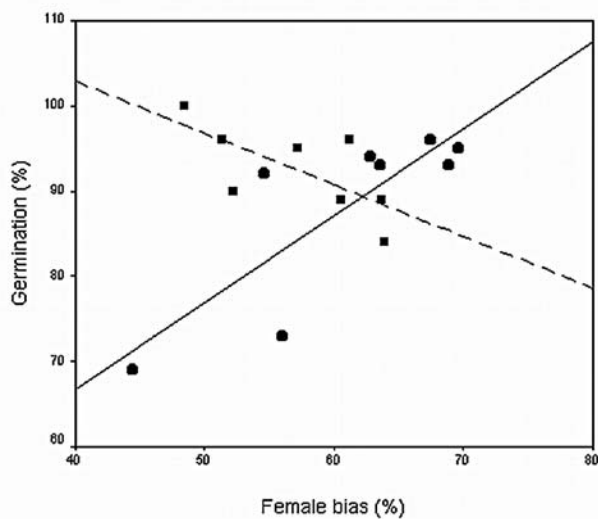


Figure 2 – Relationship between *Viscum album* germination percentages and proportion of females in each population. Circles represent Voeren populations; squares are Leuven populations; dashed line is a regression-based fit through the Leuven data; the solid line is a fit through Voeren populations.

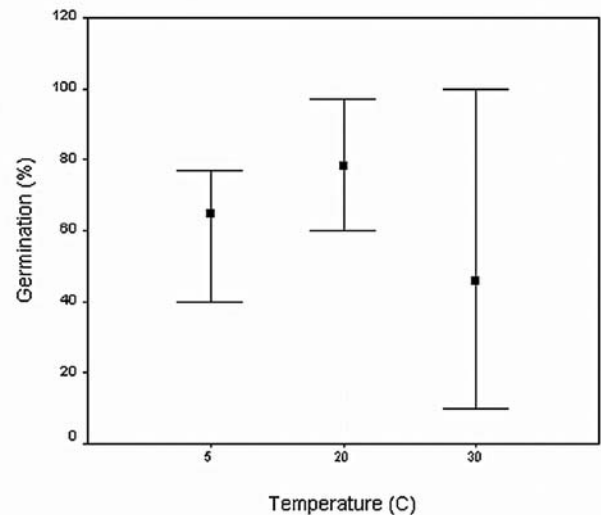


Figure 3 – Germination percentages (mean and range of three populations combined) for *Viscum album* seeds at three different temperature treatments. N = 270 for each treatment.

(population size, area and isolation, number of males, females, juveniles, density, fragment area) between the two sampling regions. Spearman rank correlations were used to identify relationships between population variables, inbreeding, and germination percentage, and Mann-Whitney U-tests to compare germination results between the two regions. We used a Kruskal-Wallis test to compare germination success among the three temperature treatments.

RESULTS

Population germination percentages at 20°C ranged from 69–100%, with 50% of germinated seeds having more than one embryo (table 2). We found no significant correlations ($p > 0.05$) between germination percentages and any of the population variables (population size, density, isolation, area, # juveniles, % female) for the pooled data set (Spearman correlation coefficient r_s ranging from -0.213 to 0.169). Among the population variables, we found significant correlations between % female and population size ($r_s = 0.50$, $P = 0.041$) and # juveniles with # males ($r_s = 0.79$, $P = 0.001$, as well as # juveniles with # females ($r_s = 0.68$, $P = 0.003$). After dividing the data set according to the two sampling regions we found a significant relationship between the proportion of female plants and germination percentage, which was negative for Leuven ($r_s = -0.77$, $P = 0.025$) and positive for Voeren ($r_s = 0.79$, $P = 0.020$) (fig. 2).

Germination percentages under the three temperature treatments ranged from 46 to 65%. There was no significant

difference ($p > 0.05$) in mean germination percentages or average number of radicles among the three temperature treatments, but the high temperature samples (30°C) exhibited the highest variation in germination success (fig. 3).

DISCUSSION

This is the first study of which we are aware that investigates the relationship between germination and population characteristics of *Viscum album*. We expected to find decreased germination success in small, isolated populations growing in fragmented habitats at the edge of its range. Stanton et al. (2009) found evidence of increased genetic differentiation and inbreeding among *V. album* populations growing in severely fragmented habitats in Belgium. Increased inbreeding and small population size typically have a negative effect on population fitness (Keller & Waller 2002, Reed & Frankham 2003), but we found no evidence of decreased germination success in small, isolated *V. album* populations.

We sampled sixteen *V. album* populations to identify the effects of population variables (size, isolation, density) on germination success. Surprisingly, we found no significant relationships between germination percentage or number of embryos and population size, area, isolation, or density. Other researchers have found positive correlations between population size and reproductive success of nonparasitic species as measured by seed set and mass, but no relationship with germination success (Luijten et al. 2000, Hensen & Wensche 2006). We did not measure seed set because of the

Table 2 – Population characteristics and germination results for each study population of *Viscum album* from two study regions in Belgium (L = Leuven and V = Voeren).

‘Pop’ is population name; ‘Pop size’ is the total number of *V. album* individuals in the population; ‘Density’ is the average number of *V. album* individuals host tree; ‘% germ’ is percentage of germination among all seeds sampled from each population; ‘Avg. # embryos’ is the mean number of embryos from germinated seeds; and % > 1 embryo is the percentage of germinated seeds that included more than one embryo.

*Data from Stanton et al. (2009).

Pop	Pop size*	Density*	% germ (n)	Avg. # embryos	% > 1 embryo
L1	372	8.86	96 (186)	1.5	50
L2	314	20.93	84 (183)	1.5	45
L3	105	15.00	100 (209)	1.5	52
L4	193	6.66	89 (203)	1.5	44
L5	386	11.03	95 (105)	1.5	43
L6	182	6.07	90 (180)	1.7	43
L7	93	5.81	96 (120)	1.5	43
L8	113	5.14	89 (90)	1.8	75
V1	523	9.87	93 (216)	1.5	49
V2	292	18.25	73 (139)	1.4	40
V3	932	21.67	93 (131)	1.7	61
V4	124	24.80	94 (90)	1.7	66
V5	545	13.63	96 (150)	1.7	67
V6	1539	29.60	95 (212)	1.6	52
V7	538	7.17	92 (108)	1.8	69
V8	497	9.75	69 (111)	1.8	68
L mean (s.e.)	220 (43)	9.94 (1.96)	92 (1.8)	1.5 (0.04)	49 (3.8)
V mean (s.e.)	624 (154)	16.84 (2.84)	88 (3.8)	1.6 (0.05)	59 (3.8)

difficulty in accessing *V. album* plants growing in host trees. Seed mass is also difficult to assess for *V. album* because of the fleshy, viscous layer surrounding each seed, but we did record the number of embryos per seed and found no significant relationships with population variables.

Despite sampling a number of relatively small and isolated *V. album* populations, germination success was remarkably high among the majority of populations we sampled (70% of populations germinated at percentages above 90%). Previous research noted high germination percentages among parasitic plant species (Wicker 1974, Lamont 1983, Livingston & Blanchette 1986, Norton et al. 2002), while others found a wide range (Dawson & Ehleringer 1991, Brandt et al. 2005). *Viscum album* seeds are large and photosynthetic, increasing the likelihood of germination as a means to compensate for the low probability of being in a suitable position to effectively establish on a host plant. This indicates that establishment on a host plant is the limiting factor for regeneration of these parasitic plants, rather than seed set or germination success (Zuber 2004). Establishment of haustoria in living host tissue would be a better measure of fitness for future research with *V. album*, but such tests are more difficult to control and may require at least four months before the parasite leaves emerge. Our results highlight the need for further research on the ecology of parasitic plants and the effects of habitat change on their population genetics and fitness. Traditional approaches to understanding germination ecology and population dynamics are not appropriate for aerial parasites such as *V. album*.

It can be expected that small plant populations may suffer from a sex bias through demographic stochasticities (Lande 1988), which may in turn negatively affect reproductive success through reductions in mate availability and sexual recombination (Hilfiker et al. 2004). Female bias is commonly observed in *V. album* populations (Wiens et al. 1996) and we expected to find that populations with more females would exhibit lower germination percentages. All but two populations we sampled exhibited a proportion of females greater than 50%, and a female bias did influence germination success, but this relationship was not consistent between study regions. Populations in Voeren with a higher proportion of females exhibited higher germination percentages, probably because populations are larger, denser, and less isolated when compared to Leuven. In contrast, germination percentages were lower in populations from Leuven with a stronger female bias. Leuven populations were significantly smaller and more isolated, reducing the likelihood of successful cross-pollination between individuals or populations. The occurrence of a sex bias in small populations of dioecious plant species often reduces mate availability and sexual recombination, resulting in lower genetic diversity and fitness (Hilfiker et al. 2004). Populations of *V. album* in Leuven were more likely to suffer from low mate availability because they were relatively small and occurred at low densities. We also found that the positive relationship between the number of juveniles and adults was stronger for adult males compared to females, suggesting the availability of male mates plays an important role in reproductive success for *V. album*. The inconsistent results from the two study regions may be related to the fact that female bias in *V. album* results from gametic selection,

where female gametes are favoured at fertilization (Wiens & Barlow 1979, Wiens et al. 1996). It is unknown why this type of selection occurs in *V. album* or how it may be influenced by environmental or population factors.

In addition to habitat and population variables, we tested the effects of temperature variation on seed germination. While previous studies have identified germination temperature ranges for *V. album*, the aim of our tests was to identify ideal germination conditions for seeds collected at the northern edge of the *V. album* range in order to assess the ability of this species to respond to potential shifts in climate. On average, the samples from the temperature tests experienced lower germination percentages than those used to compare all populations and our results were inconclusive. Lower germination success was likely the result of lower light levels available in the incubators used for the temperature experiments, as sufficient light is critical for *V. album* germination (von Tubeuf 1923). Lamont (1983) reported that *V. album* germination can start at 8–10°C, with ideal temperatures from 15 to 20°C (95% germination). The maximum temperature reported for *V. album* germination was 30°C (Wangerin 1937). We found equal success at 5°C, 20°C, and 30°C. Samples incubated at 30°C lost moisture more quickly than the other treatments, but were prone to fungal infection if moisture levels were maintained at levels consistent with the other treatments. The Intergovernmental Panel on Climate Change predicted a temperature rise of 2–5°C and an increase in precipitation up to 21% in northern Europe (IPCC 2007). Our results may indicate that warmer climates could lead to decreased germination success for *V. album* in Belgium. On the other hand, the wide range of germination temperatures and long maturation time of seeds may allow this species to persist through variations in climate regime, especially if an increase in precipitation accompanies any increase in temperature. The effects of climate change on the behaviour of bird dispersers and pollinators could create additional challenges for *V. album*, making it difficult to assess the long-term success of this species at the edge of its range.

Our results support previous reports that obligate hemiparasites evolved high germination success to compensate for the low probability that seeds will successfully establish connections with host plants. Therefore it is possible that we found no relationship between population size and germination of *V. album* in Belgium as a consequence of high germination success. It is also possible that the differences between the populations we sampled were not extreme enough to result in differences in germination success. More data are needed to compare very small, isolated populations with large, continuous ones.

We found that the number of females in a population influences germination percentages, with the degree of habitat fragmentation and connectivity appearing to interact with the proportion of female plants. Smaller, more fragmented populations with a strong female bias may experience low mate availability, leading to reduced pollination and high levels of inbreeding, resulting in the negative correlation we found between the proportion of females and germination success. While other factors besides habitat fragmentation (e.g. differences in climate or pollinator guilds) may be responsible for some of the differences we see between the two study

regions, our results indicate that further habitat loss and climate change may reduce the reproductive success of *V. album* in northern Europe. Our results indicate that germination can occur under a wide variety of conditions, which will help *V. album* adjust to changes in habitat, but it may be limited by its dependence on host plants, insect pollinators, and bird dispersers.

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Manuscript received for *Belgian Journal of Botany* 15 Dec. 2008; accepted in revised version 12 Feb. 2010.

Communicating Editor: Elmar Robbrecht.