

Significance of time of attachment, host type, and neighbouring hemiparasites in determining fitness in two endangered grassland hemiparasites

Brita M. Svensson* & Bengt Å. Carlsson

Department of Plant Ecology, Evolutionary Biology Centre, Uppsala University, Villavägen 14, SE-752 36 Uppsala, Sweden (*e-mail: brita.svensson@ebc.uu.se)

Received 27 Mar. 2003, revised version received 4 Aug. 2003, accepted 6 Aug. 2003

Svensson, B. M. & Carlsson, B. Å. 2004: Significance of time of attachment, host type, and neighbouring hemiparasites in determining fitness in two endangered grassland hemiparasites. — *Ann. Bot. Fennici* 41: 63–75.

The early-flowering, annual hemiparasite *Euphrasia stricta* var. *suecica* is a small and delicate plant, sensitive to competition for light from neighbouring plants in its grassland habitat. Today this variety of *E. stricta* is highly endangered due to the abandonment of traditional management and increased air-borne nitrogen deposition. As hemiparasites reduce vegetation height, we hypothesized a positive effect of the immediate presence of another endangered and much larger hemiparasite, *Rhinanthus serotinus* ssp. *vernalis*, on *Euphrasia* growth and fitness. In an outdoor pot experiment with the two hemiparasites grown in combination with different hosts no such effect could be detected. However, we found that early attachment to a host greatly enhanced fitness measured as net reproductive rate and seed viability, which was true for both hemiparasites. Host type also mattered: in pots with the leguminous *Trifolium pratense*, growth was augmented for both hemiparasites. When both leguminous and graminoid hosts were present, *Rhinanthus* had a higher net reproductive rate.

Key words: cohort, *Euphrasia stricta* var. *suecica*, meadow, net reproductive rate, *Rhinanthus serotinus* ssp. *vernalis*, seed viability, semi-natural grassland

Introduction

A suggested reason for the high species diversity found in semi-natural grasslands in Europe (Kull & Zobel 1991, Zobel 1992) is related to competition. Small and slow-growing plants are often inferior competitors for light, but in these grasslands where strongly competitive species are suppressed by grazing or mowing, the otherwise inferior competitors persist (Zobel 1993), thus increasing diversity and species richness (Wahl-

man & Milberg 2002). For example, Kull and Zobel (1991) reported that the highest species richness in an Estonian wooded meadow was found in areas with the highest light penetration.

One intriguing group of plants that often are found in semi-natural European grasslands are the hemiparasites within the Scrophulariaceae (Fritsch & Salisbury 1946). Such plants could, due to their ability to feed on a host plant, be expected to be less prone to competitive exclusion as they are less dependent on their own pho-

tosynthetic ability (Smith 2000). However, survivorship of the annual hemiparasite *Rhinanthus minor* in Canadian hayfields was still found to be negatively correlated with surrounding biomass (van Hulst *et al.* 1987). Also, increased sward density negatively affected seedling survival of *Rhinanthus angustifolius* in a Dutch study (de Hullu 1985). In fact, Karlsson (1982) reports that one of the largest threats to the endangered *Euphrasia rostkoviana* in Sweden is that it is becoming increasingly over-grown due to decreased management intensity in semi-natural grasslands.

In Sweden, remnants of semi-natural grasslands are particularly common on the Baltic island of Gotland, often as traditionally managed wooded hay meadows. In these, the meadow is raked and cleared from fallen branches and leaf litter in spring, and in late July mowing takes place (Ekstam *et al.* 1988). Tree branches are cut in autumn for leaf fodder, and livestock graze the aftermath crop. All these activities can be expected to result in a constant outflow of nutrients from the meadow. Competitive species are kept at bay, and small and slender species are common (Ekstam *et al.* 1988). Furthermore, patches where the annual hemiparasite *Rhinanthus serotinus* var. *vernalis* (*Rhinanthus* hereafter) is abundant often have a lower-statured vegetation (pers. obs.), suggesting that the feeding behaviour of this species negatively affects its hosts, as has been shown for this and several other related hemiparasitic plant species (Matthies 1995a, 1995b, 1998, Joshi *et al.* 2000, Mutikainen *et al.* 2000). Gibson and Watkinson (1991) suggested that since *Rhinanthus minor* drew more resources from *Trifolium repens* than from *Lolium perenne*, and reduced growth more in *T. repens* than in *L. perenne*, the presence of the hemiparasite could mediate competition and significantly influence community structure. Such an effect was indeed found for the parasitic *Cuscuta salina* which preferably parasitized *Salicornia virginica* thereby opening up gaps for *Arthrocnemum subterminale* (Callaway & Penning 1998).

We hypothesize, therefore, that when *Rhinanthus* is present in the community it may, through its strong parasitic influence, check the growth of otherwise highly competitive species result-

ing in a lower average vegetation height. This would enable small-statured species to coexist in the community. One such small plant species is another annual hemiparasite, *Euphrasia stricta* var. *suecica* (*Euphrasia* hereafter). Both *Rhinanthus* and *Euphrasia* were once common in wooded hay meadows on Gotland. Since the 1970s, *Euphrasia*, especially, has decreased dramatically (Karlsson 1984, Petersson 1999) and is now found only at a few sites.

Some time-consuming elements in the traditional management of wooded hay meadows have gradually become neglected over the last few decades. This is especially true for spring raking. This neglect impairs seedling establishment leading to delays in host attachment. We hypothesize that such late-attaching cohorts of the hemiparasites have lower net reproductive rates and lower fitness as compared with early-attaching cohorts, in accordance with results found for another annual hemiparasite, *Euphrasia frigida*, in subarctic Sweden (Svensson *et al.* 2001). We hypothesize, further, that the presence of early-attaching cohorts of *Rhinanthus* influences the performance of *Euphrasia* in a positive manner as the early cohorts of *Rhinanthus* probably will have a more negative impact on the tall-growing hosts as compared with later cohorts.

Many studies on the fitness of plant populations measure seed production, but most studies neglect the important next stage of a species' life-cycle, i.e. the ability of the seeds to produce viable seedlings (but see van Hulst *et al.* 1987, Oostermeijer *et al.* 1996). Many hemiparasites within the Scrophulariaceae either lack or have very short-lived seed banks — this is true for both *Rhinanthus* (ter Borg 1972) and *Euphrasia* (Karlsson 1982) — and seedling emergence in these annuals is especially important as they completely rely on only one season's seed production and survival. We therefore include next year's seedling emergence in our analyses.

The influence of host identity on the growth of hemiparasitic species has been demonstrated frequently (e.g., Yeo 1964, Atsatt & Strong 1970, ter Borg & Bastiaans 1973, Snogerup 1982, de Hullu 1984, Seel *et al.* 1993, Matthies 1996, 1997, 1998, Svensson *et al.* 2001). The most effective hosts are generally found among the nitrogen-fixing Fabaceae. In the field, several

hosts are often parasitized upon simultaneously (Musselman & Mann 1977, Gibson & Watkinson 1989), and many hemiparasites have a broad host range (Gibson & Watkinson 1989, Nilsson & Svensson 1997). Such a broad host range may suggest that different types and quantities of compounds are obtained from the different hosts (Govier *et al.* 1967). Improved hemiparasite fitness as a result of the mixed diet served by several hosts was, therefore, suggested by Govier *et al.* (1967) and was shown by Marvier (1998) and Joshi *et al.* (2000), but *see* Matthies (1996). We explored this further in this study.

We experimentally studied the influence of *Rhinanthus* on the growth, fitness and seed viability of *Euphrasia*. To do this we grew grass and clover plants as hosts in different combinations to create simple vegetation communities. We then planted *Rhinanthus* and *Euphrasia* seedlings into these communities with the aim that the hemiparasites (especially *Rhinanthus*) would influence the growth of the hosts. We posed the following specific hypotheses:

1. Early-attaching cohorts of *Euphrasia* and *Rhinanthus* perform better than later cohorts and reduce host growth more.
2. Due to its smaller size, *Euphrasia* reduces host growth less than *Rhinanthus*. Therefore, individuals of *Euphrasia* perform better when growing with *Rhinanthus* nearby. Specifically, *Euphrasia* plants growing in pots with early cohorts of *Rhinanthus* will perform better than those growing with later cohorts of *Rhinanthus*.
3. A host combination of clover and grasses is more favourable for hemiparasitic growth than clover alone and, especially, grasses alone.

Materials and methods

Species

Euphrasia stricta var. *suecica* (Scrophulariaceae) is an early-flowering variety of the much more common *E. stricta* (Karlsson 1984). It is an annual, hemiparasitic herb presently found only in a few wooded hay meadows on the Baltic

island of Gotland, Sweden (Petersson 1999). Karlsson (1984) suggests that the variety has originated on the island, with hay-making as the main selection factor, from the late-flowering *E. stricta* var. *brevipila*. Seedlings emerge in spring, mainly during April and May, but sometimes as late as in June (pers. obs.). The seedlings can remain in this stage for several weeks, before attachment to a host and subsequent growth takes place with the formation of true leaves and a sudden stem elongation. There is no seed bank (Karlsson 1982). Flowering starts in mid-June and continues until mowing in mid- to late July.

Most fruits are mature at this time, but usually both vegetative and flowering individuals are still present (Svensson 2000). The plants vary in height, depending on age and nourishment, from a few centimetres up to 20 cm, and bear from one to ten flowers. Each fruit contains a maximum of 16 seeds. Occasionally, branches are produced, increasing the number of flowers and fruits. The plant is sensitive to reduced irradiance, as growth more or less ceases if it is over-grown, even when attached to a host (Svensson 2000).

Rhinanthus serotinus ssp. *vernalis* (Scrophulariaceae) is also an annual, early-flowering hemiparasite. It is found mainly in wooded hay meadows, but also in other unfertilized seminatural grasslands on Gotland. Seedlings emerge in March–May, sometimes also in autumn the year before (Svensson 2000). As for *Euphrasia*, the seedlings can remain as such for several weeks before attachment and rapid growth take place. The seeds do not form a seed bank (ter Borg 1972). Flowering and seed-set is finished by mid-July, before mowing (Svensson 2000). Individuals of this species are usually ca. 20 cm in height with ca. 10 flowers each, but successful individuals can be up to 40 cm high, branched, and bear 80–100 flowers. Because of its much larger size, *Rhinanthus* can be expected to be less sensitive to light competition from neighbouring vegetation than *Euphrasia*. Each fruit contains a maximum of eight seeds.

Outdoor pot experiment

Seeds of ca. 100 individuals of the two hemiparasites were collected just before mowing

(mid-July) in 1996 in two wooded hay meadows on Gotland, Anga prästänge (57°32'N, 18°43'E, *Euphrasia*) and Bendes strandänge (57°30'N, 18°46'E, *Rhinanthus*), and immediately sown outdoors 45 km south of the two meadows on a bed of sand and garden *Sphagnum* peat (50%: 50% vol.). The peat moss was enriched with: 8 kg garden lime, 180 g N, 80 g P, 250 g K, and 1 g Mg per m³, and traces of Cu, Zn, Fe, B, Mn, and Mo. pH (H₂O) was ca. 6, slightly lower than in the meadows and conductivity around 100 μ S cm⁻¹, the same as in the meadows. Host seeds (commercial seeds of red clover, *Trifolium pratense*, and two grasses, *Poa pratensis* and *Festuca rubra*) were sown in pots (11 × 11 cm, height 15 cm) in early March 1997, in the above mixture of sand and peat moss, and placed in a non-heated greenhouse. Host seeds were sown in a regular pattern, and in three combinations: clover alone (10 seeds); the two grass species alone (20 seeds), and clover + grasses (10 plus 20 seeds). When the seeds had germinated the pots were placed outdoors and the number of plants reduced to create pots with similar density and vegetation cover. All three host species are common in meadows on Gotland. The commercial varieties used here were morphologically and phenologically similar to the wild-growing varieties.

In April 1997, seedlings of *Euphrasia* and *Rhinanthus* were transplanted from the outdoor bed to the pots. The following combinations were used: *Euphrasia* alone, *Euphrasia* together with *Rhinanthus*, and *Rhinanthus* alone (Table 1). Also, for each host type, pots were left without any hemiparasites at all, and, in addition, the hemiparasites were planted without any hosts. With 20 replicates (= pots) for each

combination, the experiment included 300 pots in total (Table 1). Three individuals of *Euphrasia* and eight individuals of *Rhinanthus* were planted to mimic proportions typically found in the field and to ensure a negative impact from *Rhinanthus* on the surrounding hosts. The same number of hemiparasite seedlings was used in the host free pots. The seedlings were planted in the same regular pattern in all treatments. The pots were placed outdoors within a fenced area, monitored daily, watered regularly, and the few seedlings that died during the first 10 days were replaced. After this, and until seeds started to be produced, we weekly kept track of the development of the hemiparasites to be able to classify them into cohorts. Also, host sward height was measured weekly.

After the eighth census (21 July), plants were monitored every other day, and seeds collected as they matured (plants were naturally pollinated). We observed slight but non-significant seed predation on *Rhinanthus*. The seeds were then stored at room temperature. When all seeds on an individual had been collected, plant height was measured and the plant harvested. All plants were dried in 70 °C for 72 h and weighed. Plants that died before the last census (21 July) were treated in the same way. When all hemiparasites in a pot had been collected, total host above-ground biomass was collected, dried and weighed as above. Leaf area measurements (Li-Cor 3100 Area Meter) were carried out for the largest *Trifolium* plant in each pot.

To get a measure of seed viability we constructed a fresh sand/peat bed within the fenced area and divided the bed into 240 plots, each ca. 15 × 10 cm. In each plot the collected parasite seeds from the corresponding pot were sown in

Table 1. Number of pots for each combination of host and hemiparasite. The number of parasites of each species per pot is given within parentheses.

Hemiparasite	Host			
	Clover	Grasses	Clover + Grasses	No host
<i>Euphrasia</i> (3)	20	20	20	20
<i>Rhinanthus</i> (8)	20	20	20	20
<i>Euphrasia</i> (3) + <i>Rhinanthus</i> (8)	20	20	20	20
No hemiparasite	20	20	20	—

August 1997. In the following spring, on 1 May 1998, we counted the number of seedlings. No traces of seed predation were noted.

Statistical analyses

As pots are used as replicates, we calculated a mean pot value for all variables measured. Hemiparasite dry weight was not used in the statistical analyses as this variable showed little variation between treatments since by the time of seed maturation (and our harvest) these species have lost nearly all their leaves and have started to senesce. Instead, plant height at the end of the experiment was used as an important performance variable as this should relate directly to competitive ability for these hemiparasites. Net reproductive rate (R_0) was calculated for each species and pot by multiplying mean number of seeds per plant with survival. This equals the finite rate of population increase (λ) for these species. Data were log-transformed before analysis, when necessary, to improve homoscedasticity (Zar 1999). Proportional data (e.g., seed viability) were arcsine square root-transformed before analysis. Data manipulations and statistical analyses were done with SPSS for Windows 10.0.5.

We assigned each individual of *Euphrasia* and *Rhinanthus* to one of five developmental cohorts indicating when the plants left the seedling stage and started to elongate rapidly and produce true leaves (cf. Klaren & Janssen 1978, Svensson *et al.* 2001). This corresponds to time of attachment to a host, an important stage in the life-cycle of parasitic plants, and found to be of greater significance for later development than emergence time (Svensson *et al.* 2001, Svensson & Carlsson unpubl. data) Cohort one (C1) left the seedling stage after one week, whereas C5 left the seedling stage after five weeks.

The first hypothesis was tested by regressing data for plant height, R_0 , and seed viability against cohort, and by regressing host weight at harvest against hemiparasite cohort. The second hypothesis was tested using analysis of covariance for the relationships between host weight and no. of hemiparasites. *Euphrasia* per-

formance (i.e. height) in relation to the development of *Rhinanthus* was tested using regression analysis. The third hypothesis was tested using analysis of variance for data for plant height, R_0 , and seed viability.

Results

Benefits of early attachment

The importance of an early attachment to a host was evident: *Euphrasia* plants belonging to C1 were three times taller than plants belonging to C5 (Fig. 1a and Table 2a). Similarly, for *Rhinanthus*, plants in the first cohort were almost four times taller than plants belonging to the last cohort (Fig. 1b and Table 2b).

Time of attachment was important for fitness as plants belonging to the earlier cohorts had significantly higher net reproductive rates (R_0 ; Fig. 1c and Table 2c). This was a result of both higher survival and more seed produced by the early cohorts. The higher number of seeds produced by plants in the early cohorts was, in turn, a result of both a higher no. of fruits and a higher no. of seeds per fruit; *Euphrasia* plants in C1 had more mature fruits at harvest, 2.9 ± 1.8 , $n = 39$ (mean $\pm$ S.D.) per plant as compared with 1.2 ± 0.38 , $n = 19$, for C5 ($F_{4,153} = 12.4$, $P < 0.001$). Number of seeds per fruit was 3.1 ± 2.1 , $n = 39$, in plants belonging to C1, and 1.0 ± 1.2 , $n = 22$ in plants belonging to C5 ($F_{4,158} = 9.22$, $P < 0.001$).

Also for *Rhinanthus*, plants belonging to the early cohorts had significantly higher net reproductive rates than later cohorts (Fig. 1d and Table 2d), as predicted. *Rhinanthus* produced more fruits at harvest as compared with *Euphrasia*, particularly plants belonging to the three earliest cohorts (4.9 ± 1.6 , $n = 12$, for C1; and 0.6 ± 0.4 , $n = 21$ for C5, $F_{4,142} = 23.4$, $P < 0.001$). Number of seeds per fruit was highest for the earliest cohort (4.6 ± 1.5 , $n = 12$) and decreased with each cohort class (C5: 0.2 ± 0.4 , $n = 26$, $F_{4,152} = 68.4$, $P < 0.001$).

Seed viability for both *Euphrasia* and *Rhinanthus* was lower for seeds produced by plants belonging to later cohorts (Fig. 1e and f); however, this measure of plant performance was

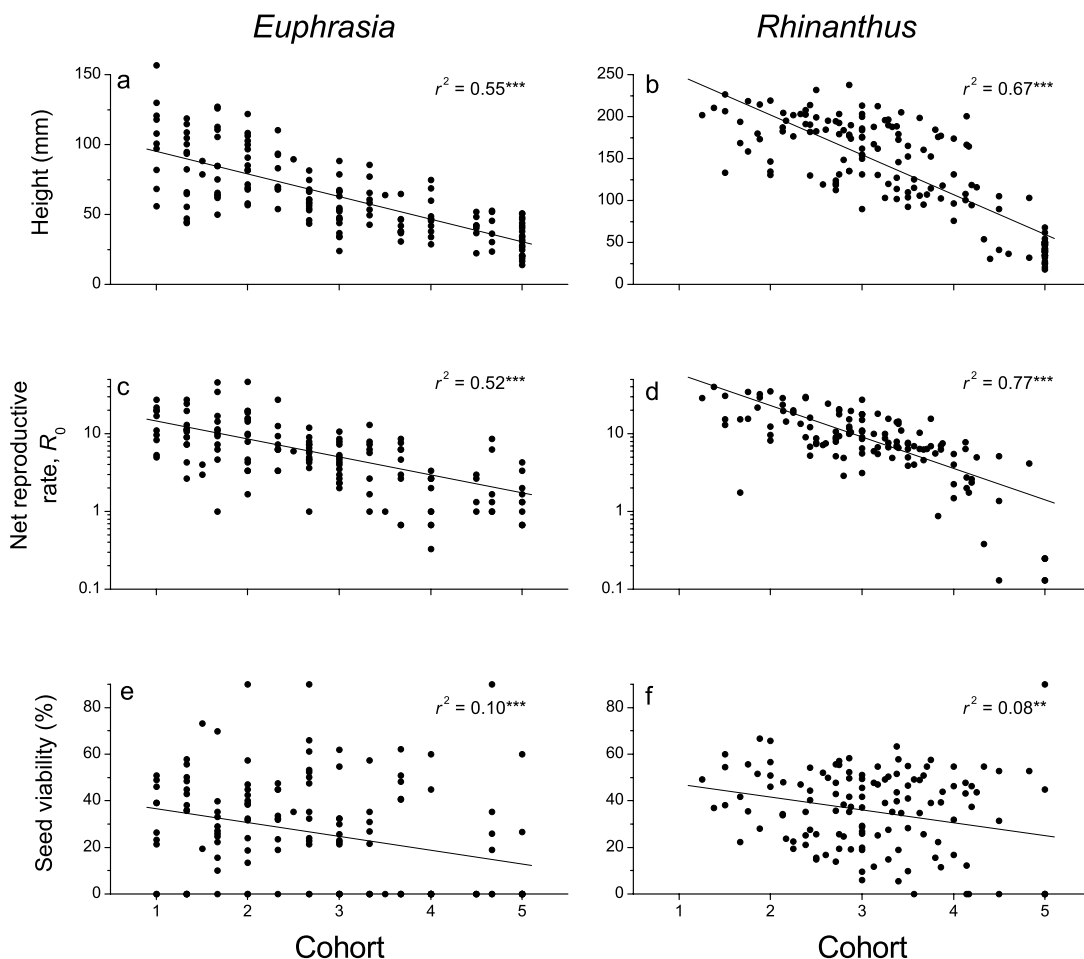


Fig. 1. Height per plant (a, b); net reproductive rate (c, d, note logarithmic scale); and seed viability (e, f, arcsine transformed data), for *Euphrasia* (a, c, e); and *Rhinanthus* (b, d, f) in relation to cohort. Each point represents one pot, pooled over treatments. Regression lines are shown for statistically significant relationships, **: $P < 0.01$, ***: $P < 0.001$. For statistical tests see Table 2.

not as strongly dependent on the developmental cohort as plant height and R_0 . The negative effect of cohort on seed viability was reduced when host type and parasite combination were included in the model (Table 2e and f).

We predicted that the presence of early cohorts of the hemiparasites in the pots should reduce host weight more than later cohorts. This was not evident for *Euphrasia* (Fig. 2a, host $\times$ cohort: $F_{2,114} = 0.23$, $P = 0.79$). When parasitized by early cohorts of *Rhinanthus*, the two host types including clover showed reduced weight (Fig. 2b, host $\times$ cohort: $F_{2,114} = 16.5$, $P < 0.0001$).

Influence of *Rhinanthus* and *Euphrasia* on host growth

The more hemiparasites there were in the pots, the more was host weight at harvest reduced (Fig. 3), particularly when clover was involved (Fig. 3a and b). In pots with clover + grass, *Rhinanthus* and *Rhinanthus* + *Euphrasia* both reduced host weight, and by the same amount (parasite combination $\times$ no. of hemiparasites: $F_{1,49} = 1.13$, $P = 0.29$, Analysis of covariance, Fig. 3a). The same was true for pots with clover; here the three hemiparasite combinations reduced host weight, with similar amounts

($F_{2,74} = 1.76$, $P = 0.18$, Fig. 3b). In pots with grass, host weight was reduced only slightly, by the two hemiparasites together ($F_{2,49} = 1.13$, $P = 0.29$, Fig. 3c). No effect was seen on sward height (measured as the height of the tallest host specimen) or leaf area, ($P > 0.5$, data not shown).

Influence of *Rhinanthus* on *Euphrasia* performance in relation to host growth

The hypothesis that the presence of *Rhinanthus* should benefit *Euphrasia* growth was not supported (Fig. 4a, c and Table 2a). In pots with grasses, *Euphrasia* seed viability was higher

Table 2. Analysis of variance of parasite harvest height (**a, b**); net reproductive rate (R_0) (**c, d**, log transformed) and seed viability (**e, f**, arcsine transformed) for **a, c**, and **e** *Euphrasia stricta* var. *suecica* and **b, d**, and **f** *Rhinanthus serotinus* ssp. *vernalis* growing in four host combinations ("Host", random factor) and with or without *Rhinanthus* or *Euphrasia*, respectively ("Parasite combination", fixed factor) and belonging to five different cohorts ("Cohort", co-variable) in a pot experiment.

Factor	SS	df	MS	F	P
a & b Harvest height					
a Euphrasia					
Host	15736	3	5245	32.4	< 0.0001
Parasite combination	122	1	122	0.877	0.610
Cohort	17409	1	17409	67.1	< 0.0001
Host × Parasite comb.	422	3	141	0.541	0.655
Error	38672	149	260		
b Rhinanthus					
Host	160856	3	50285	374	< 0.0001
Parasite combination	2.08	1	2.08	0.022	0.891
Cohort	11202	1	11202	39.2	< 0.0001
Host × Parasite comb.	282	3	93.9	0.329	0.805
Error	41736	146	286		
c & d R_0					
c Euphrasia					
Host	0.817	3	0.272	3.744	0.101
Parasite combination	0.059	1	0.059	0.825	0.432
Cohort	3.73	1	3.73	47.1	< 0.0001
Host × Parasite comb.	0.214	1	0.214	0.901	0.442
Error	11.9	150	0.079		
d Rhinanthus					
Host	3.45	3	1.15	58.5	< 0.0001
Parasite combination	0.016	1	0.016	1.04	0.384
Cohort	2.40	1	2.40	70.0	< 0.0001
Host × Parasite comb.	0.046	3	0.016	0.454	0.715
Error	5.10	149	0.034		
e & f Seed viability					
e Euphrasia					
Host	13709	3	4570	6.35	0.068
Parasite combination	781	1	781	1.05	0.377
Cohort	778	1	778	2.07	0.153
Host × Parasite comb.	2299	3	766	2.04	0.112
Error	48508	129	376		
f Rhinanthus					
Host	14512	3	4837	12.4	0.026
Parasite combination	1.75	1	1.75	0.005	0.948
Cohort	1219	1	1219	6.03	0.015
Host × Parasite comb.	1232	3	411	2.03	0.113
Error	24664	122	202		

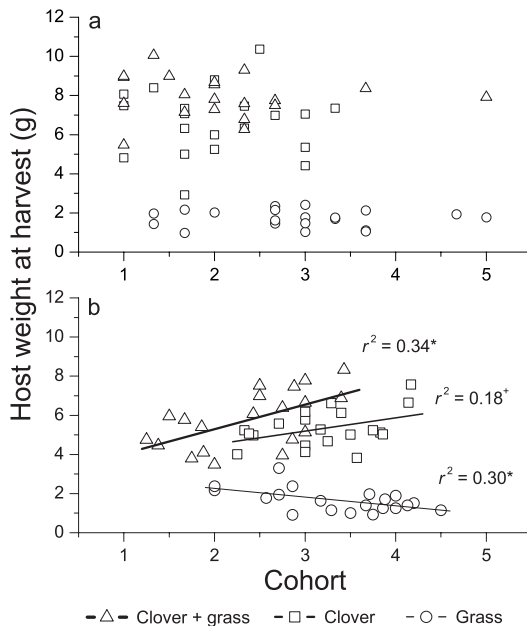


Fig. 2. Host weight at harvest in pots with (a) *Euphrasia*, and (b) *Rhinanthus* belonging to different cohorts, and growing with different host types. Each point represents one pot. Regression lines are shown for significant relationships for each host type, $N = 20$, *: $P < 0.1$, **: $P < 0.05$. The host $\times$ cohort effect is significant for *Rhinanthus*: $F_{2,54} = 8.42$, $P < 0.0001$.

when *Rhinanthus* was present (Fig. 4e and Table 2e); however, the difference was not statistically significant.

When analysing height at harvest of *Euphrasia* in relation to cohort of *Rhinanthus*, a significant negative relationship was found ($P < 0.001$, $N = 76$, Fig. 5), in concordance with our hypothesis that *Euphrasia* would benefit from growing together with early cohorts of *Rhinanthus*. However, after excluding pots without hosts, no relationship between *Euphrasia* harvest height and cohort of *Rhinanthus* remained ($P = 0.60$, $N = 60$, Fig. 5).

Host influence

As expected, clover was the superior host for *Euphrasia* (Fig. 4a and Table 2a). No additional effect on *Euphrasia* of including grasses in the pots could be seen, not supporting the hypothesis that a combination of hosts would be beneficial

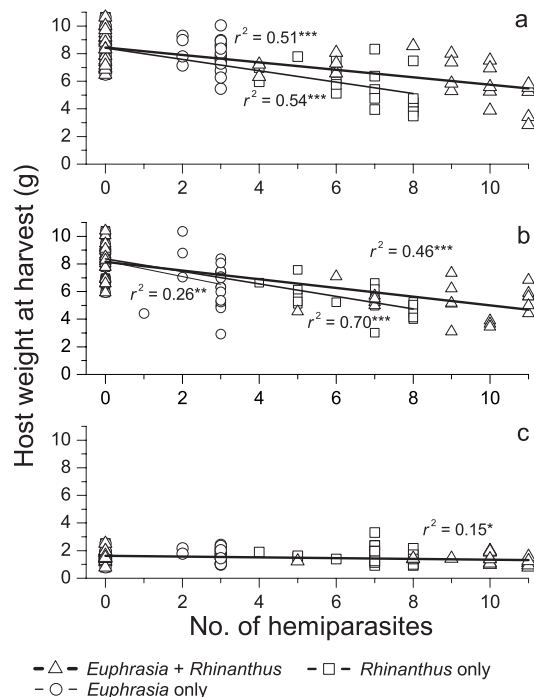


Fig. 3. Host weight at harvest in pots with (a) clover + grass, (b) clover, and (c) grass, in relation to the number of living hemiparasites per pot at harvest. Each point represents one pot, pooled over cohorts. Significant regression lines are shown, $N = 20$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. For statistical tests, see text.

to parasite growth. A strong host effect was obvious also for *Rhinanthus* growth (Fig. 4b and Table 2b). *Rhinanthus* plants were generally taller than the *Euphrasia* plants: in pots with clover, *Rhinanthus* was just below 200 mm, whereas *Euphrasia* plants were around 80 mm high (Fig. 4a and b).

As an effect of both lower survival and lower seed production in pots without hosts, net reproductive rate, R_0 , of *Euphrasia* was much lower than in pots with hosts (Fig. 4c and Table 2c). The host effect on R_0 of *Rhinanthus* was equally clear; here plants growing in pots with clover + grasses performed best (Fig. 4d and Table 2d) supporting our hypothesis. When growing without hosts, R_0 of *Rhinanthus* was very low (Fig. 4d), and seemingly suffers more than *Euphrasia*.

Seed viability was also influenced by the different hosts, and for *Euphrasia*, plants grown with clover + grasses produced the most viable

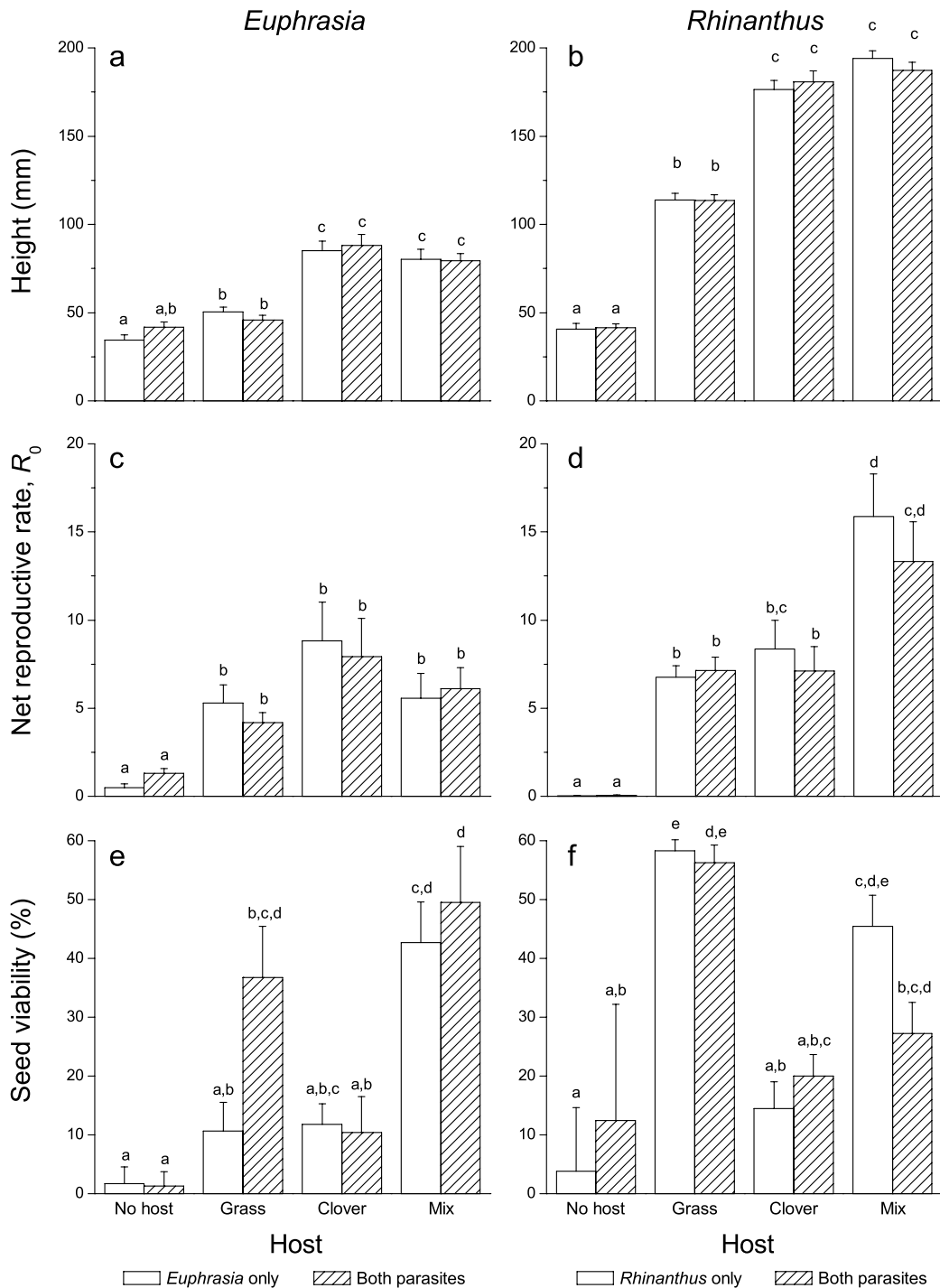


Fig. 4. Height per plant (a, b); net reproductive rate (c, d); and seed viability (e, f), for *Euphrasia* (a, c, e); and *Rhinanthus* (b, d, f) grown in pots with different hosts and with or without *Rhinanthus* and *Euphrasia*, respectively. Values are means of pot means + 1 S.E., pooled over cohorts. Different letters denote differences at $P < 0.05$ (Tukey HSD). For statistical tests see Table 2. $N = 20$, except for: a: no host (both parasites) ($N = 18$); b: no host (*Rhinanthus* only, both parasites) ($N = 16, 19$); c: no host (*Rhinanthus* only, both parasites) ($N = 4, 7$); and e: no host (*Euphrasia* only, both parasites), clover (both parasites), mix (*Euphrasia* only) ($N = 7, 16, 17, 19$).

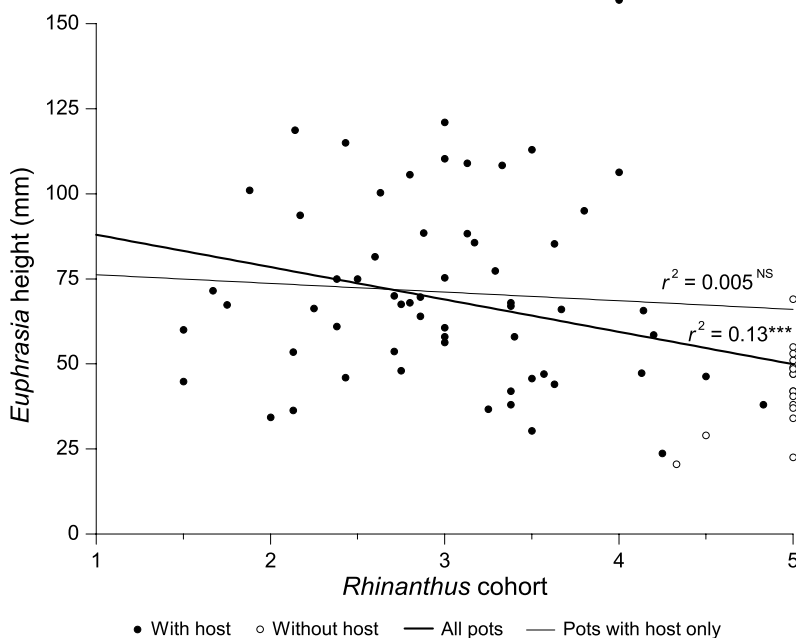


Fig. 5. Harvest height of *Euphrasia* in relation to *Rhinanthus* cohort when growing without hosts (open symbols, $N = 19$), and with three host types (closed symbols, $N = 60$). Values are pot means. The regression lines show the relationship between *Euphrasia* height and *Rhinanthus* cohort using all pots (thick line) and using only pots with hosts (thin line).

seed (Fig. 4e and Table 2e). Viability of *Rhinanthus* seeds originating from plants grown in pots with grasses alone was, surprisingly, higher than from plants grown with the other two host combinations (Fig. 4f and Table 2f).

Discussion

Early attachment to a host was strongly beneficial for parasite performance in the present study, as was detected also for *Euphrasia frigida* by Svensson *et al.* (2001). Not surprisingly, host identity was also important; clover was a particularly good host, boosting parasite growth and fitness. This is most probably an effect of a nitrogenous benefit to the hemiparasites as shown by Govier *et al.* (1967). The hypothesized increased performance of *Euphrasia* in the presence of *Rhinanthus* was, however, not detected. These were the most important findings and we will discuss them in more detail below.

The expected reduction in host growth due to the presence of the hemiparasites was evident as both hemiparasites significantly decreased host weight at harvest. In pots where most individuals of *Rhinanthus* had started to develop quite early, host growth was reduced more than in

pots with later cohort values, indicating that it indeed was the hemiparasite that reduced host growth. The effect was, however, seen only in pots with clover, and clover + grasses. A similar response was found by Matthies (1996); a maximum reduction of a leguminous host species, as compared with a non-leguminous herb or a grass host, when parasitized by *Melampyrum arvense*, and by Gibson and Watkinson (1991) for *Rhinanthus minor*. In fact, in pots with grasses, hosts were larger in pots with early-developed *Rhinanthus* than in pots with later-developed *Rhinanthus*. Maybe, that when the grass is growing well, seedlings of *Rhinanthus* were more successful in finding host roots, and, so, started to develop earlier. Further on, since the *Rhinanthus* plants in these pots were not that large, they did not manage to reduce the growth of the grasses.

Host growth was, not surprisingly, reduced more when there were more *Rhinanthus* present, especially when clover was involved. This supports our field observation of the low stature of the vegetation when there is a high abundance of *Rhinanthus*. This should facilitate growth of other low-growing species and increase species richness, as was found by Pennings and Callaway (1996). Such facilitation by *Rhinanthus* was, however, not found on *Euphrasia* perform-

ance in the present study. This may indicate either that the level of competition was not high enough in pots where *Rhinanthus* was not present, or that *Rhinanthus* instead was shading *Euphrasia* and, therefore, actually had a negative effect on *Euphrasia*. It could also be that we did not study the relevant mechanism involved. A crucial stage for all plant species is that of germination and seedling growth (Grubb 1977), and in the case of *Euphrasia* it may be that this stage is facilitated by the presence of *Rhinanthus*. This will be explored in a forthcoming field study. Mixed results were found also by Gibson and Watkinson (1992), in that the removal of *Rhinanthus minor* resulted in increased diversity at three sites but decreased diversity at the fourth. We also found that *Euphrasia* could reduce host growth, something we did not anticipate. Clearly, the interaction patterns between parasites and other community members are complex (Smith 2000).

Seed production in *Euphrasia* was mainly dependent on cohort membership and plant size. Plants belonging to earlier cohorts had more seed than plants belonging to later cohorts, and the larger the plant the more seed it produced. The same effect, but even stronger, was found for a field population of *Euphrasia frigida* in northern Sweden (Svensson *et al.* 2001). The small advantage of a few days' lead early in the season thus eventually results in a much higher fitness (*see e.g.*, Ross & Harper 1972). For plants that begin growth just a few days later, even a superior host environment cannot remedy their eventual performance.

The importance of suitable hosts for the growth and development of hemiparasitic plants is well known, (*e.g.*, Snogerup 1982, Seel *et al.* 1993, Matthies 1996, 1998). Joshi *et al.* (2000) showed that the growth of the hemiparasitic *Rhinanthus alecterolophus* increased when growing in vegetation composed of three, rather than one or two functional groups. The lack of the hypothesized increase in hemiparasite growth in the present study when both host types were present was presumably due to the overwhelming influence from clover, as indicated in a study by Seel *et al.* (1993).

For species such as *Euphrasia*, with no or short-lived seed banks, successful seed germina-

tion and emergence the following spring is crucial. Furthermore, considering the fragmented nature of its habitat, there is only a minimal chance of seed transfer between *Euphrasia* localities, emphasizing the importance of germination each spring at each locality, as noted already by Karlsson (1982). The two most important factors for seed viability in the present study were time of attachment and host identity. For both hemiparasites, more seeds emerged from early-cohort plants than from late-cohort plants, which, together with the higher net reproductive rate for these plants, emphasizes the importance of an early start in spring. The response pattern for seed emergence, especially for *Rhinanthus*, did not follow the pattern found for net reproductive rate or plant height. Even if we could not detect any seed predation over winter (between seed production and seedling emergence) this, may, of course have happened. If palatability differs in the direction that nitrogen-rich mother plants produce tastier seed, this could lead to increased seed predation of the seeds from clover plants and subsequent lower (apparent) viability. The advantage of a high R_0 will be diminished or even lost under such a scenario. In any case, our results stress the importance of following the fate of the population over the plants' whole life-cycle.

Euphrasia stricta var. *suecica* is highly threatened, both due to ceased management of the wooded hay meadows, and to air-borne nitrogen deposition. It has disappeared completely from mainland Sweden and Denmark, and from Öland and Saaremaa (two other Baltic islands) (Karlsson 1982, Th. Karlsson, pers. comm.) and is now endemic to Gotland. The taxon is included in the Swedish Red List as "endangered" (Gärdenfors 2000). Even the traditionally managed meadows are slowly becoming overgrown, both as a result of increased nitrogen deposition (Aronsson 1999), but especially as the practice of pollarding is greatly reduced (Martinsson 1999), and we need to take measures to halt this negative trend if we do not want to lose the many species connected to this species-rich habitat. Among our results, the importance of an early start in spring indicates that we should perhaps intensify spring raking. This would facilitate seedling growth and development, thus increasing seed production

and population viability. Furthermore, even if many meadow species are well-adapted to yearly mowing (Karlsson 1982, 1984, Zopfi 1998), this adaptation is not perfect: *Euphrasia* often still produces mature fruits later than the usual time for mowing (Svensson 2000). This indicates that we must treat each meadow independently, and check the plants' phenological status before mowing, when trying to preserve this little meadow gem.

Acknowledgements

We are indebted to Thomas Karlsson for knowledgeable advice on how to collect, store and sow hemiparasite seeds, to Henrik Bjersing and Maria Wallin for doing census measurements, to Siv Billberg for leaf area measurements, to Mikael Mildén for typing data into the computer, to Håkan Rydin for useful comments on an earlier version of the manuscript, and to the Foundation for Strategic Environmental Research, World Wildlife Fund, Swedish Environmental Protection Agency, and Uppsala University for financial support.

References

- Aronsson, M. 1999: Bevara våra sista ängar. — *Fakta*: 28. Svenska Naturskyddsföreningen, Sverige.
- Atsatt, P. R. & Strong, D. R. 1970: The population biology of annual grassland hemiparasites. I. The host environment. — *Evolution* 24: 278–291.
- Callaway, R. M. & Pennings, S. C. 1998: Impact of a parasitic plant on the zonation of two salt marsh perennials. — *Oecologia* 114: 100–105.
- de Hullu, E. 1984: The distribution of *Rhinanthus angustifolius* in relation to host plant species. — In: Parker, C., Musselman, L. J., Polhill, R. M. & Wilson, A. K. (eds.), *Proceedings of the Third International Symposium on Parasitic Weeds. Icarda, 7–9 May 1984, Aleppo, Syria*: 43–53.
- de Hullu, E. 1985: The influence of sward density on the population dynamics of *Rhinanthus angustifolius* in a grassland succession. — *Acta Bot. Neerl.* 34: 23–32.
- Ekstam, U., Aronsson, M. & Forshed, N. 1988: *Ängar*. — LTs Förlag, Stockholm.
- Fritsch, F. E. & Salisbury, E. 1946: *Plant form and function*. — Bell & Sons, London.
- Gärdenfors, U. (ed.) 2000: *Rödlistade arter i Sverige 2000*. — Artdatabanken, SLU, Uppsala.
- Gibson, C. C. & Watkinson, A. R. 1989: The host range and selectivity of a parasitic plant: *Rhinanthus minor* L. — *Oecologia* 78: 401–406.
- Gibson, C. C. & Watkinson, A. R. 1991: Host selectivity and the mediation of competition by the root hemiparasite *Rhinanthus minor*. — *Oecologia* 86: 81–87.
- Gibson, C. C. & Watkinson, A. R. 1992: The role of the hemiparasitic annual *Rhinanthus minor* in determining grassland community structure. — *Oecologia* 89: 62–68.
- Govier, R. N., Nelson, M. D. & Pate, J. S. 1967: Hemiparasitic nutrition in angiosperms. I. The transfer of organic compounds from host to *Odontites verna* (Bell.) Dum. (Scrophulariaceae). — *New Phytol.* 66: 285–297.
- Grubb, P. J. 1977: The maintenance of species richness in plant communities: the importance of the regeneration niche. — *Biol. Rev.* 52: 107–114.
- Joshi, J., Matthies, D. & Schmid, B. 2000: Root hemiparasites and plant diversity in experimental grassland communities. — *J. Ecol.* 88: 634–644.
- Karlsson, T. 1982: *Euphrasia rostkoviana* in Sweden. — *Växtekologiska studier* 15: 1–116.
- Karlsson, T. 1984: Early-flowering taxa of *Euphrasia* (Scrophulariaceae) on Gotland, Sweden. — *Nordic J. Bot.* 4: 303–326.
- Klaren, C. H. & Janssen, G. 1978: Physiological changes in the hemiparasite *Rhinanthus serotinus* before and after attachment. — *Physiol. Plant.* 42: 151–155.
- Kull, K. & Zobel, M. 1991: High species richness in an Estonian wooded meadow. — *J. Veg. Sci.* 2: 711–714.
- Martinsson, M. 1999: *Böisorkar u daldargras. Naturvärden och vård i gotländska odlingslandskap*. — Länsstyrelsen i Gotlands län, Visby.
- Marvier, M. 1998: A mixed diet improves performance and herbivore resistance of a parasitic plant. — *Ecology* 79: 1272–1280.
- Matthies, D. 1995a: Parasitic and competitive interactions between the hemiparasites *Rhinanthus serotinus* and *Odontites rubra* and their host *Medicago sativa*. — *J. Ecol.* 83: 245–251.
- Matthies, D. 1995b: Host-parasite relations in the root hemiparasite *Melampyrum arvense*. — *Flora* 190: 383–394.
- Matthies, D. 1996: Interactions between the root hemiparasite *Melampyrum arvense* and mixtures of host plants: heterotrophic benefit and parasite-mediated competition. — *Oikos* 75: 118–124.
- Matthies, D. 1997: Parasite–host interactions in *Castilleja* and *Orthocarpus*. — *Can. J. Bot.* 75: 1252–1260.
- Matthies, D. 1998: Influence of the host on growth and biomass allocation in the two facultative root hemiparasites *Odontites vulgaris* and *Euphrasia minima*. — *Flora* 193: 187–193.
- Musselman, L. J. & Mann, W. F. 1977: Host plants of some Rhinanthoidae (Scrophulariaceae) of eastern North America. — *Plant Syst. Evol.* 127: 45–53.
- Mutikainen, P., Salonen, V., Puustinen, S. & Koskela, T. 2000: Local adaptation, resistance, and virulence in a hemiparasitic plant–host plant interaction. — *Evolution* 54: 433–440.
- Nilsson, C. N. & Svensson, B. M. 1997: Host affiliation in two subarctic hemiparasitic plants: *Bartsia alpina* and *Pedicularis lapponica*. — *Écoscience* 4: 80–85.
- Oostermeijer, J. G. B., Brugman, M. L., de Boer, E. R. & den Nijs, H. C. M. 1996: Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. — *J. Ecol.* 84: 153–166.
- Pennings, S. C. & Callaway, R. M. 1996: Impact of a para-

- sitic plant on the structure and dynamics of salt marsh vegetation. — *Ecology* 77: 1410–1419.
- Peterson, J. 1999: Hotade växter på Gotland. Del 2: Sårbara arter. — *Rindi* 2–3: 59–118.
- Ross, M. A. & Harper, J. L. 1972: Occupation of biological space during seedling establishment. — *J. Ecol.* 60: 77–88.
- Seel, W. E., Cooper, R. E. & Press, M. C. 1993: Growth, gas exchange and water use efficiency of the facultative hemiparasite *Rhinanthus minor* associated with hosts differing in foliar nitrogen concentration. — *Physiol. Plant.* 89: 64–70.
- Smith, D. 2000: The population dynamics and community ecology of root hemiparasitic plants. — *Am. Nat.* 155: 13–23.
- Snogerup, B. 1982: Host influence on northwest European taxa of *Odontites* (Scrophulariaceae). — *Ann. Bot. Fennici* 19: 17–30.
- Svensson, B. M. 2000: *Ekologiska processer som påverkar artdiversiteten i traditionellt brukade betes- och slåttermarker: Halvparasitiska växters betydelse*. — Report, Swedish Foundation for Strategic Environmental Research, Stockholm.
- Svensson, B. M., Seel, W. E., Nilsson, C. N. & Carlsson, B. Å. 2001: Roles played by timing of seedling development and host identity in determining fitness of an annual, subarctic, hemiparasitic plant. — *Arct. Ant. Alp. Res.* 33: 299–305.
- ter Borg, S. J. 1972: *Variability of Rhinanthus serotinus* (Schönh.) Oborny in relation to the environment. — Ph.D. thesis, Univ. Groningen.
- ter Borg, S. J. & Bastiaans, J. C. 1973: Host-parasite relations in *Rhinanthus serotinus*. I. The effect of growth conditions and host; a preliminary report. — In: *Symposium on parasitic weeds, Malta 1973*: 235–246. European Weed Research Council, Wageningen.
- van Hulst, R., Shipley, B. & Thériault, A. 1987: Why is *Rhinanthus minor* (Scrophulariaceae) such a good invader? — *Can. J. Bot.* 65: 2373–2379.
- Wahlman, H. & Milberg, P. 2002: Management of semi-natural grassland vegetation: evaluation of a long-term experiment in southern Sweden. — *Ann. Bot. Fennici* 39: 159–166.
- Yeo, P. F. 1964: The growth of *Euphrasia* in cultivation. — *Watsonia* 6: 1–24.
- Zar, J. H. 1999: *Biostatistical analysis*. 4th ed. — Prentice-Hall, Upper Saddle River.
- Zobel, M. 1992: Plant species coexistence — the role of historical, evolutionary and ecological factors. — *Oikos* 65: 314–320.
- Zobel, M. 1993: Theories concerning the coexistence of plant species. — In: Kull, K. & Tiivel, T. (eds.), *Lectures in theoretical biology: the second stage*: 153–168. Estonian Acad. Sci., Tallinn.
- Zopfi, H.-J. 1998: Life-history variation among populations of *Euphrasia rostkoviana* Hayne (Scrophulariaceae) in relation to grassland management. — *Biol. J. Linn. Soc.* 64: 179–205.