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Influence of Host Condition on the Performance of *Rhopalomyia* n. sp. (Diptera: Cecidomyiidae), a Biological Control Agent for Scentless Chamomile, *Tripleurospermum perforatum*

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The gall midge *Rhopalomyia* n. sp. has been used as a biological control agent for the weed scentless chamomile, *Tripleurospermum perforatum* (=*Matricaria perforata*), in Canada since spring 1999. A series of three experiments was carried out in 1996 and 1997 to investigate the effect of three nutrient and two water levels on *T. perforatum*, on the performance of *Rhopalomyia* n. sp., and on the interaction between scentless chamomile and the gall midge. The hypotheses were that (1) the performance of *Rhopalomyia* n. sp. is favored when larvae develop on fertilized, well-watered plants of scentless chamomile (vigor hypothesis) and that (2) fertilized plants compensate for gall midge attack. Two different seed origins of tetraploid *T. perforatum*, Canadian or European, which resulted in two different phenostages, rosettes or bolting/flowering plants, respectively, were used. Weekly application of nutrients resulted in a 64–85% increase in the nitrogen level of plants and in increased plant vigor. On rosettes, the addition of nutrients did not influence gall midge performance, whereas on bolting/flowering plants, more galls were induced, and potential fecundity of females was increased by 17–19%. Within the low nutrient level, reduced water supply increased the nitrogen concentration of plants in one of the experiments, but decreased plant vigor. The potential fecundity of gall midge females was reduced by water shortage in one of the experiments. The results of this study generally support the vigor hypothesis. The height of the main shoot was always reduced in midge-attacked compared to unattacked plants, irrespective of nutrient treatment, whereas the impact of *Rhopalomyia* attack on average shoot height and on the number of shoots produced per plant varied with nutrient treatment. We recommend the release of *Rhopalomyia* n. sp. preferentially onto scentless chamomile plants at nutrient-rich, moist sites. © 2000 Academic Press

Key Words: ***Rhopalomyia* n. sp.; *Tripleurospermum perforatum;* gall former; nitrogen; water stress; biological control of weeds.**

INTRODUCTION

Nitrogen is often thought to be the key factor limiting the performance of insect herbivores (Mattson, 1980; McNeill and Southwood, 1978; Strong *et al.*, 1984). Effects of increased availability of nitrogen include improved larval survival and growth rate (Scriber and Slansky, 1981; Tabashnik, 1982; Wheeler and Center, 1997) and increased pupal weight and female fecundity (Minkenberg and Ottenheim, 1990; Myers and Post, 1981; Sands *et al.*, 1986). In some programs of biological weed control, nitrogen fertilization of the target weed has been found to facilitate and improve the establishment of biological control agents and to trigger mass outbreaks of agent populations, a prerequisite for successful control (Myers, 1987; Myers and Post, 1981; Room and Thomas, 1985). However, elevated nitrogen levels in plants have also been reported to have no (Abrahamson and McCrea, 1986; Auerbach and Strong, 1981) or even negative (Scriber, 1984; Waring and Cobb, 1992) effects on herbivores. Increased nitrogen levels can result in lower survival rates (Hartley and Lawton, 1992), decreased establishment rates (Carrow and Graham, 1968; Mitchell and Paul, 1974), and lower pupal weight (Birch *et al.*, 1992) of associated insect herbivores.

Plants generally profit from fertilization at least up to a certain threshold (Börner, 1983; Finck, 1982). Therefore, fertilized plants often compensate or even overcompensate for herbivore damage (Julien *et al.*, 1987; Maschinski and Whitham, 1989; Steinger and Müller-Schärer, 1992).

Another important limiting factor for herbivores is the water status of plants. Again, positive and negative effects of water deficiency have been reported. The outcome depends on the plant species investigated, the plant's response to water shortage, the feeding habit (guild) of the insect involved, and even the type of water stress inflicted (experimental versus natural drought or chronic water stress) (De Bruyn, 1995;

Koricheva *et al.*, 1998; Waring and Cobb, 1992). Although the majority of gall formers, for instance, are negatively affected when developing on water-stressed plants (Waring and Cobb, 1992), water deficit also has been argued to promote outbreaks of insect herbivores through elevated nitrogen levels and reduced plant defenses, especially of sucking insects on conifers (Mattson and Haack, 1987; White, 1984). Thus, the joint effect of both nutrient and water levels on herbivores is further complicated because the two factors are interactive (Waring and Cobb, 1992).

Scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (Asteraceae), is a plant of European origin that became naturalized in North America at the end of the 19th century (Woo *et al.*, 1991). It is a very adaptable plant, which can grow as a summer or winter annual, a biennial, or a short-lived perennial (Hegi, 1987; Woo *et al.*, 1991). Two different cytotypes, diploid and tetraploid, exist, which differ in their geographic distribution, but cannot be distinguished morphologically (Kay, 1969). The tetraploid cytotype, in particular, developed into a serious weed of various agricultural crops (Woo *et al.*, 1991). Similar to many other weeds of arable land, scentless chamomile depends on periodic disturbance for establishment and responds rapidly to increased soil nutrient levels by increased growth (Kay, 1994). Therefore, the weed can form dense, monospecific stands in field depressions with high soil moisture and fertility. However, the plant also grows in ruderal habitats, such as wasteland, farmyards, industrial areas, and roadsides, which typically exhibit lower soil nutrient and water levels (Douglas, D. W., 1989, Agriculture Canada, Regina, SK, unpublished report). As chemical and mechanical control measures often prove unsuccessful or uneconomical, a biological control program was initiated in 1990 with the aim of reducing the density and spread of scentless chamomile in North America (Peschken *et al.*, 1990).

The gall midge species *Rhopalomyia* n. sp. (Diptera: Cecidomyiidae) was investigated for its suitability as a biological control agent for scentless chamomile from 1995 to 1997 at the CABI Bioscience Centre Switzerland (Hinz, 1998). In March, 1999, permission for field release was obtained after host-specificity tests had revealed that it develops only on species in genus *Tripleurospermum* (Hinz, 1998). In Central Europe, *Rhopalomyia* n. sp. develops four generations per year and induces galls in different meristematic tissues of *T. perforatum*, including the apical meristems of rosettes and bolting plants, leaf axils, buds, and flowers (Hinz, 1998). Galls consist of 1–80 chambers, each containing one larva. Potential fecundity (defined as the number of eggs present in the ovaries at time of emergence, i.e., available for oviposition) was found to vary considerably between females (range 46–164) and was linearly related to body size.

To optimize release strategies it would be useful to know how nutrient and water levels influence the interaction of scentless chamomile with this biocontrol agent. Therefore, we investigated in this study the effects of four treatments, three nutrient levels and low nutrients in combination with reduced water supply, on the performance of *T. perforatum*, on different life history traits of *Rhopalomyia* n. sp., and on the interaction between the scentless chamomile and the gall midge. The hypotheses were that (1) the performance of *Rhopalomyia* n. sp. is favored when larvae develop on fertilized, well-watered plants of scentless chamomile (vigor hypothesis; see Price 1991) and that (2) fertilized plants compensate for gall midge attack.

MATERIALS AND METHODS

Plant and Insect Material

In Experiment 1, *T. perforatum* seeds collected in August, 1993 from Canadian tetraploid plants at Vegreville, Alberta were used, while in Experiments 2 and 3, seeds from European tetraploid plants collected in September, 1995 in Eastern Austria near Vienna were employed. The seeds were sown into plastic trays, which were kept in an unheated greenhouse. In contrast to the Canadian tetraploid plants, which stayed in the rosette stage throughout the experimental period, the European plants started to bolt 5–6 weeks after sowing, and nearly all flowered and produced seeds by the end of the experiment. All *Rhopalomyia* n. sp. specimens used in the experiments originated from a rearing colony established at the CAB1 Bioscience Centre Switzerland.

Experimental Design

Experiment 1. On 15 June, 1996, seeds from Canadian tetraploid scentless chamomile plants were sown, and on 4 July, 1996, 30 rosettes were transplanted singly into clay pots (13 cm diameter) in a mixture of soil (Capriflor Premium, 90% peat, 10% compost) and sand (2:1), with polystyrene pellets added for aeration. Three different nutrient levels, high, medium, and low, were randomly assigned, each to 10 rosettes. Plants in the high nutrient treatment received 50 ml of a commercial fertilizer (Wuxal; Maag AG, Dielsdorf, Switzerland; 100 g N, 100 g P_2O_2, 75 g K_2O per L) once a week per pot. Rosettes assigned to the medium nutrient treatment were treated with the same amount of fertilizer once every 14 days, and those at the low nutrient treatment were given water only. High and medium nutrient treatments were equivalent to adding a total of 380 and 190 kg N/ha, respectively, which is slightly higher than recommended rates for agricultural crops in Canada and Europe (Fowler, 1982; Riklin, 1995). The rosettes were placed in an unheated greenhouse under natural light conditions, watered daily, and randomized once a week to avoid differences in illumination. Fertilization was continued until 24 September. Between 29 and 31 July, 100 newly laid eggs of *Rhopalomyia* n. sp. were transferred with a paintbrush onto each rosette under a dissecting microscope. Plants were regularly examined for gall formation. Prior to gall midge emergence, the plants were spaced to facilitate the collection of gall midges and to avoid gall midge movement between plants. Whereas newly emerged gall midge females initially remain on the plants from which they emerge, males leave the plants shortly after emergence in search of females (Hinz, 1998). Therefore, only gall midge females were collected. To determine potential fecundity, gall midge females were preserved in 70% ethanol separately for each plant. To avoid oviposition prior to collection, females were collected early in the morning at temperatures below 15°C (Hinz, 1998). Five to seven females were randomly selected per plant, dissected, and examined for eggs under a dissecting microscope. Development time was measured as the number of days from egg transfer until 50% of all females from one plant had emerged.

The number of leaves and the length of the longest leaf were recorded on 15 September, 1996, when most gall midges had emerged. On the same date, two leaves of similar age were sampled per plant. The leaves were dried for 24 h at 70°C and milled and their nitrogen content was determined as a percentage of dry weight using a CHN analyzer (CHN(O)S 900/932, LECO, MI).

Experiment 2. Seeds from European tetraploid plants were sown on 15 June, 1996, and on 9 July, 40 rosettes were transplanted into pots. The same three nutrient treatments were applied as in Experiment 1. In addition, a fourth treatment which combined low nutrient level with reduced water supply was included; i.e., plants were watered only every 3rd day. Ten rosettes were randomly assigned to each of the four treatments. On 1 August, 1996, the rosettes, which were just starting to bolt, were covered with gauze bags (0.25 mm), and two *Rhopalomyia* pairs each were released. After 2 days, the gauze bags were removed, and attack was verified by checking plants for egg batches. Thereafter, plants were treated and gall midges collected as described for Experiment 1. On 15 September, 1996, number of galls induced, number of shoots, height of the main shoot, shoot base diameter of the main shoot, and numbers of flower heads (immature seed heads) and ripe (mature) seed heads produced per plant were recorded. The third and fourth leaf from the shoot base were sampled from each plant and the nitrogen content was determined as in Experiment 1.

Experiment 3. Seeds from European tetraploid plants were sown on 9 June, 1997. On 1 July, 80 rosettes were transplanted into pots. Twenty rosettes were randomly assigned to each of the same four treatments as in Experiment 2. To investigate the impact of *Rhopalomyia* n. sp. on its host plant, half of the plants in each treatment were infested with two gall midge pairs (as described under Experiment 2) on 18 July, 1997. The other 10 plants served as controls. As nitrogen content is known to vary between plant parts (McNeill and Southwood, 1978), an attempt was made to determine the plant part (gall type) from which females emerged. This allows a more precise test of the hypothesis that nitrogen content influences gall midge performance. Three gall types were distinguished: galls induced in apical meristems, those in leaf axils, and those in flowers. Where possible, five females were dissected per gall type. Otherwise, at least five females were dissected per plant. To determine the total number of gall midges that developed per plant, all galls were dissected after emergence had ceased, and the total number of gall chambers induced was recorded per plant.

On 29 August, 1997, the number and height of shoots and the number of ripe (mature) seed heads produced per plant were recorded. Because temperatures were higher during the experimental period in 1997 than in 1996, flower heads matured more quickly, and only ripe seed heads were present at the end of the experiment. In contrast to Experiments 1 and 2, in which the nitrogen content of leaves was determined, the plant parts in which the females actually developed were analyzed. One gall, formed in either the apical meristem, a leaf axil, or a flower, was sampled from each attacked plant on 30 August, 1997. The remaining gall midge larvae or pupae were removed under a dissecting microscope. An equivalent unattacked plant part was sampled from control plants. Depending on the availability of galls and corresponding unattacked tissue, six to nine samples were analyzed per treatment in attacked and unattacked plants. The nitrogen content was determined as in Experiment 1.

Data Analysis

Variables were analyzed by one- or two-way ANOVA and treatment means were compared with Tukey HSD tests ($P < 0.05$) (Experiment 1) or with orthogonal contrasts (Experiments 2 and 3). Nested ANOVA was carried out to test for differences in potential fecundity of gall midges in plants within treatments. Variances were tested for heterogeneity using Levene or Cochran tests. For all analyses, the statistical software package SPSS 6.1 (SPSS Inc., 1994) was used.

RESULTS

Experiment 1

The three nutrient treatments resulted in different leaf nitrogen concentrations of *T. perforatum* rosettes

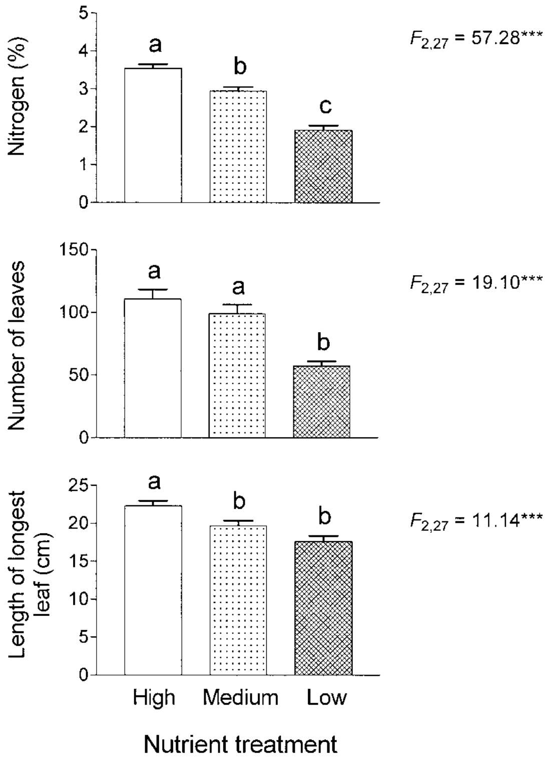

FIG. 1. The effect of three nutrient treatments on rosettes of Canadian tetraploid *Tripleurospermum perforatum* infested by *Rhopalomyia* n. sp. (Experiment 1, 1996). For details of nutrient treatments see Materials and Methods. Means + SE. Bars denoted by different letters are significantly different (Tukey's HSD test, $P < 0.05$). F ratios of one-way ANOVA with nutrient treatment as factor are given. ***$P < 0.001$.

(Fig. 1). The nitrogen concentration in leaves of plants in the medium and high nutrient treatments was 54 and 85% higher than in leaves of plants that received no additional nutrients (low nutrient level). The number of leaves per plant was nearly doubled in the high nutrient level, and the length of the longest leaf increased by 5 cm (Fig. 1).

None of the life history traits of *Rhopalomyia* n. sp. that were recorded were significantly affected by fertilization. After 42 ± 0.6 days (mean ± SE), an average of 13.0 ± 1.6 females emerged per plant with a potential fecundity of 127.0 ± 1.8 eggs. Differences in potential fecundity of females on different plants were marginally significant ($P = 0.05$, see Table 1).

Experiment 2

In flowering plants of *T. perforatum*, the four treatments (three nutrient levels and low nutrients combined with low water level) resulted in different leaf nitrogen concentrations (Fig. 2). Nitrogen increased by 49% in the medium and by 78% in the high nutrient treatment. The nitrogen concentration of plants not provided with additional nutrients and watered only every 3rd day was 23% higher than in those plants watered daily (Fig. 2). The application of nutrients and daily watering increased the height and shoot base diameter of the main shoot, but did not significantly influence the number of shoots per plant (Fig. 2). The number of flower and seed heads was nearly tripled by adding nutrients, but not significantly influenced by water level (Fig. 2). Except for nitrogen concentration, none of the other plant parameters differed significantly between the high and medium nutrient levels (Fig. 2).

Potential fecundity of gall midge females that developed on plants in the high and medium nutrient levels was 17% higher than on plants that had not received additional nutrients (Fig. 3). Potential fecundity of females was also higher when developing on plants that were watered daily compared to plants that were watered only every 3rd day (Fig. 3). Although the main effect for the number of females that emerged was not significant (Fig. 3), orthogonal contrasts revealed that more females emerged from plants in the high and medium nutrient treatments than from plants not provided with additional nutrients ($F_{1,34} = 5.71$, $P < 0.01$). The number of galls induced and development time were not affected by nutrient or water level (Fig. 3). The potential fecundity of females differed significantly between individual plants (Table 1).

Experiment 3

The addition of nutrients, averaged over attacked and unattacked plants, resulted in increased nitrogen levels of flowering plants of *T. perforatum* (Fig. 4). The high nutrient treatment increased the nitrogen concentration by 64% and the medium nutrient treatment by 21%. The nitrogen concentration was not significantly affected by water level (Fig. 4), and it did not differ between plant parts ($F_{2,76} = 2.79$, $P = 0.067$; means ±

TABLE 1

Results of Nested ANOVA for the Effects of Treatment and Plants within Treatments on the Potential Fecundity of *Rhopalomyia* n. sp.

Experiment[a]	Source of variation	*df*	*F* ratio	*P* value
1	Treatment (T)[a]	2	0.51	0.61
	Plants within T	26	1.59	0.05
	Within plants	125		
2	Treatment	3	7.72	<0.001
	Plants within T	34	2.66	<0.001
	Within plants	190		
3	Treatment	3	12.14	<0.001
	Plants within T	33	5.48	<0.001
	Within plants	296		

[a] Treatments: Experiment 1, three nutrient levels, high, medium, and low; Experiments 2 and 3, three nutrient levels and low nutrient level combined with low water level.

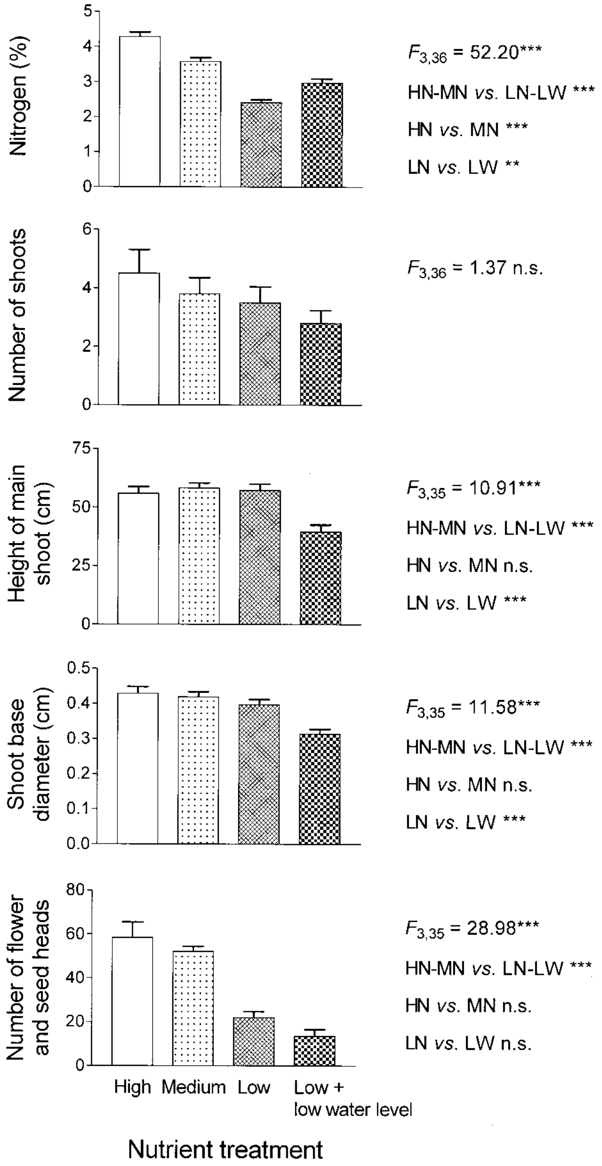

FIG. 2. The effect of three nutrient treatments and low nutrients combined with low water level on bolting/flowering plants of European tetraploid *Tripleurospermum perforatum* infested by *Rhopalomyia* n. sp. (Experiment 2, 1996). For details of treatments see Materials and Methods. Means + SE. *F* ratios of one-way ANOVA with nutrient treatment as factor are given. Treatment means were compared with orthogonal contrasts (HN, high nutrient treatment; MN, medium nutrient treatment; LN, low nutrient treatment; LW, low nutrient treatment combined with low water level). Contrasts are given only when the main effect was significant; n.s., not significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

SE: apical meristem, 3.32 ± 0.25, $n = 18$; leaf axils, 3.22 ± 0.13, $n = 40$; flowers, 2.74 ± 0.12, $n = 20$).

Plants in the high nutrient treatment produced on average two more shoots per plant than plants in the low nutrient treatment (Fig. 4, and see below). The height of the main shoot and mean shoot height were increased by about 18 and 13 cm, respectively, by adding nutrients (Fig. 4). Within plants in the low nutrient level, low water supply reduced the height of the main shoot by 18 cm and mean shoot height by about 12 cm. The number of seed heads was, similar as in Experiment 2, nearly tripled by adding nutrients, but was again not significantly influenced by water level (Fig. 4).

The number of galls induced was nearly doubled on plants that received additional nutrients, whereas the total number of gall chambers per plant was not significantly increased (Fig. 5). The potential fecundity of females that developed on plants in the high and medium nutrient treatments was 19% higher than on plants that had no nutrients added (Fig. 5). Potential

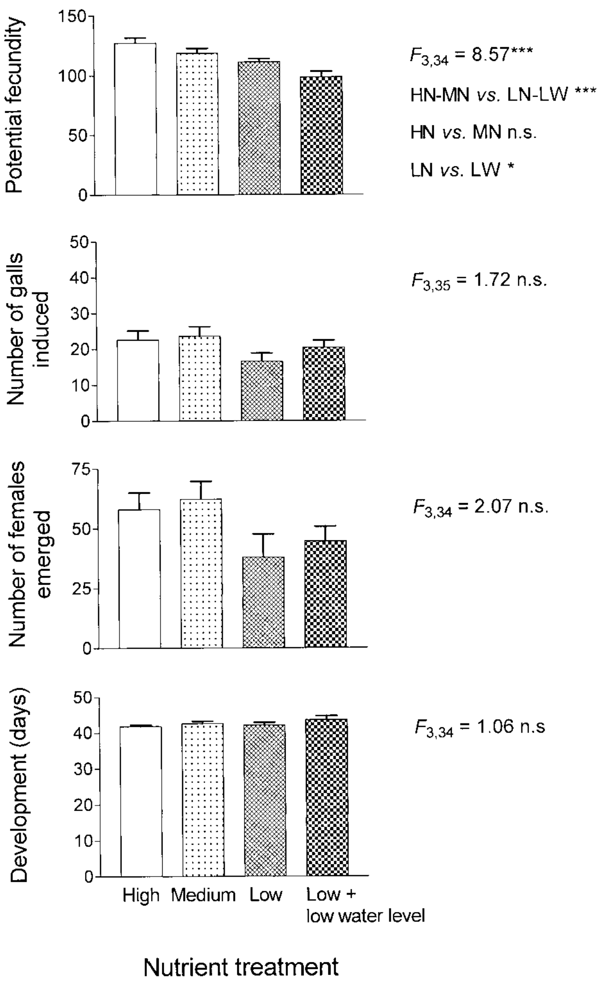

FIG. 3. The effect of three nutrient treatments and low nutrients combined with low water level on life history traits of *Rhopalomyia* n. sp. offspring (Experiment 2, 1996). For details of treatments see Materials and Methods. Means + SE. *F* ratios of one-way ANOVA with nutrient treatment as factor are given. Treatment means were compared with orthogonal contrasts (HN, high nutrient treatment; MN, medium nutrient treatment; LN, low nutrient treatment; LW, low nutrient treatment combined with low water level). Contrasts are given only when the main effect was significant; n.s., not significant; $^{*}P < 0.05$; $^{***}P < 0.001$.

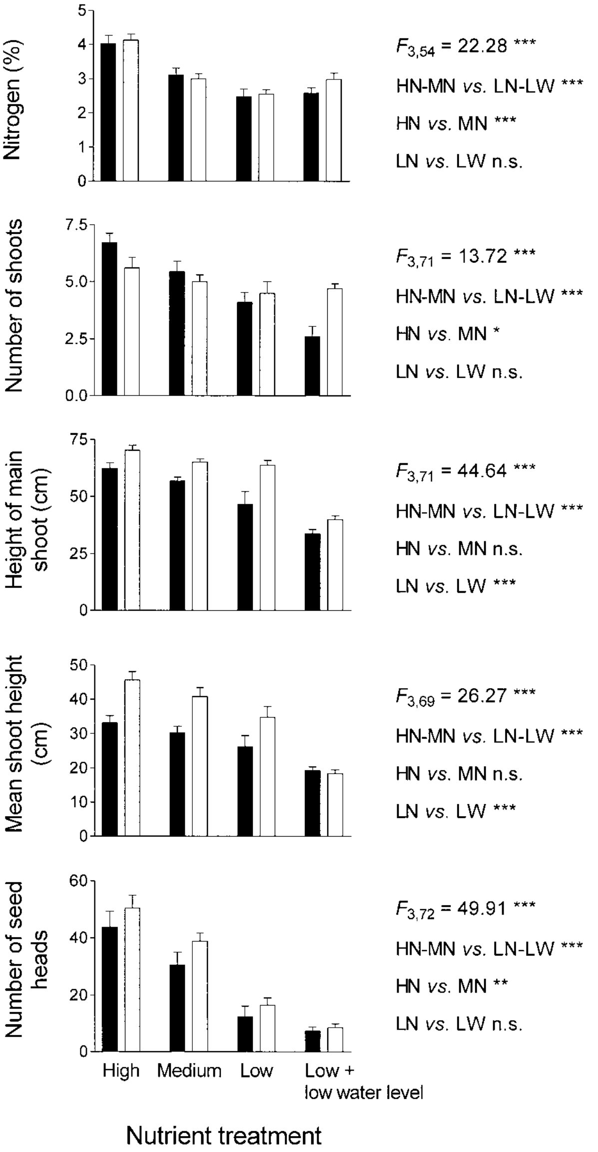

FIG. 4. The effect of three nutrient treatments and low nutrients combined with low water level on bolting/flowering plants of European tetraploid *Tripleurospermum perforatum* infested (black bars) and uninfested (white bars) by *Rhopalomyia* n. sp. (Experiment 3, 1997). For details of treatments see Materials and Methods. Means + SE. Only F ratios for nutrient treatment as factor are given (also see Table 2). Treatment means were compared with orthogonal contrasts (HN, high nutrient treatment; MN, medium nutrient treatment; LN, low nutrient treatment; LW, low nutrient treatment combined with low water level); n.s., not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Detailed statistics of two-way ANOVA with nutrient treatment and herbivory as factors are given in Table 2.

fecundity was not significantly affected by water level or by gall location ($F_{2,35} = 0.59$, $P = 0.558$). However, as in Experiment 2, potential fecundity differed between plants (Table 1).

The nitrogen concentration did not differ significantly between galled and ungalled tissue (Table 2, Fig. 4). Although herbivory by *Rhopalomyia* n. sp. did not explain a significant proportion of the variation in the number of shoots per plant, the interaction between treatments and herbivory was significant (Table 2). This was due to the fact that in plants within the low nutrient treatment that were watered only every third day, attack by *Rhopalomyia* n. sp. significantly reduced the number of shoots that developed compared to unattacked plants ($F_{1,18} = 17.64$, $P < 0.001$), whereas herbivory had no significant influence on the number of shoots produced within the other nutrient treatments (Fig. 4). Attack by *Rhopalomyia* n. sp. reduced the height of the main shoot by about 10 cm and mean shoot height by about 8 cm compared to unattacked plants (Fig. 4, Table 2). The interaction between treatments and herbivory for mean shoot height was marginally significant ($P = 0.05$; see Table 2), because herbivory significantly reduced mean shoot height in the high ($F_{1,18} = 10.55$, $P = 0.005$) and medium ($F_{1,18} = 15.30$, $P = 0.001$) nutrient treatment, but not in the low nutrient treatment (Fig. 4). The reduction by 18% in the number of seed heads produced by gall midge at-

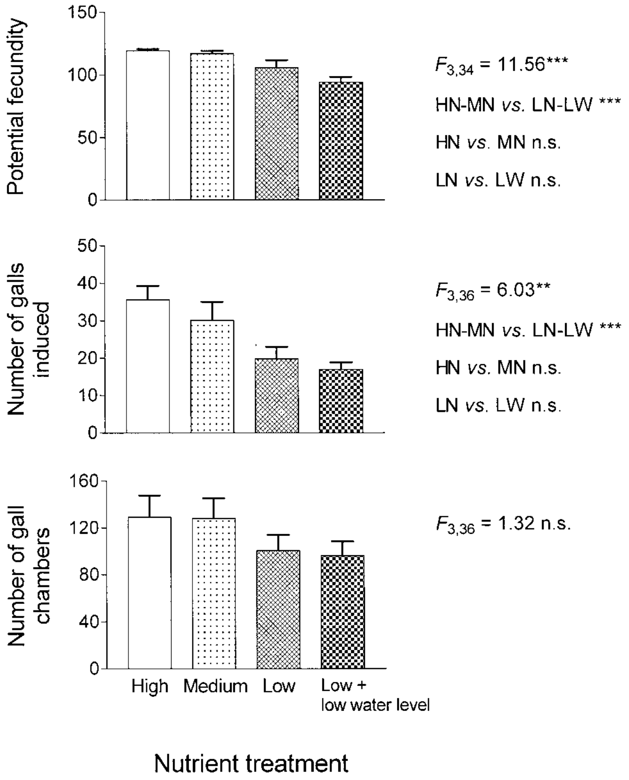

FIG. 5. The effect of three nutrient treatments and low nutrients combined with low water level on life history traits of *Rhopalomyia* n. sp. offspring (Experiment 3, 1997). For details of treatments see Materials and Methods. Means + SE. F ratios of one-way ANOVA with nutrient treatment as factor are given. Treatment means were compared with orthogonal contrasts (HN, high nutrient treatment; MN, medium nutrient treatment; LN, low nutrient treatment; LW, low nutrient treatment combined with low water level). Contrasts are given only when the main effect was significant; n.s., not significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

TABLE 2

Results of Two-Way ANOVA for the Effects of Four Nutrient Treatments and Herbivory by *Rhopalomyia* n. sp. on European Tetraploid *Tripleurospermum perforatum* (Experiment 3)

Response variable	Source of variation	*df*	*F* ratio	*P* value
Nitrogen (%)	Treatment (T)[a]	3	22.28	<0.001
	Herbivory (H)[b]	1	0.83	0.397
	T × H	1	0.55	0.648
	Error	54		
Number of shoots	Treatment	3	13.72	<0.001
	Herbivory	1	0.70	0.405
	T × H	1	5.52	0.002
	Error	71		
Height of main shoot	Treatment	3	44.64	<0.001
	Herbivory	1	26.48	<0.001
	T × H	1	1.64	0.187
	Error	71		
Mean shoot height	Treatment	3	26.27	<0.001
	Herbivory	1	22.50	<0.001
	T × H	1	2.74	0.05
	Error	69		
Number of seed heads	Treatment	3	49.91	<0.001
	Herbivory	1	3.93	0.051
	T × H	1	0.36	0.780
	Error	72		

[a] Treatments: three nutrient levels, high, medium, and low, and low nutrient level combined with low water level.

[b] Herbivory: present or absent.

tack was marginally significant ($P = 0.051$) (Fig. 4, Table 2). Five attacked plants had not produced any seed heads by the end of the experiment at the beginning of September.

DISCUSSION

Effect of Nutrient Treatments

The application of nutrients significantly increased nitrogen concentrations in all three experiments, and, as expected, positively influenced plant vigor. Nitrogen levels were similar in Experiment 2, in which the nitrogen concentration of leaves was determined, and in Experiment 3, in which the nitrogen concentration of the plant parts supporting gall development was analyzed. Although these were different experiments, and a statistical comparison is not possible, this suggests that the nitrogen content of leaves provided a valid estimate of the nitrogen available for insect development (see also McClure, 1983).

The addition of nutrients significantly increased the potential fecundity of *Rhopalomyia* n. sp. offspring in Experiments 2 and 3 and the number of galls induced in Experiment 3. Scentless chamomile plants provided with additional nutrients generally produced more secondary shoots than unfertilized plants and therefore offered more meristematic tissue for gall midge development. However, potential fecundity of females was increased by only 17–19% within the three nutrient levels. One reason for this result could be that nitrogen levels were still too high in the low nutrient level to cause a greater difference in gall midge performance. This possibility is supported by the fact that average potential fecundity was still relatively high, even at the low nutrient level, i.e., 111 eggs in Experiment 2 and 105 eggs in Experiment 3 (see Hinz, 1998). For instance, the growth rate and development time of larvae of the chrysomelid *Paropsis atomaria* Olivier were not affected by nitrogen concentrations of *Eucalyptus blakelyi* Maiden foliage until levels fell below 1.7% (Ohmart, 1991). Nitrogen levels during this study were generally above 2%. In contrast, the fecundity of *Liriomyza trifolii* Burg. females was increased by over 200% by an increase in nitrogen concentration of tomato leaves from 3.4 to 4.9% (Minkenberg and Ottenheim, 1990). The outcome presumably depends on the nutrient requirements of the insect species investigated. It also has been argued that total nitrogen levels can give misleading results, as not all the nitrogen present may be available to the insect, or the balance between the various amino acids may be unsatisfactory (Birch *et al.*, 1992; Mattson, 1980; McNeill and Southwood, 1978).

Effect of Plant Phenostage and Variation between Plants on Gall Midge Performance

On rosettes of scentless chamomile (Experiment 1), the potential fecundity of *Rhopalomyia* n. sp. females was not significantly increased by addition of nutrients. Gall formers are dependent on young, undifferentiated plant organs, in which development of tissue can still be redirected and manipulated by the insect (Abrahamson and Weis, 1987; Weis *et al.*, 1988). Such tissues offer a rich food source, because nutrients are directed to places of active growth (Mattson, 1980; McNeill and Southwood, 1978). It is hypothesized that the apical meristem of rosettes presents an optimal resource to gall midge larvae so that additional nutrient supply did not further increase performance.

The potential fecundity of gall midge females was higher when they developed in rosettes (127 ± 1.8; Experiment 1) than in bolting or flowering plants of scentless chamomile (119 ± 2.5 and 114 ± 2.1; Experiments 2 and 3), irrespective of the nutrient level. Unfortunately, the differences observed between the two phenostages (rosettes and flowering plants) are biased by the fact that these were associated with two different plant origins of scentless chamomile, tetraploid cytotypes from Canada and Europe, and slightly different experimental designs. However, the trend that larger, i.e., more fecund females (see Hinz, 1998), emerged more often from rosettes, while smaller, i.e., less fecund females, emerged more often from flowering plants of scentless chamomile of the same plant origin had been observed before (Hinz, personal observation). Therefore, it is assumed that plant phenostage, not plant origin, caused the differences noted in potential fecundity.

An analysis of the variability of gall midge performance on different plants indicated that individual plants varied in their suitability for gall midge development. Investigations on other gall formers yielded similar results, and it was concluded that plant genotypes varied in their resistance to attack by gall formers (Hartley and Lawton, 1992; Lym *et al.*, 1996; McCrea and Abrahamson, 1987). Because gall formers have a particularly intimate relationship with their host plant, it is assumed that genetic differences between plants may have a stronger influence on them than on other herbivores.

Nitrogen Concentration in Galled and Ungalled Tissue

During this study, no significant differences were found in the nitrogen concentration of tissue that was attacked or unattacked by *Rhopalomyia* n. sp. Other investigations found lower, equal, or higher nitrogen levels of galled tissue compared to corresponding ungalled tissue (Abrahamson and McCrea, 1986; Brewer *et al.*, 1987; Harris and Shorthouse, 1996; Hartley, 1998; Hartley and Lawton, 1992; Paclt and Hässler, 1967; Skuhravy *et al.*, 1980). Higher nitrogen levels in galled tissue usually indicate that the gall former draws nutrients from other plant parts, i.e., it acts as a mobilizing sink, a feature considered advantagous for a gall former to become an effective biocontrol agent (Harris and Shorthouse, 1996). However, McCrea *et al.* (1985) argued that the intercepting character of stem galls induced by *Eurosta solidaginis* (Fitch), for instance, makes it unnecessary for the gall former to actively draw nutrients from other plant parts. A similar mechanism may apply for *Rhopalomyia* n. sp., particularly in the case of galls induced in the apical meristem of rosettes or bolting plants, because nutrients are directed toward these young, actively growing meristematic tissues. However, it could also be that nitrogen levels in galled tissue were underestimated during this study. First, the galls analyzed were relatively mature. Other studies have shown that nutrient levels are lower in mature than in immature galls (Harris and Shorthouse, 1996; Hartley, 1998). Second, results depend on how detailed is the analysis that is carried out. Since the nutritive layer on which gall midges feed is only a small fraction of the total gall mass, and only these cells show the high levels of physiological and metabolic activity, analysis of the whole gall, as carried out during this study, may give misleading results (Abrahamson and McCrea, 1986; Bronner, 1992; Harris and Shorthouse, 1996). Finally, nitrogen levels in galls may have been underestimated, because some of the nitrogen is incorporated into the gall midge larvae, but these were removed prior to analysis (Abrahamson and McCrea, 1986; Hartley, 1998; White, 1993).

Effect of Water Shortage

There are contradicting reports in the literature on the effect of water stress on gall insects, but most suggest that this stress is detrimental, if the gall insect has not developed some kind of adaptation (Harris and Shorthouse, 1996; Koricheva *et al.*, 1998; Waring and Cobb, 1992). Although the performance of *Rhopalomyia* n. sp. was negatively affected by reduced water supply, differences were slight and significant for potential fecundity only in Experiment 2. This suggests that *Rhopalomyia* n. sp. is adapted to water shortage to a certain degree. Possible mechanisms that reduce transpiration within gall tissue include the reduction of the number of stomata, an increase in the number of hairs, and the accumulation of soluble minerals to increase the water content (Birch *et al.*, 1992; Harris and Shorthouse, 1996). The hypothesis that stressed food plants, through an increase in nitrogen concentrations, facilitate insect outbreaks (White, 1984) could not be supported. We also did not find that stressed scentless chamomile plants are more susceptible to gall former attack through, for instance, reduced plant defenses, as documented by other studies (De Bruyn, 1995; Tscharntke, 1988).

Impact of Gall Midges in Relation to Nutrient and Water Levels

Reduced plant height was the most prominent effect of attack by *Rhopalomyia* n. sp. on scentless chamomile plants. Several other authors report that gall midge-infested plants are stunted (Birch *et al.*, 1992; Caresche and Wapshere, 1975; Wehling and Piper, 1988). When galls are induced in the apical meristem of rosettes of scentless chamomile, the apical dominance is often broken, and plants may produce, depending on the severity of attack (i.e., gall size), several side shoots, thereby compensating for gall midge attack. However, these shoots are usually shorter than shoots of unattacked plants. The height of the main shoot was always reduced in attacked compared to unattacked plants (Table 2, Fig. 4), which indicates that fertilized plants were not able to escape the effect of gall midge attack. In contrast, mean shoot height was significantly reduced by herbivory in plants only in the high and medium nutrient treatments. This is assumed to be linked to the number of shoots produced. Attacked plants provided with additional nutrients produced more shoots than unattacked plants (though this difference was not significant), which could have resulted in a reduction in average shoot height. The number of shoots was significantly reduced by herbivory in only the low nutrient level combined with low water supply. Presumably these plants lacked sufficient resources to compensate for gall midge attack. The number of seed heads, and thus seeds produced, was, as the height of the main shoot, always reduced in plants attacked by *Rhopalomyia* n. sp. compared to unattacked plants, but the effect was only marginally significant (see Table 2). Overall, the impact of gall midge attack on scentless chamomile was not as strong as expected probably because the European tetraploid plants were already starting to bolt at the time when gall midges were released, and plants were growing very quickly. In addition, the experiments presented in this study followed only one generation of *Rhopalomyia* n. sp., and females were not given the choice on which plants to oviposit. Because *Rhopalomyia* n. sp. produces up to four generations per year, and females prefer larger plants for oviposition (Hinz, 1998), it is assumed that plants which grow in nutrient-rich conditions receive more eggs from females of subsequent generations and thus sustain more damage, similar to the findings of Room *et al.* (1989) and Hatcher *et al.* (1997) for coleopterans on *Salvinia molesta* Mitchell and *Rumex obtusifolius* L., respectively.

Implications for Biocontrol

In general, this study supports the vigor hypothesis, which predicts that, in particular, the performance of insect herbivores closely associated with plant growth processes, such as gall formers, is favored when developing on vigorously growing plants or plant parts (Price, 1991). However, the results also suggest that plant phenostage and variation in suitability might play a role in offspring fitness. In addition, the performance of the gall midge was not as strongly negatively influenced by low nutrient and water levels as expected. It is therefore assumed that *Rhopalomyia* n. sp. will be able to establish over a wide range of habitats. However, establishment and population growth should be enhanced by preferentially releasing gall midges onto scentless chamomile plants at nutrient-rich, moist sites.

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