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Non-Host Plant Odor (*Tanacetum vulgare*; Asteracea) Affects the Reproductive Behavior of *Lobesia botrana* Den. et Schiff (Lepidoptera: Tortricidae)

Bruno Gabel[1,2] and Denis Thiéry[1,3]

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Females of Lobesia botrana *Den. et Schiff. (Lepidoptera Tortricidae) are attracted in natural conditions by volatiles released by a nonhost plant: tansy (*Tanacetum vulgare *L.; Asteracea). We have shown that both tansy flowers and their odor inhibit oviposition behavior and mating behavior and reduce adult longevity. The mean number of eggs laid per female isolated with tansy flowers was reduced by up to 50% every 2 days during the 6 days of exposure. This reduction was maintained after the tansy was removed. In the presence of tansy essential oil, the egg-laying reduction ranged from about 30 to 80% according to the odor concentration. The number of spermatophores found in females isolated with tansy flowers was also reduced twofold compared to the control treatment, indicating that the presence of tansy reduced mating activity. This mating activity is strongly reduced, by two-thirds, when adults face the highest dose of essential oil compared to controls. The number of eggs laid by the controls cannot be explained by the number of spermatophores. Therefore, the reduction in oviposition has been attributed to the presence of tansy flowers or to the tansy odor. Tansy flowers and tansy odor increased male mortality during the exposure (10% in the control, 50% in the tansy treatment, and up to 98% in the odor treatment). The highest rates of male mortality occurred during the 4- to 6-day period of exposure to flowers or odor. Repellence resulting in sustained locomotor activity is a possible cause of such a mortality. Female mor-*

[1]Laboratoire de Neurobiologie Comparée des Invertébrés, INRA-CNRS, BP 23, 91440 Bures sur Yvette, France.
[2]Present address: Research Institute for Viticulture and Enology, Matuskova 25, 833 11 Bratislava, Slovakia.
[3]To whom correspondence should be addressed.

tality was increased only in response to the highest dose of odor. This increase might be due to egg retention, and not directly to a plant effect. We discuss the effects of tansy flower odor on different patterns relative to the reproductive behavior of L. botrana *and, especially, on oviposition behavior in the ecological context of plant selection and polyphagy.*

KEY WORDS: *Tanacetum vulgare;* Tansy; *Lobesia botrana;* European grapevine moth; Asteracea; Lepidoptera; Tortricidae; oviposition; behavior; nonhost plant; semiochemicals; plant odor; olfaction.

INTRODUCTION

The European grapevine moth (EGVM), *Lobesia botrana* Den. & Schiff. (Lepidoptera, Tortricidae, Olethreutini), is considered a polyphagous insect that develops on plants from different families (Bovey, 1966; Stoeva, 1982). This species is a serious pest in European paleartic vine-growing areas that can complete two to four generations on grapevine fructiferous organs. The abundance of EGVM in various areas is not uniform and can change within a relatively short distance: Typically, higher population densities occur at sunny exposures and during hotter seasons (Roehrich and Boller, 1991). In Slovakian vineyard agrosystems, large numbers of almost exclusively EGVM females can be observed on different flowering stages of *Tanacetum vulgare* L. (Asteracea) (Gabel, 1992). Dissections of females caught on tansy flowers revealed high ratios of young fertilized females and females ready to oviposit (Gabel, 1992). The attraction to tansy flowers can be mimicked in olfactometers using steam distillates of tansy (Gabel *et al.*, 1991) and in semiopen conditions using sticky traps baited with either a steam distillate of tansy flowers or a blend of nine monoterpenes produced by the flowers and placed within the vinegrape foliage (Gabel *et al.*, 1992). Despite this strong attraction of females, oviposition has never been reported on any part of tansy, and feeding acceptance by larvae has been observed only with the latest larval instars (Lüstner, 1914). Tansy is therefore not considered a host plant of *L. botrana* or a "rendezvous" site for mating, since males are seldom observed on this plant (Gabel, 1992).

To understand the role of an attractive nonhost plant in the biology of the EGVM, we have studied the influence of tansy flowers and tansy odor on different life criteria: mating efficiency, fecundity of females (defined as the number of eggs laid during a limited period of time), and adult longevity. This work documents the role of a nonhost plant in the reproductive activity of a polyphagous phytophagous insect.

MATERIALS AND METHODS

Plants, Odors, and Insects

The tansy flowers harvested in July in Gif sur Yvette (France) were of thujone chemotype according to classification based on terpenic production (Dembitsky *et al.*, 1984; Holopainen *et al.*, 1987): The ratio of α to β thujone was verified by gas chromatographic analyses (1% α thujone vs 99% β thujone). This chemotype was identical to that previously shown to be attractive to females (Gabel *et al.*, 1992). The tansy essential oil was obtained by steam distillation of flowers according to the method described by Gabel *et al.* (1992).

The insects came from the INRA strain of EGVM (INRA, Station de Zoologie, Pont-de-la-Maye, France) annually infused with wild insects. It was reared on a semisynthetic diet under controlled conditions (16 L:8 D, 22 ± 1°C, 60% RH). We used 1-day-old virgin females and males.

Experimental Setup

Each female was placed with two males in a plexiglass tube (6.5-cm diameter × 13.6-cm length) closed with a grid. Water was provided for the females by means of moistened cotton wool. Four groups of experiments were performed: (1) control treatment without flowers or odor (N = 30); (2) tansy treatment, where insects were isolated with tansy flowers (N = 20); (3) essential oil treatments, where flowers were replaced by essential oil dispensors at a dose of 20 μl (N = 30); and (4) essential oil at a dose of 100 μl (N = 22). In the control treatment, adults were left in the arenas for 7 days. In the tansy treatment, adults were kept for 6 days each with a branch carrying between 20 and 30 heads of tansy: The stems of flowers were put in water and replaced every day. After 6 days, females were transferred into plexiglass tubes without tansy flowers, to determine the number of eggs laid following the exposure to tansy flowers (seventh day). We used the same procedure for the essential oil treatments, flowers being replaced by an odor dispenser below the isolator. We used as odors dispensers Ependorf tubes filled with 200 ml of paraffin oil containing, respectively, 20 and 100 μl of essential oil.

The eggs were laid on the surface of the plexiglass tubes. Three parameters were measured: (a) the number of eggs laid per female every 2 days during the exposure to tansy, the number of eggs laid on the seventh day, and the total number of eggs laid by each female; (b) the number of spermatophores present in the *bursa copulatrix* (females dissected 1 day following experiments); and (c) the mortality of males and females every 2 days.

Data are presented as mean ± standard deviation. Parameters a and b were subjected to a nonparametric ranking test (Mann–Whitney *U* test); parameter c, to a chi-square analysis; and the correlation between parameter a and parameter b, to the Spearman rank correlation in the control treatment (Siegel, 1956).

RESULTS

Isolation of Adults with Tansy Flowers

The mean number of eggs laid per female every 2 days in each treatment (control and adults with tansy flowers) are presented in Fig. 1. The exposure to tansy led to a reduction by about 40% in the total number of eggs laid during the 6 days of exposure, i.e., 96.1 ± 38.5 (tansy) vs 180.7 ± 43.5 (control) ($P < 0.0001$, Mann–Whitney *U* test). A reduction of ca. 50% in the number of eggs had already appeared by 2–4 days of exposure (Fig. 1). This reduction was maintained for 1 day after the flowers had been removed from the tansy treatment: 0.6 ± 1.9 eggs per female (females isolated 6 days with tansy) vs 5.3 ± 6.7 eggs per female (control) ($P < 0.001$, Mann–Whitney *U* test).

Females isolated with tansy had fewer spermatophores (1.3 ± 0.6) than control females (2.1 ± 0.7) (Mann–Whitney *U* test, $P < 0.0001$). To check if the number of spermatophores influenced the number of eggs laid, we calculated the correlation (Spearman rank correlation) between these two parameters in

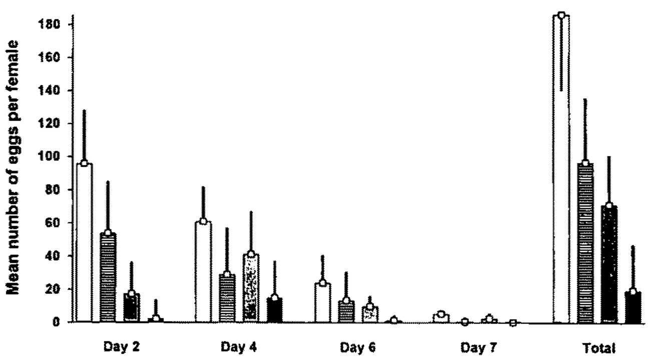

Fig. 1. Oviposition of *Lobesia botrana* females exposed to tansy flowers or tansy odor. Bars represent mean numbers of eggs per female every 2 days (except day 7): white, control; horizontal striped, tansy flowers; gray, tansy essential oil, 20 μl in paraffin oil; black, tansy essential oil, 100 μl in paraffin oil. Vertical lines represent standard deviations. Within each day all differences are significant at $P < 0.001$ (Mann–Whitney *U* test) [except day 6, between tansy flowers (striped) and tansy essential oil, 20 μl (gray), $P < 0.05$; and day 7, where no statistics were calculated (too few eggs)]. Differences between treatments in total, $P < 0.0001$ (Mann–Whitney *U* test).

each of the two groups of females (isolated with or without flowers). Considering exclusively females alive at the end of the control treatment, we found no significant correlation between the number of spermatophores and the total number of eggs laid per female ($r_s = -0.19$). Therefore, the number of eggs laid by females cannot be explained by the number of spermatophores present in the *bursa copulatrix*.

The isolation with tansy flowers decreased female mortality, 33% for control females and 20% for females in the tansy treatment (χ^2, $P < 0.001$), while it strongly increased the mortality of males, from 10% (control) to 50% (tansy) (χ^2, $P < 0.001$) (Table I). Almost all the dead males were found between day 4 and day 6 of the experiment.

Isolation of Adults with Tansy Odors

The mean total number of eggs laid during the 6 days of exposure was reduced compared to the control group according to the dose used. This reduction was even stronger than that observed in the tansy flower treatment. The reduction was 60% at the 20-μl dose, 68.4 ± 28.8 (tansy odor) vs 180.7 ± 43.5 (control), and about 90% at the 100-μl dose (19.1 ± 27.3) (both P's < 0.0001, Mann–Whitney U test). This reduction was maintained the day following the exposure to odor, the mean number of eggs laid by females exposed to tansy odor being very low, 2.6 (20-μl dose) and 0.05 (100-μl) eggs per female. In the latter treatment most of the females' oviducts were full of mature eggs.

The exposure to tansy odor also reduced the mean number of spermato-

Table I. Percentage Mortality of Adults in the Presence and Absence of Tansy Flowers and Tansy Odor*

	Number of replicates	Females: Days of exposure 2	4	6	Total	Males: Days of exposure 2	4	6	Total
Control	30	0	0	33 a	33 a	0	0 a	10 a	10 a
Tansy flowers	20	0	0	20 b	20 b	0	5 a	45 b	50 b
Essential oil, 20 μl	30	0	0	13 b	13 b	5	18 b	67 c	90 c
Essential oil, 100 μl	22	0	4	82 c	86 c	0	55 c	43 b	98 c

*Different letters within columns indicate significant differences (χ^2 analysis, $P < 0.001$) (no letter indicates no difference).

phores per female. At a dilution of 20 μl, we found 1.3 ± 0.7 spermatophore per female, which is almost equivalent to that observed in the tansy flower treatment (nonsignificant, Mann–Whitney *U* test). The exposure to the 100-μl dose caused an ever greater reduction (0.7 ± 0.6 spermatophore per female: different from control and from the previous dose, $P < 0.0001$, Mann–Whitney *U* test).

High rates of male mortality were observed after exposure to tansy odor, between 90 and 98% according to the essential oil dose (Table I). These rates were significantly higher than that obtained in controls (χ^2, $P < 0.001$) and in the tansy treatment (χ^2, $P < 0.001$). Male mortality also started earlier (day 4) than in the control and the tansy flower treatment (Table I). The 20-μl dose had an effect on female mortality similar to that of tansy flowers. A significant increase of female mortality is obtained at the dose of 100 μl of essential oil (Table I).

DISCUSSION

The effect of host-plant odors on the oviposition behavior of phytophagous insects is well documented. The modulations of oviposition preferences elicited by host-plant allelochemicals has been reported in different Lepidoptera species (Auger and Thibout, 1979; Fatzinger and Merkel, 1985; Feeny *et al.*, 1989; Mitchell *et al.*, 1990). Nonhost plants, too, can affect the oviposition behavior of phytophagous insects (Städler, 1974; Lundgren, 1975; Dover, 1985; Rothschild *et al.*, 1988). In our experiments, the presence of tansy flowers and also of tansy odor clearly reduced the number of eggs laid by the females during the 6 days of exposure, but also during the following day, after flowers or odor dispensers were removed. Flowers reduced the number of eggs laid up to about 47%, while the reduction due to odor ranged from 30 to 81% according to the dose. Physiological and behavioral mechanisms directly related to oviposition can be affected by the presence of tansy odor. The facts that the reduction in the number of eggs laid occurred even during the first 2 days of exposure to the flowers and that the reduction was almost constant support the behavioral modification hypothesis. This must be confirmed in further experiments, however, by checking the oocyte production of females.

In the presence of tansy, the number of spermatophores per female was reduced nearly twofold. In Lepidoptera, the number of spermatophores found in the *bursa copulatrix* is often correlated with the number of matings (Thibout, 1978). Therefore, we conclude that the mating activity of males and females isolated with tansy flowers was reduced. This result confirms previous observations (Gabel, 1992) that tansy is probably not a "rendezvous" site for *L. botrana*.

Mating can increase female fecundity in certain insect species (Engelman, 1970; Leopold, 1976). It has been demonstrated in Lepidoptera species that the presence of spermatophores in the *bursa copulatrix* may stimulate oviposition (Norris, 1933; Benz, 1969; 1970; Stockel, 1972; Thibout, 1978). In our experiments, the lack of correlation between the number of spermatophores and the total number of eggs laid per female indicates that the depression in the number of eggs laid by *L. botrana* females cannot be explained by different mating activities. Earlier data report that the number of spermatophores and their size have no influence on the number of eggs laid by *L. botrana* females (Durand, 1983; Torres Villa, 1991). However, the presence of spermatozoa in the spermatheca can stimulate oviposition (Torres Villa, 1991). Most of the females isolated with tansy had spermatophores full of sperm. Therefore, the reduction in the number of eggs in the presence of tansy flowers is probably due to a direct effect on the female, and not to differential mating. This regulation in oviposition can be attributed to volatile chemicals released by tansy essential oil.

Tansy flowers increased male mortality 50% and tansy odor increased it up to 98%. This reduction was not caused by a higher level of sexual activity of males since there was a reduction in the number of spermatophores found in females. Different hypotheses can be formulated to interpret this increase. It could be caused either by chemical toxicity of the flowers or by energy expenditure linked to increased activity. We cannot draw any conclusions concerning eventual plant toxicity and males. However, the hypothesis that tansy flowers repel males can be formulated on the basis of field observations (Gabel, 1992). The fact that male mortality is strongly increased in the presence of odor led us to propose that it is a consequence of sustained locomotor activity of the males provoked by tansy flower odor. Torres Villa (1991) observed increased mortality in males exposed to synthetic sex pheromone; he attributed this effect to an increase in locomotor activity. Sustained locomotor activity could also interfere with mating behavior and be responsible for the reduction in mating activity. The effect of tansy flowers and tansy odor on females mortality is less clear. The presence of tansy flowers and the lowest dose of tansy essential oil slightly reduced female mortality, while the highest concentration caused a mortality of 86%. Data relating egg retention duration and oviposition with female longevity in this species could help with interpretation.

Nonhost plants may represent various ecological values for phytophagous insects. Massive occurrences of *L. botrana* adults (both males and females) on *Taxus baccata* L. have already been observed during the first flight (Paillot, 1913), adults probably searching for refuge during the period of low-density foliage within vineyards or mating sites. In the case of tansy, the hypothesis of a refuge plant or mating site can be rejected for two reasons: (a) Only females occur on the plants, which excludes the role of tansy as a mating site; and (b) flowering tansy occurs in the period July–August, corresponding to a foliage density of vine grapes suitable for EGVM adults. We apparently face a paradox in the biology of *L. botrana*. On the one hand, flowers produce volatiles that attract females (Gabel *et al.*, 1992), and on the other hand, the odor released by flowers reduces oviposition and also mating activity. We could therefore expect *L. botrana* females to visit nonhost plants for feeding or pharmacophagy before ovipositing on vine grapes. In such cases, mechanisms that reduce mating activity or suppress egg laying on a toxic nonhost plant could have adaptive value. We are currently attempting to understand the reasons tansy attracts EGVM females.

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