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Aggregation of *Thaumatomyia glabra* (Diptera: Chloropidae) Males on *Iris* spp. Flowers Releasing Methyl Anthranilate

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Abstract

Aggregations of *Thaumatomyia glabra* (Diptera: Chloropidae) were observed on flowers of *Iris pallida* Lamarck (Asparagales: Iridaceae), whereas no *T. glabra* (Meigen) were observed on nearby *Iris germanica* L. flowers. Sampling of *T. glabra* on *I. pallida* flowers revealed the presence of males only. In a previous study, *T. glabra* males were attracted to methyl anthranilate. We found methyl anthranilate in extracts of *I. pallida* flowers on which *T. glabra* aggregated, but not in extracts of *I. germanica* flowers. Applying methyl anthranilate to *I. germanica* flowers elicited attraction of *T. glabra* to the flowers. This study suggests that *I. pallida* flowers may attract *T. glabra* males to aggregate because they release the known attractant, methyl anthranilate, whereas *I. germanica* flowers may not be attractive because they do not release methyl anthranilate.

Key words: methyl 2-aminobenzoate, floral attractant

Thaumatomyia glabra (Meigen) (Diptera: Chloropidae) is widely distributed throughout North America (Sabrosky 1943). In May 2009, large aggregations of these small flies were observed on flowers of *Iris pallida* Lamarck. (Asparagales: Iridaceae), variegated iris, outside the USDA-ARS Yakima Agricultural Research Laboratory, near Yakima, WA.

Landolt et al. (2000) reported that *T. glabra* males were attracted to blueberry-scented candles. Methyl anthranilate (methyl 2-aminobenzoate) was isolated and identified from the headspace of these candles, and field-trapping experiments showed that traps baited with methyl anthranilate were more effective at capturing *T. glabra* than traps baited with pieces of the scented candle. Both types of traps captured males exclusively, suggesting that methyl anthranilate is a sex attractant for *T. glabra* males.

Preliminary observations indicated that the flies observed on *I. pallida* were all males and that they were aggregated on *I. pallida* flowers and not on nearby *I. germanica* L. flowers. *Iris pallida* flowers have a distinct "grape/berry" odor, reminiscent of methyl anthranilate, suggesting that these flowers might produce methyl anthranilate, and that methyl anthranilate might be responsible for the aggregation of *T. glabra* on *I. pallida* flowers.

The larvae of *T. glabra* are major predators of sugar beet root aphids (Parker 1918, Harper 1961), which are pests of sugar beets, *Beta vulgaris* L. (Caryophyllales: Chenopodiaceae), throughout North America (Harper 1961), causing significant economic loss (Hutchison and Campbell 1994, Summers and Newton 1989). The adult fly emerges during May and June in Montana, at least a month before they begin to lay eggs (Parker 1918). Not much is known about the behavior of *T. glabra* outside of the sugar beet system, and very little is known about the mating behavior and chemical ecology of this aphid predator.

This study investigated whether the flies present on *I. pallida* flowers are indeed exclusively males, whether they are attracted to *I. pallida* flowers and not to *I. germanica*, and whether the presence of these flies on these flowers can be explained by the emission of methyl anthranilate by the flowers.

Materials and Methods

Insect Observations

Iris spp. flowers were inspected at five different locations between May 4 and 15, 2009, and between May 14 and 18, 2012. The flower garden located at the USDA-ARS Yakima Agricultural Research Laboratory (46° 28′13″ N, 120° 22′41″ W) contained *I. pallida*, but not other *Iris* spp. The Yakima Area Arboretum (46° 35′13″ N, 120° 28′20″ W) has an extensive collection with different varieties of *I. germanica*. Three private gardens in the Yakima area, one containing both species (46° 28′27″ N, 120° 22′30″ W), one with only *I. pallida* (46° 35′47″ N, 120° 32′45″ W), and one with only *I. germanica* (46° 35′53″ N, 120° 32′42″ W), were also sampled. Ten flowers per *Iris* cluster were checked for the presence or

absence of *T. glabra*, and the number of flies per flower, date, location, and flower variety were recorded. In the presence of *T. glabra*, flies were observed for evidence of mating behaviors and of interactions between flies, and a sample of flies at each location was captured with a sweep net. Flies were preserved in 70% ethanol for confirmation of identity and determination of sex. Preserved flies were identified and sexed by comparison with specimens in the M. T. James Entomological Collection at Washington State University Entomology Department, Pullman, Washington. Observations documenting fly behavior on *Iris* flowers were conducted for 10 min at 8:00 and again at 16:30 each day between May 4 and 8, 2009. Counts of number of flies per flower were conducted on May 18, 2012.

To confirm that methyl anthranilate can attract *T. glabra* to *I. pallida* flowers, neat methyl anthranilate (purity >98%) from Bedoukian Research, Inc. (Danbury, CT, USA) was applied with a glass micropipette to the beard of *I. germanica* flowers and the number of flies present on flowers was recorded between 14:30 and 15:00 on May 18, 2012. Five pairs of open flowers were selected from a cluster of *I. germanica* flowers. In each of the five pairs, one flower was randomly selected to receive one drop (~20 mg) of methyl anthranilate, whereas the other flower received one drop of deionized water (control). Fifteen minutes after the treatment and control were applied, flowers were observed and the number of flies present on each flower was recorded. During this experiment, *T. glabra* were observed on nearby *I. pallida* flowers, confirming that *T. glabra* were nearby.

Chemical Analysis

Extraction

Iris pallida and *I. germanica* flowers were cut with scissors with 5 cm of stem from a private flower garden near the USDA-ARS laboratory at 9:30 (P.S.T.) on May 15, 2009, and placed in individual polyethylene bags. Picked flowers were taken to the laboratory where each flower was rinsed with 5 ml of toluene through a glass funnel into a 5-ml volumetric flask. Each extraction set consisted of one *I. pallida* flower sample, one *I. germanica* flower sample, and one control, with three replicates of a complete extraction set. The control was toluene run through the glassware in the same manner as for the flower samples. The flower washes were then concentrated at room temperature to 0.5 ml under nitrogen and stored in 1-ml glass vials at −20 °C.

Gas Chromatography with Mass Spectrometry

One microliter aliquots of each sample were analyzed using an Agilent 6890 chromatograph with a 5973 electron impact mass selective detector (Agilent Technologies, Palo Alto, California). This gas chromatograph was equipped with a DB-1 fused silica capillary column (60 m length by 0.25 mm i.d. by 0.25 µm film thickness; J & W Scientific, Folsom, California). The analysis was conducted in splitless injection mode with an inlet temperature of 250 °C. The oven temperature started at 40 °C for 1 min, ramped at 5 °C/min to 200 °C, and held for 7 min. Flower rinses were compared with standards prepared from methyl anthranilate. Retention times as well as mass spectra were compared with purchased standards and NIST library for identity confirmation. MS diagnostic ions (m/z, relative abundance) were 92 (49), 119 (100), 151 (68). Quantification was done by comparison with a four-point calibration curve constructed from injections of 1, 5, 10, and 20 ng of methyl anthranilate. Detection limit was estimated using injections of 1 ng prepared standards.

Statistical Analyses

Statistical analyses were performed using SAS Version 9.1 for Windows (SAS Institute 2002). The numbers of flies present on *Iris* flowers were analyzed with a nonparametric Kruskal–Wallis test. The amount of methyl anthranilate per flower was compared among the two *Iris* species and the control and analyzed with a nonparametric Kruskal–Wallis test.

Results

During this study, *T. glabra* were observed at each of the sites on *I. pallida* flowers (Fig. 1) but never on *I. germanica* flowers, both in 2009 and in 2012. In 2012, the number of *T. glabra* present on *I. pallida* flowers was significantly higher than that found on *I. germanica* flowers for each location (Table 1; $H = 38.42$; $df = 1$; $P < 0.0001$). The flies netted at each site were all confirmed to be *T. glabra* males, both in 2009 and in 2012 (numbers provided only for 2012). No *T. glabra* females were captured during these observations, nor any other fly species. *Thaumatomyia glabra* males did not display any obvious mating or feeding behavior on the flowers, nor did they interact with each other. Flies were observed landing on flower petals and resting their abdomen tips on the petals (Fig. 1). When resting their abdomen tip on the petal, male flies everted, at least partially, their postabdomen, pressing the apex and possibly the post-abdominal eversible vesicles on the flower petal (Fig. 2). The flies took flight when disturbed and then returned to rest on the flower petals.

Fifteen minutes following the application of either methyl anthranilate or water to *I. germanica* flowers, the number of *T. glabra* present on flowers that received methyl anthranilate was significantly higher (mean ± SEM; 3.8 ± 1.0) compared with flowers that received water (0.2 ± 0.2; $H = 4.5$; $df = 1$; $P = 0.03$).

Chemical analyses of *Iris* spp. flower extracts revealed the presence of methyl anthranilate (Fig. 3) in *I. pallida* flowers but not in *I. germanica* flowers or in the control (estimated detection limit = 0.14 µg/flower). The amount of methyl anthranilate extracted from *I. pallida* flowers was 8.9 ± 1.7 µg/flower (mean ± SEM) and was significantly higher than that found in *I. germanica* flowers (0 µg/flower) or control samples (0 µg/sample; $H = 7.6$; $df = 2$; $P = 0.02$).

Discussion

This study showed that *T. glabra* aggregate on *I. pallida* flowers and that these flowers release methyl anthranilate. In contrast, *T. glabra* did not aggregate on *I. germanica* flowers and *I. germanica* did not release methyl anthranilate. However, *I. germanica* flowers onto

Fig. 1. *Thaumatomyia glabra* males on *Iris pallida* flower, May 15, 2009, Yakima County, Washington.

Table 1. Mean (± SEM) number of *Thaumatomyia glabra* observed on open *Iris pallida* and *Iris germanica* flowers and percent of *T. glabra* males netted above *Iris pallida* flowers (May 18, 2012)

	No. of flies per flower		% males netted above
Observation site	*I. germanica*	*I. pallida*	*I. pallida*
USDA-ARS	–	10.7 ± 1.8	100 ($n = 15$)
Yakima 1	0.0 ± 0.0	2.6 ± 0.5	100 ($n = 3$)
Yakima 2	0.0 ± 0.0	–	
Yakima 3	–	1.4 ± 0.4	100 ($n = 18$)
Arboretum	0.0 ± 0.0	–	

Fig. 2. *Thaumatomyia glabra* male showing everted postabdomen with paired eversible vesicles (ev), May 18, 2012, Yakima County, Washington.

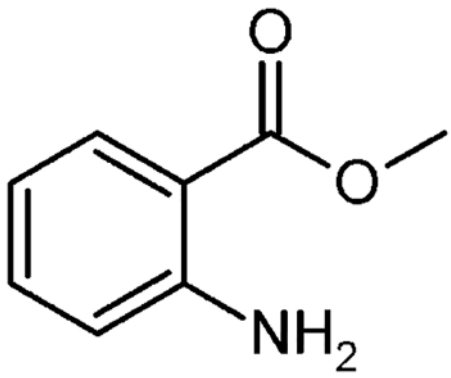

Fig. 3. Structure of methyl anthranilate (methyl 2-aminobenzoate) CAS 134-20-3.

which methyl anthranilate was applied quickly became attractive to *T. glabra* compared with flowers that received water, strongly suggesting that this chemical is responsible for the observed aggregation of *T. glabra* on *Iris* flowers, probably following their attraction to the flower odor. Landolt et al. (2000) showed that methyl anthranilate was an effective sex attractant for *T. glabra* males and also observed the zigzagging upwind flights of the flies toward the odor source. James (2005) also showed a strong attraction of *T. glabra* to traps baited with methyl anthranilate. We speculate that the aggregations of *T. glabra* males are probably the result of attraction of those males to methyl anthranilate in the odor of *I. pallida* flowers. Methyl anthranilate is also an attractant for other insect species, including the soybean beetle, *Anomala rufocuprea* Motschulsky (Imai et al. 1997), brachonid wasps (James 2005), and two species of *Thrips*, *Thripshawaiiensis* Morgan and *Thrips coloratus* Schmutz, along with the *Thrips* spp. parasitoid *Ceranisus menes* (Walker) (Murai et al. 2000). Both sexes of the soybean beetle were attracted to methyl anthranilate (Imai et al. 1997), whereas only *T. glabra* males are attracted to the chemical.

Sorensen and Sorensen (1997) reported observing *T. glabra* males aggregating in flower clusters of *Wisteria sinensis* in April in California. They reported observing one male fly occupying each flower for a 2-wk period and suggested that it may be a lekking behavior. *Wisteria sinensis* is, however, native to China, whereas *T. glabra* has a western Nearctic distribution (Sabrosky 1943), and an evolutionary behavioral association could not be suggested. At that time, the chemical properties of the flowers had not been investigated. Similarly, it is unlikely that *T. glabra* play an evolutionarily significant role in the pollination of *I. pallida* flowers due to nonoverlapping native ranges and the fact that males were not observed feeding on the flowers or contacting the reproductive organs of the flowers. Methyl anthranilate has been reported as being found in the odor profile of several flowers, including those of *Angraecum* (Orchidaceae), *Cimicifuga* (Ranunculaceae), *Citrus* (Rutaceae), *Jasminum* (Oleaceae), *Polianthes* (Asparagaceae), *Robinia* (Fabaceae), and *Spartium* (Fabaceae) (Knudsen et al. 1993). None of these flowers has known associations with *T. glabra*, and it is unknown whether *T. glabra* males are attracted to, or aggregate, on these flowers.

It is still unknown why *T. glabra* males are attracted to methyl anthranilate. In this study, only *T. glabra* males were observed on *I. pallida* flowers, supporting the findings of Landolt et al. (2000), who reported that only *T. glabra* males were attracted to methyl anthranilate-baited traps. Sorensen and Sorensen (1997) also found only males at *W. sinensis* flowers. One possibility is that methyl anthranilate is a component of a female-produced sex pheromone. Another is that male flies are attracted to this chemical to acquire it as a precursor to a male-produced chemical; however, male flies were not observed feeding on the flowers. Sorensen and Sorensen (1997) also suggested that *W. sinensis* flowers may, in some way, indicate to males, a resource where females may be anticipated. According to Parker (1918), female *T. glabra* are capable of discriminating plants infested with sugar beet root aphids from un-infested plants. Males may detect odors that allow them to anticipate females in search of oviposition sites. However, no female *T. glabra* were captured or observed during the course of this study.

During this study, *T. glabra* males were observed everting and pressing the posterior of the abdomen against the petal surface, revealing the eversible vesicles. Post-abdominal eversible vesicles were described by Kotrba for *Thaumatomyia notata* (2009). Kotrba observed possible male calling behavior and suggested that these eversible vesicles may be pheromone glands used to attract females for mating and also both sexes of conspecifics during aggregation. In this study, *T. glabra* males were not observed performing any type of calling behavior, such as raising their abdomen and fanning their wings, as reported for *T. notata* (Kotrba 2009), nor were they observed attempting to mate with other males. Future studies should address the significance of the behavior displayed by male *T. glabra* observed in this study.

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