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Research article

Aggregation Behavior and a Putative Aggregation Pheromone in Sugar Beet Root Maggot Flies (Diptera: Ulidiidae)

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Abstract

Male-biased aggregations of sugar beet root maggot, *Tetanops myopaeformis* (Röder) (Diptera: Ulidiidae), flies were observed on utility poles near sugar beet (*Beta vulgaris* L. [Chenopodiaceae]) fields in southern Idaho; this contrasts with the approximately equal sex ratio typically observed within fields. Peak observation of mating pairs coincided with peak diurnal abundance of flies. Volatiles released by individual male and female flies were sampled from 08:00 to 24:00 hours in the laboratory using solid-phase microextraction and analyzed using gas chromatography/mass spectrometry (GC/MS). Eleven compounds were uniquely detected from males. Three of these compounds (2-undecanol, 2-decanol, and sec-nonyl acetate) were detected in greater quantities during 12:00–24:00 hours than during 08:00–12:00 hours. The remaining eight compounds uniquely detected from males did not exhibit temporal trends in release. Both sexes produced 2-nonanol, but males produced substantially higher (ca. 80-fold) concentrations of this compound than females, again peaking after 12:00 hours. The temporal synchrony among male aggregation behavior, peak mating rates, and release of certain volatile compounds by males suggest that *T. myopaeformis* flies exhibit lekking behavior and produce an associated pheromone. Field assays using synthetic blends of the putative aggregation pheromone showed evidence of attraction in both females and males.

Key words: *Tetanops myopaeformis*, sex pheromone, sugar beet root maggot fly, lekking, *Beta vulgaris*

The sugar beet root maggot, *Tetanops myopaeformis* (Röder) (Diptera: Ulidiidae), is an important pest of sugar beet, *Beta vulgaris* L. (Chenopodiaceae), in the United States and Canada. Larvae feed on roots and root hairs, causing damage that can lead to significant losses in yield of sugar beet roots and allow for secondary infections by pathogens (Hawley 1922, Harper 1962, Bechinski et al. 1993). Management of *T. myopaeformis* primarily targets larvae in the soil, relying heavily upon the use of carbamate and organophosphate insecticides, both of which have worker safety issues and environmental risks (Gupta 2006). Moreover, the carbamate insecticide that has been widely used in Idaho for root maggot management (i.e., aldicarb) is scheduled to be phased out of use by 2018 (Anonymous 2010), and the remaining organophosphates may be at high risk of removal from registration in relation to ongoing enforcement of the Food Quality Protection Act of 1996. As such, there exists an urgent need to develop alternative strategies to manage this pest.

Possible pheromone-based options for improving *T. myopaeformis* management methodology could include the development of attract-and-kill strategies and more effective monitoring tools to optimize the timing and efficacy of conventional and alternative control tools. Aggregations of *T. myopaeformis* flies often are observed on utility poles and other vertical surfaces near sugar beet fields during the afternoon hours; this phenomenon is familiar to both sugar beet producers and scientists studying *T. myopaeformis* (personal observations; Anderson et al. 1994, Chirumamilla et al. 2008), but its underlying mechanisms have not been studied. While collecting flies in large numbers from such aggregations on utility poles in Minidoka County, Idaho, we noted that the flies produced an odor that was detectable to the human observer, prompting an investigation into the possible existence of sex or aggregation pheromones produced by these flies. Neither aggregation behavior nor pheromone production have been studied for any member of the family

Ulidiidae, but sex or aggregation pheromones have been described and exploited in attract-and-kill and mass trapping tactics for some Tephritidae (flies within the superfamily Tephritoidea, which also includes Ulidiidae). For example, a blend of male-specific pheromones that is attractive to females in laboratory bioassays and field trials has been identified for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Baker et al. 1990, Heath et al. 1991, Jang et al. 1994, Howse and Knapp 1996, Jang and Light 1996). In addition, a male-produced pheromone of the papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker (Diptera: Tephritidae), is attractive to conspecific females in the field (Landolt et al. 1988, 1991), and male-produced pheromones have been investigated for use in attract-and-kill or mass trapping control of the Caribbean fruit fly, *Anastrepha suspense* (Loew) (Diptera: Tephritidae) (Nation 1989, Heath et al. 1993). Moreover, a male sex pheromone in combination with a female aggregation pheromone plus a food attractant and phagostimulant has been effective in mass trapping the olive fruit fly, *Bactrocera oleae* (Rossi) (formerly *Dacus oleae*) (Diptera: Tephritidae), in Greece (Haniotakis et al. 1991).

The goals of this study included describing aggregation and mating behavior of *T. myopaeformis* in the field, identifying potential pheromones produced by male and female flies, and testing the attractiveness of a synthetic putative pheromone blend in the field. Over 3 years, we performed observations in commercial sugar beet fields in southern Idaho to document the aggregation and mating behavior of *T. myopaeformis* adults, including the sex ratios and timing of aggregations as well as the onset of mating during the day. Subsequent laboratory experiments focused on identifying the volatile compounds released by both male and female flies and characterizing the timing of their release. Finally, we evaluated responses of female and male flies to synthetic lures containing blends of the putative male-produced pheromone in commercial sugar beet fields in Idaho and North Dakota.

Materials and Methods

Field Observations

Field observations were conducted during 2001, 2003, and 2006 in commercial sugar beet fields in Minidoka County, Idaho. This area historically has experienced the greatest pressure from *T. myopaeformis* in Idaho (Bechinski et al. 1993). Wheat fields nearby and adjacent to the study fields had been planted with sugar beet the previous year and, as such, were probable sources of *T. myopaeformis* flies for our study (Pontius et al. 1983). *Tetanops myopaeformis* is univoltine, overwintering as ultimate-stage larvae, approximately 20 cm deep in the soil. As soil temperatures warm in the spring, larvae migrate to within a few centimeters of the surface to pupate. In Idaho, peak adult emergence typically begins during mid-May to mid-June, which coincides with emergence of sugar beet seedlings and stand establishment (Bechinski et al. 1993).

Observations of *T. myopaeformis* flies within beet fields were conducted throughout full-day periods during 1 June 2001 and 2 June 2003. A single observer walked a 100-m section of crop row at intervals over the day, recording observations on *T. myopaeformis*. Here we report only the sex of the flies observed; their behavioral patterns have been reported elsewhere (Emmert 2003). While slowly walking the 100-m transect, a single observer identified and recorded the sex of *T. myopaeformis* flies encountered based on the shape of the abdomen (Gojmerac 1956). Each fly was followed to observe its behavior until it flew out of sight, at which point the observer continued walking the transect until another fly was located. In 2001, observations were taken hourly from 09:00 to 17:00 hours, and in 2003, observations were taken every two hours from 09:00 to 15:00 hours. Each row observed was selected randomly.

In 2003 and 2006, *T. myopaeformis* flies were observed on utility poles bordering sugar beet fields. In 2003, observations were made on 31 May, 1 June, and 2 June—the days of peak fly activity that year. In 2006, observations were made on 24 and 31 May, and 13 and 23 June; these observations were timed to encompass the *T. myopaeformis* mating period in order to determine if there was a seasonal trend in aggregation behavior. On each observation date, we recorded the number of individual males and females observed and the number of mating pairs observed every 2 h from 08:00 (in 2003) or 10:00 (in 2006) to 20:00 hours. In 2003, observations were made on seven utility poles spaced at approximately 100-m intervals along the perimeter of a single sugar beet field. In 2006, observations were expanded to include five beet fields, with six utility poles sampled in one field and seven in the other four fields (i.e., a total of 34 poles). In 2006, the poles from one field were not sampled at 20:00 on 13 June due to a technical issue. Poles were similarly spaced, and all fields were within an approximate radius of 4 km.

Collection of Maggots and Rearing of Adult Flies

Third-instar *T. myopaeformis* were collected from sugar beet fields in Minidoka County, Idaho during July and August of 2001 and March 2002 for the use in the laboratory during the spring and the summer of 2002. By mid-July, *T. myopaeformis* larvae reach maturity, cease feeding, and enter diapause (Bechinski et al. 1993). Larvae were collected by digging to a depth of approximately 0.5 m and separating them from the excavated soil. During the mid- and late-summer collections, it was often possible to pull up individual standing beet plants and collect larvae from the roots and adjacent soil. After transfer to the laboratory, larvae were cleaned with distilled water and placed onto moist cotton pads in Petri dishes. Larvae were stored in dishes in total darkness at 4 °C until they could be reared to the adult stage for the bioassay. Larvae collected during the summer were stored for a minimum of 6 months to allow for completion of diapause (Callenbach et al. 1957). Larvae collected during the spring were stored for a minimum of one week and up to 6 months before being reared to the adult stage. Previous research has shown that *T. myopaeformis* larvae can be held in cold storage for at least 4 years with no adverse effects on fecundity or fertility (Chirumamilla et al. 2008). To generate adults, we removed larvae from storage at 4 °C and placed them into a growth chamber (Percival, Boone, IA) that was maintained at 20 °C and 16:8 L:D. After larvae pupated, they were placed into individual 30-ml plastic rearing cups to prevent mating from occurring after eclosion. Pupae and adult flies were kept in the same growth chamber. Adults were fed a 10% sucrose solution offered on cotton wicks and were maintained in rearing cups for no more than 8 days before use in experiments.

Fly Volatile Collection and Analysis

Volatiles from the headspace of individual flies were sampled using solid-phase microextraction (SPME) (Supelco, Bellefonte, PA; 65μm polydimethylsiloxane coating). During methods development, SPME fibers were tested for capacity to adsorb 2-nonanol (i.e., the predominant component in headspace of the flies) for over 4 h to confirm that the capacity of the fiber exceeded what was detectable from individual flies. We placed each fly into a glass vial

(25 mm × 95 mm) with a polytetrafluorethylene septum cap and then inserted a SPME fiber into the vial through the septum. A screen inside the vial prevented the fly from contacting the SPME fiber. A new vial was used for each individual fly sampled. Flies in vials were held at 20 °C within a growth chamber (16:8 L:D) during the SPME assays. Volatiles were collected from each fly during four 4-h blocks of time: 08:00–12:00, 12:00–16:00, 16:00–20:00, and 20:00–24:00 hours. Each SPME fiber was left in the vial for the entire 4-h sampling period, then removed and immediately inserted into the injector of a gas chromatograph. We sampled seven male flies and five females. Gas chromatography/mass spectrometry (GC/MS) was performed on all SPME fiber samples using a Hewlett-Packard 6890 Series gas chromatograph and a Hewlett-Packard 5973 Mass Selective Detector (Agilent Technologies, San Jose, CA). Compounds were tentatively identified using coupled GC/MS and spectral matches (> 90%) with the NIST library of mass spectra. Confirmation of 2-nonanol, pentadecane, and tetradecane spectra was achieved by comparison with commercial standards (Sigma-Aldrich, St. Louis, MO). The GC was fitted with a split/splitless injector operated in splitless mode and a Hewlett Packard-5MS 5% phenyl methyl siloxane capillary column (30 m × 250 μm × 0.25 μm). The GC oven temperature was programmed to increase from 50 to 300°C at a rate of 20°C/min, and the injector temperature was set to 250°C. Helium was used as the carrier gas with a flow rate of 2.0 ml per minute. To calculate concentrations of the compounds within samples, we used an external standard of racemic 2-nonanol and a previously calculated standard curve for 2-nonanol. The enantiomeric identity of 2-nonanol detected from flies was established based on the retention time of authentic standards of the *R* and *S* forms. This was carried out in a separate analysis with the same instrument and conditions, but using a chiral column (Agilent Cyclodex-B, J&W chiral column, 30 m × 0.25 mm, 0.25 μm film [10.5% Beta-Cyclodextrin], Agilent Technologies, Santa Clara, CA). SPME extracts of headspace from a group of six additional males were used to confirm that *T. myopaeformis* flies emitted only the *R* form.

Field Testing of Putative Pheromone

During 2016, field trials were conducted in Idaho and North Dakota to evaluate attractiveness of different doses of a synthetic blend of the male-produced putative pheromone. We made pheromone blends using all commercially available compounds that we collected from males, including compounds that were exclusively male-produced as well as those that were produced by both sexes. Blends were composed of the following compounds (relative percentage by mass in parentheses, based on relative quantity collected from male-produced volatiles): (*R*)-(−)-2-nonanol (93.9%), 2-nonanone (2.4%), dodecanal (1.2%), 1-nonanol (1.0%), 6,10-dimethyl-5,9-undecadien-2-one (0.8%), 2-dodecanol (0.1%), decanal (0.2%), 2-decanol (0.1%), and undecanal (0.1%). All compounds were mixed thoroughly using mineral oil as a carrier and dispensed onto cotton dental wicks (8 mm diam. × 38 mm long) (e.g., Leskey et al. 2001). The following doses were tested: 0, 8.3 (low), 16.5 (medium), and 33.1 (high) μg of total pheromone. Non-baited orange sticky traps on wooden stakes have been used widely for monitoring *T. myopaeformis* in Idaho and the Red River Valley of North Dakota and Minnesota (Blickenstaff and Peckenpaugh 1976). However, we used a trap design that featured a low-profile white sticky card in order to reduce interference with farming operations in the field as well as to limit the influence of color preferences on trap captures. Dental wick lures were affixed to white sticky cards (76 mm × 127 mm; Great Lakes IPM, Inc., Vestaburg, MI) by inserting each wick partially (i.e., about 4 mm) into a 7-mm diameter hole ca. 2 cm from a short edge of the card. We created the holes for wicks using a conventional paper punch. Traps were oriented with a long edge parallel to the ground and each trap was clipped to a wooden stake (30 cm long × 2.9 cm wide × 0.3 cm thick) by using a binder clip. We placed traps within sugar beet rows between two plants. Cards were fastened to the stakes such that their bottom edge was positioned just above the plant canopy and the side of the card from which the long end of the dental wick protruded faced northward. Traps were arranged in a randomized complete block design in a roughly 30 × 30-m grid with six or eight replicates, depending on the availability of space at each site. We deployed traps before noon on each sampling day and retrieved them 24 h later. All *T. myopaeformis* flies captured on cards were identified subsequently to gender in the laboratory. We ran the experiment on two dates in both Idaho (20 and 22 June 2016) and North Dakota (17 and 27 June 2016).

Data Analysis

All analyses were carried out using SAS (SAS Institute, 2015). Using analysis of variance (ANOVA), separately for each year, we compared the number of flies of each gender, the proportion of males, and the number of mating pairs for the effect of observation time (08:00–20:00 hours in 2003 and 10:00–20:00 hours in 2006). For this analysis, the effects of date and observation time (nested within date) were examined. For both study years, each utility pole sampled was considered a replicate ($n = 7$ in 2003; $n = 34$ in 2006). A repeated-measures ANOVA (split plot in time) was used to examine the effect of gender on concentration of 2-nonanol and 2-nonanone, with gender as the between-subjects factor and time block and its interaction with gender as within-subject factors (PROC GLM, SAS Institute 2015). Mean separation was carried out using Fisher's Protected Least Significant Difference (LSD) test. To achieve normality and equal variance, data were ln transformed. Non-transformed data for less abundant 2-undecanol, 2-decanol, and secnonyl acetate (only detected from males) were subjected to ANOVA, and means were separated using Fisher's LSD test (PROC GLM, SAS Institute 2015).

Sticky trap data were analyzed using two-way ANOVA (PROC GLM, SAS Institute 2015), which was conducted separately for females and males at each trial location (i.e., Idaho and North Dakota). Lure dose, date, and their interaction were included in the model. Data were square root-transformed to achieve normality and equal variance. Where ANOVA showed significant differences, Fisher's LSD test was used to discriminate among treatments. For all analyses, the significance level was set at $\alpha = 0.05$.

Results

Field Observations

On each sample date, the overall male:female ratio of flies in sugar beet fields was slightly female-biased. In the 2001 sample, which included a total of 270 flies, the overall male:female ratio was 0.78:1; in the 2003 sample, which included a total of 80 flies, the male:female ratio was 0.67:1. In each sample, the male:female ratio fluctuated but was only rarely ≥ 1.0 (Table 1).

Fly densities per utility pole changed significantly over time (Tables 2 and 3), increasing throughout each day to a maximum at 16:00 hours in 2003 (32.8 flies per pole) and at 14:00 hours in 2006 (8.5 flies per pole); thereafter, the numbers of flies observed on poles

Table 1. Sex ratios (male:female) of *T. myopaeformis* flies observed on plants or the soil surface within sugar beet fields over time in single-day observations on 1 June 2001 and 2 June 2003

Time (hours)	Year	
	2001	2003
09:00	2.17	0.63
10:00	0.77	–
11:00	0.76	0.30
12:00	1.00	–
13:00	0.44	0.64
14:00	0.45	–
15:00	0.00	1.00
16:00	0.14	–

Table 2. ANOVA for the effect of time of day (nested within sample date) on observations of *T. myopaeformis* flies on utility poles in 2003

Source of variation	df	*F*	*P*
Total flies			
Model	10	11.2	<0.0001
Error	26		
Date	2	3.6	0.042
Time (date)	8	11.0	<0.0001
Mating pairs			
Model	10	1.2	0.355
Error	26		
Date	2	0.4	0.701
Time (date)	8	1.3	0.292
Proportion of males			
Model	10	12.9	<0.0001
Error	26		
Date	2	12.2	0.0002
Time (date)	8	12.3	<0.0001

declined (Fig. 1). The male:female ratio on poles was always >1, with maximum proportions coinciding with the periods of greatest total fly numbers and maximum mating rates (14:00–18:00 hours in both years; Fig. 1). The effect of time of day on the proportion of males observed was statistically significant during both study years (Tables 2 and 3). The number of mating pairs observed changed significantly over time during 2006 (Table 3), increasing after 14:00 hours, peaking at 18:00 hours, and declining by 20:00 hours (Fig. 1); patterns were similar during 2003 (Fig. 1), but not statistically significant (Table 2). The proportion of females observed to be mating on utility poles peaked at ca. 93% during 14:00–16:00 hours during both years (data not shown).

Fly Volatile Collection and Analysis

Volatiles were detected in headspace of both male and female flies. Eleven compounds, eight of which were identifiable based on their mass spectra, were uniquely present in male fly headspace (Table 4). These compounds varied with regard to the levels detected from males over time (Table 5 and Figs. 2 and 3). Of the nine compounds detected in the headspace of both sexes (Table 4), two were much more abundant in male headspace (Fig. 2). The compound 2-nonanol was present in approximately 80-fold greater quantities in male headspace (Fig. 2A). Production of 2-nonanol by males changed significantly ($F = 21.1$; df = 3,24; $P = 0.0001$) over time, being much greater in samples after 12:00 hours; in contrast, production of this

Table 3. ANOVA for the effect of time of day (nested within sample date) on observations of *T. myopaeformis* flies on utility poles in 2006

Source of variation	df	*F*	*P*
Total flies			
Model	21	11.9	<0.0001
Error	719		
Date	3	0.9	0.431
Time (date)	18	9.2	<0.0001
Mating pairs			
Model	21	8.1	<0.0001
Error	719		
Date	3	0.2	0.916
Time (date)	18	7.9	<0.0001
Proportion of males			
Model	21	9.2	<0.0001
Error	643		
Date	3	3.2	0.023
Time (date)	18	10.6	<0.0001

compound by female flies remained similar across all sample periods. Release of 2-nonanol from male flies peaked during the 16:00–20:00 hours time period (Fig. 2A). Patterns in production of 2-nonanone were similar to 2-nonanol (Fig. 2B), but differences between male and female flies and change in the production by males at different times were not statistically significant (gender: $F = 0.28$; df = 1,38; $P = 0.601$; time: $F = 1.57$; df = 3,38; $P = 0.300$).

Temporal trends also occurred in the pattern of production of 2-undecanol, 2-decanol, and sec-nonyl acetate by males. For example, these compounds were released at low levels during morning time intervals and at much higher levels (7-fold) during the afternoon and evening (Fig. 3). These three compounds were not detected from females. For the following compounds, release rates from males varied among individuals (Table 5), but did not change significantly over time: tetradecane; dodecanal; 6,10-dimethyl-5,9-undecadien-2-one; pentadecane; 2-dodecanol; 1-nonenol or 2-nonenol; and the three other unidentified compounds detected only from male flies (data not shown).

Field Testing of Putative Pheromone

In the Idaho trial, female trap captures differed significantly among doses of the putative pheromone as well as between dates and by the dose × date interaction (Table 6). On both dates, females were significantly more abundant on all traps baited with the putative pheromone relative to the check (Fig. 4). On the first date, the high and medium doses captured more flies than the low dose; on the second date, the number of females captured did not differ among the three doses of putative pheromone (Fig. 4). Male trap captures in the Idaho trial showed a response that was similar to that of females, with trap captures differing significantly by dose, date, and their interaction (Table 6). As was the case with females, males were significantly more abundant on all traps baited with the putative pheromone relative to the check treatment on both sampling dates (Fig. 4). On the first date, trap captures did not differ among the three doses of putative pheromone; on the second date, the low-dose traps captured more than high-dose traps, and captures on the medium dose did not differ between the other two pheromone doses (Fig. 4).

In the North Dakota trial, female trap captures differed significantly by dose, date, and their interaction (Table 6). On the first date, no response to pheromone doses was evident; trap captures did

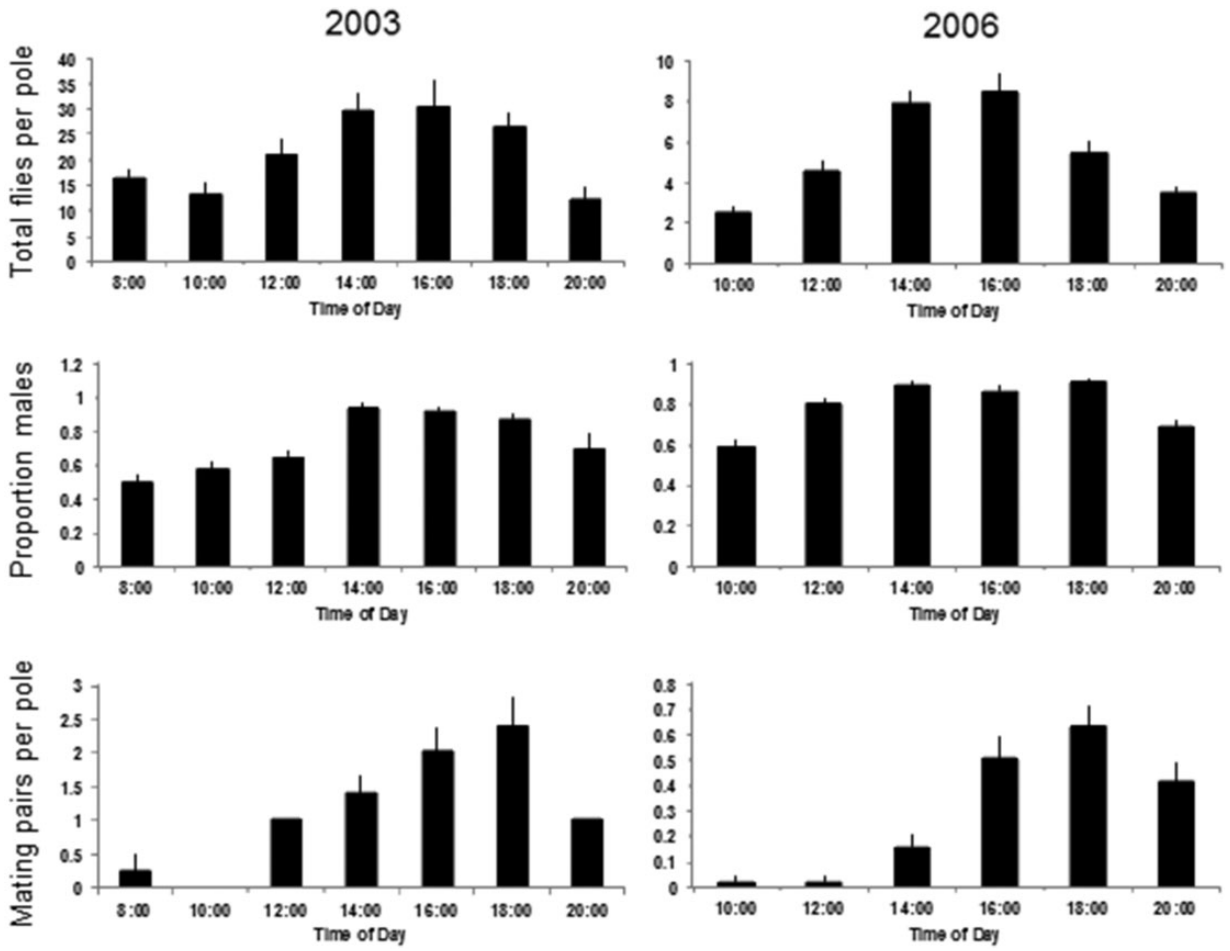

Fig. 1. Field observations of *T. myopaeformis* flies on utility poles adjacent to sugar beet fields of the mean ± SEM number of flies per pole, proportion of male flies, and number of mating pairs from 08:00 to 20:00 hours (2003) and from 10:00 to 20:00 hours (2006).

Table 4. Volatile compounds detected in headspace of *T. myopaeformis* flies

Elution order	Compound	Diagnostic ions[a]	Males	Females
1	Octanal		×	×
2	Undecane		×	×
3	Unknown	124(7), 98(32), 83(8), 69(45), 56(75), 45(100), 39(16), 32(4)	×	
4	Unknown	124(8), 109(3), 98(34), 83(8), 69(43), 56(75), 45(100), 32(3)	×	
5	2-Nonanone		×	×
6	Nonanal		×	×
7	1-Nonanol		×	×
8	(*R*)-(−)-2-nonanol		×	×
9	1-Nonenol or 2-nonenol		×	
10	Decanal		×	×
11	2-Decanol		×	
12	2-Undecanol		×	
13	Sec-nonyl acetate		×	
14	2-Dodecanol		×	
15	Undecanal		×	×
16	Tetradecane		×	
17	Dodecanal		×	×
18	Unknown	141(14), 127(13), 113(23), 99(18), 85(44), 78(14), 71(74), 57(100), 43(59), 32(13)	×	
19	6,10-Dimethyl-5,9-undecadien-2-one		×	
20	Pentadecane		×	

[a] Provided only for unknowns.

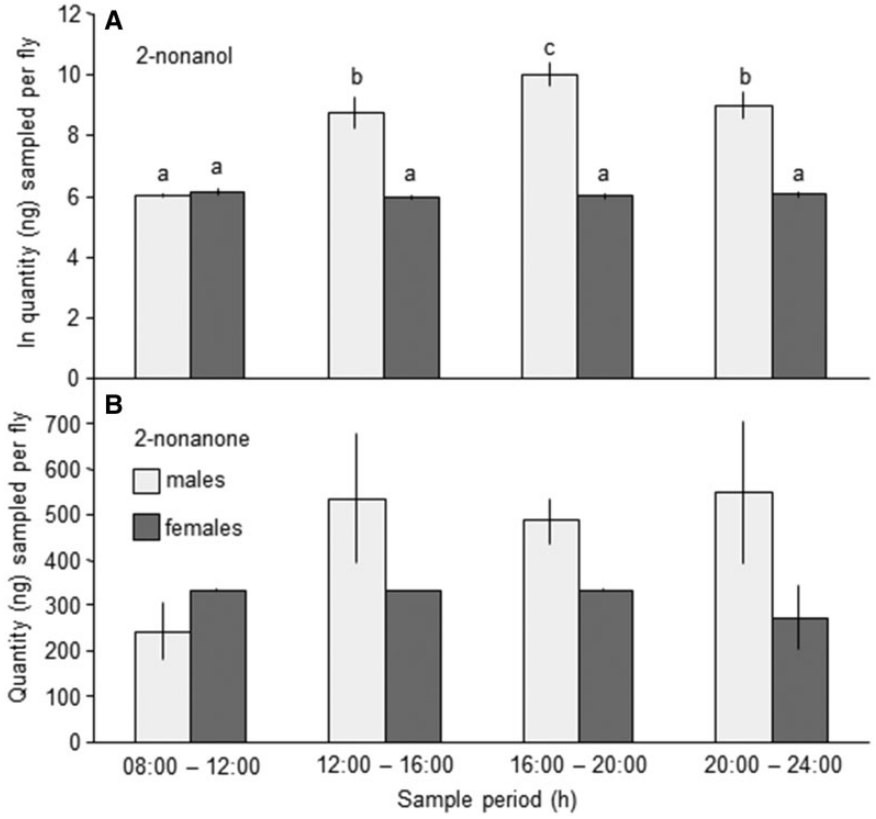

Fig. 2. Quantity of 2-nonanol (A) and 2-nonanone (B) sampled in headspace volatiles from male and female *T. myopaeformis* flies in the laboratory over 4-h time intervals throughout the day. Error bars are standard errors of means. Means sharing a letter are not significantly different (Fisher's LSD test; $\alpha = 0.05$). Data were ln-transformed for analyses. No significant differences were observed for 2-nonanone. $N = 7$ male flies; $N = 5$ female flies.

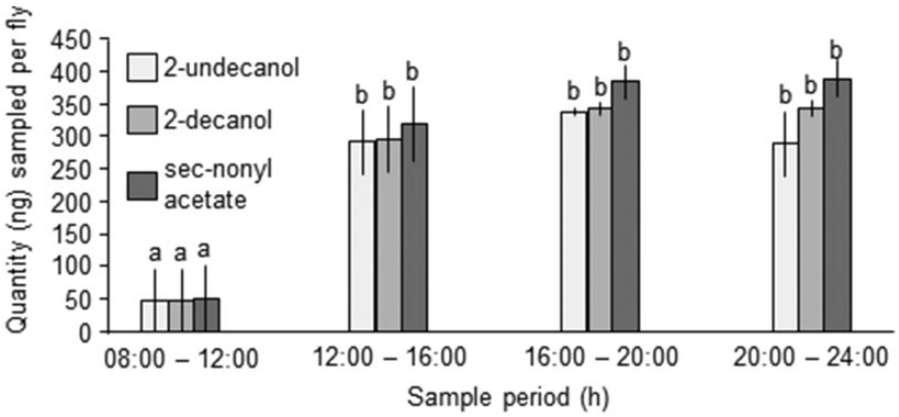

Fig. 3. Quantity of 2-undecanol, 2-decanol, and sec-nonyl acetate sampled in headspace volatiles from male *T. myopaeformis* flies in the laboratory over 4-h time intervals throughout the day. Error bars are standard errors of means. Means sharing a letter are not significantly different (Fisher's LSD test; $\alpha = 0.05$). $N = 7$ male flies. Females produced no detectable levels of any of these compounds.

not differ among the check treatment and the low and medium dose treatments, but captures on the high treatments were significantly lower than those on the medium treatment (Fig. 5). On the second date, female captures were significantly higher on the three pheromone doses (which did not differ among each other) than on the check treatment (Fig. 5). Males in the North Dakota trial only showed a statistically significant response with respect to date (Table 6), which reflected differences in overall trap captures between the two sample dates (Fig. 5).

Discussion

The tendency of *T. myopaeformis* flies to congregate on vertical objects has been well known by sugar beet producers and entomologists for decades and is still widely exploited in Idaho and North Dakota to monitor fly populations based on captures on sticky stake traps (Blickenstaff et al. 1981, Bechinski et al. 1993). Our observations show that these aggregations were predominantly males, with male:female ratios increasing on utility poles in the afternoon. In contrast, the male:female ratio for flies within sugar beet fields remained near or below unity. The

Table 5. Range of quantities (ng per fly) of some minor components sampled from the headspace of male *T. myopaeformis* flies over time

Peak	Retention time (min)	08:00–12:00 hours	12:00–16:00 hours	16:00–20:00 hours	20:00–24:00 hours
Unknown 1	7.540	0–5.71	0–6.10	0–2.85	0–13.01
Unknown 2	7.692	0–10.69	0–50.21	0–59.52	0–38.15
Tetradecane	13.042	0–5.07	0–17.97	0–4.25	0–14.29
Dodecanal	13.375	0–2.33	0–9.47	0–7.65	0–36.03
Unknown 3	13.800	0	0	0–5.34	0–25.28
6,10-Dimethyl-5,9-undecadien-2-one	14.217	0–5.71	0–20.15	0–6.72	0–15.00
Pentadecane	14.600	0–13.39	0–40.49	0–10.27	0–53.62

Table 6. ANOVAs comparing white sticky trap captures of female or male *T. myopaeformis* (square root-transformed) among putative pheromone dose treatments over time for Idaho and North Dakota field trials

Source of variation	Numerator df	Denominator df	*F*	*P*
Idaho trial: female trap captures				
Block	7	41	0.95	0.480
Pheromone dose	3	41	70.5	<0.0001
Date	1	41	20.5	<0.0001
Pheromone dose × Date	3	41	6.6	0.001
Male trap captures				
Block	7	41	0.85	0.552
Pheromone dose	3	41	26.7	<0.0001
Date	1	41	17.0	0.0002
Pheromone dose × Date	3	41	3.3	0.031
North Dakota trial: female trap captures				
Block	5	35	3.3	0.016
Pheromone dose	3	35	7.7	0.0005
Date	1	35	463.3	<0.0001
Pheromone dose × Date	3	35	11.5	<0.0001
Male trap captures				
Block	5	35	1.7	0.153
Pheromone dose	3	35	2.3	0.097
Date	1	35	216.0	<0.0001
Pheromone dose × Date	3	35	1.7	0.194

females present in these male-dominated groups were observed to be mating at higher rates during the afternoon hours. Harper (1962) reported that *T. myopaeformis* flies in southern Alberta were detectable in the field after 10:00 hours, with a peak activity between 12:00 and 13:00 hours. Emmert (2003) found a similar diurnal pattern in fly activity in Idaho sugar beet fields and also found that within-field mating occurs more frequently during afternoon hours. Thus, the increased number of flies on utility poles and increased incidence of mating in the afternoon parallels a general increase in *T. myopaeformis* fly activity as the day progresses. In the sugar beet growing regions of Idaho and North Dakota—vast, agriculturally dominated, level valleys—utility poles often represent the most distinctive vertical features of the landscape. Male-biased aggregations of flies may also be found on tree boles, fence posts, and similar objects during the afternoon hours (E.J.W. and M.A.B., personal observations). The importance of such vertical structures in facilitating the formation of aggregations remains to be investigated. Although aggregations and high densities of mating pairs often are observed on such vertical structures, mating pairs also can be observed in the field on sugar beet plants (Emmert 2003). Other more subtle features of the landscape also might attract aggregations. For example, during afternoon hours, male flies can sometimes be seen gathered in small groups on the soil surface (E.J.W., personal observations), possibly responding to currently undetermined visual or physical cues.

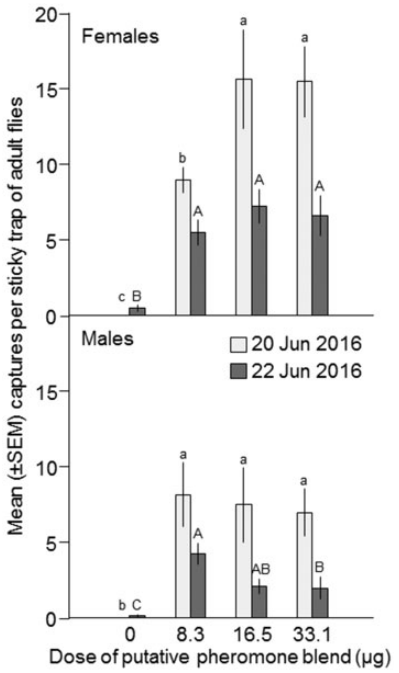

Fig. 4. Responses of female and male *T. myopaeformis* flies in the field to different doses of a nine-component putative pheromone blend on white sticky cards placed in a sugar beet field on two different dates in Idaho. Means for each date within each panel sharing a letter are not significantly different (Fisher's LSD test; $\alpha = 0.05$). Data were square root-transformed for analyses; non-transformed values are shown. $N = 6$ or 8 replicates per treatment on the first and second date, respectively.

The patterns described here suggest that *T. myopaeformis* flies produce an aggregation pheromone and develop collective male territories (i.e., leks) to which females may be attracted for mating (Emlen and Oring 1977). Lekking behavior is reported for several species of Tephritidae, including the Mediterranean fruit fly (*C. capitata*), the Mexican fruit fly (*A. ludens*), the Caribbean fruit fly (*Anastrepha suspensa*), and the South American fruit fly (*A. fraterculus*) (Prokopy and Hendrichs 1979, Burk 1983, Malavasi et al. 1983, Morgante et al. 1983, Robacker and Hart 1985). Lekking behavior sometimes is associated with production of male pheromones, particularly in the Diptera (e.g., Nation 1990, Jones and Hamilton 1998, Widemo and Johansson 2006, Cabrera and Jaffe 2007, Segura et al. 2007). Although most male-produced sex attractants bring females to a resource, such as an oviposition site, leks are aggregations that are not associated with a resource, serving primarily as a means of mate location and mate selection (Landolt 1997). Observations from our studies are consistent with a pheromone-mediated diurnal lekking phenomenon in *T. myopaeformis* flies. In particular, we observed male-only release of certain volatile

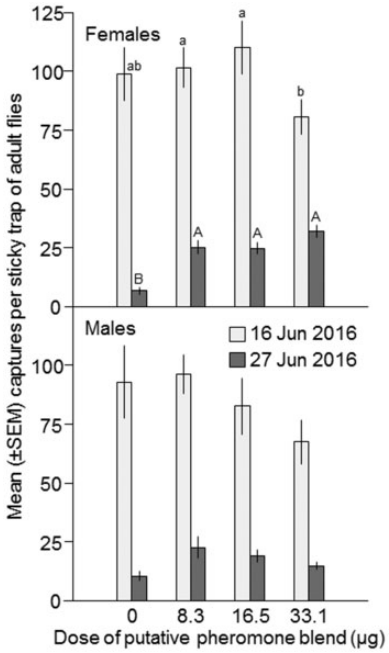

Fig. 5. Responses of female and male *T. myopaeformis* flies in the field to different doses of a nine-component putative pheromone blend on white sticky cards placed in a sugar beet field on two different dates in North Dakota. Means for each date within each panel sharing a letter are not significantly different (Fisher's LSD test; $\alpha = 0.05$). Data were square root-transformed for analyses; non-transformed values are shown. $N = 6$ replicates per treatment.

compounds as well as an increased release of some of these compounds during afternoon hours when male aggregations and increased mating rates occur. Similar diurnal peaks of mating behaviors have been reported in several tephritid flies (Prokopy et al. 1972, Kaspi and Yuval 1999, Aluja et al. 2001, Meats et al. 2003, Castrejón-Gómez et al. 2007).

Our field assays using a synthetic blend of the putative pheromone showed evidence of male attraction only in the Idaho trails. Fly abundance was considerably higher at the North Dakota site, so it is possible that the high density of flies and associated high concentrations of naturally produced pheromone overwhelmed any attractive effect that the synthetic pheromone lures may have had. The putative pheromone blend used in our field assays was based on sampling of volatiles from male flies collected in Idaho; therefore, it could be that the different responses between states reflect population-level differences in pheromone production or in how the flies respond to the pheromone. In any event, females in both Idaho and North Dakota populations showed clear evidence of attraction to the blend that we tested in the field.

The behavioral and volatile release patterns we observed in *T. myopaeformis* provide strong evidence for a pheromone-mediated mating system in this species, which represents the first recorded evidence of pheromones in the family Ulidiidae. Our results with fly headspace analysis suggest a putative pheromone blend for *T. myopaeformis* that includes (*R*)-(−)-2-nonanol, 2-undecanol, 2-decanol, and sec-nonyl acetate in the ratio in which they occur in male headspace during the afternoon: 104:1:1:1. Field assays using a blend of these and five other minor components of male-produced volatiles showed evidence of female attraction across three of the four site and date combinations. We observed female attraction to the synthetic lures for all dates and sites except for the date on which fly abundance at the North Dakota site was very high. As speculated above for the lack of male response in North Dakota, it is possible that the high fly populations on this date overwhelmed any attractiveness of our synthetic lures. It also is possible that female attraction to males is reduced when males are extremely abundant; in some insects, an abundance of males can be associated with "harassment" for additional copulations that can reduce female longevity and fecundity (e.g., Wenninger and Hall 2008). All pheromone doses tested exhibited similar levels of attraction of females, with no apparent dose–response observed, so future studies should evaluate whether captures increase with higher doses, especially at sites with high population densities of *T. myopaeformis*.

The results presented here demonstrate attraction of females and—at least in Idaho—males to a synthetic blend of volatiles identified from males. Future research should clarify factors that affect attractiveness of the synthetic pheromone blend as well as its potential practical use in management strategies. For example, our lures were composed of all of the male-produced volatiles that were commercially available; however, it is possible that all components within our pheromone blend are not necessary to achieve attraction. Moreover, our traps were designed to limit the influence of color preferences; exploiting known color preferences of *T. myopaeformis* (Blickenstaff and Peckenpaugh 1976) in a pheromone trap almost certainly would increase trap captures. Increasing captures of female flies by improving trap design, as well as optimizing the dose and the blend of pheromone lures, likely will lead to the development of better tools to monitor and manage this important pest of sugar beet.

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References Cited

Aluja, M., N. Lozada, J. Piñero, A. Birke, V. Hernández-Ortiz, and F. Díaz-Fleischer.2001. Basic behavior of *Rhagoletis turpiniae* (Diptera: Tephritidae) with comparative notes on the sexual behavior of *Rhagoletis pomonella* and *Rhagoletis zoqui*. Ann. Entomol. Soc. Am. 94: 268–274.

Anderson, A. W., R. B. Carlson, R. Dregseth, A. Schroeder, and L. J. Smith.1994. Control of the sugarbeet root maggot in the Red River Valley. 1994 Sugarbeet Res. Ext. Rep. 25: 131–163.

Anonymous. 2010., Agreement to terminate all uses of aldicarb. Available online at: https://archive.epa.gov/pesticides/reregistration/web/html/aldicarb_fs.html.

Baker, P. S., P. E. Howse, R. N. Ondarza, and J. Reyes.1990. Field trials of synthetic sex pheromone components of the male Mediterranean fruit fly (Diptera: Tephritidae) in southern Mexico. J. Econ. Entomol. 83: 2235–2245.

Bechinski, E. J., R. L. Stoltz, and J. J. Gallian.1993. Integrated pest management guide for sugarbeet root maggot. *In* University of Idaho Current Information Series, vol 999, pp. 1–8.

Blickenstaff, C. C., and R. E. Peckenpaugh.1976. Sticky stake traps for monitoring fly populations of the sugarbeet root maggot and predicting maggot populations and damage ratings. J. Am. Soc. Sugar Beet Technol. 19: 112–117.

Blickenstaff, C. C., R. E. Peckenpaugh, D. Traveller, and J. D. Stallings.1981. Insecticide tests for control of the sugarbeet root maggot, 1968–78. *In* USDA-SEA, Agricultural Research Results, vol. 18, pp. 1–75.

Burk, T.1983. Behavioral ecology of mating in the Caribbean fruit fly, *Anastrepha suspensa* (Loew), (Diptera: Tephritidae). Fla. Entomol. 66: 330–344.

Cabrera, M., and K. Jaffe.2007. An aggregation pheromone modulates lekking behavior in the vector mosquito *Aedes aegypti* (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 23: 1–10.

Callenbach, J. A., W. L. Gojmerac, and D. B. Ogden.1957. The sugarbeet root maggot in North Dakota. J. Am. Soc. Sugar Beet Technol. 9: 300–304.

Castrejón-Gómez, V. R., S. Láscares, E. A. Malo, J. Toledo, and J. C. Rojas.2007. Calling behavior of mass-reared and wild *Anastrepha serpentina* (Diptera: Tephritidae). J. Econ. Entomol. 100: 1173–1179.

Chirumamilla, A., G. D. Yocum, M. A. Boetel, and R. J. Dregseth.2008. Multi-year survival of sugarbeet root maggot (*Tetanops myopaeformis*) larvae in cold storage. J. Insect Physiol. 54: 691–699.

Emlen, S. T., and L. W. Oring.1977. Ecology, sexual selection and the evolution of mating systems. Science. 197: 215–223.

Emmert, S. Y.2003. Volatile attractants for the sugar beet root maggot fly, *Tetanops mypaeformis* (von Röder). M.S. thesis, University of Idaho, Moscow.

Gojmerac, W. L.1956. Description of the sugar beet root maggot, *Tetanops myopaeformis* (von Roder), with observations on reproductive capacity. Entomol. News. 6: 203–210.

Gupta, R. C.2006. Toxicity of organophosphate and carbamate compounds. Elsevier Academic Press, London, UK.

Haniotakis, G., M. Kozyrakis, T. Fitsakis, and A. Antonidakim.1991. An effective mass trapping method for the control of *Dacus oleae* (Diptera: Tephritidae). J. Econ. Entomol. 84: 564–569.

Harper, A. M.1962. Life history of the sugar beet root maggot *Tetanops myopaeformis* (Röder) (Diptera: Otitidae) in southern Alberta. Can. Entomol. 94: 1334–1340.

Hawley, I. M.1922. The sugar-beet root maggott *Tetanops aldrichi* (Hendel), a new pest of sugar-beets. J. Econ. Entomol. 15: 388–391.

Heath, R. R., P. J. Landolt, J. H. Tumlinson, D. L. Chambers, R. E. Murphy, R. E. Doolittle, B. D. Dueben, J. Sivinski, and C. O. Calkins.1991. Analysis, synthesis, formulation, and field testing of three major components of male Mediterranean fruit fly pheromone. J. Chem. Ecol. 17: 1925–1940.

Heath, R. R., N. D. Epsky, P. J. Landolt, and J. Sivinski.1993. Development of attractants for monitoring Caribbean fruit flies (Diptera: Tephritidae). Fla. Entomol. 72: 233–244.

Howse, P. E., and J. J. Knapp.1996. Pheromones of Mediterranean fruit fly: presumed mode of action and implications for improved trapping techniques, pp. 91–9. *In* B. A. McPheron and G. J. Steck (eds.), Fruit fly pests a world assessment of their biology and management. St. Lucie Press, Florida.

Jang, E. B., and D. M. Light.1996. Attraction of female Mediterranean fruit flies to identify components of the male-produced pheromone: qualitative aspects of major, intermediate, and minor components, pp.115–21. *In* B. A. McPheron and G. J. Steck (eds.), Fruit fly pests a world assessment of their biology and management. St. Lucie Press, Florida.

Jang, E. B., D. M. Light, R. G. Binder, R. A. Flath, and L. A. Carvalho.1994. Attraction of female Mediterranean fruit flies to the five major components in a laboratory flight tunnel. J. Chem. Ecol. 20: 9–20.

Jones, T. M., and G. C. Hamilton.1998. A role for pheromones in mate choice in a lekking sandfly. Anim. Behav. 56: 891–898.

Kaspi, R., and B. Yuval.1999. Mediterranean fruit fly leks: factors affecting male location. Funct. Ecol. 13: 539–545.

Landolt, P. J.1997. Sex attractant and aggregation pheromones of male phytophagous insects. Amer. Entomol. 43: 12–22.

Landolt, P. J., R. R. Heath, H. R. Agee, J. H. Tumlinson, and C. O. Calkins.1988. Sex pheromone-based trapping system for papaya fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 81: 1163–1169.

Landolt, P. J., M. Gonzalez, D. L. Chambers, and R. R. Heath.1991. Comparison of field observations and trapping of papaya fruit fly in papaya plantings in Central America and Florida. Fla. Entomol. 74: 408–414.

Leskey, T. C., R. J. Prokopy, S. E. Wright, P. L. Phelan, and L. W. Haynes.2001. Evaluation of individual components of plum odor as potential attractants for adult plum curculios. J. Chem. Ecol. 27: 1–17.

Malavasi, A., J. S. Morgante, and R. J. Prokopy.1983. Distribution and activities of *Anastrepha fraterculus* (Diptera: Tephritidae) flies on host and nonhost trees. Ann. Entomol. Soc. Am. 76: 286–292.

Meats, A., N. Pike, X. An, K. Raphael, and W. Y. S. Wang.2003. The effects of selection for early (day) and late (dusk) mating lines of hybrids of *Bactrocera tryoni* and *Bactrocera neohumeralis*. Genetica. 119: 283–293.

Morgante, J. S., A. Malavasi, and R. J. Prokopy.1983. Mating behavior of wild *Anastrepha fraterculus* (Diptera: Tephritidae) on a caged host tree. Fla. Entomol. 66: 234–241.

Nation, J. L.1989. The role of pheromones in the mating system of *Anastrepha* fruit flies, pp. 189–205. *In* A.S. Robinson, and G. Hooper (eds.), Fruit flies: their biology, natural enemies and control. Elsevier, Amsterdam.

Nation, J. L.1990. Biology of pheromone release by male Caribbean fruit flies, *Anastrepha suspensa* (Diptera: Tephritidae). J. Chem. Ecol. 16: 553–572.

Pontius, J. S., R. B. Carlson, and A. W. Anderson.1983. Diel periodicity of adult flight of the sugarbeet root maggot, *Tetanops myopaeformis* (von Röder). J. Kans. Entomol. Soc. 56: 99.

Prokopy, R. J., and J. Hendrichs.1979. Mating behavior of *Ceratitis capitata* on a field-caged host tree. Ann. Entomol. Soc. Am. 72: 642–648.

Prokopy, R. J., E. W. Bennett, and G. L. Bush.1972. Mating behavior in *Rhagoletis pomonella* (Diptera: Tephritidae). II. Temporal organization. Can. Entomol. 104: 97–104.

Robacker, D. C., and W. G. Hart.1985. Courtship and territoriality of laboratory reared Mexican fruit flies, *Anastrepha ludens* (Diptera: Tephritidae), in cages containing host and nonhost trees. Ann. Entomol. Soc. Am. 78: 488–494.

Segura, D., N. Petit-Marty, R. Sciurano, T. Vera, and G. Calcagno.2007. Lekking behavior of *Anastrepha fraterculus* (Diptera: Tephritidae). Fla. Entomol. 90: 154–162.

SAS Institute 2015. SAS statistical software, version 9.4. SAS Institute, Cary, NC, USA.

Wenninger, E. J., and D. G. Hall.2008. Importance of multiple mating to female reproductive output in *Diaphorina citri*. Physiol. Entomol. 33: 316–321.

Widemo, F., and B. G. Johansson.2006. Male–male pheromone signaling in a lekking *Drosophila*. Proc. R. Soc. B. 273: 713–717.