

J.D. NELSON, P.L. LAMBDIN, J. F. GRANT, Z. MENDEL

**Predators attracted to the sex pheromones of *Matsucoccus* spp.
(Margarodidae) in pine dominated forests**

Abstract: Predators attracted to the sex pheromones of *Matsucoccus josephi* Bodenheimer & Harpaz, *M. feytaudi* Ducasse, *M. matsumurae* Kuwana, and controls (without pheromones) in east Tennessee were collected from May 1999 through July 2000. Delta style sticky traps (n=60) baited with the sex pheromones of *Matsucoccus* spp. were placed in 5 pine sites along elevation gradients in the Great Smoky Mountains National Park. From 468 sticky inserts processed, 7,972 insect specimens representing 112 species, 83 families, and 12 orders were identified. Some 99% (n= 546) of all neuropterans and 88% (n =72) of all hemipterans were captured from pheromone traps, compared to the control traps. Hemerobiidae was represented by 510 specimens of *Hemerobius stigma* Stephens (= *stigmaterus* Fitch), two specimens of *Sypherobius barberi* Banks, and three specimens of *Micromus posticus* Walker. Highest numbers of individuals were captured in the sex pheromone traps of *M. josephi* (267), *M. feytaudi* (224), *M. matsumurae* (25), and the control (1), respectively. Significantly high numbers of predators were captured at pine sites at Little Greenbriar Trail and Bullhead Trail on Mt. LeConte in traps baited with pheromones of *M. feytaudi* and *M. josephi* in stands of *Pinus pungens*, on dry, rocky ridges between 914m and 1392m. Twenty-five specimens of *Elatophilus inimica* were also captured from sites at Little Greenbriar Trail and Bullhead Trail on Mt. LeConte, University of Tennessee Arboretum, and Foothills Parkway sites. Of these, 14 were captured from pine site at Little Greenbriar Trail in traps baited with pheromones of *M. matsumurae* and *M. feytaudi*. These data suggest that *E. inimica*, like *H. stigma*, were most abundant in stands of *P. pungens* on dry, rocky ridges between 914m and 1392m. Collection of *E. inimica* represents new county and state records, and the southern-most distribution recorded for this species.

Key words: Sex pheromones, *Matsucoccus*, anthocorid, lacewing.

INTRODUCTION

The ecosystems of the southern Appalachian region in eastern Tennessee contain a diversity of plant and animal species comprising over 1,600 plant species (King

& Stupka, 1950). The rich biodiversity, spread across a varying topography, provides a natural beauty enjoyed by more than 14 million tourists annually. Protection of these ecosystems from the introduction of invasive, often destructive, exotic insects is necessary to prevent altering the composition of the flora and fauna, which may result in the loss of entire assemblages of species.

Introduced forest pests are a major threat and responsible for the decline in populations of several native species, including beech, Fraser fir, hemlock, and pine. The use of environmentally compatible natural enemies to suppress pest species is an integral component in the implementation of a forest management strategy. Populations of Fraser fir (*Abies fraseri* (Pursh) Poire), a native species endemic to high elevations in the southern Appalachians, have been reduced by over 90% by the balsam woolly adelgid (*Adelges tsugae* Annand) (Nicholas et al., 1992). Control efforts since the detection of the adelgid in 1957 have proved ineffective in protecting trees on a forest wide scale due to cost and logistics.

Pheromones are substances that serve as chemical signals to members of the same species, and are the primary communication medium among insects. Predators may exploit the semiochemical pathways between phytophagous insects to find food, locate breeding sites, or to stimulate oviposition. While communicating through pheromones, herbivores are often much more conspicuous to their natural enemies that exploit these pheromones as kairomones in long-distance herbivore location (Haynes & Birch, 1985). These semiochemicals include kairomones that regulate prey finding and selection. All long-range kairomones thus far identified are sex or aggregation pheromones of the hosts. This sex pheromone/kairomonal system constitutes a special predator-prey relationship.

Noldus *et al.* (1991) describe how *Trichogramma* parasitoids use moth sex pheromones as kairomones. McClain *et al.* (1990) reported the attraction of aphelinid parasitoids to the sex pheromone of Diaspididae. Boo *et al.* (1998) demonstrated that aphid sex pheromone components attract the polyphagous predator, *Chrysopa cognata*. A predator of the larger grain borer, *Teretriosoma nigrescens* Lewis, is attracted to the aggregation pheromone of *Prostephanus truncatus* Horn, and can be lured to traps baited with its synthetic components (Tigar *et al.*, 1993). Studies on attraction of natural enemies to sex pheromones of scale insects are rare (McClain *et al.*, 1990). However, the sex pheromones of *Matsucoccus matsumurae* Kuwana, *M. feytaudi* Ducasse, and *M. josephi* Bodenheimer & Harpaz have been demonstrated to be effective in attracting potential predators to areas infested by these scale insects (Mendel *et. al.*, 1997). Researchers have recently shown for the first time that *Hemerobius stigma* Stephens and *Elatophilus* spp. display a kairomonal response to the sex pheromones of these scales. The attraction of *Hemerobius* and *Elatophilus* to these sex pheromones may be based on the key chemical configurations within the pheromones, or that kairomonal response of the predators evolved during speciation within *Matsucoccus*. The objective of this study was to establish if

predators can be lured in sufficient numbers by the sex pheromones of *Matsucoccus* spp. into pest-infested areas to suppress pest populations.

MATERIALS AND METHODS

A study was initiated to assess potential predators of *Matsucoccus* spp. in east Tennessee and the use of synthetic pheromones to attract predators to *Matsucoccus*-infested hosts. In 1999, delta style sticky traps ($n=60$; each 10cm x 10cm x 17.5cm) baited with the sex pheromones of *Matsucoccus* spp. were placed in five pine sites established along an elevation gradient in the Great Smoky Mountains National Park (GRSM) (Table 1), and 12 traps in one site at the University of Tennessee (U.T.) Arboretum located in Anderson Co., TN. The latter site was abandoned in 2000 due to the destruction of the test trees by the southern pine bark beetle, *Dendroctonus frontalis* Zimmermann. A replacement site was established on the Foothills Parkway in May 2000.

Twelve traps, consisting of three traps each of pheromones for *M. josephi*, *M. feytaudi*, and *M. matsumurae*, and three controls (without pheromones), were placed in each of the pine sites and maintained monthly from May to October 1999, and May to July 2000. Traps were suspended individually on limbs ca. 3 to 6 m. by wire hooks in trees randomly selected ca. 30 to 50 m. apart. The plastic traps were each equipped with a rubber dispenser saturated with sex-pheromones of either *M. feytaudi*, *M. matsumurae*, or *M. josephi*.

Pheromone dispensers and sticky inserts were replaced on each sampling date. Sticky inserts with trapped insects were collected and taken to the laboratory where specimens were removed using Histoclear® and placed in glass vials containing 70% ethyl alcohol. Specimens were sorted and identified to family and species, when possible. Data (GIS location, site number, date, pheromone type, species, family, and number of specimens) were entered into Microsoft Excel® and Biota® databases, and subsequently analyzed using Statistical Analysis Software (SAS) to perform Chi-Square analysis to compare captured predators among pheromone types, pine sites, and dates (SAS Institute, 1997).

RESULTS AND DISCUSSION

General Collection. Some 468 sticky inserts were processed in 1999 and 2000 from which 7,972 insect specimens representing 112 species in 12 orders representing 83 families. Total insect species collected averaged 12.9 specimens per trap representing 6.8 species. The dominant orders collected were Diptera ($n=3,269$), Colembola ($n=1,674$), Hymenoptera ($n=721$), Thysanoptera ($n=638$), and Neuroptera ($n=547$), respectively. Other orders represented were Psocoptera ($n=338$),

Table 1 Pine sites established for the exposure of *Matsucoccus* spp. sex pheromone traps.

| Site | Location / UTM Coordinates | Description |
|-------------------|--|--|
| U.T. Arboretum | 15-year-old, 0.8 ha, stand of <i>Pinus taeda</i> Miller. Anderson Co., TN. 3988129 N, 17 750536 E | This site was used in 1999, but abandoned in 2000 due to destruction by southern pine bark beetle. ~292m in elevation. |
| Pine 1 | Near Greenbriar entrance to the GRSM Sevier Co., TN 3957716 N, 17 281620 E | Mixed stand of mature <i>Pinus virginiana</i> Miller and Miller hardwoods, along the middle prong of Little Pigeon River. ~300 m in elevation. |
| Pine 2 | The Sugarlands Trail, near CCC camp Sevier Co., TN 3950572 N, 17 272381 E | Mixed stand of mature <i>Pinus virginiana</i> and mesic hardwoods. ~600 m in elevation. |
| Pine 3 | Little Greenbriar Trail, Sevier Co., TN 3953154 N, 17 263520 E With southern exposure. ~900 m in elevation. | Located on Chinquapin Ridge on Cove Mt. In mixed stand of mature <i>Pinus pungens</i> Lamb. and xeric oak near crest of a ridgeline. |
| Pine 4 | The Bullhead Trail on Mt. LeConte Sevier Co., TN 3949252 N, 17 274740 E | Mixed stand of mature <i>Pinus pungens</i> Lamb. and oak-hickory along a dry, rocky ridgeline, western exposure. ~ 1200 m in elevation. |
| Pine 5 | The Bullhead trial on Mt. LeConte Sevier Co., TN 3947887 N, 17 275595 E | Spruce-Fir dominated forest. Northeastern exposure near Balsam Point. Elevation~1500 m. |
| Foothills Parkway | Eastern section, ~2.5 km from the U.S. 321 entrance in Cocke Co., TN 3965350 N, 296900 E | Primarily <i>Pinus rigida</i> Miller and Miller with southern exposure. Elevation ~600 m. |

Homoptera (n=300), Coleoptera (n=159), Orthoptera (n=113), Lepidoptera (n=83), Hemiptera (n=80), and Trichoptera (50). Approximately, 99% (n= 546) of all neuropterans were captured in pheromone traps, compared to <1% (n = 1) captured in control traps. Also, 88% of all hemipterans (n =80) were captured in pheromone traps, compared to 12% captured in control traps. These data suggest that Neuroptera and most Hemiptera captured were attracted to the sex pheromones of *Matsucoccus* spp.

Neuroptera. The highest numbers of specimens captured were in the families Hemerobiidae (n = 514), Chrysopidae (n = 20), and Coniopterygidae (n = 13), respectively. Hemerobiidae was represented by three species: *Hemerobius stigma* Stephens (= *H. stigmaterus* Fitch) (n=50), *Symppherobius barberi* Banks (n=2), and *Micromus posticus* Walker (n=3). *S. barberi* and *M. posticus* were captured in pine site 4 and the U.T. Arboretum site in traps baited with *M. feytaudi*. However, these two species represented less than 1% of the captured Hemerobiidae. *Symppherobius barberi* and *M. posticus* were captured in traps baited with the sex pheromones of *Matsucoccus* spp. for the first time. All captured Chrysopidae were in the genus *Chrysoperla*.

The highest numbers of specimens were captured in traps baited with *M. josephi* (267), and *M. feytaudi* (224) compared to *M. matsumurae* (25) and the control (1). The difference in numbers captured may be attributed to the presence of certain chemical groups within the pheromones unique to *M. feytaudi* and *M. josephi*, or because of decreased activity of the *M. matsumurae* pheromone due its greater volatility. All three pheromones have the presence of ketodiene moiety with the (R) configuration, a precondition for pheromonal activity. However, other parts of these molecules may differ and affect the attractiveness of the pheromone to different predators.

The lowest numbers of predators were captured at pine sites 1, 2, 5, and the U.T. Arboretum site, while statistically higher numbers ($P < 0.05$) of pred4.

A significant ($P < 0.05$) inverse relationship between hemerobiids captured in traps baited with the pheromones of *M. feytaudi* and *M. josephi* occurred in all pine sites, except pine site 2. Fewer hemerobiids were captured in traps baited with *M. feytaudi*, while more specimens were captured in traps baited with *M. josephi* in pine sites 1, 4, and 5. The opposite occurred in pine site 3, where more were captured than in traps baited with *M. feytaudi*, and fewer were captured in traps baited with *M. josephi*. A Chi-square goodness to fit test (Zar, 1974) on individuals of *H. stigma* did not infer a significance difference in sexual bias (222 males:198 females).

Peak density of captured Hemerobiidae for the Arboretum site and pine sites 1 and 2 was greatest from May to June, while peak hemerobiid density was greatest from July to August for pine sites 3, 4, and 5. The difference in hemerobiid density is attributed to the increased elevation, and cooler climate of the higher pine sites.

Although adult hemerobiids exhibit high cold hardiness and are active year round, their development is still regulated by a minimal thermal threshold (Canard, 1997). The warmer temperatures of lower elevations allow for faster development, and may explain the capture of more Hemerobiidae earlier in the season. No significant ($P < 0.05$) differences were found between collections of hemerobiids from traps baited with racemic mixtures and natural stereoisomers of *Matsucoccus* spp. sex pheromones. The analogue of *M. feytaudi* and *M. josephi* was significantly ($P < 0.05$) less active than the corresponding natural pheromones.

Coniopterygidae. Four coniopterygids (*Coniopteryx* sp.) were captured during August and September 1999 from pine site 4 in traps baited with *M. feytaudi*. During May and June 2000, nine specimens were captured from the Foothills Parkway site and pine site 4 in traps baited with *M. feytaudi* and *M. josephi*. The capture of *Coniopteryx* sp. from these two sites suggests this predator is associated with *Pinus* spp. This collection represents the first record of *Coniopteryx* sp. captured in traps baited with the sex pheromones of *Matsucoccus* spp., and infers this predator responds to kairomones to find its prey.

Anthocoridae. Twenty-five adult anthocorids (*Elatophilus inimica* D. & H.) were captured from pine sites 3 and 4, the U.T. Arboretum, and the Foothills Parkway. Of these, 14 were captured from pine site 3 in traps baited with *M. matsumurae* and *M. feytaudi*. Only three *E. inimica* specimens were captured during the 2000 field season in traps baited with *M. matsumurae* and *M. josephi* compared to 22 during 1999 in traps with each of the three pheromones. The low number of captured *E. inimica* suggests that either the population is small within the area, perhaps due to the lack of preferred prey, or that they are not attracted to these pheromones in mass. These data suggest that *E. inimica*, like *H. stigma*, were most abundant in stands of table mountain pine on dry, rocky ridges between 914m and 1392m. These data represent a new state record as well as the southern most distribution recorded for this species, and suggest a relationship with habitats dominated by table mountain pine.

Matsucoccus. Males captured during this study were represented by four specimens of *M. gallicolus* at pine site 4 in a trap baited with *M. josephi*. The lack of capture of male scales of *M. feytaudi*, *M. josephi*, and *M. matsumurae* infers that these species are uncommon or absent within the range of these study sites. The capture of *M. gallicolus* males may indicate that this species exhibits cross-attraction to the sex pheromones of other *Matsucoccus* species. Previous field trials exposing the sex pheromones of *Matsucoccus* spp. revealed that the sex pheromones attract only conspecific males with the exception of *M. matsumurae* and *M. thunbergianae* (Dunkelblum, 1999). The females of these species appear to produce the same sex pheromone (Lanier et al., 1989). Adult males of *Matsucoccus* spp. live about a day,

and undergo only two generations each year; thus, the opportunity for attraction and capture is highly limited in areas with small, isolated populations (Beardsley, 1968). Therefore, it is not surprising that more males of *Matsucoccus* spp. were not captured.

Pheromone Activity. The low number of captured predators compared to other field trials exposing the sex pheromones of *Matsucoccus* spp. may be due to decreased activity of the pheromones. The pheromone dispensers remain active from 2 to 4 weeks in the field depending on ambient conditions, and may have become less active due to the time required for shipping. The difference in volatility between the pheromones of *M. feytaudi*, *M. josephi*, and *M. matsumurae* may have influenced the duration of attractiveness, and subsequent capture of predators, even though the pheromones with lower volatilities were compensated with increased amounts of pheromone. The kairomonal response of native predators to these pheromones may encourage the use of these pheromones in biological control programs directed towards managing exotic soft-bodied pests in Southern Appalachian forests.

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