

Parasitoids of the Conspersus Stink Bug (Hemiptera: Pentatomidae) in North Central Washington and Attractiveness of a Host-Produced Pheromone Component¹

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Abstract The parasitoid complex of the conspersus stink bug, *Euschistus conspersus* Uhler, was investigated in a series of field experiments conducted in native vegetation surrounding commercial apple orchards in the state of Washington. Rearing of parasitoids from adult *E. conspersus* confirmed the presence of two tachinid species, *Gymnosoma filiola* Loew and *Gymnocyttia occidentalis* Townsend. Three species of scelionids were reared from fresh egg masses placed on mullein plants (*Verbascum thapsus* L.) with *Trissolcus utahensis* (Ashmead) being the most common species. Though some parasitism was recorded in all study sites by both tachinids and scelionids, overall levels of parasitism were low (<10%). Predation comprised the major source of egg mortality in the field. Bucket traps baited with the male-produced aggregation pheromone component, methyl (2*E*,4*Z*)-decadienoate, captured significantly more *G. occidentalis* than unbaited controls, suggesting that it may use this compound as a host-finding kairomone. A test comparing *E. conspersus* egg masses placed on baited vs. unbaited *V. thapsus* revealed no differences in the rate of parasitism by scelionid parasitoids.

Key Words parasitoids, *Euschistus conspersus*, kairomone, Tachinidae, Scelionidae, Pentatomidae

Stink bugs (Heteroptera: Pentatomidae) are recognized as pests of an array of economically important crops, including cotton (Toscano and Stern 1976), soybeans (Daugherty et al. 1964), and a variety of tree fruits (Madsen 1950, Borden et al. 1952). In the fruit production areas of central Washington State, stink bugs have historically been regarded as sporadic, secondary pests (Beers et al. 1993). However, they have become increasingly important in some regions as pest management for key orchard pests shifts away from the use of broad-spectrum organophosphate insecticides.

The most abundant stink bug species, and the one implicated in most fruit damage attributed to pentatomids in commercial orchards in central Washington State, is the conspersus stink bug, *Euschistus conspersus* Uhler (McGhee 1997). It is native to North America and feeds on a variety of plants commonly found along orchard borders including mullein (*Verbascum thapsus* L.), bitterbrush (*Purshia tridentata* (Pursh)), red-osier dogwood (*Cornus stolonifera* Michx) and currant (*Rhus trilobata*

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Nutt.) (McGhee 1997). One of the shortcomings in management of the consperse stink bug is the dearth of information on its ecology in its natural habitat.

This study contributes information on an aspect of this ecology by identifying the parasitoid complex associated with it in areas adjacent to commercial apple orchards. Similarly, we examine the attractiveness of a host-produced volatile pheromone component (Aldrich et al. 1991, Krupke and Brunner 2001) to the parasitoids in question. Identification of the complex of natural enemies affecting stink bug populations is a necessary first step in assessing the impact of these natural enemies on stink bug populations and planning appropriate management programs that target the pest, while preserving natural enemy populations.

Materials and Methods

Stink bug collection and colony rearing. All experiments were conducted along orchard borders from May through September 1999 and April through September 2000 in Chelan and Douglas counties, WA. *Euschistus conspersus* adults were collected by hand, and were transferred to a greenhouse where a colony was maintained within screen cages. Each screen cage consisted of a 2.2 cm plastic piping frame measuring 3 × 3 × 4 m (l × h × w) and was covered with nylon organdy cloth. Inside each cage were 6 to 8 potted mullein plants that served as the basic nutrient source and oviposition substrate. The mullein food source was supplemented with raw, shelled, organic peanuts (*Arachis hypogaea* L.) and raw, shelled, organic sunflower seeds (*Helianthus* sp.) (McGhee 1997) that were glued on a piece of cardboard and set on the mullein plants. Insects were allowed to feed, mate and oviposit freely within each cage. Long-day conditions (16:8 h L:D) were maintained in the greenhouse by supplementing natural light with artificial lighting and temperatures were 22 to 27°C with a system of fans and water-cooled air.

Tachinid rearing and identification. Adult male and female *E. conspersus* were collected by hand from orchard border vegetation at weekly intervals throughout the spring and summer of 1999 and 2000. *Euschistus conspersus* were collected at random, i.e., no attempt was made to determine if parasitoid eggs were present on *E. conspersus* at the time of collection. *Euschistus conspersus* were held at ambient conditions in the laboratory, ranging from 16 to 22°C in individual 58-mL cups with a 2-cm piece of cut green bean (*Phaenolus vulgaris* L.) as a nutrient and water source. *Euschistus conspersus* were checked daily for emergence of parasitoid larvae and beans were replaced at 48 h intervals. Parasitoid larvae were removed from cups and allowed to pupate in 50 × 9 mm Petri dishes (Falcon® brand, Becton Dickinson and Co., Franklin Lakes, NJ) containing slightly moistened sand. Following their emergence flies were killed and mounted on insect pins for identification.

Scelionid rearing, identification and predation rates. Mullein plants in colony were examined daily for the presence of *E. conspersus* egg masses. The section of leaf including and immediately surrounding the egg mass was cut out with scissors, and the resulting leaf/egg mass section was attached to a 5 × 2 cm paper tab using a small amount of Elmer's white glue (Elmer's Products Inc., Columbus, OH). No glue was applied to the surface of the leaf in the vicinity of the egg mass. This method allowed for a natural oviposition substrate, minimal disturbance of the egg mass, and preservation of any volatile compounds left by the female, including the egg adhesive. The paper tabs with freshly laid egg masses were individually numbered and attached, using a stapler, to the undersides of mullein leaves of plants on orchard

borders, a preferred oviposition site of the bugs (McGhee 1997). A total of 26 sentinel masses were placed in the field each week, with mullein plants flagged and egg masses collected after 7 d. The average development time of eggs in the field at 21°C is 12.96 d (McGhee 1997), so the 7-d interval insured that no eggs would hatch before being collected. A total of 208 egg masses were placed in this manner, with a mean (\pm SE) of 13.6 ± 0.12 eggs per mass. The placement of egg masses was timed to coincide with the natural oviposition period of *E. conspersus*, from 11 May to 6 July, 2000 (McGhee 1997, Krupke and Brunner 2001). Following collection, paper tabs were brought back to the laboratory and egg masses were characterized in the following manner: Eggs were classified as being found by predators if at least one egg in the mass was partially consumed (Yeargan 1979). The percentage of eggs consumed by predators once found was also recorded. Similarly, the percentage of eggs found by parasites was determined by parasitism of at least one egg in the mass, and the percentage of eggs parasitized in each mass was recorded as well. Eggs were classified as normal if they continued to develop normally until hatch of stink bug nymphs. In some cases, parasitized masses, noted because they turned black prior to parasitoid emergence, were not immediately distinguishable from normally developing ones. In these cases, egg masses were held in 50 × 9 mm Petri dishes at 21°C until a clear diagnosis could be made. Missing egg masses that could not be associated with any kind of predation were not included in estimates of natural enemy activity.

Parasitoid response to host-produced pheromone. Response of tachinid flies to host-produced pheromone was tested using bucket traps (Unitrap, Pherotech Inc., Delta, BC) baited with a component of *E. conspersus* male-produced aggregation pheromone (Aldrich et al. 1991), methyl (2*E*,4*Z*)-decadienoate (Bedoukian Research Inc. Danbury, CT) or left unbaited (no pheromone) from 18 May to 10 August 2000. Pheromone was released from hollow polyethylene caps (Kimble Glass Co., Part No. 60975D-3) containing 0.2 ml of undiluted pheromone. Lures were replaced at 2-wk intervals throughout the trapping period. Traps were rotated after each lure change. All traps were affixed to PVC stakes inserted into the ground in native vegetation bordering commercial apple orchards so that the bottom edge of the traps hung at a final height of 0.6 to 1.0 m above ground level, the approximate mean height of the dominant vegetation in the area. Each trap contained a 2 cm square "kill strip" (Hercov Vaportape, DDVP toxicant strip, Great Lakes IPM) to kill captured insects. Traps were emptied of all accumulated insects at weekly intervals and taken to the laboratory for sorting. Numbers of stink bugs and/or tachinid flies captured were recorded, and tachinid flies were preserved for later identification. Any bucket traps that captured stink bugs were not included in the analysis of tachinid response to pheromone, removing the possibility of bug-produced volatiles attracting flies.

Scelionid response to host-produced pheromone. Methods of egg collection and placement used in this experiment were identical to those in the scelionid rearing and identification section above, with the exception that plants were baited with a polyethylene lure containing methyl (2*E*,4*Z*)-decadienoate. Baited plants were paired with unbaited controls and lures were rotated at 2-wk intervals from 11 May through 6 July 2000. Paired baited and unbaited plants were placed a minimum of 50 m apart. Each pair of baited plants was located on a different orchard border. A total of 25 pairs of plants were used in the study. Eggs were collected and replaced at weekly intervals throughout the stink bug oviposition period and evaluated for parasitism.

Statistical analyses. Analysis of variance and a least significant difference (LSD)

means separation test were used to test the hypothesis that pheromone-baited bucket traps captured more tachinids than unbaited traps (Zar 1999). The same analysis was used to determine whether the addition of pheromone to host plants increased parasitism rates of *E. conspersus* eggs.

Results

Parasitoid identification. Two tachinid species, *Gymnocyttia occidentalis* Townsend and *Gymnosoma filiola* Loew, were reared from field-collected *E. conspersus* (Table 1). *Gymnocyttia occidentalis* accounted for 70% of all parasitoids reared.

Four parasitoid species were identified from *E. conspersus* egg masses (Table 1). Of the parasitoids collected, one was considered a hyperparasitoid, *Ooencyrtus* sp., and the species could not readily be determined. *Ooencyrtus* sp. was collected in only one instance (i.e., one egg mass). Of the other three primary parasitoid species, *Trissolcus utahensis* (Ashmead) was most common accounting for 72% of all parasitoids (Table 1) and appears to be the key species attacking *E. conspersus* egg masses at the locations sampled.

Parasitism/predation rates. Overall parasitism rates recorded for tachinids were very low. Less than 2% of all *E. conspersus* collected ($n = 1197$) yielded a tachinid parasitoid. Of the 208 egg masses (2819 eggs) placed in the field, 22.8% of the egg masses were missing, i.e., lost without evidence of the cause of their demise. Scelionid parasitism rates peaked near 18% on 29 June, late in the oviposition period of *E. conspersus*. The scelionid parasitoids typically attacked the entire egg mass with the exception of cases where some of the eggs had been consumed by predators. Predation was the major source of egg mortality in the field. Predators attacked nearly 40% of the egg masses placed in the field and consumed an average of 47.7% of those eggs (Table 2). Degree-days above the 12°C lower developmental threshold for *E. conspersus* (McGhee 1997, Cullen and Zalom 2000) were calculated (Baskerville and Emin 1969) for 1999 and 2000 using maximum and minimum temperatures from the region. Accumulated degree-days for both years were similar and found to be within 5% of the 30-yr average temperatures for the region. Therefore, differences in

Table 1. Tachinid flies emerging from *E. conspersus* adults collected in orchards and parasitic Hymenoptera reared from *E. conspersus* eggs placed in orchards

	# Total hosts examined	# Parasitoids reared
<i>Gymnosoma filiola</i> Loew	1197 adults	6
<i>Gymnocyttia occidentalis</i> Townsend	1197 adults	14
<i>Trissolcus utahensis</i> (Ashmead)	2819 eggs	76
<i>Trissolcus euschisti</i> Ashmead	2819 eggs	13
<i>Telenomus podisi</i>	2819 eggs	16
<i>Ooencyrtus</i> sp.	2819 eggs	13

Table 2. Parasitism and predation of *E. conspersus* egg masses placed in the field in from 11 May to 6 July, 2000. Initial egg masses = 26 at each sampling date

Sampling date	% Masses recovered	% Masses found by predators	% Eggs eaten once found	% Eggs found by parasitoids	% Parasitized once found
18 May	91.6	20.8	18.0	0.0	—
25 May	76.9	65.0	62.7	5.0	100
1 June	73.0	42.1	67.2	0.0	—
8 June	76.9	25.0	31.3	10.0	100
15 June	91.6	37.5	39.1	8.3	96.1
22 June	76.9	60.0	42.5	10.0	92.3
29 June	65.3	23.5	31.0	17.6	53.3
6 July	65.3	29.4	69.1	0.0	—
MEAN(SE)	77.2 (3.6)	37.9 (5.9)	47.6 (5.7)	6.4 (2.2)	88.3 (8.9)

weather between the two years would not be expected to have influenced rates of predation or parasitism of *E. conspersus*.

Parasitoid response to pheromone. Significantly more *G. occidentalis* were captured in pheromone-baited bucket traps (57 flies) than unbaited traps (3 flies) ($F = 17.47$; $df = 1, 274$; $P = 0.0001$). The other species of tachinid fly captured, *G. filiola*, exhibited no preference for pheromone-baited (3 flies captured) vs. unbaited traps (3 flies captured) ($F = 0.2$; $df = 1, 274$; $P = 0.65$). When *E. conspersus* were found in traps with tachinids the bugs typically had multiple tachinid eggs attached to their dorsum.

The addition of methyl (2*E*,4*Z*)-decadienoate to host plants did not significantly alter parasitism rates of the scelionid parasitoids found in this study. Parasitism rates were similar on pheromone-baited ($7.1\% \pm 1.8\%$) and unbaited plants ($3.3\% \pm 1.9\%$) ($F = 1.22$; $df = 1, 159$; $P = 0.27$).

Discussion

This study is the first to attempt to identify parasitoids of *E. conspersus* in areas adjacent to orchards in North Central Washington and demonstrates the presence of two species of tachinid parasitoid and three species of scelionid egg parasitoid. There is very little literature documenting the tachinid parasitoids of *E. conspersus*. Arnaud (1978) listed both *G. filiola* and *G. occidentalis* as parasitoids of *Euschistus* spp., including *Euschistus variolarius* (Palisot de Veauvois), a species present in North Central Washington albeit in far lower densities than *E. conspersus* (Krupke and Brunner, unpubl. data). The dominant egg parasitoid detected in our study, *T. utahensis*, had previously been reported as a key egg parasitoid of *E. conspersus* (Zalom 1992, Weber et al. 1996) in California and of two other stink bug species commonly found in our area, *Chlorochroa sayi* Stal and *C. ligata* (Say) (Jubb and Watson 1971, Johnson 1985). It is important to note here that this listing of parasitoids may not be

exhaustive. For example, no nymphs were included in the study. Although the tachinid parasitoids described here have been observed ovipositing on nymphal *E. conspersus*, we have never reared a parasitoid from nymphs (Krupke, unpubl. data), and thus collected only adults for our parasitoid rearing. However, it is possible that a detailed examination of nymphal stages may reveal other important parasitoid species or guilds.

Although parasitism rates were relatively low for both tachinids and scelionids, the study area was in most cases within 10 m of commercial apple orchard borders and was, thus, under the influence of the insecticide program targeting orchard pests. The negative impact of agricultural pesticides on biological control agents is well-documented (Croft and Brown 1975, Croft 1990) and it is likely that pesticides affected the levels of parasitism reported in this study. Future studies should compare parasitoid activity in undisturbed habitats with those adjacent to commercial orchards. The data presented by other researchers do suggest that *T. utahensis* may have the potential to become a key parasitoid of *E. conspersus* (Zalom 1992, Weber et al. 1996). *Telenomus podisi* Ashmead has been reported as a parasitoid of *E. conspersus* (McGhee 1997) and *E. variolarius* (Yeargan 1979), and *Trissolcus euschisti* (Ashmead) has been reared from *E. conspersus* eggs in soybeans (Yeargan 1979).

A major source of stink bug egg mortality in our study was predation. Although no formal experiments were conducted to identify the predatory species responsible for egg mortality, in several instances ants, earwigs, nabids, and even adult *E. conspersus* were observed feeding on egg masses in the field. Predation levels were consistently high throughout the study, although there was no evidence of dominance by a single predator type. It is not surprising that we found generalist predators of *E. conspersus* eggs. In a similar study, Yeargan (1979) pointed out that predators likely destroyed both parasitized and unparasitized egg masses. Predators have been shown to be the major source of egg mortality in *N. viridula* populations in Hawaii (Jones 1995), and the identification of specific predatory species would be valuable information in the study of the ecology of *E. conspersus*. Egg masses that were missing after one week in the field may also have been removed by predators such as ants, that could be capable of manipulating the egg masses to remove them from their mullein leaf substrate.

The attraction of the tachinid parasitoid, *Gymnoclytia occidentalis*, to bucket traps baited with methyl (2E,4Z)-decadienoate indicates that the flies may use this compound as a host-finding kairomone. This attraction has been documented previously in two other tachinid parasitoids of *Euschistus* spp. in the eastern United States, *Gymnosoma occidua* (Walker) and *Euthera* spp. (Aldrich et al. 1991), as well as in a tachinid parasitoid of the southern green stink bug, *Nezara viridula* (L.), *Trichopoda pennipes* (F.) (Mitchell and Mau 1972). *Gymnosoma filiola* does not appear to use the aggregation pheromone component examined in this study to locate hosts. However, this result is based upon a relatively small number of *G. filiola* captured in bucket traps.

The attraction of *G. occidentalis* to traps baited with the stink bug aggregation pheromone and the fact that *E. conspersus* adults in traps were covered with tachinid eggs suggests a strategy to enhance *E. conspersus* parasitism in the field. We have previously shown that baiting native plants will aggregate *E. conspersus* in higher than normal numbers on mullein (Krupke and Brunner 2001). It may be possible to increase parasitism of *E. conspersus* by *G. occidentalis* by baiting mullein plants in a region, thus reducing densities of *E. conspersus* without the reliance on pesticides.

The use of host-produced volatiles by scelionid egg parasitoids has been previously reported for *T. basalis*, a parasitoid of *N. viridula* (Colazza et al. 1999). However, this study was confined to laboratory conditions using Y-tube olfactometers and experimental arenas. Our experiments attempted to quantify attraction to host-produced compounds using a field assay of pheromone-baited plants. Although there were no significant differences noted in egg parasitism between methyl (2E,4Z)-decadienoate baited and unbaited host plants, this result may not necessarily indicate that scelionids do not use this compound as a host-finding kairomone. The presence of the pheromone component on the baited plants has been shown to aggregate significant numbers of reproductively active *E. conspersus* (Krupke and Brunner 2001). This may have led to a larger number of mated females and egg masses being present on the baited plants—leading to inequalities in the numbers of egg masses that female parasitoids had to choose from. To test the hypothesis that these egg parasitoids use the male-produced compound as a host-finding kairomone, stink bugs would need to be excluded from both the baited and unbaited plants for the duration of the experiment and sentinel egg masses used to assess parasitism. What can be concluded from our experiments is that the response (i.e., attraction) of scelionids to baited plants was at least proportional to the increased recruitment of *E. conspersus* in the field.

Tachinid and scelionid parasitoids egg predators have a slight impact on populations of *E. conspersus* in areas immediately adjacent to commercial Washington orchards that are under conventional management regimes. In a study of rates of parasitism by *T. basalis* (Wollaston), a parasitoid of *N. viridula*, parasitism was shown to be significantly higher in the vegetation outside macadamia orchards than within the orchard canopy (Jones 1995). While similar comparisons of parasitism inside and outside the orchard were not made in our study it seems reasonable that the best hope for a biologically-based management program of *E. conspersus* lies far from the orchard/border interface in native host plants where the potential negative influence of pesticides on natural enemies is eliminated. The mere presence of biological control agents in areas bordering commercial orchards in Washington is encouraging from a management perspective. The current pest management trend in Washington apple orchards is a move toward alternate technologies such as mating disruption and selective insecticides and away from conventional controls, such as organophosphate insecticides (Brunner et al. 1996, Gut et al. 1996, Calkins 1998). This change may allow natural enemies of stink bugs to play an increased role in reducing bug populations in and around commercial orchards.

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