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NOTES ON INCIDENCE AND BIOLOGY OF THE PREDOMINANT PARASITOIDs ATTACKING THE COTTONWOOD LEAF BEETLE IN MINNESOTA

Alexander P. Kendrick¹, Kenneth F. Raffa¹, Steven J. Krauth¹, and Norman E. Woodley²

ABSTRACT

Populations of the cottonwood leaf beetle, *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae), in Minnesota were examined for the presence of parasitoids over a period of two years. Field samples of larvae and pre-pupae yielded two parasitoids: a tachinid fly, *Cleonice setosa* (Reinhard), and a pteromalid wasp, *Schizonotus sieboldi* (Ratzeburg). Incidence of parasitism by the tachinid was substantially higher than in previous reports, with 32.9% of third instar larvae and 81.4% of pre-pupae being parasitized. The pteromalid was present in 7.0% of pre-pupae. Both parasitoids were reared to adulthood in the laboratory, with the pteromalid exhibiting continued generations, but the tachinid completing development only after a cold treatment to break diapause.

The cottonwood leaf beetle, *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae), is the most important arthropod pest affecting hybrid poplar plantations (Coyle et al. 2005). Both larval and adult feeding can result in growth loss and destruction of leaders and shoots (Caldbeck et al. 1978, Bassman et al. 1982, Coyle et al. 2002). Management options are limited, so growers rely on insecticides as their major control option. Insecticides, however, can be financially costly, environmentally damaging, harmful to natural enemies, and can also lead to resistance (Head et al. 1977a).

Future management strategies need to include natural enemies such as predators, pathogens and parasitoids as an integral component (Bauer and Pankratz 1993, Coyle et al. 2005). There is currently some knowledge about predators and pathogens of cottonwood leaf beetle, including their biologies and population dynamics (Coyle et al. 2005). Unfortunately, knowledge about the parasitoid component of the natural enemy complex affecting *C. scripta* is very limited. Before parasitoids can be implemented into Integrated Pest Management programs it is necessary to understand their biology and behavior, and before these elements can be investigated it is essential to determine the predominant species affecting *C. scripta*.

Previous reports of parasitoids attacking the cottonwood leaf beetle include a pteromalid wasp and a tachinid fly. Head et al. (1977b) reported the presence of the wasp *Schizonotus latus* (Walker) (Hymenoptera: Pteromalidae) and an unidentified tachinid (Diptera: Tachinidae) in Mississippi. Reports of *C. scripta* pupal parasitism attributed to *S. latus* are as high as 8.6% in Iowa (Jarrard 1997) and 25.8% in Wisconsin (Burkot and Benjamin 1979). Jarrard (1997) also reported the presence of an unidentified tachinid and estimated parasitism of late-third instars to be 4.2%.

The purpose of this study was to identify the major parasitoids attacking *C. scripta* in *Populus* plantations in Minnesota, evaluate their incidence, and conduct laboratory observations on life history parameters.

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METHODS

Identification and incidence of major parasitoids. Field sites were located in hybrid poplar plantations near Carlos, MN (Site 1: 45°N 56’ 30”, 95°W 07´ 00´”; Site 2: 45°N 59´ 30”, 95°W 06´ 00´”). Site 1 was 23.1 ha and comprised of clone DN2 (*Populus deltoides* × *nigra*) planted on an 8 × 10 ft. (2.44 × 3.05 m) spacing. These trees were entering their fifth year of growth in 2004. Site 2 was 24.3 ha and comprised of clone NM6 (*P. nigra* × *maximowiczii*) on a 10 × 10 ft. (3.05 × 3.05 m) spacing. It was entering its third year of growth in 2004. Both sites were sampled in 2004, and site 2 was sampled again in 2005. Both sites contained populations of the cottonwood leaf beetle at the time of sampling.

On August 23, 2004, actively feeding larvae were collected haphazardly from feeding sites on branch terminals and separated into first, second, and third instars. We also collected pre-pupal larvae from the lower canopy in 2004. Only third instar larvae were collected in 2005; the sampling date was August 28. Larvae were placed on field-collected foliage from non-infested sites and reared to pupation in plastic boxes (#173-C, Pioneer Plastics, Dixon KY) held at 23 °C and 14L:10D photoperiod. Parasitism was determined based on presence of fly or wasp larvae and pupae.

Ptéromalid adults emerged from pupation after several days at 23 °C, and were mounted for identification. Tachinid puparia were allowed to sclerotize for a period of two to six weeks. During this period they were housed in plastic boxes (#173-C, Pioneer Plastics, Dixon, KY) at 23 °C and 70% relative humidity. Puparia were then placed into autoclaved potting soil (Metro-Mix 300, Sungro, Bellevue WA) that had been moistened with distilled water, and exposed to three different treatments in order to obtain adults. The treatments were: a) on 1 September, 40 puparia were placed directly into a refrigerator in the above boxes with no additional preparation treatment, and stored for 8 months at 6 °C; b) on 1 October, 56 puparia were placed in a sanitizing solution of 1% bleach and 5% ethanol for one minute, and then rinsed three times with autoclaved distilled water. These puparia were divided into two groups of 28, placed in two separate plastic boxes (#195 C, Pioneer Plastics, Dixon, KY) in autoclaved soil moistened with autoclaved distilled water, and held in an incubator at 10 °C for two weeks. One box (b1) was removed on 15 October, and held for slightly more than 6 months at ambient winter temperatures in a three-walled outdoor building, and brought inside on 8 April. The average high and low temperatures during this period were 16.1 °C and 5.39 °C for October, 8.9 °C and 0.1 °C for November, 1.1 °C and -7.7 °C for December, -3.1 °C and -10.8 °C for January, 2.7 °C and -5.3 °C for February, 5.3 °C and -5.2 °C for March, 17.6 °C and 3.5 °C for April (NOAA, 2005). The other box (b2) remained in the incubator at 3 °C for 5 months, then at 6 °C for 5 months, and was removed from the incubator on 29 July.

Once removed from cold treatment, puparia were brought to room temperature and then sifted from the soil. Individual puparia were placed in 1-ounce condiment cups (Dixie Brand, Koch Industries, Inc., Wichita, KS) with a hole in the lid to allow airflow. Cups were housed in a plastic incubation box (#295 C, Pioneer Plastics, Dixon, KY) containing a moist paper towel to prevent desiccation. Boxes were kept at room temperature and exposed to indirect natural light for approximately 14 hours each day. Puparia were monitored daily, and any emerged insects were recorded and preserved for identification.

Rearing. Adult wasps were kept in a portable insect tent (#1462W, BioQuip, Rancho Dominguez, CA) that housed between 1 and 30 individuals. They were fed a dilute solution of sugar water (1/8 tsp (625µl) of sucrose in 25 ml of tap water) placed in a film canister and dispensed with a felt wick. Hosts were offered in batches of 20 to 50 pre-pupal cottonwood leaf beetles. Beetles were exposed to the wasps for 48 hours before transferal to a plastic box containing a moistened paper towel (#295 C, Pioneer Plastics, Dixon, KY). Longer
exposure to wasps resulted in superparasitism of beetle hosts. When wasp pupae became evident, they were transferred to the insect tent for emergence.

Adult flies were placed in a Plexiglas box (32 × 29 × 37 cm) with a mesh-tube access port. They were fed a dilute solution of sugar water similar to that used for the wasps. The maximum number of flies housed in the box was five. Approximately two dozen actively feeding 3rd instar leaf beetles were offered as hosts for the remainder of their larval development. After beetle larvae pupated, they were removed to a plastic box containing a moist paper towel.

RESULTS

Two parasitoid species were reared from field-collected *C. scripta* larvae and pupae: a tachinid fly *Cleonice setosa* (Reinhard), and a pteromalid wasp, *Schizonotus sieboldi* (Ratzeburg). Based on pooled field data, tachinid flies parasitized no first instars, 10.2% of second instars, 32.9% of third instars, and 81.4% of pre-pupae (Table 1). Parasitism of third instar larvae by this tachinid varied between sites during 2004 (Site 1: 55.0%; Site 2: 27.8%) and between years (Site 2, 2005: 16.4%) (Kendrick 2006). The incidence of parasitism by the pteromalid was 7.0% of pre-pupal larvae collected (Table 1). The pteromalid was not present in any other life stage collected and was only found at Site 1.

No tachinid flies emerged during the two to six week period preceding cold treatments. The highest emergence was realized when puparia were sanitized and placed in ambient winter conditions (Table 2: b2). In this treatment, 60.7% of the initial puparia produced an adult fly. Overwintering without sanitation resulted in the emergence of only one tachinid. We also recorded the presence of a hyperparasitoid wasp, *Perilampus hyalinus* Say (Hymenoptera: Perilampidae), emerging from tachinid puparia (Table 2).

The development time from egg to adult for the pteromalid wasp was approximately 15 days at 23 °C. Wasps were maintained in the laboratory for multiple generations. In contrast, no flies were observed to mate or oviposit in the laboratory and no offspring were produced.

DISCUSSION

As with previous studies, the predominant parasitoids of *C. scripta* were a tachinid and a pteromalid. Our results suggest, however, that parasitism can be substantially higher than previously estimated. For example, Jarrard (1997) reported 4.2% parasitism of late-third instar larvae of *C. scripta* by an unidentified tachinid in Iowa, compared to 32.9% by *C. setosa* in our study. Additionally, 81.4% of pre-pupae were parasitized by *C. setosa*. Prepupal parasitism may be over-estimated due to the cessation of development by hosts harboring *C. setosa*, which could result in an accumulation of parasitized individuals in this stage.

Based on field data, third instars have the highest incidence of parasitization by *C. setosa*. Second instars appear less susceptible, while no first instars were parasitized (Table 1). The higher rate of parasitism of third instars could include bias due to the cumulative effect of a more prolonged exposure. Laboratory observations suggest that *C. setosa* is a univoltine, solitary endoparasitoid, which agrees with initial observations of an unidentified tachinid reared from *C. scripta* in Mississippi (Head et al. 1977b).

*Cleonice setosa* has been recorded from Canada: Alberta, Ontario; and the USA: Maryland, Nebraska, North Carolina, South Dakota, and now Minnesota (O’Hara and Wood 2004). A specimen of *C. setosa* in the Canadian National Collection (CNC), Ottawa, was reared from *Chrysomela tremulae* (Fabricius) from Ontario, and a second specimen (also CNC) was reared from *C. scripta*
Table 1. Incidence of parasitism for various life stages of *Chrysomela scripta* collected in hybrid poplar plantations near Carlos, MN in 2004-2005. L1-3 = 1st - 3rd instars, PP = pre-pupal, N = total number collected.

<table>
<thead>
<tr>
<th>Life Stage</th>
<th>N</th>
<th>No Parasitism</th>
<th>Tachinidae</th>
<th>Pteromalidae</th>
<th>Missing</th>
<th>% Parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>38</td>
<td>38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>L2</td>
<td>60</td>
<td>44</td>
<td>5</td>
<td>0</td>
<td>11</td>
<td>8.3</td>
</tr>
<tr>
<td>L3</td>
<td>336</td>
<td>218</td>
<td>107</td>
<td>0</td>
<td>11</td>
<td>32.9</td>
</tr>
<tr>
<td>PP</td>
<td>80</td>
<td>15</td>
<td>35</td>
<td>3</td>
<td>37</td>
<td>47.5</td>
</tr>
</tbody>
</table>

Table 2. *C. setosa* emergence from sterile soil exposed to different overwintering treatments. N = number of puparia initially placed in the soil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Sanitation</td>
</tr>
<tr>
<td>a</td>
<td>no</td>
</tr>
<tr>
<td>b1</td>
<td>yes</td>
</tr>
<tr>
<td>b2</td>
<td>yes</td>
</tr>
</tbody>
</table>
from Alberta. The genus has been previously reported under the synonym *Grisdalemyia* as being a parasitoid of *Chrysomela crotchi* Brown (Smereka 1965).

We estimate the incidence of parasitism of pre-pupal *C. scripta* by *S. sieboldi* to be 7.0% based on our field rearings. European reports of parasitism attributed to *S. sieboldi* on *Chrysomela vigintipunctata* (Scopoli) range from less than 3% (Lays 2002) to 45% (Urban 1998). Based on our laboratory observations, both the pre-pupal and pupal stages of *C. scripta* are susceptible to attack by *S. sieboldi*. Our observations indicate that *S. sieboldi* is an endoparasitoid, differing from a previous report from Europe on *C. vigintipunctata* (Urban 1998), but agreeing with North American observations attributed to *S. latus* on *C. scripta* (Head et al. 1977b). Each *C. scripta* host supported multiple pteromalid larvae, initially feeding internally but becoming visible externally prior to pupation. It is possible that the appearance of late-instar parasitoid larvae externally on *C. vigintipunctata* led to the interpretation that *S. sieboldi* is an ectoparasitoid.

There is some confusion surrounding the identity of *Schizonotus* spp. The wasps emerging from *C. scripta* key to *S. sieboldi*, a European species, and are identical to European specimens of *S. sieboldi* deposited in the National Museum of Natural History (USNM), Washington. However, Nearctic specimens that have been identified as *S. latus* do not appear to differ from *S. sieboldi*. It is possible that these names are synonymous, or that the specimens of *S. latus* in the USNM have been misidentified. Bouèek and Heydon (1997) list *S. sieboldi*, but not *S. latus*, as occurring in the Nearctic region. Perhaps *S. latus* is a junior synonym of *S. sieboldi*. Pending revision of the genus, *S. sieboldi* appears to be the most appropriate name for our specimens.

Future work is needed to quantify the impacts and life history of these parasitoids. In particular, a better understanding of sources of variation due to location and time, both within and among years, is required. Improved sampling procedures and detailed information on host-finding and oviposition behavior in the field would also be useful to improve biological control of *C. scripta*.

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**LITERATURE CITED**


