Reduction in feeding by diapausing and postdiapause pear psylla (Homoptera: Psyllidae) caused by extract from buffalo gourd (Cucurbitaceae)

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ABSTRACT

Rates of honeydew production were lower in diapausing winterform pear psylla, *Cacopsylla pyricola* (Foerster), than in psylla brought out of diapause by exposure either to long-day conditions or to an insect growth regulator (fenoxycarb). An extract obtained from a nonhost species, buffalo gourd (*Cucurbita foetidissima* HBK.), caused reduced honeydew production when misted onto pear shoots. Reductions in feeding were as pronounced in diapausing insects as in psylla that were in a postdiapause condition. Ovarian development scores were positively correlated with honeydew production, indicating that feeding deterrents may be useful for delaying the onset of egglaying in the field.

Key words: Insecta, *Cacopsylla pyricola*, diapause, honeydew production, feeding deterrent.

INTRODUCTION

Pear psylla, *Cacopsylla pyricola* (Foerster), is an important pest of commercial pears in both North America and Europe. The species is seasonally dimorphic, producing a large, dark overwintering form (winterform) in fall. This morphotype overwinters in a photoperiod-induced reproductive diapause, characterized by immature ovaries and a reduction or absence of mating (Krysan and Higbee 1990). Large numbers leave the pear orchard in fall to overwinter on nonhost species. Reentry into the orchard and egglaying begin in late winter.

The winterform morphotype has been the focus of a great deal of research, both because control of this stage prevents problems later in the season (Westigard and Zwick 1972), and because aspects of its life history (especially diapause) may make it vulnerable to new control technologies (e.g., Krysan 1990). There is a complex relationship between diapause status of psylla and its host plants. Overwintering psylla require access to a moisture source (Kaloostian 1970, Hodgson and Mustafa 1984). A number of species, including pear, appear to satisfy this requirement (Horton *et al.* 1994). The fact that many winterform psylla survive the winter outside of the pear orchard indicates that diapausing insects must be somewhat generalized feeders. Conversely, late postdiapause and reproductive insects are uncommon outside of the pear orchard. Thus, nonpear species may become less acceptable or unacceptable to pyslla coinciding with the end of diapause.

We tested three hypotheses dealing with pear psylla diapause, feeding, and their interaction. We first tested whether feeding by winterform psylla is reduced in the presence of an extract obtained from a nonhost plant species. Secondly, we tested whether feeding rates are higher in postdiapause psylla than in diapausing psylla. One common characteristic of diapause in other insects is a reduction in feeding (Tauber *et al.* 1986). Thirdly, we compared feeding rates of diapausing and postdiapause psylla in the presence and absence of a putative feeding deterrent, and hypothesized that the deterrent would have less effect on diapausing insects, due to their generalist feeding habits, than on postdiapause insects.

MATERIALS AND METHODS

Female winterform pear psylla were collected from commercial pear orchards near Yakima, WA on three dates: 14 Dec. 1993, 28 Dec. 1993, and 23 Nov. 1994. Psylla were exposed to one of three conditioning treatments designed either to maintain diapause or to end it: (a) control (short-day [10:14 L:D]); (b) long-day (16:8 L:D); (c) short-day; shoots misted with an insect growth regulator (fenoxycarb; obtained from Maag Agrochemicals, Vero Beach, FL). The insects were kept in three screened plastic cages (1 cage per conditioning treatment) containing dormant pear shoots from an unsprayed orchard; shoots had their cut ends placed in jars of water. Control (short-day) conditions maintain diapause, whereas both long-day conditions and contact with fenoxycarb break diapause (Krysan 1990). For the fenoxycarb treatment, the pear shoots were sprayed to runoff with fenoxycarb at the rate of 0.1 g [AI] per liter of tap water. Shoots were allowed to dry, and psylla were added to the cage. Psylla were conditioned for 1 week at 20°C in three separate environmental chambers (1 chamber per conditioning treatment).

After seven days of conditioning, the feeding portion of the experiment was conducted. Conditioned psylla were moved to 135 ml screened feeding arenas, each arena containing a single cut shoot of dormant pear from an unsprayed orchard. The cut end of the shoot was kept in tap water. Four females from the same conditioning treatment were placed in each arena. There were 46-48 arenas per conditioning treatment (summed over the three collection dates). The insects were kept at room temperature (22°C) for the duration of the feeding trial.

Half of the pear shoots for each conditioning treatment (N = 23-24) were misted to runoff with an extract known to cause reduced egglaying by psylla (Weissling unpublished data). The extract was made from the tap roots of buffalo gourd, *Cucurbita foetidissima* HBK., obtained in the summer of 1988 from plants growing near Parks, Nebraska. The plant material was air-dried and ground to a fine powder (60-80 mesh), and then stored at room temperature in a sealed jar until use. One part root powder was mixed with 10 parts water and allowed to sit for 5 min. The mixture was then poured through a coarse filter to remove root particles. The supernatant was put in an atomizer and misted on pear shoots before introducing the psylla to the feeding arena.

Feeding was allowed for 6 days. On days 1, 3, and 6 of the trial, the number of insects on each shoot (vs on the arena wall) was determined. After 6 days of feeding, the psylla were removed from the arenas and frozen for later dissection and determination of ovarian development. Adult winterform psylla produce honeydew in solid, generally oval-shaped pellets, and the number of pellets was determined in each feeding arena. A preliminary experiment in which ninhydrin was used to stain honeydew (Paguia *et al.* 1980) verified that our counting methods were accurate (correlation between counts of honeydew before and after staining was 0.98 [N = 48]).

We also compared the size of pellets among treatments (third collection date only). After the November, 1994 feeding trial, five feeding arenas from each treatment combination were randomly selected and the honeydew was brushed onto petri dishes. Between 5-20 pellets in each petri dish were randomly chosen, and maximum length and width measured using a dissecting microscope equipped with an ocular micrometer.

The feeding experiment was analyzed as a two-factor (conditioning x shoot treatment) randomized block design, with collection date included as a blocking factor. The response variable was the number of honeydew pellets per arena. Interactions involving collection date were not significant ($p > 0.25$); thus, sums of squares associated with these interactions were combined with residual sums of squares, and the new residual term was used as the error for all tests (Bancroft 1964). Numbers of insects in contact with the pear shoot on days 1, 3, and 6 were compared among treatments with a two factor (conditioning x shoot treatment) repeated measures ANOVA. Size (length; width) of honeydew pellets was compared among treatments with a two factor (conditioning x shoot treatment) multivariate analysis of variance (MANOVA).

All insects were dissected after the feeding trials to determine ovarian development. Development was scored using stages described in Krysan and Higbee (1990), in which 1 is most immature and 7 is mature (>50% of ovarioles with mature eggs). Stage 4 is considered to be the first clear indication of postdiapause development (Krysan and Higbee 1990). Stage 5 is the category at which the first mature egg is present. Scores were averaged for the four insects in a feeding arena, and mean scores compared among treatments with ANOVA.

Spearman's rank correlation was used to determine whether ovarian development and honeydew production were associated. Ovarian development varied among the three collection dates (see below). We therefore removed the effects of collection date from the ovarian scores with ANOVA before estimating correlation. A one-way ANOVA was calculated using mean ovarian score as the dependent variable and collection date (with 3 levels) as the independent variable. Residuals obtained from the ANOVA were then used in the rank correlation analyses.

RESULTS

Conditioning significantly affected ovarian development (Fig. 1). Averaged over the three collection dates, development was most advanced in fenoxycarb-treated insects (mean ovarian score = 5.2; N = 48), intermediate in long-day insects (mean = 4.4; N = 48), and least advanced in control (short-day) insects (mean = 3.2; N = 46). Comparisons among pairs of means were all significant ($p < 0.001$; by single df contrasts). Effects were not constant across collection dates (significant collection date x conditioning interaction [$p < 0.001$]). Although the dates in Fig. 1 refer to two different years, the medians suggest that fenoxycarb had more effect on younger insects (those collected in November) than those presumably more deeply in diapause (December insects). By the late December collection, long-day conditions and fenoxycarb produced almost identical median scores (Fig. 1).

The effects of the cucurbit extract on honeydew production were the same in all three conditioning treatments (Fig. 2; nonsignificant shoot treatment x conditioning effect [$p = 0.45$]). This result indicates that diapausing and postdiapause psylla were affected similarly by the extract. The extract significantly reduced honeydew production in all of the insects. Averaged over conditioning treatments, mean [±SE] honeydew production was 8.9 [2.1] pellets (N = 71) on treated shoots and 14.9 [2.1] pellets (N = 71) on untreated shoots ($p = 0.04$).

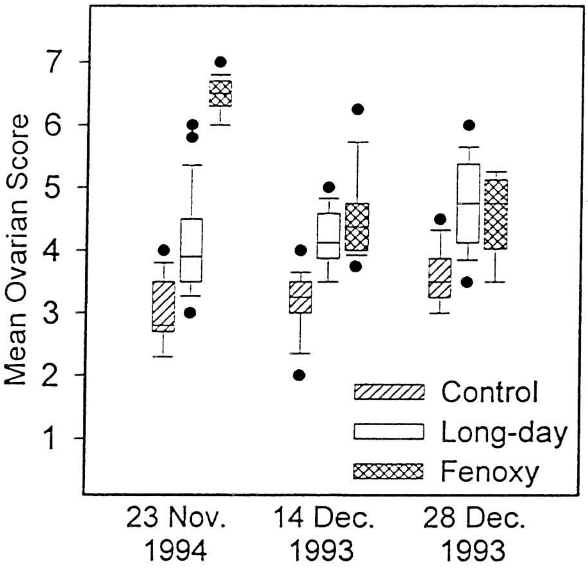

Figure 1. Box plots of effects of conditioning and collection date on ovarian scores; shoot treatments had no effect on mean score ($p = 0.42$). Upper and lower boundaries of boxes 75th and 25th percentiles, respectively. Line in box shows median. Error bars 10th and 90th percentiles. Solid circles are outlying points.

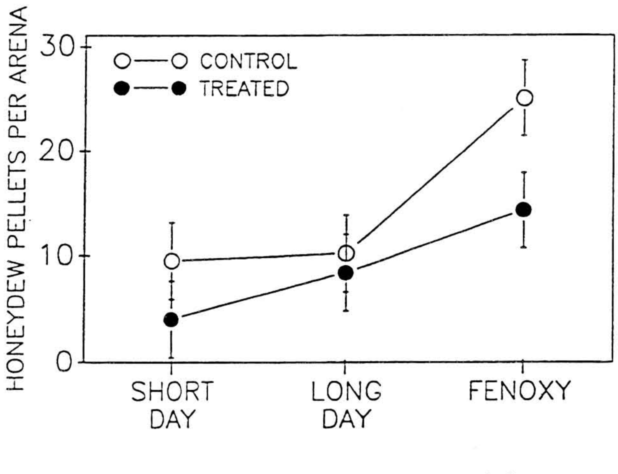

Figure 2. Mean (±SE) honeydew production as a function of shoot treatment and conditioning. Open circles, untreated; filled circles, treated with extract.

Conditioning also significantly affected honeydew production (Fig. 2; $p = 0.001$). Averaged over shoot treatment, mean [±SE] honeydew production was 6.8 [2.6] pellets for the control (N = 46), 9.3 [2.6] pellets for long-day insects (N = 48), and 19.7 [2.6] pellets for fenoxycarb-treated insects (N = 48). Single df contrasts indicated that the control differed significantly from the fenoxycarb treatment ($p < 0.001$) but was similar to the long-day treatment ($p = 0.48$).

Pellet size also differed significantly ($p < 0.001$) among conditioning treatments (shoot treatment effects were nonsignificant). Single df contrasts indicated that pellets were significantly larger in long-day and fenoxycarb-treated insects than in control insects ($p < 0.001$ for both contrasts). Pellets were similar in size between long-day and fenoxycarb-treated insects ($p = 0.19$).

Numbers of psylla in contact with the pear shoot on days 1, 3, and 6 were not affected by shoot treatment ($p = 0.95$) or conditioning ($p = 0.80$). This result suggests that the differences in honeydew production among treatments were not due to differences in the amount of time the psylla were in contact with the pear shoot.

Rank correlation analysis showed that ovarian scores and honeydew production were positively associated (Table 1). That is, insects that produced larger amounts of honeydew tended also to have more advanced ovaries, even within a conditioning treatment.

Table 1.

Correlation (Spearman's rank) between ovarian scores and honeydew production. Effects of collection date removed from scores before estimating correlation.

	Control	Long-day	Fenoxycarb
Untreated shoots	0.46 ($p = 0.026$) (N = 23)	0.22 ($p = 0.29$) (N = 24)	0.52 ($p = 0.010$) (N = 24)
Treated shoots	0.22 ($p = 0.31$) (N = 23)	0.49 ($p = 0.016$) (N = 24)	0.44 ($p = 0.033$) (N = 24)
Combined	0.29 ($p = 0.047$) (N = 46)	0.35 ($p = 0.016$) (N = 48)	0.54 ($p < 0.001$) (N = 48)

DISCUSSION

The winter form of pear psylla is an important target for management, because control of this morph often prevents problems later in the year (Westigard and Zwick 1972). Current difficulties in controlling this stage with broad-spectrum insecticides have forced growers to consider other methods, including techniques that reduce feeding and oviposition (e.g., by applying oil in spring [Zwick and Westigard 1978] or, potentially, by using unpalatable pear varieties [Stuart *et al.* 1989, Bell and Stuart 1990, Puterka *et al.* 1993]).

The specific plant cues that cause probing and continuous feeding in psylla are unknown but, as in other Homoptera (Walker 1987, Walker and Gordh 1989), may include cues received at the plant surface (Ullman and McLean 1988, Horton and Krysan 1990). Quantification of honeydew production is commonly used in studies of Homoptera to screen plant varieties (Padgham and Woodhead 1988) or otherwise determine host suitability (Blua and Toscano 1994). We used this method to show that an extract from buffalo gourd, a nonhost species, caused reductions in feeding. Extracts from buffalo gourd have been shown to cause reduced feeding in other insect species (Weissling *et al.* 1991), perhaps because of the presence of cucurbitacins (Metcalf *et al.* 1982). Weissling (unpublished data) showed that the extract also caused about a 50% reduction in oviposition by pear psylla in choice tests.

A number of physiological and behavioral characteristics are associated with diapause, including delayed ovarian development and reduced feeding (Tauber *et al.* 1986). We tested two specific hypotheses related to diapause in pear psylla. First, we tested whether feeding rates were higher in postdiapause psylla than in diapausing insects. Second, we tested whether feeding by diapausing insects was affected less by a nonhost extract than feeding by postdiapause insects. Numbers and size of honeydew pellets were both larger in fenoxycarb-treated insects than in short-day, diapausing insects. Long-day and short-day insects produced similar numbers of pellets, but pellets were larger for the long-day insects. These results support our first hypothesis.

Our second hypothesis was prompted by the observation that diapausing psylla are numerous outside of the pear orchard, whereas late postdiapause psylla or reproductive psylla are rarely encountered outside of the orchard. Thus, psylla evidently is a more generalized feeder in diapause than when out of diapause. Support for our second hypothesis would have been provided by a significant shoot treatment x conditioning effect in the analysis of variance. No such effect was noted; rather, the extract caused significantly reduced feeding in all insects, independent of diapause status.

Finally, there was evidence that feeding rates and ovarian development were associated (Table 1). Compounds that strongly deter feeding might therefore be used to cause a delay in oviposition. Horton *et al.* (1994) showed that postdiapause development of winterform psylla that overwintered on nonhost species was slower than that of insects that overwintered on pear, results that appear to be consistent with observations in this study. Growers regularly use a spring oil application to delay egglaying by winterform psylla. Our results show that compounds other than oil might also prove to be useful as feeding deterrents or for delaying oviposition.

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