Effect of host plant makeup through nitrogen fertilization and growth regulators on the pear psylla population

Liora Shalitei-Harpaz, a,*, Rika Kedoshim, a, Dovik Openheim, c, Raphael Stern, a and Moshe Coll, c

aNorthern R and D, P.O. Box 831, Kiryat Shmona 11016, Israel
bAgricultural Extension Service, Ministry of Agriculture, Kiryat Shmona 10200 Israel
cDepartment of Entomology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

(Received August 10, 2009; accepted in revised form January 6, 2010)

ABSTRACT

The pear psylla, Cacopsylla bidens (Sulc), is one of the most damaging pests of commercial pear orchards in Israel. However, growers today have only two pesticides left to control the pear psylla, therefore alternative control methods are needed. Our goal was to find ways to control the psylla population through rational use of cultural control methods: manipulating the levels of nitrogen fertilization, and using growth regulators. Our objectives were to determine the impact of nitrogen fertilization level on pear psylla populations, the impact of application of plant growth regulators (clormequat chloride and prohexadion-calcium) on psylla populations, and whether there is an interaction between the two factors in a semi-field-scale and in field-scale experiments. Higher oviposition and nymph development rates were found in trees that had higher leaf-nitrogen contents. Lower oviposition, nymph survival, and nymph development rates were found in trees that were treated with growth regulators. The suppressive impact of growth regulators was expressed even in trees with high nitrogen levels. We concluded that psylla population levels in pear trees can be reduced by using lower levels of nitrogen fertilization and that growth regulators impair psylla growth and development, and may possibly be used to reduce psylla populations.

Key words: pear psylla, cultural control, growth regulators, nitrogen fertilization

INTRODUCTION

The psyllids (Hemiptera: Psylloidae) are monophagous phloem- and xylem-feeding insects (Gullan and Martin, 2003). Seven species of pear-feeding psyllids are present in North America, Europe, and other temperate and subtropical regions (Burckhardt and Hodkinson, 1986) of which most notably Cacopsylla pyricola (Forster), C. pyri (L.), and C. bidens (Sulc) are pests of commercial pears. High-density populations of these insects can cause premature leaf and fruit drop, diminish plant growth, and reduce fruit size, and in addition their honey dew promotes sooty mold on leaves and russetting on fruits (Pfeiffer and Burts, 1983). Pear psylla are also considered vectors of pear pathogens such as the bacterium Erwinia amylovora (Burrill) that causes fire blight (Emmett and Baker, 1971).

*Author to whom correspondence should be addressed.
E-mail: lioraamit@bezeqint.net
Cacopsylla bidens is the common psylla in Israel, and was reported also in Lebanon, Moldova, Romania, France, and Italy (Zeidan-Geze and Burckhardt, 1998). In the climatic conditions of Israel C. bidens can breed 9–11 generations per year, and in warm winters it does not undergo reproductive diapause. Since the pear psylla rapidly acquires inherited resistance to pesticides (Riedl et al., 1981), the range of efficient chemicals for its control is narrowing down, the applied concentrations are constantly increasing, and there is a growing demand for alternative control methods.

The N content of a plant is a typical phenotypic trait, which is determined by the interaction between the plant’s inherent requirements and its environment, and by the substantial changes in phenotype that accompany seasonal and ontogenetic development. The N content of various plant tissues ranges from 0.03 to 7% of their dry weight; the higher concentrations (3-7%) are found in young, actively growing tissues and in storage tissues. Yet plant sap is extremely poor in N (Redak et al., 2004): xylem and phloem sap have N contents of around 0.0002 and 0.004%, respectively (Mattson, 1980). Therefore, phloem- and xylem-feeding insects are highly affected by host-plant quality, as manifested in N content (Awmack and Leather, 2002) and fluctuations in plant sap chemistry appear to determine the diurnal and seasonal variations in their host plant exploitation (Redak et al., 2004). Accordingly, it was reported that increased nitrogen applications enhanced the feeding and development rates of the psylla species C. pyricola on pear trees (Pyrus communis cv. ‘Anjou’ and cv. ‘Bartlett’), leading to larger populations and increased fruit damage (Pfeiffer and Burts, 1983, 1984).

In contrast, application of plant growth regulators such as daminozide or prohexadione-calcium suppressed C. pyricola populations and decreased damage on ‘Bartlett’ pear (Westigard et al., 1980; Lombard et al., 1982; Paulson et al., 2005).

In Israel the main commercial pear variety is ‘Spadona’, for which growers apply a wide range of nitrogen fertilization—from 50 to 200 kg/hectare.

Another common agricultural practice in commercial pear growing is the use of plant growth regulators, aimed to reduce plant vegetative growth in favor of increased yields (Stern and Flaishman, 2003).

So far, it is not fully understood why these plant growth regulators inhibit psylla (Paulson et al., 2005). Our hypothesis was that growth regulators decrease the psylla population by reducing the nitrogen content of the growing shoots.

In the present work our objectives were: (1) to determine the impact of nitrogen fertilization level on C. bidens oviposition, growth rate, and survival; and (2) to determine the impact of growth regulators on C. bidens oviposition, growth rate, and survival; and (3) to determine whether there is an interaction between these two factors.

MATERIALS AND METHODS

The research took place during 2005–2008 in northern Israel at the Matityahu Experiment Station using pear saplings, and in a commercial pear orchard at Kibbutz Yonatan using mature trees.

Experimental set-up

Pear saplings

In August 2005, sixty 1-year-old ‘Spadona’ saplings, grafted on quince rootstock and kept in soil for the first year, were replanted in 25-L pots with perlite soil (Agrekal-Habonim Industries, Kibbutz Habonim, Israel). The saplings were divided into three groups, of 13, 13, and 34 trees, with stem-diameter distributed equally among the groups (3.3 ± 0.29 cm. average ± SD). The saplings were kept in a plot at Matityahu Experimental Station in a complete randomized block (four rows with 15 trees in each row, 1 m between adjacent trees). Three irrigation pipes ran along the rows and each tree received drip irrigation via a 2 L/h garden dripper from one of the systems designed to supply 3 different levels of nitrogen fertilization: 0, 50, and 100 ppm N, applied as ammonium nitrate. Apart from nitrogen, all other macro and micro nutrients were equally applied among the groups in the form of “Sarit and Bar Koret” fertilizers (Fertilizers and Chemicals Ltd., Haifa, Israel). Irrigation was applied five times a day, to a total of 4 L per day. The trees were treated for 8 months at the different nitrogen levels before the first trial started.

Perlite was chosen as the growing substrate because of its lack of nutrients, but growing pears in perlite is a new technique on which information is sparse, therefore we monitored nitrogen levels regularly three times a year along the pear growing season in March, August and November in the dripper, in the drain water, in the branches, and in the leaves. The tests of nitrogen level in the tree parts were carried out in the Extension Service laboratories of MIGAL (Kiriath Shemona, Israel) and Zemach Laboratories (Zemach, Israel). Leaf nitrogen content was measured in the 5th leaf of a young shoot from each tree, using wet digestion with H₂O₂ and sulfuric acid, and a colorimetric analysis with Nessler reagent. The results are presented as nitrogen percentage in the dry weight.

In order to ensure naturally occurring psylla infestation on the pear sapling, this experimental plot was located 0.5 km away from a pesticide-free pear orchard.
that hosted a rich pear psylla population, and no pesticides were used on the saplings.

**The commercial orchard**
A 7-year-old commercial ‘Spadona’ pear orchard planted in 1999 at a density of 1100 trees per hectare was used to assess the impact of two plant growth regulators on the psylla population. The orchard was irrigated, fertilized, and treated against pests according to the grower’s commercial practice.

**Pear psylla**
Pear psylla were obtained from a pesticide-free pear orchard in the Matityahu Experiment Station. Adults for use in the experiments were collected early in the morning by the beating tray method (Burts and Retan, 1973), sexed, and placed in the experiment on the same day. Nymphs were collected from freshly cut branches that were brought to the laboratory and gently brushed off the branch with a delicate brush. Their instar was determined, and they were transferred with the brush to the experimental plants.

**Effect of plant nitrogen level on psylla population**

**Experiment 1: Effects of nitrogen level on C. bidens oviposition and development in a free-choice semi-field experiment**

In order to determine the free-choice oviposition preference and nymphs’ development rate among naturally occurring psylla, 13 trees of each nitrogen-level treatment in the Matityahu plot were selected. On each tree one young shoot was selected, carefully examined, and all adult psylla and natural enemies were removed by gently shaking and brushing them off, while taking care that no nymphs or eggs fell off. The adult-less shoots were then caged by tying a 15 × 25 cm white paper bag over the top 20 cm of the shoot. After 2 weeks, the young shoots covered with the bags were taken to the laboratory and the number of eggs, nymphs of each instar, and adults was recorded.

**Experiment 2: Effects of nitrogen level on C. bidens oviposition and development in a non-choice semi-field experiment**

In order to discover whether the tree nitrogen level affected the oviposition rate under controlled, no-choice conditions, 13 trees from each nitrogen-level treatment were selected. From each tree the 5th leaf of a young shoot was cut, including a 1-cm length of shoot on each side. Each leaf was washed to remove all arthropods, dried, and placed in a 50-ml plastic test tube. Adult psylla (2 females and 2 males) were released into each test tube, and the test tubes were kept in a controlled-environment room (16:8 L:D; 23 ± 2 °C) for 1 week. The test tubes were then opened, the adults were removed, and the numbers of eggs were recorded. The leaves were then returned to the test tubes for another week, after which the numbers of eggs and nymphs of each instar were recorded. Egg-hatch success rates and nymph development rates were calculated.

**Effects of plant growth regulators on pear psylla population**

Three plant growth regulator treatments were applied in the commercial orchard:

1. CCC (clormequat chloride) 1% sprayed at full bloom (FB), plus three applications at 0.5%, sprayed 1, 2, and 3 weeks after FB;
2. Regalis (prohexadion-calcium) at 300 ppm, sprayed at FB;
3. Control of water sprayed at FB.

Each treatment was applied to six blocks in a complete random block design. Each replication comprised one tree separated from the adjacent treatment by a border tree. Growth regulators were applied with a “gun sprayer”. To measure the effect of growth regulators on tree growth, the lengths of six young shoots on each tree were measured every 2 weeks. The effects of the growth regulators on the psylla population were assessed at three different time points, according to the pear shoot growth as described below.

**Zero count**

Seven days after application of the growth regulators, when there was still no significant difference in shoot length between the treatments, the natural distribution of pear psylla was assessed. The number of nymphs and eggs was determined by collecting five shoots from each tree and counting eggs and nymphs with a stereo microscope. Adults were determined by beating two branches of each tree above a tray (Burts and Retan, 1973).
Experiment 4: Effect of plant growth regulators on C. bidens oviposition and development in a non-choice field experiment

Three weeks after application of the growth regulators, when the effects on shoot growth were visible and significant, young shoots of the same size, and from the same height on each tree in every block, were cleared of all arthropods and enclosed in paper cages with four female and two male adults, as in Experiment 2. After 2 weeks, the shoots covered with the bags were taken to the laboratory and the numbers of eggs, nymphs of each instar, and adults were recorded. We repeated this experiment 2 months later, after termination of growth regulator applications, when tree growth rates had become similar in all treatments.

Interaction between nitrogen level and growth regulators

Experiment 5: Effects of plant growth regulator on C. bidens oviposition and development in a free-choice semi-field experiment on trees with a high nitrogen level

Twenty potted saplings that received the higher level of nitrogen (100 ppm) were divided into two groups, which were treated with CCC at two levels (2% and 0) in a complete randomized design. The experimental setup was as in Experiment 1. After 2 weeks the bag-covered shoots were taken to the laboratory where the numbers of eggs, nymphs of each instar, and adults were recorded. The leaf nitrogen levels in the growth regulator and the control treatments were compared.

Experiment 6: Effect of plant growth regulators on survival of C. bidens nymphs on trees with high nitrogen level

Twenty-one potted trees that had received nitrogen at 100 ppm were divided into three groups of similar size distributions, which received the following treatments: 1% CCC; Regalis at 200 ppm; and water (control). After 2 weeks, three shoots from each tree were selected and the 5th leaf was cut from each of them (i.e., 21 leaves from each treatment), as in Experiment 3. Each leaf was washed to remove all arthropods, dried, and placed in a 50-mL plastic test tube. Ten 1st instar psylla nymphs were gently placed on each leaf (i.e., a total of 630 nymphs) and the test tubes were kept in a controlled environment (16:8 L:D; 23 ± 2 °C) for 1 week, after which the test tubes were opened and the numbers of live nymphs from each instar was recorded.

Data analysis

The total number of offspring was compared among treatments with one-way ANOVA. Significant differences between means were identified with Tukey HSD posteriori test \((p < 0.05)\) using the JMP software, version 5.0.1.1 (SAS Institute Inc., USA). In order to evaluate the effect of the plant growth regulators on nymph development, the relative abundance of eggs and each nymph instar were compared among treatments with one-way ANOVA. To meet the assumptions of the test data were \([\sqrt{p}]\) transformed. In the choice experiments (Experiments 1 and 5) the Chi-squared goodness of fit test was used. In the survival experiment (Experiment 6) we calculated the survival rate of psylla nymphs on each leaf, and compared them between treatments by using one-way nested ANOVA after applying arcsine \([\sqrt{p}]\) transformation (Zar, 1996).

RESULTS

Effect of plant nitrogen level on C. bidens population

Experiment 1: Effects of nitrogen level on C. bidens oviposition and development in a free-choice semi-field experiment

Nitrogen level assessments of the treated trees in March 2006 (prior to bloom) found nitrogen contents of 2.95, 2.45, and 1.90%, in the trees that had received nitrogen fertilization at 100, 50, and 0 ppm, respectively. The total number of psylla offspring was higher on the trees that received more nitrogen than on those that received none \((\chi^2 = 95.5; p < 0.001; \text{Fig. 1})\). The proportion of 1st instar nymphs among the total number of offspring also increased with increasing nitrogen in the treatment \((0, 8 ± 5%, \text{and } 13 ± 6%, \text{in the } 0, 50, \text{and } 100 \text{ ppm treatments, respectively})\) indicating a more rapid development as nitrogen application increased \((F_{2,32} = 4.22; p = 0.023)\).

Fig. 1. Total numbers of psylla offspring (average ± S.E.) (eggs + nymphs) on pear trees under various N treatments in a free-choice experiment \((\chi^2 = 95.5, p < 0.001)\)
Experiment 2: Effect of nitrogen level on C. bidens oviposition and development in a non-choice semi-field experiment

The total number of psylla offspring was higher on the trees that received the higher nitrogen concentration than on those that received less or no nitrogen. The main contribution to this difference was the significantly larger number of eggs found in the high-nitrogen treatment (Fig. 2).

Experiment 3: Effect of nitrogen level on C. bidens oviposition and development in a non-choice laboratory experiment

The oviposition rate under the higher nitrogen treatment was twice as much as that under the lower and zero nitrogen treatments (30.7 ± 10.22, 13.5 ± 6.28, and 9.2 ± 4.15, respectively) although the difference was not significant. Egg hatch success was significantly higher in the two nitrogen treatments than in the no nitrogen treatment: 45 ± 10, 47 ± 9, and 9 ± 10%, respectively (F₁,₁₃ = 4.1; p = 0.04). Nymph numbers and development rates were positively correlated with nitrogen level: only at the higher nitrogen level did the nymphs reach the 3rd instar within a week, whereas at the lower levels they reached only the 2nd instar (Fig. 3).

Effects of plant growth regulators on C. bidens population

The effect of plant growth regulators on plant growth was significant 3 weeks after application and ceased to be significant by 11 weeks after application. By then, the shoot growth rate in treated trees was similar to that of the control trees (Fig. 4). We assessed the effects of plant growth regulators on the psylla population at three points in time: before the effect was visible on the trees (zero count), when the effect started to be significant, and when the effect had disappeared (Experiment 4).

Zero count

Seven days after applications of plant growth regulators, there were no differences between the psylla populations (eggs, nymphs, and adults) found on the treated trees and those on the control trees.

Experiment 4: Effect of plant growth regulators on C. bidens oviposition and development in a non-choice field experiment

Twenty-four days after application of growth regulators, the differences in tree growth had become significant (F₂,₁₀ = 7.20; p = 0.015; Fig. 4) there were more psylla offspring on the control trees than on the treated trees (eggs: F₁,₁₀ = 4.56, p = 0.042; nymphs: F₁,₁₀ = 5.31, p = 0.029; total offspring: F₁,₁₄ = 4.16, p = 0.026; Fig. 5). By 87 days after regulator application, when differences in growth rate had disappeared, there were fewer offspring in all the treatments, and no significant differences between the treatments and the control (Regalis 25 ± 25; CCC 56 ± 26; control 89 ± 25. Average ± S.E.)

Interaction between effects of nitrogen level and growth regulators on C. bidens population

Experiment 5: Effect of plant growth regulators on C. bidens oviposition and development in a free-choice semi-field experiment on trees with high nitrogen level

There were significantly fewer psylla offspring on trees that received a high level of nitrogen and were treated with 2% CCC than on trees that received a high level of nitrogen but were not treated with CCC (Fig. 6). Leaf nitrogen levels in the CCC-treated and control trees were 3.07 ± 0.16 and 2.88 ± 0.13%, respectively, but this difference was not significant. The same pattern of
a slightly but not significantly higher nitrogen level in the leaves of the growth regulator-treated trees appeared in the commercial orchard (Regalis 2.5 ± 0.07%; CCC 2.38 ± 0.05%; control 2.28 ± 0.05%).

**Experiment 6: Effect of plant-growth regulators on survival of C. bidens nymphs in laboratory experiment on trees treated at high nitrogen level**

The survival rate of psylla nymphs was significantly lower on leaves from growth regulator-treated trees than on the control trees (\( F_{2,40} = 4.24; p = 0.021 \); Fig. 7)

**DISCUSSION**

The pear psylla *C. bidens* responded positively to plant quality, by exhibiting oviposition preference and achieving higher oviposition rates on nitrogen-rich pear trees. Egg hatch success was higher and nymph development rate was accelerated in nitrogen-rich trees. These results are consistent with findings that other species of pear psylla responded to plant nitrogen level (Lombard et al., 1982; Pfeiffer & Burts 1983, 1984; Daugherty et al., 2007). In addition, there is ample evidence of the effect of elevated level of nitrogen on reducing the concentration of secondary products that, in turn, improve the performance of herbivores (Mattson, 1980; Bryant et al., 1983; Waterman and Mole, 1989; Kytö et al., 1996; Koricheva et al., 1998; Herms, 2002).

Several studies have demonstrated that the use of growth regulators reduced pear psylla populations and their damage (Lombard et al., 1982; Paulson et al., 2005). We found that oviposition rate, nymph survival and development rates, were lower in trees that had been treated with growth regulators. The effect was limited in time; it appeared approximately 2 weeks after application and disappeared after 3 months. These findings suggest that the effect was not related to better pesticide coverage, but to some interaction between the plant and the pest.

In looking for interaction between nitrogen level and the use of growth regulators, we found that nitrogen...
levels in growth-regulated trees were not lower than in untreated trees. Therefore, the preliminary hypothesis that the effect of plant regulators is related to lower leaf-nitrogen content was not supported. Several studies have demonstrated that the use of the growth regulator clormequat chloride caused the production of polyphenols in treated trees (Haque et al., 2007). Roemmelta et al. (2003) showed that novel flavonoids were formed in young leaves of apple (Malus domestica) after treatment with prohexadione-Ca. The compounds were identified as luteoliflavan, luteoliflavan 5-glucoside, eriodictyol 7-glucoside, and 6′-trans-p-coumarylerydicitol 3′-glucoside. Although these metabolites are not known for their insecticidal properties, it is possible that they might be involved in the negative effects on psylla, through their impact on psylla endosymbionts.

The results of the present study support earlier suggestions that management of plant quality may be an important factor in pear psylla biocontrol (Mcmullen and Jong, 1972; Pfeiffer and Burts, 1983). We conclude that in Israel pear psylla management on the pear cv. ‘Spadona’ would be improved by avoiding excessive fertilization or by applying plant growth regulators. More research is needed to optimize nitrogen use in pear growing, in order to minimize the amount of nitrogen available to the psylla while maintaining a sufficient level for pear production. Also, further investigation is needed into fine tuning the use of plant growth regulators during the course of the season to achieve biocontrol of psylla without impairing yields.

ACKNOWLEDGMENTS

We thank Moshe Agiv for technical assistance, and Yonatan Ratner and Moti Malol from Kibbutz Yonatan orchard team for allowing us to run experiments in their orchard. This study was supported by the Israeli Fruit Board.

REFERENCES


Roemmelta, S., Zimmermann, N., Rademacher, W., Treutter, D. 2003. Formation of novel flavonoids in apple (Malus domestica) treated with the 2-oxoglutarate-dependent


