Effect of tillage on abundance of Japanese beetle, *Popillia japonica* Newman (Col., Scarabaeidae), larvae and adults in highbush blueberry fields

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Abstract: At 15 commercial highbush blueberry farms, fields where row middles were tilled had 72% lower larval density of *Popillia japonica* than fields with grass in row middles. *Popillia japonica* larval populations were similar in the perimeters of tilled and untilled fields. Soil parameters measured in these sites were not correlated with larval density of *P. japonica*. Samples of adult beetles on bushes showed that they were significantly less abundant in tilled fields compared with fields with grass in the row middles. The abundance of larvae inside fields during the spring was significantly correlated with early, but not late summer abundance of adult beetles on bushes. Comparisons of different tillage timings showed that grassy plots between rows of blueberry bushes that were tilled in spring and kept bare all year round had lower abundance of *P. japonica* larvae than those that retained perennial ryegrass. The effect of tillage timing on larval abundance was not consistent across 2 years, but most timings caused similar reduction in *P. japonica* larval density in the row middles. Tillage in the spring and in the autumn caused 50.5% and 68.8% reduction of larval density in each year respectively. These results indicate that tillage can reduce *P. japonica* larval and adult abundance in highbush blueberry fields.

Key words: *Vaccinium corymbosum*, adult density, cultural control, larval density, rotovation, white grub

1 Introduction

The Japanese beetle, *Popillia japonica* Newman (Col., Scarabaeidae), is an invasive insect with potential for range expansion into many of the major agricultural production regions of the world (Allsopp, 1996). *Popillia japonica* is currently the most important pest of highbush blueberry, *Vaccinium corymbosum* L., in the midwestern US (Anon, 2001; Isaacs et al., 2004). It is a univoltine insect, with greatest adult abundance from mid-July to August (Fleming, 1972), when beetles feed on leaf tissue and ripe blueberry fruits. If beetles are not controlled, fruit can be contaminated when harvesting machines knock beetles off the bushes. Over 70% of Michigan's 7285 ha of blueberry crop is harvested mechanically (Kleweno and Matthews, 2002), and because the market demands fruit completely free of insect contamination, strategies are needed to minimize the risk of adult beetles being present during harvest. Foliar insecticide applications continue to be the foundation of *P. japonica* management in blueberries and many other fruit crops, as growers strive to meet exacting quality standards. Additional strategies targeting larvae, which develop in the soil, may help growers reduce populations of *P. japonica* within infested blueberry production regions and minimize the risk of beetles spreading into uninfested areas. Such strategies could also have the long-term benefit of reducing the number of foliar applications of insecticides.

Insect larval populations are influenced by initial adult distribution and density, oviposition preference and host plant acceptability, egg survival, and host-plant suitability (Singer, 1986; Renwick, 1989). Conditions in and around crop fields can favour or inhibit development of *P. japonica* depending on whether requirements for population development are met (Vittum et al., 1999). Within many Michigan blueberry fields and along the perimeter of these fields, ground covers of seeded grass or the naturally invading mix of grass and broad-leaved weeds are commonly used to maintain soil structure, provide conditions where agricultural machinery can be driven during wet conditions, reduce soil erosion, and prevent pesticide and fertilizer runoff (Priggs and Hancock, 1992). Fields are often irrigated to minimize crop stress and to maintain fruit quality and yield during periods without sufficient rainfall. Together, these factors provide ideal habitat for oviposition, egg hatch, growth and survival of *P. japonica* larvae (Régnière et al., 1981; Allsopp et al., 1992; Potter et al., 1996).
Cultural practices such as tillage can have strong negative effects on arthropod pests, by modifying the soil habitat where many insects reside during at least a part of their life cycle (Funderburk et al., 1990). As immature stages of *P. japonica* feed on the roots of grasses, mechanical manipulation of their habitat may alter their survival and development (Fleming, 1976). Previous studies have investigated the potential for control of white grubs through some cultural practices (mowing height, irrigation, application of organic fertilizer, aerification, heavy rolling) that are appropriate in turf systems (Potter et al., 1996). Tonskas and Stinner (1991) explored the effect of tillage on adult *P. japonica* abundance in soy bean fields, but there is a lack of information on cultural manipulation of *P. japonica* in perennial fruit crops and woody ornamentals.

To determine the response of *P. japonica* to tillage in the row middles of highbush blueberry fields, we first compared the relative abundance of larvae at 15 commercial blueberry fields in row middles that were either tilled or covered with mowed permanent sod comprised of seeded grass and local weed populations. The relationship between larval density and some soil parameters was examined to determine whether variation in grub abundance is explained by soil characteristics. Selected parameters describe conditions for nutrient uptake in the soil for the plants that serve as food sources for the grubs. Thus, these factors could have an indirect effect on herbivorous insect abundance. At six commercial blueberry fields (three tilled and three grassy), larval and adult densities were compared at the perimeter and interior of the fields. Spring larval populations were compared with subsequent summer adult populations, to determine whether there is a relationship between the two life stages within blueberry fields. In addition to these population studies, the effects of: (1) the presence or absence of a grass ground cover and (2) different tillage timings on larval abundance of *P. japonica* were investigated experimentally over two growing seasons in a research planting of highbush blueberry.

2 Methods

2.1 Larval density in commercial fields

The density of larval *P. japonica* populations was determined across the primary blueberry production region of south-west Michigan (Ottawa, Allegan, Van Buren, and Berrien counties) in 2001 and 2002. Fifteen fields in 13 commercial blueberry farms with a history of *P. japonica* infestation were sampled. The sizes of the fields ranged from 1 to 5 ha.

Larval density was determined in April 2001, September 2001, May 2002, and September 2002 by taking 15-cm-deep soil cores from the perimeter of each field and from the area between the rows of blueberry bushes (row middles) of each field. This was done using a cylindrical golf cup cutter (area = 95 cm²) (Parmenter & Andre Inc., Grand Rapids, MI). In each field, 80 samples were taken approximately 1 m from the edge of the blueberry field (in the drive lane around the field), by sampling in 20 positions spread out along each of the four sides of the field. Ten row middles were selected that were spaced evenly across the fields, but more than 5 m away from the field border. In each selected row, six samples were taken equidistant between rows of bushes along the length of the field without sampling within 5 m from the row ends (60 interior samples per field). Soil cores were examined in the field, and all beetle larvae were placed in plastic bags with a small amount of soil. The bags were labelled with date, location, and number of larvae and transported back to the laboratory in a cooler containing an icepack. Larvae were identified to species using the diagnostic rastral patterns and other morphological features (Vittum et al., 1999). For each field and sampling date, whether the fields were tilled or had grassy row middles was recorded. Because row middles were tilled based on growers’ management decisions, the number of fields that had tilled or grassy row middles varied by sampling date. Field perimeters were not tilled and had a grass–weed mix cover throughout the study, regardless of the type of row middle management within the field. Foliar insecticides were applied to the bushes according to standard practices (Wise et al., 2003) at all the farms.

The average numbers of *P. japonica* larvae in samples taken in the row middles and in the perimeter of the 15 fields were compared between tilled and non-tilled fields. Data were square-root transformed, (sqrt(x + 0.5)), before analysis to meet normality assumptions, and were analysed with a two-way ANOVA (tillage and date as factors) (SAS Institute, 1999). t-tests were used at each sample date to compare samples from tilled and untilled fields. In cases where the population variance of the two compared groups were different, the Satterthwaite’s approximation was used for calculating degrees of freedom for the t-tests (SAS Institute, 1999).

2.2 Soil analysis

Soil cores (area 95 cm², 15 cm deep) were taken for soil-quality analysis from all 15 fields in autumn 2002. One sample was taken from each of the four sides of each field, and four samples were taken from row middles, distributed across the field. For each sample, six parameters describing soil characteristics were examined: soil pH, organic matter (%), and the concentration of P, K, Mg, Ca (all in p.p.m.). Samples were analysed by A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN). Multiple regression was used to determine the relationship between the six measured soil parameters and average larval densities at the 15 fields in autumn 2002 (SAS Institute, 1999). Samples taken from the interiors and perimeters of the fields were analysed separately.

2.3 Relationship between larval and adult populations

In 2003, fields from the study described above that had consistent row middle management history during 2001–2002 were selected, comprising three tilled fields and three with grass in their row middles. Larval populations of *P. japonica* were sampled at all six fields in May using the methods described above, taking 80 samples from the interior and 80 from the perimeter of fields. Perimeter samples were taken approximately 1 m away from the bushes, with 20 samples distributed evenly on each of the four sides of the field. From the row middles of fields, eight samples were taken from 10 row middles in a similar manner as described in the previous larval study.

The number of adult *P. japonica* per bush was sampled once in July, and once in August at each field by counting the number of adult beetles on 80 bushes on the perimeter (20 on each side of the field) and on 80 inside (10 bushes from eight rows). All inside samples were taken at least 5 m from the
edges of the field and the 10 sampling points were evenly spread out through the rows. Adult beetles were counted on sunny days with low wind velocity, and observers moved carefully through the field so as not to disturb the beetles.

The abundance of adult *P. japonica* in July and in August was compared between grassy and tilled fields with the Mann–Whitney *U*-test (SAS Institute, 1999). Multiple regression analysis was used to determine relationships between larval and adult populations of *P. japonica* within fields (SAS Institute, 1999). The regression model consisted of larval and adult numbers as variables and position as an indicator variable (perimeter = 0, interior = 1). Statistical analyses were performed on average numbers of adults per bush and average numbers of larvae per sample and were considered significant at *α* = 0.05.

### 2.4 Effect of tillage on larval establishment

This experiment was conducted in a 4-year-old 0.4-ha field of *V. corymbosum* cv. Rubel, at the Trevor Nichols Research Complex (TNRC) in Fennville, MI. The planting was established 2.5 m row middles before the start of this experiment, on a 3.6 m x 1.2 m spacing with 12 bushes in each row, during which time the row middles were kept clean of plants. *Popillia japonica* larvae were not found in soil samples taken before the experiment in April, 2002. Ten row middles (14 x 2.5 m area between two rows of bushes) were tilled in 10 May 2002 and assigned to be grass or bare ground treatments. Treatments were assigned within the planting in a randomized complete block design with five replicates. Grass plots received perennial ryegrass seed at 27 kg/ha (Lolium perenne L., Michigan State Seed Solutions, Grand Ledge, MI) on 23 May 2002, which was rolled after planting to improve germination. This grass species was chosen because of its tolerance to soil acidity. Bare ground plots received further treatments of herbicide (Roundup Ultra 4WSL (Monsanto, St. Louis, MO, USA) at 31.3 l/ha) spot-applied with a 3.78-l hand-held sprayer when needed, to keep the soil free of vegetation during the growing seasons. Fertilizers and pesticides were not used in the plots during this experiment. Samples were taken in September 2002, May and October 2003 to assess larval density of *P. japonica*. Five soil cores were taken with a golf cup cutter (area = 95 cm²) from each plot, and collected larvae were identified to species level as described above.

Larval densities in the two treatments were compared for each sample date with a Mann–Whitney *U*-test, as normality assumptions were not met even after transformation (SAS Institute, 1999; Ott and Longnecker, 2001).

### 2.5 Effect of tillage timing in infested plots

This study was conducted in two 0.07-ha sections of a 1-ha field of *V. corymbosum* cv. Rubel, at TNRC. The blueberry plants were 10 years old on a 3.6 m x 1.2 m plant spacing, with 12 bushes in each row. The 14 m x 2.5 m row middles between the rows of blueberry were comprised of naturally occurring weeds dominated by grass species, and were kept mowed. The experiment was conducted in 21 adjacent row middles in 2002 and was repeated in 2003 in 21 adjacent row middles located in a different section of the same blueberry field. These two sections of the field were selected based on prior sampling to verify the presence of *P. japonica* larvae across the field sections. The following three treatments were applied during both years to the different sections: tillage once in the spring, tillage once in the autumn, or tillage once in the spring and once in the autumn. Treatments were assigned to the row middles in a completely randomized design with seven replicates. To apply tillage treatments, a tractor-powered BushHog rotovator (Model H72; Allied Products Corporation, Selma, AL) was driven between the bush rows twice, at 15 cm depth. Plots that were tilled in the spring were kept weed-free in the growing season by applying herbicide (as described above). Tillage treatments were applied on 10 May and 9 September in 2002 and on 2 May and 10 October in 2003. All plots were sampled with a golf cup cutter as described above in the spring and the autumn, before and after tillage treatments. *Popillia japonica* larvae were sampled 2–4 days before and 10–14 days after tillage. Three samples were taken per row middle in 2002 and six in 2003, and collected larvae were identified to species.

Larval count data were Poisson distributed, so pre- and post-tillage densities were compared using a log-linear model (PROC GENMOD; SAS Institute, 1999). Comparison of the percent reduction in *P. japonica* density between treatments was performed after arcsine transformation with a one-way ANOVA procedure followed by Tukey’s mean separation (SAS Institute, 1999).

### 3 Results

#### 3.1 Larval density in commercial fields

In spring 2001, the average density of *P. japonica* larvae at commercial blueberry farms was 0.28 ± 0.01 larvae per sample. This increased slightly in autumn 2001 and then decreased at each sampling time to 0.14 ± 0.01 larvae per sample by autumn 2002. Larval density was greater on the perimeter than in the row middles of fields throughout this study, regardless of whether the row middles were tilled in the field. During the two sampling seasons in 2001, larval densities at the perimeter were approximately four-fold greater than those in the interior, and by spring 2002 there was a three-fold difference between these positions which decreased to 0.4-fold in autumn 2002. When comparing the four sampling times, the highest average larval density in the perimeter of fields occurred in autumn 2001 (0.48 ± 0.02 larva per sample) and the lowest in autumn 2002 (0.17 ± 0.01 larva per sample). Eighty-seven per cent of all the larvae were found in fields with grassy row middles compared with those that were cultivated, with the largest difference between the two treatments in spring 2001 (0.14 grubs per sample) and the smallest difference in autumn 2001 (0.09 grubs per sample) (fig. 1). Larval density was significantly lower in tilled fields than in those with grassy row middles in spring 2001 (*t* = −3.41, *P* < 0.01) and autumn 2001 (*t* = −2.94, *P* < 0.01) (fig. 1a). At the other two sampling times, the differences between the two types of row middle management were not statistically significant, even though, on average, there were twice as many larvae in grassy row middles compared with tilled ones in autumn 2001 and 13 times more in spring 2002. Some growers changed row middle management practices during the study, so between six and 13 of the 15 fields sampled had a grass ground cover, depending on the date of the sample (fig. 1).

On average, larval density was 12 times greater in the perimeter than in the row middles of tilled fields, whereas density was only 2.4 times greater at the
perimeter of grassy fields. Across the whole study, 78.5% of the larvae detected in soil samples were found in the perimeter of the fields, with the highest being 85% in autumn 2001, and the lowest value being 65% in autumn 2002. 

Popillia japonica larval density at field perimeters was not significantly different between fields with grassy or tilled interiors (F_{1,3} = 3.44, NS), but in all of the samples, a greater density of larvae was found in the perimeter of grassy fields (fig. 1b). In addition to P. japonica, larvae of European chafer (Rhizotrogus majalis Razoumowsky, Col., Scarabaeidae) and Asiatic garden beetle (Maldera castanea Arrow, Col., Scarabaeidae) were also found in the samples, but in very low numbers.

### 3.2 Soil analysis

Average organic matter composition was similar in samples taken from the row middles and from field perimeters, at 3.3 ± 0.1% and 3.5 ± 0.2% respectively. The largest difference between positions was found for calcium, which was 668 p.p.m. in the perimeter on average, compared with 558 p.p.m. in the row middles, but this difference was not statistically significant. The average concentrations of potassium and magnesium ions were not significantly different between the perimeter and the row middles. There was no significant relationship between the measured soil parameters and larval densities, either for the soil samples taken in the row middles (F_{6,8} = 0.57, R^2 = 0.3, NS) or in the perimeters (F_{6,8} = 2.85, R^2 = 0.68, NS).

### 3.3 Relationship between larval and adult populations

Adult beetles were less abundant in July (0.88 ± 0.41 beetles per bush) than in August (1.68 ± 0.59 beetles per bush). At both sampling times, greater numbers of beetles were detected on bushes in blueberry fields with permanent sod than in tilled fields (fig. 2). This pattern was consistent across the positions of the sampled fields, but statistically significant differences in average adult beetle numbers were only detected in the perimeters (Z = −1.96, n = 3, P = 0.049 for both sample times). Fields that had greater larval abundance in the spring generally had more adult beetles on bushes in July, regardless of the position in the field (fig. 3). Regression analyses showed that larval populations measured in the spring predicted 84% of the variability in July adult populations (F_{2,9} = 25.19, P < 0.01). In contrast to the July adult samples, beetle abundance in August was not correlated with earlier larval abundance (fig. 3) (F_{2,9} = 0.60, NS).

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**Fig. 1.** Density of Popillia japonica larvae (average + SE) in samples taken from (a) row middles and (b) perimeters of commercial highbush blueberry fields with row middles that were either tilled or grassy. An asterisk above the bars indicates a significant difference between tillage treatments (α = 0.05). Numbers on the lower bars show the number of farms in each treatment during each date for (a) and (b).

**Fig. 2.** Average number (+SE) of adult Japanese beetles per blueberry bush in July and August in fields with grass (n = 3) or tillage in the row middles (n = 3). Values are presented for samples taken from (a) the field interior and (b) the field perimeter. Bars with an asterisk are significantly different (α = 0.05).
3.4 Effect of tillage on larval establishment

Densities of *P. japonica* larvae increased during this experiment, with the greatest increase occurring in plots that were planted with ryegrass (fig. 4). Comparison among the different sampling times showed that larvae in ryegrass plots were three times more abundant in autumn 2003 than in the previous autumn ($Z = -3.6, n = 10, P < 0.01$). During each of the three sampling dates, significantly fewer larvae were found in plots that were kept free of plants compared with those with ryegrass. The largest difference between the two treatments in the average number of larvae was in autumn 2003 ($Z = -3.8, n = 10, P < 0.01$).

3.5 Effect of tillage timing in infested plots

The change in *P. japonica* larval abundance due to tillage in the spring was not consistent over the two years; this treatment caused a significant decline in larval density in 2003, but not in 2002 (table 1). Tillage in the autumn caused a decrease in *P. japonica* during both years when comparing pre- and post-tillage larval densities, but this trend was significant only in 2003 (table 1). Tillage of the same plot twice, in the spring and in the autumn, significantly reduced larval density in both years, when comparing overall reduction from spring to autumn; by $50.5 \pm 11.45\%$ in 2002 and $68.8 \pm 14.6\%$ in 2003. The effect of tilling plots once was not consistent across the 2 years: although most of the treatments caused a similar reduction in *P. japonica* larval density in the row middles, in each year 1 of the treatments did not provide any control. The percent control values were not significantly different in either year ($F_{3,20} = 0.65, P = \text{NS} in 2002; F_{3,20} = 0.13, P = \text{NS} in 2003$).

4 Discussion

This study demonstrates that tillage is an effective cultural control for managing larval and adult abundance of *P. japonica* in highbush blueberry fields. At commercial farms, tilled row middles had lower larval and adult density of *P. japonica* than row middles with grass cover. Smitley (1996) found lower densities of *P. japonica* larvae and adults in nurseries with clean fields compared with weedy ones, and this pattern is expected to be a general response of *P. japonica* to the lack of suitable hosts, leading to lower rates of oviposition and/or larval survival.

Tillage of the row middles did not significantly reduce *P. japonica* larval populations in the uncultivated perimeters. Therefore additional control strategies, such as targeting early instars with soil-applied insecticides (Mannion et al., 2001) could be used to control *P. japonica* in regions of the farm that are not tilled. As the results depicted in fig. 4 suggest, the removal of grassy ground cover should reduce the number of beetles emerging in the field, providing fewer gravid females to move to the perimeter for oviposition. The higher larval density of *P. japonica* in the perimeters of fields, independent of the ground cover management regime, could be because gravid females arriving from outside the field are arrested at the field perimeters, and/or because females may leave insecticide-treated areas inside the field to lay eggs in the untreated soil around the fields.

The significant relationship between larval populations in the soil during the spring and adult
populations on bushes in July (fig. 3) suggests that spring larval sampling can be used to estimate local adult populations. This may allow growers to predict the risk of *P. japonica* adult presence across their farm and provide information to focus management inputs to fields with the greatest spring larval populations. Smitley (1996) found a positive correlation between the number of *P. japonica* adults trapped and the number of larvae in nursery fields. Choo et al. (2002) found a similar positive relationship between adult and larval populations of oriental beetle (*Exomala orientalis* Waterhouse) in golf course fairways in Korea, with higher adult abundance in fairways that had high larval densities. In their study of *P. japonica* spatial dispersion in golf courses, Dalthorp et al. (2000) suggested that grub dispersion patterns are dependent on location of adult feeding aggregations. *Popillia japonica* emerge from the soil in early July in Michigan, and the newly emerged adults may initially feed and aggregate close to their larval habitat if there is suitable food nearby. This may explain the greater predictability of adults in July compared to August. After 4–6 weeks of adult emergence and movement between fields, spring larval density no longer predicted the number of adult beetles on bushes.

The reduction of adult *P. japonica* densities by tillage may be the result of fewer larvae emerging from these fields, but it could also be the case that fewer adult beetles may be attracted to blueberry fields with bare soil in the row middles. In the small-plot trials, tillage in row middles proved to be effective in preventing the establishment of *P. japonica* larval populations (fig. 4). Tillage both in the spring and autumn in row middles reduced larval populations by 59%. In 1955, Cory and Langford (in Fleming, 1976) reported that rototilling destroyed up to 90% of the *P. japonica* grubs. Fleming (1976 and references therein) reported that reduction of larval population in fallow and sodlands was greatest when the soil was rototilled in early autumn and late spring, when grubs are near the soil surface. Our study seems to confirm these findings, though the mechanism(s) remain undetermined.

The lack of relationship between soil parameters and larval density indicates that these factors do not affect the suitability of the environment in a blueberry field for *P. japonica*. Previous findings by Dalthorp et al. (2000) in turf systems found no relationship between mean *P. japonica* larval density and soil organic matter, but perennially low grub densities were associated with high organic matter content. Potter et al. (1996) have shown that the response of larval populations to spatial gradients in soil moisture may override the effects caused by soil pH, making soil micronutrient parameters of low value for predicting risk from *P. japonica*.

Targeting management tactics to the parts of the field or farm with the highest *P. japonica* larval population may be an effective and efficient way to help manage the adult pest populations. There are several advantages to controlling larvae in addition to adults. First, larval *P. japonica* are present for a longer period than adults, so there may be more opportunities for control. Secondly, larvae are not capable of moving large distances, so it would be easier to target controls against them, compared with the mobile adults. A recently available option for controlling *P. japonica* larvae in blueberry fields is the use of imidacloprid (Bayer CropSciences, Kansas City, MO) applied to the soil during the egg-laying period (Mannion et al., 2001). Biological control methods are also being investigated, but parasites and pathogens of *P. japonica* are currently at low levels and cause only minor mortality in Michigan populations (Campaert and Smitley, 2002). The presence of a ground cover, and the type of plant, have been shown to be important in influencing larval *P. japonica* populations in turf (Potter et al., 1992; Crutchfield and Potter, 1995), nursery (Smitley, 1996) and in Christmas tree plantations (Kard and Hain, 1988). In sites where tillage is not appropriate, cover crops may be an alternative for management of *P. japonica*. Cover crops that are tolerant of the acidic soil conditions of blueberry fields are also being evaluated in our laboratory for their potential in reducing *P. japonica* populations.

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**Table 1. Effect of tillage timing on Popillia japonica larval density in plots within a blueberry planting**

<table>
<thead>
<tr>
<th>Tillage program</th>
<th>Sample timing</th>
<th>Average larvae per sample ± SE</th>
<th>Pre-tillage</th>
<th>Post-tillage</th>
<th>( \chi^2 )</th>
<th>P-value</th>
<th>% control ± SE</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Spring</td>
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<td>0.87 ± 0.29</td>
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<td>0.00</td>
<td>3.33</td>
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<td>33.33 ± 21.08</td>
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<tr>
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<td>3.40</td>
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<td>3.60</td>
<td>&lt;0.01</td>
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</table>

Comparisons of larval densities before and after tillage are represented by \( \chi^2 \) values (NS = not significant). Percent control values show average reduction in larval density by treatment. Values in the last column are not significantly different from each other within years (\( \alpha = 0.05 \)).
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