# Systemic Applications of Azadirachtin in the Control of *Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae), a Pest of *Platanus* sp.

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**Abstracts**

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In 2010 and 2011, the efficiency of azadirachtin, applied as systemic trunk injections in the trunks of *Platanus* sp., was tested against *Corythucha ciliata*. Azadirachtin in the doses of 0.1 and 0.05 g of active ingredient per cm of diameter at breast height was applied in April. It was found that after application of both doses, the count of *C. ciliata* in plane-tree leaves significantly decreased. In 2010 and 2011, the average counted number of *C. ciliata* individuals in trees treated with the dose 0.1 g a.i./cm of dbh was 12.9 and 4.9, respectively, and 29.1 and 6.5 individuals, respectively, in trees treated with the dose 0.05 g a.i./cm of dbh, in the control it was 152.3 and 105.8 individuals, respectively.

**Keywords:** *Azadirachta indica*; insecticidal activity; trunk injection; NeemAzal; botanical insecticides

The sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae), has been documented as an invasive exotic pest in many countries. In North America, the area of occurrence of the sycamore lace bug extends throughout the eastern US and eastern Canada (Halbert & Meeker 1998; Poland & McCullough 2006), and the species was introduced and is successfully established in Europe and Asia. The first European record originated from Italy (Padova, in 1964), and the species is currently documented in several Central and Southern European countries, namely Austria, Bulgaria, Croatia, Czech Republic, Germany, Hungary, Greece, Serbia and Montenegro, Slovakia, Slovenia, Spain, Switzerland, and Turkey (Őszi *et al.* 2005; Mutun 2009). According to Gninenko & Orlinskii (2004) it was found in southern Russia (Krasnodar) in 1997, and it was also very recently reported from China and Korea (Anselmi *et al.* 1994; Chung *et al.* 1996; Streito 2006; Izhevskii 2008).

*C. ciliata* is recognised as an invasive pest in urban areas, where its host range is essentially restricted to plants of the genus *Platanus* sp. (Ju *et al.* 2009; Ju & Li 2010). Heavy infestations have been concentrated in the region of Krasnodar. This insect feeds on the underside of leaves and produces small chlorotic stippling on the upper leaf surfaces. The resulting injury reduces tree photosynthesis and respiration and also degrades the aesthetic value of the trees (Grosso-Silva & Aguiar 2007; Ju & Li 2010). As a result of this feeding, the foliage becomes bronzed, and leaves may fall in late summer rather than in autumn. Several years of severe damage by *C. ciliata*, combined with the effects of other environmental factors, may kill the host trees (Halbert & Meeker

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1998). Management of this pest includes repeated applications of organophosphorus, synthetic pyrethroid, imidacloprid, thiamethoxam, or acetamiprid insecticides (Maceljki 1986; Ju *et al.* 2009; Ju & Li 2010).

Protection of the trees through the standard spraying application of insecticides is costly due to the need for special application technology; in addition, this method of application often causes problems in enclosed urban agglomerations because of the need to preserve the environmental and health safety of humans and pets. This is why the possible application of some synthetic insecticides had previously been studied using the method of injecting active ingredients against some pests, including *Corythucha ciliata*, into tree trunks (Baseggio 1990; Vai 2003; Pavela & Bárnet 2005). It was found during experiments that the distribution of active ingredients through the conductive tissues of trees was more advantageous economically, and the efficiency of such applications was higher than that of standard sprays (Vai *et al.* 2000; Vai 2003).

However, with the use of systemic trunk injections, the residues of active ingredients contained in synthetic insecticides can become deposited in leaves, where they become subject to very slow decomposition. Fallen leaves may thus become a source of environmental contamination; in particular, leaves in the process of decomposition may have a negative effect on nontarget organisms. In contrast, the application of botanical insecticides may provide a suitable alternative to synthetic insecticides; in addition, since these products contain natural plant metabolites, their active ingredients are easily decomposed through common biochemical processes in the soil. Azadirachtin is a natural tetranortriterpenoid compound derived from seed kernels of the neem tree (*Azadirachta indica* Juss) and has been shown to express insecticidal activity and be highly effective against a number of insect pests (Mordue & Blackwell 1993; Pavela *et al.* 2004, 2009). This compound displays an array of effects on insects acting as a phago- and oviposition deterrent, repellent, antifeedant, growth retardant, moulting inhibitor, sterilant, and preventing insect larvae from developing into adults. Its principal mechanism of effect is expected to consist in impairing the homeostasis of insect hormones (Mordue & Blackwell 1993).

Our prior studies determined the efficiency of applications of azadirachtin-based botanical insecticides against *Cameraria ohridella* (Pavela & Bárnet 2005). The plants receive azadirachtin very easily and store it as a natural metabolite in their leaves without changing its biological effect (Pavela *et al.* 2004; Cevenini & Minelli 2010).

This study determined the efficiency of azadirachtin applied using the injection method in *Platanus* sp. tree trunks against *Corythucha ciliata* nymphs and adults. This is the first study of azadirachtin efficiency against this pest, conducted directly within an urban agglomeration, and focused in particular on obtaining practical knowledge with regard to the potential of this type of application. Therefore the study was focused predominantly on assessing visual plane leaf damage and *C. ciliata* nymph and adult counts at the time of their peak occurrence.

## MATERIAL AND METHODS

The experiment was conducted in a tree alley near Slavyansk na Kubani (45°15'N, 38°07'E), Russia, which included about 50 trees. The experiment was performed on ten plane trees (*Platanus* × *acerifolia* (Aiton) Willd); five other trees were used as a control. The treatments were assigned randomly to trees within the alley. Neem was applied at two concentrations, 0.10 and 0.05 g of active ingredient per cm of diameter at breast height (g a.i./cm dbh), on 20$^{th}$ April 2010 and 30$^{th}$ April 2011. Five trees randomly distributed throughout the alley were always selected for each dose. The diameter of the tree trunks ranged between 25 and 38 cm, and their height was estimated at 5–10 m. Each year, the experiment was done in a different part of the alley.

The commercial insecticide NeemAzal-U a.i. azadirachtin A 170 g/kg (Trifolio-M GmbH, Lahnau, Germany) was used for treatment. The insecticide was applied by systemic tree injection. The water-dissolved substance was injected into the tree at a concentration of 1:20 (g substance to ml water) for 10 hours. This was done using a tree injector according to Helson *et al.* (2001). The ‘injection’ consisted of a maple sap spile, a piece of plastic tubing cut to the length needed to accommodate the required volume of insecticide, a tubeless automobile tire stem with a valve, and two hose clamps. The equipment required to set up the device on a tree included a drill with a 9.5 mm bit, a hammer, and a screwdriver for tightening the hose clamps. A 6-cm-deep hole was drilled at the base of the tree at a slight downward angle. The maple sap spile was hammered into the hole to within about 1 cm of the base of the spile, until there was solid resistance to further hammering. The plastic tube was filled with the desired volume of fluid, and the tire valve stem was inserted into the top of the tubing and sealed with a hose clamp. A bicycle air pump was connected to the tire valve, and the injection tube was pressurised to 250 kPa. The pump was disconnected rapidly to prevent depressurisation.

The plastic tubing was checked for rigidity to ensure that the system was pressurised. The required amount of insecticide was put into each tube, and the system was pressurised.

The trees received the dose of the solution very rapidly, and the devices could be disconnected 1 h after application. Subsequently, the holes remaining after application were treated using grafting wax.

The population dynamics of *Corythucha ciliata* in the planes was determined weekly on a regular basis, from the beginning of April until the end of September, and it was expressed as an average count of individuals (adults and nymphs) per 100 randomly selected plane leaves growing up to 4 m.

Efficiency was assessed in the second half of August in both years (Figure 2); maximum pest occurrence was found in untreated trees. Ten branches were randomly selected in each assessed tree, and 10 leaves were again randomly selected in each branch (approximately every third leaf). Only the number of nymphs and adults present in the leaves was assessed, and at the same time, the degree of visible leaf damage was estimated using the scheme below:

1= 0–15%; 2 = 15–25%; 3 = 25–50%; 4 = 50–75%, and 5 = more than 75% of leaf area damaged by the sucking pest.

The treatment efficiency was calculated using the formula:

$$ET (\%) = [(C - T)/(C + T)] \times 100$$

where:
C – nymph and adult count in untreated plants
T – nymph and adult count in treated plants

Data with numbers and percentages were subjected to the HOVTEST = LEVENE option of SAS to account for homogeneity of variance and normality. In the case of non-homogeneity, percent values were transformed using the arcsine square root (arcsin √) transformation, and insect count values were transformed by the square root (√) transformation before running an ANOVA (Steel & Torrie 1980; Gomez & Gomez 1984). Treatment differences in the number of insects and in damage were determined by Tukey's test. Differences between means were considered significant at $P \leq 0.05$ (SAS Institute 1999).

## RESULTS

The series of average monthly temperatures and precipitation amounts determined in the course of the experiment are presented in Figure 1. The series of the population dynamics of *Corythucha ciliata* is shown in Figure 2. Two generations, which mingled with each other, were observed in both years. The first generation was found from June to July, while the maximum occurrence of

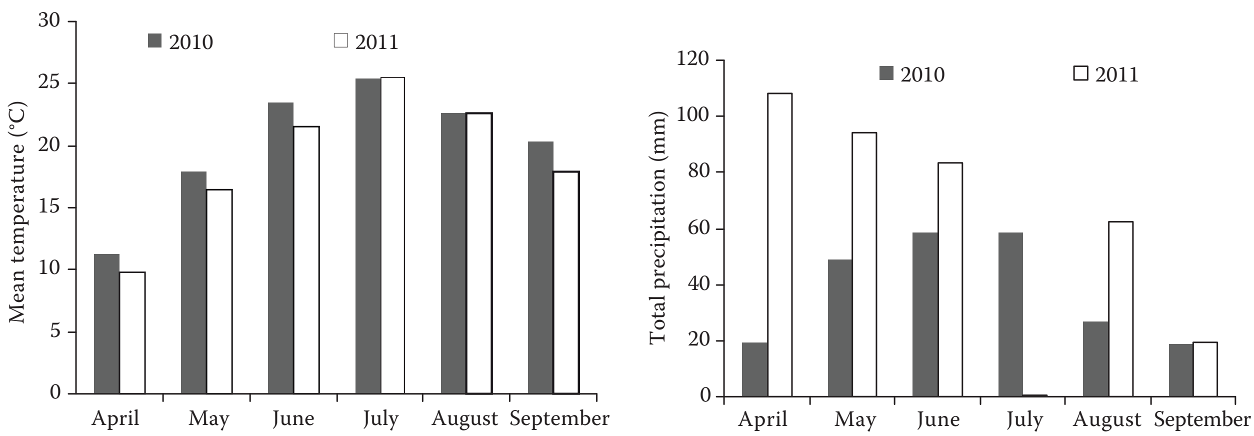


Figure 1. Monthly total precipitation and mean temperatures for the experimental years (2010 and 2011)

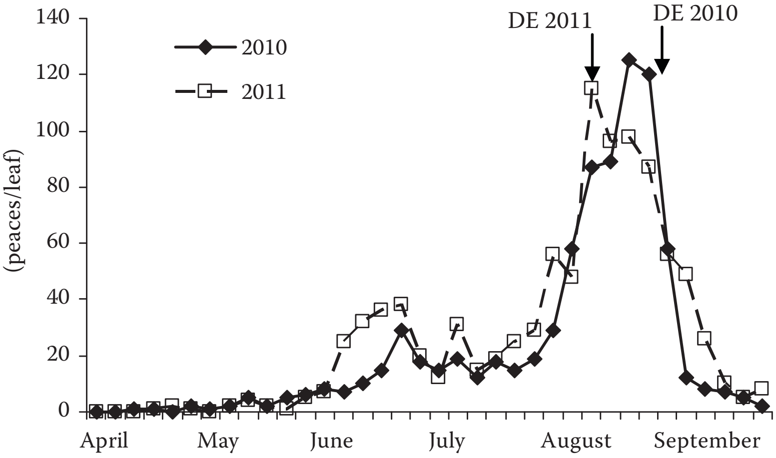


Figure 2. The average density of the *C. ciliata* population on a leaf at a given point of time (measured in insects/leaf)

DE – date the treatment efficiency was examined

the second generation was observed from the first half of August, when also biological efficiency was assessed. It was found that although *C. ciliata* nymphs and adults occurred in the treated trees, their counts were significantly lower ($P < 0.0001$) in both years compared to untreated trees (Table 1). The difference between the applied doses of 0.1 and 0.05 g a.i./cm dbh was significant only for 2010 (Table 1), with the determined average count of nymphs and adults of *C. ciliata* being equal to 12.9 and 29.1, respectively. In 2011, a difference in the count of *C. ciliata* nymphs and adults was also found (Table 2) between the doses 0.1 and 0.05 g a.i./cm dbh; however, this difference (4.9 and 6.5, respectively) was not significant ($P < 0.05$). In 2010 and 2011, the average counts of 152 and 105 *C. ciliata* individuals, respectively, were found in untreated trees, and leaf area damage due to sucking was estimated at approximately 75%. Overall leaf damage was estimated at ≤ 15% of leaf area, with no significant difference found between the doses used (Table 1). A favourable aesthetic impression was observed in the treated trees in both years; the trees showed deep green colour and were healthy at first sight. On the other hand, the leaves of the untreated trees showed a yellow to yellowish-brown colour, and some severely damaged leaves were starting to fall, resulting in a poor aesthetic impression.

The efficiency of systemic trunk injections in the control of *C. ciliata* was calculated based on the determined counts of individuals in the plane leaves (Figure 3). The efficiency ranged between 80 and 95% in both years, while a significant difference

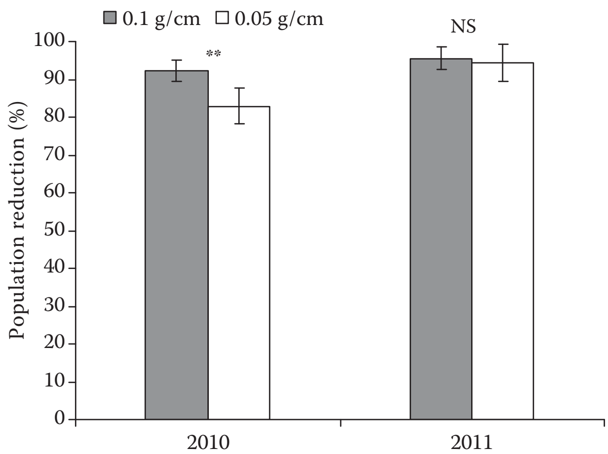


Figure 3. The treatment efficiency of systemic trunk injections against *C. ciliata* based on insects samples

*indicates significant difference ($P < 0.01$) from doses; NS – not significant difference ($P < 0.05$) from doses

Table 1. Mean number (± SE) of *Corythucha ciliata* (Say) individuals per leaf of *Platanus* during the study period 2010 and 2011

| Treatment (g/cm dbh) | 20100 | | | | 2011 | | | |
|---|---|---|---|---|---|---|---|---|
| | nymphs | adults | total | damage of leaves | nymphs | adults | total | damage of leaves |
| 0.10 | 3.1 ± 0.1[a] | 9.8 ± 3.1[a] | 12.9 ± 2.6[a] | 1.1 ± 0.1[a] | 3.8 ± 3.7[a] | 1.1 ± 1.9[a] | 4.9 ± 5.2[a] | 1.3 ± 0.5[a] |
| 0.05 | 11.9 ± 2.5[b] | 17.2 ± 8.3[a] | 29.1 ± 7.2[b] | 1.2 ± 0.2[a] | 2.9 ± 5.8[a] | 3.6 ± 5.1[a] | 6.5 ± 10.3[a] | 1.6 ± 0.6[a] |
| Control | 88.2 ± 18.1[c] | 64.1 ± 12.9[b] | 152.3 ± 15.2[c] | 4.1 ± 0.32[b] | 76.7 ± 16.9[b] | 28.5 ± 9.9[b] | 105.8 ± 17.6[b] | 3.7 ± 1.2[b] |
| *P*; *F* | 0.0001; 26.95 | 0.0001; 32.98 | 0.0001; 58.33 | 0.0001; 84.12 | 0.0001; 16.62 | 0.0001; 17.92 | 0.0001; 18.93 | 0.0001; 54.12 |

dbh – diameter at breast height; values with the same lowercase letter do not differ significantly (Turkey's HSD test, $P < 0.05$)

($P < 0.01$) in terms of efficiency was found between the doses of 0.1 and 0.05 g a.i./cm dbh.

## DISCUSSION

As follows clearly from our observations, systemic trunk injections may cause a significant reduction of the *C. ciliata* population density in plane trees, and thus be an important preventative measure against leaf damage caused by this sucking pest. The applied dose stems from our prior research focused on the damage done to *Aesculus hipocastaneum* trees by the mining larvae of *Cameraria ohridella* (Pavela & Barnet 2005). That paper presented the results of azadirachtin efficiency, applied in the doses of 0.25–0.08 g a.i./cm to chestnut trees to control *C. ohridella*. Systemic trunk injections in higher dosages (0.25 and 0.15 g a.i./cm dbh) were found to protect the trees for the entire season and increase their aesthetic value significantly. On the other hand, the dose of 0.08 g a.i./cm dbh showed significantly lower efficiency, and leaf damage by *C. ohridella* mining caterpillars was visible. In the present research, an even lower dose was used (0.05 g a.i./cm dbh), and the difference in efficiency compared to the higher dose was manifested in the first year only. However, this difference in efficiency was not reflected in the assessment of overall visible damage to the leaves. It is thus clear that *C. ciliata* is more sensitive to a.i. azadirachtin compared to *Cameraria ohridella*.

Some other authors have also focused on the efficiency of synthetic insecticides against *C. ciliata* applied via injections in plane trunks (Baseggio 1990). The efficiency of imidacloprid, acephate, hexaflumuron and oxidemeton-metile in the control of *C. ciliata* infesting *Platanus* was evaluated using three methods of application, namely spraying of the leaves, pressure injection, and "gravitated" injection of the trunk, in a field experiment conducted during 1998–1999 (Vai 2003). In some trials, with different levels of damage, imidacloprid, acephate, and oxidemeton-metile were very effective, while hexaflumuron failed to give satisfactory results. Injections of the trunk were more effective compared with the spraying of the leaves.

Injecting urban plane trees in Switzerland with a.i. demeton-S-methyl, as Inject-a-cide from Mauget, was effective in controlling the tingid *Corythucha ciliata*. A single treatment in June when nymphs first appeared prevented or greatly reduced damage throughout the vegetation season (Mauri 1989).

Botanical insecticides based on azadirachtin have been considered safe for the environment and the health of people. However, their application in a common spray could provide low efficiency because of rapid degradation of active ingredients due to weather effects and UV radiation (Mordue & Blackwell 1993). Thus azadirachtin application using the root system of plants (Pavela *et al.* 2004; Pavela & Teixeira da Silva 2006) or using injections (Pavela & Bárnet 2004; Kreutzweiser *et al.* 2011) seems to be much more advantageous in terms of both efficiency and persistence, as well as the simple mode of application. In addition, there is no risk that the plant residues will have a significant negative impact on non-target organisms, provided that dosages lower than 0.2 g a.i./cm dbh are adhered to (Stark & Walter 1995; Kreutzweiser *et al.* 2011). In general, some of the synthetic insecticides have been recommended for systemic applications. For example, the chloroneonicotinyl insecticide imidacloprid has been approved in the US and recommended for systemic application (Kreutzweiser *et al.* 2007; Smitley *et al.* 2010) against some tree pests. However, as mentioned above, imidacloprid applied to trees for the wood-borer control resulted in foliar concentrations at senescence that significantly reduced the decomposition of leaf litter in terrestrial and aquatic microcosms (Kreutzweiser *et al.* 2007, 2008). It was determined that the reduced litter decomposition resulted from sub-lethal, antifeedant effects on litter-dwelling earthworms and leaf-shredding aquatic insects, and from the significant inhibition of terrestrial and aquatic microbial decomposition activity (Kreutzweiser *et al.* 2009). Those studies suggested that imidacloprid may not be the best option for a systemic insecticide in environmentally sensitive areas because of its potential adverse effects on decomposition processes.

As clearly follows from our results, systemic applications of botanical insecticides based on azadirachtin could lead to a significant reduction of plane-tree damage caused by the *Corythucha ciliata* sucking nymphs and adults. A single application at the beginning of the vegetation period can protect the trees for the entire season, which significantly increases not only their aesthetic value but also their lifespan. Based on this study and on the studies of other authors, botanical insecticides based on azadirachtin can be recommended as suitable for systemic trunk injections against sucking and munching tree pests.

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