

## Spatial distribution and sampling of *Corythucha ciliata* (Hemiptera: Tingidae) in London plane trees

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Taylor's power law and Iwao's patchiness regression were used to describe the dispersion patterns for overwintering and wandering stages of *Corythucha ciliata* on the London plane trees, *Platanus × acerifolia* (Ait.) Willd. Both Taylor's and Iwao's tests fit the distribution data for the overwintering stage. The overwintering adults were spatially aggregated. In the wandering stage, Taylor's power law consistently fit the data, whereas the fit of Iwao's patchiness regression was erratic. Both Iwao's and Taylor's indices indicated a clumped distribution pattern for eggs, nymphs, and wandering adults. Trunk was identified as the best sampling target for the overwintering stage whereas twig was the best for the wandering stage. In order to determine the sample size for evaluating whether the population has reached the control threshold, the sampling of 35 and 7 trunks for the overwintering stage and 32 and 8 twigs per tree for the wandering stage would provide 0.5- and 0.25-precision levels, respectively.

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### 1. Introduction

The sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae), is a relatively recent, invasive, and exotic pest of *Platanus* trees (Platanaceae) in China (Ju & Li 2010). This herbivore originated from North America (Halbert & Meeker 1998) and is now widely spread in many countries throughout Europe (Maceljski 1986, Mutun 2009), South and North America (Halbert & Meeker 1998), Australia, and Asia (Chung *et al.* 1996, Ju *et al.* 2009, Ju & Li 2010). In China, *C. ciliata* was first reported in 2002 in Changsha of Hunan Province (Streito 2006), and was reported in 11 other provinces (Shanghai, Zhejiang,

Anhui, Jiangsu, Guizhou, Sichuan, Chongqing, Hunan, Hubei, Henan, and Shandong) over the last decade (Ju *et al.* 2009, Ju & Li 2010). It causes serious damage in the Yangtze River basin, and particularly in Shanghai, Jiangsu and Hubei where almost 70% of the host trees are currently infested (Ju *et al.* 2009).

*Corythucha ciliata* has a short life cycle and it is oligophagous. Its hosts, *Platanus* spp., are widely distributed in China, because they are commonly planted along city streets (Ju *et al.* 2010a, 2011a). The insect completes five generations per year in Wuhan and Shanghai, with five immature instars per generation (Xia *et al.* 2007, Xiao *et al.* 2010, Ju *et al.* 2011a). Its piercing and

Table 1. Locations with plot numbers, approximate tree ages (years) and months of investigation in 2010 of *Corythucha ciliata*.

Plot	Location in Shanghai	Tree age	Month
1	Zhaojiabang Road, Xuhui District	50	February
2	People Square, Huangpu District	50	March
3	Renmin Park, Huangpu District	50	February
4	West Yan'an Road, Changning District	50	March
5	Dongfang Road, Pudong District	45	March
6	Longwu Road, Xuhui District	50	July
7	West Longhua Road, Xuhui District	50	August
8	Qiujiang Road, Songjiang District	50	August

sucking feeding habits initially cause white stippling on the leaves, which reduces photosynthesis and respiration and also reduces the aesthetic value of the trees (Ju *et al.* 2010b). The stippled foliage then becomes chlorotic and bronzed, and the affected trees experience premature leaf drop (Halbert & Meeker 1998). Several years of severe damage by *C. ciliata* combined with the effects of other environmental stresses may kill the host trees (Halbert & Meeker 1998).

Because of serious damage caused by *C. ciliata* in many parts of the world, the pest has been frequently studied. Researchers have investigated its geographical spread (Öszi *et al.* 2005, Chung *et al.* 1996, Mizuno *et al.* 2004, Li *et al.* 2008), population dynamics (Öszi *et al.* 2005), biological characteristics (Battisti *et al.* 1985, Cheol & Kwang 2000, Xia *et al.* 2007, Ju *et al.* 2010a), thermal tolerance (Ju *et al.* 2010c, 2011b, c), natural enemies (Balarin & Maceljski, 1986a,b), and control (Jasinka & Bozsits 1977, Jasinka 1981, Wicki 1984, Reiderné & Ripka 1990, Cheol & Kwang 2000). Management of *C. ciliata* includes repeated applications of organophosphorous, synthetic pyrethroid, imidacloprid, thiamethoxam, or acetamiprid insecticides (Ju *et al.* 2009, Xiao *et al.* 2010, Chen *et al.* 2011). Basic information concerning *C. ciliata* ecology, however, remains insufficient. Because efficient pest control requires basic ecological information, more studies on *C. ciliata* ecology are needed.

The spatial distribution of a pest is one of its most important ecological properties (Taylor 1984), and information concerning spatial distribution is essential for pest management in the field (Bechinski & Pedigo 1981). The objectives

of this paper were: (1) to determine the distribution pattern of various stages of *C. ciliata* among and within London plane trees, and (2) to determine the optimal sampling units for estimating *C. ciliata* population densities.

## 2. Material and methods

### 2.1. Location and investigation periods

*Corythucha ciliata* populations were examined on London plane trees, *Platanus × acerifolia* (Ait.) Willd., at eight locations or plots (Table 1) in Shanghai, China (31.2°N, 121.5°E) during two periods in 2010: February–March (adults were overwintering) and July–August (adults and nymphs of the second and third generations were in their wandering stage). Each of the eight plots contained 100–120 trees.

### 2.2. Distribution patterns of overwintering adults

Adult *C. ciliata* overwinters under the bark of their host (Xia *et al.* 2007). To explore the population distribution pattern for overwintering adults, trees in five plots (plots 1–5 in Table 1) were sampled. The trees in these plots were about 8–10 m tall, about 20–25 cm in diameter at breast height, and were not treated with pesticide or pruned during the sampling period. In each plot, eight trees were randomly selected, and samples were collected separately from the trunk and from the branches of each tree. The adults were collected by removing all of the barks on the trunk

from the soil surface to the first branch and by removing all the barks from all the branches for a distance of 50 cm from the trunk. The adults were counted separately for trunk and branches.

Means and variances of *C. ciliata* numbers were calculated from tree-specific numbers of 40 trees, based on bug numbers on trunks and their numbers on branches. The means were compared with a t-test ( $P < 0.05$ ). The analyses were performed with the statistical package SPSS NLN, 13.0 (SPSS 2006).

### 2.3. Distribution patterns for eggs, nymphs, or wandering stage adults

To explore the population distribution pattern for eggs, nymphs, and wandering adults, trees in three plots (plots 6–8 in Table 1) were sampled. These trees were about 8–10 m tall, about 20–25 cm in diameter at breast height, and they were also not treated with pesticide or pruned during the sampling period. From each plot, 10 trees were randomly selected, and the canopy of each tree was divided into an upper layer (that received substantial sun light) and a lower layer (that was shaded). Both layers were divided into four directions (east, west, south, and north). Four twigs were cut out from every direction. Each twig bore ten leaves. The numbers of the eggs, nymphs, and adults were counted per leaf, per twig, per layer, and per tree (based on 8 twigs and 80 leaves per layer and 16 twigs and 160 leaves per tree). Adults and nymphs were observed directly and eggs were detected with a dissecting microscope. The twigs were placed into a plastic bag for bringing to the laboratory. They were stored at 5 °C and examined within two days.

Statistical analyses were the same as described in the previous section, but the means were compared with the Tukey's test ( $P < 0.05$ ) (SPSS 2006).

### 2.4. Analysis of spatial distribution

Analysis of the spatial distribution of overwintering adults was based on the means and variances of the numbers per tree, per trunk, and per branch. Analysis of the spatial distribution of

eggs, nymphs, and the wandering stage of adults was based on the means and variances of the numbers per tree, per twig, and per leaf. Two classical dispersion models, i.e., Taylor's power law (Taylor 1961) and Iwao's regression (Iwao 1968), were used to evaluate the distribution of *C. ciliata* among the different parts of the tree.

The formula for Taylor's power law is:

$$s^2 = am^b \quad (1)$$

where  $s^2$  is the variance of insect numbers in the sampling unit,  $m$  is the mean number of the insects in the sampling unit,  $a$  is the sampling factor that changes with the sampling unit, and  $b$  is the dispersion index of a certain species. The population has a random or Poisson distribution when  $s^2 = m$ , a uniform distribution when  $s^2 < m$ , and an aggregated distribution when  $s^2 > m$  (Yamamura 2000). The coefficients  $a$  and  $b$  were estimated from the following equation (Southwood 1978):

$$\text{Log}s^2 = \text{Log}a + b(\text{Log}m) \quad (2)$$

Iwao's patchiness regression was simulated by the following equation (Iwao 1968):

$$M^* = \alpha + \beta m \quad (3)$$

where  $M^*$  is the mean crowding,  $\alpha$  is the index of basic contagion and  $\beta$  is density-contagiousness coefficient. When  $\alpha = 0$ , a single individual is the basic component of the distribution, and when  $\alpha > 0$  or  $\alpha < 0$ , there is a positive or negative association between individuals. The density-contagiousness coefficient ( $\beta$ ) indicates how the basic components are distributed in space. When  $\beta < 1$ , the distribution is regular; when  $\beta = 1$ , the basic components are randomly distributed and when  $\beta > 1$ , the basic components are distributed contagiously (Iwao 1968).

The mean crowding  $M^*$  was derived from Lloyd's formula (Lloyd 1967):

$$M^* = m + S^2/m - 1 \quad (4)$$

where  $s^2$  and  $m$  were substituted by the variance and mean, respectively, of the insects in the sampling unit.

Table 2. Taylor's power law and Iwao's patchiness regression indices for overwintering adults of *Corythucha ciliata* among trees, trunks, and branches.

Sampling unit	Taylor's power law			Iwao's patchiness regression		
	$m$	$S^2$	$R^2$	$\alpha$	$\beta$	$R^2$
Among trees	1.13–87.25	2.98–7849.64	0.99**	1.75	2.00	1.00**
Among trunks	0.38–47.88	0.55–3627.84	0.99**	–0.78	2.55	1.00**
Among branches	0.75–39.38	1.93–1978.84	0.99**	–0.15	2.26	1.00**

\*\* Significance with  $P < 0.001$  by linear regression.

## 2.5. Selecting the appropriate sampling unit and sampling stage

For the overwintering stage, the appropriate sampling unit, i.e., either branches or trunks, was determined based on the adult numbers per trunk or per branch. The part of the tree with the higher insect density was selected as the sampling unit.

For the eggs, nymphs, and wandering stage of adults, the twigs growing in a specific layer (upper or lower) and in a specific direction (east, south, west, or north) with the highest insect density were regarded as the appropriate sampling unit.

The “appropriate representative sampling stage” was then determined by correlation of each developmental stage to the whole population (i.e. the total of eggs, nymphs and adults). A specific developmental stage, whose numbers were best correlated with the total numbers of insect individuals on each twig, was chosen as the appropriate representative sampling stage.

## 2.6. Sample size

After the best regression model was determined, two methods were used to calculate the number of samples required to estimate the mean with a given level of precision.

(1) Iwao and Kuno (1968) and Kuno (1969) presented a sample size method that applied  $\alpha$  and  $\beta$  indices from Iwao's regression to determine the optimal number of samples ( $N$ ) for estimating the mean density ( $m$ ) with a given level of precision ( $D$ ). The equation for this method is:

$$N = [(\alpha + 1) / m + (\beta - 1)] / D \quad (5)$$

(2) Wilson and Room (1982) incorporated  $a$  and

$b$  indices derived from Taylor's power law to calculate the optimal number of samples ( $N$ ) necessary to estimate the mean density ( $m$ ) with a given level of precision ( $D$ ). Their equation is:

$$N = (Z_{\alpha/2} / D)^2 \times (S / m)^2 \quad (6)$$

where  $Z$  is the standard normal deviate (Type I error rate ( $\alpha$ ) = 0.1, then  $Z$  is 1.64 in two tails with  $df = \infty$ ). Before sampling in the field, precision levels ( $D$ ) were set as 0.25 and 0.5 for both of these methods. Then optimal numbers ( $N$ ) were calculated. For example, when  $N = 500$  and  $D = 0.25$ , then the actual number cannot be greater than 625 (i.e.  $500 \times 0.25 + 500$ ) or less than 375 (i.e.  $500 - 500 \times 0.25$ ).

## 3. Results

### 3.1. Spatial distribution

As shown in Table 2 for overwintering adults, in Taylor's law, all  $s^2$  were higher than  $m$ , and Iwao's indices of dispersion ( $\beta$ ) were above 1 among trees, trunks and branches. These data indicated that overwintering adults were aggregated among all the studied sampling levels. Both Taylor's power law regression and Iwao's patchiness regression fit the linear law (Taylor's:  $R^2 = 0.99$ ; Iwao's:  $R^2 = 1.00$ ; for both  $P < 0.001$ ).

For the eggs, nymphs and wandering-adult stages of *C. ciliata*, both indices also indicated that all the three stages were aggregated ( $S^2 > m$  for Taylor's,  $\beta > 1$  for Iwao's). In linear regressions, Taylor's power law fit the data more consistently than Iwao's patchiness regression (Taylor's:  $R^2 = 0.88-0.99$ ; Iwao's:  $R^2 = 0.51-1.00$ ; for both  $P < 0.001$ ) (Table 3).

Table 3. Taylor's power law (A) and Iwao's patchiness regression (B) for *Corythucha ciliata* eggs, nymphs, and adults (wandering stage) on trees, twigs, and leaves.

A. Taylor's power law

Sampling unit	Developmental stage	<i>m</i>	<i>S</i> <sup>2</sup>	<i>R</i> <sup>2</sup>
Among trees	Egg	420.10–928.00	72853.21–195773.33	0.99**
	Nymph	123.60–192.50	5516.93–9733.79	0.88**
	Adult	53.50–209.20	3332.50–12533.95	0.99**
Among twigs	Egg	13.90–156.80	475.21–23078.18	0.97**
	Nymph	6.80–27.40	66.18–1597.57	0.92**
	Adult	2.70–37.20	5.56–751.96	0.96**
Among leaves	Egg	0.10–25.79	0.22–1265.13	0.98**
	Nymph	0.30–4.55	1.33–161.46	0.96**
	Adult	0.11–4.85	0.18–55.02	0.97**

\*\* Significance with *P* < 0.001 by linear regression.

B. Iwao's patchiness regression

Sampling unit	Developmental stage	<i>α</i>	<i>β</i>	<i>R</i> <sup>2</sup>
Among trees	Egg	171.31	1.04	1.00**
	Nymph	52.37	1.02	0.97**
	Adult	55.07	1.03	1.00**
Among twigs	Egg	–514.65	22.87	0.51
	Nymph	8.59	1.77	0.86**
	Adult	3.77	1.36	0.94**
Among leaves	Egg	16.25	1.23	0.69**
	Nymph	11.65	2.57	0.69**
	Adult	3.83	2.77	0.85**

\*\* Significance with *P* < 0.001 by linear regression.

Table 4. Numbers of *Corythucha ciliata* eggs, nymphs, and wandering adults per twig (mean±SE) in relation to tree layer and branch direction.

Layer	Direction	Eggs	Nymphs	Adults
Upper	East	19.6 ± 15.1	11.7 ± 7.6	51.6 ± 16.9
	South	20.8 ± 15.5	12.0 ± 7.4	52.0 ± 18.7
	West	22.4 ± 17.7	11.5 ± 6.7	49.0 ± 18.8
	North	22.6 ± 16.2	10.3 ± 5.2	53.2 ± 17.4
Lower	East	21.5 ± 17.4	12.6 ± 6.2	51.3 ± 17.1
	South	19.6 ± 16.7	12.3 ± 8.4	54.1 ± 18.0
	West	20.7 ± 15.7	13.9 ± 9.3	49.9 ± 17.5
	North	18.3 ± 17.7	12.2 ± 9.1	50.2 ± 16.8

Values within a column are not significantly different (Tukey's test: *P* < 0.05).

### 3.2. Selection of the appropriate sampling unit and sampling stage

In the overwintering stage, 59% of the adults were on trunks and 41% were on branches. Adult densities were significantly higher on trunks ( $45.76 \pm 9.56$  per trunk) than on branches ( $34.12 \pm 9.78$  per branch) ( $t_{39} = 10.03$ ;  $P < 0.0001$ ). Thus, the trunk was used as the sampling target for investigating the distribution of overwintering adults.

For eggs, nymphs, and wandering adult stages of *C. ciliata*, densities on twigs were not significantly affected by the layer of the canopy ( $F_{1, 239} = 0.19-3.09$ ,  $P = 0.17-0.69$ ) or by the direction ( $F_{2, 239} = 0.31-3.76$ ,  $P = 0.15-0.81$ ) (Table 4). Thus, twigs were used as the sampling target without regard to layer or direction.

To determine the representative developmental stage for the sampling unit during the wandering period, the numbers of each developmental stage were regressed against the total numbers of all stages from each twig. The regression coefficient was much higher for adults ( $R^2 = 0.92$ ) than for other stages (Fig. 1), which indicated that adult stage could be recommended as the sampling target for representing *C. ciliata* populations in field investigations during the wandering period.

### 3.3. Sample size

For the overwintering adults, both Taylor's power law regression and Iwao's patchiness regression fit the data well. Here, we estimated the optimal sample size according to the assumption of Iwao's regression. The  $\alpha$  and  $\beta$  indices of the target sampling unit (trunks) were  $-0.78$  and  $2.55$ , respectively. Based on Equation (5), the optimal number of trunks that should be sampled for a given number of overwintering adults per trunk is indicated in Fig. 2a. Ószi et al. (2005) reported that 48 overwintering adults per trunk would cause serious damage to the tree in late spring. According to the Technical Regulation of the Shanghai Gardening Plant Protection (TRSGPP) published by the Shanghai Administration Department of Afforestation in 2007, controls should be applied when the number of adults

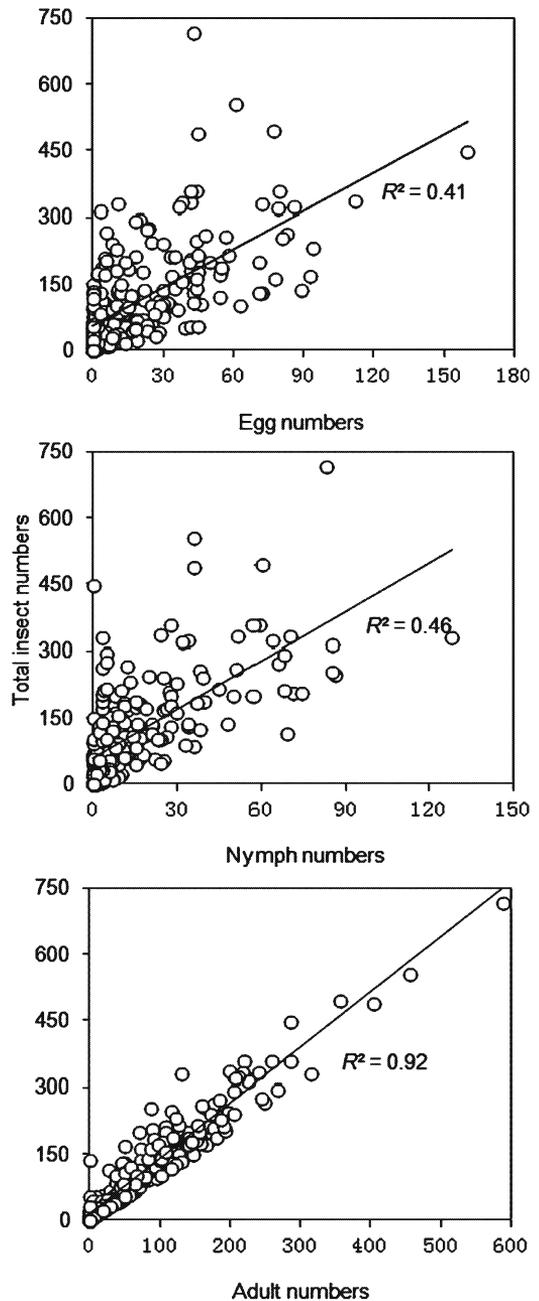


Fig. 1. Linear regressions of sample mean densities of different stages during wandering period against total numbers (sum of all stages) of *Corythucha ciliata* on London plane trees.

reaches one-quarter of the damaging level, i.e., when there are 12 overwintering adults per trunk. In Shanghai, therefore, estimating the number of overwintering adults per trunk with a precision

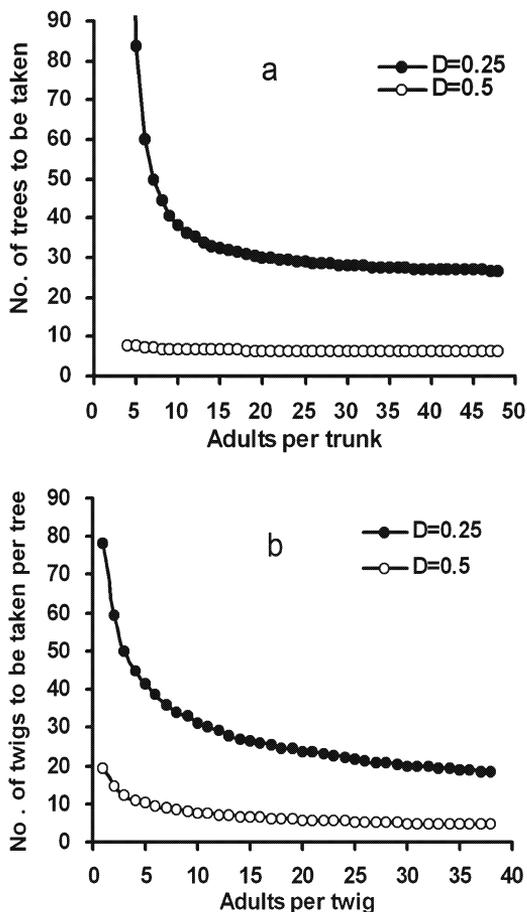


Fig. 2. Optimal number of trunks to be sampled for estimating population density of *Corythucha ciliata* with reliability levels of  $D = 0.25$  and  $D = 0.50$ . – a. Overwintering adults. – b. Wandering adults.

level of 0.25 or 0.5 would require the sampling of 35 or 7 trunks, respectively (Fig. 2a).

In the wandering stage, the Taylor's power law regression fit the data better than Iwao's patchiness regression, and the optimal sample size was therefore determined by Taylor's power law. The  $a$  and  $b$  indices of the sampling target twigs were 1.82 and 1.60, respectively. Based on Equation (6), the optimal number of trunks that should be sampled for a given number of wandering adults is indicated in Fig. 2b. According to a recent study, 38 wandering adults per twig caused serious damage (Ju *et al.* 2010b). Based on TRSGPP in 2007, controls should be applied when there are 10 wandering adults per trunk (38/4). In Shanghai, therefore, estimating the

number of wandering adults per trunk with a precision level of 0.25 or 0.5 would require the sampling of 32 or 8 trunks, respectively (Fig. 2b).

#### 4. Discussion

Many researchers have used Iwao's or Taylor's equations to determine the distribution pattern of insect pests in crops (Taylor 1961, Iwao 1968, Peng & Brewer 1994, Jiang *et al.* 1995, Xue *et al.* 2000, Hsu *et al.* 2001, Wang *et al.* 2006, Wang 2011). In this study of *C. ciliata*, both Iwao's and Taylor's indices of dispersion were  $> 1$ , suggesting (see Southwood 1978) that this pest has an aggregated distribution among and within *P. × acerifolia* trees. Clumped or aggregated distribution patterns were detected when the pest was highly active in its wandering stage between July and August and also when it was inactive in its overwintering stage between February and March. Ószi *et al.* (2005) reported that *C. ciliata* nymphs were spatially aggregated, while the adults tended to have more uniform and balanced distribution patterns.

According to Vasic (1975) and Battisti *et al.* (1985), the overwintering adults prefer the lower parts of the trunk (under 4.8 m of an 11 to 16 m high trunk), and most overwintering adults occur under the bark on the northeast and northwest side of the trunk. Our investigation demonstrated that, in addition to the trunks, the branches near the trunk were suitable overwintering sites for adults. A previous study determined that the wandering stage of *C. ciliata* mainly damages the lower crown of the tree, and the author inferred that the wandering adults were therefore distributed mainly in the lower crown (Zangheri 1984). In the current study, in contrast, the densities of the wandering stages of *C. ciliata* on twigs did not differ with crown layer (upper vs. lower) or direction. This difference in results may be due to differences in environmental conditions between the two regions.

*Corythucha ciliata* mainly infests *Platanus occidentalis* L. in its native land, North America (Halbert & Meeker 1998), but prefers to feed on *P. × acerifolia* in China. Because *C. ciliata* has seriously damaged *P. × acerifolia* in Shanghai, we are now trying to reduce its number and slow

its spread by insecticide application. Large-scale application of insecticides, however, would be environmentally undesirable, especially in the commercial districts where the London plane trees grow. Hence, pesticide applications must be restricted in space and time. By indicating effective methods for sampling *C. ciliata* on London plane trees, the results in this paper should help pest managers determine when pesticides or other control should be applied. The results should also indicate where pesticides should be applied. For example, the results showed that most overwintering *C. ciliata* adults occurred on the trunk and on the branches near the trunk. It follows that, in winter, insecticides could be restricted to these parts of the tree. In the wandering stage, however, adults and nymphs on twigs are aggregated but not related to crown layer or directions. During the wandering period, therefore, all twigs should be sprayed with insecticides. The results presented here should also help with assessing the efficacy of *C. ciliata* control.

*Corythucha ciliata* has natural predators, including bedbugs, spiders, crickets, locusts (Arzone 1984), and pathogens, including viruses, nematodes, and fungi (Sidor 1985). In urban areas, biological control would be preferred to chemical ones, because it would likely be less harmful to humans and other non-target organisms. As with application of insecticides, application of biological control agents could benefit from the results of this study in deciding when treatments are needed and which parts of trees should be treated.

Although we investigated *C. ciliata* populations during two periods (corresponding to the insect's overwintering and wandering stages), *C. ciliata* has five generations per year in the Yangze River basin of China (Xiao et al. 2010). For a more complete understanding of the spatial distribution of *C. ciliata* and of the sampling methods required to describe that distribution, the pest should be sampled at monthly intervals during periods of high and low activity.

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