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BRIEF REPORT

Effects of neonicotinoid insecticide trunk injections on non-target arboreal ants, potential biological control agents for invasive longhorn beetle *Aromia bungii* on cherry trees

Eiriki Sunamura[1] · Shigeaki Tamura[1] · Hisatomo Taki[1] · Grzegorz Buczkowski[2] · Etsuko Shoda-Kagaya[1]

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Abstract

Trunk injection is a relatively new, environmentally friendly method to apply insecticides to trees which does not cause insecticide drift and environmental runoff. However, little is known about the effect of insecticide trunk injection on non-target arboreal ants (Hymenoptera: Formicidae) which can act as biological control agents of target tree pests. This study evaluated the effect of trunk injections on arboreal ants found on cherry trees treated with neonicotinoids (dinotefuran and thiamethoxam) for controlling the invasive longhorn beetle *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae). Arboreal ants represented by *Crematogaster matsumurai* Forel can prey on *A. bungii* eggs. Results of visual sampling 1 and 3 months after injections showed that injections did not reduce the number of ant species occurring on tree trunks. Additionally, injections did not eliminate 3 of 4 most abundant species on tree trunks or extrafloral nectaries including *C. matsumurai*. However, a decline of *Lasius japonicus* Santschi was observed on injected trees. Our preliminary short-term survey suggests the possibility that chemical control by trunk injection and biological control by arboreal ants are compatible in *A. bungii* management. However, further research is needed to clarify the mechanism of *L. japonicus* decline and long-term consequences of trunk injection on arboreal ant composition.

Keywords Alien species · Biotic resistance · Predator · Side effect · Wood-boring pest

Introduction

Trunk injection is a relatively novel method to apply insecticides to trees whereby the insecticide solution is injected into the tree trunk and transported throughout the tissues via xylem water flow (Berger and Laurent 2019; Doccola and Wild 2012). Trunk injection offers several advantages over traditional broadcast spray and soil drenching methods including higher efficiency of product delivery, reduced risk for worker exposure, and reduced harm to the environment and non-target organisms (Berger and Laurent 2019; Doccola and Wild 2012). Pests are killed by feeding on insecticide-permeated tree tissues and trunk injection has proven effective against various tree pests including wood borers, phloem feeders, and defoliators (Haack et al. 2010; Herns and McCullough 2014; Sadof et al. 2022; Urban and Leach 2023).

Although concerns have been expressed about the impact of tree-injected plant protection products on non-target organisms such as pollinators, little is known about the effects of trunk injections on non-target organisms that use the injected trees as habitat and/or food source (Berger and Laurent 2019). Additionally, trunk injections can also cause unintended and unexpected ecological shifts and secondary pest outbreaks. For example, in the Asian long-horned beetle control program in North America, trunk injection of imidacloprid into elm trees caused outbreaks of spider mites by reducing their insect predators (Szczepaniec et al. 2011).

Arboreal ants that nest or forage on trees are an important group of beneficial non-target organisms in the management of tree pests. Indeed, numerous studies demonstrate that arboreal ants can be highly valuable components of biological control in a variety of agroecosystems (Blaise et al. 2021;

✉ Eiriki Sunamura
esunamura@ffpri.affrc.go.jp

[1] Department of Forest Entomology, Forestry and Forest Products Research Institute, Forest Research and Management Organization, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan

[2] Department of Entomology, Purdue University, 901 W. State St., West Lafayette, IN 47907, USA

Morris et al. 2015; Offenberg 2015; Schifani et al. 2020). They are ubiquitously distributed across various tree species, dominate the tree canopy in numbers and biomass, and are key predators of tree-dwelling arthropods (Hölldobler and Wilson 1990; Schläppi et al. 2021). Numerous tree species actively attract arboreal ants for protection against herbivorous insects by secreting extrafloral nectar (Bezerra et al. 2021; Jones et al. 2017). Therefore, tree ecosystem services provided by ants might be disrupted in situations where the ants are affected by insecticide applications (Schläppi et al. 2021).

This study was conducted to determine if trunk injections unexpectedly affect arboreal ant communities that serve as natural enemies. To this end, the effects of trunk injection on arboreal ants were evaluated using cherry trees injected with neonicotinoid insecticides to control the larvae of the alien red-necked longhorn beetle *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae). *Aromia bungii* is native to China and the surrounding countries (EPPO 2015; Iwata 2018) but is invasive in Europe and Japan since 2011 (Russo et al. 2020; Tamura and Shoda-Kagaya 2022) and causes severe damage to Amygdaloideae (Rosaceae) stone fruit trees (Yamamoto et al. 2022a; Urano et al. 2022). In Japan, 2 neonicotinoid products containing dinotefuran and thiamethoxam as active ingredients have become commercially available for trunk injection against *A. bungii* larvae in cherry trees. These products are highly effective, and thus major options for treating infested trees (Kawashima 2021; Sunamura et al. 2020a, 2021; Yamamoto et al. 2022b). Meanwhile, a previous study suggested that some species of Japanese arboreal ants can function as predators of *A. bungii* eggs and neonates (Sunamura et al. 2020b). Dinotefuran and thiamethoxam are toxic to ants by contact and ingestion (Buczkowski 2021; Milosavljević and Hoddle 2021; Sakamoto and Goka 2021; Sunamura et al. 2022) and ants might be negatively affected by trunk injection of these insecticides. Therefore, arboreal ant fauna was investigated by visual sampling of injected *vs*. un-injected cherry trees.

Materials and methods

Field surveys were conducted in Yanagiyama Park and the adjacent promenade, Fussa City, Tokyo Metropolis, Japan (35.73°N, 139.32°E). The study site was located on the riverside bank in an urban residential area and hosted 294 *Cerasus* × *yedoensis* 'Somei-yoshino' cherry trees. Establishment of *A. bungii* was detected in 2015 and 21% of the cherry trees had been infested by September 2019 (Tamura et al. 2021). Chemical control (aerosol treatment) and physical control (stripping bark and crushing detected larvae) were attempted to eliminate *A. bungii* larvae in these trees.

In this study, 18 cherry trees were used in total (mean ± SD root collar length of 263 ± 69 cm). A preliminary survey in May 2020 detected 13 infested trees with diagnostic frass excretion (Iwata 2018). Five of them were assigned to dinotefuran injection, 4 to thiamethoxam injection, and the remaining 4 were used as controls. The test trees were distributed roughly linearly over 500 m (Online Resource 1). Trees with different treatments were randomly ordered. A later survey detected 5 more infested trees and these were added as controls to increase replications as much as possible. Trunk injections were performed on 15 June 2020. Dinotefuran injections were made using Wood-Star (8% liquid solution of dinotefuran; Sankei Chemical Co., Ltd., Kagoshima, Japan) following label instructions of 4 mL injection at 10 cm intervals along root collar (64–128 mL, depending on tree size). Thiamethoxam injections were made using ATTRAC (4% liquid solution of thiamethoxam; Syngenta Japan Co., Ltd., Tokyo, Japan) following label instructions of 60 mL injection at 30 cm intervals along root collar (600–660 mL). In August 2020, heavy occurrence of *A. bungii* frass ejection was observed on one of the control trees, and a dinotefuran injection was made on 31 August in the same way as described above, to control *A. bungii* larvae inside. This tree was regarded as dinotefuran-injected tree in the subsequent survey. Additionally, Matsu Green Ekizai2 was sprayed (2.0% acetamiprid solution; Nisso Green Co., Ltd., Tokyo, Japan) on the trunks of some study trees on 18 June and 1 July 2020 for adult *A. bungii* control, following label instructions of 20-fold dilution and 20–70 L/10a spraying. However, the effect of acetamiprid on ants was likely minimal because acetamiprid shows low toxicity to hymenopteran insects (Jiang et al. 2019; Khan et al. 2021; Yang et al. 2020). For example, all of the 4 control trees sprayed with acetamiprid maintained *Lasius japonicus* Santschi nests and trunk trails with numerous active workers (Online Resource 2).

Efficacy of trunk injections against *A. bungii* larvae was evaluated following the method of Yamamoto et al. (2022b). Activity of frass holes was used as the index of control efficacy. Number and position of actively frass-excreting holes were recorded on the day of injection (15 June) and 1-month post-treatment (13 July). The surveys were conducted for the 13 initially selected test trees, and the 5 later-added trees were not included.

Ant fauna on the study trees was surveyed over three periods: before treatment (5 and 15 June 2020), approximately 1 month after the treatment (15 and 20 July 2020), and approximately 3 months after the treatment (17 and 21 September 2020). In the pre-treatment survey, 4 trees were used as controls and 5 control trees were added in post-treatment surveys (total 9), as aforementioned.

Ant fauna was sampled using two independent methods. First, a time-unit sampling on the tree trunk was conducted.

All ants detected during a 10-min visual inspection on the tree trunk up to 2 m in height were captured with an aspirator. The ants were placed in 70% ethanol, brought back to the laboratory, and identified under a stereoscope using taxonomic keys (Terayama et al. 2014). However, when more than 100 trailing ants were observed, trailing activity was recorded, and only several representative individuals were collected for identification. Additionally, the presence of nest entrance was recorded. Second, ants visiting extrafloral nectaries were sampled. At each tree, 10 branches with extrafloral nectaries were randomly chosen and the number and species of ants tending the extrafloral nectaries were recorded. Cherry trees had 0–2 extrafloral nectaries on the petiole or basal part of individual leaves; thus, there were several extrafloral nectaries on each branch (Online Resource 1). When there were less than 10 branches with active extrafloral nectaries, ants were recorded from only the available branches. Active extrafloral nectaries were wet and green, whereas old and inactive extrafloral nectaries were dry and dark colored. Ant counts at extrafloral nectaries were recorded only during the post-treatment surveys.

Statistical analyses were performed using R Version 4.2.2 (R Core Team 2022). *P* values of ≤ 0.05 were considered statistically significant. Regarding the efficacy of trunk injections against *A. bungii*, ratio of the number of active frass holes at 1-month post-treatment to that at the initial injection was compared among treated and untreated trees using Fisher's exact test with Holm adjustment (accumulated data from 4 to 5 trees in each treatment). For ant samples collected from tree trunks, the effect of treatment on the number of species and individuals were evaluated. The effect on the number of species was analyzed by constructing generalized linear models (GLMs) for pre-treatment, 1-month post-treatment, and 3-month post-treatment, respectively. In the GLMs, the response variable was ant species number, and the explanatory variable was treatment kind (dinotefuran, thiamethoxam, and control). Poisson distribution was assumed. The effect on the number of individual ants was evaluated only for abundant species that occurred on ≥ 50% of study trees in the pre-treatment survey (4 species). For each species, ANOVA was performed for pre-treatment, 1-month post-treatment, and 3-month post-treatment, respectively, using logarithm of individual number plus 1 as the response variable and treatment (dinotefuran, thiamethoxam, and control) as the explanatory variable. When the effect of treatment was significant in ANOVA, multiple comparisons were further performed by Tukey–Kramer's method. Poisson distribution was assumed. When more than 100 trailing ants were recorded, the individual number was treated as 100. Regarding ants detected on extrafloral nectaries, GLMs were constructed for 1-month post-treatment and 3-month post-treatment separately using the proportion of extrafloral nectaries-present branch visited by ants (total branch number and ant-present branch number paired by "cbind" function of R) as the response variable and treatment (dinotefuran, thiamethoxam, and control) as the explanatory variable. Binomial distribution was assumed. GLM analyses were performed for 3 groups of ants: (1) all ants recorded in the study, (2) *L. japonicus*, which was the only ant species that occurred at a high frequency and was present on ≥ 50% of study trees and ≥ 50% of extrafloral nectaries-present branches, and (3) all ants except *L. japonicus*. To obtain the correct GLM estimate in analyses for *L. japonicus*, 1 was added to *L. japonicus*-counts, as it was completely absent on dinotefuran-injected trees.

Results

At the time of injections, the total number of active *A. bungii* frass holes was 14, 21, and 12 for untreated control, dinotefuran-treated, and thiamethoxam-treated trees, respectively (Fig. 1). At 1-month post-treatment, it decreased by 29%, 90%, and 92%, respectively. Meanwhile, there was 50%, 5%, and 17% increase owing to the emergence of new frass holes. Taken together, number of active frass holes increased in

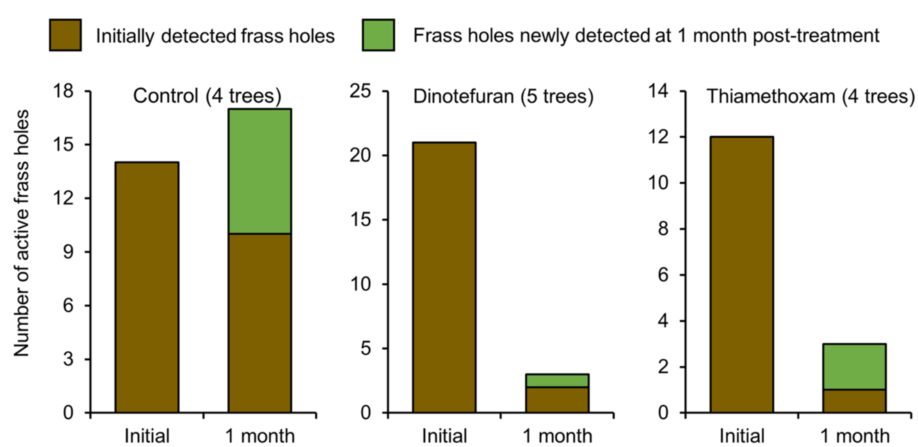

Fig. 1 Number of active *Aromia bungii* frass holes on cherry trees injected with dinotefuran or thiamethoxam versus untreated controls at the time of injection and 1-month post-treatment

control but decreased in dinotefuran- and thiamethoxam-treated trees. Ratio of the number at 1-month post-treatment to the initial number was significantly different between control and dinotefuran-treated trees (Fisher's exact test, $p < 0.01$), nearly significant between control and thiamethoxam-treated trees ($p = 0.06$), and not significant between dinotefuran- and thiamethoxam-treated trees ($p = 0.66$).

In total, 15 species of ants were collected from cherry tree trunks. Among them, 4 species occurred on ≥ 50% of 13 study trees in the pre-treatment survey, which were *L. japonicus* (Formicinae: 100%), *Formica japonica* Motschoulsky (Formicinae: 85%), *Crematogaster matsumurai* Forel (Myrmicinae: 62%), and *Temnothorax congruous* (Smith) (Myrmicinae: 85%). *Lasius japonicus* is an omnivorous species which nests in the rotten tree parts or basal soil (Terayama et al. 2014). *Formica japonica* is a ground nesting, omnivorous species which forages both on the ground and tree (Terayama et al. 2014). *Crematogaster matsumurai* and *T. congruus* are omnivorous species which nest in the rotten parts of trees (Terayama et al. 2014). The most numerically abundant was *L. japonicus*, with more than 100 trailing individuals observed on 54% trees, followed by *F. japonica* (mean ± SD of 7.9 ± 5.4 individuals per tree), *C. matsumurai* (6.4 ± 5.2 individuals), and *T. congruus* (1.7 ± 0.8 individuals). Less abundant species are detailed in Online Resource 2.

The number of ant species recorded on the trees did not differ between control and treated trees across all time periods including pre-, 1-month post-, and 3-month post-treatment surveys (GLM, $p > 0.05$; Online Resource 3) (Fig. 2). Additionally, the number of individual ants of the 4 most abundant species did not differ between control and treated trees in the pre-treatment survey (ANOVA, $p > 0.05$; Online Resource 4) (Fig. 3). However, at 1-month post-treatment, the number of *L. japonicus* was lower on dinotefuran-injected trees relative to control trees (Tukey–Kramer test, $p < 0.001$). Indeed, *L. japonicus* was present on all of the 9 control trees and more than 100 trailing individuals were observed on 7 of them. Furthermore, *L. japonicus* nest entrance was found on 6 control trees. In contrast, *L. japonicus* was detected from 2 of 5 dinotefuran-treated trees and only 1 and 3 individuals were collected from respective trees. More than 100 individuals were found from 2 of the thiamethoxam-treated trees. Three months post-treatment, the number of *L. japonicus* was lower on dinotefuran-injected and thiamethoxam-injected trees relative to control trees (Tukey–Kramer test, $p < 0.005$ for both). In this case, *L. japonicus* was detected from 6 of 8 control trees and more than 100 trailing individuals were found on respective trees. In contrast, *L. japonicus* was detected from none of the dinotefuran- and thiamethoxam-injected trees, including the former control tree which received dinotefuran injection in August (actually 3 weeks post-treatment in September survey). Regarding *F. japonica*, its number was higher on thiamethoxam-injected trees relative to control trees at 1-month post-treatment (Tukey–Kramer test, $p < 0.05$). Additionally, the number of *F. japonica* was higher on thiamethoxam-injected trees than dinotefuran-injected trees at 3-month post-treatment (Tukey–Kramer test, $p < 0.05$). The number of *C. matsumurai* was not different between control and treated trees at 1- and 3-month post-treatment (Online Resource 4). The number of *T. congruus* became lower on dinotefuran-injected trees at 1-month post-treatment (Tukey–Kramer test, $p < 0.05$) but was not significantly different from the control trees at 3-month post-treatment (Online Resource 4).

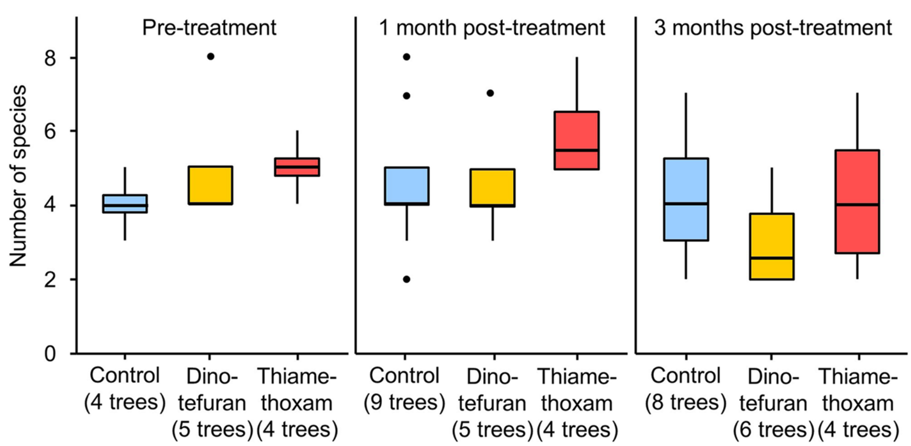

Fig. 2 Box plots on the number of arboreal ant species collected from trunks of cherry trees which received no treatment (control), dinotefuran trunk injection, or thiamethoxam trunk injection. Results for pre-treatment, 1-month post-treatment, and 3-month post-treatment surveys are shown in separate panels. Note that 5 control trees were added at 1-month post-treatment survey. Also note that 1 original control tree received dinotefuran injection after 1-month post-treatment and the tree is included in dinotefuran-treated trees in 3-month post-treatment survey, although the survey period actually corresponds to 3-week post-treatment for this tree

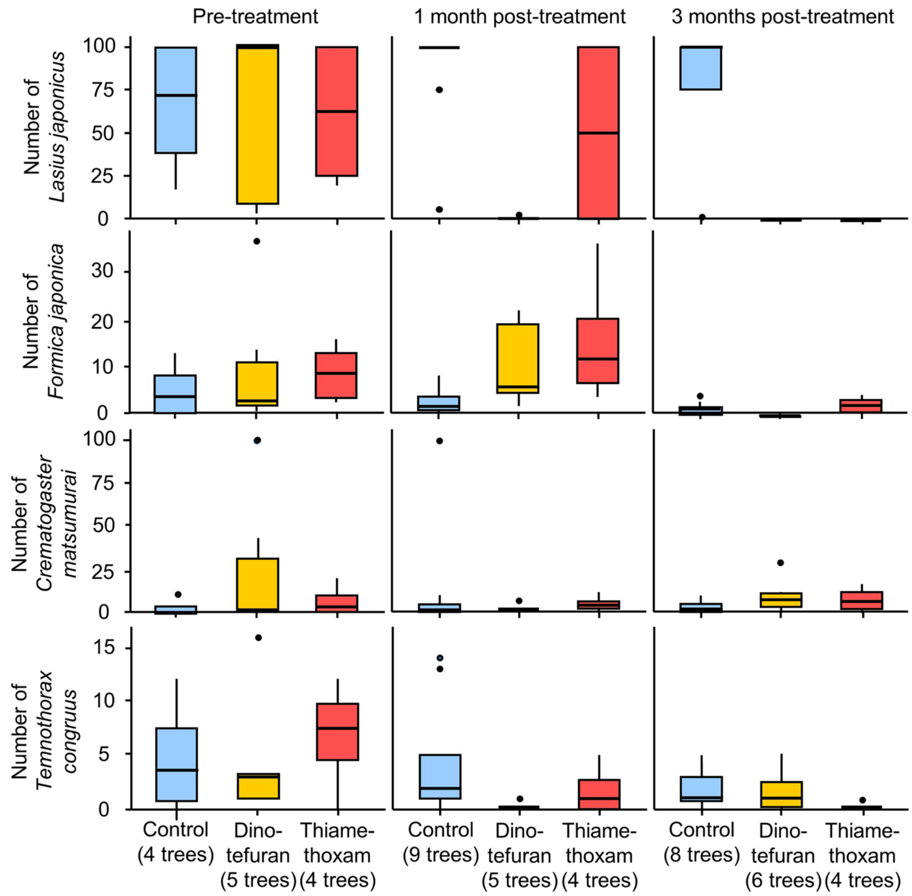

Fig. 3 Box plots on the individual number of 4 arboreal ant species collected from trunks of cherry trees which received no treatment (control), dinotefuran trunk injection, or thiamethoxam trunk injection. Results for pre-treatment, 1-month post-treatment, and 3-month post-treatment surveys are shown in separate panels. See explanations for the change of tree numbers in the legend of Fig. 2

In the extrafloral nectar survey, a total of 8 species of ants were recorded tending the extrafloral nectaries. They were a subset of ant species collected from the trunks (detaled in Online Resource 5). Fewer ants were collected from extrafloral nectaries of dinotefuran- and thiamethoxam-injected trees than control trees at 1-month post-treatment (all ant species pooled; GLM, $p < 0.05$; Online Resource 6; Fig. 4). This was due to a decline of *L. japonicus* on the injected trees (GLM, $p < 0.05$ for both insecticides). This species was present on 49/90 branches of the control trees but 0/50 and 2/40 branches of dinotefuran- and thiamethoxam-injected trees, respectively. In contrast, when *L. japonicus* was excluded from the analysis, the frequency of ants on extrafloral nectaries did not differ

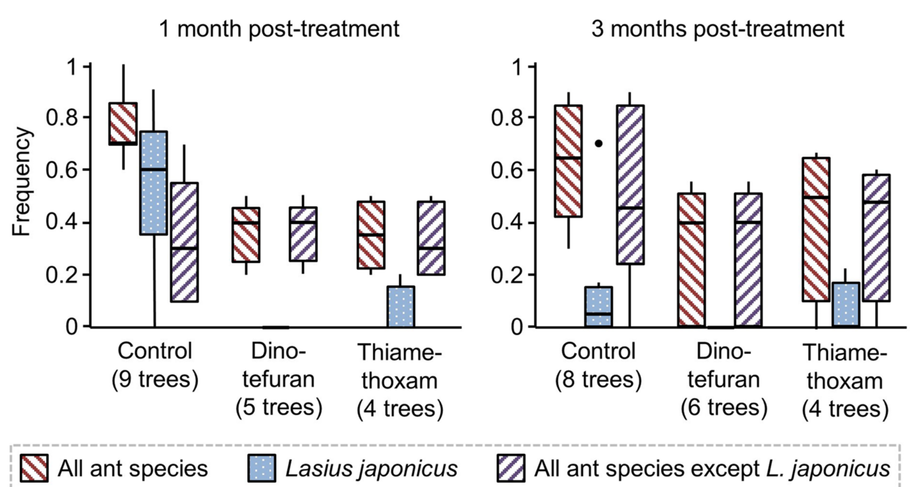

Fig. 4 Box plots on the frequency of arboreal ant species on extrafloral nectar of cherry trees which received no treatment (control), dinotefuran trunk injection, or thiamethoxam trunk injection. Results for 1-month post-treatment and 3-month post-treatment surveys are shown in separate panels. See explanations for the change of tree numbers in the legend of Fig. 2

among injected trees and controls (GLM, $p > 0.05$; Online Resource 6). At 3-month post-treatment, ant frequency on extrafloral nectaries was not different among injected and control trees in all 3 ant groups (i.e., all ant species, *L. japonicus*, and all ant species except *L. japonicus*) (GLM, $p > 0.05$; Online Resource 6). The 3-month post-treatment survey was conducted in late summer, when the number of active extrafloral nectaries was relatively low (Online Resource 5), and thus *L. japonicus* activity was relatively low on control trees (Fig. 4).

Discussion

Results of the current study suggest that trunk injections with neonicotinoid insecticides effectively reduced populations of *A. bungii* larvae in cherry trees. In contrast, arboreal ant species richness was not affected by the injections within 3 months. Additionally, species composition was not noticeably altered by the injections, except for *L. japonicus*. Arboreal ant communities are generally structured by food availability, nesting site availability, and species interactions (Blüthgen and Feldhaar 2010; Parr and Gibb 2010) and the results suggest that insecticide trunk injections do not strongly impact any of these factors.

However, the observed decline of *L. japonicus* suggests that neonicotinoids negatively affected this species, and the effect of dinotefuran was stronger than that of thiamethoxam. Further detailed studies are necessary to clarify the mechanism of decline.

In contrast to *L. japonicus*, trunk injections did not reduce or only weakly reduced *C. matsumurai* and *T. congruus*. In terms of ecosystem services, *C. matsumurai* may be highly beneficial for controlling *A. bungii* and a previous laboratory study demonstrated that it actively preys on *A. bungii* eggs and neonates (Sunamura et al. 2020b). In contrast, *L. japonicus* showed much less interest in *A. bungii* eggs and neonates (Sunamura et al. 2020b). Thus, our findings of neonicotinoid trunk injections affecting *L. japonicus* but not *C. matsumurai* suggest that trunk injections likely do not hinder predation of *A. bungii* by native arboreal ants at least in a short term. However, further studies are necessary to evaluate if chemical control of *A bungii* by trunk injection and biological control by native arboreal ants can be compatible in a long term, because trunk injections might affect competition among arboreal ants, and thus their composition. Indeed, the number of *F. japonica* on the treated trees increased, possibly to compensate for the vacant niche previously occupied by *L. japonicus*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13355-023-00844-7.

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Data availability Raw data are available either from the supplementary materials or from the corresponding author on reasonable request.

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