The Role of Predators and Attendant Ants in the Regulation and Persistence of a Population of the Citrus Mealybug *Pseudococcus citriculus* in a Satsuma Orange Orchard

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The role of predation in regulating a population of the citrus mealybug, *Pseudococcus citriculus*, and the effects of ant-attendance on the predator-prey interactions were studied in a pesticide-reduced Satsuma orange orchard in Wakayama Prefecture, Japan. The population of the first generation increased by more than 5 fold when predaceous insects were excluded, whereas it did not show a notable change in size under natural conditions where enemies naturally attacked mealybugs and some mealybugs were well protected by a honeydew-collecting ant, *Lasius niger*. The mealybug population showed a drastic decrease by 94% when the attendant ants (*L. niger*) were excluded. Attending ants (*L. niger*) were observed to protect *P. citriculus* by aggressive behaviour towards its natural enemies, the common coccidophagous ladybird beetle *Chilocorus kuwanae* and a lacewing species (Chrysopidae sp.). Field censuses and laboratory rearing suggested that the two abundant predaceous insects are the effective agents regulating the mealybug population at low density. These results demonstrate that predators regulate the population of *P. citriculus* and that ant-attendance is indispensable to the persistence of the mealybug population.

Key words: population regulation, natural enemies, ant-attendance, trophobiosis, *Lasius niger*

INTRODUCTION

The citrus mealybug, *Pseudococcus citriculus* GREEN (Homoptera: Pseudococcidae), is an insect pest of citrus in Japan (MATSUURA and HATTA, 1976; KAWAI, 1980; KORENAGA et al., 1992). This pest causes damage to citrus trees through sucking the plant sap and producing honeydew which serves as nutrition to harmful sooty mold (KAWAI, 1980; KORENAGA et al., 1992). The mealybug is known to occasionally increase exponentially to a level at which it causes economic damage (MATSUURA and HATTA, 1976; KAWAI, 1980). Various control programs of the pest have been carried out (MATSUURA and HATTA, 1976; KORENAGA et al., 1992), but few investigations have been carried out on the ecological mechanisms involved in the growth and regulation of mealybug populations in the field. ITIOKA et al. (1992) reported that the mealybug population had remained unharmed at low density for more than 10 years in a pesticide-reduced orchard where predators appeared to be abundant. In addition, MATSUURA and HATTA (1976) noted that a species of predaceous gall midge regulates the population of *P. citriculus*, recording seasonal changes in the number of the mealybug and its mortality due to predators. These results suggested that natural enemies of

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the mealybug play an important role in the population regulation. However, there is not sufficient evidence of the role of predation.

P. citriculus excretes honeydew that some ant species collect as their food (ITIOKA, 1993). Since, as many researchers have mentioned (ADDICOTT, 1984; BUCKLEY, 1987; HÖLLDOBLER and WILSON, 1990; WAY, 1963, etc.), attending ants may protect the trophobiont homopterans against their natural enemies, it is necessary to consider ant-attendance when the regulation by natural enemies is analyzed. Preceding studies have suggested that ant-attendance is expected to affect positively mealybug population growth (ITIOKA, 1993).

In this study, to assess the roles of predation and ant-attendance in the regulation and persistence of a population of *P. citriculus*, we conducted predator-excluded and ant-excluded experiments in the field. We also made field observations on the behavioural responses of a honeydew-collecting ant *Lasius niger* (LINNAEUS) to experimental interaction with adults of a coccidophagous ladybird beetle *Chilocorus kuwanae* SILVESTRI.

MATERIALS AND METHODS

The mealybug. *P. citriculus* has three to four generations per year (MATSUURA and HATTA, 1976). In the study area, most individuals of not in the adult stage are not able to overwinter. Adult females of the mealybug begin to lay eggs at the end of May and 1st-instar nymphs of the first generation appear in early to late June. This study focuses on population growth in one generation from the reproducing adult stage of the overwintering generation to that of the first generation. In the study area, *P. citriculus* were tended by at least four species of ants (ITIOKA, 1993), but mainly by the most common *L. niger*.

The predators. From our preliminary study, it was confirmed that two predaceous insects, *C. kuwanae* and a lacewing Chrysopidae sp. were the most dominant predators of *P. citriculus* in the study area. Larvae and adults of *C. kuwanae* and larvae of the lacewing are easily observed feeding on the mealybug both in the laboratory and in the field. The laboratory rearing revealed that, by feeding only on *P. citriculus*, *C. kuwanae* is able to complete its life cycle and the lacewing is able to grow from at least the latter half of larval stage to the adult.

All field experiments and censuses were carried out at a pesticide-reduced Satsuma orange (*Citrus unshiu* MARC.) orchard in Shimotsu Town, Wakayama Pref. in Japan (see ITIOKA et al., 1992 for more details).

Bag experiment. On 7 June 1990, ten trees on which *L. niger* was abundantly observed were randomly chosen, then almost all herbivorous insects were removed from the trees. On each tree, we randomly selected two 3- or 4-year-old branches, which were on the opposite sides of the tree. At least, ≥ 50 leaves, $\geq$ ten 0-year-old twigs, and $\geq$ five 1-year-old twigs were found on each of the 3- or 4-year-old branches (we hereafter call the entire foliage and stem on one 3- or 4-year-old branch as "shoot-cluster"). Twenty adult females of the overwintering generation of *P. citriculus*, collected from an aggregation on another tree, were placed on an 1-year-old leaf in each of the selected shoot-clusters. After three days, we confirmed the establishment of mealybugs, and enclosed one of the pair shoot-clusters with a bag of Tetron gauze (c. $100 \times 50 \times 50$ cm, hereafter called "bagged shoot-cluster") to prevent attacks by predators and parasitoids, but we did not bag the other of the pair shoot-clusters (hereafter called "control shoot-cluster"). On 3 August, when the mean generation period of the mealybug in the first generation had elapsed, the numbers of large-sized (≥ 3.0 mm in body length) mealybugs, which consisted of third-instar (pre-adult stage)

nymphs and adult females on both shoot-clusters were recorded. Although we monitored the distribution of mealybugs within a study tree by weekly observation, we did not find any mealybugs outside the study shoot-clusters.

Ant-exclusion experiment. The preparation of the study trees and shoot-clusters was the same as the bag experiment. Ten and five trees on which *L. niger* was abundant were randomly chosen in 1990 and 1991, respectively. After all the herbivorous insects were removed from the selected trees in mid-June, the basal stem surface of one of the shoot-cluster pair ("ant-excluded shoot-cluster") of each tree was coated with tanglefoot, an adhesive substance (FUJITANGURU, Fuji-yakuhin-kôgyô Co. Ltd., Tokyo), so that ants were unable to reach any mealybugs in the ant-excluded shoot-cluster while they could freely visit and attend the mealybugs on the other, untreated shoot-cluster ("control shoot-cluster"). Seventy adult females of *P. citriculus* of the overwintering generation were introduced into each shoot-cluster on 15 June in 1990 and on 25 June in 1991, respectively. We also confirmed that mealybugs had formed aggregations around the places of the introduction three days later. The numbers of large-sized (≥ 3.0 mm in body length) mealybugs of the first generation were recorded on 28 July in 1990 and on 15 August in 1991. We checked the movement of mealybugs in the same way as in the bag experiment, but no emigration of mealybugs out of the study shoot-clusters was detected in this experiment either.

On 17 June and 6 July 1990, predaceous insects that were thought to prey on mealybugs were censused at all the study shoot-clusters for the two field experiments shown above. On the latter date, the number of the ants attending mealybugs was also censused. About 10 min per shoot-cluster was spent on the census.

Field experiment on ants attacking against predators. On 11 July 1990, an adult of *C. kuwanae* was released near each of 20 mealybugs' aggregations (range of mealybug numbers: 21–57) which were attended by *L. niger* and randomly selected in the study site. *C. kuwanae* were placed on twigs at c. 10 cm distance from the mealybugs' aggregation after they were anesthetized with ethyl ether in plastic cubes so that the disturbance on their behaviour was minimized. The cases where released beetles showed no access to aggregations were omitted from the analysis. Behavioural response of ants to beetles' access and time from ant's encountering the beetle till the beetle fell down or ran away from the mealybug aggregation were recorded.

RESULTS

Bagging effects on the population of mealybugs

To determine bagging effects on the mealybug population, we compared the population growth rates per generation between bagged and control treatment. Here we defined the growth rate of mealybug population of the first generation as the number of large-sized mealybugs at the end of the field rearing divided by the number of introduced adult females. The population growth rate of mealybugs was significantly higher in bagged shoot-clusters than in unbagged (control) shoot-clusters; on average, the population increased as much as about five times in one generation when natural enemies were excluded, whereas the population decreased by 29% when natural enemies were not excluded (Table 1). On average, 12 ants of *L. niger*, 1.1 larvae and 1.5 adults of *C. kuwanae* and 0.5 larvae of Chrysopidae sp. were observed per shoot-cluster around aggregations of mealybug in control shoot-clusters (Table 1).

Table 1. Population growth rates of first generation *Pseudococcus citriculus* with bagged and control (unbagged) treatments, and the numbers of *Lasius niger* (on 6 July 1990) and two predators, *Chilocorus kuwanae* and Chrysopidae sp. (on 17 June 1990 and 6 July 1990) on the control treatment in a bag experiment. See text for the details of this experiment.

Treatment	No. of shoot-clusters sampled	Population growth rate of mealybugs per shoot-cluster ^a	No. of insects observed per shoot-cluster			
			<i>L. niger</i>	<i>C. kuwanae</i> larva	<i>C. kuwanae</i> adult	Other predators
Bagged	10	5.53 a	—	—	—	—
Control (unbagged)	10	0.71 b	12.0	1.1	1.5	0.1

^a Means followed by different letters in the same column differed at $p < 0.01$ by WILCOXON-test.

Table 2. Population growth rate of first generation *Pseudococcus citriculus* with ant-excluded and control treatments in 1990 and 1991, and the numbers of *Lasius niger* (on 6 July 1990) and two predators, *Chilocorus kuwanae* and Chrysopidae sp. (on 17 June 1990 and 6 July 1990) on the control treatment in an ant-exclusion experiment

Treatment	No. of shoot-clusters sampled	Population growth rate of mealybugs per shoot-cluster ^a	No. of insects observed per shoot-cluster				
			<i>L. niger</i>	<i>C. kuwanae</i> larva ^a	<i>C. kuwanae</i> adult ^a	Lacewing larva	Other predators
1990							
Ant-excluded	10	0.057 a	—	2.0 a	1.8 a	0.4	0.0
Control	10	1.20 b	17.2	0.6 b	0.9 b	0.3	0.0
1991							
Ant-excluded	5	0.069	—	—	—	—	—
Control	5	1.21	—	—	—	—	—

^a Means followed by different letters in the same column differed at $p < 0.05$ by WILCOXON-test.

Table 3. Protection of mealybugs *Pseudococcus citriculus* by attending ants *Lasius niger* against an enemy beetle *Chilocorus kuwanae*

Ant behaviour ^a	No. of aggregations observed	No. of mealybugs ^b	No. of attending ants ^b	No. of attacking ants ^b	Elapsed time from encountering ants till falling or running away from the aggregation (seconds) ^b
Bite	9	35.3 (3.75)	12.4 (1.51)	1.67 (0.24)	3.44 (0.47)
Bite with formic acid	9	33.9 (2.95)	13.7 (1.85)	3.00 (0.24)	6.44 (0.50)
Touch and bite	2	26.5 (0.50)	11.0 (2.00)	4.50 (0.50)	12.00 (3.00)

^a Ants showed three types of behaviours immediately after encountering the beetle. Bite: ants were biting the beetle with their mandible but not using the formic acid. Bite with formic acid: ants were biting with their mandible and spraying the formic acid against the beetle. Touch and bite: ants touched the beetle for several minutes and then bit it with their mandible.

^b Means with standard errors in parentheses.

Effects of ant-attendance on the population growth of mealybugs

Population growth rate per generation of mealybugs was significantly lower in ant-excluded shoot-clusters than in control shoot-clusters (Table 2) (the definition of the population growth rate here was the same as the bag experiment). The mean population growth rate of the ant-excluded shoot-clusters was 0.057 in 1990, which was about 1/21 of that of control shoot-clusters. There was a similar difference in the population growth rate between treatments also in 1991, although the sample size was not large enough for statistical test. Both in 1990 and 1991, the population of the control shoot-clusters increased by about 20%. In 1990, on average, 17.2 workers of *L. niger* per shoot-cluster per 10 min were observed. The numbers of larvae and adults of *C. kuwanae* in 1990 were both significantly higher in ant-excluded treatment than in control (Table 2). The number of lacewing larvae was higher in the ant-excluded treatment than in control, though the difference was not significant.

Ant attack against predators

L. niger showed harshly aggressive behaviour towards beetles accessing the mealybug aggregations that they attended (Table 3). Except for two cases, immediately after encountering the beetle, ants were observed biting the legs and/or the elytra of the beetle with their mandible or spraying formic acid against the beetle. On occasion, four or five ants enveloped the beetle. In all the trials, ladybird beetles were finally, within 15 s, forced by attacking ants to fall down or run away from the mealybug aggregation and showed no success in foraging on mealybugs.

DISCUSSION

The results of the bag experiment suggest that *P. citriculus* increases rapidly to a level where it causes economic damage to citrus trees when no natural enemy occurs, and that the mealybug population could be regulated by natural enemies under natural conditions. As our field censuses demonstrated, a ladybird beetle *C. kuwanae* and a lacewing (Chrysopidae sp.) are the most abundant predators of *P. citriculus* in the study site, while the densities of other predators are rather low. In our preliminary study, it was confirmed that *P. citriculus* is a suitable food for the two predators. These facts suggest that *C. kuwanae* and the lacewing

have a major role in regulating the population growth of the first generation of *P. citriculus*. *C. kuwanae* is known to be an effective predator of various species of scale insects and mealybugs (KATO, 1968; NOHARA, 1970, etc.), and many species of Chrysopidae have been reported to be predators of scale insects and aphids (MIYANOSHITA and KAWAI, 1992; TACHIKAWA, 1963, etc.). These results also support the effectiveness as a predator of the beetle and lacewing. Although the present study does not deal with the predators of the 2nd–4th generations of *P. citriculus*, the dominance of the two predators among enemies in the study site is likely to continue during the rest of the season, because, throughout the entire growth and reproductive seasons of *P. citriculus*, the two predators forage (personal observations) and the densities of other enemies including parasitoids are extremely low, compared with *C. kuwanae* (unpub. data). On the other hand, MATSUURA and HATTA (1976) reported that a species of predaceous gall midge *Nipponodiplosis hirticornis* regulated the population of *P. citriculus*. In the study site, however, the midge seems to have little effect on the mealybug population, not only because the midge was not censused in the present study, but also because it had been rarely observed in the study site (personal observations).

The ant-exclusion experiment and the field experiment on behavioural responses of attending ants to the ladybird beetle suggest that the regulation effect of predation is interfered with by *L. niger* attending the mealybug, and that unattended mealybug populations are eliminated by predators. Thus, the mealybug population is unlikely to persist without ant-attendance in this study site. Of course, as some literature has indicated (CUSHMAN and WHITHAM, 1989, 1991; BRETON and ADDICOTT, 1992), attending ants may enhance the population growth of *P. citriculus* in a manner other than protecting the mealybug against its enemies. However, it is reasonable to say that such an effect, if any, would be small because the population of *P. citriculus* was observed to persist at relatively high density for at least two years in an orchard where both enemies and attending ants had been controlled at extremely low levels by spraying pesticides (personal observation). Since our attention has been focused on the first generation *P. citriculus* in the present study, effects of ant-attendance on the population growth of the other generations remain to be determined. But it is probable that ant-attendance is a necessary factor for the persistence of *P. citriculus* population of all the generations. There are three indirect pieces of evidence to support this inference. First, we made an observation that the population growth rate of an aggregation of the third generation *P. citriculus* with bagging treatment was c. 90 times as high as that of a control (unbagged) aggregation (unpub. data). Second, it is difficult to find aggregations of *P. citriculus* on trees on which honeydew-eating ants do not have any foraging areas. Third, almost all aggregations of *P. citriculus* are attended by ants except during overwintering seasons (unpub. data).

ITIOKA et al. (1992) reported that *P. citriculus* population persisted at low density levels for > 10 years in the study site. How are the effect of the two predators and the attendance effect by *L. niger* involved in the population dynamics? In a few species of honeydew-producing homopterans, it is known that protective effect of attending ant decreases as the homopteran density increases. In such situations, the homopteran populations stop increasing at some threshold and then persist at that density level for long time with ant-attendance (ADDICOTT, 1979; CUSHMAN and WHITHAM, 1991; BRETON and ADDICOTT, 1992; SAKATA, 1994, 1995), because, they argued, excess homopterans are abandoned and not protected by ants. Similarly, the upper limit of the population size of the mealybug may be determined by the limit of the effect of ant-attendance. Population growth rates in the control shoot-clusters in the two field experiments, 0.7 and 1.2, suggest the possibility that the

mealybug population in the field might tend not to exponentially increase. In the study system, the protective effect of ant-attendance may decrease with the population size of the mealybug. If so, the long-term persistence of *P. citriculus* population at the low density can be consistently explained; ant-attendance prevents the extinction of *P. citriculus* by reducing the predation pressure, and exponential population increase is regulated by predators such as *C. kuwanae* and the lacewing. Further investigation is necessary to determine the relationships between the population size of the mealybug and the intensity of the ant-attendance effect.

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