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ANTS ALTER INSECTICIDE EFFICACY ON APHIDS IN THE YARD-LONG BEAN AGROECOSYSTEM ?

F.X. Susilo[1], Dewi Rosmawati[1], dan Nur Yasin[1]

ABSTRACT

Ants Alter Insecticide Efficacy on Aphids in the Yard-Long Bean Agroecosystem? This study was aimed to detect the relationship between *Aphis* sp. and ants and to show whether ants could alter insecticide efficacy on *Aphis* sp. A survey was conducted in the yard-long bean agroecosystems that have been frequently attacked by aphids and for years treated with imidacloprid insecticide (in Mulyosari Village, intensive sample) versus those barely treated in Ganjar Agung Village (non-intensive sample), both in the West Metro municipal area, Lampung. Two sampling occasions were made (in March and November 2005) where 35 plant parts (i.e. 35 flowers and 35 pods) per sample were randomly observed to record the number of *Aphis* sp. and ants. The efficacy of the insecticide treatment was determined by comparing the mean number of aphids or ants from intensive versus non intensive samples using a t-test at the 0.05 level. The relationships between ant and aphid numbers were determined by calculating their coefficients of correlation and testing them using a t-test at the 0.01 or 0.05 level. The study showed that the long-term application of the insecticide imidacloprid in the yard-long bean agroecosystem 1) might strengthen the relationship between *Aphis* sp. and ants (especially *Solenopsis* sp., *Camponotus* sp. and *Paratopula* sp.) and 2) was not effective to suppress *Aphis* sp. number. Stronger *Aphis* sp. — ant symbiotic relationship might alter the insecticide efficacy on *Aphis* sp. in the agroecosystem.

Key words: ant, aphid, symbiotic, insecticide, yard-long bean

INTRODUCTION

Besides their function as predators of various plant pests, ants also live in a symbiotic relationship with aphids (Susilo *et al*., 2004). Ants often feed on eggs and other stages of phytophagous insects and deter others out of their foraging areas. This gives them ability to suppress the pest number as such that fruit-growing farmers may use them as biological control agents (van Mele & Cuc, 2004). However, ants also feed on honeydew secreted by aphids (Kalshoven, 1981). Some ants are so dependent on this juicy and highly nutritious substance that they attend to and stimulate the aphids to secrete it more. These ants also help disperse the aphid neonates and protect them against natural enemies and competitors. That way, the presence of ants is beneficial for the survival of aphids in agroecosystems, and *vice versa*. More aphids produce more honeydew and more honeydew invites more ants to come in. It is then challenging to argue whether ants might be the driving-force of the aphid population increase in agroecosystems, and *vice versa*. Aphids have been known to associate with various crops, including legumes (Kalshoven, 1981). Does (or how does) ant-aphid symbiotic relationship change when those crops are disturbed, for instance through the application of insecticide?

Insecticides are often used to combat insect pests in agroecosystems. But these efforts do not always successful. It was known in the 1980s that intensive use of insecticides was ineffective and caused pest resurgence (Nakasuji *et al*., 1986) leading to considerable decline in legume productivity in Lampung, Sumatra (Kuwatsuka *et al*., 1985). In the 1990s, the ecological mechanisms of the pest resurgence in legume agroecosystems were elucidated (Susilo *et al*., 1994). In undisturbed monoculture legume agroecosystems, the natural enemies might have more preference to feed on other herbivorous arthropods (pest competitors) than on the pests themselves. That was indicated by a strong competitor-enemy correlation and weak pest-enemy correlation. As the field was disturbed with insecticide treatments, the competitor-enemy correlation weakened. In this situation the pest competitors were more suppressed by the insecticide. Consequently, the natural enemies switched host (prey) preference from the pest competitors to the pests, as indicated by stronger pest-enemy correlation. Such a relationship implied either one of two situations: 1) when the natural enemies were able to keep the pest population in check then pest outbreak would not likely occur, or 2) when predation or parasitization were ineffective then the pest number

[1] Department of Plant Protection, Faculty of Agriculture, Universitas Lampung, Jl. Prof. Sumantri Brojonegoro No. 1, Bandar Lampung, 35145

might explode following insecticide application. Nakasuji *et al*. (1986) refered the second case as the pest resurgence. Meanwhile, the correlation between pest and pest competitors, between pest and natural enemies, and between natural enemies and pest competitors were not significant in polyculture legume agroecosystems that have not been treated with insecticide. As the agroecosystem was treated with insecticide, the correlation between pest and their competitors was strengthened, implying that the occurrence of pest outbreak in the agroecosystem would be determined by the ability (inability) of the pest competitors to regulate the pest number. In other words, pest resurgence would likely occur when insecticide applications strengthened the association between the pests and their natural enemies in monoculture agroecosystems (enemy hypothesis) or strengthened the association between the pests and their herbivorous competitors in polyculture agroecosystems (competition hypothesis).

Additional ecological mechanism of pest resurgence, i.e. the symbiotic hypothesis, was implied ten years later by Yasin *et al*. (2004) when they studied the effect of insecticide use on aphids, their predators, and competitors in a leguminous vegetable agroecosystem, the yard-long bean. Their data did not support the symbiotic hypothesis but implied that a resurgence of pestiferous aphids could occur in the yard-long bean fields treated with excessive insecticide if their symbionts (i.e. ants) were strong enough to promote considerable increase in aphid number. A prolonged and intensive use of insecticide could ecologically induce aphid resurgence, or at least render it ineffective against aphids when it strengthens the association between aphids and ants. This study was therefore set to test the symbiotic hypothesis, i.e. to determine how the presence of ants, as the aphid symbionts, contributes to the ineffectivity of insecticide treatment against aphid in the yard-long been agroecosystems.

MATERIALS AND METHODS

This survey study was conducted in the farmer-owned yard-long bean agroecosystems in Mulyosari Village and Ganjar Agung Village, West Metro municipal area, located 45 km Northeast of Bandar Lampung, that have been frequently attacked by aphids. Aphids and ants were collected from yard-long bean fields with two different intensity of insecticide applications, i.e. intensive and non-intensive. The field in Mulyosari Village sized ca. 2500 m^2 has been managed for years along dikes surrounding rice paddy and sprayed intensively with neuro-active imidacloprid 20% at 2 ml/ 15 l rate and 2-3 time interval a week. In contrast, the field in Ganjar Agung Village sized ca. 2600 m^2 for years has not or only rarely sprayed with MIPC 50% at 4 g / 15 l rate. The observations were made in two sampling occasions (March and November 2005). In each sampling occasion, i.e. in the 9th week of the corresponding plant age, 35 plants per field were randomly selected.

In these selected plants ant and aphid numbers were recorded. From each selected yard-long bean plant, one pod and one flower (regardless of ant and/or aphid presence) were taken randomly and then secured into separate glass vials containing ethyl-alcohol 70% for preservation and identification. Specimen identification and counting were done in the Laboratory of Pest Arthropods, Faculty of Agriculture, Universitas Lampung at Bandar Lampung. The aphids were identified to generic level using Cottier (1953), while ants were identified to generic level using Bolton (1994), Hashimoto (2003), and Alpert & Susilo (2005). The identified aphid and ant specimens were counted using a hand-tally counter. The effectivity (ineffectivity) of the insecticide treatment was determined by comparing the mean number of aphids from intensive samples with that from non-intensive samples using a t-test at the 0.05 level of significance (Snedecor & Cochran, 1980). Similar test was also done to the ant number. Homogeneity of variance and normality assumptions of the data were tested using F-test (Snedecor & Cochran, 1980) and Kolmogorov-Smirnov test, respectively. The relationships between ant and aphid numbers were determined by calculating their corresponding Pearson coefficients of correlation (by samples) and testing them using a t-test at the 0.01 or 0.05 level of significance (Snedecor & Cochran, 1980). The changes (if any) in the relationship between the two populations were assessed by comparing the coefficients of correlation in the non-intensive samples against those in the intensive samples.

RESULTS AND DISCUSSION

All collected aphid specimens were identified as *Aphis* sp. (Hemiptera: Aphididae), characterized by their elongate cauda (both in the alate and wingless forms) dan twice-forked media (only in the alate form). Most individuals in the yard-long bean plants were found in the wingless form and they mostly attacked pods and flowers. The number of *Aphis* sp. in intensive and non-intensive samples were depicted in Table 1. It shows that the *Aphis* sp. mean number in the intensive samples did not differ significantly ($P > 0.05$) from that in the non-intensive samples. This means that the prolonged and excessive uses of insecticide in the yard-long bean fields rendered them ineffective to control the aphid number. Table 2 shows similar pattern of ant number. The excessive use of insecticide did not affect the total number of ants that were co-located with *Aphis* sp.

All collected and identified ant specimens were designated into five genera, i.e. *Camponotus* sp. (Formicinae), *Gauromyrmex* sp. (Myrmicinae), *Paratopula* sp. (Myrmicinae), *Solenopsis* sp. (Myrmicinae), and *Tapinoma* sp. (Dolichoderinae). The number was dominated by *Solenopsis* sp., *Paratopula* sp., and *Tapinoma* sp. Did (how did) the number of each ant genus correlate with the number of *Aphis* sp.?

Table 3, 4, 5, and 6 show the relationship between the number of ants and *Aphis* sp. The relationships (if any) were not significant or not defined in all observations in the yard-long bean flower or pods that have been untreated or barely treated with insecticides. In contrast, the relationships were significant in some observations in the fields that have been treated excessively with insecticides, especially in the flower observed in March 2005 (*Aphis* sp. – total ants, *Aphis* sp. – *Paratopula* sp., and *Aphis* sp. – *Solenopsis* sp.) (Table 3), on the pods observed in March 2005 (*Aphis* sp. – *Solenopsis* sp., Table 5), and on the pods observed in November 2005 (*Aphis* sp. – total ants, Table 6). In other words, there was some evidence that the *Aphis* sp.— ant relationships were strengthened as insecticide was intensively applied in the yard-long bean fields.

Previous study by Yasin *et al.* (2004) showed contrasting results. They found that *Aphis* sp. were very susceptible to insecticide and the insecticide application did not affect the relationship between *Aphis* sp. and ant numbers. Yasin *et al*. (2004) used profenophos while farmers in this study used imidacloprid insecticide. In Yasin *et al.* (2004) the term 'intensive' referred to a maximum of four times of insecticide applications in the pertinent growing season with no record of previous yard-long bean agroecosystem in their study site. Meanwhile, the yard-long bean agroecosystem have

Table 1. The mean number of *Aphis* sp. in the yard-long bean fields in West Metro Municipal Area—Lampung, treated with insecticide of differing intensity (2005)

Samples and dates	Number of *Aphis* sp. per sample		t-statistics
	Non-intensive	Intensive	
Pod (in March) (individuals / pod)	192.1	154.9	0.0507^{ns}
Pod (in November) (individuals / pod)	253.3	150.7	0.4723^{ns}
Flower (in March) (individuals / flower)	75.7	109.9	-0.8796^{ns}
Flower (in November)(individuals / flower)	105.4	187.3	-1.1181^{ns}

Notes: ns = two mean values in the same row are not significantly different at the 0.05 level, t-test was done using log-transformed data to normalize the distribution (based on Kolmogorov-Smirnov test) and equalize the variance (based on F-test), sample size = 35 (pods or flowers)

Table 2. The average number of ants in the yard-long bean fields in West Metro Municipal Area—Lampung, treated with insecticide of differing intensity (2005)

Samples and dates	Number of ants per sample		t-statistics
	Non-intensive	Intensive	
Pod (in March) (individuals / pod)	7.4	9.7	-1.47607^{ns}
Pod (in November) (individuals / pod)	11.3	9.0	1.872068^{ns}
Flower (in March) (individuals / flower)	6.5	8.3	-0.78148^{ns}
Flower (in November) (individuals / flower)	10.1	9.5	1.333851^{ns}

Notes: ns = two mean values in the same row are not significantly different at the 0.05 level, t-test was done using log-transformed data to normalize the distribution (based on Kolmogorov-Smirnov test) and equalize the variance (based on F-test), sample size = 35 (pods or flowers)

Table 3. The Pearson coefficients of correlation between *Aphis* sp. number and ant number in the yard-long bean flowers in West Metro Municipal Area—Lampung, treated with insecticide of differing intensity (March 2005)

Ant genera	Pearson coefficients	
	Non-intensive	Intensive
Camponotus sp.	n/d	n/d
Gauromyrmex sp.	n/d	n/d
Paratopula sp.	-0.040^{ns}	-0.159^{ns}
Solenopsis sp.	-0.092^{ns}	0.606**
Tapinoma sp.	n/d	n/d
Total ants	-0.120^{ns}	0.439**

Notes: ns = not significantly different from zero at the 0.05 level, ** = significantly different from zero at the 0.01 level, t-test was performed using original (untransformed) data, n/d = not defined (ant number was constant), sample size = 35 (pods or flowers)

Table 4. The Pearson coefficients of correlation between *Aphis* sp. number and ant number in the yard-long bean flowers in West Metro Municipal Area-Lampung, treated with insecticide of differing intensity (November 2005)

Ant genera	Pearson coefficients	
	Non-intensive	Intensive
Camponotus sp.	n/d	n/d
Gauromyrmex sp.	n/d	n/d
Paratopula sp.	-0.259ns	-0.080ns
Solenopsis sp.	0.167ns	0.293ns
Tapinoma sp.	-0.065ns	-0.041ns
Total ants	-0.177^{ns}	0.028^{ns}

Notes: ns = not significantly different from zero at the 0.05 level, t-test was performed using original (untransformed) data, n/d = not defined (ant number was constant), sample size = 35 (pods or flowers)

existed for decades in this study site (in Mulyosari and Ganjar Agung). Furthermore, the farmers have used the insecticide intensively ever since and did so at least eight times during the two-month period of sampling in Mulyosari Village versus no sprays in Ganjar Agung Village. Thus, Yasin *et al.* (2004) described the short-term effects while this study dealt with the long-term effects of intensive use of insecticide. If profenophos was applied excessively in the long run, would it not exert impact similarly to imidacloprid, as was the case in this study?

Ants and *Aphis* sp. apparently engaged in a dynamic relationship. Under a normal situation ants seemed to be indifferent to *Aphis* sp., as was indicated by lack of correlation in non-intensive samples (Tables 3, 4, 5, and 6). In that situation ants were probably in their mode of foraging for prey and nectarines (or other plant-originated juicy substance) in flowers and pods. Ants did not acutely attend to aphids for honeydew. But then insecticide application disturbed the system. The disturbance might have induced the aphid resistance as showed by lack of difference in *Aphis* sp. number between non-intensive and intensive samples (Table 1), but it nevertheless put the aphid under remarkable stress. The tense aphid might feed more for compensation, leading to more honeydew excretion which in turn invited

Table 5. The Pearson coefficients of correlation between *Aphis* sp. number and ant number in the yard-long bean pods in West Metro Municipal Area—Lampung, treated with insecticide of differing intensity (March 2005)

Ant genera	Pearson coefficients	
	Non-intensive	Intensive
Camponotus sp.	-0.069^{ns}	-0.078^{ns}
Gauromyrmex sp.	-0.111^{ns}	n/d
Paratopula sp.	-0.128^{ns}	0.248^{ns}
Solenopsis sp.	n/d	0.599**
Tapinoma sp.	n/d	n/d
Total ants	-0.204^{ns}	0.318^{ns}

Notes: ns = not significantly different from zero at the 0.05 level, ** = significantly different from zero at the 0.01 level, t-test was performed using original (untransformed) data, n/d = not defined (ant number was constant), sample size = 35 (pods or flowers)

Table 6. The Pearson coefficients of correlation between *Aphis* sp. number and ant number in the yard-long bean pods in West Metro Municipal Area—Lampung, treated with insecticide of differing intensity (November 2005)

Ant genera	Pearson coefficients	
	Non-intensive	Intensive
Camponotus sp.	n/d	-0.091^{ns}
Gauromyrmex sp.	n/d	n/d
Paratopula sp.	-.235^{ns}	0.173^{ns}
Solenopsis sp.	n/d	n/d
Tapinoma sp.	n/d	n/d
Total ants	-0.261^{ns}	0.369*

Notes: ns = not significantly different from zero at the 0.05 level, * = significantly different from zero at the 0.05 level, t-test was performed using original (untransformed) data, n/d = not defined (ant number was constant), sample size = 35 (pods or flowers)

more ants to come. The new situation with abundant honeydew might facilitate some genera of ants to shift their feeding mode from prey foraging to aphid attendance. With their agility, ants could evade insecticide as—or even better than— *Aphis* sp. did, resulting in their unsuppressed number in the insecticide-treated samples (Table 2). Ants could also choose to visit and stay in other micro niches (refuges) with less insecticide residue. With more cryptic folds, creases, or grooves, the yard-long bean flowers tended to offer more morphological refuges than the pods. Ants could carry away and place the aphid from the more to the less intoxicated micro niches within the niche breadth in flowers or pods. In these refuges *Aphis* sp. could then resettle their colonies and number (Table 1) and set up stronger relationship with some groups of ants, especially with *Solenopsis* sp. (Tables 3 and 5) and apparently with the pooled *Camponotus* sp. – *Paratopula* sp. (Table 6). In other words, stronger symbiotic relationship between the ants and *Aphis* sp. as induced by the long-term insecticide application might in fact contribute to the insecticide ineffectivity on the aphid in the yard-long bean agroecosystem. A similar phenomenon was reported by Rohamah *et al*. (2006) in citrus orchards where long-term applications of an organophosphate insecticide, methidathion, strengthened the correlation between the ant *Dolichoderus* sp. (Dolichoderinae) and the citrus aphid (*Toxoptera* sp., Hemiptera: Aphididae) which might contribute to the insecticide inability to control the aphid.

CONCLUSIONS

It could be concluded that the long-term application of the insecticide imidacloprid in the yard-long bean agroecosystem strengthened the relationship between *Aphis* sp. and ants (especially *Solenopsis* sp., *Camponotus* sp. and *Paratopula* sp.). In such agroecosystem the ants might alter the insecticide efficacy on the aphid. It remains to be investigated further, however, why and how the insecticide becomes ineffective, and whether it is a case of pest resurgence or resistance.

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