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Multitrophic Interaction between Cucumber Moth Diaphania indica Saunders, (Lepidoptera: Crambidae) and Its Natural **Enemies**

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Abstract. Multitrophic interactions provide an information on how plant-insects interact in the field mediated by biotic and/or abiotic factors. This study measures the effect of Apanteles taragamae parasitism toward Diaphania indica, and the rate of A. taragamae themselves being parasitized by hyperparasitoid. The insects were collected periodically at 3, 4, and 5 weeks after planting from cucumber fields in Bogor, Indonesia during the growing season in 2014 and 2015. Sampled insects were reared in the lab and the incidence of parasitism and hyperparasitism was calculated. The relationship between D. indica, its primary parasitoids and hyperparasitoids, was also analyzed. Older cucumber plants harbored larger populations of D. indica and higher rates of primary parasitism by A. taragamae. Other D. indica parasitoids, Ichneumon sp., and Elasmus sp., showed lower parasitism rates. The hyperparasitoid species found in greatest abundance were Stictopisthus sp. and Ceraphron sp., with other hyperparasitoids present i.e., Tetrastichus sp., Eurytoma sp., Orasemasp., and Brachymeria sp. The overall population of D. indica found in Bogor was low. A. taragamae have a significant suppressive effect on the population of D. indica. But, the presence of hyperparasitoids may disrupt parasitization of D. indica by A. taragamae thereby reducing the suppressive effect. indica, host-parasitoid interaction, Keywords: Apanteles taragamae, Diaphania hyperparasitoid, trophic interaction

1. Introduction

There are systems of self-regulation in nature that can suppress the outbreak of pest populations. Hostparasitoid interactions have been shown to be one such system, i.e. certain pest herbivores are suppressed by their parasitoids. The dynamic role of parasitoids in natural host-parasitoid interactions can be resolved into two components i.e., the degree of depression in the host population density caused by the observed level of parasitism and the degree of stability conferred on the interaction in the long term [1-3] which together determine the success of a successful biological control program. In conditions where the natural enemies are able to suppress its host, we usually do not see major

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outbreaks, and the pests remain "minor". Such species are recorded feeding or ovipositing on crop plants but usually do not inflict economically important damage; often their effect on the plant is indiscernible. They may be confined to particular crop plants or may prefer various plants as hosts [4]. When host-parasitoid interactions are disrupted, the systemic balance can break down, allowing a minor pest to become problematic. For example, the brown planthopper was a relatively minor rice pest prior to the green revolution, when increased use of insecticides disrupted the ecological balance between the brown planthopper and its natural enemies [4]. Similarly, mirid bugs were only a minor pest of cotton in China but became a major pest due to the introduction of genetically engineered cotton plants[5]. Any pest has potential to be a major pest. Many species that are major pests for one crop may have only minor effects on other crops, or even the same crop in a different part of the world [6].

Diaphania indica Saunders (Lepidoptera: Crambidae) is an occasionally serious pest of certain cucurbitaceae species such as cucumber, bittergourd, and snakegourd [7,8] and is reported as a major pest in Ceylon [9]. However, this pest has so far been recognized as minor pest in Indonesia [10]. In fact, the actual condition of infestation level of *D. indica* in the field is relatively unknown. The presence of minor pests in the fields provides an interesting phenomenon to study, i.e. what causes the minor status? Is it due to the slow reproductive capacity causing low population density (intrinsic factor); or is it because there are external factors that suppress the pest population buildup? These questions are important to pursue since understanding the causal factor of minor pest incidence the system can provide important information that can enhance our understanding to pest outbreaks.

Previous studies of the community structure of natural enemies of the order Hymenoptera in cucumber fields found that *Apanteles taragamae* Viereck (Hymenoptera: Braconidae), a larval parasitoid of *D. indica*, can cause a mortality rate of up to 35% [11]. Generally, the effectiveness of a parasitoid in regulating pest populations will depend on the parasitism rate, its success to adulthood, and the ability of the parasitoid in finding and attacking its hosts[12,13]. When the parasitism rate is high, it can be expected that the parasitoid is effective [14]. More information is needed to understand the interaction dynamic of the two species. Do the interactions provide key results toward the host-parasitoid relationship? Can the presence of *A. taragamae* affect the existence of *D. indica* such that it suppresses the host population overall?

Hyperparasitism or parasistim of *A. taragamae* by a secondary parasite also occurs in the field and can cause the decrease of parasitoid's performance [15]. By considering a third trophic level (natural enemies of the herbivore pest, and hyperparasitoids of those enemies), and examining the tritrophic interaction in the field, this study attempts to understand the interactions of cucumber-*D. indica-A. taragamae* in natural conditions, and the multiple factors that regulate the suppression of pest populations. This study is expected to provides important baseline information on the situation of *D. indica* in Indonesia and thus can provide a complete information on the management of the pest.

2. Materials and methods

2.1 Pilot study

Initial field surveys were carried out as pilot studies in several sites in Bogor, Indonesia (table 1). Farmers in Bogor mainly planted cucumber (*Cucumis sativus*), bittergourd (*Momordica charantia*), and snakegourd (*Luffa acutangula*). The pilot studies were conducted as preliminary quick survey in 2014 to understand the diversity and parasitism rate of *D. indica* in those different host plants. Within these fields, the larvae and pupae of *D. indica*, and *A. taragamae* cocoons, were collected via direct sampling methods twice during the sampling period when the plants are 5 - 7 weeks old.

In the lab, larvae of *D. indica* samples were reared in a transparent plastic container (15 x 10 x 5 cm) containing cucumber leaves provided as a food source, for 7 days until the formation of pupae or the emergence of parasitoid and or hyperparasitoid adults. The pupae of *D. indica* were reared in Petri dishes (86 mm x 13 mm), and placed in a transparent cylindrical plastic rearing cage (10 cm in diam., 30 cm in height) until emergence of adults. *A. taragamae* cocoon masses were placed in a glass tube

(2 cm diam., 15 cm in height) until emergence of parasitoid and/ or hyperparasitoid adults. Honey droplets were provided as food. All insect rearing occurred in a rearing cabinet in laboratory conditions (25 ± 1 °C, 90 ± 10 % RH, and L16:D8 photoperiod).

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Table 1	Description	of samn	ling	locations
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Location	Field site	Year	Coordinate	Host Plant
Ciomas*	1	2014	6°34'56.48"S, 106°45'18.98"E	Cucumber
Cibeureum*	2	2014	6°35'11.47"S, 106°43'58.94"E	Bittergourd
Cinangneng*	3	2014	6°34'01.83"'S, 106°42'36.74"'E	Snakegourd
Dramaga**	4	2014	6°32'57.57"S, 106°44'33.32"E	Cucumber
Cihideung1**	5	2014	6°35'14.83"S, 106°43'43.73"E	Cucumber
Cikarawang**	6	2015	6°32'56.13"S, 106°43'39.63"E	Cucumber
Cihideung2**	7	2015	6°35'18.77"S, 106°43'01.31"E	Cucumber

*site location for primary study

**site location for study the diversity and parasitism rate of *D. indica* in different age of cucumber plant

2.2 Population density and parasitism rate of D. indica in different age of cucumber plant

The survey activity was then continued by undergoing a study to understand the diversity and parasitism rate of *D. indica* in different age of cucumber plant. Sites were selected based on accessibility and plantation area (25x15 m). Each field was divided into 4 plots with plants selected systematically within the plots for sampling. Insect sampling was conducted using the direct sampling method during a 3-week period in both 2014 and 2015. Observations were carried out weekly in the same plots each week. Insect sampling and pest incidence measurements began when plants were 3 weeks old after planting, and continued until the plants were 5 weeks old, the time at which farmers applied pesticides to the field. The sampled insects were taken to the laboratory in a transparent plastic container (15 x10 x 5 cm), and reared using the same methods as in the pilot study.

2.3 Insect identification

Parasitoids and hyperparasitoids were identified at the Laboratory of Biological Control, Department of Plant Protection, Bogor Agricultural University. All insect identifications were confirmed by the Laboratory of Entomology, Indonesian Institute of Science (LIPI), Cibinong, Indonesia.

2.4 Statistical analysis

The number of *D. indica* (larvae and pupae), the parasitization rate of the larvae, and the hyperparasitization rate of the parasitoids, were analyzed by Analysis of Variance (One-way ANOVA) for Complete Random Design in Time as weekly observation for each host plant species and for each cucumber plant age group sampled in 2014 and 2015. Means from those of data were separated usingTukey Honest Significant Difference test (Tukey HSD test) using SAS Statistic Analytical Software version 9.4. Parasitoid and hyperparasitoid community composition was analyzed descriptively in relation to host plant species or to cucumber host plant age. The *D. indica* - primary parasitoid relationship, and the primary parasitoid - hyperparasitoid relationship on cucumber host plants was analyzed using simple linear regression. The 3-way relationship between *D. indica*, primary parasitoids, and hyperparasitoids on cucumber host plants was analyzed by path analysis using SPSS statistical program version 19. Finally, trophic interactions between host and parasitoid, and primary parasitoid and hyperparasitoid, for each species of host plant, were calculated using R Statistic 3.1.3 for Windows with bipartite package.

3. Results

3.1 Pilot study

From the initial surveys, *D. indica* found to be present in all three crops studied, with the highest population density in snakegourd. Results from the pilot studies showed that the population density of *D. indica* larvae per plant (F = 74.42, df = 2, P<0.0001) as well as the parasitism rate (F = 8.02, df = 2, P<0.05) were significantly different between plots planted with different host plants (table 2). The population density of *D. indica* is higher insnakegourd plot than inbittergourd or cucumber plots.

The parasitoid community composition in all the fields was dominated by *A. taragamae*, with 56% parasitism rate in cucumber plots, 30% parasitism rate in bittergourd plots, and 40% parasitism rate in snakegourd plots. Other larval parasitoid species including *Elasmus* sp., a gregarious ectoparasitoid which was found on *D. indica* at levels of 5%, 4%, and 0.5% parasitism rate in cucumber, bittergourd, and snakegourd plots, respectively (figure 1A). There was no significant difference in hyperparasitism rates of *A. taragamae* between the different host-plant plots (F = 3.11, df = 2, P > 0.05) (table 2).

Table 2. Population density of *D. indica* larvae, parasitism and hyperparasitism rates for *A. taragamae* in different host plants.

Parameter	Cucumber	Bittergourd	Snakegourd
Larval density/plant	$0.69 \pm 00.1c$	$0.80\pm0.05b$	$1.08\pm0.08a$
Parasitism rate (%)	$63.32\pm5.53a$	$35.13\pm448b$	$41.15\pm7.88ab$
Hyperparasitism rate (%)	$7.43 \pm 1.18a$	$12.00 \pm 3.25a$	$3.64 \pm 1.32a$

Figure followed by the different letter in a column is significantly different using Tukey (HSD) Test (α =5%). The parasitism rate shows combined parasitism rate of *D. indica* by all parasitoid species, and hyperparasitism rate shows combined parasitism of *A. taragamae* by all hyperparasitoids species



Figure 1. Community composition of *D. indica* parasitoid (a) and community composition of *A. taragamae* parasitoids (b) for different host plant species (plots 1-3).

Hyperparasitoid community composition was dominated by *Stictopisthus* sp. in all pilot study plots, and *Ceraphron* sp. was also observed for all host plant species. *Tetrastichus* sp. was found in the bittergourd and snakegourd plots; *Eurytoma* sp., *Brachymeria* sp., and *Orasema* sp. was found in cucumber plots only (figure 1B). The complexity of structure in host-parasitoid trophic relations is similar in plots with all three types of host plants. However, the complexity of structure for parasitoid/hyperparasitoid trophic relations on cucumber plants is different compared to bitter gourd or snake gourd (figure 2).

3.2 Diversity and parasitism rate of D. indica in different age of cucumber plant

Populations of *D. indica* were significantly different between 2014 and 2015 (F = 67.24, df = 1, P < 0.01), and between plots of cucumber plants of different ages (F = 94.3, df = 2, P < 0.0001) (figure 3). The population density of *D. indica* larvae per plant increased in cucumber plots with 4 week old plants compared to 3 week old plants, and then decreased in plots with 5 week old plants in 2014, so

also in 2015. Total parasitism rates were also significantly different between 2014 and 2015 (F = 41.47, df = 1, P < 0.01), and between plots of cucumber plants of different ages (F = 101.63, df = 2, P < 0.0001). Parasitism rate in 2015 was 20% higher than in 2014. The majority of parasitism rate increase as the age of plant increase. However, there was not a significant difference in total hyperparasitism rates in 2014 vs. 2015 (F = 8.16, df = 1, P > 0.05), nor between plots of cucumber plants of different ages (F = 1.76, df = 2, P > 0.05) (figure 3).



Figure 2. Trophic interaction between *D. indica* – parasitoids and parasitoid – hyperparasitoids for each species of host plant. 1. *D. indica* from Cucumber, 2. *D. indica* from bittergourd, 3. *D. indica* from snakegourd, 4. *Elasmus* sp., 5. *A. taragamae*, 6. *Ichneumon* sp., 7. *Stictopisthus* sp., 8. *Tetrastichus* sp., 9. *Eurytoma* sp., 10. *Orasema* sp., 11. *Brachymeria* sp., 12. *Ceraphron* sp., Black bars represent relative abundances of *D. indica* (lower bars) in different host plant, parasitoids (middle bars), and hyperparasitoids (upper bars) drawn to different scale.

The parasitoid community composition was dominated by *A. taragamae* in both 2014 and 2015. Other parasitoid species such as *Ichneumon* sp., a solitary larval endoparasitoid which exhibited a less than 1% parasitism rate and only in plants 3 weeks old in 2015. Meanwhile, the hyperparasitoid community composition was comprised only of *Stictopisthus* sp. and *Ceraphron* sp. in both 2014 and 2015 (figure 4).

Regression analyses revealed a significant inverse relationship between the population density of *D. indica* and its parasitism rate for plants 3 and 4 weeks old in 2014, and a positive slope between them for plants 5 weeks old in 2014. However, this latter result did not prove significant. In 2015, the same regression analysis showed a positive slope and significant relationship between population density of *D. indica* and its parasitism rate for plants of all ages (3, 4, and 5 weeks old) (figure 5).

There was no hyperparasitism by *Stictopisthus* sp. observed in plants 4 and 5 weeks old in 2014. Hyperparasitism incidence in plants 3 weeks old in 2014 showed an inverse relationship between *A*.

IOP Conf. Series: Earth and Environmental Science **197** (2018) 012026 doi:10.1088/1755-1315/197/1/012026

taragamae population and hyperparasitism rates by *Stictopisthus* sp. (figure 6A). In contrast, hyperparasitism incidence by *Stictopisthus* sp. was observed in all plants of all ages in 2015 with a positive slope, but results were only significant for plants 5 weeks old 2015 (figure 6B). Meanwhile, hyperparasitism by *Ceraphron* sp. was not observed in plants 3 and 4 weeks old in 2014. Hyperparasitism incidence in plants 5 weeks old that year showed an inverse relationship between *A. taragamae* population and hyperparasitism rates by *Ceraphron* sp. (figure 7A). Hyperparasitism incidence by *Ceraphron* sp. was observed in plants of all ages in 2015 with a positive slope, but the results were significant only for plants 4 weeks old (figure 7B).



Figure 3. Parasitism rate of *D. indica* and parasitism rate of *A. taragamae*, plotted together with population density of *D. indica* larvae per plant. Solid line shows combined parasitism rate of *D. indica* by all parasitoid species, and dashed line shows combined hyperparasitism of *A. taragamae* by all hyperparasitoids species (right y-axis). The bars represent population density of *D. indica* (left y-axis). Figure followed by different letter is significantly different using Tukey (HSD) Test (α =5%).



Figure 4. Community composition of *D. indica* parasitoids (a) and *A.taragamae*parasitoids (b) for different age of cucumber plants (plots 4-7).

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Figure 5. Linear regression of percent parasitism of *A. taragamae* vs. host density of *D. indica* in2014 (a) and in 2015 (b) (plots 4-7).



Figure 6. Linear regression of percent *Stictopisthus* sp. hyperparasitism vs. number of host *A. taragamae* in 2014 (a) and in 2015 (b) (plots 4-7).

Path analysis showed that the population size of *A. taragamae* has a significant negative effect on the population size of *D. indica* (P<0.05), as does the population size of hyperparasitoids on the population size of *A. taragamae* (P<0.05). However, the population size of hyperparasitoids has no significant effect on the population size of *D. indica* (P>0.05) (table 2).

4. Discussion

In the 2 year studies, the density of D. *indica* is found to be quite low i.e., 1-3 larvae per entire plant. The density seems to be similar in both years 2014 and 2015. Pilot study showed that the population size of D. *indica* differs according to host plant species. The population size of D. *indica* is higher in snakegourd plots than in bittergourd or cucumber plots. This stand in contrast with the result of

Pandey[7] who reported the population density of *D. indica* i.e., 8 larvae per plant in snakegourd, and no larvae found infested on bittergourd in Bharwari, India. Schreiner [16] found that yield loss due to *D. indica* predation is approximately 10%, once population density of *D. indica* reaches one larva per cucumber leaf in Inarajan, Guam; a much higher density than was observed in the current studies. The low population density of *D. indica* in this study indicate that *D. indica* in Bogor, Indonesia can be categorized as a minor pest at this time. The difference in those infestation level of *D. indica* is consistent with the conclusion of Hill [6] who said that species that are major pests for one crop may have only minor effects on other crops, or even the same crop in a different part of the world.



Figure 7. Linear regression of percent *Ceraphron* sp. hyperparasitism vs. number of host *A*. *taragamae* in 2014 (a) and in 2015 (b) (plots 4-7).

Regarding the effect of host plant age, the population density of *D. indica* increased in cucumber plots with 4 week old plants compared to 3 week old plants, and then decreased in plots with 5 week old plants. Cucumber plants enter the generative phase at 4 weeks old, and Ganehiarachchi [17] found that *D. indica* causes the greatest damage when its host plants are in the generative phase. Similary, Vanisree *et al.* [18] and Ulina [19] observed that the population size of *D. indica* is greater in the generative phase than in the vegetative phase. The majority of *D. indica* remained unparasitized when the plants at younger plants, but as they grow older, more parasitism was found. Lizmah [11] also reported the higher parasitism rate of *D. indica* in older cucumber plant. Similarly, *Diadegma semiclausum*, a parasitoid of Diamond Back Moth *Plutella xylostella* showed the lower parasitism rate after the cabbage planted but increased as the plant grew older [20]. This is a common phenomenon in the field, as the plant growing older, they may experience more pest infestation and severely damage, thus inviting more parasitoid to attack their host.

Fitriyana [10] reported that the life cycle of *D. indica* is known to be 26 days in Indonesia, with a net reproduction rate (Ro) of 50 eggs/adult. In India the life cycle of *D. indica* is known to be 39 days, with a similar net reproduction rate of 56 eggs/adult at 30 °C [21]. In addition, the net reproduction rate of *D. indica* can increase from 68 eggs/adult at 20 °C to 120 eggs/adult and 25 °C in Iran [22]. This indicates that the population growth rate of *D. indica* are influenced by the climate of the area, and where it is favorable, *D. indica* has the potential to build a bigger population size, and hence could become a major pest. However, studies done by Fitriyana[10] showed the total mortality of *D. indica* in the fields can reach as high as 51% due to external factors such as entomopathogen (21%), predator (1%), as well parasitoids (29%). In our studies, parasitism rate of *D. indica* was also dominated by *A. taragamae* in both 2014 with 17-46% parasitism rate and 2015 with 26-69% parasitism rate. This clearly showed the external factors to be a dominant factor in the suppression of *D. indica* population

build up in Bogor, thus may be one cause why D. indicia does not become a major pest in Bogor, Indonesia.

A. taragamae was parasitized by six hyperparasitoids species. The highest hyperparasitism rates overall were observed for Stictopisthus sp. (Ichneumonidae) and Ceraphron sp. (Ceraphronidae). The other hyperparasitoid species found were *Eurytoma* sp. (Eurytomidae). *Tetrastichus* sp. (Eulophidae). Orasema sp. (Eucharitidae), and Brachymeria sp. (Chalcididae). However, these other species were only observed during the pilot study. For this reason, and because hyperparasitism rates were less than 2% for these species, it was deemed to be un-influential in the community, and focused the analysis on Stictopisthus sp. and Ceraphron sp. only. The hyperparasitism rate by Stictopisthus sp. was higher than that of Ceraphron sp. However, relative abundance of Ceraphron sp. was higher than Stictopisthus sp. Parasitoid abundance depends in part on the behavioral characteristics of the parasitoid itself, as a solitary or gregarious parasitoid. Stictopisthus sp. is a solitary hyperparasitoid and *Ceraphron* sp. is a gregarious hyperparasitoid, which may explain the difference in abundance for the two species. However, Stictopisthus sp. is still remain unknown whether it is a direct or indirect secondary parasite of A. taragamae. Stictopisthus sp. individuals emerged with or without the presence of the assumed primary host A. taragamae. Meanwhile, Ceraphron sp. is indisputably a direct secondary parasitoid because it parasitizes the pre-pupal or pupal stage of A. taragamae. Fitrivana[10] also reported 14% hyperparasitism rate of A. taragamae by Stictopisthus sp. and Ceraphron sp. Moreover, Lizmah [11] reported 50% hyperparasitism rate of A. taragamae by Stictopisthus sp. In India, Peter and David [15] reported six hyperparasitoid species of A. taragamae i.e., Stictopisthus srinaraini, Aphanogmus fijiensis, Eurvtoma braconidis, Elasmus brevicornis, B. Apantelesi, and Tetrastichus pantnagarensis. Hyperparasitoid communities have been found to differ in different locations. Noves [23] said that different biotic and abiotic conditions, such as latitude, climate, rainfall, temperature, and vegetation habitats as well as different topography, can affect the diversity of parasitoids. Parasitoid species adapt variably to different environments, so that their diversity also varies regionally [24].

The slope of the regression line representing the effect of plant age, gave inconsistent results regarding the relationship between population density of *D. indica* and its parasitism rate by *A. taragamae*. Similarly, regression analysis of the effect of plant age also showed inconsistent relationships between hyperparasitism rate and the population of *A. taragamae*. These inconsistent effects may be caused by the different cultivation methods between sampling location which we do not measure as parameters in this study. The 3-way relationship between *D. indica* – primary parasitoid (*A. taragamae*) – and hyperparasitoid on cucumber host plant may explain the relationship between each trophic level. The relationship between *D. indica* and parasitoid *A. taragamae* we regard as a direct interaction because the population size of *A. taragamae* has a significant negative effect on *D. indica* population, serving to depress the parasite's numbers and impact. Meanwhile, the relationship between hyperparasitoids and *D. indica* we count as indirect interaction mediated by *A. taragamae*, because the population size of hyperparasitoids has a significant negative effect on the number of *A. taragamae* which in turn, disrupts the role of *A. taragamae* in controlling population of *D. indica*.

This study implies that information about the diversity of natural enemies of *D. indica* can be used as an effort in planning for the implementation of biological control program. Parasitoid will show its function in regulating pest population if their population in the field is sufficient. However, parasitoid population in the field is often low, causing they lose their function. Parasitoid population in the field can be improved through mass rearing in lab which further can be used for parasitoid will improve their efficiency. The abundance of parasitoid in the field can also be improved through habitat manipulation, such as land management or landscape modification. Increasing the diversity of habitat vegetation will increase the diversity of parasitoids and its host. In addition, uncultivated plants around plantation area need to be maintained and managed as one conservation effort. The existence of uncultivated plants can be used as refuge for parasitoids when their host does not present.

5. Conclusion

The overall population of *D. indica* found in study sites in Bogor was low. Therefore, this species may be categorized as a minor pest in this region in 2014 and 2015. The minor pest status for *D. indica* in Indonesia is caused by the low population density in the field, and the high parasitism rate by *A. taragamae* as external factor in regulating their population in the field. The high parasitism rate of *D. indica* by *A. taragamae* indicates that the latter species could be a good candidate for biological control agent against *D. indica* in the future. Further study of the key aspects of biology and ecology of *A. taragamae* is needed to ensure the success of any biological control program using *D. indica*.

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