Journal of Engineering Science and Technology
Special Issue on ISCoE2022 Vol. 18, No. 3 (2023) 129 - 136

FUNCTIONAL RESPONSE OF ENDOPARASITIC WASP, *Anagyrus lopezi* ON CASSAVA MEALYBUG, *Phenacoccus manihoti* BY PARASITISM AND HOST-FEEDING

MUHAMMAD ZAINAL FANANI[1,*], AUNU RAUF[2], NINA MARYANA[2], ALI NURMANSYAH[2], DADAN HINDAYANA[2], NUR ROCHMAN[1]

[1]Universitas Djuanda, Jl Tol Ciawi No. 1, Bogor, Indonesia
[2]Institut Pertanian Bogor, Jl Cikamper, Dramaga, Bogor, Indonesia
*Corresponding Author: muhammad.zainal@unida.ac.id

Abstract

Anagyrus lopezi (De Santis) is an endo-specific parasitoid of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero. The objective of the study was to determine the efficiency and effectiveness of parasitoids by estimating the parameters of functional response. A fertile female of *A. lopezi* was tested on the 2nd and 3rd instar nymphs of *P. manihoti* with six groups of densities (2, 5, 10, 20, 50, and 100 mealybugs) in the laboratory. The estimation of the logistic regression analysis showed *A. lopezi* exhibited type II functional response on the 2nd or 3rd instar, both for parasitism and host-feeding. A random parasitoid equation model was used to fit the data. The search rate of *A. lopezi* (a) was 0.0332 and 0.0281 per hour on 2nd instar nymphs, 0.0565 and 0.0068 per hour on 3rd instar nymphs for parasitism and host-feeding, respectively. The estimated handling times (Th) on the 2nd instar were 1.9998 and 0.4557 h on the 2nd instar, and 1.205 and 4.1265 h on the 3rd instar mealybugs for parasitism and host-feeding, respectively. The parasitism of *A. lopezi* showed a significantly different in the 95% confidence interval for the parameter a and Th among 2nd-instar and 3rd-instar nymphs. Our study contributed to a better understanding insight of the host-parasitoid relationships and can be used for mass-rearing purposes than for *A. lopezi* mass-release programs under field conditions.

Keywords: Biological control, Cassava pest, Handling time, Host-density, Parasitoid insect.

1. Introduction

The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), is a native pest to South America, invaded the African continent widely, and became the most important pest insect on cassava in the early 1970s [1]. This pest spreads rapidly and widely in Asia. This pest relates to the people how to treat such as using chemicals or pesticides [2-7], while they can create issues in environment [8]. This pest poses a clear and present danger to food production for many poorest farmers in the world [9]. Based on Barilli et al. [10], *P. manihoti* finishes its life cycle in approximately 45 days, over than 80% of the eggs *P. manihoti* are viable, and its fecundity exceeds 240 eggs per female in every generation, allowing for a population increase significantly damaging the cassava crop. *P. manihoti* was first detected in Indonesia in 2010 and spread widely into several provinces [11].

Parasitoid *Anagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) is the most essential wasp of *P. manihoti*. In early 2014, *A. lopezi* was introduced to Indonesia to control *P. manihoti*. This biological control agent was successfully worked, established, and spread well through several Southeast Asia countries, including Indonesia [12]. It suppresses the mealybug populations and attains a level of 8-59% in different locations [9]. The maximum number of mealybugs parasitized by *A. lopezi* over a 24-h period was 20.2 mealybugs [13]. However, synorogenic parasitoid kills hosts through parasitism and host-feeding [14]. Therefore, it is important to study the efficiency and effectiveness of *A. lopezi* as a biocontrol tool for *P. manihoti*.

One way to assess the successful parameter of this parasitoid in suppressing *P. manihoti* can be determined using functional response studies. Three functional responses have described the arthropod biological agent to its target species. Type I shows that the proportion of parasitized hosts is constant or linear between host density and the number of parasitized hosts. The host density and attack efficiency in type II are curvilinear, whereas type III shows a sigmoid curve type [15]. The success of parasitoid insects is greatly influenced by their behavior and population, especially concerning the time of host handling. This is an important key to success in biological control.

The effectiveness of parasitoids depends on the ability to find and handle their hosts in various conditions such as temperature, humidity, rainfall, quality, quantity, and density of the host [16]. Host density is an important aspect that influences the high level of parasitism [17]. The efficiency of parasitoid wasps can be explained through the estimation value of functional response parameters [18]. Parameters used to evaluate the effectiveness of parasitoids are the instantaneous search rate (a) and handling time (*Th*) as measured by parasitoid or predator functional responses [15]. This study aimed to determine the type of functional response and its parameters and evaluate the parasitism and host-feeding pattern by *A. lopezi* on the 2nd and the 3rd instar nymphs of *P. manihoti* with different host densities.

2. Method

2.1. Host-plants, Mealybugs and parasitoids

Cassava cuttings of *Manihot esculenta* were collected from the field and maintained in plastic containers for the rearing of the mealybug [19]. Waterleaf, *Talinum* *triangular* was used as an alternate host plant for experiments. The rearing of insects was carried out in a laboratory. The leaves that were attacked by *P. manihoti* were cut into pieces and transferred to other cassava cuttings. Two weeks later, the 1st became the 2nd instar, and the 3rd instar nymphs were ready to be used as hosts for the culture of *A. lopezi* and experiments.

2.2. Functional response

A female parasitoid was exposed to the 2nd and 3rd instar *P. manihoti* with six different host densities (2, 5, 10, 20, 50, and 100) mealybugs. Leaf water was placed in a petri dish. A few drops of 10% honey solution were utilized for parasitoid feed. A ventilation hole of gauze with a diameter of 3 cm was made in each lid of a petri dish. Each parasitoid was fasted 24 hours before exposure and then exposed to the nymph of *P. manihoti* for 24 hours; after that, parasitoids were removed. The mealybug mortality by host feeding was observed for 7-8 days. The mealybug was killed by parasitism visually detected on the mealybug as mummies, while a sign for the hosts killed by host feeding was flattened, desiccated, and then drying [20].

2.3. Data analysis

The proportion of parasitized hosts vs the initial host number is the best effective way to identify the functional response [21]. In the first step, the polynomial function was used to find the relationship between *Na/No* and *No* in Eq. (1).

$$\frac{N_a}{N_0} = \frac{\exp\,(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + \exp\,(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)} \qquad (1)$$

where *P0*, *P1*, *P2*, and *P3* are respectively the constants for the parameters of intercept, and coefficients of linear, quadratic, and cubic are estimated through non-linear logistic regression. *Na* is the total number of parasitized or injured mealybug nymphs, and *N0* is the initial total number of mealybugs. These parameters were estimated using the PROC CATMOD SAS procedure in SAS 9.4 software.

The signs *P1* and *P2* were utilized to distinguish the type of functional response curve. The linear coefficient P1 with a negative value indicates the type II functional response and the proportion of hosts being parasitized decreased with an increase in the host density, whereas if the coefficient *P1* is positive and *P2* is negative indicates a type III functional response, the proportion of hosts being parasitized previously increases.

The cubic equation resulted in a non-significant cubic parameter (P3), the model was reduced by eliminating the cubic term from the equation, and then the other parameters were retested [18]. The Levenberg-Marquardt method of Curve Expert 1.4 was used to draw the functional response curve. The parameters of the handling time (T_h) and the instantaneous search rate (*a*) were estimated using the least square non-linear regression based on the parameters of Rogers [19] in Eq. (2).

$$N_a = N_0\left[1 - \exp\left(-\frac{aT}{1 + aT_hN_0}\right)\right] \qquad (2)$$

where *Na* is the total number of hosts killed due to host-feeding or parasitism, N_0 is host density, and *T* is the time duration of each experiment (24 hours).

3. Results and Discussion

Parasitoid *A. lopezi* showed parasitism behavior by laying its eggs at all stages (instar) of the mealybug (Fig. 1a), although instar-3 and adult *P. manihoti* were preferred as the primary host. This is due to the availability of adequate quality and quantity of host nutrition [16]. *A. lopezi* can also cause mortality in *P. manihoti* by host-feeding behavior, namely the symptoms of shrinkage on the body of the mealybug due to the insertion of the ovipositor *A. lopezi* followed by the activity of sucking fluid from the host's body by this parasitoid adult (Fig. 1b). The most host-feeding occurs in instar-1 nymphs *P. manihoti*. In addition to parasitizing the host, *A. lopezi* can also invade the host by taking about 6-22% of the host's body fluids (regarding host feeding behavior), then the parasitoids invade a further 11-34% with mechanical injuries caused by laying eggs by using the ovipositor [19].

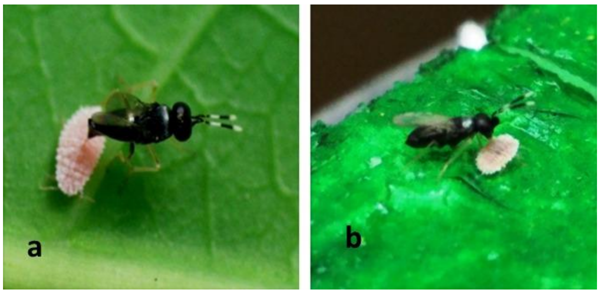

Fig. 1. Parasitism (a) and host-feeding (b) behavior on mealybug *P. manihoti.*

The functional response contains useful information for explaining the efficiency of parasitoids in suppressing insect pest populations [16]. Several studies reported that the female wasps not only become strongly synorogenic and destructive host feeders by parasitism but also co-exhibiting various host-killing behaviors by host-feeding [17]. The results of polynomial logistic regression analysis between the proportion of hosts parasitized (Na/No) and initial host density (No) provided positive quadratic coefficients linear and significant negative (Table 1), showing the significant difference of linear coefficient (P1) with the estimated value being negative by parasitism and host-feeding in difference instar of *P. manihoti* nymph (Table 1). These results indicate that *A. lopezi* exhibited a functional response type II. Thus, the functional response type is not affected by differences in host instar in both cases of parasitism and host feeding. Parasitoid functional responses can be influenced by the parasitoid's density and its hosts [16]. The functional response of *Anagyrus kamali* (Hym.: Encyrtidae) on *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) shows type II. In addition, this parasitoid was reported to be a successful biological control agent of *M. hirsutus* [21]. In general, type II functional responses show a decreasing pattern in the proportion of parasitism or host-feeding rate by parasitoids with increasing host density [15]. Therefore, *A. lopezi* can be more efficient in controlling *P. manihoti* at the low insect pest population in the field. The effort to release this parasitoid will be more effective and efficient at the initial time when the mealybug population is still low. The type II functional response possessed by *A. lopezi* illustrated the role of the parasitoid in suppressing the cassava pest to maintain the low pest population. This functional response model can be expected to be able to show that

A. lopezi is a promising biological agent to control *P. manihoti* remained the low population in the field.

The proportion of *P. manihoti* nymph was successfully killed by *A. lopezi* decreased sharply with increasing host density then the model approached at the end almost achieve the constant level, Figs. 2a-d showed that parasitism and host feeding increased with increasing host density until the parasitoid reaches its maximum reproductive capacity. But the activity of host-feeding by parasitoid on the 2nd instar nymph based on the predicted model (line in Fig. 2b) still showed a slight decrease even at the highest host density. This means that parasitoids prefer to feed on smaller hosts than bigger ones. Host-feeding by a parasitoid aims to obtain additional protein and increase egg production [17]. In contrast, a study found that *A. lopezi* prefers to paralyze its host in the 3rd instar nymph with a larger body size. Likewise, the opposite of the 2nd instar nymph with a smaller body size becomes preferable to be chosen by this parasitoid for host feeding. This is following the study of Adriani et al. [21] which stated that the parasitism of *A. lopezi* is more common in larger hosts while host-feeding is more common in smaller hosts. The results of NLIN regression showed that both parameters of the instantaneous searching rate (a) and handling time (*Th*) were significantly different in parasitism and host-feeding on the 2nd or 3rd instar nymph of *P. manihoti*, respectively (Table 2). The parameter a (searching time) indicated that the proportion of the total area was successfully explored per unit of time. Parameter a shows how quickly the functional response curve reaches the asymptote. It can describe the farthest distance of the parasitoids to detect the host, the movement speed of the parasitoids to the host, and the proportion of successfully parasitized hosts [22].

Table 1. Logistic regression of proportion of the 2nd and 3rd instar nymph *P. manihoti* in different densities killed by *A. lopezi* due to parasitism and host-feeding.

Host	Behavior	Coefficient	Estimated	SE	χ^2 value	P
2nd Instar	Parasitism	Constant	-0.392600	0.131000	8.9895	0.0027
		Linier	-0.025000	0.005910	17.9234	<0.0001
		Quadratic	-0.000063	0.000050	1.5558	0.2123
	Host-feeding	Constant	0.301700	0.226400	1.7756	0.1827
		Linier	-0.100600	0.024500	16.7884	<0.0001
		Quadratic	0.002550	0.000602	17.9397	<0.0001
		Cubic	-0.000020	3.80E-06	19.7110	<0.0001
3rd Instar	Parasitism	Constant	0.476200	0.123700	14.8224	0.0001
		Linier	-0.037500	0.005390	48.4191	<0.0001
		Quadratic	-0.000160	0.000045	12.6549	0.0004
	Host-feeding	Constant	-2.372100	0.368700	41.3924	<0.0001
		Linier	-0.011400	0.001890	36.4216	<0.0001
		Quadratic	-0.001570	0.000942	2.7643	0.0964

The searching rate (a) of *A. lopezi* on the 2nd instar nymph was 0.0332 and 0.0281, while on the 3rd instar nymph was 0.0565 and 0.0068 for parasitism and host-feeding, respectively. The value of handling time (*Th*) on the 2nd instar was

1.9998 and 0.4557, whereas on the 3rd instar was 1.2050 and 4.1265, for parasitism and host-feeding, respectively (Table 2). A theoretical maximum of 20.2 mealybugs could be parasitized by a single *A. lopezi* female within 24 h [23]. Based on the 95% confidence interval, the parameters of *Th* and *a* for host-feeding and parameter of *Th* for parasitism, the data observed was significantly different on both hosts of instar nymph *P. manihoti.* There was indicated no overlapping line among them in the predicted model. The difference in parameter values between this study and the other studies might have occurred due to the differences between the parasitoid species and its host being tested. The result of the study on the functional response of parasitoid *Eretmocerus delhiensis* Mani on *Trialeurodes vaporariorum* Westwood shows that the parameters *Th* and a resulting in parasitic behavior are significantly different at the level of 95% [24]. *A. lopezi* responded in a density-dependent manner that should contribute to the suppression of the pest populations before they reach economically damaging levels. Finally, the natural enemy is one of the main components of integrated pest control systems.

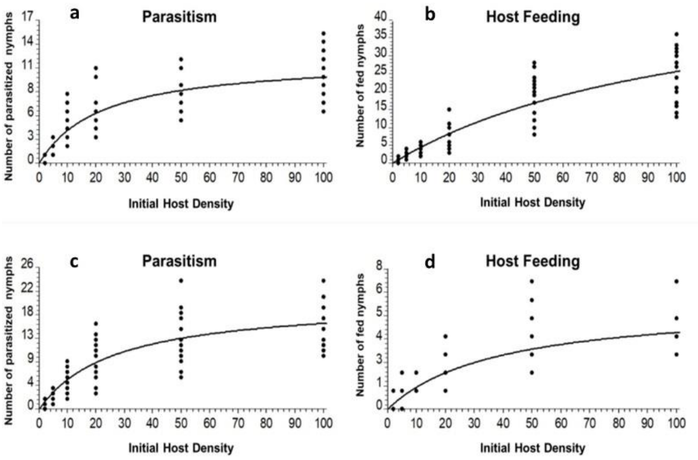

Fig. 2. Functional response *A. lopezi* on 2nd instar (a, b) and 3rd instar (c, d) nymphs of *P. manihoti* with different densities (symbols: parasitism or host-feeding observed, line: predicted by the model).

Table 2. The functional response of *A. lopezi* on the 2nd and 3rd instar nymphs of *P. manihoti* by parasitism and host-feeding.

Host stage	Behavior	Type	Parameter	Estimated	SE	Confident level 95%	
						Bottom	Upper
2nd Instar	Parasitism	II	A	0.0332	0.00653	0.0203	0.0462
			T_h	1.9998	0.1401	1.7223	2.2773
	Host-feeding	II	a	0.0281	0.00412	0.0199	0.0362
			T_h	0.4557	0.0699	0.3173	0.5940
3rd Instar	Parasitism	II	A	0.0565	0.0115	0.0337	0.0793
			T_h	1.205	0.0761	1.0543	1.3558
	Host-feeding	II	A	0.0068	0.00101	0.0048	0.0088
			T_h	4.1265	0.3883	3.3577	4.8954

4. Conclusion

The finding of the present study was *A. lopezi* showed higher parasitism on the big mealybug, whereas host-feeding was higher on the smaller host. *A. lopezi* exhibited Type II functional response, both for parasitism and host-feeding. Our study contributed to a better understanding insight of the host-parasitoid relationships and can be used for mass-rearing purposes and then for *A. lopezi* mass-release programs under field conditions.

Acknowledgments

We thank the General Directorate of Higher Education of the Republic of Indonesia through the PMDSU scholarship for funding this research.

References

1. Bellotti, A.; Herrera Campo, B.V.; and Hyman, G. (2012). Cassava production and pest management: present and potential threats in a changing environment. *Tropical Plant Biology*, 5(1), 39-72.
2. Latiza, J.A.Z., Pasawilan, A.C., Gacoscos, G., Bangeles, D.L., Caas, C.G., Valdez, A., and Abusama, H. (2022). Bignay (*Antidesma Bunius*) leaf extract stands as an organic pesticide against rice black bugs (*Scotinophara Coarctata*). *ASEAN Journal of Science and Engineering*, 2(1), 101-104.
3. Valdez, A., Abusama, H., Lapuz, N., Martinez, S., Pineda, S. and Tolentino, A. (2021). Insecticidal effects of blue ternate (*Clitoria ternatea*) leaf extract against mosquito wrigglers. *ASEAN Journal of Science and Engineering*, 1(3), 141-144.
4. Rosales, R. (2021). Hypocholesterolemic effect of mature leaf extract of sugarcane, *Saccharum officinarum* (Linnaeus, 1753), in induced rats. *ASEAN Journal of Science and Engineering*, 1(3), 199-206.
5. Magno, I.T., Esmail, R.P., Lim, M.S.S., Padua, J.N.J.D., Reyes, K.L., Tugom, L.A.G., Valdez, A.G., and Abusama, H.P. (2022). Antibacterial effect of calabash (*Crescentia cujete*) leaf and fruit extract on preservation of lettuce (lactuca sativa) leaves with escherichia coli. *ASEAN Journal of Science and Engineering*, 2(1), 91-94.
6. Sari, D.P., Yuniar, S., Fadillah, S.A.N., Mutiarani, A., and Kusumawaty, D. (2022). The effectiveness of mugwort leaf extract and gotu kola leaf extract against acne bacterial activity. *ASEAN Journal of Science and Engineering*, 2(3), 247-254.
7. Ebulue, M.M. (2023). Bioactive compounds and antioxidant activity of ethanol leaf extract of eucalyptus tereticornis. *ASEAN Journal of Science and Engineering*, 3*(*1), 69-78.
8. Altaf, R., Ullah, Z., Darko, D.A., Iqbal, A., Khan, M.S., and Asif, M. (2022). Molecularly imprinted polymers for the detection of chlorpyrifos (an organophosphate pesticide). *ASEAN Journal of Science and Engineering*, 2(3), 257-266.
9. Yonow, T.; Kriticos, D.J.; and Ota, N. (2017). The potential distribution of cassava mealybug (*Phenacoccus manihoti*), A threat to food security for the poor. *PLoS One*, 12(3), 1-16.

10. Barilli, D.R.; Pietrowski, V.; Wengrat, A.P.G.S; Gazola, D.; and Ringenberg, R. (2014). Biological characteristics of the cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae). *Revista Colombiana de Entomología*, 40(1), 21-24.
11. Fanani, M.Z.; Rauf, A.; Maryana, N.; Nurmansyah, A.; and Hindayana, D. (2019). Geographic distribution of the invasive mealybug *Phenacoccus manihoti* and its introduced parasitoid *Anagyrus lopezi* in parts of Indonesia. *Biodiversitas Journal of Biological Diversity*, 20(12), 3751-3757.
12. Wyckhuys, K.A.; Wongtiem, P.; Rauf, A.; Thancharoen, A.; Heimpel, G.E.; Le, N.T.,. and Neuenschwander, P. (2018). Continental-scale suppression of an invasive pest by a host-specific parasitoid underlines both environmental and economic benefits of arthropod biological control. *PeerJ*, 6, 1-14.
13. Fanani, M.Z.; Rauf, A.; Maryana, N.; Nurmansyah, A.; and Hindayana, D. (2020a). Parasitism disruption by ants of *Anagyrus lopezi* (Hymenoptera: Encyrtidae), parasitoid of cassava mealybug. *Biodiversitas Journal of Biological Diversity*, 21(6). 2337-2343.
14. Jervis, M.A.; and Kidd, N.A.C. (1986). Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, 61(4), 395-434.
15. Holling, C.S. (1959). Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, 91(7), 385-398.
16. Fernández-arhex, V.; and Corley, J.C. (2003). The functional response of parasitoids and its implications for biological control. *Biocontrol Science and Technology*, 13(4), 403-413.
17. Liu, W.X.; Wang, W.X.; Zhang, Y.B.; Wang, W.; Lu, S.L.; and Wan, F.H. (2015). Adult diet affects the life history and host-killing behavior of a host-feeding parasitoid. *Biological Control*, 81, 58-64.
18. Oaten, A.; and Murdoch, W.W. (1975). Functional response and stability in predator-prey systems. *The American Naturalist*, 109(967), 289-298.
19. Rogers, D. (1972). Random search and insect population models. *The Journal of Animal Ecology*, 41(2), 369-383.
20. Ebrahimifar, J.; Jamshidnia, A.; and Allahyari, H. (2017). Functional response of *Eretmocerus delhiensis* (Hymenoptera: Aphelinidae) on *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) by parasitism and host feeding. *Journal of Insect Science*, 17(2), 56, 1-5.
21. Adriani, E.; Rauf, A.; and Pudjianto, P. (2017). Laju enkapsulasi parasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) oleh kutu putih singkong *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae). *Indonesian Journal of Entomology*, 13(3), 147-155.
22. Allahyari, H.; Fard, P.A.; and Nozari, J. (2004). Effects of host on functional response of offspring in two populations of *Trissolcus grandis* on the sunn pest. *Journal of Applied Entomology*, 128(1), 39-43.
23. Fanani, M.Z.; Rauf, A.; Maryana, N.; Nurmansyah, A.; and Hindayana, D. (2020b). Parasitism of cassava mealybug by *Anagyrus lopezi* : Effects of varying host and parasitoid densities. *Biodiversitas Journal of Biological Diversity*, 21(10). 4973-4980.
24. Mills, N.J.; and Lacan, I. (2004). Ratio dependence in the functional response of insect parasitoids: evidence from *Trichogramma minutum* foraging for eggs in small host patches. *Ecological Entomology*, 29(2), 208-216.