Parasitism of cassava mealybug by *Anagyrus lopezi*: Effects of varying host and parasitoid densities

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Abstract. *Fanani MZ, Rauf A, Maryana N, Nurmansyah A, Hindayana D. 2020. Parasitism of cassava mealybug by* Anagyrus lopezi: *Effects of varying host and parasitoid densities. Biodiversitas 21: 4973-4980.* The solitary endoparasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) was introduced into Indonesia in 2014 to control the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae). The objectives of this study were to understand the influence of host and parasitoid densities on functional response and mutual interference of the parasitoid, by altering either the host or parasitoid density while keeping the other constant. The effects of host and parasitoid densities on parasitism, superparasitism, progeny production, and sex ratio were also assessed. Logistic regression was used to determine the shape of the functional response. Nonlinear least-squares regression was used to estimate the attack rate (a) and handling time (T_h). Nicholson's model and linear regression were used to determine per capita searching efficiency and interference coefficient, respectively. The logistic regression results suggested a Holling type II functional response to changing host densities, with an estimated attack rate (*a*) and handling time (T_h) were 0.037 h^{-1} and 1.19 h, respectively. The estimated maximum number of mealybugs parasitized over 24-h period was 20.2 mealybugs. The wasp-mealybug ratio and offspring production increased significantly from 1:2 to 1:100 and from 1.35 to 10.45, respectively. However, the per capita number of parasitization decreased significantly from 14.20 to 4.37, with the increase in parasitoid density from 1 to 8. Searching efficiency of the parasitoid decreased significantly with increasing parasitoid density, with a mutual interference constant (m) of -0.52.

Keywords: *Anagyrus lopezi,* functional response**,** mutual interference, parasitoid

INTRODUCTION

The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), is the most important insect pest of cassava in the world (Bellotti et al. 2012), causing a threat to the food and livelihood of poor farmers (Yonow et al. 2017). The pest is native to South America, but it was accidentally introduced into Africa in the early 1970s (Bellotti et al. 2012) and Asia in 2008 (Winotai et al. 2010; Parsa et al. 2012; Graziosi et al. 2015), including Indonesia (Muniappan et al. 2011). To control the pest, the host-specific solitary endoparasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) was introduced from Paraguay to Nigeria in 1981 (Bellotti et al. 2012), and subsequently to Thailand in 2009 (Winotai et al. 2010) and to Indonesia in 2014 (Wyckhuys et al. 2014). The introduction of this parasitoid into Africa reduced yield losses from as high as 80% to 5-10%, becoming a highly-successful case of classical biological control (Bellotti et al. 2012). A similar outcome was also reported from its introductions to Southeast Asian countries (Wyckhuys et al. 2018). The parasitoid successfully established and spread through several countries in Southeast Asia, suppressing mealybug populations and attaining a parasitism level of 10-57% (Le et al. 2018; Thancharoen et al. 2018; Wyckhuys et al. 2018; Fanani et al. 2019).

The success of a parasitoid as an efficient biocontrol agent depends on its density responsiveness to the target species, which is related to the searching efficiency of the parasitoid (Saini and Sharma 2018). One of the best ways to determine the searching abilities of a parasitoid is to study its functional response to the target species (Luo et al. 2014; Poncio et al. 2016). Functional response characterizes a relationship between the rate of attack by a single parasitoid and its host density. Thus the knowledge of functional response is important to understand the parasitoid regulatory effect on the host population (Feng et al. 2014; Luo et al. 2014; Saini and Sharma 2018). The functional response depends on handling time (T_h: the time that a parasitoid needs to parasitize a single host) and searching efficiency (a: the rate at which a parasitoid searches). Three basic types of functional responses have been described for arthropods. The type I response is a linear increase in host numbers attacked with an increase in host population density. The relationship between the attack efficiency and host population density in the type II response is curvilinear, and in type III takes the form of a sigmoid curve (Hassell 2000; Rosenbaum and Rall 2018; Tazerouni et al. 2019; Dunn and Hovel 2020).

Besides information on how individual parasitoids respond to host density, it is also important to know how they affect each other while searching for suitable hosts (Skovgard and Nachman 2015). Thus, when the number of conspecific females occurring in a patch with a fixed number of hosts increases, it is expected that the time each individual parasitoid spends searching on the patch will decline, thereby reducing their attack efficiency (Hassell 2000). Consequently, this will cause a reduction in the number of hosts parasitized per female parasitoid. Such density-dependent interactions and behavioral responses caused by interference among natural enemies of the same species leading to a reduction in the per capita attack rate are known as mutual interference (Hassell 2000).

While functional response describes the density responsiveness of the parasitoid to its host, mutual interference describes the extrinsic competition among the foraging conspecific female parasitoids (Saini and Sharma 2018). Studies of the functional response and mutual interference of various parasitoids have been reported, such as for *Dolichogenidea tasmanica* Cameron (Hymenoptera: Braconidae) attacking *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) (Yazdani and Keller 2015), *Goniozus nephantidis* Muesebeck (Hymenoptera: Bethylidae) attacking *Opisina arenosella* Walker (Lepidoptera: Oeccophoridae) (Sreenivas and Hardy 2016), *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) attacking *Stomoxys calcitrans* L. (Diptera: Muscidae) (Skovgard and Nachman 2015). With respect to *A. lopezi*, the functional response and mutual interference have not been studied. The objectives of this study were to investigate the functional response and mutual interference of *A. lopezi* by either altering the parasitoid or host density while maintaining the other constantly. The effect of parasitoid and host densities on progeny production, sex ratio, parasitism, and superparasitism of *A. lopezi* were also evaluated. The findings of the present study may provide a better understanding of host-parasitoid interactions to improve biological control of the cassava mealybug.

MATERIALS AND METHODS

Studies were conducted from August 2018 until January 2019 in the Laboratory of Insect Bionomy and Ecology, Faculty of Agriculture, IPB University. Experiments were carried out at 27 ± 2 °C, 60 ± 3% relative humidity (RH), and under a photoperiod of 12: 12 h (L:D).

Rearing of mealybugs and parasitoids

Anagyrus lopezi was reared on cassava plants, supporting the third instar population of *P. manihoti* as described by Fanani et al. (2020). Ten days after the release of the parasitoids, mummies were collected from the cassava plants and placed individually in gelatin capsules. Mummies were observed daily, and emerged parasitoids were used in experiments.

Effect of host density on parasitism, progeny, and sex ratio

Third instar mealybug nymphs at densities of 2, 5, 10, 20, 50, or 100 and placed on a leaf of waterleaf (*Talinum triangulare* (Jack.) Wild) were exposed to a single two-day-old mated *A. lopezi* female in a petri dish (d = 9 cm, h = 1.5 cm) and fed with a drop of 10% honey on the base of the petri dish. The parasitoids were allowed to forage and oviposit for 24 h, and then the adult parasitoids were removed. The exposed mealybugs were then transferred onto waterleaf cutting and placed into a cage made of a transparent plastic cylinder (d = 3 cm, h = 3.5 cm) with the top covered with nylon mesh. Seven days later, the number of mealybugs mummified (parasitized) were counted and collected. Each mummy was placed inside a gelatin capsule for adult emergence. The number of parasitoid adults emerged was recorded and separated based on sex. Each treatment was replicated 10 times. To determine the type of functional response, the data were fitted to the logistic regression as follows:

$$\frac{N_a}{N_0} = \frac{\exp{(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}}{1 + \exp{(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}}$$

Where N_a is the number of hosts parasitized, N_o is the initial host density, N_a/N_o is the proportion of the total mealybugs parasitized, and P_0, P_1, P_2, and P_3 are the parameters to be estimated. These parameters were calculated using the PROC CATMOD in SAS software (SAS Institute 2015). Where the cubic equation resulted in a nonsignificant cubic parameter (P_3), the model was reduced by eliminating the cubic term from the equation, and the other parameters were retested (Juliano 2001). A significant negative or positive linear coefficient (P_1) of the logistic regression model indicates type II or III of functional response, respectively.

Following this analysis and since our data fit a type II functional response (see Results and Discussion), we used a Holling's disc equation (Hassell 2000) as follows:

$$Na = aNoT/(1 + aT_h No)$$

Where Na is the number of hosts parasitized, No is the initial host density, a is a constant expressing attack efficiency, T is the duration of the experiment (i.e., 24 h), and T_h is the handling time (h) per host. The parameters a and T_h were estimated by non-linear regression using the Levenberg-Marquardt method of CurveExpert 1.4 (Hyams 2010). The effect of varying mealybug density on the number of parasitized hosts, the proportion of parasitized hosts per capita, number of offspring produced, and sex ratio was analyzed with ANOVA, and means were separated with Tukey tests using SAS 9.4 (SAS Institute 2015).

Effect of parasitoid density on their fecundity

Third instar mealybugs were collected from rearing cages and placed in groups of 120 on a leaf of waterleaf (*T. triangulare*). The leaf then was placed inside a petri dish (d = 9 cm, t = 1.5 cm). Parasitoid females aged two days old

with the treatment of densities (1, 2, 4, 6, or 8) were gently introduced into each petri dish and fed with a drop of 10% honey on the base of the petridish. The parasitoids were allowed to forage and oviposit for 24 h, and then the adult parasitoids were removed. Mealybugs were dissected on an object-glass in a drop of physiological solution of NaCl. The number of parasitoid eggs found in each host was recorded. Treatments in this experiment were replicated 10 times. The total number of eggs oviposited and the number of eggs oviposited per female, as well as the level of superparasitism, were analyzed with ANOVA, and means were separated with Tukey tests using SAS 9.4 (SAS Institute 2015).

Effect of parasitoid density on parasitism, progeny production and sex ratio

Petri dishes of 120 mealybugs and parasitoids were prepared as described above. Each group of the exposed mealybugs was transferred onto waterleaf cutting and placed into a cage made of a transparent plastic cylinder (d = 3 cm, h = 3.5 cm) with the top covered with nylon mesh. Ten days later, the number of mealybugs parasitized (mummies) were recorded. After two weeks, the cages were checked on a daily basis to collect and record progeny emergence and sex ratio (proportion of females). The total number of parasitized mealybugs and number of parasitized mealybugs per parasitoid were analyzed with ANOVA and means were separated with Tukey tests using SAS 9.4 (SAS Institute 2015).

The per capita searching efficiency (a) of the parasitoids at different parasitoid densities was estimated per replicate according to the Nicholson equation (Hassell 2000):

$$a = (1/PT) \ln [N_t/(N_t - N_a]$$

where P is the number of parasitoids, T is the duration of experiment (i.e. 24 h), N_t is the total number of mealybugs available (=120) and Na is the total number of mealybugs parasitized. Searching efficiency was fitted to linear regression by the least square method using the inductive model of Hassell and Varley (Hassell 2000):

$$a = QP^{-m} \text{ or } \log a = \log Q - m \log P$$

A is the searching efficiency of the parasitoid, Q is the quest constant (intercept of the regression line), and m is the mutual interference (slope of the regression line). Regression analysis was performed using MINITAB version 17.1.0 (Minitab 2013).

RESULTS AND DISCUSSION

Effect of host density on parasitism, progeny, and sex ratio

The total number of mealybugs parasitized by single *A. lopezi* females increased significantly (F $_{5, 114}$=18.46; P<0.001) from 1.35 to 15.80 mealybugs with an increasing host density from 2 to 100 mealybugs, respectively (Table 1). In contrast, the proportion of hosts parasitized decreased monotonically with the increase in host density (F $_{5, 114}$=4.94; P<0.001). The wasp-mealybug ratio and the offspring production increased significantly from 1:2 to 1:100 (F $_{5, 114}$=10.71; P<0.001) and from 1.35 up to 10.45, respectively. The sex ratios at different host densities did not differ significantly (F $_{5, 114}$=0.42; P=0.9916), but at the density of 100 nymphs, the sex ratio tended to be slightly female-biased (67%). Similar results of the effect of host density on parasitism, progeny, and sex ratio were reported for *Anagyrus kamali* Moursi parasitizing *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) (Sagarra et al. 2000a).

Further analysis using polynomial regression between initial host density (No) and the proportion of hosts parasitized (Na/No) provided significant negative linear and positive quadratic coefficients (Table 2), indicating a type II functional response of *A. lopezi* to third instar nymphs of *P. manihoti*. The functional response curve shows that parasitism increases with increasing host density until the parasitoid reaches its maximum reproductive capacity (Figure 1). By fitting the data to the Holling disc equation, the searching rate (a) and handling time (T_h) were 0.03 h^{-1} and 1.19 h, respectively. Handling time is defined as the time spent handling and parasitizing the host, and also the time spent cleaning and resting. Hence, the handling time is an important attribute for the reproductive success of a parasitoid (Hassell 2000; Beltra et al. 2015). The ratio of exposure time to handling time (T/T_h) is a theoretical maximum number of hosts parasitized per parasitoid female per unit of time. In our experiment, a single *A. lopezi* female could parasitize a theoretical maximum of 20.2 mealybugs within 24 h. This predicted value was close to the observed maximum value of 24 mealybugs per day.

The type II functional response is the characteristics of many parasitoids, though the type III functional response has also been reported (Fernandez-Arhex and Corley 2003; Tazerouni et al. 2019). The functional response of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) to different densities of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has been determined to be of type II (Feng et al. 2014; Joodaki et al. 2018). Also, type II functional response has been reported for other parasitoids, such as *Praon volucre* (Haliday) (Hymenoptera: Braconidae) attacking *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) (Farhad et al. 2011) and *Eretmocerus warrae* Naumann and Schmidt (Hymenoptera: Aphelenidae) attacking *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Hanan et al. 2017). Type III functional response has been reported for several parasitoids, such as *Coccidoxenoides perminutus* Girault attacking *Planococcus citri* (Risso) (de Menezes et al. 2017) and *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) attacking the potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae) (Yang et al. 2015).

Table 1. Mean (± SE) of the number and proportion of parasitized mealybugs, number, and sex ratio of progeny produced by *Anagyrus lopezi* at various host densities

Parasitoid density	Host density	Total number of parasitized mealybugs	Proportion of mealybugs parasitized	Number of progenies	Sex ratio (proportion of females)
1	2	1.35 ± 0.13a*	0.67 ± 0.06a	1.35 ± 0.13a	0.58 ± 0.09a
1	5	2.30 ± 0.24a	0.46 ± 0.04b	1.80 ± 0.19a	0.57 ± 0.09a
1	10	5.80 ± 0.50b	0.58 ± 0.05ab	4.50 ± 0.34b	0.58 ± 0.03a
1	20	9.00 ± 0.77c	0.45 ± 0.03b	6.70 ± 0.63c	0.58 ± 0.05a
1	50	13.35 ± 0.95d	0.26 ± 0.01c	8.00 ± 0.79c	0.62 ± 0.05a
1	100	15.80 ± 0.85d	0.15 ± 0.01c	10.45 ± 0.55d	0.67 ± 0.03a

Note: *Mean in a column with the same letters are not significantly different at $p \leq 0.05$

Table 2. Result of logistic regression analysis of the proportion of *Phenacoccus manihoti* third instars parasitized by *Anagyrus lopezi* to initial host numbers

Parameters	Estimate ±SE	χ^2	P
P_0 (Intercept)	0.6360 ± 0.1770	12.9241	0.0003
P_1 (Linear)	-0.0408 ± 0.0076	28.4141	<0.001
P_2 (Quadratic)	0.00018 ± 0.00006	7.7588	0.0053
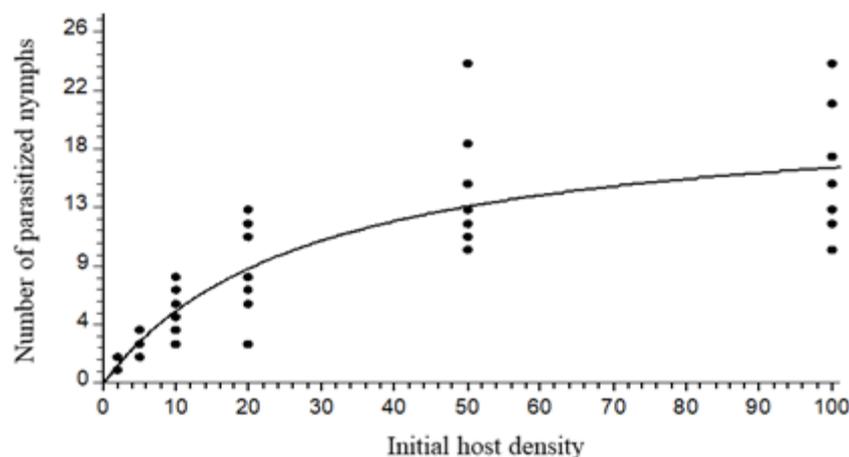
Figure 1. Functional response of *Anagyrus lopezi* to various host densities

Parasitoid with a type II functional response exerts an inverse, density-dependent action on the pest, whereas a parasitoid with a type III functional response acts in a positive, density-dependent manner. Therefore, a parasitoid with a type III functional response could be a better regulator agent than one having a type II functional response, which can destabilize the pest-natural enemy interaction (Dunn and Hovel 2020). The relative rarity of type III functional response may be an experimental artifact (van Lenteren and Bakker 1978). In natural field conditions, natural enemies can move freely to patches with high densities of hosts, but, in laboratory conditions, natural enemies are forced to remain in a patch for a fixed length of time; therefore, under laboratory conditions, the type III functional response is less common than the type II (He and Wang 2014). *Anagyrus kamali* exhibited type III functional response when the parasitoids were allowed to determine their residence in the experimental arenas

(Sagarra et al. 2000a). In contrast, the parasitoids enclosed within the arenas for the entire experimental duration showed a type II functional response. He and Wang (2014) demonstrated that parasitoid *Platygaster demades* Walker (Hymenoptera: Platygastridae) attacking *Dasineura mali* Kieffer (Diptera: Cecodomyiidae) displayed a type II functional response in the laboratory, but showed a type III in the field where females were able to freely search and disperse. The reports of type II functional response in *A. lopezi* and other parasitoid species (Farhad et al. 2011; Hanan et al. 2017) might be due to a restrictive experimental arena (van Lenteren and Bakker 1978). The form of functional response on its own does not determine the success or failure of parasitoids in biological control (Fernandez-Arhex and Corley 2003; Tazerouni et al. 2019). Other factors like a numerical response, intrinsic growth rates, host patchiness, competition, as well as abiotic and biotic factors such as temperature and host plant also affect

Effect of parasitoid density on fecundity, parasitism, and progeny production

The total number of eggs oviposited increased significantly (F $_{4, 45}$=92.49; P<0.001) from the female density of one to eight (Table 3). At a density of eight female parasitoids, an average of 49.30 ± 2.24 eggs was laid by the eight parasitoids. This was significantly greater than the total oviposition at the density of six females (average of 37.20 ± 0.82 eggs), which was significantly greater than oviposition at the density of four females (average of 25.70 ± 1.78 eggs). At the density of one female, an average of 14.30 ± 1.15 eggs was oviposited over a 24 h period. This was not significantly different from the total number of eggs oviposited by two females, but it was significantly less than oviposition at the densities of 4, 6, and 8 parasitoids. The number of eggs oviposited per female *A. lopezi* differed significantly (F$_{4, 45}$=36.19; P<0.001) among densities. A decrease in the oviposition rate per female parasitoid occurred with the increase in female density, dropping from 14.30 ± 1.16 eggs/female at the density of one female to 6.42 ± 0.44 eggs/female at the density of four females. Sagarra et al. (2000b) reported similarly that fewer eggs per female were laid by *Anagyrus kamali* Moursi as the number of wasps increased in a patch. The number of eggs oviposited per parasitized mealybug was also significantly (F$_{4, 45}$=13.15; P<0.001) affected by female densities. The number of eggs oviposited per parasitized mealybug was not significantly different between densities of one and two parasitoids, which were 1.05 ± 0.02 and 1.04 ± 0.01, respectively. However, at the density of 4, 6, and 8 female parasitoids, the number of eggs per parasitized mealybug was significantly higher than at densities of 1 to 2, which were 1.27 ± 0.07, 1.26 ± 0.04, and 1.37 ± 0.03, respectively. Female parasitoid density can also affect the timing of parasitism and development duration of progeny as reported for *Sclerodermus pupariae* Yang et Yao (Hymenoptera: Bethylidae), a parasitoid of *Agrilus planipennis* Fairmaire (Coleoptera: Cerambycidae) (Gao et al. 2016).

Intraspecific competition between parasitoids can occur at the larval stage or the adult stage with different consequences for the host-parasitoid interaction. Since *A. lopezi* is a solitary endoparasitoid, competition between larvae in superparasitized hosts results in all but one dying, and thus high levels of superparasitism would be a disadvantage for mass production of the parasitoid (Lou et al. 2014). In our study, superparasitism of *A. lopezi* increased significantly (F$_{4, 45}$=18.89; P<0.001) with the increasing density of conspecific parasitoids. Single parasitoid female caused on average 5.34 % superparasitism, whereas at densities of eight females, superparasitism was significantly (F $_{4, 45}$=18.80; P<0.001) higher (33.73%). Superparasitism occurred regardless of host density, as also reported for *A. kamali* (Sagarra et al. 2000a). Female wasps have a propensity to superparasitize their hosts as the average number of eggs per parasitized

mealybug was greater than one egg (Table 3, column 5), as previously reported by Adriani et al. (2016). Suma et al. (2012) also found that *Anagyrus vladimiri* (Triapitsyn) (=*Anagyrus* sp. nr. *pseudococci*) oviposited on average more than one egg per parasitized mealybug. They found an apparent relationship between the number of eggs laid by the parasitoid and the level of resistance of the host mealybugs, as revealed by the number of encapsulated eggs. This supports the hypothesis that superparasitism might be used by the parasitoid as a strategy for overcoming host immune response (encapsulation) (Suma et al 2012; Luna et al. 2016).

Intraspecific competition among searching wasps can lead to a direct density-dependent reduction in parasitism (Luo et al. 2014; Eliopoulos et al. 2017). The results of the present study show that parasitism by single female parasitoids decreased with increasing parasitoid density (Table 4). The average number of mealybugs parasitized per individual *A. lopezi* female decreased significantly (F $_{4, 45}$=20.32; P<0.001) from 14.20 to 4.37 when parasitoid densities increased from 1 to 8, respectively. Such reduction in the number of mealybugs parasitized is indicative of mutual interference among foraging parasitoids in the same arena (Feng et al. 2014; Lin et al. 2018). Accordingly, the per capita searching efficiency of the parasitoid also reduced significantly (F $_{4, 45}$ =14.40; P<0.001) from 0.0053 to 0.0019 with the increase in parasitoid density from 1 to 8. By fitting data to the equation log a = logQ - mlogP), the searching efficiency of the parasitoid (a) was negatively related to parasitoid density (P) as log a = - 2.3349-0.5208 log P. The mutual interference coefficient (slope of the regression line) was estimated to be m = -0.52. The negative value of the regression slope indicates an inverse relationship between parasitoid density and per capita searching efficiency, or searching efficiency decreases with increasing parasitoid density. Mutual interference appears when competition for a common resource leads to a decrease in searching efficiency of the individual parasitoid (Hassell 2000; Skovgard and Nachman 2015; Yazdani and Keller 2015). Iranipour et al. (2020) reported a decrease in searching rate of parasitoid *Trissolcus vassilievi* (Mayr) (Hymenoptera: Platygastridae) and a 2-fold increase in host survival of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) at higher wasp densities.

Increasing conspecific numbers had a significant influence on the reproduction patterns of *A. lopezi*. Progeny production increased significantly (F $_{4, 45}$=10.78; P<0.001) from the female density of 1 to 8. The progeny from the 6 and 8 females parasitoids were about 3-4 higher than those from 1 female (Table 5), suggesting a marginal decrease in the efficiency of the individual parasitoid at high density. Due to high mutual interference at higher parasitoid densities, the progeny per female decreased with increasing parasitoid density. Repeated puncturing of hosts might induce more mortality in the mealybug population, causing a decrease in the progeny emergence as also reported for *A. kamali* (Sagarra et al. 2000b). Parasitoids foraging alone produced an average progeny of 6.41, whereas parasitoids foraging in groups of 8 produced only 3.05. The sex ratio

of progenies was not significantly different (F $_{4, 45}$=1.21; P=0.321) across female densities, varying from 0.75 ± 0.04 (density = one parasitoid) to 0.57 ± 0.01 (density = 8 parasitoids). The stability of the sex ratio, regardless of the female wasp density, was reported previously by van Dijken et al. (1989), who concluded that local mate competition (LMC) does not occur in *A. lopezi*. Sagarra et al. (2000b) reported a similar phenomenon for *A. kamali*.

Table 3. Mean (± SE) of the number of total eggs oviposited, eggs oviposited by each female, eggs per parasitized mealybug and rate of superparasitism at various densities of *Anagyrus lopezi*

Parasitoid density	Host density	Total number of eggs oviposited	Number of eggs oviposited per parasitoid	Number of eggs per parasitized mealybug	Rate of superparasitism (%)
1	120	14.30 ± 1.15a*	14.30 ± 1.16a	1.05± 0.02a	5.34± 2.30a
2	120	19.70 ± 0.66a	9.85 ± 0.33b	1.04 ±0.01a	5.73±1.75a
4	120	25.70 ± 1.78b	6.42 ± 0.44c	1.27 ±0.07b	22.48± 4.08b
6	120	37.20 ± 0.82c	6.20 ± 0.13c	1.26± 0.04b	21.12± 3.06b
8	120	49.30 ± 2.24d	6.16 ± 0.28c	1.37 ±0.03b	33.73±2.15 c

Note: *Mean in a column with the same letters are not significantly different at p ≤ 0.05

Table 4. Mean (± SE) of the number of total parasitized mealybugs, mealybugs parasitized per wasp, and searching efficiency of *Anagyrus lopezi* female at various wasp densities

Parasitoid density	Host density	Total number of parasitized mealybugs	Number of parasitized mealybugs per parasitoid	Searching efficiency per parasitoid
1	120	14.20 ± 1.06a	14.20 ± 1.06a	0.0053 ± 0.0006a
2	120	17.40 ± 1.50a	8.70 ± 0.75b	0.0033 ± 0.0004b
4	120	20.70 ± 1.28ab	5.17 ± 0.32bc	0.0021 ± 0.0006b
6	120	30.40 ± 2.16bc	5.06 ± 0.36c	0.0020 ± 0.0008b
8	120	35.01 ± 3.05c	4.37 ± 0.38c	0.0019 ± 0.0009b

Note: *Mean in a column with the same letters are not significantly different at p ≤ 0.05
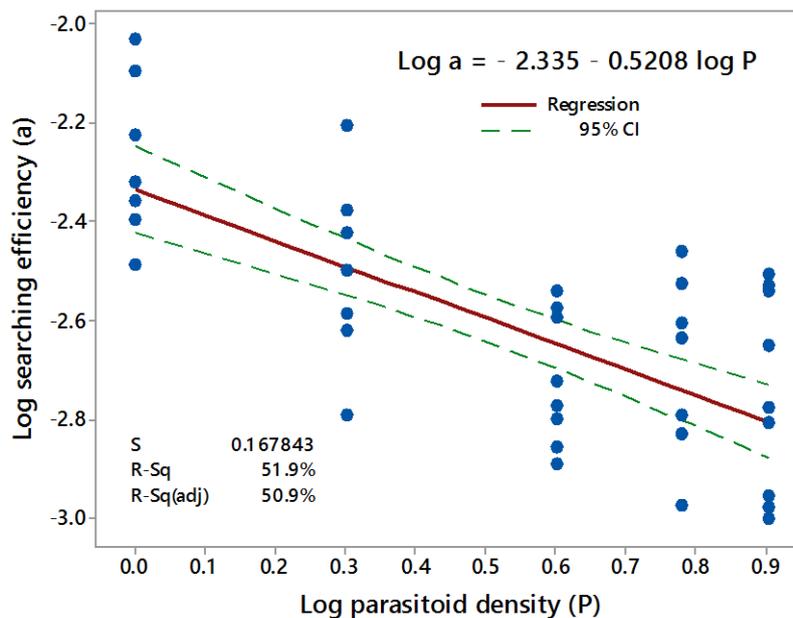

Figure 2. Mutual interference among foraging *Anagyrus lopezi* females

Table 5. Mean (± SE) of the number offspring and sex ratio of *Anagyrus lopezi* at various wasp densities

Parasitoid density	Host density	Total number of progenies	Number of progenies per parasitoid	Sex ratio (proportion of females)
1	120	6.40 ± 0.91a*	6.41 ± 0.91a	0.75 ± 0.04a
2	120	9.40 ± 1.75a	4.70 ± 0.87a	0.74 ± 0.04a
4	120	12.20 ± 1.08ab	3.86 ± 0.27a	0.69 ± 0.04a
6	120	23.20 ± 1.85bc	3.38 ± 0.01a	0.67 ± 0.04a
8	120	27.10 ± 3.20c	3.05 ± 0.40a	0.57 ± 0.01a

Note: *Mean in a column with the same letters are not significantly different at p ≤ 0.05

The results of our study indicate that there are mutual interferences when two or more *A. lopezi* females forage in the same patch. As the density of parasitoids increases in an arena, the searching female spends more time interacting with the conspecific than searching the host (Hassell 2000; Henne and Johnson 2010; Saini and Sharma 2018). Intraspecific competition among the foraging parasitoids can reduce the parasitization level and, ultimately, the efficacy of the given parasitoids (Lou et al. 2014; Poncio et al. 2016; Eliopoulos et al. 2017). Our study reveals that the searching efficiency of *A. lopezi* decreased significantly with the increase in parasitoid density, and the competition was higher at higher parasitoid densities. The negative effect of mutual interference on the progeny production of the parasitoid would undermine the efficiency of the mass-rearing system of *A. lopezi* in the laboratory. In cassava fields, the variation in host density among patches is much more variable than in laboratory arenas. This is because natural systems include more patches, hosts are present at various developmental stages, and the profitability of patches varies with both in space and in time (Yazdani and Keller 2015). The wasps would have the chance to disperse into rewarding patches and result in a more homogenous distribution among patches, which subsequently affects interference among parasitoids (Okuyama 2016; Iranipour et al. 2020). We suspect that interference would not have a pronounced effect on the efficiency of *A. lopezi* in the fields. DeLong and Vasseur (2011) suggested that mutual interference is common and mostly intermediate in magnitude.

In conclusion, this study has provided important insights into *A. lopezi* functional response and mutual interference that is unknown until now. Under laboratory conditions, *A. lopezi* showed a type II functional response to increasing host density. The number of parasitized nymph and offspring per capita females *A. lopezi* decreased with an increasing parasitoid density. Consequently, higher parasitoid densities should be avoided when mass producing *A. lopezi*.

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