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Effects of nitrogen fertilization on the biology of the cassava pink mealybug *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae)

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Abstract
The effects of nitrogen fertilization on the biology of the cassava pink mealybug, *Phenacoccus manihoti* Matile-Ferrero, were investigated in the laboratory. The experiment was carried out on cassava potted plants at three nitrogen fertilization rates: 0, 90 and 180 kg N ha^{-1}. The results showed that mealybugs grown on cassava plants receiving the highest nitrogen fertilization rate had a significantly shorter development time, longer longevity and higher fecundity than mealybugs in the other treatments. The survival rate of the total nymphal stages was not significantly different among nitrogen fertilization treatments. The mealybugs that fed on plants fertilized at the highest nitrogen fertilization level had highest body weight and size than mealybugs in the other treatments. The net reproduction (*Ro*), generation time (*T*) and intrinsic rate of natural increase (r_m) of mealybugs feeding on plants treated with the highest nitrogen dose were higher than those for mealybugs in the other treatments. Thus, an appropriate nitrogen fertilization level for minimum mealybug damage should be applied since increasing the nitrogen application level leads to an increase in the *P. manohoti* population on cassava.

Keywords: cassava; intrinsic rate of increase; life cycle; net reproduction; nitrogen fertilizers

1. Introduction
The cassava pink mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), a native of South America, is one of the most serious cassava pests, *Manihot esculenta* Crantz, in the world (Herren, 1987; Bellotti *et al.*, 1999) [14, 3]. It possesses a piercing-sucking mouth-part that removes plant fluids, causing severe distortion of terminal shoots, yellowing and curling of leaves, reduced internodes, stunting, and weakening of stems used for crop propagation (Parsa *et al.*, 2012) [22].

Sap-feeding insects (e.g., mealybugs) show a strong response to nitrogen levels in their host plants (Rae and Jones, 1992; Hogendrop *et al.*, 2015) [24, 15] due to the scarcity of nitrogenous compounds in plant tissues, especially in phloem sap (Mattson, 1980) [20]. Nitrogen is absorbed by the plant and is used to synthesize amino acids, proteins and other complex nitrogenous compounds (Singh and Sood, 2017) [28]. An appropriate supply of nitrogen is associated with high photosynthetic activity, vigorous vegetative growth and dark leaf colour (Zafar *et al.*, 2010) [31] as well as with the foliar nitrogen level (Schutz *et al.*, 2008) [27]. The nitrogen level in the diet of herbivorous insects is the most important factor affecting their performance (Awmack and Leather, 2002). Nitrogen deficiency has been shown to lower the population growth of herbivorous insects, and increased nitrogen application to crop plants has a positive effect on the growth and fecundity of the grain aphid, *Sitobion avenae* (F.), and the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) (Aqueel and Leather, 2011) [1]. These effects were also observed in the citrus mealybug, *Planococcus citri* Risso, and the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Hogendorp *et al.*, 2006; Cocco *et al.*, 2015) [15, 8]. Although it is well know that increased fertilizer application to crops affect the performance of herbivores, the influence of varying nitrogen fertilizer levels on cassava plants on the cassava pink mealybug is not well understood. The objective of this study was to determine the effect of different nitrogen fertilization levels on the development, survival and population growth of *P. manihoti*.

2. Materials and Methods
2.1. Plant cultivation and insect rearing
Cassava plants and *P. manihoti* were cultivated in the same manner as described by Wykhuys *et al.* (2017) [30]. A single vegetative cutting (approx. 20 cm in length) of cassava (variety KM94, a commonly used variety in Vietnam) was planted in a pot (30 cm diameter x 20 cm deep) in approximately 10 kg of a sandy loam soil with pH = 5.2, an organic carbon content of 1.5%, and available concentrations of K_2O, N and P_2O_5 of 4.59 mg, 0.65 mg and 10.5 mg, respectively, per 100 g of soil. After two weeks, plants were randomly assigned to one of three fertilizer treatments: 1) no nitrogen fertilizer (0N), 2) low N addition (90N, 90 kg N ha^{-1}) and 3) high N addition (180N, 180 kg N ha^{-1}). This was equivalent to application rate of 0, 0.65 and 1.30 g N per pot, respectively for no, low and high nitrogen addition). Nitrogen (as urea) was dissolved in water and applied in liquid form. The potted plants were kept in the net house. After six weeks, the plants were moved into a climate-controlled chamber (60-70% humidity, 30 ± 1.0°C and 12 L: 12 D photoperiod).

Phenacoccus manihoti used for the present study were collected by removing ovisacs from from a cassava field in Quang Tri Province, Central Vietnam, and reared on potted cassava plants with a diluted fertilizer solution inside a cage (60 × 160 × 180 cm). Three sub-colonies of *P. manihoti* were established in a climate-controlled chamber (30 ± 1°C and 12 L:12 D photoperiod) on plants subjected to each of

the above three experimental nitrogen fertilizer treatments. The sub-colonies were maintained on these plants for two to three generations prior to their use in experiments.

2.2. Immature development and mortality

The development of *P. manihoti* reared on cassava plants under different nitrogen fertilization conditions was investigated. One newly laid ovisac was collected from each sub-colony of *P. manihoti* and transferred to a plastic clip-cage (5 × 10 × 20 cm) covered with a fine nylon mesh on the third-youngest leaf of cassava plants grown in the same fertilizer treatment as the sub-colony. After hatching, only 10 first-instar nymphs were maintained in the clip-cage. Ten plants were used for each treatment. All plants with the clip-cages were maintained in a climate-controlled chamber (60–70% humidity, 30 ± 1.0°C and 12 L:12 D photoperiod) until adult emergence. The moulting and survival of the mealybug were recorded daily to determine development time and mortality.

2.3. Adult weight, size, longevity and fecundity

The weight of a batch of 10 newly emerged adults was recorded. A total of 50 adults were weighed for each treatment. The length and width of 50 newly emerged adults per each treatment were measured under a binocular microscope.

Ten newly emerged adults were collected from the sub-colonies and were placed in a clip-cage on the third-youngest leaf of cassava plants with the same fertilizer treatment as the sub-colony. Ten plants were used for each treatment. All plants with the clip-cages were maintained in a climate-controlled chamber (60-70% humidity, 30 ± 1.0°C and 12 L:12 D photoperiod). The reproduction and mortality of females were recorded daily until all adults died. The fecundity of each adult was recorded daily by removing newly laid ovisacs from the clip-cage and counting the number of eggs under a stereomicroscope.

2.4. The rate of population increase

The net reproduction rate (*Ro*), mean generation time (T) and intrinsic rate of increase (r_m) were calculated according to the equations given by Birch (1948) [5],

$Ro = \sum l_x m_x$; $T = \sum x l_x m_x \,/\, \sum l_x m_x$; $\sum \left(\exp(-r_m x) l_x m_x\right) = 1$; where x is female age, l_x is the proportion of females surviving to age x, and m_x is the expected number of daughters produced per female alive at age x.

Statistical analysis

All statistical procedures were carried out using StatView (SAS Institute, 1998) [26]. The effects of fertilizer treatment on immature developmental time, adult size and weight, fecundity and longevity were analysed using Tukey's HSD test after one-way ANOVA. Kaplan-Meier survival analyses were used with the log-rank test.

3. Results

3.1. Effects of nitrogen fertilization on mealybug development and survivorship

The development time of *P. manihoti* was significantly affected by the levels of nitrogen fertilization. The mealybugs feeding on nitrogen fertilized plants developed more rapidly than those reared on the untreated control (Table 1). The durations of the first instar and second instar stages were the shortest on plants treated with the highest nitrogen level (180N). The third instar stage was the longest on untreated plants (0N), and no significant difference in developmental stage lengths was observed between 90N and 180N. The total development time from first instar to adult emergence of mealybugs fed on plants treated with the highest nitrogen level (180N) was shorter (14.7 days) than that on plants fertilized at lower nitrogen levels (F=50.12; df=2,219; P<0.0001) (Table 1). The survival rate of the total nymphal stages was not significantly different among nitrogen fertilization treatments (χ^2= 0.122; df=2; P=0.941).

3.2. Effects of nitrogen fertilization on mealybug adult weight, size, longevity and fecundity

The nitrogen fertilization level had a significant effect on both the adult weight and size of *P. manihoti*. The mealybugs fed on plants fertilized with the highest nitrogen fertilization levels had higher body weight (6.96 mg) than those fed on the lower nitrogen (6.50 mg) and control plants (5.86 mg) (F=71.81; df=2,147; P<0.001). Low nitrogen fertilization had a negative effect on both insect length and width (Table 2).

The mean longevity of mealybugs feeding on 180N plants was longer than that in mealybugs feeding on untreated control plants (F=3.78; df=2,199; P=0.0256) (Table 3). The longevities were 22.5, 22.8 and 26.0 days for the 0, 90 and 180N nitrogen treatments, respectively. There was no significant difference between the mean longevities of mealybugs feeding on the plants treated with 0N and 90N (P>0.05).

The level of nitrogen fertilization had a significant effect on *P. manihoti* reproduction. The mealybugs that fed on plants treated with 180N had both the longest oviposition time (F=4.42; df=2,119; P=0,0141*)* and the largest fecundity (F=14.97; df=2,119; P<0.001*)*. The fecundities were 295.6, 373.6 and 454.6 eggs at the 0, 90 and 180N fertilization levels, respectively. There was no significant difference between the mean oviposition rates of mealybugs feeding on the plants treated with 90N and 180N (P>0.05). The pre-oviposition period did not vary among treatments (P>0.05) (Table 3).

3.3. Effects of nitrogen fertilization on mealybug population growth rate

Nitrogen application had a positive impact on the population increase of *P. manihoti*. There were differences in the net reproduction (*Ro*), generation time (*T*) and intrinsic rate of increase (r_m) of mealybugs feeding on plants treated with different nitrogen doses. The mean net reproductive rates were 314.490, 319.545 and 388.885 at the nitrogen fertilization levels of 0N, 90N and 180N, respectively. The mean generation times were 12.227, 13.353 and 13.276 days at the nitrogen fertilization levels of 0N, 90N and 180N, respectively. The intrinsic rates of increase were 0.665, 0.713 and 0.734 ♀/♀/day at the nitrogen fertilization levels of 0N, 90N and 180N, respectively (Table 4).

Table 1: Mean (± SE) developmental times (days) of *Phenacoccus manihoti* reared on cassava plants fertilized with different nitrogen levels

Developmental stage (days)	Nitrogen level (kg N ha^{-1})			ANOVA parameters		
	0N	90N	180N	*F*	*df*	*P*
First instar	6.1 ± 0.04 a	5.6 ± 0.08 b	5.3 ± 0.08 c	31.67	2,219	<0.001
Second instar	5.0 ± 0.08 a	4.7 ± 0.08 b	4.4 ± 0.07 c	11.96	2,219	<0.001
Third instar	5.5 ± 0.07 a	5.0 ± 0.07 b	5.0 ± 0.08 b	13.55	2,219	<0.001
First instar - adult	16.5 ± 0.12 a	15.3 ± 0.14 b	14.7 ± 0.13 c	50.12	2,219	<0.001
N	74	76	72			

Means with the same letters within the same stage are not significantly different by Tukey's HSD test after one-way ANOVA, $P<0.05$.
N is the number of tested individuals developing until adult emergence

Table 2: Mean (± SE) weight and size of *Phenacoccus manihoti* adults reared on cassava plants fertilized with different nitrogen levels

Parameter		Nitrogen level (kg N ha^{-1})			ANOVA parameters		
		0N	90N	180N	*F*	*df*	*P*
Weight (mg/10 adults)		5.86 ± 0.23 c	6.50 ± 0.25 b	6.96 ± 0.19 a	71.81	2,147	<0.001
Size (mm)	Length	1.91 ± 0.03 c	2.16 ± 0.02 b	2.23 ± 0.03 a	43.94	2,147	<0.001
	Width	0.99 ± 0.01 b	1.12 ± 0.03 a	1.23 ± 0.03 a	34.27	2,147	<0.001

Means with the same letters within the same stage are not significantly different by Tukey's HSD test after one-way ANOVA, $P<0.05$.

Table 3: Mean (± SE) longevity and fecundity of *Phenacoccus manihoti* reared on cassava plants fertilized with different nitrogen levels

Parameter	Nitrogen level (kg N ha^{-1})			ANOVA parameters		
	0N	90N	180N	*F*	*df*	*P*
Longevity (days)	22.5 ± 0.83 b	22.8 ± 1.09 ab	26.0 ± 0.92 a	3.78	2,119	0.0256
Pre-oviposition time (days)	6.5 ± 0.25 a	6.5 ± 0.20 a	6.4 ± 0.20 a	0.14	2,119	0.8696
Oviposition time (days)	16.0 ± 0.79 b	16.4 ± 1.02 b	19.6 ± 0.92 a	4.42	2,119	0.0141
Fecundity (no. eggs)	295.6 ± 17.78 c	373.6 ± 20.75 b	454.6 ± 20.58 a	14.97	2,119	<0.001
Oviposition rate (no. eggs/day)	18.2 ± 0.66 b	23.7 ± 0.60 a	23.8 ± 0.70 a	23.27	2,119	<0.001
Post-oviposition (days)	0.0	0.0	0.0	-	-	-
N	37	45	40			

Means with the same letters within the same stage are not significantly different by Tukey's HSD test after one-way ANOVA, $P<0.05$.
N is the number of tested individuals

Table 4: Population growth parameters of *Phenacoccus manihoti* reared on cassava plants at different nitrogen fertilization levels

Fertilization level (kg N ha^{-1})	Net reproduction (*Ro*) (♀/♀)	Generation time (*T*) (days)	Intrinsic rate of increase (r_m) (♀/♀/day)
0N	314.490	12.227	0.665
90N	319.545	12.353	0.713
180N	388.885	13.276	0.734

4. Discussion

The nutritional content of the host plant has an important influence on the growth and reproduction of herbivores (Dixon, 1977) [9]. Nitrogen is a constituent of amino acids, proteins and chlorophyll. It is a critical nutrient for plants and thus in turn is critical for phytophagous insects. Applying high levels of nitrogen to plants changes plant biochemistry, leading to a high incidence of herbivorous insects, especially hemipterans (Jansson *et al.*, 1991; Bi *et al.*, 2001) [18, 4]. Increasing nitrogen fertilization was reported to enhance the growth and reproduction of many sap-sucking insects, such as *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae) (Bi *et al.*, 2001) [4], *P. citri* and *P. ficus* (Hogendrop *et al.*, 2006; Cocco *et al.*, 2015) [15, 8], *S. avenae*, *R. padi*, and *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) (Aqueel and Leather, 2011; Lama *et al.*, 2019) [1, 19]. The results of our study also provide evidence that increased nitrogen fertilization of cassava plants has a positive effect on the growth and fecundity of *P. manihoti*.

Our study showed that higher nitrogen fertilizer levels resulted in a shorter developmental time in *P. manihoti* (Table 1). This result is consistent with previous studies on *P. manihoti* (Wyckyus *et al.*, 2017) [30] and other hemipterans such as *P. citri* and *P. ficus* (Hogendrops *et al.*, 2006; Cocco *et al*,, 2015) [15, 8], *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) (Wang *et al.*, 2006) [29], *M. sacchari* (Lama *et al.*, 2019) [19], *S. avenae* and *R. padi* (Aqueel and Leather, 2011) [1], and *Aphis gossypii* Glover (Hemiptera: Aphididae) (Hosseini *et al.*, 2010) [17]. However, a previous study reported that there was no significant effect of nitrogen fertilization on the developmental time of *Stephanitis pyrioides* (Scott) (Hemiptera: Tingidae) (Casey and Raupp, 1999) [7]. On the other hand, Pfeiffer and Burts (1983) [23] reported that the developmental time of *Cacopsylla pyricola* (Foerster) (Hemiptera: Psyllidae) decreased initially and then increased as the nitrogen fertilization rate increased.

Some studies have shown that insect survival is higher when insects feed on plants fertilized at moderate nitrogen levels, rather than at higher nitrogen fertilization rates (Rae and Jones, 1992; Gash, 2012) [24, 12]. Meanwhile, Cocco *et al.* (2015) [8] reported that the vine mealybug, *P. ficus*, exhibited higher survival on plants supplied with higher nitrogen fertilization rates. However, in our study, the nymphal survival of *P. manihoti* was not affected by nitrogen

fertilization, in accordance with the findings of Wyckuys *et al.* (2017) [30], who also found no significant effect of nitrogen fertilization on the immature survival of *P. manihoti*, and Hosseini *et al.* (2010) [17], who found that the survivorship of juvenile *A. gossypii* on cucumber was not affected by nitrogen fertilization.

There was also a significant difference in the adult body size of *P. manihoti* at various nitrogen fertilization levels, with the highest weight (6.96 mg/10 adults) in the 180N treatment. Similarly, Rae and Jones (1992) [24] reported that the body sizes of sugarcane mealybugs, *Saccharicoccus sacchari* (Cockerell) (Hemiptera: Pseudococcidae), reared on plants supplied with higher nitrogen regimes were significantly larger. Wang *et al*. (2006) [29] also indicated that *P. maidis* feeding on leaf tissue with higher nitrogen levels had heavier body weights. Adult size is one of the most frequently used indicators of insect fitness. It has been shown that female fitness increased with body size. Larger individuals have higher potential fecundity and greater longevity (Honěk, 1993; Ellers *et al.,* 1998) [16, 10].

In our study, we found that nitrogen fertilization resulted in significantly longer adult longevity and oviposition periods. This finding is in agreement with the results reported by other authors for the oviposition period of adult *P. manihoti* (Wyckuys *et al.,* 2017) [30] and for the longevity of other insects, such as *P. maidis* on corn (Wang *et al.,* 2006) [29], *A. gossypii* on cucumber (Hosseini *et al.,* 2010) [17], and *S. avenae* and *R. padi* on wheat (Aqueel and Leather, 2011) [1]. In contrast, nitrogen fertilization had no effect on adult longevity or the reproductive period of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) (Moon *et al.*, 1995) [21].

In this study, nitrogen fertilization had the most consistent benefit for the fecundity of *P. manihoti*, which was 1.2-fold and 1.5-fold greater in plants fertilized with 90 and 180 kg N ha^{-1}, respectively. Similar effects on adult fecundity have been demonstrated for other phloem-feeding insects. For example, positive effects of nitrogen on fecundity were reported for *P. citri* (Hogendorp *et al.,* 2006) [15], *P. ficus* (Cocco *et al.,* 2015) [8], *A. gossypii* (Hosseini *et al.,* 2010) [17], *H. setariae* (Gary *et al.,* 2005) [11], *S. avenae* and *R. padi* (Aqueel and Leather, 2011) [1], and *M. sacchari* (Lama *et al.,* 2019) [19]. In accordance with many studies reporting that adult fecundity is correlated with body size and weight (Honěk, 1993; Rosenheim *et al.,* 1994; Ellers *et al.,* 1998; Hogendorp *et al.,* 2006; Cocco *et al.,* 2015) [16, 25, 10, 15, 8], our study indicated that *P. manihoti* reared on plants with higher nitrogen levels were significantly larger and heavier, and the body size and weight may be correlated with fecundity.

The intrinsic rate of increase (r_m) of *P. manihoti* was significantly affected by nitrogen fertilization and increased with increasing nitrogen levels (Table 4). The intrinsic rate of increase is a measure of how quickly insect populations increase. Variation in the intrinsic rate of increase could be attributed to three main factors: development rate, fecundity and longevity (Dixon, 1987) [9]. Our study demonstrated that nitrogen fertilization of cassava enhanced the developmental rate of nymphs and the fecundity and longevity of adults, which increased the intrinsic rate of increase of the *P. manihoti* population. Zarghami *et al.* (2010) [32] reported that *Brevicoryne brassicae* L. (Hemiptera: Aphididae) feeding on oilseed rape receiving the highest nitrogen fertilization level had the greatest r_m in that study. Similar effects on r_m have been demonstrated for other insects, such as *P. maidis* on corn (Wang *et al.,* 2006) [29] and *A. gossypii* on cucumber (Hosseini *et al.,* 2010) [17].

Previous studies indicated that higher nitrogen treatment resulted in higher nitrogen concentrations in the plants (Wang *et al.,* 2006; Hogendorp *et al.,* 2006; Cocco *et al.,* 2015) [29, 15, 8]. Plant nitrogen content is an appropriate indicator to assess the quality of plants for many insects, as most herbivore insects generally prefer plants with high nitrogen content to be their food (Singh and Sood, 2017) [28]. When nitrogen content in food is abundant and growing conditions are favourable, the insect population has the potential to increase in number from generation to generation. An increase in *P. manihoti* can also be the result of a reduction in cassava plant defences. Plant secondary compounds provide plants with protection against attack by pest insects and pathogen attacks. For example, Calatayud (2000) [6] described the inhibitory effects of rutin, a flavonoid glycoside, on the growth and development of *P. manihoti*. Gazola *et al.* (2018) [13] indicated that 15 days after applying 90 kg N ha^{-1}, the concentration of rutin in cassava apical leaves was significantly higher than that at lower doses (e.g., 0, 30 and 60 kg N ha^{-1}); the lower doses, in turn, reduced plant defences. Therefore, the lack of rutin increased *P. manihoti* performance.

5. Conclusion

It is concluded from this study that an appropriate nitrogen fertilization level should be applied for minimum mealybug damage because increasing the nitrogen application leads to an increase in the *P. manihoti* population on cassava. Therefore, nitrogen fertilizer should be used as an integrated pest management measure in the control of *P. manihoti* on cassava crops.

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7. References

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