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Geographic distribution of the invasive mealybug *Phenacoccus manihoti* and its introduced parasitoid *Anagyrus lopezi* in parts of Indonesia

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Abstract. *Fanani Z. M., Rauf A, Maryana N, Nurmansyah A, Hindayana D. 2019. Geographic distribution of the invasive mealybug* Phenacoccus manihoti *and its introduced parasitoid* Anagyrus lopezi *in parts of Indonesia. Biodiversitas 20: 3751-3757.* Cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), is an invasive pest detected for the first time in Indonesia in 2010. An exotic parasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) was introduced and released in 2014 to control the pest. Study was conducted with the objective to determine the geographic distribution of *P. manihoti* and spread of *A. lopezi*. Field surveys were conducted on cassava fields in various locations in Lampung, Java, and Nusa Tenggara. Our studies showed that *P. manihoti* was found to be widely distributed in Lampung, Banten, West Java, Central Java, East Java, West Nusa Tenggara and East Nusa Tenggara. In each location visited, symptoms of *P. manihoti* infestation as indicated by internode distortion and bunchy top were prominent. Three years following release, parasitoid *A. lopezi* has established and spread into several cassava growing areas, except East Nusa Tenggara. Parasitism rates varied from 1.50% in West Nusa Tenggara up to 59.18% in East Java. Logistic regression revealed that probability of severe damage by the cassava mealybug was significantly ($P<0.05$) increased with the increasing abundance of ants.

Keywords: *Anagyrus lopezi*, geographic distribution, *Phenacoccus manihoti*

INTRODUCTION

Cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), is one the most important pests of cassava in the world (Bellotti et al. 1999). It is native to South America, but it was accidentally introduced into Africa in early 1970s (Nwanze 1982; Hennessey et al. 1990). *Phenacoccus manihoti* is parthenogenic, producing only female offspring. Hence, a single immature or adult is sufficient to start an outbreak. The pest spread throughout the cassava belt of Africa, sharply reducing cassava yields (Norgaard 1988). In the field, the mealybug spread by the wind and over large distances through the movement of infested plant materials. The pest feeds on the leaf near the growing point of the cassava plant. During feeding, the mealybug injects a toxin that causes leaf curling, slowing of shoot growth, and eventual leaf withering. Reduction of tuber yield loss of infested plants up to 84.4% and 54.4% were reported in late- and early- planted cassava, respectively (Nwanze 1982). To control the pest, a host-specific solitary endoparasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) was imported from Paraguay and released in Nigeria in 1981 (Herren and Lema 1982). The introduction of this parasitoid into Africa reduced mealybug infestation by 90%, becoming a highly-successful case of classical biological control (Norgaard 1988).

Phenacoccus manihoti was not known to occur in Asia until 2008, when it was first detected in Thailand (Winotai et al. 2010; Parsa et al. 2012). Since that year, it has spread to others countries in Southeast Asia including Indonesia (Muniappan et al. 2011), Cambodia and Vietnam (Parsa et al. 2012), and Malaysia (Sartiami et al. 2015), caused serious damage to cassava production (Winotai et al. 2010; Bellotti et al. 2012). *Phenacoccus manihoti* poses a threat to the food security and livelihood of the poor farmers (Yonow et al. 2017). Following the success of classical biological control of the cassava mealybug in Africa, the parasitoid *A. lopezi* then was introduced into Thailand in 2009 and released at a nationwide scale in mid-2010 (Winotai et al. 2010). *Anagyrus lopezi* was subsequently introduced into neighboring countries, such as Laos and Cambodia (in 2011), Vietnam (in 2013) and Indonesia (in 2014) (Wyckhuys et al. 2014; Wyckhuys et al. 2018b).

Five years following the first detection in Bogor in 2010, *P. manihoti* has spread to almost all cassava growing areas in Java as well as in Lampung (Abdulchalek et al. 2017). Meanwhile, the introduced parasitoid *A. lopezi* which was released in Bogor in September 2014 has successfully established and survived under local climatic conditions. However, there is no information available on the distribution of *P. manihoti* outside of Java till now; and so establishment and spread of *A. lopezi* following field release have not been evaluated. Based on this background, research was conducted with the objectives to determine the geographic distribution of *P. manihoti* and its parasitoid *A. lopezi* in some of the major cassava growing areas in Indonesia.

MATERIALS AND METHODS

Field survey

The field survey of *P. manihoti* and *A. lopezi* was carried out during the dry season of 2017 (October-November) and 2018 (September-October) in 80 cassava fields in Lampung (n = 11 fields), Banten (n = 9 fields), West Java (n = 9 fields), Central Java (n = 9 fields), East Java (n = 12 fields), West Nusa Tenggara (n = 9 fields), and East Nusa Tenggara (n = 21 fields). Survey protocols followed those that have been used in the region (Graziosi et al. 2016; Wyckhuys et al. 2018a; Le et al. 2018), and was focused on cassava fields having characteristics of monoculture, uniform age and a continuous planting throughout the year. We selected older fields (7-10 months of age) with a minimum size of 0.25 ha in the main cassava-growing areas of each province, with individual sites located at least 1 km apart. Five linear transects were randomly chosen per site, with ten plants (spaced about 0.8 m) sampled in each transect. By doing so, a total of 50 plants per field were assessed for the presence of mealybugs, parasitoids, predators, and ants as well as level of infestation. Field-level *P. manihoti* infestation was expressed as the proportion of plants infested (mealybug incidence), average number of *P. manihoti* per tip (mealybug abundance), and tip damage. In field-identification of mealybugs was based on morphological characteristics such as coloration and length of abdominal waxy filaments (Parsa et al. 2012). Ants were identified using the keys provided by Hashimoto (2010). Tip damage was categorized as follows: score 0 = healthy plants, score 1 = slight curling of leaf margins, score 2 = slight bunching of the tip, score 3 = leaves curling and distortion of the tip (bunchy top), and 4 = severe defoliation (Neuenschwander et al. 1989). Location and elevation of each cassava field were recorded using a handheld GPS unit (Garmin Ltd, Olathe, KS). Information on cassava varieties, field size, and age of plants were obtained by interviewing local field owners or farmers.

To asses parasitism rates of *A. lopezi*, a total of 20 mealybug-infested tips or bunchy tops were randomly collected from each field. The tips (approximately 10 cm each) were broken off and placed in sealed paper bags and stored in a cooler box and brought to laboratories (University of Flores, University of Cendana, University of Mataram, and the Bogor Agricultural University). Upon arrival to the laboratory, cassava tips were carefully examined, and number of nymphal instar-2, -3, adults, and mummies of *P. manihoti* were counted. Further, cassava tips were placed separately within transparent plastic *polyvinyl chloride* (PVC) container (d = 8 cm, t = 11.3 cm) and covered with a gauze cover. Samples were held in the laboratory at temperature 27-30 ºC, relative humidity 65-70 %, 12:12 h light-dark cycle, and observed daily for 3-4 weeks for parasitoid or hyperparasitoid emergence. Specimens were stored in Eppendorf tube with 70% alcohol and labeled according to the alphanumeric code of the field samples for subsequent identification. Hyperparasitoid generic determinations were made using Hayat (1998). Level of parasitism was calculated by dividing the number of emerged parasitoids by the number of mealybugs per tip, and level hyperparasitism was calculated as the number of emerged hyperparasitoids divided by number of emerged primary parasitoids. Parasitism rates were computed for individual cassava tips, and average-field-level parasitism rates were calculated for each province. The sex of emerged *A. lopezi* wasps was determined and sex ratio was expressed as proportion of adults that were males.

Data analysis

All data from the fields were tabulated in MS. Excel consisting of latitude, longitude, elevation, abundance of mealybugs, predators, ants, parasitoids, the number of plants infested. Using Arc GIS version 10.41, a map of geographical distribution of *P. manihoti* and parasitoid *A. lopezi* was drawn. Field-level crop damage (I) by *P. manihoti* was calculated by the formula (Townsend and Heuberger 1943):

$$I = \frac{\sum_{i=1}^{n=5} n_i \times v_i}{N \times V} \times 100\%$$

Where n_i is the number of cassava tips with score i, v_i is the value of score-i, N is the total number of cassava tips observed, and V is the highest score.

Following the calculation of I, field-level of crop damage is grouped into two categories: low-moderate damage (I = 1 - 50%) and severe damage (I = 51-100%). Logistic regression analysis was then used to model the relationship of a binary dependent variable (low-moderate or severe damage) to independent variables using equation:

$$P(I) = \frac{e^{b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4 + b_5 x_5}}{1 + e^{b_0 + b_1 x_1 + + b_2 x_2 + b_3 x_3 + b_4 x_4 + b_5 x_5}}$$

Where: P (I) is the probability of crop damage (0 = low to moderate; 1 = severe), *x* is independent variables, b_0 is intercept and b is coefficient of regression. The dependent variables incorporated into the models were elevation, abundance of *P. manihoti*, ants, predator *Plesiochrysa ramburi* Schneider (Neuroptera: Chrysopidae), and level of parasitism by *A. lopezi*. Logistic regression analysis was performed using SAS version 9.4.

RESULTS AND DISCUSSION

Distribution and infestation of *P. manihoti*

Based on the field survey, a total of four different mealybug species were recorded from cassava fields: *P. manihoti*, *Paracoccus marginatus* Williams & Granara de Willink, *Pseudococcus jackbeardsleyi* Gimpel-Miller, and *Ferrisia virgata* Cockerell. Across sites, a total of 98.949 mealybugs were counted, with *P. manihoti* constituted 55.36%, *P. marginatus* 31.89%, *F. virgata* 8.67%, and *P. jackbeardsleyi* 4.06%. *Phenacoccus manihoti* has been found in all provinces surveyed (Figure 1).

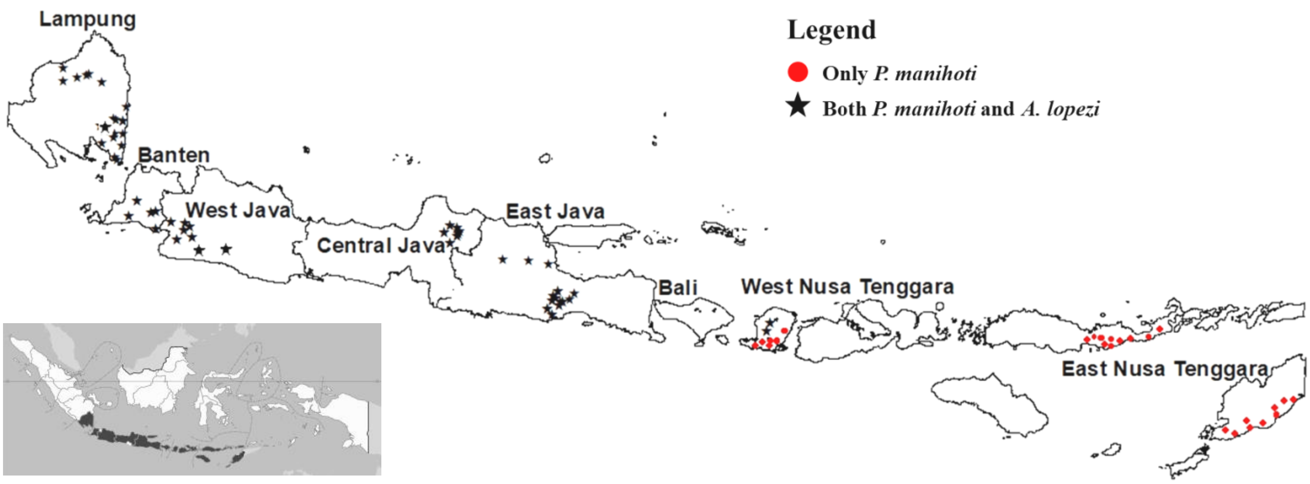

Figure 1. Map showing the geographical distribution of *Phenacoccus manihoti* and *Anagyrus lopezi*

Figure 2. Cassava field severely infested by *Phenacoccus manihoti* with symptom of bunchy tops

Among 80 cassava field sites, the *P. manihoti* infestations were found in 77 sites (95%) distributed in Lampung, Banten, West Java, Central Java, East Java, West Nusa Tenggara, and East Nusa Tenggara. More recently, *P. manihoti* was reported being present in Bangka (Nopriawansyah et al. 2019). Personal communication with other scientists (Hidrayani of Andalas University, West Sumatra and Adriani Ichsan of State University of Gorontalo, Gorontalo) suggested that *P. manihoti* has been found in West Sumatra as well as North Sulawesi.

The cassava mealybug attacked the terminal ends of cassava shoots, caused symptom of clustering and curling of top new leaves called "*bunchy top*". Further symptoms are stem distortion (shortening of the internodes) and severe defoliation. The symptoms of *P. manihoti* infestation were more commonly found in Lampung, East Nusa Tenggara and in West Nusa Tenggara, and occurred in a large area (± 5 ha) at each location. Relatively higher infestation was noticed in East Nusa Tenggara were fields with bunchy tops (Figure 2) were widespread, and almost all of the plants had only a few leaves left intact.

Field-level incidence of *P. manihoti* (i.e. proportion of mealybug-affected tips) was high and ranged from 35.50% in Central Java up to 74.18% in Lampung (Table 1). Level of crop damage by the cassava mealybug varied from 15.12% in Central Java up to 44.05% in West Nusa Tenggara. Abundance of cassava mealybug ranged from 10.21 insects per tip in Lampung up to 43.89 insects per tip in East Nusa Tenggara. Le et al. (2018) reported that field-level incidence of *P. manihoti* in Vietnam averaged 24.8% and mealybug abundance averaged 5.56 insects per tip. Higher infestation in some locations, especially in East Nusa Tenggara, was allegedly due to hot weather conditions and lack of intensive cassava cultivation. Ezumah and Knight (1978) demonstrated that dry weather, soil moisture stress, and soil erosion are some of the environmental factors that enhance the population build-up of *P. manihoti*. According to Iheagwam and Eluwa (1983), the population density of *P. manihoti* was usually high during the dry season and markedly reduced during rainy season. Rainfall is a key determinant of *P. manihoti* abundance and population dynamics: dry regions, years and seasons favor outbreaks (Gutierrez et al. 1988). In addition to hot weather conditions and low rainfall, population build-up of *P. manihoti* was also triggered by nutrient-deficient soil as result of lack of fertilization (Schulthess et al. 1997).

Distribution and parasitism rate of *A. lopezi*

Three years following the release, *A. lopezi* was found in Lampung, Java, and Lombok (see Figure 1). It can be assumed that *A. lopezi* has spread on its own or through winds from the release site in Bogor and passive transport by man of parasitized cassava mealybug on cassava cuttings from one district to another. Herren et al. (1987) reported that seven years following release in Nigeria, the parasitoid has established in 19 African countries and has spread over an area of over 1.5 million km^2. The speed of dispersal of *A. lopezi* in Africa was 50-100 km in one dry season (5-8 months) (Herren et al. 1987). Lema and Herren (1985) reported that parasitoid *A. lopezi* has spread over a distance of about 150 km in 14 months.

Rate of parasitism of *A. lopezi* in each province varied, ranging from 1.50 % (West Nusa Tenggara), up to 59.18% (East Java) (Table 2). The highest parasitism rate (81.25%) was detected in Mojokerto Regency (East Java), as indicated by large number of mummified mealybugs found at the end of the dry season. Le et al. (2018) reported seasonal rate of parasitism in Vietnam averaged 49.9-52.1%. Sex ratio of *A. lopezi* in the field was generally male-biased. The proportion of male wasps was 0.69 in Lampung, 0.57 in Banten, 0.52 in West Java, 0.56 in Central Java, 0.61 in East Java, but 0.4 in Lombok (West Nusa Tenggara). van Dijken et al. (1991) reported field sex ratio of *A. lopezi* in Africa varied from 0.44 at low cassava mealybug densities to highly male-biased of 0.70 at high host densities. Small hosts (2^{nd} instar nymph) are mainly used for the production of male offspring, whereas large hosts (3^{rd} instar nymph) are used for production of female offspring. Therefore, sex ratio (males to total parasitoids) increases with host density, since proportion of small hosts encountered in the field increases with increasing host density (van Dijken et al. 1991).

A total of 150 hyperparasitoids emerged from field-collected tips held in the laboratory, consisting of 56.7% *Chartocerus* sp. (Hymenoptera: Signiphoridae) and 43.3% *Prochiloneurus* sp. (Hymenoptera: Encyrtidae). Average hyperparasitism rates (x±SE) were 5.93±1.46% (Lampung), 0.13±0.12% (Banten), 3.29±1.10% (West Java), 2.16±0.91% (Central Java), 4.89±1.32% (East Java), and 2.57±1.97% (West Nusa Tenggara). Le et al. (2018) reported three species of hyperparasitoids: *Chartocerus* sp. near *walkeri* Hayat, *Prochiloneurus* sp., and *Promuscidea unfasciativentris* Girault (Hymenoptera: Eriaporidae). In Vietnam, hyperparasitism rates were on average 2.79±5.38%, with a maximum hyperparasitism rate of 26.4% (Le et al. 2018). Hyperparasitoids associated with *A. lopezi* in Africa were *Prochiloneurus insolitus* (Alam) and *Chartocerus hyalipennis* (Hayat) (Neuenschwander et al. 1987). The rate of hyperparasitism in Togo varied considerably between 20-90% (Agricola and Fischer 1991). In spite of high hyperparasitism, no detrimental effect on the control efficiency of *A. lopezi* was noticed (Neuenschwander and Hammond 1988; Agricola and Fischer 1991). Cage experiment showed that adding hyperparasitoids did not significantly reduce efficiency of *A. lopezi* in preventing plant damage (Goergen and Neuenschwander 1992).

Table 1. Infestation of cassava mealybug *Phenacoccus manihoti* at various field sites

Provinces	Number of field sites	Mealybug incidence (%)*	Level of crop damage (%)*	Mealybug abundance (insects / tip) *
Lampung	11	74.18 ± 4.30	25.57 ± 1.87	10.21 ± 2.44
Banten	9	36.00 ± 9.67	28.19 ± 7.68	17.78 ± 4.14
West Java	9	47.00 ± 8.95	18.69 ± 2.33	38.96 ± 8.26
Central Java	9	35.50 ± 9.07	15.12 ± 5.51	21.99 ± 8.33
East Java	12	38.67 ± 6.20	16.83 ± 2.93	21.61 ± 4.86
West Nusa Tenggara	9	57.56 ± 5.57	44.05 ± 5.26	37.33 ± 3.58
East Nusa Tenggara	21	54.67 ± 7.10	36.57 ± 4.25	43.89 ± 4.67

Note: *Mean ± SE

Table 2. Parasitism rate of *Anagyrus lopezi* and abundance of *Phenacoccus manihoti* and ants

Province	Number of field sites	Parasitism rate (%)*	Abundance of *P. ramburi* (insects/tip)*	Abundance of ants (insects/tip)*
Lampung	11	50.85 ± 3.67	0.13 ± 0.03	1.04 ± 0.42
Banten	9	22.15 ± 1.54	0.10 ± 0.03	4.19 ± 1.57
West Java	9	17.01 ± 4.01	0.06 ± 0.01	4.06 ± 2.51
Central Java	9	24.17 ± 7.92	0.45 ± 0.03	1.01 ± 0.04
East Java	12	59.18 ± 4.80	0.26 ± 0.09	5.35 ± 2.15
West Nusa Tenggara	9	1.50 ± 0.13	0.19 ± 0.04	4.03 ± 1.12
East Nusa Tenggara	21	0.00 ± 0.00	0.29 ± 0.06	3.66 ± 1.28

Note: *Mean ± SE

Table 3. Logistic regression analysis of the effect of elevation and biotic factors on level of crop damage by the cassava mealybug

Parameter	Independent variable	Estimated parameter	SE	Wald χ^2	P value
b_0	Intercept	-5.51	1.53	56.91	0.000
b_1	Elevation	-0.00309	0.00245	1.72	0.190
b_2	*P. manihoti*	0.1498	0.0418	32.92	0.000
b_3	Ants	0.188	0.101	4.04	0.044
b_4	*A. lopezi*	-0.0122	0.0201	0.38	0.539
b_5	*P. ramburi*	-0.68	1.68	0.16	0.685

Green lacewing and ants

Predatory insect generally found in the cassava fields during the surveys was a green lacewing *Plesiochrysa ramburi* (Walker) (Neuroptera: Chrysopidae). The density of larvae was low ranged 0.06-0.45 individuals per tip (Table 2). Numerous eggs of *P. ramburi* were often observed on mealybug infested shoots. Wardani (2015) reported that *P . ramburi* is a natural enemy of cassava mealybug which was commonly found in cassava fields in Bogor. Sattayawong et al. (2016) also found *P. ramburi* as a dominant predatory insect in cassava fields in Thailand. Mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is often found in cassava fields infested by mealybugs, as previously reported by Wahyuningsih et al. (2019). Other insect species associated with cassava mealybug-infested plants were ants, which are attracted to honeydew excreted by mealybugs. The ants were identified as *Dolichoderus thoracicus* Smith, *Anoplolepis gracilipes* Smith, *Tapinoma melanocephalum* Fabricius, *Camponotus* sp., and *Crematogaster* sp. The density of ants varied between 1-5 individuals per tip (Table 2). In Africa, species of ants associated with the cassava mealybug were *Camponotus*, *Crematogaster*, and *Pheidole* (Cudjoe et al. 1993).

Logistic regression

The logistic regression analysis shows the coefficients b_1 (elevation), b_4 (parasitism rate of *A. lopezi*), and b_5 (abundance of *P. ramburi*) each with a negative sign (Table 3). This indicates that the probability of severe crop damage by *P. manihoti* decreases with the increasing of elevation, parasitism rate of *A. lopezi*, and abundance of predator *P. ramburi*. However, their effects are not statistically significant ($p > 0.05$). This might be due to low abundance and high variability of *P. ramburi* larval population in the fields. In addition, *P. ramburi* generally arrived later on the growing *P. manihoti* populations when the pest has reached a peak and caused severe damage to cassava plants as previously observed by Wardani (2015). Meanwhile, *A. lopezi* is reported to be an effective and efficient parasitoid in reducing population of *P. manihoti* (Neuenschwander et al. 1989; Hammond and Neuenschwander 1990). *A. lopezi* is attracted to the mealybug even at the lowest host densities and reacted strongly to an increase in host density (Neuenschwander et al. 1989). The lack of significant effect of parasitoid *A. lopezi* on mealybug damage was due to the fact that our observations were limited only to parasitoid emergence (parasitism rate), whereas mortality of mealybug due to host-feeding and mutilation by *A. lopezi* was not observed. Neuenschwander and Madojemu (1986) reported that mealybug mortality caused by parasitoid host-feeding and mutilation generally occurs on the 1st and 2nd instar of mealybug nymphs. Therefore, although the parasitism rate in the field rarely exceeds 30% (Lema and Herren 1985), mealybug mortality can be higher as a result of host-feeding behavior of the parasitoid (Neuenschwander and Sullivan 1987). According to Neuenschwander and Madojemu (1986) mealybug mortality caused by host feeding and mutilation is a major component of the effectiveness of *A. lopezi* as a biological control agent for *P. manihoti*. However, this effect could not be measured in the field.

In contrast to the effect of *P. ramburi* and *A. lopezi*, the coefficient b_2 (abundance of *P. manihoti*) and b_3 (abundance of ants) each has a positive sign (Table 3), indicating that the probability of severe crop damage increases with the increasing abundance of *P. manihoti* as well as ants. Ants and mealybug form a mutualistic relationship. Mealybug produces honeydew as a food source for ants. In turn, ants visiting mealybug protect it against natural enemies such as predator and parasitoid (Helms and Vinson 2008). Therefore, a high population abundance of ants can indirectly increase the mealybug population, and subsequently, cause severe damage to plants.

Challenges

To date, several provinces have been invaded by *P. manihoti*, and it could impose a serious threat to the cassava production in yet uninvaded provinces. *P. manihoti* has thelytokous parthenogenesis, so introduction of a single individual could be sufficient to lead to successful invasion. Moreover, ovisacs or eggs adhere to carriers, while nymphs and adults can spread with wind, facilitating long-distance dispersal (Parsa et al. 2012). In Africa, *P. manihoti* has been spreading at an alarming rate of 150 km per year (Winotai et al. 2010), compared to less than 30 km per year for other invasive hemipterans (Liebhol and Tobin 2008). Risk analysis for biological invasion indicates that almost all provinces in Indonesia are climatically suitable for *P. manihoti* (Yonow et al. 2017). Therefore, further surveys in other cassava growing provinces such as in Sumatra, Sulawesi, Maluku, and Papua are needed to map this pest distribution at a national scale. In addition to that, since *A.lopezi* has not been found in East Nusa Tenggara, it would be necessary to introduce the parasitoid into this area to control *P. manihoti*. Particularly for invasive pests, biological control constitutes an environmentally sound and cost-effective management option (Tancharoen et al. 2018). Parasitoid *A. lopezi* has exceptional dispersal potential, a high degree of adaptation to varying agro-ecological conditions, host-feeding capabilities and ability to persist at low *P. manihoti* densities (Neuenschwander et al. 1989), and thus is well-adapted to provide effective biological control in various settings of cassava productions (Le et al. 2018). Furthermore, especially in intensified cassava production, good agricultural practices should be followed. It has been shown that fertilizer inputs and suitable water management likely benefit biological control by boosting *A. lopezi* development and fitness (Wyckhuys et al. 2017).

In conclusion, *P. manihoti* has been spreading throughout Java, Sumatra and Nusa Tenggara. The introduced parasitoid *A. lopezi* has successfully established and spread to Lampung and Lombok. Relatively higher *P. manihoti* population and crop damage in East Nusa Tenggara were due to the absence of parasitoid *A. lopezi* in this region. Probability of severe damage was more in cassava fields inhabited by higher ant population.

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