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Continental-scale suppression of an invasive pest by a host-specific parasitoid heralds a new era for arthropod biological control

Kris Wyckhuys Corresp., [1, 2, 3] , **Prapit Wongtiem** [4] , **Aunu Rauf** [5] , **Anchana Thancharoen** [6] , **George Heimpel** [7] , **Nhung Le** [8] , **Muhammad Zainal Fanani** [5] , **Geoff Gurr** [9] , **Jonathan Lundgren** [10] , **Dharani D Burra** [11] , **Leo Palao** [12] , **Glenn Hyman** [13] , **Ignazio Graziosi** [14] , **Vi Le Xuan** [8] , **Matthew Cock** [15] , **Teja Tscharntke** [16] , **Steve Wratten** [17] , **Liem V Nguyen** [8] , **Minsheng You** [2] , **Yanhui Lu** [18] , **Johannes Ketelaar** [19] , **Georg Goergen** [20] , **Peter Neuenschwander** [20]

1 CGIAR Program on Roots, Tubers and Banana, International Center for Tropical Agriculture, Hanoi, Vietnam
2 Fujian Agriculture and Forestry University, Fuzhou, China
3 School of Biological Sciences, University of Queensland, Brisbane, Australia
4 Rayong Field Crops Research Center, Thai Department of Agriculture, Rayong, Thailand
5 Bogor Agricultural University, Bogor, Indonesia
6 Kasetsart University, Bangkok, Thailand
7 University of Minnesota, Minneapolis, United States
8 Plant Protection Research Institute, Hanoi, Việt Nam
9 Charles Sturt University, Orange, Australia
10 Ecdysis Foundation, Estelline, United States
11 International Center for Tropical Agriculture CIAT, Hanoi, Viet Nam
12 International Center for Tropical Agriculture, Los Banos, Philippines
13 International Center for Tropical Agriculture, Cali, Colombia
14 University of Kentucky, Lexington, United States
15 CABI, Wallingford, United Kingdom
16 University of Goettingen, Goettingen, Germany
17 Lincoln University, Christchurch, New Zealand
18 China Academy of Agricultural Sciences, Beijing, China
19 Food and Agriculture Organization, Bangkok, Thailand
20 International Institute for Tropical Agriculture, Cotonou, Benin

Corresponding Author: Kris Wyckhuys
Email address: kagwyckhuys@gmail.com

Biological control constitutes one of the world's prime ecosystems services, and can provide long-term and broad-scale suppression of invasive pests, weeds and pathogens in both natural and agricultural environments. Following (very few) widely-documented historic cases that led to sizeable environmental up-sets, the discipline of insect biological control has -over the past three decades- gone through much-needed reform. Now, by deliberately taking into account the ecological risks associated with insect biological control, immense environmental and societal benefits can be gained. In this study, we document and analyze a rare, successful case of biological control against the invasive mealybug, *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) which invaded Southeast Asia in 2008, where it caused substantial crop losses and triggered 2- to 3-fold surges in

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agricultural commodity prices. In 2009, the host-specific parasitoid *Anagyrus lopezi* (Hymenoptera: Encyrtidae) was released in Thailand and subsequently introduced into neighboring Asian countries. Drawing upon continental-scale insect surveys, multi-year population studies and (field-level) experimental assays, we show how *A. lopezi* attained intermediate to high parasitism rates across diverse agro-ecological contexts. Driving mealybug populations below non-damaging levels at a continental scale, *A. lopezi* allowed yield recoveries up to 10.0 t/ha and provided biological control services worth several hundred dollars per ha (at local farm-gate prices) in Asia's 4-million ha cassava crop. Our work provides lessons to invasion science and crop protection worldwide, heralds a new era for insect biological control, and highlights its potentially large socio-economic benefits to agricultural sustainability in the face of a debilitating invasive pest. In times of unrelenting insect invasions, surging pesticide use and accelerating (invertebrate) biodiversity loss across the globe, this study unequivocally demonstrates how biological control – as a pure public good – constitutes a powerful, cost-effective and environmentally-responsible solution for invasive species mitigation.

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Send correspondence to:
Kris A. G. Wyckhuys
Chinese Academy of Agricultural Sciences
2 West Yuanmingyuan Rd., Beijing, 100193, P. R. China
Tel:+86-10-62813685
Contact: kagwyckhuys@gmail.com

Continental-scale suppression of an invasive pest by a host-specific parasitoid heralds a new era for arthropod biological control

Running head: Biocontrol of the cassava mealybug in Asia

Wyckhuys, K.A.G.[1, 2*], Wongtiem, P.[3], Rauf, A.[4], Thancharoen, A.[5], Heimpel, G.E.[6], Le, T.T.N.[7], Fanani, M.Z.[4], Gurr, G.M.[2], Lundgren, J.G.[8], Burra, D.D.[9],, Palao, L.K.[9], Hyman, G.[9], Graziosi, I.[10], Le, X.V.[7],Cock, M.J.W.[11], Tscharntke, T.[12], Wratten, S.D.[2], Nguyen, V.L.[6], You, M.S.[2], Lu, Y.H.[13], Ketelaar, J.W.[14], Goergen, G.[15], Neuenschwander, P.[16]

1. CGIAR Program on Roots, Tubers and Banana (CRP-RTB), International Center for Tropical Agriculture CIAT, Hanoi, Vietnam;
2. International Joint Research Laboratory on Ecological Pest Management, Fuzhou, China;
3. Thai Department of Agriculture (DoA), Bangkok, Thailand;
4. Bogor Agricultural University, Bogor, Indonesia
5. Kasetsart University, Bangkok, Thailand;
6. University of Minnesota, Minneapolis, Minnesota (USA);
7. Plant Protection Research Institute (PPRI), Vietnam Academy of Agricultural Sciences, Hanoi, Vietnam;
8. Ecdysis Foundation, Estelline, South Dakota (USA);
9. International Center for Tropical Agriculture CIAT, Cali, Colombia;
10. University of Kentucky, Lexington, Kentucky (USA), World Agroforestry Center (ICRAF), Nairobi, Kenya;
11. CABI, Egham, UK
12. Gottingen University, Gottingen, Germany;
13. China Academy of Agricultural Sciences IPP-CAAS, Beijing, China;
14. Food and Agriculture Organization FAO, Bangkok, Thailand;
15. International Institute for Tropical Agriculture IITA, Cotonou, Benin;

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Abstract

Biological control features as one of the world's prime ecosystems services, and can provide long-term and broad-scale suppression of invasive pests, weeds and pathogens in both natural and agricultural environments. Following (very few) widely-documented historic cases that led to sizeable environmental up-sets, the discipline of arthropod biological control has -over the past three decades- gone through much-needed reform. Now, by deliberately taking into account the ecological risks associated with the deliberate introduction of insect natural enemies, immense environmental and societal benefits can be gained. In this study, we document and analyze a rare, successful case of biological control against the invasive cassava mealybug, *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) which invaded Southeast Asia in 2008, where it caused substantial crop losses and triggered 2- to 3-fold surges in agricultural commodity prices. In 2009, the host-specific parasitoid *Anagyrus lopezi* (Hymenoptera: Encyrtidae) was released in Thailand and subsequently introduced into neighboring Asian countries. Drawing upon continental-scale insect surveys, multi-year population studies and (field-level) experimental assays, we show how *A. lopezi* attained intermediate to high parasitism rates across diverse agro-ecological contexts. Driving mealybug populations below non-damaging levels at a continental scale, *A. lopezi* allowed yield recoveries up to 10.0 t/h and provided biological control services worth several hundred dollars per ha (at local farm-gate prices) in Asia's 4-million ha cassava crop. Our work provides lessons to invasion science and crop protection worldwide, heralds a new era for insect biological control, and highlights its potentially large socio-economic benefits to agricultural sustainability in the face of a debilitating invasive pest. In times of unrelenting insect invasions, surging pesticide use and accelerating (invertebrate) biodiversity loss across the globe, this study unequivocally demonstrates how

biological control – as a pure public good – constitutes a powerful, cost-effective and environmentally-responsible solution for invasive species mitigation.

Introduction

Biological control is one of the world's prime ecosystem services, and plays a pivotal role in the functioning and broader resilience of agricultural and natural ecosystems alike (Costanza *et al.* 1997). For US agriculture alone, insect-mediated biological control is conservatively valued at $4.5 billion per year, and a diversity of natural enemies helps alleviate pressure from herbivores and other crop antagonists (Losey and Vaughan, 2006). However, rapid depletion of animal populations and progressive ecosystem simplification compromise the strength and stability of this vital ecosystem service (Oliver *et al.,* 2015; Hallmann *et al.,* 2017). In tropical terrestrial ecosystems, these trends might be even more pronounced though they routinely remain un-documented (Melo *et al.,* 2013; Barnes *et al.,* 2014).

Across the globe, arthropod pests reduce agricultural productivity by 10-16% and constitute key impediments to food security and (indirectly) poverty alleviation (Oerke, 2006; Bebber *et al.,* 2013). Though native pests continue to pose major problems for the world's agriculture, non-native species are of increasing significance as a result of trade globalization and human movement (Bradshaw *et al.,* 2016; Paini *et al.,* 2016). Importation biological control (IBC; also known as 'classical biological control'), or the judicious selection and subsequent introduction of a specialized natural enemy from the pest's region of origin, has been repeatedly shown to effectively reduce invasive pests (van Driesche *et al.,* 2008; Heimpel and Mills, 2017).

Particularly in the developing-world tropics, IBC can be a “silver bullet” option for destructive agricultural pests, being largely self-sustaining and requiring little or no stakeholder intervention (Andrews *et al.,* 1992). Since the late 1800s, more than 2,000 natural enemy species have been released against approximately 400 invasive pests worldwide, occasionally resulting in complete pest control but regularly causing limited or no impact (van Lenteren *et al.,* 2006; Cock *et al.,* 2016b). Though economic impacts are not routinely assessed for IBC, levels of pest suppression and ensuing benefit:cost ratios can be exceptionally favorable (5:1 to >1,000:1) (Heimpel and Mills, 2017; Gutierez *et al.,* 1999; Naranjo *et al.,* 2015). Yet, IBC is marred with remarkably low rates of success (Greathead & Greathead, 1992; Cock *et al.*, 2016a), and consequently biological control as a whole is habitually undervalued and all too often taken for granted (Daily *et al.,* 2009). Furthermore, over the past three decades, IBC initiatives have been met with stringent regulations and a heightened emphasis on potential ecological risks or unintended side-effects (Heimpel & Cock, 2018). The latter was triggered by a provocative yet necessary account by Howarth (1983, 1991), built around misguided biological control releases that were conducted decades earlier, and in which the long-established paradigm of IBC as ‘ecologically-safe’ practice was challenged.

One widely-acclaimed IBC program is the Africa-wide initiative targeting the invasive cassava mealybug, *Phenacoccus manihoti* (Hemiptera: Pseudococcidae), which led to a 50% yield recovery resulting in long-term economic benefits up to US $20.2 billion as well as the likely avoidance of widespread famine without negative side effects (Neuenschwander *et al.,* 1989; Herren and Neuenschwander, 1991; Zeddies *et al.,* 2001). Key to the success of this program was the carefully-selected host-specific and environmentally-adaptable parasitic wasp *Anagyrus lopezi* (Hymenoptera: Encyrtidae), recovered in 1981 after foreign exploration from South

America, and introduced into Nigeria soon thereafter. As the *A. lopezi* wasp is considered to be a specialist internal feeder on *P. manihoti*, no detrimental ecological impacts resulted from its continent-wide release (Neuenschwander, 2001). Following its devastating passage through Africa's cassava belt in the 1970s and 80s, *P. manihoti* was inadvertently introduced into Thailand in 2008, spread through mainland Southeast Asia, and had made its appearance in insular Indonesia by 2010 (Graziosi *et al.,* 2016). As cassava is grown on >4 million ha by an estimated 8 million farming families throughout tropical Asia, this pest had ample potential to cause massive socio-economic impacts. As part of an internationally-coordinated management campaign for *P. manihoti*, *A. lopezi* was promptly sourced from Benin, West Africa and 500 adults of this wasp were introduced into Thailand in 2009 (Winotai *et al.,* 2010). Parasitoid wasps were subsequently mass-reared by multiple Thai institutions, released across the country during 2010-2012 (some by airplane) and introduced into neighboring Laos, Cambodia (in 2011), Vietnam (in 2013) and Indonesia (in 2014) (Wyckhuys *et al.*, 2015).

In this study, we characterized the degree to which *A. lopezi* has established in the highly-heterogeneous cassava cropping environments of Southeast Asia. Field research was carried out over the course of 2014-2017 by various country teams, each pursuing different objectives as outlined below. We employed seasonal population surveys that extended from Myanmar's Ayeyawaddy River delta to the uplands of Timor in eastern Indonesia, to quantify magnitude and spatial extent of parasitoid-induced *P. manihoti* population suppression (*section i, ii*). Furthermore, we employed well-established manipulative protocols to assess the effectiveness of *A. lopezi* and subsequent yield benefits of biological control (De Bach *et al.,* 1971; van Lenteren *et al.,* 1980; Luck *et al.,* 1988) (*section iii*). Finally, we conducted an analysis of production statistics and cassava prices in one of Asia's main cassava-growing countries (Thailand) over a time period spanning the 2008 *P. manihoti* invasion, the 2009 parasitoid introduction into Thailand and the subsequent (natural, and human-aided) continent-wide distribution of *A. lopezi* (*section iv*).

Our work uses original datasets to present a rare, continental-scale and multi-year assessment of IBC-mediated insect pest suppression, and the cascading trophic and socioeconomic effects on cassava yield loss reduction and commodity prices. We present a data-rich body of information on the benefits of *A. lopezi* as a biological control agent, and lay the basis for further econometric investigations. This study illustrates the potential value of an insect-driven ecosystem service to agricultural sustainability, in the face of a potentially devastating invasive pest.

Materials & Methods

i. Multi-country pest & natural enemy survey

From early 2014 until late 2017, insect surveys were carried out in 634 cassava fields in Myanmar, Thailand, Lao PDR, Cambodia, Vietnam, southern China and Indonesia. Survey protocols are described in detail in Graziosi *et al.* (2016). In brief, we selected older fields (i.e., 8-10 months of age) in the main cassava-growing areas of each country, with individual sites located at least 1 km apart. Five linear transects were randomly chosen per site, with ten plants (routinely spaced at 0.8-1.2 m) sampled in each transect. By doing so, a total of 50 plants per field were assessed for *P. manihoti* infestation and per-plant mealybug abundance. In-field identification of mealybugs was based on morphological characteristics such as coloration and presence or length of abdominal waxy filaments, while samples were also taken to the laboratory for identification by specialist taxonomists. Following transect walks we calculated average *P. manihoti* abundance (number of individuals per infested tip) and field-level incidence (proportion of *P. manihoti*-infested tips per field).

To assess local *A. lopezi* establishment and parasitism rates, we conducted dry-season sampling from 2014 to 2017 at sub-sets of mealybug-invaded sites in Thailand (*n*= 20), Cambodia (*n* = 10, 15), southern Vietnam (*n*= 20, 20, 6) and Indonesia (*n* = 10, 9, 21) (total *n*= 131). Sampling consisted of collecting 20 mealybug-infested tips from local fields and transferring them to a laboratory to monitor subsequent parasitoid emergence (Neuenschwander *et al.,* 1989). Surveys were carried out during January-May 2014 (dry season), October-November 2014 (late rainy season), January-March 2015 (dry season) in mainland Southeast Asia, and during October-November 2014 and 2017 (dry season) in insular Indonesia. Locations were recorded using a handheld GPS unit (Garmin Ltd, Olathe, KS). In-field identification of mealybugs was based on morphological characters, while samples were also transferred to the laboratory for further taxonomic identification. Voucher specimens of *P. manihoti* were equally deposited at the Thai Department of Agriculture (Bangkok, Thailand), Bogor Agricultural University (Bogor, Indonesia) and Plant Protection Research Institute (Hanoi, Vietnam).

To assess local *A. lopezi* establishment and parasitism rates, mealybug-infected tips were collected in the field and transferred to a laboratory. Upon arrival in the laboratory, each tip was carefully examined, predators were removed and the total number of *P. manihoti* was determined. Tips were then placed singly into transparent polyvinyl chloride (PVC) containers, closed with fine cotton fabric mesh. Over the course of three weeks, containers were inspected on a daily basis for emergence of parasitic wasps and *A. lopezi* parasitism levels (per tip and field) were computed. Next, for fields where presence of *A. lopezi* was reported, we carried out a regression analysis to relate field-level mealybug abundance with parasitism rate. Mealybug infestation levels and parasitism rates were log-transformed to meet assumptions of normality and homoscedasticity, and all statistical analyses were conducted using SPSS.

ii. Multi-year mealybug and parasitoid population assessment in Vietnam

From July 2013 until July 2015, we conducted population surveys in Tay Ninh province, Vietnam; an area with near-continuous, all-year cassava cultivation (see also Le et al., 2018). The cassava mealybug is assumed to have arrived in southern Vietnam during 2011-2012, and *A. lopezi* was first detected from Tay Ninh province in early 2013. Eight newly-planted cassava fields were selected of uniform age, crop variety, developmental stage and management. Every two months, insect surveys were done within these fields, to characterize *P. manihoti* incidence, infestation pressure and *A. lopezi* parasitism rate. In each field, a total of five linear 10-15 m transects were screened (plants routinely spaced at 0.8-1.2 m) and, 50 plants were thus carefully inspected for *P. manihoti*. *Phenacoccus manihoti* infestation was recorded as field-level abundance (number of individuals per infected tip) and field-level incidence (proportion of mealybug-affected tips) at each sampling date and location. To assess *A. lopezi* parasitism rates, 20 mealybug-infested tips were randomly collected from each field by breaking off the top parts of individual plants, and transferred to the laboratory. Parasitism rates were estimated from these samples as described above, and parasitism levels were computed for each individual field and sampling date. We used analysis of variance (PROC MIXED, SAS version 9.1; SAS Institute, Cary, NC) with field as random factor, and tested the effect of cassava age, sampling date and

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year for *P. manihoti* incidence, abundance and *A. lopezi* parasitism. Means were compared with least squares means approach. Mealybug abundance data were log-transformed while incidence, parasitism and hyperparasitism data were arcsine-transformed to meet normality.

The intrinsic rate of mealybug population increase, *r*, over two months was calculated over subsequent sampling events as $\ln(m_{t+1}/m_t)$ where m = the per-tip mealybug density. This growth rate was regressed against the mealybug parasitism rate as a means of assessing the role of the parasitoids in suppressing mealybug population growth rates and also to estimate the parasitism level needed to suppress population growth rate. The statistical significance of the relationship between parasitism rate and mealybug population growth was assessed using a generalized linear model incorporating normal error distribution with *r* as the response variable and parasitism level and field identity as independent variables.

iii. Exclusion cage assays

In August 2014, a field study was initiated at the Rayong Field Crops Research Center in Rayong, Thailand (Thancharoen et al., under review). To assess the relative contribution of natural enemies such as *A. lopezi* to pest control, we employed exclusion assays (Snyder and Wise, 2001; Costamagna *et al.,* 2007). More specifically, to determine separate and joint effects of *P. manihoti* and *A. lopezi* on cassava crop yield, three different treatments were established using two common cassava varieties: Kasetsart 50 (KU50) and Rayong 72 (R72). Treatments consisted of the following: 1) 'full cage' assays, in which a plant was entirely covered by a mesh screen cage to exclude all natural enemies; 2) 'sham' cage assays, in which a plant was covered by a screen cage to provide a microhabitat similar to that of the 'full cage', but left open at the sides to allow natural enemy access; 3) 'no cage' assays, in which a plant was kept without a cage, as a 'real-world' benchmark. Each treatment was established with four replicates. The experimental field was established using locally-sourced stem cuttings of KU50 or R72, planted at 1-m distances within plots. In experimental plots, weeding was done manually, fertilizer was used at conventional rates and insecticide use was avoided throughout the assay.

Once plants had reached 4.5 months of age, 2 x 2 x 2 m polyvinylchloride (PVC) frame cages were deployed, with four plants contained within each cage. Cages were covered with fine nylon mesh screen to prevent entry by insects, including *A. lopezi* parasitoids. In January 2015, 10 adult female *P. manihoti* were gently brushed onto plants within each treatment (shared among the four plants). Mealybug adults were obtained from a laboratory colony at Rayong Field Crops Research Center that had been started in early 2014, in which *P. manihoti* were maintained on potted cassava plants within a screen-house that were regularly supplemented with field-collected individuals. Visual observations were carried out within the cages on a monthly basis and *P. manihoti* abundance was recorded on each plant. On September 7, 2015, once the crop had reached 12 months of age, cages were removed and plants within the different experimental treatments were harvested manually. At harvest, fresh root yield (FRY) was determined for each plant: (Karlstrom *et al.,* 2016).

Mealybug population build-up under each experimental treatment was calculated, by converting the average number of mealybugs per plants on a given sampling date to cumulative mealybug-days (CMD) (Ragsdale *et al.,* 2007):

$$\sum_{n=1}^{\infty} = \left(\frac{x_{i-1} + x_i}{2}\right) \times \left(t_i - t_{i-1}\right)$$

where n is the total number of days over which sampling took place, x_i is the number of mealybugs counted on day i and t_i is the number of days since the initiation of sampling on day i.

Mealybug population build-up under each experimental treatment was computed, and average CMD measures were compared between the respective treatments using a mixed modeling approach with plot as random factor and time as repeated measure. A mixed modeling approach was equally used to compare different yield parameters, using treatment and variety as fixed factors. Plant survival rates were compared between treatments, using a Chi-square analysis. Where necessary and feasible, data were transformed to meet assumptions of normality and homoscedasticity, and all statistical analyses were conducted using SPSS.

iv. Country-wide yield changes

Crop production statistics were obtained through the Office of Agricultural Economics, Ministry of Agriculture & Cooperatives (Bangkok, Thailand). Yield measures were computed for 2006-2016, for a total of 51 cassava-growing provinces within Thailand, and annual weighted means were compared between successive years. Cassava crop yield can be impacted by agro-climatic conditions (e.g., temperature-related variables) and by attack of pests such as *P. manihoti*. To assess the impact of the sustained, broad-scale *A. lopezi* releases from the 2011 cropping season onward, mean values of yields across all the cassava-growing provinces were regressed with explanatory variables which included rainfall, minimum and maximum temperature (obtained from Thai Meteorological Department, Bangkok, Thailand) and time (year). In addition, a categorical variable representing the introduction of *A. lopezi* ('present' for the 2011 and 2012 growing seasons, and 'absent' for growing seasons 2008, 2009 and 2010) was equaled entered as an explanatory variable in the regression model. Specifically for regression analysis, the distribution of the response variable (i.e. yield) was identified to be normal (Shapiro test $p < 0.05$). A step-wise regression approach (forward and backward) using a linear modeling approach was used to identify the model that best explains variation in yield. The model with the lowest Akaike information criterion (AIC) was selected. In the next step, the model with the lowest AIC score was compared with models containing interaction terms between time and the remaining explanatory variables (i.e. temperature minimum, rainfall and *A. lopezi* introduction) separately. The regression analysis was performed in R (v 3.4.1) statistical computing environment. Additionally, R package “gvlma” was used to assess if the assumptions of regression were met by the selected model. Additional diagnostics of the selected model, such as determination of variance inflation factor (VIF) for detection of multicollinearity, the Non-constant Variance Score Test (i.e. test for heteroscedasticity of residuals over fitted values) was performed using R package “MASS” and “car” respectively. Significant variables, as identified by the selected model were visualized using the “effects” package in R statistical computing environment.

Results

i. Multi-country pest & natural enemy survey

During continental-scale insect surveys from 2014 until 2017 (i.e., 5-8 years following the initial *A. lopezi* introduction), the mealybug complex on cassava largely comprised four non-native species: (1) *P. manihoti*; (2) the papaya mealybug *Paracoccus marginatus* Williams & Granara

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de Willink; (3) *Pseudococcus jackbeardsleyi* Gimpel & Miller; and (4) the striped mealybug *Ferrisia virgata* Cockerell. *Phenacoccus manihoti* was the most abundant and widespread mealybug species, and was reported from 37.0% (*n*= 582) and 100% fields (*n*= 52) in mainland Southeast Asia and Indonesia, respectively. Among sites, *P. manihoti* reached field-level incidence of 7.4 ± 15.8% (mean ± SD; i.e., proportion mealybug-affected tips) and abundance of 14.3 ± 30.8 insects per infested tip in mainland Southeast Asia, and incidence rates of 52.7 ± 30.9% and 42.5 ± 67.7 individuals per tip in Indonesia. Field-level incidence and population abundance were highly variable among settings and countries, reaching respective maxima of 100%, and 412.0 individuals per tip (Fig. 1).

When examining *P. manihoti* parasitism rates from a select set of sites, *A. lopezi* wasps were present in 96.9% of mealybug-affected fields (n= 97) in mainland Southeast Asia, yet were only found in 27.5% sites (*n*= 40) across Indonesia. Among sites, highly variable parasitism rates were evident with dry-season rates of 16.3 ± 3.4% in coastal Vietnam, versus 52.9 ± 4.3% in intensified systems of Tay Ninh (also in Vietnam). In Indonesia, *A. lopezi* was found in 22.0% fields in Lombok (*n*= 9) and was absent from prime growing areas in Nusa Tenggara Timur (NTT). In sites where *A. lopezi* had successfully established, dry-season parasitism ranged from 0% to 97.4%, averaging 30.0 ± 24.0% (*n*= 110) (Fig. S1). In fields where *A. lopezi* had effectively established, mealybug pest pressure was lower at increasing levels of parasitism ($F_{1,98}$= 13.162, p< 0.001; R^2= 0.118).

ii. Multi-year mealybug and parasitoid population assessment in Vietnam

Over the course of three years, we monitored *P. manihoti* abundance, field-level incidence and associated *A. lopezi* parasitism rates in Tay Ninh, southern Vietnam. Field-level incidence of *P. manihoti* ranged from 0% to 82%, averaging 24.8 ± 17.7% (mean ± SD) plants infested over two consecutive crop cycles. Mealybug incidence was significantly higher on older crops ($F_{7,57} = 9.9$; $p<0.0001$), and rapidly increased during the dry season. Similarly, mealybug abundance (average 5.6 ± 5.0 individuals per tip) was higher during the dry season ($F_{1,63} = 9.10$; $P = 0.0037$), and in crops older than six months compared to younger crops ($F_{7,57}$ =269 4.06; $P < 0.0001$). Mealybug population levels were comparable to those in Nigeria in 1982 (Fig. 2a), where *P. manihoti* attained 23% incidence and field-level abundance <10 individuals per tip soon after the release of *A. lopezi* (Hammond & Neuenschwander, 1990) (Fig. 2b). In Tay Ninh, *A. lopezi* attained mean parasitism rates of 42.3 ± 21.7%, with maxima of 76.7 ± 28.9% during the early rainy season (Fig. 2b). Overall, parasitism gradually increased over the dry season, up until crops were 4-6 months old.

Mealybug growth rates were significantly negatively correlated with parasitism levels across the 8 sites studied (GLM w/ Normal error distribution and corrected for field: $\chi^2_{87} = 125.4$; $P = 0.0017$; the field term was not significant) (Fig. 3). The x-intercept of each per-field regression represents the parasitism level above which mealybug growth rates are negative and this value ranged between 0.38 and 0.69 for the 8 sites (average = 0.47 ± 0.09) (Fig. 3). Whilst *A. lopezi* was the sole primary parasitoid at this location, three hyperparasitoid species attacked it at 2.79 ± 5.38% levels (as % parasitized hosts).

iii. Exclusion cage assays

Over the entire assay, *P. manihoti* populations under 'full cage' attained 48,318 ± 51,425 (*n*= 4; mean ± SD) and 7,256 ± 8,581 cumulative mealybug days (CMD) in 'sham cage' for one popular variety (i.e., R72) (Fig. 4). For a second variety, KU50, *P. manihoti* attained 28,125 ± 32,456 CMD in a 'full cage' treatment, and 1,782 ± 1,073 CMD in 'sham cage'. This compared to CMD measures in a 'no cage' control of 1,378 ± 1,039 and 342 ± 252, for R72 and KU50 respectively. CMD measures were significantly affected by treatment ($F_{3,189}$= 240.752, p< 0.001) and time ($F_{6,189}$= 113.347, p< 0.001), and the interaction term time x treatment ($F_{18,189}$= 2.012, p= 0.011). Furthermore, total CMD measures at the end of the trial significantly differed between treatments for both R72 and KU 50 ($F_{3,12}$= 6.767, p= 0.006; $F_{3,12}$= 11.152, p= 0.001, respectively).

Cassava yield parameters varied substantially under the four experimental treatments, and for both crop varieties (see also Thancharoen et al., *under review*). For Rayong 72, plant survival attained 37.5% under a 'full cage' set-up as compared to 75% and 87.5% under 'no cage' or 'sham cage' (Chi square, χ^2= 10.473, p= 0.015). Fresh root yield (FRY) was significantly affected by treatment ($F_{3,27}$= 4.104, p= 0.016) and variety ($F_{1,27}$= 4.364, p= 0.046). For R72 and KU50, FRY under 'full cage' was 74.6% or 71.2% lower than under 'sham cage'(Kruskal-Wallis, χ^2= 8.344, p= 0.039; χ^2= 19.134, p< 0.001, respectively), and respective yield reductions for both varieties were 77.2% and 67.8% compared to 'no cage' treatments.

iv. Country-wide yield changes

During the 2009 dry season, *P. manihoti* attained its peak population in Thailand, with field-level incidence near 100% and abundance rates of hundreds of *P. manihoti* per plant on at least

230,000 ha (Rojanaridpiched *et al.* 2013). Over the subsequent 2009-10 cropping season, province-level crop yields dropped by 12.59 ± 9.78% nationwide (weighted mean: -18.2%) (Fig. 5). Furthermore, country-wide aggregate yields declined from 22.67 t/ha to 18.57 t/ha, and total production dropped by 26.86% to 22,005,740 tonnes of fresh root. Following the lowered crop output, prices for Thai cassava starch increased 2.38-fold at domestic prices in Thailand, and 2.62-fold at export prices (US$ FOB) (Fig. S2). To differentiate *P. manihoti*-induced yield drops from climatic impacts, regression analyses were carried out. Multiple regression analysis revealed that a model with interaction terms between time and all other explanatory variables, i.e. time of introduction of *A. lopezi* and rainfall had the lowest AIC score and lowest residual deviance values. The model showed a significantly positive effect ($F_{7,183}$= 8.641) of the interaction term Time x Presence (i.e. 'presence' of *A. lopezi* and time , $p < 0.01$) on observed yields. Over 2009-2010, annual yield shifts in 51 cassava-growing provinces were not affected by changes in average monthly temperature and rainfall ($F_{3,33}$= 0.036, p= 0.991). By 2012, province-level yields were partially restored and then steadily increased to 21.42 ± 1.96 t/ha in 2015.

Discussion

In 2008, the invasive mealybug *P. manihoti* made its accidental arrival into Thailand. Through its extensive spatial spread, rapid population build-up and unrestricted feeding on plants (this leading to stunting and plant death), *P. manihoti* caused significant yield declines and a 27% drop in the nation's cassava production. This study shows how the neotropical wasp *A. lopezi*, released for mealybug control in 2010, had effectively established in 97% mealybug-affected fields in mainland Southeast Asia by 2014, and colonized 27% sites across insular Indonesia by late 2017. Attaining average dry-season parasitism rates of 30% across sites, *A. lopezi* populations readily oscillate with those of its mealybug host and suppress *P. manihoti* to incidence levels of 7% and background infestation pressure of a mere 14 individuals per infested tip. Experimental assays using two widely-grown cassava varieties reveal how biological control secures approximate yield gains of 5.3-9.4 t/ha. Our work clearly demonstrates how *A. lopezi* downgrades the invasive *P. manihoti* to non-economic status at a continental scale and enables a lasting yield-loss recovery. Offering a quantitative assessment of IBC's contribution to (the restoration of) primary productivity in Asia's expansive cassava crop, our work illuminates the broader societal value of biological control in a geographical region where there is heavy and increasing use of pesticides (Schreinemachers *et al.,* 2015).

Aside from featuring as 'beacon of hope' in Asia's pesticide-tainted agro-landscapes, our work heralds a new era for the discipline of insect biological control. Since the late 1800s, biological control has permitted the complete or partial suppression of 226 debilitating insect pests globally, formed the crux of transformative ecological theories (e.g., Hairston et al., 1960), and was widely deemed to be a safe, dependable and preferred means for (invasive) pest control. Following the release of Rachel Carson's 1962 *Silent Spring*, biological control was met with unrestrained enthusiasm and a firm belief in its potential as a reliable alternative to pesticide-centered practices. Yet, as concerns over its ecological risks rose following Howarth's (1983, 1991) denunciation of few cases of historic malpractice, regulatory hurdles surfaced, public funding lowered and the practice of insect biological control went through trying yet necessary reform (Strong & Pemberton, 2000; Hoddle, 2004; Messing & Brodeur, 2018). Over the past decades, IBC implementation has centered on ecological safety and increasingly strives to balance environmental benefits and risks (Heimpel & Cock, 2018). Though weed biological control has a 99% safety record (Suckling & Sforza, 2014), scientists are conscious that ecological risk will never be zero and certain factors are difficult to anticipate (Crooks & Soule, 1999; Sexton et al., 2017). Also, invasive pests routinely present far higher threats to native biota than judiciously-selected natural enemies with a narrow dietary breadth (Culliney, 2005). Though the 1980s Africa campaign against *P. manihoti* was implemented during times when the primary focus of insect biological control was on benefits (but see Neuenschwander, 2001), risks were considered minimal and did not delay implementation. The fact that *A. lopezi* was both effective and highly host-specific vindicated this. As a result, the implementation of IBC in Southeast Asia more than 30 years later was greatly facilitated by recognizing that (i) IBC had been effective across Africa's cassava-belt, and (ii) widespread benefits were gained in the overall absence of negative side-effects.

In light of the above, *A. lopezi* attained consistently high parasitism rates across most of the *P. manihoti* range of climatic suitability in tropical Asia (Yonow and Kriticos, 2017), except for Indonesia where it was only introduced at one site in late 2014. The far superior *P. manihoti* infestation pressure in eastern Indonesia (i.e., NTT, Lombok), where *A. lopezi* waits to be introduced, further accentuate the role of the parasitoid in suppressing cassava mealybug. Across locations, *A. lopezi* reached maximum parasitism levels of 98% (in late dry season, at Tay Ninh), which greatly surpassed the established threshold of 33-36% maximum parasitism rate for successful biological control (Hawkins and Cornell, 1994). At multiple sites, parasitism rates equally surpassed (max. 30%) levels from smallholder plots in Africa's savanna (Hammond and Neuenschwander, 1990). Factors ensuring this exceptional parasitoid efficacy and resulting pest control are a) unique features of the cassava crop, including prolonged durational stability, vegetational complexity and a constitutive secretion of energy-rich nectar for foraging parasitoids (Pinto-Zevallos *et al.,* 2016); b) spatio-temporal continuity of mealybug-infested crops at a landscape level (Schellhorn *et al.,* 2014), especially in sites where farmers employ staggered planting and piece-meal harvesting; c) favorable ecological traits of *A. lopezi*, including high dispersal ability, environmental adaptability and density-dependent parasitism (Neuenschwander *et al.,* 1989); d) non-usage of (prophylactic) insecticides, except for Thailand and parts of southern Vietnam; and e) the important human-assisted dispersal of A. lopezi, by mealybug-infested planting material (Herren *et al.*, 1987). Furthermore, substantial fertilizer inputs and suitable water management in areas with intensified cassava production -e.g., Vietnam's Tay Ninh province- likely benefited parasitoids further by boosting *A. lopezi* development and fitness (Wyckhuys *et al.,* 2017a). All of the above factors may have resulted in *P. manihoti* pest pressure that is largely identical to that observed during the Africa campaign, in which mealybug populations stabilized following the *A. lopezi* release at 23% incidence and field-level abundance below 10 individuals per tip (Hammond and Neuenschwander, 1990).

Exclusion cage assays illustrated how biological control enabled a root yield recovery of 5.3-10.0 t/ha in two main cassava varieties and how 2015 yields under 'no cage' ('real-world') conditions were in line with historic in-country yield tendencies. Though no direct field-level measurements were made of *A. lopezi* parasitism during the cage trials, biological control was found to occupy a central role in downgrading *P. manihoti* populations (Thancharoen *et al.*, under review), and *A. lopezi* is a determining factor in ensuring mealybug suppression in a similar fashion as in southern Vietnam (Le et al., 2018). Cage trials also revealed large variability in responses between the two cassava clones, likely reflective of differences in plant vigor and a clone's photosynthetic capability (Connor et al., 1981; Cock et al., 2012). The cassava plant possesses a unique set of features to sustain root production under (a)biotic stress, including the adaptive mobilization of biomass and a highly-effective use of resources (Cock et al. 2012). Yet, the pronounced production losses can be ascribed to continuous (unrestrained) attack of the active apex, direct damage to stems and high rates of plant death, especially for R72.

As *P. manihoti* currently occurs at low infestation pressure across mainland Southeast Asia, we believe that the above cage assays lend themselves to further extrapolation to a far broader geographical scale. Yet, slightly higher population levels were recorded in settings with sandy, low-fertile soils (Wyckhuys *et al.*, 2017a) and in Indonesian sites where *A. lopezi* had not yet made its arrival. The latter can now constitute a 'natural laboratory' to refine and validate existing projections on *A. lopezi*-mediated yield gain. Also, as landscape composition and plant disease infection status equally shape *P. manihoti* performance and efficacy of biological control at local scale (Wyckhuys *et al.,* 2017b; Le *et al.,* 2018), further replicated trials could be warranted to validate the robustness of our findings under varying agro-ecological contexts. Despite the above shortcomings, careful analysis of production statistics and commodity market fluxes (as in *section iv*) do lend support to our empirical results.

In tropical Asia, cassava underpins a multi-billion dollar starch sector, constitutes a key source of farm income and provides an (oftentimes indirect) means to food security for poor, under-privileged populations (Howeler, 2014; Delaquis *et al.,* 2017). On the one hand, the *P. manihoti*-induced yield shocks, as recorded during 2009-2011, can have major implications for rural livelihoods. Sustained pest attack can aggravate food security issues in areas where cassava is a prime food staple or progress into chronic 'poverty traps' (Tittonell *et al.,* 2013), all of which is counteracted through *A. lopezi*-mediated biological control. Aside from restoring FRY, *A. lopezi* equally helped recover a plant's total dry matter or 'biological yield' (Thancharoen *et al.,* under review), which is highly relevant as cassava leaves and shoots are widely consumed in tropical Asia. On the other hand, the net productivity loss of 5.14 million ton of fresh root equaled a respective loss of revenue of US$ 267.5-591.7 million (at 2009-10 factory price) for Thailand's cassava sector and the Asia-based starch industry. In any case, socio-economic impacts of the *P. manihoti* campaign are deemed to be substantial and potentially equal or even surpass those recorded in Africa (Zeddies *et al.,* 2001).

Yield recovery level in our cage assays were substantially higher than the 2.48 t/ha yield increase recorded through on-farm measurements in sub-Saharan Africa (Neuenschwander *et al.,* 1989). At Thai farm-gate prices, *A. lopezi*-mediated yield recovery equals to US$200-704 per ha (Thancharoen *et al.*, under review), although this does not take into account changes in production costs, local elasticities of supply and demand, or insecticide expenditures. Though we call for caution in extrapolating our findings, the approximate value of *P. manihoti* biological control could be hundreds of dollars higher than estimates of $63 ha^{-1} year^{-1} across global biomes including natural systems (Costanza *et al.* 1997), $33 ha^{-1} year^{-1} for (natural) biological control of the soybean aphid in the US Midwest (Landis *et al.,* 2008), or $75 to $310 ha^{-1}year^{-1} for bird-mediated pest control in Costa Rican coffee (Karp *et al.,* 2013). This strengthens arguments by Landis *et al.* (2008) and Naranjo *et al.* (2015) that the potential of insect biological control has been significantly under-valued, and that comprehensive cost-benefit analyses are urgently needed to raise (or restore) societal recognition of this prime ecosystem service.

These substantial economic benefits of (naturally-occurring, cost-free) biological control need to be contrasted with the unrelenting global increase in the use of chemically-synthesized insecticides for the mitigation of (domestic and, increasingly invasive) pests (Enserink et al., 2013). Following the *P. manihoti* invasion, pesticides have equally become pervasive in Thailand's cassava crop and growers have embraced the (prophylactic) use of neonicotinoid insecticides. Yet, given the omnipresence of *A. lopezi* and the largely low mealybug population levels across Southeast Asia, cost-effectiveness of such approaches needs closer scrutiny. Though pesticides do bring great benefits to society, they tend to simplify ecological communities, impact natural enemies and accelerate further pest proliferation (Lundgren and Fausti, 2015). On the other hand, our work shows that a carefully-selected, host-specific parasitoid constitutes a viable, most lucrative alternative to insecticide-centered approaches. Hence, potential (non-target ecological) risks of classical biological control clearly have to be viewed in terms of refraining from action and thus creating room for far-less environmentally-friendly tactics (Messing and Wright, 2006; Suckling and Sforza, 2014; Hajek *et al.,* 2016).

Conclusions

This study provides a quantitative assessment of how importation biological control helps restore primary productivity in Asia's cassava crop, following the arrival and extensive spread of an invasive sap-feeding pest. Our work reminds the reader of how IBC can provide durable and cost-effective control of an invasive pest such as *P. manihoti*, and deliver huge socio-economic and environmental benefits (Bale *et al.,* 2008). Aside from the concerns over its unintended ecological impacts, disciplinary silos and attitudinal factors have prevented routine (economic) valuation of biological control and a far broader recognition of its societal contributions (Naranjo *et al.,* 2015; Bale *et al.,* 2008). Hence, our characterization and (approximate) valuation of *P. manihoti* biological control is clearly not an end in itself, but should now become a starting point for further awareness-raising, and efforts to guide and inform policy and agile decision-making (Daily *et al.,* 2009). In a world typified by massive declines in insect numbers, extreme biodiversity loss, and dwindling public interest in biological control (Bale *et al.,* 2008; Hallmann *et al.,* 2017; Warner *et al.,* 2012), our research underlines the immense yet largely untapped potential of ecologically-based approaches to resolve invasive species problems, intensify global agriculture and feed a growing world population in the 21st century.

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Author contributions

KAGW, AR, TTNL, AT and PW conceived and designed the experiments; AT, MZF, IG, TTNL, performed trials and collected the data; KAGW, MZF, IG, AT, LKP, DDB and GH analyzed the data; all authors co-wrote the paper

Competing interests

There are no competing interests.

Figure legends:

Figure 1. Map of Southeast Asia, depicting *P. manihoti* spatial distribution, infestation pressure and *A. lopezi* parasitism rates. Doughnut charts in the left and right margins represent field-level incidence (i.e., red portion reflecting the proportion of *P. manihoti* affected tips, ranging from 0 to 1 for full circumference), and are complemented with bar charts indicative of plant-level *P. manihoti* abundance (i.e., average number of individuals per tip). The number inside each doughnut reflects the number of fields sampled per locale. Doughnut charts in the lower panel indicate average *A. lopezi* parasitism rate at six selected sites (depicted by the dark green section, reflecting proportion parasitism ranging from 0 to 1 for full circumference). The distribution map is created as overlay on a 2005 cassava cropping area (MapSpam, 2017).

Figure 2. Bi-monthly mealybug population fluctuations in southern Vietnam, as contrasted with those in 1982 Nigeria. Vietnam's *P. manihoti* dynamics (*panel a*) are contrasted with those in Nigeria following the 1982 release of *A. lopezi*. In *Panel b*, field-level *P. manihoti* abundance (n= 8) is contrasted with respective *A. lopezi* parasitism rates, from July 2013 until July 2015.

Figure 3. Effect of cassava mealybug parasitism rate on intrinsic rate of mealybug increase over consecutive 2-month periods in Tay Ninh, Vietnam. Lines are linear regressions per each of the eight sites monitored. The red dotted line shows r=0; values above this on the y axis indicate positive growth of mealybug populations and below it indicate negative population growth. Parasitism level above which *P. manihoti* growth rates are negative ranged between 0.38 and 0.69 for the 8 sites. See text for statistical details.

Figure 4. Mealybug abundance and subsequent yield parameters for two cassava varieties under an exclusion cage assay at Rayong, Thailand. Six weeks after experimental set-up, mealybug abundance (n = 16; mean ± SE) is compared between treatments for two common varieties (R72, KU50), and is significantly higher under 'full cage' conditions (i.e., exclusion of natural enemies, incl. *A. lopezi*), as compared to 'sham cage' and un-caged controls (ANOVA, $F_{2,45}$= 50.289; P< 0.001). For each treatment, fresh root yield is determined at time of harvest, on a 12-month old crop.

Figure 5. **Annual percent shifts in crop yield for 51 cassava-growing provinces in Thailand, reflective of the mealybug invasion and ensuing biological control.** Shifts cover the country-wide spread of *P. manihoti* from late 2008 until 2011, the first release of *A. lopezi* (Nov. 2009) and subsequent nation-wide distribution of the parasitoid from June 2010 onward. Province-level yield shifts depict the percent change of crop yield in one given year, as compared to the previous year.

Figure 1

Map of Southeast Asia, depicting *P. manihoti* spatial distribution, infestation pressure and *A. lopezi* parasitism rates.

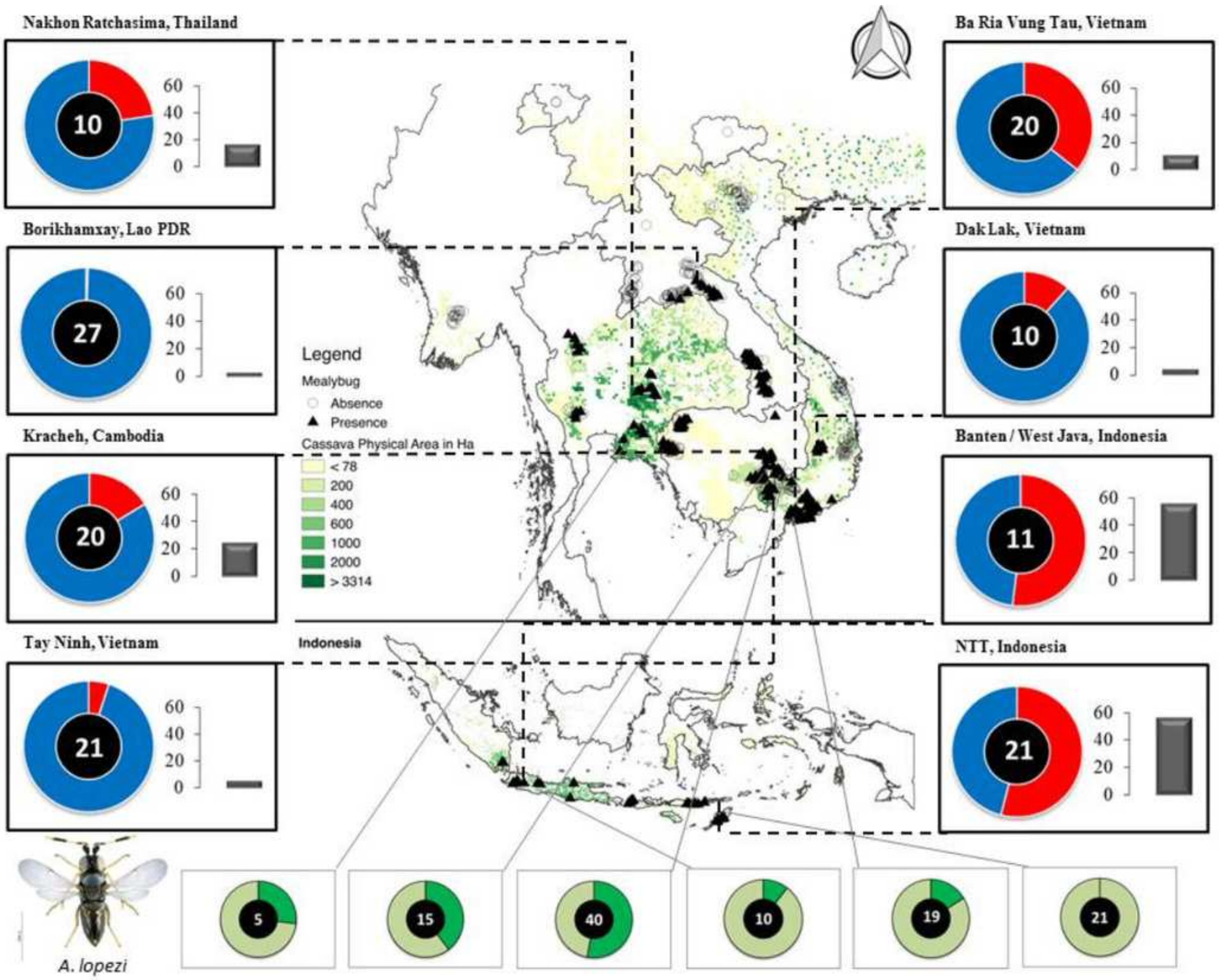

Figure 2

Bi-monthly mealybug population fluctuations in southern Vietnam, as contrasted with those in 1982 Nigeria.

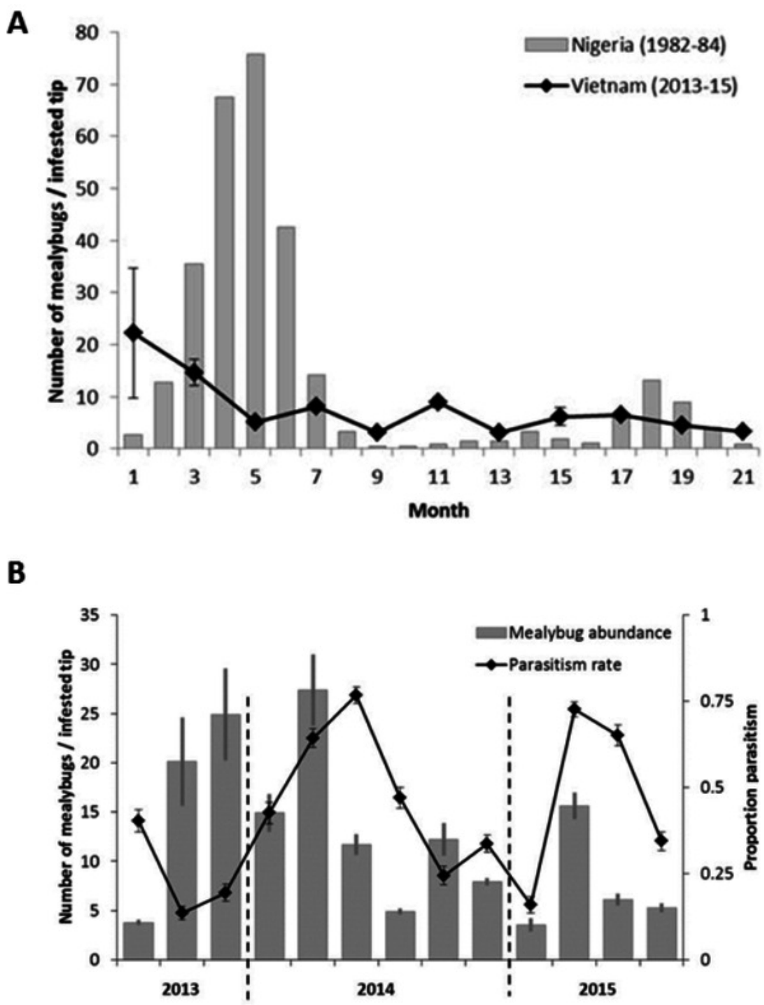

Figure 3

Effect of cassava mealybug parasitism rate on intrinsic rate of mealybug increase over consecutive 2-month periods in Tay Ninh, Vietnam.

Lines are linear regressions per each of the eight sites monitored. The red dotted line shows r=0; values above this on the y axis indicate positive growth of mealybug populations and below it indicate negative population growth. Parasitism level above which *P. manihoti* growth rates are negative ranged between 0.38 and 0.69 for the 8 sites. See text for statistical details.

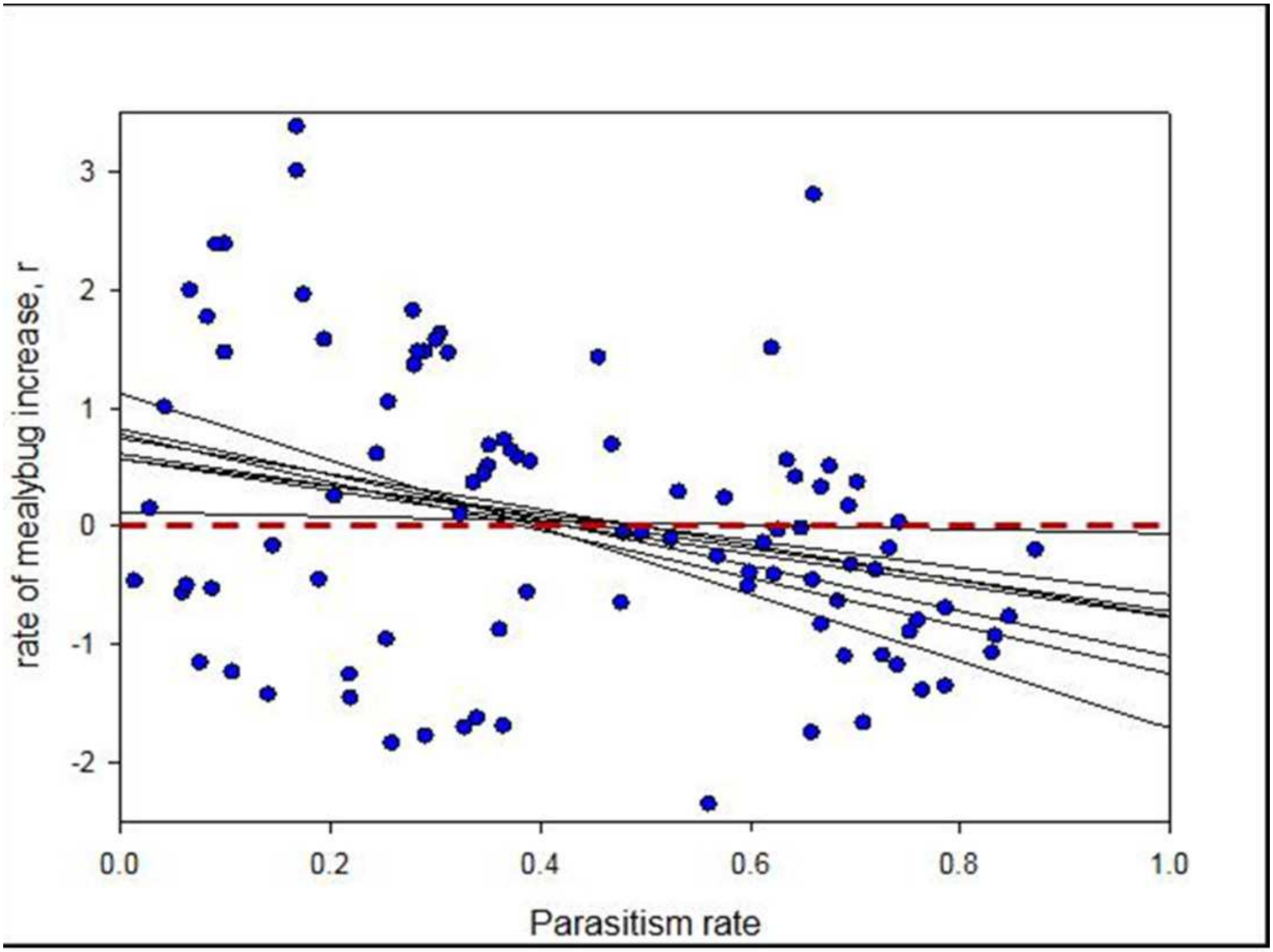

Figure 4

Mealybug abundance and subsequent yield parameters for two cassava varieties under an exclusion cage assay at Rayong, Thailand.

Six weeks after experimental set-up, mealybug abundance (n = 16; mean ± SE) is compared between treatments for two common varieties (R72, KU50), and is significantly higher under 'full cage' conditions (i.e., exclusion of natural enemies, incl. *A. lopezi*), as compared to 'sham cage' and un-caged controls (ANOVA, $F_{2,45}$= 50.289; P< 0.001). For each treatment, fresh root yield is determined at time of harvest, on a 12-month old crop.

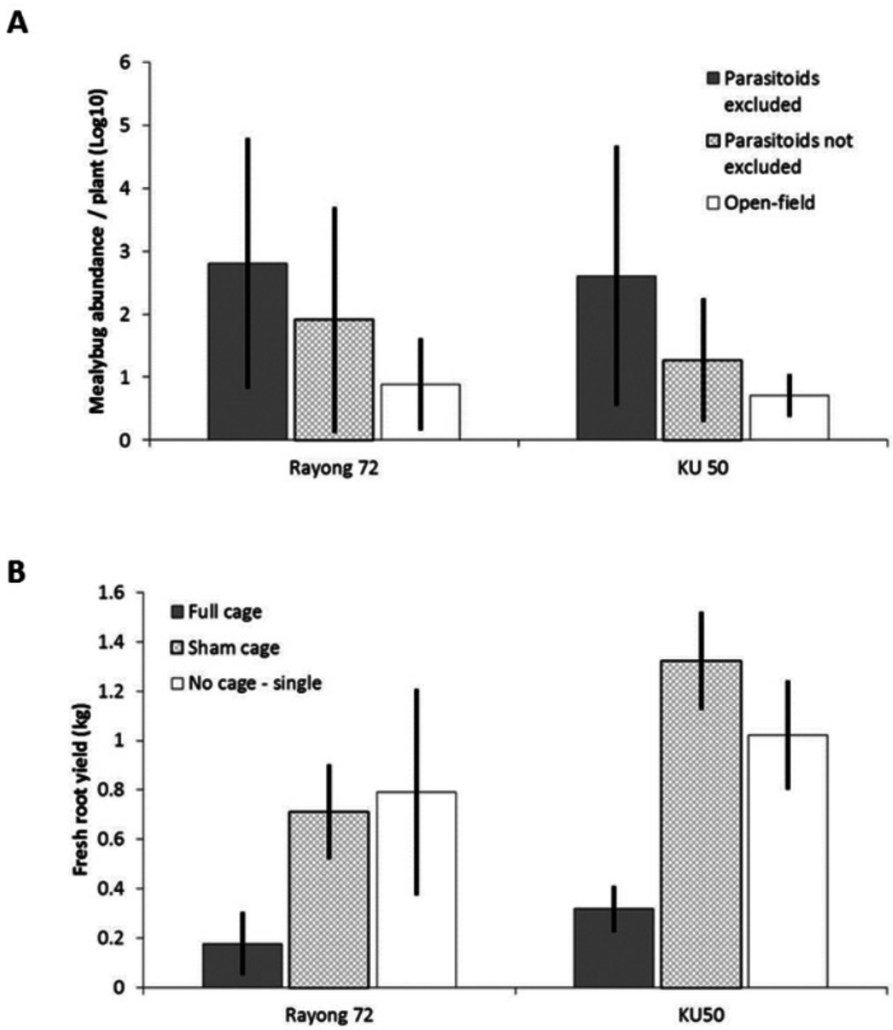

Figure 5

Annual percent shifts in crop yield for 51 cassava-growing provinces in Thailand, reflective of the mealybug invasion and ensuing biological control.

Shifts cover the country-wide spread of *P. manihoti* from late 2008 until 2011, the first release of *A. lopezi* (Nov. 2009) and subsequent nation-wide distribution of the parasitoid from June 2010 onward. Province-level yield shifts depict the percent change of crop yield in one given year, as compared to the previous year.