RESEARCH

e-Xtra*

Impact of Insecticide Applications on Arthropod Predators and Plant Feeders in Cambodian Rice Fields

Chhay Ngin, Department of Rice Crop, General Directorate of Agriculture, Ministry of Agriculture, Forestry and Fisheries, Phnom Penh, Cambodia, and Nagoya University Asian Satellite Campus-Cambodia, Royal University of Agriculture, Phnom Penh, Cambodia; **Seng Suon**, Center for Development-Oriented Research in Agriculture and Livelihood Systems, Phnom Penh, Cambodia; **Toshiharu Tanaka**, Nagoya University Asian Satellite Campus-Cambodia, Royal University of Agriculture, Phnom Penh, Cambodia; **Akira Yamauchi** and **Kazuhiro Kawakita**, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan; and **Sotaro Chiba**,[†] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan, and Asian Satellite Campuses Institute, Nagoya University, Nagoya 464-8601, Japan

Accepted for publication 11 August 2017.

ABSTRACT

Rice (*Oryza sativa*) is the staple crop of Cambodia. The heavy use of pesticides has caused significant financial losses to local farmers. This study examined the necessity and economic viability of insecticide use for rice cultivation, and the multiple interactions between the populations of both insect pests and natural enemies in dry-season rice fields. The outcomes of calendar-based insecticide-sprayed and non-sprayed rice fields were compared at three provincial research stations in Cambodia in terms of rice growth, economic returns, damage caused by insect pests, and population dynamics of insect pests and arthropod natural enemies. The results showed that rice fields without insecticide application did not suffer significant damage by insect pests or by diseases and had similar yields to those treated with insecticide application in two provinces. Although the occurrence of caseworm

was observed in the third province, this was successfully controlled by an integrated pest management (IPM) treatment without using insecticides. Insufficient densities of natural enemies appeared to correlate with the caseworm outbreak in this location, whereas high densities of natural enemies relative to those of insect pests contributed to control potential pest expansion in the other two provinces. Natural enemy populations were significantly decreased by insecticide applications. Reduced insecticide input in the insecticide-free control and IPM plots resulted in higher net profits than those in insecticide-applied plots, except for plots in the region with a caseworm outbreak (insecticide-free). Overall, this study provides encouraging insight into cost effective and environmentally friendly pest management in Cambodian rice fields.

A holistic understanding of biological and environmental interactions within agriculture, which collectively form a phytobiome, is becoming an important focus for the development of sustainable crop production (Bianchi et al. 2006; Kathiresan 2007; Way and Heong 1994). This approach advocates maintaining soil health, strengthening plant tolerance to environmental stresses and the natural control of numerous pests in the fields. However, within modern agriculture, many chemical fertilizers and pesticides such as insecticides, fungicides, and herbicides, biological control agents, and plant growth promoting microorganisms are introduced to the

crops (Paredes and Lebeis 2016). Although these artificial inputs can be useful, they are also considered the main cause of biodiversity loss in the field (Wilby and Thomas 2002). Therefore, it is clear that new approaches toward modernizing agriculture are required, and to this end, fundamental knowledge must be accumulated to determine the required levels of biodiversity in agricultural crops and to understand the interactions between species, particularly in conjunction with human activities (Ooi and Kenmore 2005; Savary et al. 2012).

To resolve the food crisis in the near future, the roadmap to solve this problem has been debated from an array of aspects (Fraser et al. 2016). Since rice (*Oryza sativa*) is the most important cereal and supports food demand in most Asian countries, phytobiome studies in rice fields will contribute to food security in Asia where population and food demand are growing most rapidly. Even though rice fields are anthropogenic ecosystems, these accommodate a rich biodiversity, including diverse insect pests and natural enemies (including parasitoids, pathogens to insect pests, and predators such as spiders, fishes,

[†]Corresponding author: S. Chiba; E-mail: chiba@agr.nagoya-u.ac.jp

*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary figures and five supplementary tables are published online.

frogs, and snakes) (Shepard et al. 1987). Those pests demonstrate rapid colonization, reproduction, and growth in response to changes in the ecosystem (Bambaradeniya et al. 2004). Complex interactions exist among biotic and abiotic agents in such ecosystems, and these are easily influenced by a wide range of factors, including seasonal changes, various agricultural practices (field preparation, crop varieties, agro-chemicals, etc.), and geographical factors.

To intensify rice production, irrigated areas have been expanded and modern improved cropping practices have been adapted, which entailed the greater use of insecticides, and this has had a serious impact on the health of the farmers and the environment, and safer alternatives, including integrated pest management (IPM), aquaponics, and organic farming are now recommended (Nguyen-Vaucheret et al. 2002). In Vietnam and the Philippines, a high proportion of insecticide sprays target leaf feeding insects, which accounted for 42% and 28% of the total insecticide sprays used, respectively (Heong et al. 1994). However, much of these insecticides may be unnecessary because the control of leaf feeders generally does not increase rice yields. This assertion was supported in part by our previous study which found that insecticide application did not increase the rice yield, but rather decreased the net income of farmers in Cambodia (Chhay et al. 2016). Moreover, it has been reported that most Cambodian farmers are inclined to follow the instructions of pesticide retailers, thus establishing routine calendar-based pesticide applications (Matsukawa et al. 2015; Seng and Chhunhy 2012). Such a farmers' attitude generally escalates the indiscriminate use of pesticides by paying scant regard to target pests, chemical dosage and timing of applications (Heong et al. 1995; Pretty and Hine 2005). IPM was introduced to Cambodia in 1993, and promotes a holistic approach to crop production and protection to provide greater integration of management practices, thereby enabling informed decisions for pest management that go beyond simple scouting and economic threshold levels (Chhay et al. 2016; Gallagher et al. 2005).

Early-season insecticide applications are generally considered effective but this approach destroys the ecological balance and kills predators and parasitoids, thus, leaving the crop open to potential pests (IRRI 2016; Matteson 2000). A study by the International Rice Research Institute (IRRI) previously found that the arthropod biodiversity could be restored by decreasing insecticide usage by 95%, eventually preventing planthopper outbreaks because of rejuvenated ecosystem services through the important roles played by parasitoids and predators (Heong 2009). Natural enemies are ubiquitous in rice fields and are responsible for maintaining populations of rice herbivores at a low level in tropical Asia (Heong et al. 1991, 1992).

Cambodia has a tropical monsoon climate with two distinct seasons: wet (May to October) and dry (November to April). Rice is the main crop covering 3.05 million ha, which is approximately 80% of the total agricultural area (Ministry of Agriculture, Forestry and Fisheries 2015; Nesbitt 1997; Sarom 2007). Of this area, 15% is used for fully irrigated rice, whereas the remainder is used for rain-fed lowland rice (Makara and Chhay 2014). During the wet season in Cambodia, there is a rich biodiversity in rice fields and severe damages associated with insect-pests are rarely observed even in insecticide-free fields, thereby the ecosystem services seemingly prevents serious yield losses (Chhay et al. 2016; Huan et al. 2005). In contrast, rice cropping is restricted to irrigated areas during the dry season, and it is possible that biodiversity maybe reduced. The effectiveness of this ecosystem functions during the dry season has not been investigated.

It is anticipated that biologically-balanced pest management is one of the best options to maintain the ecosystem services. In this sense, the Cambodian rice field provides an attractive model because it has shown good potential for rice ecosystem functions for the natural biocontrol of insect pests, at least during the wet season. In the present study, we conducted arthropod phytobiome studies in

Cambodian rice fields by specifically focusing on the dry season when the biodiversity and ecosystem services may be reduced. The population dynamics of insect pests and their natural enemies, rice yield and its components, and the economic viability were compared between non-insecticide application plots and insecticide-treated plots in three separate provinces in Cambodia.

MATERIALS AND METHODS

Research locations. Field studies were conducted at sites located at three agricultural research stations: (1) Koktrap Agricultural Research Station located in Svay Rieng Province (location 1); (2) Polors Agricultural Research Station located in Prey Veng Province (location 2), and; (3) Tonle Bati Agricultural Development Center located in Takeo Province (location 3). These locations were selected for three main reasons: (i) they are located in major rice producing provinces containing large rice cultivation areas, particularly during the dry season (Ministry of Agriculture, Forestry and Fisheries 2015); (ii) many rice farmers in these areas apply large quantities of insecticides to control insect pests with the perception that this approach increases the yield in the dry season, particularly in the areas bordering Vietnam; and (iii) the locations have different soil types; therefore, the effects of the study treatments can be compared for different soil groups in Cambodia.

In general, the majority of soil types in Svay Rieng are poorer soil fertility compared with the soil types in Takeo and Prey Veng, which both have a good potential to produce high rice yields, particularly during the dry season (Nesbitt 1997; White et al. 1997). Koktrap soils are prevalent in location 1 on old alluvial terraces, and have a dark gray or very dark brown to black topsoil with a clay or loam texture over a light gray or light brown loamy or clay subsoil. Prateah Lang soils are prevalent in location 2 on the old alluvial terraces or colluvial-alluvial plains and have a sandy topsoil <40 cm deep over a subsoil that has a loam or clay texture. Toul Samroung soils are prevalent in location 3 on the old alluvial terraces or colluvial-alluvial plains. They have a clay or loam topsoil which forms wide cracks that penetrate more than 3 to 4 cm into the soil over a clay or loam subsoil (White et al. 1997). The geographic locations of the three study sites are shown in Figure 1.

Most of the rice-growing areas in the three study locations receive between 1,107 and 1,370 mm of rainfall annually. Rainfall varies widely by season (particularly in July, August, October, and November) and by province. During July 2015, the total amount of rainfall was 204.8 mm in Svay Rieng but only 67.8 mm in Takeo. During November, the rainfall in Takeo was 329.5 mm and only 18.2 mm in Prey Veng (Supplementary Fig. S1). Rainfall tends to peak in August, September, and October, although small droughts often continue for three weeks or more during any of these months, and crops suffered without some form of supplementary irrigation. Although some rainfall was recorded in Svay Rieng and Takeo, the three study locations did not receive any rainfall during the study period from January to early April. Temperatures did not vary significantly between provinces. The peak of the hot humid season occurs during April when temperatures reach a maximum of 39°C and drop in the cool season to a minimum of 19°C in January. However, the average monthly temperatures are 31°C in April and 26°C in January (Ministry of Water Resource and Meteorology 2015).

Treatments in experimental rice fields. The present study was conducted during the dry season from January to April 2015. The four experimental treatments used were as follows:

1. Treatment 1 (T1): No use of insecticides nor IPM techniques (control).

2. Treatment 2 (T2): Application of IPM techniques when necessary without the use of insecticides. In location 1, action was taken to control caseworms by maintaining dryness of the T2 plot for 5 days, following which irrigation was resumed. These activities were not carried out in locations 2 and 3.
3. Treatment 3 (T3): Sprayed with insecticides three times at 15, 30, and 45 days after transplanting (DAT), following an established spraying calendar.
4. Treatment 4 (T4): Sprayed with insecticides four times at 15, 30, 45, and 60 DAT.

For T3 and T4, the insecticide Cyperan 10EC (active ingredient: Cypermethrin) was used to spray the fields at a dosage of 2.0 liter/ha per application based on the calendar schedule. This insecticide is commonly used by farmers to control insect pests in Cambodian rice fields and it is on the list of recommended insecticides for rice pest control of the Plant Protection, Sanitary, and Phyto-Sanitary Department under the Ministry of Agriculture, Forestry and Fisheries (MAFF) (Chhunhy et al. 2014).

Field design and cultivation techniques. A complete block design with three replicates was used within the present experiment (Gomez and Gomez 1984). A 4-m buffer zone of rice crops between the treatments was established to minimize the effects of the insecticide on the insect pests and natural enemies across the different treatments when it was applied. Moreover, the treatment blocks were orientated perpendicular to the wind direction and the insecticide-free treatments were placed in the upwind position to avoid insecticide drift from sprayed treatments to non-sprayed treatments. The design of the field experiment is shown in Figure 2. The plot size is 30 m², which is in a range suggested by IRRI for testing insecticides on rice (Heinrichs et al. 1981). The IR66 variety with 18-day-old seedlings was transplanted at a spacing of 20 by 20 cm². This variety is commonly grown by farmers during the dry season. Fertilizer application was based on Cambodian Agricultural Research and Development Institute (CARDI) recommendations in compliance with the soil type (White et al. 1997). Disease and weed management was similarly applied in all plots across the three locations when necessary.

Data collection and collection methods. Data for insect pests and natural enemies were collected weekly for 8 weeks starting at 15 DAT and finishing at 67 DAT. To observe these organisms, the rice fields were covered by net cages (60 × 60 cm) to facilitate a visual count (three samples per plot). The cages were first placed in the fields in all treatments and then the visual count was conducted. The cages were removed from the fields after count was completed. For the purpose of the present study, we observed six major insect pests (caseworms, stem borers, leafroller, gall midges, brown planthopper (BPH), and rice bugs) and seven groups of predators (spiders, dragonflies/damselflies, lady beetles, ground beetles/earwigs, long-horned grasshoppers/crickets, mirid bugs, and water bugs). In addition, visible parasitoids were also counted to assess their population dynamics in the insecticide-free and insecticide-treated plots. The scientific names of these arthropods are written in Supplementary Table S1. It is likely that not all flying and fast moving natural enemies such as dragonflies, ground beetles, and earwigs were caught in the cages during data collection; therefore, the counts are probably underestimated. Because parasitoids are small and species identification is very difficult by eye in the field, particularly within the parasitic wasps, they were observed and counted as one group. Thus, their numbers are likely underestimated. The insect pests and their natural enemies commonly found in Cambodian rice field are summarized in Supplementary Table S1 (Department of Rice Crop 2011).

The damage caused by different insects was assessed in accordance with the IRRI standard evaluation system for rice (International Rice Research Institute 2002) for each stage of the crop (recorded weekly over 8 weeks); a sum of damage scores was considered the total injury by insect pests and compared between treatments.

Data on yield components including the numbers of tillers, panicles, and full grains/panicle, the weight/1,000 grains, and grain yield were collected based on the following methods:

1. Rice was harvested from three 1-m² sample quadrats (yield, kg/m²).
2. The number of tillers was collected from one hill in each sample quadrat at the maximum tilling stage.

Fig. 1. Map of locations of research stations where the experiments were conducted. Exact location of research stations in Svay Rieng, Prey Veng, and Takeo provinces was recorded by GPS coordinate. The soil types and fertilities of the research stations are different from each other (Cambodia–IRRI–Australia Project, 2000; Cambodia Agricultural Research and Development Institute).

3. The number of panicles was collected from one hill in each sample quadrat before harvesting.
4. The number of full grains per panicle was counted from three panicles for each sample quadrat after harvesting.
5. The grain weight (g) of 1,000 full grains was weighed for each sample quadrat.
6. Economic data (4,000 Riel to 1 U.S. dollar, USD): net profit was calculated as follows: revenue (USD/m²) – cost (USD/m²); revenue was calculated as follows: yield (kg/m²) × selling price (2.25 USD/kg).

In the net profit comparison between treatments, only the costs related to insect-pest management were variable but the other production costs including the abovementioned factors were kept constant for all treatments. The total cost of insecticide application was calculated by summing up the cost of insecticide (7.5 USD/liter) and hiring labors to do the spraying (6.25 USD/labor/day) in T3 and T4.

Data analysis. An analysis of variance (ANOVA) was first used for mean yield comparisons to determine if there were any significant differences between treatments. If a significant difference was detected, then a mean comparison of the yield components was performed to determine which component contributed to the yield difference. Mean comparisons were also performed on data from the three experimental sites to assess consistency and evaluate whether there were significant differences among the three locations through one-way and two-way ANOVA. Moreover, the populations of insect pests and natural enemies and levels of damage were analyzed and evaluated. All statistical tests were performed using the Statistical Package for Social Sciences (IBM SPSS Statistics, ver. 22.0).

RESULTS

Differences in yield and its components between insecticide-free and insecticide-treated rice plots. Five rice yield components among treatments in the three study locations were compared: (i) rice yield (kg/m²); (ii) tiller number (number/m²); (iii) panicle

number (number/m²); (iv) full-grain number (full-grain/panicle); and (v) grain weight (g/1,000 grains). The yield comparison across the three locations showed a significant difference in the order of location 3 (Tonle Bati station, Takeo Province) > location 2 (Polors station, Prey Veng Province) > location 1 (Koktrap station, Svay Rieng Province) ($P < 0.01$) (Figs. 1 and 3A). Interestingly, similar yields were obtained among treatments in both locations 2 and 3, indicating no obvious impact of insecticide (T3 and T4) and IPM (T2) in these locations. However, in location 1, the yield of T1 (non-treatment control plot) was significantly lower than those of the other treatments T2, T3, and T4 (Fig. 3). The numbers of tillers and panicles showed the same trend as yields (Fig. 3B and C), whereas the full-grain number and 1,000 grain weight were not significantly different among all treatments across three locations (data not shown). Indeed, even when the insecticide was not applied, a pest-associated yield reduction was suspected only in location 1 but not in locations 2 and 3.

Higher economic returns in insecticide-free fields were observed when ecosystem services worked well. The net profit (USD/m²) in all treatments was analyzed. When the same treatments were compared across locations, location 3 had the highest profit, whereas location 1 had the lowest, thereby same profitability trend was observed in Farmer Field Schools in these three Cambodian provinces (Chhay et al. 2016). In most cases, T4 produced significantly lower profit than those of the other treatments, and this was commonly followed by T3 (Fig. 3D). However, T1 in location 1 showed a low profit, similar to T3, due to possible pest-associated damages and low yield (Fig. 3A and D). With regard to overall cost factors, the cost for insecticide application was the major difference between non-chemical (T1, T2) and chemically treated (T3, T4) plots (data not shown). T2 under IPM practices always produced the highest net profit in all locations. Nevertheless, insecticide-free T1, with no serious pest-associated damage in locations 2 and 3, achieved similar profits and yields to T2 (Fig. 3A). Therefore, the change from chemical insecticide application to IPM is promising for the maintenance of the yield, increase the incomes of farmers,

Fig. 2. Experimental design for insecticide-sprayed and non-sprayed trials in rice fields. T1, T2, T3, and T4 represent treatments 1, 2, 3, and 4, respectively, whereas R1, R2, and R3 represent replicates 1, 2, and 3, respectively (described in methodology). White open boxes represent experimental plots, and the gray highlighted region represents the buffer space between each treatment. Direction of the seasonal wind during dry season (northward-blowing) is shown in relative to plot design.

and the effort required from farmers might be minimal under well-organized ecosystems as such in locations 2 and 3.

Overall damage by insect pests. The T1 plot in location 1 was expected to incur damage by insect pests and its yield was accordingly reduced. The score of damages on rice plants due to six major pests was recorded weekly at eight time points. A comparison of total pest-associated damages revealed that only T1 had damages at a significantly higher level than those in the other three treatments in location 1 (Fig. 4A). The damage caused by caseworm in T1 was significantly higher than those in T2, T3, and T4 (Fig. 4B), indicating that caseworms were the cause of the rice yield reduction in T1. Caseworms were obviously under control by insecticide applications in T3 and T4, and IPM practices in T2. Damages caused by caseworm in locations 2 and 3, and by the other five pests in all locations, were all similar (data not shown). These results suggested that insect pests were naturally less abundant in locations 2 and 3, thereby being consistent with the high yields of rice, regardless of insecticide application (Fig. 3A). It should be noted that any serious disease-associated damages were not observed in the present study.

Comparison of total numbers of insect pests and natural enemies in rice fields. We had hypothesized that the natural rice farm ecosystem in locations 2 and 3 accommodated sufficiently rich biodiversity to control insect pests, whereas that in location 1 was poor. The total populations of selected insect pests and natural enemies were counted at 15, 22, 30, 37, 45, 52, 60, and 67 DAT (eight times) to understand biodiversity in three locations. Insecticide was applied to T3 and T4 plots at 15, 30, and 45 DAT and additionally to T4 at 60 DAT in this study, according to the instructions of pesticide retailer (during vegetative to ripening stages; see red arrowhead in Fig. 5D). It is important to note that the insecticide was applied after the counting of arthropods at 15, 30, 45, and 60 DAT so the effects of the insecticide appeared on the following counting days, namely 22, 37, 52, and 67 DAT.

It was noted that total insect pest numbers were not statistically different among the four treatments in each of the three locations, even if the insecticide was sprayed three times and four times in T3 and T4, respectively (Supplementary Tables S2 and S3). This result

Fig. 4. Comparison of damage levels of rice caused by insect pests. Total damage scores of **A**, six major pests and **B**, that of caseworm. Sum damage values of eight observation time points are compared. Letters A to D indicate a significant difference at $P < 0.01$.

Fig. 3. Yield components and economic returns for four different treatments in three research stations. **A**, Rice yield (kg/m^2), **B**, tiller numbers/ m^2 (tiller/ m^2), **C**, panicle numbers/ m^2 (panicle/ m^2), and **D**, total net profit (USD/m^2) for T1, T2, T3, and T4 were compared by research station. Letters A to D indicate a significant difference at $P < 0.01$.

indicated that insect pests recovered rapidly after insecticide application, and pest populations were not significantly decreased as a whole. Secondly, the numbers of total natural enemies in T3 and T4 were significantly lower than those in T1 and T2 in all three locations due to the impact of insecticide treatments. In that respect, the repeated insecticide applications resulted in reduced populations of pests and natural enemies in insecticide-treated plots (T3 and T4) after every spraying compared with those in the non-treated control plot (T1) and the insecticide-free IPM plot (T2) (Fig. 5A to C).

Fig. 5. Population dynamics of total insect pests (solid line) and total natural enemies (dotted line). The total number of insect pests and natural enemies in locations 1, 2, and 3 are presented in **A**, **B**, and **C**, respectively. A schematic representation of the different phases and growth stages of a rice crop (IR66 variety) is shown in **D**. Red arrowheads indicate dates of insecticide applications in T3 and T4. DAT, day after transplanting.

These population reductions by the insecticide were significantly more apparent in natural enemies (Fig. 5, dotted lines) than those for insect pests (Fig. 5, solid lines), as supported by statistical analyses. These data indicate that the insecticide was more effective at killing natural enemies than insect pests, highlighting a negative impact of pesticides on ecosystem services.

Balance of insect pest and natural enemy populations in nature. The insect/arthropod population data from T1 can be considered the standard structure of rice ecosystems (control). Therefore, we compared those across the locations to understand why more pest damage was experienced in location 1 compared with the other locations. As shown in Supplementary Figure S2, the total number of insect pests in location 1 was obviously high from 15 DAT (the beginning of the experiment) until 52 DAT; however, those in locations 2 and 3 were very low (compare solid lines in Supplementary Fig. S2). On the other hand, natural enemies were abundant in locations 1 and 3, and moderately abundant in location 2. Thus, the assertion that the large population gap between insect pests and natural enemies in location 3 resulted in successful natural biocontrol is reasonable. Although a relatively low density of natural enemies was observed in location 2, the original numbers of pests were also at low levels, and these were kept under control. However, the pests with high population numbers in location 1 were not successfully controlled by natural enemies, despite the population densities exceeding that of the insect pests.

The gap between the number of natural enemies and insect pests at location 1 was small at 30 DAT when the damage caused by caseworms started becoming evident. During the same period, the gap in the number of natural enemies in locations 2 and 3 was large, and this may have contributed to the suppression of caseworm multiplication to a level that yield was not affected (compare insect pest versus natural enemies at 30 DAT in locations 1, 2, and 3, respectively). Thus, an understanding of both the environmental conditions and the trends of biodiversity (arthropod phytobiome information) in regional rice fields may have been important to estimate the efficacy of ecosystem services in natural biocontrol.

Population dynamics of insect pests and natural enemies in Svay Rieng Province (location 1). Finally, we analyzed the population dynamics of arthropods in location 1. Under the natural condition (T1), the pest arthropods prevalent in the vegetative phase until 30 DAT were caseworms, gall midges, and BPH. By 30 DAT, there was an increase in the populations of stem borers and leaffolders, whereas the maximum number of caseworms occurred at 37 DAT, but suddenly dropped subsequently, whereas the population densities of the remaining four pests were maintained until 45 DAT, after which they started to decrease (Fig. 6A). The numbers of rice bugs increased at 30 DAT and 52 DAT, and were present at the ripening phase together with leaffolders. Similar trends of pest population dynamics were observed in the IPM plot (T2), except for the successful prevention of the caseworm (Fig. 6A, black lines) outbreak at 37 DAT (water removal was conducted) (Fig. 6A, T2, blue arrowhead). The numbers of rice pests in insecticide treated plots T3 and T4 drifted slightly below the levels in T1 and T2. The caseworms and BPH were decreased after insecticide treatments (applications are indicated by red arrowheads in Fig. 6A). The number of caseworms in T1 was statistically different to those in other treatments particularly at 37 and 45 DAT when the caseworm outbreak was observed in T1 (Supplementary Table S4), thereby further indicating that T1 sustained considerable damage by caseworms, resulting in yield reduction (Fig. 3A and B).

Natural enemies started to colonize the fields at the beginning of the tillering stage, and their numbers increased until the end of the reproductive phase before declining at the end of the ripening phase. Populations of spiders, mirid bugs, dragonflies, and water bugs were high during the early rice crop stages, whereas populations of ground

beetles, lady beetles, and long-horned grasshoppers increased during the later stages, and populations of all species declined toward the end of the ripening phase (Fig. 6B). The most striking result was that the numbers of parasitoids in T3 and T4 were significantly low, indicating that they were very sensitive to insecticide applications (Fig. 6B, brown lines) as supported by statistical analyses (Supplementary Table S5).

DISCUSSION

From comparisons of arthropod population dynamics across Cambodian rice fields under different insect pest management, we

generally found similar trends in population shifts; however, the outbreak in caseworms in the plot with non-insecticide treatment in location 1 resulted in a significant loss in yield (Figs. 3, 5, and 6). In the rice fields under non-insecticide treatment, major factors influencing pest-associated rice damages were considered to be the basal populations of insect pests and the gap between insect pest populations and those of natural enemies. Thus, we were reminded of the importance of careful observation of pests and rice damage in insecticide-free cultivation because of the caseworm outbreak in T1 of location 1 (Figs. 4 and 6). On the other hand, we also confirmed our long-term experience that natural enemies generally are able to

Fig. 6. Population dynamics for each insect pest and each natural enemy in four treatments in location 1. **A**, Number of different insect pests in four treatments, and **B**, total number of different natural enemies in four treatments. Red and blue arrowheads indicate dates of insecticide application and integrated pest management (IPM)-based action, respectively.

control insect pests, both in the wet and dry seasons, illustrating encouraging examples of insecticide-free crop production. Altogether, the unnecessary application of chemical insecticide resulted in increased production costs responsible for low profits (Fig. 3D) as well as adverse effects on human health (FAO 2013) and the biodiversity in rice ecosystems (Figs. 5 and 6). In contrast, naturally occurring rich biodiversity could facilitate, at least in part, pest control measures in Cambodian rice fields. This could be assisted by non-chemical IPM application, in the case of T2 in location 1. As efficiency of biological control is varied by different landscapes and populations of pests and natural enemies, careful observation of given rice fields is essential particularly in much larger fields than the plots in this study. An improvement of the skills of farmers to monitor pest/natural enemy populations and behaviors is the next challenge to harness these effective ecosystem services in natural rice fields and to sustain high yields and income, particularly for farmers of smallholdings.

Profit is the result of the balance between yield and expenses. Heong et al. (1995) suggested that many poor farmers do not financially benefit from the heavy use of pesticides. For example, there was a 10-fold overestimation of the potential loss of profit due to stem borer infestations by farmers in the Philippines, resulting in the money spent on insecticides being more than double their actual loss to pests. In addition, Cambodian farmers are becoming trapped on the “pesticide treadmill” that has been observed for a long period worldwide (Heong and Escalada 1997, 1999). This term describes the use of increasing pesticide volumes to control perceived threats by insect pests. The cost of spraying pesticides could outweigh the benefit for farmers of smallholdings (Hamilton 2008); therefore, a reduction in pesticide use is a key option to secure their incomes. A recent study comparing technically recommended practices with common practices by farmers in Cambodia strongly supported this notion (Chhay et al. 2016). This is why phytobiome studies in the economic and ecologic interface are important.

The yield from T1 in location 1 was significantly lower than that from other the three treatments because T1 experienced a higher number of caseworms, which caused more severe damage (Figs. 4 and 6). Numbers of caseworms in T2 (IPM) was decreased by keeping the field dry for 5 days, resulting in fine damage control and no yield loss. The rice plants were able to recover from moderate damage if there were no other defoliators present (Chhay et al. 2014; Chhunhy et al. 2014; IRRI 2016). For locations 2 and 3, the yields were not significantly different among the four treatments, although the insecticide was not applied in T1 and T2 (Fig. 3A). Although direct evidence of insects/arthropods interplays between pests and biocontrol agents was not provided in the current study, this probably means that the natural enemies controlled the populations of insect pests and balanced the system, thereby preventing severe damage caused by high pest populations (Figs. 4 and 5). Several studies have also shown that in tropical rice fields, there is a rich and dynamic array of natural enemies that controls the pest populations under most agroecosystems in Asia (Kenmore et al. 1984; Ooi 1996). In addition, when pesticides were used less frequently, ecosystem services in the form of predators and parasitoids were able to control pests (Heong 2009; Way and Heong 1994). These natural enemies in rice fields exist during every stage of rice crops and attack insect pests at every stage of insect growth. However, the reason for the high pest population under similar population dynamics of natural enemies in location 1 remains unknown. One possible explanation is that during the dry season, there were small numbers of cultivated rice fields around the research area due to water shortage. This situation might have resulted in mobile pest adults such as Lepidoptera flying into the field, since location 1 is located near rice fields of Vietnam in the Mekong Delta region where rice cultivation had started (Fig. 1). It is unclear

whether the soil types (rich fertility in location 3; poor fertility in location 1) have a causal connection with insect pest occurrences under a similar population density of natural enemies. Further phytobiome studies incorporating a survey of arthropod diversity and plant physiological analyses with different soil conditions are required to clarify this point. The natural enemies in T3 and T4 were lower than those in T1 and T2 due to the insecticide application. The high populations of natural enemies in T1 and T2 likely played a key role in maintaining the populations of pests. This result is consistent with an experiment in Jinhua, China over five years, which indicated that both predators and parasitoids significantly increased in density in ecologically engineered fields where pesticides were not applied compared with those in the control fields with pesticide applications (Lu et al. 2015). In this regard, the application of chemical insecticides is not recommended because they would result in the termination of abundant and well-distributed populations of natural enemies, resulting in resurgence of pest populations later in the season (FAO 2011; Matsukawa et al. 2014; Settle et al. 1996). A study of the community ecology of irrigated tropical rice fields in Java, Indonesia, demonstrated that abundant and well-distributed populations of generalist predators could be found in most early-season tropical rice fields, and their populations were reduced with insecticide applications, leading to a resurgence of pest populations later in the season, which eventually required more insecticide applications (Gallagher et al. 2005; Settle et al. 1996).

In general, the populations of insect pests in insecticide-treated sites were not significantly lower than those in untreated sites. This means that the natural enemies played an effective role in controlling the populations of insect pests. Ecological engineering projects in Vietnam and Thailand showed a reduction in yield losses by rice pests. In those countries, local communities successfully controlled pest populations to levels that do not result in major damage by maintaining the abundance of natural enemies, including parasitoids (Gurr et al. 2011). As a wide-ranging assemblage of predatory spiders, beetles, bugs, and wasps hunt insect pests throughout the growing season, both the diversity and complexity of species interactions contribute to the robustness of natural pest control (Chen 2008). In most seasons, insect pest populations did not reach threshold levels despite the fact that no insecticides were used (Heong 2009). After 14 years of research, pesticide use on the farm had decreased by a staggering 87.5%, and the use of insecticides had fallen by 95.8% (Hamilton 2008). Several studies had contributed evidence to show that in tropical rice fields, appropriate management of these diversity components together with the ability of plants to compensate for damage can confer stability, resulting in the control of pests to levels that do not justify the use of insecticides (Ooi and Shepard 1994; Way and Heong 1994). Ironically however, most farmers are unaware of the role of these natural enemies, particularly of predators in rice fields that protect their crops, and they therefore incur expenses due to insecticide applications. Farmer education was important to facilitate the understanding of the worth of conserving these natural enemies (Chhay et al. 2016; Kenmore 1996; Matteson et al. 1994). Of equal importance, arthropod phytobiome studies by researchers are required to be conducted more widely and deeply for developing better pest management practices.

Conclusions and perspectives. Even during the dry season, the calendar-based application of chemical insecticides to control insect pests was not appropriate for rice production because an increase in yield as compared to insecticide-free plots was not achieved. Insecticide applications could lead to not only an unnecessary financial burden on farmers and impacts on their health, but also lead to the destruction of rice ecosystems and the development of pest insecticide resistance, with all of these concerns being very serious within sustainable crop intensification paradigms.

As we demonstrated in the arthropod phytobiome study, environmentally friendly IPM practices could provide an alternative to the use of chemical insecticides, thereby sustainably maintaining efficient ecosystem services. To this end, the capacity and knowledge of farmers to examine the pest populations should be developed by regional experts, incorporating a long-term approach to sustainable crop production for smallholder farmers.

With indiscriminate use, insecticide application kills both pests and natural enemies concurrently and results in the resurgence of non-target insects, and the misuse and overuse of insecticides can harm farmers, consumers, and the environment. Particularly for farmers of smallholdings, the financial costs incurred by spraying insecticides can critically outweigh the benefits. Ecosystems can be sustainably maintained only when farmers become aware that chemical pesticide application can result in more harm than good. One of the most difficult challenges ahead will be to convince policymakers, researchers, technicians, extension agents, and farmers of the efficacy and robustness of natural biological control, underpinned by the amazingly rich biodiversity present in Cambodian rice fields during the dry season that can control the pest populations, thus avoiding unnecessary insecticide application. As the price of Cambodian rice decreases and the costs of pesticides/fertilizers increase or vice versa, clarifying the above challenge will immediately benefit farmers.

ACKNOWLEDGMENTS

This study was supported in part by Nagoya University Asian Satellite Campuses Institute under its Transnational Ph.D. Program, the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA), and the JSPS KAKENHI grant number 15H02644. We are grateful to the staffs of the National IPM Program and three research stations in Cambodia who assisted in conducting field experiment and data collection. We thank E. C. Cedicol, J. W. Ketelaar, D. Mole, and B. Hadi for critical reading and fruitful comments on the manuscript.

LITERATURE CITED

- Bambaradeniya, C. N. B., Edirisinghe, J. P., De Silva, D. N., Gunatilleke, C. V. S., Ranawana, K. B., and Wijekoo, S. 2004. Biodiversity associated with an irrigated rice agro-ecosystem in Sri Lanka. *Biodivers. Conserv.* 13: 1715-1753.
- Bianchi, F. J. J. A., Booij, C. J. H., and Tschamtker, T. 2006. Sustainable pest regulation in agricultural landscapes: A review on landscape composition, biodiversity and natural pest control. *Proc. Roy. Soc. B* 273:1715-1727.
- Chen, Y. 2008. The unsung heroes of the rice field. *Rice Today* 7:30-31.
- Chhay, N., Hak, K. L., Kea, K., Cheythyrit, C., and Ratana, H. 2014. Book on Integrated Technology for Rice Intensification. Department of Rice Crop, GDA, MAFF, Phnom Penh, Cambodia. (in Kumer).
- Chhay, N., Seng, S., Tanaka, T., Yamauchi, A., Cedicol, E. C., Kawakita, K., and Chiba, S. 2016. Rice productivity improvement in Cambodia through the application of technical recommendation in a farmer field school. *Int. J. Agric. Sustain.* 15:54-69.
- Chhunhy, H., Sreivuth, L., Chatna, M., Sareth, K., Thavarith, S., Chanthy, S., Sophal, K., Monivath, M., and Socheata, L. 2014. Technical book on pest management on rice crop. General Directorate of Agriculture, Phnom Penh, Cambodia.
- Department of Rice Crop. 2011. Field Guide for FFS on Rice Crop. Department of Rice Crop and Cambodian National IPM Program, Phnom Penh, Cambodia.
- FAO. 2011. Save and Grow: A Policymakers' Guide to the Sustainable Intensification of Smallholder Crop Production. Food and Agriculture Organization of the United Nations, Rome, Italy.
- FAO. 2013. Empowering Farmers to Reduce Pesticide Risks. Food and Agriculture Organization Regional Office for Asia and the Pacific, Bangkok, Thailand.
- Fraser, E., Legwegoh, A., Krishna, K. C., CoDyre, M., Dias, G., Hazen, S., Johnson, R., Martin, R., Ohberg, L., Sethuratnam, S., Sneyd, L., Smithers, J., Van Acker, R., Vansteenkiste, J., Wittman, H., and Yada, R. 2016. Biotechnology or organic? Extensive or intensive? Global or local? A critical review of potential pathways to resolve the global food crisis. *Trends Food Sci. Technol.* 48:78-87.
- Gallagher, K., Ooi, P. A. C., Mew, T., Borromeo, E., Kemore, P. E., and Ketelaar, J. W. 2005. Ecological basis for low-toxicity integrated pest management in rice and vegetable. Pages 116-134 in: *The Pesticide Detox: Towards a more sustainable agriculture*. J. Pretty, ed. Earthscan, London.
- Gomez, K. A., and Gomez, A. A. 1984. *Statistical Procedures for Agricultural Research*, 2nd ed. Wiley-Interscience, Manila, Philippines.
- Gurr, G. M., Liu, J., Read, D. M. Y., Catindig, J. L. A., Cheng, J. A., Lan, L. P., and Heong, K. L. 2011. Parasitoids of Asian rice planthopper (Hemiptera: Delphacidae) pests and prospects for enhancing biological control by ecological engineering. *Ann. Appl. Biol.* 158:149-176.
- Hamilton, H. S. 2008. The pesticide paradox. *Rice Today* 7:32-33.
- Heinrichs, E. A., Chellah, S., Valencia, S. L., Fabellar, L. T., Aquino, G. B., and Pickin, S. 1981. *Manual for testing insecticides on rice*. International Rice Research Institute, Manila, Philippines.
- Heong, K. L., Aquino, G. B., and Barrion, A. T. 1991. Arthropod community structures of rice ecosystems in the Philippines. *Bull. Entomol. Res.* 81: 407-416.
- Heong, K. L., Aquino, G. B., and Barrion, A. T. 1992. Population dynamics of plant- and leafhoppers and their natural enemies in rice ecosystems in the Philippines. *Crop Prot.* 11:371-379.
- Heong, K. L., Escalada, M., and Mai, V. 1994. An analysis of insecticide use in rice: Case studies in the Philippines and Vietnam. *Int. J. Pest Manage.* 40: 173-178.
- Heong, K. L., Escalada, M. M., and Lazaro, A. A. 1995. Misuse of pesticides among rice farmers in Leyte, Philippines. Pages 97-108 in: *Impact of Pesticides on Farmer Health and the Rice Environment*. Kluwer Academic, Norwell, MA.
- Heong, K. L., and Escalada, M. M. 1997. Perception change in rice pest management: A case study of farmers' evaluation of conflict information. *J. Appl. Commun.* 81:3-17.
- Heong, K. L., and Escalada, M. M. 1999. Quantifying rice farmers' pest management decisions: beliefs and subjective norms in stem borer control. *Crop Protection*. 18:315-322.
- Heong, K. L. 2009. Are planthopper problems caused by a breakdown in ecosystem services? Pages 221-232 in: *Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia*. K. L. Heong and B. Hardy, eds. International Rice Research Institute, Manila, Philippines.
- Huan, N. H., Thiet, L. V., Chien, H. V., and Heong, K. L. 2005. Farmers' participatory evaluation of reducing pesticides, fertilizers and seed rates in rice farming in the Mekong Delta, Vietnam. *Crop Prot.* 24:457-464.
- International Rice Research Institute. 2002. *Standard evaluation system for rice*. International Rice Research Institute (IRRI), Manila, Philippines.
- IRRI. 2016. Rice caseworm. Manila, Philippines: International Rice Research Institute. (Accessed on 2016 January 10). <http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/insects/item/rice-caseworm>
- Kathiresan, R. M. 2007. Integration of elements of a farming system for suitable weed and pest management in the tropics. *Crop Prot.* 26:424-429.
- Kenmore, P. E. 1996. Integrated pest management in rice. Pages 214-232 in: *Biotechnology and Integrated Pest Management*. G. J. Persley, ed. CAB International, Wallingford, U.K.
- Kenmore, P. E., Carino, F., Perez, C., Dyck, V. A., and Gutierrez, A. 1984. Population regulation of the rice brown planthopper (*Nilaparvata lugens* Stål) within rice fields in the Philippines. *J. Plant Prot. Tropics* 1:19-37.
- Lu, Z., Zhu, P., Gurr, G. M., Zheng, X., Chen, G., and Heong, K. L. 2015. Rice pest management by ecological engineering: A pioneering attempt in China. Pages 163-180 in: *Rice Planthoppers: Ecology, Management, Socio Economics and Policy*. K. L. Heong, J. A. Cheng, and M. M. Escalada, eds. Springer, Dordrecht, Germany.
- Ministry of Agriculture, Forestry and Fisheries. 2015. Annual conference report on agriculture, forestry and fisheries. Ministry of Agriculture, Forestry and Fisheries (MAFF), Phnom Penh, Cambodia.
- Makara, O., and Chhay, N. 2014. Cambodian rice development strategy. Paper presented at the CORRA-GRiSP Workshop on Asian National Rice Strategy. Regional Workshop of IRRI, Hyderabad, India.
- Matsukawa, M., Ito, K., Kawakita, K., and Tanaka, T. 2014. Current status of the occurrence and farmer perceptions of rice planthopper in Cambodia. *Jpn. Agric. Res. Q.* 48:167-173.
- Matsukawa, M., Ito, K., Kawakita, K., and Tanaka, T. 2015. Farmer perceptions and management of rice planthoppers in Cambodia. *Jpn. Agric. Res. Q.* 49:103-109.
- Matteson, P., Gallagher, K., and Kenmore, P. E. 1994. Extension of integrated pest management for planthoppers in Asian irrigated rice: Empowering the

- user. Pages 656-685 in: Planthoppers. R. F. Denno and T. J. Perfect, eds. Springer, U.S.
- Matteson, P. C. 2000. Insect pest management in tropical Asian irrigated rice. *Annu. Rev. Entomol.* 45:549-574.
- Ministry of Water Resource and Meteorology. 2015. Report on annual rainfalls and temperatures. Ministry of Water Resource and Meteorology (MOWRAM), Phnom Penh, Cambodia.
- Nesbitt, H. J. 1997. Rice Production in Cambodia. International Rice Research Institute, Manila, Philippines.
- Nguyen-Vaucheret, S., Shanahan, M., Williams, J., and Trent, S. 2002. Death in small doses: Cambodia's pesticide problems and solutions. Environmental Justice Foundation, London, U.K.
- Ooi, P. A. C. 1996. Experiences in educating farmers to understand biological control. *Entomophaga* 41:375-385.
- Ooi, P. A. C., and Kenmore, P. E. 2005. Impact of educating farmers about biological control in farmer field schools. Presented at the Second International Symposium on Biological Control of Arthropods. The Bugwood Network, Davos, Switzerland.
- Ooi, P. A. C., and Shepard, B. M. 1994. Predators and parasitoids of rice insect pests. Pages 585-612 in: *Biology and Management of Rice Insects*. E. A. Heinrichs, ed. Wiley Eastern, New Delhi, India.
- Paredes, S. H., and Lebeis, S. L. 2016. Giving back to the community: Microbial mechanisms of plant-soil interactions. *Funct. Ecol.* 30:1043-1052.
- Pretty, J., and Hine, R. 2005. Pesticide use and environment. Pages 1-22 in: *The Pesticide Detox: Towards a More Sustainable Agriculture*. J. Pretty, ed. Earthscan, London, UK.
- Sarom, M. 2007. Rice Crop in Cambodia. Cambodian Agricultural Research and Development Institute, Phnom Penh, Cambodia.
- Savary, S., Horgan, F., Willocquet, L., and Heong, K. L. 2012. A review of principles for sustainable pest management in rice. *Crop Prot.* 32:54-63.
- Seng, S., and Chhunhy, H. 2012. Pesticide supply chain and brown planthopper study in Cambodia. Cambodian National IPM Program, Phnom Penh, Cambodia.
- Settle, W. H., Ariawan, H., Astuti, E. T., Cahyana, W., Hakim, A. L., Hindayana, D., and Lestari, A. S. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. *Ecology* 77:1975-1988.
- Shepard, B. M., Barrion, A. T., and Litsinger, J. A. 1987. Helpful insects, spiders, and pathogens. International Rice Research Institute, Manila, Philippines.
- Way, M. J., and Heong, K. L. 1994. The role of biodiversity in the dynamics and management of insect pests of tropical irrigated rice—A review. *Bull. Entomol. Res.* 84:567-587.
- White, P. F., Oberthur, T., and Sovuth, P. 1997. The soils used for rice production in Cambodia. International Rice Research Institute, Manila, Philippines.
- Wilby, A., and Thomas, M. B. 2002. Natural enemy diversity and pest control: Patterns of pest emergence with agricultural intensification. *Ecol. Lett.* 5:353-360.