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Influence of humidity, rainfall, and fipronil toxicity on rice leaffolder (*Cnaphalocrocis medinalis*)

Jakarpong Supawan, Wiboon Chongrattanameteekul*

Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900 Thailand

*Corresponding author, e-mail: wiboon.c@ku.ac.th

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ABSTRACT: The relationship between weather and rice leaffolder population in Kamphaeng Phet and Phichit provinces, Thailand from February 2013 to February 2014 was evaluated. The results showed that the numbers of rice leaffolder larvae were significantly correlated with maximum temperature ($r = 0.474$) and average temperature ($r = 0.375$). Increasing temperature within the upper threshold of the species generally promoted insect population growth. Although statistically non-significant, relative humidity and rainfall produced negative correlation with rice leaffolder population ($r = -0.249$ and -0.091, respectively). Both factors might reduce leaffolder population via promoting activity of microbial control agents and physically dislodging eggs and larvae of rice leaffolder. When the insecticide fipronil was tested against populations of rice leaffolder in conventional and organic paddy fields, the lethal concentration (LC_{50}) value from toxicity tests with a population from a conventional field was significantly higher than with that of the organic field (63.3 versus 51.1 ppm). With continued and prolonged use of fipronil in a rice field, there is a tendency of that rice leaffolder to develop resistance to this insecticide. Resistance management and resistance monitoring should to be seriously considered if effective control is desired.

KEYWORDS: weather components, rice leaffolder, organic rice

INTRODUCTION

Rice (*Oryza sativa*), the staple diet of more than half of the world's population, is grown over 120 million hectares to produce 600 million tons of grain with an average productivity of 3.4 Mt/ha[1]. The world rice crop is attacked by more than 100 species of insects; 20 of them can cause economic damage during the different stages of the crop. Insect pests that can cause significant yield losses are stem borers, leafhoppers and planthoppers (which cause direct damage by feeding as well as by transmitting viruses), gall midges (a group of defoliating species, mainly lepidopterans), and a grain-sucking bug complex[2,3].

The rice leaffolder (RLF), *Cnaphalocrocis medinalis* (Guenee), was considered a minor pest of rice, but it has become increasingly important with the spread of high-yielding rice varieties and the accompanying changes in cultural practices[4]. Serious outbreaks of RLF have been reported in many Asian countries including India, Korea, Japan, China, Malaysia, Sri Lanka, Thailand, and Vietnam[5,6]. The larvae feed by scraping the green mesophyll tissues from rice leaves, thus producing areas of damage appearing as linear, pale white stripes. The damaged leaves serve as entry points for fungal and bacterial infections[7].

Temperature is an important abiotic factor that regulates the development, phenology, and population dynamics of insects. Understanding the relationship between temperature and the developmental rate of a target pest is important in predicting the seasonal occurrence of the species and to establish environment-friendly pest management strategies[8]. Management of RLF using synthetic chemicals has failed because of insecticide resistance, pesticide residues in food, pest resurgence, toxic effects on human beings, and environmental pollution[9,10]. This study investigates the relationship between RLF population and weather components in lower northern Thailand and the toxicity of the insecticide fipronil in order to provide information for effective pest management strategy for this insect in the region.

MATERIALS AND METHODS

Field experiment

The experiments were conducted in the paddy fields at Kamphaeng Phet and Phichit provinces, Thailand during 3 cropping seasons between 2013 and 2014.

The first crop (January–April 2013) and the second crop (May–August 2013) of both locations were grown approximately at the same period. However, the third crop in Kamphaeng Phet province was grown during November 2013 to February 2014, and the third crop in Phichit province was grown during August–November 2013. Each location consisted of 3 plots (replications) within the same vicinity with plot size of 40 m × 40 m. Field observations were recorded once every 2 weeks, starting from approximately 30 days after transplanting until harvest. The observations were taken by counting the total number of larvae from rice plants within a quadrat (0.5 m × 0.5 m) using line transects method. Rice plants from 5 quadrats (spots) were observed on each diagonal line of each plot. The average population per quadrat was calculated.

Weather data including maximum temperature, minimum temperature, average temperature, relative humidity, and amount of rainfall from the nearest weather stations were obtained from the Thai meteorological department. All weather parameters except rainfall were averaged from weather data within 14 days prior to the specified insect sampling dates. For rainfall, the values were accumulative rainfall of 14 days prior to the specified sampling dates. The effects of weather components on RLF population were calculated by Spearman's correlation coefficient.

Mass rearing of RLF in the laboratory

The experiment was conducted on 'Phitsanulok 2' rice variety. Rice seeds were sown in plastic trays (30 cm × 40 cm) in the greenhouse. The 20 days old seedlings were transferred into pots and prepared for rearing the rice leaffolder. The adult RLFs were collected from conventional and organic rice fields in Kamphaeng Phet province. Adults from different locations were separated into cages kept in the shade of a screened and roofed insectary and allowed to complete development. Adults were fed with honey solution following the techniques by Waldbauer and Marciano[11]. The third-instar larvae were collected from rice leaves to Petri dishes for insecticide resistance testing.

Fipronil toxicity against RLF larvae

Different concentrations of fipronil 5% SC were prepared from commercial formulation. The insecticide was diluted with distilled water to prepare a range of concentrations from 0, 20, 40, 60, 80, and 100 ppm. The rice leaves were treated by dipping with different concentrations of insecticide. Untreated control leaves were treated with distilled water. The leaves cut were allowed to dry at room temperature for 10 min and then placed in 15 cm diameter Petri dishes with moistened paper. Ten third-instar larvae were placed on the treated leaves and kept in environmental chambers at 25 ± 1 °C, 70 ± 10% RH. Each treatment was replicated 3 times. The larvae which did not respond to pencil tip prodding were considered dead. Mortality was recorded at 24 h after the treatment.

Statistical analysis

Mortality data were subject to analysis using dose-mortality regression. The LC_{50} and LC_{90} values were computed by probit analysis[12] with corrected mortality using Abbott's formula[13]. The LC_{50} values obtained from conventional and organic rice fields were compared by independent sample *t*-test.

RESULTS

Relationship between weather components and RLF population

The correlations of weather components and larval population of RLF are shown in Table 1. The results showed that the number of RLF larvae in the field had significant positive correlation with maximum temperature and average temperature ($r = 0.474$ and 0.375, respectively). Although other weather parameters including minimum temperature, relative humidity, and rainfall did not show significant results ($p > 0.05$), it should be noted that both relative humidity and rainfall produced a negative correlation with the number of RLF larvae ($r = -0.249$ and −0.091, respectively).

Toxicity of fipronil against RLF larvae

Laboratory tests to evaluate the LC_{50} and LC_{90} values of insecticide fipronil against RLF were performed using insect populations from conventional and organic rice fields. The results showed that the regression lines fitted very well with the mortality data with the coefficient of determination (R^2) ranging between 0.81 and 0.96 (Tables 2 and 3). The progenies of leaffolder collected from conventional field had higher LC_{50} and LC_{90} values in all 3 tests conducted. The LC_{50} values from the 1st, 2nd, and 3rd tests were 64.7, 60.1, and 65.2 ppm, respectively (Table 2), while those from the organic field were 52.0, 50.6, and 50.7 ppm, respectively (Table 3). Similarly, the LC_{90} of the 1st, 2nd, and 3rd tests from conventional field were also higher

Table 1 Correlation of weather components and mean number of *C. medinalis*.

Observation date	Location	Weather components†					Mean no. of RLF larvae per 0.25 m^2 quadrat
		Maximum temp. (°C)	Minimum temp. (°C)	Average temp. (°C)	Relative humidity (%)	Rainfall (mm)	
26/2/2013	Kamphaeng Phet	35.0	22.4	28.3	67.8	1.6	0.43
	Phichit	34.3	22.3	28.2	73.9	0	0
12/3/2013	Kamphaeng Phet	34.9	23.1	28.4	70.5	24.8	0.67
	Phichit	33.3	22.5	28.0	76.7	55.6	0.33
26/3/2013	Kamphaeng Phet	37.5	21.5	29.4	59.3	0	0.33
	Phichit	35.5	22.7	29.1	72.8	0	0.07
11/4/2013	Kamphaeng Phet	38.6	24.7	31.5	60.8	0	1.70
	Phichit	37.2	23.5	30.6	66.1	0	1.40
8/6/2013	Kamphaeng Phet	34.8	25.1	29.1	78.8	121.7	0
	Phichit	35.2	23.2	29.5	74.2	62.2	0
21/6/2013	Kamphaeng Phet	33.3	25.2	28.6	82.8	93.9	0.40
	Phichit	33.5	22.2	28.6	82.1	240.1	0.23
5/7/2013	Kamphaeng Phet	32.7	25.1	28.3	82.4	40.6	0
	Phichit	32.9	23.8	28.7	82.3	51.6	0
18/7/2013	Kamphaeng Phet	32.6	24.6	28.0	82.6	135.4	0.13
	Phichit	32.4	23.2	27.9	83.9	93.8	0.20
1/8/2013	Kamphaeng Phet	31.3	24.8	27.2	86.6	96.7	0
	Phichit	31.2	23.0	27.5	86.8	43.6	0.17
1/10/2013	Phichit	30.6	23.4	27.1	87.7	179.2	0.07
16/10/2013	Phichit	33.1	23.4	29.1	76.8	9.0	0.03
31/10/2013	Phichit	31.5	24.0	27.4	82.3	52.2	0.03
16/11/2013	Phichit	32.8	23.4	28.0	76.5	52.2	1.77
28/11/2013	Phichit	31.2	23.5	27.6	78.7	0.9	3.70
15/12/2013	Kamphaeng Phet	30.3	19.4	24.2	76.2	2.7	0
2/1/2014	Kamphaeng Phet	27.0	14.4	20.0	74.3	0	0
17/1/2014	Kamphaeng Phet	30.4	18.1	23.7	71.9	0	0
30/1/2014	Kamphaeng Phet	28.9	14.0	20.9	67.3	0	0
16/2/2014	Kamphaeng Phet	33.6	19.7	26.2	65.2	0	0.10
Spearman's corr.‡		0.474	0.164	0.375	−0.249	−0.091	
p-value		0.011*	0.405	0.050*	0.200	0.645	

† Maximum temperature, minimum temperature, average temperature, and relative humidity were averaged from data within 14 days prior to insect sampling date; rainfall data were cumulative rainfall within 14 days prior to insect sampling date.
‡ Spearman's correlation coefficient between weather components and mean number of RLF larvae recorded within the date.
* Significant at $\alpha = 0.05$.

with the values of 125.3, 112.2, and 114.6 ppm, respectively, as compared to 94.6, 87.9, and 95.1 ppm, respectively, for those from the organic field. The higher lethal concentration values indicated that the population of RLF from the conventional field could tolerate higher concentrations of fipronil. This may be the result of selection pressure from applying insecticides including fipronil which had been imposed on the RLF population. Although the recommendation rate for RLF control in Thailand (equivalent to 125 ppm) is roughly the same as the LC_{90} value and is still effective, there is a tendency that continued application of fipronil may cause resistance development in RLF.

When the LC_{50} values obtained from RLF population collected from conventional field were compared with those from organic field using independent sample *t*-test, the result was statistically significant with $p = 0.001$ (mean difference: 12.256; standard error of the difference: 1.698; lower: 7.542;

Table 2 Lethal concentration (LC) and regression parameter estimates of fipronil tested against RLF collected from conventional rice field at 24 h after treatment.

Treated/Parameter*	1st test	2nd test	3rd test
LC_{50} (ppm)†	64.7	60.1	65.2
Range of LC_{50}	55.2–76.2	36.8–89.6	48.7–86.9
LC_{90} (ppm)†	125.3	112.2	114.6
Range of LC_{90}	106.9–158.3	84.9–223.0	91.3–182.1
A	−1.370	−1.478	−1.689
B (slope)	0.021	0.025	0.026
R^2	0.91	0.81	0.87

† LC_{50} and LC_{90} are the lethal concentrations (ppm) that kill 50% and 90%, respectively, of RLF from the average of three replications.
* Parameter estimates for regression line $Y = A + BX$.

Table 3 Lethal concentration (LC) and regression parameter estimates of fipronil tested against RLF.

Treated/Parameter*	1st test	2nd test	3rd test
LC_{50} (ppm) †	52.0	50.6	50.7
Range of LC50	35.0–68.9	43.7–57.3	42.9–58.3
LC_{90} (ppm) †	94.6	87.9	95.1
Range of LC_{90}	75.8–143.0	78.5–101.7	84.0–112.3
A	−1.796	−1.736	−1.459
B (slope)	0.023	0.034	0.029
R^2	0.91	0.94	0.96

† LC_{50} and LC_{90} are the lethal concentrations (ppm) that kill 50% and 90%, respectively, of RLF from the average of three replications.
* Parameter estimates for regression line $Y = A + BX$.

upper: 16.969; t: 7.219; degrees of freedom: 4; significat differences at the 5% level (1-tailed): 0.001). This result confirmed that population of RLF from the conventional rice field was more resistant to fipronil than the population from the organic rice field.

DISCUSSION

Weather components and insect population

An insect population always fluctuates according to the dynamic condition of its environment. Abiotic and biotic factors are believed to be responsible for the population change[14]. Weather components such as temperature, rainfall, and relative humidity have known to greatly influence the insect population directly by limiting or expanding their distribution, growth, reproduction, diapause, dispersal, and indirectly through plant mechanisms and natural enemies that regulate the insect population[15–19]. The seasonal effects of weather and ongoing changes in climatic conditions will directly lead to modifications in dispersal and development of insect species. The changes in surrounding temperature regimes certainly alter developmental rates and survival of insects, and subsequently act upon size and density of the population[20].

With respect to the survival of the different stages of the RLF, *C. medinalis* was greatly affected at 35 °C and the upper temperature threshold for the survival of this species appears to lie between 30 °C and 35 °C, with the outbreak usually following a prolonged drought[19,21]. The significant positive correlations between larval population and maximum and average temperatures in this study (Table 1) correspond with the report that the temperatures within daily minimum temperature of 15 °C and maximum temperatures of 39 °C within rice growing season can affect distribution, developmental rate, and phenology of rice insect pests[19]. The results also agree with the reports[3,22,23] which indicated that maximum temperature, minimum temperature and sunshine hours had significant positive correlation with larval population of RLF. However, few reports showed that the maximum temperature did not affect the infestation level significantly whereas the minimum temperature showed a significant and negative correlation with larval population[24].

Insect abundance is certainly linked to seasonal variations in rainfall, with some species being more abundant in the dry season, whereas others proliferate only during the rains. This is generally due to direct effects of rain on insects, and indirect effects mediated through plant quality and the efficacy of natural enemies affected by rainfall[25]. Although non-significant, the negative correlation between RLF population and rainfall and relative humidity ($r = -0.091$ and -0.249) in the present study indicate that increased rainfall and humidity might reduce the population of RLF. This observation is supported by previous studies[24,26]. The explanation for negative effects of rainfall and humidity is probably related to the increased virulence of microbial control agents under high humidity and physical dislodging of eggs or larvae from rice or host plant by heavy rainfall[19,27].

Toxicity of fipronil against rice insect pest

Fipronil is highly toxic to both piercing-sucking and chewing insects and has been shown to possess excellent activity against a broad spectrum of insect order. However, due to the increased frequency and years of use, fipronil resistance started to appear in insects[28,29]. Many studies reported fipronil resistance in various insect pests such as rice stem borer, house fly, and diamondback moth[29–32]. In this study we found that the average LC_{50} values from toxicity tests of fipronil against RLF population collected from conventional paddy field was 63.3 ppm (Table 2), which is comparable to that of a previous study in Sri Lanka in 2001 that reported the LC_{50} values of 60 ppm for the same insect and insecticide[33]. When comparing the LC_{50} values of fipronil between populations of RLF from organic and conventional paddy field, the result showed that the average LC_{50} value from the tests with population from organic paddy field was significantly lower (51.1 ppm, Table 3). This result is in accordance with a previous study in China in 2005[29] about fipronil resistance in rice stem borer. They found that rice stem borers from location with frequent insecticide application were more resistant to fipronil than the susceptible strain (reared under laboratory condition without any contact to insecticide). The higher LC_{50} in RLF population from conventional field indicated the tendency of fipronil resistance and might be the result from RLF and rice stem borer control during the early growth stage of rice. Overuse and frequent exposure to insecticides is often a precursor to the development of insecticide resistance[34]. Thus fipronil resistance monitoring should be conducted regularly in order to maintain appropriate and effective use of this insecticide.

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