# Quantitative measurement of the influence of repeated pesticide application on food intake of *Ummeliata* insecticeps by the fluorescence labeling method

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**Abstract**: By applying a patented technique, the quantitative analysis of food intake using the fluorescence tracer as it is transferred in the food chain, we studied the food intake of Ummeliata insecticeps (Bosenberg et Strand) when exposed to pesticide buprofezin repeatedly. The results showed that food intakes of the surviving spiders in all treated groups were much less than that in the control group. Food intake of surviving U. insecticeps sprayed with buprofezin for the first time was less than that of the surviving spiders sprayed with the pesticide for the second or third time when buprofezin was diluted. It took a long time for the surviving spiders to recover when sprayed with the pesticide at high concentrations. Since the applying pesticides could bring high mortality to the spider, food intake of U. insecticeps population greatly decreased in 13 days after being sprayed with buprofezin. Though the surviving spiders in the treated groups at even more than the control individuals on the 13th day, no pesticide was worth spraying because food intake of the population in the treated groups was less than that in the control group due to the high mortality of the population.

**Key words**: Ummeliata insecticeps; Sogatella furcifera; fluorescence labeling method; food intake; repeated pesticide exposure

Spiders are among the essential predators which prey on insects in rice paddies. There are more than 370 spider species in Chinese rice planting areas, accounting for 51.2% to 89.5% of the total predator species (Wang et al., 1999). Spiders are effective predators because of several traits, including their vigorous predation habits, strong starvation endurance, and large population sizes in a variety of species (Wang, 1981; Reichert and Lockley, 1984; Sunderland et al., 1986; Nyffeler and Benz, 1987; Bogya and Mols, 1996; Marc et al., 1999; Xiao et al., 2006). Ummeliata insecticeps (Boesenberg et Strand) is one of the constant dominant spider species in most of the Chinese rice planting areas. Because applying pesticides to control pests is prevalent nowadays, predators like spiders in rice paddies suffer greatly from the chemicals. Pesticides affect the spider disturbing their survival, growth, fitness development, immunity, activity, metabolism and so on ( Xu et al., 1984; Baatrup and Bayley, 1993; Punzo, 1997; Jagers et al., 1995). Suffering from pesticides, the predator populations will lighten the predatory pressure on pest populations in the rice ecosystem, which may cause the resurgence of the target pests and the eruption of the non-target pests, and make the rice planting depend on pesticides even more. Therefore, studying the influence of pesticides on the predators food intake is one of the most important items to comprehensively evaluate the ecological effect of pesticides. Due to lack of effective techniques, however, there were just a few reports on qualitative measurement of the food intake of predators exposed to pesticides ( Xu et al., 2000; Widiarta et al., 2001).

By using the quantitative analysis technique (Wang et al., 2001), the food intake of U. insecticeps exposed to the pesticide repeatedly was quantitatively determined, and a theoretical basis was proved for intelligent use of pesticides and allows us to integrate pest management in rice cultivation. The lanthanide chosen for the experiment, europium, can be absorbed by the rice plant from the soil. Sogatella furcifera eats the plants, while U. insecticeps preys on

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 $S.\ furcifera$  for food. Therefore, the europium enters into the  $S.\ furcifera$  bodies and accumulates in  $U.\ insecticeps$  bodies. By applying a series of chemical analyses such as extraction/invert extraction to the treated  $U.\ insecticeps$  and  $S.\ furcifera$  specimens, the food intake of  $U.\ insecticeps$ , which has been sprayed with pesticides for three times, can be measured. By taking the mortality of the spiders into account, the food intake of the experimental spider population exposed to pesticides can also be measured.

#### 1 MATERIALS AND METHODS

#### 1.1 Materials

Rice variety Jinyou 974 was used , which was one of early-season hybrids. The test pest and spider species was S. furcifera and U. insecticeps , respectively. Both U. insecticeps and S. furcifera were collected from a rice paddy in a western suburb of Changsha , Hunan Province , China. Only healthy , uninjured individuals were collected for the test.

Pesticide buprofezin (  $25\,\%$  wettable powder , Pesticide Factory of Jilin Chemical Corporation ) , whose molecular formula is  $C_{16}\,H_{23}\,N_3\,OS$  , was used in the study. It is a low-toxic chemical that kills the target insects by preventing the synthesis of chitin , and routinely applied at 20-30 g/667 m² . The recommended concentration is buprofezin : water = 1:2~000-3~000 .

#### 1.2 Apparatus and Chemicals

Apparatus: Fluorescence spectrophotometer ( RF-5301PC Shimadzu, Japan), Automatic Double Pure Water Distiller (BSZ-2, Shanghai Zhisun Instruments Co., Ltd.), Electronic Analytical Balance (FA1104, Shanghai Balance Instrument Factory), Cabinet-type Resistance Furnace (SRJX-3-9, Changsha Electric Furnace Factory), PH Meter (PHS-3C, Shanghai Zhisun Instruments Co., Ltd.), Conviron Plant Growth ( LRH-250-GS, Chamber Guangdong Medical Appliance Factory ), Constant Temperature Drying Oven Shanghai ( DHG-9031-A , Jinghong Instrument Co., Ltd.), Stereomicroscope, Separatory Funnel, Plastic Planting Tub, Gauze hood;

Chemicals:  $HNO_3$ , HCl,  $H_2O_2$ ,  $Eu_2O_3$ , etc.

#### 1.3 Methods

**1.3.1** Sample collection: The rice sprouts were transplanted into plastic tubs covered by gauze hoods on April 28 , 2006. A solution of  $Eu_2O_3$  dissolved in water at a concentration of 40 mg/kg was poured into the planting tubs on May 10 so that the  $Eu^{3+}$  could be absorbed by the rice roots.

The S. furcifera adults were collected from a rice paddy in a west suburb of Changsha during the rice

maturing stage. The *S. furcifera* were put into the gauze hood and allowed to feed on the rice plants for 72 h, after which they were recollected. Among the recollected *S. furcifera*, 19 individuals were used for measuring europium content in their bodies and others were offered to the treated spiders for food. The 19 individuals were separated into 8 portions. Each portion was tested twice. The average europium content of the 19 individuals was taken as the europium content in an individual *S. furcifera* body.

The *U*. insecticeps adults were collected at the same time as the S. furcifera adults were collected, but they were taken from a fallow field, which had not been sprayed with any chemicals for over 3 years. Each spider was weighed with the electronic analytical balance and then put into a 30 ml cuvette with a little wet cotton on the bottom to provide suitable humidity. No food was offered to the spiders for 48 h. The spiders were separated into four groups: three treated groups and one control group, each group with 20 individuals. The 3 diluted buprofezin at concentrations of 1:5 000, 2:5 000 and 3:5 000 were applied to the treated groups, while pure water to the control group. The treated groups were sprayed 3 times with the pesticide, on June 15, June 30, and July 15, respectively. A mini atomizer was used to spray the buprofezin solutions on the spider backs until their backs were wet (Fabellar and Heinrich, 1984). Then each spider was removed to a 250 ml beaker with wet cotton on the bottom and all the beakers were put into the plant growth chamber (  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$  ). On the 2nd day , the 5th day , the 9th day and the 13th day after each spray of the pesticide solutions or water, two steps were taken: First, the number of dead U. insecticeps individuals in each group were checked, recorded, and the individuals were eliminated. Second, each surviving spider was supplied with 10 S. furcifera individuals, which had been fed on the europium-marked rice for 72 h. All the spiders were collected again 24 h later. The spiders collected at the same time and belonging to the same group were put together for measuring europium content in their bodies. The experiment was replicated 3 times. The mean value was taken as the europium of U. insecticepsindividuals content corresponding group.

1.3.2 Measurement and analysis of the europium content: According to the technique patented by our laboratory, the quantitative analysis of food intake by the fluorescence tracer transferred into the food chain (Wang et al., 2001), the recollected fly louses and spiders were processed by steps as follows to get the europium content: (1) Cineration and nitration of the samples and its dissolution (2) Extraction and invert

extraction of europium from the solution to form the europium-complex; (3) The europium-complex is excited by the wavelength of 390 nm with RF-5301PC fluorescence spectrophotometer and emits fluorescence at the wavelength of about 612 nm; (4) Calculation of europium content in the plant hoppers or spider bodies according to the standard curve equation of the fluorescent tracing method.

### 1.4 Data analysis

Standard curve equation (Wang et al., 2001): y = -0.8344 + 177.0988x, x is the europium content ( $\mu$ g) and y is value of the fluorescent spike.

Europium content (  $\mu$ g ) = ( fluorescent spike value + 0.8344 ) /177.0988

Food intake of U. insecticeps (ind.) = Europium content of U. insecticeps ( $\mu g/\text{ind.}$ ) /Europium content of S. furcifera ( $\mu g/\text{ind.}$ )

Mortality = the number of dead individuals/the number of treated individuals  $\times~100\,\%$ 

Food intake rate of the spider community = [ mean food intake of the individuals in the treated group  $\times$  (1 – mortality, 1)  $\mathcal{N}$  mean food intake of the individuals in the control group  $\times$  (1 – mortality, 1)]

The data accorded with the normal distribution. The

variances of all the data groups were homogeneous according to the methods of the *Bartlett test* for homogeneity of several variances (Neter *et al.*, 1996). The variance analysis was conducted according to the fixed model of three-factor analysis of variance. Multiple comparison of the means was carried out by the method of LSD (least significant difference) (Thomas and Kevin, 2001). All the analyses were carried out with SPSS 11.5 software.

### 2 RESULTS AND ANALYSIS

## 2.1 Food intake of U. insecticeps individuals exposed to the pesticide repeatedly

**2.1.1** Europium contents in S. furcifera and U. insecticeps individuals: The europium contents of S. furcifera and U. insecticeps specimens were determined by testing fluorescent intensities in their bodies. The results are listed in Table 1 and 2.

Dividing the total europium contents ( $0.166~\mu g$ ) by the total number of individuals (19), we got S. furcifera individual content of europium ( $0.009~\mu g$ ) averagely.

Table 1 Contents of Eu<sup>3+</sup> in Sogatella furcifera individuals

Number of $S$ . furcifera treated ( ind. )	Total dry weight ( mg )	Intensity of fluorescence	Contents of Eu <sup>3+</sup> ( μg )	Eu <sup>3+</sup> content per individual ( $\mu g/\text{ind.}$ )
3	1.000	2.708	0.020	0.007
2	0.800	3.062	0.022	0.011
2	0.700	3.062	0.022	0.011
3	1.800	3.770	0.026	0.009
3	2.400	1.999	0.016	0.005
2	1.650	3.239	0.023	0.012
2	1.550	2.530	0.019	0.010
2	1.200	2.353	0.018	0.009
$\Sigma$ 19	$\bar{x}$ 0.584		$\Sigma$ 0.166	$\bar{x} = 0.009$

Table 2 Contents of Eu<sup>3+</sup> in the *Ummeliata insecticeps* survivals exposed to the pesticide buprofezin

Concentration Spraying			Mean value			
of pesticide	Spraying	2nd day	5th day	9th day	13th day	Mean value
0:5 000	CK	0.024	0.016	0.017	0.019	0.019
	1st	0.005	0.006	0.009	0.008	0.007
1:5 000	2nd	0.008	0.014	0.014	0.015	0.013
	3rd	0.010	0.015	0.015	0.015	0.014
	Mean value	0.008	0.012	0.013	0.013	
	1st	0.006	0.006	0.009	0.013	0.008
2:5 000	2nd	0.011	0.016	0.017	0.020	0.016
	3rd	0.009	0.015	0.017	0.017	0.015
	Mean value	0.008	0.013	0.014	0.017	
	1st	0.004	0.010	0.013	0.023	0.013
3:5 000	2nd	0.007	0.009	0.011	0.020	0.012
	3rd	0.009	0.011	0.011	0.023	0.013
	Mean value	0.007	0.010	0.012	0.022	

**2.1.2** Food intake trend of U. insecticeps individuals sprayed with buprofezin for three times: According to the data of Europium contents in U. insecticeps and S. furcifera individuals, food intake of U. insecticeps individuals exposed to buprofezin is calculated. Food

intake of the U. insecticeps surviving individuals sprayed 3 times with three concentrations of the pesticide are shown in Fig. 1, where the y-axis represents food intake of the surviving spiders, and x-axis respresents the day after spraying buprofezin.

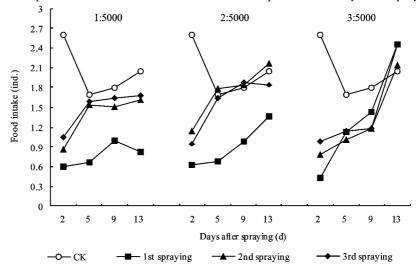


Fig. 1 Food intake of U. insecticeps survivals which were exposed 3 times to the pesticide

From Fig. 1 we notice that the food intake curve of the control group is concave. They preyed food most on the 2nd day and preyed least on the 5th day. Reasons could be that the spiders were starving on the 2nd day because no food had been supplied for 48 h before experiment. When they gorged themselves with S. furcifera for the first time they relaxed and didn 't prey actively in the following couple of days. Then their food intake amount would get back to normal. It can also be seen from Fig. 1 that the food intake of the spiders sprayed with the pesticide for the first time was much less than those of the spiders sprayed with the

pesticide for the second and the third time when the buprofezin diluted with water was 1 to 5 000 or 2 to 5 000. When spiders sprayed with buprofezin diluted in water at a concentration of 3 to 5000, food intakes of the treated groups were all greater than that of the control group on the 13th day.

**2.1.3** Variance analysis on food intake of U. insecticeps groups: Taking three-way-variance analysis on the average food intake within 13 days of the control group and the treated groups which were sprayed with buprofezin three times, the results are shown in Table 3.

Table 3 Three-way-variance analysis on food intake of the spiders which were exposed to the pesticide three times

Source	Sum of squares	df	Mean square	F	Significance
Corrected model	8.829 <sup>a</sup>	8	1.104	6.194	0.000
Intercept	108.478	1	108.478	608.796	0.000
Concentration of the pesticide	4.782	3	1.594	8.946**	0.000
Spray times	1.316	2	0.658	3.692*	0.034
The day after spraying	2.731	3	0.910	5.110**	0.004
Error	6.949	39	0.178		
Total	124.256	48			
Corrected total	15.778	47			

a: R Squared = 0.560 (Adjusted R Squared = 0.469). \*: The difference is significant at the 0.05 level. \*\*: The difference is significant at the 0.01 level.

It can be seen from Table 3 that among the three factors, influence of the spraying time on the food intake of U. insecticeps is statistically significant, while influences of concentration of the pesticide and

the day after spraying are both extremely significant. Results of multiple comparisons on individual food intake of the groups are listed in Table 4.

Table 4 Multiple comparison on food intake of the spiders which were exposed to the pesticide the
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Commonina itomo	Comparing groups	Compared groups	Mean difference	Standard error	Significance	95% confidence interval	
Comparing items						Lower bound	Upper bound
Concentrations of the pesticide		1:5 000	0.825**	0.172	0.000	0.477	1.174
	CK	2:5 000	0.628**	0.172	0.001	0.279	0.977
		3:5 000	0.675**	0.172	0.000	0.326	1.023
	1:5 000	2:5 000	- 0.197	0.172	0.260	- 0.546	0.151
		3:5 000	-0.151	0.172	0.388	- 0.499	0.198
	2:5 000	3:5 000	0.045	0.172	0.788	- 0.302	0.395
Spray times	1st time	2nd time	- 0.335 *	0.149	0.030	- 0.637	-0.033
		3rd time	- 0.365 *	0.149	0.019	- 0.667	-0.063
	2nd time	3rd time	-0.030	0.149	0.842	-0.332	0.272
Days after spraying	2nd day	5th day	-0.082	0.172	0.637	- 0.431	0.267
		9th day	-0.232	0.172	0.187	-0.580	0.117
		13th day	- 0.621 *	0.172	0.001	- 0.970	-0.272
	5th day	9th day	- 0.149	0.172	0.391	- 0.498	0.199
		13th day	- 0.539 *	0.172	0.003	-0.888	-0.190
	9th day	13th day	- 0.340*	0.172	0.029	- 0.738	-0.041

<sup>\* :</sup> The mean difference is significant at the 0.05 level. \*\* : The difference is significant at the 0.01 level.

According to the results of Table 4, food intake of the control group is obviously greater than that of the treated groups, in which buprofezin concentrations are 1:5000, 2:5000 and 3:5000, respectively. The difference between food intake of the control group and that of the treated groups is extremely significant. However, the food intake differences among the three treated groups are not statistically significant. As far as the spray times are concerned, food intake of the spiders sprayed with buprofezin for the first time is less than those of the spiders sprayed with buprofezin for two or three times, and the difference between them is

statistically significant. When it comes to the day after spraying, it can be seen that food intake of the treated spiders increased with time. A significant difference exists between food intake of the spiders on the 13th day after spraying buprofezin and on other days.

### 2.2 Food intake of U. insecticeps population exposed to the pesticide repeatedly

**2.2.1** Lethality of the pesticide to U. insecticeps: The mortality rates of the spider groups are shown in Fig. 2. The y-axis representatives the mortality and the x-axis representatives the day after spraying.

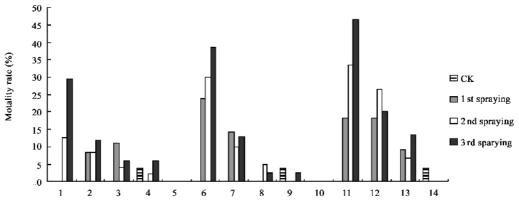


Fig. 2 Mortality rates of the treated groups

It can be seen that high death rates of the treated groups occurred in 2-5 days after spraying buprofezin. As the time interval increases , the mortality decreased gradually. Unlike the fact that the first time spraying effects the food intake of U. insecticeps surviving individuals more than that of the second or third time , the mortality of the spiders that suffered from buprofezin for the first time is relatively low. The death number became greater and greater as the treated groups

repeatedly suffered form buprofezin and as the pesticide concentration became higher. Within 13 days , the total mortality of the treated groups sprayed with the solution of  $1:5\ 000$  ,  $2:5\ 000$  and  $3:5\ 000$  are 34.33% , 46.25% and 76.31% , respectively , which are much more than that of the control group ( 3.85% ). It can be concluded that the toxicity of the pesticide caused high mortality to the treated spider groups .

**2.2.2** Food intake of *U*. insecticeps population

exposed to the pesticide repeatedly: Based on the data of mortality and the individual food intake of the treated groups and the control group, the population food intakes of the three treated groups is calculated in comparison with that of the control group (Table 5).

Table 5 Population food intake rate of the treated groups compared to the control group

- ·	Population	M 1		
Spraying	1:5 000	2:5 000	3:5 000	Mean values
1st	31.58	34.03	32.81	32.81
2nd	43.56	48.63	25.80	39.33
3rd	40.93	26.90	14.71	27.51
Mean values	38.69	36.52	24.44	

If the mortalities of the spider groups are taken into account, food intake of the population in the treated groups is much less than that of the control group during the 13 days being exposed to the pesticide. When spiders were sprayed with the pesticide repeatedly, the population food intake of the group sprayed with buprofezin of high concentration (diluting 3 to 5 000) decreased the most, only about a quarter of the food intake of the control population (24.44%), while the population food intake of the groups sprayed buprofezin at recommended concentrations (diluting 1 or 2 to 5 000) also decreased to about one third of food intake of the control population (38.69% and 36.52%). Therefore, application of the pesticide buprofezin affects the control capacity of U. insecticeps on S. furcifera.

### 3 DISCUSSION

### 3.1 Spider food intake can be quantitatively measured by the fluorescent labeling method

It is a challenge to measure the food intake of natural predators on target pests quantitatively. Some new techniques such as the serological method (Zhou, 1989) and enzyme-linked immunosorbent assay (Zhang et al., 1997; Lim and Lee, 1999) were applied to evaluate the natural enemy predation instead of traditional "eyeballing". However, these methods can only determine whether the predator eats the target insects or not. The fluorescent labeling method is one of the desired techniques for quantitatively analyzing food intake of the small predators. Some theses applying this method to measure spider food intake have been published in China ( Hu et al., 2002; He et al., 2003 ; Wen et al., 2003 ; Xiao et al., 2006 ). By applying the fluorescent tracer into the food chain, this experiment determines the consumption rate of U. insecticeps, which were sprayed with the pesticide 3 times at various concentrations, on S. furcifera quantitatively. The fluorescent labeling method can be

one of the quantitative techniques for evaluating the influences of pesticides on predator food intake.

### 3.2 Effects of concentration and spraying times of the pesticide on spiders food intake

In this experiment, food intakes of the surviving spiders in all treated groups are much less than that of the control group on the 2nd day after spiders were sprayed the pesticide solutions. That is, no matter which spraying time or what the concentration is, the pesticide affects the predation of U. insecticeps on S. furcifera in a short time. It can be deduced that the U. insecticeps survivals were poisoned when exposed to the pesticide. The pesticide would have decreased the spider metabolic rate or disordered their other physiological functions. Therefore, the survivals were not able to catch the prey successfully or probably they lost their appetite because of the toxicity of the pesticide.

Food intake of surviving U. insecticeps which were sprayed with the pesticide for the first time was less than that of the survivals sprayed for the second or third time when the pesticide was diluted in relatively low concentrations. It can be seen from Fig. 1 that at the first time the spiders suffered from the pesticide (in the diluted concentrations of  $1:5\ 000$  and  $2:5\ 000$ ), their food intake did not recovered within 13 days. When they were exposed to the pesticide for the second or the third time, however, it just took 5 days for their food intake to recover. This indicates that U. insecticeps individuals are hypersensitive when they contact the pesticide for the first time. They have strengthened their resilience when they were sprayed the pesticide repeatedly. That is why they can recover more quickly than the first time they were exposed to the pesticide.

It took a long time for the surviving spiders to recover when they were sprayed with the pesticide at high concentration (diluted in 3 to 5 000). But once they recovered they preyed on more *S. furcifera* for food than the individuals in the control group. The reason could be the two sides of the coin. On one hand, the physiological function of the surviving spiders is damaged a lot by the pesticide at high concentrations. They lost their appetite to the prey or their vigor and other capabilities in capturing their prey for a long time period. On the other hand, because the surviving spiders had been in the state of hunger or semi-hunger for a long time, they were hungry for food and would prey more actively when they recovered.

However, food intake of U. insecticeps which was exposed to the pesticide buprofezin was just measured in the laboratory. It is worthwhile to compare the results from the laboratory experiment to those from paddy

fields to further study the effects of pesticides on spider food intake.

### 3.3 Not to apply the pesticide rashly

The chosen pesticide buprofezin is one of the insect growth regulators. It works by preventing the insects from synthesizing chitin and disturbing their metabolism. The target insects are killed by touching the poison or by the gastric toxicity created by the insecticide. The buprofezin concentrations used in the experiment are around 50%, 100% and 150% of the recommended concentration. The results showed that the population food intakes of all the treated groups decreased sharply after spraying the insecticide. The phenomenon that some surviving spiders ate even more than the control individuals on the 13th day after being sprayed with the insecticide is not worthwhile because it is at cost of some other individuals 'death in the population. Besides, the pesticide decreased the predatory capacity of U. insecticeps population for about 2 – 9 days after spraying the pesticide, even if at the recommended concentration. The higher the concentration is , the more times U. insecticeps suffers from the pesticide, and the higher the spider mortality will be and the longer time the surviving individuals will need to recover their predatory capacity. Pests below economic threshold will not hurt the rice but a favorable condition is created for the stability of the predator populations. Integrative effect of predators on the pests should be take into account when dealing with the insect pest in paddy ecosystem. It is unadvisable to apply pesticides hastily as soon as the pests show up. The interval between the two times of applying pesticides should be as long as possible so that the predator population could rebuild and reactivate.

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### 荧光物示踪法测定重复施药胁迫 下食虫沟瘤蛛的摄食量动态

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摘要:定量评价农药对天敌的影响是IPM 研究的重要内容之一,由于对天敌的捕食量缺乏有效的定量测量方法,农药对天敌摄食功能影响仍处于定性水平的研究,误差基数较大。本文采用一种定量的测定方法——荧光物示踪法,以稀土元素铕作为荧光示踪物质,定量地测定了食虫沟瘤蛛在重复接受噻嗪酮喷施后,其存活个体在药后 13 天内摄食量的变化趋势,且把药后食虫沟瘤蛛的死亡率加以考虑,计算药后整个试验食虫沟瘤蛛群体的摄食量变化。结果表明:任何浓度任何一次施药后2天左右食虫沟瘤蛛存活个体的捕食量均急剧下降,药物浓度较低时,第1次施药对食虫沟瘤蛛的影响最大,药物浓度较高时,食虫沟瘤蛛存活个体药后摄食量恢复周期较长。药后一段时间内整个食虫沟瘤蛛种群的摄食率相当低下,高浓度农药处理的食虫沟瘤蛛组3次施药后13天内的平均种群摄食率只有正常状况下的1/4左右(24.44%),推荐浓度及低于推荐浓度的食虫沟瘤蛛种群摄食率也仅为对照组的1/3左右(38.69%36.52%)。因此在对水稻虫害防治时,应当尽可能地避免使用化学农药,充分发挥天敌对害虫数量的调控作用,当害虫数量超过经济阈值而必须施药时,也应当掌握农药用药剂量和次数的尺度,以利于食虫沟瘤蛛等稻田天敌的生理恢复和群落重建。

关键词:食虫沟瘤蛛;白背飞虱;荧光物示踪;摄食量;重复施药

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