

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

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**Abstract:** This paper focuses on studying the influence of herbicides on the food intake of *Ummeliata insecticeps* (Bosenberg et Strand) by a novel quantitative method – the fluorescence labeling method. Europium, the lanthanide chosen for the experiment, was dissolved in the soil and then absorbed by the rice plants. *Sogatella furcifera* eats the plants and *U. insecticeps* preys on *S. furcifera* for food. Thus, by applying a series of chemical analytical methods of extracting and invert extracting on the tested fly louse and spider samples, the food intake of *U. insecticeps* could be known. The results showed that each surviving spider individual sprayed with Simetryne preyed on 1.784 *S. furcifera* individuals per day over the course of 12 days. If sprayed with Starane, spiders preyed on 2.678 *S. furcifera* individuals/d, and with Gallant-S, 2.856 individuals/d, on average. The individual food intakes of the three test groups were all less than that of the control group, which preyed on 3.321 *S. furcifera* individuals/d. The curves of the food intakes of the spiders in the different groups are all inverted parabolas, which can be fitted by quadratic equations. If mortality is taken into account, for the first 8 days after the application of herbicides, the population food intakes of the test groups are all much less than that of the control group. The average predatory ratios of the three test groups in the 2nd day, the 5th day and the 8th day are 52.11%, 36.45% and 50.27% of the control group's population food intake, respectively. In the 12th day, however, the population food intake of the test groups is 131.84% of that of the control group. It could be concluded that all of the herbicides tested do harm to the spiders' food intake capacity within 8 days of exposure. Therefore, it is unadvisable to apply herbicides when the paddy field is rife with pests because the herbicides would weaken the spiders' control over the pests. It is also unadvisable to apply herbicides during the harvest season because the spiders migrate at this time and the lethality of the herbicides will disturb the rebuilding of the spider communities.

**Key words:** *Ummeliata insecticeps*; *Sogatella furcifera*; lanthanide; fluorescent tracking; herbicides; food intake

## 1 INTRODUCTION

People have been using the herbicide 2,4-D to kill unwanted weeds in paddy fields for more than 60 years. Herbicides are now used worldwide, and the consumption of and application areas of herbicides have become even greater than that of pesticides and bactericides (Zhang *et al.*, 1997a; Guo, 2001). Although herbicides are low-toxic chemicals, it is inevitable that they will negatively affect the beneficial organisms in the paddy ecosystem while killing off paddy weeds (Wright and Verkerk, 1995). There have been numerous studies on the lethality and other negative effects of herbicides on natural predators (Brust, 1990; Torres, 1992; Ding *et al.*, 1998; Tang, 1998). However, there are just a few reports that qualitatively measure the influence of herbicides

upon the predators' food intake (Li *et al.*, 2000).

*Ummeliata insecticeps* is the dominant species of spider population in most planting area in China. By adopting europium as a fluorescent tracer, this paper focuses primarily on the quantitative measurement of food intake of *U. insecticeps* exposed to herbicides. This paper aims to provide the further theoretical basis for the intelligent use of herbicides and to integrate pest management in rice cultivation.

The lanthanide chosen for the experiment, europium, can be absorbed by the rice plant after it has dissolved in the soil. *Sogatella furcifera* eats the plants and *U. insecticeps* preys on *S. furcifera* for food. Therefore, the europium accumulates in the spiders' bodies as they consume *S. furcifera*. By applying a series of chemical analytical methods of extracting and invert extracting on the tested fly louse and spider

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samples( Wang *et al.* ,2001 ;Hu *et al.* ,2002 ;He *et al.* ,2003 ;Wen *et al.* ,2003 ), the food intake of *U. insecticeps* that have been sprayed with herbicides could be known.

## 2 MATERIALS AND METHODS

### 2.1 Materials

The paddy rice :Jinyou 974 ;the pest species : *S. furcifera* ;the spider species : *U. insecticeps* .

The herbicide :Simetryne( 25% wettable powder , made by Pesticide Factory of Chemical Co. , Jilin , the recommended concentration is Simetryne : water = 1 : 400 ) ; Starane ( 25% emulsion , made by Dow Agrosiences , America , the recommended concentration is Starane : water = 1 : 900 ) ; Gallant-S ( 10.8% emulsion , made by Dow Agrosiences , America , the recommended concentration is Simetryne : water = 1 : 1 000 )

### 2.2 Apparatus and chemicals

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 Chamber ( LRH-250-GS , Guangdong Medical Appliance Factory ), Constant Temperature Drying Oven ( DHG-9031-A , Shanghai Jing Hong Laboratory Instrument Co. , Ltd. ), Stereomicroscope , Separatory Funnel , Plastic Planting Tub , Gauze hood ;  $\text{HNO}_3$  ,  $\text{HCl}$  ,  $\text{H}_2\text{O}_2$  ,  $\text{Eu}_2\text{O}_3$  , *etc.*

### 2.3 Methods

**2.3.1 Specimen collection :** The rice sprouts were transplanted in plastic tubs covered by a gauze hood on April 28 , 2003 . A solution of  $\text{Eu}_2\text{O}_3$  dissolved in water at a concentration of 40 mg/kg was poured into the planting tubs on May 10 .

The *S. furcifera* adults were collected from a paddy field in a western suburb of Changsha in the maturing stage of the rice . The *S. furcifera* were put into the gauze hood and allowed to eat the rice plants for 72 hours , after which they were recollected . Among the recollected *S. furcifera* , 19 individuals were used for measuring the europium content in their bodies ; the others were offered to the spiders for food . The 19 individuals were separated into 8 units each unit comprised of either 2 or 3 individuals . Each unit was tested 2 times . 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 , but they were

picked up from a fallow field which had not been sprayed with any chemicals for about 3 years . Each spider was put into a 30 mL cuvette with a little wet cotton to provide suitable humidity , and no food was offered for 48 hours . The spiders were then separated into four groups : three test groups and one control group . Each group had 60 individuals in total , and the experiment was carried out 3 times with each group , using 20 specimens each time . Each test group was sprayed with one kind of herbicide in a solution diluted to the recommended concentration and the control group was sprayed with pure water . A mini atomizer was used to spray each spider 's back until it was wet . On the 2nd day , the 5th day , the 8th day and the 12th day after the application of herbicides , two things were done : First , the number of dead *U. insecticeps* was checked and recorded , and the dead were eliminated . Second , each surviving spider was moved to a 250 mL beaker and was supplied with 10 *S. furcifera* individuals which had been fed on the europium-marked rice for 72 hours . Twenty four hours later , the spiders were recollected . The spiders that recollected at the same time and belonged to the same group were put together for measuring the europium content in their bodies . Each group 's europium content was tested 2 times . The average value was taken as the europium content of *U. insecticeps* individuals of the corresponding group .

**2.3.2 Measurement and analysis of the europium content :** To find their europium contents , the recollected fly louses and spiders were processed according to the technique patented by our laboratory , the quantitative analysis of food intake by the fluorescence tracer transferred in the food chain ( Wang *et al.* , 2001 ). The steps were as follows : ( 1 ) Cineration and nitration of the samples to form a solution ; ( 2 ) Extraction and invert extraction of europium from the solution to form the europium-complex ; ( 3 ) Excitation of the europium complex with a light of 390 nm in the RF-5301PC fluorescence spectrophotometer and examination of the intensity of the fluorescence that the excited europium-complex emitted ( at the wavelength of approximately 612 nm ) ; ( 4 ) Calculation of the europium content in the fly louse and spider bodies according to the standard curve equation of the fluorescent tracing method .

**2.3.3 Data analysis :** Standard curve equation ( Wang *et al.* , 2001 ) :  $y = -0.8344 + 177.0988 x$  (  $x$  is  $\mu\text{g}$  of the europium content and  $y$  is value of the fluorescent peak )

Europium content (  $\mu\text{g}$  ) = ( fluorescent peak value + 0.8344 ) / 177.0988

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

Mortality rate = the number dead / the number tested  $\times 100\%$

Food intake ratio of the spider population =  $\frac{[\text{mean food intake of the individuals in the test group} \times (1 - \text{mortality}_{(t)})]}{[\text{mean food intake of the individuals in the control group} \times (1 - \text{mortality}_{(c)})]}$

The data accord with the normal distribution. The variances of all the data groups are homogeneous according to the method of Bartlett test for homogeneity of several variances (Neter *et al.*, 1996). The variance analysis was taken according to the two-factor fixed effect model because there was no interaction between factor A and B according with the test method for interaction presented by Tukey (Tukey, 1949). The multiple comparison is carried out by Duncan multiple

range test (Li, 2002). The analysis of variance, the calculation of curve equations and correlation coefficients were all completed by the software EXCEL.

### 3 RESULTS AND ANALYSIS

#### 3.1 The lethalties of the herbicides to *U. insecticeps*

The mortality rates of the spider groups at different times are calculated in Table 1. It can be seen that the mortality rates of the test groups within 12 days are about 20% – 30%, which is much more than that of the control group's rate of 3.85%. It can be concluded that the toxicity of the herbicides brought high death rates to the test groups.

Table 1 Mortality rates of the spider groups

Time after spraying ( days )	Mortality rates ( % )			
	Simetryne	Starane	Gallant-S	Control group
2	0.00	10.71	10.71	0.00
5	0.00	7.14	7.14	0.00
8	13.33	7.14	0.00	0.00
12	6.67	3.57	3.57	3.85
$\Sigma$	20.00	28.56	21.42	3.85

#### 3.2 Food intake of *U. insecticeps* individuals sprayed with herbicides

3.2.1 The europium contents in *S. furcifera* and *U. insecticeps* individuals: The europium contents of *S. furcifera* and *U. insecticeps* samples were determined by testing for fluorescent intensities. The results are

shown in Table 2 and Table 3.

Dividing the total europium content, 0.166  $\mu\text{g}$ , by the total number of the individuals, 19, each *Sogatella furcifera* individual had 0.009  $\mu\text{g}$  of europium, on average.

Table 2 Contents of  $\text{Eu}^{3+}$  in *Sogatella furcifera* individuals

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

Table 3 Contents of  $\text{Eu}^{3+}$  in *Ummeliata insecticeps* individuals

Time after spraying ( days )	Europium contents ( $\mu\text{g}/\text{ind.}$ )				
	Simetryne	Starane	Gallant-S	Mean value	Control group
2	0.012	0.038	0.019	0.023	0.040
5	0.008	0.010	0.008	0.009	0.023
8	0.010	0.011	0.023	0.015	0.028
12	0.036	0.040	0.055	0.044	0.032
$\bar{x}$	0.016	0.025	0.026	0.022	0.031

3.2.2 The food intake of *U. insecticeps* individuals: The food intake of *U. insecticeps* individuals and the food intake ratio of the test spiders compared with the

control spiders are calculated in Table 4 according to the  $\text{Eu}^{3+}$  contents in the spider and the fly louse individuals.

**Table 4 Food intake of *Ummeliata insecticeps* individuals and the food intake ratio of the test individuals compared with the control individuals**

Time after spraying ( days )	Food intakes ( ind. )				Food intake ratios ( in terms of time )( % )	
	Simetryne	Starane	Gallant-S	Mean values	Control group	
2	1.294	4.159	2.035	2.496	4.365	57.18
5	0.918	1.033	0.909	0.953	2.502	38.09
8	1.037	1.182	2.504	1.574	2.984	52.75
12	3.886	4.339	5.977	4.734	3.432	137.94
$\bar{x}$	1.784	2.678	2.856		3.321	
Food intake ratios ( % ) ( in terms of herbicide )	53.72	80.64	86.00			

The data in Table 4 shows that within 8 days of herbicide exposure , the food intakes of the test spiders were all less than that of the control spiders. This indicates that all of the herbicides tested do harm to the spider 's food intake capacity during this period. In the 12th day , however , the food intakes of the spiders in the three test groups were all more than that of the spiders in the control group. The food intake of the group sprayed with Gallant-S was particularly highly , almost two times that of in the control group (  $5.977/3.432 = 1.74$  ). The reasons could be that the herbicide activated the spiders ' resistance to its toxicity gradually ; the toxic ingredient in the herbicide could

have also been decomposed gradually by the spider 's metabolism. The appetite and predatory capacity of the spiders recovered about 12 days after being sprayed with herbicide. During this time , the surviving spiders had been in a starving or semi-starving state. Thus , after they recovered from the herbicide exposure , they preyed on many more insects to relieve their hunger.

The food intakes of the test and control groups were compared by two-factor analysis of variance , in which factor A represents the time after spraying the herbicides , and factor B represents the type of the herbicide. The results are shown in Table 5.

**Table 5 Two-factor analysis of variance on food intake of different spider groups on different days**

Sources of variation	Sum of squares	Degrees of freedom	Mean squares	<i>F</i>	<i>F</i> <sub>0.05</sub>	<i>F</i> <sub>0.01</sub>
Factor A	24.675	3	8.225	13.418**	3.863	6.992
Factor B	2.643	3	0.881	1.437		
Error	5.517	9	0.613			
Total	32.835	15				

\*\* indicates significant difference at  $P < 0.01$  level.

The results show that the time after spraying the herbicides is an essential factor that influenced the food intakes of the test groups. The type of the herbicides , however , is not so important. It can be seen that the food intakes of the spiders fluctuated a lot in the period of time after they were sprayed with herbicides. This implies that during this time the herbicides exerted a certain influence on the spiders ' physiologic functions , such as homostasis and behavior , *etc.* Therefore , the predatory capacities of the spiders were unstable and in a stage of auto-regulation.

Taking the time as abscissa and the food intake as ordinate , the food intake trends of the spider groups are displayed in Fig. 1.

All the curves are concave lines and the food intake curves of three test groups sink much more than that of the control group. It could be deduced that the herbicides negatively affected the food intake capacities of the test groups in 2 - 8 days. In the 12th day , however , the predatory capacities of the test spiders rebounded and they preyed even more insects than the

control spiders. Fitting the curves by dualistic parabolas , the equations and correlation coefficients are as follows :

$$y_c = 0.578 x^2 - 3.119 x + 6.787 ( r = 0.9218^{**} )$$

$$y_{Si} = 0.806 x^2 - 3.241 x + 3.841 ( r = 0.9788^{**} )$$

$$y_{Si} = 1.571 x^2 - 7.785 x + 10.360 ( r = 0.9998^{**} )$$

$$y_{Ga} = 1.150 x^2 - 4.406 x + 5.249 ( r = 0.9987^{**} )$$

The correlation between the food intake  $y$  and the time  $x$  is extremely remarkable no matter what spider group is concerned - the relationship between the food intake of the spider and the time after being sprayed herbicides all accord with inverted parabolas. Therefore , the food intakes of the test spiders can be predicted approximately by the dualistic equations.

### 3.3 Population food intake ratios of the spider groups sprayed with herbicides

Using the data in Table 1 and Table 4 , the population food intakes of three test groups in comparison with that of the control group were calculated in Table 6.

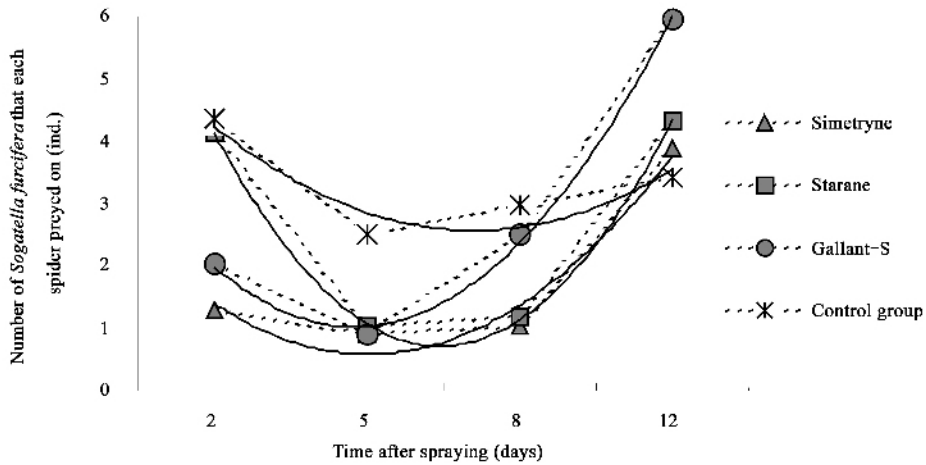


Fig. 1 Trend curves of food intakes of the spider groups

Table 6 Population food intake ratios of the test groups compared with the control group

Time after spraying ( days )	Population food intake ratios compared to the control group ( % )			
	Simetryne	Starane	Gallant-S	Mean values
2	29.64	85.07	41.63	52.11
5	36.69	38.34	33.74	36.25
8	30.12	36.78	83.91	50.27
12	105.68	121.91	167.94	131.84
$\bar{x}$	50.53	70.53	81.80	67.62

If mortality is taken into account, within 12 days of herbicide exposure, the population food intakes of the test groups are all much less than that of the control group. The population food intake of the group sprayed with Simetryne decreased the most, nearly 50%, while the population food intake of the groups sprayed with Starane and Gallant-S descended 20% to 30%. This clearly shows the negative impact of the herbicides on the predatory capacity of *U. insecticeps*.

#### 4 DISCUSSION

It is a challenge to measure the food intake of natural predators on their target pests quantitatively. Some new techniques, such as the serological method (Zhou, 1989) and enzyme-linked immunosorbent assay (Zhang *et al.*, 1997b; Lim and Lee, 1999) have been applied to evaluate predation instead of the traditional "eyeballing" method. However, these methods can only determine that the predator eats or does not eat the target insects. By applying the fluorescent tracer into the food chain, this experiment quantitatively determined the consumption rate of *U. insecticeps* on *S. furcifera*.

Because of their toxic selectivity, herbicides have traditionally been considered harmless for arthropods (Zhang, 1988). However, the chosen herbicides did cause high mortality in the spider population in about 8 days after spraying the herbicides, even though they were applied in the recommended dosages. During the

harvest season, spider populations are unstable and fragile because they have to migrate to the ridges of the field temporarily, rebuilding their populations in the late-rice season. Therefore, it is advisable not to apply the herbicides in harvest season.

The chosen herbicides all do certain harm to the spider's predatory capacity for about 8 days after application. Therefore, it is unadvisable to apply herbicides for weeding when the paddy field is rife with pests. If herbicides are applied at this time, the spider's control over the pests will be weakened and the paddy rice would suffer more from the paddy pests. This would increase the possibility that the farmers would have to resort to using chemical pesticides.

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## 荧光物示踪法定测定除草剂对食虫沟瘤蛛摄食量的影响

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摘要: 采用一种全新的测定方法——荧光物示踪法, 以稀土元素钬作为荧光示踪物质, 以“水稻-白背飞虱 *Sogatella furcifera* Horvath-食虫沟瘤蛛 *Ummeliata insecticeps* Bosenberg et Strand”这一食物链中的水稻和白背飞虱作为钬的传递者, 经萃取和反萃取等一系列化学分析方法, 定量地测定了 3 种除草剂胁迫下食虫沟瘤蛛的摄食量。结果表明: 施用除草剂西草净、使它隆和高效盖草能以后, 食虫沟瘤蛛的存活个体 12 天内平均每天捕食白背飞虱分别为 1.784、2.678 和 2.856 头, 均低于对照组的平均个体摄食量 3.321 头/d; 各组 12 天内个体摄食量的趋势线均为开口向上的抛物线, 可用二次方程进行拟合; 同时除草剂的喷施亦对食虫沟瘤蛛造成一定的致死率, 因而造成施药后食虫沟瘤蛛的种群摄食率减退, 第 2、5、8 天的平均摄食率分别为对照组的 52.11%、36.45% 和 50.27%; 药后第 12 天 3 个处理组的摄食量均超过了正常值, 平均摄食率达到对照组的 131.84%。因此在稻田中害虫大发生或天敌群落迁移时应慎用除草剂, 以免食虫沟瘤蛛药后摄食量减少导致对目标害虫控制力减弱或由于除草剂的致死力而对食虫沟瘤蛛的群落重建构成威胁。

关键词: 食虫沟瘤蛛; 白背飞虱; 稀土元素; 荧光物示踪; 除草剂; 摄食量

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