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# Appraisal of the impact of three insecticides on the principal rice pests and their predators in China

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## Abstract

Chemical control is an effective measure for decreasing the numbers of rice planthoppers (*Nilaparvata lugens* [Stål] and *Sogatella furcifera* [Horváth]; Hemiptera: Delphacidae) and rice leaffolders (*Cnaphalocrocis medinalis* Guenée; Lepidoptera: Crambidae), which have caused substantial yield losses of rice in China in recent years. Virtako is a new mixture of insecticides that has low mammalian toxicity and high toxicity to insect pests. We conducted a study of the effectiveness of Virtako (a mixture of chlorantraniliprole and thiamethoxam), versus chlorantraniliprole alone and thiamethoxam alone, for control of rice planthoppers and rice leaffolders, as well as the impact of these insecticides on predator diversity. One and 28 d after application, Virtako treatment (36–60 g a.i./ha) reduced the numbers of planthoppers to 46 to 60% and 59 to 66%, respectively, of the control levels. Virtako also suppressed damage by rice leaffolders, resulting in leaf protection rates of 11 to 46% and 37 to 76% at 7 and 28 d after application, respectively. Both 1 and 2 applications of Virtako per crop cycle caused significant short-term reductions in insect predator populations. However, 21 d after the application of Virtako (36 g a.i./ha), the diversity indices and the total number of predators were similar to those in untreated plots. A single application of Virtako provides good control of insect pests in paddy fields, and predator populations recovered quickly after the Virtako application. Our studies indicated that Virtako might be an effective alternative for the control of planthoppers and rice leaffolders in paddy fields.

Key Words: insecticide; planthopper; rice leaffolder; predator; community

## Resumen

El control químico es una medida eficaz para disminuir el número de saltadores de plantas de arroz (*Nilaparvata lugens* [Stål] y *Sogatella furcifera* [Horváth]; Hemiptera: Delphacidae) y dobladores de hojas de arroz (*Cnaphalocrocis medinalis* Guenée; Lepidoptera: Crambidae), que han causado pérdidas de rendimiento sustanciales de arroz en la China en los últimos años. Virtako es una nueva mezcla de insecticidas que tiene una baja toxicidad en mamíferos y alta toxicidad para las plagas de insectos. Realizamos un estudio de la eficacia de Virtako (una mezcla de clorantraniliprol y tiametoxam), frente a clorantraniliprol solo y tiametoxam solo, para el control de saltadores de las plantas de arroz y dobladores de las hojas de arroz, así como el impacto de estos insecticidas sobre la diversidad de depredadores. Uno y 28 días después de la aplicación, el tratamiento Virtako (36-60 g i.a./ha) redujo el número de saltadores de las plantas a 46-60% y 59-66%, respectivamente, de los niveles de control. Virtako también suprimió daños por los dobladores de hojas de arroz, lo que resulta en tasas de protección de la hoja del 11 al 46% y un 37 a un 76% a las 7 y 28 días después de la aplicación, respectivamente. Una o 2 aplicaciones de Virtako por ciclo de cultivo causó reducciones significativas a corto plazo en las poblaciones de depredadores de insectos. Sin embargo, 21 días después de la aplicación de Virtako (36 g i.a./ha), los índices de diversidad y el número total de los depredadores fueron similares a los de las parcelas no tratadas. Una sola aplicación de Virtako ofrece un buen control de plagas de insectos en los campos de arroz, y las poblaciones de depredadores se recuperó rápidamente después de la aplicación Virtako. Nuestros estudios indican que Virtako podría ser una alternativa efectiva para el control de saltadores de las plantas y dobladores de las hojas de arroz en arrozales.

Palabras Clave: insecticidas; saltador de plantas; dobladores de hojas de arroz; depredador; comunidad

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More than 3.5 billion people depend on rice (*Oryza sativa* L.; Poales: Poaceae) as their food staple, and more than 90% of the world's rice is produced and consumed in Asia (Sigsgaard 2000; Bashir et al. 2010). The green revolution has led to considerable progress in improving rice production in the last 5 decades. However, due to the planting of various rice varieties and the overuse of fertilizers and pesticides, rice planthoppers [particularly the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), and the white-backed planthop-

per, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae)], and the rice leaffolder, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Crambidae), have become serious pests in most rice-producing countries in Asia (Heong 1993; Karim & Riazuddin 1999; Sigsgaard 2000, 2007; Matsumura et al. 2009).

In China, insecticides are still the first-line measure for controlling rice insect pests because they are rapid, efficient, easy to use, and cost-effective. However, since 2003, insecticide resistant rice plan-

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thoppers (particularly the brown planthopper) and the rice leafhopper have caused severe losses in the southern and Yangtze River regions of China (Dale 1994; Yang et al. 2004; Zhai & Cheng 2006; Yin et al. 2008; Zheng et al. 2011).

In China, although rice is attacked by both planthoppers and leafhoppers, these insects do not necessarily occur together. Even though generations may overlap, these insects exploit different niches on the rice plant and feed in different ways (Ye et al. 2007). In order to reduce the effects of pesticides on non-target insects, rice pest control methods need to be improved. Application of insecticide mixtures is an effective strategy to manage several insects simultaneously and possibly to reduce total insecticide use (Han et al. 1993; Corbel et al. 2004; Peng et al. 2010). Such mixtures have been widely used to manage pests in agriculture. For example, more than 2,200 pesticide mixtures are registered for use in China (Peng et al. 2010).

Virtako is a new insecticide mixture developed by Syngenta during the 2000s. It contains 20% chlorantraniliprole and 20% thiamethoxam, and it has low toxicity to mammals and high toxicity to pests in the orders Lepidoptera, Coleoptera, and Diptera. Our previous studies found that Virtako might be an effective alternative for the control of the brown planthopper by delaying the development of resistance to thiamethoxam (Chen et al. 2013, 2014). However, only a limited amount of research exists on the field efficacy of Virtako in controlling rice insect pests or their natural enemies (Yang et al. 2010; Geng et al. 2011). In particular, the effects of Virtako on natural enemies are poorly known. Also, assessment of parameters such as the Shannon–Weaver diversity index  $H'$ , Pielou evenness index  $J$ , Simpson dominance index  $C$ , and species richness  $S$  should provide important appraisal of the overall impact of this insecticide. There have been reports that insect diversity was distinctly influenced after insecticide application (Jiang et al. 2006; Dupo & Barrion 2009; Xu et al. 2012). In order to determine whether Virtako may be used to effectively control rice insect pests, we conducted a field appraisal of Virtako against planthoppers and the rice leafhopper, and we examined its impact on predators in the rice paddy.

## Materials and Methods

### EXPERIMENTAL SITE

The study was conducted from 27 May to 25 Jun 2012. Field trials were performed based on the Pesticide Guidelines for Field Efficacy Trials (I) (Chen et al. 2000; Wang et al. 2000). The experimental site was located in Huizhou City of Guangdong Province, China (23.1638889°N, 114.4861111°E), which receives approximately 1,630

mm of annual rainfall and has an annual average temperature of 20 to 22 °C. The site had red soil and the dominant natural vegetation was barnyard grass. The rice cultivar 'Xiangya Zhan' was planted on 15 Mar 2012, with rows and plants spaced 20 cm and 15 cm apart, respectively.

### APPLICATION OF INSECTICIDES

Over the course of 2 experiments (see below), we tested 10 formulations, which included 3 rates of each of 3 products (Virtako, chlorantraniliprole, and thiamethoxam) plus an equivalent amount of water as a control (Table 1). There were 33 plots, 3 × 20 m each, separated by ridges of soil approximately 0.30 m high to prevent water flow between the adjacent treatment plots. A solution containing the insecticide was diluted to 750 kg/ha and sprayed on rice seedlings by using a hand-pumped pressurized sprayer (SX-LK16J, SeeSa, Huangyan, Zhejiang, China).

*Experiment 1: One application per crop cycle.* Insecticide applications were made on 27 May 2012. Treatments 1 to 5 and treatment 11 (see Table 1) were randomly arranged in plots, and each treatment was repeated in 3 plots.

*Experiment 2: Two applications per crop cycle.* Treatments 6 to 10 (see Table 1) were double-application experiments. The 1st applications were made on 27 May 2012. Each treatment was repeated in 3 plots, and all treatment plots were randomly arranged. On 3 Jun 2012, each treatment plot received a 2nd application of the same insecticide.

### INSECT SAMPLING

The number of rice planthoppers (adults and nymphs), the predator species, and the number of predators (the number of individual predators of all taxa together) were investigated before pesticide application and 1, 7, 14, 21, and 28 d after application. The number of rolled leaves (50 hills per plot, where each hill equals 1 rice plant) was used as an indicator of rice leafhopper damage and was determined before application, and again 7, 14, 21, and 28 d after application.

Five points per plot were investigated according to the equidistant sampling method. Briefly, we established the 1st point in the center of the habitat, at least 3 m from the edge, and then walked about 3 m along the row to start the next point. Ten hills were sampled at each point. The leafhopper damage was recorded by counting the number of rolled leaves. Rice planthoppers and predators were collected by using white porcelain basins, with each basin containing water (0.5 cm deep) to trap the rice planthoppers and predators from 1 hill that measured 30 × 45 cm (length × width). The insects were knocked from the rice plants into the water by hand (shaken 3–5 times). The trapped

**Table 1.** Evaluation of field efficacy of insecticides against rice insect pests.

| Treatment | Insecticide                  | Rate<br>(g a.i./ ha) | No. of applications | Producer |
|-----------|------------------------------|----------------------|---------------------|----------|
| 1         | Virtako (WG)                 | 36.0                 | 1                   | Syngenta |
| 2         | Virtako (WG)                 | 48.0                 | 1                   | Syngenta |
| 3         | Virtako (WG)                 | 60.0                 | 1                   | Syngenta |
| 4         | Thiamethoxam (25% WG)        | 22.5                 | 1                   | Syngenta |
| 5         | Chlorantraniliprole (25% SC) | 30.0                 | 1                   | Dupont   |
| 6         | Virtako (WG)                 | 36.0                 | 2                   | Syngenta |
| 7         | Virtako (WG)                 | 48.0                 | 2                   | Syngenta |
| 8         | Virtako (WG)                 | 60.0                 | 2                   | Syngenta |
| 9         | Thiamethoxam (25% WG)        | 22.5                 | 2                   | Syngenta |
| 10        | Chlorantraniliprole (25% SC) | 30.0                 | 2                   | Dupont   |
| 11        | Control                      | —                    | —                   | —        |

insects were counted and unknown predator species were transferred to sample jars containing 70% ethanol and returned to the laboratory for identification. Observations were recorded 1, 7, 14, 21, and 28 d after insecticide application.

DATA ANALYSES

For each treatment and replicate, the control effect (%) on planthoppers was calculated by the following equation:

Control (%) = {[(Nt0 - NtX) / Nt0] - [(Nc0 - NcX) / Nc0] / [1 - (Nc0 - NcX) / Nc0]} × 100

Where Nc is the planthopper number from water-treated samples and Nt is the planthopper number from insecticide-treated samples. The number 0 means before application of insecticide and X means days after application of insecticide.

The rolled leaf growth rate (Rr) and the leaf protection rate (Ptr) were calculated by the following equations:

Rr (%) = [(Pr - Cr) / Pr] × 100;

Ptr (%) = (Rt - Rc) / (100 - Rc) × 100

Where Rr (%) is the percentage of increase in damage, Ptr (%) is the percentage of control, Pr is the prior number of rolled leaves, Cr is the current number of rolled leaves, Rc is the rolled leaf growth rate with water-treatment control, and Rt is the rolled leaf growth rate with insecticide treatment.

Most predators sampled were identified to the species level. Alpha species diversity was calculated using the Shannon–Weaver diversity index *H'*, Pielou evenness index *J*, Simpson dominance index *C*, and species richness *S* (Zhang et al. 2013). All data on population densities of predators (the number of individual predators of all taxa together) from the treatments in the field were analyzed using 1-way analysis of variance (ANOVA). Percentage data (except for the rolled leaf growth rate) were arcsine square-root transformed to reduce the heterogeneity of variance (Peck et al. 2008; Zar 2009) and analyzed with standard ANOVA. When significant differences were found, a Tukey's honest significant difference (HSD) test for multiple comparisons of means was carried out. Arcsine square-root transformed data were back transformed after analysis for presentation in the text, figures, and tables.

All analyses were done using SPSS software (version 17.0; SPSS, Inc., Chicago, Illinois). Throughout the text, results are shown as means ± SE based on data from replicate experiments.

Results

The key insect pests of rice were found to be the white-backed plant hopper, brown planthopper, and rice leaffolder. In the control (untreated) plots, the populations of the white-backed plant hopper, brown planthopper, and rice leaffolder reached peaks 1, 14, and 21 d after application, respectively, and then decreased.

FIELD EFFICACY OF THREE INSECTICIDES AGAINST PLANTHOPPERS

In the single insecticide application experiments at 1 d after application, the percentage of control of planthoppers in plots treated with low, medium, or high rates of Virtako was 45.92, 56.42, and 60.12%, respectively (Table 2), showing a positive response rate. With the passage of time, Virtako's efficacy increased. At 7 d after the application of

Table 2. Planthopper abundance (number per 50 hills) and efficacy of Virtako WG for suppression of planthoppers after using a single application of insecticide.

|           | Days after application                     |                                 |                            |                                 |                            |                                 |
|-----------|--------------------------------------------|---------------------------------|----------------------------|---------------------------------|----------------------------|---------------------------------|
|           | 1                                          | 7                               | 14                         | 21                              | 28                         |                                 |
| Treatment | Pretreatment<br>planthoppers<br>(no. ± SE) | Plant-<br>hoppers<br>(no. ± SE) | Control effect<br>(% ± SE) | Plant-<br>hoppers<br>(no. ± SE) | Control effect<br>(% ± SE) | Plant-<br>hoppers<br>(no. ± SE) |
| 1         | 2,482.3 ± 77.8 aA                          | 1,370.3 ± 38.0                  | 45.92 ± 1.50 cC            | 1,370.3 ± 38.0                  | 45.92 ± 1.50 cC            | 1,140.7 ± 6.4                   |
| 2         | 2,554.7 ± 57.2 aA                          | 1,136.3 ± 29.4                  | 56.42 ± 1.13 dD            | 672.3 ± 20.4                    | 80.73 ± 0.58 cDc           | 1,121.3 ± 13.0                  |
| 3         | 2,594.3 ± 93.4 aA                          | 1,056.0 ± 20.0                  | 60.12 ± 0.75 dD            | 610.0 ± 12.9                    | 82.78 ± 0.36 dC            | 985.3 ± 10.1                    |
| 4         | 2,617.3 ± 80.3 aA                          | 1,775.7 ± 53.0                  | 33.53 ± 1.98 bB            | 1,054.0 ± 36.0                  | 70.51 ± 1.01 bB            | 1,343.7 ± 22.3                  |
| 5         | 2,579.0 ± 120.5 aA                         | 2,222.3 ± 33.0                  | 15.58 ± 1.25 aA            | 2,690.3 ± 42.9                  | 23.60 ± 1.22 aA            | 2,109.7 ± 87.4                  |
| Control   | 2,545.7 ± 65.4 aA                          | 2,598.3 ± 5.8                   | —                          | 408.7 ± 8.0                     | —                          | 2,831.0 ± 97.6                  |

Means within a column not followed by the same lowercase letter are significantly different by ANOVA and Tukey's HSD test at *P* < 0.05, and means within a column not followed by the same capital letter are significantly different by ANOVA and Tukey's HSD test at *P* < 0.01.

the high rate of Virtako, planthopper numbers were less than 10% of the populations in control plots (Table 2).

Overall, Virtako treatment had a greater effect in controlling the planthopper population than treatments with either thiamethoxam alone or chlorantraniliprole alone ( $F = 8.74$ ;  $df = 107$ ;  $P < 0.01$ ). At 14 d after application, the best control of planthoppers (>81% decrease in the insect population) was achieved with Virtako (medium and high rates) and thiamethoxam, and there was no difference among the 3 rates of treatment. At 21 d after application, all 3 rates of Virtako showed a greater level of control than thiamethoxam or chlorantraniliprole ( $F = 6.76$ ;  $df = 107$ ;  $P < 0.01$ ). At 28 d after application, the high rate of Virtako still showed better levels of control (66%) than thiamethoxam (54%) or chlorantraniliprole (26%) ( $F = 12.74$ ;  $df = 107$ ;  $P < 0.01$ ). The low rate (36 g active ingredient [a.i.] per ha) of Virtako also had a significant effect on the insects (59% reduction in numbers) but was not significantly different from thiamethoxam.

In the treatments with 2 separate applications (Table 3), at 14 d after the 1st application, Virtako (all 3 rates) and thiamethoxam showed superior levels of insect control (83–89%) compared with chlorantraniliprole, and treatments with Virtako showed a positive response rate. However, at 21 and 28 d after the 1st application, Virtako gave inconsistent results, with 85.80, 89.84, and 86.80% (after 21 d) and 64.57, 61.49, and 51.32% (after 28 d) control efficacy for the low, medium, and high rates, respectively. Thus, the high rate (60 g a.i./ha) gave a lower level of control than the medium and low rates 28 d after the 1st application ( $F = 3.79$ ;  $df = 107$ ;  $P < 0.01$ ).

Compared with the control treatments, at 14 and 21 d after the 1st application, a single application of Virtako (regardless of application rate) showed a greater level of planthopper control than the double application (Fig. 1). In contrast, the high rate of Virtako in the double application experiment gave a lower level of control at 28 d from the 1st application than in the single application experiment (Fig. 1).

#### FIELD EFFICACY OF THREE INSECTICIDES AGAINST THE RICE LEAFFOLDER

In the single application treatments (Table 4), at 7 d after application, the 3 rates of Virtako (low, medium, and high) resulted in rolled leaf growth rates of 124.53, 95.62, and 75.26%, respectively, and leaf protection rates of 10.62, 31.37, and 45.98%, respectively. This indicates a positive response rate. The chlorantraniliprole treatment gave the lowest (64%) rolled leaf growth rate; however, there was no significant difference when compared with the high-

rate application of Virtako. At 14 d after application, the levels of control reached a peak value and then decreased. The rolled leaf growth rates in all insecticide treatments were higher than those in the water control 21 d after application ( $F = 29.74$ ;  $df = 107$ ;  $P < 0.01$ ). At 28 d after application, Virtako (high and medium rates) showed the best level of insect control (leaf protection rate >70%). The rolled leaf growth rate (10–12%) was significantly lower than in the chlorantraniliprole treatment (23%) ( $F = 11.68$ ;  $df = 107$ ;  $P < 0.05$ ).

In the double application treatments (Table 5), the 3 rates of Virtako had greater levels of leaffolder control compared with water-treated control samples, but there was a negative rate response 14, 21, and 28 d from the 1st application. At 28 d after the 1st application, the plants treated with the low rate of Virtako showed the lowest rolled leaf growth rate (3%) and had a higher rate of leaf protection than all the other treatments ( $F = 6.16$ ;  $df = 107$ ;  $P < 0.01$ ). The thiamethoxam treatment had a significantly lower leaf protection rate (–276%) compared with all other treatments. In the single and double application experiments involving the same concentrations of insecticides (Fig. 2) at 14, 21, and 28 d after the 1st application, the double application of the low rate of Virtako controlled rolled leaf growth better than the single application. In contrast, the high rate of Virtako in the double application experiment had a lower level of control of rolled leaf growth than in the single application experiment.

#### EFFECTS OF THREE INSECTICIDES ON PREDATORY INSECTS IN RICE PADDIES

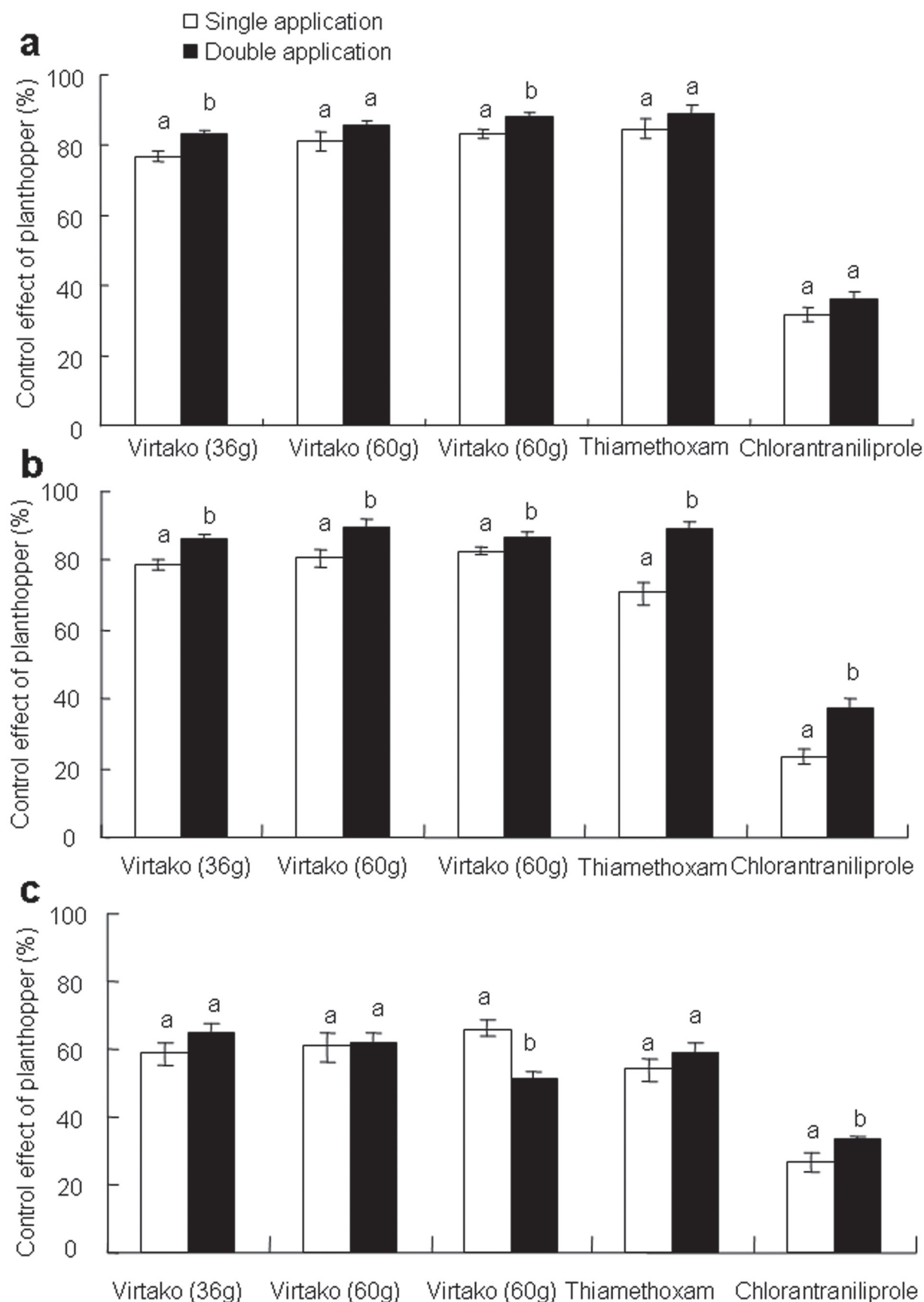
We collected 56 predator species (including 41 species of spiders and 15 species of predatory insects) in the rice fields. The dominant spider species were *Tetragnatha shikokiana* Yaginuma (Araneae: Tetragnathidae) and *Hylyphantes graminicola* (Sundevall) (Araneae: Linyphiidae), and the dominant predatory insect species were *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) and *Paederus fuscipes* Curtis (Coleoptera: Staphylinidae).

In both single and double application treatments of insecticides (Fig. 3), the predator numbers showed a decline until the insecticide residues ceased to be toxic; then, insect numbers rose again, in contrast to the water-treatment control. Chlorantraniliprole showed the least damage to predatory insects, whereas the high rate of Virtako showed the greatest damage to the total number of predators. Among the 3 rates of Virtako tested, the high rate led to a higher mortality of the individual predator species. However, at 21 d after application, the total number of predators at the low rate of Virtako was similar to that

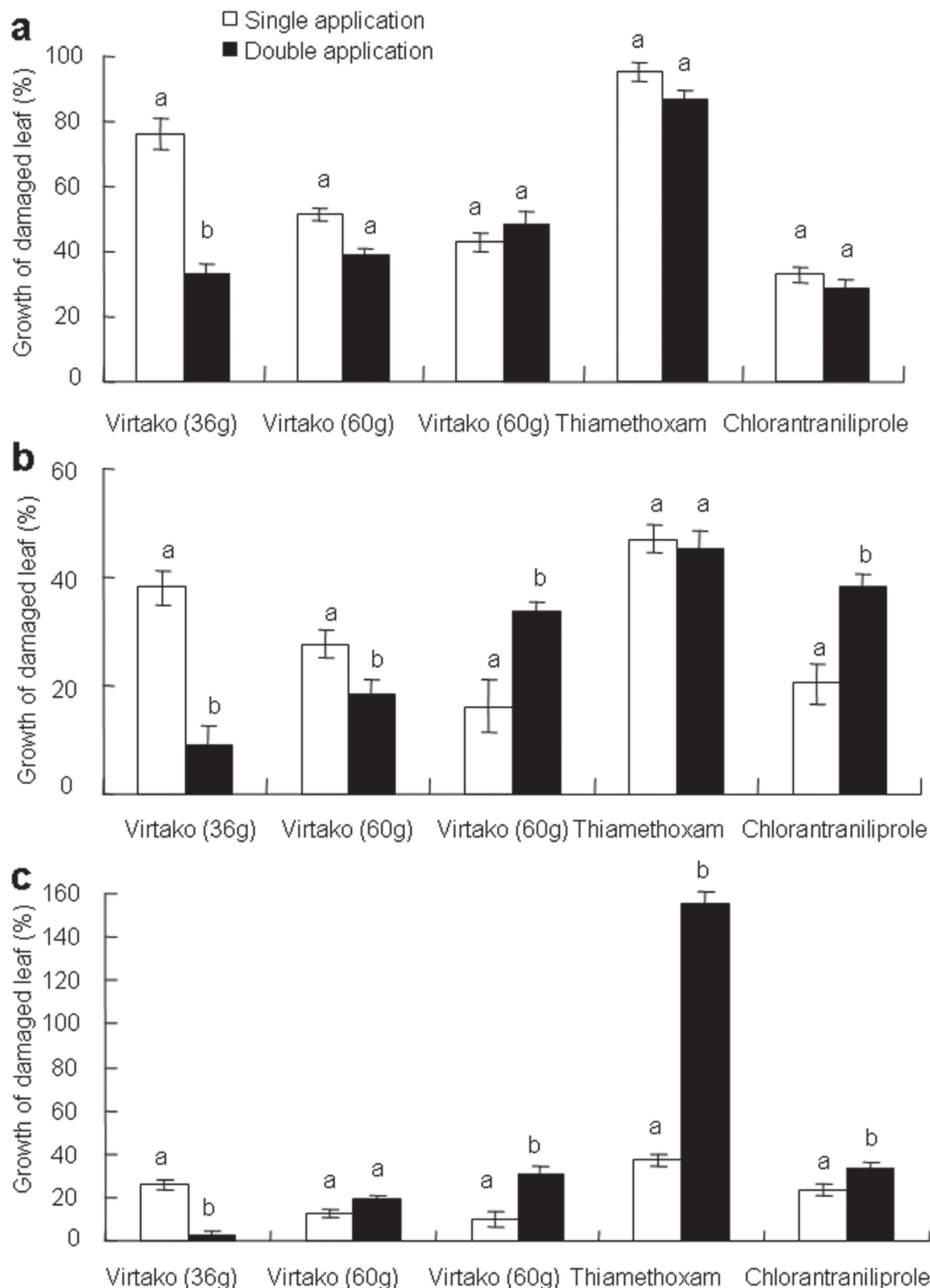
**Table 3.** Planthopper abundance (number per 50 hills) and efficacy of Virtako WG for suppression of planthoppers after using 2 applications of insecticide.

| Treatment | Pretreatment planthoppers<br>(no. ± SE) | Days after the first application |                            |                            |                            |                            |                            |
|-----------|-----------------------------------------|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|           |                                         | 14                               |                            | 21                         |                            | 28                         |                            |
|           |                                         | Planthoppers<br>(no. ± SE)       | Control effect<br>(% ± SE) | Planthoppers<br>(no. ± SE) | Control effect<br>(% ± SE) | Planthoppers<br>(no. ± SE) | Control effect<br>(% ± SE) |
| 6         | 2,489.0 ± 34.5 aA                       | 67.7 ± 4.2                       | 83.07 ± 1.05 cC            | 482.7 ± 21.1               | 85.80 ± 0.62 bB            | 980.7 ± 26.0               | 64.57 ± 0.94 cC            |
| 7         | 2,587.3 ± 62.7 aA                       | 59.3 ± 1.5                       | 85.72 ± 0.35 dD            | 359.0 ± 11.5               | 89.84 ± 0.33 dC            | 1,108.0 ± 34.0             | 61.49 ± 1.18 cC            |
| 8         | 2,497.3 ± 194.8 aA                      | 46.7 ± 3.2                       | 88.36 ± 0.79 dD            | 450.0 ± 17.7               | 86.80 ± 0.52 bcBC          | 1,352.0 ± 25.7             | 51.32 ± 0.92 bB            |
| 9         | 2,579.0 ± 54.3 aA                       | 45.3 ± 2.6                       | 89.05 ± 0.63 bB            | 387.3 ± 2.7                | 89.0 ± 0.08 cdBC           | 1,177.0 ± 51.8             | 58.96 ± 1.81 cC            |
| 10        | 2,585.3 ± 60.3 aA                       | 266.0 ± 9.6                      | 35.91 ± 2.32 aA            | 2,223.7 ± 37.1             | 37.01 ± 1.05 aA            | 1,909.7 ± 31.7             | 33.58 ± 1.10 aA            |
| Control   | 2,545.7 ± 65.4 aA                       | 408.7 ± 8.0                      | —                          | 3,476.0 ± 89.5             | —                          | 2,831.0 ± 97.6             | —                          |

Means within a column not followed by the same lowercase letter are significantly different by ANOVA and Tukey's HSD test at  $P < 0.05$ , and means within a column not followed by the same capital letter are significantly different by ANOVA and Tukey's HSD test at  $P < 0.01$ .



**Fig. 1.** Effects of Virtako on planthopper abundance (mean number  $\pm$  SE). Means followed by the same lowercase letter are not significantly different (ANOVA and Tukey's HSD test,  $P > 0.05$ ).



**Fig. 2.** Effects of Virtako on rice leaffolder abundance (mean number  $\pm$  SE). Means followed by the same lowercase letter are not significantly different (ANOVA and Tukey's HSD test,  $P > 0.05$ ).

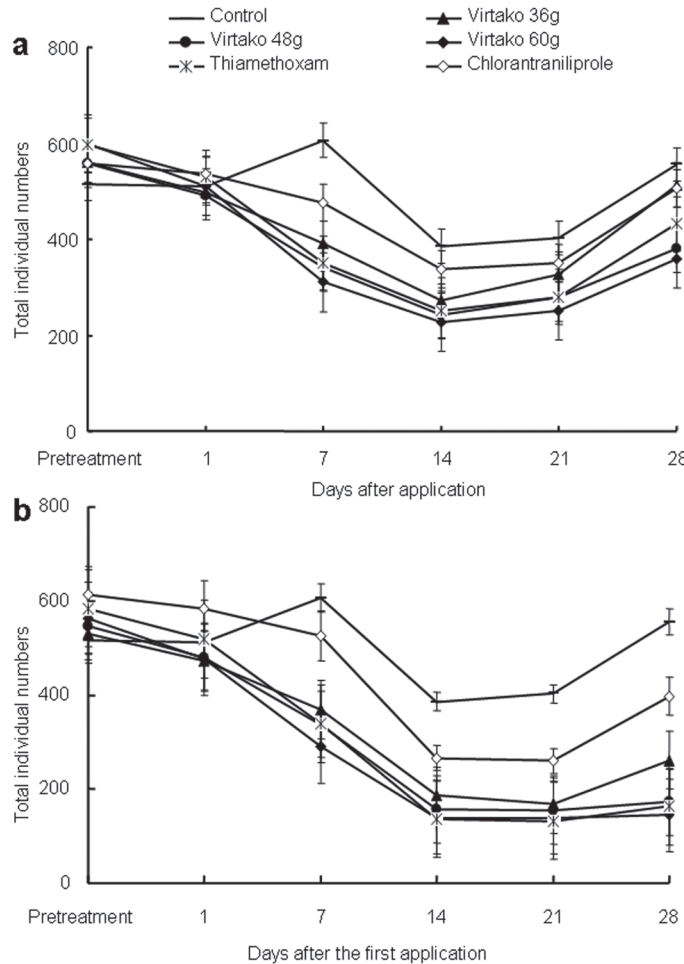
**Table 4.** Rice leaf folder damage and efficacy of Virtako WG for suppression of damage by rice leafrollers after using a single application of insecticide.

| Treatment | Days after application                                             |                                      |                                     |                                      |                                     |                                      |                                     |                                      |                                     |                                      |
|-----------|--------------------------------------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|
|           | 7                                                                  |                                      |                                     | 14                                   |                                     |                                      | 21                                  |                                      |                                     | 28                                   |
|           | Pretreatment<br>count of rolled leaves<br>(no. per 50 hills, ± SE) | Roll<br>leaf growth rate<br>(% ± SE) | Leaf protection<br>rate<br>(% ± SE) | Roll<br>leaf growth rate<br>(% ± SE) | Leaf protection<br>rate<br>(% ± SE) | Roll<br>leaf growth rate<br>(% ± SE) | Leaf protection<br>rate<br>(% ± SE) | Roll<br>leaf growth rate<br>(% ± SE) | Leaf protection<br>rate<br>(% ± SE) | Roll<br>leaf growth rate<br>(% ± SE) |
| 1         | 106.0 ± 7.0 aA                                                     | 124.53 ± 3.31 cBC                    | 10.62 ± 2.38                        | 76.10 ± 4.40 cB                      | 57.47 ± 2.46                        | 38.05 ± 1.13 cCD                     | 37.58 ± 1.86                        | 26.10 ± 2.27 bCD                     | 36.79 ± 5.49                        |                                      |
| 2         | 121.7 ± 2.2 aA                                                     | 95.62 ± 2.61 bAB                     | 31.37 ± 1.88                        | 51.51 ± 2.24 bA                      | 71.21 ± 1.25                        | 27.67 ± 1.45 bBC                     | 54.60 ± 2.38                        | 12.33 ± 1.71 aAB                     | 70.14 ± 4.14                        |                                      |
| 3         | 126.7 ± 7.2 aA                                                     | 75.26 ± 3.03 abA                     | 45.98 ± 2.18                        | 42.63 ± 2.99 abA                     | 76.17 ± 1.67                        | 16.05 ± 1.84 aA                      | 73.67 ± 3.02                        | 10.00 ± 1.84 aA                      | 75.78 ± 4.46                        |                                      |
| 4         | 120.3 ± 1.9 aA                                                     | 135.73 ± 1.21 cC                     | 2.58 ± 0.87                         | 95.29 ± 2.89 dB                      | 46.74 ± 1.62                        | 47.09 ± 1.54 dD                      | 22.74 ± 2.53                        | 37.12 ± 2.62 cDE                     | 10.11 ± 5.96                        |                                      |
| 5         | 116.7 ± 3.9 aA                                                     | 65.14 ± 0.49 aA                      | 53.24 ± 0.36                        | 33.14 ± 2.23 aA                      | 81.48 ± 1.25                        | 20.29 ± 1.87 abAB                    | 66.72 ± 3.07                        | 23.43 ± 1.74 bBC                     | 43.26 ± 4.21                        |                                      |
| Control   | 118.7 ± 5.5 aA                                                     | 139.33 ± 12.22 cC                    | —                                   | 178.93 ± 4.08 eC                     | —                                   | 60.96 ± 2.19 eE                      | —                                   | 36.18 ± 6.98 cE                      | —                                   |                                      |

Means within a column not followed by the same lowercase letter are significantly different by ANOVA and Tukey's HSD test at  $P < 0.05$ , and means within a column not followed by the same capital letter are significantly different by ANOVA and Tukey's HSD test at  $P < 0.01$ .

of the water control. The double application of Virtako yielded higher predator mortality than the single application at all 3 rates. The total numbers of predators were significantly lower in all insecticide treatments than in the water control.

Several diversity indices (Shannon–Weaver diversity index  $H'$ , Pielou evenness index  $J$ , Simpson dominance index  $C$ , and species richness  $S$ ) were used to evaluate the insecticide damage to the predators. In the single insecticide application treatments, the indices  $H'$  and  $J$  with the 3 rates of Virtako and with thiamethoxam rose until the insecticide residues ceased to be toxic, then they declined again. With the chlorantraniliprole treatments and the water control, the indices showed an ascending-descending-ascending-descending pattern (Fig. 4a, b), which was different from the pattern seen with the Virtako and thiamethoxam treatments. The indices  $H'$  and  $J$  with Virtako (medium and high rates) treatments were much higher than those of the water control ( $F = 9.61$ ;  $df = 197$ ;  $P < 0.05$ ), but there was no significant differences compared with the thiamethoxam treatment ( $F = 0.79$ ;  $df = 197$ ;  $P > 0.05$ ) (Table 6). The indices  $C$  and  $S$  for the Virtako (3 rates) and thiamethoxam treatments declined until the insecticide residues ceased to be toxic, then they rose again. The  $C$  and  $S$  indices for treatments with chlorantraniliprole and the water control differed from these as they showed a descending-ascending-descending-ascending pattern (Fig. 4c, d). The index  $C$  for all insecticide treatments (except for chlorantraniliprole) was significantly lower than for the water



**Fig. 3.** Temporal dynamics of total numbers of predators per 50 hills where insecticide was applied (a) as a single application or (b) as 2 applications to rice plots.

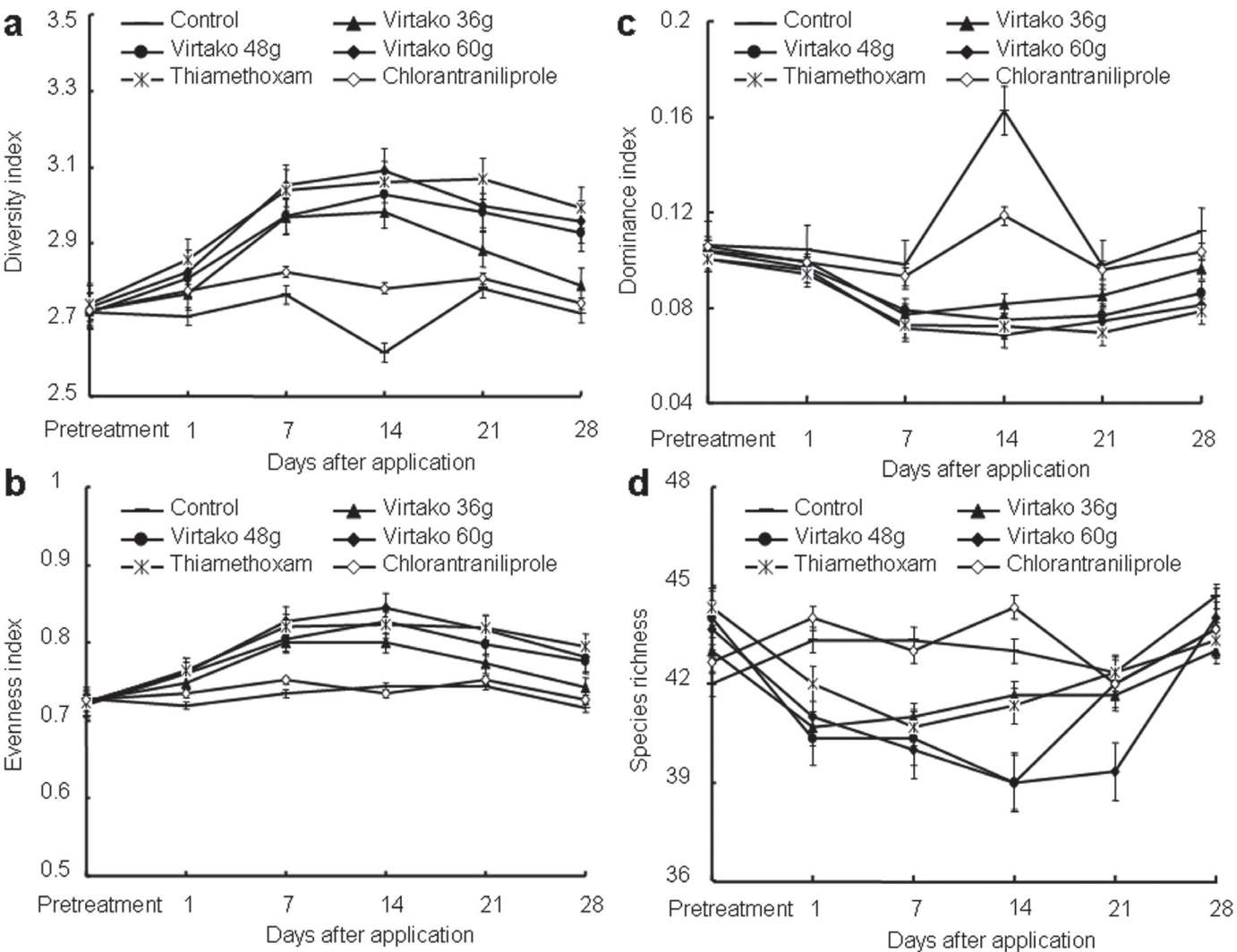
**Table 5.** Rice leaffolder damage and efficacy of Virtako WG for suppression of damage by rice leaffolders after using 2 applications of insecticide.

| Treatment | Pretreatment<br>count of rolled leaves<br>(no. per 50 hills, $\pm$ SE) | Days after the first application           |                                         |                                            |                                         |                                            |                                         |
|-----------|------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------|--------------------------------------------|-----------------------------------------|--------------------------------------------|-----------------------------------------|
|           |                                                                        | 14                                         |                                         | 21                                         |                                         | 28                                         |                                         |
|           |                                                                        | Rolled leaf<br>growth rate<br>(% $\pm$ SE) | Leaf protection<br>rate<br>(% $\pm$ SE) | Rolled leaf<br>growth rate<br>(% $\pm$ SE) | Leaf protection<br>rate<br>(% $\pm$ SE) | Rolled leaf<br>growth rate<br>(% $\pm$ SE) | Leaf protection<br>rate<br>(% $\pm$ SE) |
| 6         | 110.7 $\pm$ 4.3 aA                                                     | 33.73 $\pm$ 2.63 aAB                       | 81.15 $\pm$ 1.47                        | 9.34 $\pm$ 1.09 aA                         | 84.68 $\pm$ 1.78                        | 3.01 $\pm$ 0.80 aA                         | 92.71 $\pm$ 1.93                        |
| 7         | 128.3 $\pm$ 4.3 aA                                                     | 38.96 $\pm$ 2.38 abAB                      | 78.23 $\pm$ 1.33                        | 18.44 $\pm$ 0.94 bA                        | 69.75 $\pm$ 1.54                        | 18.96 $\pm$ 1.58 bB                        | 54.08 $\pm$ 3.83                        |
| 8         | 123.3 $\pm$ 2.3 aA                                                     | 48.38 $\pm$ 1.95 bB                        | 72.96 $\pm$ 1.09                        | 33.78 $\pm$ 1.50 cB                        | 44.58 $\pm$ 2.47                        | 30.54 $\pm$ 0.72 cC                        | 26.04 $\pm$ 1.73                        |
| 9         | 125.3 $\pm$ 1.9 aA                                                     | 86.44 $\pm$ 2.78 cC                        | 51.69 $\pm$ 1.55                        | 45.21 $\pm$ 1.48 dC                        | 25.83 $\pm$ 2.43                        | 155.32 $\pm$ 2.27 eE                       | -276.15 $\pm$ 5.50                      |
| 10        | 111.0 $\pm$ 4.9 aA                                                     | 28.23 $\pm$ 1.08 aA                        | 84.22 $\pm$ 0.61                        | 38.44 $\pm$ 2.10 cdBC                      | 33.94 $\pm$ 3.45                        | 33.33 $\pm$ 1.04 bcCD                      | 19.27 $\pm$ 2.52                        |
| Control   | 118.7 $\pm$ 5.5                                                        | 178.93 $\pm$ 4.08 dC                       | —                                       | 60.96 $\pm$ 2.19 eD                        | —                                       | 36.18 $\pm$ 6.98 dD                        | —                                       |

Means within a column not followed by the same lowercase letter are significantly different by ANOVA and Tukey's HSD test at  $P < 0.05$ , and means within a column not followed by the same capital letter are significantly different by ANOVA and Tukey's HSD test at  $P < 0.01$ .

control ( $F = 11.68$ ;  $df = 197$ ;  $P < 0.05$ ); however, the level of  $S$  was not significantly different from that of the water control ( $F = 0.86$ ;  $df = 197$ ;  $P > 0.05$ ) (Table 6).

In the double application experiment, the indices  $H'$ ,  $J$ ,  $C$ , and  $S$  showed similar patterns as those for the single application (Fig. 5). Overall, all indices from the insecticide treatments (except for chloran-



**Fig. 4.** Temporal dynamics of predator insect diversity (a, diversity index; b, evenness index; c, dominance index; and d, species richness) in rice plots with various treatments (1 application per season).

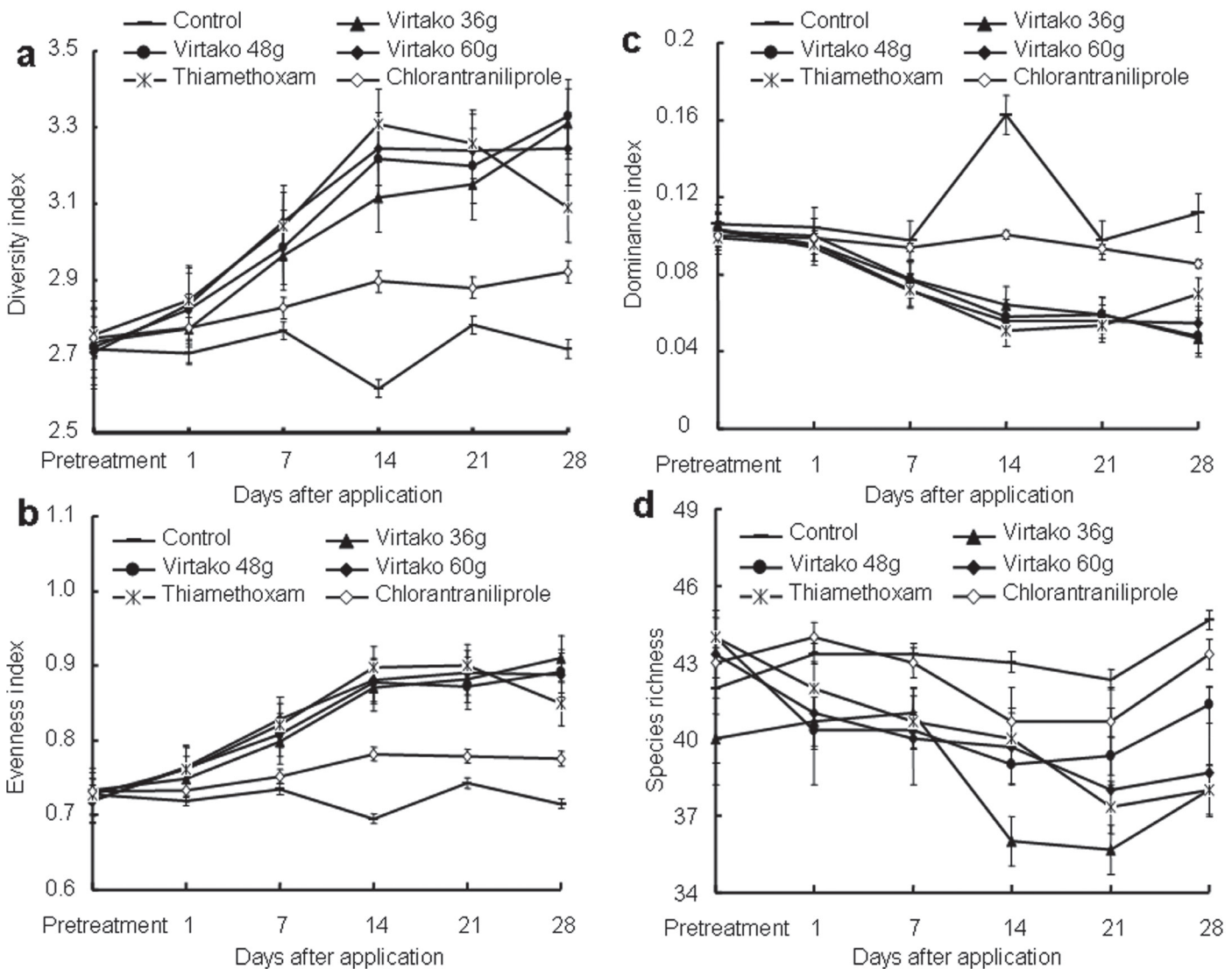


Fig. 5. Temporal dynamics of predator insect diversity (a, diversity index; b, evenness index; c, dominance index; and d, species richness) in rice plots with various treatments (2 applications per season).

traniliprole) were significantly different from those of the water control ( $F = 12.16$ ;  $df = 197$ ;  $P < 0.05$ ) (Table 6).

## Discussion

Fifty-one predator species were found in this study. Pest suppression by predators is often cited as an important ecosystem service provided by natural enemies, but this service is threatened by pesticide use. Several previous studies have shown that thiamethoxam was mildly harmful (20% mortality) to spiders, *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae), and *Paederus affterii* Koch (Coleoptera: Staphylinidae) in plantations (Al-Kherb 2011; Jiang et al. 2011) but highly toxic to *C. lividipennis* and *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) (Sun et al. 2008; Preetha et al. 2009). The results of Preetha et al. (2009) showed that chlorantraniliprole was highly toxic to *T. chilonis* in laboratory tests. Although chlorantraniliprole did not harm spiders and *C. lividipennis* (Liu et al. 2009; Xu et al. 2012) in rice fields, our results indicated that the application of Virtako, regardless of rate, distinctly reduced the total number of predators 7 and 14 d after application (both

single and double applications) and affected the diversity indices  $H'$ ,  $J$ , and  $C$ . This is likely because Virtako is a combination of the highly toxic thiamethoxam and the less toxic chlorantraniliprole. However, the total numbers of predators in the Virtako treatment plots were not significantly different from those in the plots treated with thiamethoxam ( $P > 0.05$ ), and the  $J$  and  $C$  indices with the low rate of Virtako (36 g a.i./ha) were also not significantly different from those of the water control 21 d after application. Moreover, the level of species richness with Virtako (single application) treatment was not significantly different from that of the water control. Our results suggest that the toxicity of Virtako to predators is higher than that of chlorantraniliprole but similar to that of thiamethoxam, which could be acceptable when applied in a single dose at low rates in rice paddy fields. It is possible that Virtako has higher toxicity to other predators when used in other areas, but this will need to be tested by future research.

Based on the present results, although the total number of predators and the diversity indices ( $H'$ ,  $C$ , and  $J$ ) were greatly reduced 7 and 14 d after application, Virtako still showed great efficacy against planthoppers and leafhoppers in a single application treatment. All 3 rates of Virtako reduced the numbers of planthoppers by 46 to 60% at 1 d af-

**Table 6.** Average diversity indices of predatory insects in rice plots with various insecticide treatments.

| Treatment | H'                | J                 | C                  | S                  |
|-----------|-------------------|-------------------|--------------------|--------------------|
| 1         | 2.854 ± 0.026 abc | 0.765 ± 0.007 abc | 0.091 ± 0.002 bcd  | 41.833 ± 0.506 bc  |
| 2         | 2.907 ± 0.028 bcd | 0.781 ± 0.009 bcd | 0.086 ± 0.003 abcd | 41.556 ± 0.590 bc  |
| 3         | 2.943 ± 0.032 bcd | 0.793 ± 0.010 cd  | 0.082 ± 0.003 abc  | 41.167 ± 0.601 abc |
| 4         | 2.960 ± 0.031 cd  | 0.791 ± 0.009 cd  | 0.081 ± 0.003 abc  | 42.333 ± 0.388 bc  |
| 5         | 2.777 ± 0.010 ab  | 0.738 ± 0.003 ab  | 0.103 ± 0.002 de   | 43.278 ± 0.609 c   |
| 6         | 3.008 ± 0.051 cd  | 0.824 ± 0.017 d   | 0.075 ± 0.005 ab   | 38.556 ± 0.677 a   |
| 7         | 3.046 ± 0.054 d   | 0.822 ± 0.016 d   | 0.074 ± 0.005 ab   | 40.722 ± 0.470 abc |
| 8         | 3.054 ± 0.053 d   | 0.828 ± 0.016 d   | 0.072 ± 0.005 a    | 40.111 ± 0.554 ab  |
| 9         | 3.049 ± 0.050 d   | 0.826 ± 0.016 d   | 0.073 ± 0.005 ab   | 40.333 ± 0.605 ab  |
| 10        | 2.841 ± 0.020 abc | 0.759 ± 0.006 abc | 0.095 ± 0.002 cd   | 42.444 ± 0.584 bc  |
| Control   | 2.718 ± 0.018 a   | 0.723 ± 0.005 a   | 0.113 ± 0.006 e    | 43.111 ± 0.631 c   |

Means within a column not followed by the same lowercase letter are significantly different (ANOVA and Tukey's HSD test,  $P < 0.05$ ). H': Shannon–Weaver diversity index; J: Pielou evenness index; C: Simpson dominance index; S: species richness.

ter application, which was more than 1.35-fold more efficient than the thiamethoxam treatment. The high rate of Virtako application still gave 65.85% planthopper control 28 d after application, which was 1.24-fold more efficient than thiamethoxam alone. In addition, our results indicate that the effect of Virtako (48–60 g a.i./ha) against the rice leaffolder lasts longer than that of chlorantraniliprole (30 g a.i./ha). Its leaffolder control percentage was 1.62- to 1.75-fold greater than that of the single chlorantraniliprole treatment after 28 d. These results, together with the total number of predators and the diversity indices after Virtako treatment being quickly restored to normal levels and being similar to those of the water control 21 d after application, suggest that Virtako against planthoppers and the rice leaffolder is rapid and longer lasting compared with thiamethoxam and chlorantraniliprole, and could be used to control planthoppers and the rice leaffolder in paddy fields.

The overuse of organophosphorus, carbamate, and pyrethroid insecticides on rice pests throughout Asia has been cited as a major cause of planthopper and rice leaffolder outbreaks, as excessive spraying with insecticides disrupts the natural biological control of these pests (Dale 1994; Hemingway et al. 1999; Yea et al. 2003; Nathan et al. 2006; He et al. 2008). Our results indicate that a double application of thiamethoxam in plots had significant negative effects on rice leaffolder control. As thiamethoxam is not recommended for rice leaffolder control and is highly toxic to some predators, it likely disrupts the natural biological control of these pests. The total number of predators and the diversity indices in the double Virtako treatments were greatly reduced and returned to normal levels more slowly as compared with the single application. Furthermore, the high rate of Virtako in the double application showed lower control efficiency than in the single application 28 d after the 1st application. These results indicate that higher rates and double application led to higher mortality of predators and had a negative impact on pest control (Lee et al. 1993; Yu et al. 2011). The use of Virtako must therefore be minimized. This minimization is also necessary because of the high costs and harmful effects of Virtako on human health and the environment. We suggest that Virtako be applied in a single dose in the range of 36 to 48 g a.i./ha 7 d before insects reach peak numbers.

We acknowledge several limitations to our study. First, we did not study the effect of Virtako on parasitic natural enemies living in the rice paddy. Second, the effects of Virtako on pests and predators were conducted only in 1 yr. However, the generality of our results will be tested by additional future experiments.

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