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TECHNICAL NOTE

Effect of acetone solution in a topical application method on mortality of rice planthoppers, *Nilaparvata lugens*, *Sogatella furcifera*, and *Laodelphax striatellus* (Homoptera: Delphacidae)

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Abstract The effect of acetone solution on the mortality of rice planthoppers, *Nilaparvata lugens* (Stål), *Sogatella furcifera* (Horváth), and *Laodelphax striatellus* (Fallén), was examined, as it is widely used as a solvent solution in a topical application method for monitoring insecticide susceptibility. The mortality of *N. lugens* and *S. furcifera* was significantly higher at 0.32 and 0.28 µl or more acetone droplets per insect than in the control treatment (without acetone), respectively. The mortality of *L. striatellus* was significantly higher at 0.24 µl or more acetone droplets per insect than in the control treatment. Another commonly used solvent solution, methanol, had a similar effect on *L. striatellus*. The most standard topical application method uses a hand-operated micro-applicator (Burkard Manufacturing Co., Ltd., UK) equipped with a 50-µl micro-syringe, which can deliver a very small amount of droplets of insecticide/acetone solution per insect, 0.08 µl, so there is no effect of acetone solution on the mortality of *N. lugens*, *S. furcifera*, and *L. striatellus*. However, other equipment for topical application, a repeating dispenser (Hamilton Co., USA) attached with a 10-µl micro-syringe, can deliver a 0.24-µl droplet at a minimum. This equipment can be used for *N. lugens* and *S. furcifera*, but not for *L. striatellus*. It is necessary to choose appropriate equipment for each rice planthopper so that they are not affected by the solvent solution.

Keywords Insecticide resistance · *Laodelphax striatellus* · *Nilaparvata lugens* · *Sogatella furcifera* · Topical application

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Introduction

The brown planthopper *Nilaparvata lugens* (Stål), the whitebacked planthopper *Sogatella furcifera* (Horváth), and the small brown planthopper *Laodelphax striatellus* (Fallén) are the three most important insect pests of rice in Asia. To control these planthoppers, many groups of insecticides (such as organophosphates, carbamates, and pyrethroids) have been developed and widely used in this region of the world. Long-term decreases in susceptibility to these insecticides were detected in East-Asian populations of *N. lugens* and *S. furcifera* from the 1970s through the early 1990s (Fukuda and Nagata 1969; Hosoda 1983, 1989; Endo et al. 1988; Endo and Tsurumachi 2001; Nagata 2002; Nagata et al. 2002). New types of insecticides (such as neonicotinoids and phenylpyrazoles) were developed in the 1990s, and the population densities of *N. lugens* and *S. furcifera* have decreased in Japan and other East-Asian countries (Matsumura and Sanada-Morimura 2010). However, since 2005, insecticide resistance against some neonicotinoids and phenylpyrazoles (such as imidacloprid and fipronil) has been detected in the three rice planthopper species in East Asia and the Indochina Peninsula (Ma et al. 2007; Matsumura et al. 2008; Otuka et al. 2010). Because rice planthoppers are highly mobile in these areas (Kisimoto 1976; Otuka et al. 2010), nationwide monitoring of insecticide susceptibility should be established as a component of pest management strategies.

Topical application is a common method for monitoring insecticide susceptibility in rice planthoppers (Fukuda and Nagata 1969; Nagata 2002; Nagata et al. 2002; Matsumura et al. 2008). A topical applicator (e.g., hand micro-applicator produced by the Burkard Manufacturing Co., Ltd., Hertfordshire, UK) equipped with a micro-syringe is most commonly used in the standard topical application method for monitoring insecticide susceptibility of rice planthoppers around the world (Nagata 2002; Matsumura et al. 2008; Gao et al. 2008). This hand micro-applicator is a very precise instrument, but it is expensive (ca. $2,800), preventing its wide use in many Asian countries. Simple and low cost equipment is required for nationwide monitoring of insecticide susceptibility in rice planthoppers. A repeating dispenser (e.g., Hamilton Repeating Dispenser; Hamilton Co., Reno, NV) equipped with a micro-syringe is a simple piece of equipment used for topical application for many insect species such as *Hericoverpa armigera* (Hubner), *Aedes aegypti* (L.), and house flies (e.g., Kranthi et al. 2002; Al-Badry and Knowles 1980; Pridgeon et al. 2007). Although a similar method is also used for topical application for *N. lugens* (Vontas et al. 2001) and *S. furcifera* (Nakao et al. 2010), it has been used only for investigating the mechanisms of insecticide resistance, but not for monitoring insecticide resistance. Therefore, it is important to promote simple and low cost equipment for monitoring insecticide resistance for as many researchers as possible in Asian countries to enable them to compare monitoring data of insecticide susceptibility among countries from past to present. However, a simple topical method faces some issues related to the accuracy of the delivery solution and mortality control because simple equipment applies much more insecticide/acetone solution to each insect than the standard method delivering 0.08-µl droplets. It is still unclear how the amount of acetone solution affects the mortality of rice planthoppers. Thus, we examined the effect of acetone solution on the mortality of insects for monitoring insecticide resistance in rice planthoppers.

Materials and methods

Equipment

A hand micro-applicator (Burkard Manufacturing Co.) equipped with a 50-µl micro-syringe (MS-N50; Ito Corp., Shizuoka, Japan), which is able to deliver a 0.08-µl insecticide/acetone solution droplet (hereafter referred to as the "standard method"), was used to examine the effect of acetone solution on the mortality of insects (Fig. 1). A repeating dispenser (PB600-1; Hamilton Co.) equipped with a 10-µl micro-syringe (MS-N10; Ito Corp., Shizuoka, Japan) was used for simple topical application (hereafter referred to as the "simple method") (Fig. 1). This equipment delivers a 0.24-µl insecticide/acetone solution droplet with a single push on the dispenser. The accuracy of this equipment was compared to that of the standard method. The volumes of insecticide/acetone droplets by the two methods were calibrated using mercury.

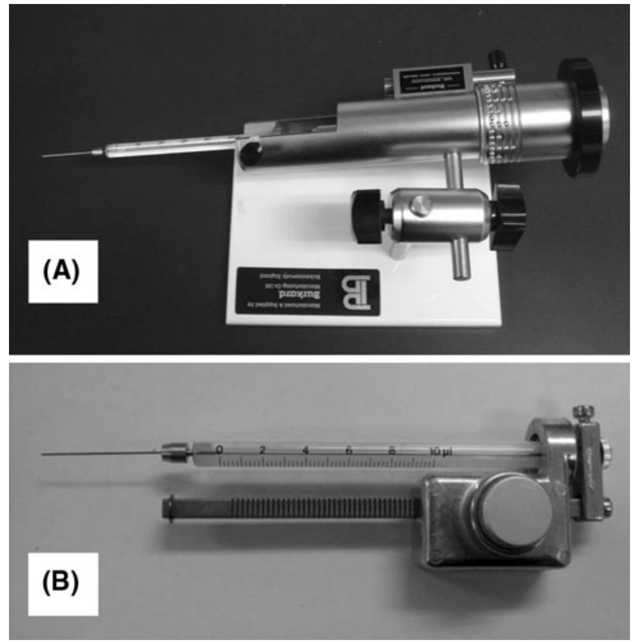

Fig. 1 Equipment for topical insecticide application. **a** Standard method: hand micro-applicator (Burkard Manufacturing Co., Ltd., Hertfordshire, UK) equipped with a 50-µl micro-syringe (MS-N50; Ito Corp., Shizuoka, Japan). **b** Simple method: repeating dispenser (PB600-1; Hamilton Co., Reno, NV) equipped with a 10-µl micro-syringe (MS-N10; Ito Corp.)

Insects

Insecticide susceptibilities of the three rice planthopper species were determined using the simple and standard methods. *Nilaparvata lugens* was collected in Ureshino, Saga Prefecture, Japan (N33.08, E129.93) on 13 July 2007. *Sogatella furcifera* was collected in Tu Liem, Ha Noi City, Vietnam (N21.07, E105.77) on 7 September 2007. *Laodelphax striatellus* was collected in An Lao, Hai Phong Province, Vietnam (N20.77, E106.58) on 5 September 2007 and in Checheng Township, Pingtung County, Taiwan (N22.08, E120.71) on 20 May 2010. The samples were maintained in the laboratory on rice seedlings (var. Reihou) growing at 25°C under a 16L:8D photoperiod.

Effect of acetone on mortality of adult females

The drop sizes of acetone delivered by the standard method are usually in the range of 0.05–0.1 µl per insect (Fukuda and Nagata 1969; Matsumura et al. 2008). This amount of acetone has no harmful effect on the tested insects. The acetone droplet size of the simple method was 0.24 µl from a single push of the dispenser, which is larger than the droplets produced by the standard method, and the possibility of harmful effects arises. Thus, we examined the relationship between the size of acetone droplets applied to single insects and the mortality of adult females.

Long-winged adult females of the three species within 7 days after emergence were anesthetized with carbon dioxide for about 5 s prior to treatment. For *N. lugens*, 0.08, 0.16, 0.2, 0.24, 0.28, 0.32, 0.36, and 0.40 µl acetone droplets were applied individually to the dorsal surface of the thorax by the Burkard micro-applicator. In the case of *S. furcifera* and *L. striatellus*, 0.08, 0.16, 0.2, 0.24, 0.28, and 0.32 µl acetone droplets were applied in the same manner. Controls were not treated with acetone (0 µl). The treated insects were kept in a transparent plastic box (5 cm diameter, 10 cm high) with rice seedlings at a temperature of 25°C and a relative humidity of 50–60% under a 16L:8D photoperiod. Mortality was determined 24 h after treatment. A minimum of 45 females were tested for each treatment. The difference in percent mortality between acetone treatments and controls was tested with a Dunnett-type multiple comparison test for proportions (Zar 2010). When the mortality with a 0.24-µl droplet of acetone, the same volume as in the simple method, was higher than that of the control treatment, another solution in common use, methanol, was tested in the same manner as described above.

Topical application

Insecticide susceptibility of the sampled planthopper populations was determined by the simple and standard methods using a carbamate insecticide BPMC (96.9%) provided by Sumitomo Chemical Co., Ltd. (Tokyo, Japan). Long-winged female adults within 7 days after emergence were anesthetized with carbon dioxide for about 5 s prior to treatments. The 0.08- and 0.24-µl droplets of BPMC-acetone solution were applied topically to the dorsal surface of the thorax by the simple and standard methods, respectively. The treated insects were kept in a transparent plastic box as described above. Mortality was determined 24 h after treatment. A minimum of 42 females were treated at each insecticide concentration, and five concentrations were tested. The LD_{50} values (µg/g), 95% confidence intervals, and the slopes of the regression lines were calculated by Bliss's (1935) probit method using PoloPlus software (LeOra Software 2003). To compare LD_{50} values between the two methods, likelihood ratio tests of equality (slopes and intercepts) between probit regression lines calculated for the two methods were conducted with PoloPlus software (LeOra Software 2003; Robertson et al. 2007).

Results and discussion

Percent mortality for the three rice planthoppers was 0% when no acetone (0 µl) was applied (control), and increased with an increasing amount of acetone per insect (Fig. 2). When 0.28 and 0.32 µl or more acetone solution was applied, percent mortality for *S. furcifera* and *N. lugens* was significantly higher than with the control treatment (0% acetone) (Fig. 2). In *L. striatellus*, mortality was significantly higher at 0.24 µl or more droplets than with the control treatment (Fig. 2). When the simple application method was used, i.e., 0.24 µl acetone was applied, percent mortalities for *S. furcifera* and *N. lugens* were 7.1 and 4.9%, respectively, and were not significantly different from the zero acetone control (0%) (Dunnett-type multiple comparison test for proportions). In contrast,

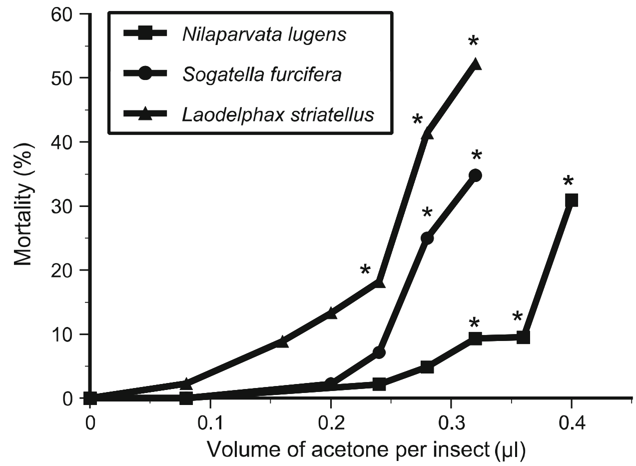

Fig. 2 Relationship between amount of acetone applied to each insect species (*Nilaparvata lugens*, *Sogatella furcifera*, and *Laodelphax striatellus*) and the mortality of adult females 24 h after treatment. *Asterisks* indicate significant mortality differences between treatments and control (0 µl) (Dunnett-type multiple comparison test for proportions, $p < 0.05$)

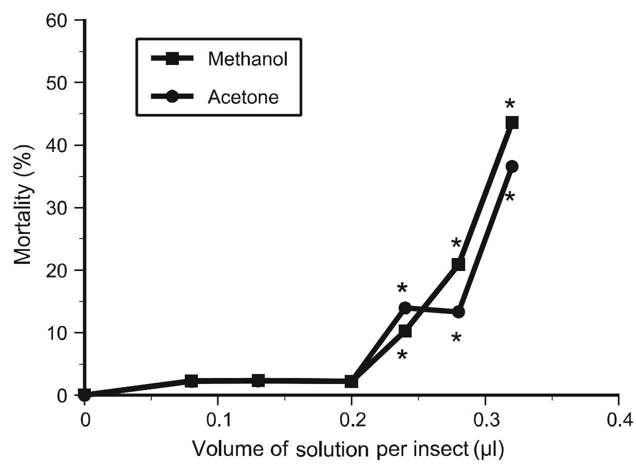

Fig. 3 Relationship between amount of acetone and methanol applied to *Laodelphax striatellus* and mortality of adult females 24 h after treatment. *Asterisks* indicate significant mortality differences between treatments and control (0 µl) (Dunnett-type multiple comparison test for proportions, $p < 0.05$)

Table 1 Comparison of LD_{50} values for the three rice planthoppers by the simple and standard topical application methods (Macropter females)

Species	Method[a]	Mortality in control (%)[b]		LD_{50} value of BPMC			Slope (±SE)[d]	
				(μg/g)[c]		(95% range)		
Nilaparvata lugens	Simple	2.0	ns	13.9	ns	8.1–20.7	3.0 ± 0.4	ns
	Standard	4.1		18.1		12.5–24.6	3.4 ± 0.4	
Sogatella furcifera	Simple	7.1	ns	12.3	ns	9.5–15.0	3.2 ± 0.5	ns
	Standard	2.3		9.8		7.8–11.9	2.8 ± 0.4	
Laodelphax striatellus	Simple	18.6	*	89.1	ns	55.1–126	2.1 ± 0.5	ns
	Standard	4.2		114.5		91.3–142	2.6 ± 0.4	

[a] Simple: Hamilton dispensor + 10 μl syringe; Standard: Burkard hand microapplicator + 50 μl syringe

[b] 24 h after treatment. Fisher's exact probability test; *$p < 0.05$; *ns* not significant

[c] Likelihood ratio tests of equality; *ns* not significant

[d] Slope of regression line. Likelihood ratio tests of equality; *ns* not significant

percent mortality of *L. striatellus* treated with a 0.24 μl droplet of acetone (18.2%) was significantly higher than the zero acetone controls (0%) (Dunnett-type multiple comparison test for proportions, $p < 0.05$). Percent mortality with another solvent solution, methanol, was also significantly higher at 0.24 μl and higher droplets than the control treatment (methanol 0%) in *L. striatellus* (Fig. 3).

The LD_{50} values of BPMC determined for the three species using the simple and standard methods are presented in Table 1. There were no significant differences in LD_{50} values or slopes of regression lines between the two methods; this was the case for all three planthopper species (likelihood ratio tests of equality, NS). Percent mortalities in the controls (acetone-only treatment) when using the simple method were less than 10% for *N. lugens* and *S. furcifera*, and not significantly different from the standard method (Fisher's exact probability test, NS) (Table 1). In contrast, percent mortality in *L. striatellus* was 18.6%, significantly higher than the mortality when using the standard method (Fisher's exact probability test, $p = 0.042$). Hence, the simple method can be used with the same level of accuracy as the standard method for monitoring insecticide susceptibility in *N. lugens* and *S. furcifera*. However, the control mortality should be corrected carefully during calculations of LD_{50} values for *S. furcifera* because of slightly high mortality (7.1%) when 0.24 μl acetone was applied. For *L. striatellus*, the simple method is discouraged for determining LD_{50} values because the control mortality was high and not negligible for both the acetone and methanol solutions. When percent mortality in the control is more than ca. 20%, PoloPlus software is not able to correct for such high control mortality when calculating LD_{50} values (LeOra Software 2003; Robertson et al. 2007). The cost of the equipment for the simple method is around $200, which is <10% standard method equipment. The simple method could be widely used for monitoring insecticide susceptibility in rice planthoppers, especially *N. lugens* and *S. furcifera*, in many Asian countries.

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References

Al-Badry MS, Knowles CO (1980) Phthalate-organophosphate interactions: toxicity, penetration, and metabolism studies with house flies. Arch Environ Contam Toxicol 9:147–161

Bliss CI (1935) The calculation of the dosage-mortality curve. Ann Appl Biol 22:134–167

Endo S, Tsurumachi S (2001) Insecticide susceptibility of the brown planthopper and the white-backed planthopper collected from Southeast Asia. J Pesticide Sci 26:82–86

Endo S, Nagata T, Kawabe S, Kazano H (1988) Changes of insecticide susceptibility of the white-backed planthopper *Sogatella furcifera* Horváth (Homoptera: Delphacidae) and the brown planthopper *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Appl Entomol Zool 23:417–421

Fukuda H, Nagata T (1969) Selective toxicity of several insecticides on three planthoppers. Jpn J Appl Entomol Zool 13:142–149 (in Japanese with English summary)

Gao B, Wu J, Huang S, Mu L, Han Z (2008) Insecticide resistance in field populations of *Laodelphax striatellus* Fallén (Homoptera: Delphacidae) in China and its possible mechanisms. Int J Pest Manag 54:13–19

Hosoda A (1983) Decrease in susceptibility to organophosphorus and carbamate insecticides in the brown planhhopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Jpn J Appl Entomol Zool 27:55–62 (in Japanese with English summary)

Hosoda A (1989) Incidence of insecticide resistance in the white-backed planthopper, *Sogatella furcifera* Horváth (Homoptera: Delphacidae) to organophosphates. Jpn J Appl Entomol Zool 33:193–197 (in Japanese with English summary)

Kisimoto R (1976) Synoptic weather conditions inducing long-distance immigration of planthoppers, *Sogatella furcifera* Horváth and *Nilaparvata lugens* Stål. Ecol Entomol 1:95–109

Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Ali SS, Russelll DA (2002) Insecticide resistance in five major insect pests of cotton in India. Crop Prot 21:449–460

LeOra Software (2003) PoloPlus: probit and logit analysis. User's guide. Version 2.0. LeOra Software, Berkeley

Ma CY, Gao CF, Wei HJ, Shen JL (2007) Resistance and susceptibility to several groups of insecticides in the small brown planthopper, *Laodelphax striatellus* (Homoptera: Delphacidae). Chinese J Rice Sci 21:555–558 (in Chinese with English summary)

Matsumura M, Sanada-Morimura S (2010) Recent status of insecticide resistance in Asian rice planthoppers. JARQ 44(3):225–230

Matsumura M, Takeuchi H, Satoh M, Sanada-Morimura S, Otuka A, Watanabe T, Thanh DV (2008) Species-specific insecticide resistance to imidacloprid and fipronil in the rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera* in East and Southeast Asia. Pest Manag Sci 64:1115–1121

Nagata T (2002) Monitoring on insecticide resistance of the brown planthopper and the white backed planthopper in Asia. J Asia-Pacific Entomol 5:103–111

Nagata T, Kamimuro T, Wang YC, Han SG, Noor NM (2002) Recent status of insecticide resistance of long-distance migrating rice planthoppers monitored in Japan, China and Malaysia. J Asia-Pacific Entomol 5:113–116

Nakao T, Naoi A, Kawahara N, Hirase K (2010) Mutation of the GABA receptor associated with fipronil resistance in the whitebacked planthopper, *Sogatella furcifera*. Pest Biochem Physiol 97(3):262–266

Otuka A, Matsumura M, Sanada-Morimura S, Takeuchi H, Watanabe T, Ohtsu R, Inoue H (2010) The 2008 overseas mass migration of the small brown planthopper, *Laodelphax striatellus*, and subsequent outbreak of rice stripe disease in western Japan. Appl Entomol Zool 45:259–266

Pridgeon JW, Meepagala KM, Becnel JJ, Clark GG, Pereira RM, Linthicum KJ (2007) Structure-activity relationships of 33 piperidines as toxicants against female adults of *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 44:263–269

Robertson JL, Russel RM, Preisler HK, Savin NE (2007) Bioassays with arthropods, 2nd edn. CRC Press, New York

Vontas JG, Small GJ, Hemingway J (2001) Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. Biochem J 357:65–72

Zar JH (2010) Biostatistical analysis, 5th edn. Prentice Hall, New Jersey