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ARTICLE

Effect of imidacloprid and fipronil pesticide application on *Sympetrum infuscatum* (Libellulidae: Odonata) larvae and adults

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Abstract The effect of imidacloprid and fipronil on *Sympetrum infuscatum* larvae and adults during the rice cultivation period was monitored using an experimental micro-paddy lysimeter (MPL) system. Twenty-two hatched larvae were laid on the soil surface of each MPL. MPLs were treated with imidacloprid, fipronil, and the control MPL was left untreated. The pesticide concentration, *S. infuscatum* larval and adult populations, and larval emergence time were monitored in each MPL. The maximum imidacloprid and fipronil concentration in paddy water was 52.8 μg/l at 1 day, and 1.3 μg/l at 6 h, respectively, after the pesticide application. Both pesticides dissipated quickly in paddy water, with half-lives of 8.8 and 5.4 days for imidacloprid and fipronil, respectively. The absence of *S. infuscatum* larvae and exuviae in the fipronil-treated MPL was remarkable. The larval survival decreased to 63.6 ± 18.2, 15.2 ± 2.6, and 0% in the control, imidacloprid-treated, and fipronil-treated MPLs, respectively, by 9 days after pesticide application. Emergence in the imidacloprid-treated MPL was also significantly lower than that in the control MPL. The observed decrease in the abundances of *S. infuscatum* larvae and adults in MPLs seems to be both directly and indirectly associated with nursery-box application of fipronil and imidacloprid.

Keywords Imidacloprid · Fipronil · Sympetrum infuscatum · Micro paddy lysimeter

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Introduction

Maintaining biodiversity in agricultural environments is important for agronomic sustainability (Swift and Anderson 1994; Matson et al. 1997). Both aquatic and terrestrial components of rice paddy fields typically support high levels of biodiversity, which is essential for agricultural productivity (Cohen et al. 1994; Schoenly et al. 1998). Many studies have shown that the impact of pests in rice paddy fields is often reduced to negligible levels when predator communities are conserved through reducing the use of pesticides (Way and Heong 1994; Settle et al. 1996; Schoenly et al. 1998).

About 20 species of dragonflies belonging to the genus *Sympetrum* have been identified in Japan, most of which utilize rice fields during some portion of their life cycle. *Sympetrum* spp. larvae and adults are considered useful insects because they prey on harmful insects in rice paddy fields. *Sympetrum infuscatum* has a wide distribution, and is commonly found in rice paddies in Japan, Korea, and China (Sugimura et al. 1999; Han et al. 2010). *S. infuscatum* is one of the most effective predators of pests that infest rice, in part because their density in rice fields increases through the growing season (Nakano et al. 1977). *S. infuscatum* deposits its eggs on the soil surface of rice fields before harvest. Eggs overwinter on the soil surface and hatch immediately upon filling the paddies with water in the spring. Larvae develop to imagoes in approximately

2 months. After emergence, the adults enter the forests near the paddies, where they remain throughout the sexually immature stages, and after maturation, they return to the paddies for oviposition (Watanabe et al. 2005). The presence of *S. infuscatum* in rice paddy fields is limited during the egg, larval, and adult stages.

The use of nursery-boxes for rice cultivation is popular in Japan and East Asia. The application of pesticides to nursery-boxes before transplantation to protect rice plants from pests during the early growth stage has been practiced in Japan since the 1970s (Asaka et al. 1978). Insecticides are applied to the nursery-box either immediately before transplanting or at sowing (Thuyet et al. 2011b), depending on farmer practice and target pests.

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylidene-amine) and fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile) are systemic insecticides that have been widely used worldwide for broad spectrum insect control (Liu et al. 2002; Aajoud et al. 2003). Imidacloprid has a low mammalian toxicity but is highly effective as an insecticide (Fossen 2006), while fipronil has a high efficacy, even at low field application rates (Aajoud et al. 2003). These advantages have contributed to the rise in popularity of imidacloprid and fipronil as insecticides for use in rice cultivation in Japan, particularly for nursery-box application. While imidacloprid and fipronil use has increased in recent years, populations of *Sympetrum* spp. have declined (Uéda 2008a, b), yet there have been few reports on the cause of this decline or on the ecological impacts of rice pesticides on *Sympetrum* spp. larvae inhabiting rice paddies. Thus, an ecotoxicological assessment of the effect of pesticides on *Sympetrum* spp., particularly *S. infuscatum*, is necessary for agronomic sustainability. The hatching speed rate of *S. infuscatum* eggs tends to increase proportionately with increases in water temperature (Jinguji et al. 2010). Thus, larvae that hatch immediately after irrigation water is flooded into a paddy might be exposed to concentrations of pesticide that impact their survival.

Field ecotoxicological assessments are important for clarifying the environmental impact of pesticides. However, such assessments are usually expensive and labor intensive. The micro-paddy lysimeter (MPL) was developed as an alternative portable ecotoxicological testing system, and has proven to be an effective and convenient tool for simulating solute transport in paddy environments (Thuyet et al. 2010a). The MPL system has also been used to evaluate the behavior of broadcast granular herbicides (Nhung et al. 2009) and nursery-box-applied granular insecticides in rice paddies (Thuyet et al. 2012). In this study, an experimental MPL system was employed to evaluate the effects of rice nursery-box-applied imidacloprid and fipronil insecticides on *S. infuscatum* from larvae to emergence under a typical rice paddy water management scenario. The evaluation was based on the monitoring of pesticide concentrations in paddy water and the survival of larvae and adult *S. infuscatum*.

Materials and methods

MPL experiment

The MPLs used in this study (350 × 500 × 300 mm^3) are shown in Fig. 1. To eliminate any effects due to residual insecticides from previous applications, soil was taken from a rice paddy to which no insecticide or chemical fertilizer had been applied during the previous 2 years. The MPL was packed to a depth of 26 cm with undisturbed paddy soil. The water balance components in the lysimeter such as irrigation, percolation, and surface drainage were adjusted to simulate actual conditions used in water management in typical paddies in northern Japan. Groundwater was initially added to the MPL as a source of irrigation water to a depth of approximately 5 cm. After recording the daily evapotranspiration (ET), appropriate volumes (depth) of percolation, and surface runoff were discharged at once daily from drain pipes installed on the side and the bottom of MPL. The designed percolation and irrigation rates were set to about 10 and 20 mm/day, respectively. Experiments were conducted outdoors, and the MPLs were protected from precipitation by placing them under the eaves of the laboratory building.

Imidacloprid and fipronil were applied as the commercial granular formulations Admire® Box Granule (2% imidacloprid; Bayer Cropscience K.K., Japan) and Prince® (1.0% fipronil, BASF Agro Ltd.), respectively, to nursery-boxes containing 32 day-old rice seedlings (*Oryza sativa*

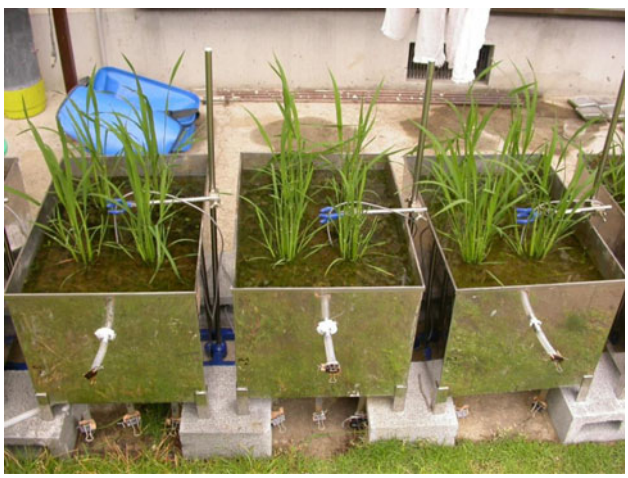

Fig. 1 Layout of micro-paddy lysimeter

cv. var. Hitomebore). The application rate for both imidacloprid and fipronil was 50 g of granules per nursery-box, as recommended for field use. The pesticide product was first applied homogeneously over the rice seedlings. Immediately after pesticide application, four rice seedlings to which pesticides had been applied were transplanted by hand on 19 May 2008 to a depth of approximately 3 cm in each MPL with a spacing of 12 × 20 cm to achieve the recommended densities. Three treatments with three replicates for each treatment were set in MPLs: imidacloprid treatment, fipronil treatment, and a control without insecticide application were applied. Entire experiment lasted until 1 August 2008 for final observation on emerged adults.

Chemicals and materials

Imidacloprid and fipronil standards (>99% purity) and analytical grade solvents used for chemical analyses were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was produced using a Milli-Q Water Purification System (Millipore, Billerica, MA, USA). The solid-phase extraction cartridges used for water extraction were Supelclean ENVI-18 (500 mg/6 ml; Supelco, MA, USA).

Water sampling

Paddy water samples were taken at 6 h, 12 h, 24 h, 48 h, 7 days , 14 days, and 30 days after transplantation (DAT). At each sampling, five 100 ml samples of paddy water taken from five randomly selected locations were mixed to form one composite water sample for each MPL. The samples were then frozen until chemical analysis.

Chemical analysis of water samples

Water samples for fipronil analysis were thawed at ambient temperature and filtered through 1.2 μm glass-fiber filters (GF/C, Whatman, UK) before extraction. Each water sample was analyzed using a solid-phase ENVI C18 Superclean cartridge. Each cartridge was preconditioned with 5 ml of acetonitrile and then washed with 5 ml of water. 500 ml of each water sample was passed through the cartridge at a flow rate of 3 ml/min without allowing the cartridge bed to dry; the eluate was discarded. Chemicals adsorbed to the cartridge were eluted with 6.0 ml of acetonitrile and the eluate was evaporated under vacuum. The resulting residue was redissolved in 1 ml of acetonitrile/water (20:80, v/v) and the sample was filtered through a 0.2 μm syringe filter (Whatman, Maidstone, UK) and kept at 4°C for HPLC analysis.

HPLC analyses were performed on a Shimadzu HPLC System consisting of an LC20AD Separations Module and an SPD-M20A photodiode array detector. The system was controlled using LCSolution software. The analytical column was a Shimadzu C-18 (150 mm × 4.6 mm × 4.6 μm particle size; Shimadzu Corporation, Kyoto, Japan) which was kept at 40°C during analytical runs. The detector operated at a fixed wavelength of 280 nm. The pump was set in isocratic mode at a flow rate of 1 ml/min with the mobile phase consisting of acetonitrile/water (60:40, v/v). The volume of the sample injection was 20 μl. The detection limit and recovery for fipronil were 0.05 μg/l and 93.0 ± 4.6% ($n = 3$), respectively.

Water samples containing imidacloprid were analyzed similarly by following the method of Thuyet et al. (2011b). The detection limit and recovery for imidacloprid were 0.05 μg/l and 90.0 ± 5.6% ($n = 3$), respectively.

Egg collection and larvae rearing

Collection of eggs from sexually mature *S. infuscatum* females was carried out in a paddy field at Miyagi University in the Miyagi Prefecture, Japan (38°13′N, 140°49′E). Eleven females were captured while ovipositing. A total of 2,018 eggs were collected by holding each insect's wings and dipping the tip of the abdomen into a dry glass tube. All eggs were combined at the end of collection. The eggs were then divided into water-permeable packs (50 eggs per pack) containing soil that had been oven-dried at 110°C for 24 h. These packs were placed on the surface of a paddy at the Miyagi University farm on 30 September 2007 and left to overwinter in order to allow the eggs to complete diapause under natural conditions. The packs were removed from the paddy and transported to the laboratory on 15 May 2008, and 50 eggs and the soil in each pack was transferred into a square plastic tray ($L = 10$ cm, $W = 10$ cm, $H = 3$ cm). The eggs were then covered with distilled water to a depth of 2 cm and the trays were maintained in an incubator (GC351, Sanyo, Japan) at 23°C with a photoperiod of 14L:10D (relative light intensity = 3,000 lux). Beginning on 16 May 2008, the eggs were examined daily under a binocular microscope (SZ60, Olympus, Japan) at 30× magnification. Newly hatched larvae were counted and removed.

Larvae sampling

Immediately after rice seedlings were transplanted into each MPL, 22 *S. infuscatum* larvae that had been reared in the incubator for 4 days were placed in the center of each MPL by a pipette in order to prevent larvae from directly touching the insecticide granules on the surface of the transplanted rice seedlings. Quantitative sampling of larvae was conducted at 9 DAT and then every 7 days until 52 DAT for a total of seven times. In the first two sampling

events, 5 cm-tall stainless steel cores were driven about 1 cm into the soil at five randomly chosen spots in the MPL. Next, the water and about 1 cm of the surface soil were collected from inside the core using a pipette. The number of larvae in the water and soil samples was counted in a Petri dish under a binocular microscope, and the number of larvae per unit area in the MPL was extrapolated from observations of the core samples. From the second sampling event, the number of larvae in each MPL was visually confirmed. Survival here was defined as a percentage of living larvae relative to initial released larvae in MPL and it was calculated from the following equation:

$$\text{Survival}\,(\%) = 100 \times n/N \quad (1)$$

where N represents the initial number of larvae ($N = 22$) and n represents the number of surviving larvae at each weekly sampling.

Exuviae and adult sampling

Exuviae and adults were collected by covering each MPL with a net and the collected specimens were then stored individually in paraffin paper. The day of emergence, the condition of each adult, and the number of dead adults observed during emergence were recorded. Exuviae and adult sampling was conducted every day from 50 to 70 DAT.

Statistical analyses

One-way ANOVA was followed by a multiple comparison test (Tukey HSD post hoc test) to determine whether treatment results were significantly different. All statistical analyses were performed using SPSS statistics software (Ver. 18.0, SPSS Institute Inc., Japan). Survival data were arcsine transformed and analyzed using ANOVA.

Results

MPL conditions

The paddy water temperature was similar among the MPLs and was dependent on weather patterns. Although the MPL was slightly shaded in the morning time, the paddy water temperature remained at 18.5 ± 3.7°C during the first month; however, it increased to 21.5 ± 2.5°C toward the end of the rainy season in late June. During the emergence period in mid-July the paddy water temperature was 22.3 ± 2.8°C and remained at or near this temperature throughout most of the summer. The ET was similar among the MPLs and ranged from 5 to 8 mm/day. Electrical conductivity (EC) of MPL paddy water during the trial was generally low. Mean EC were 105.1 μS cm^{-1} and ranged from 72 to 124 μS cm^{-1}.

Dissipation of fipronil and imidacloprid in paddy water

The dissipation curve for fipronil in MPL water is shown in Fig. 2. Fipronil reached a maximum concentration of 1.3 μg/l at 6 h after the seedlings were transplanted, and then dissipated exponentially to <0.5 μg/l by 7 DAT. Fipronil remained in this concentration range until the end of the monitoring period. During the first 7 days of the monitoring period, the fipronil DT50 value in paddy water was determined to be 5.4 days ($r^2 = 0.92$).

The dissipation curve for imidacloprid in MPL water is shown in Fig. 3. Imidacloprid reached a maximum concentration of 52.8 μg/l at 1 DAT. The initial imidacloprid concentration was approximately 50 times higher than that of fipronil. The imidacloprid concentration decreased to 13.2 μg/l by 14 DAT and dropped to 4.9 μg/l after 1 month. During the first 14 days of the monitoring period, the imidacloprid DT50 value in paddy water was determined to be 8.8 days ($r^2 = 0.98$).

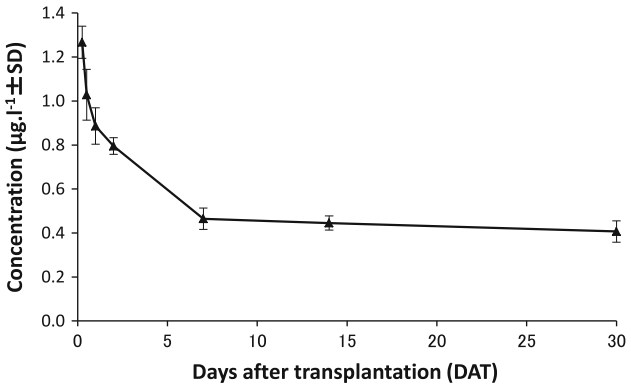

Fig. 2 Dissipation of fipronil in MPL surface water

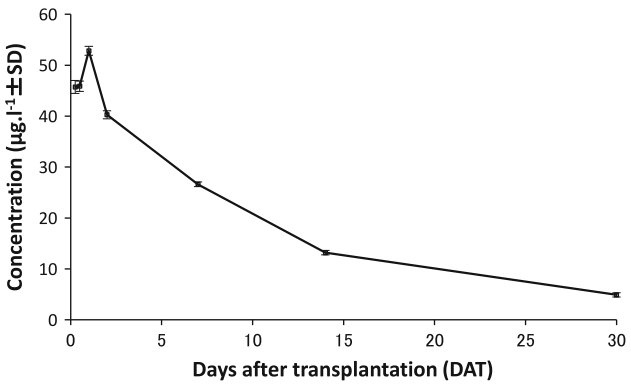

Fig. 3 Dissipation of imidacloprid in MPL surface water

Larvae survival

Figure 4 shows the survival of *S. infuscatum* larvae during the rice cultivation season for the three MPL treatments. Survival decreased sharply immediately after transplantation, especially in the fipronil-treated MPL. By 9 DAT, the larvae survival dropped to 63.6 ± 18.2, 15.2 ± 2.6, and 0% in the control, imidacloprid-, and fipronil-treated MPLs, respectively. After 9 DAT, the larvae survival in the imidacloprid-treated MPL was significantly lower than in the control MPL (ANOVA, $P < 0.01$, $P < 0.001$). At 9 DAT, no larvae were observed during the remainder of the experimental period in the fipronil-treated MPL. Note that the variation of estimated survival rate was large and the temporal increase in mean survival rate at 30 DAT in the control MPL may be the result of heterogeneous distribution of larvae in MPL. By 52 DAT, the larvae survival decreased to 12.1 and 53% in the imidacloprid-treated and controls MPLs, respectively.

Successful emergence and daily emergence patterns

Figure 5 shows the mean emergence percentage of successful *S. infuscatum* emergence in the MPLs. No exuviae or adults were captured in the fipronil-treated MPL. The mean emergence percentage in the control MPL was 66.7 ± 2.6, 12.1 ± 2.6% in the imidacloprid-treated MPL, and 0% in the fipronil-treated MPL. The emergence percentage in the imidacloprid-treated MPL was significantly lower than it was in the control (ANOVA, $P < 0.05$). The proportion of larvae that did not emerge into adults was 1.3% in the imidacloprid-treated MPL.

Figure 6 shows the daily emergence pattern in the MPLs during the experimental period. Emergence began on 17 July (55 DAT) in the control MPL and on 21 July (58 DAT) in the imidacloprid-treated MPL. The mean total period of emergence in the control and imidacloprid-treated MPLs was 14.7 and 8.7 days, respectively.

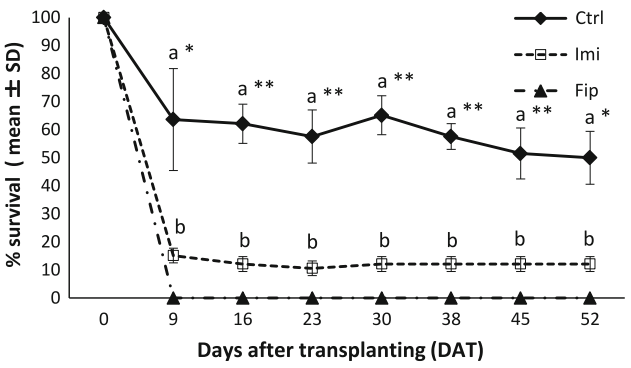

Fig. 4 Survival of *S.infuscatum* larvae during the rice cultivation season in *Imi* imidacloprid-treated, *Fip* Fipronil-treated, and *Ctrl* control MPLs. Both treatments started on the day of planting. *Letters* denote significant differences compared to the control (*$P < 0.01$, **$P < 0.001$). *Error bars* indicate standard deviation

Discussion

Dissipation of fipronil and imidacloprid in paddy water

Fipronil appeared at a low range of concentrations from about 1.3 µg/l to about 0.5 µg/l for the first week and became relatively stable afterwards (Fig. 2). The maximum mass of fipronil in paddy water peaked at 1 DAT and accounted for 0.43% of the applied mass. Fipronil is very sensitive to sunlight, and its photolysis half-life has been reported to be 0.33 days (Gunasekara et al. 2007), and 1.5 days (Thuyet et al. 2011a). In an actual rice paddy field monitoring study, Thuyet et al. (2010b) found the maximum concentration of fipronil was 2.5 µg/l at 1 DAT, and it dissipated quickly in paddy water, with a DT50 of 0.9 days.

Imidacloprid tended to dissipate in a manner similar to reported paddy field experiments. The initial concentration of imidacloprid in paddy water was varied depending on

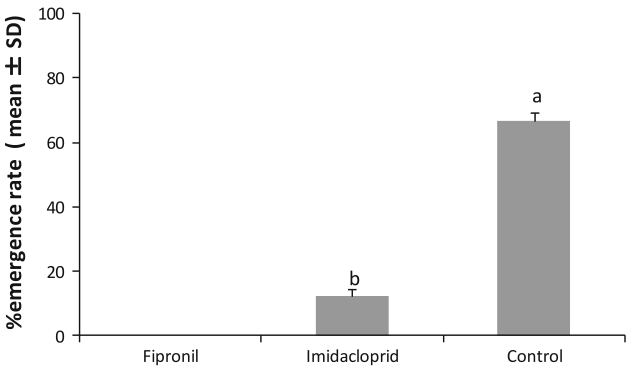

Fig. 5 *S. infuscatum* successful emergence rate. *Letters* denote significant difference compared to control ($P < 0.05$)

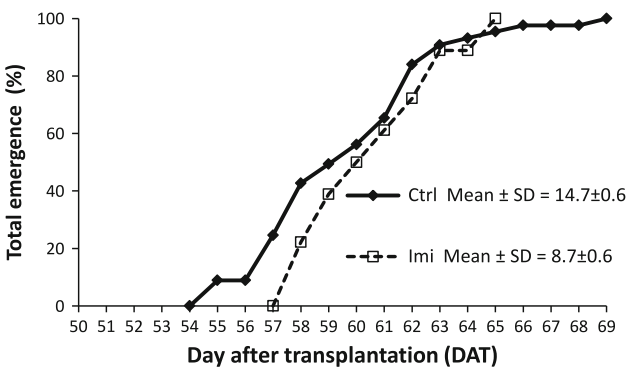

Fig. 6 Emergence pattern of *S. infuscatum* during the rice cultivation season in *Imi* imidacloprid-treated and *Ctrl* control lysimeters

experiments and sample time, e.g., 58.6 μg/l at 1 DAT (Phong et al. 2009), 30.2 μg/l at 0.5 DAT (Thuyet et al. 2011b), and 240 μg/l at 2 h after transplantation (Sanchez-Bayo and Goka 2006a) with a similar application rate, however, it was around 1.0 μg/l after 1 month (Sanchez-Bayo and Goka 2006a). DT50 of imidacloprid was almost similar to previously reported values of, 1.9–2.0 days (Phong et al. 2009), 2.0 days (Thuyet et al. 2011b), and 4 days (Sanchez-Bayo and Goka 2006a). As is the case with fipronil, imidacloprid is reportedly sensitive to photodegradation, its half-life in paddy water exposed to natural sunlight in October being reduced to 1.0 day (Thuyet et al. 2010a).

The DT50 of fipronil and imidacloprid in this study was longer than those reported in other studies, probably due to differences in experimental variables such as water, soil properties, and solar radiation intensity. Fate of insecticides in rice paddy may highly depend upon environmental factors as well as management factors (Thuyet et al. 2011b). The field dissipation of both compounds encompasses three major fate processes such as photolysis, biochemical degradation, and soil/water partitioning. The latter process could be more prevalent in the case of fipronil due to its higher adsorption properties (Gunasekara et al. 2007). Considering the micro habitat (water and soil) of larvae, monitoring of concentrations in paddy soil surface in addition to paddy water may give realistic assessment on their toxicological response although the data for paddy soil is not available in this study. The period of insecticide monitoring were conducted for 30 days by assuming the effects in later period is negligible, however, the appropriate period of chemical monitoring may be depend on the sensitivities of test species.

Effect of fipronil and imidacloprid on *S. infuscatum*

Our data indicate that nursery-box-application of fipronil and imidacloprid cause significant mortality to *S. infuscatum* larvae. Fipronil at ppb levels (0.4–1.3 μg/l; Fig. 2) was found to be very toxic to young *S. infuscatum* larvae. *S. infuscatum* larvae were completely eliminated in the fipronil-treated MPL. The survival of *S. infuscatum* decreased to 0% in the first 9 days after pesticide application. No adults or exuviae were captured during the experimental period. Jinguji et al. (2009) reported a similar early decline in the larvae of *S. frequens* in MPLs; they demonstrated that *S. frequens* larvae were eliminated by 14 DAT in fipronil-treated lysimeters. In addition, no exuviae or adults were observed during their experiment. These results suggest that application of fipronil may be the cause of early decline of larvae and may lead to the extinction of *S. infuscatum* and *S. frequens* in treated rice paddies. Fipronil is highly toxic to many aquatic species even at low concentrations (Gunasekara et al. 2007) and has been reported to be highly effective against several mosquito species. Its reported 24 h LC_{90} and 24 h LC_{50} for larvae of the mosquito *Culex quinquefasciatus* are 0.90 and 0.35 μg/l, respectively (Ali et al. 1999). Moreover, the 24 and 48 h LC_{50s} for larvae of the mosquito *Aedes aegypti* are 24.8 nM (~11.7 μg/l) and 15.1 nM (~7.14 μg/l), respectively (Aajoud et al. 2003). Fipronil is also highly toxic to midges (*Chironomus tepperi*), which are common pests in rice fields; the LC_{50} and LC_{90} values for midges are 0.43 and 1.05 μg/l, respectively (Stevens et al. 1998).

In this study, the maximum fipronil concentration of 1.3 μg/l, which correspond to 0.43% of the applied mass, was observed at 1 DAT, after which the concentration decreased exponentially. The half-life of fipronil in our study was 5.4 days. The fipronil concentration decreased to less than 0.5 μg/l by 7 DAT and remained in this range until the end of the monitoring period. A second stadium *S. infuscatum* larva needs around 30 days to develop into a six stadium larva (Jinguji and Tsuyuzaki 2008). Thus, it is reasonable to suggest that released second stadium larvae were exposed to fipronil concentrations of 0.4–1.5 μg/l, which are close to the LC_{50} for *C. quinquefasciatus* and the LC_{90} for *C. tepperi*. It is certain that *S. infuscatum* larvae are sensitive to fipronil concentrations in this range as are *C. quinquefasciatus* and *C. tepperi*, and that this sensitivity is responsible for the sharp decline in the number of individual larvae observed just after rice transplantation.

Imidacloprid is reportedly less toxic to young *S. frequens* larvae than fipronil (Jinguji et al. 2009). Jinguji et al. (2009) reported a 60% survival for *S. frequens* larvae at 9 DAT in an imidacloprid-treated MPL. In this study, the survival of *S. infuscatum* larvae decreased to 15.2% by 9 DAT, and the mean emergence percentage was 12.1%. These results suggest that *S. infuscatum* is more susceptible to imidacloprid than is *S. frequens*. The diet of many odonates, although encompassing many insect taxa, consists predominantly of small *Diptera*, among which *Chironomidae* and *Culicidae* are well-represented (Corbet 1999). Zooplankton and midges are important because they serve as food sources during the early stages of larval development. Sanchez-Bayo and Goka (2006a) found that zooplankton species were absent from imidacloprid-treated rice fields during the first 2 months following application, when the concentration of imidacloprid was greater than 1 μg/l, and the recovery of zooplankton populations was slow and never returned to the levels found in untreated fields. A similar effect was observed in this study. Many zooplankton and midges were observed in the paddy water of the control MPL; however, crystal-clear water indicative of few zooplankton or midges was observed in the imidacloprid-treated MPL. This suggests that populations of zooplankton and midges might have been impacted by

imidacloprid. As an indirect effect of imidacloprid application, the lack of zooplankton and midges might have caused a delay and shortened the emergence period in the imidacloprid-treated MPL. Delay of emergence for larvae makes them more vulnerable to drying by the mid-summer due to drainage. Moreover, lack of food due to the use of imidacloprid is likely to cause incomplete emergence. Toxicological data suggest that imidacloprid is not very toxic to fish or Daphnia, but it is very toxic to chironomids and all other crustaceans, particularly ostracods, amphipods, and crayfish (Stoughton et al. 2008; Overmyer et al. 2005; Sanchez-Bayo and Goka 2006b). The 48 h LC_{50} for *Daphnia magna* is reportedly 17–85 mg/l (Song et al. 1997; Iwaya and Kagabu 1998), whereas imidacloprid concentrations in rice paddies have been reported to be quite low: 240 μg/l at 2 h after transplantation (Sanchez-Bayo and Goka 2006a), 30.2 μg/l at 0.5 DAT (Thuyet et al. 2011b), and 58.6 μg/l at 1 DAT (Phong et al. 2009). However, the absence of typical paddy ostracods and other microcrustaceans from imidacloprid-treated fields (Sanchez-Bayo and Goka 2006a) is relevant to the survival of *S. infuscatum* larvae, which may be indirectly affected in imidacroprid-treated rice paddies. Susceptibility to imidacloprid appears to differ among aquatic species; while some organisms are relatively unimpacted, *S. infuscatum* larvae are highly susceptible to low imidacloprid concentrations.

Conclusion

We investigated the effect of nursery-box-applied fipronil and imidacloprid pesticides on *S. infuscatum* in rice paddies using MPLs. Fipronil completely eliminated young *S. infuscatum* larvae at concentrations of 0.4–1.3 μg/l (ppb levels) in the first 9 DAT. The effect of imidacloprid on larvae right after hatching was not as great as that of fipronil, however, the impact of imidacloprid was not negligible, as indicated by the low survival during emergence as compared to the control. Imidacloprid is likely to produce an indirect effect by diminishing prey availability. Therefore, growers should be aware that when nursery-box-applied pesticides are used in rice paddies, *Sympetrum* larvae will be exposed to pesticide immediately after hatching upon transplantation of the rice seedlings. Decreases in the abundance of *S. infuscatum* larvae and adults appear to be both directly and indirectly associated with nursery-box application of fipronil and imidacloprid in MPLs. Our research has demonstrated an applicability and usefulness of MPL for ecotoxicological assessments of nursery-box-applied pesticides for rice paddy field ecosystems. Such microcosm-based approaches establish a relevant context for faunal assessments, and complement traditional experimental methods, including laboratory toxicology studies.

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