1st June 2015

Can the use of more selective insecticides promote the conservation of *Sympetrum frequens* in Japanese rice paddy fields (Odonata: Libellulidae)?

Hiroshi Jinguji[1] & Tetsuyuki Uéda[2]

[1] School of Food, Agricultural and Environmental Sciences, Miyagi University,
2-2-1 Hatatate, Taihaku-ku, Sendai, Miyagi 982-0215, Japan;
<jinguji@myu.ac.jp>

[2] Environmental Sciences, Ishikawa Prefectural University, 1 Suematsu,
Nonoshi, Ishikawa 921-8836, Japan

Received 7th November 2014; revised and accepted 15th January 2015

Abstract. The effect of two relatively selective nursery-box-applied insecticides on *Sympetrum frequens* larvae and adults as substitutes for the commonly used insecticides, imidacloprid and fipronil, was examined using an experimental micro-paddy lysimeter (MPL) system. Fifty hatched larvae were placed on the soil surface of separate MPLs that had been treated with imidacloprid, fipronil, dinotefuran, and cartap hydrochloride, as well as an untreated control MPL. At 30 days after transplantation, the complete absence of *S. frequens* larvae and exuviae in the imidacloprid and fipronil-treated MPLs was remarkable. In the control, cartap- and dinotefuran-treated MPLs, the mean number of larvae was 31.0 ± 6.0, 27.0 ± 6.0, and 6.3 ± 1.5, respectively. No *S. frequens* adults were observed later in the imidacloprid- and fipronil-treated MPLs. The rate of emergence did not differ significantly among the control, cartap- and dinotefuran-treated MPLs. However, the mean head width of *S. frequens* in the dinotefuran-treated MPL was significantly narrower than that of *S. frequens* in the control and cartap-treated MPLs. The mean EM_{50} in the cartap-treated MPL was significantly longer than that in the control- and dinotefuran-treated MPLs. The findings showed that the ecological impact of cartap on *S. frequens* was slightly less than the application of fipronil, imidacloprid and dinotefuran to rice paddy fields.

Key words. Dragonfly, Anisoptera, microcosm, cartap, imidacloprid, fipronil

Introduction

Of the approximately 20 dragonfly species belonging to the genus *Sympetrum* in Japan, seven utilize rice fields during their life cycle (Uéda 1998). *Sympetrum* spp. larvae and adults are considered to be beneficial insects because they prey on insect pests in rice paddy fields. *Sympetrum frequens*

(Selys, 1883) has a wide distribution in Japan and is commonly found in rice paddies (Sugimura et al. 1999), where it deposits its eggs on the soil surface after harvesting. The eggs overwinter on the soil surface and hatch immediately upon contact with water when the paddies are inundated in spring. Larval development is completed in approximately two months. After emerging, the tenerals migrate to mountainous regions where they remain until they are sexually mature. Once mature, they return to the paddies to copulate and lay eggs (Uéda 1988, 1993).

Sympetrum frequens is considered to be important for several reasons. The species is one of the most effective predators of rice insect pests, such as the beetles *Lissorhoptrus oryzophilus* and *Oulema oryzae*, partly because of the high density of this dragonfly species in rice fields during the rice-growing season (Urabe & Ikemoto 1986). Furthermore, since *S. frequens* is a major predator of the mosquito *Anopheles sinensis*, a known malaria vector (Urabe & Ikemoto 1986), increased predation pressure by *S. frequens* may reduce the potential for malaria transmission in areas where the disease is endemic (e.g., Southeast Asia). Further, the many brands of 'red dragonfly rice' on the market attest to the fact that *S. frequens* is symbolic of the Japanese countryside (Fig. 1).

The use of nursery boxes for rice cultivation is popular in Japan and East Asia, and the application of insecticides to these nursery boxes prior to transplantation to protect rice plants from pests during the early growth stage has been practiced in Japan since the 1970s (Asaka et al. 1978). Depending on the farmer and the pest species being targeted, insecticides are typically applied to the nursery-box immediately before transplanting or at the time of sowing (Thuyet et al. 2011b).

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 are widely used around the world for broad-spectrum insect control (Liu et al. 2002; Aajoud et al. 2003). The neonicotinoid, imidacloprid, has a low mammalian toxicity but is highly effective as an insecticide (Fossen 2006), while the phenylpyrazole, fipronil, has a high efficacy, even at low field application rates (Aajoud et al. 2003; Gunasekara 2007). These advantages have contributed to the increased popularity of both compounds for use in rice cultivation in Japan, particularly for application to nursery boxes (Fig. 2). However, the increased usage of these insecticides for rice cultivation since ca 2000 has had a detrimental effect on populations of *Sympetrum* spp. (Uéda 2008a, 2008b). In addition, outbreaks of infectious diseases in honey bee, fish, amphibian, bat, and bird populations during the last two decades have coincided with the increased use of systemic insecticides, particularly neonicotinoids and fipronil (Mason 2012).

Figure 1. 'Red dragonfly rice' is a symbol for the socio-cultural importance of *Sympetrum frequens* in the Japanese countryside. The dragonfly illustrated on the package of this organically grown rice in the picture is *S. frequens*.

Imidacloprid is susceptible to runoff, and maximum concentrations have been reported in paddy water at 0.5 days after transplanting plants from treated nursery boxes (Thuyet et al. 2011a). Fipronil has also been detected in rivers receiving rice field tailwater (Scott 2008). However, very little toxicity data exists for these chemicals on non-target organisms, particularly on the odonates that inhabit rice fields. Jinguji et al. (2009, 2013) reported that even low concentrations of fipronil completely eliminated *S. frequens* and *S. infuscatum* larvae immediately after rice transplantation (Fig. 3). While the effect of imidacloprid on larvae immediately after hatching was not as marked as that of fipronil, the impact of imidacloprid was not negligible, as indicated by the low rates of survival during adult emergence (Jinguji et al. 2009).

The purpose of this study was therefore to clarify the effects of two more selective nursery-box-applied insecticides on *S. frequens* larvae in the field, i.e., the systemic neonicotinoid, dinotefuran (1-methyl-2-nitro-3-[tetrahydro-3-furylmethyl] guanidine), which has accounted for an increasingly large share of the market since 2002, and cartap hydrochloride, (S,S'-2-(dimethylaminotrimethylene)-bis(thiocarbamate), that was registered as a nursery-box insecticide in 1976. An experimental micro-paddy lysimeter (MPL) system was employed to evaluate the effects of nursery-box-applied imidacloprid, fipronil, dinotefuran, and cartap on *S. frequens* under a water management regime typically used in rice paddies (Fig. 4). The MPL was developed for use as a 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; Jinguji et al. 2013).

Materials and methods

MPL experimental design

Fifteen MPLs (350 × 500 × 300 mm) were used in this study. To eliminate the possible contribution of residual insecticides from previous applications, soil samples were collected from a rice paddy to which no insecticide or chemical fertilizer had been applied for the previous four years. The MPL was packed to a depth of 25 cm with undisturbed paddy soil. After tilling, the paddy soil in the MPLs, a 5 cm-deep layer of surface soil was removed from all MPLs. This soil was then combined, mixed, divided into 15 equal parts and then returned to the 15 MPLs. The reason for this mixing and redistribution step was to ensure that the zooplankton and microinvertebrates that are preyed upon by *Sympetrum* spp. would be homogenously distributed among all MPLs.

The parameters affecting water balance in the lysimeter, such as irrigation, percolation and surface drainage, were adjusted to simulate the actual water management conditions employed in paddies in northern Japan. Groundwater was initially added to a depth of approximately 5 cm in the MPL as a source of irrigation water. After recording daily evapotranspiration rates, appropriate percolation and surface runoff regimes were implemented on a daily basis using drainage pipes installed on the side and the bottom of each MPL. In this way, the percolation and irrigation rates were set to about 10 mm/day and 20 mm/day, respectively. The water temperature in each MPL was recorded using a temperature logger (SK-L200TII, Sato Keiryoki Mfg. Co., Ltd., Japan). Experiments were conducted outdoors and the MPLs were shielded from precipitation by placing them under the eaves of a laboratory building at Miyagi University.The microcosm system consisted of 15 independent MPLs with five different treatments: a control MPL, and imidacloprid-, fipronil-, dinotefuran- and cartap-treated MPLs, with three replicates for each treatment. Granular formulations of four commercial insecticides commonly applied to nursery boxes were used: Admire® Box Granule, (2 % imidacloprid; Bayer CropScience K.K., Japan), Prince® (1.0 % fipronil; BASF Agro Ltd., Japan), Starkle® (2 % dinotefuran; Mitsui Chemical and Agro, Japan) and Padan® (4 % cartap; Kyoyu Agri Co., Japan), respectively. The granulates were applied to nursery boxes containing 32-day-old rice seedlings (*Oryza sativa* var. Hitomebore).

The application rate for all insecticides was 50 g of granules per nursery box, which is the amount recommended for field application by the manufacturers. The pesticide was initially applied homogeneously over the rice seedlings on 14-v-2010. Immediately after pesticide application, four of the rice seedlings to which pesticides had been applied were transplanted by hand to a depth of approximately 3 cm in each MPL at a spacing of 12 × 20 cm to achieve an optimal density (Fig. 4). The experiment was continued until 20-ix-2010 to permit final observations of the emerged adults.

Egg collection and larval rearing

Eggs were obtained from sexually mature *S. frequens* females that were collected from a paddy field at Miyagi University in Miyagi Prefecture, Japan (38°13'N, 140°49'E). Eleven females were captured while ovipositing and a total of 4,280 eggs were obtained by holding an individual by the wings and dipping the tip of the abdomen into a test tube containing distilled water. All of the eggs were pooled into a single batch after collection. The eggs were then divided into batches of 50 and placed into water-permeable bags containing soil that had been oven-dried at 110°C for 24 h. These bags were placed on the surface of a paddy field at the Miyagi University farm on

Figure 2. Nursery boxes for rice cultivation (top) and rice seedlings separated for the micro-paddy lysimeter experiment (bottom).

Figure 3. Rice transplantation from nursery boxes into the paddy field. The small white box at right contains insecticide which is dusted on rice seedlings at the time of planting.

28-x-2009 and left to overwinter so that the eggs could complete diapause under natural conditions. The bags were then collected from the paddy and transported to the laboratory on 10-v-2010 where the 50 eggs and the soil in each bag were transferred to a square plastic tray (10 × 10 × 3 cm). The eggs were then covered with distilled water to a depth of 2 cm and the trays were placed in an incubator (GC351, Sanyo, Japan) at 23°C under a photoperiod of 14L:10D (relative light intensity = 3,000 lux). From 12-v-2010 onward, the eggs were examined daily under a binocular microscope (SZ60, Olympus, Japan) at 30× magnification. Newly hatched larvae were counted and placed in a Petri dish containing distilled water until the next stage of the experiment.

Population size

Immediately after the rice seedlings were transplanted into each MPL, 50 *S. frequens* larvae that had been reared in the incubator for two days were placed in the center of each MPL using a pipette in order to prevent the larvae from coming into direct contact with any of the insecticide granules on the surface of the transplanted rice. The number of remaining larvae

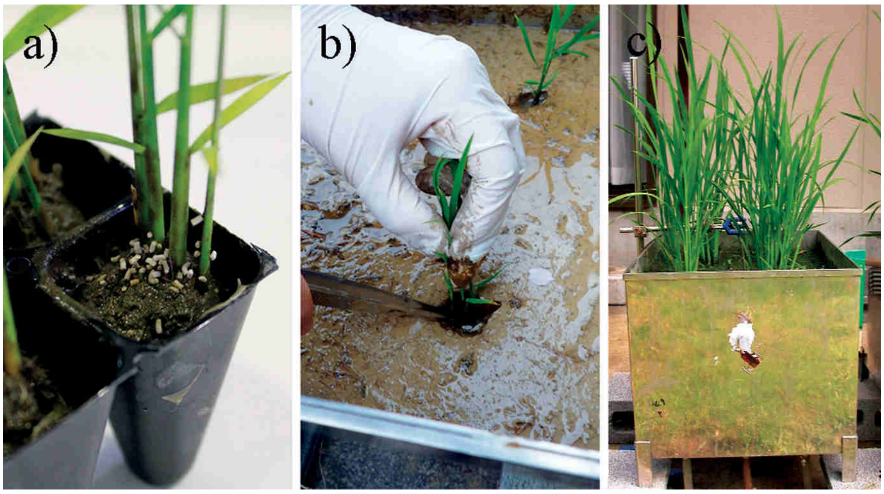

Figure 4. (a) Separated rice seedlings with insecticides for the micro-paddy lysimeter (MPL) experiment; (b) transplantation of rice seedlings into a MPL; (c) MPL at 50 days after transplantation.

was estimated in each MPL at 30 days after transplantation (DAT) by visual confirmation and removal of larvae by two researchers over a 10-minute period with a stainless steel spoon. The surfaces of the soil, rice plants and weeds were carefully examined for larvae, taking great care not to disturb the soil surface and make the water cloudy when removing the larvae with the spoon. The reason for this cautious approach was to ensure that dissolution of the insecticides would not be promoted due to stirring of the water in the MPL. This sampling methodology was replicated in triplicate at 10-min intervals. The head width of each larva (*sensu* Corbet 1999) was measured to the nearest 0.1 mm with an ocular micrometer (GS-E, Uchida, Japan) under a binocular microscope (SZ60, Olympus) at 30× magnification.

Exuviae and adult sampling

Exuviae and adults were collected by covering each MPL with a net. The collected specimens were then stored individually in paraffin paper. The condition of each adult and the number of dead adults observed during emergence were recorded on the day of emergence. Exuviae and adult collection was performed every day from 10-vii- to 21-ix-2010. The shape of the emergence curve was affected by the cumulative percentile of emergence, and EM_{50} was determined to compare differences in the emergence patterns; EM_{50} is the time (expressed as days elapsed since emergence began) taken for 50 % of the total population to emerge during the experimental period.

Statistical analysis

A one-way ANOVA followed by a multiple comparison test (Tukey's HSD *post-hoc* test) was performed to determine whether the obtained results were significantly different. All statistical analyses were performed using SPSS statistical software (Ver. 18.0, SPSS Institute Inc., Japan) and survival data were arcsine transformed and analyzed by ANOVA.

Results

MPL conditions

The paddy water temperature was dependent on the ambient weather and did not vary markedly among the control, cartap-, dinotefuran-, imidacloprid-, and fipronil-treated MPLs; the mean water temperature in each treatment was 23.6 ± 4.2°C (mean ± SD), 25.3 ± 4.4°C, 24.2 ± 4.5°C, 25.3 ± 4.5°C, and 24.1 ± 4.5°C, respectively. Although the MPLs were slightly shaded in the morning, the average paddy water temperature was 18.6 ± 3.0°C during the first month; however, the water temperature increased to 23.8 ± 1.8°C toward the end of the rainy season in late June. During the emergence period in mid-July, the paddy water temperature was 27.7 ± 3.0°C and it remained at or near this temperature for most of the summer. The maximum paddy water temperature was 40.6 ± 0.8°C in mid-August. The daily evapotranspiration was similar among the MPLs and ranged from 5 to 10 mm/day.

Larval survival

Figure 5 shows the number of *Sympetrum frequens* larvae counted at 30 DAT in each of the five MPL treatments. The number of larvae was 31.0 ± 6.0 in the control MPL, 27.0 ± 6.0 in the cartap-treated MPL, 6.3 ± 1.5 in the dinotefuran-treated MPL, and 0 in both the fipronil and imidacloprid-treated MPLs at 30 DAT. Larval survival in the dinotefuran-treated MPLs

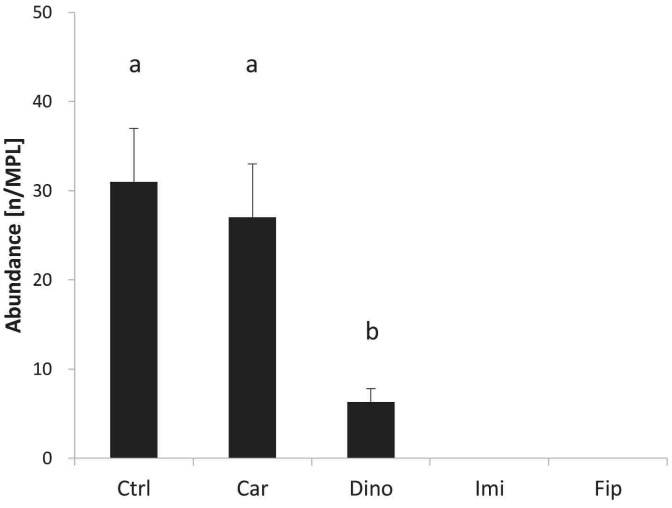

Figure 5. Mean number of *Sympetrum frequens* larvae at 30 DAT in control (Ctrl) and cartap- (Car), dinotefuran- (Dino), imidacloprid- (Imi), and fipronil-treated (Fip) micro paddy lysimeters. All treatments started on the day of planting. Different letters denote significant differences compared to the control (*P* <0.001). Error bars indicate standard deviation.

was significantly lower than in the control and cartap MPLs (P <0.001, $F_{2,6} = 21.219$). By 30 DAT, no larvae were observed in the imidacloprid- and fipronil-treated MPLs for the remainder of the experimental period.

Larval growth

Larval growth at 30 DAT differed significantly between the four experimental MPLs (Fig. 6). The mean head width of *S. frequens* in the control, cartap- and dinotefuran-treated MPLs was 1.9 ± 0.3 mm, 1.6 ± 0.3 mm and 1.2 ± 0.2 mm, respectively. The mean head width of *S. frequens* in the dinotefuran-treated MPL was significantly narrower than the head width of *S. frequens* in the cartap-treated MPL and in the control MPL (P <0.01, $F_{2,6} = 5.45$).

Successful emergence rate and EM_{50}

Figure 7 shows the mean rate of successful *S. frequens* emergence in the MPLs. No exuviae or adults were observed or captured in the fipronil- and imidacloprid-treated MPLs. The mean emergence rate was 14.7 ± 4.6 % in the control MPL, 13.3 ± 2.3 % in the cartap-treated MPL, 7.3 ± 7.0 % in the dinotefuran-treated MPL, and 0 % in both the fipronil and imidacloprid-treated MPLs. The rate of emergence was not significantly different among the control, cartap- and dinotefuran-treated MPLs ($P = 0.262$, $F_{2,6} = 1.688$).

Figure 8 shows the daily *S. frequens* emergence patterns observed in all MPLs over the experimental period. No *S. frequens* adults were observed in the imidacloprid- and fipronil-treated MPLs. Emergence started on 02-viii--2010 (79 DAT) in the control and cartap-treated MPLs, and on 05-viii--2010 (82 DAT) in the dinotefuran-treated MPLs. The mean EM_{50} for the control, cartap- and dinotefuran-treated MPLs was 7.3 ± 3.5, 26.0 ± 7.8 and 11.3 ± 3.5 days, respectively. The mean EM_{50} in the cartap-treated MPL was significantly longer than that in the control- and dinotefuran-treated MPLs (P<0.01, $F_{2,6} = 10.1$).

Discussion

In this study, clear differences in *Sympetrum frequens* abundance, larval growth, and emergence patterns were observed using a long-term MPL experiment. Our findings showed that nursery-box application of fipronil and

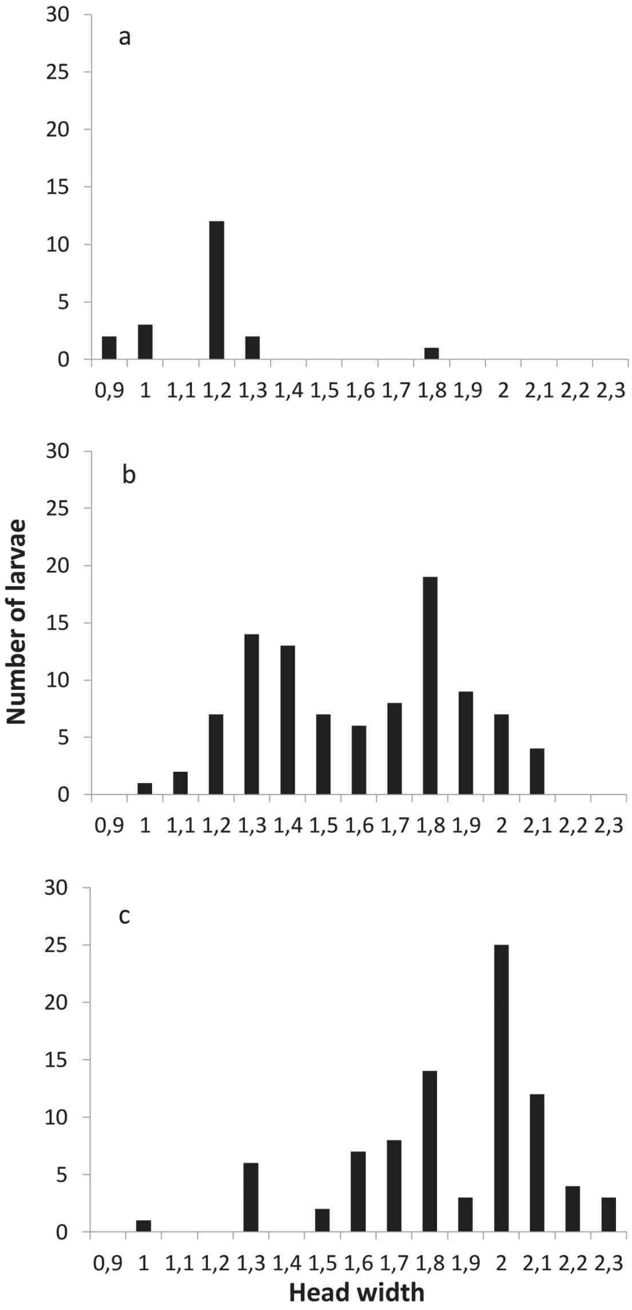

Figure 6. Frequency distributions of head width in *Sympetrum frequens* at 30 days after transplantation in (a) dinotefuran, (b) cartap, and (c) control treatments. Data for all insecticide and control treatments were pooled. Mean head width of *S. frequens* in the dinotefuran treatment was significantly narrower than in the cartap-treated and control treatments ($P < 0.01$).

imidacloprid was associated with very high *S. frequens* larval mortality. Indeed, all of the larvae were eliminated in the fipronil and imidacloprid treatments by 30 DAT (Fig. 5). Although *S. frequens* larval emergence occurred within three months in the cartap, dinotefuran, and control treatments, no larvae emerged from the fipronil- and imidacloprid-treated MPLs until the end of the experimental period (Fig. 7). Jinguji et al. (2009, 2013) reported

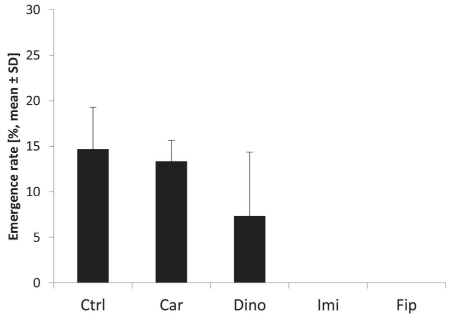

Figure 7. Successful emergence of *Sympetrum frequens* in control (Ctrl) and cartap- (Car), dinotefuran- (Dino), imidacloprid- (Imi), and fipronil-treated (Fip) micro-paddy lysimeters, from 50 larvae released in each treatment in three replications, respectively. Error bars indicate standard deviation.

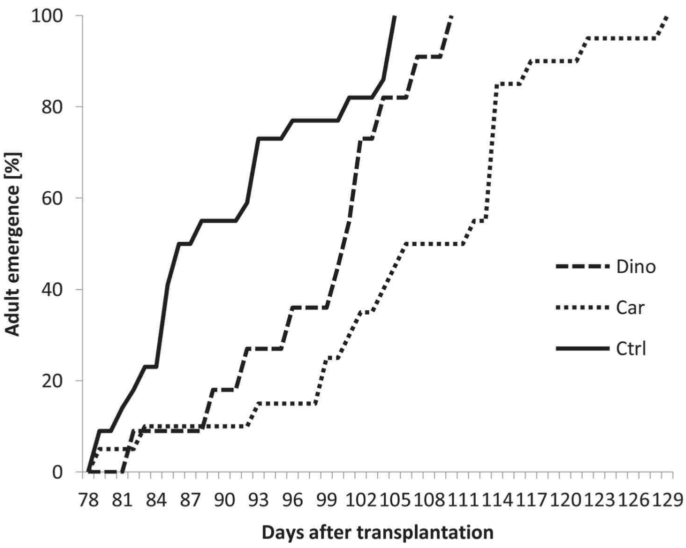

Figure 8. Emergence pattern of *Sympetrum frequens* in dinotefuran (Dino), cartap (Car) and control (Ctrl) treatments. Data for all insecticide and control treatments were pooled. 100 % emergence in dinotefuran-treated micro-paddy lysimeters represents 11, in cartap 20, and in control 22 individuals.

a similar decline in *S. frequens* and *S. infuscatum* (Selys, 1883) larvae, with larvae of both species eliminated by 9 and 14 DAT in fipronil-treated lysimeters, respectively, and no adults observed during their experiments. Although imidacloprid is reportedly less toxic than fipronil in *S. frequens* and *S. infuscatum* larvae, the mean emergence percentages of *S. frequens* and *S. infuscatum* in previous MPL experiments were 3.3 % and 12.1 %, respectively (Jinguji et al. 2009, 2013). In this study, the impact of imidacloprid was severe, as indicated by the low survival during emergence. Our findings are consistent with numerous previous studies in which imidacloprid insecticides had a negative effect on many non-target species. Flying insects with an aquatic larval stage appeared to be the most vulnerable to imidacloprid (Overmyer 2005; Beketov 2008; Stoughton et al. 2008; Van Dijk et al. 2013; Roessink 2013).

In a study using a paddy mesocosm, Hayasaka et al. (2011) demonstrated that nekton communities of long-lived species, such as dragonfly larvae, did not recover within three months after exposure to imidacloprid and fipronil. Moreover, the impact of fipronil on aquatic arthropods was more pronounced after a second application in the following year (Hayasaka et al. 2012), indicating the high persistence of this insecticide in rice paddies. These results suggest that the application of fipronil and imidacloprid at the recommended commercial rates is likely to have a long-term impact on *Sympetrum* spp.

Zooplankton and midges are important sources of food during the early stages of odonate larval development. Sanchez-Bayo & Goka (2006a, 2006b) found that zooplankton species were absent from imidacloprid-treated rice fields for the first two months after application when the concentration of imidacloprid exceeded 1 mg/l. In addition, their study showed that the recovery of zooplankton populations was slow and that they never reached the levels found in untreated fields. A similar effect was observed in this study, with many zooplankton taxa and midges observed in the paddy water of the control MPLs, and very few zooplankton taxa or midges observed in the imidacloprid- and dinotefuran-treated MPLs. Although the direct effect of dinotefuran on larval and adult emergence was not as marked as that of imidacloprid, it was not negligible, as indicated by the low larval growth compared to the control (Figs 5 and 6). As with imidacloprid, dinotefuran is also likely to have an indirect effect on reducing prey availability.

Our study also showed that cartap is relatively less toxic to *S. frequens*. The rates of adult survival and emergence in cartap-treated MPLs were similar to those observed in the control MPLs. In addition, no significant differences in larval head width were observed between cartap and control treatments at 30 DAT, suggesting that exposure to cartap at the recommended commercial application rates does not have any acute or long-term influence on the life cycle of *S. frequens*. Further, except for *Anotogaster sieboldii* (Selys, 1854), laboratory experiments have shown that cartap does not have a marked detrimental effect on the larvae of the families Coenagrionidae and Libellulidae (Ishida & Murata 1992). On the other hand, cartap does have a negative effect on several hymenopteran species, including aphid parasitoids that are used to control crop pests (Takeda et al. 2001; Kobori & Amano 2004; Ateyyat 2012). In addition, in rice paddies, cartap hydrochloride has been reported to reduce populations of coccinellid beetles, carabid beetles, dragonflies, and damselflies by 20 to 50 % (Srinivas & Madhumathi 2005). In this study, EM_{50} in cartap-treated MPLs was longer than that in the dinotefuran-treated and control MPLs (Fig. 8), suggesting that populations of prey species, such as zooplankton and midges might have been impacted by cartap. As an indirect effect of cartap application, the relative absence of zooplankton and midges may have delayed adult emergence in the cartap-treated MPLs (Fig. 8). Studies clarifying the effect of cartap on zooplankton and midges should be undertaken in the future.

In rice paddy fields in Ishikawa Prefecture, adults of the *Sympetrum* species did not emerge from fields to which fipronil has been applied, and adult emergence from paddy fields to which cartap had been applied and fields to which no pesticide had been applied was not significantly different (Uéda & Jinguji 2013). A similar effect was observed in our microcosm experiment, suggesting that our observations closely reflect the actual situation in paddy fields.

In Japan, cartap first entered widespread use as an insecticide for nursery-box applications_in 1976, primarily because of its high solubility in water (Uneme 2003). It has been reported that cartap is highly effective for controlling the rice water weevil, *Oulema oryzae*, which has developed resistance to fipronil in recent years (Ishimoto et al. 2004).

Cartap is also toxic to fish. However, with 3-h LC_{50} values in the range 0.02 to 6.8 mg/L, the direct toxicity of cartap to fish species is not as high as that of other neurotoxic insecticides (LANNACONE et al. 2007; ZHOU et al. 2009). Laboratory experiments have shown that cartap sprayed on the water surface of a small container at the recommended application rates had no impact on the paddy field loach, *Misgurnus anguillicaudatus* (OZAWA 1982). On the other hand, recent findings of endocrine disrupting effects and developmental neurotoxicity have raised concerns about the potential ecological impacts of cartap. The incidence of larval fish deformities in *Oryzias latipes*, increased significantly after exposure to cartap concentrations of 250 µg/L for periods as short as 96 hours (KIM et al. 2008).

It therefore appears that the ecological impact of cartap on *S. frequens* is relatively less than the impacts associated with fipronil, imidacloprid and dinotefuran. Imidacloprid and fipronil are very effective for controlling insects, particularly sucking pests such as aphids, while being quite innocuous to fish and vertebrates in general (TOMIZAWA et al. 2000). Many rice farmers in Japan employ images of the red dragonfly, *S. frequens,* on their products, as the species is synonymous with the rice fields that have been treated with imidacloprid and fipronil insecticide. However, in addition to the targeted pests, the chronic toxicity of imidacloprid and fipronil means that they also affect non-target insects, not only at the time of application, but for several months thereafter. Therefore, farmers should be aware of the fact that *S. frequens* will be eliminated if imidacloprid and fipronil are applied to their nursery boxes. Our findings showed that the ecological impact associated with cartap application on *S. frequens* populations was relatively lower than the impacts associated with fipronil, imidacloprid, and dinotefuran. However cartap delayed the emergence of *S. frequens* in MPL experiments. In addition, even low concentrations of cartap can result in the abnormal development of fish larvae. It is therefore considered that using cartap insecticides that are relatively less toxic will contribute positively to the conservation of *S. frequens*, in those cases where farmers insist on using insecticides. However, there is a need to further examine the effect of cartap on non-target, aquatic species so that it can be recommended for ‘red dragonfly rice’ branded products on the market. In addition, it is desirable that farmers employ organic farming practices to better conserve *S. frequens.*

Acknowledgements

We thank K. Goka, T. Tsubaki, and K. Miyai for their valuable contributions to the study. This work was primarily supported by a Grant-in-Aid for Biological Studies on Wildlife under the Framework of ExTEND2005, and a Grant-in-Aid for Scientific Research (No. 22580278) from Japan Society for the Promotion of Science.

References

Aajoud A., Ravanel P. & Tissut M. 2003. Fipronil metabolism and dissipation in a simplified aquatic ecosystem. *Journal of agricultural and Food Chemistry* 51: 1347-1352

Asaka S., Kawauchi N., Koyama S. & Emura K. 1978. Fate of Propaphos (Kayaphos) applied on seedling box. *Journal of Pesticide Science* 3: 305-310

Ateyyat M. 2012. Selectivity of four insecticides to wooly apple aphid, *Eriosoma lanigerum* (Hausmann) and its sole parasitoid, *Aphelinus mali* (Hald.). *World applied Sciences Journal* 16: 1060-1064.

Beketov M.A. & Liess M. 2008. Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. *Archives of environmental Contamination and Toxicology* 55: 247-253.

Gunasekara A.S., Truong T., Goh K.S., Spurlock F. & Tjeerdema R.S. 2007. Environmental fate and toxicology of fipronil. *Journal of Pesticide Science* 32: 189-199

Hayasaka D., Korenaga T., Sanchez-Bayo F. & Goka K. 2011. Differences in ecological impacts of systemic insecticides with different physicochemical properties on biocenosis of experimental paddy fields. *Ecotoxicology* 21: 191-201

Hayasaka D., Korenaga T., Suzuki K., Saito F., Sanchez-Bayo F. & Goka K. 2012. Cumulative ecological impacts of two succesive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicology and environmental Safety* 80: 355-362

Ishimoto M., Nagase A., Nishido K., Kanbayashi T. & Nishizawa Y. 2004. Resistance to fipronil in the rice leaf beetle, *Oulema oryzae*, in Niigata prefecture. *Proceedings of the Association for Plant Protection of Hokuriku* 53: 37-42 [In Japanese, English abstract]

Ishida K. & Murata M. 1992. Effects of pesticides application for the paddy field on the larvae of Odonata. *Scientific Reports of the Faculty of Agriculture, Meijo University* 28: 1-12

Jinguji H., Uéda T., Goka K., Hidaka K. & Matsura T. 2009. [Effects of imidacroprid and fipronil insecticide application on the larvae and adults of *Sympetrum frequens*]. *Transactions of the Japanese Society of Irrigation, Drainage and Rural Engineering* 259: 355-360 [In Japanese]

Jinguji H., Thuyet D.Q., Uéda T. & Watanabe H. 2013. Effect of imidacloprid and fipronil pesticide application on *Sympetrum infuscatum* (Libellulidae: Odonata) larvae and adults. *Paddy and Water Environment* 11: 277-284

Jinguji H., Uéda T., Tsunoda M., Aihara S. & Saito M. 2010. [Effects of fipronil insecticide application on *Sympetrum* spp. larvae and adults in experimental rice paddy field]. *Transactions of the Japanese Society of Irrigation, Drainage and Rural Engineering* 267: 79-86 [In Japanese]

Kim Y., Jung J., Oh S., Choi K. 2008. Aquatic toxicity of cartap and cypermethrin to different life stages of *Daphnia magna* and *Oryzias latipes*. *Journal of environmental Science and Health*, *Part B: Pesticides, Food Contaminants, and Agricultural Wastes* 43: 56-64

Kobori Y. & Amano H. 2004. Effects of agrochemicals on life-history parameters of *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae). *Applied Entomology and Zoology* 39: 255-261

Liu W.P., Zheng W. & Gan J.Y. 2002. Competitive sorption between imidacloprid and imidacloprid-urea on soil clay minerals and humic acids. *Journal of agricultural and Food Chemistry* 50: 6823-6827

Lannacone J., Onofre R. & Huanquj O. 2007. Ecotoxicological effects of cartap on *Poecilia reticulata* "guppy" (Poecilidae) and *Paracheirodon innesi* "Neon Tetra" (Characidae). *Gayana* 71: 170-177

Mason R., Tennekes H., Sanchez-Bayo F. & Jepsen P.U. 2012. Immune Suppression by Neonicotinoid insecticides at the root of grobal wildlife declines. *Journal of environmental Immunology and Toxicology* 1: 3-12

Overmyer J.P., Mason B.N. & Armbrust K.L. 2005. Acute toxicity of imidacloprid and fipronil to a nontarget aquatic insect, *Simulium vittatum* Zetterstedt cytospecies IS-7. *Bulletin of environmental Contamination and Toxicology* 74: 872-879

Ozawa T. 1982. [Residue and fish toxity of submerged application of agricultural chemicals in paddy fields]. *Bulletin of the Iwate-ken Agricultural Experiment Station* 23: 91-104 [In Japanese]

Roessink I., Merga L.B., Zweers H.J. & Van den Brink P.J. 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Environmental Toxicology and Chemistry* 32: 1096-1100

Sanchez-Bayo F. & Goka K. 2006a. Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields. *Environmental Toxicolology and Chemistry* 25: 1677-1687

Sanchez-Bayo F. & Goka K. 2006b. Influence of light in acute toxicity bioassays of imidacloprid and zinc pyrithione to zooplankton crustaceans. *Aquatic Toxicology* 78: 262-271

Scott V.M., Stephen D.P. & Dennis K.D. 2008. Influence of fipronil compounds and rice-cultivation land-use intensity on macroinvertebrate communities in streams of southwestern Louisiana, USA. *Environmental Pollution* 152: 491-503

Srinivas K. & Madhumathi T. 2005. Effect of insecticide applications on the predator population in rice ecosystem of Andhra Pradesh. *Pest Management and economic Zoology* 13: 71-75

Stoughton S.J., Liber K., Culp J. & Cessna A. 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions. *Archives of environmental Contamination and Toxicology* 54: 662-673

Sugimura M., Ishida S., Kojima K,. Ishida K. & Aoki H. 1999. [Dragonflies of the Japanese archipelago in color]. Hokkaido University Press, Sapporo [In Japanese]

Takeda Y., Kawamura S. & Tanaka T. 2001. Effects of various insecticides on the development of the egg parasitoid *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae). *Journal of economic Entomology* 94: 1340-1343

Thuyet D.Q., Hien T.Q., Watanabe H., Saito H., Phong T.K. & Nishimura T. 2010. Micro paddy lysimeter for monitoring solute transport in paddy environment. *Paddy and Water Environment* 8: 235-245

Thuyet D.Q., Watanabe H., Yamazaki K. & Takagi K. 2011a. Photodegradation of imidacloprid and fipronil in rice–paddy water. *Bulletin of environmental Contamination and Toxicology* 86: 548-553

Thuyet D.Q., Watanabe H. & Motobayashi T. 2011b. Effect of formulations and treatment methods of nursery boxes applied with insecticide on the behavior of imidacloprid in rice paddy fields. *Journal of Pesticide Science* 36: 9-15

Tomizawa M., Lee D.L. & Casida J.E. 2000. Neonicotinoid insecticide: molecular features conferring selectivity for insect versus mammalian nicotinic receptors. *Journal of agricultural and Food Chemistry* 48: 6016-6024

Uéda T. 1988. [Diversity in the life history of the dragonfly *Sympetrum frequens* (Odonata: Insecta)]. *Bulletin of Ishikawa Agricultural College* 17: 41-51 [In Japanese]

Uéda T. 1993. [Questions on the life history of *Sympetrum frequens* (1)]. *Insectarium* 30: 292-299 [In Japanese]

Uéda T. 1998. [Dragonfly communities in paddy fields]. In: Ezaki Y. & Tanaka T. (Eds), [Conservation in wetlands]: 93-110. Asakura Syoten, Tokyo [In Japanese]

Uéda T. 2008a. [A recent striking tendency to decrease in *Sympetrum frequens* and the estimated year when it started from the survey using questionnaires to the members of Aka-tombo network]. *Symnet* 10: 6-7 [In Japanese]

Uéda T. 2008b. [The results of 2007 *Sympetrum* censuses at various localities in Japan by the members of Aka-tombo network (prompt report)]. *Symnet* 10: 8 -10 [In Japanese]

Uéda T. & Jinguji H. 2013. [Ecological impact of insecticides, fipronil and imidacloprid, on *Sympetrum frequens* in Japan.] *Tombo*, Fukui 55: 1-12 [In Japanese]

Uneme H. 2003. Cartap. In: Sasaki M., Umetsu N., Saka H., Nakamura K & Hamada K. (Eds), [Development of agrochemicals in Japan]: 116-130. Pesticide Science Society of Japan, Tokyo [In Japanese]

Urabe K. & Ikemoto T. 1986. [*Sympetrum frequens* (Odonata: Libellulidae) nymphs as natural enemies of the mosquito larvae, *Anopheles sinensis*, in rice fields. III. Estimation of the prey consumption rate in the rice fields]. *Japanese Journal of applied Entomology and Zoology* 30: 129-135 [In Japanese]

Van Dijk T.C., Van Staalduinen M.A. & Van der Sluijs J.P. 2013. Macro-invertebrate decline in surface water polluted with imidacloprid. *PLOS ONE* 8(5):e62374. doi:10.1371/journal.pone.0062374

Zhou S., Dong Q., Li S., Guo J., Wang X. & Zhu G. 2009. Developmental toxicity of cartap on zebrafish embryos. *Aquatic Toxicology* 95: 339-346