Original Article

Selectivity and Absorption of the Herbicide Naproanilide in Rice Plants (*Oryza sativa* L.) and *Sagittaria pygmaea* MiQ.*

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The selectivity of naproanilide [2-(2-naphthoxy)propionanilide] in rice plants, broad-leaf and cyperaceous weeds was studied through both pre- and post-emergence tests. Rice plants were found extensively tolerant to naproanilide, while *Cyperus difformis* L., *Monochoria vaginalis* BURM. f., *Rotala indica* WILLD., *Sagittaria pygmaea* MiQ. and *Scirpus juncoides* ROXB. were very sensitive. The absorption rate of ¹⁴C-naproanilide in rice plants was much lower than in *Sag. pygmaea*. Moreover, in rice plants the translocation from root to upper portion was retarded, but in *Sag. pygmaea*, radioactivity was distributed uniformly in the shoot, tuber and root.

INTRODUCTION

In recent years, the continuous use of herbicides effective against annual weeds has caused the spread of perennial weeds such as *Sagittaria pygmaea* MiQ., *Cyperus serotinus* ROTH. and *Sagittaria trifolia* L. in paddy fields in Japan. Various herbicides more effective against a wide spectrum of weeds are required. In order to avoid the undesirable succession of weeds, the development of new herbicides effective for a wide spectrum of weeds has been promoted. Through the efforts, the herbicide naproanilide was developed to control perennial weeds in paddy fields.

Field and greenhouse studies by Takasawa¹⁾ showed that naproanilide was effective to selectively control broad-leaf and cyperaceous weeds by both pre- and post-emergence treatments in rice fields. Takasawa *et al.*²⁾ also

showed that naproanilide *per se.* had no phytotoxicity but was activated through its conversion to 2-(2-naphthoxy)propionic acid (NOP). Furthermore, they reported that the selective herbicidal activity of NOP was similar to that of naproanilide.

Oyamada and Kuwatsuka³⁾ also studied the absorption, translocation and metabolism of ¹⁴C-naproanilide in rice seedlings. ¹⁴C-Naproanilide was absorbed through the root, but it was hardly translocated to the upper part.

Studying the selectivity mechanism of naproanilide, Kobayashi *et al.*^{4,5)} found that naproanilide remarkably stimulated RNA synthesis in *Cyperus difformis* L. (a susceptible species), and suggested that the disturbance of RNA metabolism might induce the observed abnormal growth and subsequent suppression of plant growth. On the other hand, RNA synthesis in rice plants (a tolerant species) was hardly stimulated.

The objectives of this study are to define the selectivity of naproanilide in tolerant rice plants and some susceptible plants, and to

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clarify the contribution of absorption and translocation to it.

MATERIALS AND METHODS

1. Radioactive Naproanilide and NOP

^{14}C -Naproanilide and ^{14}C -NOP, uniformly labelled at the naphthalene ring (specific radioactivity: naproanilide 1.0, NOP 1.6 mCi/mmol; radioactive purity: more than 99%) were used.

2. Radioassay

Whole plant and top, root and tuber were dried, weighed and analyzed to determine radioactivity by combustion using a Model ASC-113 Aloka sample oxidizer. The trapped $^{14}\text{CO}_2$ was measured by a Model LSC-903 Aloka liquid scintillation spectrometer.

3. Selectivity

3.1 Pre-emergence treatments

Seeds of the rice plant (*Oryza sativa* L. cv. Nihonbare) and the *Scirpus juncooides* ROXB. (Hotarui in Japanese), and tubers of the *Sagittaria pygmaea* MIQ. (Urikawa in Japanese) were planted at the surface of quartz sand in petri dishes (90 mm in diameter, 90 mm in height) containing 0.03 μM to 40 μM of naproanilide solution, NAA (α -naphthaleneacetic acid) or 2,4-D (2,4-dichlorophenoxyacetic acid). The plants were grown in a chamber at a range from 23°C to 27°C. Artificial light of 5000 lux was provided by cool-white fluorescent lamps. Twenty days after initiating the treatment, the plants were harvested and dry weight of the whole plant was determined. Each treatment was triplicated.

3.2 Post-emergence treatments

Rice seedlings at the 2.5 leaf-stage, and tubers and seeds of *Sagittaria pygmaea* MIQ., *Monochoria vaginalis* BURM. f. (Konagi in Japanese), *Scirpus juncooides* ROXB., *Rotala indica* WILLD. (Kikashigusa in Japanese), and *Cyperus difformis* L. (Smallflower umbrellaplant) were transplanted or sown into 1/5000 are-sized Wagner's pots containing an alluvial soil submerged up to 2 cm deep. The alluvial soil sample was collected from the arable layer of a rice field in Chigasaki, Kanagawa Prefecture. To treat the rice seedlings, naproanilide dissolved in one ml of acetone solution was applied at the rates of 7.5, 15, 30 and 60 g/a

a.i. to the flooded water for five days after the transplanting. When *C. difformis*, *M. vaginalis*, *R. indica* and *Sci. juncooides* were grown to the 1.5- to 2.0-leaf stage and *Sag. pygmaea* to the 2.0- to 2.5-leaf stage, the naproanilide solution was treated as described above. Each treatment was triplicated.

The plants were grown in a greenhouse at 18°C to 30°C under supplemental light by cool-white fluorescent lamps with a 14-hr photoperiod. Thirty days after the treatment with the herbicide, the plants were harvested and the dry weight was determined.

4. Absorption of ^{14}C -Naproanilide and ^{14}C -NOP in Water Culture

Seeds of rice plants and tubers of *Sagittaria pygmaea* were germinated in an incubator at 30°C for 3 days. The germinated seeds were transplanted into square plastic pots containing Kasugai's nutrient solution or the soil under paddy field conditions, until they grew to the 2.5-leaf stage in a greenhouse. The roots and basal parts of rice seedling shoots and whole plants of *S. pygmaea* were dipped in the 4-time-diluted Kasugai's solution containing 0.05 μM of either ^{14}C -naproanilide or ^{14}C -NOP. The plants were grown according to the practice in rice paddy fields except that they were put in a chamber (Koitozon® HNB-20) under a light intensity of 5000 lux provided by wide spectrum lamps with a 12-hr photoperiod at 25°C by day and 20°C by night. The initial level of the nutrient solution was maintained by supplying water periodically. The plants were harvested at designated intervals and washed with water. The total radioactivity of the whole plant was measured as described above.

5. Absorption of ^{14}C -Naproanilide in Flooded-Soil Culture

Rice seedlings at the 2.5-leaf stage and sprouted tubers of *Sagittaria pygmaea* were transplanted to flooded soil (submerged up to 2 cm deep) in a glass pot (150 mm in diameter, 90 mm in height). ^{14}C -Naproanilide (2.5 mg) (corresponding to 1.5 g/are a.i.) dissolved in 0.25 ml of acetone was applied to the flooded water 5 days after the transplanting of rice seedlings or at the 2 to 2.5-leaf stage of *S.*

Table 1 ED₅₀ levels (the chemical concentration required to reduce the dry weight increase to 50% of the control) μM .

Chemicals	Rice plant (<i>Oryza sativa</i> L.)	<i>Scirpus juncooides</i> ROXB.	<i>Sagittaria pygmaea</i> MIQ.
Naproanilide	19.2	0.15	0.05
2,4-D	1.49	3.57	—

pygmaea. The plants were grown in a chamber (Koitoiron® HNB-20) under the conditions described above, and harvested at designated intervals. The whole plants were washed with water to remove soil particles and radioactive chemicals adhering to the surface. They were divided into shoots, roots and tubers, and then the total radioactivity of each part was measured as described above.

6. Radioautogram

The harvested plants were spread out and pressed between two sheets of filter paper. Then they were exposed to X-ray film (Fuji Rx Medical, Fuji Photo Film Co., Ltd.) for several weeks to obtain the radioautograms.

RESULTS AND DISCUSSION

1. Selectivity

To compare the selectivity of naproanilide with that of 2,4-D, each chemical was applied to rice plants, *Scirpus juncooides* and *Sagittaria pygmaea*. The growth of both weeds was considerably stunted when treated with naproanilide at the germinating or sprouting stage, but rice plants at the same stage were hardly affected even when treated at about $5 \mu\text{M}$ as shown in Fig. 1. Rice plants were rated tolerant. Naproanilide at a concentration of about $10 \mu\text{M}$ inhibited the growth 30%, but it did not kill even at $35 \mu\text{M}$. Both *Sci. juncooides* and *Sag. pygmaea* died 20 days after the treatment at concentrations exceeding about 1 and $3 \mu\text{M}$, respectively. Although the mechanism of this inhibition in *Sci. juncooides* and *Sag. pygmaea* has not been made clear, Kobayashi *et al.*⁽⁴⁾ recently found that naproanilide stimulated RNA synthesis in susceptible *C. diffinis* and suppressed its growth. In fact, rice plants were killed by the 2,4-D treatment at about $4.5 \mu\text{M}$. The response of *Sci. juncooides* and rice plants to 2,4-D was the same, but *Sag.*

Fig. 1 Dose-response of rice plants and two plant species at germinating or sprouting stage to naproanilide and 2,4-D in the petri dish assay.

○: Rice plant, □: *Scirpus juncooides* ROXB., △: *Sagittaria pygmaea* MIQ.

pygmaea was less sensitive. In rice plants, $0.45 \mu\text{M}$ of 2,4-D resulted in strong malformation in both shoot and root, whereas naproanilide did not even at $3.4 \mu\text{M}$.

The concentrations of naproanilide and 2,4-D causing 50% inhibition of whole dry weight are given in Table 1. The results showed the extremely high sensitivity of *Sci. juncooides* and *Sag. pygmaea* and the remarkable tolerance of rice plants to naproanilide. In contrast, 2,4-D was highly susceptible to rice plants as well as to *Sci. juncooides*. The tolerance of rice plants to naproanilide contrasted with their high sensitivity to 2,4-D, demonstrating a marked selectivity difference between these two chemicals.

Data showing the effect of post-emergence treatments on weed control and the growth of rice plants are presented in Fig. 2. Naproanilide at 30 g/a a.i. showed high herbicidal activity against these five species of weeds, causing severely abnormal growth and finally resulting in the death of all the seedlings 2 or 3 weeks after the treatment. In contrast, rice plants were very tolerant to naproanilide, showing little inhibitory effect on the growth at 60 g/a a.i. Thus, naproanilide at 30 and

Fig. 2 Dose-response of rice plants and five weed species to surface water-applied naproanilide under paddy field conditions.

○: Rice plant (*Oryza sativa* L.), △: *Cyperus difformis* L., □: *Monochoria vaginalis* BURM. f., ▲: *Scirpus juncoides* ROXB., ■: *Rotala indica* WILLD., ●: *Sagittaria pygmaea* MIQ.

60 g/a a.i. proved to selectively control annual and perennial weeds such as *C. difformis*, *M. vaginalis*, *R. indica*, *Sag. pygmaea* and *Sci. juncoides* in rice paddy fields. The data in this study emphasize that naproanilide can be effective to selectively control weeds by both pre- and post-emergence applications in transplanted rice cultivation.

2. Absorption of ^{14}C -Naproanilide and ^{14}C -NOP in Water Culture

The patterns of absorption of radioactivity in rice plants and *Sagittaria pygmaea* at various intervals after the treatment with ^{14}C -naproanilide and ^{14}C -NOP are shown in Fig. 3. Although naproanilide was absorbed more rapidly by *S. pygmaea* than by rice plants, the radioactivity per unit weight of both plants increased for 1 to 3 days after naproanilide treatment, but did not increase after that period. The absorption of naproanilide by *S. pygmaea* declined for 1 to 7 days due to an increase of the weight, causing the dilution of radioactivity. Three days after the treatment with ^{14}C -naproanilide, the radioactivity absorbed was about 120 dpm/mg in rice plants and about 200 dpm/mg in *S. pygmaea*. *S. pygmaea*, on an average, absorbed ^{14}C -naproanilide twice as much as rice plants. The absorption rate of naproanilide was greater by *S. pygmaea* than by rice plants. But this probably reflected the application difference,

Fig. 3 Absorption of ^{14}C -naproanilide and ^{14}C -NOP in plants in water culture.

○: Naproanilide, ●: NOP.

because only the root and basal part of rice seedling shoots were soaked in the naproanilide solution while the whole plant of *S. pygmaea* was soaked. The application, however, was done in imitation of field practices, so this difference in absorption may cause the herbicidal selectivity of naproanilide in rice plants and *S. pygmaea* in paddy fields.

Oyamada *et al.*⁶⁾ reported that a large amount of NOP was produced when naproanilide was degraded more rapidly in flooded soils, and that the phytotoxicity of NOP was almost the same as that of naproanilide. Our study showed that the absorption of ^{14}C -NOP by both plants was rapid during the initial 3 days and thereafter it continued at a slower speed (Fig. 3). A larger amount of NOP was absorbed by *S. pygmaea* than by rice plants, and so was in the case of naproanilide. The rate of absorption of NOP, however, was lower than that of naproanilide in both plants. Takasawa *et al.*¹⁾ reported that cyperaceous and broad-leaf weeds were very sensitive to NOP and rice plants tolerant. The difference in the absorption of NOP by these plants appears to be one of the factors which determines the herbicidal selectivity of naproanilide in paddy fields.

3. Absorption and Translocation of ^{14}C -Naproanilide in Flooded-Soil Culture

The radioautograms of rice seedlings and *Sagittaria pygmaea* treated with ^{14}C -naproanilide in the surface water of flooded soil are shown in Figs. 4 and 5, indicating the presence

Fig. 4 Radioautogram of rice seedlings 3 days after treatment under paddy field conditions. ^{14}C -Naproanilide was applied to the flooded water.

of radioactivity in shoots and roots. But most of the radioactivity in rice plants was distributed only in the basal parts of shoots and roots which were immersed in the treated surface water. The results were similar to those obtained in the previous experiments by Oyama *et al.*³⁾ This may be due to the limited translocation of naproanilide and/or its metabolic products from the root and basal part of shoots to the upper part of rice plants. In *S. pygmaea*, the radioactivity was distributed uniformly in the shoot and root, though it was not clear whether naproanilide or its metabolites were translocated.

Changes in the distribution of radioactivity in both plants after the treatment with ^{14}C -naproanilide are shown in Fig. 6. The radioactivity in shoots of both plants greatly increased during the initial 7 days after the treatment, although the radioactivity in roots and tubers increased gradually. In rice seedlings, the radioactivity of shoots and roots was about 100 and 40 dpm/mg, respectively, 14 days after the treatment. In *S. pygmaea* it was about 200, 35 and 15 dpm/mg in shoots, tubers and roots, respectively. The radioactivity equivalents in the plant tissue (dpm/

Fig. 5 Radioautogram of *Sagittaria pygmaea* M19. 3 days after treatment under paddy field conditions. ^{14}C -Naproanilide was applied to the flooded water.

Fig. 6 Absorption and translocation of ^{14}C -naproanilide applied at a rate of 1.5 g/a a.i. in rice and *Sagittaria pygmaea* Miq. seedlings under paddy field conditions.

○: Shoots, □: Roots, △: Tuber.

mg dry weight) were: 6.7 and 2.7 in the shoot and root of rice seedlings; 13.3, 1.0 and 2.3 in the shoot, root and tuber of *S. pygmaea*, respectively. A substantial portion of the radioactivity in both plants was found to be distributed in the shoot. Although the radioactive distribution showed a similar trend in both plants, the radioactivity in the shoot of *S. pygmaea* was about two fold that of rice seedlings 7 and 14 days after the treatment. It is presumed that there was a marked difference in the absorption patterns of the two plants, because the shoot area of *S. pygmaea* was bigger than that of rice plants immersed in the treatment solution. Although the metabolic pathways of naproanilide in *S. pygmaea* have not been established, it was concluded that the difference in the absorption rates of both plants may contribute to the herbicidal selectivity of naproanilide treated to surface water. Moreover, although the radioactivity accumulated in rice shoots was half of that in *S. pygmaea*, it was hardly translocated to the upper parts, as shown in the radioautogram of rice seedlings in Fig. 4. In contrast, naproanilide and/or its metabolites are readily accumulated in the whole shoots of *S. pygmaea*.

One of possible mechanisms of herbicidal selectivity in rice plants and *S. pygmaea* obtained under flooded soil conditions, was attributed to the difference in the absorption and translocation of naproanilide. The metabolism of naproanilide in rice plants and *S. pygmaea* will be reported in our subsequent paper.

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要 約

水稻(*Oryza sativa* L.)とウリカワ(*Sagittaria pygmaea* Miq.)におけるナプロアニリドの選択性と吸収性

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ナプロアニリドによる水稻と広葉, カヤツリグサ科雑草間の選択殺草作用, および実用的使用場面を考慮した試験で水稻およびウリカワの吸収性の差について検討した。出芽前処理におけるナプロアニリドによる水稻の ED_{50} は, ホタルイ, ウリカワに比べおのおの約 130 倍および 380 倍高かった。出芽後処理において, ナプロアニリドはホタルイ, ウリカワ, タマガヤツリ, コナギおよびキカングサに対して 15 g/a a.i. でほぼ 90% の阻害を示し, 30 g/a a.i. では完全に枯死した。これに対して水稻では 60 g/a a.i. までもほとんど抑制作用は見られなかった。 ^{14}C -ナプロアニリドは, 水稻よりウリカワで 2 倍吸収された。化合物の吸収は, 水稻では茎基部と根部により吸収するが, 上部への移行は著しく制限された。ウリカワでは茎葉, 塊茎および根部に化合物が一樣に分布した。