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Root growth inhibition of rice by bensulfuron

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Summary

Bensulfuron inhibited root growth of water-seeded rice (*Oryza sativa* L.) plants in a solution culture system. Rice root growth was more sensitive to bensulfuron than was shoot growth. At 2.5×10^{-9} M bensulfuron, plant height, number of leaves and number of roots were not significantly affected, while root growth was reduced. Seedlings treated 3 or 4 days after emergence showed a significant reduction of total root length by the second day after treatment. Root growth inhibition was proportional to duration of treatment; however, treatment for 2 days was necessary to affect root growth. Bensulfuron decreased the mitotic index and the mitotic height in root tips. At 5 days after treatment, only 0.7% of the cells were dividing and mitotic height was 68% of control.

Introduction

Bensulfuron, a sulfonylurea herbicide, is characterized as a broad-spectrum herbicide that is effective for controlling broad-leaved weeds and *Carex* spp. at very low use rates in rice fields (Takeda *et al.*, 1985). Sulfonylurea herbicides are inhibitors of acetolactate synthase, thereby blocking the biosynthesis of the branched amino acids, isoleucine, leucine and valine, which leads to rapid inhibition of plant cell division and growth (Brown, 1990). In pea plants (*Pisum sativum* L.) inhibition of plant growth occurs within 1–2 h after treatment with chlorsulfuron, another sulfonylurea herbicide, and the effect is specific to plant cell division (Ray, 1982). Chlorsulfuron rapidly blocks the cell cycle in the G_1 and/or G_2 phase but does not have a direct effect on the mitotic apparatus (Rost, 1984). Rice plants, which are known to be relatively tolerant to bensulfuron, inactivate bensulfuron rapidly while susceptible weedy plants metabolize the herbicide much more slowly, which provides for the basis of selectivity. Bensulfuron is metabolized by *japonica* rice with a half-life between 2.6 and 9 h, while for *indica* rice the half-life is 1.1–5.8 h (Takeda *et al.*, 1986). Plants susceptible to chlorsulfuron have metabolic half-lives of 50 h or more (Ray, 1982).

Phytotoxicity of bensulfuron has been reported on *japonica* varieties of rice which are usually transplanted in Far-East Asia (Yuyama *et al.*, 1986). The symptoms observed in rice plants were inhibition of root elongation and a decrease in the number of roots (Fujita & Shibayama, 1989). The risk of herbicide injury to rice is greater in water-seeded rice than dry-seeded or transplanted rice (Smith, 1988). Selectivity is more difficult to achieve under these conditions, and herbicide injury to rice plants is common. It was observed in California rice fields treated with bensulfuron that the roots of rice often grew along the soil surface and failed to penetrate the herbicide-treated soil, often enhancing 'stilting', a condition in which the crown of the rice plant is raised above the soil surface 1–3 cm or more and is supported only by the roots (Bayer & Hill, 1993). These symptoms are different from temporary root growth inhibition, in that they decrease the number of established seedlings and lead to lodging and a reduction in yield.

The objectives of this investigation were to evaluate the effect of bensulfuron on root growth of rice plants in an early growth stage, using a

water solution culture system to facilitate accurate measurements of root growth, and to study the inhibition of cell division in the rice root meristem.

Materials and methods

Growth analysis

Rice cv. 'S-201' seeds were imbibed for 2 days at 28±1°C in a growth chamber with 16 h daylight. A 2.5 cm×35 cm acrylic bar (3 mm thick) was wrapped with a sheet of glass fibre filter paper and the filter paper was saturated with distilled water. One pre-germinated seed with a 2- to 3-mm-long coleoptile was selected and placed near the top of the filter paper and held in place with a strip of tissue paper. The acrylic bar with the seed was placed in a test tube (30 cm×3 cm) and the test tube was filled with 1/4-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950) until the entire seed was slightly immersed. The test tubes were wrapped with aluminium foil to reduce algal growth. The tubes were transferred to a growth chamber with 28/23°C day/night temperature. Photoperiod was 16 h daylight with a photosynthetic photon flux density of 220 μmol m^{-2} s^{-1} at plant level. The day the pre-germinated seed was transferred to the tube was counted as 0 day for emergence. Bensulfuron, recrystallized from the commercial formulation, was applied at concentrations ranging from 2.5×10^{-6} M to 2.5×10^{-9} M on indicated dates by replacing the nutrient solution without the herbicide with nutrient solution containing the herbicide. A 2.5×10^{-5} M stock solution of the herbicide was prepared with 1/4-strength Hoagland nutrient solution and serially diluted to the indicated concentration with 1/4-strength Hoagland solution. The experiment was terminated 8 days after emergence (DAE): at this time the longest root in the control was close to the bottom of the tube.

Root growth was recorded daily by marking on the glass fibre filter with a soft lead pencil along the growing path of the roots. On the eighth day after emergence seedlings were harvested and number of roots and leaves and plant height was measured. The root growth as recorded on the filter paper was measured for each date, and daily increments were accumulated to obtain total root length. For the experiment on duration of bensulfuron effects, seedlings were transferred from the 2.5×10^{-9} M bensulfuron solution 3 DAE to 1/4-strength Hoagland's nutrient solution at 4, 5 or 6 DAE to provide seedlings that were treated with bensulfuron for 1, 2 or 3 days. Three seedlings were used for each treatment and all experiments were repeated three separate times.

Mitotic index and mitotic height

Rice seedlings were grown as previously described for growth analysis and treated with 2.5×10^{-9} M bensulfuron at 3 DAE. Seedlings were harvested daily until 5 days after the treatment (8 DAE) and approximately two root tips per seedling were excised from 8- to 12-cm-long roots. The root tips were fixed in absolute ethanol and glacial acetic acid (3:1, V/V) for approximately 24 h. The fixed root tips were washed in distilled water and hydrolysed in 1 N HCl at 55°C for 15 min. After rinsing in distilled water, the samples were stained in Schiff's reagent using the Feulgen method (Rost & Morrison, 1984). The terminal 3 mm of each stained root tip was excised, placed on a glass slide in a drop of 45% acetic acid and macerated thoroughly with a round-tipped glass rod. The slide with a cover glass was lightly pressed between two sheets of filter paper to spread cells and absorb excess moisture. To affix the dispersed cells on to the glass slide, the slide with the cover glass was placed on dry ice for several minutes until the cells were completely frozen. The cover glass was removed while the slide was still frozen. The slide was then placed into absolute ethanol and mounted with alcohol-soluble mounting medium and a new cover glass. The number of dividing cells in approximately 300 cells were counted under a microscope and the mitotic index, the number of dividing cells out of the total number of cells, was expressed as a percentage. The number of dividing cells was counted from 25 different root tips per treatment and the experiment was repeated three times.

Mitotic height was measured as the length of the meristematic region of the root tips. Root tips were fixed and stained as above. The root tips were pressed lightly between a glass slide and a cover glass. The distance from the terminal end to the furthest point of the darkly stained meristematic region of the root tip was measured under a microscope. Mitotic height was meas-

ured in more than 25 different root tips and the experiment was repeated three times.

All results were combined and subjected to analysis of variance (split-plot ANOVA) and followed by a comparison of means using the least significant difference (LSD). Because the experiments were re-randomized each time, time was used as the block for the analysis. The statements made in text are based on differences which were statistically significant using LSD at $P<0.05$. Treatment means are presented with their standard errors.

Results and discussion

Concentration response

All treatments inhibited root growth significantly from 2 days after treatment. Root growth reduction at 2.5×10^{-9} M, 2.5×10^{-8} M and 2.5×10^{-7} M concentration was similar, while the 2.5×10^{-6} M concentration reduced root growth more than other treatments at 5 DAE (Fig. 1). Two days after treatment was initiated, root growth became significantly reduced in all concentrations. Shirakura *et al.* (1988) reported that root growth of transplanted *japonica* type rice plants was inhibited by 50% by 0.41 mg L^{-1}, (approximately 10^{-6} M) bensulfuron 2 days after treatment. The herbicide-treated seedlings had a similar number of roots as the control plants except for seedlings treated with 2.5×10^{-6} M at 5 days after treatment (Table 1). This suggests that bensulfuron primarily inhibits growth of roots rather than the initiation of new roots in young rice plants. Research by Shirakura *et al.* (1988) showed that the herbicidal effect on shoots was less than on the roots because the bensulfuron was metabolized more rapidly in the leaves. They reported that approximately 60% of the absorbed bensulfuron was not metabolized in the roots, while only 30% of absorbed bensulfuron was not metabolized in the leaves, after 24 h exposure to the herbicide. This may result in an underestimation of the injury effects of bensulfuron on rice plants in field conditions. In the field, use rates approximate 2.5×10^{-9} M, at which concentration plant height, numbers of leaves and numbers of roots did not differ significantly from the values in control plants (Table 1). However, the total root length was significantly reduced by 2.5×10^{-9} M treatment (Fig. 1). Because the 2.5×10^{-9} M concentration effectively reduced root growth and approximated the concentration commonly found in water from field application made during the early cropping period, this concentration was used in all further investigations.

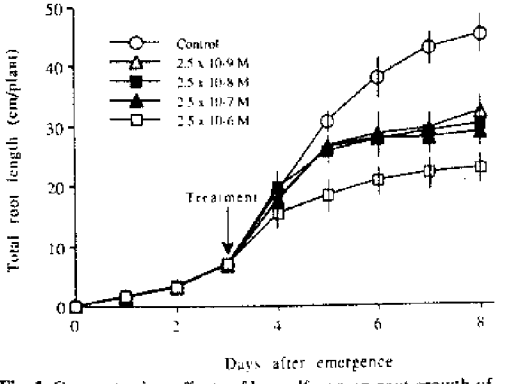

Fig. 1. Concentration effects of bensulfuron on root growth of rice seedlings. Rice seedlings were treated at 3 days after emergence (DAE) and harvested at 8 DAE (5 days of treatment). Vertical bars indicate the standard error of the means.

Timing of treatment

To evaluate herbicide effects, the timing of application is important because plants respond to a herbicide differently at different stages of growth. In the control plants, root growth of rice seedlings was relatively slow for the first 3 DAE and increased noticeably thereafter (Fig. 2). The total root length of the seedlings treated with bensulfuron at 2 DAE showed a significant reduction 3 days after treatment and did not recover by the end of this experiment. The seedlings treated at 3 or 4 DAE showed a reduction in total root length by the second day after treatment. Around 3 DAE, the second leaf was emerging in this system, which is close to the stage that bensulfuron is usually applied in direct

Table 1. Concentration effects of bensulfuron on plant height and leaf and root number of rice seedlings*

Concentration (M)	Plant height† (cm)	Number†	
		Leaves	Roots
0	15.0 (2.0)	3.8 (0.5)	11.2 (2.0)
2.5×10^{-9}	14.4 (1.8)	3.8 (0.5)	10.3 (1.0)
2.5×10^{-8}	12.5 (1.6)	3.8 (0.5)	10.3 (1.0)
2.5×10^{-7}	11.3 (1.9)	3.0 (0.5)	10.7 (1.0)
2.5×10^{-6}	7.0 (2.1)	3.0 (0.5)	6.7 (2.0)

*Rice seedlings were treated 3 days after emergence and seedlings were harvested 5 days after treatment.
†Standard error of the mean indicated in parenthesis.

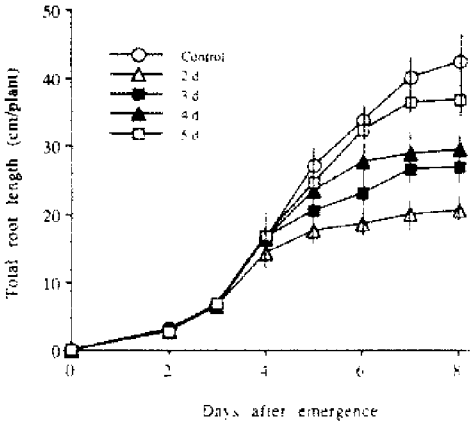

Fig. 2. Effects of 2.5×10^{-9} M bensulfuron on various days (d) after emergence. Rice seedlings were treated with bensulfuron at each day after emergence and grown in bensulfuron solution until harvested. Vertical bars indicate the standard error of the means.

seeded rice. Treatment at 5 DAE did not show a significant response by the termination of the experiment because there was insufficient time to detect a growth response. Roots were affected more by bensulfuron than were shoots (Table 2; Fig. 2). Shirakura *et al.* (1988) reported that rice seedlings at approximately the three-leaf stage whose roots had been cut 1 cm below the crown and treated with 0.41 mg L^{-1} [^{14}C]bensulfuron in a nutrient culture system metabolized bensulfuron more rapidly in the shoots than in the roots. Taketomi *et al.* (1989), using a similar growing technique with rice seedlings approximately the same size, showed that acetolactate synthase extracted from rice shoots was only inhibited 50% when treated with 5×10^{-7} M bensulfuron. These results suggest that sensitivity to bensulfuron is quite different in the roots and shoots of young rice plants. The different metabolic activity between shoots and roots of rice plants can explain the difference in sensitivity to bensulfuron of various plant tissues. In this experiment only the treatment at 2 DAE resulted in a significant reduction in plant height. The number of leaves and roots was not reduced by the herbicide treatments (Table 2). The inhibition of total root length was a function of reduced root length rather than root numbers when treatments were applied at 3 and 4 DAE. The longer treatment caused more root growth inhibition (Fig. 2).

Table 2. Plant height, number of leaves and roots of rice seedlings 8 days after emergence*

Treatment DAE	Plant height† (cm)	Number†	
		Leaves	Roots
0	15.2 (1.3)	3.8 (0.5)	11.0 (1.0)
2	12.6 (1.0)	3.2 (0.7)	9.8 (1.0)
3	14.4 (2.0)	3.6 (0.7)	10.5 (1.5)
4	14.9 (0.9)	3.6 (0.5)	10.7 (1.0)
5	15.2 (0.9)	3.8 (0.5)	10.8 (1.0)

*Bensulfuron (2.5×10^{-9} M) treatment was initiated 2, 3, 4, and 5 days after emergence of rice seedlings.
†Standard error of the mean is indicated in parenthesis.
DAE, days after emergence.

Duration of treatment

Indica rice can metabolize bensulfuron relatively rapidly, with a half-life of 1.1–5.8 h (Takeda *et al.*, 1986). These data suggest that the duration of the herbicide treatment can be critical for phytotoxicity to rice plants. Rice seedlings were treated 3 DAE with 2.5×10^{-9} M bensulfuron and seedlings were transferred to nutrient solution without the herbicide 1, 2 or 3 days after treatment (Fig. 3). Treatment for 1 day reduced root growth at 2 days after treatment, but total root length recovered from 3 days after treatment. The reduction in total root length was not significant 1 day after treatment in either the concentration study (Fig. 1) or the timing study (Fig. 2). Removing the rice seedlings from the bensulfuron solution 2 or 3 days after treatment or continuing treatment reduced root growth and the inhibition was proportional to the duration of treatment. A similar experiment in which rice seedlings were treated with bensulfuron on the fourth day after emergence and removed from the treatment so-

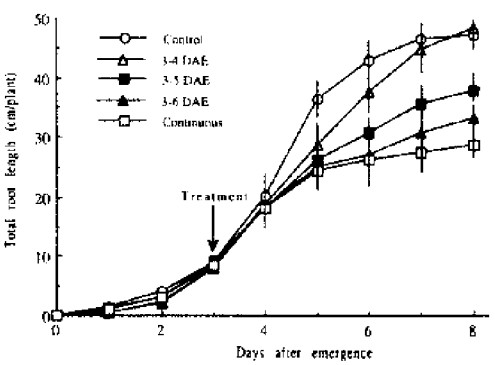

Fig. 3. Effects of duration of bensulfuron (2.5×10^{-9} M) treatment on rice seedlings. The herbicide treatment was initiated at 3 days after emergence (DAE) and rice seedlings were transferred to nutrient solution without herbicide at each indicated date. Vertical bars indicate the standard error of means.

Table 3. Change in mitotic index of rice seedlings treated 3 days after emergence with bensulfuron (2.5×10^{-9} M)*

Treatment (M)	Change in mitotic index (%)†					
	3 DAE†	4 DAE	5 DAE	6 DAE	7 DAE	8 DAE
0	2.9 (0.4)	3.5 (0.3)	3.3 (0.3)	3.0 (0.4)	2.7 (0.5)	2.2 (0.4)
2.5×10^{-9}	2.9 (0.3)	3.2 (0.3)	2.2 (0.4)	1.8 (0.5)	1.1 (0.6)	0.7 (0.3)

*Root tips of bensulfuron-treated seedlings were harvested daily and mitotic indices were compared with those in control plants at each date as indicated.
†Standard error of the mean is indicated in the parenthesis.
DAE, days after emergence.

Table 4. Changes in mitotic height (length of meristematic region) of rice seedlings treated 3 days after emergence with bensulfuron (2.5×10^{-9} M)*

Treatment (M)	Change in mitotic height (mm)†					
	3 DAE†	4 DAE	5 DAE	6 DAE	7 DAE	8 DAE
0	1.7 (0.3)	1.9 (0.4)	1.9 (0.3)	1.8 (0.3)	1.7 (0.2)	1.7 (0.3)
2.5×10^{-9}	1.7 (0.3)	1.5 (0.3)	1.3 (0.2)	1.2 (0.3)	1.2 (0.3)	1.2 (0.2)

* Root tips of bensulfuron-treated rice seedlings were harvested daily after treatment.
†Standard error of the mean is indicated in the parenthesis.
DAE, days after emergence.

lution 1, 2 or 3 days after initial exposure showed similar patterns as Fig. 3 (data not shown).

Mitotic index and mitotic height

Sulfonylurea herbicides are known to inhibit acetolactate synthase, the key enzyme of the branched amino acid synthesis pathway, which results in a cessation of the cell cycle. Pea roots treated with chlorsulfuron, another sulfonylurea herbicide, are blocked in the G_2 and G_1 transition points of the cell cycle (Rost, 1984). In this study 2.5×10^{-9} M bensulfuron caused a significant reduction in total root length, but the numbers of roots were not affected (Fig. 1, Table 1). Bensulfuron caused a significant reduction in the mitotic index in the meristem of rice roots 5 DAE (2 days after treatment) (Table 3). The number of dividing cells was significantly reduced by bensulfuron and the reduction pattern correlated with the pattern of root growth inhibition shown in Fig. 1. Only 0.7% of the cells were dividing 5 days after treatment. In comparison, chlorsulfuron (2.8×10^{-9} M) significantly reduced mitotic index 8 h after treatment in pea roots (Rost, 1984). Root growth in relatively tolerant rice plants was not significantly inhibited by bensulfuron until 2 days after treatment.

Bensulfuron also caused a 32% reduction in mitotic height (Table 4). A large meristematic area and high meristematic activity usually result in increased meristematic height (Barlow & Adam, 1989). In root tips, cells outside the meristematic region participate in the growth cycle through differentiation and enlargement (Rost, 1984). Cells in the growth cycle stain lightly with Schiff's reagent because of their enlarged vacuoles. If the mitotic progression in the meristem is inhibited the cells become differentiated and vacuolized prematurely (Evans, 1965). These data suggest that bensulfuron inhibited cell cycling in the meristem and caused premature maturation of meristematic cells by differentiation and vacuolization, which led to a reduction in the mitotic height in the rice roots. However, the deceased meristematic height caused by the bensulfuron treatment was probably the result of inhibition of mitotic cell cycling because chlorosulfuron, another sulfonylurea herbicide, does not inhibit other important physiological processes such as respiration, photosynthesis, protein synthesis or hormone-induced cell enlargement (Ray, 1982).

Bensulfuron inhibited root growth of rice seedlings by reducing the number of dividing cells in the meristematic area. Rice roots were more sensitive to growth inhibition by bensulfuron than were shoots, as measured by root length, cell division and mitotic cycle. This may be because of differential metabolism of bensulfuron in rice roots and shoots; a point which remains to be investigated.

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