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Basis for Differential Susceptibility of Rice (*Oryza sativa*), Wild Rice (*Zizania palustris*), and Giant Burreed (*Sparganium eurycarpum*) to Bentazon[1]

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Abstract. The basis for differential susceptibility of tolerant rice (*Oryza sativa* L.), susceptible wild rice (*Zizania palustris* L.), and susceptible giant burreed (*Sparganium eurycarpum* Engelm. #[3] SPGEU) to foliar application of 1.1 kg ai/ha of bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] was investigated by evaluating herbicide absorption, translocation, and metabolism. Giant burreed and wild rice absorbed more bentazon than rice at similar growth stages. Less than 10% of the absorbed bentazon was translocated out of the treated leaf of any of the species. Differential tolerance of bentazon among the three species was due to differences in the rate of bentazon metabolism. Rice metabolized 98% of the bentazon retained in the treated leaf 1 day after treatment (DAT), while giant burreed and wild rice metabolized less than 2% of the bentazon retained in the treated leaf 5 DAT.

Additional index words. Absorption, translocation, metabolism, SPGEU.

INTRODUCTION

Wild rice is an important specialty grain grown on 10 000 ha in northern Minnesota, 6400 ha in northern California, and limited areas of Wisconsin, northern Idaho, and southern Manitoba, Canada. Wild rice is grown in an aquatic environment similar to that of rice and many of the weed problems in wild rice are similar to those encountered in rice production. Obtaining registration for use of a herbicide in wild rice is difficult because of the limited acreage grown. Herbicides registered for use on rice, including propanil [*N*-(3,4-dichlorophenyl)propanamide], bentazon, 2,4-D [(2,4-dichlorophenoxy)acetic acid], and MCPA [(4-chloro-2-methylphenoxy)acetic acid], have been tested on wild rice (10)[4,5]. The U.S. Environmental Protection Agency (EPA) allows use of registered rice herbicides in wild rice if the application rate on wild rice does not exceed registered rates for rice. Registered rice herbicides, however, must be tested for wild rice tolerance and application rates and timing revised to obtain maximum weed control.

Giant burreed is a perennial aquatic monocot that infests 90% of the wild rice fields in Minnesota. Giant burreed interferes with penetration of photosynthetically active radiation through the wild rice canopy. Wild rice yield and panicle number can be reduced by 60% when giant burreed shoot density is 40/m^2 (3).

Bentazon applied at 2.2 kg/ha, the highest registered rate for rice, has controlled giant burreed[6]. However, application of 1.2 and 2.2 kg/ha of bentazon to wild rice at the two-aerial-leaf (four-leaf) growth stage resulted in 60 and 70% crop injury, respectively, 4 weeks after application[6]. Because of unacceptable crop injury, use of bentazon in wild rice is feasible only as a spot treatment for areas heavily infested with giant burreed.

Differential spray retention (4, 5), chemical absorption and translocation of the herbicide within the plant (11), and herbicide metabolism (1, 2, 6, 7, 8, 9) are factors that contribute to differential responses to foliar herbicide applications. Rapid metabolism of bentazon has been cited as a major cause for tolerance for several species including rice (7), hot pepper (*Capsicum chinense* L.) (1), and soybean [*Glycine max* (L.) Merr.] (2, 9), while slower rates of metabolism have been observed for susceptible species including sweet pepper (*Capsicum annuum* L.) (1) and Canada thistle [*Cirsium arvense* (L.) Scop. # CIRAR] (9). Mine and Matsunaka (7) and Mine et al. (8) found that rice metabolized 85% of the applied bentazon to a nonphytotoxic *O*-glucoside within 24 h.

The objective of this study was to determine the mechanism(s) responsible for the differential susceptibility of rice, wild rice, and giant burreed to bentazon.

MATERIALS AND METHODS

Cultural practices. Giant burreed corms were collected from a field in Aitkin County, MN, in September 1985 and were stored in mesh bags at 2 C until planting. Two months of cold storage were required before dormancy ceased. Wild rice (cultivar 'K2') was harvested from the same area and stored in water for at least 3 months at 2 C to release dormancy. Rice (cultivar 'M2'), a medium grain rice, was used as a bentazon-tolerant species. Plants were grown in the glasshouse at 21 ± 5 C with 16 h of supplemental fluorescent light with a photosynthetic photon flux of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Ten-cm-diameter pots were filled to within 2 cm of the top with a greenhouse soil consisting of soil

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[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

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(Waukegan silt loam; Typic Hapludoll), sand, peat, and manure (6:6:5:2, v/v/v/v). The 10-cm-diameter pots were placed in plastic-lined 30-cm-diameter pots and these larger pots were filled with enough water to cover the smaller pots by 4 cm. Planting dates were staggered at 1-week intervals in order to treat plants at the desired growth stage on the same day.

All treatments were replicated three times in a completely random design. The absorption and translocation and metabolism studies were conducted twice. All data were combined and subjected to regression analysis or analysis of variance. Treatment means were compared using Fisher's Protected Least Significant Difference (LSD) Test at the 5% level of significance.

Absorption and translocation. The sodium salt of ^{14}C-bentazon [(U-phenyl ^{14}C) bentazon, specific activity 45.5 mCi/mmol, purity >99%] was mixed with water and technical grade sodium salt of bentazon (purity >98%) to form a solution equal to the concentration of bentazon applied at 1.1 kg/ha in a spray volume of 185 L/ha. Wild rice, rice, and giant burreed plants were treated with technical grade sodium salt of bentazon applied with a stationary sprayer at the rate of 1.1 kg/ha in a volume of 185 L/ha and with a pressure of 206 kPa. Eighteen 0.3-μl drops (0.3 μCi) of ^{14}C-bentazon solution were applied to the youngest fully expanded (third-aerial) leaf of wild rice, and the third leaf of giant burreed and rice. Octonoxyl [α-(p-1,1,3,3-tetramethyl butyl phenyl)-ω-hydroxypoly (oxyethylene)] was added (0.01%, v/v) to the ^{14}C-bentazon solution to increase surface wetting of the treated leaves. Rice and giant burreed were treated at the three-leaf stage and wild rice was treated at the five-leaf (three-aerial-leaf) stage. Rice and giant burreed do not have submersed leaves, while wild rice has one or two leaves that stay submersed, a floating leaf, and finally true aerial leaves. The submersed leaves and floating leaf of wild rice had senesced or were removed at the time of treatment. Glass slide controls were treated at the time of the plant application to make sure that bentazon did not volatilize or degrade under the lights in the growth chamber.

Treated plants and glass slides were placed in a growth chamber having a 16-h day, a day/night temperature regime of 20/15 C, and a relative humidity of 84%. Light was provided by fluorescent and incandescent bulbs with an intensity of 950 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR. Plants were harvested 1, 2, and 5 days after treatment (DAT). At harvest, rice and wild rice were sectioned into: 1) treated leaf, 2) shoot above treated leaf, 3) leaves below the treated leaf, 4) stem section from treated leaf to next oldest leaf, 5) remainder of the stem, and 6) roots. Giant burreed has linear leaves that have sheaths at the base; therefore, giant burreed was sectioned into: 1) treated leaf, 2) leaves younger than the treated leaf, 3) leaves older than the treated leaf, 4) base of plant below soil level, 5) roots, and 6) planted corm.

The treated leaf of each species was placed in a test tube and agitated with a vortex mixer for 30 s, once with 10 ml of 10% v/v ethanol plus 0.01% octonoxyl (v/v), and twice with 7.5 ml of 95% ethanol. The 95% ethanol solutions were combined. A 1-ml aliquot from the 10% ethanol leaf wash was frozen and later chromatographed to determine if metabolites were present on the leaf surface. The remaining leaf wash solutions from 95 and 10% wash were evaporated at 30 C under a stream of air to approximately 1 ml. Glass slide controls were washed and treated in a similar manner. ^{14}C in the leaf washes was quantified using liquid scintillation spectrometry, and counts were combined.

Plant sections were frozen and lyophilized. Sections were weighed and samples greater than 250 mg were ground to pass through a 50-mesh screen. Entire plant sections less than 250 mg or a 150-mg subsample of the ground tissue were used for further analysis. The plant samples (except treated leaves that were used for the metabolism study) were combusted in a sample oxidizer; $^{14}CO_2$ was trapped with 7 ml of carbo-sorb[7], and then 14 ml of permafluor[7], a scintillation solution, was added. The ^{14}C was quantified using liquid scintillation spectrometry. These data were used to determine the percent of absorbed ^{14}C translocated out of the treated leaf, and individually as a measure of specific activity (dpm/mg dry weight) of each plant part.

Metabolism. The treated leaf from each plant was extracted for metabolism studies by grinding with a tissue homogenizer for 45 s with 5 ml of cold (2 C), absolute methanol (5) in a 15-ml centrifuge tube. The sample extract and two 2.5-ml methanol washes of the tissue homogenizer were combined and centrifuged at 8000 g for 10 min. The supernatant was removed from the pellet and brought up to a volume of 10 ml. The pellet was removed from the centrifuge tube, dried, and oxidized as described above to determine remaining ^{14}C. A 1-ml aliquot of the supernatant was used for ^{14}C quantification via liquid scintillation as described above. Thin-layer chromatography (TLC) plates[8] were spotted with 200 μl (containing approximately 5000 dpm) of the supernatant in 10, 20-μl aliquots. The plates were developed in a solvent system of ethyl acetate:glacial acetic acid:water (8:1:1, v/v/v) to a height of 15 cm. The relative amount of bentazon and metabolites in the extract was determined on a TLC linear analyzer[9]. Technical grade bentazon (>98% purity) and extract from ^{14}C-bentazon-treated soybean[10] were cochromatographed with the supernatant from the treated leaf. Radioactivity migrating to the same height as bentazon was assumed to be bentazon. All other peaks were assumed to be metabolites and not identified further.

RESULTS AND DISCUSSION

Absorption and translocation. Average recovery of ^{14}C, expressed as a percentage of the total applied, ranged from

[7] Packard Instrument Co., Inc., Downers Grove, IL.

[8] Baker Si.250 F-PA (19C) TLC plates. J. T. Baker Chem. Co., Phillipsburg, NJ.

[9] LB 2832 Analyzer. Berthold Instruments, Pittsburg, PA.

[10] Obtained from M. D. Johnson, Former Grad. Res. Asst., Univ. Minnesota.

85% for giant burreed and wild rice to 96% for rice and glass slide controls. Generally, increased amounts of ^{14}C absorbed or translocated, or both, decreased the amount of radioactivity recovered in the plants. More than 80% of the ^{14}C recovered from the leaf surface was in the first (10% ethanol) leaf wash.

The amount of ^{14}C absorbed by rice, wild rice, and giant burreed 1 DAT was 21, 50, and 64% of the total ^{14}C applied, respectively (Figure 1). No additional absorption of ^{14}C-bentazon occurred during the 5-day period in giant burreed and wild rice. Absorption of bentazon in rice increased during the same 5-day period. However, the amount of ^{14}C absorbed by rice at the last harvest date was significantly less than the amount absorbed by wild rice or giant burreed. Absorption of bentazon may be a factor in tolerance or susceptibility of the three species to bentazon.

The total amount of ^{14}C translocated from the treated leaf was less than 7% of the amount absorbed by each species 1, 2, and 5 DAT (Tables 1 and 2). Differences among species in the amount of ^{14}C translocated out of the treated leaf over time were not detected. Distribution of ^{14}C material among plant parts was similar among species (Tables 1 and 2). Specific activity of giant burreed was lower than that of rice and wild rice because of greater plant dry weight rather than less total bentazon absorption. Harvest date by plant part interaction was not observed for plant part accumulation of ^{14}C. Since translocated amounts and accumulation of ^{14}C were similar in the three species, it is unlikely that translocation had an impact on the species tolerance or susceptibility to bentazon.

Metabolism. Metabolism of bentazon has been shown to be an important factor in tolerance of bentazon in several species (1, 2, 8, 9). Metabolism of bentazon was different among rice and wild rice and giant burreed (Figure 2). Bentazon metabolites were not found on the leaf surface of any of the studied species, indicating that metabolism occurred within the leaf. The ^{14}C-bentazon standard had an R_f value of 0.96. Leaf extracts from wild rice and giant burreed treated leaves had 98% of the ^{14}C cochromatographing with ^{14}C-bentazon at all harvest dates (Table 3). Extracts from the treated rice leaf had virtually no radioactivity cochromatographing with ^{14}C-bentazon. Peak radioactivity for rice extracts was observed at $R_f = 0.43$ for all three harvest dates. Soybean leaf extracts from plants treated with 0.71 μCi of ^{14}C-bentazon[11] that were cochromatographed with rice extracts indicated that the metabolite in rice corresponded to a soybean metabolite. Mine et al. (8) reported that 85% of the applied bentazon was metabolized by rice (cultivar Nihonbare) 24 h after treatment. The major rice metabolite was identified as 6-(3-isopropyl-2,1,3-benzothiadiazin-4-one-2,2-dioxide)-*O*-B-glucopyranoside. In the study by Mine et al. (8), bentazon and the major metabolite had R_f values of 0.59 and 0.24, respectively, when chromatographed with $CHCl_3$:methanol (7:3, v/v) solution. Therefore, further studies would need to be conducted to determine if the metabolite described in this study is the same as the metabolite described by Mine et al. (8).

Table 1. Specific activity of plant parts of rice and wild rice, after application of 0.3 μCi of ^{14}C-bentazon to the third leaf of rice and the third aerial leaf of wild rice[a].

Plant part	Plant species	
	Rice	Wild rice
	(dpm/mg dry weight)	
Treated leaf	15 580	16 204
Stem portion[b]	687	485
Shoot above treated leaf	166	185
Stem portion[c]	166	171
Leaves below treated leaf	84	39
Roots	27	6
LSD (0.05)	1 442	1 539

[a]Means of each plant part averaged over the 1-, 2-, and 5-day harvest dates.

[b]Stem portion from the treated leaf to the next oldest leaf.

[c]Stem portion from leaf below treated leaf to base of plant.

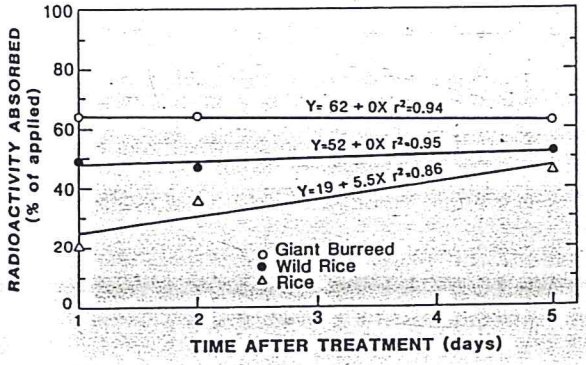

Figure 1. Absorption of ^{14}C by giant burreed, wild rice, and rice 1, 2, and 5 days after application of 0.3 μCi ^{14}C-bentazon. Data points represent the mean of six replications.

Table 2. Specific activity of plant parts of giant burreed after application of 0.3 μCi of ^{14}C-bentazon to the third fully developed leaf[a].

Plant part	Specific activity
	(dpm/mg dry weight)
Treated leaf	3050
Leaves younger than treated leaf	73
Base of plant below soil	58
Leaves older than treated leaf	15
Roots	12
Planted corm	T[b]
LSD (0.05)	345

[a]Means of each plant part averaged over the 1-, 2-, and 5-day harvest dates.

[b]Trace of radioactivity.

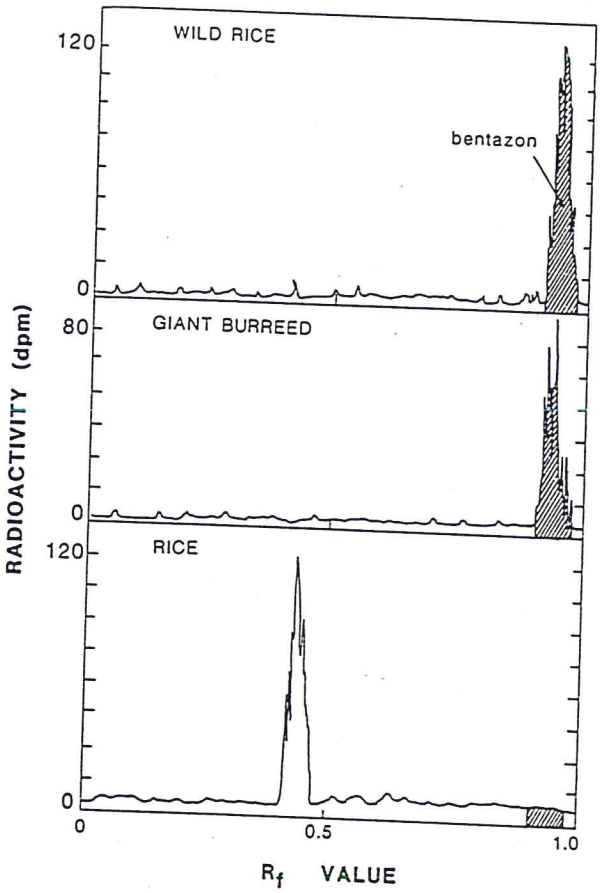

Figure 2. Graph of TLC plate showing the radioactive compounds extracted from treated leaves of wild rice and giant burreed 5 days after treatment, and rice 1 day after treatment with 0.3 μCi of ^{14}C-bentazon. The bentazon-treated leaves were extracted with cold, absolute methanol and chromatographed on a silica gel thin-layer plate, and radioactivity was detected with a TLC linear analyzer.

Apparently the inability of wild rice to metabolize bentazon is the mechanism that causes wild rice to be susceptible to bentazon injury. Soybean cultivars that were susceptible to bentazon could not metabolize bentazon (2). Bernard and Wax (2) reported that the inability of soybean to metabolize bentazon was controlled by one recessive gene, which they designated as hb. At present, screening of wild rice for bentazon-tolerant plants and backcrossing the ability to metabolize bentazon into desirable cultivars may be the most promising course of action. In the future, techniques to transfer genetic material from one species to another may also be used to transfer the dominant (Hb) gene from soybean or rice to wild rice.

Table 3. Metabolism of ^{14}C-bentazon in giant burreed, wild rice, and rice after application of 0.3 μCi ^{14}C-bentazon[a].

Species	Bentazon metabolized
	(% of absorbed)
Giant burreed	2
Wild rice	2
Rice	98
LSD (0.05)	2

[a]Means for each species averaged over the 1-, 2-, and 5-day harvest dates.

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