INHIBITION OF RICE WEED GERMINATION AND SEEDLING GROWTH WITH METABOLITES FROM *PSEUDOMONAS SYRINGAE*-STRAIN 3366

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Summary Natural herbicidal products are present in the culture broth from aerobic shake cultures of the rhizobacterium *Pseudomonas syringae* strain 3366. Inhibitory activity of these metabolites against Katy, a cultivated rice cultivar (*Oryza sativa*), and several weeds common in rice-soybean rotations, including red rice (*Oryza sativa*), barnyard grass (*Echinochloa crus-galli*), pitted morning glory (*Ipomoea lacunosa*), bearded sprangletop (*Leptochloa fasicularis*), and hemp sesbania (*Sesbania exaltata*), was evaluated in seed germination bioassays in agar. Ethyl acetate extracts from aerobic shake cultures of strain 3366 inhibited root growth (radicle elongation) strongly in all species tested. Concentrations that inhibited root growth by 50% (I_{50}) were less than 10 mg L^{-1} for red rice and barnyard grass. Root growth always was inhibited at lower concentrations than was shoot growth or germination. Root growth was most sensitive in Katy, red rice, barnyard grass and bearded sprangletop which were inhibited at least 67% at 40 mg L^{-1} of strain 3366 extract. Root growth was least sensitive in hemp sesbania and pitted morning glory which were inhibited 67% or less at 400 mg L^{-1}. Surprisingly, shoot growth was most sensitive in these two species and was more than 35% inhibited at 40 mg L^{-1}. Shoot growth in all the other species was not inhibited at concentrations less than 400 mg L^{-1}. Germination was unaffected by strain 3366 extracts in all species except for bearded sprangletop, barnyard grass, and red rice which were inhibited more than 28% at 400 mg L^{-1}. Overall, bearded sprangletop appeared to be the species most sensitive to strain 3366 extracts. Although strain 3366 extracts appeared to inhibit Katy somewhat less than most of the weed species, the margin of selectivity was small and not adequate for effective weed control in rice. Active metabolites from strain 3366 were identified previously as phenazine-1-carboxylic acid, 2-amino phenoxazone, and 2-amino phenol.

INTRODUCTION

In recent years, research has been conducted to develop alternative methods of weed control in rice and in many other agronomic crops. Such efforts have been, in part, driven by increased incidences of herbicide-resistant weeds (Carey *et al.* 1995) and a perception among the public that traditional chemically-based weed control systems are environmentally unsound and too expensive. Drill-seeded rice fields in the southern United States often are heavily infested by barnyard grass (*Echinochloa crus-galli*) which has been controlled effectively in rice with propanil and other herbicides (Smith and Khodayari 1985). The recent discovery of propanil-resistant barnyard grass populations in Arkansas rice fields (Carey *et al.* 1995) is a serious reminder of the real need for additional or alternative weed management systems in rice. Red rice (*Oryza sativa*) is an extremely serious weed problem in drill-seeded rice which currently can not be controlled in the rice crop (Kwon *et al.* 1991).

Biologically-based weed control in rice has been investigated with some success. The most well known of these has been the use of inundative inoculations of the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* (COLLEGO™) for control of northern jointvetch (*Aeschynomene virginica*) (Templeton *et al.* 1986). Other biologically-based weed control efforts for weeds of rice also are underway (Boyette *et al.* 1993).

Rhizobacteria and their metabolites have been evaluated as weed control agents in non-rice systems (Kennedy *et al.* 1991, Mazzola *et al.* 1995, Gealy *et al.* 1996a, Norman *et al.* 1994, Tranel *et al.* 1993). Live cultures of *Pseudomonas syringae* strain 3366 (strain 3366) have sometimes reduced weed root growth in controlled-environments (Johnson *et al.* 1993) and in field studies (Kennedy *et al.* 1991). Ethyl acetate extracts from strain 3366 have dramatically reduced weed root and shoot growth under field conditions in the Pacific Northwest (Gealy *et al.* 1996a). In these studies, root growth was inhibited substantially more than shoot growth, downy brome (*Bromus tectorum*) was inhibited substantially more than winter wheat, and inhibitory activity was greater in a pH 5.8, 3.6% organic matter-silt loam than in a pH 9.0, 0.8% organic matter-silt loam. Phenazine-1-carboxylic acid, the major identifiable metabolite (20 % by weight) present in ethyl acetate extracts of strain 3366, inhibited downy brome root growth by 99% at concentrations of 5.7 mg L^{-1} (Gealy *et al.* 1996a). Strain 3366 extracts also have controlled corn spurry (*Spergula arvensis* L.) and fireweed (*Epilobium angustifolium* L.) selectively in non-rooted cranberry (*Vaccinium macrocarpon* Ait.) cuttings at concentrations of 10 ppm in sand culture (Norman *et al.* 1994).

The objectives of this research were to determine the levels of ethyl acetate extract derived from *Pseudomonas syringae* strain 3366 culture broth that inhibit the growth of roots and shoots of germinating seeds and seedlings of Katy, a cultivated rice cultivar and several weeds common in rice-soybean rotations, including red rice, barnyard grass, pitted morning glory, bearded sprangletop, and hemp sesbania, and to determine the degree of selectivity between rice and these weed species.

MATERIALS AND METHODS

Originally isolated from the rhizosphere of wheat (Kennedy *et al.* 1991), strain 3366 bacteria were grown on a shaker at 24–26°C for about 48 h on semi-synthetic King's B media (Gealy *et al.* 1996a) (Figure 1). Phytotoxin extraction was performed as described previously (Gealy *et al.* 1996a). Briefly, equal volumes of ethyl acetate and shake-culture broth were combined and mixed overnight to extract the active compounds from the aqueous phase. The organic phase was separated from the aqueous phase and concentrated in a rotoevaporator. The ethyl acetate extract was resuspended in a small volume of acetone, placed in a glass vial, and evaporated to dryness under hood. Total weight of the extracted phytotoxins was determined by weighing the glass vial with and without the extracts. A phytotoxin stock solution of 10 mg mL^{-1} was prepared in acetone. Phytotoxin solutions of 0, 10, 20, 40 and 400 mg L^{-1} were prepared in acetone by serial dilution of the stock solution. Inhibition of seed germination and growth was determined in an agar assay (Gealy *et al.* 1996a). In the assay, 0.8 mL of the various phytotoxin solutions was spread evenly on the surface of 20 mL of solidified 0.8% agar in the appropriate dish. Dishes prepared similarly, and treated only with acetone, were included as non-treated controls. Petri dishes were placed under hood for approximately two hours to evaporate the acetone completely. Twenty-five seeds of Katy rice, red rice, barnyard grass, pitted morning glory and hemp sesbania, and 50 seeds of bearded sprangletop were placed on the surface of the agar in the petri plates and plates were sealed with parafilm. The plates were incubated for eight days in a controlled-environment chamber at 25°C with a photoperiod of 14 hours. Only Katy rice, red rice and barnyard grass were tested at all five concentrations of phytotoxin. At the end of the incubation period, germination was determined and seedlings were extracted from the agar. Root and shoot lengths were measured to the nearest mm with a caliper. The experiment was conducted as a randomized complete block with four replications, and was repeated. Data for Katy rice, red rice and barnyard grass were transformed with Log 10 of extract concentration and inverse normal of % of control values to linearize dose response curves. Regression analyses were conducted on transformed data to compare the species statistically.

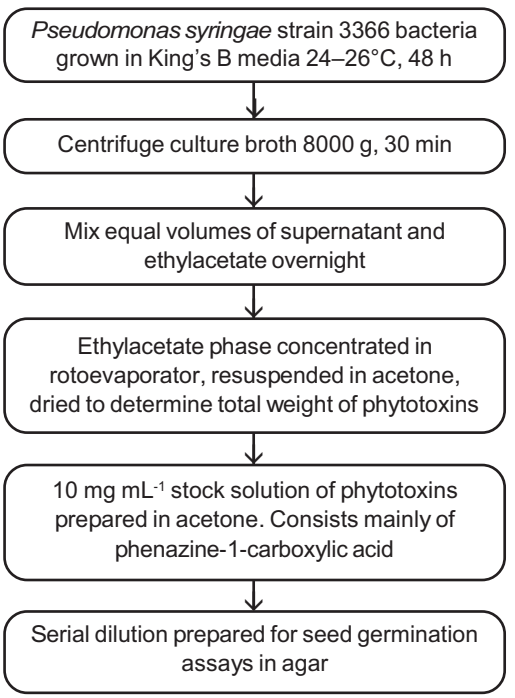

Figure 1. Production and extraction of *Pseudomonas syringae* strain 3366 phytotoxins.

RESULTS AND DISCUSSION

Most species tested were highly sensitive to strain 3366 toxin extracts in agar germination assays. I_{50} concentrations for root length were below 10 mg L^{-1} for red rice and barnyard grass, and were near 10 mg L^{-1} for Katy rice (Table 1). Bearded sprangletop appeared to be similarly sensitive, but was not tested at concentrations below 40 mg L^{-1}. Roots of hemp sesbania and pitted morning glory were less sensitive than the other species and were inhibited 67% or less at 400 mg L^{-1} with apparent I_{50} concentrations of greater than 40 mg L^{-1}. Regression analysis indicated that root growth dose-responses of Katy rice, red rice and barnyard grass all differed from one another (i.e. intercepts and/or slopes differ).

Strain 3366 extracts inhibited shoot growth less strongly than root growth (Table 2). Generally, I_{50} concentrations for shoots were greater than 400 mg L^{-1}, with the exception of that for pitted morning glory which was near 40 mg L^{-1}. Interestingly, the two broadleaved weeds, hemp sesbania and pitted morning glory, were the species with the most sensitive shoots and the least sensitive roots. Regression analysis indicated that shoot growth

dose-responses of red rice differed from those of Katy rice and barnyard grass.

Germination, as defined by radicle protrusion, was the least sensitive plant process evaluated. That is, seeds tended to germinate at relatively high concentrations of strain 3366 extract, but produced very short radicles. Germination was unaffected in all species except for barnyard grass, red rice, and bearded sprangletop which were inhibited 29, 32 and 82%, respectively at 400 mg L^{-1} (detailed data not shown). Regression analysis indicated that germination dose-responses of Katy rice, red rice and barnyard grass all differed from one another (i.e. intercepts differ) (data not shown).

The relatively high, moderate, and low activities against root growth, shoot growth and germination, respectively, were similar to those reported in earlier studies with strain 3366 (Gealy *et al.* 1996a) and a related rhizobacterium (Gealy *et al.* 1996b). These results suggest that the weed control niche for strain 3366 could be as a soil-applied root inhibitor. However, because there was little or no selectivity between the Katy rice and the weeds included in the present study, the future commercialization of strain 3366 in rice is unlikely. Presently, we are evaluating the response of rice and rice weeds to more than 20 additional microbial extracts that previously have proven to be highly active against downy

Table 1. Effect of ethyl acetate extracts from *Pseudomonas syringae*-strain 3366 on root length of germinating rice and weed species in an agar assay[A].

Species	Extract concentration (mg L^{-1})					Transformed regression values	
	10	20	40	400	Control value	Intercept (b_0)	Slope (b_1)
	% of control				mm seedling^{-1}		
Katy rice	55	39	33	15	33.0	0.52 b	-0.59 b
Red rice	34	33	26	11	33.6	0.27 c	-0.59 b
Barnyard grass	43	30	21	2	18.2	0.88 a	-1.09 a
Hemp sesbania	–	–	57	33	26.6	–	–
Pitted morning glory	–	–	63	51	15.6	–	–
Bearded sprangletop	–	–	24	1	6.7	–	–
Overall LSD (P=0.05)			11				

[A] Per cent of control mean values were separated with a protected LSD which applies across all species and concentrations. Actual control values were included for species size comparisons. Regression analysis for % of control vs. extract concentration was conducted for Katy rice, red rice and barnyard grass on data transformed as follows: Log 10 (extract concentration) and inverse normal of (% of control). Within columns, values followed by the different letters indicate a difference between regression lines at the 0.05 level. Dashes indicate that the concentration was not included and that regression analysis was not conducted for these species. Information in this footnote also applies to Table 2.

Table 2. Effect of ethyl acetate extracts from *Pseudomonas syringae*-strain 3366 on shoot length of germinating rice and weed species in an agar assay[A].

Species	Extract concentration (mg L^{-1})					Transformed regression values	
	10	20	40	400	Control value	Intercept (b_0)	Slope (b_1)
	% of control				mm seedling^{-1}		
Katy rice	91	78	87	77	18.7	1.36 b	-0.21 b
Red rice	109	108	90	62	24.2	2.45 a	-0.90 a
Barnyard grass	99	95	96	77	34.3	1.36 b	-0.21 b
Hemp sesbania	–	–	63	56	23.1	–	–
Pitted morning glory	–	–	47	45	27.1	–	–
Bearded sprangletop	–	–	94	74	4.9	–	–
Overall LSD (P=0.05)			22				

[A] Refer to Table 1.

brome. The emphasis of these studies will be to determine the acute toxicity and selectivity of these extracts to roots and foliage.

ACKNOWLEDGMENTS

Authors thank Ann Kennedy for providing the original strain 3366 cultures, Delvar Peterson for assistance with statistical design and analysis, and Dawn Novak and Howard Black for their technical support on this project.

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