Weed Science Society of America

Metabolites from Pseudomonas fluorescens Strain D7 Inhibit Downy Brome (Bromus tectorum) Seedling Growth

Author(s): David R. Gealy, S. Gurusiddaiah, Alex G. Ogg, Jr. and Ann C. Kennedy

Reviewed work(s):
Source: *Weed Technology*, Vol. 10, No. 2 (Apr. - Jun., 1996), pp. 282-287
Published by: Weed Science Society of America and Allen Press
Stable URL: http://www.jstor.org/stable/3988057
Accessed: 12/09/2012 17:55

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at http://www.jstor.org/page/info/about/policies/terms.jsp

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Weed Science Society of America and *Allen Press* are collaborating with JSTOR to digitize, preserve and extend access to *Weed Technology*.

http://www.jstor.org

Metabolites from *Pseudomonas fluorescens* Strain D7 Inhibit Downy Brome (*Bromus tectorum*) Seedling Growth[1]

DAVID R. GEALY, S. GURUSIDDAIAH, ALEX G. OGG, JR., and ANN C. KENNEDY[2]

Abstract. Phytotoxins produced by the naturally-occurring rhizobacterium, *Pseudomonas fluorescens* strain D7 (strain D7), in shake cultures from several media sources adversely affected downy brome at several growth stages and affected seeds, whole plants, and isolated cells. These uncharacterized phytotoxins inhibited root and shoot growth of downy brome by 80% in a seed germination agar assay at 2 and 400 ng total solids/ml, respectively. In a hydroponics assay, downy brome and winter wheat dry weights were reduced 50% by cell-free broth concentrations of 15 and 26% of the initial broth concentration, respectively, indicating an almost 2× selectivity between winter wheat and downy brome. Plant water use was reduced similarly. Plasma membranes of isolated protoplasts of downy brome were damaged within 2 h by diluted cell-free broth. Cold-precipitated phytotoxins of strain D7 produced in semi-synthetic and synthetic media reduced dry weight of downy brome seedlings by 100 and 89%, respectively, and killed plants rapidly. **Nomenclature:** Downy brome, *Bromus tectorum* L. #[3] BROTE; wheat, *Triticum aestivum* L.

Additional index words: Bioassay, bioherbicide, biological control, protoplast, root, BROTE.

INTRODUCTION

Downy brome and related *Bromus* spp. are troublesome grass weeds that infest substantial portions of the winter wheat in the western United States. With sufficient post-planting fall moisture, downy brome typically emerges simultaneously or within about 3 wk after wheat emerges. Under these conditions, downy brome competes vigorously with winter wheat because it becomes established near the time of wheat emergence, has more vigorous root growth in the winter months, and completes its life cycle early in the spring, thereby depleting limited soil water and nutrient supplies before wheat can use them (11). Herbicides and cultural practices do not control downy brome consistently in winter wheat (11, 13, 18), and metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one], the only herbicide available for postemergence application in this crop, frequently injures wheat (13).

Poor grass weed control has limited successful implementation of conservation tillage systems in wheat rotations of the Pacific Northwest (12). Biological control of downy brome in winter wheat fields could hasten the adoption of conservation tillage systems which reduce soil erosion and preserve the soil resource. Recently, the rhizobacterial isolate *Pseudomonas fluorescens* strain D7 (strain D7[4]) was shown to inhibit root growth of downy brome but not winter wheat in controlled-environment and field experiments in the Pacific Northwest (3, 7, 8). Use of rhizobacteria as potential biocontrol agents of weeds has been reviewed (10).

Cell-free culture filtrate of strain D7 inhibited germination and root growth of downy brome in agar diffusion assays (8). A partially-purified, active complex (low-temperature precipitate, LTP[4]), which was crystallized from cell-free broth, contained chromopeptides and other peptides, fatty acid esters, and a lipopolysaccharide matrix (5).

[1]Received for publication Sept. 5, 1995 and in revised form Nov. 29, 1995. Contribution from Agric. Res. Serv., U.S. Dep. Agric. in cooperation with Coll. of Agric. and Home. Econ. Res. Ctr., Washington State Univ., Pullman, WA 99164-6421 and Bioanalytical Ctr., Washington State Univ., Pullman, WA 99164-4235.

[2]Plant Physiol., U.S. Dep. Agric., Agric. Res. Serv., NRGEEC, P.O. Box 287, Stuttgart, AR 72160, Assoc. Dir. Bioanalytical Ctr., Washington State Univ., 514 Science Hall, Pullman, WA 99164-4235, Plant Physiol., USDA, Agric. Res. Serv., 165 Johnson Hall, Washington State Univ., Pullman, WA 99164-6416, and Soil Microbiol. USDA, Agric. Res. Serv., 215A Johnson Hall, Washington State Univ., Pullman, WA 99164-6421.

[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 West University Ave., Champaign, IL 61821-3133.

[4]Abbreviations: BSA, bovine serum albumin; CFB, cell-free-broth; HEPES, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]; I_{50} and I_{80}, concentration causing 50 and 80% reductions, respectively, in growth parameters or physiological processes; KB, King's B media; KB-LTP, low temperature precipitate derived from KB media; LTP, low temperature precipitate (partially purified crystalline form of strain D7 phytotoxins); PMS, *Pseudomonas* minimal salts media; PMS-LTP, low temperature precipitate derived from PMS media; strain D7, *Pseudomonas fluorescens* strain D7 bacteria; strain 3366, *Pseudomonas syringae* strain 3366.

Apparently all of these components were necessary for downy brome-inhibitory activity, because purified forms of each component were inactive against downy brome when applied separately (5). These phytotoxins stopped downy brome seedling root growth within several hours (17), because they inhibited lipid synthesis and disrupted membrane integrity (16).

The objectives of this research were to quantify the levels of metabolites produced by strain D7 bacteria required to inhibit the growth of roots and shoots of germinating seeds and seedlings, and to disrupt protoplast membranes of downy brome; to evaluate the phytotoxic effects of a partially-purified, low-temperature precipitate form of these metabolites; and to determine the degree of selectivity of these metabolites between downy brome and winter wheat.

MATERIALS AND METHODS

Organism and production of phytotoxic metabolites. Strain D7 was isolated originally from the rhizosphere of wheat (8) and stored at –80 C in 6.8 M glycerol. Cultures from cryostorage were plated onto an agar medium (15) and grown at 25 C for 48 h. These cultures were maintained at 4 C and served as the inoculum for shake cultures of strain D7 which were grown at 22 to 25 C for 24 to 48 h on a chemically defined *Pseudomonas* minimal salts (PMS[4]) medium (1) or on a semi-synthetic (King's B; KB[4]) medium (9) as described previously (5, 16).

Preparation of phytotoxic metabolites for bioassays. Phytotoxic metabolites used in bioassays were prepared by centrifugation (8000 *g* for 30 min) of the culture broth or by low-temperature precipitation of the centrifuged culture broth (5). The resultant products were 'cell-free broth' (CFB[4]) and partially-purified LTP, respectively. LTP has the appearance of a fine crystal although it consists of several distinct chemical components (5). Inhibitory properties of these products were determined in several bioassays.

Many attempts have been made without success to prepare pure, active forms of the specific phytotoxins responsible for downy brome activity (5). Therefore, the experiments described herein were conducted using only the CFB and partially pure LTP forms of these phytotoxins. The inherent limitations of interpreting the effects of these impure phytotoxins on plants is acknowledged.

Dose response of root and shoot growth in an agar bioassay. Effects of PMS-grown strain D7 cell-free broth on root and shoot growth of germinating downy brome seedlings were determined in a petri plate agar assay (8). A PMS control and serial dilutions of cell-free broth ranging from 0.1 to 2×10^{-7} of the original concentration were prepared in 19 ml of 0.8% (w/v) agar and poured into petri plates. The 0.1× concentration of the original broth contained 100 μg total solids per ml (5). Fifteen downy brome seeds per petri dish were placed on the surface of the agar and incubated for 5 d at 15 C. Downy brome seeds from near Pullman, WA were stored dry at 20 to 25 C before use. Root and shoot lengths were measured after incubation. Only radicles ≥ 0.5 mm were measured. The experiment was conducted as a completely random design. Each treatment was replicated two or three times and the experiment was conducted four times. Data were subjected to regression analysis[5] and 95% confidence limits were calculated. I_{50} and I_{80}[4] values for root and shoot growth were estimated by interpolation.

Dose response of downy brome and wheat to strain D7 cell-free broth in hydroponics. Seeds of downy brome and 'Hill 81' winter wheat were incubated at 20 C in rolls of moistened germination paper. Because wheat seeds germinate and elongate roots more rapidly than downy brome, downy brome seeds were moistened 3 d before wheat so that seedlings would be similar in size when treated. Wheat (two plants) and downy brome (four plants) were transferred after 5 and 8 d, respectively, to 15-ml graduated centrifuge tubes containing 14 ml of various concentrations of strain D7 cell-free broth in nutrient solution (6), and placed into a growth chamber maintained at 20 C with a 12-h photoperiod and PPFD of 400 $\mu E/m^2/s$. Tubes were plugged at the top with soft styrofoam to reduce evaporation of water from the tubes. Treatments consisted of six concentrations of strain D7 cell-free broth ranging from 5 to 25% of the original, and a nontreated nutrient solution control. An additional control treatment of nutrient solution in a plugged tube without plants was included to provide a guide for adjusting for evaporative losses from the tubes. Every other day for 8 d, nutrient solution consumption (water use) was recorded and all treatment solutions were replaced with fresh preparations. Eight days after being transferred to nutrient solutions, plants were harvested and total dry weight was determined by drying at 55 to 60 C for about 48 h. The experiment was conducted as a completely random design with four and six replications, respectively, in the two separate runs of

[5]Statistical Analysis System. 1989. SAS Institute, Cary, NC 27511-8000.

the experiment. Regression analysis[5] was used to determine dose response curves for wheat and downy brome. Slopes and intercepts of the response curves of the two species were compared with F-tests using the general linear model regression techniques for comparing individual parameters between regression lines. I_{50} concentrations for each curve were estimated by interpolation.

Response of downy brome to partially-purified strain D7 low-temperature precipitate (LTP) in hydroponics. Strain D7 LTP was precipitated as a fine crystal-like material from 50 ml of cell-free broth that had been cultured in KB or PMS media as described previously (5). The final phytotoxin concentration of both LTP preparations in hydroponics was equivalent to 87% of the original CFB concentration. Methods of germination, hydroponic culture, and measurement of water use were described previously. Shoot, root, and total dry weights were determined separately. Growth was calculated as the increase in plant weight during the 8-d treatment period. Replenishment of solutions was the same as before except that the depleted volumes of the various solutions were only supplemented with fresh solutions instead of being replaced entirely. The experiment was conducted as a completely random design with four replications. An analysis of variance was performed and means were compared with Duncan's multiple range test at $P \geq 0.05$.

Effects of cell-free broth on disruption of downy brome protoplasts. Intact protoplasts were isolated from 3-wk-old downy brome shoot tissue after cellulase digestion (2). Isolated protoplasts were kept on ice in a buffered osmoticum (0.525 M sorbitol, 0.3% BSA[4], 25 mM HEPES[4], 10 mM $MgCl_2$, 10 mM KH_2PO_4, and 1.0 mM $CaCl_2$ adjusted to pH 7.4). Chlorophyll content of protoplasts in the osmoticum was 0.07 to 0.10 mg chlorophyll per ml and was determined spectrophotometrically (19). Cell-free broths from strain D7 (5), two inactive auxotrophs of strain D7 (strain D7-6C1 and strain D7-DM5) (8), and *Pseudomonas syringae* strain 3366[4] (4) were produced separately from PMS media. Strain D7 cell-free broth preparations that initially were concentrated 11:1 by evaporation, purified partially on G10 or G50 Sephadex gels (5), and then rediluted, were included also.

To measure protoplast disruption, droplets of the various cell-free-broth preparations (or a PMS control) and the protoplast osmoticum were mixed on a microscope slide to achieve a final phytotoxin concentration for each treatment of 50% (v/v) 'original cell-free broth equivalent' (a 1:1 mixture of 'original cell-free broth equivalent' and 'protoplast osmoticum'). Slides were maintained at about 20 C for 2 h. Protoplasts were observed for disruption under a microscope (lens 6.3/0.16; eye piece 10×) and representative areas were photographed for later quantification. Protoplasts in photographs were scored as 'intact' (appearing spherical and turgid) or 'disrupted' (appearing somewhat flaccid and often ruptured). Percent disruption within the field of view of the microscope was calculated as (100)(1 – [(no. intact protoplasts)/(total no. of protoplasts)]). The total number of protoplasts in the field of view in the nontreated controls ranged generally between 30 and 40. There were five to eight replicate slides per treatment and the experimental design was a randomized complete block. The experiment comparing strain D7 cell-free broth treatment with the PMS control was conducted twice, but in the first experiment, disruption was evaluated qualitatively only. In the second experiment, disruption was evaluated both qualitatively and quantitatively with results for strain D7 cell-free broth similar to those from the first experiment. Quantitative data from the second experiment were subjected to analysis of variance and means were compared with Duncan's multiple range test at $P \geq 0.05$.

RESULTS AND DISCUSSION

Dose response of root and shoot growth in an agar bioassay. Downy brome was highly sensitive to dilute D7 cell-free broth phytotoxins in agar germination assays. Strain D7 cell-free broth inhibited root growth more strongly than shoot growth (Figure 1). I_{80} concentrations for root and shoot length were 0.0002 and 0.04% of cell-free broth, respectively. These concentrations were estimated to be 2 and 400 ng total solids per ml cell-free broth based on a typical yield of 1000 mg total solids per L cell-free broth (5). I_{50} for shoot length was 2×10^{-5} of the cell-free broth and was less than 10^{-7} for root length (Figure 1). Similarly, roots of downy brome and winter wheat were more sensitive than shoots to phytotoxins produced by *Pseudomonas syringae* strain 3366 (strain 3366) (4).

Dose response of downy brome and wheat to strain D7 cell-free broth in hydroponics. I_{50} for downy brome and winter wheat in hydroponics were 11 and 22%, respectively, for transpiration, and 15 and 26%, respectively, for dry weight (Figure 2). This nearly 2× margin of selectivity between winter wheat and downy brome growth inhibition is consistent with the bioassays which were used successfully to screen for selective activity against downy brome

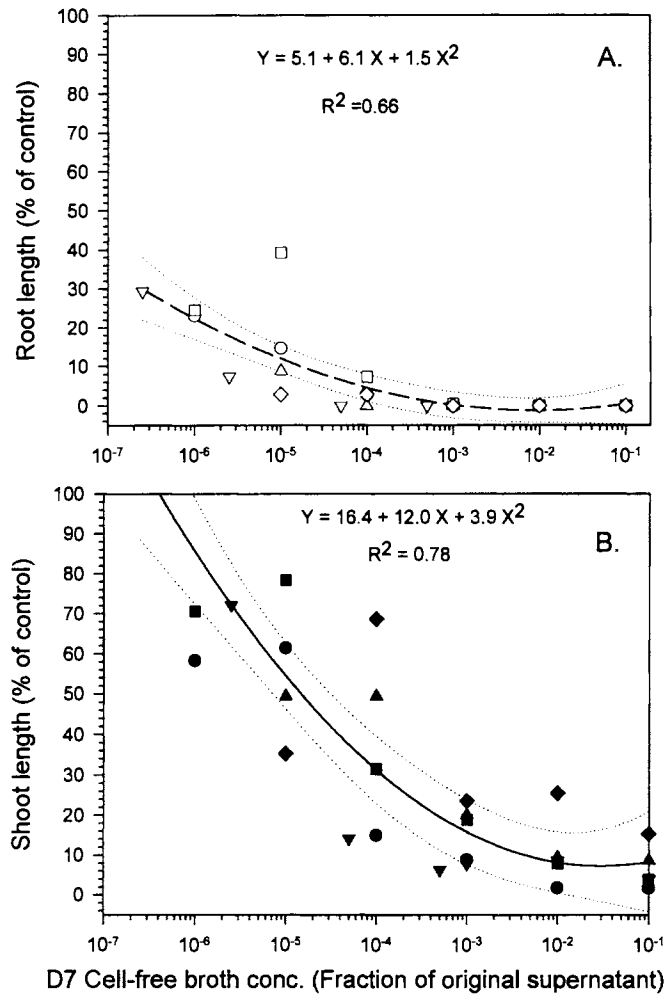

Figure 1. Dose-response of root (A) and shoot (B) growth of downy brome to strain D7 cell-free broth in an agar seed germination bioassay. Different symbols represent means from separate experiments. Dotted lines represent 95% confidence limits for regression curves. Estimated I_{80} and I_{50} concentrations (expressed as fraction of original cell-free broth) were as follows. I_{80} was 2×10^{-6} for roots and 4×10^{-4} for shoots. I_{50} was 10^{-5} for shoots but was too low to be estimated accurately for roots. X values in regression equations are log base 10 concentration values.

(8). Furthermore, seedlings (Figure 2) were less sensitive to strain D7 cell-free broth than were germinating seeds (Figure 1).

Therefore, these phytotoxins reduce plant growth (especially roots) and water absorption. These responses are consistent with the membrane disrupting ability shown previously for these phytotoxins (17). Similarly, supernatant from liquid cultures of *Pseudomonas fluorescens* strain 52 reduced water uptake by cut rose (*Rosa hybrida* cv. Sonia) stems, because exopolysaccharides produced by these bacteria blocked or damaged rose vessels (14). Polysaccharide materials are a significant component of the downy brome-inhibiting phytotoxins produced by strain D7 (5).

Response of downy brome to partially-purified strain D7 low-temperature precipitate (LTP) in hydroponics. LTP derived from both PMS and KB media inhibited downy brome growth in hydroponics (Table 1). Root dry weight, shoot dry weight, total dry weight and water use were inhibited usually > 85% by LTP in hydroponics. KB-LTP[4] was more inhibitory to growth than the PMS-LTP and it killed plants sooner. However, KB-LTP appeared to be less inhibitory than PMS-LTP to water use.

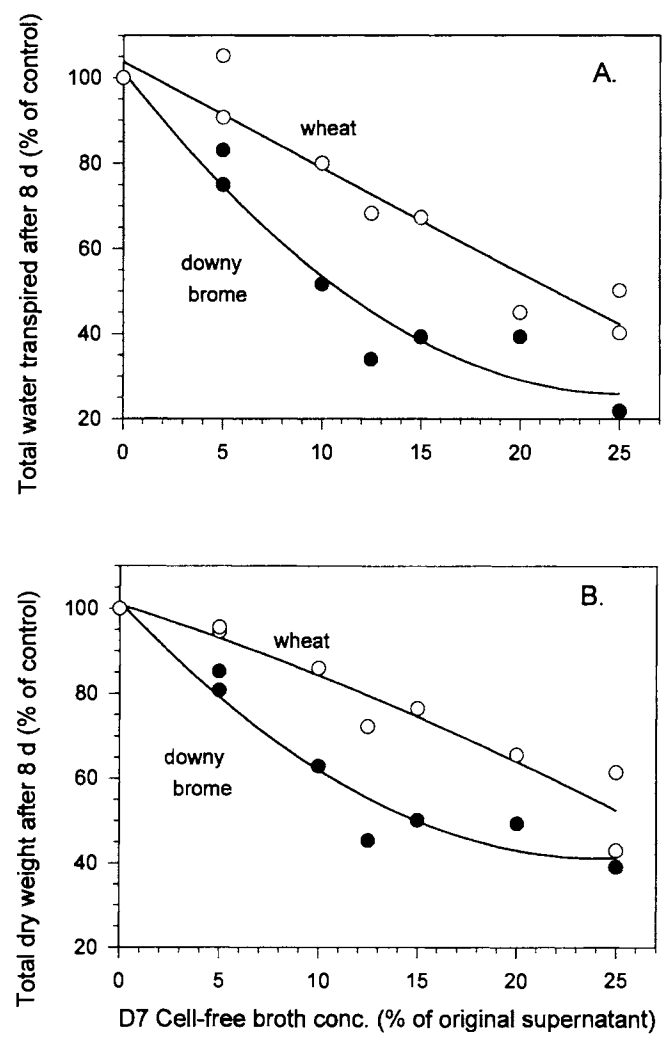

Figure 2. Dose-response of downy brome and wheat seedling transpiration (A) and growth (B) to strain D7 cell-free broth in a hydroponics assay. Roots of plants in the one-leaf stage were exposed to strain D7 cell-free broth for 8 d. Regression analysis of both transpiration and growth data indicated that response curves for wheat and downy brome were different at P = 0.05.

Table 1. Effect of low temperature precipitate (LTP) from King's B and *Pseudomonas* minimal salts media on growth and water use of downy brome after 8 d[a].

Media source for low temperature precipitate	Increase in root dry weight per four plants		Increase in shoot dry weight per four plants		Increase in total dry weight per four plants		Cumulative water use per four plants	
	% of control	mg	% of control	mg	% of control	mg	% of control	mg
Control	100 a	3.0 a	100 a	16.0 a	100 a	19.0 a	100 a	3.3 a
King's B	–9 b	–0.3 b	1 b	0.2 b	0 c	–0.1 c	12 b	0.4 b
Pseudomonas minimal salts	17 b	0.5 b	10 b	1.6 b	11 b	2.1 b	2 c	0.1 c

[a]Means within the same column followed by the same letter are not different at P = 0.05 according to Duncan' s Multiple Range Test. Growth was calculated as the increase in plant weight during the 8 d treatment period.

These results suggest that LTP materials derived from the two media may elicit somewhat different physiological responses. In earlier research, the composition of the polypeptide components of the phytotoxin was affected by the culture media from which it was derived, and the KB-LTP dry weight yields were approximately five times that of PMS-LTP yields (40 vs. 8 mg LTP per ml) (5). Therefore, the actual dosage of active phytotoxin in the PMS-LTP was probably somewhat less than the KB-LTP. In addition, there are large amounts of inorganic salts present in PMS media. Thus, much of the LTP derived from this medium may be present as nonphytotoxic inorganic precipitates which become mixed with the phytotoxin precipitate. This dilution of phytotoxic precipitates with nonphytotoxic precipitates in PMS-LTP may reduce the potency of LTP when expressed on a per weight basis. Even in the hydroponics solution, much phytotoxin material precipitated out of the PMS-LTP, whereas most of the KB-LTP material remained in solution.

Downy brome roots in LTP turned dark within 2 d of exposure to LTP. Strain D7 phytotoxins also darkened downy brome root tips in earlier research (16). After 8 d, plants in both KB and PMS media had died and both roots and shoots appeared necrotic. These results are in contrast to the effects of strain D7 phytotoxins reported in other bioassays in which shoots and roots of germinating seeds and seedlings were inhibited, but generally plants were not killed (5, 16) (Figures 1 and 2).

Effects of cell-free broth on disruption of downy brome protoplasts. Strain D7 phytotoxins disrupted downy brome protoplasts within 2 h (Figure 3). Disrupted protoplasts were flaccid and nonspherical to varying degrees and often had ruptured, releasing chloroplasts into the surrounding solution. Nearly four times as many protoplasts were disrupted by strain D7 than by the PMS control. Degree of disruption was similar for strains D7 and 3366. These results substantiate that D7 increased membrane disruption of downy brome roots in hydroponics (17). The exopolysaccharide-containing supernatant from shake cultures of *Pseudomonas fluorescens* strain 52 disrupted beet (*Beta vulgaria* L.) root membranes similarly (14). Consistent with the reduced phytotoxicity of noninhibitory auxotrophs of strain D7 (8) and their metabolites (16), phytotoxins from strain D7-6C1 and D7-DM5 auxotrophs disrupted protoplasts less (about 30%) than those from the strain D7 'wild type' (Figure 3). Sephadex gel preparations and the concentrated (11:1) strain D7 cell-free broth solutions from which they were prepared were only about half as disruptive to protoplasts as the original strain D7, suggesting that certain factor(s) involved in disruption of the plasmalemma may have been altered by the concentration procedure or removed by the Sephadex gels. Some of the

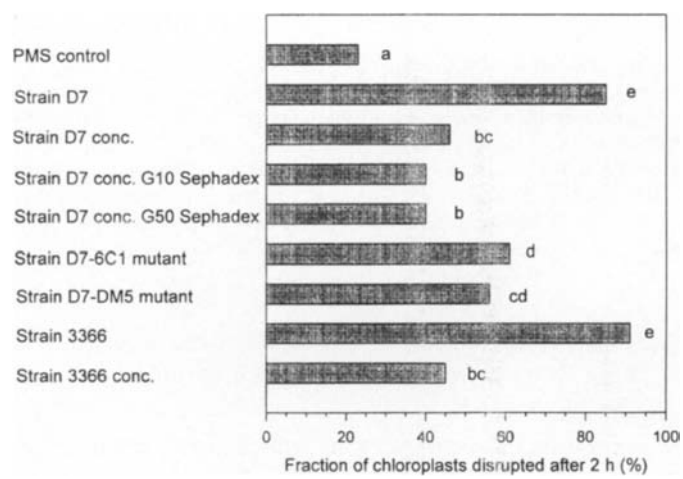

Figure 3. Effect of rhizobacterial cell-free broth on disruption of protoplasts isolated from downy brome leaves. Horizontal bars followed by the same letter indicate that means are not different at P = 0.05 according to Duncan's Multiple Range Test. All treatments were adjusted to a final concentration equivalent to 50% (v/v) of original cell-free broth. The cell-free broth in strain D7 conc., strain 3366 conc. treatments, and in Sephadex treatments was concentrated 11:1 initially and then diluted to achieve the constant final cell-free broth concentration of 50%.

active compounds may have precipitated or volatilized from solution at the high concentration. Previous research indicated that the 'complex' of downy brome-inhibitory phytotoxins is unstable and can be rendered inactive against downy brome by numerous physical and chemical processes (5).

Phytotoxins in strain D7 cell-free broth are potent inhibitors of germinating downy brome seedlings. Root growth was about 200 times more sensitive to strain D7 than shoot growth. In older seedlings, strain D7 cell-free broth was several orders of magnitude less inhibitory to plant growth. Strain D7 cell-free broth was nearly twice as inhibitory to downy brome as it was to wheat, and inhibition of water absorption paralleled that of growth. LTP phytotoxins rapidly desiccated downy brome seedlings and killed plants. However, plants often did not die when exposed to strain D7 phytotoxins (5, 16), and roots can resume growth after removing them from the phytotoxic materials (16).

ACKNOWLEDGMENTS

We thank Steven Seefeldt, Pat Tranel, and Shelly Crites for technical assistance and Delvar Peterson for assistance with statistical design and analysis.

LITERATURE CITED

1. Bolton, H., Jr. and L. F. Elliott. 1989. Toxin production by a rhizobacterial *Pseudomonas* sp. that inhibits wheat root growth. Plant Soil 114:269–278.
2. Buman, R. A., D. R. Gealy, and A. G. Ogg Jr. 1991. Inhibition of photosynthesis in downy brome (*Bromus tectorum*) and jointed goatgrass (*Aegilops cylindrica*) protoplasts by metribuzin and its ethylthio analog. Weed Sci. 39:13–17.
3. Elliott, L. F. and A. C. Kennedy. 1991. Method for screening bacteria and application thereof for field control of the weed downy brome. United States Patent no. 5,030,562. SN 07/207,592. Granted July 9, 1991.
4. Gealy, D. R., S. Gurusiddaiah, and A. G. Ogg, Jr. 1996. Isolation and characterization of metabolites from *Pseudomonas syringae*-strain 3366 and their phytotoxicity against certain weed and crop species. Weed Sci. 44:(In press).
5. Gurusiddaiah, S., D. R. Gealy, A. C. Kennedy, and A. G. Ogg, Jr. 1994. Isolation and characterization of metabolites from *Pseudomonas fluorescens*-D7 for control of downy brome (*Bromus tectorum* L.). Weed Sci. 42:492–501.
6. Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. California Agric. Exp. Stn. Cir. 347. 32 p.
7. Johnson, B. N., A. C. Kennedy, and A. G. Ogg, Jr. 1993. Suppression of downy brome growth by a rhizobacterium in controlled environments. Soil Sci. Soc. Am. J. 57:73–77.
8. Kennedy, A. C., L. F. Elliott, F. L. Young, and C. L. Douglas. 1991. Rhizobacteria suppressive to the weed downy brome. Soil Sci. Soc. Am. J. 55:722–727.
9. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration-of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301–307.
10. Kremer, R. J. and A. C. Kennedy. 1996. Rhizobacteria as biocontrol agents of weeds. Weed Technol. 10:(In press).
11. Morrow, L. A. and P. W. Stahlman. 1984. The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Sci. 32(Suppl. 1):2–6.
12. Papendick, R. I. and D. E. Miller. 1977. Conservation tillage in the Pacific Northwest. J. Soil Water Conserv. 32:49–56.
13. Peeper, T. F. 1984. Integrated systems for control and management of downy brome (*Bromus tectorum*) in wheat and alfalfa in North America. Weed Sci. 32(Suppl. 1):18–25.
14. Put, H.M.C. and W. Klop. 1990. The effects of microbial exopolysaccharides (EPS) in vase water on the water relations and the vase life of *Rosa* cv. Sonia. J. Appl. Bacteriol. 68:367–384.
15. Sands, D. C. and A. D. Rovira. 1970. Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20:513–514.
16. Tranel, P. J., D. R. Gealy, and G. P. Irzyk. 1993. Physiological responses of downy brome (*Bromus tectorum*) roots to *Pseudomonas fluorescens* strain D7 phytotoxin. Weed Sci. 41:483–489.
17. Tranel, P. J., D. R. Gealy, and A. C . Kennedy. 1993. Inhibition of downy brome (*Bromus tectorum*) root growth by a phytotoxin from *Pseudomonas fluorescens* strain D7. Weed Technol. 7:134–139.
18. Wicks, G. A. 1984. Integrated systems for control and management of downy brome (*Bromus tectorum*) in cropland. Weed Sci. 32(Suppl. 1):26–31.
19. Wintermans, J.F.G.M. and A. DeMots. 1965. Spectrophotometric characteristics of chlorophyll a and b and their pheophytins in ethanol. Biochim. Biophys. Acta 109:448–453.