Influence of Environment on Albizzia julibrissin Root Exudation and Exudate Effect on Fusarium oxysporum f. sp. perniciosum in Soil

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ABSTRACT

The effect of various combinations of light, temperature, and humidity on carbohydrate exudation of sterile mimosa roots was studied. Glucose and galactose were present in root exudates under all conditions. Neither light nor humidity alone had a marked effect on glucose or galactose exudation. Increasing temperature from 17 to 33 C increased the exudation rate of both sugars. The light-high temperature (33 C) interaction significantly increased glucose exudation; however, there was no significant light-low temperature (17 C) interaction on glucose exudation. The light-low temperature interaction (17 C) significantly increased galactose exudation, but there was no significant light-high temperature (33 C) interaction on galactose exudation. Maltose, another exudate component, was not detected at low levels of light, temperature, and humidity. Increasing the levels of these factors from medium to high levels increased maltose exudation.

Spore germination and fungus growth were greatest in glucose and maltose, and least with galactose. Sugars supplied to spores in amounts greater than the calculated amounts released by mimosa roots were necessary to significantly increase spore germination in water or soil. The influence of temperature and sugar amount on spore germination increase was greater in water than soil. Phytopathology 61: 812-815.

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The response of a plant to the environment may be reflected in the composition of root exudates. These exert an influence upon soil-borne plant pathogens (3, 11, 12). Few papers have dealt with the influence of specific levels of light, temperature, or humidity on root exudation (10, 14, 17). None has attempted to determine whether any interactions between light, temperature, and humidity influence the qualitative and quantitative nature of root exudation. It is hoped that answers to such questions will be helpful in better understanding the behavior of plant pathogens in the rhizosphere and mechanisms operating in exudation. Fusarium wilt of mimosa (*Albizzia julibrissin* Durazz.), caused by *Fusarium oxysporum* f. sp. *perniciosum* (Hept.) Toole, offers an opportunity to study the dynamic relationship between root exudate and a rhizosphere pathogen. This paper reports the effect of certain environmental factors on root exudation, and the influence of the kinds and amounts of sugars exuded by mimosa roots on spore germination and growth of the pathogen.

MATERIALS AND METHODS.—For the qualitative and quantitative analysis of root exudates, the following procedures were used. Mimosa seeds were scarified in concentrated sulfuric acid for 10 min and washed in sterile water, surface-sterilized in 0.1% $HgCl_2$, and again washed in sterile water. The seeds were then germinated aseptically in petri dishes containing moistened filter paper discs. Five-day-old seedlings were placed in French square bottles containing 30 ml of sterile tap water. A complete nutrient solution was not used because cotyledons can redistribute essential minerals to other parts of young, developing seedlings. It has been shown that clover roots release the same carbohydrates whether grown in water or in a complete nutrient solution (15). No nutrient deficiency symptoms were observed after 3 weeks of growth in nonaerated water. The roots were maintained sterile in the tap water while the tops were exposed. At the end of each experiment, roots were plated on potato-dextrose agar and incubated at 25 C to test for contamination.

The experimental design was a 2^3 factorial augmented with four center points. Temperature, humidity, and light were simultaneously varied in nine different treatments as follows: 33 C, 60 relative humidity (RH), 2,054 ft-c; 17 C, 95 RH, 2,054 ft-c; 17 C, 60 RH, 2,846 ft-c; 33 C, 95 RH, 2,846 ft-c; 17 C, 60 RH, 2,054 ft-c; 33 C, 95 RH, 2,054 ft-c; 33 C, 60 RH, 2,846 ft-c; 17 C, 95 RH, 2,846 ft-c; 24 C, 77 RH, 2,450 ft-c. The last treatment combination or center point represents a medium level of treatment variables repeated 4 times to obtain an estimate of experimental error. RH varied ±2%; and temperature, ±1 C. Forty plants were included in each treatment, and subjected to 14 hr of light and 10 hr of dark for 14 days in growth chambers.

After the 14-day growth period, solutions from the 40 plants were harvested, combined, filtered, immediately placed in 300 ml round-bottom flasks, and concentrated under vacuum to 4 ml. The necessity of desalting prior to chromatographic separation was avoided by using tap water as the growth medium.

Carbohydrates were separated by triple, descending, unidimensional paper chromatography in an *n*-butanol: acetic acid:water (4:1:5, v/v epiphase) solvent system. Sugars were detected with aniline hydrogen

phthalate. Final identification of sugars was based on standard R glucose values, spot color, and comparison of the unknown spot patterns to known patterns.

Sugar quantities were estimated by eluting detected sugar spots with 0.7 N hydrochloric acid in 80% ethanol and measuring the absorbance for hexoses at 390 mμ and oligosaccharides at 380 mμ (18). The sugar concentration was estimated by the regression equation of the known standard concentrations.

Fusarium oxysporum f. sp. *perniciosum* spore response to sugars was evaluated by amending infested soil with different levels and kinds of sugar exuded from mimosa roots. Soil (sandy loam) used was collected from the top 12 inches of the surface layer at the base of mimosa trees growing in Cape May County, N. J.

The rhizosphere of 20-day-old mimosa plants was estimated by weighing the amount of soil remaining on roots after loose, clinging soil was gently removed. Approximately 0.41 g of air-dried soil represented the amount of rhizosphere soil per plant.

For spore production, the fungus was grown on a nutrient medium for 10 days at 24 C. The surface of the mycelial mat was then flooded with deionized distilled water and scraped with a plastic policeman to dislodge microspores. This organism produces few macrospores in culture. The spore suspension was then filtered through sterile cheesecloth to remove mycelial fragments. The spore suspension was centrifuged at 18,000 rpm for 5 min in an International centrifuge. The spores were resuspended and centrifuged twice in sterile distilled water. The number of spores was adjusted to ca. 5×10^5 spores/ml.

To each spot plate well, containing 0.82 g of air-dried soil, 0.25 ml of the spore suspension containing 1, 4, 10, and 16 μg of the respective sugars glucose, galactose, and maltose were added. The addition of 0.25 ml of liquid to 0.82 g of air-dried soil approximated the original moist wt of the rhizosphere soil. Evaluating the influence of sugars alone on spore response required a second series of spot plates which contained only deionized water, spores, and a carbon source. Controls were established to measure per cent spore germination in (i) deionized distilled water; and (ii) soil alone. Spot plates were placed in stender dishes with moist paper towels to keep evaporation at a minimum and incubated for 8 hr at 17, 24, and 32 C. Contents of the spot plates were then washed into 50-ml flasks containing 8.5 ml of deionized distilled water. These flasks were shaken for 1 min to suspend the spores and then allowed to stand for 5 min to settle out the soil. Slides were covered with 0.2 ml of the supernatant and slowly dried on a hot plate. The microspores were fixed in 40% glacial acetic acid and stained with rose-bengal. Spore germination was evaluated by counting a total of 100 spores on four replicate slides. A spore was considered germinated if the germ tube length was at least twice the diam of the spore. The data were statistically analyzed and presented using the arcsin transformation recommended by Steel & Torrie (16).

To evaluate the influence of exuded carbohydrates on fungus growth, the following procedure was used. Spore inoculum was obtained by growing the fungus on an agar medium (KNO_3, 10 g; K_2HPO_4, 5 g; $MgSO_4$, 2.5 g; $FeCl_3$, 0.02 g; carbon, 6 g/liter) for 12 days at 24 C. Sugars (glucose, galactose, or maltose) used in the medium were sterilized separately by passage through a sterile Millipore filter (0.45 μ ± 0.02 μ). Mycelial mats were flooded with sterile deionized water, then scraped with a plastic policeman to dislodge microspores. The solutions were filtered and spores were washed as mentioned above. The final spore suspension was adjusted to 5×10^6 spores/ml.

The liquid growth medium was prepared as above without agar and adjusted to pH 6. The medium was distributed in 50 ml quantities in 250 ml Erlenmeyer flasks previously cleaned by a 5-day soak in 50% HNO_3. Each flask received 0.5 ml of the spore suspension and was oscillated on a rotary shaker at 240 rpm at 24 C. A carbonless treatment was included as a control. For each of 6 days, two flasks from each of the four treatments were harvested. The solutions were filtered through tared, acid-cleaned, fritted glass crucibles and washed of any impurities with deionized distilled water. The fungus material remaining in the crucible was air-dried for 24 hr at 65 C and weighed.

RESULTS.—*Qualitative evaluation of root exudates.*—Glucose, galactose, and maltose were identified as carbohydrate fractions of mimosa root exudates. Glucose and galactose were exuded from roots under all conditions tested. Maltose was exuded under only two sets of conditions, a medium level (2,450 ft-c, 77 ± 2% RH, 24 ± 1 C) and a high level (2,846 ft-c, 95 ± 2% RH, 33 ± 1 C).

Quantitative evaluation of root exudates.—Humidity and light alone had no effect on the amount of glucose or galactose exuded. Temperature alone had a significant effect; on the average, glucose increased from 0.740 to 1.508 μg/plant and galactose increased from 0.732 to 0.991 μg/plant with increasing temperature (17 to 33 C). Temperature and light interacted in the production of glucose and galactose (Fig. 1). The light-

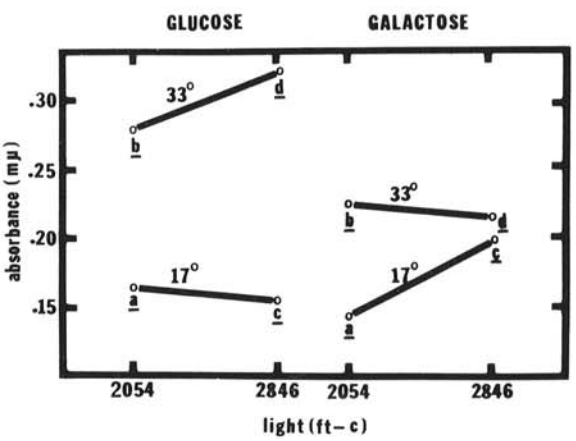

Fig. 1. Influence of light and temperature on sugar exudation from mimosa roots. Points a, b, c, and d represent treatments at the indicated light and temperature levels. LSD for absorbance was .035.

high temperature (33 C) interaction increased the amount of glucose exuded from 1.407 to 1.609 μg/plant. There was no significant effect on glucose exudation by a light-low temperature (17 C) interaction. The form of the interaction was different for galactose and glucose. A light-low temperature (17 C) interaction increased the amount of galactose exuded from 0.574 to 0.889 μg/plant. The light-high temperature (33 C) interaction did not increase galactose exudation. Maltose exudation significantly increased from 0.880 at medium levels to 1.572 μg/plant at higher levels of light, temperature, and humidity. No maltose was detected at low levels of light, temperature, and humidity.

Effect of soil, water, sugar type and amount, and temperature on spore germination.—Both substrate (soil and water) and temperature had a significant effect at the 1% probability level on spore germination at the lower temperatures. Germination increased from 35% at 17 C to 46% at 24 C in soil. Germination also increased from 4% at 17 C to 16% at 24 C in water. There was no significant difference in spore germination at 24 and 32 C in either soil or water.

Sugars added in amounts up to 4 μg/0.8 g air-dried soil did not significantly increase the spore germination rate above the 40% average recorded for nonemended soil at all temperatures tested. Spore germination was significantly increased to a 45% average when 10 and 16 μg of sugar were added to the soil at all temperatures tested. Sugars added to the water-spore suspension at the 10- and 16-μg levels raised the germination rate to an 18% average at 24 and 32 C. Germination in the nonemended water-spore suspension averaged 4%. Glucose and maltose stimulated germination more effectively than did galactose in both soil and water.

An increase in temperature from 17 to 24 C increased per cent germination of spores with all sugars added to both soil and water (Table 1). Glucose and maltose stimulated germination more than galactose at higher temperatures (24 and 32 C).

Influence of root exudate sugars on fungus growth.—*Fusarium oxysporum* f. sp. *perniciosum* used glucose, galactose, and maltose, identified as components of mimosa root exudate, as carbon sources for growth (Fig. 2). The fungus dry wt was greatest with glucose, less with maltose, and lowest with galactose. Fungus growth in the no-carbon control was negligible.

TABLE 1. Influence of temperature and sugar type on germination of *Fusarium oxysporum* f. *perniciosum* microspores

	% Germination		
	Temperature (C)		
Sugar	17	24	32
Glucose	18.1[a]	31.0	31.9
Maltose	19.6	30.0	30.0
Galactose	17.4	25.6	26.4
No sugar	15.1	22.8	23.5

[a] Values represent cumulative germination average at varying concentrations of sugar in soil and water, expressed in per cent germination.

Values for glucose and maltose at 24 and 32 C were significantly greater than galactose at the 1% probability level.

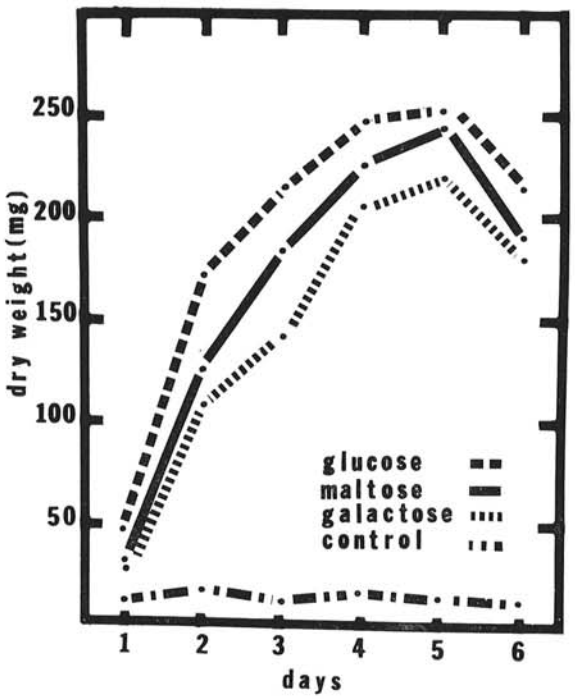

Fig. 2. Growth of *Fusarium oxysporum* f. sp. *perniciosum* in liquid shake cultures with exudate sugars as the carbon source.

DISCUSSION.—Environmental factors influence carbohydrate component of root exudates. The increase in sugar exudation with temperature is consistent with the findings of Vancura (17). Since temperature influences the uptake of mineral nutrients in plants, possibly through enzyme-mediated carriers, a similar mechanism may operate in root exudation, as suggested by Rovira (10).

Light had no significant effect on glucose exudation at low temperatures (17 C), however, a light-high temperature (33 C) interaction significantly increased glucose exudation. Apparently, low light (2,054 ft-c) at the high temperature was limiting for photosynthesis in mimosa, since increased light significantly increased exudation. The effect of photosynthetic rate on root exudation is largely unknown.

Greater galactose exudation at high (33 C) rather than at low temperatures (17 C) may suggest a temperature effect on the inversion of glucose to galactose. The rate of this inversion occurs maximally at 37 C, and only 25% of the glucose is inverted to galactose (7). This may explain generally lower amounts of galactose than glucose present in root exudation throughout the experiment. Linder & Mitchell (9) demonstrated that ^{14}C-tagged materials would move down stems and appear in root exudates of beans grown in light, but not in exudates of light-deficient plants. If increasing light from 2,054 to 2,846 ft-c at 17 C would substantially increase carbohydrate mobility in stems, then an at-

tempt could be made to relate an inversion of glucose to galactose to increased galactose exudation.

Apparently combinations of high light and temperature may influence maltose exudation. Amylase activity in soybean, wheat, and barley is greater at temperatures above 20 C than below (4), thus possibly accounting for greater amounts of maltose at 33 than at 17 C.

Significantly greater spore germination in soil than in water was consistent with the findings of Griffin (5). Plain water or water amended with small amounts of sugar probably does not supply the nutrients for spore germination supplied by soil. As reported by others (1, 2, 13), increasing sugar amounts in water and soil increased per cent germination. Sugars added to the soil, however, did not greatly increase spore germination. This may be due either to a not substantial increase in available carbon sources; or to soil microorganisms in competition for the added sugars, thus removing much of their effect. Consistent with other findings (6, 13, 15), galactose was least effective in promoting spore germination.

It was necessary to add sugars at a higher rate than that calculated to be released in a given amount of rhizosphere soil to significantly increase spore germination. However, it is possible that sugars do not diffuse very far into the rhizosphere. It may be that roots exude substances mainly into the mucilaginous layer (composed mostly of water) surrounding the root, where they exert an influence on spore germination. In the present investigation, increased sugar amounts had a greater effect on spore germination rate in water than soil.

All three sugars supported vegetative growth of the fungus. That galactose did not support vegetative growth to the same extent as glucose or maltose is consistent with the findings of Lindberg (8).

These findings indicate the importance of environmental factors which influence plant metabolism resulting in exudation of sugars beneficial to plant pathogens. These sugars stimulate spore germination, and support vegetative growth of the pathogen in the rhizosphere. However, conditions which support germination and vegetative growth of the fungus may not insure pathogenesis. More studies are needed to determine to what extent the kinds and levels of sugars released by plant roots influence pathogenesis.

LITERATURE CITED

1. Adams, P. B., J. A. Lewis, & G. C. Papavizas. 1968. Survival of root-infecting fungi in soil. IV. The nature of fungistasis in natural and cellulose-amended soil on chlamydospores of Fusarium solani f. sp. phaseoli. Phytopathology 58:378-383.
2. Adams, P. B., G. C. Papavizas, & J. A. Lewis. 1968. Survival of root-infecting fungi in soil. III. The effects of cellulose-amendment on chlamydospore germination of Fusarium solani f. sp. phaseoli in soil. Phytopathology 58:373-377.
3. Barton, R. 1957. Germination of Pythium mamillatum in response to exudates from living seedlings. Nature 180:613-614.
4. French, D. 1960. 6-amylases, p. 345-368. *In* P. D. Boyer, H. Lardy, & K. Myrback [ed.]. The Enzymes, Vol. IV. Academic Press, N.Y.
5. Griffin, G. J. 1962. Production of a fungistatic effect by soil microflora in autoclaved soil. Phytopathology 52:90-91.
6. Jackson, R. M. 1957. Fungistasis as a factor in the rhizosphere phenomenon. Nature 180:96-97.
7. Leloir, L. F. 1951. The enzymatic transformation of uridine diphosphate glucose into a galactose derivative. Arch. Biochem. Biophys. 33:186-190.
8. Lindberg, M. 1967. Stimulating effect of xylose on the utilization of galactose in some fungi. Nature 213:395-396.
9. Linder, P., & J. Mitchell. 1960. Rapid transport of α-methoxyphenylacetic acid introduced directly into the water stream of bean plants. Bot. Gaz. 121:139-142.
10. Rovira, A. D. 1959. Root excretions in relation to the rhizosphere effect. IV. Influence of plant species, age of plant, light, temperature, and calcium nutrition on exudation. Plant Soil 11:53-64.
11. Schroth, M. N., & F. F. Hendrix. 1962. Influence of nonsusceptible plants on the survival of Fusarium solani f. phaseoli in soil. Phytopathology 52:906-909.
12. Schroth, M. N., & W. C. Snyder. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus, Fusarium solani f. phaseoli. Phytopathology 51:389-393.
13. Schulz, F. A. 1968. Untersuchungen über den einflus der grundungung auf die Halmbruchkrankheit des weizens und ihrer erreger Cercosporella herpotrichoides Fron. III. Ursachliche Zusammenhange zwischen pflanzenwachstum, grundungung und konidienkeimung. Phytopathol. Z. 62:205-219.
14. Segeta, V., & M. Stanek. 1967. Root exudates of plants. Plant Soil 27:319-328.
15. Smith, W. H., & J. L. Peterson. 1966. The influence of the carbohydrate fraction of the root exudates of red clover, Trifolium pratense L., on Fusarium sp. isolated from the clover root rhizosphere. Plant Soil 25:413-424.
16. Steel, R. G. D., & J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Co., N.Y. 481 p.
17. Vancura, V. 1967. Root exudates of plants. III. Effect of temperature and 'cold shock' on the exudation of various compounds from seeds and seedlings of maize and cucumber. Plant Soil 27:319-328.
18. Wilson, C. M. 1959. Quantitative determination of sugars on paper chromatograms. Anal. Chem. 31: 1199-1201.