Rhizosphere Competition in Model Soil Systems

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ABSTRACT

Model soil systems were used to measure competition in conditions known to promote biological control of bean root rot. Glucose (5,000 ppm C) was added to the soil systems (to simulate addition of a carbon source in the rhizosphere) and its rate of utilization assayed. Rate of utilization of glucose in cellulose (4500 ppm C)-amended soils decreased in 6-8 days, correlating with N immobilization. The addition of KNO_3 (1,225 ppm) nullified the effect. Lignin and chitin together increased rate of utilization of glucose in comparison with raw soil. In general, glutamic acid utilization with these amendments was similar to glucose utilization. This contributes further evidence that biological control with lignin-chitin amendments results from competition for carbon.

There was no increase in total CO_2 evolution from soil amended with NO_3-N compared with raw soil. CO_2 evolution from glucose-amended soil was rapid, and carbon became limiting after 12 days. With cellulose amendments, however, N was the limiting factor in its decomposition. Lignin and chitin amendments increased CO_2 evolution as compared with raw soil. Addition of NO_3-N to this system failed to alter significantly the increased CO_2 evolution noted with these amendments. Phytopathology 60:1058-1061.

Additional key words: Biological control, soil microbiology.

Biological control of soil-borne plant pathogens has been defined by Garrett (8) as the reduction of disease incidence by living organisms other than the host, man, or the pathogen itself. Biological control may occur when demand exceeds the supply resulting in competition for a limiting factor (6). Usually a large active soil population creates this condition by immobilizing available nutrients and thus limiting the pathogen. Hence, pathogens requiring exogenous sources of carbon and nitrogen for spore germination and penetration could be controlled through competition (2).

The incitant of bean root rot, *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk) Snyd. & Hans. exists as chlamydospores in field soil (14). It requires an exogenous source of carbon and N for germination (7, 10) and penetration (17) that is met by exudates in the bean root rhizosphere (15). Soil amendments of mature barley straw, cellulose, and glucose have reduced severity of disease (13, 16). Hence, competition for soil N has been proposed as the mechanism of control. Similarly, soil amendments of lignin and chitin (12) reduced disease expression, but carbon rather than N was suggested to be limiting pathogenesis.

Model soil systems were used to study the mechanisms of competition. First, substances representative of compounds exuded by plants into the rhizosphere (15) were added to soils amended with relatively complex compounds characteristic of the constituents of crop residues (12, 13). The persistence of these simple representative substances in the amended soils was determined. The rate at which they were mineralized was assumed to indicate the amount of competition in the model system for that particular substance. Secondly, the activity of the microflora in the model soil systems was measured by CO_2 production to indicate the rate at which the complex amendments were decomposed.

MATERIALS AND METHODS.—A loam soil collected near Fort Collins, having the following characteristics, was used: organic matter, 1.4%; N, 0.069%; lime, 2.4% soluble salts, 5.0 mmhos/cm; pH, 7.8; cation exchange capacity, 15.7 meq./100 g; P_2O_5, 17 ppm; and K_2O, 215 ppm. The soil moisture content was 18% in all experiments.

Cellulose, 4,500 ppm C; lignin, 4,500 ppm C; and chitin, 1,000 ppm C were added to the model soil systems. The lignin was derived from Kraft pine that had been precipitated and washed free of sugars and inorganic salts. Potassium nitrate, 1225 ppm, was added as a N source.

Each soil system was set up by mixing the amendment and soil in a separate plastic bag. A positive air pressure was created in the bag and the amendment and soil were mixed thoroughly by rotating. The soils were then transferred to pint size mason jars with non-treated cellophane covers which allowed for CO_2 and O_2 diffusion but inhibited moisture changes. An equilibration period of 7 days at 25 C followed for soils with cellulose before glucose or glutamic acid were added. Similarly for lignin-chitin systems, this period was 14 days.

The effect of the amendments on the rate of glucose utilization (5,000 ppm C), and rate of glutamic acid utilization (100 ppm) was determined by an extraction and chromatography technique. To recover free glucose from the model soil systems, 50-g soil samples were extracted with 50 ml of an 80% ethanol solution for 5-10 min in 250 ml Erlenmeyer flasks and then filtered (11). The filtrate was evaporated to dryness by a continuous air stream, and the residue redissolved in 5 ml

of the ethanol solution. This sample was placed in an 11- × 100-mm test tube to allow undissolved fractions to settle out, leaving a clear supernatant for chromatography. A 2-μliter sample was spotted on chromatography paper and run in an isopropanol:water solvent (4:1) for 24 hr. The chromatograms were dried, dipped in aniline hydrogen phthalate reagent, and developed in an oven at 105 C for 3 min. A standard curve for known concentrations of glucose was developed from a Beckman Spinco Analytrol Model RB densitometer to find the concentration of the unknown glucose sample. The glutamic acid samples were analyzed similarly as outlined by Gilbert & Altman (9).

No free glucose was detected in the nontreated soil using the method described above. Amino-N in this case was 10.7×10^{-6} g/g of oven dry soil.

The second model soil system measured CO_2 evolution from amended soil in a specially designed biometer flask (4). To insure sufficient O_2 in the flask during decomposition of the amendments, CO_2 free air was circulated through the flask each time the base was changed and titrated. A set of flasks containing no soil samples was used as the control. All treatments were replicated three times, and each experiment was repeated twice.

RESULTS.—*Effect of cellulose amendments.*—Glucose utilization was similar in both cellulose-amended soil and raw soil during the first 6 days (Fig. 1). In the raw soil system, glucose was used at constant rate, becoming undetectable 13 days after application. However, in cellulose-amended soil, the rate of utilization of glucose was not rapid during 6 to 13 days but became undetectable after 20 days. Addition of KNO_3 soil amended with cellulose resulted in rapid rate of utilization of glucose. With KNO_3-amended soil, glucose was used even more rapidly than in raw soil, becoming undetectable after 3 days. The rates of utilization of glucose in soil amended with KNO_3 or cellulose with KNO_3 were similar.

In raw soil, there was a 39% reduction in detectable glutamic acid in 24 hr. Addition of KNO_3 to raw soil increased the rate of glutamic acid utilization (66% in 24 hr). The rate of utilization of glutamic acid in cellulose-amended soil (63% in 24 hr) was similar to that in KNO_3-amended soil (Fig. 2).

Carbon dioxide evolution from raw soil was minimal (Fig. 3). Addition of KNO_3 to raw soil did not alter the rate of CO_2 evolution. Cellulose-amended soil showed greatest activity 7-13 days after the soil was amended. Addition of KNO_3 to cellulose-amended soil lengthened this period of activity to 20 days.

The addition of glucose (5,000 ppm C) to soil produced large amounts of CO_2 through the first 9 days of this experiment. Since decreased activity followed, experiments were designed to determine whether carbon or N was limiting activity. The total CO_2 evolution from glucose-amended soil was similar to that from soil amended with both glucose and KNO_3. A second addition of KNO_3 (1,225 ppm) at 12 days to glucose-KNO_3-amended soil did not increase CO_2 evolution significantly, indicating that N was not limiting (Fig. 3). In contrast, addition of glucose (5,000 ppm C) to soil already amended with glucose for 12 days promoted increased CO_2 evolution indicating that carbon was limiting activity.

Effect of lignin-chitin amendments.—A second set of experiments designed to evaluate the effect of lignin and chitin amendments was analyzed with the same procedures used for the cellulose amendments. The rate of utilization of glucose was much more rapid in soils when chitin-lignin was added than in raw soil; glucose was not detected after 3-4 days (Fig. 4). Addition of KNO_3 with the chitin-lignin did not alter the rate of utilization of glucose significantly.

Glutamic acid utilization in the lignin-chitin-amended soil was much more rapid (71% in 24 hr) than its utilization in raw soil (39% in 24 hr, Fig. 5). Addition of KNO_3 to lignin-chitin amended soil did not alter the rate of glutamic acid utilization.

The evolution of CO_2 from lignin-chitin, lignin-chitin-KNO_3, chitin, and chitin-KNO_3 amended soils was initially rapid (Fig. 6). After about 11 days, the rate of CO_2 evolution decreased in all treatments, but was still greater than the rate for raw soil over the 29-day period. In all treatments, the addition of KNO_3 slightly inhibited the total amount of CO_2 evolved. This effect was more pronounced in the soil amended with chitin and with lignin-chitin than in soil with lignin (Fig. 6). Utilization of lignin and lignin with KNO_3 were intermediate.

A smaller amount of chitin (1,000 ppm C) produced more CO_2 than a larger amount of lignin (4,500 ppm C). For soils with chitin and chitin-KNO_3, the total CO_2 evolution was 1.330 mmoles and 1.086 mmoles, respectively. The total CO_2 evolved from soil amended with lignin and lignin-KNO_3 was 0.608 mmoles and 0.558 mmoles, respectively. In the case of lignin-chitin and lignin-chitin-KNO_3, the amount of CO_2 evolved was 1.913 mmoles and 1.700 mmoles, respectively. By summing the total CO_2 evolved for lignin-amended and chitin-amended soil (1.938 mmoles collectively after subtracting that evolved from raw soil, 0.331 mmoles/g, Fig. 3) a close relationship was found to the total CO_2 evolved from lignin-chitin amended soil (1.913 mmoles). Likewise for lignin-KNO_3-amended soil and chitin-KNO_3-amended soil the total was 1.644 mmoles, as compared to 1.700 mmoles for lignin-chitin-KNO_3-amended soil. The effect of these amendments on total CO_2 evolution appeared to be additive rather than synergistic, as would be the case if one of the amendments was favoring a population that could in turn better utilize the other amendment (18).

DISCUSSION.—Biological control by competition essentially alters the availability of essential nutrients in the rhizosphere without reduction in inoculum density (except when it influences survival), so that germination and penetration by a pathogen adjacent to the infection court is reduced (2, 3). Thus, it is of interest to know how rapidly these compounds are utilized in the rhizosphere under the influence of various organic residue treatments to ascertain the available supply of essential nutrients. The models in this paper attempt to demonstrate the rate at which glutamic acid and glucose, present in host exudates (15), may be utilized

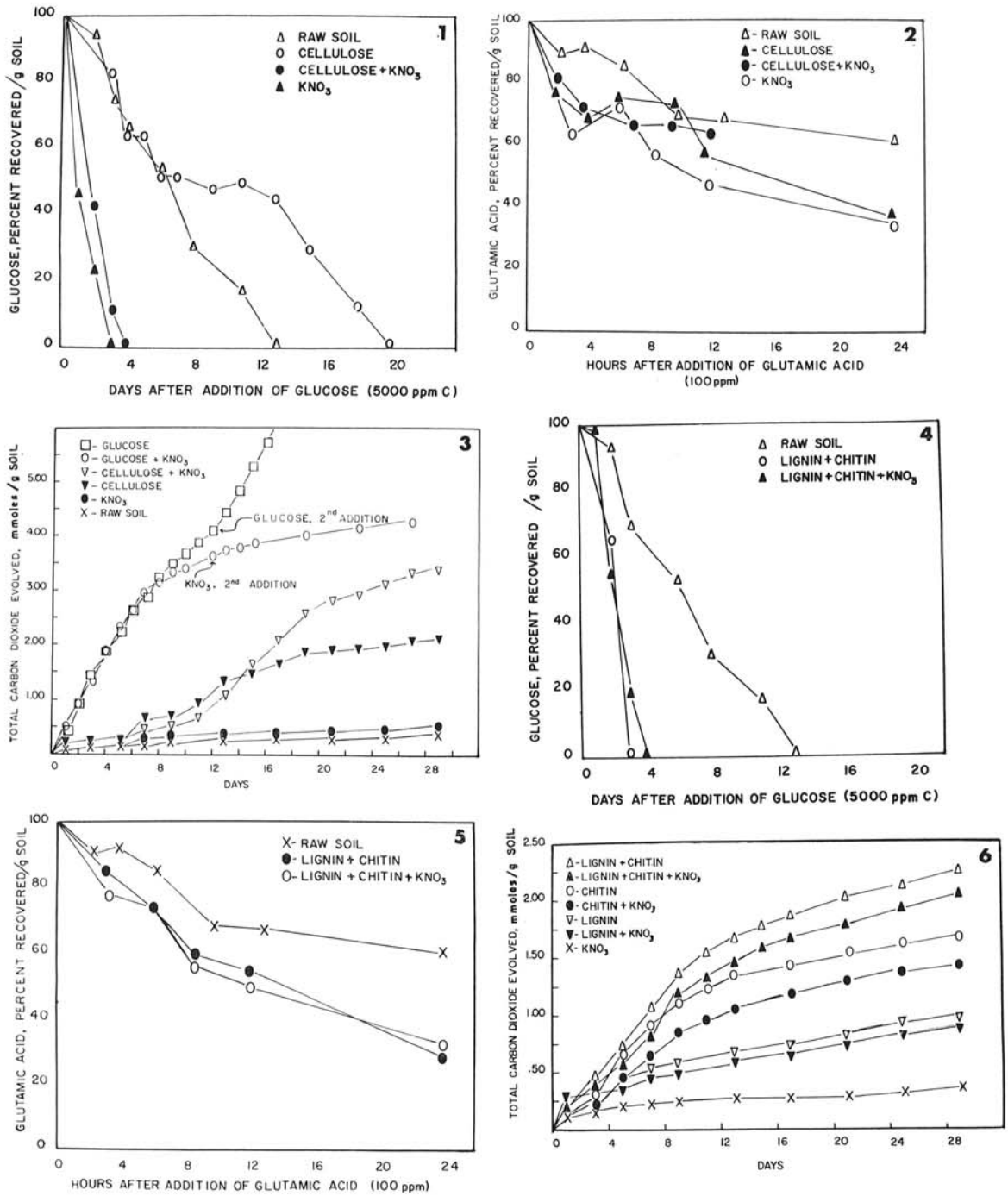

Fig. 1-6. **1)** Rate of glucose utilization in raw soil and soil amended with cellulose, cellulose-KNO_3, and KNO_3. **2)** Rate of glutamic acid utilization in raw soil and soil amended with cellulose, cellulose-KNO_3, and KNO_3. **3)** Total carbon dioxide evolution from raw soil and soil amended with glucose, glucose-KNO_3, cellulose, cellulose-KNO_3, and KNO_3. **4)** Rate of glucose utilization in raw soil and soil amended with lignin-chitin and lignin-chitin-KNO_3. **5)** Rate of glutamic acid utilization in raw soil and soil amended with lignin-chitin and lignin-chitin-KNO_3. **6)** Total carbon dioxide evolution from soil amended with lignin-chitin, lignin-chitin-KNO_3, chitin, chitin-KNO_3, lignin, lignin-KNO_3, and KNO_3.

when various treatments reported to promote biological control (12, 13) were applied. While compounds in the rhizosphere released by the host may be more constantly produced, these models may give an indication of the relative rates of utilization of various nutrients.

In biological control, one factor has been added to soil so that another essential nutrient is dissipated or immobilized (2). This was demonstrated in the control of bean root rot when soil N was immobilized within 7 days due to the addition of amendments with a C:N ratio over 25 (13). When this occurs, however, other compounds may not be utilized so rapidly. Glucose utilization was slowed 8 and 12 days after application to soil containing cellulose in contrast to raw soil (Fig. 1). There was a slight tendency for the reverse to be true soon after the glucose was added, which may confirm the observation of Adams et al. (1) that anthrone positive substances were decomposed more rapidly in cellulose-amended soil supplemented with glucose during a 10-hr period following addition of amendments.

When N (the limiting factor) was added with the cellulose, glucose utilization was more rapid and was not detected after 4 days. This was also true for glutamic acid which contains N in the molecule (Fig. 2).

Glucose or glutamic acid were rapidly utilized when only N was added to the soil (Fig. 1, 2). A small degree of control of bean root rot has been consistently noted with simple additions of nitrate N (13). This could be a result of more rapid utilization of simple essential carbon compounds in the rhizosphere, although an alternate or supplemental explanation is that the pathogen does not use the nitrate form of N as efficiently as other soil microorganisms (5).

Biological control with chitin-lignin amendments has been postulated to be due, at least partially, to competition for simple carbon substrates in the rhizosphere (12). In this paper, both glutamic acid and glucose were utilized more rapidly in the presence of chitin-lignin than in raw soil (Fig. 4, 5). Total CO_2 evolved also increased, suggesting a large population actively capable of competing for simple compounds like glucose and glutamic acid.

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