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Contribution of soil-borne bacteria to the rotation effect in corn

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Abstract

Few efforts have been directed at understanding how the rhizosphere microbiology of continuous corn may effect crop yields. This relationship may explain, in part, the decreases in yield associated with continuous corn as compared to the corn in rotation with a second crop. This study was conducted to determine the importance of soil-borne microorganisms to yield declines in long term continuous corn. Continuous corn (*Zea mays* L.) or rotated corn-soybean (*Glycine max* L.) field plots, established in 1975, under either fall plowing or no-till tillage treatments were used. Treatments consisted of methyl bromide applied at 48.8 g m^{-2} 3 days prior to planting in all four combinations. Total plant samples from both the fumigated and non-fumigated areas were collected 14 days after planting. Rhizosphere bacteria were recovered and tested for their ability to impact plant growth. Bacterial assessments were made in a test tube bioassay where germinated corn was transported in to agar containing a bacterial isolate. In the first year of the study a highly significant interaction of fumigation and rotation was indicated. With fumigation continuous corn yields were similar to that of rotated corn-bean. Rotated corn yields were less affected by fumigation. In the second year, the effects were similar but less significant. Over 130 bacterial isolates were tested for their effect on plant growth. Approximately 22% were able to inhibit plant growth. Of these, 72% were from the continuous corn system. Clearly, the interaction of rotation and yield is at a microbiological level. The suggestion that microorganisms similar to those isolated are responsible for controlling early plant growth in the continuous corn system is indicated.

Introduction

Long term continuous cultivation of a single species of crop plant can result in yield reductions when compared to the same species grown in a crop rotation. Following an 11 year field study, Griffith *et al.* (1988) reported that both crop rotation and tillage method inpacted corn yields; average yields in continuous corn rotation were suppressed by 0.52 or 1.21 Mg ha^{-1} compared to corn in rotation with soybean in either a plowed or no-till tillage system, respectively. Dick and Van Doreen (1985) reported a similar finding for a 20-year study. In both studies, poorly drained soils were more susceptible to continuous corn yield declines. A corn-soybean rotation on the same soils limit the degree of yield reduction for no-till planting. Using various corn rotations and N combinations, Baldock *et al.* (1981) showed that rotation effects are independent of N from the alternated crop and suggested some type of deleterious microbial involvement in the process.

The exact mechanism responsible for the yield reduction in continuous cropping systems is unclear. However, it has been attributed to allellopathic effects in corn have centered on allellopathic toxins derived from decomposing residues alleopathic effects in corn have centered on alleopathic toxins derived from decomposing residues (Bhowmik and Doll, 1982; Guenzi *et al.* 1967; Yakle and Cruse, 1984). Our efforts have shown that materials originating directly from corn residue decomposing under field or laboratory conditions is of limited consequence to corn growth

(Breakwell and Turco, 1990). This indicates that the rotation effect is more likely an adjustment of the soil-root environment and the organisms present. These organisms, in turn, influence plant growth. In continuous wheat and sugar beets, yield reductions have been attributed to a build up of deleterious rhizobacteria (Fredrickson and Elliott, 1985; Suslow and Schroth, 1982). These deleterious bacteria, primarily Pseudomonas, are thought to lessen plant vigor, shortened root length and increased the plants sensitivity to fungal pathogens.

O'Sullivan and Reyes (1980) reported that the use of methyl bromide (MB) as a fumigant in soils continuously planted to a single plant species, simulated the effects of a crop rotation. Use of MB serves as a non-selective biocide that reduces the overall microbial population. Fumigation reduces resident microbial activity, without altering materials (toxins) that may be present. In essence it provides a way to fractionate active effects from effects such as toxin build up that might occur during residue decomposition. Because methyl bromide fumigation is non-selective, qualifications of changes in specific populations are generally not done. However, Cook and Baker (1983) showed that fumigation in dry soil will result in up to a 3 log unit reduction in both bacteria and fungi. Millhouse and Munnecke, (1981) reported a significant reduction in soil fungi and *Pseudomonas* sp. after 16 hr of exposure to MB. Other organisms were less susceptible, reductions took as long as 64 hr to occur.

The importance of the microbial population as related to rotation of corn is unclear. How deleterious the rhizosphere microorganisms are to the growth of corn is also unknown. This report presents the findings of a study that shows yield reductions in continuous corn may reflect deleterious microbial activity. One aspect of this reduction may be the contribution of Pseudomonas.

Methods

Field site

The long term tillage plots (Griffith *et al.* 1988) at the Purdue Agronomy farm were utilized in all studies. Briefly, the plots have been maintained in continuous corn or soybean or rotated corn-soybean for 12 years, and are replicated 3 times. The soils are a nearly level, tile drained Chalmers silty loam, (fine-silty, mixed, mesic Typic Haplaquoll). Organic matter content in the surface 30 cm is 40 g kg^{-1} (Griffith *et al.*, 1988).

We considered only the continuous corn and rotated corn-soybean (corn portion) cropping sequence under either fall plow or no-till tillage. Fall moldboard plowing to 20 cm followed by one disking and one field cultivation to 10 cm in the spring, was conducted prior to either fumigation or planting. A 2.5 cm wide fluted counter to cut an opening in the standing residue was used to plant no-till corn (Griffith *et al.*, 1988). Fumigation was through the standing residue in the no-till plots. Becks brand '65X' corn seed was used in field as well as laboratory portions of the study. Detailed description of fertilizer, and pesticide application is given by Griffith *et al.* (1988).

Fumigation

Three days prior to scheduled planting in 1986 and 1987 a 27.8 m^2 area that would contain four rows of corn, was trenched on four sides and covered with heavy weight clear plastic. The cover was held in place by returning the soil to the trenches. Bromagas containing 98% methyl bromide along with 2% chloropicran (Great Lakes Chemicals, W. Lafayette In.) was injected under the plastic using a hand-held injector. A final concentration of 48.8 g bromogas m^{-2} was achieved. The injector tubes were removed and plastic covers left in place until planting. In 1987, a rain occurred during cover removal and planting was delayed two days.

Yield was determined by hand harvesting the middle two rows of the fumigated areas. Responses were evaluated by comparing areas to a non-fumigated hand harvested area. Statistical analysis (SAS, Systems Inc.) was made with rotation, tillage and fumigation as main effects.

Bacterial enumeration and isolation

In order to quantify the presence of deleterious resident microflora, bacterial isolates were

recovered from corn rhizosphere in both years. Corn plants were collected from no-till and plowed continuous, as well as rotated corn-soybean plots 14 days after planting. In both years plants (15–20 cm tall) were removed at random from the non-fumigated area of the plots. In order to assess the effects of fumigation in 1986, plants were also removed from the fumigated areas. Rhizosphere soil was collected and the plants transferred to plastic bags. Bulk soil and surface residues were collected from random sites in each plot.

The rhizosphere (rhizoplane) was established by removing loose soil by shaking and washing the roots with a stream of water. Roots from two plants were transferred to 99 ml sterile water blanks and shaken for two hours. Roots were strained from the dilution bottle and dried in a forced air oven (60°C) for 24 hr and weighed. A serial dilution onto Sands and Rovira and Kings B media (Sands and Rovira, 1970) was carried out. The plates were incubated at 25°C for 24–48 hr and the colonies counted. Bacterial counts are expressed on a gram-root basis.

Bulk soil (10 g dry weight equivalent) was serially diluted onto Sands and Rovira and Kings B media. The plates were treated as above. Bacterial counts were expressed on a gram dry weight soil basis. Residue was extracted in a manner similar to the soil.

Isolates from either rhizosphere, bulk soil or residue were selected at random from the plates and transferred to 40% glycerol and stored at -10°C.

Bioassay

Isolates were recovered from the glycerol by streaking onto Sands and Rovira agar and were assumed to be *Pseudomonas* sp. (Elliott and Lynch, 1984). Pure colony isolates were recovered and spread on to a second plate of Sands and Rovira. The plate was grown for 24 hr at 25°C. The cells were transferred into sterile water, washed once and diluted to an optical density of 0.2 (660 nm) corresponding to a population of 1.19×10^8 cfu ml^{-1}. Two milliliters of the cell suspension were transferred to a 20 × 2.5 cm test tube and 38 ml of molten (45°C) 0.9% agar (Difco). The tubes were swirled and allowed to solidify. Final concentration of cells was approximately 5.95×10^6 cfu ml^{-1}.

Using a procedure similar to Elliott and Lynch (1984) Beck 65X corn (with captan coating) was pregerminated (30 h) on moist, sterile, filter paper in petri dishes maintained at 30°C. Pregerminated seeds with approximately the same length radical were placed into the solidified agar. The tubes were loosely covered with a plastic film and transferred to a growth chamber. The chamber was maintained at 18°C on a 12 h photoperiod. Controls were formulated using sterile water and no bacteria. Ten corn replicates were used for each bacterial isolate.

Following a ten day incubation, the entire plant was harvested from the agar using forceps. The tap root was measured and the seed piece, roots and shoots separated from each other. The roots and shoots were dried in a forced air oven (60°C) and the weight determined.

Isolate toxin production in corn residue

Following initial testing, strain 1040 was identified as able to reduce root growth to less than 50% of control. Its ability to produce a toxin when grown on a corn residue was assessed in sterile and non-sterile residue. The sterile residue was formulated by exposing 90 g of dry corn residue to 2 Mrad ^{60}Co. Residue was divided between individual containers, allowed to wet with sterile water overnight ($4\,g\,H_2O\,g^{-1}$ residue) and inoculated (4×10^{-7} cfu g^{-1} residue). As a comparison, non-inoculated residue was included. The containers were incubated at 26°C with variable or constant moisture. The variable moisture condition was imposed by allowing the sample to undergo repeated wet and drying cycles. The variable samples were returned to their original wet weight at each sampling. Samples were destructively taken biweekly by adding 100 ml of cold water to the containers and shaking for 1 h. The extracts were filtered (0.22 μm) and 2 ml used in a plant bioassay. The study was repeated three times.

Results

Bacterial counts and fumigation

Kings B agar, less restrictive than Sands and Rovira (SR), gave higher recoveries of bacteria in both years (Table 1). After 14 days of growth,

Table 1. Bacterial counts (log CFU g^{-1} root) from corn rhizosphere 14 days after planting

Rotation/ tillage	Year			
	1986		1987	
	SR[a]	KB	SR	KB
CC-p[b]	8.4(6.8)[c]	9.3(8.1)	7.5	8.8
CC-n	9.7(9.3)	9.6(9.2)	nd[d]	nd
CB-p	8.5(7.2)	8.9(8.0)	7.8	9.3
CB-n	6.7(bd)[d]	7.3(bd)	nd	nd

[a] SR = Sands and Rovaria agar; KB = Kings B agar.
[b] CC = Continuous Corn; CB = Corn-Soybean; p = plowed; n = notill.
[c] Bracketed values for fumigated treatments.
[d] nd = not determined; bd = below detection limit.

rhizosphere populations from the fumigated soils remain suppressed, as indicated by either Kings B or SR agar (Table 1).

Fumigation, corn yield and rotation

Hand harvesting of the subplot areas had no significant effect on yield, relative to the larger machine harvested areas (Table 2). Our hand harvested fields compare favorably to the 12 year average (Table 2). For the 1986 data, statistical analysis indicated fumigation significantly ($p < 0.05$) increased yield (Fig. 1a) in the continuous corn systems. The fumigation affect was partitioned to a significant ($p < 0.05$) interaction with rotation and no effect from tillage. For both the no-till and tilled system, fumigation increased average yields 2.84 and 0.43 Mg ha^{-1} for the Corn-Corn and Soybean-Corn sequence, respectively. The 1987 yields on the rotated plots also interacted significantly with the fumigation process and no effect from tillage was indicated. However, the level of significance ($p < 0.1$) was lower. This can be attributed to high coefficients of variation among the average yields for the two harvested rows and may reflect the rain forced delay in planting. In 1987, fumigation increased average yields 0.73 Mg ha^{-1} on the continuous corn plots. This contrasts with the average 0.74 Mg ha^{-1} decrease on the rotated corn plots (Fig. 1b).

Table 2. Effect of harvesting method, crop rotation and tillage on yield (Mg ha^{-1})

	1986		1987		12-Yr[a] ave
	Mach[b]	Hand	Mach	Hand	Mach
No-till					
Continuous corn[c]	9.4	9.1	10.2	10.0	9.6
Rotated corn[d]	11.2	11.4	11.4	11.6	10.8
Plowed					
Continuous corn	10.6	9.1	10.9	10.4	10.6
Rotated corn	12.0	12.4	11.7	11.5	11.2

[a] 12-year average corn yields for Purdue Tillage Study (Griffith and West, unpublished).
[b] M = average for machine harvested four middle rows of the overall plot (Griffith *et al.*, 1988). Machine harvested data for 1987 from Griffith and West, unpublished; H = hand harvested two middle rows from fumigated area.
[c] Continuous corn for 11 or 12 years, respectively.
[d] Rotated corn to soybean, in the corn portion.

Bacterial isolates

Over 130 bacterial isolates from two years of field investigations were evaluated using the bioassay system. Because of the varied sources and times over which the isolates were taken, their impact on plant growth was assessed at a level of 50% reduction from control indicating a significant effect. At this level, approximately 5% of the tested isolates reduced shoot weight while between 10 and 16% effected the rooting system (Table 3). It is interesting to note that over 33% of the isolates enhanced either the roots or shoot formation. The majority of

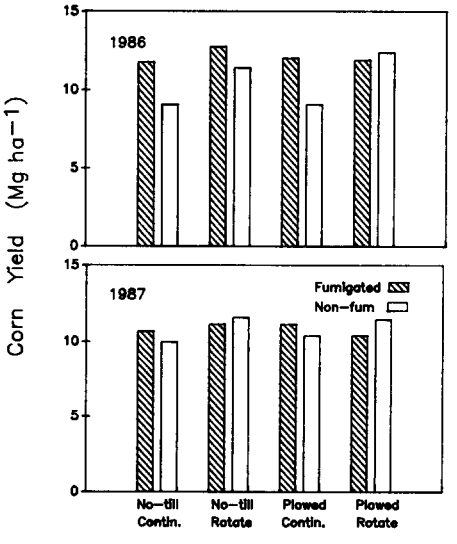

Fig. 1. Effect of fumigation, rotation and tillage on yields of corn for years 1986 (**a**) and 1987 (**b**).

Table 3. Effect of bacteria on plant response

Control[b] (%)	Plant response (%)[a]		
	Root length	Root weight	Shoot weight
0–25	4.93	0.61	0.61
26–50	12.96	9.87	4.32
51–75	18.51	13.58	12.96
76–100	30.24	39.50	43.82
> 100	33.36	36.44	38.29

[a] % of the tested isolates capable of affecting plant parts as compared to control. Isolate source: 36.4% from Rhizosphere, 56.1% from bulk soil and 7.4% from residue. Isolate source: 61% collected in 1986, 38.8% in 1987.

[b] Percentage of control, 0 = no growth; 100 = growth equal to control.

the isolates lessened the growth, but not to the established threshold. Of the isolates that inhibited root to the 50% level, 72% were recovered from the continuous corn system.

Inoculation of residue materials with strain 1043, previously shown to inhibit root growth to greater than 50%, did not result in production of toxic materials under any of the test conditions. Reduction in root growth from the viable cell inoculation was limited to 20% of the control.

Discussion

The interaction of crop yield and rotation has been established. Voss and Shrader (1984) as well as Langer and Randall (1981) have suggested that the contribution of rotation is not entirely from N-fixation, but results from a change in plant species and most likely the associated rhizosphere. They attributed the difference to either reductions in autotoxic substances coming from the residue or to disease reductions. Our work, (Breakwell and Turco, 1989) has shown that the influence of residue source materials has a limited impact on corn growth. This implies that factors other than a reduction in residue decomposition allelopathic substances result in a rotation effect.

Soil fumigation acts in a non-specific manner to reduce microbial populations in soil. Sullivan and Reyes (1980) demonstrated that following fumigation, potatoes grown in continuous cropping systems responded as if they were in a crop rotation. They attributed the effect to a reduction in Verticillium wilt. In our plots, corn lacked any indications of obvious plant disease. However, the detection of sub-clinical or low level infection is difficult even though significant reductions in yield may result (Suslow and Schroth, 1982). Fredrickson and Elliot (1985) isolated from fields that showed no apparent plant disease, a root-colonizing Pseudomonas capable of reducing root growth. They indicated that bacteria may contribute to a disease complex.

While fumigation randomly suppresses the organisms present, the effect is transient since the number of bacteria in soil samples taken from the fumigated area after one month, had returned to normal (data not shown). As microbial numbers remained suppressed up to 2 weeks following fumigation, increased yields in corn following fumigation may reflect an enhanced early reason root vigor. Suslow and Schroth (1982) had indicated that rhizosphere colonization by deleterious bacteria within the first 4 weeks of plant growth impacted plant yield. It is clear from even our limited collection of bacterial isolates that the potential for microbial reductions in root vigor, resulting in an increased opportunity for disease, is indicated for continuous corn.

For the virulent strain tested, toxin production is not enhanced by growth in plant residue materials. This contrasts the work of Stroo *et al.* (1988) who had reported increased toxin from Pseudomonas into wheat straw (assessed using a bacterial indicator). They indicated that maximum production of toxin was found when the bacteria was in log phase. This may indicate that for corn, active rhizosphere colonization is needed for toxin production.

Clearly, methyl bromide is not a selective inhibitor of Pseudomonas. Secondly, we are not suggesting that Pseudomonas are the only microorganism contributing to the rotation effect. Rather the fumigant retards biological activity. Toxins that are present in the system at fumigation, as would be the case with residue source materials, would still be expressed. Expression of biological processes in the active rhizosphere would be suppressed following fumigation. Our findings show that in combination with other factors, Pseudomonas may contribute to declines reported in continuous corn systems much like the declines reported in continuous wheat (Fredrickson and Elliott, 1985). These declines are associated with active microbial processes in the rhizosphere, not a build up of residue source toxins

in soil. Baldock *et al.* (1981) showed clearly that rotation effect in corn was not related to nitrogen. The rotation effect appears to result from shifting the type of rhizosphere microorganisms in the system.

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