Relationship between a greenhouse and field assay for biological control of common root rot of spring wheat and barley

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Bacteria and fungi, isolated from coleoptiles of seedlings of spring wheat and barley collected from commercial fields in Saskatchewan, were evaluated as seed treatments to reduce common root rot. There was no relationship between greenhouse and field trials in effectiveness of isolates in controlling common root rot. Some isolates reduced symptoms in the field, with the best showing a 61% decrease in disease compared to the control. Some isolates also reduced symptoms in the greenhouse assay. As currently described, the greenhouse assay is unsuitable as a screening technique to evaluate potential efficacy in the field.

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Des bactéries et des champignons, isoles a partir de coleoptiles de semis de ble de printemps et d'orge cueillis dans des champs commerciaux de la Saskatchewan, ont ete evalues comme traitement de semence en vue de reduire le pietin commun. Aucun lien n'a ete etabli entre les essais faits au champs et ceux en serre pour ce qui est de l'efficacite des isolats dans la lutte contre le pietin commun. Certains isolats ont reduit les symptômes au champ et, au mieux, ont fait chuter les cas de maladie jusqu'a 61 % comparativement aux sujets temoins. Des isolats ont egalement reduit les symptômes dans les essais en serre. Selon les donnees que nous possedons, l'essai en serre ne convient pas en tant que technique de depistage pour evaluer le rendement au champ.

Introduction

In the Canadian prairies, common root rot, caused mainly by *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur, anamorph *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., has been estimated to annually reduce yield of spring wheat (*Triticum aestivum* L.) by 6% *(5)* and spring barley (*Hordeum vulgare* L.) by 10% **(9).** Antagonistic microorganisms have been evaluated as biological controls for this disease. Porter (10) reported a reduction of disease in aseptically grown seedlings with a bacterium that also inhibited the growth of C. *sativus* in culture. Later reports indicated that some bacteria and/or fungi were antagonistic to C. *sativus* in vitro (1,2,4,8,11), but these organisms were not evaluated in the field.

Although several reviews indicate organisms selected from in vitro tests generally fail as biological control agents under field conditions (6,7), a rapid and reliable screening test would permit an efficient evaluation of large numbers of microorganisms. The purpose of this study was to compare the results of greenhouse screening tests with field evaluations to determine if a greenhouse test is a suitable assay.

Materials and methods

Fungi and bacteria were isolated from plants collected from commercial spring wheat or spring barley fields in Saskatchewan. In each field, plants were removed from four sites 10 m apart. In **1990,** plants were collected at the two-leaf to tillering stage (June 6-21) from 79 fields. In **1991,** plants were collected over the same growth stages (June **5–19)** from 44 fields. The coleoptiles were removed from the plants, washed in running tap water, and surface treated for 2 min in 0.6% sodium hypochlorite, and rinsed twice in sterile distilled water. For the isolation of fungi, coleoptiles were plated on Difco Potato Dextrose Agar (PDA) supplemented with 100 mg/L streptomycin sulphate and 50 mg/L of vancomycin hydrochloride. Plates were incubated at 20°C in the dark for 2 to 6 days and then hyphal tips of randomly selected fungi were transferred onto slants of PDA. Cultures of known pathogens and of mucoraceous fungi were discarded and 177 isolates from the 1990 collection and 101 isolates from **1991** were retained for the assays.

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Bacteria were isolated from coleoptiles by grinding ten washed coleoptiles in 2 mL of sterile distilled water in a glass tube and pestle tissue grinder. The homogenate was poured into 100 mL of sterile distilled water, mixed by shaking and 4 mL was pipetted into 100 mL of warm molten Difco Nutrient Agar (NA). The agar-homogenate suspension was poured into six plates. After one or two days of incubation at 20°C, individual randomly selected colonies were transferred to NA slants. Eighty-two unidentified bacterial isolates were selected from the 1991 plant collections and maintained for the assays.

For the greenhouse assays, inocula from the fungal slants were placed on PDA plates and incubated for about four weeks. Most of the fungi produced myceliogenic colonies. A 2.5 mL aliquot of 2% methocel and 80 seeds of the spring barley cultivar cv. Melvin were added to a single plate of each isolate. The surface colony was scraped with a spatula and the fungal suspension was mixed with the seeds. Bacterial isolates were grown for about one week on NA plates and 80 barley seeds were added to each plate and stirred with a spatula. The seeds inoculated with the fungal or bacterial suspensions were air dried for 16 h before planting. Twenty seeds were placed at a 6 cm depth in 13-cm diameter pots containing a soilless mix (12) and covered with a 6 cm layer of the *C. sativus* inoculated sand and soilless mix. The *C. sativus* culture was isolated from a naturally infected subcrown internode of wheat. The pathogen was incubated for 10 or more days on minimal medium agar (13) in 9-cm petri plates. A 0.1% Tween 20 solution was added to each plate and the colony was scraped with a bent glass rod. The suspension was filtered through two layers of cheesecloth and the spore concentration estimated with a haemocytometer. Conidia were sprayed onto a soilless mix and sand (2:1 ratio) mixture as it was being tumbled in an electric cement mixer to a concentration of 200 *C. sativus* conidia/cm^3. There were four replicate pots of each test isolate. Because of space limitations, 20 isolates were screened concurrently, along with controls (untreated or methocel treated seed) in a greenhouse at 15–25°C with 16 h of natural light supplemented with high pressure sodium lighting at 9000 lux. After five weeks, disease symptoms on subcrown internodes were rated using the Horsfall-Barratt rating system (3).

The viability of isolates on seeds was only determined for some of the isolates by plating treated seeds on PDA for fungal isolates and NA for bacterial isolates. Only isolates which grew from all seeds were retained for further testing.

From the 1990 collection, 24 fungal isolates which showed a wide range of reaction$ in the greenhouse assays were selected for testing in the field in 1991 and of the 1991 collection, all 101 isolates were tested in the field in 1992. All 82 bacterial isolates from the 1991 collection were tested in the greenhouse and then in the field in 1992.

For the 1991 field test, 3 mL of 2% methocel along with 250 seeds of the spring wheat cv. Leader were added to each of two plates of each fungal isolate. The seeds were coated as previously described, air-dried for one to three days and 125 seeds were planted on May 30 in a single 2-m row plot. There were four replicates of seeds inoculated with each organism in a completely randomized block design. Seed was planted about 6 cm deep in a common root rot disease nursery site at the Saskatoon Research Centre farm. Untreated seed and methocel treated seed were planted as controls. At the firm dough stage (August 26–29), subcrown internodes of 40 plants per plot were rated for disease. Disease reaction was expressed as the percentage of plants with more than 50% of the subcrown internode covered with brown lesions typical of *C. sativus.* A similar test using seeds of the barley cv. Melvin was included in the field assays.

For the 1992 field test, only the wheat cv. Leader was used. Because of the large number of isolates, both the fungal and bacterial isolates were divided among three tests, each with an untreated control and an additional methocel control in the case of the fungal tests. Planting occurred on May 14, 1992 and subcrown internodes were rated August 13–26 at the soft dough stage.

Data were analyzed using the analysis of variance procedure and the least significant difference was used to determine isolates which differed significantly from the control (SAS Statistics, Version 6.07, SAS Institute, Box 8000, Cary NC 27511-8000). Isolates of both fungi and bacteria were then ranked based on the percentage control over the untreated control for bacteria and the methocel control for fungi. Values obtained from the greenhouse tests were compared with the results from the field tests using Pearson product-moment correlation.

Results and discussion

In the greenhouse assay, values for disease severity varied from 6 to 143% of the methocel control for the 1990 collection of fungal isolates (Fig. 1). For isolates 441A, 441B, 443-2, 443A, 444C, 445A, 447A and 471, disease severity was significantly ($P=0.05$ or 0.01) lower than the methocel control. In the 1991 field tests, disease severity values ranged from 68–128% of the control on wheat and from 89 to 99% on barley. There were no differences between the disease rating of the methocel control to that of any of the isolates in the field tests. The correlation between the greenhouse barley assay and the field test was -0.274 for wheat and 0.027 for barley ($n=24$). These correlations were not significant.

This study and others (Duczek unpublished) show a narrow range of disease reaction to C. *sativus* on barley in the field relative to wheat. In contrast, disease reaction in the greenhouse was more consistent on barley, and barley checks had higher levels of disease than wheat checks. For this reason in further studies, barley was used in the greenhouse and wheat in the field.

In the greenhouse assay, values for disease severity ranged from 27 to 165% of the methocel control for fungal isolates from 1991 (Fig. 2). Disease severity for 17 isolates was significantly (P=0.05) lower than the methocel control and for three isolates disease severity was significantly higher. Significance and level of significance does not appear consistent by rank in Fig. 2 perhaps because isolates were measured in several separate tests. For example, isolate 541, which was used in one test, was not significantly lower than the control, whereas isolate 546, which was used in another test, was significantly lower but its value was higher than for isolate 541. In the field tests of the same isolates, the disease severity range was 39 to 211%. For one isolate, 544A, disease levels were significantly (P=0.05) lower than the methocel control and for seven isolates significantly higher. Disease was significantly reduced compared to the methocel control in both the greenhouse and the field tests for only isolate 544A. For isolates 528A and 543, disease was significantly reduced in the greenhouse compared to the control but significantly increased in the field. The correlation between the greenhouse assay and the field test was -0.061 (n=101). This correlation was not significant. The overall mean disease rating of the methocel control in all the greenhouse tests was 43 and in the three field tests it was 24. The difference between the untreated and the methocel treated control was not significant.

For the bacterial isolates, disease severity values ranged from 42 to 151% of the controls in the greenhouse assay and from 51 to 191% in the field (Fig. 3). For six isolates disease severity was significantly (P=0.05) lower and for one isolate significantly higher than the control in the greenhouse assay. In the field, only isolate B553B, was significantly lower than the control and three isolates were significantly higher. For two isolates, B554B and B562, disease levels were significantly lower than the control in the greenhouse but significantly higher in the field. The correlation between the greenhouse assay and the field test was -0.144 (n=82). This correlation was not significant. The mean disease rating of the methocel control overall greenhouse tests was 43 and overall three field tests it was 28.

To confirm the causal organism in the field tests, five discoloured subcrown internodes were taken from randomly selected plots in each test; C. *sativus* was isolated from over 90% of the internodes.

Candidate organisms for biocontrol were isolated from the coleoptiles of young plants, since this is the site where interactions will determine success of primary infections by C. *safivus*. Some of the isolates of bacteria and fungi significantly decreased common root rot symptoms in the greenhouse. However, there was no relationship between the reaction in the greenhouse with that in the field, indicating that the greenhouse assay was unsuitable as a primary screening technique. In the field, some isolates also reduced common root rot and the lowest rating in 1991 was 68% of the control in 1991, while in 1992 it was 39%. Based on these results, all future screening will be done in the field until an appropriate greenhouse or laboratory test is developed.

Acknowledgements

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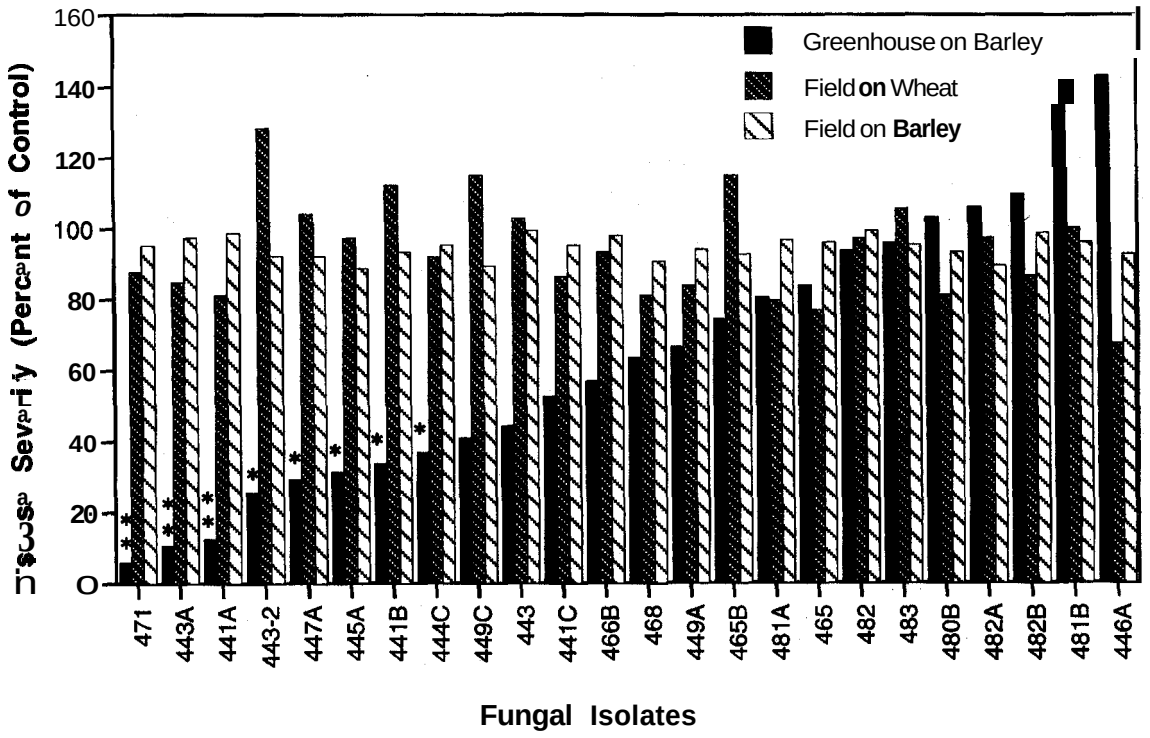

Figure 1. Effect of fungi isolated from cereal coleoptiles in 1990 on common root rot of wheat and barley in the greenhouse, and in the field in 1991. Fungi were applied to the seed. Asterisks over columns indicate a significant difference from the methocel control (* indicates P=0.05, ** indicates P=0.01).

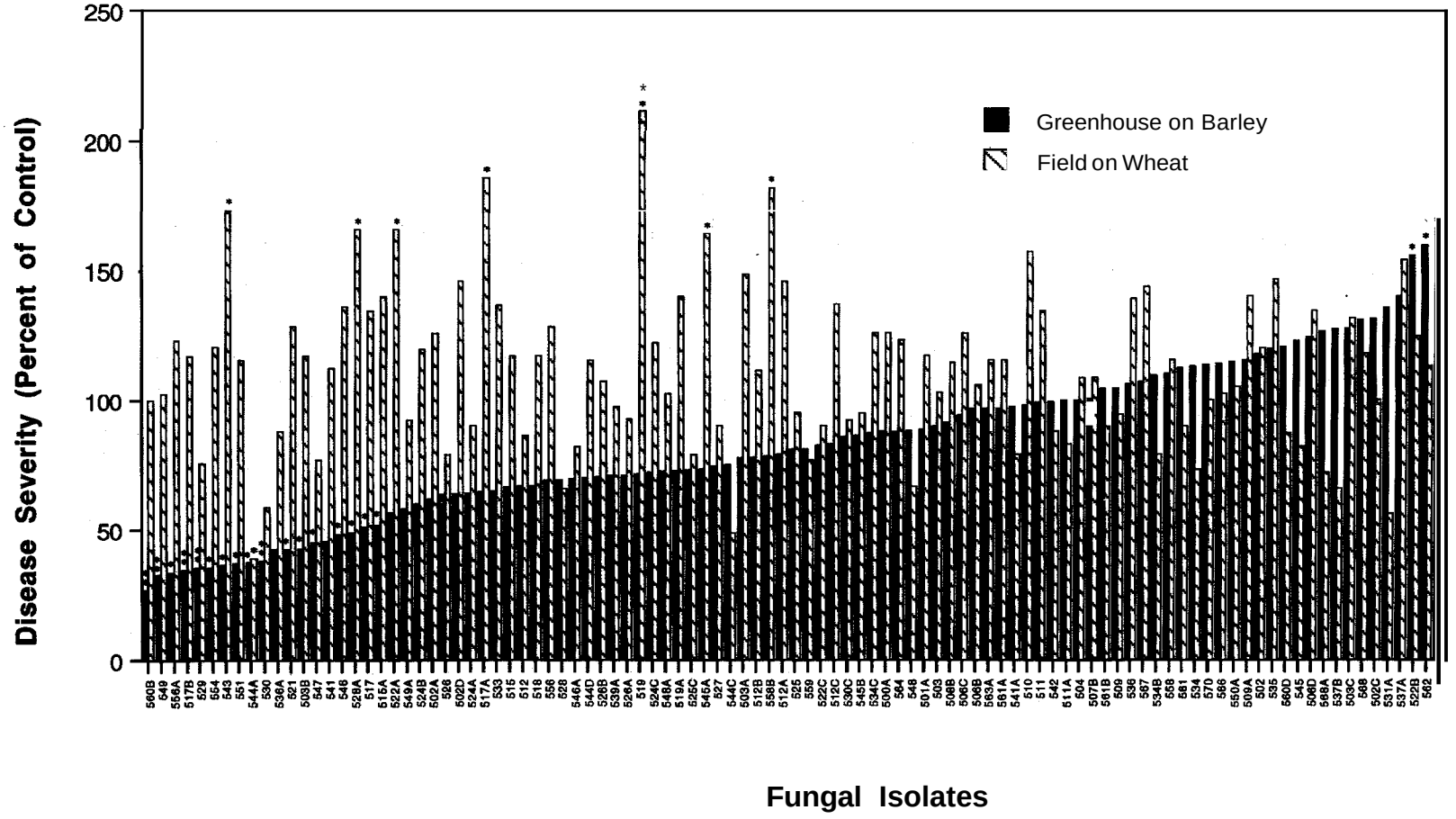

Figure 2. Effect of fungi isolated from cereal coleoptiles in 199 on common root rot of wheat and barley in the greenhouse, and in the field in 1992. Fungi were applied to the seed. Asterisks over columns indicate a significant difference from the methocel control (* indicates P=0.05, ** indicates P=0.01).

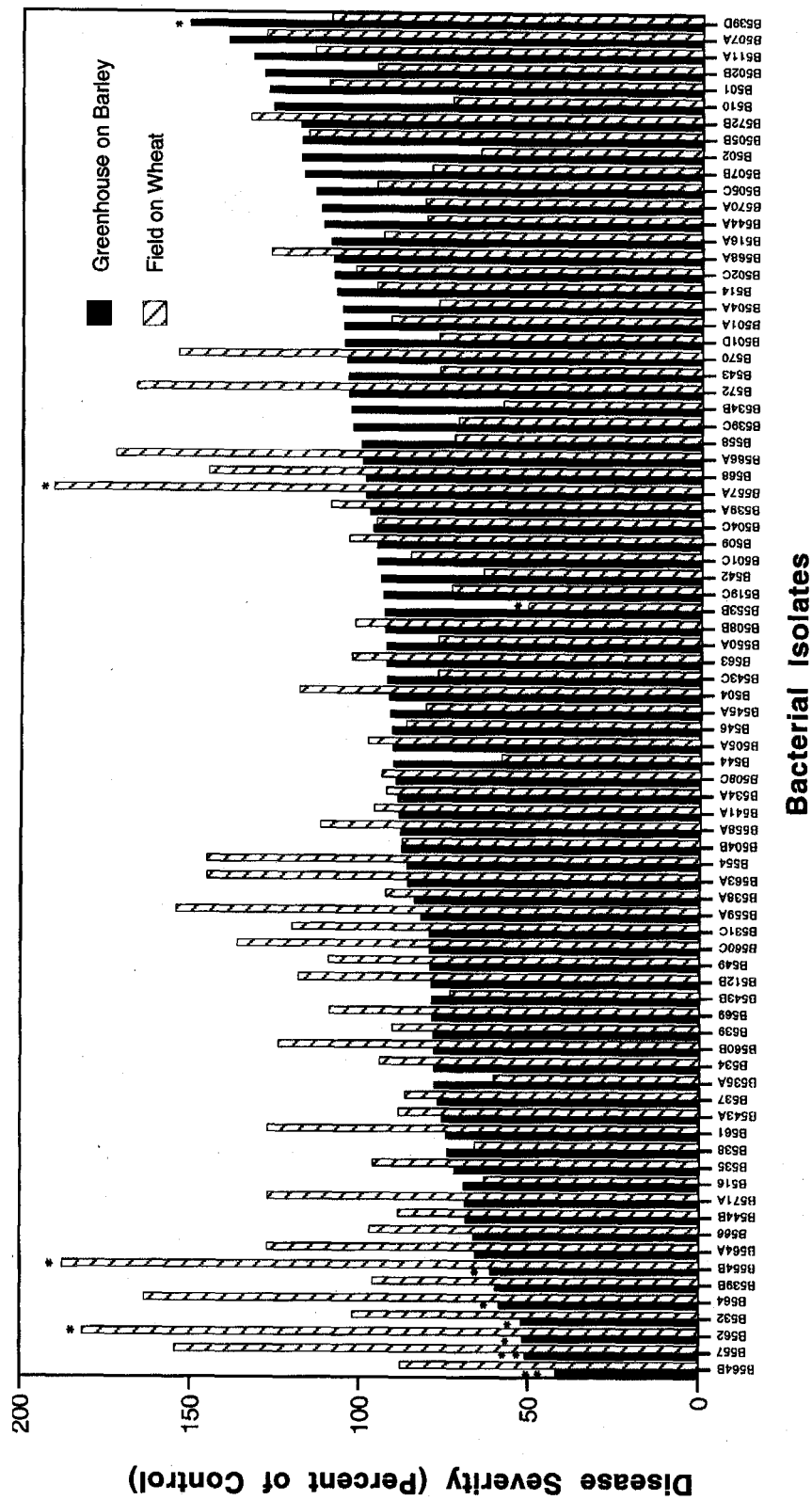

Figure 3. Effect of bacteria isolated from cereal coleoptiles in 1991 on common root rot of wheat and barley in the greenhouse, and in the field in 1992. Bacteria were applied to the seed. Asterisks over columns indicate a significant difference from the untreated control (* indicates P=0.05, ** indicates P=0.01).