The accuracy of identifying *Bipolaris sorokiniana* conidia extracted from soils in Saskatchewan'.

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The assignment of Bipolaris-type conidia extracted from Saskatchewan soils using the flotation technique, as *B.* sorokiniana, was 99% reliable. Single spore isolates of Bipolaris-type conidia obtained from naturally infested soil in Saskatchewan were compared to single conidium isolates of 6. sorokiniana derived from colonies growing out of naturally infected subcrown internodes of wheat. With two exceptions the severity of spot blotch symptoms on Betzes barley as well as length of conidia was similar for isolates from each source. Of 181 isolates from soil, 179 were 6. sorokiniana and the two exceptions were identified as Curvularia spicata.

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L'identification comme Bipolaris sorokiniana des conidies de types Bipolaris extraites d'echantillons de sol de la Saskatchewan à l'aide d'une technique de flottaison, s'est révélée exacte à 99%. Des isolats de conidies uniques de type Bipolaris extraites de sol infecté naturellement en Saskatchewan ont ete compares à des isolats de conidies uniques de 6. sorokiniana provenant de colonies croissant sur du ble infect6 naturellement au niveau des entrenoeuds sous le collet. Sauf pour deux exceptions, la severite des symptômes de tache helminthosporienne sur l'orge Betzes, de même que la longueur des conidies se sont averees similaires quel que soit la provenance des isolats. Des 181 isolats de type Bipolaris, 179 se sont révélés être 6. sorokiniana tandis que les deux exceptions ont été identifiees comme etant Curvularia *spicata*.

Introduction

Bipolaris sorokiniana (Sacc. in Sorok.) Shoem. (syn. *Helminthosporium* sativum Pamm., King & Bakke; H. *sorokinianum* Sacc. in Sorok.), perfect stage *Cochliobolus sativus* (Ito & Kurib). Drechsl. ex Dastur, is the main cause of common root rot of wheat and barley in the Canadian prairies. Primary infections are initiated generally by soil-borne conidia. Ledingham and Chinn (14) developed a technique to extract conidia from soil and then modified it to determine conidial viability as well as total population (7). This technique, or one slightly modified, was used to study the relationship between conidial population and severity of common root rot (8), the conidial population in different soil profiles (9), the change in conidial populations in soil over time (1), the change in conidial populations following rapeseed (3) and cereal crops (4), and the effect of fungicide sprays on conidial populations in soil (5).

The procedure involved shaking soil, oil, and water in a tube, pipetting an aliquot of the oil-emulsion phase containing conidia onto a slide, counting and then determining the number per gram by using the appropriate dilution factor. Conidia conforming morphologically to that of **6.** *sorokiniana* were identified at 50-100X using a compound or binocular microscope. This technique has also been used to recover conidia of **6.** oryzae (Breda de Haan) Shoem. (16) and large spores of other fungi (2, 7, 14). The similarity of *B. sorokiniana* conidia to those of other multi-septate dematiaceous hyphomycetes could result in inaccurate counts if a mixed population was extracted. Therefore, the purpose of this study was to determine the accuracy of assigning all the Bipolaris-type conidia extracted from soil as **6.** *sorokiniana*. Cultures derived from single conidia extracted from naturally infested soil were compared to cultures originating from single conidium isolations made from naturally infected plant tissue. The two variables used were conidial length and the production of leaf spot lesions on barley. These were chosen because this fungus, in addition to causing symptoms on subcrown internodes, crowns, and basal stems, causes spot blotch on leaves of cereals, and conidial size is one of the important criterion used in separating species of dematiaceous hyphomycetes (11).

Materials and methods

Conidia were recovered from soil by the oil-flotation method (9). Three or four millilitres of the oil emulsion layer were mixed with a sufficient amount of warm melted potato dextrose agar (Difco), supplemented with 1% molasses, 100 ppm streptomycin sulfate, and 50 ppm vancomycin hydrochloride, to cover the bottom of a 9 cm diameter plastic petri plate. After incubation for 8-16 hr at 20-22°C, single germinated Bipolaris-type conidia, identified with a binocular microscope at 50-100X, were plated on minimal agar medium (18). After a further 16-24 hr, hyphal tips were transferred to the medium in test tubes. Each culture originated from a separate single conidium. Isolates from a plant source originated from single conidium cultures obtained from B. *sorokiniana* colonies growing from naturally infected subcrown internodes of wheat plated on minimal agar medium (12). Isolates from both sources were from various locations throughout the agricultural area of Saskatchewan. Soil was collected in 1979 and 1980, and plants (internodes) were collected in 1979.

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For the leaf spot test, 15 seeds of Betzes barley (*Hordeum vulgare* L.) were planted 2 cm deep in soil-free mix (17) in a 10 cm square plastic pot. Pots were placed in a growth chamber where the regime was 20°C, 16 hr light and 15°C, 8 hr dark. Plants were inoculated 11 days later at the early three leaf growth stage. Inoculum was prepared by scraping and suspending spores of 10 + day old cultures, grown on minimal agar medium, in 0.1% Tween 20 solution, filtering through four layers of cheesecloth, and adjusting the concentration using a haemocytorneter. For each isolate, plants in four pots were sprayed simultaneously with 30 ml of a 2 X 10^4 spore/ml suspension using a soda straw sprayer (10). The spray mist was delivered from a 1 m distance with the 10 cm cut end of the straw immersed in the 50 ml erylenmeyer flask spray vessel. Pots were turned 180" when half the spore suspension remained. For the control only 0.1% Tween 20 solution was used. A plastic bag (13 X 8 X 34 cm) was placed over the plants in a pot to form a humidity chamber. The bag was supported by a wire frame and secured to the pot exterior by an elastic band. The plastic bags were removed after 3 days. The severity of spot blotch (percentage of leaf area lesioned) was estimated on the first leaf of 10 plants/pot 7 days after inoculation. Leaf tip discoloration was not included in the estimate of coverage since some leaf tip discoloration occurred in the control as well as the other treatments

One hundred and ten isolates from soil and from plants were compared. Eleven trials each of 20 isolates comprising 10 from each source were conducted. The plant and soil isolates originates from 85 and 75 locations, respectively. Subsequently, an additional six isolates from plants and 81 from soil were examined. A control (0.1% Tween 20 solution) and one standard culture (No. 1133) were also included in each trial.

Two to five leaves infected by the individual isolates were removed, placed in a 9 cm diameter glass petri plate fitted with moist filter paper and incubated at 20-22°C. After 3-4 days, sporulation occurred on leaf tips, and the length of 20 randomly selected spores was measured at 250X. No sporulation occurred on the leaves of plants sprayed with 0.1% Tween 20 solution.

Results and discussion

The mean ± standard error for percent leaf spot and conidial length for the standard culture (No. 1133) used in all 11 tests was 3.8 ± 0.91 and 80.4 μ ± 4.98, respectively. The variation between trials was not significant for percent leaf spot, but the variation between trials was significant at the 0.1% level for conidial length.

For percent leaf spot on Betzes barley the mean ± standard error for the 110 isolates from each of the plant source and soil source was 2.8 ± 1.37 and 2.7 ± 1.12, respectively. Values from both sources were normally distributed according to the Kolmogorov-SmirovTest for goodness of fit. Data were analyzed as a three-level nested analysis of variance (Table 1). This shows that there was a significant added variance among isolates within trials and among trials within the source (plant or soil), but no significant difference between the plant or soil source.

The mean ± standard error for conidium length for the 110 isolates from the plant and the soil source was 76.5 μ ± 6.16 and 77.1 μ ± 5.65, respectively. The range for the means of isolates and for readings, given in brackets, was 63.8 to 97.5 μ (49.4 to 106.4 μ) and 64.0 to 93.9 μ (41.8 to 117.8 μ), respectively, for the plant and soil source. The means of isolates from both sources were normally distributed according to the Kolmogorov-Smirov Test for goodness of fit. Data were analyzed as a three-level nested analysis of variance (Table 2). This shows that there was a significant added variance among isolates within trials and among trials within the source (plant or soil), but no significant difference between the plant or soil source.

The correlation coefficient **of** spore length and leaf spot based on means of all isolates (pairs = 220) was -0.1 10. This value was not significant.

The additional six plant derived isolates and 81 soil derived isolates formed colonies typical of *B.* ***sorokiniana.*** All but two isolates from soil produced spot blotch symptoms on Betzes barley and conidia having the typical *B.* ***sorokiniana*** shape, dimension, and coloration.

The conidium length of isolates was within the range for *B.* ***sorokiniana*** given by Ellis as 40-120 μ (11) and by Luttrell as 36 to 129 μ (15). **All** but two isolates caused spot blotch of barley. The data show that isolates from soil are similar to isolates from infected plant tissue.

Considerable variability occurred between trials for both variables. No doubt conditions varied between trials over the time period of the study even though procedures were standardized. Harding (13) noted conidium size was influenced by different cultural conditions.

Table 1. Three-level nested analysis of variance table for percent leaf spot on Betzes barley for 11 trials of 10 isolates of ***Bipolaris sorokiniana*** from two sources (plant or soil) with 4 readings per isolate

Sources	df	SS	MS	Fs
Among source (plant-soil)	1	2.397	2.397	0.095n.s.
Among trials within source	20	504.434	25.222	5.830"""
Among isolates within trials	198	856.481	4.326	6.686""*
Error	660	427.181	0.647	
Total	879	1790.493		

***Significant at **the** 0.1% **level.**

Table 2. Three-level nested analysis of variance table for conidial length for 11 trials of 10 isolates of ***Bipolaris sorokiniana*** from two sources (plant or soil) with 20 readings per isolate

Source	df	SS	MS	Fs
Among source (plant-soil)	1	118.871	118.871	0.186n.s.
Among trials within source	20	12801.754	640.087	4.651***
Among isolates within trials	198	27248.071	137.617	8.915***
Error	4180	64527.610	15.437	
Total	4399	104696.286		

***Significant at the 0.1% level.

Two of the isolates derived from soil were different from the rest. In culture, the colonies of both of these isolates, which were alike, were similar to those of *B.* ***sorokiniana.*** They did not produce leaf spots on barley, but they did sporulate on the necrotic leaf tips. The conidia produced in culture or on senescent leaf tissue differed from those of *B.* ***sorokiniana*** in shape, septation, and coloration. Although conidial length was within the range recorded for *B.* ***sorokiniana,*** the mean length of 44 μ was less than for *B.* ***sorokiniana.*** These isolates were identified as ***Curvularia spicata*** (Bainer) Boedijn.

Individual conidia of C. ***spicara*** extracted from soil might be confused as those of *B.* ***sorokiniana,*** particularly in view of the variability in *B.* ***sorokiniana.*** In culture, however, C. ***spicara*** is easily differentiated on the basis of conidial morphology. Of 181 isolates from soil that were selected as *B.* ***sorokiniana,*** 179 isolates (99%) were *B.* ***sorokiniana*** and ***2*** isolates **(1%)** were C. ***spicara.*** This level of reliability indicates that the oil and water flotation technique is a valuable tool for estimating *B.* ***sorokiniana*** conidial numbers in naturally infested Canadian prairie soils.

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