CANADIAN JOURNAL OF PLANT PATHOLOGY 7: 382-386, 1985

Effect of inoculum density of *Cochliobolus sativus* on common root rot of wheat and barley

L.J. Duczek, P.R. Verma, and D.T. Spurr

Research Station, Agriculture Canada, 107 Science Crescent, Saskatoon, Saskatchewan S7N 0X2. Contribution No. 902.

Accepted for publication 1985 09 09

In a growth room study, the incidence (percentage of plants with symptoms) and intensity (severity of symptoms) of common root rot in the artificially inoculated spring wheat, cultivars Neepawa (moderately resistant) and Cypress (susceptible) and the spring barley, cultivars Bonanza (moderately resistant) and Melvin (susceptible) increased as the number of soilborne conidia of *Cochliobolus sativus* increased. Maximum disease intensity levels in the wheat and barley cultivars were attained with 10–60 and 50–120 conidia/cm^3, respectively. In spite of disease incidence reaching 100% in many cases, the disease intensity was never higher than 75%. As inoculum density increased, the susceptible cultivars became relatively more severely diseased than the moderately resistant cultivars.

Duczek, L.J., Verma, P.R., and Spurr, D.T. 1985. Effect of inoculum density of *Cochliobolus sativus* on common root rot of wheat and barley. Can. J. Plant Pathol. 7: 382-386.

Au cours d'une étude de culture en chambre, la fréquence (pourcentage des plantes présentant des symptômes) et l'intensité (gravité des symptômes) du piétin commun dans le cas des céréales suivantes artificiellement inoculées augmentaient à mesure que le nombre des conidies externes de *Cochliobolus sativus* s'accroissait : blé de printemps, cultivars Neepawa (résiste modérément) et Cypress (sensible); orge de printemps, cultivars Bonanza (résiste modérément) et Melvin (sensible). Dans le cas des cultivars de blé et d'orge, les niveaux d'intensité maximums de la maladie ont été atteints avec des conidies allant respectivement de 10-60 et de 50-120 par cm^3. Bien que la fréquence de la maladie ait atteint 100 pour cent dans de nombreux cas, l'intensité de la maladie n'a jamais dépassé 75 pour cent. À mesure que la densité de l'inoculum augmente, la maladie atteint relativement plus gravement les cultivars sensibles que ceux qui offrent une résistance modérée.

Cochliobolus sativus (Ito & Kurib.) Drechsl. ex Dastur [anamorph *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. = *Helminthosporium sativum* P.K. & B.] is the major cause of common root rot of spring wheat and barley in the Canadian prairies (8,11). *Fusarium* spp., particularly, *F. culmorum* (W.G.Sm.) Sacc., are involved to a lesser extent. Soilborne conidia of *C. sativus* are the most important source of infection, but the effect of inoculum density on disease has been examined only incidentally in other studies.

Chinn et al. (5) found that viable conidia in field soils ranged from fewer than 8 to 253 per gram of soil, with a mean number of 56. In greenhouse tests using soil from those fields, disease intensity on wheat seedlings varied according to the logarithm of spore number, but disease intensity was not related to spore number on mature plants harvested in the field near the soil collection site. The results on seedlings confirmed their earlier greenhouse study (4), where a significant correlation was recorded between numbers of conida and disease intensity in wheat seedlings. Ledingham (7) found that 20 to 50 conidia per gram of soil caused considerable infection and that disease intensity at crop maturity was as great in plots with low numbers as in those with high numbers of conidia. Chinn (1) also found disease intensity to be similar over a wide range of conidial concentrations, with the lowest number being 27 viable conidia per gram. Nesterov (10) showed that as numbers of conidia increased from 36 to 84 per gram of soil, root rot incidence at the tillering stage increased from 12.6 to 33.2. The relationship appeared to be linear but other factors such as the amount of soil moisture also varied. In a preliminary report (16), disease intensity on hard red winter wheat in the greenhouse reached a plateau at 250 to 1000 conidia per gram of soil.

The purpose of this study was to determine the effect of inoculum density on disease incidence and disease intensity when conidia of a similar age were uniformly distributed at specific densities in the planting medium. This approach was followed to minimize the variability encountered in previous studies where field soil with indigenous inoculum densities were used.

Materials and methods

Two spring wheat (*Triticum aestivum* L.) cultivars, Neepawa (moderately resistant) and Cypress (susceptible), and two spring barley (*Hordeum vulgare* L.) cultivars, Bonanza (moderately resistant) and Melvin (susceptible), were used. Seeds of all cultivars were surface disinfected with 0.1% $HgCl_2$ for 10 min. In some tests, seeds were also treated with "Vitaflo Drill Box" (carbathiin;thiram) before sowing in soilless mix (12) in 13-cm-diameter plas-

tic pots. Fifteen seeds were planted in each pot and the number of plants then thinned to 10 after emergence. The seeds in each pot were covered to a depth of 6 cm with 500 cm^3 of inoculating medium (2 soilless mix:1 sand) infested with the desired number of conidia. The tests for wheat and barley cultivars and for the three ranges of inoculum density (0–256, 0–1024, 0–2560) were conducted separately. The design was a randomized complete block with either two wheat cultivars or two barley cultivars. In the high range, 10 inoculum densities were used, ranging from 0 to 2560, namely 0, 10, 20, 40, 80, 160, 320, 640, 1280, and 2560 conidia/cm^3 of inoculating medium. In the low range, the 10 inoculum densities were 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256. Five replicates (pots) were used per inoculum density. Each barley test was repeated twice and each wheat test was repeated once. Results are given as means for all tests. Plants were grown in a growth room maintained at 20°C during 16 h of light and at 15°C during 8 h of dark. For inoculum densities between 0 and 1024 conidia/cm^3, 12 densities were used, namely, 0, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024, with six replicates (pots) per density. These tests were done in a greenhouse in which the daylength was extended to 16 h light at 18-30°C. Both the barley and the wheat tests were repeated twice, but results of only one test for each are presented.

The culture of *C. sativus* used to produce the inoculum originated from a single conidium obtained from a colony growing out of a naturally infected subcrown internode of wheat. Inoculum was prepared by scraping conidia from 10-day or older cultures of *C. sativus* grown on minimal medium (14) and suspending them in a 0.1% Tween 20 solution. The suspension was filtered through four layers of cheesecloth and the concentration of conidia was adjusted using a hemocytometer. A 100 mL suspension containing the desired amount of inoculum was sprayed in four aliquots onto the screened (1-cm mesh) inoculating medium described earlier. The medium was mixed and screened between each application of the inoculum aliquots.

Subcrown internodes were rated after flowering (Feekes' scale 10.51) for the presence of brown lesions typical of those caused by *C. sativus*. For tests with 0–256 and 0–2560 conidia/cm^3, the four-category system was used (8) and disease intensity was calculated using the formula of Tinline and Ledingham (13). For the tests with 0–1024 conidia/cm^3, a Horsfall and Barrett system was used (6). Disease incidence refers to the percentage of infected plants based on the presence or absence of discoloration on the subcrown internodes; disease intensity is a measure of the severity of disease and relates to the amount of discoloration on the sub-

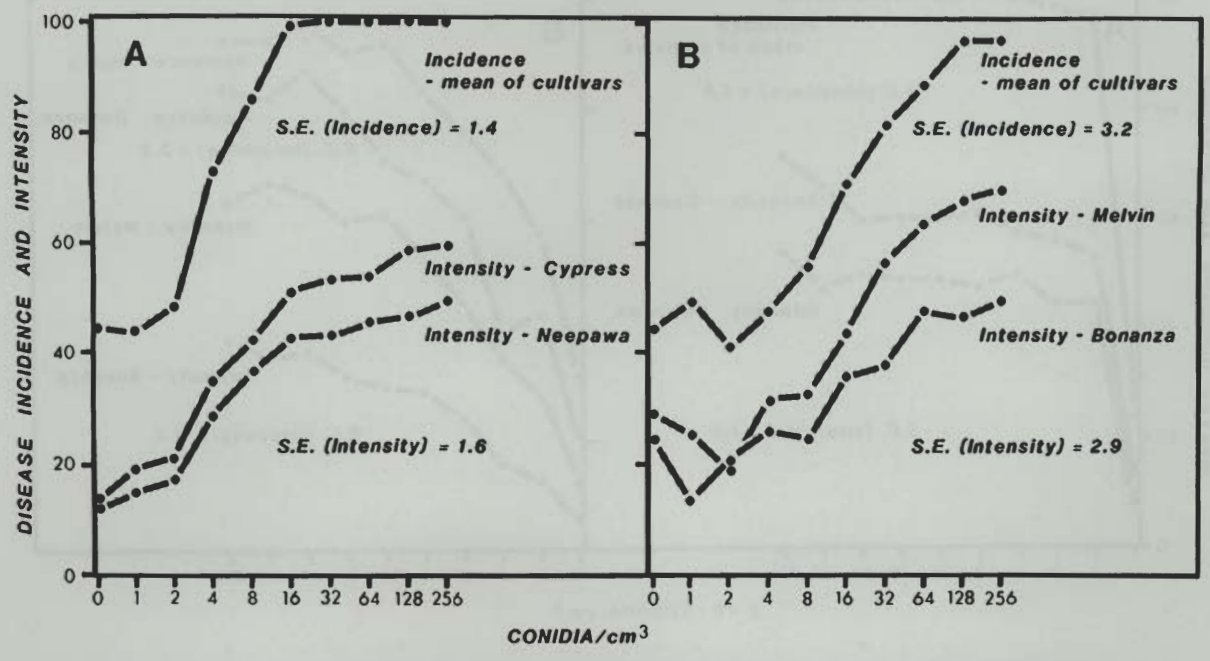

Figure 1. The relationship between inoculum density of 0–256 conidia/cm^3 of *Cochliobolus sativus* and common root rot disease incidence and disease intensity in **A)** Neepawa and Cypress wheat and **B)** Bonanza and Melvin barley. Standard errors (SE) are for means at inoculum levels.

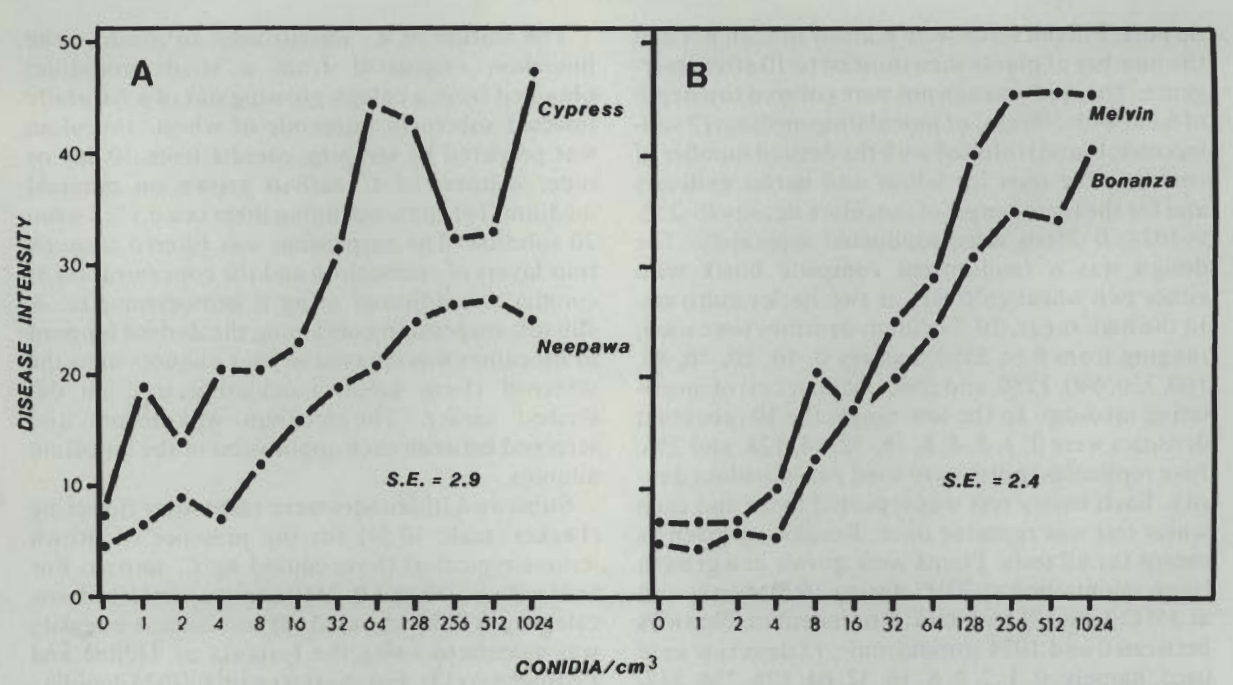

Figure 2. The relationship between inoculum density of 0–1024 conidia/cm³ of *Cochliobolus sativus* and common root rot disease intensity in **A)** Neepawa and Cypress wheat, and **B)** Bonanza and Melvin barley. Standard errors (SE) are for means at inoculum levels.

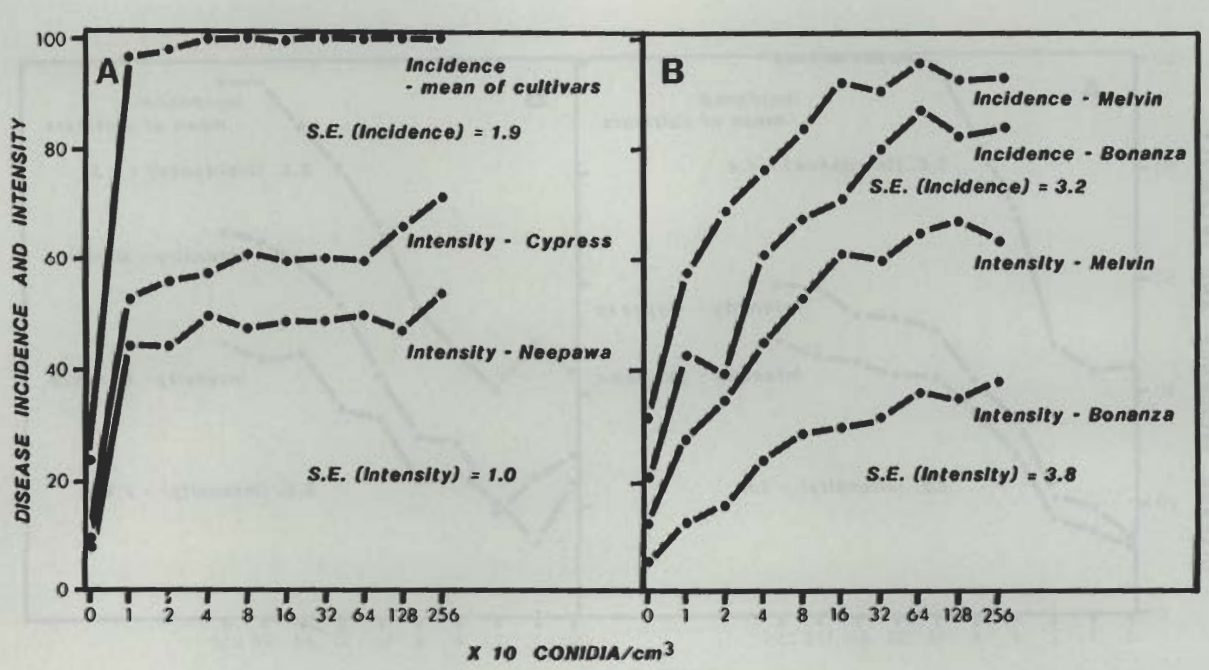

Figure 3. The relationship between inoculum density of 0–2560 conidia/cm³ of *Cochliobolus sativus* and common root rot disease incidence and disease intensity in **A)** Neepawa and Cypress wheat and **B)** Bonanza and Melvin barley. Standard errors (SE) are for means at inoculum levels.

crown internodes. Isolations were made from discolored internodes to confirm the presence of *C. sativus*.

Results and discussion

As the number of conidia increased, the incidence and intensity of disease also increased in both wheat and barley (Figs. 1-3). In the growth room, maximum incidence and intensity were reached with about 20 conidia for the wheat cultivars and with about 160 conidia for the barley cultivars in the tests with 0–256 and 0–2560 conidia/cm^3 (Figs. 1,3). In the greenhouse tests with 12 densities between 0–1024 conidia/cm^3, disease intensity was at a maximum at about 120 conidia for wheat and 250 conidia for barley (Fig. 2). Differences might be expected between tests conducted in the growth room and those in the greenhouse because environmental conditions would be different and might influence disease expression.

Although disease incidence approached or reached 100% (all plants with symptoms), disease intensity only reached 70%, indicating that perhaps even the susceptible cultivars have a level of resistance that limits the increase in lesion size (Figs. 1,3). Disease incidence is not shown in the test with 0–1024 conidia/cm^3 (Fig. 2). Cypress was more susceptible than Neepawa, and Melvin was more susceptible than Bonanza (Figs. 1-3); similar rankings of these cultivars occur in the field (13).

Considerable variability occurred in the data. An analysis of variance showed that the repeated tests for each inoculum range were generally significantly different from one another. This indicates that the effects of inoculum density on disease intensity and disease incidence were different in tests repeated under fairly similar conditions. Even in a growth room it was difficult to achieve similar amounts of disease each time a test was run. The incidence of disease for the wheat cultivars was similar in experiments with 0–256 and 0–2560 conidia/cm^3, but in barley the cultivars reacted similarly for disease incidence only in the experiments with 0–256 conidia/cm^3 (Figs. 1,3). The inoculum × cultivar interaction was not significantly different for disease incidence, indicating that as inoculum density increased disease incidence remained proportionally the same between susceptible and resistant cultivars. On the other hand, the disease intensity for cultivars and for the inoculum × cultivar interaction was significantly different ($P < 0.01$) both for wheat and for barley (Figs. 1,3). This indicates that as inoculum increased, disease intensity increased proportionally more on susceptible cultivars than on the moderately resistant cultivars. These results confirm those of Verma (15) who found that lesions developed faster in susceptible cultivars than in resistant cultivars.

In the uninoculated treatments disease incidence ranged from 12 to 44% and disease intensity from 6 to 29% (Figs. 1-3). *C. sativus* was isolated from some of these plants although not all discoloration, especially small lesions, was caused by *C. sativus*. The planting medium was free of inoculum. Further, plating of disinfected seed showed that Bonanza, Melvin, and Neepawa were also free of inoculum, although 1-3% of the Cypress seed was contaminated. Spore trapping with slides covered in petroleum jelly indicated the presence of airborne conidia of *C. sativus*. For instance in the test with 0–1024 conidia/cm^3 (Fig. 2) 1.9 conidia per slide (6 × 2.6 cm area) were found in the barley test and 4.9 conidia per slide in the wheat test. These airborne conidia possibly were from infected plants (2,9), or they may have come from plant and soil material that is constantly being handled in the greenhouse area. This latter inoculum source possibly explains why uninoculated treatments may become infected. Such extraneous inoculum also complicates the results because it increases the error when establishing a series of inoculum density experiments. However this additional inoculum source contributes relatively few conidia compared to what was intentionally added; even at the lowest density, 500 conidia were added to each pot.

The relatively low inoculum densities needed to cause the maximum amount of disease might help explain why there was little relationship between the amount of inoculum and the intensity of disease in previous studies (1,4,5,7). Probably even the lowest inoculum density was high enough to cause the maximum amount of disease.

These results, coupled with the fact that conidia remain viable for a long time in soil (3,7), explain why short-term crop rotations are not adequate for disease control. Even though Ledingham (7) found that the spore number decreased during the winter from 88 to 58 spores per gram, and Chinn and Ledingham (3) reported a 40% viability of spores in soil after 2 years, these populations would still exceed the threshold level necessary to cause maximum disease.

The authors wish to thank R. Kloster, M. Bahrey, and L. Jackson for technical assistance.

1. **Chinn, S.H.F.** 1976. Influence of rape in a rotation on prevalence of *Cochliobolus sativus* conidia and common root rot of wheat. Can. J. Plant Sci. 56: 199-201.
2. **Chinn, S.H.F.** 1977. Influence of fungicide sprays on sporulation of *Cochliobolus sativus* on Cypress wheat and on conidial populations in soil. Phytopathology 67: 133-138.

3. **Chinn, S.H.F.,** and **R.J. Ledingham.** 1958. Application of a new laboratory method for the determination of the survival of *Helminthosporium sativum* spores in soil. Can. J. Bot. 36: 289-295.

4. **Chinn, S.H.F.,** and **R.J. Ledingham.** 1961. Mechanisms contributing to the eradication of spores of *Helminthosporium sativum* from amended soil. Can. J. Bot. 39: 739-748.

5. **Chinn, S.H.F., B.J. Sallans,** and **R.J. Ledingham.** 1962. Spore populations of *Helminthosporium sativum* in soils in relation to the occurence of common rootrot of wheat. Can. J. Plant Sci. 42: 720-727.

6. **Couture, L.** 1980. Assessment of severity of foliage diseases of cereals in cooperative evaluation tests. Can. Plant Dis. Surv. 60: 8-10.

7. **Ledingham, R.J.** 1961. Crop rotations and common root rot in wheat. Can. J. Plant Sci. 41: 479-486.

8. **Ledingham, R.J., T.G. Atkinson, J.S. Horricks, J.T. Mills, L.J. Piening,** and **R.D. Tinline.** 1973. Wheat losses due to common root rot in the prairie provinces of Canada, 1969-71. Can. Plan Dis. Surv. 53: 113-122.

9. **Nair, N.G.** 1962. Behavior of *Bipolaris sorokiniana* in the rhizosphere of wheat. Proc. Indian Acad. Sci. B, 55: 290-295.

10. **Nesterov, A.N.** 1975. [Effect of forest belts on the increase and maintenance of soil moisture and limiting the damage to spring wheat by root rot.]. Byull. Vses. Nauchno-Issled. Inst. Zashch. Rast. 32: 51-53 (In Russian).

11. **Piening, L.J., T.G. Atkinson, J.S. Horricks, R.J. Ledingham, J.T. Mills,** and **R.D. Tinline.** 1976. Barley losses due to common root rot in the prairie provinces of Canada, 1970-72. Can. Plant. Dis. Surv. 56: 41-45.

12. **Stringham, G.R.** 1971. Genetics of four hypocotyl mutants in *Brassica campestris* L. J. Hered. 62: 248-250.

13. **Tinline, R.D.,** and **R.J. Ledingham.** 1979. Yield losses in wheat and barley cultivars from common root rot in field tests. Can. J. Plant Sci. 59: 313-320.

14. **Tinline, R.D., J.F. Stauffer,** and **J.G. Dickson.** 1960. *Cochliobolus sativus* III. Effect of ultraviolet radiation. Can. J. Bot. 38: 275-282.

15. **Verma, P.R.** 1982. Temporal progression of common root rot (*Cochliobolus sativus*) lesions on subcrown internodes of wheat and barley cultivars. Can. J. Plant Pathol. 4: 349-352.

16. **Umechuruba, C.I.,** and **L.L. Singleton.** 1982. Inoculum-density-level study of *Helminthosporium sativum* and *Fusarium graminorum* and the incidence of wheat root rot disease in Oklahoma. Phytopathology 72: 948 (Abstr.).