

# Surface Disinfestation of Wheat Seed and Inoculation of Seedling Roots with Single Macroconidia of *Fusarium acuminatum*

J. P. HILL, Associate Professor, C. R. ARMITAGE, Former Graduate Student, D. KAUTZMAN-EADES, Research Assistant, and P. HANCHEY, Professor, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins 80523

## ABSTRACT

Hill, J. P., Armitage, C. R., Kautzman-Eades, D., and Hanchey, P. 1987. Surface disinfestation of wheat seed and inoculation of seedling roots with single macroconidia of *Fusarium acuminatum*. Plant Disease 71:130-131.

Seed of seven wheat cultivars were surface-disinfested with sequential treatments of detergent, Tween 20, ETOH, NaOCl, and mercuric chloride under negative pressure. The surface-disinfested seed were germinated in petri dishes containing potato-dextrose agar. Five days later, uncontaminated wheat seedlings were transferred to test tubes containing water agar and the largest root was inoculated with a small piece of water agar containing a single, germinated macroconidium of *Fusarium acuminatum*. After 6 days, inoculated roots were excised, surface-disinfested for 1 min in a solution of ETOH and NaOCl, and placed in petri dishes containing PDA. Five days later, roots were assayed for the presence of *F. acuminatum*. The pathogen was isolated from almost all inoculated roots, and there were no significant differences among cultivars. Most inoculated roots were symptomless. Staining and microscopic examination revealed that the fungus grew profusely over the entire root and repeatedly penetrated between epidermal cells. Hyphae grew intercellularly within the cortex. No evidence of vascular tissue penetration was found.

Additional key words: foot and root rot, *Fusarium culmorum*, *F. graminearum*, winter wheat

Cereal foot and root rots, commonly occurring throughout the principal wheat-growing areas of the world (2), are major yield constraints in Colorado and Wyoming (8,9). In some regions, *Cochliobolus sativus* is the major pathogen (7,14,16,17,20-22). In other regions, foot and root rot may be induced by certain species of *Fusarium* (3,4), either by themselves or in a complex with *C. sativus* (2,5,9,13,15,18). *Fusarium*

spp. most often associated with common foot and root rot of wheat are *F. culmorum* (Smith) Sacc., *F. graminearum* Schwabe, and *F. acuminatum* Ell. & Ev. (3,4,23). *F. avenaceum* (Fr.) Sacc. and *F. poae* (Peck) Wollenw. are less virulent and more localized as root pathogens (23). General resistance to certain common foot and root rot pathogens is reported (10,20), but effects can be subtle and cultivar differences are not always expressed clearly in yield responses.

A major restraint in the search for general resistance and its subsequent incorporation into agronomically acceptable cultivars has been the inconsistency in wheat-plant inoculations and accurate quantitative disease measurement. The purpose of this study was to develop a method to efficiently inoculate wheat seedlings with defined inoculum and quantitatively measure disease response to infection by the root-rot organisms.

## MATERIALS AND METHODS

Surface disinfestation of wheat seed for 1-5 min in 95% ethanol and 5.3% NaOCl (1:1, v/v) followed by two sterile distilled water (SDW) rinses did not adequately free the seed from contamination by species of *Fusarium* in

sections Discolor, Gibbosum, Roseum, and Sporotrichiella (12), and resulted in low seed viability (1). To reduce seed mortality and natural contamination, elements of a seed-disinfestation method developed for cereal tissue culture were used (11). Seed of wheat cultivars Vic or Calvin (*Triticum turgidum* L. var. *durum*) or Baca, Duke, Newton, Scout 66 or Vona (*T. aestivum* L.) were placed in a 250-ml flask containing 100 ml of SDW and two drops of commercial dish-washing detergent, shaken for 3 min, rinsed with SDW until suds were removed, and covered with 50 ml of 20% NaOCl solution and two drops of Tween 20. The flask was evacuated to -7.0 to -8.4 kg/m<sup>2</sup>. After 30 min on a stir plate, the flask was removed, the NaOCl solution replaced with a solution of 0.1% HgCl<sub>2</sub> in SDW, and the negative pressure resumed. After 3 min, the HgCl<sub>2</sub> solution was removed and the seed rinsed four to six times with SDW. Seed were transferred to petri dishes containing potato-dextrose agar (PDA; three per dish) and placed in the dark. After 5 days, only germinated seedlings, which appeared uncontaminated, were aseptically transferred to water agar test tube slants and placed under daily regimes of 18 hr of light followed by 6 hr of darkness. After 5 days on the water agar slants, the roots were excised and placed on PDA plates at room temperature. Excised roots were assessed for contamination after 5 days.

In subsequent inoculation experiments, seeds were disinfested, as described, and placed on petri dishes containing PDA. After 5 days, each germinated seedling, which appeared uncontaminated, was aseptically transferred to a water agar test tube slant. Inoculum was prepared by placing macroconidia in petri dishes containing water agar, and after 12 hr, germinated spores were collected, along with a portion of agar, under a dissecting microscope. Seedlings were inoculated immediately after transfer by placing a single, germinated macroconidium of *F. acuminatum* on the largest root 10-15 mm below the seed. After 6 days,

Some of the information presented herein is a portion of the second author's M.S. thesis.

Supported in part by the Colorado Agricultural Experiment Station and a grant by the Colorado Wheat Administrative Committee.

Published with the approval of the director, Colorado Agricultural Experiment Station, as Scientific Series Paper 1-53121.

Accepted for publication 18 September 1986 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

© 1987 The American Phytopathological Society

inoculated roots were excised, cut into three or four segments, surface-disinfested for 1 min in the ETOH/NaOCl solution, and placed in petri dishes containing PDA. Five days later, root segments were assayed for presence of *F. acuminatum*. The total number of root segments and numbers with colonies of *F. acuminatum* were recorded. Tests were conducted on all cultivars. Tests also were conducted on cultivars Vona and Newton with macroconidia of *F. graminearum* or *F. culmorum* as inoculum. Observations on presence of *F. acuminatum* also were made when cultivar Vona roots were excised 3 and 5 days after inoculation.

Microscopic observations were made on seedling roots 5 days after inoculation with a single, germinated macroconidium of *F. acuminatum*. Inoculated roots, many showing no symptoms, were excised and stained by a modification of the procedure of Schans et al (19). Fresh, unfixated root segments 2 cm long were stained for 2 min in 0.5% aqueous malachite green to suppress host tissue autofluorescence and counterstained 20 min with 0.001% acridine orange in a boric acid-borate buffer, pH 8.6. Segments were mounted on glass slides and examined with a Leitz Dialux 20 EB microscope equipped with a Pleomopak vertical illuminator and a 50W, ultra-high-pressure mercury lamp at wavelengths of 390–490  $\mu\text{m}$  (Leitz narrow-band filter H2).

## RESULTS AND DISCUSSION

The seed surface-disinfestation procedure resulted from a compromise between effectiveness and retention of seed viability. The germination of seed on PDA in petri dishes permitted detection and elimination of the remaining infested seed. Few seedlings were found to be contaminated after 5 days of growth on water agar in test tube slants. Between 50 and 75% of the disinfested seed germinated and produced uncontaminated seedlings.

Root inoculation of uncontaminated seedlings with a single, nongerminated macroconidium resulted in high infection (or colonization) efficiency, but variation among tests of the same cultivar persisted. This variation was thought to be due, in part, to variation in the viability of macroconidia. When only germinated macroconidia were used as inoculum, most of this variation was eliminated and *F. acuminatum* was recovered from almost all inoculated roots.

When inoculated Vona roots were excised 3 days after inoculation, few (10–15%) root segments contained *F. acuminatum*. As time between inoculation and excision increased, the number of root segments containing *F. acuminatum* increased. Excision 6 days after inoculation resulted in recovery of the pathogen from more than 50% of the segments, and at least one segment of

each inoculated root contained *F. acuminatum* in almost all tests. This may indicate that under these experimental conditions, it takes at least 5–6 days for the fungus to penetrate and ramify through the root or that the younger, smaller roots permitted the surface disinfectant to penetrate and kill the fungus within the tissue. No observable differences in *F. acuminatum* recovery were recorded among cultivars.

When germinated macroconidia of *F. graminearum* or *F. culmorum* were used as inoculum, the pathogen was recovered from almost all root segments. This is consistent with observations that these two *Fusarium* spp. are more virulent than *F. acuminatum*. More roots showed visual symptoms (hyphal growth on root or seed and a slight darkening of roots) when inoculated with *F. graminearum* or *F. culmorum* than those inoculated with *F. acuminatum*. However, most of the inoculated roots showed no symptoms after inoculation with any of the *Fusarium* spp. No pathogen recovery differences were observed among cultivars.

Microscopic examinations of Vona and Newton roots 5 days after inoculation revealed orange-fluorescing hyphae growing over the entire root surface. By focusing down toward the cortex through epidermal cells, hyphae were observed growing intercellularly along the direction of the root axis between epidermal and cortex cells and, in a few cases, between the first and second cortical layer of cells. Hyphae were observed most readily at cut surfaces, presumably because acridine orange penetration was facilitated at those sites. Examination of squash-mount preparations failed to reveal hyphal penetration into the vascular system. It seems that certain *Fusarium* spp. can invade wheat seedling roots and remain viable but do not produce visible symptoms. Disease symptoms may appear only after infected plants are stressed by drought, cold, or other factors (3,4,6).

Although the seed surface-disinfestation procedure and seedling root inoculations with a single, germinated macroconidium did not reveal cultivar differences in resistance to infection or colonization by each of the three *Fusarium* spp., the procedure may be useful in subsequent studies. Interaction studies are in progress to determine the effect of exposure to "saprophytic" fungi or weak pathogens before challenging with *F. acuminatum*. These procedures also may be valuable in efficacy studies with fungicides and in delineating biological control mechanisms of root pathogens. Uncontaminated seedling roots of wheat seem to be universally susceptible to infection by a single macroconidium of *F. acuminatum*, *F. graminearum*, or *F. culmorum*, and no significant cultivar differences seem to exist. There may be

some microbial interaction that influences infection, which can be explored with this technique. Also, root-inoculated seedlings could be transplanted to sterilized soil and grown under various stress conditions to investigate the effect of infection from defined inoculum.

## LITERATURE CITED

- Armitage, C. R. 1984. Quantitative assessment of wheat seedling root infection by *Fusarium acuminatum*. M.S. thesis. Colorado State University, Fort Collins. 40 pp.
- Butler, F. C. 1961. Root and foot rot diseases of wheat. N.S.W. Dep. Agric. Bull. 77. 98 pp.
- Cook, R. J. 1968. *Fusarium* root and foot rot of cereals in the Pacific Northwest. *Phytopathology* 58:127-131.
- Cook, R. J. 1980. *Fusarium* foot rot of wheat and its control in the Pacific Northwest. *Plant Dis.* 64:1061-1066.
- Fenster, C. R., Boosalis, M. G., and Weihing, J. L. 1972. Date of planting studies of winter wheat and winter barley in relation to root and crown rot, grain yields, and quality. *Nebr. Agric. Exp. Res. Bull.* 250.
- Fernandez, J. A., Wofford, D. S., and Horton, J. L. 1985. Interactive effects of freezing and common root rot fungi on winter wheat. *Phytopathology* 75:845-847.
- Harding, H. 1973. Fungi associated with subcrown internodes of wheat (*Triticum aestivum*). *Can. J. Bot.* 51:2514-2516.
- Henderson, W. J. 1958. *Colorado Handbook of Plant Disease*. 3rd ed. Colorado State University Extension Service, Fort Collins. 230 pp.
- Hill, J. P., Fernandez, J. A., and McShane, M. S. 1983. Fungi associated with common root rot of winter wheat in Colorado and Wyoming. *Plant Dis.* 67:795-797.
- Larson, R. I., and Atkinson, T. G. 1970. A cytogenetic analysis of reaction to common root rot in some hard spring wheats. *Can. J. Bot.* 48:2059-2067.
- Nabors, M. W., Heyser, J. W., Dykes, T. A., and DeMott, J. J. 1983. Long-duration, high-frequency plant regeneration from cereal tissue cultures. *Planta* 157:385-391.
- Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park. 193 pp.
- Oswald, J. W. 1950. Etiology of cereal root rots in California. *Hilgardia* 19:447-462.
- Purss, G. S. 1970. Resistance to common root rot (*C. sativus*) in wheat in Queensland. *Aust. J. Exp. Agric. Anim. Husb.* 10:497-501.
- Saari, E. E., and Wilcoxson, R. D. 1974. Plant disease situation of high-yielding dwarf wheats in Asia and Africa. *Annu. Rev. Phytopathol.* 12:49-68.
- Sallins, B. J. 1965. Root rots of cereals III. *Bot. Rev.* 31:505-799.
- Sallins, B. J., and Tinline, R. D. 1965. Resistance in wheat to *Cochliobolus sativus*, a cause of common root rot. *Can. J. Plant Sci.* 45:343-351.
- Scardaci, S. C., and Webster, R. K. 1982. Common root rot of cereals in California. *Plant Dis.* 66:31-34.
- Schans, J., Mills, J. T., and Van Caeseele, L. 1982. Fluorescence microscopy of rapeseeds invaded by fungi. *Phytopathology* 72:1582-1586.
- Statler, G. D., and Darlington, L. C. 1972. Resistance of hard red spring wheat and durum wheat to seedling blight and crown rot. *Plant Dis. Rep.* 56:788-791.
- Sprague, R. 1950. *Diseases of Cereals and Grasses in North America*. Ronald Press, New York. 538 pp.
- Verma, P. R., Morral, R. A. A., and Tinline, R. D. 1974. The epidemiology of common root rot in Manitowish wheat: Disease progression during the growing season. *Can. J. Bot.* 52:1757-1764.
- Wiese, M. V. 1977. *Compendium of Wheat Diseases*. American Phytopathological Society, St. Paul, MN. 106 pp.