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Seed transmission of *Pyrenophora tritici-repentis*, causal fungus of tan spot of wheat

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Abstract

Seed transmission of *Pyrenophora tritici-repentis*, a common foliar pathogen of wheat, was investigated in soft white winter wheat cv. Frankenmuth and found to be non-systemic; the emerging coleoptile was infected externally by hyphal growth from the infected pericarp. Hyphae from the infected coleoptile then infected the first and second seedling leaves as they emerged. Coleoptile symptoms ranged from tiny brown streaks or spots to large, brown necrotic areas accompanied by cracking and distortion of the coleoptile. Small brown spots sometimes occurred on the first and rarely on the second seedling leaves, often accompanied by leaf distortion. Pseudothecial initials of the fungus were present within or on the seed remnants. Seed transmission efficiency was as high as 92% *in vitro* and 60% in potting soil outdoors. Seed infection did not affect germination *in vitro*, but slightly reduced emergence in potting soil. Seedling weight and height were reduced significantly. In potting soil, seed infection also resulted in delayed plant growth and increased tan spot severity at later stages of plant development. Under controlled conditions, seed transmission efficiency and incidence of pseudothecia on seed were negatively correlated with seed germination temperature in the range of 9 to 21 °C, whereas frequency of recovery of the fungus from symptomatic coleoptiles and leaves was positively correlated with seed germination temperature.

These results suggest that infected seed may serve as a source of inoculum for tan spot epidemics and for dispersal of strains of the fungus to new areas.

Introduction

Many plant pathogens perpetuate themselves by infecting or infesting seeds of their host plants and are therefore termed seedborne [Neergaard, 1977]. After germination, seed transmission may occur by passage of seedborne inoculum to the developing plant, which may serve as a source of primary inoculum for epidemic development. The mechanism of seed transmission depends on specific host-pathogen interactions, whereas the rate of seed transmission also may be influenced by environmental conditions [Agarwal and Sinclair, 1987a]. Not all seedborne pathogens are seed-transmissible; those that are may pose a threat to crop production [Neergaard, 1977].

Wheat (*Triticum aestivum* L.) is attacked by several seedborne pathogens, among which is *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.), the causal fungus of a worldwide foliar disease called tan spot or yellow spot [de Tempe, 1964; Duff, 1954; Galloway, 1936; Hyltén-Cavallius, 1984; Obst, 1989; Schilder and Bergstrom, 1993; Shaw and Valder, 1952; Tekauz et al., 1982; Vanterpool, 1963]. The fungus is

present as mycelium within the pericarp of the wheat seed [Schilder and Bergstrom, 1994]. Reports conflict, however, regarding seed transmissibility of the fungus [Duff, 1954; Hyltén-Cavallius, 1984; Kolk, 1970; Obst, 1989; Shaw and Valder, 1952].

P. tritici-repentis has been isolated from certified winter wheat seed in New York [Schilder and Bergstrom, 1994]. Da Luz and Bergstrom [1986] noted excellent control of tan spot in New York spring wheat by the seed treatment fungicide triadimenol in the apparent absence of other sources of inoculum, indicating that seedborne inoculum may be important in the epidemiology of tan spot. To evaluate the potential role of seedborne *P. tritici-repentis* as primary inoculum for tan spot epidemics, we set out to determine if seed transmission of the fungus occurs under local conditions, to elucidate the mechanism of seed transmission, and to study the effect of temperature on aspects of seed transmission.

Materials and methods

Seed transmission in vitro

A representative sample of 400 seeds was taken from a seed lot of soft white winter wheat cv. Frankenmuth containing 32% naturally infected seed as detected by the freezing blotter method [Limonard, 1966]. A subsample of 200 seeds was surface-sterilized by soaking in 95% ethanol for 30 sec and in 1% sodium hypochlorite for 20 sec, followed by rinsing in sterile, distilled water. The other 200 seeds were not surface-sterilized. Both groups of seeds were placed on sterile, 1%-water agar slants in test tubes (15 mm diam, 15 cm long), one seed per tube. The tubes were plugged with sterile cotton and placed in racks under 12 h flourescent light at 21 °C. After one week, the cotton plugs were removed to allow the seedlings to expand, and after 15 d the seedlings were removed and examined by means a dissecting microscope. Fungi occurring on the seeds and seedlings were identified by means of a compound microscope. One-sided t-tests were applied to the data using the Minitab statistical computer program (Minitab Inc., State College, PA, USA).

Seed transmission in potting soil

Two seed lots of soft white winter wheat cv. Frankenmuth were obtained from glasshouse-grown plants, half of which had been inoculated with a single isolate of *P. tritici-repentis* (Ptr23NY86). The lots contained 0% and 75% infected seeds, respectively. On October 12, 1991, fifty seeds of each seed lot were planted equidistantly at a depth of 1–1.5 cm in large plastic pots (21 cm diam, 22 cm deep) in moist peat lite mix (50% peat moss, 50% #2 vermiculite, 3 kg/m^3 Osmocote, 3 kg/m^3 limestone, 0.6 kg/m^3 superphosphate, 0.9 kg/m^3 potassium nitrate, 0.9 kg/m^3 Micromax trace elements, 0.6 l/m^3 Aqua-gro wetting agent). The experiment was set up according to a completely randomized design, with six replications per seed lot. The pots were placed in an uncovered cold frame and exposed to ambient outdoor conditions. Plants were not watered until dry periods necessitated it during late spring and summer. After two weeks the two groups of plants were placed at a distance of 2 m from each other in the same cold frame to avoid possible cross-contamination. In the spring, they were placed in two adjacent, uncovered cold frames.

On October 31, 1991, ten seedlings at Zadoks growth stage (ZGS) 12 (two-leaf stage) were removed at random from each pot [Zadoks et al., 1974]. They were examined with the naked eye and with a dissecting microscope for symptoms and signs of infection. On January 14, 1992, ten random plants at ZGS 21–22 (one to two tillers) were removed from each pot for examination. The roots were cut off and the shoots were weighed and examined with the naked eye for signs and symptoms of infection. The coleoptile and leaves of each plant were cut off and surface-sterlized by soaking in 95% ethanol for 15 sec and in 1% sodium hypochlorite for 15 sec, followed by rinsing in sterile, distilled water. They were placed on moist filter paper in 9-cm Petri plates under 12 h near-ultraviolet (NUV) light at 21–25 °C. This was done for all replicates of the infected seed lot and for one replicate of the uninfected seed lot. After 7 d incubation, the coleoptiles and leaves were examined for signs of *P. tritici-repentis*.

Leaf spot severity was estimated visually on all leaves at ZGS 31 (first node detectable), on the

upper four leaves at ZGS 55 (heading), and on the upper three leaves on ZGS 83 (early dough). In addition, plant height was measured on 10 random tillers at ZGS 31 and the number of heads that emerged completely was determined at ZGS 55. To verify the presence of *P. tritici-repentis*, 12 to 23 distinct leaf spots per replicate were cut out of leaf samples taken at ZGS 55 and surface-sterilized as above. Leaf sections were placed on moist filter paper in 9-cm Petri plates under 12 h NUV light at 20–25 °C for 8 d, after which they were examined for the presence of fungal structures.

Disease incidence data were arcsine-transformed and disease severity data were square-root-transformed before analysis with one-side t-tests and confidence intervals using Minitab. Weather data were obtained from the Ithaca weather station, approximately 1.5 km from the experimental site.

Microscopic examination of infected seedlings

Ten seedlings exhibiting coleoptile lesions and pseudothecia on the seed remnants were selected from the *in vitro* seed transmission assay and cut longitudinally into 20–25 µm thick sections with a freezing microtome (Model 880, American Optical Corporation, Buffalo, NY, USA). Sections were stained in 0.1% aqueous aniline blue in lactic acid, rinsed in water, and mounted in 50% glycerol on microscope slides before examination with dissecting and compound microscopes.

Whole mounts of two seedlings which exhibited prominent coleoptile and leaf lesions in the seed transmission test in potting soil were prepared by cutting the coleoptile and leaf blade into 1.5-cm long sections. The coleoptile and leaf sections were stained in 0.1% aqueous aniline blue in lactic acid, rinsed in water, and mounted in 50% glycerol on microscope slides before examination with dissecting and compound microscopes.

Effect of temperature on seed transmission

A seed lot of soft white winter wheat cv. Frankenmuth containing 64% infected seed, obtained from glasshouse-grown and -inoculated (at anthesis to early milk stage) plants was selected for this study. An uninfected seed lot of the same cultivar was used as a control. Three temperature treatments were selected: 9 °C, 15 °C, and 21 °C. For each temperature treatment, three replications of one-hundred seeds per seed lot were planted in flats of plastic cone cells (cell dimensions 2.5 × 2.5 × 7.5 cm) containing moist Metro Mix (W. R. Grace Co., Allentown, PA, USA) one seed per cell, at a depth of 2.5 cm. The flats were placed in an incubator (Seed Germinator 30, Hoffman Manufacturing Company, Albany, OR, USA) at the desired temperature with 12 h light supplied by three vertically placed, cool white fluorescent bulbs (General Electric F40CW) on either side of the shelves. The flats were watered evenly to keep the potting mix moist, but not overly wet. The seedlings were removed from the flats at the one-and-a-half-leaf stage (one leaf fully expanded, the second half expanded) for visual examination of the coleoptile and leaves for lesions, and of the seed for the presence of pseudothecia of the fungus. From each replicate, all coleoptiles showing lesions as well as ten symptomless coleoptiles of seedlings which had pseudothecia on the seed, ten coleoptiles of apparently healthy seedlings, and ten coleoptiles of the control seedlings were excised and surface-sterilized as above. Leaf pieces which showed necrosis, cracking, or malformation were similarly excised and surface-sterilized. They were aseptically placed on V8 medium (200 ml V8 juice, 8 g Gelrite gellan gum, 3 g $CaCO_3$, and 300 mg streptomycin per liter) and incubated in a controlled-temperature room under ambient light at 21 °C for 2 d, followed by 12 h NUV light at 21–25 °C for 5 d. Fungi recovered from coleoptiles and leaf pieces were identified by characteristic spore and colony morphology by means of dissecting and compound microscopes. Data were arcsine-transformed and subjected to linear regression analysis using the SAS REG procedure (SAS Institute Inc., Carey, NC, USA).

Results

Seed transmission in vitro

The humid environment created by the water agar in test tubes favored abundant fungal growth, while the percentage germination was low for both surface-sterilized and nontreated seed (Table 1).

Table 1. Seed germination and recovery of *P. tritici-repentis* (*P. t.-r.*) from wheat seeds (cv. Frankenmuth) in an *in vitro* seed transmission assay

Seed Treatment	Seed % germination[a]	% Seeds with *P. t.-r.*[a,b]	% Viable seeds with *P. t.-r.*[a,b]
Surface-sterilized	65	40	25
Not surface-sterilized	72	36	25

[a] Based on 200 seeds per treatment.
[b] Based on presence of characteristic pseudothecia, mycelium, and/or pigment of *P. tritici-repentis*.

Surface-sterilization may have reduced germination, but it actually enhanced recovery of *P. tritici-repentis* from seeds and symptom development (Tables 1 and 2) in seedlings infected by this fungus. *P. tritici-repentis* was recovered from a higher proportion of the seed in this assay than in a freezing blotter test of the same seed lot. The fungus was associated with nonviable seeds in proportion to its occurrence in the whole seed lot, indicating no specific effect on seed viability. Many seedlings showed coleoptile lesions, the majority of which were associated with infection by *P. tritici-repentis*. The fungus produced a characteristic orange pigment and large, black pseudothecia on the seeds and sometimes on the upper roots. Often, the coleorhiza and upper roots also exhibited a dark brown discoloration. Characteristic symptoms on the shoot included numerous small brown spots and streaks, sometimes coalescing to form large necrotic areas, on the base of the coleoptile and extending upward. Sometimes lesions were situated in the middle or at the tip of the coleoptile. In several cases the coleoptile was malformed and cracked with necrotic margins. External mycelium was observed on some coleoptiles, at times extending onto and causing lesions on the sheath of the first leaf. Only rarely were lesions present on the leaf blade. A number of seedlings showed coleoptile lesions without evidence of infection by *P. tritici-repentis*. In some cases *S. nodorum, Fusarium* spp., *Alternaria* spp., or *Drechslera biseptata* (Sacc. & Roum.) Richardson & Fraser were associated with the affected seedlings. More often, seedlings showed a nondescript, brown or yellow discoloration of the roots or coleoptile base, associated with heavy fungal or bacterial growth. Seedlings showing no symptoms or obvious discoloration were considered healthy. Seedlings infected by *P. tritici-repentis* were significantly shorter and lighter than apparently healthy seedlings (Table 2).

Table 2. Symptom development and measurements on wheat seedlings (cv. Frankenmuth) in an *in vitro* assay for seed transmission of *P. tritici-repentis* (*P. t. r.*)

Seedlings	% Seedlings with lesions on		Average shoot length (cm)	Average shoot weight (g)
	Coleoptile	First leaf		
		Seed surface-sterilized		
Apparently healthy[a]	0	0	16.5 a[e]	0.23 a[e]
P. t.-r.-infected[b,d]	92	14	12.5 b	0.19 b
		Seed not surface-sterilized		
Apparently healthy[c]	0	0	16.5 a	0.23 a
P. t.-r.- infected[b,d]	66	2	12.2 b	0.20 a

[a] Sample of 34 seedlings.
[b] Sample of 50 seedlings.
[c] Sample of 30 seedlings.
[d] Based on presence of characteristic pseudothecia, mycelium, and/or pigment of *P. tritici-repentis* on seed, roots, or in the agar.
[e] Values followed by the same letter are not significantly different from each other according to a one-sided t-test at the 5% level.

Seed transmission in potting soil

Emergence was slightly, but significantly, lower in the seed lot infected by *P. tritici-repentis* compared to the uninfected seed lot (Table 3). Pseudothecia of the fungus were observed on the seed remnants of many seedlings grown from the infected lot. Somewhat less than half of the seedlings grown from infected seed had lesions, ranging from tiny brown stripes to large necrotic areas on the coleoptile (Fig. 1, Table 3). In addition, foliar lesions, up to five per leaf, were also evident on some of the seedlings grown from infected seed, whereas seedlings grown from uninfected seed showed no foliar lesions. Most of the lesions were located on the first seedling leaf and only a few on the second seedling leaf. This translates to a transmission efficiency to the coleoptile of 60% and to the leaves of 20%. Many of the seedlings that originated from infected seed were visibly stunted and some had distorted coleoptiles and leaves.

At the tillering stage (ZGS 21–22), plants grown from *P. tritici-repentis*-infected seed were delayed in their development in terms of shoot weight and number of tillers compared to plants grown from uninfected seed (Table 4). Pseudothecia were still evident on seed remnants of 62% of the plants grown from infected seed and were not observed on remnants of uninfected seed.

The average incidence of plants with recognizable lesions on the coleoptile, however, was less than at the first sampling date. The average inci-

Table 3. Observations of wheat seedlings (cv. Frankenmuth) at ZGS 12 (two-leaf stage) in a potting soil assay for seed transmission of *P. tritici-repentis*

Seed lot	% Seed infection[a]	% Emergence[b]	% Seedlings with pseudothecia on seed[c]	% Seedlings with lesions on coleoptile[c]	1st leaf[c]	2nd leaf[c]
Healthy	0	99 a[d]	0 a[e]	2 a[d]	0 a[e]	0 a[e]
Infected	75	93 b	60 b	45 b	13 b	5 b

[a] Tested by the freezing blotter method, 100 seeds each.
[b] Percentage of total seeds planted; mean of six replicates of 50 seeds.
[c] Mean of six replicate samples of 10 seedlings each.
[d] Values followed by the same letter are not significantly different from each other based on a one-sided t-test at the 5% level of the arcsine-tranformed data.
[e] Values followed by the same letter are not significantly different from each other based on recalculated 95% confidence intervals of the arcsine-transformed data.

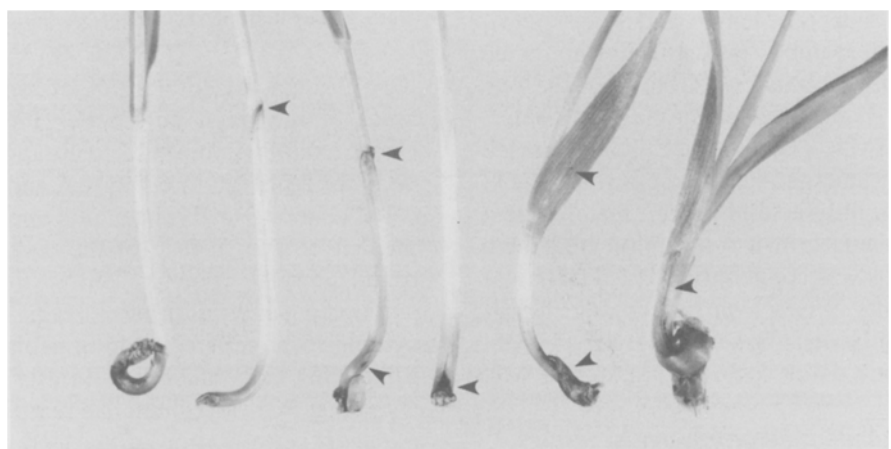


Fig. 1. Range of symptoms on wheat seedlings (cv. Frankenmuth) grown from *P. tritici-repentis*-infected seed, including necrotic lesions on the upper and lower coleoptile (all seedlings), a small foliar lesion (second seedling from the right), and coleoptile distortion and cracking, general stunting (first and second seedling from the right).

Table 4. Observations of wheat plants (cv. Frankenmuth) at ZGS 21–22 (tillering) in a potting soil assay for seed transmission of *P. tritici-repentis* (*P.t.r.*)

Seed lot	Shoot weight (g)[a]	No. tillers[a]	% Plants w/ lesions on coleoptile[a]	% Plants w/ lesions on leaves[a]	% Plants from which *P. t.r.* was isolated[b]
Healthy	0.56 a[c]	1.5 a[c]	2 a[c]	7 a[c]	0 a[d]
Infected	0.48 a	1.2 b	27 b	32 b	8 b

[a] Mean of six replicate samples of 10 plants each.
[b] Mean of six replicate samples of 10 plants each, except for healthy seed lot (one replicate sample of 10 plants).
[c] Values followed by the same letter are not significantly different from each other based on a one-sided t-test at the 5% level of the arcsine-transformed data.
[d] Values followed by the same letter are not significantly different from each other based on recalculated 95% confidence intervals of the arcsine-transformed data.

dence of plants with recognizable foliar lesions had increased somewhat. *P. tritici-repentis* was isolated to a limited extent from coleoptiles and first leaves at this stage of growth, and was not isolated from second or higher leaves. A few lesions were also evident on plants grown from uninfected seed, but *P. tritici-repentis* could not be isolated from them. Other fungi, such as *Phoma* spp., *Septoria* sp., *Fusarium* spp., *Ascochyta* sp., *Alternaria* spp., *Epicoccum* sp., and *Cladosporium* spp. were commonly isolated from leaves and coleoptiles of plants from both treatments.

At the first node detectable (ZGS 31), heading (ZGS 55), and early dough (ZGS 83) stages of crop development, a significantly higher percentage of the leaf area of plants grown from infected seed was affected by tan spot-like lesions than of plants grown from uninfected seed (Fig. 2). At ZGS 31, plants grown from infected seed also had more senescent leaves and were significantly shorter than plants grown from uninfected seeds. The difference in height between the two treatments persisted until about flag leaf emergence. At ZGS 55, plants grown from infected seed had fewer emerged heads and, even at ZGS 83, were still slightly behind in their development compared to plants grown from uninfected seed. *P. tritici-repentis* was commonly isolated from leaf lesions on plants grown from infected seed, but was isolated only once from plants grown from uninfected seed.

Microscopic examination of infected seedlings

Longitudinal sections of seedlings grown from infected seed on water agar in test tubes showed

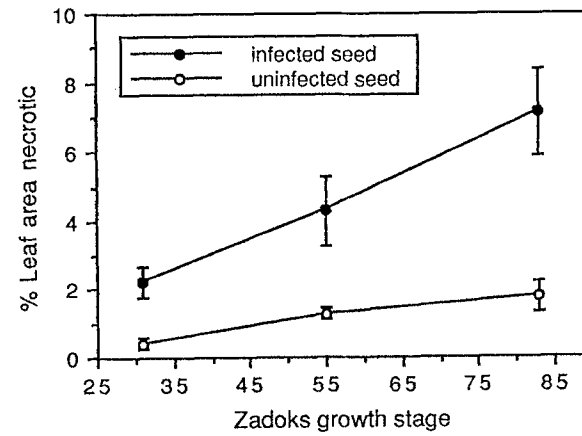


Fig. 2. Leaf spot severity at three stages of crop development in wheat plants (cv. Frankenmuth) grown from *P. tritici-repentis*-infected seed and from uninfected seed. All leaves rated at ZGS 31, the upper four leaves rated at ZGS 55, and the upper three leaves rated at ZGS 83 (Zadoks et al., 1974). Vertical bars indicate standard errors (n = 6).

dark brown discoloration of small to large areas of the outer coleoptile (Fig. 3A). Under closer examination, the discoloration appeared to be limited mainly to epidermal and subepidermal cells (Fig. 3B). Hyphae and appressoria were present on the surface of the lesions, and dark, round papillae were present in the cells below attempted penetration sites (Figs. 3B, 3C). The cytoplasm of cells containing papillae and of the subtending cells had taken on a granular appearance (Fig. 3C). Mycelium had ramified throughout the endosperm portion of the seed remnants (Fig. 3D). An immature pseudothecium was visible between the outer and inner pericarp of the seed remnant, and the upper portion of the coleorhiza

also exhibited a brown discoloration (Fig. 3D). Some hyphae were observed on the surface of the coleorhiza as well as on the upper roots.

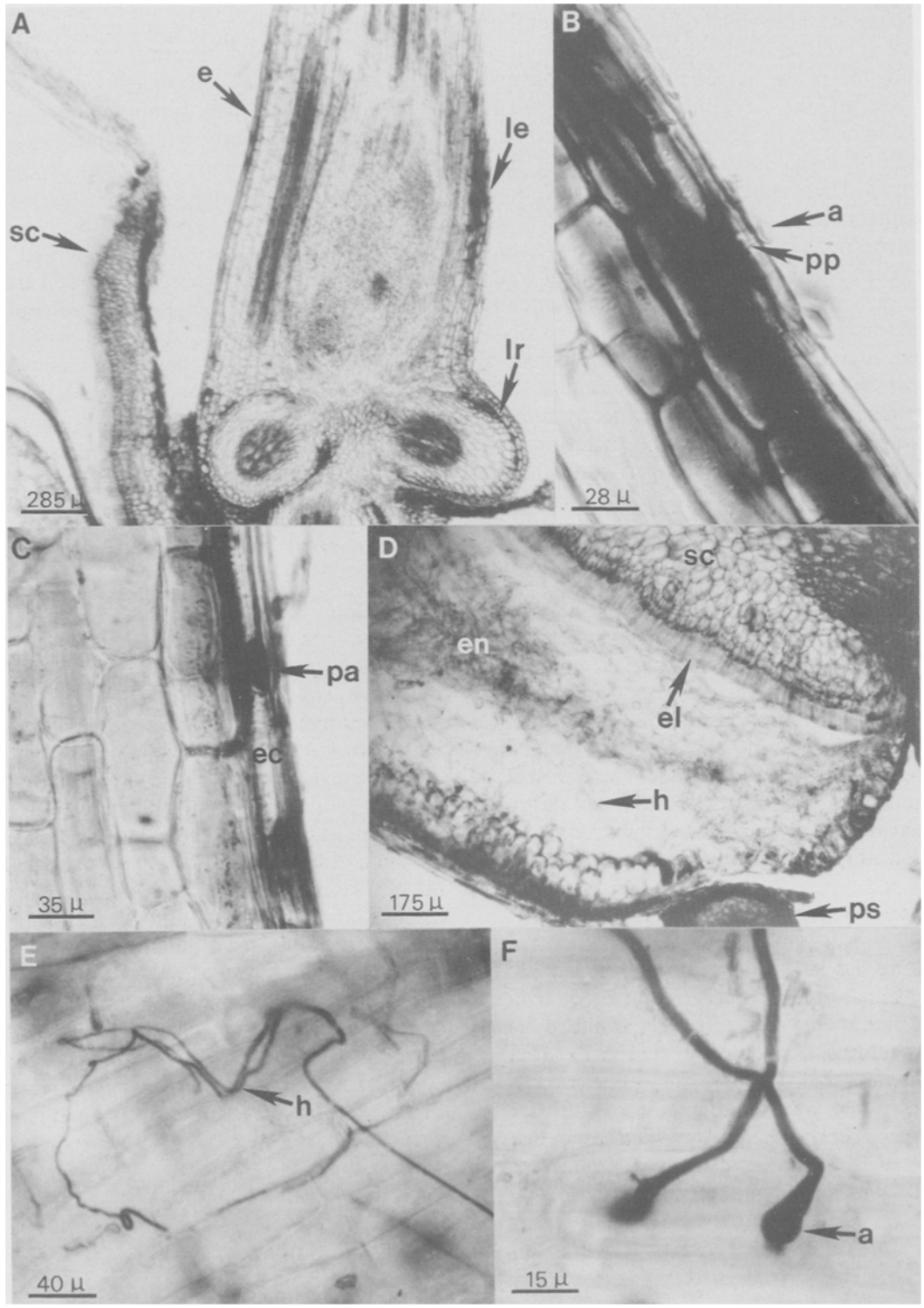
Examination of the necrotic tip of the coleoptile of one of the seedlings from the seed transmission assay in potting soil revealed an abundance of mycelium on the surface of the affected area. There appeared to be no connection with mycelial patches in the middle and at the base of the coleoptile. Many appressoria were present on the coleoptile epidermis, especially at cell wall junctions, near the tip. The cells in this area had collapsed, and they exhibited a dark brown discoloration. Some cells at the base of the coleoptile also were dark brown. Mycelium was observed on the epidermis of the leaf sheath directly below the coleoptile as well. Examination of lesions at the tip of the first leaf revealed superficial hyphae (cells approximately 2–3 × 30–70 µm) growing in the lesion area (Fig. 3E). Several appressoria (4–8 µm wide) were observed at cell junctions, as well as over the guard cells of a stomate (Fig. 3F). No hyphae were observed on other areas of the affected leaf. A severely affected seedling showed mycelium all over the cracked, twisted, and necrotic coleoptile. There were several lesions on the distorted first leaf. Superficial mycelium was observed in several places along the leaf blade, even in areas where no lesions were present, as well as along the edge of the leaf tip and on the leaf sheath. No mycelium was observed on the blade or sheath of the second leaf.

Effect of temperature on seed transmission

Temperature did not significantly affect the percentage of seedlings that emerged, but affected the length of time to emergence. At 9, 15, and 21 °C, it took seedlings 12, 7, and 4 days to emerge, respectively. A significant, negative linear correlation was noted between temperature and incidence of pseudothecia on the seed remnants, incidence of lesions on the coleoptile, and incidence of lesions on the upper portion of the coleoptile (above the soil line) (Fig. 4A). Most of the coleoptile lesions were tiny brown streaks, but moderate to severe lesions (affecting ≥ 15% of the coleoptile) occurred on 3.7%, 0.7%, and 1.3% of the seedlings at 9, 15, and 21 °C, respectively. Lesions on the first seedling leaf were observed on 1.7, 0.3, and 1.7% of the seedlings at 9, 15, and 21 °C, respectively, while lesions on the second seedling leaf only occurred on 0.7% of the seedlings at 9 °C. The frequency of recovery of *P. tritici-repentis* from symptomatic coleoptiles increased significantly with an increase in seed germination temperature (Fig. 4B). Recovery of the fungus from symptomatic leaves was more frequent than from symptomatic coleoptiles. Under the assumption that all observed lesions were caused by *P. tritici-repentis*, seed transmission efficiency of the fungus to the coleoptile was calculated to be 83, 47, and 27% at 9, 15, and 21 °C, respectively. At those same temperatures, seed transmission efficiency of the fungus to the foliage was 2.7, 0.5, and 2.6%. In comparison, transmission efficiency to the coleoptile was 60% in potting soil under outdoor temperatures which averaged 6 °C for the first 11 d, followed by an average of 13 °C for the next 9 d.

Discussion

The present study shows that seed infected by *P. tritici-repentis* can be a source of inoculum for tan spot development. This has consequences for both wheat production and exchange and trade of seed at a national and international level. De Tempe [1964] postulated that movement of infected seed was responsible for the worldwide occurrence of tan spot. *P. tritici-repentis* was transmitted from infected seed to seedlings with relatively high efficiency, although only those lesions above the soil line may contribute inoculum for secondary spread of the disease. A comparable seed transmission rate for seedlings grown in potting soil was reported by Hyltén-Cavallius [1984], whereas Obst [1989] reported a much lower seed transmission rate of 13%. Seed transmission efficiency was higher *in vitro* than in potting soil, since the humid environment created by water agar in test tubes was very conducive to both growth and transmission of *P. tritici-repentis*. Seed infection by *P. tritici-repentis* reduced emergence only slightly, but resulted in a marked and long-lasting reduction in plant vigor, similar to what has been reported for seedborne *Stagonospora nodorum* (Berk.) Cast. & Germ. [Kietreiber, 1961]. Hyltén-Cavallius [1984] also reported stunting of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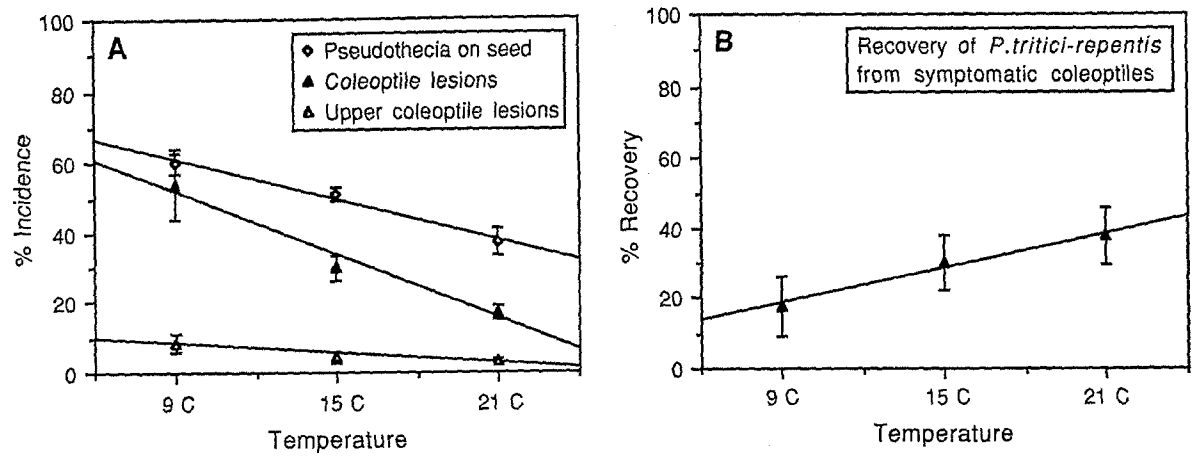

Fig. 4. (A) Effect of seed germination temperature on the incidence of symptoms and signs of *P. tritici-repentis* on wheat seedlings (cv. Frankenmuth) grown from seed with an infection incidence of 64%. Regression equations, regression coefficients, and probability values for each regression are, in order of listing in figure: $y = 71.9–11.5x$, $r^2 = 0.99$, $P = 0.0001$; $y = 69.7–18.2x$, $r^2 = 0.97$, $P = 0.0001$; $y = 10.8–2.7x$, $r^2 = 0.88$, $P = 0.007$. Vertical bars indicate standard errors (n = 3). (B) Effect of seed germination temperature on recovery of *P. tritici-repentis* from symptomatic coleoptiles on V8 medium. The regression equation, regression coefficient, and probability value for the regression are: $y = 8.5 + 10x$, $r^2 = 0.98$, $P = 0.0171$. Vertical bars indicate standard errors (n = 3).

seedlings grown from *P. tritici-repentis*-infected seed. This could affect winter survival, susceptibility to other pathogens, and possibly even yield. Activity of *P. tritici-repentis* within the seed remnants was evident by the formation of immature pseudothecia in the pericarp. Competition with the seedling for the nutrients stored in the endosperm and aleurone layer may explain the general stunting observed in plants grown from infected seed.

Microscopic examination of infected seedlings provided information on the probable path followed by the fungus during seed-to-seedling transmission. *P. tritici-repentis* externally infected the coleoptile as it emerged during germination by hyphal growth from the pericarp. The first leaf apparently became infected by contact with hyphae from an infected coleoptile as the leaf pushed through its tip. A similar transmission pathway was reported for *Pyrenophora teres* Drechs. in barley (*Hordeum vulgare* L.) [Jordan, 1981].

Environmental conditions and cultural practices may affect the degree of seed transmission of *P. tritici-repentis*. In the present study, seed transmission efficiency of *P. tritici-repentis* was reduced by an increase in soil temperature, a phenomenon also observed for *Pyrenophora graminea* Ito & Kurib. in barley [Isenbeck, 1938; Johnson, 1925; Leukel et al., 1933; Teviotdale and Hall, 1976]. The fungus may have been at a relative advantage due to slow growth of the seedling at lower temperatures. A delay of seed germination by means of an osmotic sugar solution enhanced symptom development on coleptiles of seedlings grown from *P. tritici-repentis*-infected wheat seed [Schilder, unpublished]. The effect of temperature on seed transmission of *P. tritici-repentis* may

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Fig. 3. Longitudinal sections and whole mounts of symptomatic wheat seedlings (cv. Frankenmuth) grown from seeds infected by *P. tritici-repentis*. (A) Large, dark brown lesion on seedling coleoptile, (B) Hyphae and appressoria on coleoptile epidermis, (C) Papillae in epidermal cells at attempted penetration sites on the coleoptile, (D) Hyphae ramifying through seed endosperm; immature pseudothecium between layers of seed pericarp, (E) Hyphae on surface of lesion on first seedling leaf, (F) Appressoria on stomate in lesion on first seedling leaf. a = appressorium, e = epidermis, ec = epidermal cell, el = epithelium, en = endosperm, h = hypha, le = lesion, lr = lateral root, pa = papilla, pp = penetration peg, ps = pseudothecium, sc = scutellum.

explain why Shaw and Valder [1952] did not observe symptoms in a seed transmission test carried out at 20–30 °C.

The incidence of lesions on the upper coleoptile was much lower than the incidence of lesions on the entire coleoptile and also exhibited a negative linear response to an increase in temperature in the range of 9 to 21 °C. Proximity of the fungus within the pericarp to the emerging coleoptile may have determined the location of lesions on the coleoptile, and indirectly the incidence of foliar lesions, which apparently resulted from infection by hyphae on lesions at the coleoptile tip or along cracks in the coleoptile.

In contrast to seed transmission efficiency, recovery of *P. tritici-repentis* from symptomatic coleoptiles and leaves improved with an increase in seed germination temperature. More thorough colonization of plant tissues probably occurred as the optimum temperature for growth of *P. tritici-repentis* (20–25 °C [Summerell and Burgess, 1988]) was approached. In general, the fungus was more frequently isolated from large lesions than from small lesions.

The effects of soil moisture, planting depth, wheat cultivar, fungus isolate, and degree of seed colonization on seed transmission of *P. tritici-repentis* should be investigated further.

The increased recovery and transmission of the fungus from infected seed after removal of seed surface contaminants in the *in vitro* assay indicated possible interactions with antagonistic microorganisms. Da Luz [1992] reported good control of *P. tritici-repentis* on wheat seeds by various microorganisms.

Important epidemiological measures that determine the impact of seedborne inoculum on subsequent disease development in the field are incidence of seed infection, the efficiency of seed-to-seedling transmission, and the rate of disease development from the initial focus of inoculum. The threshold incidence of seedborne inoculum below which crop damage is acceptable is not constant, since it is greatly affected by environmental factors [Agarwal and Sinclair, 1987b]. Even though seed transmission rates of *P. tritici-repentis* have been established under laboratory and outdoor conditions, the relative contribution of seedborne *P. tritici-repentis* to tan spot development under actual field conditions remains unclear and should be investigated further. Piening [1968] stated that even one percent of seed infected with *P. teres* could be significant in the development of later infections. Even if seedborne inoculum turns out to play a minor role in the development of tan spot epidemics, it may still provide additional inoculum, possibly with a different array of virulence phenotypes than already exists in a field. Fungal populations that are normally isolated in space and time may get a chance to interact through the planting of infected seed and may constitute, in effect, a single, large population. This possibility raises intriguing questions about the population dynamics of *P. tritici-repentis* and, indeed, other seedborne pathogens.

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