Infection of Wheat Seedlings by Ascospores of *Tapesia yallundae*: Morphology of the Infection Process and Evidence for Recombination

Alison Daniels, Michalis Papaikonomou, Paul S. Dyer, and John A. Lucas

Department of Life Science, University of Nottingham, University Park, Notts NG7 2RD, UK.
Current address of A. Daniels: AgrEvo UK Ltd., Chesterford Park, Saffron Walden, Essex CB10 1XL, UK. Current address of J. A. Lucas: IACR—Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS18 9AF, UK.
A. Daniels thanks Hoechst-Schering AgrEvo GmbH for financial support. M. Papaikonomou acknowledges AgrEvo UK Ltd. for an SERC CASE award. P. S. Dyer and J. A. Lucas thank the Biotechnology and Biological Sciences Research Council, U.K., for a research support grant (PO1440).
We thank B. V. Case and D. West (Department of Life Science, University of Nottingham) for photographic and technical assistance, respectively, and J. E. Hardy for assistance with plant trials.
Accepted for publication 8 May 1995.

ABSTRACT

Daniels, A., Papaikonomou, M., Dyer, P. S., and Lucas, J. A. 1995. Infection of wheat seedlings by ascospores of *Tapesia yallundae*: Morphology of the infection process and evidence for recombination. Phytopathology 85:918-927.

The infectivity to wheat seedlings of ascospores from apothecia of *Tapesia yallundae* grown in vitro was demonstrated for the first time. The infection process from spore adhesion to lesion formation was monitored using low-temperature scanning electron microscopy and was similar to that observed for conidia of the anamorph, *Pseudocercosporella herpotrichoides*. Mycelium isolated from lesions was characterized using randomly amplified polymorphic DNA (RAPD) markers and was compared to DNA amplification patterns obtained for parental isolates from which the teleomorph was induced. Recombination was demonstrated in all but one lesion from which mycelium was successfully reisolated. A high proportion of reisolates had unique RAPD profiles, indicative of novel genotypes. The implications for pathogenic variation and the development of fungicide resistance in the field are discussed.

Pseudocercosporella herpotrichoides (Fron) Deighton causes cereal eyespot disease, in particular of wheat and barley, often resulting in considerable yield reduction. Using recently developed molecular fingerprinting techniques, *Tapesia yallundae* Wallwork & Spooner has been confirmed as the teleomorph of the two major pathotypes of *P. herpotrichoides*, known as the wheat (W) and rye (R) types (9,10,23). These pathotypes are routinely isolated from lesions in the field and may be differentiated on the basis of a variety of cultural characteristics and biochemical tests (4,16,21,24,28).

Recently, apothecia of *T. yallundae* have been found on infected straw stubble in most major wheat-growing areas of Europe (8,9,10,14,17,18,20,23), Australasia (26,30), and South Africa (25). Since in other Ascomycetes ascospores are forcibly ejected from mature apothecia (15) and active airborne ascospore discharge has been observed in *T. yallundae* (18), there is speculation concerning ascospores as a potential inoculum source in the eyespot disease cycle. It has been assumed that the primary inoculum in disease outbreaks is conidia that are produced on crop debris and rain-splashed over short distances (11). Airborne ascospores might provide an alternative means of long-range dispersal and infection. Furthermore, the capacity for genetic recombination in the sexual stage (23) might extend the range of variation present in field populations of the eyespot pathogen, with important implications for characteristics such as pathogenicity and fungicide resistance. To date, however, the infectivity of *T. yallundae* ascospores to cereal hosts has not been demonstrated.

This paper presents the first conclusive evidence that ascospores of *T. yallundae* can infect wheat seedlings in a manner similar to infection from rain-splash–dispersed conidia. The infection process can occur under environmental conditions similar to those required for seedling infection by the anamorph. Molecular analysis of isolates derived from lesions initiated from ascospore inoculum using randomly amplified polymorphic DNA (RAPD) markers confirmed the occurrence of recombination.

Corresponding author: A. Daniels; E-mail address (c/o): johnlucas@bbsrc.ac.uk

MATERIALS AND METHODS

Fungal culture and production of apothecia. Maintenance of isolates and induction of the sexual cycle were performed as described previously (9). The parental isolates selected from the Nottingham University culture collection, Nottingham, U.K., were the fertile strains 22-432 (mating group MAT1-1) and 22-433 (mating group MAT1-2). Both belong to the W-pathotype and originated from single spores.

Seedling inoculation and plant growth. Assessment of ascospore infectivity was carried out on 23-day-old wheat seedlings, cv. Avalon, at the two-leaf stage. Plants were grown as described previously (6) in 9-cm^2 pots, with nine seeds per pot in a 3:1 (vol/vol) peat and loam mixture, and were maintained in a growth room (18-h photoperiod, 220 μmol m^{-2} s^{-1}, day temperature 19 ± 2°C, night temperature 15 ± 2°C). Two inoculation methods were used. In the first method, dissected straw segments bearing apothecia were mounted with petroleum jelly on the inner surface of plastic Pasteur pipette bulbs that were used to shroud the base of wheat seedlings as illustrated in Figure 1A. The stem and tip of the pipette were cut off, and the bulb was slit longitudinally to allow it to be wrapped around the seedling base. Apothecia were thus positioned approximately 0.5 cm from the seedling and 2 cm from the soil surface. In the second inoculation method, entire

straw segments bearing apothecia were placed onto cocktail sticks positioned upright in the soil approximately 1 cm from stem bases and approximately 2 cm from the soil surface (Fig. 1B). In each case, controls were prepared using uninfected straw segments. Twenty pots were used for each treatment. These were randomly positioned to eliminate experimental bias. Pots with plants were individually enclosed in polyethylene tubes to maintain humidity and prevent cross-infection. Plants were watered daily from the base. Straws bearing apothecia and uninfected straws were removed after 7 days when infection was judged to have occurred.

Microscopy. Low-temperature scanning electron microscopy (LTSEM) of dissected coleoptiles and leaf sheath bases was performed as described previously (6). The progress of leaf sheath infection was monitored by a simultaneous clearing and staining technique using lactic acid and trypan blue (5).

Pathogenicity testing. Disease was assessed visually by the method of Scott (27), in which 0 = no infection, 1 = coleoptile infected, 2 = coleoptile penetrated, 3 = first leaf sheath infected, 4 = first leaf sheath penetrated, etc. Visual recognition of symptoms such as pigmented infection plaques was facilitated by use of a stereo-microscope.

Fungal reisolation. Infected tissue fragments from lesions were carefully dissected and rinsed in sterile distilled water (SDW) amended with 16 µg of penicillin and 74 µg of streptomycin sulfate per ml (SDW+). A proportion of samples was surface-sterilized by brief immersion in sodium hypochlorite solution (1% [vol/vol] available chlorine) followed by two rinses in SDW+. After air-drying in a laminar flow cabinet, lesion fragments were plated on 20% (wt/vol) tap water agar or 20% (wt/vol) potato-dextrose agar (PDA) containing the same antibiotic amendment as above. Cultures were incubated under continuous white light (25 to 40 µmol m^{-2} s^{-1}) at 20 ± 2°C. Putative *T. yallundae* mycelium was subcultured every 3 to 5 days on fresh medium until pure cultures were obtained. These were maintained on 20% (wt/vol) PDA.

DNA extraction and RAPD analysis. RAPD markers (31) were used to differentiate isolates of *T. yallundae*. Mycelium (area 2 to 3 cm^2), scraped off 4- to 6-week-old cultures, was suspended in 0.6 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and boiled for 5 min. Samples were cooled on ice for 5 min, and 0.6 ml of a 25:24:1 (vol/vol) mixture of TE-saturated phenol/chloroform/isoamyl alcohol was added. The resulting suspensions were vortex mixed and centrifuged (11,600 × *g* for 5 min), and the supernatant was removed. An equal volume of chloroform/isoamyl alcohol (24:1) was added to the new solution, which was mixed in a vortex and centrifuged (11,600 × *g* for 5 min). The supernatant was removed and stored at 4°C for future use.

RAPD analysis was performed as previously described (22,31). Aliquots of the supernatant (1 µl) were added to 49 µl of reaction volumes containing 100 µmol each of dATP, dCTP, dGTP, and dTTP (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ), 0.5 µl each of a Tween 20 (0.05 ml ml^{-1}) and Nonidet P-40 (0.05 ml ml^{-1}) solution (Sigma Chemical Company, St. Louis), 10 pmoles of a single 10-base primer, 1 unit of *Taq* DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, IN), and 5 µl of a 10-fold polymerase chain reaction (PCR) buffer (supplied with the enzyme). Screening of 40 different 10-mer primers (OPA and OPB kit, Operon Technologies, Alameda, CA) showed that primers OPA-11 (CAATCGCCGT) and OPA-9 (GGGTAACGCC) gave discriminatory banding profiles for the two parental strains (22-432 and 22-433) and, thus, could reveal recombination in the ascospore progeny.

T. yallundae genomic DNA was amplified using a Perkin-Elmer 480 thermocycler (Norwalk, CT) for PCR, with 45 cycles of 94°C for 15 s (ramp rate 30°C min^{-1}), 36°C for 1 min (ramp rate 30°C min^{-1}) and 72°C for 1 min (ramp rate 60°C min^{-1}) before a final elongation stage of 5 min at 72°C. Amplification products were separated by electrophoresis in 1.5% agarose gels and visualized by staining with ethidium bromide.

RESULTS

Ascospore discharge. Coleoptiles of seedling bases were examined by LTSEM at daily intervals to assess when apothecial discharge had occurred. This was synchronous and occurred between 3 and 4 days under the experimental conditions adopted in this study. By simultaneous examination of the inoculum source, discharge was found to coincide with the stage at which apothecia reached maximum concavity during the maturation process, before opening out to form a flat platelike structure. At this stage, the marginal cells flanking the hymenium were still positioned above the level of the hymenium surface (Fig. 2A). Papillate asci protruded above the paraphyses of the hymenium (Fig. 2B); the whole surface was often covered with a thick mucilage just prior to the active discharge of ascospores (Fig. 2A).

In the majority of cases, ascospores did not appear to be forcibly ejected onto seedling bases, because examination of the coleoptile or leaf sheath base surface directly opposite the inoculum source generally failed to reveal adherent ascospores. Instead, these commonly were found at positions below the inoculum source and in highest concentrations on the coleoptile about 3 mm above the soil surface. Asexual spores were not observed in any of the seedling bases examined microscopically. Thus, it is assumed that in the absence of air turbulence, discharged ascospores drift downward from the apothecium. Evidence of forcible discharge of ascospores directly onto the coleoptile was found in less than 1% of 50 plants examined microscopically. This likely reflected instances in which the hymenial surface of an open-cup apothecium was oriented exactly parallel to the coleoptile surface. In this case, hundreds of ascospores were forcibly propelled onto the coleoptile surface in a large volume of mucilaginous matrix, which initially obscured underlying ascospores but subsequently dried out and revealed individual profiles (Fig. 3A and B). The "impact zone" of such discharges in some cases could be observed after partial freeze-drying of specimens (Fig. 3C). Water-soluble extracellular mucilage, normally extracted during conventional SEM processing schedules, was retained in a frozen-hydrated state by LTSEM. In this case, mucilaginous material obscuring underlying cellular detail was gradually etched away by controlled sublimation of ice using a conductive heating device.

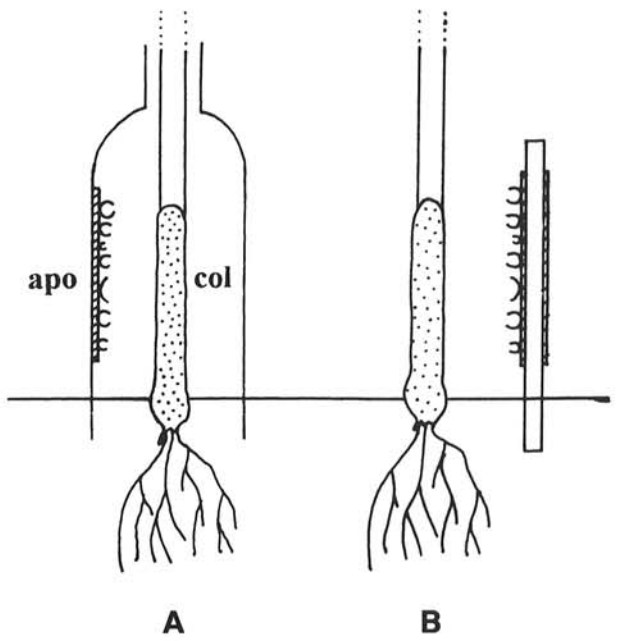

Fig. 1. Diagrammatic representation of inoculation techniques, **A** and **B**, used to promote natural ascospore ejection of *Tapesia yallundae* onto soil-grown wheat-seedling bases. col = coleoptile surrounding seedling base, apo = straw segment bearing apothecia mounted with petroleum jelly **A,** on the inside surface of a cut-off plastic Pasteur pipette bulb or **B,** over a cocktail stick.

Ascospore germination and host infection. Ascospore germination and development on coleoptile tissue was highly synchronous, both on individual seedlings and between plants. This was most likely due to the similar developmental stage of the in vitro-generated apothecia used to inoculate seedlings, so ascospore discharge occurred at the same time in different samples. Evidence for successive discharges on individual seedling bases from 0 to 7 days was not observed, because apothecia were removed after this time to prevent this occurring at a later stage.

Ascospores observed on the coleoptile surface were straight filiform 2-celled structures (approximately 2 × 6 to 8 μm) with blunt or tapered ends. These closely adhered to the host cell surface; attachment apparently was mediated by extracellular mucilage (Fig. 3A). Germination was usually from a single terminal germ tube that rapidly extended to form a superficial hypha, invariably showing alignment with anticlinal coleoptile cell wall grooves (Fig. 4A). Lateral branches were produced from superficial hyphae that either formed extensions of the latter or bulbous appressorium-like structures (Fig. 4A). Intramural growth was often evident from such structures, seen as a swelling of the underlying host cell wall (Fig. 4A). Mycelial ramification on the coleoptile cell surface and underlying host cell colonization was evident 24 to 48 h after ascospore germination (Fig. 4B).

Between 10 and 17 days after ascospore discharge, infection of the first and successive leaf sheaths was evident in about 20% of plants. This coincided with the onset of visible symptoms, recorded as darkly pigmented infection plaques with or without associated discrete areas of leaf sheath browning. Characteristic infection plaques (Fig. 5A and B) were produced from runner hyphae in a manner identical to that recorded for *P. herpotrichoides* in a previous study (6). Infection plaques were formed from differentiation of runner hypha tip cells, forming localized aggregations of expanded parenchyma-like cells, commonly localized above vascular bundles (Fig. 5A). Since these cells were encased in a thick extracellular mucilage, distinction of individual composite cells was difficult. Adventitious runner hyphae were produced at the peripheries of infection plaques, which, in turn, initiated new infection plaques, thus extending the spread of infection (Fig. 5A and B). These eventually overgrew each other to give the appearance of a mat of mycelium (Fig. 5C). As detailed in Daniels et al. (6), each infection plaque functions as a compound appressorium, with multiple infection hyphae produced on its underside. Infection hyphae penetrate the leaf sheath epidermal cells, presumably with the aid of extracellular cell wall-degrading enzymes (3), and the fungus then colonizes vascular parenchyma cells, eventually emerging on the successive leaf sheath as runner hyphae. This process continues on to the stem in time.

Visible lesions produced after 6 weeks were characteristic of the cereal eyespot disease, with lens-shaped, dark-brown borders surrounding a central mass of darkly pigmented mycelium (Fig. 6A and B). Seminal tillers were readily infected (Fig. 6A). Lesions were invariably found at the soil surface (Fig. 6B), rather than some distance above it, as seen for the anamorph.

Pathogenicity. Infection scores derived from the means of 20 plants assessed by the method of Scott (27) were 6.2 ± 1.09 (inoculation method 1) and 9.5 ± 1.6 (inoculation method 2). These values are within the normal range recorded elsewhere for W-type isolates (16). A lower infection score was achieved for inoculation method 1, which was thought to be due to extensive tillering that retarded leaf sheath infection.

Phenotypic variation. The cultural morphology of parental strains and selected reisolates is shown in Figure 6C. Although colony morphology and growth rate of all isolates is characteristic of the W-pathotype, in that cultures have a fast-growing (range 1.8 to 2.1 mm/day), even-edged growth habit (M. Papaikonomou, *personal communication*), variation in colony texture and pigmentation is apparent in reisolates.

RAPD analysis. The results of RAPD analysis of *T. yallundae* mycelium reisolated from lesions on wheat seedlings is shown in Figure 7A and B. For easier interpretation, the RAPD bands scored for the progeny and parental isolates are shown in Tables 1 and 2 for primers OPA-09 and OPA-11, respectively. Recombination was demonstrated at multiple loci in all but one isolate (ALR 14), which showed two new bands (1,290 and 1,235 bp) with primer OPA-11 (Table 2). The majority of isolates (AD1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, and 17) exhibited recombination by the presence and absence of the 1,520- and 1,300-bp bands, respectively, of 22-433 produced by OPA-09 (Table 1). Recombination also was observed for other loci for both primers OPA-09 (Table 1) and OPA-11 (Table 2). It is unknown how or if

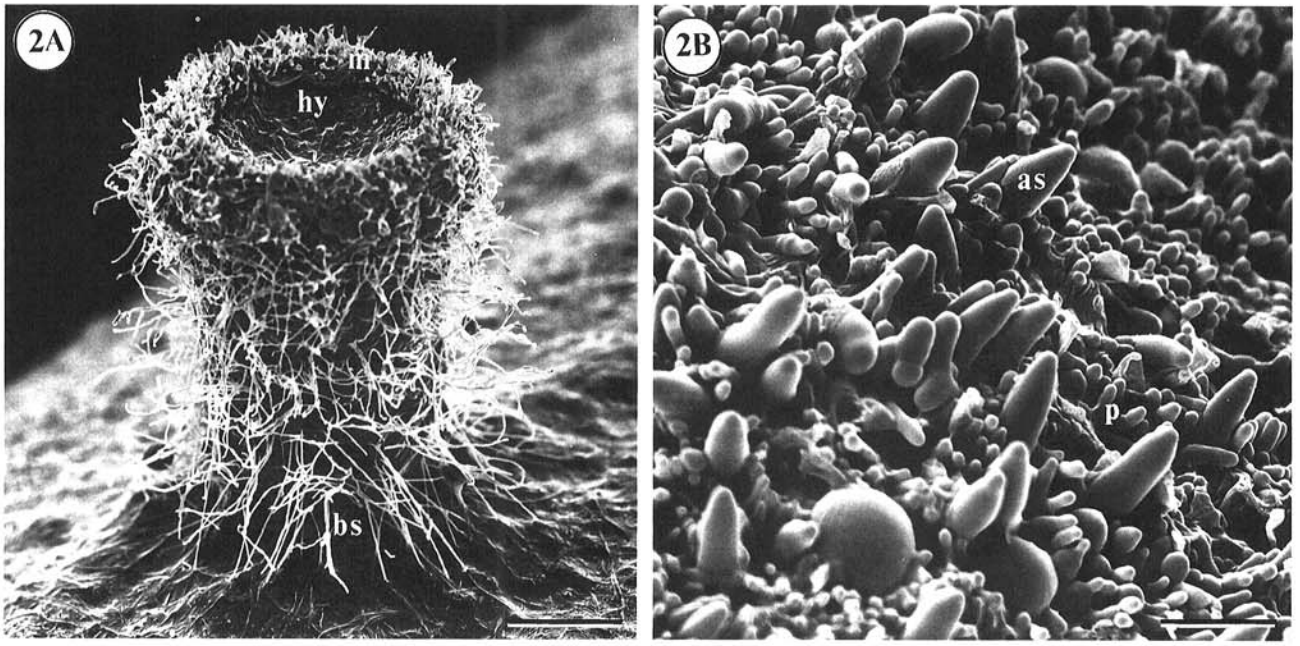

Fig. 2A. Open-cup apothecium of *Tapesia yallundae* just prior to ascospore discharge. m = marginal cells, hy = hymenium, bs = basal stroma. There is a mucilaginous layer over the hymenium surface. Scale marker = 20 μm. **B,** Detail of ascocarp surface showing asci (as) protruding above paraphyses (p) within the hymenium. Scale marker = 20 μm.

the observed genetic variation correlates with the phenotypic variation shown in Figure 6C.

DISCUSSION

The discovery in recent years of apothecia of *T. yallundae* at stubble sites in European, Australasian, and South African locations indicates that sexual reproduction may often be an integral part of the life cycle of the fungus (10). This has various implications for the epidemiology and biology of *T. yallundae*. Of particular significance is the possibility that ascospores may provide a genetically varied source of airborne inoculum for long-range dispersal of the cereal eyespot pathogen.

The major finding from the current study is the demonstration, for the first time, that ascospores were able to infect wheat seedlings and produce lesions characteristic of cereal eyespot disease over a 6-week period. The infection process followed a pattern similar to that demonstrated for conidia of the anamorph (6) and occurred under similar controlled environmental conditions. After colonization of the coleoptile, infection plaques that functioned as

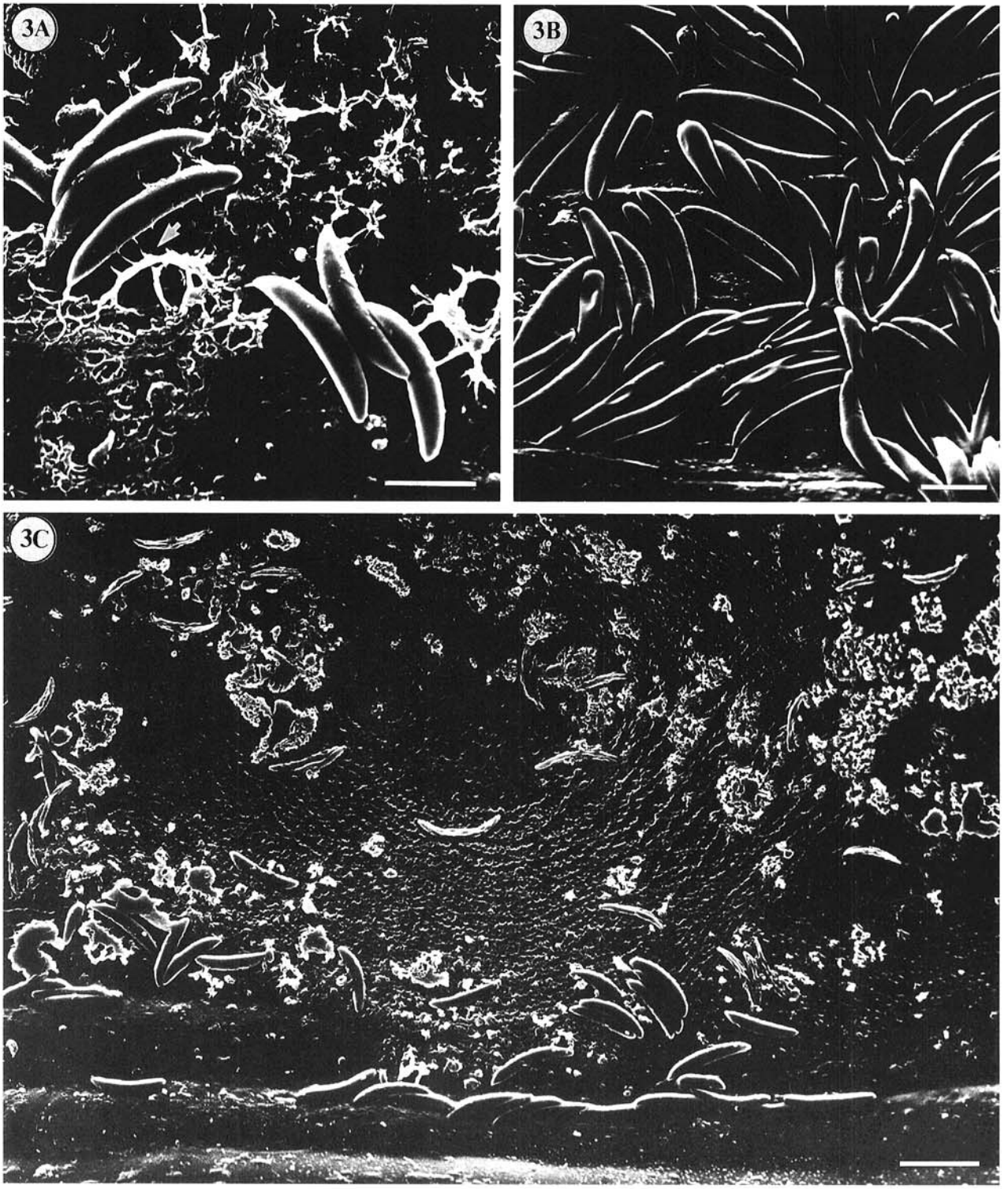

Fig. 3A. Ascospores of *Tapesia yallundae* forcibly propelled onto wheat coleoptile, showing remains of extracellular mucilage (arrow). Scale marker = 10 µm. **B,** Ascospores with multiple associations on the coleoptile surface after desiccation of extracellular mucilage. Scale marker = 10 µm. **C,** Part of an "impact zone" after ascospore discharge onto the coleoptile that was revealed after partial freeze-drying of extracellular mucilage. Scale marker = 20 µm.

compound appressoria were formed on successive underlying leaf sheath surfaces, facilitating colonization of host tissue and leading to lesion formation. Lesions produced under the experimental conditions adopted in the present study were formed low on the stem base at the soil level. This was assumed to be due to actively discharged ascospores drifting downward in the absence of air turbulence, although the role of microconvection currents in this experimental system has yet to be investigated.

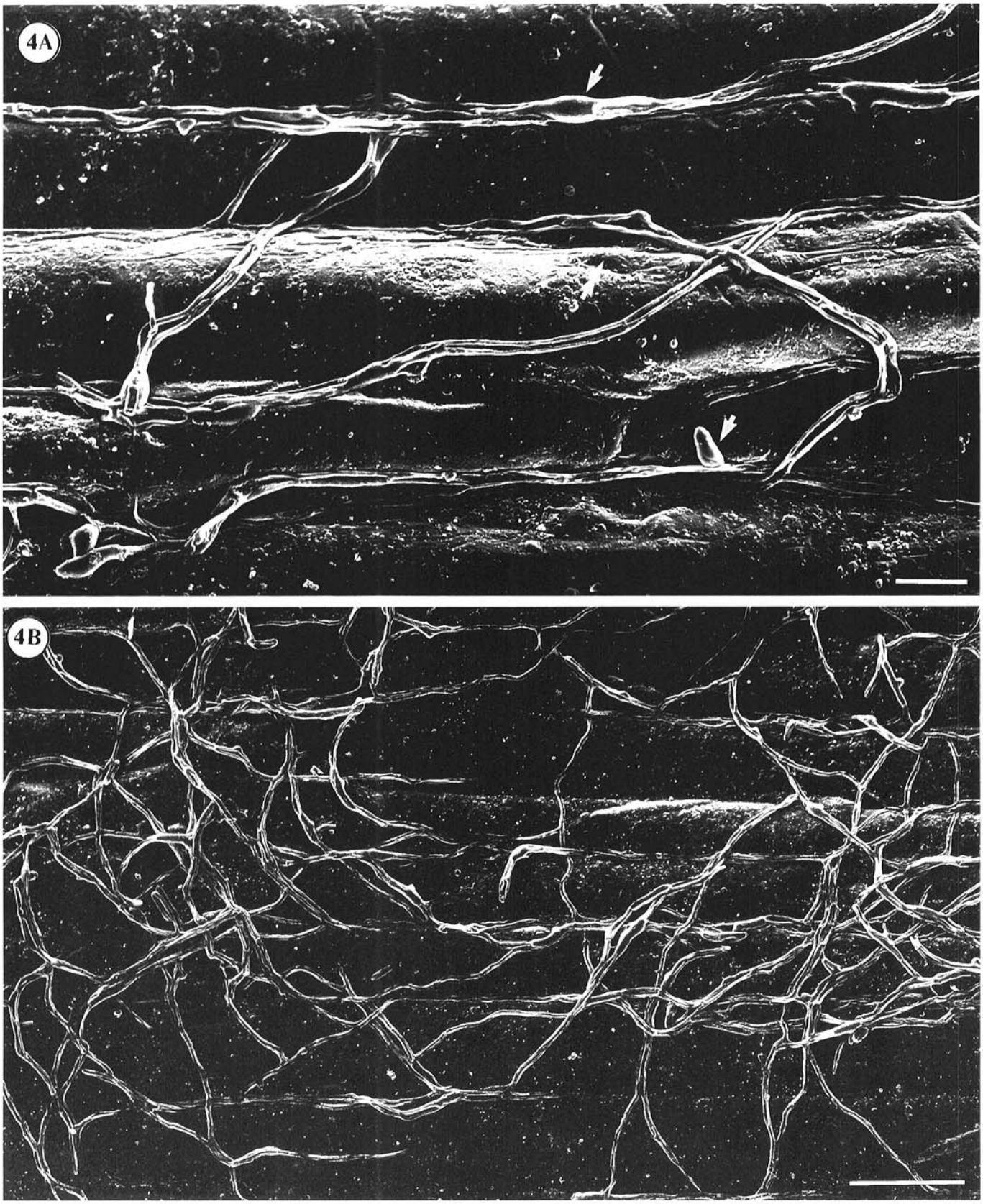

Fig. 4A. Superficial hyphae of *Tapesia yallundae* formed from ascospore germlings showing growth alignment with anticlinal host cell wall grooves. Single arrows indicate appressoria-like swellings. Double arrows indicate cell wall swellings indicative of intramural growth of infection hyphae. Scale marker = 10 μm. **B,** Proliferation of surface mycelium after intramural growth. Scale marker = 50 μm.

Violent forcible ejection onto adjacent seedlings rarely was observed, probably because there is only a remote chance of a 'ripe' apothecium orientating precisely toward the stem base. Ascospore liberation was thought to be achieved by instantaneous rupture of asci that had attained a critical state of turgidity. It was thought that during a prolonged still period, asci become highly turgid and unstable (15); some environmental stimulus, such as strong light, dry air or temperature fluctuation, then triggers ascospore discharge or "puffing." An extracellular mucilaginous matrix appears to be involved in the generation of this high water potential, with a possible antidesiccant role. The stimulus for this event is unlikely to be a drying-out process as reported previously (18),

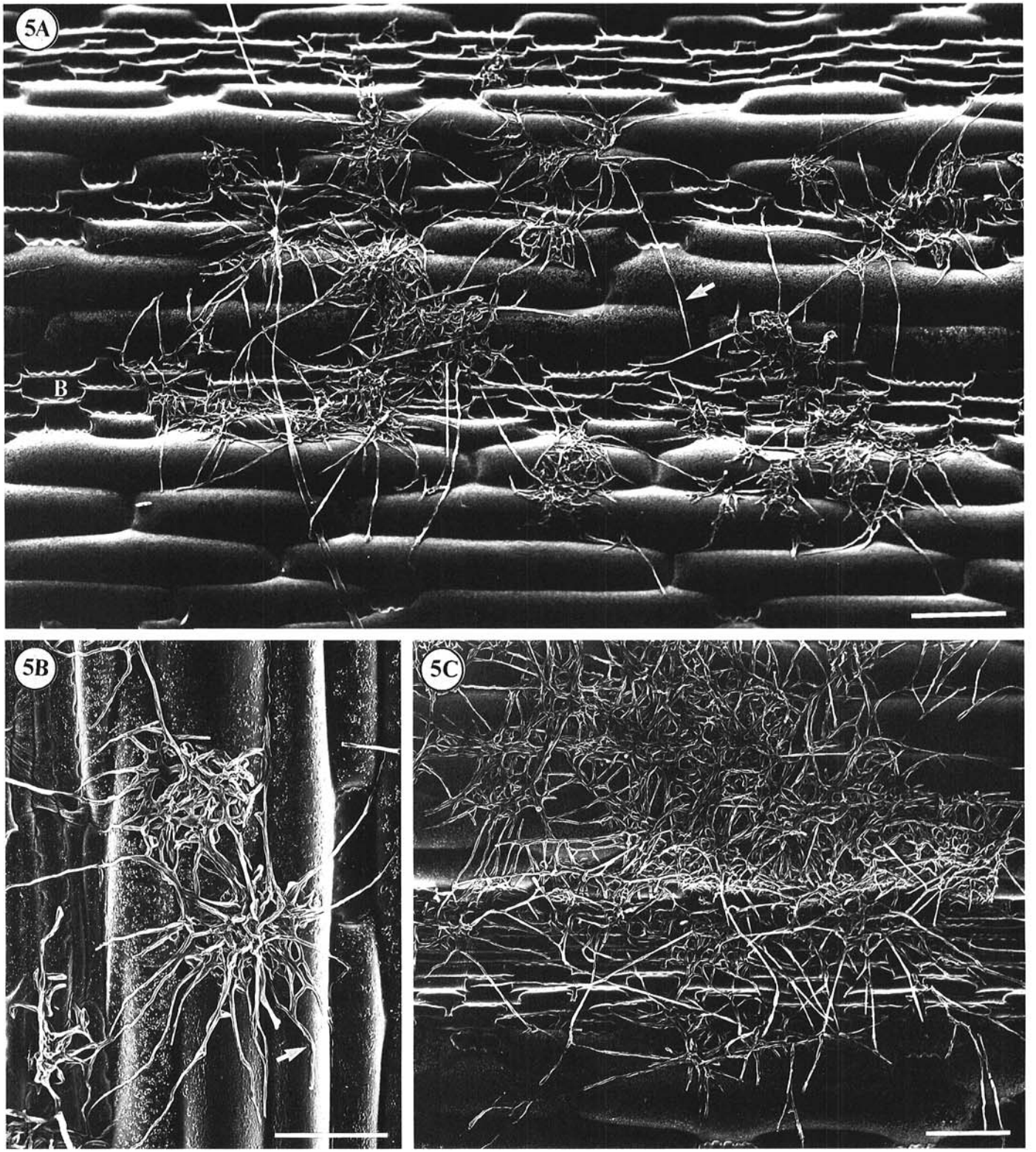

Fig. 5A. Multiple infection plaques of *Tapesia yallundae* formed on the first wheat leaf sheath, showing preferential localization over bulliform cells (B) in grooves above vascular bundles. Runner hyphae interconnect (arrow). Scale marker = 100 μm. **B,** Detail of two adjacent infection plaques on the second leaf sheath showing formation of secondary runner hyphae from peripheral cells (arrow) that subsequently form new infection plaques. Scale marker = 50 μm. **C,** Overgrowth of infection plaques to form a "mat" on the third leaf sheath; small lesions are evident. Scale marker = 100 μm.

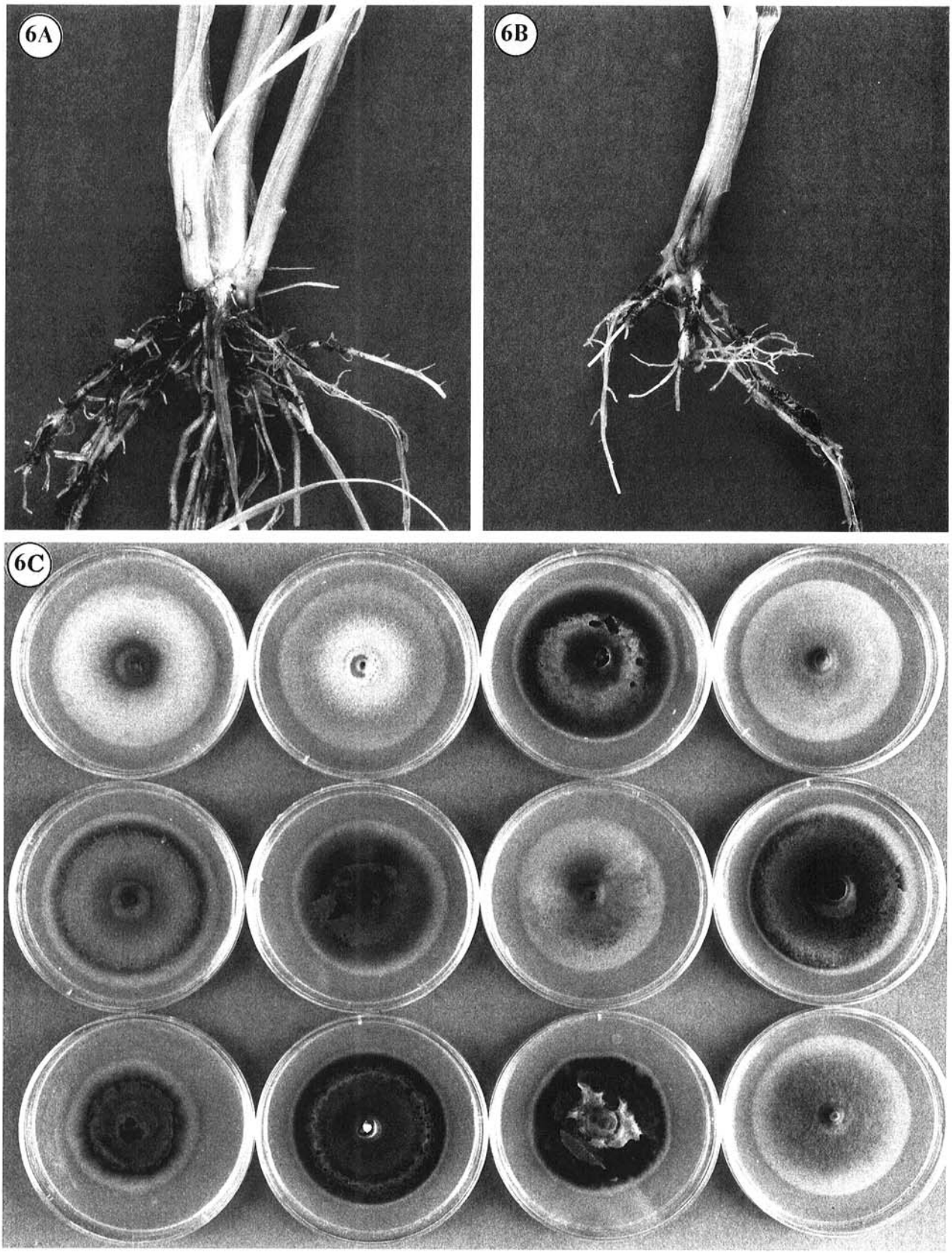

Fig. 6A. Small lesions formed by *Tapesia yallundae* on wheat seminal tillers. **B,** Typical *T. yallundae* seedling infection with lesion just above the soil level. **C,** Phenotypic variation in *T. yallundae* mycelium reisolated from lesions and grown on half-strength potato-dextrose agar. First two plates from left in top row are parental strains 22-432 and 22-433. The remainder are a selection of reisolated mycelia showing variation in colony pigmentation and texture.

since high-humidity conditions were maintained throughout the period of active ascospore discharge. In addition, the thick layer of extracellular mucilage seen on the hymenium surface was not reduced prior to spore discharge, which might facilitate dehydration. A recent study by Dyer et al. (8) indicates that in the field, rainfall stimulates maturation of *T. yallundae* apothecia and, by inference, ascospore discharge. High humidity also is required for *Pyrenopeziza brassicae* ascospore release in the field (2,19). Fluctuations in temperature and light levels are known to trigger ascospore discharge in other Ascomycetes (15), and these seem to be more likely stimuli for the process in *T. yallundae* than periods of desiccation.

Another important observation was that mycelium reisolated from lesions resulting from ascospore infection exhibited variation in colony morphology and pigmentation. RAPD analysis using primers able to discriminate between the parental strains confirmed that genetic recombination had occurred in the reisolates. Although some individual amplicons (e.g., 1,300 with OPA-9) did not segregate in a Mendelian fashion, the average genetic ratio of the discriminatory parental amplicons was 1:1. This is expected of a haploid organism showing Mendelian segregation. Dyer et al. (9,10) also observed novel RAPD profiles in ascospore progeny recovered after apothecial discharge in vitro from controlled crosses using known parents. However, this is the first demonstration of genetic reassortment in the eyespot pathogen resulting from infection of the host by ascospores.

Confirmation of a sexual cycle in both the W- and R-pathotypes and demonstration of the infectivity of ascospores to a cereal host have wide-ranging implications for the population biology of a pathogen previously believed to be spread exclusively by means of asexual conidia. Infection by ascospores still has to be demonstrated under field conditions. Ascospores of *Gaeuman-*

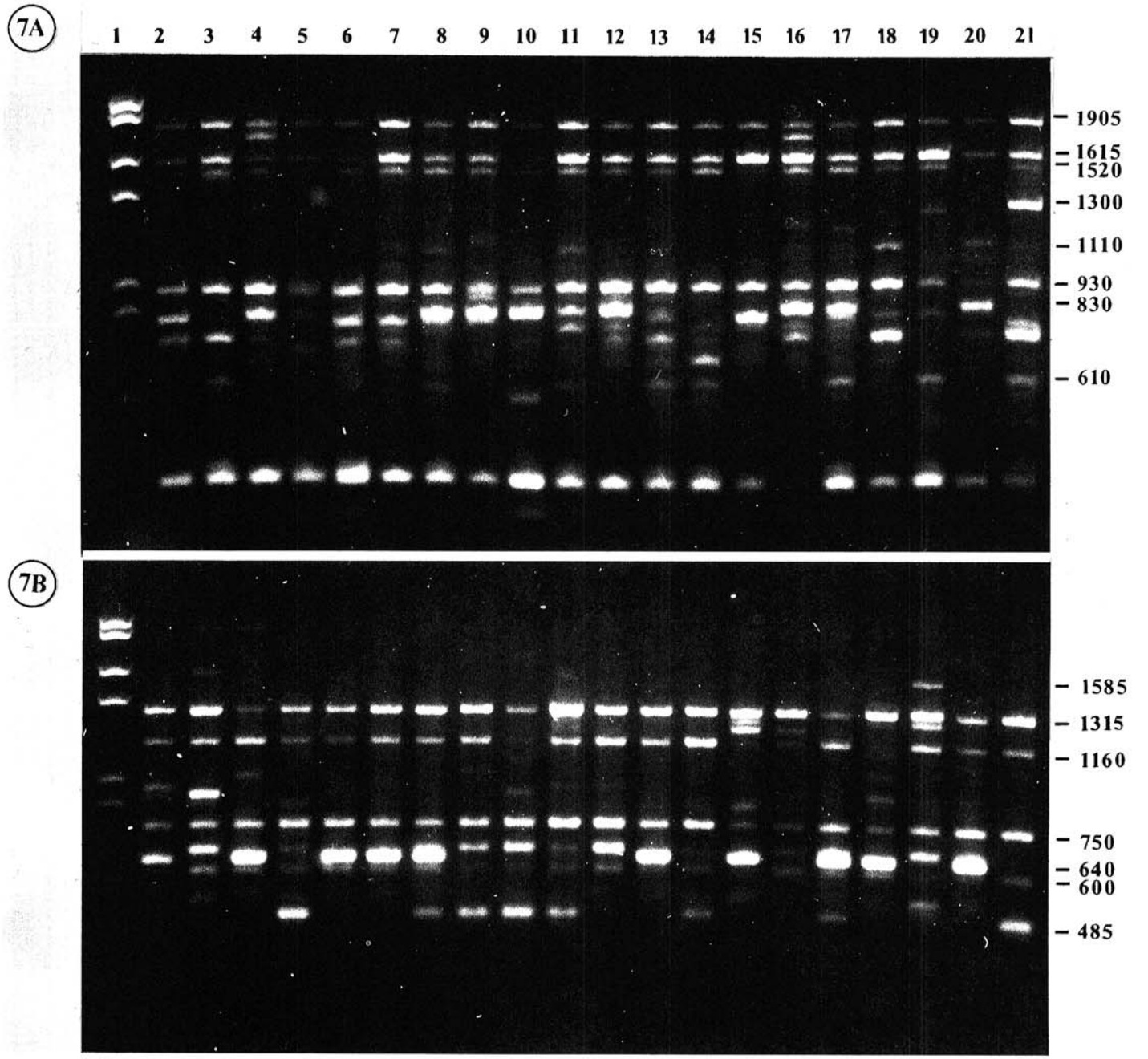

Fig. 7A and B. Randomly amplified polymorphic DNA bands generated by primers OPA–09 (**A**) and OPA–11 (**B**) from DNA extracted from *Tapesia yallundae* isolates 22–432 and 22–433 (lanes 20 and 21) and a series of ascospore progeny (lanes 2 through 19) after reisolation from lesions formed on wheat seedlings. Water and no primer controls gave no bands (not included). Lane 1 is *Eco*RI- and *Hin*dIII-digested lambda DNA used as a size marker.

nomyces graminis also have been infective in laboratory tests but are thought to be important only rarely in the spread of disease in the field (13). However, ascospores of *T. yallundae* would be expected to become airborne in the presence of air turbulence, since ascospore discharge over a 2-cm radius in still air was observed in the present study. Apothecia have been detected at numerous stubble sites in field surveys in Germany and England (18; P. S. Dyer and J. A. Lucas, *unpublished data*), suggesting that ascospore inoculum may be present for a significant proportion of the season for winter cereals in Europe, with clear potential for infection. In France in particular, eyespot is being viewed increasingly as a tricyclic disease, with three successive infection cycles reported from October through January, February through April, and May through July (7). It is possible that ascospore inoculum may contribute to the late season cycle.

Studies on other plant pathogens in which a sexual stage is involved with epidemic development have confirmed that genetic recombination increases the range of variation present in the population (1,12,29). For instance, the recent worldwide migration of the A2 mating type of *Phytophthora infestans* has extended the degree of genetic polymorphism detected by molecular analysis of samples from the field (29). Monitoring eyespot populations at sites where the sexual stage is known to occur should indicate whether a similar increase in genetic diversity is taking place in *T. yallundae.*

TABLE 1 Summary of major bands produced by the primer OPA–09 during randomly amplified polymorphic DNA (RAPD) analysis of total genomic DNA from *Tapesia yallundae* mycelium reisolated from lesions

Isolate number	RAPD band sizes (bp) of primer OPA-09								
	610	830	930	1,110	1,300	1,520	1,615	1,750	1,905
22-432		+	+	+			+		+
22-433	+		+		+	+	+		+
ALR1			+			+	+		+
ALR2	+		+			+	+		+
ALR3		+	+			+	+	+	+
ALR4		+	+			+	+		+
ALR5		+	+			+	+		+
ALR6				+		+	+		+
ALR7		+	+	+		+	+		+
ALR8		+	+			+	+		+
ALR9	+	+	+			+	+		+
ALR10	+	+	+	+		+	+		+
ALR11		+	+			+	+		+
ALR12	+		+			+	+		+
ALR13	+		+			+	+		+
ALR14			+				+		+
ALR15		+	+	+		+	+	+	+
ALR16	+	+	+	+		+	+		+
ALR17			+	+		+	+		+
ALR18	+		+		+	+	+		+

TABLE 2. Summary of major bands produced by the primer OPA–11 during randomly amplified polymorphic DNA (RAPD) analysis of total genomic DNA from *Tapesia yallundae* mycelium reisolated from lesions

Isolate number	RAPD band sizes (bp) of primer OPA-11									
	485	600	640	750	900	1,160	1,235	1,290	1,315	1,585
22-432			+	+		+			+	
22-433	+	+		+		+			+	
ALR1			+	+	+	+			+	
ALR2		+		+	+	+			+	+
ALR3		+	+	+		+			+	
ALR4	+	+		+		+		+	+	
ALR5		+	+	+		+			+	
ALR6		+	+	+		+			+	
ALR7	+	+	+	+		+			+	
ALR8	+	+		+		+			+	
ALR9	+	+		+	+	+			+	
ALR10	+	+		+		+		+	+	
ALR11		+		+		+			+	
ALR12		+	+	+		+			+	
ALR13	+	+		+		+			+	
ALR14			+	+		+	+	+	+	
ALR15		+		+		+	+		+	
ALR16	+	+	+	+		+			+	
ALR17		+	+	+	+	+			+	
ALR18		+		+		+		+	+	+

LITERATURE CITED

1. Andrivon, D., and De Vallavieille-Pope, C. 1993. Racial diversity and complexity in regional populations of *Erisyphe graminis* f. sp. *hordei* in France over a 5-year period. Plant Pathol. 42:443-464.
2. Cheah, L.-H., Harthill, W. F. T., and Corbin, J. B. 1982. Ascospore release in *Pyrenopeziza brassicae*. Trans. Br. Mycol. Soc. 79:536-539.
3. Cooper, R. M., Longman, D., Campbell, A., Henry, M., and Lees, P. E. 1988. Enzymic adaptation of cereal pathogens to the monocotyledonous primary wall. Physiol. Mol. Plant Pathol. 32:33-47.
4. Creighton, N. F., and Bateman, G. L. 1991. Problems in identifying pathogenicity types of *Pseudocercosporella herpotrichoides* by colony morphology arising from the source and concentration of the nutrient agar. Mycol. Res. 38:253-254.
5. Daniels, A., and Lucas, J. A. 1990. *Pseudocercosporella herpotrichoides* on wheat seedlings: Effects of prochloraz on early infection of leaf sheaths. Med. Fac. Landbouww. Rijksuniv. Gent 55(3a):997-1005.
6. Daniels, A., Lucas, J. A., and Peberdy, J. F. 1991. Morphology and ultrastructure of W and R pathotypes of *Pseudocercosporella herpotrichoides* on wheat seedlings. Mycol. Res. 95:385-397.
7. Delos, M., Bruchet, S., and de la Rocque, B. 1994. Dynamique du piétin-verse dans le centre-ouest et le sud-ouest de la France. Phytoma 468:27-29.
8. Dyer, P. S., Bateman, G. L., Lucas, J. A., and Peberdy, J. F. 1994. Seasonal development of apothecia of the cereal eyespot pathogen *Tapesia yallundae* on straw stubble in the UK. Ann. Appl. Biol. 125:489-500.
9. Dyer, P. S., Nicholson, P., Rezanoor, H. N., Lucas, J. A., and Peberdy, J. F. 1993. Two-allele heterothallism in *Tapesia yallundae*, the teleomorph of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides*. Physiol. Mol. Plant Pathol. 43:403-414.
10. Dyer, P. S., Papaikonomou, M., Lucas, J. A., and Peberdy, J. F. 1994. Isolation of R-type progeny of *Tapesia yallundae* from apothecia on wheat stubble in England. Plant Pathol. 43:1039-1044.
11. Fitt, B. D. L., and Bainbridge, A. 1983. Dispersal of *Pseudocercosporella herpotrichoides* spores from infected wheat straw. Phytopathol. Z. 106:214-225.
12. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.
13. Hornby, D. 1981. Inoculum. Pages 271-293 in: Biology and Control of Take-All. M. J. C. Asher and P. J. Shipton, eds. Academic Press, London.
14. Hunter, T. 1989. Occurrence of *Tapesia yallundae*, teleomorph of *Pseudocercosporella herpotrichoides* on unharvested wheat culms in England. Plant Pathol. 38:598-603.
15. Ingold, C. T. 1966. Spore release. Pages 679-707 in: The Fungi. Vol. 2, The Fungal Organism. G. C. Ainsworth and A. S. Sussman. Academic Press, New York.
16. Julian, A. M., and Lucas, J. A. 1990. Isoenzyme polymorphism in pathotypes of *Pseudocercosporella herpotrichoides* and related species from cereals. Plant Pathol. 39:178-190.
17. King, A. C. 1990. First record of *Tapesia yallundae* as the teleomorph of *Pseudocercosporella herpotrichoides* var. *acuformis*, and its occurrence in the field in the Federal Republic of Germany. Plant Pathol. 39:44-49.
18. King, A. C. 1991. Observations of apothecia of *Tapesia yallundae* and the cultural phenotypes of their progeny. Plant Pathol. 40:367-373.
19. McCartney, H. A., and Lacey, M. E. 1990. The production and release of ascospores of *Pyrenopeziza brassicae* on oilseed rape. Plant Pathol. 39: 17-32.
20. Moreau, J. M., Van Schingen, J. C., and Maraite, H. 1989. Detection of *Tapesia yallundae*, the teleomorph of *Pseudocercosporella herpotrichoides* on wheat stubbles in Belgium. Med. Fac. Landbouww. Rijksuniv. Gent 54:555-559.
21. Nicholson, P., Hollins, T. W., Rezanoor, H. N., and Anamthawat-Jonsson, K. 1991. A comparison of cultural, morphological and DNA markers for the classification of *Pseudocercosporella herpotrichoides*. Plant Pathol. 40:554-594.
22. Nicholson, P., and Rezanoor, H. N. 1994. The use of random amplified polymorphic DNA to identify pathotype and detect variation in *Pseudocercosporella herpotrichoides*. Mycol. Res. 98:13-21.
23. Nicholson, P., Rezanoor, H. N., and Hollins, T. W. 1991. Occurrence of

Tapesia yallundae apothecia on field and laboratory inoculated material and evidence for recombination between isolates. Plant Pathol. 40:626-634.
24. Priestley, R. A., Dewey, F. M., Nicholson, P., and Rezanoor, H. N. 1992. Comparison of isoenzyme and DNA markers to differentiate W, R, and C pathotypes of *Pseudocercosporella herpotrichoides*. Plant Pathol. 41: 591-599.
25. Robbertse, B., Crous, P. W., and Holz, G. 1994. *Tapesia yallundae* collected from wheat stubble in South Africa. Mycopathologia 125:23-28.
26. Sanderson, F. R., and King, A. C. 1988. Field occurrence of *Tapesia yallundae*, the teleomorph of *Pseudocercosporella herpotrichoides*. Aust. Plant Pathol. 17:20-21.
27. Scott, P. R. 1971. The effect of temperature on eyespot in wheat samples. Ann. Appl. Biol. 68:169-175.
28. Scott, P. R., Hollins, T. W., and Muir, P. 1975. Pathogenicity of *Cercosporella herpotrichoides* to wheat, barley, oats and rye. Trans. Br. Mycol. Soc. 65:529-538.
29. Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., and Fry, W. E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84:201-207
30. Wallwork, H., and Spooner, B. 1988. *Tapesia yallundae*—The teleomorph of *Pseudocercosporella herpotrichoides*. Trans. Br. Mycol. Soc. 91:703-705.
31. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.