Directional Growth and the Perennial Characteristic of *Ustilago striiformis* in *Poa pratensis*

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ABSTRACT

Studies on directional development of *Ustilago striiformis* var. *poae* in *Poa pratensis* following coleoptile infection revealed mycelium in primary crowns and subsequent development of smutted rhizomes and tillers. Similar mycelial colonization of crowns, rhizomes, and tillers occurred in plants infected via axillary buds on rhizome nodes. Healthy primary crowns of plants with infected axillary crown buds from which smutted rhizomes had grown remained unsmutted, indicative of mycelium growth in the direction of developing axillary crown buds; i.e., primary crowns were not infected via axillary crown buds. The perennial characteristic of the stripe smut pathogen was a function of mycelial colonization of crowns. Phytopathology 60:849-851.

Perennial mycelium of *Ustilago striiformis* (West.) Niessl var. *poae* Thir. & Dick. has been reported in *Poa pratensis* L. (1, 6), and its presence in leaves, sheaths, and floral parts of grasses is documented (2, 5, 7, 8). Recent investigations show that, in addition to coleoptile infection (1, 3, 5, 8), axillary crown buds and axillary buds on rhizome nodes are also infected by the pathogen (3). Discovery of axillary buds as infection sites suggests the need for information on directional growth of mycelium following infection; studies relating directional growth to establishment of perennial mycelium have not been conducted. Furthermore, the term "perennial mycelium" is ill-defined relative to its focal point and function in the plant and its role in the epiphytology of the disease. Therefore, the purpose of this investigation was to evaluate postinfection directional growth of mycelium and the perennial characteristic of the pathogen in relation to the epiphytology of the disease.

MATERIALS AND METHODS.—Merion Kentucky bluegrass, *Poa pratensis* L., was used in all experiments. Seeds were surface-disinfected for 10 to 15 min under vacuum in a solution containing: 280 cc of H_2O, 14 ml of 5.25% sodium hypochlorite (Clorox), 2 ml of 37% formaldehyde, and 1 ml of polyoxyethylene sorbitan monolaurate (Tween 20). Seed was germinated in a steamed 2:1 (v/v) sand-soil mixture in flats in a greenhouse. Temp ranged from 12.8 to 30.0 C.

Inoculation of coleoptiles and axillary crown buds with *U. striiformis* was accomplished as previously described (3). Twenty-five plants with coleoptile infections and 50 plants with axillary crown bud infections were collected for study. Coleoptile infections were evidenced by infected primary crowns (crowns produced from a germinating seed) (3). The 25 coleoptile-infected plants were potted separately and examined for development of stripe-smutted rhizomes (extravaginal branches) and tillers (intravaginal branches); 10 additional plants were sectioned to determine directional growth of hyphae. Axillary crown bud-infected plants were selected on the basis of stripe-smutted rhizomes growing from unsmutted primary crowns (3). Development of *U. striiformis* was studied by removing stripe-smutted rhizomes from unsmutted primary crowns and planting the crowns and rhizomes separately. Development of stripe-smutted rhizomes and tillers from healthy primary crowns and smutted rhizomes was recorded. Nine additional plants were examined histologically. Axillary buds on rhizome nodes were also inoculated (3), and 15 plants were examined for development of smutted rhizomes and tillers. Six additional plants were sectioned to determine directional growth of hyphae. All plants were observed for a period of 4 to 6 months.

Histological studies were conducted to determine location and directional growth of hyphae. Plants were fixed in FAA (formalin-acetic acid-alcohol) for a minimum of 12 hr, and dehydrated in ethyl alcohol-tertiary butyl alcohol series. The tissue was then exposed to three 8-hr changes of 1:1, 55, and 61 C molten Tissuemat under vacuum at 65 C. Longitudinal sections of crowns and rhizomes were cut 10 to 12 μ thick and fixed to microslides with Haupt's adhesive. Tissuemat was removed in xylene and sections were successively hydrated and stained in modified Margolena's stain (4). Sections were stained in thionin for 10 to 30 min (0.1 g thionin in 100 cc of 5% aqueous phenol) and counterstained in orange G-erythrosin for 8 min (1:2 saturated orange G in 100% ethanol and saturated erythrosin in clove oil). Cover slips were mounted with Harleco synthetic resin.

RESULTS.—*Coleoptile infection.*—Twenty-one of the 25 infected plants survived and produced 287 rhizomes and tillers. Of this number, 278 were smutted and 9 were unsmutted (Table 1). Histological examination of coleoptile infected plants showed that primary crowns were completely colonized by intercellular mycelia (Fig. 1-A). Mycelia extended from the crowns into developing rhizomes and tillers, but was not directly associated with meristems (Fig. 1-A, B). Individual hyphae were found in young tissues shortly after differentiation.

Axillary crown bud infection.—Of the 50 infected plants separated into 50 unsmutted primary crowns and 50 smutted rhizomes detached from the crowns, all primary crowns survived and 39 rhizomes survived.

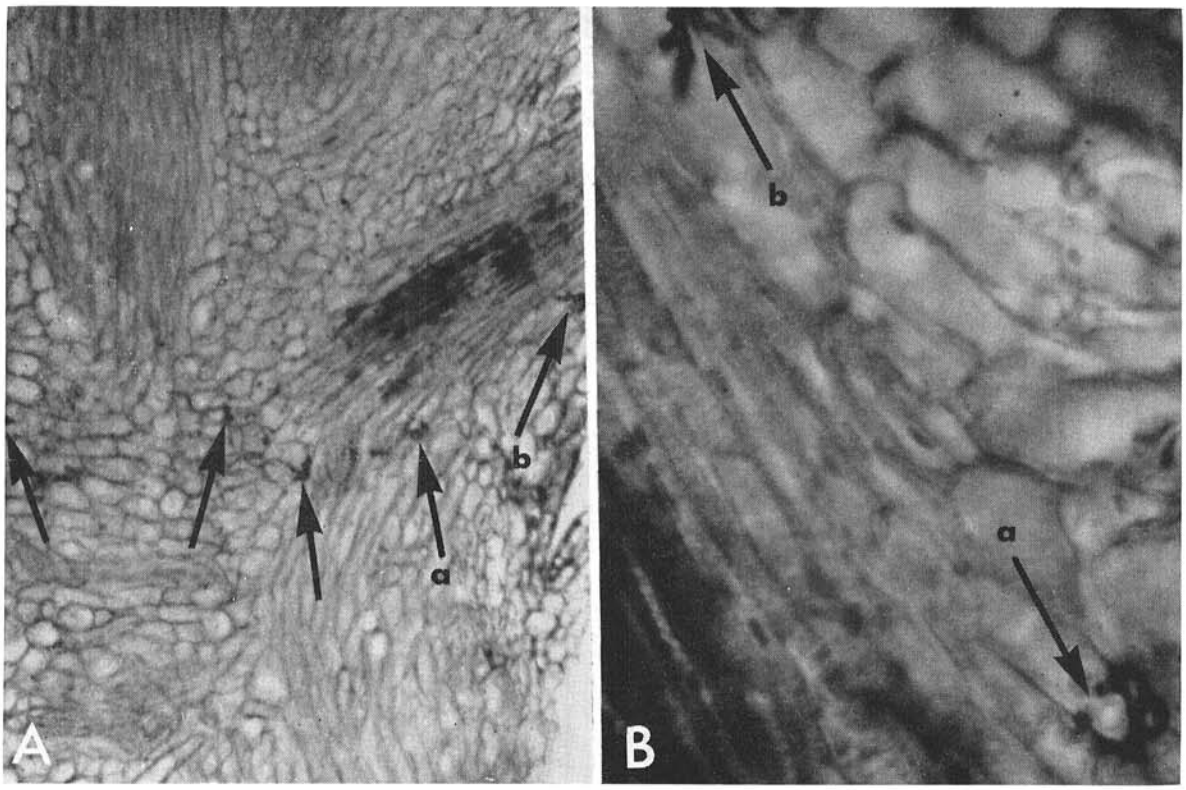

Fig. 1. Primary crowns of *Poa pratensis* colonized by *Ustilago striiformis* following coleoptile infection. **A)** Undesignated arrows show intercellular mycelium. Arrows a and b show growth of intercellular mycelium from crown into a rhizome developing from an axillary crown bud. (× 100) **B)** Arrows a and b same as a and b in A. (× 400)

The 50 unsmutted primary crowns produced 585 rhizomes and tillers, 13 of which were smutted (Table 1). The 13 smutted rhizomes and tillers were produced on 7 of the 50 crowns. Five of the 7 crowns produced only one smutted rhizome or tiller/crown; other rhizomes and tillers produced by the 5 crowns were unsmutted. The other 2 primary crowns produced only smutted rhizomes and tillers. The 39 surviving smutted rhizomes produced 166 rhizomes and tillers; 145 smutted and 21 unsmutted (Table 1).

Histological examination of primary crowns that produced healthy rhizomes disclosed complete absence of mycelium. Examination of crowns established by smutted rhizomes detached from unsmutted primary crowns disclosed mycelial development analogous to coleoptile infections (Fig. 1-A).

Infected axillary buds on rhizome nodes.—Twelve of the 15 plants infected through axillary buds on rhizome nodes survived. From the surviving plants, 120 rhizomes and tillers were produced, all stripe-smutted (Table 1). Sectioned crowns disclosed mycelial growth analogous to infected crowns resulting from coleoptile infection (Fig. 1-A). Mycelium was not found to extend into adjacent internodes.

Discussion.—Two specific characteristics of *U. striiformis* in *P. pratensis* were disclosed: (i) Growth and development of the pathogen is in the direction of plant growth; (ii) the perennial characteristic of *U. striiformis* is a function of mycelial colonization of crowns. In coleoptile-infected plants, the pathogen colonizes primary crowns formed by developing seedlings. From such perennially infected crowns, mycelia grow into the rhizomes and tillers as they develop, with exception of occasional escapes (Table 1). Such rhizomes and tillers then establish other perennially infected crowns. Development in infected axillary buds on rhizome nodes is analogous to that in coleoptile infection; i.e., the pathogen becomes perennially estab-

Table 1. Directional development of *Ustilago striiformis* in relation to the perennial characteristic of stripe smut following infection of specific meristems of *Poa pratensis*

Specific meristems of *Poa pratensis* infected by *Ustilago striiformis*[a]	Crown mortality	Unsmutted tillers and rhizomes produced	Smutted tillers and rhizomes produced
Coleoptile-infected plants	4	9	278
Axillary crown-bud-infected plants			
Unsmutted primary crowns	0	572	13
Smutted rhizomes detached from primary crowns	11	21	145
Axillary buds on rhizome nodes	3	0	120

[a] Figures for coleoptile infected plants based on 25 plants; for axillary crown-bud-infected, 50 plants; for axillary buds on rhizome nodes, 15 plants.

lished in resulting crowns, and subsequent rhizomes and tillers are smutted (Table 1).

Directional growth of pathogen and subsequent establishment of perennially infected crowns is best illustrated in axillary crown-bud-infected plants. If unsmutted primary crowns from which smutted rhizomes were detached had been infected with mycelia, they would have eventually produced stripe-smutted rhizomes and tillers (Table 1). Of the 50 primary crowns examined, however, only 7 produced smutted rhizomes and tillers. Five of the 7 crowns remained unsmutted, with only rhizomes and tillers becoming smutted. Such development indicates axillary crown-bud-infection before separation of primary crowns from the original smutted rhizomes. Only 2 of the 7 crowns were perennially infected, as indicated by presence of stripe-smutted leaves on the primary crowns, rhizomes, and tillers. It is reasonable to conclude, therefore, with only 2 of 50 unsmutted primary crowns becoming perennially infected after separation from smutted rhizomes, that growth of mycelium from the rhizome to the crown is the exception rather than the rule. Furthermore, detached stripe-smutted rhizomes produced 145 smutted rhizomes and tillers, with 21 escapes analogous to those of coleoptile infected plants. Such results indicate that, following axillary crown bud infection, the pathogen seldom grows into crowns on which buds are located; instead it grows in the direction of the rhizome or tiller produced from the buds.

Primary infections of coleoptiles, axillary crown buds, and axillary buds on rhizome nodes are of major importance in establishing stripe-smutted plants over large areas of turf and for reestablishment of diseased plants where stripe-smutted plants have succumbed to high temperature and drought. Of equal epiphytological importance is disease spread via perennially infected crowns which increase the number of diseased plants in localized areas. Such increases in number of stripe-smutted crowns from perennial development may be illustrated as follows: from one perennially infected crown, X^n infected rhizomes and tillers may be produced which establish perennially infected crowns; X^n crowns may then produce Y^n perennially infected crowns, and so on, ad infinitum. Therefore, once the pathogen is perennially established, epiphytology of individual diseased plants becomes the sum progression of perennially infected crowns. Furthermore, it is probable that the number of diseased crowns produced via perennial infection are of major importance in increasing the inoculum potential of *U. striiformis*. Increases in inoculum, in the form of teliospores, increases the number of primary infections via coleoptiles, axillary crown buds, and axillary buds on rhizome nodes, and results in an infection cycle that builds stripe smut to epiphytotic proportions.

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