Floral Induction and Development in Poa pratensis Infected with Ustilago striiformis var. poae and Urocystis agropyri

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ABSTRACT

Ustilago striiformis var. *poae* and *Urocystis agropyri* inhibited rate of appearance, number, and size of inflorescences produced on *Poa pratensis* 'Merion'. Control and smutted plants given floral induction treatments of 30, 60, 90, and 120 days at 12 C and 10-hr day-length produced increasing numbers of shorter tillers as length of induction increased. Inflorescences were produced on control plants after a minimum of 60 days' induction, and on smutted plants after a minimum of 90 days' induction. Inflorescences increased on stripe-smutted plants as induction was increased from 90 to 120 days; flag-smutted and control plants produced fewer inflorescences under the same conditions. Sori occurred in inflorescences of stripe-smutted plants subjected to a minimum of 120 days' induction; few sori were observed in inflorescences of flag smutted plants after 90 and 120 days' induction. Smutted inflorescences were often aborted; sori occurred in rachises, glumes, lemmas, ovaries, and developing caryopses. Seed set on smutted plants was greatly reduced. Seeds from smutted plants produced seedlings that were smut-free. Phytopathology 61: 1373-1376.

Additional key words: floral inhibition, inflorescence sori.

Sori of *Ustilago striiformis* var. *poae* (stripe smut) and *Urocystis agropyri* (flag smut) usually are confined to laminae and sheaths of Gramineae species. *Ustilago striiformis* var. *poae* has been observed also in inflorescences of grass hosts (2, 4, 5, 9, 10, 13, 14, 18). Most studies of stripe-smutted inflorescences have been conducted with *Agrostis alba* (redtop) and *Phleum pratense* (timothy); sorus formation in these species occurs in rachises, glumes, lemmas, paleae, stamens, and ovaries (4, 13, 18). Reports of stripe smut in inflorescences of bluegrass (usually *Poa pratensis*, but not always stated) occur in the literature, but are limited to incidental observations without developmental data (2, 14, 18). Recent observations of field-collected *P. pratensis* revealed the presence of atypical, globular sori in glumes of some stripe-smutted plants (10). *Urocystis agropyri* has been reported in inflorescences of *A. alba* (3), but development of flag-smut sori in inflorescences of *P. pratensis* has not been reported.

Several researchers have indicated that stripe- and flag-smutted grasses do not readily produce inflorescences and that, when inflorescences are produced, viable seed may not occur (4, 13, 17). Research on floral induction of stripe- and flag-smutted *P. pratensis* has not been conducted; studies pertaining to inflorescence induction and development in healthy *P. pratensis*, however, indicate that, with few exceptions (1), floral induction requires short days and low temperatures, and development requires long days and warmer temperatures (6, 7, 8, 15).

Although stripe-smutted *P. pratensis* has been observed with inflorescences (2, 10, 14, 18), the extent to which diseased plants produce inflorescences and their potential importance in dissemination of the pathogen is unknown. The research presented herein was initiated to determine if *P. pratensis* infected with *U. striiformis* or *U. agropyri* could be induced to produce inflorescences and to determine seed set, viability, and potential for dissemination of the pathogens via seed.

MATERIALS AND METHODS.—*Poa pratensis* L. 'Merion' was used in all studies. Control plants were vegetatively propagated from individual crowns of healthy stock plants; smutted plants were propagated from stock plants infected with *U. striiformis* (West.) Niessl var. *poae* Thir. & Dick. and *U. agropyri* (Preuss) Schroet. All plants were grown in 3-inch plastic pots in a steamed 2:1 (v/v) loam-peat soil mixture. Plants were grown 21 days in the greenhouse; leaves were then cut to 9.0 cm. Four floral induction treatments were initiated (32 plants/treatment) in which plants were subjected to 30, 60, 90, and 120 days' exposure to 12 C (±2) and to a 10-hr day (1,800 ft-c), respectively. After the induction treatments, plants were placed in the greenhouse at 18-30 C with an 18-hr day supplemented by incandescent lights (400 to 5,100 ft-c).

Number and maximum length of tillers per plant were recorded at the end of each induction treatment. Plants were observed for rate of inflorescence appearance at 2-day intervals after being placed in the greenhouse, and total number of inflorescences and height of mature inflorescences were recorded and measured to the nearest 0.5 cm. Presence of stripe- and flag-smut sori in inflorescences of diseased plants was observed, and developmental symptomatology of diseased inflorescences recorded.

Seeds were harvested from all plants a minimum of 30 days after expansion of the last inflorescence. Seeds from stripe- and flag-smutted plants were counted, and all bracts (glumes, paleae, lemmas) were removed from caryopses. Caryopses were then surface-sterilized 15 min in 10% Clorox (5.25% sodium hypochlorite) and germinated on sterile filter paper in petri dishes. Seedlings from germinating

caryopses were observed for stripe- and flag-smut sori. Seeds from control plants were not counted because of heavy production; seed viability was determined on the average of three 100-seed samples from each treatment. Only glumes were removed from control seeds; sterilization and plating were the same as described for seed from smutted plants. Seeds were germinated over a period of 6 weeks by subjecting them to 3-day alternating periods of cold (10 C) and warm (24 C) temperatures. Seeds were maintained in darkness during cold periods; a day-length of 14 hr (400 ft-c) was provided during warm periods. At the 2-leaf stage, seedlings were placed in the greenhouse in steamed soil (3-inch pots) and observed 30 days for development of stripe and flag smut sori.

RESULTS.—*Number and maximum length of tillers after floral induction.*—Tiller numbers on control and flag-smutted plants increased with longer periods of induction; tiller production was greatest on flag-smutted plants after 120 days' induction (Table 1). Tiller production among stripe-smutted plants did not change significantly with induction periods up to and including 90 days, but an increase in tillers occurred after 120 days' induction (Table 1).

Tiller length decreased among all plants as induction was increased. Maximum reduction in tiller length occurred among control plants after 120 days' induction (Table 1). Stripe-smutted plants showed a marked reduction in maximum tiller length after 120 days' induction.

Rate of appearance and production of inflorescences.—Inflorescences appeared on control plants at 32, 24, and 18 days after 60, 90, and 120 days' induction, respectively. No inflorescences were produced on control plants after 30 days' induction. Maximum inflorescence production occurred on control plants after 60 and 90 days' induction with 252 and 264 inflorescences, respectively. Production of inflorescence on control plants decreased significantly (.05) to 165 after 120 days' induction.

Rate of inflorescence appearance was slowed on stripe- and flag-smutted plants relative to control plants; inflorescences appeared on stripe-smutted plants 30 days after 90 and 120 days' induction. Inflorescences appeared on flag-smutted plants at 30 and 34 days after 90 and 120 days' induction, respectively. Length of induction had no effect on number of inflorescences produced on flag-smutted plants; inflorescences decreased from 3 to 1 in response to 90 and 120 days' induction, respectively. Increasing length of induction had the opposite effect on stripe-smutted plants; i.e., inflorescence numbers increased from 5 to 82 after 90 and 120 days' induction, respectively.

Seed set and viability.—Seed set was heavy on all control plants in response to all induction treatments, with the exception of the 30-day treatment, in which no inflorescences were produced. Percentage germination of control seed was 70, 69, and 77 after 60, 90, and 120 days' induction, respectively. Inflorescences on stripe-smutted plants set 89 and 29 seeds after 90 and 120 days' induction, respectively. Of these respective numbers, 72% and 77% germinated. Seed set decreased on flag-smutted plants as induction was increased from 90 to 120 days. Flag-smutted plants set 61 and 18 seeds, of which 78 and 82% germinated from plants given 90 and 120 days' induction, respectively. No smutted seedlings were produced from germinated caryopses from stripe- or flag-smutted plants.

Expression of symptoms by inflorescences produced on smutted plants.—Mean height of inflorescences on smutted plants was less than that of control plants. Mean height of control inflorescences was 27.6, 39.3, and 27.2 cm following 60, 90, and 120 days' induction, respectively. Mean height of inflorescences on stripe- and flag-smutted plants following 90 and 120 days' induction was 34.3 and 9.9 cm, and 26.3 and 15.5 cm, respectively.

No sori were observed in any of the five inflores-

TABLE 1. Number and length of tillers produced by stripe- and flag-smutted *Poa pratensis* subjected to progressively longer exposures to short days and low temperatures[a]

Treatments[b]	Tillers produced per plant			Tiller length per plant		
	Control plants[c]	Stripe-smutted plants[c]	Flag-smutted plants[c]	Control plants[c]	Stripe-smutted plants[c]	Flag-smutted plants[c]
	Mean	Mean	Mean	Mean	Mean	Mean
30 days	16.1	10.1	18.6	21.5	24.1	17.6
60 days	20.9	10.5	22.1	17.0	23.8	14.4
90 days	22.9	9.6	24.1	13.6	21.6	11.7
120 days	25.8	12.2	32.0	5.3	8.9	6.0

[a] Thirty-two plants/treatment.

[b] Within treatment, means were paired and tested (Duncan's multiple range test). Tiller production: Means differed (.05), except control and flag smut in 60- and 90-day treatments, respectively. Tiller length: Means differed (.05) except control and flag smut of 120-day treatment.

[c] Means within control, stripe-, and flag-smutted plants were paired and tested (Duncan's multiple range test), respectively. Tiller production: Control means differed (.05) except 60 and 90 days, and 90 and 120 days; stripe smut means differed (.05) for 30 and 120 days, and 90 and 120 days, all other pairs not significant; flag smut means differed (.05) except for 60 and 90 days. Tiller length: Control means differed (.05); stripe smut means differed (.05) except for 30 and 60 days; flag smut means differed (.05).

Fig. 1. Expression of symptoms on inflorescences produced on stripe- and flag-smutted plants. **A)** Aborted inflorescence. **B)** Sori in rachis of mature inflorescence. **C)** Masses of teliospores replacing ovaries (dark areas). **D)** Caryopsis replaced by teliospores.

cences produced on stripe-smutted plants after 90 days' induction. Of the three inflorescences produced on flag-smutted plants after 90 days' induction, one was partly aborted, the second had one sorus in the rachis, and the third was fully formed and devoid of sori.

All inflorescences on stripe-smutted plants subjected to 120 days' induction displayed sori. Some inflorescences were completely or partly aborted (Fig. 1-A). Unexpanded inflorescences often displayed sori adjacent to rachises that had completely replaced spikelets. Less severely diseased inflorescences were fully developed, with sori in rachises and rachillae (Fig. 1-B). Glumes of spikelets often displayed atypical globular sori (10). Commonly, lemmas were smutted, but sori were not observed in paleae. Ovaries and developing caryopses often were completely replaced by masses of teliospores (Fig. 1-C, D). Most unsmutted florets on stripe-smutted plants subjected to 120 days' induction were blind. The single inflorescence produced by flag-smutted plants after 120 days' induction displayed only one sorus, which replaced a developing caryopsis.

DISCUSSION.—*Ustilago striiformis* and *U. agropyri* inhibit the rate of appearance and number of inflorescences produced on *P. pratensis*; both pathogens also increase the time necessary to induce inflorescence production. Each pathogen shows a distinctly different type of inhibition relative to length of induction. *Ustilago striiformis* prevented inflorescence production following 30 and 60 days' induction; however, after 90 and 120 days' induction, inflorescences increased from 5 to 82, respectively. This response indicates that the inhibitory effect of *U. striiformis* can be reduced by longer induction. *Urocystis agropyri* also prevented inflorescence production after 30 and 60 days' induction; induction of 90 and 120 days resulted in 3 and 1 inflorescence, respectively. Length of induction has little effect on overcoming the inhibitory effect of *U. agropyri*; indeed, it may enhance inhibition.

Tillering of smutted plants in response to induction may, in part, provide a limited explanation for the different inhibitory characteristics of these pathogens. Total tiller production (intra- and extravaginal branching) is normally reduced in *P. pratensis* infected with these pathogens when grown under long days and variable temperatures (11). Under the conditions of short days and low temperatures of this study, stripe-smutted plants continued to produce fewer tillers than control plants; however, flag-smutted plants produced more tillers than did control plants (Table 1). Although tiller proliferation during floral induction is normal in *P. pratensis* (15), *U. agropyri* may overstimulate vegetative growth during induction, possibly at the expense of future reproductive growth. Tiller stimulation is not uncommon in the Ustilaginales (12), and although the cause is unknown it is suggestive of growth regulator imbalances; there is considerable evidence for such imbalances in plants infected by various species of the Uredinales (16).

Reduction in rate of appearance, number, and size of inflorescences on all smutted plants may, in part, be due to pathogen-induced imbalances in carbohydrate and nitrogen physiology of *P. pratensis* during floral induction and development. *Poa pratensis* responds to short days by becoming pros-

trate in growth habit, by proliferating rhizomes and tillers, and by increasing carbohydrate reserves, which can be further enhanced by low temperatures (7, 8, 15). As induction was increased from 30 to 120 days, some of these characteristics were observed on both control and smutted plants. Although not noticeably prostrate, tillers of all plants increased in number and became progressively shorter as length of induction increased (Table 1). Ultimately, however, only control plants produced large numbers of inflorescences after induction treatments. One possible explanation involves the perennial systemic habit of the pathogens and their nutritional requirements for growth. For the pathogens to be perennial and grow continuously with the host-plant's growth, they must be nourished. Under such circumstances, the pathogens are, in effect, energy sinks within the host plant; i.e., they are heterotrophic and must derive their energy from carbohydrates of the host plant. If enough carbohydrates are used by the pathogens during floral induction and development, it may become a limiting factor in total inflorescence production, and those inflorescences that are produced could be slowed in growth and reduced in size. A similar relationship may also exist between host-plant nitrogen and the pathogens. Applications of nitrogen to *P. pratensis* during induction have been shown to increase inflorescence production (15). As primary constituents of protein, nitrogen must be supplied to developing inflorescences; it must also be supplied to *U. striiformis* and *U. agropyri*. It is probable that the pathogens reduce nitrogen available for inflorescence development.

Certain practical implications relative to the epiphytology of stripe- and flag-smutted *P. pratensis* are evident from the results. It is apparent that *U. striiformis* and *U. agropyri* are not disseminated by embryo-infected seed. Furthermore, although seed set is greatly reduced on smutted plants, germination of seed produced was good. Seed germination among controls was lower than that of smutted plants; this was believed due to contaminants not killed in bracts during surface sterilization of seed. There is little doubt, however, that, under conditions of long induction periods, stripe-smutted plants will produce inflorescences with sori in bracts, ovaries, and caryopses that could function as primary sources of inoculum for dissemination to new sites. It is possible that, to a much lesser extent, flag smut may be disseminated in the same manner. It is probable that even in quality seed lots, where inert matter is minimal, seeds could still be surface-contaminated with teliospores from smutted inflorescence and leaf sori. To determine the importance and extent of dissemination by means of diseased floral structures and surface contamination, studies should be conducted under conditions comparable to those found in commercial seed fields.

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