Evidence for Developmental Control of Ustilago striiformis by Apical Dominance in Perennially Infected Stolons of Agrostis palustris

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Journal Paper No. J-7277 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project 1751.

Accepted for publication 9 August 1972.

ABSTRACT

Survival of apical and axillary buds of *Agrostis palustris* Hud. grown in organ culture was greater for healthy stolons than for stolons perennially infected with *Ustilago striiformis* (West.) Niessl. Survival of apical buds from healthy and stripe-smutted plants was greater than that of their first and second axillary buds, respectively; survival of third, fourth, and fifth axillary buds from all plants was greater than that of apical buds. Of the surviving apical buds from stripe-smutted plants, 35.8% produced stripe smutted stolons; the youngest axillary buds produced all healthy stolons; and the proportion of stripe-smutted to healthy stolons increased with each older axillary bud. The results suggest that growth of *U. striiformis* from nodes into axillary buds is controlled by apical dominance.

Phytopathology 63:146-148

Additional key words: organ culture, etiology, growth regulation.

The perennial nature of *Ustilago striiformis* (West.) Niessl is well established in numerous species of Graminae (1, 2, 5, 6, 7). Once established in *Agrostis palustris* Hud., mycelium of the pathogen colonizes and proliferates within nodes, and subsequently grows into stolons produced from adjoining axillary buds (2, 3). Development of the pathogen into axillary buds and developing stolons is influenced by temperature (3). Low temperatures (7.2-12.8 C) allow the pathogen to grow in developing stolons, but intercalary formation of teliospores is slowed; high temperatures (32.2-37.8 C) inhibit growth of the pathogen within developing axillary buds. Recent studies, in which axillary meristems from stripe-smutted stolons were grown in organ culture, established that nodes of *A. palustris* are the focal point for the perennial character of *U. striiformis* (4). Less than half (44%) the surviving meristems from infected plants produced stripe-smutted plants. The results indicated that *U. striiformis* was not directly associated with axillary meristems. They also suggested that apical dominance might control growth of mycelium from infected nodes into adjoining axillary meristems. The research herein was initiated to examine the hypothesis that apical dominance is responsible for controlling growth of *U. striiformis* into axillary meristems of *A. palustris* and the stolons produced from such meristems.

MATERIALS AND METHODS.—*Agrostis palustris* 'Old Orchard' was propagated vegetatively in the greenhouse (12-27 C). The apical meristem and first five axillary meristems were removed from stolons with a microdissecting knife. Apical meristems could not be removed directly without injury; therefore, under a dissecting microscope, a 3-mm segment of stolon was cut directly above the youngest visible node to secure the apical bud for culture. Axillary buds ranged from 1.0 to 2.4 mm long. All buds were surfaced-sterilized in 0.5% cetyl trimethyl ammonium bromide and 5.0% Clorox (5.25% sodium hypochlorite) solutions for various times, depending on bud size; i.e., stolon segments with terminal buds and first through fifth axillary buds ca. 3, 1.5, 2, 4, 4, and 5 min, respectively, in each solution. All buds were then washed in sterile, double-distilled water and placed on organ culture media.

Buds were collected from 200 healthy and stripe-smutted stolons each, and grown in 25 x 150-mm culture tubes on a medium containing White's salts solution (9), 2.0 mg/liter gibberellic acid (K salt), and 30 g/liter Difco-Bacto agar. Gibberellic acid was added with a syringe equipped with a Millipore filter to the autoclaved medium before it solidified. Cultures were grown 60 days at 24.0 C (±1.0 C) under a 14-hr day (400 ft-c) and evaluated for bud survival and relative proportions of healthy and diseased stolons produced from buds of stripe-smutted plants.

RESULTS.—*Bud survival and development.*—Survival of buds was greater for healthy stolons than for stripe-smutted stolons. Of the 1,200 buds cultured from healthy and stripe-smutted stolons each, 66.7% and 55.7% survived, respectively. Survival of buds of all ages was greater for healthy stolons, except for the fifth axillary buds from stripe-smutted plants (Fig. 1, left). Greatest differences in bud survival for healthy and stripe-smutted plants occurred among apical buds; twice the number of healthy apical buds survived (Fig. 1, left).

Although survival of buds from healthy and stripe-smutted plants differed, all buds showed similar developmental patterns. Apical bud survival for healthy and stripe-smutted stolons was greater than that of the first and second axillary buds (Fig. 1, left). The percentage of surviving axillary buds did not exceed that of the apical buds until they were in the third, fourth, and fifth positions.

Production of stripe-smutted stolons from buds of diseased plants.—Approximately three-fourths (72.6%) of the surviving buds from stripe-smutted plants produced healthy stolons. Among surviving apical buds, 64.2% produced healthy stolons (Fig. 1, right). All the surviving youngest axillary buds (No. 1) from stripe-smutted plants produced healthy stolons, and each older axillary bud produced proportionally more stripe-smutted stolons than healthy stolons (Fig. 1, right).

DISCUSSION.—The survival of apical and axillary buds from both healthy and stripe-smutted stolons warrants explanation relative to apical dominance. Because the apical buds grow continuously, it would seem that survival of such buds should have been much greater; it is probable, however, that many apical buds were lost because of the methods of securing them. Apical buds were too small to remove and culture directly; therefore, it is possible that, in the process of removing stolon segments in the region of the apical bud, many buds may have been missed or damaged. Failure of many first and second axillary buds to survive may have been due to morphological immaturity (most were ca. 1 mm long) or damage during removal for culture.

Although the methods of securing buds for culture may have influenced survival, the consistently greater survival rate of buds from healthy plants suggests that *U. striiformis* may kill some buds directly after they are placed on culture media or that the pathogen may produce inhibitory substances that slow or prevent

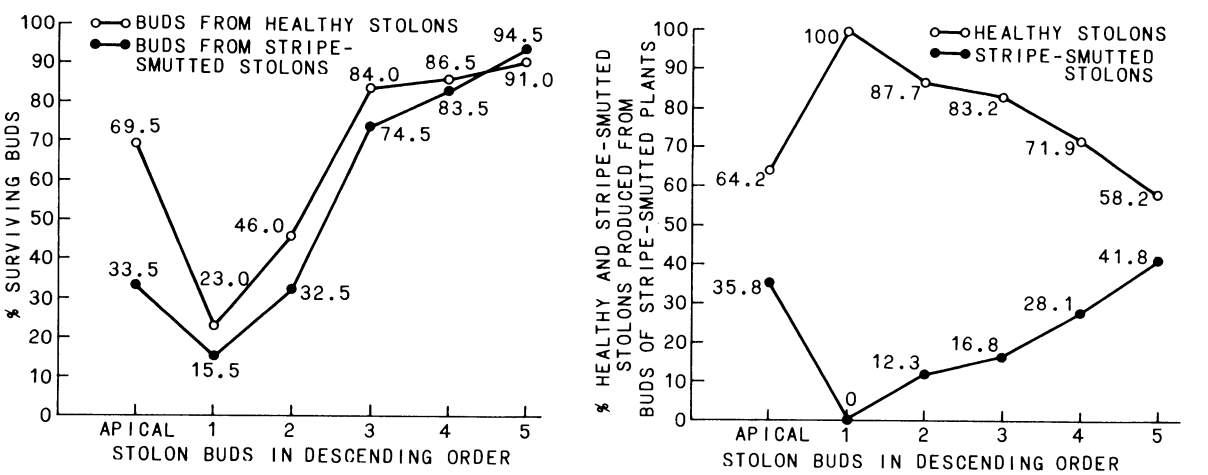

Fig. 1. (Left) Survival of apical and axillary buds removed from healthy and stripe-smutted stolons of *Agrostis palustris* and grown on an organ culture medium. (Right) Relative proportions of healthy and stripe-smutted stolons produced in organ culture from apical and axillary buds from stripe-smutted plants.

bud growth. Although direct killing of buds by *U. striiformis* is possible, it is believed not to occur commonly. The greatest differences in survival of buds from healthy and stripe-smutted plants occur between the apical buds and the first and second axillary buds (Fig. 1, left). The differences in bud survival decrease considerably among the third and fourth axillary buds, and survival of axillary buds from stripe-smutted plants in the fifth position exceeded that of buds from healthy stolons in the same position (Fig. 1, left). These characteristics of bud survival from stripe-smutted plants suggest that *U. striiformis* may have an inhibitory effect on bud growth that is progressively lost with each older bud, possibly in conjunction with the loss of apical dominance. Although the nature of this possible inhibition cannot be precisely explained, it is probable that it is affected by temperature. Plants for this study were grown at 12-27 C; in a previous study, however, in which plants were grown at 13-36 C, survival of axillary buds was greater from stripe-smutted plants (4). *U. striiformis* also is inhibited in *A. palustris* stolons above 32 C (3). Therefore, greater survival of buds from stripe-smutted plants grown at higher temperatures may be due to direct inhibition of *U. striiformis* and/or of a temperature-sensitive inhibitor produced by the pathogen.

It was originally hypothesized that, if apical dominance controlled development of *U. striiformis* into axillary buds, all apical buds from stripe-smutted plants should produce stripe-smutted stolons (because apical buds grow continuously) and each older axillary bud should produce progressively more stripe-smutted stolons as apical dominance is lost (4). The present study supports the hypothesis relative to axillary buds; i.e., the proportion of stripe-smutted stolons produced from progressively older axillary buds from stripe-smutted plants increased from 0 to 41.8% for the first through fifth axillary buds (Fig. 1, right). If the lines of the graph (Fig. 1, right) were extended, it is probable that the greater proportion of axillary buds beyond the fifth position would produce stripe-smutted stolons. The hypothesis is not supported, however, relative to production of stripe-smutted stolons from apical buds of stripe-smutted plants. The original hypothesis did not account for the fact that substances responsible for apical dominance originate in the region of the apical bud; and if these substances prevent *U. striiformis* from entering axillary buds, they also would prevent its growth into apical buds where such substances originate. This may explain why only one-third (35.8%) of the apical buds from stripe-smutted plants produced stripe-smutted stolons (Fig. 1, right).

The over-all results of the study provide reasonable evidence that the same physiological mechanisms that control axillary bud growth also control growth of *U. striiformis* from nodes of *A. palustris* into developing buds. On the basis of percentage of stripe-smutted plants produced from axillary buds, the incidence of *U. striiformis* clearly increases in progressively older buds (Fig. 1, right). It is improbable that the absence or low incidence of *U. striiformis* in the first five axillary buds is due to a growth lag of the pathogen in stolons. Mycelium is present in nodes of all ages (2, 3) and when infected plants are vegetatively propagated from nodes of any age it is extremely rare that a healthy plant is produced. These observations indicate that mycelium is present in all nodes of infected plants. Histological studies also have shown mycelium to be absent in dormant axillary buds (2). Therefore, the absence or low incidence of mycelium in the first through fifth axillary buds does not occur by chance. The growth of the pathogen into axillary buds seems to be regulated by or coordinated with those mechanisms which control bud development and growth in *A. palustris*. In this respect, the interpretation of the results is unique; however, the observation that *Ustilago nuda* initially does not keep pace with the growth rate of wheat may be related (8). The results also reconfirm and strengthen earlier findings that nodes are the focal point for the perennial character of *U. striiformis* (2, 3) and that the mycelium of the pathogen is not directly associated with meristems of *A. palustris* (4).

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