Smut Gall Development in Adult Corn Plants Inoculated with *Ustilago maydis*

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ABSTRACT

Thakur, R. P., Leonard, K. J., and Pataky, J. K. 1989. Smut gall development in adult corn plants inoculated with *Ustilago maydis*. Plant Disease 73:921-925.

Tassel galls were induced in 100% of corn plants in which the leaf whorl was injected with a suspension of sporidia of compatible isolates of *Ustilago maydis* 3-10 days before tassel emergence. Ear galls were induced at high frequency in Tastyvee sweet corn plants in which the sporidial suspension was injected between the leaf sheath and stalk at the sixth, seventh, and eighth nodes below the top of the plant 0-8 days before tassel emergence. Gall development was more variable in stalks and axillary buds than in ears. Mean incubation periods for appearance of galls in sweet corn hybrids grown in pots, greenhouse soil beds, and field plots were 17.5, 16, and 19 days, respectively. Ear galls were much larger on plants inoculated in the field than on those inoculated in the greenhouse, but the size of galls on stalks or axillary buds did not differ significantly for greenhouse- and field-grown plants. Inoculated hybrids in soil beds in the greenhouse had 27-77% fewer ears than the same hybrids left untreated or injected with water; 25% of those with galls on primary or secondary ears produced at least one normal ear, 23% of those without ear galls but with galls on stalks or axillary buds produced ears, and 32% of those with galls only on leaves or tassels produced ears. The injection technique is suitable for studies of host-pathogen interactions between corn and *U. maydis* in which typical gall development for adult plants is essential, but it may require some refinement before it can be used to screen corn genotypes for quantitative resistance to common smut.

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Journal Article 760 of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Paper No. 11396 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh.

Cooperative investigations of the USDA-ARS and the North Carolina Agricultural Research Service.

Accepted for publication 28 April 1989 (submitted for electronic processing).

Common smut of corn (*Zea mays* L.), caused by *Ustilago maydis* (DC.) Cda., occurs throughout the world nearly everywhere corn is grown. Losses from this disease vary from a trace to 10% or more in different geographic areas (1,13). Flint-dent corn hybrids grown in Poland and other northern corn-growing regions tend to be susceptible to common smut because of the extreme smut susceptibility of the early flint corn cultivars from which they were derived (1). Sweet corn is generally more susceptible to common smut than dent corn, and the destructiveness of the disease in sweet corn is even greater because it reduces quality and adds to processing costs (2).

Meristematic tissues of aboveground parts of corn plants are most susceptible to infection. Smut galls can be produced on stalks, axillary buds, ears, leaves, and tassels. Ear development on infected plants may be impaired depending on the number, size, and location of smut galls (2). Large galls, particularly those above the ear, can cause barrenness of corn plants; multiple galls per plant also often reduce yield by 100% (2,10). For sweet corn, of course, the ear galls are particularly important in reducing marketability of the crop even if the ears are not completely infected.

Infection of corn plants by *U. maydis* is not systemic. Instead, the infection of meristematic tissue occurs from sporidia either disseminated by wind and deposited in suitable infection sites on plants or produced by germinating teliospores that have been so disseminated and deposited (2). Therefore, seed treatments with fungicides are not effective in control of this disease on adult plants. Protective fungicides are unlikely to be effective, because infection occurs from sporidia lodged deep in the leaf whorls or between leaf sheaths and stalks of plants in locations not usually accessible to foliar spray applications. Disease control is best achieved through host plant resistance.

Marked differences among corn inbreds and hybrids have been reported in relation to smut incidence as well as to size and location of galls on plants (1). Early advances in breeding for smut resistance in corn were reviewed by Christensen (2), who indicated that very little is known of the basic nature of resistance

of corn to common smut or of the nature of host-parasite interactions in the disease. Only limited information is available in the literature since 1963 on evaluation of corn genotypes for susceptibility to *U. maydis*. Current breeding programs rely on natural infection of smut in field plots and elimination of the most susceptible breeding lines in order to maintain smut resistance.

Various inoculation methods have been tried by several workers with varying success. Smut galls were reported to develop in stalks, axillary buds, ears, leaves, or tassels, depending on the plant parts inoculated (2). In most early studies, however, there was not a clear distinction between infections arising from natural inoculum and those resulting from the artificial inoculation. According to Christensen (2), there is no good evidence to indicate that frequent spraying or dusting of plants with inoculum, a popular method for attempting to induce epidemics, actually increased the incidence of infection. Injection of sporidial suspensions into the leaf whorls of seedlings consistently gives high incidence of infection and has been used widely in genetic studies of *U. maydis* (2). This technique commonly results in either leaf galls or distortion of the growing point and death of the seedling. In breeding for resistance to common smut, it is important that susceptibility of corn genotypes be evaluated in terms of induction of smut galls on ears and stalks, which are more commonly seen in natural infections in the field and are much more damaging than those on leaves.

The objectives of our investigation, therefore, were to develop an inoculation method for induction of galls in adult plants, particularly in the ear shoot, to better study smut development in adult plants, and to test the reliability of the inoculation method in screening corn genotypes for susceptibility to *U. maydis*.

MATERIALS AND METHODS

Inoculation procedures. Monosporidial isolates were obtained from six teliospore collections of *U. maydis* from Raleigh, North Carolina; Clayton, North Carolina; Newfield, New York; Columbia, South Carolina; and Madison, Wisconsin (two collections). Teliospores were surface-disinfested in copper sulfate solution (5), spread on potato-dextrose agar (PDA) containing 10 g/L of dextrose in petri dishes, and incubated for 5 days at 23 C. Sporidia from the resulting colonies were spread on agar, and colonies arising from single sporidia were isolated and stored frozen in 15% glycerol at −70 C. When needed, cultures were revived by streaking a small portion of the frozen sporidial suspension on PDA for growth at 23 C.

Inoculum was prepared by flooding 5- to 7-day-old sporidial cultures on PDA with a solution containing 2 drops of polyoxyethylene sorbitan monolaurate (Tween 20) per 100 ml of distilled water and gently scraping the agar surface to release the sporidia. The suspension was filtered through four layers of cheesecloth, sporidia were counted in a hemacytometer, and the suspension was adjusted to 10^6 sporidia per milliliter. Equal volumes of suspensions of compatible sporidial lines were mixed to constitute the inoculum for various experiments. Compatibility of isolates was determined by Puhalla's (12) agar plate method.

The effects of time of inoculation relative to host plant developmental stage were tested in three greenhouse experiments. Dent corn hybrid B73 × Mo17 (first two experiments) and Tastyvee sweet corn (third experiment) were grown in 2:1 sand:soil mixture in 30-cm-diameter clay pots, five plants per pot, and fertilized as necessary. The pots were planted at intervals of 3–5 days, and all plants were inoculated when tassels first became visible in the leaf whorls of the oldest set of plants.

Plants were inoculated by using a hypodermic syringe to inject 2 ml of a mixed sporidial suspension of six sporidial isolates either into the leaf whorl (first two experiments) or between the leaf sheath and stalk at the sixth, seventh, and eighth nodes below the tassel. These nodes were chosen because previous observations had shown them to be the most likely sites for ear shoot development. We did not wait for emergence of ear shoots, because earlier experiments had shown that galls rarely developed in ear shoots inoculated after they had emerged from behind the leaf sheath.

Virulence of six monosporidial isolates was compared. Isolates were combined in nine pairs of sexually compatible isolates (Table 1), and the mixed sporidial suspensions were injected between the leaf sheath and stalk at the sixth, seventh, and eighth nodes of Tastyvee plants to induce ear gall formation. The experiment was run twice.

Comparison of sweet corn inbreds and hybrids in the greenhouse. Plants were grown in greenhouses either in pots (first experiment) or in soil beds (second experiment). Plants were inoculated at tassel emergence by injecting them between leaf sheaths and stalk with a suspension of sporidial isolates 2 and 36, which consistently induced large ear galls in preliminary tests.

Plants in pots. Plants of 140 commercial sweet corn hybrids from 15 different seed companies and 18 public inbreds were grown in Metro Mix (W. R. Grace and Co., Cambridge, MA) in 25-cm-diameter plastic pots (four plants per pot) in greenhouses at 21 ± 5 C. The hybrids and inbreds were planted in sets of 30–40 genotypes, with Tastyvee included as a check hybrid in each set. Fertilizer (N-P-K at 10:10:10) was applied at planting and again 30 days later. An average of eight or nine plants of each genotype were inoculated as described above when tassels first began to emerge. Because of space limitations, the evaluation of hybrids and inbreds in pots was not replicated, but eight hybrids were tested a second time in the experiment. The inoculation date for each plant was recorded, and the plants were examined daily thereafter for first appearance of galls. At 20–30 days after inoculation, the number, size, and location of galls and the number of ears for each plant were recorded.

Plants in soil beds. Tastyvee and 24 hybrids, selected from the group of 140 hybrids and representing 14 different commercial seed companies, were grown in soil beds in a plastic greenhouse. The 25 hybrids were planted in single, 2.4-m rows with two replications. There were 16 plants per row spaced 15 cm apart, and the rows were 30 cm apart. Soil beds were fertilized with N-P-K mixture at 100 kg N, 60 kg P, and 40 kg K per hectare before planting; topdressed with 20 kg N, 20 kg P, and 20 kg K per hectare 30 days after planting; and irrigated as needed. Eight to 10 plants in each row were inoculated with the procedure used for potted plants. For 14 of the 25 hybrids, six to eight check plants were either injected with tap water or left untreated.

Comparison of sweet corn hybrids in the field. The 140 sweet corn hybrids were planted in the field at two locations in 1987: the Agronomy/Plant Pathology South Farm at Urbana, Illinois, and the Central Crops Research Station, Clayton, North Carolina. In Illinois, the seeds were planted on 13 May in unreplicated

Table 1. Infection of ears of Tastyvee sweet corn by nine paired combinations of six sporidial isolates of *Ustilago maydis*[a]

Isolate	Mean number of ear galls per plant/Mean weight (gm) per gall[b]		
	Isolate 11	Isolate 27	Isolate 36
2	0.7/23.0	0.8/18.5	1.0/15.2
34	0.7/3.2	0.5/9.2	1.2/17.6
35	0.8/11.6	0	0.8/35.0

[a] Sources: isolate 2, Raleigh, North Carolina; isolate 11, Newfield, New York; isolate 27, Columbia, South Carolina; isolates 34 and 36, Madison, Wisconsin; isolate 35, Clayton, North Carolina.

[b] Data are means of two replications with three to five inoculated plants per treatment per replication.

single-row plots 3.4 m long and 0.76 m apart, with approximately 12 plants per row. The plants were not inoculated. Smut incidence from natural infection was rated on primary and secondary ears on 7 August.

At Clayton, the hybrids were planted on 21 April in unreplicated single row plots 4 m long and 0.9 m apart, with up to 21 plants per row. Plants were inoculated at tassel emergence as described for greenhouse experiments. Inoculations of the earliest maturing hybrids began on 8 June. Each inoculated plant was labeled with a tag recording its date of inoculation. The date of appearance of smut galls was recorded, and when the galls reached full size, the length and diameter of each ear gall and the diameter of each axillary bud gall or stalk gall were measured. Volume of galls was estimated according to the formula for a prolate spheroid ($V = 4\ \pi ld^2/3$, with l = length and d = diameter) for ear galls and according to the formula for a sphere ($V = \pi d^3/6$) for axillary bud galls and stalk galls.

RESULTS

Inoculation procedures. Tassel galls were induced in plants inoculated in the leaf whorl between 3 and 16 days before tassel emergence (Figs. 1 and 2). Ear galls developed only in those plants inoculated in the region of ear shoot initiation between 0 and 8 days before tassel emergence (Fig. 1). Two types of ear galls occurred (Fig. 3). When infection occurred early in ear shoot development, the resulting galls were long and narrow, with the host cob tissue replaced by pathogen mycelium and teliospore formation. When infection occurred later, the cob tissue developed more or less normally, but the developing florets were converted into galls.

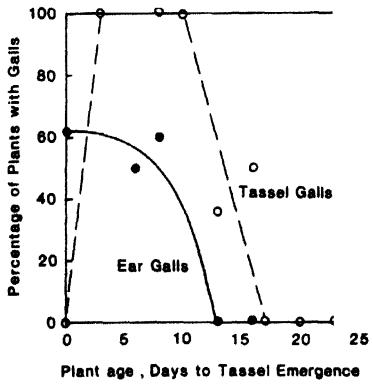

Fig. 1. Effect of corn plant development stage at inoculation with sporidial suspensions of *Ustilago maydis* on the percentage of plants in which either tassel galls (circles) or ear galls (solid dots) were induced. Data for tassel galls are combined means from two experiments with a total of 58 inoculated plants. Data for ear galls are from one experiment with a total of 31 inoculated plants.

Inoculation of Tastyvee sweet corn with the combination of monosporidial isolates 2 × 36 of *U. maydis* consistently yielded a high frequency of large smut galls (Table 1), so this paired combination was used in subsequent experiments. Differences in pathogenicity were apparent among pairs of sporidial isolates. In five additional tests of this inoculation method with eight to 80 Tastyvee plants per test, smut incidence varied from 50 to 100% (mean 78%), with an average of 0.9 ear galls per plant (Table 2).

Greenhouse evaluations of sweet corn hybrids and inbreds. *Plants in pots.* Among the 140 hybrids and 18 inbred lines evaluated in pots (total of 1,737 inoculated plants), the hybrids tended to have shorter incubation periods and developed larger galls than inbred lines (Table 3). Mean incubation periods ranged from 12 to 25 days for inbreds and from 10 to 27 days for hybrids. Inoculated plants of three of the inbreds and eight of the hybrids developed no smut symptoms. There were weak but statistically significant negative correlations ($P < 0.05$) between incubation period and smut incidence ($r = -0.199$) and between incubation period and number of ear galls per inoculated plant ($r = -0.241$). Eight of the hybrids were tested a second time in pots with correlation coefficients between runs of 0.541 for incubation period, 0.494 for smut incidence, and 0.544 for ear galls per plant. The number of stalk and axillary bud galls per plant was not correlated between runs.

Plants in soil beds. For the 25 hybrids grown both in pots and in soil beds in the greenhouse, mean smut incidence was significantly greater ($P < 0.01$) and mean incubation period significantly shorter ($P < 0.01$) for plants grown in soil beds than for those grown in pots. All 25 hybrids in soil beds had smut incidence of 39% or more, including one hybrid (73% incidence) that had no smut in the plants grown in pots. Mean values for plants in soil beds and plants in pots, respectively, were: incubation period, 15.8 and 18.2 days; smut incidence, 73.6 and 39.1%; number of ear galls per inoculated plant, 0.58 and 0.29; and number of stalk and axillary galls per plant, 0.42 and 0.10. Gall size did not differ significantly between plants grown in soil beds and pots in the greenhouse. Incubation periods were significantly correlated ($r = 0.430$, $P < 0.05$) between plants in soil beds and pots, but smut incidence and numbers of ear galls or stalk and axillary bud galls per inoculated plant were not.

For the 25 hybrids grown in soil beds, the number of ear galls per inoculated plant was significantly correlated with the number of stalk and axillary bud galls per plant ($r = 0.465$, $P < 0.05$). This was also true for these 25 hybrids grown in pots ($r = 0.508$, $P < 0.01$). There was a statistically significant negative correlation between smut incidence and number of healthy ears per plant ($r = -0.484$, $P < 0.05$). Injection of tap water between the leaf sheath and stalk had no measurable effect on ear development. For the 14 hybrids that had both inoculated and uninoculated treatments, the mean smut incidence was 70% in

Fig. 2. Tassel galls induced in a B73 × Mo17 dent corn plant injected in the leaf whorl with a mixed suspension of sporidia of isolates of *Ustilago maydis* 3 days before tassel emergence. Similar tassel galls formed on inoculated sweet corn plants.

Fig. 3. Ear galls induced in B73 × Mo17 dent corn plants in which a mixed suspension of sporidia of isolates of *Ustilago maydis* was injected between the leaf sheath and stalk at the sixth, seventh, and eighth nodes below the top of the plant at the time of tassel emergence from the leaf whorl. Similar ear galls formed on inoculated sweet corn plants.

inoculated plants and 0% in uninoculated plants, and the reduction in numbers of ears produced on inoculated plants ranged from 27 to 77%, with a mean of 56% fewer ears on inoculated plants than on uninoculated plants.

Of 426 inoculated plants in 25 hybrids, 193 (45.3%) developed galls in primary or secondary ears, and only 48 (25%) of these formed any normal ears. Only 19 (23%) of the 84 plants with galls on stalks or axillary buds but not on ears formed normal ears, and only 32% of the 57 plants without galls on ears, stalks, or axillary buds but with galls on leaves or tassels formed ears. Among the 92 inoculated plants that did not develop galls, 70% formed normal ears. The average number of normal ears per uninoculated plant ranged from 1.0 to 1.2 for the 14 hybrids.

Field evaluations of sweet corn hybrids. Smut incidence among the 1,627 inoculated plants of 140 sweet corn hybrids in the field at Clayton, North Carolina, was low (Table 3) and did not differ significantly from the incidence of ear galls on uninoculated plants of the same hybrids at Urbana, Illinois, in 1987. Apparently, the conditions at Clayton in 1987 were unfavorable for smut infection; only 1 of 368 (0.3%) uninoculated plants at Clayton developed smut symptoms, whereas 8.7% of the plants at Urbana developed ear galls. Only two hybrids, Summer Sweet 7800 (38 observed plants) and AVX 2658 (45 plants), remained free of smut at both field locations and in the greenhouse experiments.

Incubation period for smut development on hybrids at Clayton was negatively correlated with smut incidence ($r = -0.374$, $P < 0.01$) and incidence of ear galls ($r = -0.419$, $P < 0.01$) and was positively correlated with stalk and axillary bud gall volume ($r = 0.379$, $P < 0.05$). Incidence of ear galls was weakly correlated ($r = 0.225$, $P < 0.05$) with incidence of stalk and axillary bud galls at Clayton. Volume of ear galls and volume of stalk and axillary bud galls at Clayton were also weakly correlated ($r = 0.550$, $P < 0.05$). None of these parameters for hybrids at Clayton was correlated with incidence of ear smut in the uninoculated hybrids at Urbana. There also were no significant correlations for incubation period, smut incidence, or gall size between hybrids grown in pots in the greenhouse and those grown in the field.

Plants in the field at Clayton were examined at 2- or 3-day intervals, which may partly account for the longer incubation period (14–37 days) recorded for hybrids in the field than for the same hybrids grown in the greenhouse, which were examined every day after inoculation (Table 3). Ear galls on plants in the field were significantly ($P < 0.01$) larger than those on plants in the greenhouse (Table 3). The size of stalk and axillary bud galls did not differ significantly between plants grown in the field and in the greenhouse.

DISCUSSION

Although some early workers (8,11) reported success in inducing smut galls in ears inoculated at various stages of host plant development up to drying of the silks, we found little or no gall development in preliminary experiments in which we injected sporidial suspensions into ear shoots after they emerged from the subtending leaf sheath. From our attempts to induce tassel galls, it was apparent that the tassel florets were no longer susceptible to smut infection at the time when tassels began to emerge from the leaf whorl. Thus, it seemed likely that ear shoots might also be most susceptible to infection by *U. maydis* before they began to emerge from the subtending leaf sheath. This was confirmed by the results of inoculations at the sixth, seventh, and eighth nodes below the tops of the plants. For greenhouse-grown plants, the time of tassel emergence was a convenient indicator that the ear shoots were at a susceptible stage of development.

The critical importance of timing of infection is shown by the two types of ear galls that resulted from inoculations at this time. Apparently, infections that occurred before florets were sufficiently developed did not result in galls involving floret tissue, but, instead, the galls occupied the developing cob tissue. Infections that occurred after cob tissue was differentiated appeared to be confined to the florets, which at that time were sufficiently developed to be induced to form galls.

The inoculation procedure, which consistently worked well with Tastyvee sweet corn and the dent corn hybrid B73 × Mo17, also successfully induced gall formation in 94% of the sweet corn hybrids that were tested in the greenhouse. In replicated tests of eight hybrids in the greenhouse, there was no correlation in incidence of stalk and axillary bud galls in the two runs, although the incidence of ear galls was moderately correlated between runs. Davis (4) also found that incidence of galls of axillary buds was an extremely variable character. He suggested that most axillary buds that are infected with *U. maydis* do not develop noticeable galls unless some factor such as injury to the plant or failure of pollination stimulates the axillary bud to resume growth.

The percentage of smutted plants per sweet corn hybrid was not correlated between greenhouse experiments with plants in either pots or soil beds. Only the incubation period was significantly correlated between these experiments. Two hybrids developed no smut symptoms in any of the tests, but neither incubation period nor incidence of smut was significantly correlated among greenhouse and field experiments at Clayton, North Carolina, and Urbana, Illinois. The differences in apparent susceptibility of hybrids to smut in North Carolina and Illinois might be attributed to difference in virulence (3) between the isolates of

Table 2. Infection of Tastyvee sweet corn plants inoculated with *Ustilago maydis* in five experiments with plants grown in pots in the greenhouse

Expt. no.	Number of plants inoculated	Infected plants (%)	Ear galls per plant	Stalk and axillary bud galls per plant
1	8	88	1.4	0.6
2	80	86	0.7	0.6
3	11	100	1.1	0.2
4	11	64	1.0	0.3
5	12	50	0.3	0.3

Table 3. Comparison of smut development in sweet corn inbreds and hybrids grown in the greenhouse or in the field in North Carolina[a]

Experiment	Incubation period (days)[b]	Infected plants (%)	Number/plant		Volume (cm^3)	
			Ear galls	Stalk and bud galls	Ear galls	Stalk and bud galls
Greenhouse: 18 inbreds in pots	20.4	30	0.22	0.15	15	18
Greenhouse: 140 hybrids in pots	17.5	35	0.30	0.12	39	43
Greenhouse: 25 hybrids in soil beds	15.8	74	0.58	0.42	59	48
Field: 123 hybrids	18.9	12	0.06	0.06	234	61

[a] Plants were injected with sporidial suspension between leaf sheath and stalk at ear nodes. All hybrids were from a set of 140 grown in pots in the greenhouse; stands in the field of 17 of these hybrids were inadequate for testing.

[b] Days from inoculation to appearance of first galls.

U. maydis used in inoculations at Clayton and those that occurred naturally in the field at Urbana, but this could not explain the lack of correlation between greenhouse and field experiments in North Carolina, because the same pair of compatible sporidial isolates was used in inoculum for both experiments. Differences in growing conditions between experiments might account for some of the variation. Immer and Christensen (9) found a low but significant correlation of $r = 0.40$ between smut incidence from natural infection in 34 dent corn lines in the field and incidence of smut symptoms in the same lines inoculated as seedlings in the greenhouse by injection of a sporidial suspension into the leaf whorl. This may indicate a wider range of susceptibility among their lines than among the sweet corn hybrids we tested.

The inconsistency in ranking of size and incidence of smut galls in hybrids in our greenhouse and field tests casts doubt on the utility of our inoculation procedure in evaluating quantitative resistance of corn genotypes to common smut. One aspect of the inconsistency may be related to differences in growth and development of corn plants under greenhouse and field conditions. In the greenhouse, ear shoot development is usually delayed relative to tassel development. Consequently, inoculation at tassel emergence, which worked well in the greenhouse, may have been too late for maximum infection of ears of most hybrids in the field. Furthermore, it was not always possible to examine plants in the field on a daily schedule, so some plants were inoculated 1 or 2 days after emergence of tassels. The poor growing conditions in the field at Clayton in 1987 probably also interfered with smut infection. The plants suffered from drought and were stunted early in the growing season. Christensen (2) and Garber and Hoover (7) suggested that infection by *U. maydis* may be more likely in vigorous than in poorly growing plants.

Griffiths (8) and Platz (11) suggested that resistance to smut in corn may be largely due to morphological factors that tend to exclude inoculum from reaching areas of meristematic tissue in which galls are likely to result from infection. In that case, inoculation by injection of sporidial suspensions into infection courts would bypass the resistance, and little correlation could be expected between disease incidence among hybrids injected with sporidial suspensions and the same hybrids infected naturally in the field. On the other hand, purely morphological resistance should preclude the possibility of differential interactions among smut isolates and corn lines of the type reported by Christensen and Stakman (3) and Eddins (6). Our results have not clarified this issue. We can only agree with Christensen (2) that: "Little is known regarding the basic nature of resistance of corn to smut and the interaction between the pathogen and the host."

It is possible that inoculation procedures similar to ours may be developed to effectively distinguish levels of resistance of corn genotypes to common smut, but more research will be required to refine the techniques. Multiple inoculations may be necessary, or it may be preferable to inoculate plants in the field at a specific time before, rather than at or shortly after, tassel emergence. Duplicate plantings of corn genotypes approximately 1 wk apart could allow inoculations to be timed at reproducible intervals before tassel emergence. Genotypes in the second planting could be inoculated on a designated number of days after tasseling of the same genotypes in the first planting.

The incubation periods of 16–20 days in our greenhouse and field experiments seem long in relation to Christensen's (2) statement that under favorable conditions young galls may become visible within a few days and mature teliospores may be found 7–9 days after inoculation. In preliminary experiments when we injected seedlings with sporidial suspensions, we found that galls in young leaves and in the growing point of the seedlings appeared and matured quite early, as indicated by Christensen. In adult plants, however, galls in ears, axillary buds, and stalks mature more slowly and grow much larger than galls in seedlings.

We had hoped that gall size would be a valid indication of quantitative differences in resistance of corn genotypes to common smut. This appears not to have been the case, since gall size for corn genotypes was not correlated among our experiments. The size of the galls apparently depends on the physiological condition of the infected host plants as influenced by the environment in which they are grown. Ear galls and stalk and axillary bud galls on inbreds were significantly smaller than those on hybrids grown under the same conditions. The most striking difference in gall size was in the much larger ear galls found on hybrids in the field at Clayton than on the same hybrids in the greenhouse. Ear galls were four to five times larger on plants in the field than on plants in soil beds or pots in the greenhouse. It seems likely that the greater light intensity in the field and the resulting increased photosynthesis may have been responsible for this difference. This would be consistent with the hypothesis that ear galls induce a stronger sink for photosynthates than axillary bud galls and stalk galls, which were not much larger on plants in the field than on plants in the greenhouse.

In comparisons between pairs of infected and uninfected corn plants in the field, Johnson and Christensen (10) found that plants with single galls averaged 25% less grain yield than plants with no galls, and plants with multiple galls yielded an average of 50% less. Plants with large galls, particularly above the ear, were often barren. In our experiment with hybrids grown in soil beds in the greenhouse, smut infection dramatically increased the frequency of barren plants over that of the uninoculated or water injection treatments. Even plants with galls only on leaves and tassels yielded 68% fewer normal ears than plants with no visible smut infection.

Although our inoculation procedure is not sufficiently refined to serve in screening corn genotypes for quantitative resistance to common smut, it can be useful for further studies of infection processes of *U. maydis* and of the physiology of smut gall development. Perhaps with larger numbers of plants per host line, smut incidence after inoculation would provide a satisfactory measure of relative susceptibility. Based on our experience, we suggest that at least 50 plants per host genotype should be inoculated. Incidences of galls of ears and of stalks above the ear appear to be the most useful measures of resistance of host lines or virulence of pathogen isolates.

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