Techniques

# Evaluation of Inoculation Methods for Inducing Common Smut on Corn Ears

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This research was supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations.
Procedures for production of *cuitlacoche* for commercial purposes are subject to a patent pending by the University of Georgia Research Foundation.
We thank John Dixon and Jan Fowler for technical assistance.
Accepted for publication 5 June 1992.

## ABSTRACT

Pope, D. D., and McCarter, S. M. 1992. Evaluation of inoculation methods for inducing common smut on corn ears. Phytopathology 82:950-955.

Tests were conducted in the mountain, piedmont, and coastal plain areas of Georgia to develop a reliable inoculation method for inducing a high incidence and high severity of common smut of corn caused by *Ustilago maydis*. Inoculum, consisting of six mixed sporidial lines at $10^6$ cells per milliliter, was applied (3 ml per ear) to unwounded silks, to silks wounded by partial or complete removal with pruning shears, or to the exposed ends of ears from which 1.5–2.0 cm of cob was removed with pruning shears, or it was injected through the husk into the cob. Inoculation of unwounded or partially removed silks resulted in little or no disease. Inoculation of the ear tips from which silks were totally removed or where 1.5–2.0 cm of cob was removed was moderately successful. The cob injection and tip injection methods consistently resulted in the highest incidence (as high as 97%) and the highest severity of disease. Certain pairwise combinations of compatible sporidial lines were more effective than others in inducing galls. Ear-injection inoculation methods should be further evaluated for usefulness in producing smutted ears for disease screening and food purposes.

*Additional keywords*: breeding, *cuitlacoche, huitlacoche,* maize mushroom, resistance, *Zea mays.*

Common smut caused by *Ustilago maydis* (DC) Corda results in economically significant yield reductions of corn (*Zea mays* L.). Any aboveground part of the plant can become infected when airborne sporidia from germinated teliospores, or germinating teliospores, contact susceptible tissue, form dikaryotic mycelia, and penetrate directly or through wounds (6). Localized galls consisting of a mixture of hypertrophic and hyperplastic host and fungal tissue are produced, most conspicuously on the ears. Yields of individually infected plants can be reduced 40–100% if galls are large or if they are located on the ears (9,10,11,19,20). Annual yield reduction estimates in the United States usually range from 1 to 5% but may exceed 10% under epidemic conditions (19). Losses vary with the year, geographical location, and cultivar grown (3,20). Sweet corn cultivars usually sustain greater losses than field corn cultivars (1).



Host resistance offers the most economical means of control of common corn smut (3,20). Smut-resistant hybrids have been produced in breeding programs, but the nature and durability of resistance remains unknown. In some cases resistance may be a polygenic trait that involves few genes that most likely condition functional, physiological, and morphological characters (3,20) such as tightness and thickness of husks (14). Major genes for resistance exist in small grains against other smut fungi, but such genes against *U. maydis* have not been found in corn. Detailed studies of the genetic nature and mechanisms of resistance to *U. maydis* are hindered by the lack of reliable, repeatable inoculation methods for ears. This limitation also has prevented breeders from screening germ plasms for smut resistance specifically in cob and kernel tissue.

Although common smut is an economically important disease of corn, the galls are edible. Since the time of the Aztecs, the galls from naturally infected ears, known as *cuitlacoche* (renamed *huitlacoche* by the Spanish), have been highly prized (12). For generations, Mexicans and native American Indians have used

*cuitlacoche* as a seasonal food additive (24). In Mexico, it is also sold as a canned product. Recently, gourmets in the United States have recognized *cuitlacoche*, sometimes marketed as "maize mushrooms" or "Mexican truffles," as a delicacy (13), and demand for edible smutted ears greatly exceeds the supply (15). The limited availability of marketable smutted ears is related to their usually low occurrence in naturally diseased corn and the inability to harvest galls in a timely and efficient manner because of different infection times.

Smut resistance breeding programs and production of *cuitlacoche* as a cash crop both require an inoculation technique that consistently produces a high percentage of severely diseased ears. Numerous reports are available on the influence of various inoculation techniques, host developmental stages, and environmental conditions on pathogenesis (3,15,21). Attempts have been made to induce smut galls on various plant parts (including the stalk, tassel, bud, ears, and roots) of corn with both teliospores and sporidia by methods that include spraying, sandblasting, dusting, painting, dipping, injecting, and vacuum infiltrating spores onto the host (3,15,21,25). Injection of inoculum into the whorls of seedlings with a hypodermic syringe has been a common and successful method of inducing galls on meristematic tissue (3,6,21). Contradictory results have been reported when attempts have been made to produce disease on ears. In some tests (7,16), galls were produced when sporidia were introduced into the tips of developing ears, sometimes even up to the time the silks started to dry. However, other workers (21) were mostly unsuccessful in producing galls on ears if ear shoots were inoculated after emergence from subtending leaf sheaths. Galls were induced in high frequency when a 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 (21). In these experiments extensive inoculations of developing ears were not attempted, because of failure in preliminary studies and the finding that tassel florets were not infected after emergence from the leaf whorl. Presumably, the ear shoots also were most susceptible prior to emergence from the leaf shoot. In a recent study (15), sporidial suspensions injected into the sixth to eighth internodes produced ear galls, but inoculum sprayed onto the wounded tissues located in these same general locations did not. These conflicting reports on the success of various inoculation methods have precluded the identification of an efficient and completely reliable method for producing galls on ears.

The purpose of this work was to evaluate inoculation methods with the objective of developing a reliable, repeatable method for inducing smut on ears for both disease-screening and food purposes. Preliminary reports on this work have appeared (17,18).

## MATERIALS AND METHODS

**Isolation and maintenance of sporidial lines.** Teliospores from a naturally infected ear of field corn collected near Plains, Georgia, in 1987 were spread on Vogel's complete agar (VCA) (22) and incubated at 30 C. Following teliospore germination, sporidia were removed and streaked onto VCA. Resulting colonies from single haploid sporidia were removed and streaked onto VCA two more times to obtain pure-line colonies. Five such lines, designated GA1, GA2, GA3, GA4, and GA15, and a sixth (983 from Percy Thomas, Winnipeg, Manitoba) were grown on VCA at 30 C. The lines were transferred to Vogel's complete broth (VCB; VCA without agar) containing 15% glycerol and placed at −80 C for long-term storage.

**Preparation of inoculum.** Cells of each of the six lines were transferred separately to test tubes containing 3 ml of VCB and placed on an orbital shaker at 100 rpm at 30 C for 3–4 days. One milliliter of each resulting culture was transferred to a 250-ml flask containing 100 ml of VCB and shaken at 100 rpm at 30 C for 4–6 days. Cells of the six lines were mixed in equal concentrations in sterile deionized water to give $10^6$ cells per milliliter. However, in one experiment each line was either kept separate, or the lines were prepared in all possible pairwise combinations to give a final concentration of $10^6$ sporidia per milliliter. In all experiments, 1 ml $L^{-1}$ of polyoxyethylene sorbitan monooleate (Tween 80) was added to each inoculum preparation.

**Field plot location and establishment.** In 1990, field plots were located at the University of Georgia Horticultural Science Farm near Athens (a piedmont location). Early (24 April) and late (16 May) plantings were made. In 1991, plantings were made at the Horticultural Science Farm (18 April); the University of Georgia Plant Sciences Farm, also near Athens (18 April and 16 May); the Georgia Mountain Branch Station near Blairsville (15 May); and the Southeast Georgia Branch Station near Midville, in the coastal plain (2 May).

Routine land preparation practices were used on all of the plots, and the soil was fertilized according to soil test results. Atrazine (2.24 kg a.i./ha) was used for weed control. Fungicide-treated (PCNB + ethazole [1.25 g/kg]) seed of the hybrid Silver Queen were planted in rows spaced 0.97 m apart, and plants were thinned to 46 cm within rows 2 wk after planting. The field corn hybrid Pioneer 3320 was used in one test at Blairsville with similar spacing. Sprinkler irrigation was used as needed to prevent moisture stress. A completely randomized design was used in all experiments.

**Description of experiments.** Five types of experiments were conducted. In all experiments, except that in which the effect of ear development stage on pathogenesis was studied, the ears were inoculated when the silk had emerged at least 5–10 cm but had not become dry. This usually occurred 56–69 days after seeding and 7 days after initial tassel emergence. Only the two uppermost ears of each plant were inoculated. Inoculum was delivered either with a 12-ml syringe equipped with an 18-gauge needle or with a 50-ml syringe equipped with an udder infusion cannula and connected with a hose to a 2-L insulated canister reservoir carried in a backpack. The needles and cannulas used for ear injection treatments were modified to prevent obstruction by tissue and to facilitate distribution of inoculum within the ear. The tips of needles were bent, the tips of cannulas were blocked, and two small holes were drilled in the sides for inoculum release. Volume of inoculum was 3 ml per ear except where noted otherwise. Controls consisted of unwounded or wounded ears treated with diluted VCB amended with Tween 80 but without sporidia.

**Comparison of inoculation methods.** Inoculum was applied to unwounded silks, to wounded tips of silks cut closely at the point of emergence from the husk (designated as the "cut silk" method), or to wounded exposed ends of cobs from which 1.5–2.0 cm was cut (the "cut cob" method), or it was injected into the centers of intact ears through the husk tissue via a median-line needle wound (hereafter designated as the "cob injection" method). The silks and terminal cob tissues were removed with pruning shears. The tests were run at Athens (four times), Blairsville, and Midville. Inoculations of unwounded silks were made only at Athens during 1990. Treatments were replicated four times in each test, with a replication consisting of 15 m of row length, in which an average of 65 ears were inoculated.

**Silk inoculation methods.** Inoculum was applied to intact silks at the point of protrusion from the husk, to silks from which the terminal third was removed with pruning shears, and to silks from which three fourths of the length was removed. The experiment was conducted at Athens (two times), Blairsville, and Midville. Treatments were replicated five times, and each replication consisted of 15 m of row with an average of 60 inoculated ears.

**Comparison of injection methods.** The cob injection method described previously was compared with a tip injection method performed by inserting the inoculation cannula from the tip end of the ear through the silk channel. The inoculum was released into the developing ear with a minimum of wounding. Two tests were conducted at Blairsville. In the first, four replications, each with 22–25 ears of Silver Queen, were used. In the second, three replications, each with 21–22 ears of Pioneer 3320, were used. Inoculations in the second test were made 3 wk after the first.

**Effect of ear development.** Ears in different stages of development were inoculated by the cob injection method. Ear develop-

ment stages included: silks not yet visible but within 1–7 days of extrusion; silks extruded 1–4 cm; and silks extruded 5–10 cm but not yet dry. The experiment was conducted at both Athens and Blairsville in 1991. Two replications were used at each site, and each replication had an average of 25 ears.

**Effect of sporidial composition of inoculum.** The six sporidial lines that were used as a mixture in all previously described tests were tested singly and in all pairwise combinations. The cob-injection inoculation method was used. Treatments were replicated four times near Athens in 1991, and each replication had an average of 58 inoculated ears.

**Disease assessment.** Incidence (percentage of infected ears) and severity (proportion of ear with galls) were quantified 21 days after inoculation. Disease severity was recorded on a 0–6 scale for which 0 = no disease, T (trace) = 5 or fewer kernels diseased, and 1–6 = increasing proportions of ear galled (Fig. 1). The incidence of aborted ears or nonsmutted ears with microbial damage was recorded.

**Analysis of data.** Disease severity values were transformed to a continuous numerical scale from 0 to 7. Thus, disease severity ratings in the text and tables are transformed values. Disease incidence data were subjected to an angular transformation prior to analysis to improve homogeneity of variances; however, values listed in this paper are the actual percentages. In all studies except one, counts of aborted ears and ears destroyed by microorganisms were eliminated from the data before statistical analyses were performed. Counts of aborted and microbially damaged ears were included in the ear development study, as treatment significantly affected them. Data were analyzed with the Statistical Analysis System (SAS Institute, Cary, North Carolina) by the FREQ, GLM, and MEANS procedures. Differences among main-effect levels were tested by Duncan's multiple range test to accommodate unequal sample sizes. Means calculated across plot locations were weighted by number of ears rated.

## RESULTS

Excellent development of smut occurred on ears over a wide range of environmental conditions when appropriate inoculation methods were used. Although significant differences occurred among some locations and years, no location × treatment or year × treatment interaction effects were significant. Therefore, treatment means averaged over the various tests are presented. Generally, the cob-injection inoculation method resulted in both higher incidence and higher severity of disease than the cut cob or cut silk inoculation methods (Table 1). In two tests at Athens (early planting, 1990, Horticultural Science Farm, and late planting, 1991, Plant Sciences Farm) disease incidence was similar for the cob injection and cut cob methods, but disease severity was still significantly higher when the cob injection method was used instead of the cob cut method. In most cases the cut cob inoculation method resulted in higher incidence and severity of disease than the cut silk inoculation method (Table 1). However, in two tests (late planting, 1990, Horticultural Sciences Farm, and 1991, Blairsville), the cut silk method produced equal or significantly more disease and more severe disease than the cut cob method. These exceptions probably resulted from slight modifications in the way in which inoculum was applied and to weather conditions. In these tests inoculum was more forcibly injected into the cut silk tips than in the four other tests. At Blairsville, moderately heavy rain occurred during inoculation, which probably washed much of the inoculum from the flat cut surfaces on the cut cobs.

Gall development differed with the different inoculation methods. The cut silk method resulted in gall formation mostly on the uppermost third of the ear. With the cut cob method, the galls were clustered mostly on the outside of the ear at the tip (Fig. 2). The most uniform distribution of galling on ears occurred when the cob injection method was used. Ears were often totally covered with galls when sporidia were injected

TABLE 1. Effect of inoculation method on the incidence and severity of smut on the corn hybrid Silver Queen inoculated with a mixture of sporidia of *Ustilago maydis*

| Inoculation method[x] | Mean[y] | |
|---|---|---|
| | Disease incidence | Disease severity |
| Cob injection | 83.2 a[z] | 4.1 a |
| Cob injection (control) | 6.2 d | 0.2 d |
| Cut cob | 58.7 b | 1.6 b |
| Cut cob (control) | 10.5 d | 0.2 d |
| Cut silk | 40.1 c | 1.4 c |
| Cut silk (control) | 3.5 d | 0.1 d |
| Intact silk | 3.4 d | 0.1 d |
| Natural | 2.6 d | 0.1 d |

[x] Inoculum (3 ml) consisting of a $10^6$ sporidial line mixture amended with 1 ml $L^{-1}$ of Tween 80 was injected into the cob near the center of the ear (cob injection) or drenched onto the cut end of ears from which 1.5–2.0 cm of cob was removed (cut cob), onto the tips of silks removed at the point of emergence (cut silk), or onto intact silks. Controls received 3 ml of diluted Vogel's complete broth (22) amended with Tween 80 but without sporidia.

[y] Each value is a mean of four to six tests conducted at Athens, Blairsville, and Midville, Georgia. Disease incidence is the percentage of ears with galls, and disease severity is the proportion of the ears diseased based on a scale on which 0 = no disease and 7 = all of ear galled.

[z] Values with letters in common in each column do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. Root mean square error was 16.9 and 1.5 for disease incidence and disease severity readings, respectively.

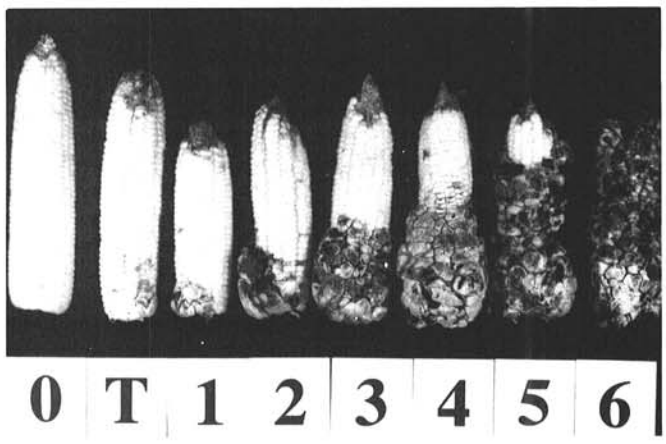


**Fig. 1.** Corn ears representing the disease severity scale (0–6) used to record smut severity. T = trace.

**Fig. 2.** Ears of corn left uninoculated (left) or inoculated by the cut cob (center) or cob injection (right) methods. The photo was taken 21 days after inoculation.

internally (Fig. 2). With the cob injection method, irregularly shaped galls were detectable to the touch through the husks 10 days after inoculation. The galls developed very rapidly after this time, and in 14–15 days they were large and white to slightly black internally but still contained by the husks. In 18–21 days the husks were breached by the rapidly expanding galls, and black masses of teliospores were clearly visible but not necessarily dry and dusty. Some secondary microbial invasion occurred through the needle wound site on the injected ears. This was most evident on ears that did not form galls. Inoculation of unwounded intact silks did not result in any more disease than occurred naturally (Table 1). Generally, galls resulting from natural infection were fewer but larger than those on inoculated ears.

In another test conducted at three locations, inoculation of unwounded intact silks or silks wounded by partial removal with pruning shears resulted in either little or no more disease than occurred naturally (Table 2).

When tested on the sweet corn hybrid Silver Queen and the field corn hybrid Pioneer 3320, the cob injection and tip injection methods resulted in similar smut incidence and smut severity (Table 3). More galling occurred on Silver Queen ears inoculated by the tip injection method than by the cob injection method. The nature of gall development was similar on ears inoculated by the two methods. However, secondary microbial invasion sometimes occurred on ears inoculated by cob injection, whereas none was observed on ears inoculated by tip injection.

Developing ears inoculated by the cob injection method when silks had emerged 5–10 cm from the ear tip but had not yet become dry had higher incidence and severity of disease than ears inoculated when silks were only 1–4 cm long or had not yet emerged (Table 4). Many of the ears inoculated in the early development stages did not mature because of microbial damage, presumably because of bacterial and fungal invasion through the needle wound made during inoculation (Table 4).

Nine of the 15 pairwise combinations of sporidial lines produced very high levels of disease when inoculum was injected into developing ears (Table 5). In all cases, these nine pairs of lines caused disease incidence levels similar to each other and to the mixed inoculum in which all six lines were combined (91.4%). However, the disease severity levels for two of the nine pairs were higher than the mixture (5.1), and four others were lower than the mixture. Also, disease severity values differed significantly among the nine pairs. A positive correlation (0.89, $P > 0.0006$) between disease incidence and disease severity was found among the nine compatible pairs of lines. Disease incidence

TABLE 2. Incidence and severity of smut on ears of the corn hybrid Silver Queen inoculated by drenching intact or wounded silks with a mixture of sporidia of *Ustilago maydis* in 1991

| | Mean[y] | |
|---|---|---|
| Inoculation method[x] | Disease incidence | Disease severity |
| Intact silk | 3.80 a[z] | 0.15 a |
| 1/3 silk removed | 2.98 a | 0.10 ab |
| 3/4 silk removed | 2.88 a | 0.08 ab |
| Natural | 2.11 a | 0.08 b |

[x]Inoculum (3 ml) consisting of a $10^6$ sporidial mixture amended with 1 ml $L^{-1}$ of Tween 80 was applied to intact silks or to silks from which portions were removed with pruning shears.
[y]Each value is a mean of three tests conducted at Athens, Blairsville, and Midville, Georgia. Disease incidence is the percentage of ears with galls, and disease severity is the proportion of ears diseased based on a scale on which 0 = no disease and 7 = all of ear galled.
[z]Values with letters in common in each column do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. Root mean square error was 29.9 and 0.6 for disease incidence and disease severity, respectively.

TABLE 3. Effect of inoculation method on incidence and severity of smut on two corn hybrids (Silver Queen and Pioneer 3320) inoculated with a mixture of sporidia of *Ustilago maydis*

| | Silver Queen | | Pioneer 3320 | |
|---|---|---|---|---|
| Inoculation method[x] | Incidence[y] (%) | Severity (0–7 scale) | Incidence (%) | Severity (0–7 scale) |
| Cob injection | 97.1 a[z] | 4.9 b | 97.1 a | 4.6 a |
| Tip injection | 92.9 a | 5.7 a | 96.6 a | 4.8 a |
| Natural | 0.0 b | 0.0 c | 0.0 b | 0.0 b |

[x]Inoculum (3 ml) consisting of a $10^6$ sporidial mixture amended with 1 ml $L^{-1}$ of Tween 80 was either injected through the husk into the cob in the center of the ear (cob injection) or through the silk channel without intentional wounding.
[y]Disease incidence is the percentage of ears with galls, and disease severity is the proportion of the ears diseased based on a scale on which 0 = no disease and 7 = all of ear galled.
[z]Values with letters in common in column do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Effect of ear development stage (in silk length) on ear abortion and incidence and severity of smut after inoculation of the corn hybrid Silver Queen with sporidia of *Ustilago maydis* by the cob injection method

| | Mean[w] | | |
|---|---|---|---|
| Silk length (cm)[v] | Aborted ears (%)[x] | Disease incidence (%)[y] | Disease severity (0–7 scale)[y] |
| 0 | 38.8 a[z] | 21.9 bc | 1.0 c |
| 0 (Control) | 41.6 a | 13.7 cd | 0.3 d |
| 1–4 | 25.4 ab | 39.1 b | 1.6 b |
| 1–4 (Control) | 22.4 ab | 4.1 d | 0.1 d |
| 5–10 | 12.2 b | 77.0 a | 3.2 a |
| 5–10 (Control) | 8.6 b | 5.4 d | 0.1 d |

[v]0 = Silks not visible but within 1–7 days of emergence. Silks 5–10 cm were not yet dry. Inoculum (3 ml) consisted of $10^6$ sporidial mixture amended with 1 ml $L^{-1}$ of Tween 80. Control ears received 3 ml of diluted Vogel's complete broth (22) amended with 1 ml $L^{-1}$ of Tween 80 but without sporidia.
[w]Each value is a mean of tests conducted at Athens and Blairsville, Georgia, in 1991.
[x]Ears did not develop, largely because of microbial deterioration.
[y]Disease incidence is the percentage of ears with galls, and disease severity is the proportion of the ears diseased based on a scale on which 0 = no disease and 7 = all of ear galled.
[z]Values for disease incidence and severity with letters in common in each column do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. Root mean square error was 16.9, 11.1, and 1.6 for percentage of aborted ears, disease incidence, and disease severity, respectively.

TABLE 5. Incidence and severity of smut on ears of the corn hybrid Silver Queen inoculated by the cob injection method with six sporidial lines in all possible pairwise combinations[w]

| | Sporidial line[x] | | | | | |
|---|---|---|---|---|---|---|
| Sporidial line | GA1 | GA15 | 983 | GA2 | GA3 | GA4 |
| GA1 | ... | 6.5[y] b | 6.4 b | 92.3 a | 88.4 a | 95.5 a |
| GA15 | 0.4 f[z] | ... | 6.8 b | 93.4 a | 96.3 a | 89.7 a |
| 983 | 0.3 f | 0.2 f | ... | 84.6 a | 90.7 a | 97.3 a |
| GA2 | 5.3 ab | 5.7 a | 4.0 e | ... | 7.3 b | 2.5 b |
| GA3 | 4.1 e | 5.8 a | 4.7 cd | 0.2 f | ... | 9.6 b |
| GA4 | 5.2 abc | 4.2 de | 5.4 ab | 0.1 f | 0.4 f | ... |

[w]Disease incidence and severity values are above and below, respectively, the diagonal running from upper left to bottom right.
[x]Sporidia were mixed to a final concentration of $10^6$ cells per milliliter and amended with 1 ml $L^{-1}$ of Tween 80. The control received 3 ml of diluted Vogel's complete broth (22) amended with 1 ml $L^{-1}$ of Tween 80 but without sporidia.
[y]Disease incidence is the percentage of ears with galls, and disease severity is the proportion of the ears diseased based on a scale on which 0 = no disease and 7 = all of ear galled.
[z]Values in each column with letters in common do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. Root mean square error was 20.5 and 0.4 for disease incidence and severity ratings, respectively.

and severity levels resulting from inoculation by six of the paired lines did not differ significantly from each other or from the control or natural background levels. The six lines used individually at $10^6$ cells per milliliter did not produce significantly more disease than the control treatment or disease resulting from natural infection (data not shown). The disease incidence and severity values for the control, 4.6 and 0.1, respectively, and for the natural treatment, 3.4 and 0.2, respectively, were not different from those of the incompatible sporidial pairs.

## DISCUSSION

The two ear injection methods (cob and tip injection) were effective in producing both a high incidence and high severity of smut on developing ears and were almost always more effective than the other methods tested. Up to 97% of ears became infected by these methods when 3 ml of compatible sporidia at $10^6$ cells per milliliter were used when the silks had emerged 5–10 cm. Injection of inoculum through the husks at stages of ear development earlier than the 5- to 10-cm silk extrusion stage were less successful, probably because of increased damage from invading secondary microorganisms that resulted in ear loss. Stage of ear development is probably not as critical with the tip injection method as with the cob injection method, because wounding is minimized with the former. The cob injection method was tested more extensively than the tip injection method. However, the limited test data available indicate that it will be as effective as the cob injection method, without the detrimental effects of wounding. In Illinois, Zimmerman and Pataky (25) recently reported 50% smutted ears when a sporidial suspension was injected through the silk channels of developing ears in full silk. This method was more effective than several others they evaluated.

The injection inoculation method shows considerable promise for the commercial production of smutted corn ears for food purposes. Preliminary observations indicate that ears would be at a peak edible stage at about 14 days after inoculation, but more studies to refine various aspects of the procedure need to be conducted. The cultivar used could influence the severity of smutting and the best harvest time. Sweet corn cultivars vary greatly in smut susceptibility, and the cultivar Silver Queen, used in most of the present studies, would probably be classified as moderately susceptible compared with some of the more susceptible genotypes tested (15). The environmental conditions after inoculation also could influence pathogenesis and the acceptable harvest time for smutted ears for food purposes. Differences in environmental conditions at different times and locations in the present work probably explains differences in disease incidence and severity among the various tests. Good to excellent disease development occurred in all our tests, suggesting that the injection inoculation method would have wide application. The injection method bypasses the critical phase of ear penetration in nature. Environmental conditions that influence the initial entrance of the smut fungus are of greater importance in causing an epidemic than those conditions that affect fungal development after infection (3).

The ear injection method also should be tested as a possible way to screen germ plasms for resistance to *U. maydis*. Apparently much of the resistance in presently grown cultivars results from ear characteristics such as thickness and tightness of husks (14). This type of resistance has been detected mostly under natural selection conditions (20). The ear injection method would not be an acceptable way to screen for such resistance. However, it should be evaluated as a means of identifying physiological resistance that may be present in the kernel and other internal ear parts. Use of combined morphological resistance associated with the external part of the ear coupled with physiological resistance in the kernel and of cob tissue could result in a higher level of resistance than presently exists.

In most of our tests, a mixture of six sporidial lines was used to ensure the presence of compatible combinations. In later in vitro mating assays using the methods of Holliday (8), two compatible groups were shown, each consisting of three of the six lines. Therefore, only nine of the 15 pairwise combinations were compatible and capable of forming pathogenic dikaryons. These nine compatible combinations produced moderate to severe smut on inoculated ears, whereas the six incompatible combinations did not cause significantly more disease than the individual lines used separately or than that occurring naturally. Significant differences occurred in disease severity ratings among the compatible lines, which suggests that variation in aggressiveness exists among the isolates used. Furthermore, the small, continuous differences among the disease severity values observed indicate that these genes probably act quantitatively to control aggressiveness of the infective dikaryons. Similar variation in aggressiveness has been reported in *U. maydis* by others (4,5,7). The positive correlation between disease incidence and severity suggests that factors controlling disease severity have pleiotropic effects on disease incidence. Linkage of factors that control both characters separately is also possible but less likely, because of the absence of significant differences in disease incidence values among compatible lines.

The sporidial line studies show the feasibility of selecting and using specific pairs of sporidial lines to maximize galling on ears for food purposes. The use of pure cultures of known sporidial lines, rather than teliospores for the production of smut for food purposes, would reduce purity problems, since teliospores are sometimes contaminated with an array of bacteria and fungi. Furthermore, inoculations with sporidia have been more successful than those with teliospores (25).

Kernel infection of corn by *U. maydis* supposedly occurs as the result of the growth of a dikaryotic mycelium through the silks (1,19). However, numerous inoculations of unwounded and wounded silks in our studies under a variety of environmental conditions were unsuccessful. Evidence that the mere spraying or dusting of inoculum on the external parts of plants increases infection over that which occurs naturally is lacking (3). Penetration of silks by *U. maydis* has been described, but growth of the fungus through the silk tissue has not (2). In another test, direct penetration of young plant tissue was reported, but no penetration of silks was observed (23). Further studies are needed to determine whether the infection of ears through the silk is a rare event strongly influenced by environmental conditions, or whether it occurs at all.

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