The Effect of Wounding and High-Pressure Spray Inoculation on the Smut Reactions of Sugarcane Clones

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ABSTRACT

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Sugarcane was inoculated with the smut fungus, *Ustilago scitaminea*, in a factorial experiment involving sugarcane clones, methods of applying inoculum, and wound states. A significant interaction of clones and wound states indicated at least two components of resistance, one that is circumvented by wounding, and one that is not. The relative importance of the two barriers varied among clones. One of the barriers to infection probably depends upon bud morphology, and the other upon host physiology. Among clones, inoculation of wounded, but not of unwounded, buds resulted in a significant negative correlation between minimum latent period and proportion of plants infected.

Additional key words: morphological resistance, physiological resistance, resistance components, *Saccharum*.

Infection of sugarcane (*Saccharum* spp. hybrids) by the smut fungus (*Ustilago scitaminea* H. Sydow and P. Sydow) occurs primarily through buds (1). Both the maturity (1) and the morphology (10) of buds affect susceptibility to infection. Hirschorn (4) increased infection by immersing bud-bearing cuttings in a spore suspension under reduced pressure to increase intrusion of inoculum under bud scales. Waller (11) confirmed this and found that appressoria formed mainly on meristematic regions at the base of inner bud scales. He showed further that when inoculum was introduced artificially to these regions, the proportion of infected plants was essentially the same for the resistant and susceptible clones in his test. He concluded that resistance depends upon the ability of cultivars to exclude the fungus from susceptible sites at the base of inner bud scales.

The most popular method of inoculation for screening sugarcane selections for resistance to smut in cultivar improvement programs is to immerse cuttings in a teliospore suspension (3); this is commonly called the "dip" method. Steiner and Byther (8) reported that reaction determined by several methods of inoculation correlated well with reaction under conditions of natural infection, but those involving inoculation of wounded buds did not. Wound inoculation has been avoided in screening trials in Hawaii (3) and elsewhere (9). Matsuoka (7), however, has used the "needle-wound-paste" method of inoculation effectively under conditions that make the dip method impractical. Matsuoka (*personal communication*) believes that wounding may circumvent a mechanical resistance barrier, but he regards this as a possible advantage because mechanical resistance may be less reliable than physiological resistance. Toffano (9), who regards inoculation of wounded buds as unsuitable for screening selections for resistance because it circumvents one infection barrier, uses the vacuum method to increase infection. If vacuum inoculation acts as supposed, then it circumvents the bud-scale barrier described by Waller (11) and should be subject to the same criticism as wounding.

It is evident in the works of Matsuoka (7) and Steiner and Byther (8) that some resistance is lost through wounding and that some remains after wounding. This suggests that there are at least two components of resistance to smut in sugarcane. The purpose of this research was to seek evidence for both components in the same set of cultivars in a single experiment. If the mechanical barrier to infection as described by Waller (11) is circumvented, leaving a second barrier (presumably physiological), and if the two barriers are at least partially independent, their presence in a set of cultivars should result in a significant interaction between cultivars and inoculation treatments in a suitable factorial experiment. Inoculations would include treatments that do, and treatments that do not, circumvent one of the postulated infection barriers. Wound state was one such factor in this research, and methods of applying inoculum posed at least the possibility of being a second such factor.

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MATERIALS AND METHODS

Smut whips were collected in paper bags from plants of many clones in a smut resistance trial in the field. The bags were closed and held for 48 hr in a forced-air seed dryer at 38 C. The paper bags were then sealed in plastic bags and held for about 4 mo at −20 C until inoculum was needed. Inoculum preparation consisted of the stepwise addition of teliospores from the stored smut whips to a tank of water containing 0.002% by volume of a nonionic surfactant. At each step the spore concentration was determined in a cell counter. Final spore concentration was 5×10^6 spores per ml. A sample of spore suspension from the tank was plated on 1% sucrose agar and the plates were held at 31 C for 6 hr. Spore germination on these plates ranged from 78 to 86%. Dip inoculation consisted of a 15-min immersion of single-budded stalk cuttings in a tank of spore suspension. Spray inoculation was accomplished by delivery of a 1-sec blast of the same inoculum from a high-pressure atomizing nozzle held almost in contact with the bud. This sprayer, an automotive paint sprayer, has been described in connection with virus inoculations (2). To produce wounds, four pinholes were punched around the periphery of each bud.

After the cuttings were inoculated, they were incubated in plastic bags at 31 C overnight, then planted in 20-cm-diameter plastic pots in the greenhouse. Inoculated plants were grown in the greenhouse for about 8 mo and monitored twice a week for infection. The criterion for infection was the production of a smut whip. When a whip was found, the date was recorded and the plant bearing the whip was destroyed.

The experiment consisted of two tests differing in a number of details, but both were three-factor factorials involving clones, methods of applying inoculum, and wound states. Test 1 was done from October through May of 1979–1980, and Test 2 was done in about the same period of 1980–1981. In the statistical analyses of the factorial effects, data representing the proportions of infected

plants were transformed to the arcsines.

Test 1. This test included five clones, (Table 1) four methods of applying inoculum (dip inoculation and three spray inoculations differing only in the angle of spray direction), and two wound states (wounded and unwounded). Because sugarcane buds are protected by overlapping scales, the angle at which a high-pressure spray is directed could affect the amount of inoculum intrusion under the scales and thus, presumably, the infection courts reached by the inoculum. Therefore, this variable was included in the test. For one treatment the spray was directed toward the apex of the bud; for another, it was directed toward the base, and for the third it was directed at a right angle to the plane of the bud scales. The 40 treatments resulting from the factorial combinations were replicated three times in a randomized complete block design. A plot comprised four pots, each containing two single-budded cuttings.

Test 2. This test was arranged in a completely random design because no significant block effect was detected in Test 1. It included 21 clones (listed in Table 2), 15 with 10 replications and six with less than 10 replications of some treatments. The use of a completely random design permitted analysis of the factorial treatment effects with unequal numbers of replications. The analysis was done both with 10 replications of 15 clones and with unequal replication of 21 clones, and the results were the same. Only one spray angle (right angle to plane of bud scales) was used in Test 2, because analysis revealed no significant effect of spray angle in Test 1.

RESULTS AND DISCUSSION

Results of analysis of variance of data from Tests 1 and 2 are shown in Table 1. In both tests, clones, wound states, and the interaction of clones and wound states were significant. In Test 2, methods of applying spores were also significant. The significance of the interaction of clones and wound states provides statistical evidence for the existence of two infection barriers, one that was breached by wounding and one that was not.

Data from Tests 1 and 2 are summarized in Tables 2 and 3, respectively. Differences between specific clones were tested by Duncan's new multiple range test and these results are indicated in the tables. Since clones interacted with wound states, clonal comparisons are valid only within wound states.

The period between inoculation and the appearance of the first smut whip in a clone, regardless of treatment, is referred to as the "minimum latent period" of that clone. In both tests, there was a significant negative correlation between minimum latent period and the percentage of plants infected by inoculation of wounded buds (Tables 1 and 2), but this correlation was not significant for unwounded buds. This implies that postinfection development of the fungus in host tissues is retarded in clones that also tend toward low whip production in spite of the presence of wounds. This relationship was obscured if whip production was further limited by the infection barrier that can be breached by wounding. Thus, the barrier that is not breached by wounding is more closely related to a clearly physiological resistance, and the barrier that is breached by wounding is less directly related to physiology.

The source of the interaction of clones and wound states can be seen in the comparison between the mean percentage of infection of plants in the wounded and unwounded states for each clone. In some clones (CP 57-603 and CP 63-588, Table 2), there is no difference and in others (CP 65-357, Table 2, and CP 72-1312, Table 3) there is a very large difference. The standard developed in Hawaii for measuring resistance in smut screening trials (5) has been tentatively adopted for use in Florida. With some contingent modifications, a clone is acceptable by this standard if no more than 30% of inoculated plants are infected. In Test 2, wounding changed the status of one-third of the clones from "acceptable" to "not acceptable" according to this standard. Most sugarcane breeding programs screen for resistance to several diseases and the combined loss of selections can seriously retard the rate of cultivar improvement. The impact of wounding on the frequency of acceptable clones is too great to be ignored in many breeding programs.

It is possible that physiological resistance is more stable under environmental influences than is mechanical resistance, but

TABLE 1. Analysis of variance of arcsine-transformed data for percent smut-infected sugarcane plants

Source	Test 1 df	Test 1 Mean square	Test 2 df	Test 2 Mean square
Clones (C)	4	7,842**[a]	20	10,198**
Wound states (WS)	1	1,390*	1	56,140**
Methods (M)	3	618	1	8,150**
C × WS	4	748*	20	1,616**
C × M	12	235	20	728
WS × M	3	357	1	0
C × WS × M	12	287	20	690
Error	78	282	696	758

[a] *Indicates $P<0.05$; ** indicates $P<0.01$.

TABLE 2. Percentage of smut infected plants resulting from spray and dip inoculation of wounded and unwounded sugarcane buds (Test 1)

Clone	Wounded Spray (%)	Wounded Dip (%)	Wounded $\bar{x}$ (%)	Unwounded Spray (%)	Unwounded Dip (%)	Unwounded $\bar{x}$ (%)	Minimum latent period[z] (days)
CP 57-603	46	40	43.0 a[y]	36	50	43.0 a[y]	69
CP 65-357	33	33	33.0 a	7	0	3.5 b	82
CP 68-1067	13	6	10.5 b	11	0	5.5 b	115
CP 70-1133	3	0	1.5 b	0	0	0.0 b	112
CP 63-588	0	0	0.0 b	0	0	0.0 b	···
$\bar{x}$	19.0	15.8		10.8	10.0		

[y] Means in the same column followed by the same letter are not significantly different according to Duncan's new multiple range test ($P<0.05$).
[z] Minimum latent period is significantly ($P<0.01$) correlated with mean percent infection ($r=-0.97$) for wounded, but not for unwounded buds.

TABLE 3. Percentage of smut-infected plants resulting from spray and dip inoculation of wounded and unwounded sugarcane buds (Test 2)

Clone	Wounded Spray (%)	Wounded Dip (%)	Wounded $\bar{x}$[y] (%)	Unwounded Spray (%)	Unwounded Dip (%)	Unwounded $\bar{x}$[y] (%)	Minimum latent period[z] (days)
CP 75-1091	80	80	80.0 a	68	57	62.5 a	61
CP 75-1693	83	67	75.0 a	50	67	58.5 ab	61
CP 75-1257	68	39	53.5 bc	19	0	9.5 ef	61
CP 65-357	50	54	52.0 bc	35	20	27.5 cde	63
CP 72-1312	62	35	48.5 bc	0	0	0.0 f	63
CP 70-1133	11	26	18.5 def	12	4	8.0 ef	63
CP 73-1547	0	17	8.5 ef	0	8	4.0 f	63
CP 56-59	13	19	16.0 ef	10	0	5.0 f	64
CP 75-1632	59	44	51.5 b	11	0	5.5 f	67
CP 75-1553	50	43	46.5 bc	38	42	40.0 abc	67
CP 72-1210	41	50	45.5 bc	25	12	18.5 def	68
CP 68-1026	35	30	32.5 cde	0	0	0.0 f	68
CP 57-603	75	58	66.5 a	63	12	37.5 bcd	70
CP 72-2083	50	53	51.5 bc	20	15	17.5 def	70
CP 70-1527	46	28	37.0 bcd	12	20	16.0 def	78
CP 68-1067	52	32	42.0 bc	40	38	39.0 abc	82
CP 74-2005	7	13	10.0 ef	0	9	4.5 f	83
CP 75-1353	8	4	6.0 f	0	4	2.0 f	91
CP 74-2013	36	4	20.0 def	8	7	7.5 ef	97
CP 75-1082	12	16	14.0 ef	4	0	2.0 f	106
CP 72-2086	30	20	10.0 ef	16	0	8.0 ef	134
$\bar{x}$	43.2	34.6		20.5	11.6		

[y] Means in the same column followed by the same letter are not significantly different according to Duncan's new multiple range test ($P<0.05$).
[z] Minimum latent period is significantly ($P<0.01$) correlated with mean percent infection ($r=-0.56$).

experimental demonstration of this apparently has not been conclusive. Physiological resistance is probably influenced by effects of environment, and theoretically is more likely to be lost through genetic change in the pathogen. For these reasons, dip inoculation of unwounded buds will remain the standard screening procedure in the Canal Point breeding program, at least until new evidence becomes available.

Since invasion of wounded and unwounded buds require the fungus to overcome different defenses, there is a chance that the variance associated with the percentage of plants infected could be different. Intuition suggests that circumventing one defense and allowing direct interaction of host and parasite physiologies will reduce the variability of the outcome. A reduction in variance due to wound inoculation could reduce the well-known excessive variability of data from resistance trials and thus could increase precision. However, analysis revealed no difference in variance in the data from wounded and unwounded buds.

In Test 2, high-pressure spray significantly increased infection. It is difficult to understand how it did so unless it breached the mechanical infection barrier to some extent. In that case, it should have led to a significant interaction of clones and methods of applying inoculum. Actually there was no evidence for that interaction (Table 1). Furthermore, if wounding and high-pressure spray tended to breach the same barrier, then high-pressure spray should have increased infection less in wounded buds than it did in unwounded buds and led to a significant interaction between wound states and methods of applying inoculum. In fact, the increase in infection due to high-pressure spray was almost exactly the same on wounded and unwounded buds in Test 2 and was slightly higher on wounded buds in Test 1 (Tables 1 and 2), and, of course, there was no interaction of wound states and methods.These results apparently are consistent with those of Toffano (9) who reported that vacuum inoculation increases infection without affecting the relative resistance ranking of clones. To explain this and the lack of an interaction between clones and methods or wound states and methods in the work reported here, it is necessary to assume that high-pressure spray or vacuum increases infection via routes already available under natural conditions, whereas wounding circumvents a different barrier. A three-barrier model is needed. The required additional barrier cannot be circumvented by forcing inoculum under bud-scales, but it can be by wounding. A recent report (6) suggests the possibility that bud scales of some clones contain a chemical inhibitor of smut spore germination. This would be consistent with a three-barrier model. The following hypothesis is proposed for whatever value it may have in suggesting further research. The first obstacle to infection is the bud-scale barrier proposed by Waller (11), which acts to reduce the amount of inoculum that reaches the susceptible sites inside the buds. Methods used thus far to increase the intrusion of inoculum under bud scales (vacuum and high pressure-spray) do not change the rank of inoculated clones because they increase infection in proportion to the amount of inoculum that naturally reaches these susceptible sites. The second obstacle is the chemical inhibitor (6), or something like it, that is separate from the bud-scale barrier, and can be circumvented by wounding. The final barrier is physiological.

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