Resistance of St. Augustine Grass to Infection by Sugarcane Mosaic Virus

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ABSTRACT

Only 10% of 176 plants of St. Augustine grass inoculated manually with sugarcane mosaic virus (SCMV) became infected, whereas 91% of 114 similarly inoculated corn seedlings became infected. Removal of epicuticular wax deposits with *n*-butanol prior to inoculations did not materially increase manual transmission rates of SCMV to St. Augustine grass. Low rates of aphid transmission of SCMV to St. Augustine grass were also noted, regardless of the aphid species tested or the virus source plant used. Significant differences in transmission rates were found, however, between two different SCMV isolates from St. Augustine grass. The generally low aphid transmission rates obtained could not be explained by the failure of aphids to probe on leaves of St. Augustine grass. Recorded probe durations were conducive to stylet-borne virus transmission, and aphid stylets were found to be inserted into St. Augustine grass leaf tissue.

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St. Augustine grass, *Stenotaphrum secundatum* (Walt.) Kuntze, a perennial lawn grass of major significance in Florida, is susceptible to sugarcane mosaic virus (SCMV), a common aphid-borne virus first reported from Florida in 1919 (2). In St. Augustine grass, SCMV induces pronounced mosaic symptoms which persist as long as infected plants are propagated. Since none of the four most important cultivars of this grass (Common, Roselawn, Bitter Blue, and Floratine) is homozygous and thus must be propogated exclusively by vegetative means, elimination of virus through seed production is precluded. The practice of cultivating St. Augustine grass near adjoining large acreages of sweet corn and sugarcane before distribution throughout the southeast as lawn sod seemingly would provide an effective means of disseminating any virus transferred to the St. Augustine grass.

These circumstances would cause one to anticipate widespread SCMV infection in St. Augustine grass plantings in Florida. Although this virus has been recovered from naturally infected plants on several occasions (5, 6, 11, 13), infected plants are not common, and the incidence of SCMV in commercial and private plantings of this grass is much less than 1% (T. E. Freeman, T. A. Zitter, J. L. Dean, *personal communications*). Furthermore, the records of the University of Florida Extension Service and of the Florida Department of Agriculture & Consumer Services at Gainesville, which date from 1959 and 1963, respectively, disclosed only two instances of virus or viruslike disorders of St. Augustine grass in Florida.

This study was designed to determine the relative susceptibility of St. Augustine grass to SCMV through inoculations by aphids and by various manual techniques. The results derived therefrom, hopefully, would divulge any resistance mechanisms operative in St. Augustine grass that would account for the unexpectedly low incidence of virus in that grass in Florida.

MATERIALS AND METHODS.—An isolate of SCMV from St. Augustine grass in southern Florida was used throughout this study. It was identified as strain E, and has been previously described and designated as "TV" (12). In experiments involving aphids, isolates collected in south Florida from St. Augustine grass ("SS-1") and from sugarcane ("POJ") were used. All isolates were transferred to and maintained in seedlings of sweet corn, *Zea mays* var. *saccharata* (Sturtev) Bailey 'Golden Cross Bantam' (GCB corn).

Manual inoculations.—We made all manual inoculations by dusting test plants with 600-mesh Carborundum and abrading their leaves with a sterile cheesecloth pad previously dipped into inoculum. We prepared inoculum by triturating diseased GCB corn leaf tissue and diluting extracts 1:10 with (i) distilled water; (ii) 0.05 M KH_2PO_4-$NaHPO_4$, pH 7.0; (iii) 0.02 M H_3BO_3-NaOH, pH 8.2; or (iv) 0.5% Na_2SO_3. In one experiment, we rubbed the adaxial surfaces of leaf blades of St. Augustine grass and GCB corn either 5 or 10 times, constituting "normal" and "severe" rubbing. In another experiment, we inoculated abaxial leaf surfaces, using the normal rubbing technique. Each potted plant in this experiment was inverted and an absorbent cloth placed under the leaf blade to prevent any inoculum from contacting the upper blade surface. This technique prevented wounding of adaxial leaf surfaces during inoculations.

At least six test plants were inoculated in every experiment. As controls, we always inoculated GCB corn plants before and after each trial to assure inoculum viability.

We attempted to determine whether epicuticular wax deposits of St. Augustine grass could impede virus transmission rates. Since SCMV does not induce local lesions on St. Augustine grass, this study was accompanied by inoculations of *Cassia tora* L. and *C. occidentalis* L. with papaya mosaic virus; both species of *Cassia* are local lesion indicators of this virus and, like St. Augustine grass, have dense epicuticular wax deposits on adaxial leaf surfaces. The isolate of papaya mosaic virus used in this study was obtained from E. Hiebert, University of Florida. Leaf surfaces (5 X 5 mm) of GCB corn, St. Augustine grass (Floratine and Roselawn), *C. tora,* and *C. occidentalis* were mounted on specimen grids coated with a silver base paint, and were examined with a Cambridge Mark II-A Stereoscan electron microscope for epicuticular wax deposits. Leaf samples were either shadowed with 20 to 30 nm of gold or were examined without shadowing. Prior to inoculations, we removed the wax deposits of leaves of St. Augustine grass, *C. tora*, and *C. occidentalis* by dipping a cotton ball into 10% *n*-butanol, then gently rubbing the upper leaf surface 5 times. In all inoculations, leaves so treated were compared with nontreated leaves. The effectiveness of the wax removal procedure was readily assessed by comparison of the contact angles of water droplets applied to leaf surfaces with an eyedropper before and after the butanol treatment.

Aphid transmissions.—All aphids tested as vectors were reared on virus-free plants in an insectary at 19 to 22 C. The aphids used were (i) the black cowpea aphid (*Aphis craccivora* Koch) reared on broadbean (*Vicia faba* L. 'Longpod'); (ii) the green peach aphid (*Myzus persicae* [Sulzer]), reared on tobacco (*Nicotiana tabacum* L. 'Samsun NN'); (iii) the corn leaf aphid (*Rhopalosiphum maidis* [Fitch]) reared on GCB corn; and (iv) the greenbug (*Schizaphis graminum* [Rondani]), reared on barley (*Hordeum vulgare* L. 'Moore'). These four aphid species were chosen for these trials because specimens of each have been collected from St. Augustine grass plants in southern Florida. Furthermore, *S. graminum* and *R. maidis* reportedly colonize St. Augustine grass during their population peaks (W. G. Genung, *personal communication*); also, *M. persicae, R. maidis*, and *S. graminum* are reportedly vectors of SCMV (1, 3, 9).

Nonviruliferous aphids were starved 1 to 4 hr, and transferred singly to a virus source. Symptomatic leaves of either GCB corn or St. Augustine grass were used as virus source plants. Aphids were allowed access periods of 16 sec to 6 min. Following test feedings of at least 12 hr, aphids were killed with liquid formulations containing either malathion or nicotine sulfate. Either GCB corn or St. Augustine grass was used as test plants. In all aphid trials, noninoculated plants were intermixed among test plants as controls.

A test was designed to determine whether probing aphids actually inserted their stylets into the leaves. In this test, 25 aphids of each species were starved 1 hr, then placed on a leaf of Floratine St. Augustine grass. After access periods of 1 to 4 hr, they were immediately frozen by insertion of the leaf and probing aphids into a beaker of acetone maintained at less than 0 C by direction of a stream of Cryokwik vapor through the acetone. The frozen aphids were then examined with a microscope, and the extended stylets measured. As a control, 25 individuals of each species, starved but not permitted access feedings, were frozen and examined for stylet extension.

In this study, all significant differences between means were identified by the analysis of variance test.

RESULTS.—*Manual inoculations.*—This study shows St. Augustine grass to be much more resistant then corn to manual inoculations of the TV isolate of SCMV regardless of (i) technique employed; (ii) diluent used; (iii) inoculum source selected; or (iv) leaf surface rubbed (Table 1). Of a total of 114 GCB corn plants inoculated, 91% became infected as compared to only 6, 8, 8, and 23% of 35, 53, 53, and 35 inoculated plants of Bitter Blue, Floratine, Roselawn, and Common St. Augustine grass, respectively. In every instance when corn was inoculated, transmission was 63% or higher. Transmission was 25% or lower in all trials except one with St. Augustine grass. In this instance, 33% transmission was obtained when leaves of 12 St. Augustine grass plants were rubbed

TABLE 1. Comparison of Golden Cross Bantam (GCB) sweet corn and cultivars of St. Augustine grass as suscepts of the TV isolate of sugarcane mosaic virus strain E by manual inoculation techniques

Techniques	Inoculum source	Diluent[a]	Leaf surface	GCB corn	Test plants St. Augustine grass cultivars: Roselawn	Floratine	Common	Bitter Blue
Normal rub[c]	St. Augustine grass	Water	Adaxial	17/18[b]	0/6	1/6		
	Corn	Water	Adaxial	37/38	1/17	1/17	5/20	1/20
	Corn	Water	Abaxial	8/12	0/6	0/6	3/15	1/15
	Corn	Phosphate	Adaxial	13/13	0/6	0/6		
	Corn	Borate	Adaxial	7/11	0/6	0/6		
	Corn	Sulfite	Adaxial	10/10	0/6	1/6		
Severe rub[c]	Corn	Water	Adaxial	12/12	3/6	1/6		

[a] All dilutions were 1:10 of extracted juice to diluent.
[b] Ratio is number of plants infected to number inoculated.
[c] Normal rub consists of 5 rubs/leaf; severe rub consists of 10 rubs/leaf.

10 times; only 6% of 34 plants became infected when rubbed only 5 times. Despite the relative resistance of St. Augustine grass to infection by manual inoculation, plants once infected always exhibited prominent and persistent mosaic symptoms, and virus was always readily recovered by back assay to GCB corn.

Common St. Augustine grass appeared to be more susceptible to SCMV than the other cultivars tested. Under identical conditions of inoculation, only 5% of 17, 17, and 20 plants of Roselawn, Floratine, and Bitter Blue St. Augustine grass, respectively, became infected, in contrast to 25% of 35 Common St. Augustine grass plants infected (Table 1).

Scanning electron micrographs of adaxial and abaxial leaf surfaces of GCB corn and Floratine and Roselawn St. Augustine grass revealed (i) deposits of wax on both surfaces of the corn leaves as described by Davis (4); (ii) pronounced, waxy, scalelike deposits on the adaxial surfaces of both St. Augustine grass cultivars (Fig. 1-A); and (iii) absence of any apparent wax deposits on the abaxial surfaces of St. Augustine grass (Fig. 1-B). Contact angle measurements verified these observations for St. Augustine grass: contact angles of ca. 135° were measured for adaxial leaf surfaces, and ca. 50° contact angles were recorded for abaxial leaf surfaces (Fig. 1-C). Contact angles of less than 90° indicate that wax is not a prominent feature of a cuticle surface, whereas contact angles of more than 90° indicate wax to be a significant feature (8).

Removal of wax deposits from adaxial leaf surfaces of St. Augustine grass, however, did not markedly increase virus transmission rates. Decreases in contact angle measurements indicated that wax was removed following butanol treatments. In three trials (10 plants each trial), a total of 7 of 30 plants inoculated with the TV isolate of SCMV on nontreated leaves became infected, whereas 11 of 30 *n*-butanol treated leaves became infected. These differences were not significant at the 5% level. Moreover, manual inoculation of the abaxial leaf surfaces of St. Augustine grass plants did not significantly (at 5% level) increase their susceptibility in these same trials; only 4 of 30 plants inoculated with the TV isolate of SCMV on abaxial leaf surfaces became infected. In this trial, 11 of 12 GCB corn plants inoculated with the TV isolate of SCMV on nontreated leaves as controls became infected.

That epicuticular wax deposits of plant leaves apparently do not materially reduce manual transmission of virus was further indicated in studies utilizing the local-lesion/host characteristic of two species of *Cassia* and the papaya mosaic virus. The number of local lesions induced by papaya mosaic virus on 13 leaves of *C. occidentalis* that had been dewaxed was 843 in contrast to 688 lesions on leaves that had not been dewaxed with *n*-butanol. This difference was not significant at the 5% level. Similarly, the number of lesions, 470, induced by this virus on 12 dewaxed leaves on *C. tora* was not significantly greater at the 5% level than the number of lesions, 445, on nontreated leaves. No significant differences in lesion numbers were observed in dewaxed and treated inoculated leaflet halves of both species of *Cassia*. On 12 leaves of *C. tora* (each leaf with 6 leaflets), the number of lesions on dewaxed leaflet halves was 242 and on nontreated halves, 207; the difference was not significant at the 5% level. Similarly, on 12 leaves of *C. occidentalis* (each leaf with 4 leaflets), 320 lesions were counted on dewaxed leaflet halves and 286, on nontreated halves; the difference was likewise not significant at the 5% level.

Aphid transmission.—Relatively low aphid transmission rates of SCMV-E were noted regardless of (i) virus source plant; (ii) test plant; (iii) virus isolate; or (iv) aphid species used.

Plants of corn and Roselawn St. Augustine grass infected with the TV isolate of SCMV were compared as virus sources for individuals of *S. graminum*. The relative susceptibility of corn and St. Augustine grass to inoculations of the TV isolate by *S. graminum* aphids was also determined in this trial. Transmission was low, regardless of test plant or virus source plant used. Transmission by single aphids of the TV isolate from corn to corn and from corn to St. Augustine grass was 2 and 0 of 40 plants inoculated, respectively. Transmission by single aphids of the TV isolate from St. Augustine grass to corn, and from St. Augustine grass to St. Augustine grass, was 1 and 0 of 40 plants inoculated, respectively.

Transmission of the TV isolate from corn to corn by individuals of *A. craccivora, M. persicae, R. maidis*, and *S. graminum* was 0, 0, 0, and 2 of 50 plants inoculated, respectively. Similarly, only 4 of 50 individuals of *A. craccivora* allowed single acquisition probes transmitted the POJ isolate of SCMV-E from corn to corn.

When the TV isolate was directly compared with the SS-1 isolate to determine the variation in transmission rates between SCMV-E isolates, transmissibility rates were low, but the SS-1 isolate was more readily transmitted than the TV isolate from corn to corn. Only 1 of 120 individuals of *S. graminum* transmitted the TV isolate; 22 of 120 aphids transmitted the SS-1 isolate. Transmission rates of the SS-1 isolate were significantly higher than that of the TV isolate. In a similar test with these two isolates, 9 of 60 individuals of *R. maidis* transmitted the SS-1 isolates, and only 1 of 60 transmitted the TV isolate.

Because of these low transmission rates, an effort was made to determine whether aphids, in fact (i) probe in St. Augustine grass or GCB corn leaves of durations conducive to the transmission of stylet-borne viruses; and (ii) whether they inserted their stylets into the leaves of these plants.

Individuals of all four aphid species used in this study made probes on St. Augustine grass and corn of at least 16 sec, a time conducive to the transmission of stylet-borne viruses. Of 109, 63, 39, and 54 probes made on Roselawn St. Augustine grass by 25 individuals of *M. persicae, A. craccivora, R. maidis*, and *S. graminum*, 16, 42, 80, and 84%, respectively, were 16 sec or longer. Similarly, of 86, 61, 36, and 56 probes made on Floratine St. Augustine grass by 25 individuals of *M. persicae, A. craccivora, R. maidis*, and *S. graminum*, 36, 76, 84, and 95%, respectively, were 16 sec or longer. Similar results were obtained for aphids probing corn. Of 63, 46, 39, and 34 probes made on the corn by 25 individuals of *M. persicae, A. craccivora, R. maidis*, and *S. graminum*, 50, 76, 84, and 74%, respectively, of the probes were 16 sec or longer.

The result of a final trial showed that individuals of all four aphid species tested actually penetrated the leaves of Floratine St. Augustine grass with their stylets: 96, 96, 96, and 88% of 25 individuals of *A. craccivora, M. persicae, R. maidis*, and *S. graminum*, respectively, had 20 μ of their stylets exposed beyond the distal portions of their labia. Measurements made of paraffin-embedded sections of Floratine St. Augustine grass leaves revealed an epidermis of ca. 15 μ in thickness for adaxial and abaxial leaf surfaces. These data suggest that the aphids penetrated the epidermis of St. Augustine grass. None of 25 individuals of each aphid species which were fasted, but not permitted to probe leaves, had their stylets exposed beyond the distal portions of their labia.

DISCUSSION.—Low aphid transmission rates of SCMV-E to St. Augustine grass were obtained regardless of (i) virus source plant; (ii) test plant; (iii) SCMV-E isolate; or (iv) aphid species used. Such low rates are not surprising in view of the low incidence of this virus strain in St. Augustine grass plantings in

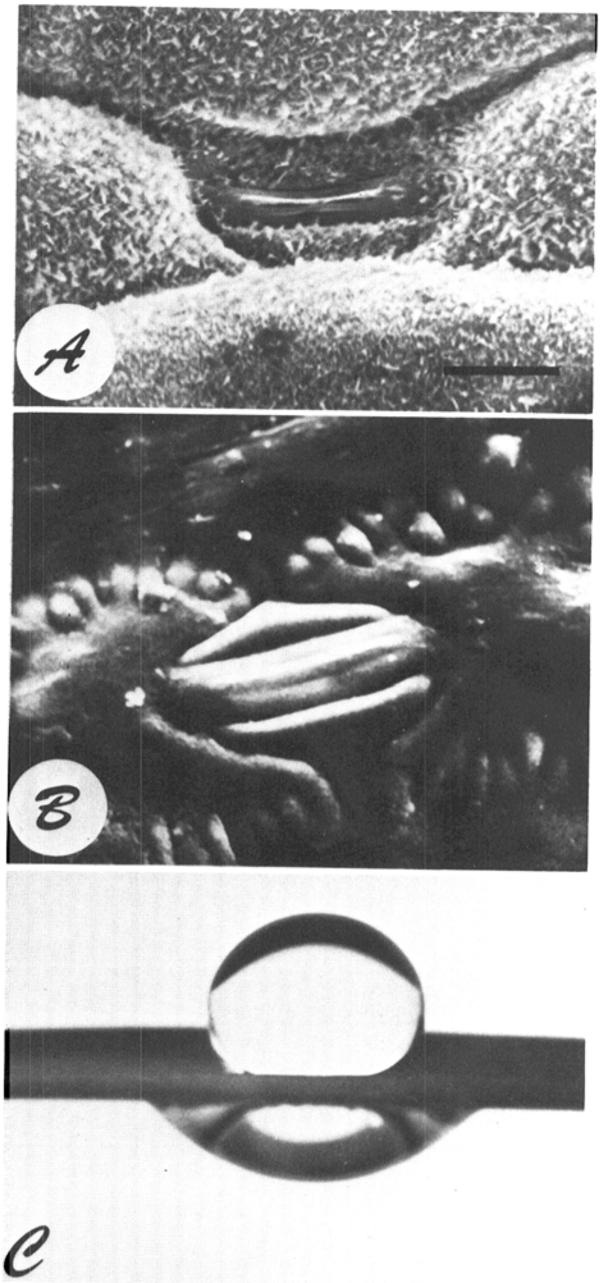

Fig. 1. A, B) Scanning electron micrographs of adaxial and abaxial leaf surfaces, respectively, of Roselawn St. Augustine grass showing wax deposits on adaxial surface (A) and absence of wax on abaxial surface (B). Scale line is 10.0 μ. **C)** Water droplets on adaxial and abaxial leaf surfaces of Roselawn St. Augustine grass. Adaxial surfaces had contact angles of $\geqslant 135°$, whereas abaxial surfaces were $\leqslant 50°$

Florida. Yet, as shown in this study, aphids probed St. Augustine grass leaves for durations conducive to virus transmission and pierced the epidermis with their stylets.

Freeman (7) reported that the bitter blue types of St. Augustine grass appear to exhibit a degree of resistance to infection by SCMV. In this study, Bitter Blue and other cultivars of St. Augustine grass were found to be susceptible to SCMV-E. However, transmission rates of SCMV-E to St. Augustine grass clones by manual means were mcuh lower than those to corn tested as comparisons in these same trials.

No significant differences in susceptibility to manual inoculations were noted among St. Augustine grass plants inoculated on (i) dewaxed adaxial leaf surfaces prior to mechanical inoculation; (ii) adaxial leaf surfaces without prior removal of wax deposits; and (iii) abaxial leaf surfaces. Moreover, the number of local lesions obtained on either *C. tora* or *C. occidentalis* leaves which had been dewaxed, then manually inoculated with papaya mosaic virus, were not significantly different than the number of local lesions obtained on untreated leaves of either species. Thus, it appears that wax is not the factor responsible for the observed resistance of St. Augustine grass to manual inoculations of SCMV. It is possible that increased abrasion during manual inoculation would improve transmission rates, as was discovered by Louie & Lorbeer (10) in their attempts to obtain dependable manual transmission of onion yellow dwarf virus, a potyvirus, to onions. They found that to obtain consistent transmissions, the basal portions of onion leaves must be rubbed firmly 10 times. They also state that the hard, intact epidermis that many monocotyledonous plants possess may act as a barrier to manual methods without a severe abrasive technique. This may be the case with St. Augustine grass, as this study indicates.

The relationship of manual inoculations of SCMV-E to inoculations by aphids is not clear; it has been assumed that each technique involves different infection sites. Manual inoculation presumably involves the periclinal walls of epidermal cells, whereas aphids presumably introduce virus into or near the anticlinal walls of epidermal cells. Thus, it is considered unlikely that the low transmission rates of SCMV by aphids noted in this study are related to the observed low rates of manual transmissions to St. Augustine grass. Transmission by aphids was low regardless of whether plants of corn or St. Augustine grass were used as test plants, whereas manual transmission of SCMV to corn was always high.

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