Differential Transmission of Mississippi and Ohio Corn Stunt Agents by Graminella nigrifrons

M. M. Choudhury and E. Rosenkranz

Graduate Research Assistant, Department of Plant Pathology and Weed Science, Mississippi State University, State College 39762; and Research Plant Pathologist, Plant Science Research Division, ARS, USDA, and Adjunct Associate Professor, Department of Plant Pathology and Weed Science, Mississippi State University, State College.

Cooperative investigations, Plant Science Research Division, ARS, USDA, and Mississippi Agricultural and Forestry Experiment Station; published as Journal Series Paper No. 2324 of the Mississippi Agricultural and Forestry Experiment Station.

Accepted for publication 1 August 1972.

ABSTRACT

Graminella nigrifrons was established as a vector of the Mississippi corn stunt agent (CSA-MS), in addition to being a vector of the Ohio corn stunt agent (CSA-OH). This leafhopper recovered CSA-MS from corn plants inoculated with CSA-MS by the more efficient vector, *Dalbulus maidis*. Adults of *G. nigrifrons* also acquired CSA-MS from corn plants naturally infected in the field in Mississippi. The shortest latent period of CSA-MS in *G. nigrifrons* was 15-18 days, compared to 12 days for the same agent in *D. maidis*. The minimum incubation periods of CSA-OH and CSA-MS in corn, infected by *G. nigrifrons*, were 11 and 15 days, respectively. The efficiency of transmission of CSA-MS by *G. nigrifrons* was 3-4%; that of CSA-OH, 34%. Beyond 24 hr, transmission efficiency of CSA-MS by this vector was independent of the length of acquisition access or the duration of transmission feeding. Female leafhoppers appeared to transmit CSA-OH more efficiently than did their male counterparts. The occurrence of CSA-OH in field corn in Mississippi was proved for the first time. When noninfective *G. nigrifrons* were exposed to field-diseased corn plants with typical corn stunt symptoms, some of the leafhoppers acquired CSA-OH, others CSA-MS from the same plants. While attempting to recover the transmitted corn stunt agents from test plants, inoculated by leafhoppers that had acquired the agents from field-diseased corn, a few *G. nigrifrons* transmitted both CSA-MS and CSA-OH from test plants that showed symptons of only Mississippi corn stunt. In serial transmission experiments, when a colony of *G. nigrifrons* contained some transmitters of CSA-OH and some of CSA-MS, such a colony would invariably transmit CSA-OH in the first 1 or 2 transfers, then CSA-MS to the subsequent test plants in the series. A few individual leafhoppers were able to transmit both CSA-OH and CSA-MS to the same test plant. Doubly infected test plants always developed symptoms first of CS-OH and later of CS-MS. When noninfective *G. nigrifrons* were exposed to such doubly diseased plants and then divided into subcolonies, some of these subcolonies transmitted CSA-OH, fewer transmitted CSA-MS, and an intermediate number transmitted both pathogens.

Phytopathology 63:127-133

Additional key words: maize, mycoplasmalike organism, leafhopper transmission.

In Mississippi, corn stunt (CS) was first observed on one farm in Yazoo County, probably as early as 1961 (W. White, *personal communication*). The following year, Maramorosch (10) confirmed the presence of corn stunt in the same county through transmission tests with the leafhopper vector *Dalbulus maidis* (De Long & Wolcott). In the ensuing years, the disease spread to all parts of the state and has continued to recur each year. It has become the most serious disease of corn (*Zea mays* L.) in Mississippi.

Several species of leafhoppers were reported as vectors of the causal agent of corn stunt, which until 1968 had been assumed to be a virus. Since then, substantial evidence has been obtained to presume that the corn stunt agent (CSA) is a mycoplasmalike organism. In 1946, Kunkel (9) disclosed the successful transmission of CSA with *D. maidis*. In 1949, Niederhauser & Cervantes (11) found that *Dalbulus elimatus* (Ball), in addition to *D. maidis*, was a vector of CSA in Mexico. However, *D. elimatus* has never been collected in the southeastern USA, where CS has had its widest distribution, and *D. maidis* has appeared too late in the season to explain the early CSA infection in corn (3). Therefore, a search for other possible leafhopper vectors was initiated at State College in 1964. In May 1965, Rosenkranz et al. (16) obtained the first indication that the indigenous leafhopper *Graminella nigrifrons* (Forbes) was probably an inefficient vector of CSA. These findings were presented at the Third Corn Virus Conference, held at Purdue University, 15-16 November 1965. In April 1966, Granados et al. (6) corroborated these preliminary results by reporting the transmission of a CSA isolate from Louisiana with *G. nigrifrons*. Since 1966, the junior author has occasionally obtained an experimental transmission of a corn-stunting agent with *G. nigrifrons*, but the resulting symptoms in test plants were not typical of CS that was being induced by infective *D. maidis*.

In a comparative study of CSA transmission by *D. maidis, D. elimatus, and G. nigrifrons*, Granados et al. (4) found that the last species was an inefficient vector of the Louisiana isolate of CSA when compared with the other two leafhopper vectors. Their data from various experiments showed that between 10.1 and 18.2% of the tested populations of *G. nigrifrons* transmitted this CSA isolate. Having tested field populations of *G. nigrifrons*, collected in stunt-diseased corn, for their natural infectivity of CSA, Boyd & Pitre (1) concluded that this leafhopper was a very inefficient vector of CSA. In their study, only a

small fraction of 1% of the tested leafhoppers was naturally infective for CSA. In 1968, another leafhopper, *Deltocephalus sonorus* (Ball), was described as a vector of CSA (5). More recently, Granados & Whitcomb (8) reported the transmission of corn stunt mycoplasma [CSA isolate from Louisiana] by a newly described leafhopper species, *Baldulus tripsaci* (Kramer & Whitcomb).

In the meantime, Rosenkranz (12, 13) discovered a new leafhopper-transmissible CSA in Ohio, and reported on the occurrence of two distinct types of corn stunt in the USA. His studies revealed that the Ohio corn stunt agent (CSA-OH) was readily transmitted by *G. nigrifrons* but not by *D. maidis*, whereas the Mississippi corn stunt agent (CSA-MS) was transmitted very efficiently by *D. maidis* but not by *G. nigrifrons*. Recently, a CSA closely resembling CSA-OH in its biology of transmission, was isolated from sorghum [*Sorghum bicolor* (L.) Moench] in Mississippi (14). *G. nigrifrons* was moderately efficient in the transmission of this CSA-isolate for which *D. maidis*, however, was not a vector. The host range of CSA was further extended with the transmission of CSA-MS by both *G. nigrifrons* and *D. maidis* from field-diseased Johnson grass [*Sorghum halepense* (L.) Pers.] to corn (15).

In view of the growing complexity of the etiology and epidemiology of corn-stunting diseases in the USA, a need was felt to further clarify the relationship of *G. nigrifrons* to corn stunt. The principal aims of this study were to determine whether *G. nigrifrons* was indeed a vector of CSA-MS and, if so, its efficiency in the transmission process. Other biological properties of the agent-vector relationship needed investigation, the results of which are also reported here. A preliminary report on this study has been published (2).

MATERIALS AND METHODS.—The leafhoppers used in this study derived from several hundred adult *G. nigrifrons* which had been collected on corn and indigenous grasses in the vicinity of State College, Miss., in 1968. Each collected specimen was checked under a stereomicroscope as to its identity. Subsequently, noninfective stock colonies were established from isolated eggs and maintained on sweet corn, barley, and rice. Periodic checks were made to ascertain the noninfective state of these stock colonies. Adults of *D. maidis* were also acquired locally, and noninfective stock colonies established in a manner similar to those of *G. nigrifrons*.

Sweet corn, cultivar Seneca Chief, was used as the test plant in all experiments. The corn plants were planted singly in peat pots (8 cm in diam) and, after treatment, were transplanted into clay pots (16 cm in diam). The substrate consisted of a 3:1 soil-sand mixture.

Acquisition of the disease agents was performed either by a random "spot feeding" over the total foliar area with the aid of a magnetic cage or by confinement of the entire source plant to an insect cage. Serial transfers of the vectors were made by insertion of a cylindrical cage over a corn seedling into the soil of the peat pot and introduction of the insects through an opening in the cage.

The source plants were inoculated with either CSA-MS or CSA-OH by *D. maidis* or *G. nigrifrons*, respectively, several weeks prior to their use, and exhibited pronounced symptoms of either the Mississippi corn stunt (CS-MS) or the Ohio corn stunt (CS-OH). Symptoms of CS-MS and CS-OH can be readily distinguished in experimentally infected test plants. CSA-MS causes plants to be disproportionally stunted, with upper internodes and upper leaves much shorter than the lower ones, whereas CSA-OH produces plants that are proportionally reduced in size resulting in miniature plants. Proliferation in the form of shoots in the leaf axils and tillers occurs with infection by CSA-MS; proliferation is absent in CSA-OH infection. Uneven growth and expansion of upper leaves leads to tearing of tissue along chlorotic leaf margins in test plants diseased with CS-MS; no splitting of leaf margins occurs with CS-OH. Infection by CSA-OH produces necrosis at the base of the stalk, leading to constriction and premature death of the plant. Such basal stalk necrosis has not been noted in the case of CSA-MS infection. Leaves of plants diseased with CS-MS appear wide, thick, and rigid whereas leaves of plants diseased with CS-OH are narrow, thin, and flexible. High incidence of "tassel seed", absent in CS-MS, characterizes CS-OH in Seneca Chief sweet corn.

CSA-MS was originally isolated with noninfective *D. maidis* from CS-diseased corn plants grown in the field at State College, Miss., and has been cultured in sweet corn and *D. maidis*. CSA-OH was isolated in the course of this study with noninfective *G. nigrifrons* from CS-diseased field corn collected in the State College area. All naturally infected corn plants, serving as source plants, showed severe symptoms of CS and were collected also in the vicinity of State College, Miss. Only adult *G. nigrifrons* were used in acquisition access feedings that varied in duration from 1 to 14 days, as described in each experiment. Serial transfers of the surviving leafhoppers in each subcolony to fresh corn seedlings were made at intervals of from 2 to 7 days, depending on the test.

The experiments were conducted in the greenhouse with a daily temperature range of 20 to 38 C and 15 to 30 C during the warm and cold months, respectively. The relative humidity was close to 100% most of the time, approaching 70% only in the early afternoon. The test plants grew under natural light, but received an ample supply of nutrient elements for normal growth during the 10-12 weeks they were kept under observation.

RESULTS.—*Attempts to transmit CSA-MS with G. nigrifrons.*—In three initial trials, some adult *G. nigrifrons* were able to acquire CSA-MS from test plants that had been inoculated several weeks earlier with CSA-MS by *D. maidis* and were exhibiting pronounced symptoms of CSA-MS. After a 48-hr acquisition access and a latent period of 16-24 days, CS-MS was transmitted by four out of eight insect colonies in trial 1, one out of eight colonies in trial 2, and one out of seven colonies in trial 3. Transmission

occurred in two to six consecutive transfers among the individual transmitting colonies. At the start of acquisition, colonies consisted of six leafhoppers in trial 1 and of five leafhoppers each in trials 2 and 3. From the transmission records, it was possible to deduce that in trial 2 only one of the 23 (4.3%) leafhoppers that had survived the acquisition period became a transmitter. In trial 3, we derived a transmission efficiency of 3.1%, or one transmitter of 32 surviving leafhoppers tested. Since the incidence of transmission in trial 1 was of such a magnitude that it has not been achieved since, the possibility of some contamination cannot be ruled out. When the acquistion access period was extended to 72 hr, no increase in the efficiency of transmission was obtained. In one representative experiment of this type, only one out of 12 colonies, each containing initially 10 leafhoppers, transmitted CSA-MS. It was estimated that in this experiment, a maximum of 3.2% of the leafhoppers became transmitters.

Effect of length of acquisition access on the efficiency of CSA-MS transmission.—Results from tests in which the acquisition feeding period was systematically increased by 24 hr indicated that exposure of *G. nigrifrons* to source plants of CSA-MS for extended periods had no effect on their efficiency of CSA-MS transmission. When three colonies of 10 leafhoppers each were allowed to feed on severely stunt-diseased plants for 1, 2, 3, 4, and 5 days, only one out of the three colonies from each of the five acquisition access periods transmitted CSA-MS. In another experiment, 180 adult leafhoppers spent 14 days on CS-MS-diseased plants; then 120 of them were transferred, in groups of 10 each, to fresh test plants every 7 days. Since four of the 12 colonies transmitted CSA-MS, the minimum transmission efficiency was four/120 or 3.3%. Twenty-eight days after the end of the acquisition access, the last of the four transmitting colonies ceased to transmit CSA-MS. By that time, all insects in two of the four colonies were dead, and there was one survivor in each of the other two colonies. Seven days later (i.e., 35 days after start of serial transmission feeding), the two surviving leafhoppers were placed together on a single corn seedling. By that time, one of the leafhoppers had not transmitted CSA-MS during two 7-day intervals; and the other one had not transmitted during three 7-day intervals. Subsequently, the two leafhoppers again transmitted consecutively CSA-MS in four weekly transfers, which is the time they remained alive. This phenomenon could be explained by the assumption that a test plant needs to be inoculated with a minimum dose of the disease agent in order to develop the disease. One "infective" leafhopper was unable to provide this dosage, but two such leafhoppers could supply the minimum dose required for CSA-MS infection.

Latent period of CSA-MS in G. nigrifrons.—Rosenkranz (12) reported that the minimum latent period of CSA-MS in *D. maidis* was 12 days, and found this period to be 5 days for CSA-OH in *G. nigrifrons*. Working with an isolate of CSA from Louisiana, Granados et al. (4) obtained a minimum incubation period in *G. nigrifrons* that ranged between 22 and 26 days. In the present work, the shortest latent period for CSA-MS in *G. nigrifrons* was 15-18 days.

Attempts to isolate CSA-MS with G. nigrifrons from corn plants naturally infected in the field.—CSA-MS had been isolated from naturally infected corn plants, collected in Mississippi and in other states, many times before, but the vector used had been usually *D. maidis*. Two corn plants, nearing maturity and exhibiting distinct symptoms of CS, were brought from a field at State College, Miss., to the greenhouse. On plant 1 and plant 2, 60 and 50 adult *G. nigrifrons*, respectively, were placed for an acquisition access lasting 7 days. The leafhoppers were divided into groups of 12 insects, and each group was confined to one magnetic cage. Each cage was attached to a different leaf on the two source plants. The identity of each of the five and four colonies on plants 1 and 2, respectively, was maintained through seven serial transfers made at weekly intervals. The results showed (Table 1) that of the five colonies from plant 1, three transmitted CSA-OH and one transmitted CSA-MS; and of the four colonies, having fed on plant 2, two transmitted CSA-OH and none transmitted CSA-MS. In another similar trial, involving a CS-diseased corn plant that was collected in a nearby field about 4 weeks later, out of five colonies of 12 leafhoppers each, only one colony transmitted CSA-OH and none transmitted CSA-MS. Transmission, whether of CSA-OH or of CSA-MS, occurred through at least three consecutive transfers. This experiment proved that *G. nigrifrons* was able to acquire CSA-MS from a field-diseased plant, but that its transmitting ability for this CSA was very low. The results of these tests also revealed, for the first time, the presence of CSA-OH in CS-diseased field corn in Mississippi. Earlier in the same season, the junior author had isolated CSA-OH from sorghum grown in the same general area (14).

Recovery of CSA-OH with G. nigrifrons from experimentally infected test plants.—Attempts were made to recover CSA-OH previously transmitted by *G. nigrifrons* from field-diseased corn plants. The source plants consisted of five test plants that had been inoculated with CSA-OH by *G. nigrifrons* several weeks earlier and were showing typical symptoms of CS-OH. In trial 1, 50 adult leafhoppers fed on each of two test plants for 3 days; in trial 2, 50 adult leafhoppers were confined on each of three test plants for 6 days. The survivors from the acquisition access feeding were divided in groups of 10 so that there were six and 10 colonies in trials 1 and 2, respectively. Transfers were made at 7-day intervals. In trial 1, four of the six colonies transmitted CSA-OH. Of the 10 colonies in trial 2, two transmitted CSA-OH in three consecutive transfers, three transmitted CSA-MS in three to five consecutive transfers, and another three colonies transmitted consecutively first CSA-OH and in later transfers, only CSA-MS. The manner in which the last three colonies transmitted the two disease agents confirms our former finding that CSA-OH has a shorter latent

TABLE 1. Attempted transmission of the corn stunt agent with *Graminella nigrifrons* from corn plants naturally infected in the field at State College, Miss.

	Colony no. [a]	Transmission during stated interval (days)					
		0-7[b]	8-14	15-21	22-28	29-35	36-42
Plant 1	1	OH(10)[c]	–(5)	–(5)	–(3)	–(1)	(0)
	2	OH(10)	OH(9)	OH(9)	OH(4)	OH(2)	OH(2)
	3	–(10)	–(5)	–(4)	–(3)	–(2)	–(2)
	4	OH(10)	OH(7)	OH(4)	OH(2)	–(1)	(0)
	5	–(7)	–(3)	MS(2)	MS(2)	MS(2)	–(1)
Plant 2	1	OH(10)	OH(6)	OH(3)	–(1)	–(1)	(0)
	2	–(10)	–(8)	–(5)	–(1)	–(1)	–(1)
	3	–(11)	–(7)	–(6)	–(4)	–(2)	–(2)
	4	OH(12)	OH(8)	OH(5)	–(3)	–(2)	(0)

[a] At start of acquisition access, which lasted 7 days, each colony consisted of 12 adult leafhoppers.

[b] Day 0 denotes last day of acquisition access and first day of exposure to test plants in the first series. Colonies were serially transferred at weekly intervals, providing 7 days of inoculation feeding to each test plant.

[c] – = healthy plants; OH = plants diseased with Ohio corn stunt; MS = plants diseased with Mississippi corn stunt: number in parentheses indicates number of survived leafhoppers placed on test plants for inoculation feeding.

period than CSA-MS in *G. nigrifrons*. CSA-MS seems to suppress CSA-OH in doubly inoculated plants, since symptoms of only CS-MS were evident in these plants. Yet, we know from experience that once a leafhopper becomes infective with CSA-OH, it retains its infectivity, with rare exceptions, for the rest of its life. The fact that, in trial 2, some of the *G. nigrifrons* were able to recover CSA-MS from test plants that showed symptoms of only CS-OH, would indicate that at least one of the three plants used as source of CSA-OH may have become accidentally inoculated with CSA-MS later in its growth by an escaped infective *D. maidis*, after CSA-OH had thoroughly established itself in it. An alternative explanation for this apparent anomaly may be that the original field-diseased corn plant contained a low concentration of CSA-MS which was picked up by one or more *G. nigrifrons* and transmitted to one of the test plants, used in this experiment, in which it remained latent. In this experiment, the shortest incubation periods in corn of CSA-OH and CSA-MS were 11 and 15 days, respectively.

Efficiency of G. nigrofrons as a vector of CSA-OH and CSA-MS.—The efficiency of acquisition of CSA by *G. nigrifrons* and its possible relationship to sex of the vector was explored. In one experiment, 150 leafhoppers were allowed to feed for 7 days on a test plant infected with CSA-OH. Among the survivors of the acquisition access, there were only 33 females which were complemented by 67 males. The 100 leafhoppers were then individually assayed for infectivity during a 14-day inoculation access period. CSA-OH was transmitted by 20 males and 14 females (Table 2). On that basis, 30% of the male and 42% of the female leafhoppers became transmitters of CSA-OH, and the total transmittion efficiency of *G. nigrifrons* was 34%. This percentage of transmitters agrees closely with that obtained by Rosenkranz (*unpublished data*), who found that 35% of adult *G. nigrifrons* were able to transmit the disease agent of CS originally isolated from corn collected in Ohio.

In another experiment, 200 noninfective adults of *G. nigrifrons* were exposed, in a single cage, to 3 test test plants infected with CSA-MS. All three source

TABLE 2. Efficiency of transmission of Ohio corn stunt agent (CSA-OH) and Mississippi corn stunt agent (CSA-MS) by sexed *Graminella nigrifrons*

Corn stunt agent (CSA)[a]	No. insects tested/sex		No. insects/sex transmitting		% Transmission/ sex/CSA	Total % transmission/CSA
	♂	♀	CSA-OH	CSA-MS		
CSA-OH	67		20		30	34
		33	14		42	
CSA-MS	50			2	4	4
		50		2	4	

[a] Adult leafhoppers were exposed to source of CSA-OH for 7 days or of CSA-MS for 14 days, then differentiated as to sex and caged individually on test plant seedlings for 14 days.

plants exhibited identical symptoms, typical of CS-MS, each having been inoculated by eight *D. maidis* approximately 6 weeks earlier. At the end of a 14-day acquisition access, 50 males and 50 females were selected at random and tested singly for their infectivity. After 14 days of transmission feeding, two leafhoppers of each sex transmitted CSA-MS. Thus, out of 100 adult *G. nigrifrons*, only four became infective with CSA-MS (Table 2). In another similar experiment, two females and one male, out of 50 leafhoppers of each sex tested, acquired and transmitted CSA-MS. These percentages of CSA-MS transmitters represent about one-tenth of the transmission affinity this leafhopper species has for CSA-OH.

Isolation of CSA with G. nigrifrons from a doubly infected corn plant.—Three hundred and fifty adult leafhoppers from a noninfective stock culture of *G. nigrifrons* were caged with a corn plant exhibiting characteristic symptoms of CS-OH and CS-MS (Fig. 1). This source plant had been inoculated by a doubly infective *G. nigrifrons* 41 days earlier. After 24, 48, and 72 hr of acquisition access, 100, 100, and 120 leafhoppers, respectively, were removed from the caged source plant, and each group was placed separately on a healthy corn plant. Following a 14-day latent period, the survivors in each group were divided into subcolonies of 10 insects each. In this way, we obtained 7, 4, and 8 subcolonies for the 24-, 48-, and 72-hr exposure periods, respectively. Serial transfers were made every 7 days. The following transmission results were obtained for the three acquisition access periods, in increasing order of duration (Table 3): of the seven subcolonies, one transmitted CSA-OH and two transmitted both CSA-OH and CSA-MS; of the four subcolonies, three transmitted CSA-OH and one transmitted both agents; and of the eight subcolonies, three transmitted CSA-OH and two transmitted both agents.

Fig. 1. Sweet corn cultivar Seneca Chief, infected with both the Ohio corn stunt agent (CSA-OH) and the Mississippi corn stunt agent (CSA-MS) by *Graminella nigrifrons*, reduced in size to about one-third of a noninoculated plant. Narrow upper leaves are due to CSA-OH, splitting of margins on the same leaves is caused by CSA-MS.

Though *G. nigrifrons* recovered both corn stunt agents from the doubly infected corn plant, the low transmissibility of CSA-MS by this vector prevented the leafhopper colonies from transmitting it separately from CSA-OH. On the other hand, when noninfective adults of *D. maidis* were exposed to a mixed infection of CSA-OH and CSA-MS, they recovered only the latter agent. If we assume that only one leafhopper in each of the five subcolonies that transmitted CSA-MS became infective with this agent, then the minimum efficiency of CSA-MS transmission by this population of *G. nigrifrons* would have been 5/190, or 2.6% – a likely frequency. The results from this experiment also indicated that the frequency of transmission of either CSA by *G. nigrifrons* is not increased by prolonging the acquisition access beyond 48 hr.

DISCUSSION.—At the time *G. nigrifrons* was reported a vector of CSA (6), it was not known that two distinct types of CSA occurred in the USA (12). Granados et al. (7) proved that *G. nigrifrons* was a vector of two isolates of CSA, one from Louisiana and the other from Mexico. Both isolates were transmitted also by *D. maidis*. In 1968, Rosenkranz (13) isolated a new CSA which he named the Ohio corn stunt agent (CSA-OH). He found that *G. nigrifrons*, but not *D. maidis*, was a vector of CSA-OH. It remained to be determined what the relationship was between *G. nigrifrons* and the prevalent CSA in Mississippi (i.e., CSA-MS) that *D. maidis* transmitted with an efficiency approaching 100%. In the process of attempting to clarify this relationship, we came to realize that the etiology of CS was more complicated than was previously assumed. We now know that at least two disease agents are associated with CS in Mississippi, and probably in most other southern states. In 1969, we found CSA-OH in association with CSA-MS in CS-diseased corn plant plants that were grown at State College, Miss. The following year, we succeeded in isolating both CSA-OH and CSA-MS from the same CS-diseased corn plant that was collected at Clemson, S.C.

Our present study established *G. nigrifrons* as a vector of CSA-MS, capable of transmitting it with an

TABLE 3. Recovery of disease agents by *Graminella nigrifrons* from a corn test plant doubly infected with Ohio corn stunt agent (CSA-OH) and Mississippi corn stunt agent (CSA-MS) using three acquisition access periods

Serial intervals (days) of inoculation feeding	Transmission by aggregate colonies after acquisition in hr[a]								
	Transmitting						Not transmitting		
	CSA-OH			CSA-OH + CSA-MS[b]					
	24	48	72	24	48	72	24	48	72
0-7[c]	10/1[d]	30/3	30/3	20/2	10/1	20/2	40/4		30/3
8-14	9/1	26/3	21/3	20/2	9/1	14/2	35/4		17/3
15-21	5/1			16/2	8/1	5/1	22/4	5/3	7/4
22-28	3/1			6/1	3/1		15/5	3/2	1/1

[a] The number of leafhoppers/colony at start of inoculation feeding (0 day) was 10; there were seven, four, and eight such colonies that had been exposed to the source plant for 24, 48, and 72 hr, respectively, followed by a 14-day incubation. The survivors of each colony were transferred to one test seedling at weekly intervals.

[b] Colonies in this category transmitted serially both CSA-OH and CSA-MS to each test plant.

[c] Day 0 indicates last day of 2-week incubation and first day of inoculation feeding.

[d] Fraction expresses aggregate number of surviving insects (numerator) on specified number of plants; number of plants equals number of colonies.

efficiency of 3-4%. Furthermore, about 35% of the adult population of *G. nigrifrons* is able to transmit CSA-OH. If the capacity of *G. nigrifrons* to transmit CSA-OH is independent of its capacity to transmit CSA-MS, then among 100 transmitters of CSA-OH there would still be, on the average, only three leafhoppers capable of transmitting CSA-MS. Our data would more closely fit this assumption than the one that would postulate the transmissibilities of CSA-MS and CSA-OH to be dependent on each other in this vector. To satisfy the second assumption, the number of double transmitters would have to be about 3 times higher than actually observed.

On the basis of the fragmentary information available at the present, we would like to propose the following explanation on the relative occurrence of the two CS agents in the USA. CSA-MS occurs primarily in the warmer regions of the country, whereas CSA-OH seems to have a wider adaptation as far as temperature is concerned. The incubation periods in the insect vectors and in the plant hosts may have a bearing on the ability of the two disease agents to survive. The more adaptable CSA-OH requires a shorter incubation than CSA-MS in both insect and plant, and is thus able to establish itself in an environment that would prove less suitable for CSA-MS. Factors other than temperature, such as presence or absence of overwintering host plants, would also be involved in delineating the area of CSA-MS distribution. In the South, corn plants diseased with CS appear to be infected with either CSA-MS alone or jointly with CSA-MS and CSA-OH. Whether infected singly or doubly, the diseased corn plants are indistinguishable by the symptoms they exhibit in the field, apparently because CSA-MS plays a dominant role in double infection. Toward the northern limits of the area of CS distribution, corn plants diseased with CS seem to be infected with either CSA-OH alone or jointly with CSA-OH and CSA-MS. At present, we do not know whether these two types of CS-diseased plants could be distinguished visually in the field.

Another factor that needs further investigation is the minimum dosage of CSA required to incite infection. Records from single-insect transmission tests with *G. nigrifrons* showed that occasionally there were individual leafhoppers that singly could not transmit CSA-MS but when placed in pairs were able to incite infection in the test plant on which they fed. This phenomenon has not been noticed with *D. maidis*. It is possible that when both leafhopper vectors are exposed to the same source of CSA-MS, the average potential *D. maidis* transmitter will carry a greater amount of inoculum than will its *G. nigrifrons* counterpart.

Our results on the efficiency of *G. nigrifrons* as a vector of CSA do not agree with those reported by Granados and coworkers (4, 7). Depending on the experiment, 10.1, 15.5, 18.2, and 40.0% of the leafhoppers from their *G. nigrifrons* cultures transmitted CSA (4). In our tests, performed with single insects, the efficiency of CSA-MS transmission by *G. nigrifrons* was 3-4%. Since the CSA isolate and the *G. nigrifrons* cultures, which Granados et al. (4, 7) employed in their studies, originated in Louisiana, and our cultures of both CSA and *G. nigrifrons* derived from neighboring Mississippi, there seems to be good reason to assume that both groups worked with the same CSA and essentially the same biotype of *G. nigrifrons*. Furthermore, symptoms in test plants of sweet corn infected with CSA from Louisiana and from Mississippi are identical. (Is it possible that our colleagues worked with a complex involving CSA-MS and CSA-OH?)

However, Granados et al. (4, 7) used nymphs whereas we used adults. Not only may nymphs of *G. nigrifrons* be more efficient in acquiring CSA than adults, but the latter, naturally, have a shorter life span; thus, some potential transmitters may die before the disease agent completes its latency in them. The sex ratio of the leafhoppers that are used in determining the efficiency of transmission may also affect the outcome of the test, as female *G. nigrifrons* live longer than males. This fact may help

to explain why we obtained a higher percent of transmitters among female than male *G. nigrifrons*. It is possible that females of *G. nigrifrons* are inherently better suited than males to become vectors of CSA, independent of longevity. This possibility is being now investigated.

LITERATURE CITED

1. BOYD, F. J., & H. N. PITRE. 1968. Studies on the field biology of Graminella nigrifrons, a vector of corn stunt virus in Mississippi. Ann. Entomol. Soc. Amer. 61:1423-1427.
2. CHOUDHURY, M. M., & E. ROSENKRANZ. 1971. Graminella nigrifrons as a vector of corn stunt agent. Phytopathology 61:888 (Abstr.).
3. DOUGLAS, W. A., W. H. WHITCOMB, L. W. HEPNER, V. M. KIRK, & R. DAVIS. 1966. Some Cicadellidae (Homoptera) collected from corn in the southeastern United States. Ann. Entomol. Soc. Amer. 59:393-396.
4. GRANADOS, R. R., JOHANNA S. GRANADOS, K. MARAMOROSCH, & J. REINITZ. 1968. Corn stunt virus: Transmission by three cicadellid vectors. J. Econ. Entomol. 61:1282-1287.
5. GRANADOS, R. R., R. D. GUSTIN, K. MARAMOROSCH, & W. N. STONER. 1968. Transmission of corn stunt virus by the leafhopper Deltocephalus sonorus (Ball). Contrib. Boyce Thompson Inst. 24:57-60.
6. GRANADOS, R. R., K. MARAMOROSCH, T. EVERETT, & T. P. PIRONE. 1966. Leafhopper transmission of a corn stunt virus from Louisiana. Phytopathology 56:584 (Abstr.).
7. GRANADOS, R. R., K. MARAMOROSCH, T. EVERETT, & T. P. PIRONE. 1966. Transmission of corn stunt virus by a new leafhopper vector, Graminella nigrifrons (Forbes). Contrib. Boyce Thompson Inst. 23:275-280.
8. GRANADOS, R. R., & R. F. WHITCOMB. 1971. Transmission of corn stunt mycoplasma by the leafhopper Baldulus tripsaci. Phytopathology 61:240-241.
9. KUNKEL, L. O. 1946. Leafhopper transmission of corn stunt. Nat. Acad. Sci. Proc. 32:246-247.
10. MARAMOROSCH, K. 1963. The occurrence in Arizona of corn stunt disease and of the leafhopper vector Dalbulus maidis. Plant Dis. Reptr. 47:858.
11. NIEDERHAUSER, J. S., & J. CERVANTES. 1950. Transmission of corn stunt in Mexico by a new insect vector, Baldulus elimatus. Phytopathology 40:20-21 (Abstr.).
12. ROSENKRANZ, E. 1969. Two types of corn stunt in the USA. Phytopathology 59:1047 (Abstr.).
13. ROSENKRANZ, E. 1969. A new leafhopper-transmissible corn stunt disease agent in Ohio. Phytopathology 59:1344-1346.
14. ROSENKRANZ, E. 1970. Corn stunt agent isolated from sorghum. Phytopathology 60:1311 (Abstr.).
15. ROSENKRANZ, E. 1971. Johnson grass, an over-wintering host of corn stunt agent. Phytopathology 61:908 (Abstr.).
16. ROSENKRANZ, E. E., W. A. DOUGLAS, & H. N. PITRE. 1968. Corn viruses in Mississippi in 1965, p. 45-47. *In* W. N. Stoner [ed.]. Corn (maize) viruses in the continental United States and Canada. U.S. Agr. Res. Serv. Special Rep. ARS 33-118.