

Simulated Intrafield Dispersal of Maize Chlorotic Dwarf Virus by *Graminella nigrifrons* With a Rubidium Marker

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ABSTRACT

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Aqueous sprays of rubidium chloride (RbCl) were applied to emergent johnsongrass to simulate an inoculum source of maize chlorotic dwarf virus (MCDV). The properties of plant uptake and translocation of Rb provided a new approach to epidemiological studies since it could be acquired by vectors feeding on the inoculum sources. Analysis of Rb contents in *Graminella nigrifrons* collected from nearby corn showed a net dispersal

rate of simulated MCDV inoculum from johnsongrass into corn of ~15 m/4 days. A preference of tagged *G. nigrifrons* for a susceptible hybrid also was demonstrated by analysis of Rb; vector dispersal into DeKalb 1214 was significantly greater ($P < 0.05$) than dispersal into Pioneer 3009. MCD incidence and severity were correlated with levels of the simulated inoculum in vectors collected at varying distances from the treated source.

Additional key words: disease severity index.

The semipersistent transmission of maize chlorotic dwarf virus (MCDV) from johnsongrass (*Sorghum halapense* [L.] Pers.), the common overwintering host, to corn (*Zea mays* L.) by *Graminella nigrifrons* (Forbes), the primary leafhopper vector, is well documented (4,10). Transfer of MCDV inoculum is dependent on the dispersal of its vectors (9). However, the epiphytotic relationship of vector, perennial host, and corn has not been thoroughly investigated, and the spatial and temporal displacement of inoculum from primary sources to corn has been difficult to study.

Berry et al (3) proposed a technique for marking phytophagous insects in their natural habitat using rubidium (Rb) as a substitute for radioisotopes and thereby avoiding the disadvantages of marking-recapture and radio-isotope tagging methods (14). Rb is rapidly absorbed by plants from aqueous sprays and is translocated throughout the plant, including new growth (8,16). We found that Rb was translocated in johnsongrass and other Gramineae and that *G. nigrifrons* could be effectively tagged by using this method (2). In the present study Rb was used to simulate virus inoculum in an area sustaining an epiphytotic of MCD. Intrafield dispersal of *G. nigrifrons* from johnsongrass was studied, and some of the factors affecting disease expression (ie, incidence, severity, vector movement) in a resistant and a susceptible hybrid were demonstrated.

MATERIALS AND METHODS

The test was conducted in Morgan County, Georgia, which has a history of severe MCD disease (1,7). In the fall of 1977, a 2 ha area that previously had been planted in corn and which was heavily infested with johnsongrass was plowed and harrowed except for four 45 × 6-m strips parallel to each other and spaced 65 m apart. The tilled portions were reharrowed and treated with Eradicane® herbicide (S-ethyl-N, N-dipropylthiocarbamate + N, N, diallyl-1, 1-dichloroacetamide) at 1.8 L/ha prior to planting.

On 12 May 1978, 32 rows 45 m long and 0.9 m apart and parallel to the johnsongrass strips were planted with one of two corn

hybrids on either side of the strips. The hybrids, DeKalb 1214 and Pioneer 3009, are, respectively, susceptible and intermediate in resistance to MCDV (1,7). Both hybrids have been used extensively in controlled tests, and Pioneer 3009 was reported to All et al (1) to show a nonpreference type of resistance to *G. nigrifrons*. The side to which either hybrid was planted was randomly selected in each of the four replications. A 10-m-wide swath of plowed, unplanted ground separated each replicate. The plots were further divided transversely and longitudinally into eight sectors for sampling (Fig. 1). The transverse sector divided the johnsongrass strip into halves, and one was randomly chosen for treatment with an aqueous spray of RbCl. The untreated portion was used to assess nonlateral movement of leafhoppers from the treated strips as opposed to movement across rows. On 15 June standard grade RbCl was applied on selected halves of the johnsongrass strips in an aqueous spray at a rate of 10.8 kg/ha (spray solution of 6 L of RbCl [18 g/L]

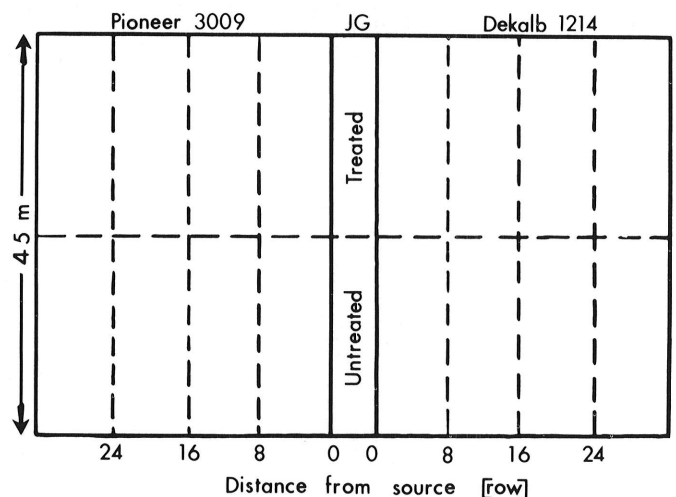


Fig. 1. Typical plot design (one of four replicates) for dispersal study using RbCl treatments on johnsongrass (JG) strips at varying distances (meters) from a source row. Dashed lines indicate sector divisions (subplots) for sampling *Graminella nigrifrons* (leafhoppers).

to each half strip). Alverson et al (2) showed no adverse effects on longevity or development of *G. nigrifrons* exposed to this level of RbCl on host plants, and Graham et al (5) detected 79 ± 9 ppm Rb in leafhoppers from plots of grain sorghum treated with 10 kg RbCl/ha.

Pretreatment samples of the vector populations were taken with a D-Vac vacuum insect collector from 180 m of row in each hybrid. The corn at this time was ~30 cm in height, and johnsongrass plants in the nontilled strips were ~1.5 m tall.

D-Vac samples were collected from four rows (90 m of row) of each sector (subplot) at 4-day intervals after treatment with RbCl and kept frozen in the laboratory until ready for separation and counting. *G. nigrifrons* were prepared for Rb analysis as described by Shepard and Waddill (13). Briefly, oven-dried samples were weighed, ashed for 8 hr at 600 C, dissolved in 5 ml 0.25 N HCl, and analyzed by atomic absorption spectroscopy with emission analysis at 780 nm.

MCD incidence was recorded by counting plants showing symptoms, and corn height was measured in whole rows at selected distances from the inoculum source (johnsongrass strip) in July - September. Yields (15% moisture) were determined by hand harvests from these rows. A disease severity index (DSI) was developed that reflected the relative times of infection and disease impact on hybrids at selected distances from the inoculum source. The severity of symptoms and degree of stunting are related to the stage of growth at which plants become infected (6,12). The DSI was calculated by the equation:

$$DSI = \left(\frac{1}{CH} \right) (PMCD)$$

in which CH = corn height and PMCD = % MCD-infected plants per row.

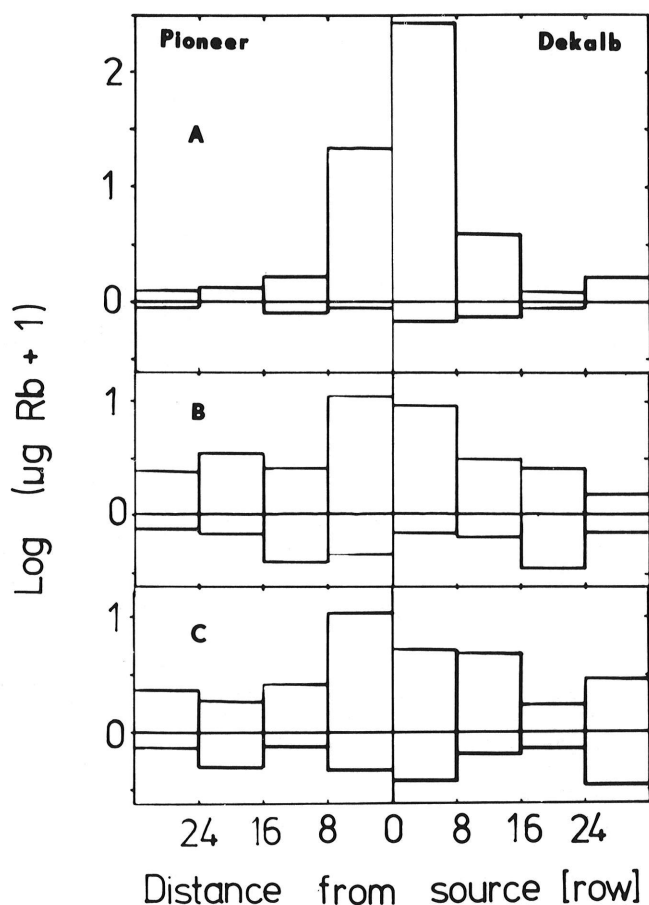


Fig. 2. Rb levels ($\mu\text{g}/100$ *G. nigrifrons*) in sectors at varying distances (meters) from inoculum source row. A, 4 days posttreatment; B, 8 days posttreatment; and C, 12 days posttreatment.

RESULTS

Pretreatment analysis. Analysis of pretreatment samples from the johnsongrass strips and surrounding corn showed a mean endogenous level of Rb in *G. nigrifrons* (\pm SE) of 0.633 ± 0.159 $\mu\text{g}/100$ leafhoppers or 12.57 ± 1.05 $\mu\text{g}/\text{g}$ of leafhoppers. However, because of the variability in size and dry weight of individual *G. nigrifrons*, particularly between sexes (15), dispersal patterns of virus inoculum and virus transmission are best presented per unit of vector numbers. The average weight of the black-faced leafhoppers collected was 51.8 mg/100 leafhoppers. The mean endogenous level of Rb was subtracted from quantities obtained posttreatment for presentation in this text. Although the Rb level in posttreatment samples ranged from 0.099 to 1.448 $\mu\text{g}/100$ *G. nigrifrons*, posttreatment samples falling at or below the mean endogenous level were considered to have obtained no Rb from the treated source.

Acquisition and movement of Rb. Acquisition and movement of Rb (simulated MCDV inoculum) from the inoculum source by *G. nigrifrons* to rows adjacent to treated plots is shown in Fig. 2 (upper histograms). The lower inverted histograms show nonlateral movement of tagged leafhoppers. The $\ln(x+1)$ transformation of data was used due to the exponential pattern of Rb dispersal away

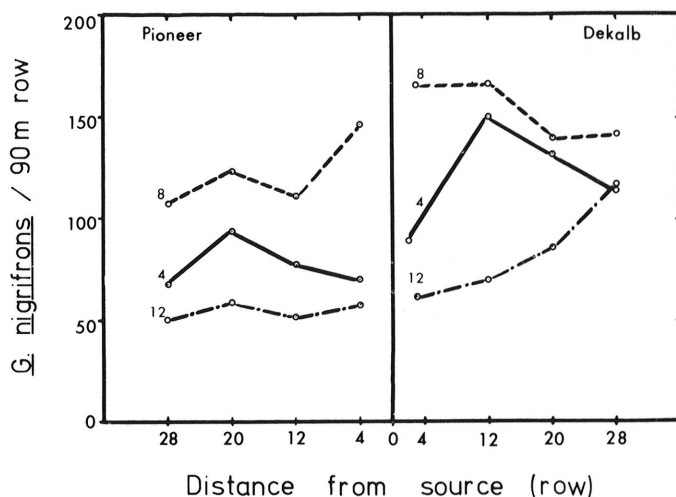


Fig. 3. Mean number of captures of *G. nigrifrons* at varying distances (meters) from johnsongrass (row 0) in plots of two corn hybrids at 4, 8, and 12 days posttreatment with RbCl.

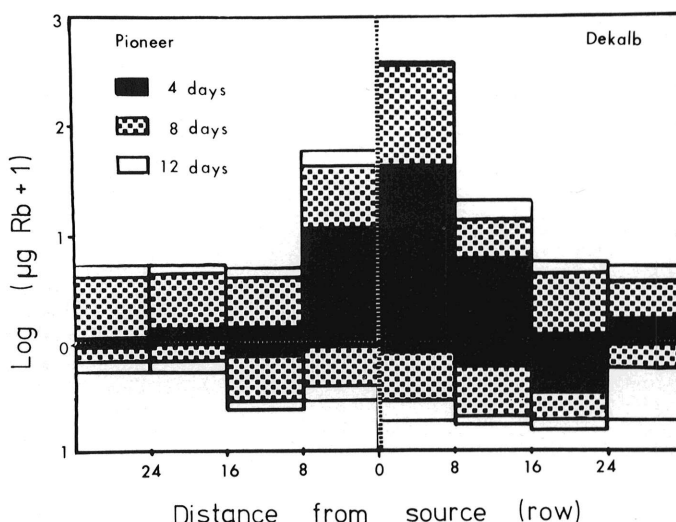


Fig. 4. Cumulative Rb levels ($\mu\text{g}/100$ *Graminella nigrifrons* \times no. of leafhoppers) at varying distances (meters) from inoculum (Rb) source (row 0) two corn hybrids through 12 days posttreatment.

from the source with levels ranging from 11.3 μg to $< 1 \mu\text{g}$ above the endogenous level.

Four days after application, the highest mean levels of Rb-simulated inoculum were found in the composite samples from the eight rows nearest the source (Fig. 2A). The amount per 100 *G. nigrifrons* was significantly higher ($P < 0.05$) in the DeKalb 1214 hybrid ($10.66 \pm 6.60 \mu\text{g}$) than in Pioneer 3009 ($2.74 \pm 1.69 \mu\text{g}$). On a per capita basis, 84% of the Rb was found within eight rows (7.2 m) of the treated source, and $\sim 80\%$ of that amount was found on the DeKalb side of the source. Significantly ($P < 0.05$) less Rb was found in rows 9–17 of either hybrid; there were approximately 13 and 10 times less than in rows 1–8 for DeKalb 1214 and Pioneer 3009, respectively. There were no significant differences in the quantity of Rb beyond the 16th row (14.3 m) for either hybrid; sample means there ranged from 0.070 ± 0.053 to $0.252 \pm 0.218 \mu\text{g}/100 G. nigrifrons$.

By 8 days posttreatment, the Rb had moved farther away from the inoculum source than at 4 days; only 40% of the Rb per 100 leafhoppers was found in rows 1–8; 22% was detected in rows 8–16; 22% was found in rows 17–24; and 16% was detected in rows 24–32. Differences between the two hybrids were not significant (Fig. 2B). By 12 days posttreatment Rb levels were rather uniformly distributed across the DeKalb 1214 (1.07 ± 0.4 to $0.58 \pm 0.2 \mu\text{g}/100$ leafhoppers); the only sector of significant difference was in rows 16–24 ($0.42 \pm 0.2 \mu\text{g}$) (Fig. 2C). In Pioneer 3009, the reduction of Rb beyond row 8 was significantly less ($P < 0.05$) than in the sector adjacent to the inoculum source ($1.91 \pm 0.13 \mu\text{g}/100$ leafhoppers). The remaining samples showed relatively equal levels of the simulated inoculum (0.328 ± 0.044 to $0.498 \pm 0.120 \mu\text{g}/100$ leafhoppers), indicating a new dispersal of at least 30 m from the source after 12 days. Considering all samples at 12 days, DeKalb 1214 had a higher level of Rb per 100 leafhoppers than did Pioneer 3009.

Distribution of leafhoppers. The actual dispersal of inoculum is dependent on the number and distribution of vectors relative to the primary source. *G. nigrifrons* numbers were not correlated with their proximity to the johnsongrass inoculum source (Fig. 3), despite the presence of this host prior to corn emergence. However,

TABLE 1. A comparison of factors affecting maize chlorotic dwarf disease severity in two corn hybrids of differing susceptibility

Factor	$\bar{x} (\pm \text{SE})^a$	
	DeKalb 1214	Pioneer 3009
% MCD	69.9 (± 1.9)	37.9 (± 3.7)
Corn height (m)	0.8 (± 0.0)	1.4 (± 0.1)
DSI	86.2 (± 4.9)	30.1 (± 3.6)

^a All means were significantly different ($P < 0.05$) between hybrids ($n = 32$).

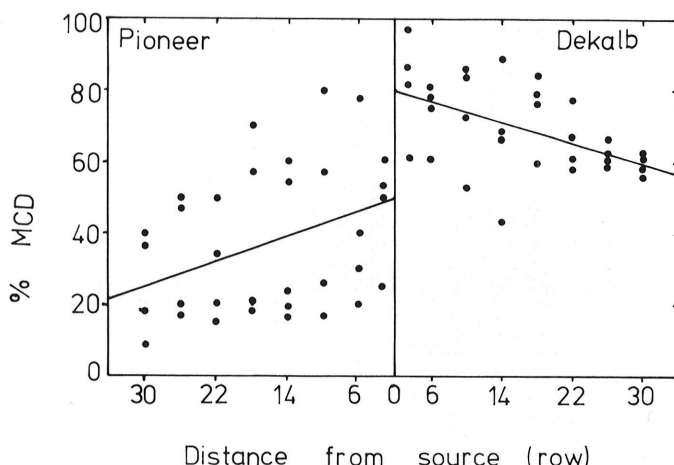


Fig. 5. Regression models of maize chlorotic dwarf (MCD) incidence in two corn hybrids with varying susceptibility as a function of distance (meters) from the inoculum source (row 0).

there were 1.4 times more *G. nigrifrons* in DeKalb 1214 than in Pioneer 3009. This difference was significant ($P = 0.05$) at the first two collection dates; ($P = 0.10$ at the third collection).

Accumulation of Rb. The total amount of simulated inoculum in test plots was dependent on the amount of Rb acquired (Fig. 2) and the number of *G. nigrifrons* present (Fig. 3). Based on these data, the accumulation of Rb through time is shown in Fig. 4. This pattern is very similar to the per capita concentrations shown in Fig. 2. At 4 days, the gradient of dispersal was highly pronounced; the range of Rb was 0.014 ± 0.046 to $9.567 \pm 4.806 \mu\text{g}/\text{per sample}$ and ~ 4.5 times more Rb was found in DeKalb 1214 than in Pioneer 3009. About 85% of the Rb was detected within the first eight rows from the source at 4 days posttreatment, but the distribution became more uniform with time.

Nonlateral movement. By splitting Rb treatments in the strips of johnsongrass, nonlateral movement also could be estimated by comparing Rb levels in the rows adjacent to treated and nontreated johnsongrass. This emigration of tagged leafhoppers is shown in the inverted histograms, below the $\log = 0$ line, in Figs. 2 and 4. At 4 days posttreatment, relatively little Rb was found in rows adjacent to untreated johnsongrass. At 8 and 12 days, there was a gradual increase of Rb, but it was not related to distance from the johnsongrass source. The net movement of Rb away from the treated source shows similar amounts along rows 1–8 adjacent to the untreated strip as is found in rows 16–24 opposite treated strips (Figs. 2 and 4). This finding is consistent with the experimental design in that the distance to row 24 is approximately the same as the length of row from the treated zone to the plot edge on the untreated side of rows 1–8.

When all sampling dates for Rb were considered, it was obvious that more Rb accumulated in the DeKalb plots (adjacent to nontreated johnsongrass) than in the Pioneer plots (Fig. 4).

Disease factors. The overall incidence of MCD was significantly greater in DeKalb 1214 than in Pioneer 3009 (Table 1), and the disease incidence at varying distances from the primary source of inoculum is shown in Fig. 5. The regression model of disease incidence with regard to distance reflects the dispersal pattern in both hybrids ($R^2 = 0.35$ for DeKalb 1214; $R^2 = 0.141$ for Pioneer 3009).

Regression coefficients for DSI vs. ln Rb were 0.85 for DeKalb 1214 and 0.79 for Pioneer 3009. With increasing severity, the reciprocal of the corn height increases, and the percentage of plants expressing symptoms increases. A comparison of these factors for the two hybrids is given in Table 1.

Pioneer 3009 produced approximately five times more grain (1164 kg/ha) than did DeKalb 1214 (208 kg/ha). In both cases, the correlations between DSI and yield were significant, $P < 0.05$ (-0.95 for Pioneer and -0.62 for DeKalb), and regression models followed the pattern of dispersal obtained by Rb simulation of inoculum. Low yields can be attributed primarily to drought conditions in 1978.

DISCUSSION

The successful use of Rb in field dispersal studies demonstrates an effective new tool for epidemiological studies of phytarbovirus diseases. The vector of MCDV, *G. nigrifrons*, acquired Rb from johnsongrass infected with the virus and then moved into adjacent plots. The dispersal pattern of leafhoppers with Rb was significantly correlated with a DSI which in turn was significantly correlated with seed yield.

This study substantiates previous results (1,7) which demonstrated a preferential migration of *G. nigrifrons* to plants of specific corn hybrids. Furthermore, Rb analysis may give more meaningful results than collection of leafhoppers. The number of *G. nigrifrons* was 1.4 times greater in DeKalb 1214 than in Pioneer 3009; however, at 4 days after the availability of Rb, there were 4.5 times more Rb in the former hybrid than in the latter. This suggests that the initial preference of the MCDV vector is very high for DeKalb 1214 in comparison to Pioneer 3009.

Dispersal of Rb-tagged *G. nigrifrons* occurred at a rate of ~ 15 m/4 days across rows spaced 0.9 m apart. Because of continual

tagging in the johnsongrass, the trend toward uniform dispersal at 8–12 days posttreatment may be indicative of random directional changes by individuals in the vector population, analogous to the diffusion of a substance by Brownian movement. The 4-day dispersal rate is more applicable to movement of inoculum by individual vectors since the ability of *G. nigrifrons* to transmit MCDV is lost within 48 hr of acquisition (11).

The accumulation gradient of Rb was higher near the source, declining exponentially as a function of distance from the primary inoculum or tagging source. In theory, secondary sources of inoculum (corn) would be established in the field and the gradient would disappear, given enough time. However, the importance of corn as a secondary source of inoculum has not been established. A disease gradient, based on disease index and/or disease incidence, usually exists in relation to a primary source of inoculum (4) (Fig. 5). Application of Rb to MCDV-infected corn could address this question.

Louie et al (9) and Damsteegt (4) discussed the limitations of using disease incidence alone to evaluate the epidemiology of plant virus diseases. They improved techniques by using trap plants (9) and by measuring the chronology of disease development (4). Rb has the potential to add a new dimension to epidemiological studies. The relative activity of leafhoppers (perhaps other insects as well) can be monitored closely and related to various factors associated with disease development.

LITERATURE CITED

1. ALL, J. N., C. W. KUHN, R. N. GALLAHER, M. D. JELLUM, and R. S. HUSSEY. 1977. Influence of no-tillage cropping, carbofuran, and hybrid resistance on dynamics of maize chlorotic dwarf and maize dwarf mosaic diseases of corn. *J. Econ. Entomol.* 70:221-225.
2. ALVERSON, D. R., J. N. ALL, and P. B. BUSH. 1980. Rubidium as a marker and simulated inoculum for the black-faced leafhopper, *Graminella nigrifrons*, the primary vector of maize chlorotic dwarf virus of corn. *Environ. Entomol.* 9:29-31.
3. BERRY, W. L., M. W. STIMMANN, and W. W. WOLF. 1972. Marking of native phytophagous insects with rubidium: a proposed technique. *Ann. Entomol. Soc. Am.* 65:236-238.
4. DAMSTEEGT, V. D. 1976. A naturally occurring corn virus epiphytotic. *Plant Dis. Rep.* 60:858-861.
5. GRAHAM, H. M., D. A. WOLFENBARGER, and J. B. NOSKY. 1978. Labeling plants and their insect fauna with rubidium. *Environ. Entomol.* 7:379-383.
6. HAO, G. T. F., and H. N. PITRE. 1970. Relationship of vector numbers and age of corn plants at inoculation to severity of corn stunt disease. *J. Econ. Entomol.* 63:924-927.
7. KUHN, C. W., M. D. JELLUM, and J. N. ALL. 1975. Effect of carbofuran treatment on corn yield, maize chlorotic dwarf and maize dwarf mosaic virus diseases, and leafhopper populations. *Phytopathology* 65:1017-1020.
8. LEVI, E. 1970. Penetration, retention, and transport of foliar applied single salts of Na, K, Rb, and Cs. *Physiol. Plant* 23:811-819.
9. LOUIE, R., J. K. KNOKE, and D. T. GORDON. 1974. Epiphytotics of maize dwarf mosaic and maize chlorotic dwarf diseases in Ohio. *Phytopathology* 64:1455-1459.
10. NAULT, L. R., D. T. GORDON, D. C. ROBERTSON, and O. E. BRADFUTE. 1976. Host range of maize chlorotic dwarf virus. *Plant Dis. Rep.* 60:374-377.
11. NAULT, L. R., W. E. STYER, J. K. KNOKE, and H. N. PITRE. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. *J. Econ. Entomol.* 66:1271-1273.
12. PITRE, H. N. 1968. A preliminary study of corn stunt vector populations in relation to corn planting dates in Mississippi. Notes on disease incidence and severity. *J. Econ. Entomol.* 61:847-849.
13. SHEPARD, M., and V. H. WADDILL. 1976. Rubidium as a marker for Mexican bean beetles, *Epilachna varivestris* (Coleoptera: Coccinellidae). *Can. Entomol.* 108:337-339.
14. SOUTHWOOD, T. R. E. 1969. Ecological methods with particular reference to the study of insect populations. Methuen and Co. Ltd., London. 391 pp.
15. STONER, W. N., and R. D. GUSTIN. 1967. Biology of *Graminella nigrifrons* (Homoptera: Cicadellidae), a vector of corn (maize) stunt virus. *Ann. Entomol. Soc. Am.* 60:496-505.
16. WALLACE, A. 1968. Retranslocation of Rb⁸⁶, Cs¹³⁷, and K to new leaf growth in bush beans. *Plant Soil* 29:184-197.