ECOLOGY AND MANAGEMENT OF THE LEAFHOPPER VECTORS

(*CICADULINA* Spp.) OF MAIZE STREAK VIRUS

by

GEORGE DIEDERICK JANSE VAN RENSBURG

Dissertation presented for the degree of

Doctor of Philosophy

in the

Department of Entomology and Nematology

University of Stellenbosch

Promoter   : Prof. J.H. Giliomee

Co-promoter   : Dr. K.L. Pringle

External examiner : Dr. Z.T. Dabrowski

August, 1990

Potchefstroom

DECLARATION

I the undersigned hereby declare that
the work contained in this thesis is my
own original work and has not previously
in its entirety or in part been submit-
ted at any university for a degree.

G.D.J. van Rensburg   1990-08-30

CONTENTS

ABSTRACT

Several Cicadulina species are important pests of maize by virtue of their role as vectors of maize streak virus (MSV). This study deals with various aspects of the vector/virus/host plant relationship.

Developmental times for all life stages of both C. anestae and C. mbila were inversely related to temperature, and the data were used to develop a degree-day (DD) model that can be used to estimate population growth in the field. The rate of development peaked at 30 ^{O}C for both species. The seasonal abundance of C. anestae and C. mbila adults at two localities were monitored by means of sticky traps. Seasonal fluctuations in leafhopper numbers were related mainly to temperature (expressed as DD).

Although C. anestae was added to the list of MSV vectors it was not an exceptionally efficient vector compared with C. mbila. MSV transmission efficiency of female leafhoppers was significantly higher that that of males. MSV incubation in maize was inversely related to post-inoculation temperature. Rapidity of MSV symptom appearance and symptom severity in maize were dependent on the number of leafhoppers feeding on the plants.

Host plant resistance in conjunction with chemical control measures was shown to offer an economical solution to the problem of maize streak disease. Maize hybrids presently grown in South Africa showed varying degrees of resistance to MSV. MSV occurred more severely in white than in yellow maize.

Feeding threshold period for MSV transmission by C. mbila was 30 min. The number of plants developing MSV symptoms increased progressively and disease symptoms appeared sooner after inoculation as the feeding time was extended beyond 30 min.

Corrective post-emergence sprays of contact and systemic insecticides were inefficient for the control of the maize leafhopper, C. mbila. Protection of maize against MSV infection could only be achieved effectively through pre-emergence preventative treatment with systemic insecticides applied in the planting furrow. The use of a broad-spectrum soil systemic insecticide for leafhopper control would eliminate additional insecticide applications for control of other maize pests such as stalk borers, thereby reducing cost of protection against MSV infection indirectly.

INTRODUCTION

The cicadellid genus Cicadulina China consists of a small number of species that are widely distributed in the tropical and warm temperate regions of the world (Ruppel, 1965; Rose, 1978; Van Rensburg, 1983; Webb, 1987). Several of these leafhopper species are important pests of maize by virtue of their role as vectors of maize streak virus (MSV). Extensive research on this vector-virus-plant relationship has been done by several workers, and only some of the key references will be mentioned here.

Apart from the description of single species, major taxonomic studies of the genus Cicadulina have been undertaken by Ruppel (1965), Nielson (1968), Van Rensburg (1983) and Webb (1987). Of the 22 recognizable species, 14 are endemic to Africa, another four are common in Africa; 11 of these species have been shown to transmit plant pathogens, the most important being MSV (Webb, 1987). The status of some species as vectors of plant pathogenic organisms are, however, still unknown. In a pioneering series of investigations various aspects of the transmission of MSV by Cicadulina have been studied by Storey (1924; 1925; 1926; 1927; 1928; 1931; 1932; 1933; 1936; 1938; 1939a; 1939b). Recently, Markham et al. (1984) and Dabrowski (1985) reported on the proportion of vectors within populations. The epidemiology of maize streak disease was reviewed by Rose (1978), and the progress in geminivirus research, the plant virus group to which MSV belongs, by Harrison (1985).

Biological studies of different Cicadulina species dealt mainly with the effect of temperature and host plants on their life-cycles (Van der Merwe, 1926; Ruppel, 1965; Rose, 1973a; Ammar 1975, 1977; Van Rensburg, 1982a,

1982b; Dabrowski, 1985 and Okoth et al., 1987). The only attempt to relate development periods to population cycles in the field was done by Rose (1973b) based on his (1973a) equation for laboratory data.

With regard to injuriousness, MSV causes the greatest yield losses in maize infected at an early growth stage (Guthrie, 1978; Soto & Buddenhagen, 1978; Van Rensburg, 1981). In fact, Van Rensburg (1981) stated that infection occurring before plants are seven weeks old would result in economically important yield losses. Chemical control of the vectors of MSV is based on the preventative application of systemic insecticides to the soil at planting (Van Rensburg & Kuhn, 1977; Van Rensburg & Walters, 1978; Drinkwater et al., 1979). Application of less-persistent insecticides seems to be inefficient in preventing MSV infection (Van Rensburg & Kuhn, 1977; Rose, 1978; Van Rensburg, 1980). The use of resistant maize cultivars is considered to be the most economical method of controlling MSV (Van Rensburg & Kuhn, 1977), and maize varieties with varying degrees of resistance to MSV have long been known (Rose, 1941; Gorter, 1953, 1959; Storey & Howland, 1967a, 1967b). Recently, plant breeders have renewed efforts to develop streak-resistant maize (Van Rensburg & Kuhn, 1977; Rose, 1978; Fourie & Pienaar, 1984). The success of resistance breeding is largely dependant on the availability of mass rearing techniques for Cicadulina (Dabrowski, 1985) and suitable artificial infestation techniques for MSV screening (Van Rensburg, 1979b).

Maize streak disease is endemic in Africa and had been recognized in South Africa as an important factor limiting maize production in Natal at the turn of the century (Fuller, 1901). The cause of the diseae was, however, unknown until Storey (1924) indentified it as a plant virus disease transmitted by Cicadulina mbila (Naudé). Prior to the 1970's, streak disease was regarded as

being restricted to the warmer parts of South Africa, such as areas of Natal and the Transvaal Lowveld, and irrigation schemes such as those at Vaalharts. Storey (1926) stated that the disease rarely occurred at altitudes above 1 200 m (4 000 ft). In the colder Highveld areas the disease was limited to isolated infected plants that occurred sporadically in maize fields. During the 1973/74 season, however, the disease reached epidemic proportions in maize plantings in the North-Western Orange Free State (Van Rensburg, 1979a). Since then upsurges in disease incidence occurred frequently in parts of the Highveld. During the 1979/80 season infection levels of up to 95 % were observed in parts of the Western Transvaal and North-Western Orange Free State, while the disease again caused great concern during 1982/83 in the latter area. Streak disease also spread to parts of the Eastern Orange Free State where severe MSV infections in several maize plantings were reported to have resulted in almost total crop losses. Various reports of severe streak infections in both maize and wheat have also been received from different parts of the Highveld during recent years, particularly in irrigation areas. Van Rensburg (1979a) ascribed the epidemic incidence of maize streak disease in the Highveld to migratory activities of the leafhopper vectors, the availability of host plants and the occurrence of conditions satisfying the ecological requirements of the insect vectors. It thus seems that during recent years Cicadulina, and thereby also MSV, have spread to and become well established in areas which were relatively free of streak disease prior to 1973.

Moran (1983), in evaluating the pest status of the various guilds of phytophagous pests of cultivated plants in South Africa, concluded that the plant disease vectors were the most important guild. Furthermore, the maize leafhopper C. mbila was ranked amongst the insects with the highest pest status

in South Africa. MSV in itself is considered to be one of the world's most destructive virus diseases of crop plants (Harrison, 1985). This vector-virus-plant relationship is therefore economically very important and presents a challenging subject for research.

The aim of the present investigation was to obtain information on the seasonal occurrence of Cicadulina populations, and to analize the role of climatic conditions in their population dynamics. Aspects of the chemical control of the leafhopper vectors, virus transmission and plant resistance to MSV were also investigated with a view to improving pest management practices. The results are presented in the form of eight papers which have been prepared for publication. The papers deal with:

- Development and reproduction as a function of temperature in Cicadulina anestae and C. mbila (Chapter 1).

- Seasonal fluctuations in the numbers of Cicadulina anestae and C. mbila caught with sticky traps at two localities in South Africa. (Chapter 2).

- Comparison of sexes and species of Cicadulina with regard to MSV transmission (Chapter 3).

- The effect of vector numbers and temperature on MSV symptom appearance and severity (Chapter 4).

- The relative status of maize hybrids regarding resistance to maize streak virus (Chapter 5).

- Chemical prevention of MSV transmission (Chapter 6).

- Pre- and post-emergence application of insecticides aimed at multiple-pest control (Chapter 7).

- The most cost effective chemical control strategy against vectors of MSV (Chapter 8).

REFERENCES

AMMAR, E.D. 1975. Biology of the leafhopper Cicadulina chinai Ghauri (Homoptera, Cicadellidae) in Giza, Egypt. Zeitschrift für angewandte Entomologie 79 : 337-345.

AMMAR, E.D. 1977. Biology of Cicadulina bipunctella zeae China in Giza, Egypt (Homoptera, Cicadellidae) . Deutsche entomologische Zeitschrift 24 : 345-352.

DABROWSKI, Z.T. 1985. The biology and behaviour of Cicadulina triangula in relation to maize streak virus resistance screening. Insect Science & its Application 6 : 417-424.

DRINKWATER, T.W., WALTERS, M.C & VAN RENSBURG, J.B.J. 1979. The application of systemic insecticides to the soil for the control of the maize stalk borer, Busseola fusca (Fuller) (Lep. : Noctuidae), and of Cicadulina mbila (Naudé) (Hem. : Cicadellidae), the vector of maize streak virus. Phytophylactica 11 : 5-12.

FOURIE, A.P. & PIENAAR, J.H. 1983. Breeding for resistance to maize streak virus : A report of the Vaalharts breeding programme. Pages 44-50 in: Proceedings of the fifth South African Maize Breeding Symposium 1982. J.G. Du Plessis, ed. Republic of South Africa, Department of Agriculture, Technical Communication 182.

FULLER, C. 1901. Mealie variegation. First Report of the Government Entomologist, Natal, pp. 17-19.

GORTER, G.J.M.A. 1953. Studies on the spread and control of the streak disease of maize. Department of Agriculture and Forestry of the Union of South Africa, Scientific Bulletin 341 : 1-20.

GORTER, G.J.M.A. 1959. Breeding maize for resistance to streak. Euphytica 8 : 234-240.

GUTHRIE, E.J. 1978. Measurement of yield losses cuased by maize streak disease. Plant Disease Reporter 62 : 839-841.

HARRISON, B.D. 1985. Advances in geminivirus research. Annual Review of Phytopathology 23 : 55-82.

MARKHAM, P.G., PINNER, M.S. & BOULTON, M.I. 1984. The transmission of maize streak virus by leafhoppers, a new look at host adaptation. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 57 : 431-432.

MORAN, V.C. 1983. The phytophagous insects and mites of cultivated plants in South Africa : Patterns and pest status. Journal of Applied Ecology 20 : 439-450.

NIELSON, M.W. 1968. The leafhopper vectors of phytopathogenic viruses (Homoptera, Cicadellidae). Taxonomy, biology and virus transmission. United States Department of Agriculture, Technical Bulletin no 1382 : 1-386.

OKOTH, V.A.O., DABROWSKI, Z.T. & VAN EMDEN, H.F. 1987. Comparative biology of some Cicadulina species and populations from various climatic zones in Nigeria (Hemiptera : Cicadellidae). Bulletin of entomological Research 77 : 1-8.

ROSE, D.J.W. 1973a. Laboratory observations on the biology of Cicadulina spp. (Hom., Cicadellidae), with particular reference to the effects of temperature. Bulletin of entomological Research 62 : 471-476.

ROSE, D.J.W. 1973b. Field studies in Rhodesia on Cicadulina spp. (Hem., Cicadellidae), vectors of maize streak disease. Bulletin of entomological Research 62 : 477-495.

ROSE, D.J.W. 1978. Epidemiology of maize streak disease. Annual Review of Entomology 23 : 259-282.

ROSE, M.F. 1941. Rotation crops. Empire Cotton Growing Association, Progress Report 1939, 40 : pp. 27-31.

RUPPEL, R.F. 1965. A review of the genus Cicadulina (Hemiptera, Cicadellidae). Publications of the Museum, Michigan State University Biological Series 2 : 387-428.

SOTO, P.E. & BUDDENHAGEN, I. 1978. Yield loss from a localized epidemic of maize streak virus in Nigeria. East African agricultural and Forestry Journal 44 : 175-177.

STOREY, H.H. 1924. The transmission of a new plant virus disease by insects. Nature (Lond.) 114 : 245.

STOREY, H.H. 1925. The transmission of streak disease of maize by the leaf-hopper Balclutha mbila Naudé. Annals of applied Biology 12 : 422-439.

STOREY, H.H. 1926. Streak disease of maize. Farming in South Africa 1 : 183-185.

STOREY, H.H. 1927. Control of streak disease on maize and sugar-cane. Proceedings of the South and East African agricultural Conference, 1926, pp. 212-213.

STOREY, H.H. 1928. Transmission studies of maize streak disease. Annals of Applied Biology 15 : 1-24.

STOREY, H.H. 1931. The inheritance by a leafhopper of the ability to transmit a plant virus. Nature (Lond.) 127 : 928.

STOREY, H.H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. Proceedings of the Royal Society of London Series B, 112 : 46-60.

STOREY, H.H. 1933. Investigations of the mechanism of the transmission of plant viruses by insect vectors. 1. Proceedings of the Royal Society of London Series B, 113 : 463-485.

STOREY, H.H. 1936. Virus diseases of East African Plants. V-Streak disease of maize. East African agricultural Journal 1 : 471-475.

STOREY, H.H. 1938. Investigations of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. Proceedings of the Royal Society of London Series B, 125 : 455-477.

STOREY, H.H. 1939a. Transmission of plant viruses by insects. Botanical Review 5 : 240-272.

STOREY, H.H. 1939b. Investigations of the mechanism of the transmission of plant viruses by insect vectors. III. The insect's saliva. Proceedings of the Royal Society of London Series B, 127 : 526-543.

STOREY, H.H & HOWLAND, A.K. 1967a. Transfer of resistance to the streak virus into East African maize. East Africa agricultural and Forestry Journal 33 : 131-135.

STOREY, H.H & HOWLAND, A.K. 1967b. Inheritance of resistance in maize to the virus of streak disease in East Africa. Annals of applied Biology 59 : 429-436.

VAN DER MERWE, C.P. 1926. The maize Jassid Balclutha mbila Naudé. Journal of the Department of Agriculture of the Union of South Africa 12 : 75-77.

VAN RENSBURG, G.D.J. 1979a. The ecology and culture of leafhoppers (Cicadulina spp.). Pages 100-103 in: Proceedings of the second South African Maize Breeding Symposium 1976. H.O. Gevers, ed. Republic of South Africa, Department of agricultural technical Services, Technical Communication 142.

VAN RENSBURG, G.D.J. 1979b. Maize streak disease : A new infection technique for use under field conditions. Pages 80-84 in: Proceedings of the third South African Maize Breeding Symposium 1978. J.G. du Plessis, ed. Republic of South Africa, Department of agricultural technical Services, Technical Communication 152.

VAN RENSBURG, G.D.J. 1980. The taxonomy of the Southern African species of the genus Cicadulina China (Homoptera : Cicadellidae), with remarks on the economic importance and ecology of the maize leafhopper, Cicadulina mbila (Naudé), a vector of maize streak disease. Unpublished M.Sc (Agric.) -thesis, University of Stellenbosch, 188 pp.

VAN RENSBURG, G.D.J. 1981. Effect of plant age at the time of infection with maize streak virus on yield of maize. Phytophylactica 13 : 197-198.

VAN RENSBURG, G.D.J. 1982a. Laboratory observations on the biology of Cicadulina mbila (Naudé), (Homoptera : Cicadellidae), a vector of maize streak disease. 1. The effect of temperature. Phytophylactica 14 : 99-107.

VAN RENSBURG, G.D.J. 1982b. Laboratory observations on the biology of Cicadulina mbila (Naudé), (Homoptera : Cicadellidae), a vector of maize streak disease. 2. The effect of selected host plants. Phytophylactica 14 : 109-111.

VAN RENSBURG, G.D.J. 1983. Southern African species of the genus Cicadulina China (Homoptera : Cicadellidae) with descriptions of new species. Department of Agriculture, Republic of South Africa, Entomology Memoir no. 57.

VAN RENSBURG, G.D.J. & KüHN, H.C. 1977. Maize streak disease. Department of agricultural technical Services of South Africa Maize Series Leaflet No. E.3.3 (Farming in South Africa), pp. 1-4.

VAN RENSBURG, J.B.J. & WALTERS, M.C. 1978. The efficacy of systemic insecticides applied to the soil for the control of Cicadulina mbila (Naudé), (Hem. : Cicadellidae), the vector of maize streak disease, and the maize stalk borer, Busseola fusca (Fuller) (Lep. : Noctuidae). Phytophylactica 10 : 49-52.

WEBB, M.D. 1987. Species recognition in Cicadulina leafhoppers (Hemiptera : Cicadellidae), vectors of pathogens of Gramineae. Bulletin of entomological Research 77 : 683-712.

CHAPTER 1

DEVELOPMENT AND REPRODUCTION AS A FUNCTION OF TEMPERATURE IN

CICADULINA ANESTAE AND C. MBILA (HOMOPTERA : CICADELLIDAE)

INTRODUCTION

Cicadulina leafhoppers are widely distributed in tropical and warm temperate regions (Van Rensburg, 1983; Webb, 1987). At least nine Cicadulina species, including C. anestae Van Rensburg and C. mbila (Naudé), are known vectors of maize streak disease (Webb, 1987; Chapter 3), which is one of the world's most serious virus diseases of crop plants (Harrison, 1985).

The first biological data on Cicadulina was the brief description by Van der Merwe (1926) of the life-cycle of C. mbila at fluctuating temperatures (natural conditions). It was the only such study of Cicadulina until Ruppel (1965) found the life-cycle of C. pastusae Ruppel & DeLong to be very similar. Subsequently Ammar (1975; 1977) studied the biology of C. bipunctella zeae China (= C. bipunctata (Melichar); Webb, 1987) and C. chinai Ghauri under semi-natural conditions.

More recently the biology of several Cicadulina species were studied under controlled conditions. Rose (1973) compared the development periods for eggs and nymphs of C. mbila, C. parazeae Ghauri and C. storeyi China at different temperatures, while Dabrowski (1985) studied the effect of temperature on the biology of C. triangula Ruppel (= C. storeyi; Webb, 1987). The effect of temperature and host plant was studied by Van Rensburg (1982 a; b) on C. mbila, and by Okoth et al. (1987) on various populations of C. mbila and

C. triangula. These studies indicated that although the life-cycles of the different species are very similar, there are differences between species and populations with regard to overall developmental periods. These differences should be considered in mass rearing programmes of Cicadulina species (Okoth et al., 1987).

Our purpose was to compare the developmental rates, fecundity and mortality of C. anestae and C. mbila at constant temperature in order to improve the understanding of the influence of the abiotic environment on these species. The developmental threshold temperature (t) as well as the number of degree-days (DD) above the threshold required to complete development was determined since it would help to understand population cycles in the field.

MATERIALS AND METHODS

The studies were conducted at Potchefstroom (26° 44' S, 27° 05' E) during 1988 and 1989. The specimens used in these studies were from stock colonies of C. anestae and C. mbila maintained in a greenhouse at 26/18 ^{O}C ($\pm$ 1.5 ^{O}C) day/night temperature with a photoperiod of 14L : 10D. The stock colonies were established from adults collected during February 1988 at the Sandvet experimental station (28° 07' S, 26° 38' E), and maintained in culture on the maize (Zea mays (L)) hybrid PNR6428 for about 10 months before the study commenced.

All experiments were carried out in constant temperature cabinets at a 14L : 10D photoperiod. The relative humidities ranged from 50 % to 80 %, and the temperature remained within $\pm$ 0.25 ^{O}C of the desired setting. Observations were made between 10:00 and 12:00 each day so that individual

leafhoppers were monitored at approximately 24-h intervals. The study was repeated at five constant temperatures, viz 18, 21, 24, 27 and 30 ^{o}C.

Development

The maize plants were grown in 19 cm diameter pots under lantern glasses to avoid uncontrolled oviposition. At each temperature groups of 20 actively ovipositing females and 20 males of each species were confined on maize seedlings for 24 h under a lantern glass, 16 cm in height. Eggs were incubated naturally in the tissues of the maize seedlings and the number hatching each day were recorded. Both species, C. anestae and C. mbila, were treated similarly throughout.

Newly hatched nymphs of each species were transferred singly onto wheat (Triticum aestivum L.) seedlings (cultivar Harts), grown individually in small containers (40 cm height x 60 cm diameter). Nymphs were confined on the wheat seedlings by means of glass tubes (14 cm height x 2 cm diameter), the tops of which were closed with fine muslin. Glass tubes were supported by polystyrene discs fitted around them and resting on the tops of the pots, which at the same time preserved soil moisture. Periodically plants showing yellowing were replaced with fresh seedlings. The duration of successive instars was confirmed by looking for cast skins, which were then removed from the plants. Completely randomized designs were used and analyses included only those individuals, reared at a specific temperature, that completed development to the adult stage. Nymphal mortalities were recorded, and the mortality analyses included all individuals reared at a specific temperature.

The number of days required by each of the two species to develop from egg to adult were computed and the relationship between development rate and temperature analysed by means of linear regression. The x-intercept of the linear regression is an estimate of the developmental threshold temperature (t) and the reciprocal of the regression coefficient an estimate of the number of degree-days (DD) above the threshold required to complete development (Campbell et al., 1974). The values of t and DD for C. anestae and C. mbila were calculated accordingly.

Pre-oviposition period and fecundity

Pre-oviposition periods were determined by pairing newly developed adults and recording the dates on which the first eggs were laid. Eggs are inserted by the female in the tissues of the host plant, but are visible from either side of the leaf when observed against a light background. Each pair of leafhoppers was transferred daily to a fresh wheat seedling until the female died; if a male died it was replaced. The number of eggs laid daily by each female was subsequently recorded. In this way the pre-oviposition period, day of peak oviposition and number of eggs per female were determined. Analyses of variance of the data for each species were carried out and means were ranked by Scheffe's LSD test at P=0.05.

Life tables

Age-specific life tables (not shown) were constructed for both species at three temperatures (24, 27, and 30 ^{o}C) according to the procedures described by Southwood (1978) and Price (1984). From these tables, the following population reproductive statistics were calculated for C. anestae and for

C. mbila : net reproductive rate (R_o) = $l_x m_x$; generation time (T) = $\Sigma x l_x m_x / \Sigma l_x m_x$; intrinsic capacity for increase (r_m) = $\ln R_o/T$; and finite rate of increase (λ) = e^{r_m} (Southwood, 1978; Price, 1984).

RESULTS

Development

Developmental times for all C. anestae and C. mbila life stages were inversely related to temperature (Table 1.1). The data on C. mbila obtained from this study were very similar to those of previous studies on the same species (Rose, 1973; Van Rensburg, 1982a; Okoth et al., 1987). Table 1.1 presents data only of those individuals which reached the adult stage. Although embryonic development was completed in both C. anestae and C. mbila at all temperatures tested, the latter species did not develop to the adult stage at 18 °C. The egg incubation period at 18 °C for C. mbila was 24.9 ± 1.2 d compared to 26.2 ± 0.4 d for C. anestae. Incubation periods for eggs of both C. anestae and C. mbila at 27 °C did not differ significantly $(P \leq 0.05)$ from those at 30 °C (Table 1.1).

With regard to nymphal development, significant decreases in the duration of all five instars were observed with increases in temperature for both species (Table 1.1). This was most marked in instars I and V and in the total development time from egg hatch to adult (I - V) of both species and for either sex. Fig. 1.1 presents comparative survivorship curves for C. anestae and C. mbila, showing the percentage of nymphs which survived to the adult stage at different constant temperatures. There was a marked increase in survival from 0 % to 55.0 % for C. mbila and from 8.3 % to 80.0 % for

C. anestae as the temperature increased from 18 ^{o}C to 21 ^{o}C. From 24 ^{o}C to 30 ^{o}C mortality in nymphs of both species was relatively constant and low.

Total development periods (egg to adult) of both C. anestae and C. mbila decreased significantly with increases in temperature (Table 1.1). Overall, females took slightly longer than males to reach the adult stage but the differences were not significant. Table 1.2 records the number of each sex for C. anestae and C. mbila surviving to the adult stage at the various temperatures. The binomial test (α = 0.05) indicated that the observed sex ratios for both species at all temperatures, except for C. mbila at 27 ^{o}C (where the ratio was 1 : 2.6), did not differ significantly from a 1 : 1 ratio. Usually more females than males reached the adult stage at a specific temperature.

Linear regressions describing the relationship between developmental rate (1/Y) and temperature (X) for eggs, nymphs, and total development time (egg to adult) of the two species are presented in Fig. 1.2. The linear regression models for total development periods were fitted using pooled developmental times for both males and females because durations of C. anestae and C. mbila life stages were not consistently different between the sexes (Table 1.1). Correlation coefficients (r) ranged from 0.90 (eggs) to 0.97 (egg to adult) for C. mbila, and from 0.92 (eggs) to 0.97 (egg to adult) for C. anestae, all being highly significant (P $\leq$ 0.01), indicating a strong linear relationship. These models were used to calculate the lower developmental threshold temperature (t) and the number of degree-days (DD) above the threshold required to complete development (Fig. 1.2). Lower threshold temperatures for eggs, nymphs and total development (egg-to-adult) were 12.14 ^{o}C, 14.65 ^{o}C and 13.81 ^{o}C respectively for C. mbila, and 11.29 ^{o}C, 12.44 ^{o}C and

12.23 $^{\circ}$C respectively for C. anestae. The corresponding DD necessary for development were 129.9, 185.2 and 312.5 respectively for C. mbila, and 153.8, 222.2 and 384.6 respectively for C. anestae.

Pre-oviposition period and fecundity

The pre-oviposition period and fecundity of C. anestae and C. mbila reared on wheat seedlings at constant temperatures of 24 $^{\circ}$C, 27 $^{\circ}$C and 30 $^{\circ}$C are given in Table 1.3. Pre-oviposition periods and day of peak oviposition decreased, while the number of eggs increased significantly ($P \leq 0.05$) with increase in temperature from 24 $^{\circ}$C to 30 $^{\circ}$C. The pre-oviposition period for C. mbila ranged between 2 and 7 days and for C. anestae between 2 and 9 days (all temperatures) with the mean 4.8 days for C. mbila and 6.4 days for C. anestae. A decrease in temperature from 30 $^{\circ}$C to 24 $^{\circ}$C affected fecundity in C. anestae more adversely than in C. mbila.

Life tables

The calculated population reproductive statistics for both species are summarized in Table 1.4. Net reproductive rates (R_o) increased with increasing temperature, indicating greater fecundity and survival at the higher temperatures. Generation times (T) decreased with increasing temperature, a result of the inverse relationship between developmental time and temperature (Table 1.1). The intrinsic capacity for increase (r_m) peaked for both species at 30 $^{\circ}$C (r_m = 0.0573 females per female per day for C. anestae, and r_m = 0.0532 for C. mbila). The finite rate of increase (λ) followed a trend similar to r_m.

DISCUSSION

The data pertaining to development of C. mbila (Sandvet population) from this study were similar to those of previous studies with other populations of this species (Rose, 1973; Van Rensburg, 1982a; Okoth et al., 1987). The development threshold temperatures of both eggs and nymphs, and consequently also for egg-to-adult were higher for C. mbila than for C. anestae (Fig. 1.2). Eggs and nymphs of C. anestae required respectively 24 and 37 DD more to complete development than the corresponding life stages of C. mbila. Overall, the lower developmental threshold temperature of eggs was lower than that of nymphs for both species, indicating that eggs can survive lower temperatures than nymphs. This means that although eggs will hatch at low temperatures, nymphal mortality might be so high that no individuals would reach the adult stage, as was indeed observed for C. mbila by Rose (1973) at 15.1 ^{o}C and in the present study at 18 ^{o}C.

The thermal constant of greatest interest is that for development from oviposition to adult emergence (Campbell et al., 1974), which was 312.5 DD for C. mbila and 384.6 DD for C. anestae. The latter thus complete development from egg to adult at a much slower rate than C. mbila. Linear regressions of development rates against temperature for C. mbila from the data of Rose (1973) (Zimbabwe population) and Van Rensburg (1982a) (Potchefstroom population) were used to calculate t and DD for comparison with our data (Sandvet population) on this species. The thermal constant calculated from the data of Van Rensburg (1982a) was the same as for this study, viz 312.5 DD, while the calculated lower threshold temperature of 14.03 ^{o}C is probably not significantly different physiologically from the present estimate. A threshold temperature of 15.3 ^{o}C and a thermal constant of 303 DD were

calculated from the data of Rose (1973), working in Zimbabwe. These differences in the values of t and DD may be ascribed to an adaptation to local climatic conditions in which the various populations of the species have evolved. Differences in t values within (and between) species were illustrated by Campbell et al. (1974) for aphids.

Development times for the different life stages of both Cicadulina species in our study did not decrease much from 27 $^{\circ}$C to 30 $^{\circ}$C. This was most apparent for the egg stage where no significant differences between the incubation periods were found at these temperatures (Table 1.1). The results of Rose (1973) for C. mbila show a similar trend. Nevertheless, no conclusions can be drawn from our results regarding the upper development threshold. Previous data for C. mbila (Van Rensburg, 1982a) together with our data, however, suggests that 31 $^{\circ}$C is probably near the upper threshold.

The observed sex ratios correspond with those previously recorded for C. mbila (Van Rensburg, 1982a), C. chinai (Ammar, 1975), and C. bipunctata (= C. bipunctella zeae) (Ammar, 1977) in that the ratios are female biased. In contrast, Rose (1973) found a preponderance of males at a wide range of temperatures, a result which still awaits a satisfactory biological explanation.

The mean pre-oviposition periods (days) of 4.8 for C. mbila and 6.4 for C. anestae (calculated over all temperatures) are quite similar to the 5.6 for C. chinai (Ammar, 1975), 5.9 for C. bipunctata (Ammar, 1977) and 5.3 for C. mbila (Van Rensburg, 1982a), but are much shorter than the 9.6 for C. mbila and 11.0 for C. storeyi (= C. triangula) recorded by Okoth et al. (1987). The survival data, coupled with the fecundity data obtained in the present study,

indicate that Cicadulina can survive during cold weather and then significantly increase reproduction when temperatures increase.

The population reproductive statistics (Table 1.4) indicate that temperatures between 27 °C and 30 °C favour the development, reproduction and survival of C. anestae and C. mbila. The optimal temperature for population increase of both species appears to be near 30 °C. Rose (1973) concluded that fecundity of C. mbila (Zimbabwe population) is near a maximum at 28 °C.

CONCLUSION

Temperature plays a significant role in the development of C. anestae and C. mbila. Despite the fact that in some insects high mortality occurs in the temperature range near the lower development threshold (Campbell et al., 1974), our results show that Cicadulina survives at low temperatures by increasing egg-to-adult development time and by slowing reproduction. Development rates at constant temperatures apparently do not differ much from those at temperatures fluctuating about the same average value (Gilbert & Gutierrez, 1973; Campbell et al., 1974). Developmental thresholds calculated from the models developed for C. mbila and C. anestae can help predict when development and growth rates of field populations may be increasing. For the purposes of degree-day calculations, a lower threshold temperature of 12 °C and 14 °C should be used for C. anestae and C. mbila, respectively.

REFERENCES

Ammar, E.D. (1975) Biology of the leafhopper Cicadulina chinai Ghauri (Homoptera, Cicadellidae) in Giza, Egypt. Zeitschrift für Angewandte Entomologie 79, 337-345.

Ammar, E.D. (1977) Biology of Cicadulina bipunctella zeae China in Giza, Egypt (Homoptera, Cicadellidae). Deutsche Entomologische Zeitschrift 24, 345-352.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P. & Mackauer, M. (1974) Temperature requirements of some aphids and their parasites. Journal of Applied Ecology 11, 431-438.

Dabrowski, Z.T. (1985) The biology and behaviour of Cicadulina triangula in relation to maize streak virus resistance screening. Insect Science & Its Application 6, 417-424.

Gilbert, N. & Gutierrez, A.P. (1973) A plant-aphid-parasite relationship. Journal of Animal Ecology 42, 323-340.

Harrison, B.D. (1985) Advances in geminivirus research. Annual Review of Phytopathology 23, 55-82.

Okoth, V.A.O., Dabrowski, Z.T. & Van Emden, H.F. (1987) Comparative biology of some Cicadulina species and populations from various climatic zones in Nigeria (Hemiptera : Cicadellidae). Bulletin of Entomological Research 77, 1-8.

Price, P.W. (1984) Insect ecology. 2 nd edn. 607 pp. New York, John Wiley & Sons.

Rose, D.J.W. (1973) Laboratory observations on the biology of Cicadulina spp. (Hom., Cicadellidae), with particular reference to the effects of temperature. Bulletin of Entomological Research 62, 471-476.

Ruppel, R.F. (1965) A review of the genus Cicadulina (Hemiptera, Cicadellidae). Publications of the Museum, Michigan State University Biological Series 2, 387-428.

Southwood, T.R.E. (1978) Ecological methods with particular reference to the study of insect populations. 2 nd edn. 524 pp. London, Chapman & Hall.

Van Der Merwe, C.P. (1926) The maize jassid Balclutha mbila Naudé. Journal of the Department of Agriculture of the Union of South Africa 12, 75-77.

Van Rensburg, G.D.J. (1982a) Laboratory observations on the biology of Cicadulina mbila (Naudé) (Homoptera : Cicadellidae), a vector of maize streak disease. 1. The effect of temperature. Phytophylactica 14, 99-107.

Van Rensburg, G.D.J. (1982b) Laboratory observations on the biology of Cicadulina mbila (Naudé) (Homoptera : Cicadellidae), a vector of maize streak disease. 2. The effect of selected host plants. Phytophylactica 14, 109-111.

Van Rensburg, G.D.J. (1983) Southern African species of the genus Cicadulina

China (Homoptera : Cicadellidae) with descriptions of new species. Department of Agriculture, Republic of South Africa, Entomology Memoir No. 57, 22 pp.

Webb, M.D. (1987) Species recognition in Cicadulina leafhoppers (Hemiptera : Cicadellidae), vectors of pathogens of Gramineae. Bulletin of Entomological Research 77, 683-712.

TABLE 1.1 Mean duration (days) $\pm$ SD of different life stages of <u>Cicadulina</u> <u>anestae</u> and <u>C</u>. <u>mbila</u> at constant temperature.

| Species | Temp °C | Egg | Nymphal instar | | | | | | Egg-Adult |
			I	II	III	IV	V	I - V	
<u>C</u>. <u>anestae</u> ♂	18	26.0+0.0 a	9.5+0.5 a	5.5+0.5 a	7.0+0.0 a	9.5+0.5 a	12.5+0.5 a	44.0+1.0 a	70.0+1.0 a
	21	15.0+0.3 b	5.6+0.3 b	2.6+0.3 b	4.2+0.2 b	4.4+0.3 b	9.6+0.4 b	26.4+0.4 b	41.4+0.3 b
	24	12.9+0.5 c	3.5+0.2 c	2.9+0.3 b	3.0+0.2 c	3.9+0.2 b	6.9+0.2 c	20.1+0.6 c	33.0+0.7 c
	27	8.2+0.1 d	3.5+0.2 c	2.2+0.1 b	2.4+0.2 c	2.7+0.2 c	4.2+0.1 d	15.0+0.3 d	23.2+0.4 d
	30	8.3+0.2 d	2.3+0.1 d	2.4+0.2 b	2.8+0.2 c	2.1+0.1 c	3.3+0.1 d	12.9+0.3 e	21.2+0.2 e
♀	18	26.3+0.3 a	8.7+0.3 a	5.3+0.3 a	7.3+0.3 a	9.3+0.3 a	11.3+0.3 a	42.0+0.6 a	68.3+0.3 a
	21	15.1+0.5 b	5.7+0.2 b	3.7+0.2 b	4.2+0.2 b	4.7+0.2 b	9.1+0.4 b	27.3+0.5 b	42.4+0.3 b
	24	13.1+0.3 c	3.7+0.3 c	3.0+0.2 bc	2.9+0.1 c	4.4+0.3 b	5.9+0.2 c	19.9+0.8 c	33.0+0.6 c
	27	9.3+0.4 c	3.7+0.2 c	2.1+0.1 c	2.6+0.2 c	2.7+0.2 c	3.7+0.3 d	14.9+0.6 d	24.1+0.3 d
	30	8.7+0.1 d	2.4+0.1 d	2.6+0.1 c	2.3+0.1 c	2.3+0.2 c	3.7+0.1 d	13.4+0.4 d	22.1+0.3 d
<u>C</u>. <u>mbila</u> ♂	21	16.0+0.5 a	5.1+0.3 a	3.6+0.3 a	5.3+0.3 a	7.4+0.6 a	11.5+1.1 a	32.9+1.2 a	48.9+1.6 a
	24	10.7+0.3 b	3.9+0.5 ab	2.8+0.2 ab	2.8+0.1 b	3.3+0.3 b	5.4+0.2 b	18.2+0.6 b	28.9+0.6 b
	27	7.7+0.3 c	3.7+0.2 b	2.3+0.2 bc	3.0+0.2 b	2.9+0.2 b	4.2+0.1 bc	16.1+0.3 b	23.8+0.4 c
	30	7.8+0.1 c	2.0+0.0 c	2.1+0.1 c	2.6+0.2 b	2.5+0.2 b	3.3+0.2 c	12.4+0.3 c	20.2+0.3 d
♀	21	15.9+0.3 a	5.6+0.4 a	3.6+0.2 a	5.6+0.5 a	6.9+0.4 a	10.8+0.3 a	32.6+1.1 a	48.5+1.1 a
	24	11.3+0.3 b	3.6+0.2 b	2.8+0.2 b	2.9+0.2 b	3.3+0.2 b	5.8+0.3 b	18.3+0.7 b	29.7+0.6 b
	27	8.5+0.2 c	3.6+0.1 b	2.4+0.1 b	2.6+0.1 b	2.9+0.1 b	4.0+0.1 c	15.5+0.3 b	23.9+0.3 c
	30	7.7+0.2 c	2.1+0.1 c	2.1+0.2 b	2.7+0.3 b	2.1+0.1 b	3.2+0.1 c	12.2+0.4 c	19.9+0.4 d

[1]Means followed by the same letter in the same column do not differ significantly (P = 0.05) according to Scheffe's LSD

TABLE 1.2 Sex ratios of <u>Cicadulina</u> <u>anestae</u> and <u>C.</u> <u>mbila</u> obtained at constant temperature.

Species	Temperature	No. surviving to adult stage		Ratio		Calculated Z value
		♂	♀	♂	♀	
<u>C.</u> <u>anestae</u>	18 °C	2	3	1	1.50	0.000 NS
	21 °C	5	9	1	1.80	-0.802 NS
	24 °C	8	14	1	1.75	-1.066 NS
	27 °C	10	7	1	0.70	-0.485 NS
	30 °C	12	18	1	1.50	-0.913 NS
<u>C.</u> <u>mbila</u>	21 °C	8	14	1	1.75	-0.066 NS
	24 °C	10	9	1	0.90	0.000 NS
	27 °C	10	26	1	2.60	-2.500 *
	30 °C	18	10	1	0.56	-1.323 NS

TABLE 1.3 The preoviposition period, day of peak oviposition and fecundity of <u>Cicadulina</u> <u>anestae</u> and <u>C. mbila</u> females reared on wheat seedlings at different temperatures[1].

		Preoviposition period (days)		Day of peak oviposition		Fecundity		Maximum No. of eggs/ female/day	
		<u>C. anestae</u>	<u>C. mbila</u>	<u>C. anestae</u>	<u>C. mbila</u>	<u>C. anestae</u>	<u>C. mbila</u>	<u>C. anestae</u>	<u>C. mbila</u>
Mean ± SD	24 °C	7.8±0.4 a	6.7±0.2 a	9.8±0.5 a	10.8±0.7 a	2.4±0.5 a	6.2±1.1 a	1.2±0.2 a	2.0±0.6 a
	27 °C	6.8±0.5 a	4.4±0.3 b	9.2±0.5 ab	7.5±0.5 b	6.5±1.3 ab	11.1±1.1 a	2.7±0.8 ab	4.3±0.6 ab
	30 °C	4.5±0.8 b	3.3±0.4 c	7.1±0.9 b	6.2±0.4 b	12.2±3.5 b	17.1±2.4 b	5.4±1.2 b	7.2±1.4 b
Range	24 °C	7 - 9	6 - 7	8 - 11	8 - 13	1 - 4	3 - 10	1 - 2	1 - 5
	27 °C	5 - 8	3 - 5	7 - 10	6 - 10	2 - 10	6 - 16	1 - 6	2 - 7
	30 °C	2 - 8	2 - 6	3 - 12	5 - 8	2 - 38	7 - 28	2 - 14	2 - 14

[1]As in table 1.

27

TABLE 1.4 Population reproductive statistics for <u>Cicadulina</u> <u>anestae</u> and <u>C.</u> <u>mbila</u> reared at three temperatures at 14:10 (L:D) photoperiod.

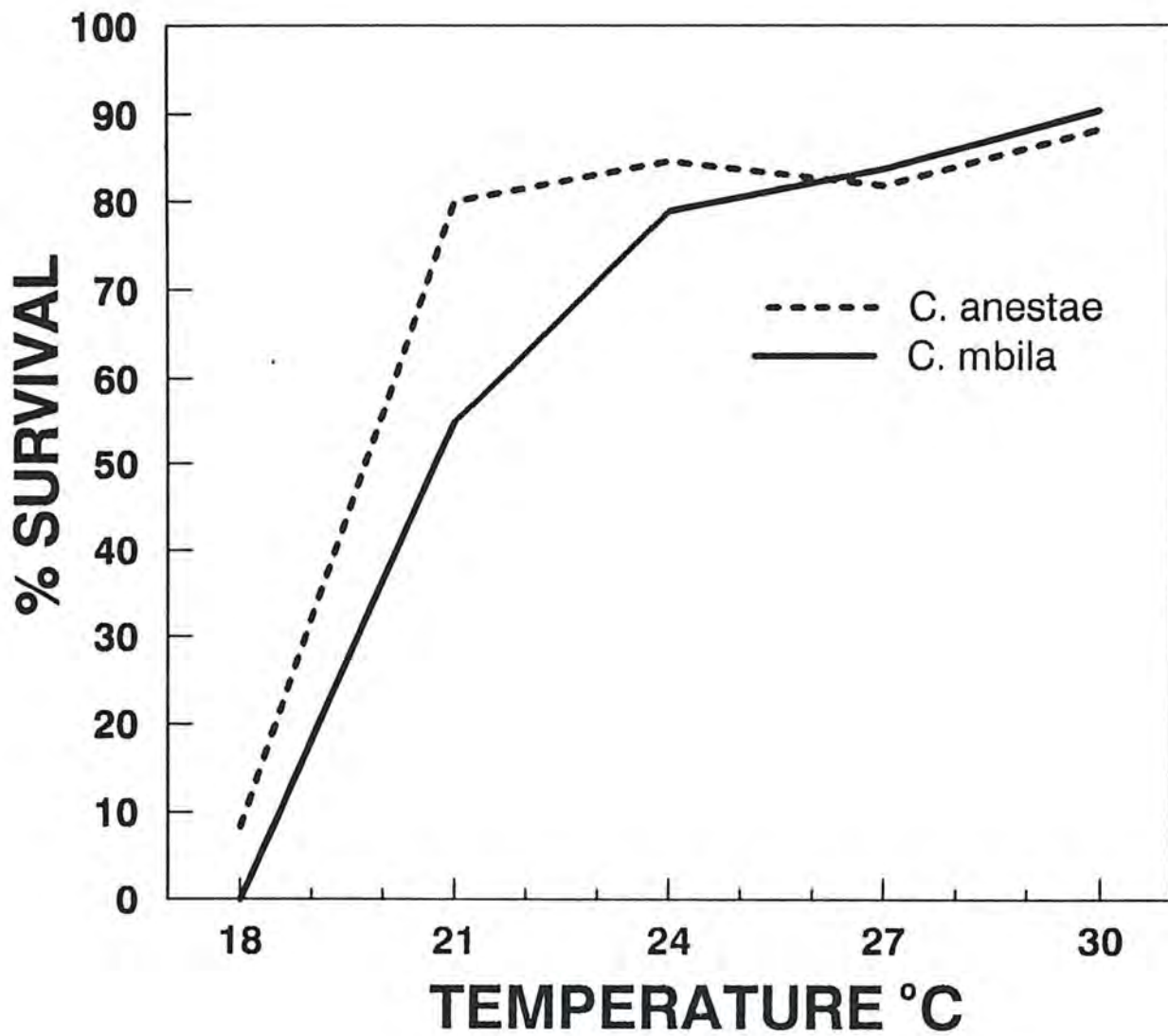| Species	Tempe-rature (°C)	Net repro-ductive rate (R_o)	Generation time (T)	Capacity for increase (r_m)	Finite rate of increase (λ)
C. anestae	24	0.71	42.08	-0.0083	0.9917
	27	1.58	32.80	0.0140	1.0141
	30	4.90	27.92	0.0573	1.0590
C. mbila	24	0.90	38.14	-0.0006	0.9994
	27	4.92	30.54	0.0522	1.0536
	30	3.88	25.56	0.0532	1.0547

FIG. 1.1. Survivorship curves for <u>Cicadulina</u> <u>anestae</u> and <u>C</u>. <u>mbila</u>, plotted as the percent individuals reaching the adult stage at the different temperatures.
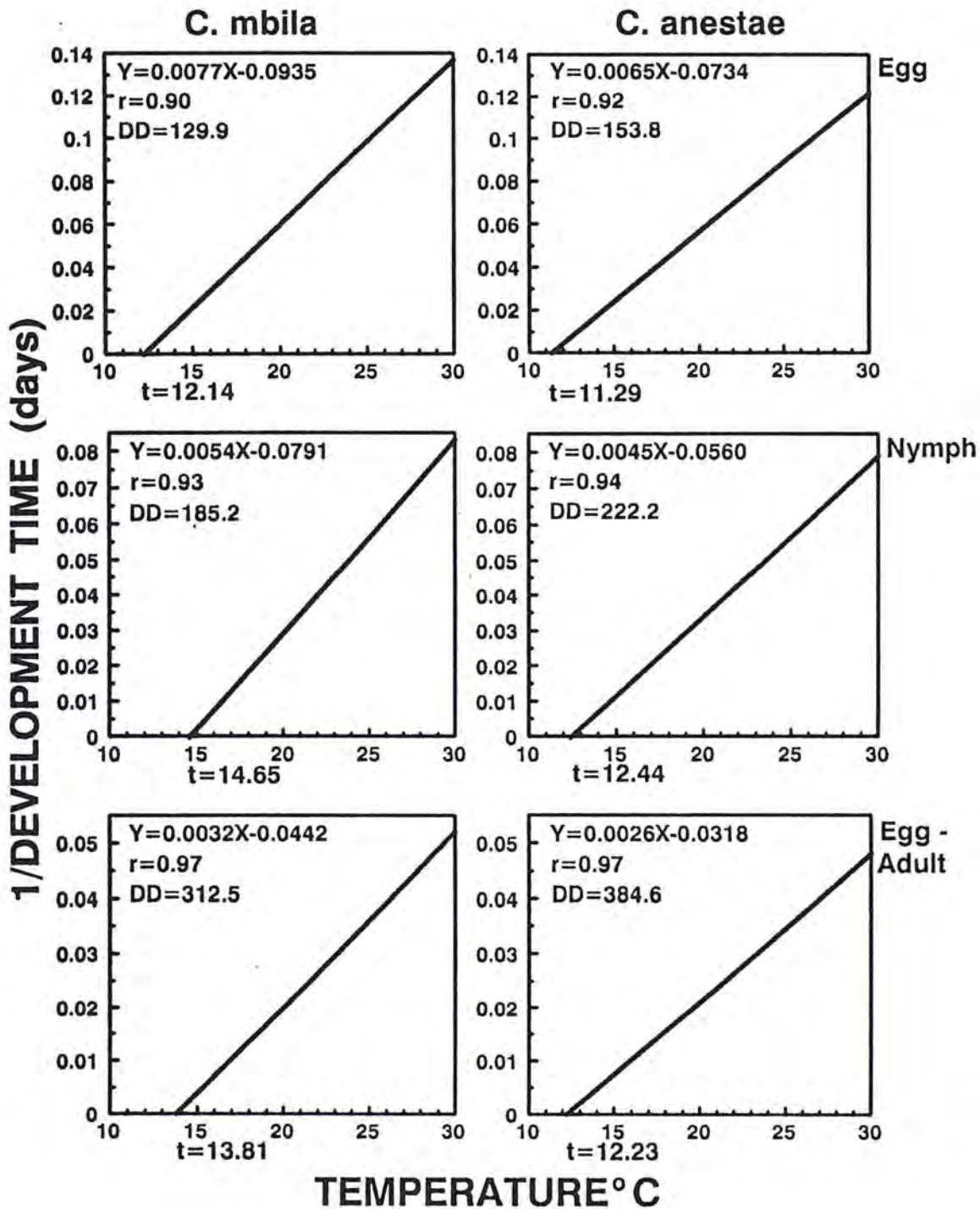
Fig. 1.2 The relationship between temperature and development rate (1/Y) for different life stages of Cicadulina mbila and C. anestae.

CHAPTER 2

SEASONAL POPULATION FLUCTUATIONS OF CICADULINA SPECIES (HOMOPTERA :
CICADELLIDAE)

INTRODUCTION

Maize streak virus (MSV) is spread by several species of the leafhopper genus
Cicadulina (Rose, 1978; Van Rensburg, 1983; Webb, 1987; Chapter 3.)
Cicadulina spp. are host-alternating pests of grain crops (Rose, 1972a;
1973a; Van Rensburg, 1979), and are generally associated with graminaceous
hosts (Rose, 1978) as is MSV (Storey & McClean, 1930; Damsteegt, 1983).

Streak disease of maize was originally limited to the warmer and low lying
parts of South Africa (Storey, 1926). In one particular area of the South
African maize production triangle, the North-Western Orange Free State, maize
streak disease reached epidemic levels during the seventies (Van Rensburg,
1979). The disease has since caused damage to maize crops at irregular
intervals. Van Rensburg (1979) concluded that Cicadulina has two major,
annual migration flights in South Africa, the one during autumn from maturing
maize to winter wheat and the second flight during spring from maturing wheat
to early planted maize.

Since MSV causes the greatest yield loss in maize infected at an early growth
stage (Guthrie, 1978; Soto & Buddenhagen, 1978; Van Rensburg, 1981; Mzira,
1984), spring migration of vectors would be very important in areas where
early planting of maize occur. Initial infection of such maize plantings

would depend on the numbers of migrant leafhoppers, the proportion of viruliferous leafhoppers and the species composition of the Cicadulina population. These have been combined in an infectivity index to give a measure of infection probability (Chapter 3), which was estimated to be at least 0,458 with a population comprising of Cicadulina anestae Van Rensburg and C. mbila (Naudé).

Population studies pertaining to Cicadulina spp. have been conducted in various parts of Africa. These studies have dealt mainly with seasonal occurrence (Rose, 1972a), population cycles in relation to host grasses (Rose, 1973a), dispersal (Rose, 1972b; 1973b), and population densities in maize (Rose, 1974) in Zimbabwe. In South Africa field observations focussed primarily on the epidemiology of maize streak disease in relation to vector migration (Van Rensburg & Kühn, 1977; Van Rensburg, 1979). Cicadulina populations from various localities in Nigeria were largely similar in their ability to acquire and transmit MSV (Okoth et al., 1987). Dabrowski (1988) illustrated that the artificial large scale release of C. triangula did not have a pronounced effect on the species composition or population density of field populations in Nigeria. However, rainfall and temperature were noted to affect species composition and abundance of Cicadulina populations in Nigeria and Togo (Anon, 1985). Leafhopper migration seems to be associated with the age and physiological condition of the host plant (Rose, 1972a; 1973a; 1973b; Van Rensburg, 1979; Okoth & Dabrowski, 1987).

The wide distribution and economic importance of Cicadulina species as vectors of MSV emphasize the need for ecological studies to complement conventional pest management strategies concerning these leafhoppers. Our objective therefore was to determine the seasonal abundance of Cicadulina populations

and to identify those climatic factors which could explain fluctuations in leafhopper numbers. An account of sticky-trap catch data obtained from 1982 to 1987 and subjected to time series analysis (Kendall, 1974), is presented.

MATERIALS AND METHODS

The seasonal incidence of leafhopper populations was studied at two localities in irrigation areas, viz. the research stations at Vaalharts (27° 57' S, 24° 51' E) and Sandvet (28° 07' S, 26° 38' E). Four sticky traps were used to trap adult leafhoppers at each locality. The traps were placed 200 m apart on the borders of cultivated fields in a more or less square pattern. Trap catches were associated with maize during the period October to April, and with winter wheat from May to September. It was very rarely necessary to move traps from their initial positions, and then at most 250 m to an adjacent field. Each trap consisted of two yellow hardboards (each 130 mm H x 250 mm W), suspended at right angles from iron reïnforcement rods 0,8 m above the ground. The full surface of each hardboard was coated with an insect adhesive (Formex) on both sides so that each trap presented sticky surfaces in all major wind directions. Sticky boards were replaced every 15 $\pm$ 1 d, which was a practical time period.

The use of wooden boxes with grooves into which hardboards fitted upright provided a convenient method of transporting laboratory-prepared hardboards to, and used hardboards from trapping sites. The used traps were placed over a template and the leafhoppers counted under a stereomicroscope, after which the traps were cleaned and prepared for re-use.

The traps were operated continuously from January 1982 to July 1987. Two

species, $\underline{C}$. $\underline{anestae}$ and $\underline{C}$. $\underline{mbila}$, were trapped at both localities. The numbers of adult leafhoppers for each species caught in each of the four traps during the 15 d periods were $\log_e (x + 1)$ transformed. Correlation between samples was determined for each locality and species by means of autocorrelation analysis in an attempt to reveal periodicities in the data. Spectral analysis was applied to the data because of the close relationship between the spectrum and correllogram (serial correlation against lag), and the fact that both provide useful information on the time series (Kendall, 1974). Peaks in the spectrum would indicate large regression sums of squares and thus the existence of cycles in the data at those frequencies.

Total number of degree-days accumulated per season were determined from the temperature records of each locality, using 14^oC and 12^oC as base temperatures for $\underline{C}$. $\underline{mbila}$ and $\underline{C}$. $\underline{anestae}$, respectively (Chapter 1). From these data, the likely number of generations per season for each species was calculated, using DD requirements for completion of development (egg to adult) by the two species, viz. 312,5 DD for $\underline{C}$. $\underline{mbila}$ and 384,6 DD for $\underline{C}$. $\underline{anestae}$ (Chapter 1).

The relationship between leafhopper catches and different climatological parameters was determined by means of lag correlation analyses. Meteorological data were obtained from records collected daily at official meteorological stations at both localities. Although trap data suggested that leafhoppers migrated from a northerly direction at both localities, trap catches associated with the main wind directions did not differ significantly, and no further mention will thus be made of wind direction. Maximum temperature, minimum temperature, mean temperature, rainfall, number of sun-hours, windspeed and degree-days were used in these analyses. Degree-days

were calculated using the mentioned development thresholds of 14 $^{\circ}$C for $\underline{C}$. $\underline{mbila}$ and 12°C for $\underline{C}$. $\underline{anestae}$. Multiple regression of lagged data of those factors indicated by correlation analysis to have been significant, were performed on leafhopper numbers.

RESULTS

The numbers of $\underline{Cicadulina}$ captured at Vaalharts and Sandvet in the sticky traps from January 1982 to June 1987 are shown separately for $\underline{C}$. $\underline{mbila}$ and $\underline{C}$. $\underline{anestae}$ and for each locality in Fig 2.1. The average seasonal flight activities calculated from these catches are presented in Fig 2.2. The inception of the flight activity period (after 1 July) was characterized by very small catches of both species irrespective of locality. The average date of first capture and of peak numbers, as well as the duration of flight activity for both species at the two localities are given in Table 2.1. $\underline{C}$. $\underline{anestae}$ commenced its "spring flight" earlier than did $\underline{C}$. $\underline{mbila}$ at both localities, the observed difference in average date of first capture between the two species being 16 d at Vaalharts and 34 d at Sandvet. In general, flight activity commenced earlier and lasted longer at Sandvet than at Vaalharts. Marked decreases in leafhopper numbers occurred during October and again in December, and numbers only increased rapidly and relatively consistently from January (Fig 2.2) to reach a peak during late summer (Table 2.1). Flight activity declined dramatically after peaks had been reached, and no flight was recorded for either species during July except during the latter half of July 1983 when a single adult of $\underline{C}$. $\underline{anestae}$ was captured.

$\underline{Cicadulina}$ numbers varied with sampling dates throughout the growing season (Fig 2.1). This may have resulted from environmental influences on the

leafhopper populations and movement among habitats. Nevertheless, two main flight-activity periods were observed for both species, the first from August to December and the second from January to June. Significantly fewer adults were captured during the first period than during the second for both species at either locality (Table 2.2). The greatest total number of adults captured during a season (July to June) occurred at Sandvet with regard to both C. mbila (N = 496; 1985/86) and C. anestae (N = 768; 1986/87) (Table 2.2). Overall, C. mbila constituted approximately 54 % of the total population at both localities. The number of generations per season calculated for each species varied from season to season and between seasons (Table 2.3). Overall, both species appear to have at least seven generations per season at Vaalharts, and five generations at Sandvet.

Fig. 2.3 shows the correlograms (serial correlation coefficient against lag) of the data of both species at Vaalharts and Sandvet. It shows the annual cycle in the catches, and although there is a hint of a cycle with wavelength about 1 480 days (four years) especially with regard to C. anestae, a longer time series data set is clearly needed to firmly establish long-term cycles. Apart from the annual cycles, the spectra did not provide additional information on periodicities of data and are therefore not shown.

The results of the cross-correlation analysis, using the leafhopper catches of each species at each locality separately as input data vectors and various environmental factors as output data vectors, are given in Table 2.4. The data on DD and rainfall were lagged as indicated in Table 2.4 in relation to Cicadulina data in order to explain the annual variation in leafhopper flight.

Linear regressions of Cicadulina numbers on lagged DD data (base temperatures

of $14^{\circ}C$ and $12^{\circ}C$ for C. mbila and C. anestae, respectively) were highly significant (Fig 2.4). By including total rainfall over each sampling period as a second variable in multiple linear regression analysis, the efficiency did not improve significantly (only 1 % improvement in explaining the variation in leafhopper numbers).

DISCUSSION

The interpretation of trap catch data is often complicated by the confounding effects of both the physical and biological environment on activity and population size. The numbers of leafhoppers caught in the traps can thus be expected to be a reflection of both flight activity and the size of the population at given times.

In spite of a large annual variation in total numbers of Cicadulina captured in the sticky traps at Vaalharts and Sandvet, the seasonal flight activities of both species tended to repeat the same trend. Some conclusions on the implications for MSV epidemiology may be deduced from the observed seasonal flight activity. The variation in the date of commencement of flights and the subsequent overlapping of generations signifies that MSV infection of maize crops can be expected at virtually any time throughout the growing season. However, since Cicadulina species and sexes differ with regard to their vectoring ability, factors such as species composition, sex ratio and population size have been indicated in Chapter 3 to play an important role in MSV epidemiology. The present study has shown that the flight-activity patterns and size of flights are significantly related to DD. Since the data were collected during a prolonged drought period, it is possible that the effect of rainfall on the seasonal abundance and activity of the leafhoppers

could have been underestimated, since Rose (1972a; 1973b) associated rainfall with the size of <u>Cicadulina</u> populations in Zimbabwe, and temperature with the duration of seasonal flight periods.

The disease-ridden area of the North-Western Orange Free State is situated in a part of the Highveld where temperatures are less severe during winter and higher during summer than localities lying further east where the disease occurs only sporadically and is limited to isolated infected plants. Furthermore, precipitation in the eastern maize producing areas is higher than in the disease-ridden area, indicating that rainfall is unlikely to be the factor which prevents epidemic outbreaks of MSV-disease in the eastern Highveld. Rose (1973a) and the present author (Chapter 1) observed high nymphal mortalities at low temperatures. The chances of survival of the leafhopper could therefore be increased by moderate winters. However, the leafhoppers are also dependent for their survival in the Highveld on the seasonal succession of maize and wheat crops, and the ready availability of alternate hosts such as annual grasses. The influence of rainfall on leafhopper numbers thus seems to be indirect since the suitability and abundance of host plants, especially natural grasses, depends on rainfall patterns. On the other hand, the effect of temperature on the activity and development of the <u>Cicadulina</u> populations is more direct. The sharp decline in numbers of leafhoppers during October can be attributed to widely fluctuating temperatures experienced during spring.

The seasonal flight activity pattern of <u>Cicadulina</u> in Zimbabwe differs somewhat from that observed in South Africa. Rose (1972a) found that the main flight period in Zimbabwe extends from March to September, while very few <u>Cicadulina</u> are captured during the summer months from October to February.

The present study revealed that the main flight period in South Africa extends from January to May, with a smaller flight from August to December. In South Africa the rainy season extends from October to March and, according to Rose (1978), from November to March in Zimbabwe. Rose (1972a) found a significant and positive correlation between the amount of rain at the end of each wet season and the numbers of Cicadulina caught in suction traps the following spring. The result was, however, attributed to the indirect effect of rainfall on population size since late rains ensures an abundance of grass suitable for development of nymphs during the winter.

Rose (1973b) found 4-5 population cycles per year in irrigated pastures and showed that the cycles were largely predictable by using ambient temperatures in equations expressing the development rate of the leafhoppers. C. chinai has nine generations (Ammar, 1975), and C. bipunctata has eight generations (Ammar, 1977) at ambient temperatures in Egypt. There was no clear distinction between the flights of the different generations of the Cicadulina species in the present study, possibly due to rapid population build-up and the consequent overlapping of generations. However, the indication of five to seven peaks occurring throughout a growing season (Fig 2.2) is in agreement with the calculated number of generations (Table 2.4). The difference between the localities can be ascribed to the cooler climate at Sandvet, since temperature expressed as degree-days appears to be the primary factor regulating the incidence and duration of seasonal flights.

The implications of the observed seasonal flight activity of Cicadulina populations for MSV epidemiology is evident. The risk of leafhopper colonization, MSV infection and spread would be greater in years when early planting of maize would result in crop emergence coinciding with the spring

flight. Severe yield losses could occur in these early plantings since yield loss is inversely related to the plant age at which infection takes place (Van Rensburg, 1981). Because flight activity declines sharply during December, maize planted during November are likely to escape serious infestation at a time when the crop is most prone to severe yield loss. At the time of the second seasonal increase in leafhopper numbers (towards the end of December), these plants would have grown past highly susceptible stages. However, very late plantings would probably be heavily infested as a result of the "early summer flight" coinciding with the incidence of relatively young plants. The patterns of MSV infection observed in maize in South Africa (Van Rensburg, 1979) can thus be explained by the seasonal flight activity of the vectors in relation to temperature.

REFERENCES

AMMAR, E.D. 1975. Biology of the leafhopper Cicadulina chinai Ghauri (Homoptera, Cicadellidae) in Giza, Egypt. Zeitschrift für Angewandte Entomologie 79, 337-345.

AMMAR, E.D. 1977. Biology of Cicadulina bipuntella zeae China in Giza, Egypt (Homoptera, Cicadellidae). Deutsche Entomologische Zeitschrift 24, 345-352.

ANON. 1985. Maize improvement program research highlights 1981-1984. IITA, Ibadan, Nigeria, 48 pp.

DAMSTEEGT, V.D. 1983. Maize streak virus : 1. Host range and vulnerability of maize germ plasm. Plant Disease 67, 734-737.

DABROWSKI, Z.T. 1988. Effect of the release of viruliferous Cicadulina triangula Ruppel adults on population density and species composition of "wild" Cicadulina spp. leafhoppers (Hemiptera, Euscelidae). Journal of Applied Entomology 105, 450-454.

GUTHRIE, E.J. 1978. Measurement of yield losses caused by maize streak disease. Plant Disease Reporter 62, 839-841.

KENDALL, M.G. 1976. Time-series. 2nd edn. 197 pp. London, Charles Griffin and Company.

MZIRA, C.N. 1984. Assessment of effects of maize streak virus on yield of maize. Zimbabwe Journal of agricultural Research 22, 141-149.

OKOTH, V.A.O. & DABROWSKI, Z.T. 1987. Population density, species composition and infectivity with maize streak virus (MSV) of Cicadulina spp. leafhoppers in some ecological zones in Nigeria. Oecologia Applicata 8, 191-200.

OKOTH, V.A.O., DABROWSKI, Z.T., THOTTAPPILLY, G. & VAN EMDEN, H.F. 1987. Comparative analysis of some parameters affecting maize streak virus (MSV) transmission of various Cicadulina spp. populations. Insect Science & Its Application 8, 295-300.

ROSE, D.J.W. 1972a. Times and sizes of dispersal flights by Cicadulina species (Homoptera : Cicadellidae), vectors of maize streak disease. Journal of Animal Ecology 41, 495-506.

ROSE, D.J.W. 1972b. Dispersal and quality in populations of Cicadulina species (Cicadellidae). Journal of Animal Ecology 41, 589-609.

ROSE, D.J.W. 1973a. Field studies in Rhodesia on Cicadulina spp. (Hem., Cicadellidae), vectors of maize streak disease. Bulletin of Entomological Research 62, 477-495.

ROSE, D.J.W. 1973b. Distances flown by Cicadulina spp. (Hem., Cicadellidae) in relation to distribution of maize streak disease in Rhodesia. Bulletin of Entomological Research 62, 497-505.

ROSE, D.J.W. 1974. The epidemiology of maize streak disease in relation to population densities of Cicadulina spp. Annals of Applied Biology 76, 199-207.

ROSE, D.J.W. 1978. Epidemiology of maize streak disease. Annual Review of Entomology 23, 259-282.

SOTO, P.E. 1978. Yield loss from a localized epidemic of maize streak virus in Nigeria. East African agricultural and Forestry Journal 44, 175-177.

STOREY, H.H. 1926. Streak disease of maize. Farming in South Africa 1, 183-185.

STOREY, H.H. & McCLEAN, A.P.D. 1930. The transmission of streak disease between maize, sugar-cane, and wild grasses. Annals of Applied Biology 17, 691-719.

VAN RENSBURG, G.D.J. 1979. The ecology and culture of leafhoppers (Cicadulina spp.). In H.O. Gevers, (Ed.). Proceedings of the second South African Maize Breeding Symposium, 1976. Republic of South Africa Department of Agricultural technical Services, Technical Communication 142, 100-103.

VAN RENSBURG, G.D.J. 1981. Effect of plant age at the time of infection with maize streak virus on yield of maize. Phytophylactica 13, 197-198.

VAN RENSBURG, G.D.J. 1983. Southern African species of the genus Cicadulina China (Homoptera : Cicadellidae) with descriptions of new species.

Department of Agriculture, Republic of South Africa, Entomology Memoir no. 57.

VAN RENSBURG, G.D.J. & KüHN, H.C. 1977. Maize streak disease. Department of agricultural technical Services of South Africa Maize Series Leaflet No. E.3..3 (Farming in South Africa), pp. 1-4.

WEBB, M.D. 1987. Species recognition in Cicadulina leafhoppers (Hemiptera : Cicadellidae), vectors of pathogens of Gramineae. Bulletin of entomological Research 77 : 683-712.

TABLE 2.1 The average number of days after 1 July for first and peak captures
of _Cicadulina_ adults, and the length of flight activity (days) at
Vaalharts and Sandvet.

	C. mbila		C. anestae	
	Vaalharts	Sandvet	Vaalharts	Sandvet
First capture:	85	79	69	45
	(23 Sept)	(17 Sept)	(7 Sept)	(14 Aug)
Peak capture:	226	259	244	254
	(11 Febr)	(16 March)	(1 March)	(11 March)
Flight activity period:	258	264	285	297

TABLE 2.2 Total numbers of <u>Cicadulina</u> captured during the two main flight periods at Vaalharts (VH) and Sandvet (SV) over the period 1982-1987.

Period	C. mbila						C. anestae					
	July - December		January - June		Total		July - December		January - June		Total	
	VH	SV	VH	SV	VH	SV	VH	SV	VH	SV	VH	SV
1982	-	-	533	479	533	479	-	-	504	412	504	412
1982/83	29	25	222	82	251	107	36	91	108	52	144	143
1983/84	35	5	149	122	184	127	4	6	20	43	24	49
1984/85	16	9	115	464	131	473	9	6	166	276	175	282
1985/86	4	4	170	492	174	496	15	37	105	210	120	247
1986/87	13	16	65	442	78	458	32	119	120	649	152	768
Total	97	59	1 254	2 081	1 351	2 140	96	259	1 023	1 642	1 119	1 901
Average	19,4	11,8	209,0	346,8	-	-	19,2	51,8	170,5	273,7	-	-

TABLE 2.3 Total degree-days accumulated per season and the calculated number of _Cicadulina_ generations per season (DD above 14 $^{\circ}$C relate to _C. mbila_ data and DD above 12 $^{\circ}$C to _C. anestae_ data).[1]

Season (July to June)	Vaalharts				Sandvet			
	DD_{14}	No of _C. mbila_ generations	DD_{12}	No of _C. anestae_ generations	DD_{14}	No of _C. mbila_ generations	DD_{12}	No of _C. anestae_ generations
1982/83	2 226,9	7,13	2 976,9	7,74	1 939,7	6,21	2 677,7	6,96
1983/84	2 173,3	6,95	2 911,3	7,57	1 801,1	5,76	2 539,1	6,60
1984/85	2 132,3	6,82	2 870,3	7,46	1 601,8	5,13	2 339,8	6,08
1985/86	2 221,1	7,11	2 959,1	7,69	1 823,5	5,84	2 561,5	6,66
1986/87	2 '231,9	7,14	2 969,9	7,72	1 797,4	5,75	2 535,4	6,59
Mean	2 197,1	7,03	2 937,5	7,64	1 792,7	5,74	2 530,7	6,58
± S.E.	19,3	0,06	20,3	0,05	54,4	0,17	54,4	0,14

[1]Calculations based on 312,5 DD required by _C. mbila_ to complete development from the egg to the adult stage, and 384,6 DD required by _C. anestae_ (Van Rensburg & Giliomee, In press).

TABLE 2.4 Serial correlation coefficients for leafhopper numbers [Ln (catch + 1)] and various factors at two localities (r_k/lag (sampling periods))

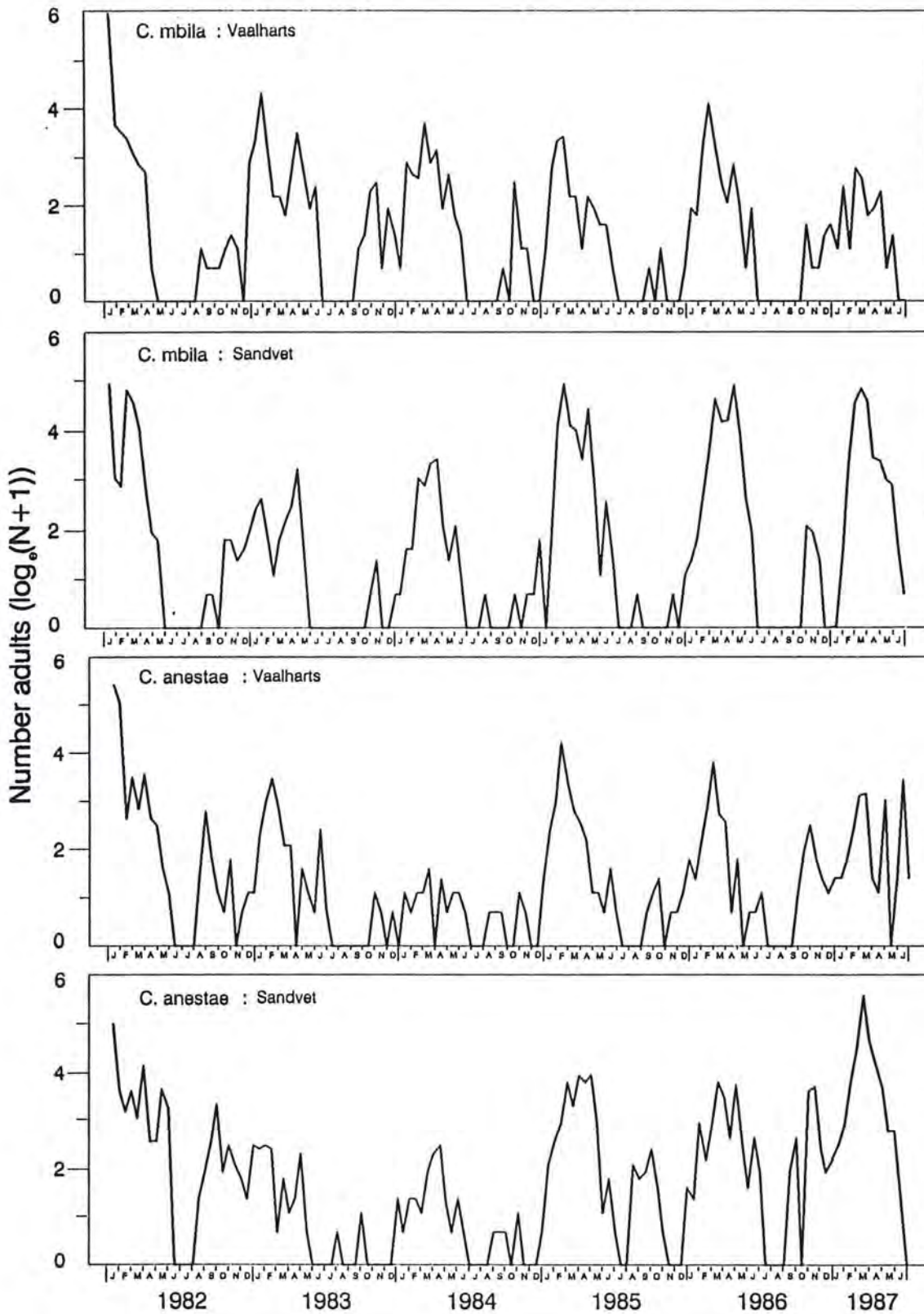| Factors	C. mbila		C. anestae	
	Vaalharts	Sandvet	Vaalharts	Sandvet
DD_{14}	0,6730/3	0,7020/3	-	-
DD_{12}	-	-	0,5225/3	0,5050/3
Ln (total rainfall + 1)	0,4550/1	0,3752/4	0,3510/1	0,3786/4

Fig 2.1 Numbers of adult <u>Cicadulina</u> <u>mbila</u> and <u>C.</u> <u>anestae</u> caught in sticky traps at 15 $\pm$ 1 d intervals from January 1982 to June 1987.
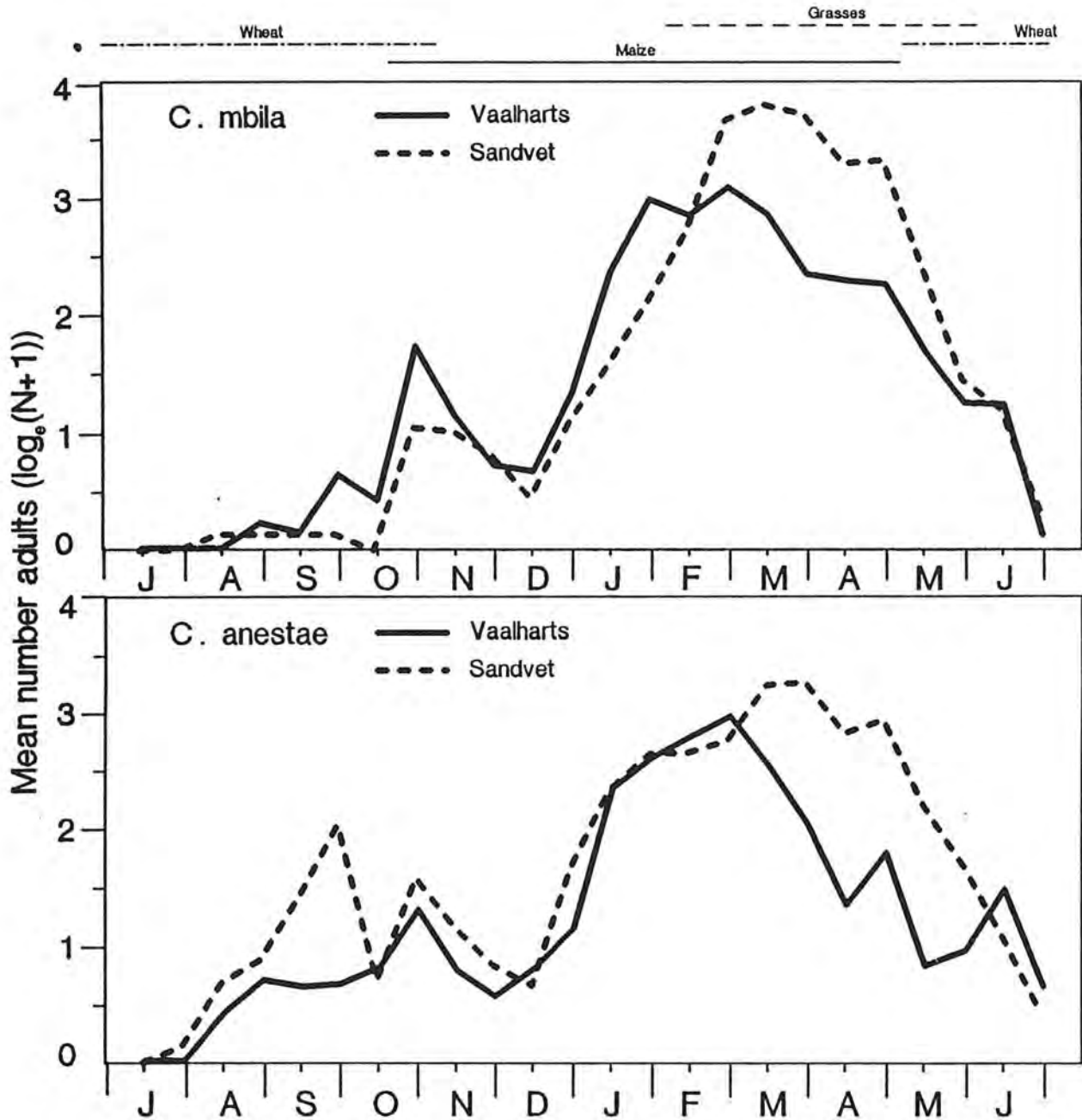
Fig 2.2 Seasonal population fluctuations of Cicadulina mbila and C. anestae at
two localities as indicated by sticky trap catches for the period
January 1982 to June 1987.
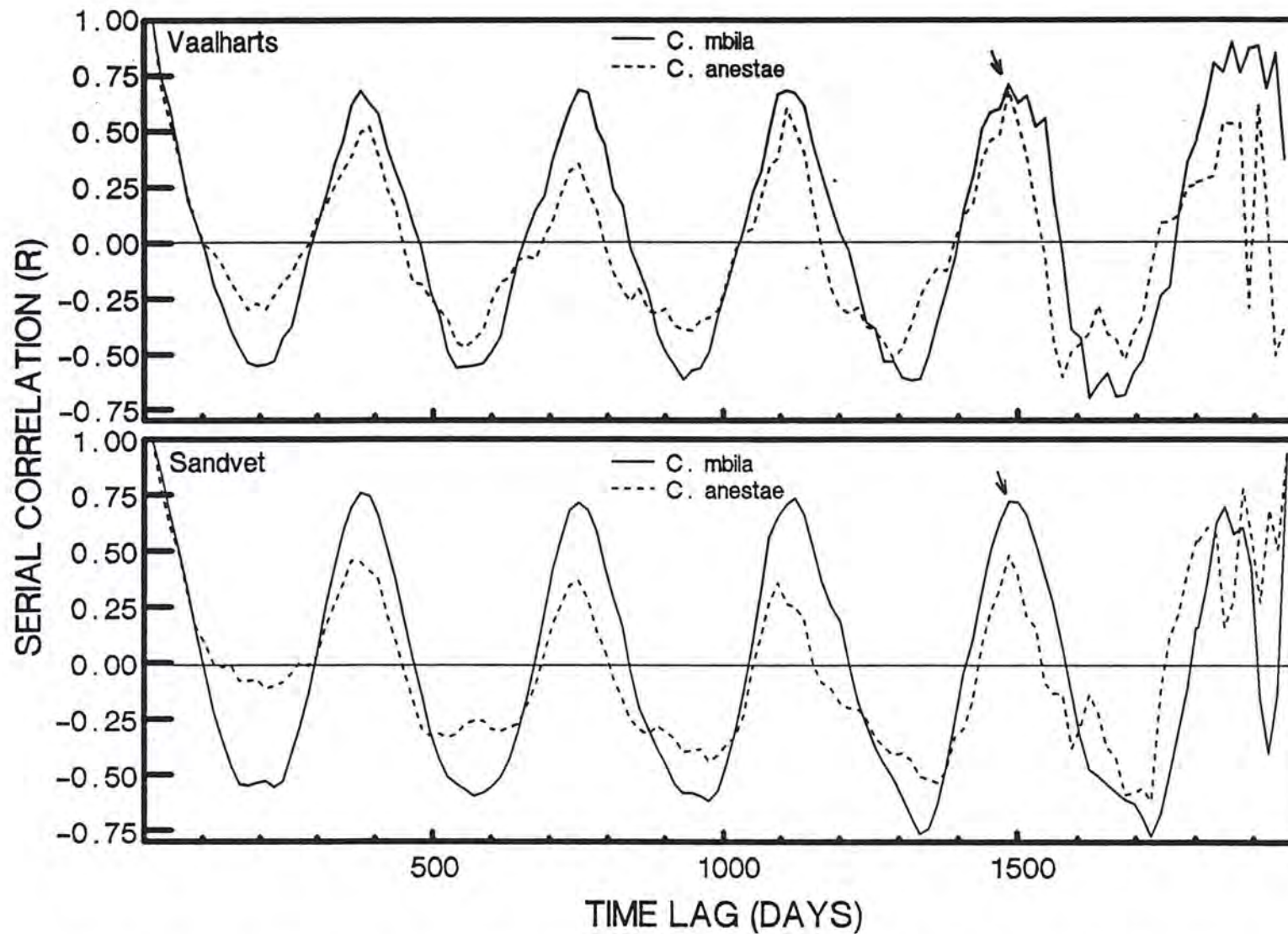
Fig 2.3 The serial correlation coefficients (R) plotted against time lag, showing the annual cycles in size of catch. A slight increase in amplitude of the coefficient at about 1480 days (arrow), especially with regard to C. anestae, suggests the existence of a four-year cycle.

Fig 2.4 The relationship between temperature (expressed as degree-days) and leafhopper numbers. (Note the difference in base temperature on X axis: Degree-days above 14 ^{o}C for _Cicadulina mbila_ and above 12 ^{o}C for _C. anestae_).

CHAPTER 3

A COMPARISON OF FEMALES AND MALES OF CICADULINA ANESTAE AND C. MBILA
(HOMOPTERA : CICADELLIDAE) AS VECTORS OF MAIZE STREAK VIRUS

INTRODUCTION

At least eight species of the cicadellid genus Cicadulina China are
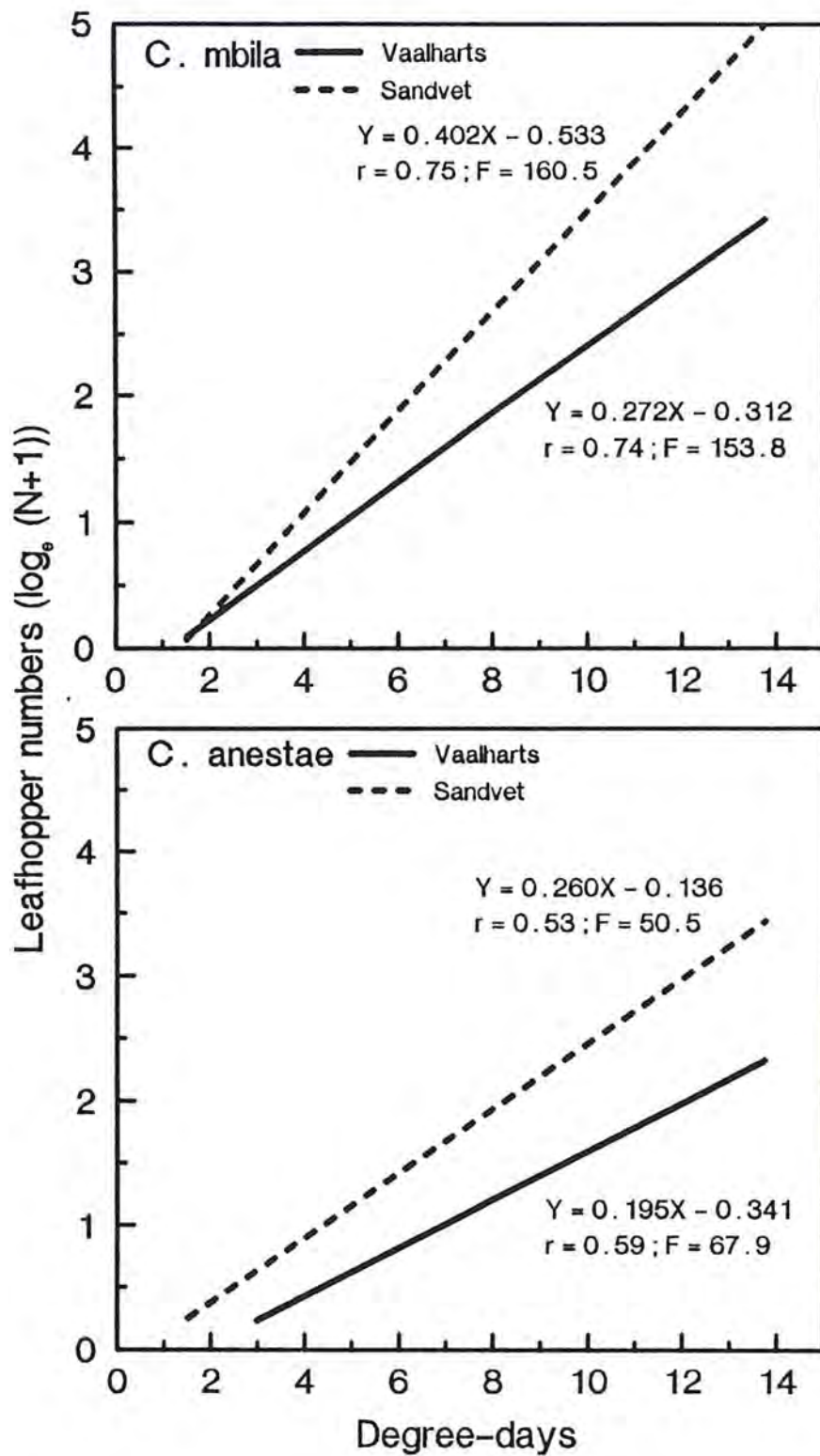vectors of maize streak virus (MSV): C. mbila (Naudé), C. storeyi China (= C.
triangula Ruppel; Webb, 1987), C. parazeae Ghauri, C. latens Fennah, C.
bipunctata (Melichar) (= C. bipunctella zeae China; Heller & Linnavuori,
1968; Vilbaste, 1976), C. arachidis China, C. similis China and C. ghaurii
Dabrowski (Fennah, 1960; Ruppel, 1965; Nielson, 1968; Ishihara, 1969;
Bock, 1974; Soto, 1978; Dabrowski, 1987). C. chinai Ghauri transmitted MSV
following artificial injection of the virus, but failed to acquire and
transmit the virus from an infected to a healthy plant (Markham et al., 1984).
Of these species, C. mbila is considered the most important MSV vector, not
only by virtue of its wide geographical distribution (Ruppel, 1965; Rose,
1978; Van Rensburg, 1983), but also because significantly larger proportions
of C. mbila populations proved to be viruliferous in comparison with other
Cicadulina species (Storey, 1928; 1933; Markham et al., 1984).

During field studies in South Africa from 1982-1987, large numbers of
C. mbila and C. anestae Van Rensburg were recorded in sticky traps in the
vicinity of maize plantings with a high incidence of maize streak disease. As
C. anestae accounted on average for 43,5 % of the Cicadulina population caught
annually in the traps, it was imperative to establish the importance of this
species in maize streak virus epidemiology.

METHODS

Adults of C. mbila and C. anestae were collected during February 1988 at the Vaalharts ($27^{o}57'S;24^{o}51'E$) and Sandvet ($28^{o}07'S;26^{o}38'E$) experimental stations and transported, using the method described by Van Rensburg & Walters (1977), to Potchefstroom ($26^{o}44'S;27^{o}05'E$) to establish colonies. The colonies were maintained in a greenhouse at a day/night temperature regime of 26/18 ^{o}C ($\pm$ 1,5 ^{o}C), using an MSV susceptible maize hybrid (PNR6428) as host plant for both the virus and the leafhopper. The identities of C. mbila and C. anestae were confirmed by comparison with type specimens and species descriptions (Van Rensburg, 1983; Webb, 1987). Voucher specimens of these greenhouse populations have been deposited in the insect collection of the Department of Entomology and Nematology, University of Stellenbosch.

Prior to transmission studies, adults were allowed acquisition feeding for 90 d on greenhouse sources of MSV-infected maize (PNR6428). Single females and males of both Cicadulina spp. were then transferred to uninfected plants of the MSV-susceptible homozygous maize line P134, grown in pots and kept under lantern glasses closed off with gauze (Van Rensburg, 1979b) to avoid extraneous MSV infection. Plants were inoculated at the one- to two-leaf stage, 8 to 10 d after emergence, by allowing the leafhoppers to feed on the plants for 24 h. The leafhoppers were then removed and the plants transferred to an insect-free greenhouse and kept at a day/night temperature of 30/23 ^{o}C for 20 d for disease symptoms to develop. A positive transmission was indicated by the appearance of typical MSV symptoms. The number of days required for symptoms to appear was also recorded. Sixty control plants, unexposed to leafhoppers, were kept under similar conditions during the period

of investigation.

The data were analyzed as a 2x2 factorial according to the methods of Cox (1970) for binary data, using linear contrasts to determine whether the species and the sexes differed from each other with regard to the ability to transmit MSV (two-tailed tests). Untreated plants were similarly compared with inoculated plants, using a right one-sided test.

RESULTS

The results and analysis of the MSV transmission tests are summarized in Fig. 3.1. Both C. mbila and C. anestae were able to transmit MSV to healthy maize plants. The species by sex interaction was significant (P=0,03) due to the greater differences in transmission between sexes for C. mbila than C. anestae. However, comparison of the 95 % confidence intervals (Fig.3.1) indicate that there is a tendency for a higher proportion of females than males to transmit the virus, and that a higher proportion of C. mbila than C. anestae transmitted the virus. None of the control plants showed MSV symptoms.

The proportion of viruliferous C. mbila females and males in the present study was very similar to the respective 85 % and 56 % reported by Storey (1928) for the same species. However, various workers found inter- and intra-specific differences in the efficiency of Cicadulina species with regard to MSV transmission. According to Markham et al. (1984) 60 % to 100 % of C. mbila populations are vectors, and 5 % to 15 % of both C. bipunctata (= C. bipunctella) and C. storeyi (= C. triangula) populations. Soto (1978) recorded 29 % for C. triangula. Storey (1932) introduced the terms "active"

and "inactive" leafhoppers to explain the variation in virus transmission results obtained with C. mbila. C. bipunctata (= C. zeae China) and C. storeyi apparently also have inactive individuals in their populations (Storey, 1939). Storey (1932) showed that the ability to transmit MSV is an inherited, dominant, and sex-linked character, with the male being heterozygous.

MSV symptoms appeared somewhat sooner on plants exposed to females (4,6 ± 1,9 d for C. mbila and 10,3 ± 3,6 d for C. anestae) than on plants exposed to males (5,9 ± 2,5 d for C. mbila and 11,8 ± 3,6 d for C. anestae). Alivizatos & Markham (1986) ascribed the differences in the ability of sexes of the leafhopper Dalbulus maidis (DeLong & Wolcott) to transmit the corn stunt spiroplasma to differential feeding behaviour. Since Cicadulina was observed to exhibit the same feeding behaviour as described for D. maidis, the MSV transmission data for Cicadulina species may also reflect the difference in feeding behaviour between the sexes.

Van Rensburg (1982) found that the sex ratio of C. mbila varied from 1:1 to 1:2 (♂ : ♀) at different temperatures. The probabilities that a maize plant could become infected with MSV following leafhopper feeding are presented in Table 3.1 for the two different sex ratios of both C. mbila and C. anestae. The proportion of C. mbila to C. anestae of 0,565 : 0,435 found in natural populations, was used to calculate the probability for either species to transmit MSV to maize. A female-biased population can increase the initial infection by at least 5 % to 7 % (Table 3.1).

DISCUSSION

Successive cultivation of winter wheat and maize allows the leafhoppers to survive by migrating to alternative host plants, with grasses playing an important role as interim hosts during the autumn (Van Rensburg, 1979a). From our results, it appears that the initial MSV infection caused by leafhopper invasion of maize, and the subsequent development of the disease will largely depend on the species composition and sex ratio of the invading Cicadulina population. However, the role of each of the sexes and species in maize streak epidemiology needs to be assessed in relation to population size and vector activity. Furthermore, MSV is not transovarially transmitted and since each leafhopper generation must acquire the virus from infected plants, the availability of streak-diseased host plants would obviously also play an important role in the development of the disease.

The progress and success of resistance breeding programmes against maize streak under conditions of natural infestation will evidently depend on the composition of the Cicadulina population. Our results suggest that localities where the population consists mainly of C. mbila should be selected for screening maize germplasms for resistance to MSV. Even in the case of controlled infection for screening of maize germplasm (Van Rensburg, 1979b), C. mbila should be preferred as the vector species to ensure a high level of success. The exclusive use of females holds additional advantage, since they proved to be 100 % more efficient regarding vectoring ability than males (Table 3.1). Instead of attempting to improve the vectoring ability of less efficient vector species as suggested by Soto (1978), the superb efficiency of C. mbila as a vector of MSV should rather be exploited in bioassay studies.

REFERENCES

ALIVIZATOS, A.S. & MARKHAM, P.G. 1986. Acquisition and transmission
of corn stunt spiroplasma by its leafhopper vector <u>Dalbulus</u> <u>maidis</u>.
<u>Annals</u> <u>of</u> <u>applied</u> <u>Biology</u> 108: 535-544.

BOCK, K.R. 1974. Maize streak virus. <u>Commonwealth</u> <u>Mycological</u>
<u>Institute/Association</u> <u>of</u> <u>Applied</u> <u>Biologists</u>: <u>Descriptions</u> <u>of</u> <u>Plant</u>
<u>Viruses</u>, <u>Set</u> 8, No. 133.

COX, D.R. 1970. Analysis of binary data. Methuen & Co. Ltd., London.

DABROWSKI, Z.T. 1987. Comparative studies of <u>Cicadulina</u> leafhop-
pers in West Africa. Pages 35-39 in: Proceedings of 2nd International
Workshop on Leafhoppers and Planthoppers of Economic Importance, Brigham
Young University, Provo, Utah, USA. Wilson, M.R. & L.R. Nault, eds. CAB
International Institute of Entomology, London.

FENNAH, R.G. 1960. A new species of <u>Cicadulina</u> (Homoptera :
Cicadellidae) from East Africa. <u>Annals</u> <u>and</u> <u>Magazine</u> <u>of</u> <u>natural</u> <u>History</u>
(<u>Series</u> 13) 11: 757-758.

HELLER, F. & LINNAVUORI, R. 1968. Cicadelliden aus Athiopien.
<u>Stuttgarter</u> <u>Beiträge</u> <u>zur</u> <u>Naturkunde</u> 186: 1-42.

ISHIHARA, T., 1969. Families and genera of leafhopper vectors.
Pages 235-254 in: Viruses, vectors, and vegetation. K. Maramorosch, ed.
Interscience Publishers, New York.

MARKHAM, P.G., PINNER, M.S. & BOULTON, M.I. 1984. The transmission of maize streak virus by leafhoppers, a new look at host adaptation. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 57: 431-432.

NIELSON, M.W. 1968. The leafhopper vectors of phytopathogenic viruses (Homoptera, Cicadellidae). Taxonomy, biology and virus transmission. United States Department of Agriculture, Technical Bulletin no 1382: 1-386.

ROSE, D.J.W. 1978. Epidemiology of maize streak disease. Annual Review of Entomology 23: 259-282.

RUPPEL, R.F. 1965. A review of the genus Cicadulina (Hemiptera, Cicadellidae). Publications of the Michigan State University Museum, Biological Series 2: 387-428.

SOTO, P.E. 1978. A new vector of maize streak virus. East African agricultural and Forestry Journal 44: 70-71.

STOREY, H.H. 1928. Transmission studies of maize streak disease. Annals of applied Biology 15: 1-24.

STOREY, H.H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. Proceedings of the Royal Society of London Series B 112: 46-60.

STOREY, H.H. 1933. Investigations of the mechanism of the transmission of plant viruses by insect vectors - I. Proceedings of the Royal

Society of London Series B 113: 463-485.

STOREY, H.H. 1939. Transmission of plant viruses by insects.
Botanical Review 5: 240-272.

VAN RENSBURG, G.D.J. 1979a. The ecology and culture of leafhoppers
(Cicadulina spp.). Pages 100-103 in: Proceedings of the second South
African Maize Breeding Symposium 1976. H.O. Gevers, ed. Republic of South
Africa, Department of agricultural technical Services, Technical
Communication 142.

VAN RENSBURG, G.D.J. 1979b. Maize streak disease : A new infection
technique for use under field conditions. Pages 80-84 in: Proceedings of
the third South African Maize Breeding Symposium 1978. J.G. du Plessis,
ed. Republic of South Africa, Depart- ment of agricultural technical
Services, Technical Communication 152.

VAN RENSBURG, G.D.J. 1982. Laboratory observations on the biology
of Cicadulina mbila (Naudé)(Homoptera : Cicadellidae), a vector of maize
streak disease. 1. The effect of temperature. Phytophylactica 14:
99-107.

VAN RENSBURG, G.D.J. 1983. Southern African species of the genus
Cicadulina China (Homoptera : Cicadellidae) with descriptions of new
species. Department of Agriculture, Republic of South Africa, Entomology
Memoir no. 57.

VAN RENSBURG, G.D.J. & WALTERS, M.C. 1977. A method for the long-distance

transport of <u>Cicadulina</u> <u>mbila</u> (Naude)(Homoptera : Cicadellidae), a vector of maize streak virus. <u>Phytophylactica</u> 9: 115-116.

VILBASTE, J. 1976. A revision of Homoptera - Cicadenea described by S. Matsumura from Europe and the Mediterranean area. I. Cicadellidae. <u>Eesti</u> <u>NSV</u> <u>Teaduste</u> <u>Akadeemia</u> <u>Toimetised</u> 25 (<u>Koide</u> <u>Bioloogia</u> Nr 1): 25-36.

WEBB, M.D. 1987. Species recognition in <u>Cicadulina</u> leafhoppers (Hemiptera : Cicadellidae), vectors of pathogens of Gramineae. <u>Bulletin</u> <u>of</u> <u>entomological</u> <u>Research</u> 77: 683-712.
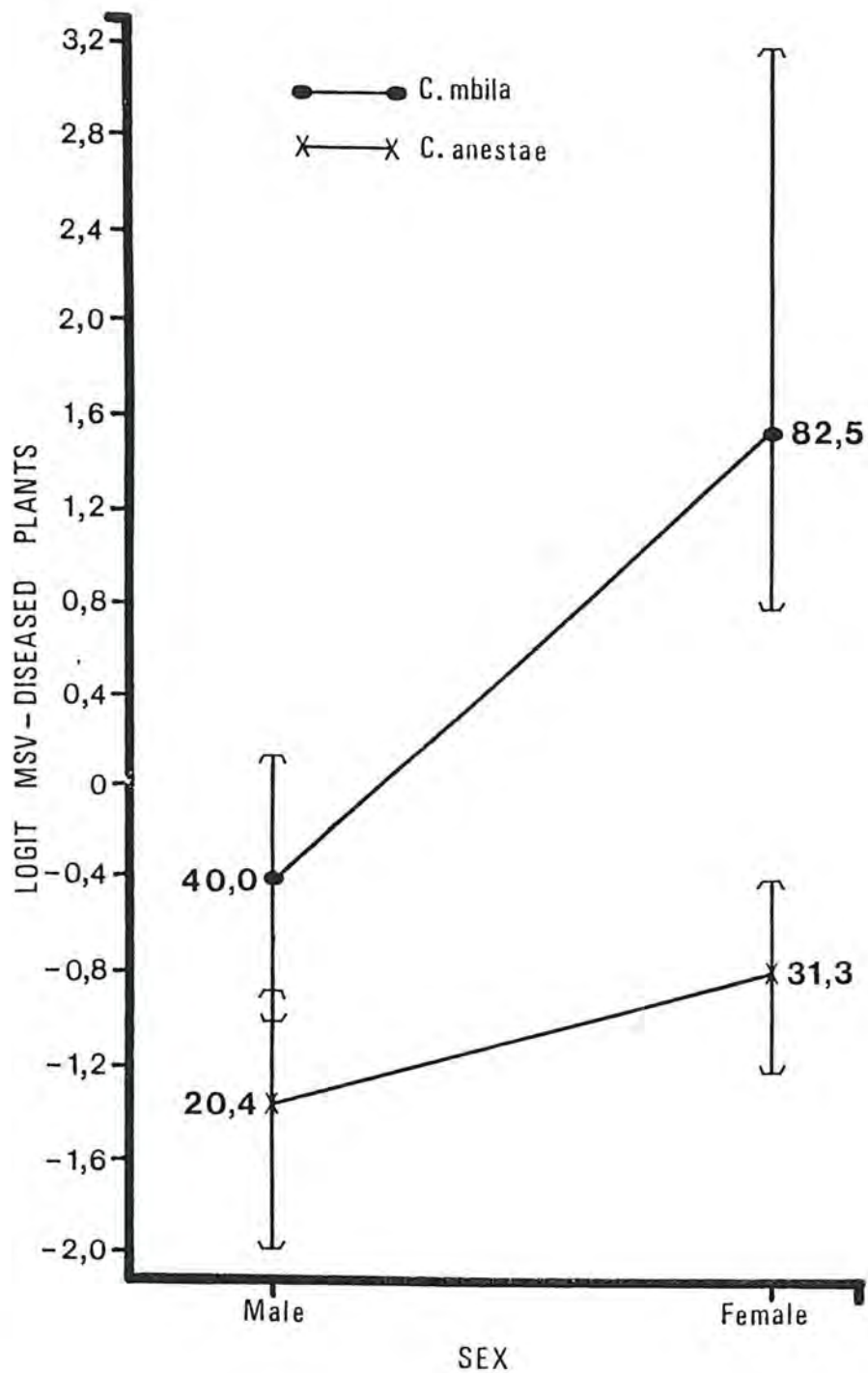
Fig. 3.1. Logit MSV diseased plants with the corresponding 95 % confidence intervals plotted against sex for the two leafhopper species. Numbers given next to each point indicate the per cent diseased plants.

TABLE 3.1 Estimated probabilities for <u>Cicadulina</u> <u>mbila</u> and
<u>C</u>. <u>anestae</u> to transmit MSV to maize

Species		Probability of transmitting MSV	
		Sex ratio	
	Sex	1 ♂ : 1 ♀	1 ♂ : 2 ♀
<u>C</u>. <u>mbila</u>	-	0,615	0,687
Female	0,825	-	-
Male	0,400	-	-
<u>C</u>. <u>anestae</u>	-	0,255	0,273
Female	0,313	-	-
Male	0,204	-	-
Either species	-	0,458	0,507

CHAPTER 4

EFFECT OF TEMPERATURE AND VECTOR NUMBERS ON MAIZE STREAK VIRUS INCUBATION AND SYMPTOM SEVERITY IN MAIZE

INTRODUCTION

Maize streak disease in maize is an important plant virus disease in some of the major maize producing areas of South Africa (Van Rensburg, 1979), and is regarded as one of the most destructive virus diseases of crop plants in the world (Harrison, 1985). Several species of the leafhopper genus Cicadulina (Homoptera : Cicadellidae) are vectors of maize streak virus (MSV), of which C. mbila (Naudé) is the most important vector (Van Rensburg, 1983; Webb, 1987).

The role of environmental factors in streak disease epidemiology is probably very complex, temperature presumably being a key factor. Van Rensburg (1979) ascribed the inability of maize streak disease to attain epidemic levels in some areas, in part, to temperature limitations. It has been reported that temperature affects maize dwarf mosaic virus (MDMV) incubation, infection, multiplication and translocation in the host plant (Tu & Ford, 1969 a; b). Damsteegt (1984), using fluctuating temperature regimes, found the time from inoculation with MSV to symptom development in maize to be inversely related to increase in temperature. MSV incubation in maize under constant conditions of temperature has, however, not been studied previously.

Dabrowski (1985) found that both height and mass of plants and MSV symptom severity were affected by C. triangula Ruppel numbers. Hao & Pitre (1970)

found that corn stunt disease symptoms increased in severity and that the incubation period decreased in maize with increasing numbers of the vector, Dalbulus maidis (DeLong & Walcott). Disease incidence and symptom severity ratings were also shown to be related to vector population density by Pitre (1968). Furthermore, patterns of MSV infection in maize fields are closely related to seasonal differences in the numbers of both C. mbila and C. storeyi populations (Rose, 1973 b; 1974).

The objective of this study was to determine the effects of temperature and vector numbers on MSV incubation and symptom severity in maize.

MATERIALS AND METHODS

An MSV-susceptible homozygous maize line, P134, was used throughout this study. The maize was planted in 19 cm pots and maintained in a greenhouse with a 26 ^{O}C/18 ^{O}C day/night setting. Lantern glasses, placed over individual plants, were used to prevent extraneous MSV inoculation. C. mbila adults from a greenhouse colony proven to be efficient vectors of MSV were used to inoculate maize seedlings in the three-leaf stage. All treatments comprised 10 plants.

Effect of post-inoculation temperature on MSV incubation

A total of 80 maize seedlings were inoculated in the greenhouse by allowing five leafhoppers per plant to feed for 24 h. The leafhoppers were then removed and the plants transferred to growth chambers at constant temperature and a photoperiod of 16 L : 8 D. Eight temperatures were used, ranging from 15 ^{O}C to 36 ^{O}C in 3 ^{O}C intervals with a deviation of $\pm$ 0,25 ^{O}C from

the desired setting. Plants were maintained in the growth chambers and checked daily for the appearance of MSV symptoms. The incubation periods were determined as the mean number of days from inoculation to the appearance of the first disease symptoms. Regression analysis of temperature on mean incubation time was performed on the data, using a model of the form $Y = \propto + ae^{bx}$.

Effect of vector numbers on MSV incubation and on symptom severity

A total of 50 plants were inoculated in the greenhouse (26 ^{o}C day/18 ^{o}C night) using 1, 2, 5, 10 and 20 C. mbila adults on each of 10 plants. The vectors were allowed to feed on seedlings for 24 h after which they were removed. The plants were then transferred to an insect-free greenhouse and maintained at a 30 ^{o}C day/ 23 ^{o}C night temperature regime. Plants were observed 12-hourly for the appearance of disease symptoms to determine the incubation periods. On days 6, 10 and 18 after inoculation, disease severity was rated on an ordinal scale from 0 to 5 (0 = no symptoms; 5 = entire leaf surface covered by chlorotic streaks).

The relationship between vector levels and mean number of days from inoculation to symptom appearance was determined by means of regression analysis. The index data pertaining to disease severity was analysed by extracting the linear effects of vector numbers and time after inoculation in a factorial analysis. Vector numbers were linearised using log (vector numbers) while time after inoculation was linearised using log (days-2). The programme, PC-PLUM (Randall, 1989), was used for the analysis. This programme uses the generalized linear model (McCullagh & Nelder, 1983) to analyse index data that is on an ordinal scale.

RESULTS

Effect of post-inoculation temperature on MSV incubation

The incubation period of MSV in maize was inversely related to post-inoculation temperature (Fig. 4.1). All the plants at the different temperatures developed disease symptoms except those at 15 ^{o}C where three plants showed no symptoms 20 d after inoculation, at which time observations were terminated.

Effect of vector numbers on MSV incubation and on symptom severity

The number of leafhopper vectors had a pronounced effect on rapidity of MSV symptom appearance (Fig. 4.2). As the number of vectors per plant increased from one to 20, the mean incubation period in maize decreased from 3,9 d to 3,0 d. The steep slope of the curve on the left hand side of the graph (Fig. 4.2), indicates that the incubation period of MSV in maize increased considerably at vector levels below five. In contrast, the incubation period at vector levels higher than five did not differ much.

The relation between number of leafhoppers and average index of symptom severity for three different times after inoculation is shown in Fig. 4.3. There was a very strong linear effect for both number of vectors and time after inoculation (P < 0,001 in both cases). This can be seen in Fig. 4.3 which shows a tendency for the index to increase with increases in both leafhopper numbers and time after inoculation. In addition, there were significant quadratic effects (P = 0,02 for vector numbers and P = 0,003 for time after inoculation), indicating curvature. The curvature was due to a

flattening off of the average index with both increasing numbers of vectors and time after inoculation. Symptom expression seemed to have almost reached a maximum 10 d after inoculation at a day/night temperature regime of 30/23 ^{o}C, except in the case where single vectors was used. In contrast to the results obtained with single vectors, the use of 20 vectors resulted in almost maximum symptom severity 6 d after inoculation.

DISCUSSION

The incubation period of MSV in maize was influenced by both post-inoculation temperature and by the number of vectors. The marked increase in incubation period with a decrease in temperature from 18 ^{o}C to 15 ^{o}C (Fig. 4.1) indicates that temperatures below 15 ^{o}C probably lie in the lower unfavourable range with regard to MSV multiplication. The fact that not all the plants developed MSV symptoms when kept at 15 ^{o}C after inoculation, is further evidence that MSV multiplication in the plant was inhibited at temperatures below 15 ^{o}C. Damsteegt (1984) found that no MSV symptoms appeared in maize infected at 5 ^{o}C day/0 ^{o}C night temperature. Our result is also in general agreement with the finding of Tu & Ford (1969 a) regarding MDMV, viz. that no virus multiplication occurred at 10 ^{o}C in maize, regardless of the fact that infection has occurred at this temperature (Tu & Ford, 1969 b).

Dabrowski (1985) found that MSV symptoms increased in severity and that both plant height and mass decreased with increasing C. triangula numbers. Furthermore, plant growth was affected by both virus free and viruliferous adult leafhoppers. Storey (1938) concluded that C. mbila inoculates MSV in distinct doses, each independent in its effect of any other doses that may be

inoculated by the same or other vectors. However, results presented in Chapter 6 suggested that MSV transmission occurs in a cumulative manner. The incubation period of MSV in maize decreased and the number of infected plants increased with increasing feeding time of C. mbila, a result which is attributed to an increase in MSV inoculum dose with increasing inoculation feeding time (Chapter 6). The marked decrease in mean MSV incubation period in maize observed for five or more vectors in comparison with the effect of one or two vectors (Fig. 4.2), and the positive relationship between vector numbers and MSV symptom severity (Fig. 4.3), support the cumulative effect hypothesis. The present results, together with those of Chapter 6, thus suggest that initial MSV titres in plants following inoculation differ and are dependent on vector numbers and inoculation feeding time.

Of all environmental factors, temperature probably has the greatest direct effect on host plant/MSV/vector relationships. As it is inversely related to the incubation period, and presumably also the virus multiplication rate, it may affect the incidence and spread of MSV. Furthermore, it also influences the development and reproduction of the leafhopper vectors of MSV (Rose, 1973 a; Van Rensburg, 1982; Dabrowski, 1985; Okoth et al., 1987; Chapter 1), so that leafhopper population size would, in a particular area or planting season, depend on prevailing temperatures. Vector numbers in turn determine incubation period and symptom severity as was shown in this study. We thus conclude that temperature is of special consequence to MSV epidemiology and plays an important role in the incidence of maize streak disease.

REFERENCES

DAMSTEEGT, V.D. 1984. Maize streak virus: Effect of temperature on vector and virus. Phytopathology 74, 1317-1319.

DABROWSKI, Z.T. 1985. The biology and behaviour of Cicadulina triangula in relation to maize streak virus resistance screening. Insect Science & Its Application 6, 417-424.

HAO, G.T. & PITRE, H.N. 1970. Relationship of vector numbers and age of corn plants at inoculation to severity of corn stunt disease. Journal of Economic Entomology 63, 924-927.

HARRISON, B.D. 1985. Advances in geminivirus research. Annual Review of Phytopathology 23, 55-82.

McCULLAGH, P. & NELDER, J.A. 1983. Generalized linear models. Chapman and Hall, London.

OKOTH, V.A.O., DABROWSKI, Z.T. & VAN EMDEN, H.F. 1987. Comparative biology of some Cicadulina species and populations from various climatic zones in Nigeria (Hemiptera : Cicadellidae). Bulletin of Entomological Research 77, 1-8.

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. Journal of Economic Entomology 61, 847-849.

RANDALL, J.H. 1989. The analysis of sensory data by generalized model. Biometrical Journal 31, 781-793.

ROSE, D.J.W. 1973 a. Laboratory observations on the biology of Cicadulina spp. (Hom., Cicadellidae), with particular reference to the effects of temperature. Bulletin of Entomological Research 62, 471-476.

ROSE, D.J.W. 1973 b. Distances flown by Cicadulina spp. (Hem., Cicadellidae) in relation to distribution of maize streak disease in Rhodesia. Bulletin of Entomological Research 62, 497-505.

ROSE, D.J.W. 1974. The epidemiology of maize streak disease in relation to population densities of Cicadulina spp. Annals of Applied Biology 76, 199-207.

STOREY, H.H. 1938. Investigations of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. Proceedings of the Royal Society of London Series B, 125, 455-477.

TU, J.C. & FORD, R.E. 1969 a. Effect of temperature on maize dwarf mosaic virus infection, incubation, and multiplication in corn. Phytopathology 59, 699-702.

TU, J.C. & FORD, R.E. 1969 b. Translocation of maize dwarf mosaic and soybean mosaic viruses from inoculated leaves. Phytopathology 59, 1158-1163.

VAN RENSBURG, G.D.J. 1979. The ecology and culture of leafhoppers (Cicadulina

spp.). Pages 100-103 in: Proceedings of the Second South African Maize Breeding Symposium 1976. H.O. Gevers, ed. Republic of South Africa, Department of agricultural technical Services, Technical Communication 142.

VAN RENSBURG, G.D.J. 1982. Laboratory observations on the biology of Cicadulina mbila (Naudé) (Homoptera : Cicadellidae), a vector of maize streak disease. 1. The effect of temperature. Phytophylactica 14, 99-107.

VAN RENSBURG, G.D.J. 1983. Southern African species of the genus Cicadulina China (Homoptera : Cicadellidae) with descriptions of new species. Department of Agriculture, Republic of South Africa, Entomology Memoir No. 57.

WEBB, M.D. 1987. Species recognition in Cicadulina leafhoppers (Hemiptera : Cicadellidae), vectors of pathogens of Gramineae. Bulletin of Entomological Research 77, 683-712.
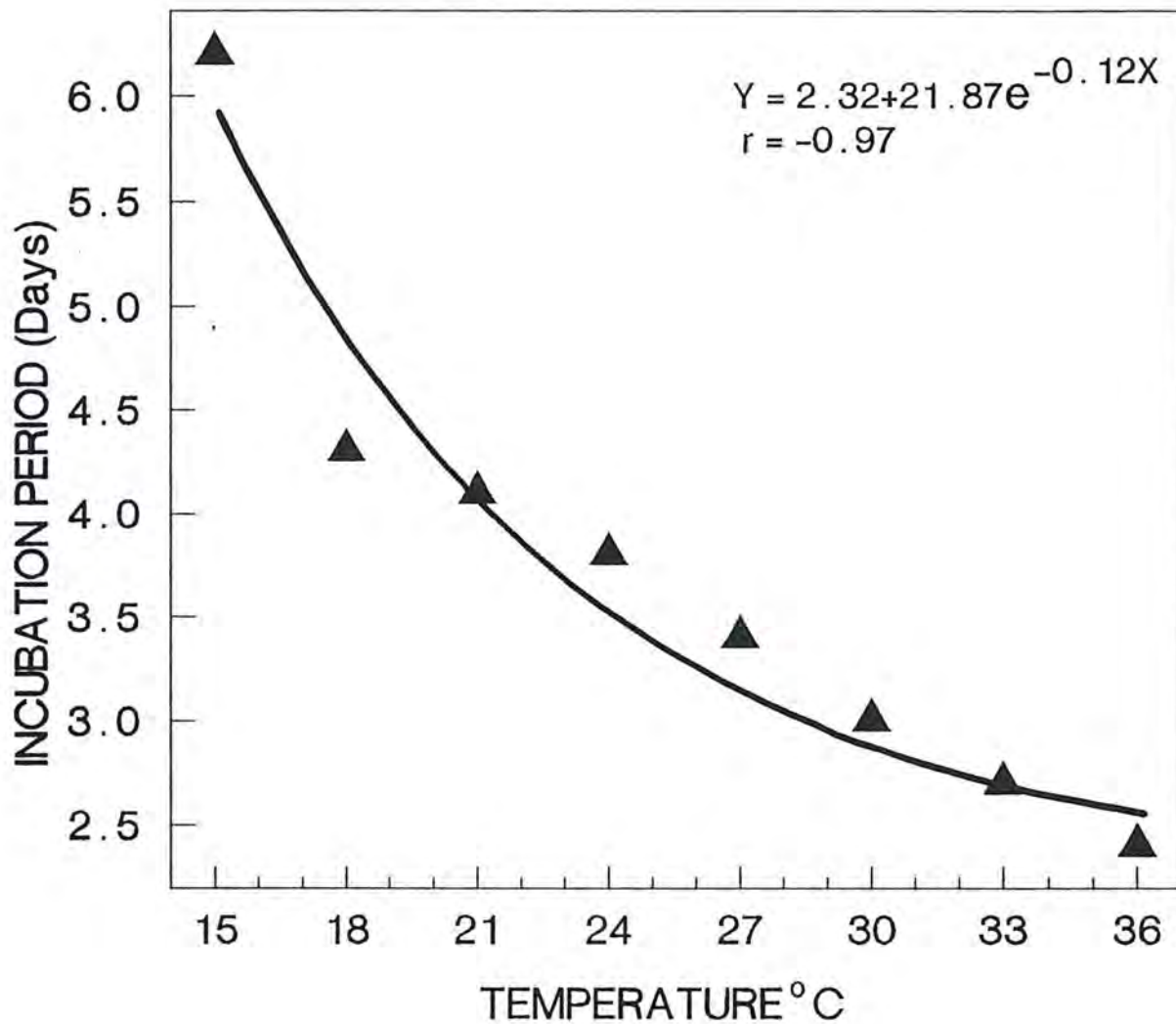
Fig. 4.1 The relationship between temperature and mean number of days from inoculation to MSV symptom appearance (incubation period) in maize, using <u>Cicadulina</u> <u>mbila</u> as vector.
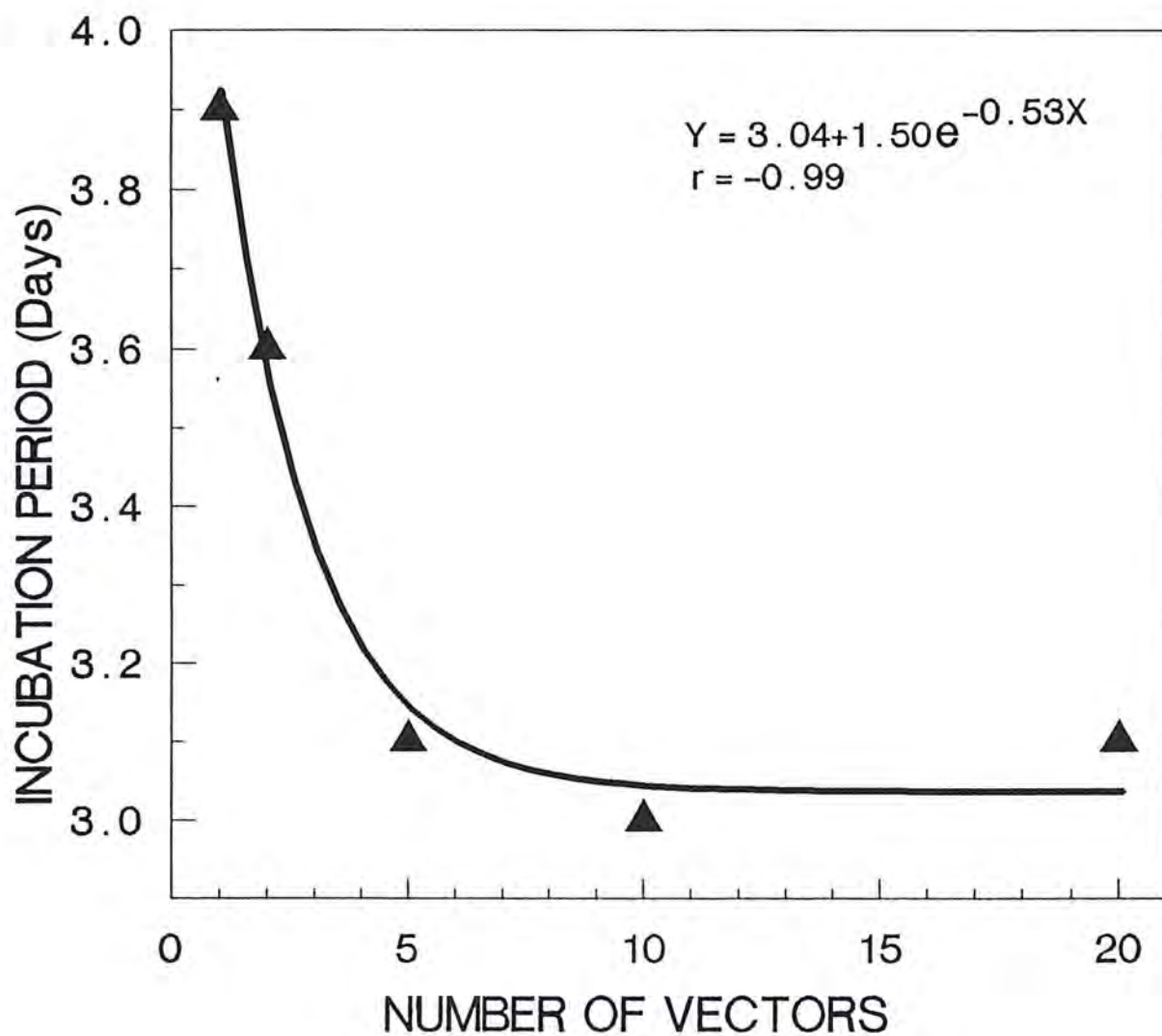
Fig. 4.2 The relationship between number of Cicadulina mbila adults, vectors of MSV, and mean number of days from inoculation to MSV symptom appearance (incubation period) in maize.
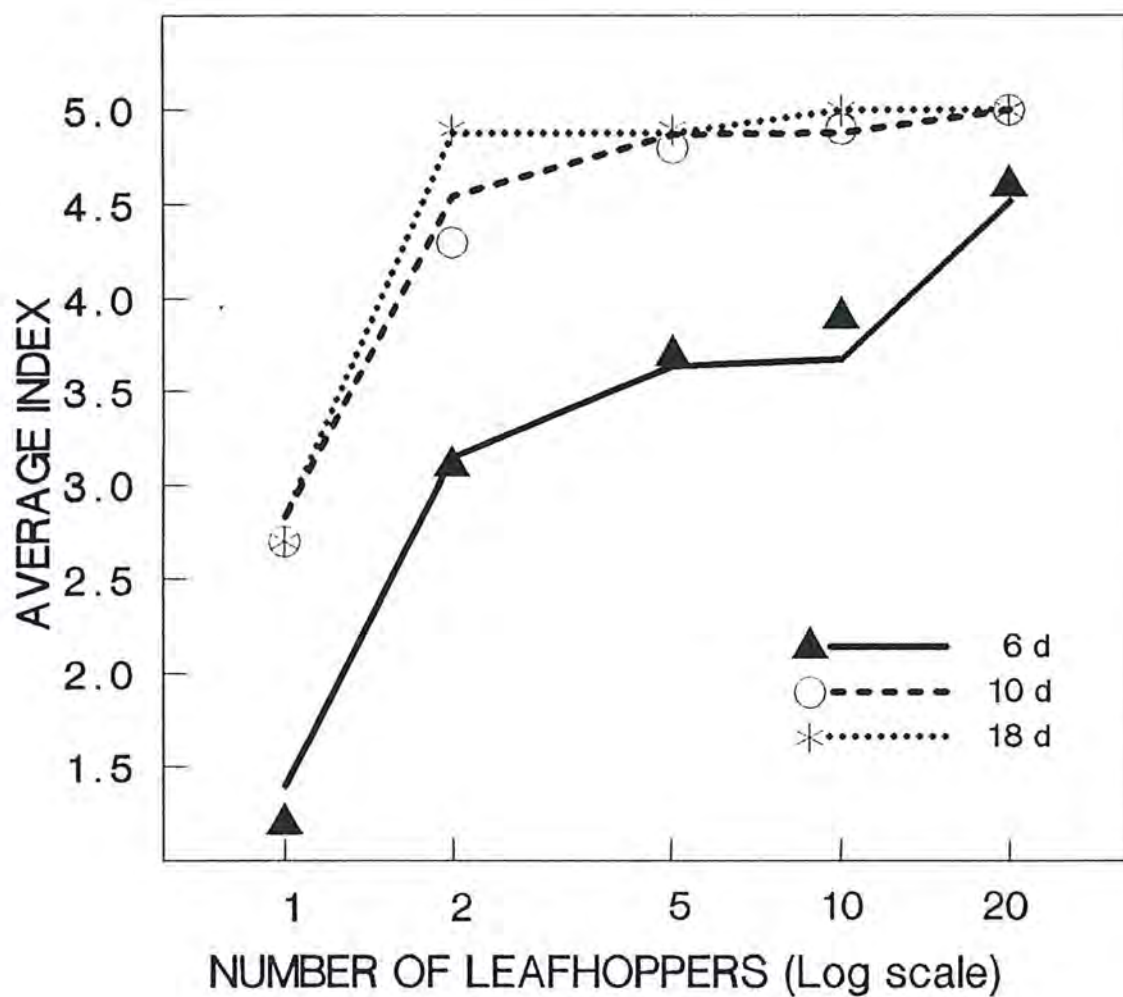
Fig. 4.3 The effect of number of Cicadulina mbila leafhoppers, vectors of MSV, on MSV symptom severity (expressed as average index on a scale of 0-5) at three different times after inoculation.

CHAPTER 5

RESISTANCE OF SOUTH AFRICAN MAIZE HYBRIDS TO MAIZE STREAK VIRUS

INTRODUCTION

Maize streak virus (MSV), transmitted by <u>Cicadulina</u> leafhoppers, may cause severe yield losses in maize especially when infection occurs during early growth stages of the plant (Guthrie, 1978; Soto & Buddenhagen, 1978; Van Rensburg, 1981; Mzira, 1984). Efficient vector control, whereby MSV spread is limited and yield losses are reduced to acceptable levels, can be achieved (Van Rensburg & Walters, 1978; Drinkwater, Walters & Van Rensburg, 1979; Van Rensburg, 1988; Chapters 7 & 8) provided that the residual activity of an insecticide is still sufficient 46 d after application to effect knockdown of the vectors within the 30 min inoculation feeding threshold (Chapter 6).

The most economical solution to the problem would be to breed MSV resistant maize cultivars adapted to the climatic conditions of the various localities. Storey & Howland (1967a; 1967b) reported that resistance in maize to MSV is controlled by a major gene, but with minor genes modifying specific reactions. Engelbrecht (1973) found MSV resistance in maize to be controlled by five dominant genes. Resistance to MSV is simply inherited (Soto, Buddenhagen & Asnani, 1982; Fourie & Pienaar, 1983). Although maize varieties tolerant or resistant to MSV have been known since the early 1930s (Rose, 1941; Fielding, 1949; Gorter, 1951; 1953; 1959; Storey & Howland, 1967a; 1967b), these varieties were not commercially successful.

Recent efforts to develop MSV resistant maize hybrids have resulted in varying degrees of success (Engelbrecht, 1973; 1975; Van Rensburg & Kühn, 1977; Soto et al., 1982; Fourie & Pienaar, 1983; Anon, 1985; Gevers, 1989). Progress is dependent on the availability of suitable techniques for artificial infection and mass rearing of Cicadulina spp. (Van Rensburg, 1979a; 1979b; Soto et al., 1982; Dabrowski, 1985; 1989). Alternately, breeding for MSV resistance may also be carried out under natural conditions at localities where MSV epiphytotics regularly occur, providing high and uniform incidence of MSV on young plants (Fourie & Pienaar, 1983; Dabrowski, 1989).

The objective of this study was to evaluate the present levels of MSV resistance in the commercial maize hybrids of South Africa, and to determine the potential value of resistance. This knowledge would be particularly useful in areas where MSV epiphytotics occur regularly.

MATERIALS AND METHODS

Data were obtained from the National Phase 2 Maize Cultivar Trials at the Vaalharts Experimental Station ($27^{o}57'$S; $24^{o}51'$E) for the five seasons from 1983/84 to 1987/88. Each trial consisted of 49 hybrids and included widely used as well as new hybrids; thus not the same hybrids were planted throughout the five years. This resulted in the same 16 hybrids being present in all five years, 22 hybrids in the last four years, 29 hybrids in the last three years, and 36 hybrids in the last two years. The trials were planted as completely randomized blocks with three replications, using 40 plants per plot with 1,0 m row widths and plant densities of approximately 50 000 plants ha^{-1}. Two guard rows were planted around the trials, but not between

plots. The soil type was a Hutton form, Mangano series, with a pH (KCl) of 6,0 $\pm$ 0,2 over the five seasons. Each year, fertilizer was applied at a rate of 500 kg 3:2:1 (N:P:K) ha^{-1} at planting followed by 300 kg ammonium sulphate ha^{-1} twice after planting. Weeds were controlled by the application of alachlor/atrazine. Stalk borer control was obtained by hand application of trichlorfon granules in the plant leaf whorls. The trials were planted during late October/early November and harvested during April/May. During each of the 1984/85, 1985/86 and 1986/87 seasons an additional trial was planted during late November/early December, and harvested during May/June. Plants were exposed to natural infestation by leafhoppers which are usually heavy at Vaalharts, especially in early plantings.

The number of plants showing no infection, light infection (very few streaks to light streaking and slight stunting), and heavy infection (severe streaking on at least 60 % of leaf area and plants severely stunted) was determined at tasseling. These data were used to calculate a resistance index (R.I.) on a rating scale of 1 to 5 as follows: R.I. = [(Number of uninfected plants) + (3X Number of lightly infected plants) + (5X Number of heavily infected plants)]/total number of plants. A rating of 1 indicates that all the plants were disease free, while a rating of 5 indicates that all plants were severely infected. Thus, hybrids with ratings between 1 and 2 were regarded as resistant and those between 4 and 5 as highly susceptible. Relative rankings of hybrids included in the trials over all five years were analysed using Spearman's rank correlation coefficient (r_s) (Snedecor & Cochran, 1980), to determine their stability over time with regard to MSV resistance. MSV ratings obtained from the early season trials were compared with those from later plantings of the corresponding seasons to determine the intra-seasonal stability of hybrids, i.e. the relative MSV resistance rankings of hybrids

within seasons.

In order to investigate the effect of MSV infection on yield loss, trials during the 1983/84 and 1987/88 planting seasons were duplicated. The duplicate trials were protected against MSV infection by applying the systemic insecticide, carbofuran, in the planting furrow at a rate of 20,0 g a.i. 100 m^{-1}. The differences in yields between the carbofuran treated trials and the untreated trials were used as an estimate of the yield loss in relation to various resistance levels. Yield data were converted to t ha^{-1} at the standard 12,5 % moisture level. The average percentage yield loss was logit transformed and subjected to regression analysis using the resistance rating as independent variable. The actual yield of the carbofuran treated and untreated maize hybrids were regressed separately on the resistance index. The difference in yield of the insecticide treated and untreated hybrids at both ends of the resistance index was used to calculate the monetary value of the present levels of MSV resistance. This would also indicate whether or not insecticidal control of the leafhopper vectors can be economically justified. These calculations were based on a producer maize prize of R239 per ton and the cost of carbofuran of R147 per ha.

RESULTS

The rankings of different numbers of hybrids according to MSV resistance indices obtained from the trials in the five years from 1983/84 were significantly correlated ($\propto < 0,01$) in all possible combinations over the five year period (Table 5.1). The maize hybrids included in the 1987/88 trial differed considerably with regard to MSV infection response, the resistance index varying from 1,35 to 4,69 (Table 5.2).

MSV resistance indices for the 49 hybrids varied from 1,25 - 3,60 and 1,85 - 4,79 for the early and late 1984/85 trials, from 3,22 - 4,93 and 4,16 - 5,00 for the 1985/86 trials, and from 1,46 - 4,75 and 3,17 - 4,90 for the 1986/87 trials, respectively. These differences can be ascribed to different vector infestation levels in the various trials. Nevertheless, the correlation between rankings of the 49 entries of each year was highly significant ($\alpha < 0,0001$ for each of the years), r_s being 0,77, 0,68 and 0,81 for rankings of the 1984/85, 1985/86 and 1986/87 plant growth seasons, respectively.

The relation between MSV resistance indices and yield loss for 1983/84 and 1987/88 is shown in Figure 5.1. Asymptotic regressions of the form $Y = \alpha + ae^{bX}$ fitted the data well. The regression lines for the two seasons follow the same trend. The positive effect of resistance on yield resulted in the most resistant hybrids suffering approximately 30 % less yield loss than the susceptible hybrids during both seasons (Figure 5.1). Figure 5.2 shows the difference in actual yield between carbofuran treated and untreated maize in relation to MSV resistance ratings. Asymptotic regressions of the form $Y = \alpha - ae^{bX}$ fitted the yield data of the untreated trials well. However, the regressions (not shown) of the carbofuran treated trials in both seasons explain only one percent of the observed variation in yield. High F-values indicate that the coefficients, and a, are highly significant and therefore approximately equal to the mean. The horizontal lines (CC) in Figure 5.2 represent the mean yields, viz 4,49 and 8,39 t ha^{-1} observed for the carbofuran treated trials of 1983/84 and 1987/88, respectively. Carbofuran application had a more pronounced effect on yield in the 1983/84 season (Figure 5.2A) than in the 1987/88 season (Figure 5.2B). The relative yield increase due to carbofuran treatment, however, was substantially greater with the susceptible hybrids than the more resistant ones in both seasons (100,0

and 48,6 % in 1983/84 and 43,3 and 7,5 % in 1987/88 for susceptible and resistant hybrids, respectively). Based on a producer maize prize of R239 per ton the present insecticide cost is equivalent to 0,62 ton grain per ha as indicated in Figure 5.2.

DISCUSSION

The fact that the Spearman rank correlation coefficients (r_s) showed a high degree of concordance between hybrid rankings of different years regardless of the sample size (Table 5.1), indicates that the level of MSV resistance in any one hybrid is probably very stable. Furthermore, the reliability of the result implies that hybrid rankings, according to their MSV resistance indices, during any year would give a true picture of the relative differences in resistance to MSV of the hybrids at that time.

According to the calculated MSV resistance indices from the different trials, hybrids also ranked consistently similar within the same year regardless of the magnitude of vector infestation. Thus, under field conditions the resistance index used in this study proved to be very useful in quantifying genetically based plant reaction to MSV infection.

Maize hybrids grown at present in South Africa showed varying degrees of genetically based reaction to MSV infection, ranging from resistant (indices between 1 and 2) to highly susceptible (indices between 4 and 5), as indicated in Table 5.2 for the 1987/88 trial. Engelbrecht (1973) stated that studies during the late 1960s showed that no commercial maize hybrid in South Africa had resistance to MSV at that time. The present results, therefore, show some progress with breeding for MSV resistance in South Africa during the last 20

years. In general, white grain hybrids were rated as susceptible to highly susceptible, the only exception being SSM2039 which rated consistently amongst the most resistant hybrids. On the other end of the R.I. scale the ten most susceptible hybrids included only two yellow grain entries. Gorter (1953) also noted that maize streak disease occurred more severely in white than in yellow maize.

Pinner, Markham, Markham & Dekker (1988) tested several isolates of MSV originating from various countries and host plants. They showed that all of these MSV isolates, including a maize isolate from South Africa, were serologically related to a Nigerian maize-isolate of MSV, and that, in general, symptoms were similar in both grass and maize hosts. Furthermore, in contrast with previous research (Storey & McClean, 1930; McClean, 1947; Bock, Guthrie & Woods, 1974), they could find no evidence that adaptation to grass hosts occurred, as all isolates could be transmitted to maize. Pinner et al. (1988) stated that Cicadulina probably transmits the entire 'population' of virus genotypes. Johnston (1983) mentioned that sources of MSV resistance from Nigeria and South Africa exhibited the same resistance under Zimbabwean conditions. Lazarowitz (1988) showed that, based on sequence analysis of the infectious clone, a South African isolate of MSV was highly homologous to Kenyan and Nigerian isolates. It can therefore be assumed that MSV resistance breeding programmes carried out under natural conditions at one locality would yield similar results at any other locality.

Knowledge of the differences in MSV resistance amongst South African commercial maize hybrids and their relationship with yield loss has a practical value in both cultivar and streak disease control recommendations in all areas where MSV epiphytotics occur regularly. Due to continual changes in

the cultivar list there is a need for regular review of the status of hybrids regarding MSV resistance. Since the ranking of cultivars according to the MSV resistance index was shown to be a reliable criterion to determine the relative levels of MSV resistance amongst hybrids, updated information in this regard can be obtained from a cultivar trial at any locality where MSV infection levels are reasonable.

The positive correlation between yield loss and resistance indices (Figure 5.1) confirms the genetic variation in hybrids with regard to MSV resistance. This implies that the adverse effect of MSV on yield can now be reduced on commercial scale by using some of the more resistant hybrids. The smaller yield increases due to carbofuran treatment in 1987/88 relative to 1983/84 (Figure 5.2) may possibly be ascribed to higher levels of MSV resistance in the hybrids used in the 1987/88 trials. If this is indeed the case, the value of the resistance observed in the 1987/88 trial was at least equal to the cost of carbofuran. Nevertheless, the results indicated that, regardless of which hybrid is used, protection of the maize crop against MSV infection by the application of carbofuran can be economically beneficial in areas where maize streak disease is a serious problem. This situation may however change, as it can be expected that levels of resistance to MSV in commercial hybrids will be further increased in future. Changes in the cost of insecticides and the producer prize of maize will obviously also determine the economic viability of chemical protection of the maize crop against MSV infection.

REFERENCES

ANON., 1985. Maize improvement program research highlights 1981-1984. IITA, Ibadan, Nigeria, 48 pp.

BOCK, K.R., GUTHRIE, E.J. & WOODS, R.D., 1974. Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugar cane and Panicum maximum. Ann. appl. Biol. 77, 289-296.

DABROWSKI, Z.T., 1985. The biology and behaviour of Cicadulina triangula in relation to maize streak virus resistance screening. Insect Sci. Applic. 6, 417-424.

DABROWSKI, Z.T., 1989. Procedures and techniques for rearing Cicadulina leafhoppers. In CIMMYT. Toward insect resistant maize for the third world : Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. Mexico, D.F. : CIMMYT, pp 84-93.

DRINKWATER, T.W., WALTERS, M.C. & VAN RENSBURG, J.B.J., 1979. The application of systemic insecticides to the soil for the control of the maize stalk borer, Busseola fusca (Fuller)(Lep.: Noctuidae), and of Cicadulina mbila (Naudé)(Hem.: Cicadellidae), the vector of maize streak virus. Phytophylactica 11, 5-12.

ENGELBRECHT, G.C., 1973. Die genetika van weerstandbiedendheid teen streepsiektevirus by Zea mays. Unpublished D.Sc. (Agric.) Thesis, University of the Orange Free State, Bloemfontein, 122 pp.

ENGELBRECHT, G.C., 1975. Streak, a major threat? In : J.G. du Plessis, C.O. Grogan, H.C. Kühn and M.C. Walters (Eds). Proceedings of the first South African Maize Breeding Symposium, 1974. Republ. S. Afr. Dept. agric. tech. Serv., Tech. Commun. 132, 101-103.

FIELDING, W.L., 1949. Research provides answer to streak virus in maize, mixed hybrid shows strong resistance to virulent disease in Baberton area. Fmr's Wkly 76, 50-51.

FOURIE, A.P. & PIENAAR, J.H., 1983. Breeding for resistance to maize streak virus : A report on the Vaalharts breeding programme. In: J.G. du Plessis (Ed.). Proceedings of the fifth South African Maize Breeding Symposium, 1982. Republ. S. Afr. Dept. Agric., Tech. Commun. 182, 44-50.

GEVERS, H.O., 1989. The ear rot epidemic. In: J.G. du Plessis (Ed.). Proceedings of the Eighth South African Maize Breeding Symposium, 1988. Republ. S. Afr. Dept. Agric. Water Supply, Tech. Commun. 222, 77-80.

GORTER, G.J.M.A., 1951. Streak disease in maize. Helpful measures for its prevention. Fmg S. Afr. 26, 361-362, 364.

GORTER, G.J.M.A., 1953. Studies on the spread and control of the streak disease of maize. Dept. Agric. For Un. S. Afr., Scien. Bull. 341, 1-20.

GORTER, G.J.M.A., 1959. Breeding maize for resistance to streak. Euphytica 8, 234-240.

GUTHRIE, E.J., 1978. Measurement of yield losses caused by maize streak

disease. <u>Pl</u>. <u>Dis</u>. <u>Reptr</u>. 62, 839-841.

JOHNSTON, L.M., 1983. The status of maize virus diseases in Zimbabwe. Pages 155-157 in D.T. Gordon, J.K. Knoke, L.R. Nault & R.M. Ritter, eds. Proceedings International Maize Virus Disease Colloquium and Workshop, 2-6 August 1982. The Ohio State University, Ohio Agricultural Research and Development Center, Wooster. 266 pp.

LAZAROWITZ, S.G., 1988. Infectivity and complete nucleotide sequence of the genome of a South African isolate of maize streak virus. <u>Nucleic</u> <u>Acids</u> <u>Res</u>. 16, 229-249.

MZIRA, C.N., 1984. Assessment of effects of maize streak virus on yield of maize. <u>Zimbabwe</u> <u>J</u>. <u>agric</u>. <u>Res</u>. 22, 141-149.

McCLEAN, A.P.D., 1947. Some forms of streak virus occurring in maize, sugarcane and wild grasses. <u>Dept</u>. <u>Agric</u>. <u>S</u>. <u>Afr</u>., <u>Scien</u>. <u>Bull</u>. 265, 39 pp..

PINNER, M.S., MARKHAM, P.G., MARKHAM, R.H. & DEKKER, E.L., 1988. Characterization of maize streak virus : description of strains; symptoms. <u>Plant</u> <u>Pathology</u> 37, 74-87

ROSE, M.F., 1941. Rotation crops. <u>Emp</u>. <u>Cott</u>. <u>Grow</u>. <u>Ass</u>., <u>Prog</u>. <u>Rep</u>. 1939-40, pp. 27-31.

SNEDECOR, G.W. & COCHRAN, W.G., 1980. Statistical Methods. 7th edn. Iowa State University Press, Ames, Iowa, 507 pp.

SOTO, P.E. & BUDDENHAGEN, I. 1978. Yield loss from a localized epidemic of maize streak virus in Nigeria. E. Afr. agric. For. J. 44 : 175-177.

SOTO, P.E., BUDDENHAGEN, I.W. & ASNANI, V.L., 1982. Development of streak virus-resistant maize populations through improved challenge and selection methods. Ann. appl. Biol. 100, 539-546.

STOREY, H.H. & HOWLAND, A.K., 1967a. Tranfer of resistance to the streak virus into East African maize. E. Afr. agric. For. J. 33, 131-135.

STOREY, H.H. & HOWLAND, A.K., 1967b. Inheritance of resistance in maize to the virus of streak disease in East Africa. Ann. appl. Biol. 59, 429-436.

STOREY, H.H. & McCLEAN, A.P.D., 1930. The transmission of streak disease between maize, sugar-cane, and wild grasses. Ann. appl. Biol. 17,691-719.

VAN RENSBURG, G.D.J., 1979a. The ecology and culture of leafhoppers (Cicadulina spp.). In H.O. Gevers, (Ed.). Proceedings of the second South African Maize Breeding Symposium, 1976. Republ. S. Afr. Dept. agric. tech. Serv., Tech. Commun. 142 : 100-103.

VAN RENSBURG, G.D.J. 1979b. Maize streak disease : A new infection technique for use under field conditions. In: J.G. du Plessis (Ed.). Proceedings of the third South African Maize Breeding Symposium, 1978. Republ. S. Afr. Dept. agric. tech. Serv., Tech. Commun. 152 : 80-84.

VAN RENSBURG, G.D.J., 1981. Effect of plant age at the time of infection with maize streak virus on yield of maize. Phytophylactica 13, 197-198.

VAN RENSBURG, G.D.J. & KüHN, H.C., 1977. Maize streak disease. Dept. agric. tech. Serv. S. Afr. Maize Ser. Leafl. No. E3.3 (Fmg S. Afr.), 4 pp.

VAN RENSBURG, J.B.J., 1988. Efficacy of cloethocarb for control of Busseola fusca, Cicadulina spp. and nematodes in maize. Appl. Pl. Sci. 2, 63-67.

VAN RENSBURG, J.B.J. & WALTERS, M.C., 1978. The efficacy of systemic insecticides applied to the soil for the control of Cicadulina mbila (Naudé) (Hem : Cicadellidae), the vector of maize streak disease, and the maize stalk borer Busseola fusca (Fuller) (Lep : Noctuidae). Phytophylactica 10, 49-52.

TABLE 5.1 Correlation between rankings of MSV resistance indices of different numbers of maize

hybrids evaluated at Vaalharts from 1983/84 to 1987/88

Plant growth season	No. of hybrids (n)	r_S $(\propto)$[a]			
		Plant growth season			
		1984/85	1985/86	1986/87	1987/88
1983/84	16	0,8433 (0,0011)	0,7167 (0,0055)	0,8315 (0,0013)	0,8712 (0,0007)
1984/85	16	—	0,7647 (0,0031)	0,6529 (0,0114)	0,7676 (0,0029)
	22	-	0,8111 (0,0002)	0,7177 (0,0010)	0,7911 (0,0003)
1985/86	16	-	-	0,7059 (0,0063)	0,8412 (0,0011)
	22	-	-	0,8156 (0,0002)	0,8760 (0,0001)
	29	-	-	0,7777 (0,0000)	0,9054 (0,0000)
1986/87	16	-	-	-	0,8500 (0,0010)
	22	-	-	-	0,8588 (0,0001)
	29	-	-	-	0,8107 (0,0000)
	36	-	-	-	0,8652 (0,0000)

[a]Spearman's rank correlation coefficient (significance level)

TABLE 5.2 MSV resistance index and rank for maize hybrids evaluated at Vaalharts in 1987/88

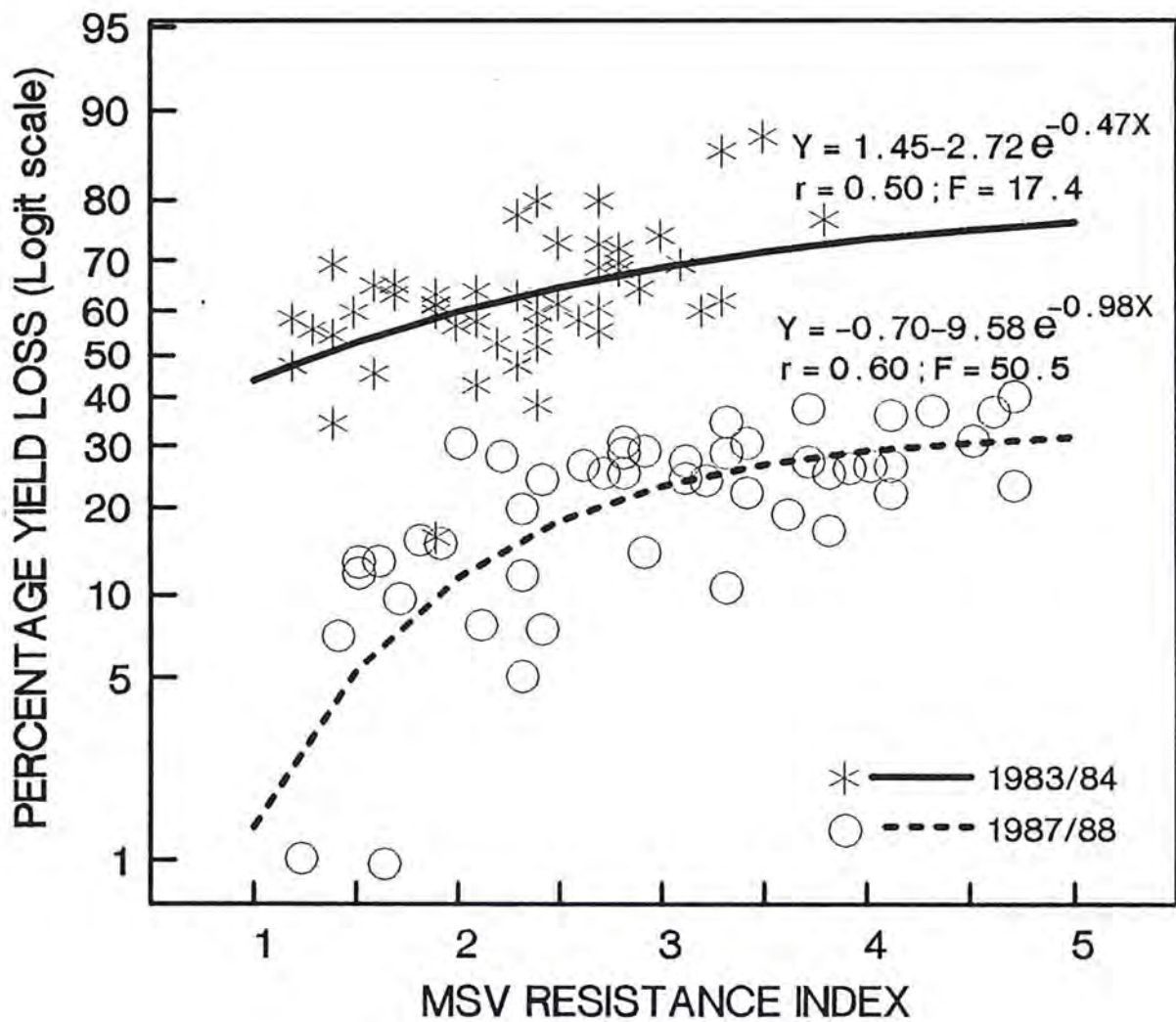| Hybrid	Resistance Index	Rank	Hybrid	Resistance Index	Rank
CRN7530	1,35	1	A475W	3,05	26
SSM2039	1,36	2	PNR6528	3,10	27
HL2(0_2)	1,48	3	SNK2338	3,25	28
PNR6552	1,52	4	A1650	3,29	29
CRN7522	1,63	5	PNR473	3,33	30
CRN7560	1,73	6	SA4MS	3,33	30
CRN4512	1,82	7	SNK2663	3,35	32
CRN7532	1,87	8	TX382	3,40	33
TX24	1,90	9	SNK2244	3,41	34
CRN308	2,03	10	PNR6549	3,57	35
CRN4526	2,19	11	SNK2232	3,69	36
AX305W	2,20	12	TX552	3,72	37
CRN4410	2,22	13	RO430	3,80	38
A1305W	2,29	14	SNK2340	3,83	39
A1600	2,29	14	PNR6429	3,87	40
PNR6334	2,34	16	A1257W	3,98	41
SNK2147	2,37	17	CRN4419	4,12	42
PNR6428	2,38	18	RO419	4,13	43
CRN4502	2,64	19	CRN4403	4,14	44
A210	2,66	20	KDU9051	4,29	45
KDU9046	2,76	21	RO405	4,51	46
SNK2771	2,82	22	TX564	4,64	47
PNR6330	2,83	23	A1849W	4,67	48
PNR394	2,86	24	RS5206	4,69	49
PNR6434	2,93	25			

Figure 5.1   Relationship between percentage yield loss in maize hybrids due to MSV infection, and MSV resistance index.
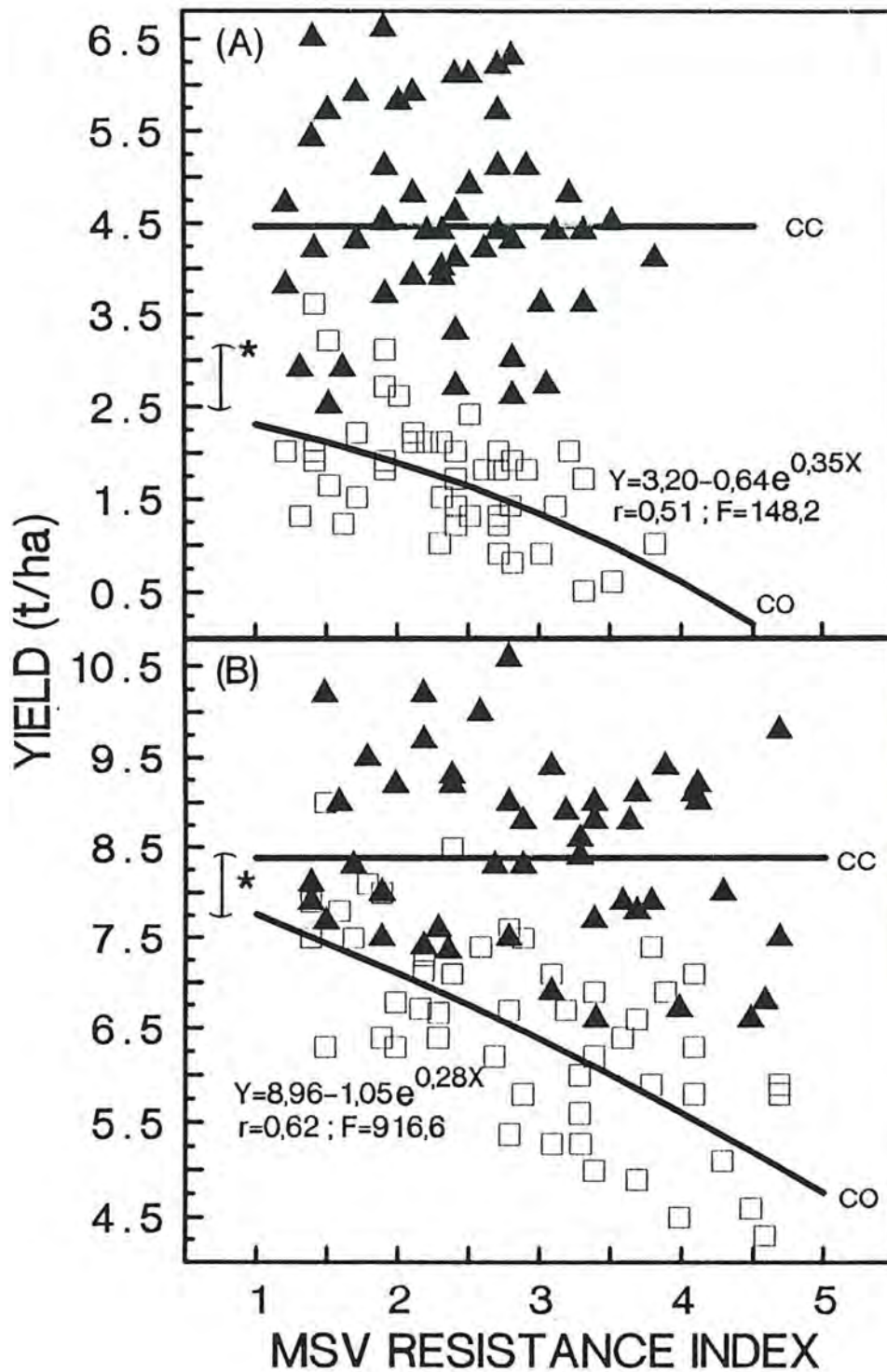
Figure 5.2   The relationship between actual yield of carbofuran treated
(CC) and untreated (CO) maize hybrids subjected to MSV
infection and MSV resistance index.   A. 1983/84 season.   B.
1987/88 season.   (*Insecticide cost = 0,62 t grain ha^{-1};
Note differences in scale on Y axis).

CHAPTER 6

CHEMICAL PREVENTION OF MAIZE STREAK VIRUS TRANSMISSION BY THE MAIZE
LEAFHOPPER, CICADULINA MBILA (NAUDé) (HOMOPTERA : CICADELLIDAE)

INTRODUCTION

Cicadulina leafhoppers, vectors of maize streak virus (MSV), are killed by
contact and systemic insecticides sprayed on green plants and by granular
systemic insecticides applied into the soil at planting (Rose, 1978). In
contrast to the findings of Rose (1978), soil applied systemic insecticides
were found to be particularly effective under very severe infection pressure
(Chapter 8). However, insecticides will not necessarily protect a maize crop
against MSV infection or prevent the spread of streak disease (Chapter 7).
This is due to the vectors having to feed on a treated plant before they can
be killed by insecticidal action. In this respect the ability of an
insecticide to effect a quick knockdown is of particular importance (Chapter
7).

Storey (1938) concluded that MSV transmission to a healthy maize plant is
achieved by feeding of C. mbila (Naudé) for at least 5 min, provided that the
insect's mouthparts penetrates to the phloem. Furthermore, the ability to
transmit MSV persisted in a viruliferous leafhopper throughout its life
(Storey, 1928). Since the feeding duration of the leafhopper vector
presumably has to be limited to five minutes to prevent MSV transmission
(Storey, 1938), the knockdown ability of an insecticide is obviously very
important in limiting maize streak disease effectively.

In view of the varying results obtained in Chapter 7 in controlling MSV with carbofuran, monocrotophos (systemic insecticides) and deltamethrin (contact insecticide), the object of this investigation was therefore to determine the knockdown of the leafhopper vectors by these insecticides in relation to the MSV inoculation threshold period.

MATERIALS AND METHODS

The study was conducted at Potchefstroom, and C. mbila adults from the greenhouse colony shown in Chapter 3 to be efficient vectors of MSV, were used.

Inoculation threshold time

The study was done in a greenhouse at a day/night temperature regime of 26/18 ^{o}C ($\pm$ 1,5 ^{o}C) and a 14 L : 10 D photoperiod. Homozygous MSV susceptible maize line P134 was grown in pots and kept under lantern glasses closed off with gauze (Van Rensburg, 1979) to avoid extraneous MSV infection. The minimum period required for virus transmission was determined by confining five C. mbila adults on the youngest fully unfolded leaf of maize plants at the two to three leaf stage by means of a clip-on cage which allowed the vectors direct access to the plant. The leafhoppers were starved for three hours before allowing them to feed on the plants for periods ranging from one minute to 24 h, using 40 plants for each inoculation feeding period except at the 25, 30 and 60 min feeding periods for which 50 plants each were used. Leafhoppers were observed continuously in the case of feeding periods up to 120 min, but for practical reasons only occasionally for feeding periods from 4 h to 24 h. The plants were then transferred to an insect-free greenhouse and kept at a day/ night temperature of 30/23 ^{o}C ($\pm$ 1,5 ^{o}C) for 20 d to

allow disease symptoms to develop. A positive transmission was indicated by the appearance of typical MSV symptoms. In this way the percentage of plants showing streak disease symptoms was determined for each inoculation feeding period.

The results were analysed using weighted regression of logit number of MSV diseased plants on feeding time (Cox, 1970) for those feeding times which resulted in positive transmission. The number of days from inoculation to MSV symptom appearance was recorded and the data subjected to one-way analysis of variance.

Knockdown ability of insecticides

Seeds of a MSV susceptible maize hybrid, PNR6428, were planted singly in pots, 190 mm in diameter with a height of 170 mm. Ten plants were treated with carbofuran (100 g/kg gran), hand-applied at planting in contact with the seed at a rate of 0,38 g insecticide per pot (=20,00 g a.i. 100 m^{-1} plant row length). Two other groups of ten plants each were sprayed three weeks after emergence with monocrotophos (400 g/ℓ ws-liq) and deltamethrin (25 g/ ℓ ec) at rates equivalent to 6,52 and 0,05 g a.i. 100 m^{-1} row length, respectively. A group of 30 plants served as untreated controls.

At each assessment of the knockdown ability of the three insecticides, three plants from each treatment and control were chosen at random to serve as experimental plants. After a starvation period of three hours adult leafhoppers were released on the crown leaves of the various plants, which is the position where they are most commonly observed on plants in the field (personal observation). Ten leafhoppers were allowed to feed on the

deltamethrin and monocrotophos sprayed plants and the untreated control plants, and 25 leafhoppers on the carbofuran treated plants. Knockdown time, i.e. time from insertion of mouthparts into plant tissue until mouthparts were withdrawn again, was recorded for individual leafhoppers. The leafhoppers almost without exception did not try to reinsert their mouthparts after withdrawal. They either flew off the plant within a few seconds after withdrawal of their mouthparts or simply remained on the leaf until they fell to the ground and were assumed dead.

Knockdown times were recorded at weekly intervals from 11 to 53 days after treatment (DAT) for carbofuran and on days 1, 2, 3, 5 and 9 after treatment for deltamethrin and monocrotophos. Feeding time on the untreated control plants was recorded at every assessment of each of the insecticides. Data were subjected to regression analysis of mean knockdown time against time after insecticidal application.

RESULTS

Inoculation threshold time

All 530 plants involved with inoculation feeding periods ≤ 25 min (1 through 10, 15, 20 and 25 min) failed to develop MSV symptoms. At an inoculation feeding period of 30 min MSV transmission occurred in 30 % of the test plants. The number of plants which developed MSV symptoms increased progressively as the inoculation feeding time was extended from 30 min to 24 h (Fig. 6.1). The weighted linear regression of logit number of plants showing MSV symptoms against the inoculation feeding times ≥ 30 min is shown in Fig. 6.1. The correlation coefficient (r) was highly significant (r = 0,90;

P < 0,001).

The relationship between feeding time and the number of days from inoculation to appearance of MSV symptoms is illustrated in Fig. 6.2. MSV symptoms appeared strikingly sooner after inoculation feeding of 1440 min than all other feeding periods. In comparison, the decrease in number of days needed for MSV symptoms to appear with increasing feeding time from 30 min to 480 min was considerably smaller. Thus it decreased from 8,8 d following 30 min inoculation feeding to 6,0 d and 3,8 d after 480 min and 1440 min, respectively (Fig. 6.2). Scheffe's multiple comparison test confirmed a significant difference (P 0,01) between the incubation periods.

Knockdown ability of insecticides

The knockdown abilities of the three insecticides used in this study are illustrated in Fig. 6.3. The knockdown times of both systemic insecticides viz. carbofuran and monocrotophos were best described by asymptotic regression models, and of the contact insecticide, deltamethrin, by linear regression. The correlation coefficients (r) were significant (P<0,05) for both deltamethrin (r = 0,950) and monocrotophos (r = 0,952) and highly significant (P<0,001) for carbofuran (r = 0,998).

The most striking difference between the insecticides was the very short residual activity of both insecticides applied as sprays compared to carbofuran which showed biological activity against the leafhoppers for almost 50 DAT. Furthermore, knockdown of the vectors by carbofuran up to 47 DAT was still sufficient to prevent MSV transmission (Fig. 6.3). In contrast, the inoculation feeding time of the vectors exceeded the minimum period required

for MSV transmission on days 1 and 3 after application for monocrotophos and deltamethrin, respectively.

C. mbila adults were reluctant to move when feeding on the untreated control plants unless disturbed. Inoculation feeding time on control plants was recorded for up to four hours after feeding commenced before observations were stopped, at which time some leafhoppers were still feeding. None of the leafhoppers fed continuously for less than two hours on control plants.

DISCUSSION

The 30 min MSV inoculation threshold recorded in this study with C. mbila as vector species differs greatly from the 5 min threshold reported by Storey (1938) which is widely quoted today, e.g. in Rose (1978) and Harrison (1985). Storey (1938) stated that although the inoculation threshold period for MSV was close to 5 min, he found that even 10 min feeding periods were rarely successful. Close scrutiny of the procedure Storey had used, reveals that he allowed a succession of specific feeding periods for a total contact duration of one hour. Furthermore, he interpreted the data on the assumption that the effect produced by one insect is independent of the effects of any others. In contrast to Storey's (1938) interpretation of an independent effect, the MSV transmission data obtained in this study (Figs. 6.1 & 6.2) suggest a cumulative effect. The present results indicate that the critical MSV titre, resulting in plants developing maize streak disease symptoms, was only reached after ca. 30 min inoculation feeding period by five C. mbila adults. Furthermore, the decrease in incubation period in the plant and the increased number of plants showing MSV symptoms with increasing feeding time, is attributable to an increase in MSV inoculum dose with increasing inoculation

feeding time. Increased transmission to plants and shortened incubation periods in plants with increasing transmission access period of the vectors were also illustrated for Dalbulus maidis (DeLong & Wolcott), the vector of corn stunt spiroplasma (Alivizatos & Markham, 1986).

Bhirud & Pitre (1972), using D. maidis as test insect, found that carbofuran was translocated to the upper portions of the maize plant, but that the highest concentration of the chemical accumulated in the foliage of the lower parts of the plant. Since C. mbila was allowed to feed on the upper foliage of carbofuran treated plants in this study, the recorded knockdown times can be considered as the maximum feeding times for the different assessments.

Apart from the fact that both monocrotophos and deltamethrin, applied as foliar full cover sprays, broke down rapidly in comparison with carbofuran, the knockdown abilities of both these insecticides were not sufficient to limit MSV transmission. These results explain the excellent control of the leafhopper vectors, as reflected in the low number of MSV diseased plants, obtained with soil-applied systemic insecticides under conditions of heavy natural infestation (Chapters 7 & 8), and the inability of foliar sprays to limit maize streak disease to economically acceptable levels (Chapter 7).

Leafhoppers are capable of becoming resistant to insecticides, including carbamates, with cross resistance to other groups of insecticides within a few years (Heinrichs, 1979). It thus seems important to have an early warning method of detecting changes in the response of leafhopper populations to insecticides. The technique described above to determine the knockdown time of an insecticide in the laboratory can be used to monitor leafhopper

populations over time for the development of resistance. Since a method for the long-distance transport of live leafhoppers is available (Van Rensburg & Walters, 1977), samples of vector populations from different localities can be taken to a central laboratory for such bioassays.

Since the relationship between knockdown time of C. mbila and time after insecticidal application was highly significant, this technique can also be employed to screen experimental insecticides at low cost for their potential to limit MSV transmission. Van Rensburg (1981) indicated that economically important yield losses result from MSV inoculation occurring before plants are seven weeks old. Insecticides should therefore be able to knock down the leafhopper vectors of MSV within 30 min for up to ca. 50 DAT to prevent significant yield losses due to MSV infection.

101

REFERENCES

ALIVIZATOS, A.S. & MARKHAM, P.G. 1986. Acquisition and transmission of corn stunt spiroplasma by its leafhopper vector Dalbulus maidis. Annals of Applied Biology 108, 535-544.

BHIRUD, K.M. & PITRE, H.N. 1972. Bioactivity of carbofuran and disulfoton in corn in greenhouse tests, particularly in relation to leaf positions on the plant. Journal of Economic Entomology 65, 1183-1184.

COX, D.R. 1970. Analysis of Binary Data. Methuen & Co. Ltd., London.

HARRISON, B.D. 1985. Advances in geminivirus research. Annual Review of Phytopathology 23,55-82.

HEINRICHS, E.A. 1979. Control of leafhopper and planthopper vectors of rice viruses. Pages 529 - 560 in: Leafhopper vectors and plant disease agents. K. Maramorosch & K.F. Harris, eds. Academic Press, New York.

ROSE, D.J.W. 1978. Epidemiology of maize streak disease. Annual Review of Entomology 23, 259-282.

STOREY, H.H. 1928. Transmission studies of maize streak disease. Annals of Applied Biology 15,1-24.

STOREY, H.H. 1938. Investigations of the mechanism of the transmission of

plant viruses by insect vectors. II. The part played by puncture in transmission. Proceedings of the Royal Society of London Series B, 125, 455-477.

✓ VAN RENSBURG, G.D.J. 1979. Maize streak disease: A new infection technique for use under field conditions. Pages 80 - 84 in: Proceedings of the Third South African Maize Breeding Symposium 1978. J.G. du Plessis, ed. Republic of South Africa, Department of Agricultural Technical Services, Technical Communication 152.

✓VAN RENSBURG, G.D.J. 1981. Effect of plant age at the time of in fection with maize streak virus on yield of maize. Phytophylactica 13, 197-198.

✓ VAN RENSBURG, G.D.J. & WALTERS, M.C. 1977. A method for the long-distance transport of Cicadulina mbila (Naudé) (Homoptera: Cicadellidae), a vector of maize streak virus. Phytophylactica 9, 115-116.
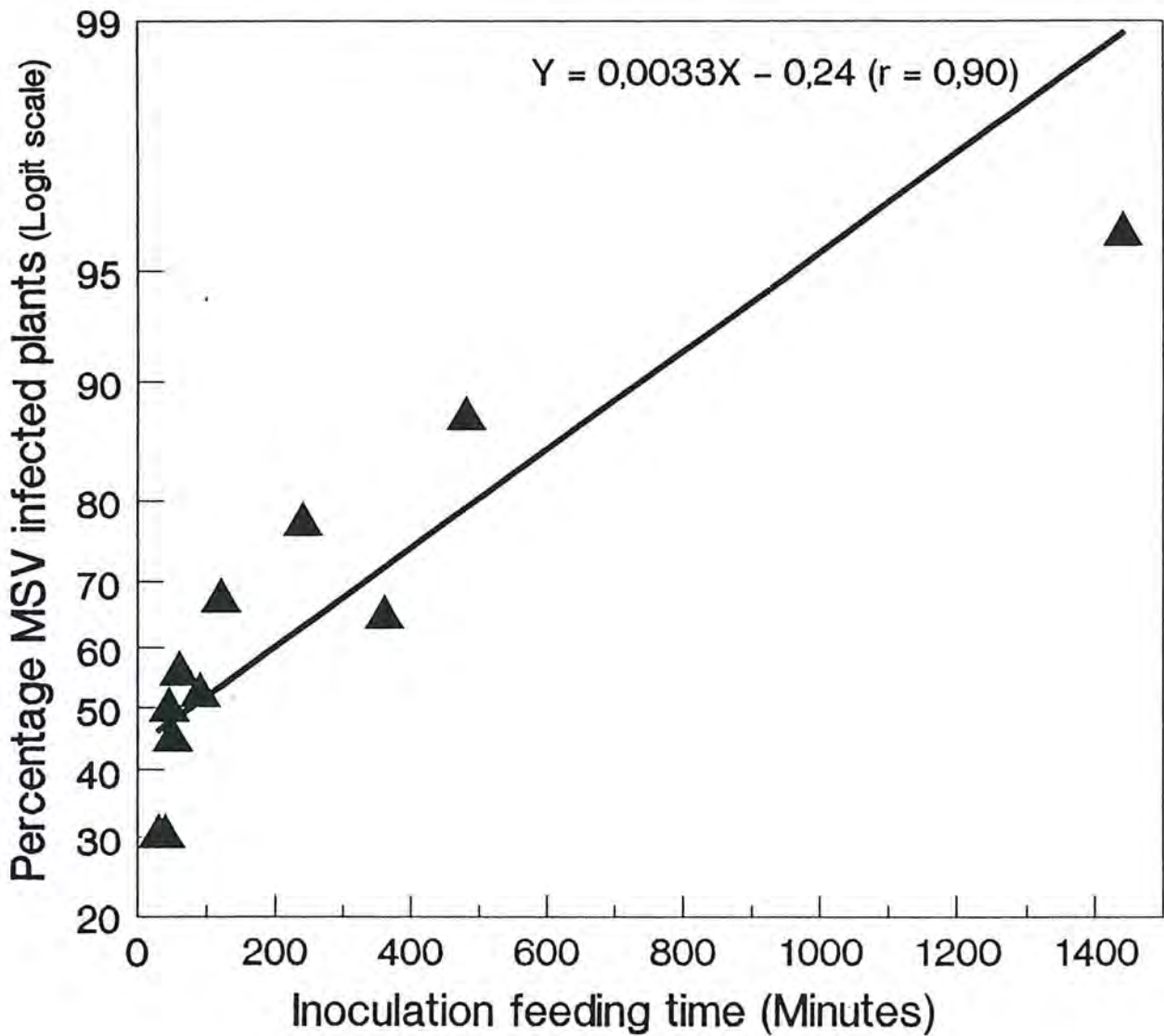
Fig. 6.1   The relationship between inoculation feeding time and the percentage of plants which developed MSV symptoms, using five *Cicadulina mbila* adults as vectors.
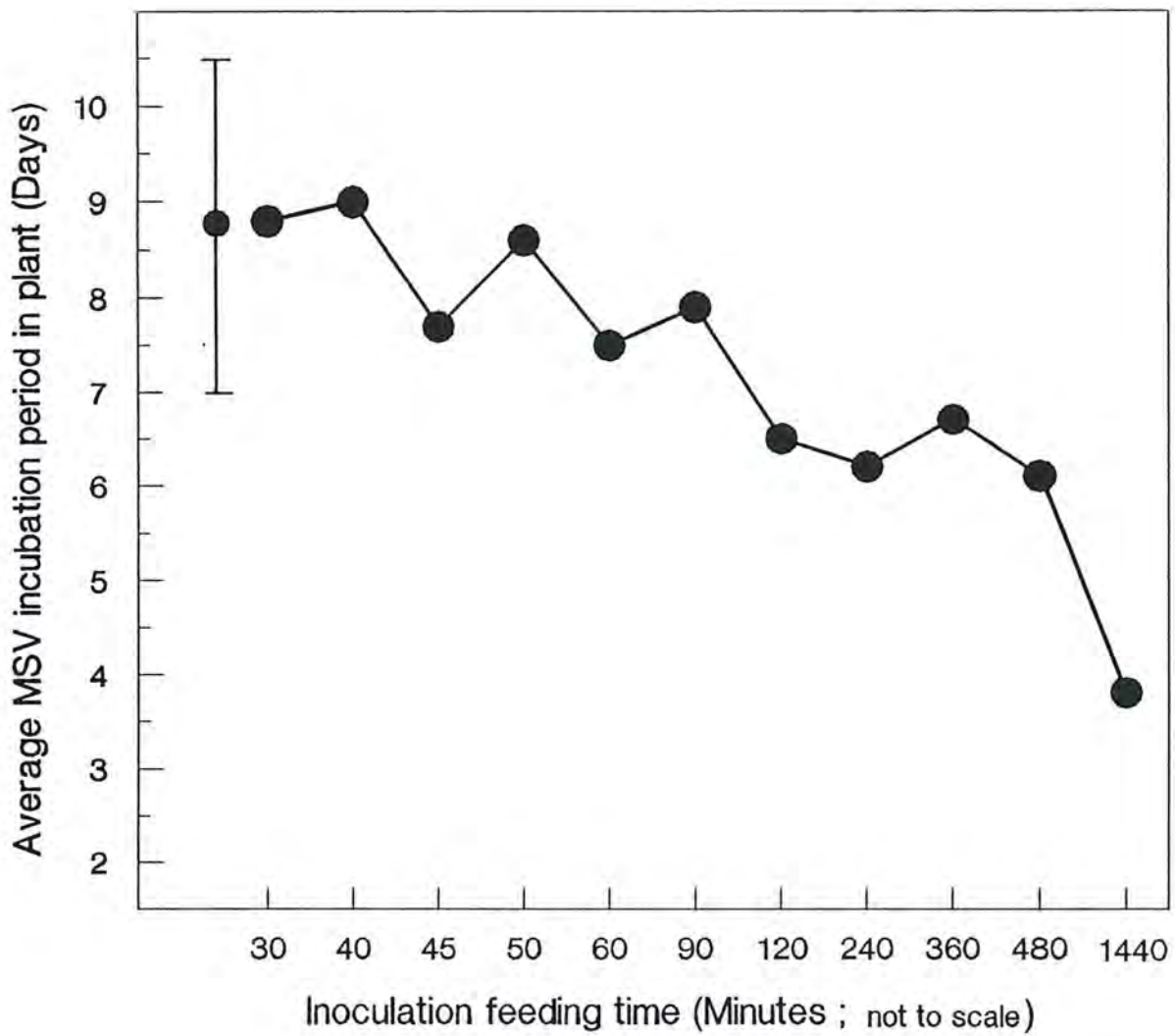
Fig. 6.2   Average number of days from inoculation feeding to MSV symptom appearance and standard deviation at different inoculation feeding times by Cicadulina mbila.

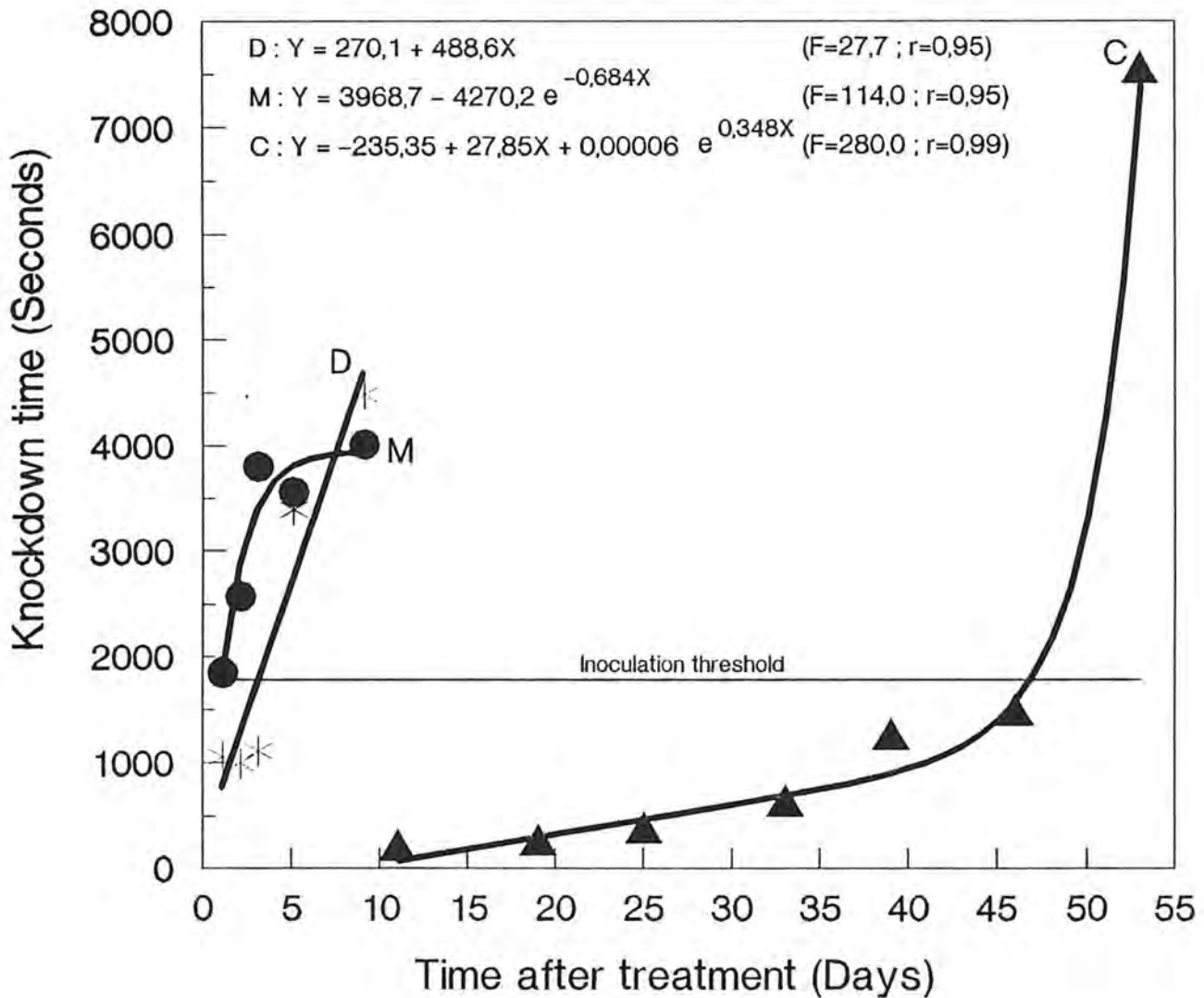Fig. 6.3   The relationship between knockdown time of <u>Cicadulina</u> <u>mbila</u> by
the insecticides deltamethrin (line D), monocrotophos (line M),
and carbofuran (line C), and time (days) after insecticidal
application in relation to the 30 min MSV inoculation threshold.

CHAPTER 7

COMPARATIVE EFFICACY OF PRE- AND POST-EMERGENCE APPLICATION OF INSECTICIDES
FOR SIMULTANEOUS CONTROL OF THE MAIZE LEAFHOPPER, CICADULINA MBILA , AND THE
STALK BORERS, BUSSEOLA FUSCA AND CHILO PARTELLUS, ON MAIZE

INTRODUCTION

Although maize streak disease caused concern amongst maize producers in Natal
towards the end of the previous century (Fuller, 1901), the disease was for
many years of little importance in the main maize producing area of South
Africa, the so-called maize triangle. However, in the early 1970s an epidemic
outbreak resulted in research again being focussed on the maize streak virus
(MSV) and its leafhopper vectors. The most important vector is Cicadulina
mbila (Naudé), one of at least three Cicadulina species known to transmit MSV
in South Africa (Van Rensburg, 1983). The occurrence of stalk borers in the
MSV-afflicted areas further aggravates the damage caused to the maize crop.
In this regard the maize stalk borer, Busseola fusca (Fuller) has for many
years been known as a major pest of maize (Mally, 1920), whereas Chilo
partellus (Swinhoe) is a recent introduction to the main maize producing area
of South Africa (Van Rensburg & Bate, 1987).

Several systemic insecticides applied in the planting furrow are registered
for the preventative control of the Cicadulina leafhoppers and the maize stalk
borer, B. fusca, in South Africa (Bot, Sweet & Hollings, 1987). Residual
activity of these insecticides extend at least up to seven weeks after crop
emergence (Van Rensburg & Walters, 1978; Drinkwater, Walters & Van Rensburg,
1979). In addition, insecticides for corrective post-emergence treatment are

registered against both stalk borer species but not for the leafhopper vectors of MSV (Bot et al., 1987).

Despite knowledge of leafhopper migration (Van Rensburg, 1979), prediction of MSV outbreaks is not possible. This necessitates the preventative use of the relatively expensive soil systemic insecticides. A systemic insecticide that can be applied as a seed dressing for the control of the MSV vectors would present a cost effective alternative to granular formulations applied in the planting furrow, whereas an effective corrective treatment would eliminate the unnecessary application of expensive soil systemic insecticides. An experiment was therefore conducted in order to evaluate a) the efficacy of a seed dressing formulation of a systemic insecticide for the control of MSV vectors; b) the possibility of leafhopper control by the application of corrective post-emergence sprays at the first signs of infestation; and c) the different control approaches for the simultaneous control of leafhoppers and stalk borers.

MATERIALS AND METHODS

In a field trial during the 1987/88 growing season at Potchefstroom ($26^o43'S$, $27^o6'E$) the efficacy of pre- and post-emergence applications of different insecticides for the simultaneous control of the maize leafhopper C. mbila and the stalk borers B. fusca and C. partellus was compared. A randomized block design was used, with five replicates per treatment. Plots consisted of two 20m rows, 2,2m apart, and separated by single guard rows to eliminate border effects. The soil type was a Bainsvlei form, Bainsvlei series with a pH(H_2O) of 6,5. Fertilizer was applied at a rate of 180 kg 2:3:2 (N:P:K) per ha at planting. The maize hybrid PNR6428 was planted with

a commercial planter on 15 January 1988. The plants emerged seven days later. The very late planting date was selected to ensure high infestation levels of both stalk borers and the leafhopper vectors of MSV. Broadleaf weeds were controlled by the application of atrazine/ bendioxide 28 days after planting.

The formulations, dosage rates and methods of application of the pesticides used are summarized in Table 7.1. Carbofuran was applied to the soil by means of a standard tractor-mounted granule-applicator. All the spray mixtures were applied by a hand-carried CO_2 sprayer, at a volume of 3 ℓ per 100m, and a pressure of 300 kPa. The sprayer was equipped with solid-cone nozzles of the single type and the disc - core sequence used was D4 - 35. The first sprays took place 35 days after planting (DAP) and the follow-up sprays 45 DAP.

The number of plants per plot was determined 17 and 90 days after planting. The effect of the different treatments on infestation by the stalk borers B. fusca and C. partellus, and on infection with MSV was evaluated at regular intervals from 32 to 75 days after planting. All plants per plot were examined, and the number of plants showing signs of fresh larval feeding activity in the whorl leaves as well as MSV symptoms were recorded.

Since the first occurrence of frost was experienced before the crop reached physiological maturity, yields were determined 14 weeks after crop emergence based on the mass of green ears after removal of the husks. The percentage of stalk borer damaged ears was determined at the same time. The mass of the green plants was determined separately. The data expressed as percentages of the total number of observations per plot were transformed (arcsin) before analysis of variance.

RESULTS AND DISCUSSION

The results are summarized in Table 7.2. Seed treatment with carbosulfan at both rates (D;E) gave significant control of the leafhopper vector as reflected in streak disease occurrence. Since the higher rate did not reduce the percentage infestation appreciably, carbosulfan at a rate of 0,8g a.i. per kg seed (D) is probably near the optimum rate for this compound when applied as a seed dressing. However, when compared with the conventional pre-emergence preventative treatments (B;C), the degree of control offered by carbosulfan could not be regarded as satisfactory. Carbofuran applied at both rates (B;C) proved to be superior to any of the other treatments, and showed biological activity against the leafhoppers over the entire pre-tasseling period. In contrast, none of the treatments applied as sprays (F;G;H;I) had any effect on MSV incidence at any stage of crop development.

With regard to stalk borer control, both carbosulfan seed treatments (D;E) had no effect on the borer infestation, but carbofuran applied in the planting furrow (treatments B;C) gave significant control of the stalk borers, with the lower rate slightly inferior to the higher rate. Biological activity of this compound extended almost to the onset of tasseling, corresponding to previous results (Van Rensburg & Walters, 1978; Drinkwater et al., 1979). The double spray applications of monocrotophos (G) and the buprofezin/deltamethrin mixture (I) gave the best control of the stalk borers, with the latter treatment superior to all other treatments. Buprofezin by itself (H) had no effect at all on the stalk borer infestation.

With regard to plant stand at 17 DAP as a measure of phytotoxicity , none of the treatments were significantly different from the control. The observed

differences in stand at 90 DAP was attributable to some plants being killed as a result of stalk borer infestation or MSV infection at an early stage of crop development. Both the carbofuran treatments (B;C) and the buprofezin/deltamethrin spray mixture (I) resulted in a significantly better stand at 90 DAP than the other treatments.

Both carbofuran applications (B;C) and the buprofezin/deltamethrin spray (I) resulted in significantly greater yields than all other treatments. Two monocrotophos treatments (G) also gave substantially better grain yield than the rest of the treatments, which did not differ from the untreated control. The major reduction in yield observed in the present study was probably attributable to borer attack, since the level of MSV incidence in the untreated control remained relatively low up to almost six weeks after emergence, which is the critical period with regard to yield loss caused by MSV (Van Rensburg, 1981). The fact that yields of the carbofuran treatments (B;C) did not differ from that of the buprofezin/deltamethrin spray (I) suggests that borer attack was the major cause of yield reduction.

Buprofezin is an insect growth regulator with biological activity against homopterous nymphs, including leafhoppers, but not against lepidopterous pests (Anon., 1985). Since nearby sticky traps indicated a continuous migration of adult leafhoppers throughout the growing season (unpublished data), the disappointing performance of buprofezin (H) in limiting MSV incidence can be ascribed to a lack in adulticidal action of this compound.

CONCLUSIONS

The prospects for using conventional insecticide sprays as corrective

post-emergence treatments to control maize streak disease when the first symptoms appear are remote. Since MSV can be vectored within five minutes after feeding commences (Storey, 1938), post-emergence applied insecticides appear to act too slowly to prevent MSV spread by the leafhopper. Unlike stalk borers which can be controlled correctively, protection of the maize crop against MSV infection can only be assured by prophylactic measures, i.e. controlling the leafhopper vectors by means of a pre-emergence treatment. Only systemic insecticides applied in the planting furrow can be used in this regard, with the additional benefit of simultaneous stalk borer control. The strikingly effective control of maize streak disease by carbofuran is difficult to explain because insects usually have to feed on a treated plant before they can be killed by systemic insecticidal action. Because the killing activity of an insecticide against the leafhopper vectors seems to be of primary importance for MSV control, studies relating to the mode of action of soil systemic insecticides and of virus transmission by the leafhopper should be conducted to gain a better understanding of the mechanism of control. Furthermore, the results of this study emphasized the need for the prediction of MSV outbreaks in order to minimize the risk of applying soil systemic insecticides uneconomically.

REFERENCES

ANON., 1985.   Applaud 25 WP on citrus scales and mealybugs. Technical information sheet. Nihon Nohyaku, Japan, 1 - 13.

BOT, J., SWEET, S. & HOLLINGS, N., 1987. A guide to the use of pesticides and fungicides in the Republic of South Africa. Department of Agriculture and Water Supply. Government Printer, Pretoria, 1 - 316.

DRINKWATER, T.W., WALTERS, M.C. & VAN RENSBURG, J.B.J., 1979. The application of systemic insecticides to the soil for the control of the maize stalk borer, Busseola fusca (Fuller)(Lep. : Noctuidae), and of Cicadulina mbila (Naudé)(Hem.: Cicadellidae), the vector of maize streak virus. Phytophylactica 11, 5 - 12.

FULLER, C., 1901. Mealie variegation. First Report of the Government Entomologist, Natal, pp 17 - 19.

MALLY, C.W., 1920. The maize stalk borer, Busseola fusca (Fuller). Bulletin of the Department of Agriculture, Union of South Africa, no 3, 111pp.

STOREY, H.H., 1938. Investigations of the mechanism of the transmission of plant viruses by insect vectors - II. The part played by puncture in transmission. Proceedings of the Royal Society of London Series B, 125, 455 - 477.

VAN RENSBURG, G.D.J.,1979. The ecology and culture of leafhoppers (Cica-

dulina spp). Proceedings Second South African Maize Breeding Sympo- sium, Pietermaritzburg, 1976. Technical Communication of the Depart- ment of Agricultural technical Services, RSA, 142, 100 - 103.

VAN RENSBURG, G.D.J.,1981. Effect of plant age at the time of infection with maize streak virus on yield of maize. Phytophylactica 13, 197 - 198.

VAN RENSBURG, G.D.J.,1983. Southern African species of the genus Cica- dulina China (Homoptera : Cicadellidae) with descriptions of new species. Entomology Memoir of the Department of Agriculture, RSA, no 57, 22pp.

VAN RENSBURG, G.D.J. & BATE, R., 1987. Preliminary studies on the re- lative abundance and distribution of the stalk borers Busseola fusca and Chilo partellus. Proceedings Seventh South African Maize Breeding Symposium, Potchefstroom, 1986. Technical Communication of the Department of Agriculture and Water Supply, RSA, 212, 49 - 52.

VAN RENSBURG, J.B.J. & WALTERS, M.C., 1978. The efficacy of systemic insecticides applied to the soil for the control of Cicadulina mbila (Naude) (Hem : Cicadellidae), the vector of maize streak disease, and the maize stalk borer Busseola fusca (Fuller) (Lep. : Noctuidae). Phytophylactica 10, 49 - 52.

TABLE 7.1 Formulations and dosage rates of pesticides, and details of treatments used in the field trial against a complex of maize pests (Potchefstroom, 1988)

Treatment	Formulation (%)	Dosage rate (g a.i. $100m^{-1}$)	Application directions	Time of application (Days after planting)
A. Control	Untreated	-	Untreated	-
B. Carbofuran	10 gran	20,00	Applied in planting furrow together with seed	0
C. Carbofuran	10 gran	15,00	Applied in planting furrow together with seed	0
D. Carbosulfan	8 sdr-liq	0,80[a]	Mixed undiluted with seed just before planting	0
E. Carbosulfan	8 sdr-liq	1,04[a]	Mixed undiluted with seed just before planting	0
F. Monocrotophos	40 ws-liq	6,52	Spray mixture applied directly into plant whorl	35
G. Monocrotophos	40 ws-liq	3,00	Spray mixture applied directly into plant whorl	35 & 45
H. Buprofezin	25 wp	0,25	Spray mixture applied directly into plant whorl	35 & 45
I. Buprofezin/	25 wp	0,125/	Spray mixture applied directly into plant whorl	35 & 45
Deltamethrin	2,5 ec	0,05		

[a] g a.i. per kg seed.

114

TABLE 7.2 Incidence of maize streak disease, stalk borers (Busseola fusca and Chilo partellus), plant stand and the corresponding yield response with various pre- and post-emergence insecticide applications (Potchefstroom, 1988)[1]

Treatment	32DAP[2]	38DAP	45DAP	53DAP	59DAP	75DAP	32DAP	38DAP	45DAP	53DAP	59DAP	17DAP	90DAP	Yield (kg plot⁻¹)	Plant mass (kg plot⁻¹)
		Maize streak disease							Stalk borer			Number of plants plot⁻¹			
A	2,6ab	8,0ab	17,7ab	34,3a	43,4a	55,7a	10,4a	25,3a	52,8a	82,1a	91,4a	210,2ab	166,0a	12,5a	53,9a
B	0,1d	1,6d	4,6d	11,6c	14,0c	14,9d	0,6c	1,4c	3,6b	22,5c	49,3d	211,4ab	202,2d	31,0c	77,2c
C	0,3d	1,2d	3,2d	12,9c	18,6c	23,3c	2,3b	4,2b	6,6b	36,4b	65,9c	207,6ab	201,8d	31,9c	76,9c
D	0,9c	3,3c	8,5c	26,6b	33,6b	41,6b	10,3a	24,1a	51,7a	86,4a	95,9a	206,8a	168,4ab	13,1a	51,8a
E	1,9b	6,0b	13,6b	28,2b	34,0b	41,4b	9,8a	20,7a	52,7a	87,1a	94,1a	207,8ab	169,2ab	13,5a	52,7a
F	2,3ab	8,1ab	17,8ab	38,7a	44,4a	52,1a	10,8a	24,1a	3,3b	38,9b	76,2b	210,0ab	189,8c	15,3ab	64,7b
G	2,7ab	8,8ab	19,3a	40,2a	46,0a	52,4a	10,4a	22,0a	7,1b	11,9d	38,1e	214,0ab	189,8c	18,7b	64,6b
H	2,6ab	7,9ab	16,8ab	35,8a	42,5a	52,5a	10,5a	24,6a	43,9a	82,1a	91,7a	215,2b	178,6bc	13,5a	54,8a
I	3,4a	9,4a	18,6a	41,7a	47,2a	50,9a	11,2a	24,6a	5,5b	2,3e	3,6f	210,6ab	202,2d	32,2c	77,1c

[1] Means within columns followed by the same letter do not differ significantly (P = 0,05) according to Duncan's Multiple Range Test.

[2] DAP = Days after planting.

CHAPTER 8

TOWARDS COST EFFECTIVE INSECTICIDAL CONTROL OF THE MAIZE LEAFHOPPER,
CICADULINA MBILA

INTRODUCTION

The leafhopper-transmitted maize streak virus (MSV) causes major yield losses
in maize (Zea mays L.) infected within seven weeks after plant emergence.
Losses are closely related to the plant growth stage at the time of infection
(Van Rensburg, 1981). Application of insecticides does not necessarily
prevent the incidence or spread of streak disease (Chapter 7). Systemic
insecticides applied in the planting furrow are, however, particularly
valuable as a control measure for the vectors of MSV in that the spread of the
disease is reduced to such an extent that economically important yield losses
are prevented (Drinkwater, Walters & Van Rensburg, 1979; Van Rensburg, 1988;
Chapter 7).

In addition to MSV vectors, soil applied systemic insecticides, especially
carbofuran, have proved to be effective for the control of the leafhopper
vectors of maize chlorotic dwarf and maize dwarf mosaic viruses (Kuhn, Jellum
& All, 1975), corn stunt (Bhirud & Pitre, 1972), and rice tungro virus
(Heinrichs, 1979). Both systemic and contact insecticides applied as foliar
sprays failed to limit the spread of corn stunt and mosaic viruses (Pitre,
1968a), and MSV (Chapter 7). Soil systemic insecticides are up to six times
more expensive than conventional insecticides used as foliar sprays. The cost
of MSV vector control could possibly be reduced indirectly by using a
broad-spectrum soil systemic insecticide, thereby eliminating and saving on

additional applications of pesticides for other pests.

The aim of this study was to identify one or more broad-spectrum insecticides, applied to the soil during planting, that would provide comprehensive control during the early stages of the maize plant. In this regard the stalk borers, Busseola fusca (Fuller) and Chilo partellus (Swinhoe), major lepidopterous pests of maize with a wide distribution, were considered the most important target pests in addition to the leafhopper, Cicadulina mbila (Naudé). The effect of the insecticides on plant stand and plant mass was also investigated.

MATERIALS AND METHODS

Seven insecticides, three of which are registered for the control of maize leafhoppers in South Africa (Bot, Sweet, Krause & Hollings, 1988), were evaluated for simultaneous control of C. mbila, B. fusca and C. partellus in two field trials at Potchefstroom ($26^{o}43'S$; $27^{o}06'E$). The formulations and dosage rates of these insecticides are shown in Table 8.1. Both trials were laid out in randomized block designs. Trial 1 comprised 11 treatments and an untreated control, each with six replications, and Trial 2 nine treatments and an untreated control with four replications. All plots consisted of two rows, each 20 m long and 2,2 m apart, planted with the maize hybrid A220. Except for two outer rows, guard rows were not used as the methods of treatment application and the wide row-spacing were regarded as sufficient to eliminate inter-plot effects. The soil type was a fine sandy clay loam (Westleigh form, Davel series) of alluvial origin, with a pH (H_2O) of 6,4. Late planting dates (first week in February) were selected to ensure severe infestations of both stalk borers and leafhopper. Weeds were controlled by the application of atrazine directly after planting.

In Trial 1, granular formulations were applied in the planting furrow with the seed by means of a standard tractor-mounted granule-applicator. Due to formulation problems with cloethocarb, only one cloethocarb treatment was included in the trial (Table 8.1). Carbosulfan (EC formulation) was applied in the planting furrow with the seed by means of a CO_2 knapsack sprayer mounted on the tractor. A spray volume of 2 ℓ per 100 m was used, with a pressure of 250 kPa. In Trial 2, the seed was planted by hand and all insecticides were applied by means of a Horstine microband granular applicator in the planting furrow before covering the seed with soil.

The number of plants per plot was determined 14 days after planting (DAP). The effect of the different treatments on infestation by the stalk borers, B. fusca and C. partellus, and on MSV infection was evaluated at 7 day intervals. All plants per plot were examined and the percentage infected plants per plot determined at each assessment. Data were transformed (arcsin) before analysis of variance. Grain yield could not be obtained as the late planting dates resulted in the killing of the plants by the first autumn frost, but the mass of green plants was determined 70 DAP in Trial 2.

RESULTS AND DISCUSSION

The average numbers of emerged plants in both trials are presented in Table 8.1. The reduced plant stand in the cloethocarb (1E) plots relative to the control (Trial 1) suggests a degree of pre-emergence phytotoxicity. This is in contrast to the results of Van Rensburg (1988) for this insecticide, although he did observe post-emergence symptoms of phytotoxicity related to cloethocarb in the form of scorched leaf edges. With the exception of both terbufos treatments (2Q; 2R) there was a general tendency for plant stand to

be increased by all compounds in Trial 2. Although the plant stand in the terbufos treated plots (2Q; 2R) did not differ significantly from that in the control (2A), the data indicate that pre-emergence phytotoxicity may occur, particularly at higher dosage rates.

The incidence of MSV and stalk borer at the time of the assessments is presented in Table 8.2 (Trial 1) and Table 8.3 (Trial 2). In trial 1 all insecticides gave significant control of the leafhopper vector up to 56 DAP as reflected in streak disease incidence (Table 8.2). The higher dosage rates of benfuracarb (1P), furathiocarb (1M), and aldicarb (1C) were, however, superior to the other treatments. In Trial 2, carbofuran (2F; 2H) provided excellent control throughout the entire pre-tasseling period, while cloethocarb (2D; 2E), benfuracarb (2N), and furathiocarb (2M) also gave significant control. Both terbufos treatments (2Q; 2R) failed to limit the spread of MSV (Table 8.3). Due to particularly high leafhopper infestation levels, streak disease increased steadily in treated and untreated plots throughout the course of both trials. Even under these conditions of severe infestation, MSV was limited to 22,6 % infected plants in the benfuracarb (1P) treated plots compared to 94,8 % diseased plants in the untreated control (1A) plots (Table 8.2), and 52,4 % in the carbofuran (2F) treated plots compared with 98,9 % diseased plants in the untreated control (2A) plots (Table 8.3).

Both aldicarb treatments (B1; C1) (Table 8.2) and terbufos treatments (Q2; R2) (Table 8.3) had little effect on stalk borer incidence at any stage of crop development. In contrast, all other insecticides in Trial 1 invariably gave significant control of stalk borers up to 42 DAP, while benfuracarb (10; 1P), furathiocarb (1M), carbosulfan (1I), and carbofuran (1G) controlled the stalk borers up to 56 DAP (Table 8.2). In Trial 2, only carbofuran (2F; 2H)

gave control of the stalk borers up to 56 DAP (Table 8.3).

The enhancement of plant growth through application of carbofuran observed by Kolbe (1975) is presumably the result of a property inherent in carbamate soil systemic insecticides, since we observed varying degrees of improved crop growth vigour in all treated plots relative to the untreated controls. The fact that a higher incidence of stalk borer occurred in aldicarb plots (1B; 1C) than in untreated control (1A) plots, must be attributed to the association of borer attack with better growing plants (Harris, 1962; Van Rensburg, Walters & Giliomee, 1989), and to the inefficacy of this compound against stalk borers.

Lack of control by terbufos (2Q; 2R) of C. mbila and the two stalk borer species was also reflected in the plant mass data (Table 8.3). The increases in plant mass attributable to control of the stalk borers and C. mbila were substantial, with the carbofuran treatment (2H) resulting in the greatest increase (62,4 %) relative to the untreated control (2A).

A single application of a soil systemic insecticide in the planting furrow has been shown to control several insect and nematode pests attacking maize in South Africa (Drinkwater et al. 1979; Van Rensburg, 1988; Chapter 7). Supplemental applications (side-dressings) have not provided additional benefit compared with a single in-furrow application for control of leafhopper vectors of plant diseases (Pitre, 1968b) and stalk borers (Egwuatu & Ita, 1982; Van Rensburg & Malan, 1982). In this experiment, the residual protection against both pests over almost the entire pre-tasseling period of maize, provided particularly by benfuracarb (1O; 1P), furathiocarb (1M), and carbofuran (2F; 2H), was such that two applications (in-furrow + side-dress)

evidently would also not have improved on the control reported in our study.

CONCLUSION

Soil systemic insecticides applied in the planting furrow can provide excellent control of maize pests. However, some of them such as aldicarb and terbufos have a limited target range. Hence, a broad-spectrum soil systemic insecticide, applied in a formulation and at a dosage rate that would control most maize pests, should be used in MSV afflicted areas. This would eliminate additional insecticide applications for control of other maize pests, thereby reducing cost of protection against MSV infection indirectly.

REFERENCES

BHIRUD, K.M. & PITRE, H.N. 1972. Bioactivity of systemic insecticides in corn: Relationship to leafhopper vector control and corn stunt disease incidence. Journal of Economic Entomology 65 : 1134-1140.

BOT, J., SWEET, S., KRAUSE, M. & HOLLINGS, N. 1988. A guide to the use of pesticides and fungicides in the Republic of South Africa. 33 rd rev. ed. Pretoria : Government Printer. 319 pp.

DRINKWATER, T.W., WALTERS, M.C. & VAN RENSBURG, J.B.J. 1979. The application of systemic insecticides to the soil for the control of the maize stalk borer, Busseola fusca (Fuller) (Lep. : Noctuidae), and of Cicadulina mbila (Naudè) (Hem. : Cicadellidae), the vector of maize streak virus. Phytophylactica 11 : 5-12.

EGWUATU, R.I. & ITA, C.B. 1982. Some effects of single and split applications of carbofuran on the incidence of and damage by Locris maculata, Busseola fusca and Sesamia calamistis on maize. Tropical Pest Management 28 : 277-283.

HARRIS, K.M. 1962. Lepidopterous stem borers of cereals in Nigeria. Bulletin of Entomological Research 53 : 139-171.

HEINRICHS, E.A. 1979. Control of leafhopper and planthopper vectors of rice viruses. Pages 529-560 in: Leafhopper vectors and plant disease agents. Maramorosch, K. & K.F. Harris, eds. New York : Academic Press, Inc.

KOLBE, W. 1975. Trials with Curaterr insecticide in maize, beets, potatoes and vegetables, with consideration to effect on yield. Pflanzenschutz Nachrichten Bayer 28 (1) : 144-151.

KUHN, C.W., JELLUM, M.D. & ALL, J.N. 1975. Effect of carbofuran treatment on corn yield, maize chlorotic dwarf and maize dwarf mosaic virus diseases, and leafhopper populations. Phytopathology 65 : 1017-1020.

PITRE, H.N. 1968a. Effect of insecticidal sprays on stunt and mosaic virus diseases of corn in small field plots in Mississippi. Journal of Economic Entomology 61 : 585-587.

PITRE, H.N. 1968b. Systemic insecticides for control of the black-faced leafhopper, Graminella nigrifrons, and effect on corn stunt disease. Journal of Economic Entomology 61 : 765-768.

VAN RENSBURG, G.D.J. 1981. Effect of plant age at the time of infection with maize streak virus on yield of maize. Phytophylactica 13 : 197-198.

VAN RENSBURG, G.D.J. & MALAN, E.M. 1982. Control of sorghum pests and phytotoxic effect of carbofuran on five hybrids of grain sorghum. Phytophylactica 14 : 159-163.

VAN RENSBURG, J.B.J. 1988. Efficacy of cloethocarb for control of Busseola fusca, Cicadulina spp. and nematodes in maize. Applied Plant Science 2 : 63-67.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. 1989. Selective oviposition by the maize stalk borer, _Busseola fusca_ (Fuller) (Lepidoptera : Noctuidae). _Journal of the Entomological Society of Southern Africa_ 52 : 105-108.

TABLE 8.1 Formulations and dosage rates of insecticides applied at planting, and the corresponding plant emergence of maize

Treatment	Formulation[1] (g a.i.kg^{-1})	Dosage rate (g a.i. 100 m^{-1})		Average number of plants emerged (14 DAP)[2]	
		Trial 1	Trial 2	Trial 1	Trial 2
A. Control	Untreated	0	0	107,7 b[3]	147,3 ab[3]
B. Aldicarb	15 G	15	-	106,5 b	-
C. Aldicarb	15 G	18	-	106,5 b	-
D. Cloethocarb	5 G	-	5	-	158,5 a
E. Cloethocarb	5 G	10	10	99,0 a	151,5 ab
F. Carbofuran	10 G	-	10	-	154,8 ab
G. Carbofuran	10 G	15	-	103,0 ab	-
H. Carbofuran	10 G	20	20	104,7 ab	163,5 a
I. Carbosulfan	25 EC	20	-	108,7 b	-
J. Carbosulfan	25 EC	28	-	105,7 b	-
K. Furathiocarb	5 G	-	10	-	154,5 ab
L. Furathiocarb	5 G	15	-	104,8 ab	-
M. Furathiocarb	5 G	20	20	104,0 ab	158,8 a
N. Benfuracarb	5 G	-	10	-	164,8 a
O. Benfuracarb	10 G	20	-	105,5 b	-
P. Benfuracarb	10 G	30	-	104,8 ab	-
Q. Terbufos	10 G	-	14	-	141,5 ab
R. Terbufos	10 G	-	28	-	125,8 b

[1] g a.i. $^{-1}$ for carbosulfan

[2] DAP = Days after planting

[3] Means within columns followed by the same letter do not differ significantly at P=0,05

TABLE 8.2 The influence of systemic insecticides applied to the soil at planting on the incidence of maize streak disease and stalk borers (Busseola fusca and Chilo partellus) in maize (Trial 1)[1]

| Treat-ment | % Infested plants | | | | | | | | | |
| | Streak disease | | | | | Stalk borers | | | | |
	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP
A	33,5 a	68,4 a	81,9 a	94,4 a	94,8 a	17,9 a	19,1 a	20,7 a	26,8 abc	27,9 ab
B	5,3 b	13,4 cde	27,3 bcd	37,5 bc	38,1 bc	13,4 b	15,3 ab	21,2 a	30,0 a	30,5 a
C	2,5 bc	10,2 de	18,8 de	25,1 de	25,9 de	9,3 c	11,6 b	18,1 a	29,8 ab	30,4 a
E	2,6 bc	16,4 bcd	25,0 bcd	38,3 bc	38,3 bc	1,7 d	4,6 c	12,9 b	21,0 cd	22,0 bc
G	5,5 b	20,1 bc	33,8 b	45,3 b	45,6 b	2,3 d	5,7 c	12,7 bc	20,3 cd	20,3 cd
H	4,8 b	22,2 b	30,1 bc	37,8 bc	37,8 bcd	0,3 d	4,8 c	10,2 bcd	20,3 cd	21,2 bc
I	4,6 bc	19,8 bc	28,5 bcd	39,9 bc	41,0 bc	0,9 d	4,5 c	10,7 bcd	19,5 de	19,8 cd
J	4,8 bc	14,2 cde	21,3 cde	30,7 cde	31,5 cde	1,1 d	6,7 c	10,9 bcd	22,7 bcd	23,2 abc
L	3,3 bc	13,2 cde	24,5 bcd	32,6 cde	32,9 cde	0,8 d	3,7 c	13,4 b	22,6 cd	23,3 abc
M	3,0 bc	8,4 e	19,9 de	29,1 cde	32,3 cde	0,0 d	3,7 c	10,4 bcd	16,3 de	16,3 cd
O	2,2 bc	11,2 de	20,4 cde	36,5 bcd	37,3 bcd	0,3 d	3,8 c	8,5 cd	17,6 de	18,1 cd
P	1,5 c	8,0 e	14,8 e	22,3 e	22,6 e	0,2 d	3,2 c	6,8 d	13,1 e	13,7 d

1. ... ... f ll d by th s m l tt r do not differ significantly at P=0.05

TABLE 8.3 The influence of systemic insecticides applied to the soil at planting on the incidence of maize streak disease and the stalk borers, _Busseola fusca_ and _Chilo partellus_, in maize and the corresponding plant mass (Trial 2)[1]

Treat-ment	% Infested plants											Plant mass (kg per 50 plants)
	Streak disease						Stalk borers					
	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	
A	4,0 a	32,1 a	63,0 a	86,6 a	95,8 a	98,9 a	7,5 a	12,4 a	22,3 a	23,0 a	29,7 a	10,1 a
D	0,2 ab	6,9 b	16,0 cd	37,6 cd	68,4 bc	75,7 bcd	1,9 ab	10,7 a	11,6 ab	17,7 ab	16,3 ab	14,2 cd
E	0,1 ab	3,1 b	11,1 cd	24,0 cd	50,5 c	55,7 d	1,5 ab	7,3 ab	8,6 ab	11,5 abc	17,1 ab	14,7 cd
F	0,2 ab	3,0 b	8,1 cd	19,9 d	44,6 c	52,4 d	0,0 b	4,1 ab	2,8 b	1,6 c	4,0 b	13,9 bcd
H	0,3 ab	1,4 b	5,1 d	21,9 d	48,8 c	55,5 d	0,5 b	5,1 ab	2,1 b	1,7 c	3,5 b	16,4 d
K	0,8 ab	7,1 b	23,7 bc	48,8 bc	78,5 abc	85,2 abc	1,1 ab	12,5 a	12,2 ab	17,1 ab	17,9 ab	12,7 abc
M	0,0 b	3,3 b	10,5 c	31,1 cd	60,9 bc	67,1 cd	1,0 ab	6,2 ab	4,1 b	3,6 bc	7,9 ab	14,9 cd
N	0,2 ab	2,8 b	10,3 cd	33,2 cd	62,2 bc	68,1 cd	0,4 b	9,0 a	7,1 ab	5,4 abc	13,3 ab	14,2 cd
Q	3,8 a	20,1 a	44,5 ab	67,1 ab	89,2 ab	93,3 ab	2,4 ab	14,6 a	17,5 ab	20,7 a	27,9 a	12,1 abc
R	4,1 a	23,8 a	47,9 ab	68,4 ab	88,6 ab	97,7 a	3,5 ab	12,8 a	13,4 ab	16,9 ab	21,2 ab	11,2 ab

[1]Means within columns followed by the same letter do not differ significantly at P = 0,05.

SUMMARY

1. The rate of development and the fecundity of C. anestae and C. mbila were determined at five constant temperatures. Developmental times for all life stages of both species were inversely related to temperature over the range 18°C to 30°C, and the data were used to calculate degree-days (DD) required for development. An estimated 312,5 DD (13,8 $^{\circ}$C lower developmental threshold) were required by C. mbila, and 384,6 DD (12,2 $^{\circ}$C lower developmental threshold) by C. anestae for development from egg to adult. At 18 $^{\circ}$C only 8,3 % of C. anestae nymphs survived to the adult stage, while all nymphs of C. mbila died before they could reach the adult stage. At temperatures above 21 $^{\circ}$C, nymphal survival increased considerably to 88,2 % and 90,3 % for C. anestae and C. mbila, respectively. Although a general trend towards a female biased sex ratio in both species was observed, only at 27 $^{\circ}$C for C. mbila did the sex ratio differ significantly from a 1 : 1 ratio (1 : 2,6). With increases in temperature pre-oviposition periods and day of peak oviposition decreased, while the number of eggs increased significantly for both species. The intrinsic capacity for increase peaked at 30 $^{\circ}$C for both species.

2. The seasonal occurrence of C. anestae and C. mbila were determined with sticky traps at two localities in South Africa from January 1982 to June 1987. Trap catches of either species were significantly correlated with degree-days lagged three sampling periods ($\pm$ 45 d) at both localities. Time series analysis of numbers of C. anestae and C. mbila adults caught in the sticky traps, were very useful in identifying sources of periodic variation in catches. Two distinct flight activity periods

were observed: a spring flight from August to December, and a late summer flight from January to May. Maximum flight activity occurred during February/March for both species. The results indicated that temperature, expressed in terms of degree-days, was the main factor which determined the seasonal occurrence of the leafhoppers. In this study rainfall had no significant direct affect on vector populations. Population densities of Cicadulina species may, however, be indirectly affected by rainfall through its influence on the incidence of suitable host plants and planting date of the maize crop.

3. Following a 90-day access period of C. mbila and C. anestae on infected maize, C. mbila was an exceptionally efficient vector of MSV compared with C. anestae. The transmission efficiency of females was significantly higher than that of males for both species, being 83 % and 40 % for C. mbila, and 31 % and 20 % for C. anestae, respectively. The importance of determining the identity of the Cicadulina species and sex ratio in studies on streak disease epidemiology is emphasized. The efficiency of C. mbila as a vector of MSV makes this species very suitable for bioassays involving virus transmission.

4. MSV incubation in maize was inversely related to post-inoculation temperature. The number of C. mbila adults feeding on the plants had a pronounced effect on rapidity of MSV symptom appearance and on symptom severity. An increase in the number of vectors from one to 20 resulted in a decrease in the mean incubation period from 3,9 d to 3,0 d at a day/night temperature of 30/23 °C, as well as in an increase in the average symptom severity index.

5. Data from maize cultivar trials at Vaalharts over a five year period were used to evaluate the level of resistance to MSV in commercial maize hybrids, and to determine the potential value of such resistance. Maize hybrids presently grown in South Africa showed varying degrees of resistance to MSV. In general, yellow maize hybrids displayed a wide range of genetic variability, ranging from resistant to highly susceptible, whereas white maize hybrids were mostly highly susceptible. Calculated resistance indices on a scale from 1 (resistant) to 5 (highly susceptible) proved very useful in quantifying genetically based plant reaction to MSV infection. Hybrids ranked consistently similar, both within and over seasons. The relationship between yield loss due to MSV infection and resistance indices was positively correlated.

6. A greenhouse experiment was conducted to determine the effect of three insecticides in preventing the maize leafhopper, C. mbila, from transmitting MSV. Feeding threshold period for MSV transmission was 30 min and the number of plants developing MSV symptoms increased progressively as the feeding time was extended beyond 30 min. MSV symptoms appeared sooner after inoculation as the feeding periods was increased. Knockdown of the leafhoppers by carbofuran 46 d after treatment was still sufficient to prevent MSV transmission. Both monocrotophos and deltamethrin, applied as sprays directed into the plant whorls, were ineffective in preventing MSV transmission since the feeding time of the vectors exceeded the minimum period required for virus transmission on the first and third days after treatment, respectively.

7. Corrective post-emergence sprays of monocrotophos, buprofezin and buprofezin/deltamethrin proved to be inefficient for the control of C. mbila, a

leafhopper vector of maize streak virus (MSV). Protection of maize against MSV infection could only be achieved through conventional pre-emergence preventative treatment with systemic insecticides applied in the planting furrow at planting. In this study the use of carbosulfan applied as a seed dressing suppressed the leafhopper population but did not control the stalk borers, Busseola fusca (Fuller) and Chilo partellus (Swinhoe). Carbofuran granules applied to the planting furrow at rates of 15g and 20g a.i. per 100m row length provided simultaneous control of the leafhopper vector and the stalk borers.

8. Granular formulations of aldicarb, benfuracarb, carbofuran, cloethocarb, furathiocarb, and terbufos, and an emulsifiable concentrate of carbosulfan were evaluated as soil applications at planting for control of C. mbila, and the stalk borers, B. fusca and C. partellus. The incidence of leafhoppers, as reflected in number of MSV infected plants, were reduced significantly by all insecticides except terbufos. Stalk borers were not effectively controlled by aldicarb and terbufos. Benfuracarb, carbofuran and furathiocarb were particularly effective against both leafhoppers and stalk borers, providing residual protection of maize over almost the entire pre-tasseling period. The use of a broad-spectrum soil systemic insecticide for leafhopper control is advocated since it would eliminate additional insecticide applications for control of other maize pests, thereby reducing cost of protection against MSV infection indirectly.

OPSOMMING

1. Die ontwikkelingstempo en die fekunditeit van C. anestae en C. mbila is
    bepaal by vyf konstante temperature. Ontwikkelingsperiodes van alle
    lewenstadia van beide spesies was omgekeerd verwant aan temperatuur oor
    die reeks $18^{O}C$ tot $30^{O}C$, en dié data is gebruik om die aantal graaddae
    (DD) te bereken wat nodig is vir ontwikkeling. 'n Beraamde 312,5 DD
    $(13,8 \,^{O}C$ onderste ontwikkelingsdrumpel) was benodig deur C. mbila, en
    384,6 DD $(12,2 \,^{O}C$ onderste ontwikkelingsdrumpel) deur C. anestae vir
    ontwikkeling van eier tot volwassene. By $18 \,^{O}C$ het slegs 8,3 %
    C. anestae nimfe die volwasse stadium bereik, terwyl alle nimfe van
    C. mbila dood is voordat hulle die volwasse stadium kon bereik. By
    temperature bokant $21 \,^{O}C$ het nimf-oorlewing aansienlik toegeneem tot
    88,2 % en 90,3 % vir C. anestae en C. mbila, onderskeidelik. Hoewel 'n
    algemene neiging tot 'n wyfie-oorheersde geslagsverhouding in beide
    spesies waargeneem is, was dit slegs by $27 \,^{O}C$ vir C. mbila dat die
    geslagsverhouding betekenisvol van 'n 1:1 verhouding verskil het (1:2,6).
    Vooreierleggingsperiodes en dae van maksimum eierlegging het afgeneem, en
    die aantal eiers het betekenisvol toegeneem vir beide spesies met toename
    in temperatuur. Die intrinsieke aanwasvermoeë het vir beide spesies by
    $30 \,^{O}C$ 'n piek bereik.

2. Die seisoenale voorkoms van C. anestae en C. mbila is met kleefvalle by
    twee lokaliteite in Suid-Afrika bepaal vanaf Januarie 1982 tot Junie
    1987. Valvangstes van beide spesies was betekenisvol gekorreleer met
    graad-dae van drie monsternemingsperiodes ($\pm$ 45 d) vroeër by beide
    lokaliteite. Tydreeksanalise van getalle C. anestae en C. mbila
    volwassenes wat in die kleefvalle gevang was, was van groot nut om bronne

van periodieke variasie in vangstes te identifiseer. Twee hoof
vlugaktiwiteit-periodes is waargeneem: 'n lentevlug vanaf Augustus tot
Desember en 'n laat-somervlug vanaf Januarie tot Mei. Piek vlugaktiwiteit
het gedurende Februarie/Maart voorgekom vir beide spesies. Die resultate
het aangetoon dat temperatuur, uitgedruk in terme van graad-dae, die
belangrikste faktor is wat die seisoenale voorkoms van die blaarspringers
bepaal. In hierdie studie het reënval nie 'n betekenisvolle direkte
invloed op vektorpopulasies uitgeoefen nie. Bevolkingsdigthede van
Cicadulina spesies mag egter indirek deur reënval bepaal word deur die
invloed daarvan op die voorkoms van geskikte voedsterplante en plantdatum
van mielies.

3. Na 'n 90 dae toegangsperiode van C. mbila en C. anestae tot besmette
mielies, was C. mbila 'n besonder doeltreffende vektor van MSV in
vergelyking met C. anestae. Die oordragingsdoeltreffendheid van wyfies
was betekenisvol hoër as die van mannetjies vir beide spesies, naamlik
83 % en 40 % vir C. mbila, en 31 % en 20 % vir C. anestae,
onderskeidelik. Die belangrikheid om die identiteit van Cicadulina
spesies en die geslagsverhouding te bepaal by studies oor streepsiekte-
epidemiologie word beklemtoon. Die doeltreffendheid van C. mbila as 'n
vektor van MSV maak hierdie spesie baie geskik vir bio-essais ten opsigte
van virusoordraging.

4. MSV inkubasie in mielies was omgekeerd verwant aan na-inokusasie
temperatuur. Die aantal C. mbila volwassenes wat op die plante gevoed
het, het 'n duidelike effek op die tempo van MSV simptoomverskyning en op
simptoomstrafheid gehad. 'n Toename in die aantal vektore van een tot 20
het 'n afname vanaf 3,9 d tot 3,0 d in die gemiddelde inkubasieperiode tot

gevolg gehad by 'n dag/nag temperatuur van 30/23 ^{O}C, asook 'n verhoging in die gemiddelde indeks van simptoomstrafheid.

5. Data van mieliecultivarproewe te Vaalharts oor 'n vyf jaar periode is gebruik om die vlak van weerstand teen MSV in kommersiële mieliebasters te evalueer, en die potensiële waarde van sodanige weerstand te bepaal. Mieliebasters wat tans in Suid Afrika verbou word, het verskillende vlakke van weerstand teen MSV getoon. In die algemeen het geelmieliebasters 'n wye reeks genetiese variasie vertoon, van weerstandbiedend tot hoogs vatbaar, terwyl witmieliebasters meestal hoogs vatbaar was. Berekende weerstandindekse op 'n skaal van 1 (weerstandbiedend) tot 5 (hoogs vatbaar) was baie nuttig om die geneties-gebaseerde plantreaksie teen MSV-infeksie te kwantifiseer. Basters het deurgaans dieselfde rangorde gehandhaaf, beide binne en oor seisoene. Die verhouding tussen oesverlies as gevolg van MSV-infeksie en weerstandindekse was positief gekorreleer.

6. 'n Glashuiseksperiment is uitgevoer om die effek van drie insekdoders op die voorkoming van die oordraging van MSV deur die mielieblaarspringer, C. mbila, te bepaal. Voedingsdrumpelperiode vir MSV-oordraging was 30 min en die aantal plante wat MSV simptome ontwikkel het, het progressief verhoog namate die voedingstyd verby 30 min verleng is. MSV simptome het vinniger na inokulasie verskyn hoe langer die voedingsperiode was. Uitklop van die blaarspringers deur karbofuran 46 d na toediening was steeds voldoende om MSV-oordraging te verhoed. Beide monokrotofos en deltametrien, toegedien as kelk-gerigte bespuitings, was ondoeltreffend in die voorkoming van MSV oordraging omdat die voedingstyd van die vektore die minimum periode wat benodig word vir virusoordraging op die eerste en derde dae na toediening, respektiewelik, oorskry het.

7. Korrektiewe na-opkom bespuitings met monokrotofos, buprofezin en buprofezin/deltametrien vir die beheer van C. mbila, 'n blaarspringervektor van mieliestreepvirus (MSV), was ondoeltreffend. Beskerming van mielies teen MSV infeksie kon slegs verkry word deur konvensionele vooropkoms-voorkomende behandeling met sistemiese insekdoders wat in die plantvoor toegedien word tydens planttyd. In hierdie ondersoek het die gebruik van karbosulfan, toegedien as 'n saadbehandeling, die blaarspringerbevolking onderdruk maar nie die stamboorders, Busseola fusca (Fuller) en Chilo partellus (Swinhoe) nie. Karbofuran-korrels toegedien in die plantvoor teen dosisse van 15g en 20g a.b. per 100m rylengte het gelyktydige beheer van die blaarspringervektor en die stamboorders verskaf.

8. Korrel-formulasies van aldikarb, benfurakarb, karbofuran, klo-ethokarb, furathiokarb en terbufos, en 'n emulgeerbare konsentraat van karbosulfan is geëvalueer as grondtoedienings tydens plant vir beheer van C. mbila, en die stamboorders, B. fusca en C. partellus. Die voorkoms van die blaarspringers, soos weerspieël in die aantal MSV-besmette plante, is betekenisvol verminder deur alle insekdoders behalwe terbufos. Stamboorders is nie doeltreffend beheer deur aldikarb en terbufos nie. Benfurakarb, karbofuran en furathiokarb was besonder doeltreffend teen beide blaarspringers en stamboorders en het residuele beskerming van die mielies oor bykans die volle voor-pluim periode verskaf. Die gebruik van 'n breë-spektrum grondsistemiese insekdoder word vir blaarspringerbeheer voorgestel aangesien dit addisionele insekdodertoedienings vir beheer van ander mielieplae sal uitskakel, en daardeur die koste van beskerming teen MSV-besmetting indirek verlaag.

ACKNOWLEDGEMENTS