SOME VECTOR, VIRUS, HOST-PLANT RELATIONSHIPS OF THE SIX-SPOTTED LEAFHOPPER, *Macrosteles fascifrons* (Stål) AND ASTER YELLOWS VIRUS IN MANITOBA.

A Thesis

Presented to

The Department of Entomology

Faculty of Agriculture and Home Economics

The University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Howard Percival Richardson

January 1966

ABSTRACT

by

Howard Percival Richardson

SOME VECTOR, VIRUS, HOST-PLANT RELATIONSHIPS OF THE SIX-SPOTTED LEAFHOPPER, Macrosteles fascifrons (Stål), AND ASTER YELLOWS VIRUS IN MANITOBA.

Contact and systemic insecticides formulated as emulsive concentrates (EC), wettable powders (WP), and granules (G) were compared at various times, rates and intervals of application to control the six-spotted leafhopper, Macrosteles fascifrons (Stål), and prevent the spread of aster yellows virus (AYV) to head lettuce and carrots. The insecticides malathion EC, Baygon EC and G and phorate G, at the rate of one pound per acre controlled the six-spotted leafhopper and prevented the spread of AYV to the head lettuce in the spring and summer crops of 1960, 1961 and 1962. The same insecticides gave only partial protection to the spring crop and failed to protect the summer crop of 1963 because of a combination of a large population of M. fascifrons with a high percentage (ten per cent) of infective leafhoppers. Other insecticides tested against M. fascifrons on head lettuce were less effective.

The start of applications of insecticides to the spring crops may be delayed for two or three weeks after the plants emerge. On summer crops, spray applications must begin at crop emergence.

DDT EC and Carbaryl WP, one pound per acre, one application per week gave a significant reduction in carrots affected by AYV when the spray applications were started within two to three weeks of crop emergence.

Less than 0.5 p.p.m. of malathion was found on lettuce heads which were analyzed nine days after the last of 15 applications.

Three strains of AYV, "A", "B", and "C", were isolated from lettuce, zinnia and celery, respectively, in Manitoba. The three strains were separated by symptoms on aster, Nicotiana rustica var. humilis and celery. The criteria: 1) plant height from first leaf node to tip; 2) plant height from first to last exposed leaf node; 3) number of exposed leaf nodes and 4) axillary growth on aster were also successfully used to separate the strains.

The transmission of the three strains of AYV by single infective M. fascifrons showed that the six-spotted leafhopper, male or female, is a consistent and reliable transmitter after a two-day inoculation feed; that aster is a poor indicator of the proportion of infective leafhoppers; that stinkweed and head lettuce are superior indicator plants; and that different species of host plants vary in their susceptibility to different strains.

The acquisition of the three strains of AYV by M. fascifrons from 14 different host plants showed that it depended on the strain of AYV and the host plant.

ACKNOWLEDGMENTS

I wish to thank Dr. A. E. Hannah, Director, Research Station, Canada Department of Agriculture, Winnipeg, Manitoba for permission to use the project as a thesis, and Dr. A. G. Robinson, Department of Entomology, University of Manitoba, for accepting the project as a thesis.

Thanks are due also to Mr. P. H. Westdal for doing the final statistical analysis of data presented in Chapters IV, V, VI, and VII; to Dr. L. B. Smith and Dr. W. Ives for advice on statistical methods; to Mr. R. Cheale for photographing the plants in Fig. 8, 10, and 19; to Dr. P. Barker, Dr. W. C. McDonald and others for reviewing the manuscript and Mr. J. Ilchyna, Mr. N. Brandt, Mr. D. Tiltman and Mr. G. Cox for assistance in carrying out the field experiments.

TABLE OF CONTENTS

APPENDIX A (Cont'd) PAGE

LIST OF TABLES

TABLE PAGE

LIST OF TABLES

APPENDIX B

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

The recognition of plant virus infections as a distinct class of disorders and of insects as vectors of the plant viruses dates back to the end of the nineteenth century (Cook, 1947). Since then, it has been shown that most plant species of economic importance are infected by one or more virus diseases, many of which are transmitted by insects (Chapman, 1959). In Manitoba, wheat is known to be infected by three insect-transmitted viruses: barley yellow dwarf, aster yellows, and wheat striate mosaic; oats by two: barley yellow dwarf and oat blue dwarf; barley by four: barley yellow dwarf, oat blue dwarf, aster yellows, and wheat striate mosaic; flax by two: oat blue dwarf and aster yellows, and most other crops, vegetables and herbaceous ornamentals by at least one: aster yellows. Barley yellow dwarf, a virus disease of world distribution, was discovered in 1951 by Oswald and Houston (Bruehl, 1961), and the oat blue dwarf as recently as 1961 (Banttari and Moore, 1961).

The protection of the crops from the insect-transmitted viruses may be based on the development of crop varieties resistant to the insect or the disease, or, on the use of chemicals. Each method has its particular advantages or disadvantages, but common to each is the necessity for a thorough understanding of the vector, virus and host-plant relationships. The complexity of the relationship is illustrated by the aster yellows virus (AYV). It is a complex of virus

strains (Granados, 1965) some with different host ranges, different host effects and different vectors in different parts of its range. Macrosteles fascifrons (Stål), the six-spotted leafhopper, is the major vector, though only one of at least 25 different species of leafhoppers which are capable of transmitting AYV. In Manitoba the migrant populations of M. fascifrons are the major source, through the eggs they deposit, of the local population that develops in Manitoba. The migrants are also the major source of AYV which they carry with them and inject into the local crop and weed plants. The diseased crop and weed plants form the major source of infection for the generations of M. fascifrons which develop in Manitoba.

The problem

The problem is the protection of the crops from infection by AYV. The first method studied was the direct destruction of the six-spotted leafhopper by chemicals. For this purpose a series of insecticides were evaluated to determine the most effective time of application, rates and interval of application.

The second method was a study to determine the relationship of the vector and virus with a number of common host plants. The purpose was to elucidate the facts that may prove effective in reducing AYV infections, possibly through the reduction of certain host plants or in the production of resistant crop plants.

Importance of the problem

The host range of M. fascifrons and AYV includes most of

the cereal crops, oil seed crops, vegetables and many of the herbaceous ornamentals grown in Manitoba. Although the losses due to AYV infection seldom exceed ten per cent of most crops, these losses have practically eliminated commercial production of head lettuce in Manitoba, and constitute serious problems on carrots and celery; in some years some growers have lost their entire celery crops. The loss in most of the other crops caused by the virus disease although low, represents another addition to the cost of production and to the cost of the final food product.

Organization of the thesis

The chemicals used in the tests are referred to by their trade names or company designations throughout the thesis. The chemical names are given in the section on Materials and Methods Chapter III. The results of the field tests are presented in Chapters IV to VII.

The laboratory tests conducted at the Canada Department of Agriculture, Research Station, Winnipeg, to differentiate three strains of aster yellows virus, and on the ability of M. fascifrons to transmit three strains of AYV to and from fourteen different host plants are given in Chapters VIII, IX and X respectively.

Two publications based on the results presented in Chapters IV, V, VI and VII are given in Appendix A. Summary tables of data that form the basis for the final Tables presented in the text are given in Appendix B.

CHAPTER II

REVIEW OF LITERATURE

Control of insect vectors of plant viruses

The control of vectors of plant viruses to prevent the spread of diseases has been attempted with insecticides, trap crops, and various types of barriers. A survey of the literature on insecticidal control of the spread of plant viruses was presented by Broadbent (1957). In this review he listed the earlier successes and failures by a number of workers attempting to reduce the spread of spotted tomato wilt on tomatoes by thrips, potato viruses by aphids, aster yellows virus on lettuce, carrots, and endive by M. fascifrons and curly top of sugar beets by the leafhopper Circulifer tenellus Baker. Broadbent (1957) concluded from the early attempts to control the spread of viruses by using such insecticides as nicotine, derris and pyrethrum, that it is not sufficient to kill the insects on the crop, that insufficient insecticide remained active on the foliage, and very frequent applications were necessary to prevent virus spread. These were expensive and often failed because incoming insects introduced virus or spread it from plant to plant in the intervals between applications. An increased incidence of potato virus Y in potato fields sprayed with insecticides has been reported by various workers (Chapman, 1951; McEwen, 1953; Roland, 1953). McEwen (1953) showed that insecticides kept the foliage green late in the season thus making the plants susceptible to late-season infection. Chapman (1951) hypothesized that parathion increased aphid activity and

Roland (1953) suggested that it attracted aphids. According to Shanks and Chapman (1965), aphids, after initial probes made longer feeding probes on parathion-treated tobacco than on untreated leaves and vice-versa on DDT-treated leaves. Neither material affected aphid acquisition of potato virus Y but parathion tended to decrease and DDT to increase the number of transmissions to treated plants. The authors found that the insecticides required 51-180 min. to kill 90 per cent of the aphids two hours after application and much longer three days after treatment, while aphids can transmit non-persistent viruses in less than a minute. They also found that the winged aphids remain longer on parathion-treated plants than on DDT-treated plants, and, that infected aphids transmitted potato virus Y slightly more often to parathion-treated than to untreated tobacco plants.

It was shown by Becker and Rich (1956) and by Morgan (1965) that the control of aphids, Pentatrichopus spp., on strawberry beds prevented the spread of virus diseases and, as a result, increased runner and fruit production. Morgan (1965) showed that one application of Meta-Systox or dimethoate during the spring to prune trees gave a season-long control of aphids and suggested that limitation of the spread of viruses in the field through control of insect vectors, whether or not the identity and behavior characteristics of such vectors are known, is feasible.

Harrison et al (1963) and Murant et al (1965) showed that the nematocide D-D effectively controlled two species of nematodes Xiphinema diversicaudatum (Micol) and Longiforus elongatus (de Mon) and thus

prevented the spread of virus diseases in strawberry beds. According to Murant et al (1965) the effects lasted throughout the four year experiment.

Strong and Rawlins (1958) tested four insecticides: DDT, demeton, malathion and parathion at various dosages for the control of M. fascifrons on lettuce. The materials were sprayed on plants growing outdoors and which were taken into the laboratory for tests after different lengths of time. Their conclusion was that none of the insecticides applied as sprays was very effective for as long as three days. Further tests were carried out by Strong and Rawlins (1958) in the field using the same four insecticides and using populations of infective insects. The authors were able to differentiate between incidence of disease on sprayed and unsprayed lettuce, and in the residual effectiveness of the insecticides tested. The differences between the insecticides were not evident and the degree of protection was not satisfactory for commercial conditions. Chiykowski (1958), found malathion effective for the control of M. fascifrons on head lettuce and prevention of infection by AYV. Miller (1960) recommended DDT and malathion for the control of M. fascifrons on carrots and lettuce in Ontario.

Thompson and Rawlins (1961) tested the systemic insecticides phorate, dimethoate and Di-Syston to control M. fascifrons and reduce the incidence of lettuce yellows. The insecticides, applied at the rate of one pound per acre at time of seeding as granules or as drenches, were more effective than seed treatments and equally or more effective than malathion applied twice weekly as a spray. Phorate was more

effective than Di-Syston or dimethoate. The systemic materials were effective four to five weeks following soil application but appeared to lose much of their effectiveness six weeks after application. The systemics gave a significant reduction in the incidence of lettuce yellows.

Thompson (1965) applied combined sprays of DDT one pound and malathion 1.25 pound per acre at weekly intervals for the control of the leafhopper vectors of lettuce yellows. The reduction in lettuce yellows was significant. Twice weekly applications of the insecticide mixture were more effective than weekly applications. Granular phorate one pound per acre at seeding time was as effective as Di-Syston and an Experimental Insecticide 43064. Control of lettuce and carrot yellows with phorate was as effective as that with malathion or Carbaryl.

The use of barriers both physical and insecticidal has been tried by a number of workers. Pepper and Haenseler (1939) used a seven foot high cloth fence to enclose a plot of lettuce. At harvest the fenced lettuce showed a one per cent infection of lettuce yellows and the check 31.8 per cent. The fence proved impractical in commercial operations. Plant barriers dusted with insecticides were tested but failed to stop migrating aphids and proved uneconomic (Crumb and McWhorter, 1948). This was again shown by Thompson (1956) in which he sprayed a barrier of sweet corn that completely surrounded lettuce and carrot beds. The barrier failed to stop the six-spotted leafhopper.

Vector, virus, and host-plant relationship

The relationship between the six-spotted leafhopper and the aster yellows virus was first discovered by Kunkel (1924). The host of the virus was the China aster, Callistephus chinensis Nees. Following this discovery, Kunkel (1926) showed that AYV could be transmitted experimentally by M. fascifrons to more than 50 different species in 23 different families of plants. In California, Severin (1929) observed celery and Zinnia elegans Jacq. infected with yellows and transmitted the yellows from Z. elegans to aster and back to Z. elegans. The symptoms in aster were similar to those of aster yellows. Kunkel (1931) failed to transmit aster yellows to celery and concluded that the two strains of aster yellows were not identical. Further evidence of the difference between the two strains was provided by Severin (1934) who used infected plants obtained from California, Idaho, Indiana, Maine, New York and Wisconsin as source of AYV, to show that celery was susceptible only to the California strain of the virus. Further tests by Kunkel (1955) showed that the two strains could be readily separated on the basis of symptoms on aster, Nicotiana rustica L., Vinca rosea L., and Z. elegans. The identification of the aster yellows and California aster yellows strains was followed by the identification of a number of additional strains of AYV (Kunkel, 1937a, 1937b, 1945; Frazier and Posnette, 1948; Freitag, 1948, 1964; Raymer and Milbrath, 1960; Lee, 1962; and Granados, 1965).

Vector-virus relationship

The relationship between M. fascifrons and the aster yellows

virus has been closely studied since Kunkel (1924) discovered that M. fascifrons was the vector of AYV. No other species has been found that transmits the Eastern strain of AYV though 25 species of leafhoppers have been recorded as vectors of the Western strains or celery-infecting strains (Wallis, 1960; Chiykowski, 1962, 1963).

The ability of the various stages of M. fascifrons to acquire AYV from infected plants was studied by Kunkel (1926) who performed 34 experiments with different ages of nymphs, and with viruses obtained from different host plants. He showed the existence and approximate length of the incubation period. He concluded that nymphs at all stages and adults of both sexes were capable of acquiring the virus. In one experiment in which 80 newly hatched nymphs were confined for successive periods on three healthy aster plants, the virus incubated in the nymphs and was transmitted by them. This result was obtained with a culture kept at a relatively low temperature. At temperatures of 70° F, or above Kunkel (1962) found that most of the insects matured before the incubation period of the virus was completed. Whipp (1951) also found that all stages of the insect were able to acquire the virus when reared on diseased plants. The proportion of the colonies which acquired the virus, however, was less with the first and second instar nymphs than with later instars. Both nymphs and adults were able to obtain the virus from both old and young leaves (Kunkel, 1926) and according to Chapman (1949) noninfective leafhoppers acquired the virus from symptomless leaves below the last evidence of the disease on aster plants as well as from distinctly symptomatic foliage.

The performance of individual M. fascifrons in acquiring and transmitting aster yellows virus was considered. Kunkel (1926) took young adults which had hatched and been reared on an infected aster and placed them individually on a succession of healthy asters with an exposure period of one to seven days. The 30 transmission records show that all the insects that lived long enough to give a transmission record were virus carriers. Both males and females were included in the group tested. According to Chapman (1949) starving the insect had no particular effect on its transmission of aster yellows, and such treatment often killed the insects in three to four hours. Various amounts of light per day did not affect the acquisition or transmission of AYV by the insect. The AYV did not affect the longevity of the leafhopper (Kunkel, 1926; Severin, 1947); and Kunkel (1929) and Dobroscky (1929, 1931) failed to find any cytological differences between infective or virus-free leafhoppers. However, Littau and Maramorosch (1956, 1960) showed cytological changes in nuclei of trophocytes or fat body cells of M. fascifrons infected with one strain of AYV but not with a second strain.

The effect of heat on the virus in M. fascifrons has been reported by several workers. Kunkel (1937) showed that infected colonies of six-spotted leafhoppers subjected to heat treatments lasting one day or longer at about 31° or 32° C lost the ability to transmit yellows either permanently or temporarily. The colonies held at these temperatures for 12 days or longer suffered permanent loss of ability to transmit AYV. Colonies treated from one to eleven days regained

ability to transmit AYV after periods varying from a few hours up to many days. Chapman (1949) found that constant temperatures of 32°C for ten days had no significant effect on the ability of the leafhoppers to transmit the virus. He found that following temperatures of 36°C for five days the leafhoppers regained their ability to transmit the virus after a post treatment period of seven to ten days, but temperatures of 40°C caused the insects to lose the virus permanently. He showed that the incubation period of the virus in the insect was longer at 18°C and 32°C than at 24°C and 28°C and insects held at 36°C did not become infective. According to Lee (1961) the thermal inactivation point of AYV in insects was higher than previously reported by Kunkel (1937) or Chapman (1949). He also found that 32°C for ten to thirteen days did not affect ability of infective colonies of six-spotted leafhoppers to transmit a celery-infecting isolate of AYV (Wis. CAYV). Colonies treated at 36°C for 10 days regained their ability to transmit the virus within six to eight days and infective colonies subjected to five-day exposures at 40°C regained their ability to transmit the virus within 18 days.

The acquisition and transmission of AYV by the six-spotted leafhopper were studied by Chapman (1949). He states that acquisition of the virus was accomplished in about 20 per cent of small colonies of six-spotted leafhoppers during infection feeding periods of two hours. One colony acquired the virus in a feeding period of only 16 minutes. A high percentage of transmission (Chapman, 1949) was affected by small colonies of viruliferous insects in test feeding periods of five to ten

hours. Single leafhoppers infected three per cent of the plants when fed on small asters for one hour and colonies of 16 leafhoppers gave 28 per cent infection in similar tests but no mass action phenomena were evident. Maramorosch (1953) used the Eastern strain of AYV and found that an occasional insect, after feeding for only ten minutes, acquired enough virus to become infective. However, with acquisition times of 8 and 24 hours, 37 per cent and 41 per cent of M. fascifrons became infective. Similarly he found that while a small number of plants could be infected during a ten minute inoculation period, the percentage of plants infected increased with feeding times of 100 and 1000 minutes. Strong and Rawlins (1958), however, found that infective insects resting on healthy plants for ten minutes transmitted Eastern AYV almost as readily as those caged on plants for six hours, but the percentage of infection was considerably higher for insects caged on healthy plants for 24 hours. Using a celery-infecting isolate of AYV and the same insect vector, Chiykowski (1958) found the shortest acquisition time to be two hours and the shortest inoculation time to be 15 minutes. Lee (1962) found that transmission of a celery-infecting isolate of AYV was an exponential function of both acquisition and inoculation periods, for feeding times arranged in a geometric progression from 0.125 to 32 hours. In a single acquisition test conducted transmission did not occur when feeding periods were less than two hours; however, transmission did occur over the entire range of inoculation times tested. The per cent transmission from a 32 hour acquisition time, or from a similar inoculation time were, 77.7 and 73.7 per cent respect-

ively. Maramorosch (1964) showed that the transmission of the Eastern strain of AYV by the six-spotted leafhopper fluctuated during a 16 hour period. Transmissions were less frequent during the first eight hours of each day and five distinct peaks, two in the morning hours and three in the afternoon, were repeatedly and consistently detected.

The ability of individual six-spotted leafhoppers to transmit AYV has been examined. Kunkel (1926) found that some individual leafhoppers transmit the Eastern strain of AYV to all plants on which they feed for as long as one day, while others transmit it with much less certainty. Chapman (1949) found that infective leafhoppers, after long periods on immune barley plants, transmitted the virus to asters rather erratically in short test-feeding periods but consistently in longer ones. He showed, also, that single insects introduced into large cages with many plants remained on one plant for as long as ten consecutive days and transmitted yellows to practically every plant on which they fed. After 18 days of incubation of the Eastern strain AYV in the six-spotted leafhopper, no differences were found in the infectivity of infective leafhoppers during infection periods of three, six and ten days on china aster (Strong and Rawlins,1958). After 18 days of virus incubation two infective insects per plant were as successful as large numbers in inoculation of all healthy plants. Infective insects which rested on plants for ten minutes transmitted virus almost as readily as those caged on plants for six hours, but per cent of infection was considerably higher for insects caged on plants for 24 hour periods.

Most infective leafhoppers inoculated only one plant each 24 hour period of individual confinement to groups of plants. After becoming infective, 70 to 78 per cent of infective leafhoppers inoculated all plants on which they were confined individually for 12 to 48 hours in serial transfers. Such consistent infection was not obtained with one hour confinement periods.

Virus host-plant relationships

Aster yellows virus has a very wide host plant range. Wallis (1960) listed 297 species of plants in 51 families which were susceptible to the virus. According to Sackston (1959), the virus has been found in 80 genera in about 30 families in Canada. To the above host lists two more hosts in the grass family have been added: barley (Banttari and Moore, 1960) and wheat (Chiykowski, 1963, 1965).

The symptoms caused by AYV on its hosts are: stunting, chlorosis and sterility (Kunkel, 1926; Chiykowski, 1964). The use of the effect of specific strains of AYV on the same host plants has been one method used to separate the strains (Kunkel, 1931; Severin, 1934; Kunkel, 1955; Freitag, 1948, 1964; and Granados, 1965). The host plants commonly used are aster C. chinensis; N. rustica; periwinkle, V. rosea; plantain, Plantago major L; and celery, Apium graveolens L. var. dulce Pers. Three strains of celery-infecting AYV were identified in California (Freitag, 1958) and Granados (1965) identified eight in Wisconsin of which two were related to the Western or celery-infecting group which either did not infect celery or infected celery occasionally.

One strain which infected celery was classified as intermediate because of symptoms resembling both Eastern and Western AYV.

Temperature affects incubation of aster yellows virus in the host plant as it does in the insect. Kunkel (1941) showed that plants of N. rustica and V. rosea infected with AYV would recover after subjecting the plants to high temperatures of 40°C for three weeks and 38° to 42°C for two weeks, respectively. Periwinkle also recovered after immersion in a water bath at 40° to 45°C for a few hours. Chapman (1949) found that the incubation period of the virus was similarly affected in insects, asters and carrots, when held at similar temperatures. The period became shorter in both host plants as the temperature was increased from 16° to 28°C but became longer at 32°C.

Resistance of the host plants to infection by aster yellows virus has been shown in carrots, sunflowers and flax. Based on the incubation period of AYV, 22.9 days in small carrots, 43.2 days in large carrots, Severin (1932) concluded that in all probability large carrots are more resistant to the disease. Putt and Sackston (1960) claimed that resistance to aster yellows had been identified in sunflowers. The resistance to aster yellows appeared to be qualitatively inherited and single cross hybrids between resistant and susceptible lines showed the resistance to be dominant.

Martin et al (1961) showed that the flax variety Abyssinian (C.I. 302) contributed a greater number of plants free from the AYV symptoms than other varieties tested. A selected strain of Abyssinian with a high degree of resistance to aster yellows was considered sat-

isfactory as a parent in a breeding program. Frederiksen (1964) found that varieties of flax selected for resistance or tolerance to aster yellows are not acceptable from an agronomic point of view.

Vector host relationship

The six-spotted leafhopper has a wide range of food plants. Osborn (1916) found it in the grasslands and oat fields of Maine and mentions it as a serious pest on wheat, oats and barley crops in the Northwest (Osborn, 1912). Kunkel (1926) listed 22 species on which the leafhoppers feed and reproduce and seven species on which they do not flourish. Wallis (1960) listed 144 species of plants as hosts of the leafhopper. Among 38 plant species, that Wallis (1962) sampled, over a period of three years, there were no leafhoppers taken on collards, eggplant or okra. He found celeriac, celery and carrot more susceptible to infestation than any of the other plants. He listed carrot, lettuce and onion in a descending order of susceptibility as did McClanahan (1962). Lee and Robinson (1958) listed lettuce, aster, parsley, carrot or flax in a descending order of preference. According to McClanahan (1962) the reason for leafhopper preference for certain plant species cannot be explained. It does not seem to be a function of physical characteristics such as leaf form, color or pubescence. The insects move about for several minutes before starting to feed, and possibly are influenced by a chemoreceptor mechanism.

CHAPTER III

GENERAL MATERIALS AND METHODS

Field experiments

In Manitoba three crops of head lettuce, a transplant crop, and two field seeded crops, and one crop of carrots are grown each year. The transplant crop is harvested by mid-June and usually escapes severe infection. The two seeded head lettuce crops spring and summer, if they are not protected may be completely destroyed.

In the experiments the head lettuce (Imperial 456) and carrots (Special Long Type Nantes) were seeded with a "Planet Junior" seeder in rows two feet apart in plots approximately 50 x 50 feet. The plots were separated from each other by seven feet of cultivated soil. The lettuce seedlings were thinned to one foot between plants.

The insecticides used in the experiments are given in the following list by the common names designated by the Entomological Society of America (Billings, 1965) or the registered trade names or Company designations for experimental materials together with their chemical names.

Names used in the thesis	Chemical names
American Cyanamid (AC) 43064	2-(diethoxyphosphinothioylimino)-1,3-dithiolane
American Cyanamid (AC) 47470	2-(diethoxyphosphinylimino-4-methyl-1,3-dithiolane)
American Cyanamid (AC) 47031	2-(diethoxyphosphinylimino)-1,3-dithiolane
American Cyanamid (AC) 47300	NOT KNOWN
Baygon R	o-isopropoxyphenyl methylcarbamate
Captan R	N-((trichloromethyl)thio)-4-cyclo hexene-1,2-dicarboximide
* Carbaryl	1-naphthyl N-methylcarbamate
* DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane
Delnav R * (dioxathion)	p-dioxane-2,3-diyl ethyl ethyl phosphorodithioate
* Dimethoate	0,0-dimethyl S-(N-methyl carbamoylmethyl) phosphorodithioate
Di-Syston R * (disulfoton)	0,0-diethyl S-(2-(ethylthio) ethyl) phosphorodithioate
* Endosulfan	6,7,8,9,10,10-hexachloro-1,5, 5a,6,9,9a-hexahydro-6,9-methano 2,4,3-benzodioxathiepin-3-oxide
* Malathion	S-(1,2-bis(ethoxycarbonyl) ethyl) 0,0-dimethyl phosphoro-dithioate
Meta-Systox R * (oxydemetonmethyl)	S-(2-(ethylsulfinyl)ethyl)0,0-dimethyl phosphorothioate
* Phorate	0,0-diethyl S-((ethylthio)methyl) phosphorodithioate
TEPP R * (sulfotepp)	ethyl thiopyrophosphate

* - common name

R - Registered product

The insecticides formulated as wettable powders or emulsive concentrates were applied at various rates in 15 gallons of water per acre with a two gallon compressed air sprayer equipped with a "T" - jet nozzle, at a pressure of 60 p.s.i.

The insecticides formulated as granules were applied in rows two feet apart with a "V" belt seeder at a depth of one inch. The lettuce seed was planted immediately afterwards on the same rows at a depth of a half inch and the carrots at a depth of a half inch.

The lettuce crops were swept with a 15 inch diameter insect net to obtain an estimate of the number of six-spotted leafhoppers on each plot. A sweep was a single stroke of the net over a distance of approximately three feet (Westdal et al, 1961).

When the head lettuce was marketable, heads were sectioned and examined for symptoms of aster yellows virus. Lesions and latex deposits (Grogan et al, 1955) on the plants were the main symptoms sought (Fig. 1). The carrots were dug up and examined for symptoms of the disease. The incidence of disease was recorded as a percentage of the total number of plants examined.

Laboratory experiments

The virus free M. fascifrons used in the laboratory experiments were obtained from a culture that originated from one female M. fascifrons. The culture was reared in a cage on oats (var. Rodney) which is not known to be susceptible to AYV. Groups of M. fascifrons from the culture were checked periodically on aster to verify their freedom from AYV.

Three strains of AYV were isolated and maintained in asters. The one non-celery infecting strain "A" was obtained from field infected lettuce at Winnipeg in 1960. Of the two celery infecting strains "B" and "C", "B" was obtained from a field infected Zinnia, Winnipeg, 1964, and "C" from a field infected celery plant, Winnipeg, 1963. Celery was used to differentiate the celery and non-celery-infecting strains. The two celery infecting strains were separated on the basis of symptoms in aster and N. rustica var. humilis.

Strain "B" in both aster and N. rustica caused a rapid initial growth producing a spindly plant. In aster the rapid growth was followed by stunting but without the formation of the terminal rosette characteristic of the strain "C" and "A". Axillary growth was a common feature of strain "B" and not of "A" or "C".

In addition to these different aster and N. rustica growth habits, three other criteria were used to identify the strains.

1) the height of the plant from the first leaf node to plant tip.
2) the distance from the first leaf node to the last observable leaf node.

3) the number of observable leaf nodes.

The above criteria were used on a series of plants for each replicate of the experiment to certify the identity of the three virus strains.

The host plants used in the experiments were either from registered seed stocks in case of crop plants or were collected from identified species in case of weeds. Fourteen different hosts were used in the laboratory experiments and are as follows:

Common name	Scientific name	Variety
Aster	*Callistephus chinensis* Nees	Giant Pink
Barley	*Hordeum vulgare* L.	Parkland
Carrot	*Daucus carota* L.	Special Long Type Nantes
Celery	*Apium graveolens* L.	Utah
Common plantain	*Plantago major* L.	-
Flax	*Linum usitatissimum* L.	Redwood
Head lettuce	*Lactuca sativa* L.	Imperial 456
Onion	*Allium cepa* L.	Ebenezer
Stinkweed	*Thlaspi arvense* L.	-
Sunflower	*Helianthus annuus* L.	Peredovik
Sunflower	*Helianthus annuus* L.	Commander
Tame buckwheat	*Fagopyrum esculentum* M.	Common
Wheat	*Triticum aestivum* L.	Selkirk
Wild buckwheat	*Polygonum convolvulus* L.	-

Seeds of species with large seeds such as: sunflower, wheat,

barley, flax and tame buckwheat were treated with Captan and sown three to four seeds per three-inch pot made of peat. On germination, all but one healthy seedling were removed. The sunflower seedlings were used within a week of germination when the two cotyledon leaves had fully expanded; wheat and barley were used within a week of germination when the seedlings were one to three inches tall, and flax and tame buckwheat were used at the four leaf stage. The seeds of the small seeded hosts as aster, lettuce, onion, carrot, celery, wild buckwheat, stinkweed and common plantain were treated with Captan and then sown in a six-inch clay pot. The seedlings less than one week old were transplanted individually to three-inch peat pots and were used at the four to six leaf stage. The times of seeding were scheduled to provide plants at the required time and stage of growth. Ninety plants of each host were required for each replicate of the test.

The leafhoppers were handled with a glass-tube aspirator. In the first half of the experiment to determine the ability of the leafhopper to transmit the aster yellows virus to aster, cylindrical cages of cellulose nitrate six inches long by two and one-half inches in diameter were used (Fig. 2). The top end and a one and one-half inch hole in the side of the cage were closed with nylon cloth. A one-half inch opening, stoppered with a cork, in the side, was used for introducing and removing leafhoppers with the aspirator.

Two types of cages were used in the second half of the experiment in which the ability of the leafhoppers to remove the aster yellows virus was determined. One was a metacrylic plastic tube cage

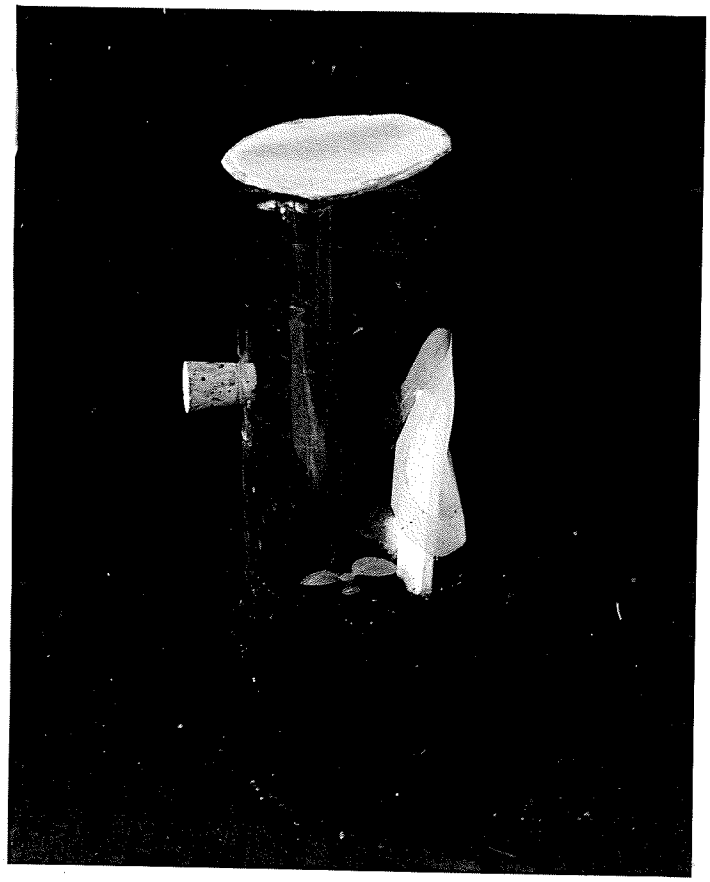

FIGURE 2

CELLULOSE NITRATE CAGE OVER AN ASTER FOUR-LEAF STAGE, GROWN IN A THREE-INCH PEAT POT.

eight inches long by four inches in diameter (Fig. 3). The top of the tube and two side holes, each two inches in diameter, were closed with nylon cloth. A one-inch opening in the side, closed with a rubber stopper was used to introduce and remove insects. The second cage was a 1 pound polyethylene bag measuring $3\frac{1}{2}$ inches by $1\frac{1}{2}$ inches by 8 inches long (Fig. 4). A sharp pointed pair of forceps was used to punch several hundred small holes in the bag to permit circulation of air.

The laboratory experiments were carried out in a greenhouse in which the temperatures were thermostatically controlled. Four compartments of a greenhouse at Canada Department of Agriculture, Winnipeg, Man., were used. One compartment, maintained at 21 ± 2°C was used to grow virus free plants. A second compartment, at 24 to 26°C was used to retain the exposed plants until symptoms appeared on them. The plants were grown on moist beds of fine gravel which provided a rooting medium and a constant source of water. These two compartments were sprayed twice weekly with TEPP for insect control. A third compartment, at 26 ± 2°C was used to produce virus-free M. fascifrons. The leafhoppers were reared on oats, in cages which were approximately three feet square and four feet high covered with 60 mesh Saran screening. A fourth compartment, at 24 ± 2°C was used to expose the infective leafhoppers to the host plants. This was done using cellulose nitrate or polyethylene bag cages. Cultures of the three virus strains were maintained in this compartment in metacrylic tube cages. All transfers of M. fascifrons were made in a room which was attached to the greenhouse. The precautions of handling only one virus strain at

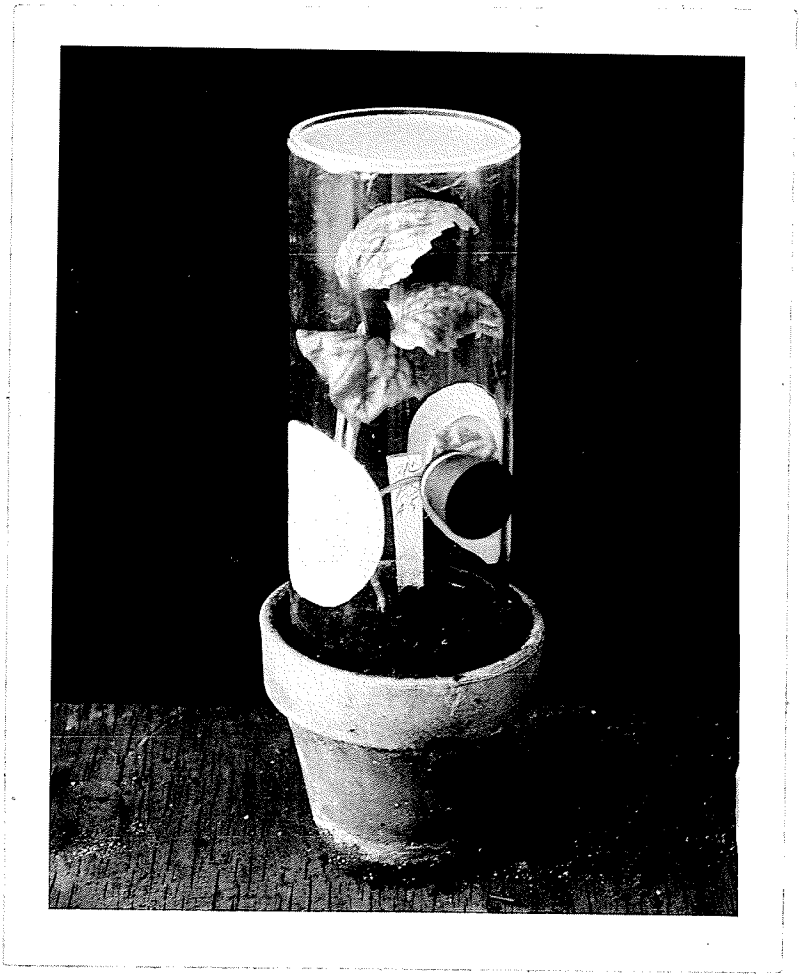

FIGURE 3

METACRYLIC PLASTIC TUBE CAGE OVER AN ASTER GROWN IN A FOUR-INCH CLAY POT.

FIGURE 4

POLYETHYLENE BAG CAGE OVER AN ASTER GROWN
IN A THREE-INCH PEAT POT.

a time and accounting for all the insects transferred eliminated possibilities of contamination. In addition room lights were left off so that any free leafhoppers would be attracted to the window, the major light source, where they could be captured.

In the experiment (Chapter IX) to determine the ability of M. fascifrons to infect the fourteen host plants with the three strains of aster yellows virus, it was necessary, due to lack of greenhouse space, to run Replicates 1, 2 and 3 successively. For each replicate of each strain of AYV approximately 150 nymphs, 2nd to 4th instar, were placed on an infected aster and retained there for ten days. Ninety nymphs for each strain of aster yellows virus were then removed and placed individually on a seedling aster at the four-leaf stage, grown in a three-inch peat pot which was covered with the plastic bag cage. The bag was pulled down over the edge of the peat pot and held in place with a rubber band. The remaining nymphs of each strain were retained on a single virus free aster. After a period of one week the cages containing the single leafhopper nymphs were examined and dead insects were replaced with live nymphs. The single leafhoppers were retained on the aster plants until the aster either showed symptoms of aster yellows or for a period which lasted until two weeks after the date on which the last plant showed symptoms. The sex of each leafhopper was noted. The leafhoppers whose host plants showed symptoms of aster yellows were considered to have shown themselves to be transmitters of aster yellows. For each strain of AYV, a group of 30 infective leafhoppers were selected at random and were individually caged on a seedling aster,

four-leaf stage, grown in a three-inch peat pot, using the cellulose nitrate cage, for a period of two days. The open end of the cage was forced into the soil in the peat pot. After a feeding period of two days, each leafhopper was transferred to a second seedling host plant for two days. The transfers were made every two days until all of the fourteen hosts were exposed. The death of a test leafhopper was recorded and the leafhopper was replaced with another infective individual which carried the same strain. After the two day feeding period, the cages and leafhoppers were removed and the plants were grown until they showed symptoms of aster yellows, or, in case of negative results, until the plant set seed.

A record was made of those leafhoppers which died during the test. The last host plant was noted and if that particular plant failed to show symptoms it was discarded because it was unknown whether or not the leafhopper fed before it died. If the plant was positive for aster yellows symptoms then the result was retained.

In the experiment (Chapter X) to determine the ability of M. fascifrons to acquire the three different strains of AYV from the infected host plants, 150 virus free nymphs, 2nd to 4th instar were caged on each host infected with each strain of the virus for a period of ten days. After the ten day period 90 nymphs, in three replicates of 30, for each host infected with each strain of AYV were caged in plastic bag cages on individual aster plants. (Fig. 5). The asters were at the four-leaf stage, grown in a peat pot. The leafhoppers were retained until the asters showed the virus or for two weeks after the

FIGURE 5

GREENHOUSE BENCH SHOWING A SERIES OF POLY-ETHYLENE BAG CAGES USED IN A TRANSMISSION TEST

last plant showed the virus symptoms. The remaining nymphs were caged on a virus-free aster plant. One week after the test was set up each cage was examined and any dead leafhopper nymphs were replaced. As soon as the nymphs became adult the sex was recorded. Any leafhopper that died before the first symptoms of AYV appeared in the test was recorded. If the plant failed to show symptoms of AYV it was discarded, but if the plant was positive the record was retained. This was done because it was impossible to determine whether or not the leafhopper had acquired sufficient virus to be infective and whether or not it would have transmitted the virus to the aster plant.

CHAPTER IV

EXPERIMENTS ON CONTROL OF *M. fascifrons* DURING 1960

Materials and methods

This experiment was designed to evaluate malathion for the control of the six-spotted leafhopper and prevention of the spread of aster yellows to the spring and summer crops of head lettuce and carrots. It was also designed to determine the most effective time, rate and interval of application of the insecticide. Malathion was found to be ineffective in New York, (Strong and Rawlins, 1958, 1959) and effective in Wisconsin, U.S.A. (Chiykowski, 1958) for the control of *M. fascifrons* on head lettuce and the prevention of infection by aster yellows virus. In Wisconsin, treatments were necessary throughout the season (Chapman, 1949) but in Manitoba the spring transplant crop, harvested about June 15, was grown without application of insecticides although the six-spotted leafhoppers arrived at least one month before harvest (Westdal *et al*, 1961).

The experiments were carried out at the Special Crops Substation, Canada Department of Agriculture, Portage la Prairie, Man. The experiments on the spring and summer crops of head lettuce and on carrots were of similar designs. The plots were arranged in a randomized split-plot design replicated three times. The subplots were treated on the following dates:

Treatment Number	Spray schedule dates Subplot A Malathion 2 lb./ac. at 2-day interval	Spray schedule dates Subplot B Malathion 2 lb./ac. at 4-day interval
1	May 27	May 27
2	June 9	June 9
3	June 21	June 21
4	July 3	July 3
5	July 15	July 15
6 (check)	-	-

Each spray schedule when commenced was continued until five days before harvest.

The summer spray schedules were the same as the spring spray schedules. They commenced on: Treatment 1-Aug. 8, T. 2-Aug. 22, T. 3-Sept. 3, T. 4-Sept. 15, T. 5-Sept.27, T. 6 was a check.

The subplots in the carrot experiment were treated on the following dates:

Treatment Number	Spray schedule dates Subplot A DDT 2 lb./ac.	Spray schedule dates Subplot B Carbaryl 2 lb./ac.
1	June 13	June 13
2	July 13	July 13
3	July 31	July 31
4	Aug. 18	Aug. 18
5 (check)	-	-

Each spray schedule was continued until five days before harvest.

Malathion and DDT were formulated as emulsive concentrates and Carbaryl as a wettable powder.

The lettuce was swept with an insect net to obtain a relative

measure of the number of the leafhoppers in each plot. Sweeps were started about three weeks after crop emergence. Fifty sweeps were made on each subplot which was sprayed at four-day intervals with malathion. The sweeps were made at eight-day intervals, three days after the last spray application.

For residue analysis, six heads of lettuce per replicate were taken at random from each of the five subplots treated with malathion at the four-day interval and from the check plots. The heads were collected at the time of harvest, five days after the last application of insectide. They were stored in plastic bags at 40°F for four days, after which they were analysed for malathion residues by the Canada Department of National Health and Welfare, Winnipeg, Man. The method of analysis was that listed by Horwitz (1960).

Results and discussion

On head lettuce, malathion applied at both two- and four-day intervals reduced the leafhopper population (Table II). This reduction of the leafhopper population brought about a reduction in the incidence of aster yellows (Table I). On the spring crop, the treatments differed at the one percent level of significance in the seasonal mean number of leafhoppers and in the per cent of heads which were infected with aster yellows virus (Tables I and II). There was no difference between the first three treatments (Tables I and II). This was because early in the year, the leafhopper population was at a low level. When the third treatment, late in June, was started, the

population was beginning to build up rapidly on lettuce and by the fourth treatment leafhopper populations were high. There was no difference between the first three treatments of the summer crop (Table III) in reduction of the incidence of aster yellows infected heads. This may have been partly because many plants had not emerged when the first treatment was applied and partly due to slow dispersal of leafhoppers to the test area while the vegetation was sparse. The leafhopper populations remained at a very low level during the entire experiment. Attempts to sweep for leafhoppers failed to give any consistent data and were abandoned.

The incidence of aster yellows, as determined by symptoms shown in the field, was higher in the spring crops than in the summer crops, particularly in the check and later treatments (Tables I and III). This was thought to be due to slow development of the virus in cool fall weather (Chapman, 1949 and Self and Darling, 1953). To determine whether or not the virus was latent, approximately 50 lettuce plants without symptoms were taken from the check plots at time of harvest and held in the greenhouse at approximately 24°C for two weeks. Virus was acquired from 66 per cent of the lettuce plants by virus-free leafhoppers and transmitted to aster.

Residue analysis showed that nine days after the last of fifteen applications of malathion at a rate of two pounds per acre at four-day intervals the residues were less than 0.5 ppm (Appendix B, Table IV). The tolerance limit was 8 ppm *. The results show that there were no

---

* Doroznski, J., Can. Dept. Natl. Health and Welfare, Winnipeg, Man. (personal communication).

accumulations of residues. There was no difference in the amount of residue on plants from plots that received fifteen applications and those that received only the last three applications.

On carrots, DDT and Carbaryl were equally effective when applied within a month of crop emergence in reducing the per cent of plants infected with aster yellows virus (Table IV). The treatments begun on July 31 or later did not prevent the spread of aster yellows virus by the six-spotted leafhopper. The low rate of infection in the check, however, places in doubt the economy of the insecticide applications. The cost of insecticide and application would probably exceed the value of the carrots saved.

TABLE I

PERCENT OF HEAD LETTUCE PLANTS INFECTED WITH ASTER YELLOWS AFTER APPLICATION OF MALATHION AT 2 LB. PER ACRE AT 2- AND 4-DAY INTERVALS WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE AGAINST *Macrosteles fascifrons*, SPRING CROP 1960 *

Treatment and date started	No. applications of insecticides 2-day	4-day	No. of plants Examined	Infected	Per cent plants infected **	
1. May 27	29	15	1381	29	2	***
2. June 9	23	12	1407	37	3	
3. June 21	17	9	1317	31	2	
4. July 5	11	6	1427	176	12	
5. July 11	5	3	1347	360	27	
6. (check)	-	-	1294	311	36	

* 2- and 4-day data combined since there was no significant difference between these treatments at the 5% level of significance.

** Angular transformations applied before analysis (Goulden, 1952)

*** Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE II

TOTAL AND SEASONAL MEAN NUMBER OF *Macrosteles fascifrons* FOR THREE REPLICATES AFTER APPLICATION OF MALATHION AT 2 LB. PER ACRE AT 4-DAY INTERVALS WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE, SPRING CROP 1960

Treatment and date started	No. applications of insecticides 4-day	Total no. *M. fascifrons*	Mean no. *M. fascifrons*	
1. May 27	15	18	6	*
2. June 9	12	17	6	
3. June 21	9	115	38	
4. July 3	6	180	60	
5. July 15	3	638	213	
6. (check)	-	2802	934	

* Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE III

PERCENTAGE OF HEAD LETTUCE PLANTS INFECTED WITH ASTER YELLOWS AFTER APPLICATION OF MALATHION AT 2 LB. PER ACRE AT 2- and 4-DAY INTERVALS WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE AGAINST *Macrosteles fascifrons*, SUMMER CROP 1960 *

Treatment and date started	No. applications of insecticides 2-day	4-day	No. of plants Examined	Infected	Per cent plants infected **	
1. Aug. 8	28	16	1082	24	2	***
2. Aug. 22	21	12	940	14	2	
3. Sept. 3	15	9	967	48	5	
4. Sept. 15	9	5	1069	101	9	
5. Sept. 27	3	2	994	98	10	
6. (check)	-	-	1086	142	13	

* 2- and 4-day data combined since there was no significant difference between these treatment intervals.

** Angular transformation applied before analysis (Goulden, 1952).

*** Any two means connected by the same line are not significantly different from each other at the 5% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE IV

PERCENTAGE OF CARROT PLANTS INFECTED WITH ASTER YELLOWS AFTER APPLICATION OF DDT AND CARBARYL, EACH AT 2 LB. PER ACRE, AT 6-DAY INTERVALS WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE AGAINST Macrosteles fascifrons

Treatment and date started	No. of carrot plants DDT Examined	DDT Infected	Carbaryl Examined	Carbaryl Infected	Per cent plants infected * DDT **		Carbaryl **	
1. June 13	1830	6	1872	9	0.3		0.5	
2. July 13	1826	11	1936	31	0.6		1.6	
3. July 31	2019	57	2089	50	2.8	***	2.4	***
4. Aug. 18	2306	89	2260	72	3.9		3.2	
5. (check)	4274	74	4274	62	3.2		3.2	

* Angular transformation applied before analysis (Goulden, 1952).

** DDT and Carbaryl not significantly different.

*** Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

CHAPTER V

EXPERIMENTS ON CONTROL OF *M. fascifrons* DURING 1961

Materials and methods

The experiments were designed to evaluate malathion EC and to test Carbaryl WP and Baygon WP, at different rates and intervals of application, for the control of *M. fascifrons* to prevent the spread of aster yellows on head lettuce. The treatments were similar to those in 1960 in which the plants were sprayed at two-week intervals in an attempt to determine the most effective time to commence treatment for economic control.

The experiments were carried out at the Special Crops Substation of the Canada Department of Agriculture, Portage la Prairie, Man. The experiments on the spring and summer crops of head lettuce were similar; the plots were arranged in a randomized split-plot design which was replicated three times. The four subplots were treated as follows:

Treatment	Spray schedule dates			
	Subplot A malathion 3-day intervals 1 lb./ac.	Subplot B malathion 6-day intervals 2 lb./ac.	Subplot C Carbaryl 6-day intervals 1 lb./ac.	Subplot D Carbaryl 6-day intervals 2 lb./ac.
		SPRING CROP		
1	May 29	May 29	May 29	May 29
2	June 13	June 13	June 13	June 13
3	June 28	June 28	June 28	June 28
4	July 13	July 13	July 13	July 13
5 (check)	-	-	-	-
Treatment	malathion 3-day intervals 1 lb./ac.	Carbaryl 6-day intervals 1 lb./ac.	Baygon 3-day intervals 1 lb./ac.	Baygon 6-day intervals 1 lb./ac.
		SUMMER CROP		
1	July 28	July 28	July 28	July 28
2	Aug. 9	Aug. 9	Aug. 9	Aug. 9
3	Aug. 21	Aug. 21	Aug. 21	Aug. 21
4	Sept. 2	Sept. 2	Sept. 2	Sept. 2
5 (check)	-	-	-	-

To obtain a relative measure of the number of M. fascifrons, fifty sweeps were made on each subplot, beginning three weeks after crop emergence. All subplots of the spring and summer crops were swept at six-day intervals. The sweeps were made two days after every second spray of the insecticides which were applied at three-day intervals, and five days after the insecticides applied at six-day intervals. Samples of heads of lettuce were sectioned to determine the per cent infection of aster yellows virus.

Results and discussions

Malathion and Carbaryl in the spring crop and malathion, Carbaryl and Baygon in the summer crop of head lettuce (Tables VI and

VIII) at the rates and intervals tested effectively reduced the populations of the six-spotted leafhopper. This reduction resulted in a lower incidence of aster yellows (Tables V and VII). The treatments differed at the one per cent level of significance in the spring and summer crops. This was probably because, as in 1960, the leafhopper population was at a low level. By the time of the third treatment, late in June the population was beginning to build up rapidly on lettuce and by the time of the fourth treatment leafhopper populations were high (Table VI). There was no difference at the one per cent level of significance between the first three treatments of the summer crop (Table VIII). As in 1960 this may have been due to the slow dispersal of the leafhoppers to the test area while vegetation was sparse. There was no difference at the five per cent level of significance in the incidence of aster yellows between plots with different insecticides rates, or intervals of application. However, in the spring crop there was a difference at the one per cent level of significance in the mean seasonal number of leafhoppers (Table IX).

Malathion applied at six-day intervals was not as effective as the other insecticides in controlling the six-spotted leafhopper; a higher incidence of aster yellows would therefore, be expected. There was a small increase in the incidence of aster yellows, but it was not statistically different, nor was it proportional to the difference in numbers of leafhoppers. It has been shown that in 1960, (Chapter IV), malathion applied at four-day intervals was as effective as at two-day intervals. It is likely that at two pounds per acre, malathion was

still effective but to a diminishing degree on the 5th and 6th days, permitting more invading leafhoppers to survive. However, the time for virus inoculation would be limited and the leafhoppers would be destroyed with the next application of insecticide on the 6th day. With a larger leafhopper population and/or a higher percentage of infective individuals than in 1961, the decreasing effectiveness of the insecticide toward the end of the six-day interval would probably be reflected in a significantly higher incidence of aster yellows.

Carbaryl and Baygon gave effective control of leafhoppers but were not satisfactory for use on lettuce. Lettuce treated with Carbaryl became heavily infested with aphids and was unmarketable. Very few aphids were noted in any of the other plots. Baygon left a heavy white deposit which detracted from the appearance of the lettuce.

In treatments 1, 2, and 3 of the spring lettuce crop of 1961 (Table V), most of the infected heads had minor aster yellows symptoms which showed only on the growing tip of the plant. This did not affect the market value of the lettuce. In contrast, most of the infected heads in treatment 4 and the check had typical advanced aster yellows symptoms. The minor symptoms were the result of a heavy influx of leafhoppers into the plots a short time before harvest.

The incidence of aster yellows, as determined by symptoms expressed in the field, was higher in the spring crops than in the summer crops, particularly in the check and later treatments (Table V). This was thought to be due to low development of the virus in cool autumn weather (Chapman, 1949, Self and Darling, 1953). To determine

whether or not the virus was latent, approximately 50 lettuce plants without symptoms were taken from the check plots at time of harvest, and held in the greenhouse at approximately 24°C for two weeks. Virus-free leafhoppers were placed on the lettuce for a period of one week, then on virus-free aster to determine rate of transmission. In 1961, 47 per cent of the lettuce plants had latent virus infections.

Malathion applied at the rate of one pound toxicant per acre at three-day intervals was the most suitable treatment for the control of the six-spotted leafhopper and aster yellows on head lettuce. The results show that treatment need not be started until about four weeks after emergence of spring-seeded crops, and for summer crops the treatments should commence at crop emergence.

TABLE V

PERCENTAGE OF HEAD LETTUCE PLANTS INFECTED WITH ASTER YELLOWS AFTER APPLICATIONS OF MALATHION AND CARBARYL, WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE AGAINST *Macrosteles fascifrons*, SPRING CROP 1961 *

Treatment and date started	Number of plants Examined	Infected	Per cent plants infected **	
1. May 29	1233	279	23	***
2. June 13	1091	238	22	
3. June 28	1171	225	20	
4. July 13	1004	619	61	
5. (check)	1079	629	58	

* Data for all insecticides, rates and intervals of application are combined.

** Angular transformation applied before analysis (Goulden, 1952)

*** Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's Multiple range test (Duncan, 1955).

TABLE VI

TOTAL AND SEASONAL MEAN NUMBER OF *Macrosteles fascifrons* AFTER APPLICATION OF MALATHION AND CARBARYL, WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE, SPRING CROP 1961

Treatment and date started	Total number *M. fascifrons*	Mean number *M. fascifrons* *	
1. May 29	6710	80	**
2. June 13	6464	78	
3. June 28	5328	64	
4. July 13	17617	210	
5. (check)	24243	289	

* Data for all insecticides, rates and intervals of application are combined.

** Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE VII

PERCENTAGE OF HEAD LETTUCE PLANTS INFECTED WITH ASTER YELLOWS AFTER APPLICATIONS OF MALATHION, CARBARYL AND BAYGON, WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE AGAINST Macrosteles fascifrons, SUMMER CROP 1961 *

Treatment and date started	Number of plants Examined	Infected	Per cent plants infected **	
1. July 28	2273	62	3	***
2. Aug. 9	2194	136	6	
3. Aug. 21	2257	243	13	
4. Sept. 2	2248	365	16	
5. (check)	2244	381	17	

* Data for all insecticides, rates and intervals of application are combined.

** Angular transformation applied before analysis (Goulden, 1952).

*** Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE VIII

TOTAL AND SEASONAL MEAN NUMBER OF Macrosteles fascifrons AFTER APPLICATION OF MALATHION, CARBARYL AND BAYGON, WITH TREATMENT 1 BEGINNING AT CROP EMERGENCE, SUMMER CROP 1961

Treatment and date started	Total number M. fascifrons	Mean number M. fascifrons *	
1. July 28	774	14	**
2. Aug. 9	775	15	
3. Aug. 21	886	17	
4. Sept. 2	5757	86	
5. (check)	6734	107	

* Data for all insecticides, rates and intervals of application are combined.

** Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE IX

SEASONAL MEAN NUMBER OF LEAFHOPPERS AFTER APPLICATION OF MALATHION AND CARBARYL AGAINST *Macrosteles fascifrons*, SPRING CROP 1961

Insecticide	Rate (lb./acre)	Application interval (days)	Mean number *M. fascifrons*	
Malathion EC	1	3	75	*
Carbaryl WP	2	6	80	
Carbaryl WP	1	6	88	
Malathion EC	2	6	187	
Check	-	-	289	

* Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

CHAPTER VI

EXPERIMENTS ON CONTROL OF *M. fascifrons* DURING 1962

Materials and methods

Systemic insecticides applied to the soil or plant, as granules at time of seeding or as sprays during the growing season, were compared with malathion the recommended insecticide. The experiments on the spring and summer crop of head lettuce were similar. The plots were arranged in a randomized block design. The insecticides which were tested, the formulations, rates and interval of application are as follows:

Insecticide	Toxicant per ac.	Interval of application
Baygon G	1 lb.	1 app. at seeding
Di-Syston G	1 lb.	1 app. at seeding
AC 43064 G	1 lb.	1 app. at seeding
Phorate G	1 lb.	1 app. at seeding
Baygon EC	1 lb.	2 app. per season
Meta-SystoxR EC	8 oz.	2 app. per season
Dimethoate EC	1 lb.	2 app. per season
AC 47300 EC	1 lb.	2 app. per season
Malathion	1 lb.	2 app. per week (3- and 4-day interval)
Delnav G	1 lb.	1 app. at seeding
Delnav EC	1 lb.	1 app. per week

The first nine materials were applied to the spring crop; all the materials were used on the summer crop.

The first applications of the insecticides which were formulated as emulsifiable concentrates were made one week after the spring

crop emerged and at crop emergence in the summer crop. The second application of the systemic insecticides was three weeks after the first application.

The lettuce crops, as in previous experiments, were swept with a net to obtain a relative measure of the number of leafhoppers on each plot. The sweeps were started about three weeks after the crop emerged. Fifty sweeps were made on the day before the four-day application of malathion. At harvest, the heads of lettuce were sectioned and examined for symptoms which were mainly lesions and latex deposits.

Results and discussion

During 1962 the populations of the six-spotted leafhopper were low (Table X and XII), and the percentage of infective leafhoppers was also low (0.2%). Subsequently, the incidence of aster yellows on the spring crop, (Table XI) was too low to show differences between the insecticides tested. On the summer crop, the incidence of aster yellows appeared negligible; the crop was not sampled to determine rate of infection by aster yellows virus. The results (Table XII) show that a number of insecticides gave a significant reduction of the population of leafhoppers. Based on the results of experiments performed during 1960 and 1961, a reduction in leafhopper population resulted in a reduction of the incidence of aster yellows on the head lettuce.

TABLE X

TOTAL AND SEASONAL MEAN NUMBER OF *Macrosteles fascifrons* FOR EACH INSECTICIDE, SPRING CROP 1962

Insecticide	No. of application insecticides	Total number of *M. fascifrons*	Seasonal mean	
Malathion EC	15	30	2	*
Phorate G	1	108	7	
Baygon G	1	128	8	
Baygon EC	2	165	10	
Meta-SystoxR EC	2	299	12	
Dimethoate EC	2	274	17	
AC 47300 EC	2	311	19	
Di-Syston G	1	352	22	
AC 43064 G	1	586	37	
Check	-	637	40	

* Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE XI

PERCENTAGE OF HEAD LETTUCE PLANTS INFECTED WITH ASTER YELLOWS VIRUS, SPRING CROP 1962

Insecticide	Number of applications insecticides	Total no. of plants Examined	Infected	Per cent infection *
Baygon EC	2	472	9	0.02 **
AC 47300 EC	2	474	9	0.02
Baygon G	1	504	15	0.03
Malathion EC	15	308	10	0.03
Phorate G	1	399	16	0.04
Meta-SystoxR EC	2	482	22	0.04
Dimethoate EC	2	475	20	0.04
Di-Syston G	1	469	21	0.04
AC 43064 G	1	480	26	0.05
Check	-	505	26	0.05

* Angular transformation applied before analysis (Goulden, 1952).

** An analysis of variance showed there was no difference between insecticides tested to reduce the incidence of aster yellows virus.

TABLE XII

TOTAL AND MEAN NUMBER OF *Macrosteles fascifrons* FOR EACH INSECTICIDE TESTED, SUMMER CROP 1962

Insecticides	No. applications insecticides	Total no. of *M. fascifrons*	Mean
Baygon G	1	21	5
Malathion EC	11	25	6
Delnav EC	6	26	6
Phorate G	1	30	8
Baygon EC	2	42	10
Meta-SystoxR EC	2	44	11
Dimethoate EC	2	53	13
AC 43064 G	1	57	14
Delnav G	1	60	15
AC 47300 EC	2	72	18
Di-Syston G	1	72	20
Check	-	89	22

* Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

CHAPTER VII

EXPERIMENTS ON CONTROL OF *M. fascifrons* DURING 1963

Materials and methods

The 1963 experiments were designed to compare the most effective insecticides of previous tests with two new compounds for the control of the *M. fascifrons* and to prevent the spread of AYV on head lettuce.

The insecticides tested with amount of toxicant per acre and interval of applications are as follows:

Insecticide	Toxicant per acre	Interval of application
Baygon G	1 lb.	at seeding
Phorate G	1 lb.	at seeding
AC 47031 G	1 lb.	at seeding
AC 47470 G	1 lb.	at seeding
Malathion EC	1 lb.	twice weekly (3- and 4-day)
Baygon EC	1 lb.	weekly
Delnav EC	1 lb.	weekly
Endosulfan EC	1 lb.	weekly

Two experiments were carried out on the spring crop, one at Portage la Prairie, and one at Winnipeg, Manitoba. A third experiment on the summer crop was carried out at Portage la Prairie. These experiments were similar, varying only in the number of insecticides tested. The plots were arranged in a randomized block design, and were replicated four times.

The first applications of the insecticides as emulsifiable

concentrates were made one to two weeks after the spring crop of lettuce emerged. The spray schedule was continued until five days before the harvest.

The lettuce crops were swept with a net to obtain a measure of the number of leafhoppers on each plot. The sweeps were started three weeks after the crop emerged. Fifty sweeps were made the day before the four-day application of malathion, and the weekly application of Baygon, Delnav and Endosulfan.

At harvest the heads of lettuce were sectioned and examined for symptoms, mainly lesions, and latex deposits of AYV infection.

Leafhoppers collected near harvest in the check plots of both spring and summer crops, were caged on asters to determine the proportion that were infective.

Results and discussion

During 1963, control of the leafhopper was not directly reflected in the incidence of AYV in lettuce (Tables XIII, XIV, XV, XVI, XVII and XVIII). Malathion was more effective than Baygon G, Phorate, and Baygon EC in reducing the numbers of leafhoppers, but was generally less effective in preventing AYV. Delnav, AC 47470 G, and Endosulfan were less effective than malathion although better than no insecticide at all. AC 47031 G appeared to act as an attractant and resulted in a higher population of both adults and nymphs than in the check (Table XIX). This population level was not reflected in a similar incidence of AYV (Table XIII). Nymphs occurred only in the

check plots and in the plots treated with AC 47031 G indicating that Baygon G, Phorate G, and AC 47470 G prevented oviposition or development of nymphs during the nine weeks of the test.

The incidence of AYV was higher on the summer crop than on the spring crop at Portage la Prairie 1963, although the seasonal mean numbers of leafhoppers were much higher in the spring crop (Table XIV) than in the summer crop (Table XVIII). However virus transmission tests showed that the percentage of infective leafhoppers in the check plots at the time of harvest was 1.3 and 10.0 per cent in the spring and summer crops, respectively. It is also apparent that given a high percentage of infective leafhoppers it is not possible to prevent a high incidence of AYV on field plots with the insecticides tested.

In 1963, malathion the recommended insecticide, was reasonably effective against the leafhopper and AYV in the spring crops, but not in the summer crop. However, under commercial conditions where the entire crop as well as border areas are sprayed, better control of the leafhopper and AYV should be obtained.

The systemic insecticides Baygon (G and EC) and phorate G were better than malathion in 1963 for the prevention of AYV in head lettuce. Similar results were reported for phorate by Thompson and Rawlins (1961), and for Baygon wettable powder by Richardson and Westdal (1963). Baygon has a much lower mammalian toxicity (Anonymous, 1963) than phorate and in granular form is more convenient and cheaper to apply than malathion. Systemic insecticides such as Baygon may prove to be suitable for reducing the leafhopper and AYV.

TABLE XIII

PERCENTAGE OF HEAD LETTUCE PLANTS EXAMINED AND FOUND INFECTED WITH ASTER YELLOWS VIRUS, PORTAGE LA PRAIRIE, SPRING CROP 1963

Insecticide	No. of applications of insecticide	Total no. plants Examined	Infected	Per cent plants infected *	
Baygon G	1	764	227	34	**
Phorate G	1	801	305	38	
Baygon EC	8	766	321	42	
Malathion EC	15	772	330	43	
AC 47470 G	1	817	418	51	
Delnav EC	8	791	524	66	
Endosulfan EC	8	751	523	70	
AC 47031 G	1	789	567	72	
Check	-	757	688	91	

* Angular transformation applied before analysis (Goulden, 1952).

** Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's multiple range test (Duncan, 1955).

TABLE XIV

TOTAL AND SEASONAL MEAN NUMBER OF *Macrosteles fascifrons*, PORTAGE LA PRAIRIE, SPRING CROP 1963

Insecticide	No. of applications of insecticide	Total number *M. fascifrons*	Mean number *M. fascifrons*	
Malathion EC	15	3161	132	*
Baygon EC	8	5297	221	
Phorate G	1	5385	224	
Baygon G	1	7825	326	
Delnav EC	8	10208	425	
Endosulfan EC	8	12103	504	
AC 47470 G	1	12260	511	
Check	-	13559	565	
AC 47031 G	1	17863	744	

* Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's multiple range test (Duncan, 1955).

TABLE XV

PERCENTAGE AND NUMBER OF HEAD LETTUCE PLANTS EXAMINED AND FOUND INFECTED WITH ASTER YELLOWS VIRUS, WINNIPEG, SPRING CROP 1963

Insecticide	No. of applications of insecticide	Total plants Examined	Infected	Per cent plants infected *	
Baygon G	1	1204	140	9	**
Malathion EC	11	1141	149	13	
Phorate G	1	1175	186	16	
Check	-	1171	760	65	

* Angular transformation applied before analysis (Goulden, 1952).

** Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's multiple range test (Duncan, 1955).

TABLE XVI

TOTAL AND SEASONAL MEAN NUMBER OF
Macrosteles fascifrons PER 50 SWEEPS PER TREATMENT,
WINNIPEG, SPRING CROP 1963

Insecticide	No. of applications of insecticide	Total number *M. fascifrons*	Mean number *M. fascifrons*
Malathion EC	11	576	32 *
Phorate G	1	1085	60
Baygon G	1	1098	61
Check	-	2401	133

* Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's multiple range test (Duncan, 1955).

TABLE XVII

PERCENTAGE AND NUMBER OF HEAD LETTUCE PLANTS EXAMINED AND FOUND INFECTED WITH ASTER YELLOWS VIRUS, PORTAGE LA PRAIRIE, SUMMER CROP 1963

Insecticide	No. of applications of insecticide	Total plants Examined	Infected	Per cent plants infected *	
Baygon G	1	980	723	74	
Baygon EC	8	946	771	82	**
Malathion EC	16	942	804	84	
Phorate G	1	973	817	85	
Check	-	952	928	97	

* Angular transformation applied before analysis (Goulden, 1952).

** Any two means connected by the same line are not significantly different from each other at the 1% level of significance by Duncan's multiple range test (Duncan, 1955).

TABLE XVIII

TOTAL AND SEASONAL MEAN NUMBER OF
Macrosteles fascifrons PER 50 SWEEPS PER TREATMENT,
PORTAGE LA PRAIRIE, SUMMER CROP 1963

Insecticides	Applications of insecticide	Total number *M. fascifrons*	Mean number *M. fascifrons*	
Malathion EC	16	584	29	*
Baygon EC	8	597	30	
Baygon G	1	1060	53	
Phorate G	1	1145	57	
Check	-	2006	100	

* Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's multiple range test (Duncan, 1955).

TABLE XIX

TOTAL NUMBER OF Macrosteles fascifrons, ADULTS AND NYMPHS, ON AC 47031 AND CHECK, PORTAGE LA PRAIRIE, SPRING CROP 1963

Date swept	AC 47031 Number of Adults	AC 47031 Number of Nymphs	Check Number of Adults	Check Number of Nymphs
June 14	194	-	286	-
June 19	311	-	239	-
June 26	831	-	746	9
July 3	4,359	1	2,628	35
July 10	6,187	4	4,923	319
July 17	4,478	1,498	3,614	760
Total	16,360	1,503	12,436	1,123

CHAPTER VIII

DIFFERENTIATION OF THREE STRAINS OF ASTER YELLOWS VIRUS

Materials and methods

Three strains of aster yellows virus, "A", "B" and "C", were isolated from lettuce, zinnia and celery and transmitted to fifteen different host plants: aster, head lettuce, celery, plantain, sunflowers var. Peredovik and Commander, N. rustica var. humilis, tame and wild buckwheat, stinkweed, onion, carrot, barley, flax, and wheat by the six-spotted leafhopper. Two methods were used to differentiate the strains: host-plant susceptibility, and the different growth habits of the infected plants. The growth habits, considered in the second method and used to differentiate the strains, were the height of infected aster plants from the first leaf node to tip, the distance from first to last leaf node, number of exposed leaf nodes and axillary growth.

Results and discussion

The different effects which each strain of the virus had on aster and N. rustica var. humilis are shown in Figures 6 and 7. Strain "A" is a non-celery infecting strain which in aster (Fig. 6) results in a stunted but sturdy plant with the terminal growth point ending in a loose rosette. It has a similar effect on N. rustica (Fig. 7). Strain "B", which is a celery-infecting strain, caused a rapid growth of aster and N. rustica (Figs. 6 and 7) resulting in a

tall spindly plant. The central terminal growth of the infected aster is stunted but does not form a rosette, and the axillary growth is a common feature of such plants (Fig. 6). N. rustica infected with strain "B" are incapable of erect growth and sprawl, unless supported, over the edge of the pot. Strain "C", a celery-infecting strain in aster (Fig. 6) and N. rustica var. humilis (Fig. 7) causes severe stunting. The plant has a weak stem and the terminal growth forms a tight rosette.

In addition to the symptoms which separate the virus strains on aster and N. rustica var. humilis, the strains may be separated on the basis of height measurements, number of exposed leaf nodes and number of plants showing axillary growth (Tables XX, XXI and XXII). The two celery infecting strains, "B" and "C", may be distinguished on the basis of the mean plant height from the first leaf node to tip, the mean plant height from the first leaf node to the last exposed leaf node, and the number of exposed leaf nodes. In Tables XX, XXI and XXII, the measurements of symptoms of strains "B" and "C" were significantly different at the one per cent level. Generally, on the basis of the criteria, it was possible to separate all three strains, but the fact that the symptoms of strains "B" and "A" were not always different at the one per cent level, was irrelevent since strain "A" and "B" could be separated on the basis of whether or not they infected celery.

A comparison of these strains with the Eastern strain of Kunkel (1955) and the three Western or California strains of Freitag (1964) shows several differences. The Eastern strain of Kunkel (1955)

TABLE XX

MEAN AND LSD FOR 40 ASTER PLANTS FOR EACH OF THREE CRITERIA AND THE PER CENT OF PLANTS SHOWING AXILLARY GROWTH, A FOURTH CRITERION USED TO DIFFERENTIATE AYV STRAINS "A", "B", "C", AND CHECK *

	Strain A	Strain B	Strain C	Check	LSD.01%
Mean plant height in cm from first leaf node to tip	20.9	23.1	17.8	28.6	1.7
Mean plant height in cm from first to last leaf node	11.4	14.9	8.7	22.7	1.7
Mean number of leaf nodes	14.5	15.6	10.8	23.9	1.8
Per cent plants showing axillary growth	40.0	67.5	2.5	15.0	-

* The aster plants four-leaf stage, were caged with six-spotted leafhoppers, which had been fed on an infected plant for ten days, on February 24, the symptoms of AYV showed on March 11, and the plants were examined on April 7.

TABLE XXI

MEAN AND LSD FOR 50 ASTER PLANTS FOR EACH OF THREE CRITERIA AND THE PER CENT OF PLANTS SHOWING AXILLARY GROWTH, A FOURTH CRITERION USED TO DIFFERENTIATE AYV STRAINS "A", "B", "C", AND CHECK *

	Strain A	Strain B	Strain C	Check	LSD.01%
Mean plant height in cm from first leaf node to tip	17.4	19.2	15.5	28.6	1.7
Mean plant height in cm from first to last leaf node	6.6	10.3	5.3	22.7	1.4
Mean number of leaf nodes	10.8	12.1	10.0	23.2	.7
Per cent plants showing axillary growth	4.0	82.0	0.0	1.4	-

* The aster plants four-leaf stage, were caged with six-spotted leafhoppers, which had been fed on an infected plant for ten days, on April 7, symptoms of AYV showed on April 25, and the plants were examined on June 24.

TABLE XXII

MEAN AND LSD FOR 25 ASTER PLANTS FOR EACH OF THREE CRITERIA AND THE PER CENT OF PLANTS SHOWING AXILLARY GROWTH, A FOURTH CRITERION USED TO DIFFERENTIATE AYV STRAINS "A", "B", "C", AND CHECK *

	Strain A	Strain B	Strain C	Check	LSD.01%
Mean plant height in cm from first leaf node to tip	8.8	9.8	7.7	13.7	1.5
Mean plant height in cm from first to last leaf node	1.4	2.5	.8	3.4	.5
Mean number of leaf nodes	8.1	9.5	7.0	10.4	1.7
Per cent plants showing axillary growth	0.0	24.0	0.0	0.0	-

* The aster plants four-leaf stage, were caged with infective six-spotted leafhoppers for two days on April 30, the symptoms showed May 12, the plants were examined June 3.

is non-celery infecting (or celery may be infected with difficulty) and produces abundant axillary growth. This is not a characteristic of strain "A" used in this study. On N. rustica (Kunkel, 1955) the Eastern strain of AYV produced upright growth with little gnarling of the leaves. This is not characteristic of strain "A" (Fig. 7). The symptom expression of the two celery infecting strains, "B" and "C" (Fig. 7) do not resemble the symptoms of the California strains, Severe, Dwarf and Tulelake, produced on N. rustica var. humilis (Freitag, 1964). A comparison of symptoms of strains "A", "B" and "C" with the descriptions for the eight strains isolated in Wisconsin by Granados (1965) indicates a number of differences. He classified three strains AYV 8, Wis. CAYV, and AYV 6, as belonging to the Eastern AYV group because infected asters were not severely stunted. The plants produced side shoots and the strains did not infect celery, or did so with difficulty. The non-celery infecting strain, "A", tested in this experiment produced a stunted plant with few if any side shoots (Fig. 6)(Tables XX, XXI, XXIII). The two strains which Granados (1965) classified as celery infecting, AYV 2 and AYV 7, produced severe stunting on aster and N. rustica. Of the two celery infecting strains which were tested in this study only strain "C" produced severe stunting on aster and N. rustica. The similarity of the symptoms may indicate that these two are the same strain. Strain "B" does not fit any of the descriptions given for celery-infecting strains by Granados (1965).

The effects of the three strains on the 13 other hosts tested

are shown in Figs. 8 to 19. Celery and plantain were susceptible to strain "B" and "C" but not "A" and wheat was susceptible to strain "B" but not to strains "A" or "C". Strains "B" and "C" could not be separated on the basis of symptoms on celery or plantain and strains "A", "B" and "C" could not be separated on the basis of symptoms on head lettuce, stinkweed, carrot, onion, sunflowers, tame and wild buckwheat, barley or flax.

Wheat would not be a suitable host to identify strain "B" because of the low rate of infection.

CHAPTER IX

TRANSMISSION BY *Macrosteles fascifrons* OF THREE STRAINS OF AYV TO FOURTEEN DIFFERENT HOST PLANTS

Materials and methods

One hundred and fifty nymphs, 2nd to 4th instar, *M. fascifrons* were placed on aster plants infected with one of the three strains of AYV. The nymphs were left for a ten-day virus acquisition feed, after which 90 nymphs, in three replicates of 30, were chosen at random and placed singly on aster plants which were at the four-leaf stage. They remained there until the plants developed symptoms of AYV. Thirty leafhoppers which had shown the ability to transmit AYV were then placed for two-day intervals on each of the 14 host plants tested, beginning with aster. In the successive two replicates the hosts used after aster were picked at random. This was repeated for each virus strain. The host plants were then held for expression symptoms. The sex of the leafhoppers and the time of symptom expression were noted.

Results and discussion

The ability of infective *M. fascifrons* to transmit three strains of AYV to 14 different hosts varied with the host plant (Table XXIII). There was no difference between AYV strains "A", "B", and "C", (Appendix B Table XXI). The fact that single infective leafhoppers were able to infect 99 and 98 per cent of stinkweed and head lettuce plants, respectively, on which they were placed for a two-day

TABLE XXIII

TOTAL AND PER CENT TRANSMISSION OF THREE STRAINS * OF ASTER YELLOWS VIRUS BY INFECTED SINGLE *Macrosteles fascifrons* TO FOURTEEN DIFFERENT HOST PLANTS

Host	Total No. *M. fascifrons* tested	Total No. *M. fascifrons* transmitted	Per cent transmission	Angular transformation **	
Stinkweed	268	265	98.9	86.5	***
Head lettuce	263	258	98.1	87.4	
Onion	261	208	79.7	66.1	
Flax	263	213	81.0	64.6	
Aster	258	207	80.2	62.1	
Carrot	260	168	64.6	53.5	
Barley	243	128	52.7	44.4	
Tame buckwheat	251	120	47.8	43.7	
Wild buckwheat	244	44	18.0	23.7	
Celery	247	44	17.8	20.3	
Sunflower (Peredovik)	257	28	10.9	18.7	
Plantain	249	23	9.2	13.5	
Sunflower (Commander)	253	16	6.3	12.9	
Wheat	264	6	2.3	4.7	

* The data for the strains were combined (Appendix B, Table XXI)

** Angular transformation applied before analysis (Goulden, 1952).

*** Any two means connected by the same line are not significantly different from each other at the 1% level by Duncan's multiple range test (Duncan, 1955).

feeding period would indicate that the infective six-spotted leafhopper is a consistent and reliable transmitter of the three strains of AYV tested. However, the similarity of results obtained on aster in this test, and by Kunkel (1926), Strong and Rawlins (1958) and Lee (1962), would indicate that the rate of transmission for different strains of AYV to aster is constant. The fact that aster was significantly less susceptible to the AYV than was stinkweed or head lettuce would indicate that aster is a poor indicator of the per cent of the leafhoppers which are infective. This interpretation questions the per cent of virus infected host plants taken from summer crops (Chapters IV and V) and the per cent of infective six-spotted leafhoppers and infected head lettuce plants would be higher.

The variability of the hosts to the strains of AYV may be explained on the basis of plant resistance or vector host-plant relationships. It has been shown by Putt and Sackston (1960) and Martin et al (1961) that resistance is present in sunflowers and in some varieties of flax, respectively. Though the vector host-plant relationship could possibly be a reason for the differences, it appears remote, because although onion is listed by Wallis (1962) and McClanahan (1962) as a poor host, and experience has shown that leafhoppers cannot survive for long or reproduce on it, the variety used in the test was more susceptible to the virus strains than celery, sunflowers, plantain and wheat. On these plants the leafhoppers survive and reproduce normally. The relative susceptibility of the varieties which were tested, indicates that it may be difficult to obtain resistance in certain crop

plants such as head lettuce. In a breeding program, however, it will be necessary to consider the susceptibility of the plants to the different strains of AYV.

In the transmission tests, the sex of each leafhopper was noted. For the experiment, in which 2225 female M. fascifrons were tested individually, 1076 transmitted AYV, a 48.36 per cent transmission. Of the 1445 male six-spotted leafhoppers tested, 686 or 47.47 per cent transmitted AYV. The near 1:1 ratio between the per cent transmission by the female and male M. fascifrons would indicate that there was no difference in the ability of the sexes to transmit AYV. The leafhoppers were unmated.

CHAPTER X

ACQUISITION BY *Macrosteles fascifrons* OF THREE STRAINS OF ASTER YELLOWS VIRUS FROM FOURTEEN DIFFERENT HOST PLANTS

Materials and methods

Infected host plants were obtained from the test described in Chapter IX. One hundred and fifty nymphs, 2nd. to 4th. instar, were placed on each host plant carrying one of the three strains of "A", "B", or "C". The nymphs were left on the plants for a ten-day infective feed then transferred individually to single four-leaf stage aster plants, in the polyethylene bag cage. The cages were examined at the end of a week and dead insects were replaced. The sex of the leafhoppers was noted when they matured. The insects were retained on the asters until the plants showed symptoms of AYV or for a period of two weeks after the date on which the last aster showed symptoms.

Results and discussion

The results of the transmission test (Chapter IX) showed that infective *M. fascifrons* were consistent and reliable transmitters of AYV, when a two-day inoculation period was used, and that aster (Table XXIII) was a poor indicator plant when compared with stinkweed or head lettuce as an indicator of the proportion of infective leafhoppers. Aster, however, does give consistent results. (Kunkel, 1926; Strong and Rawlins, 1958; Lee, 1962).

The use of aster in this experiment, as an indicator of the