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FINAL REPORT

PROJECT No. I-27-79

**Forecasting Epidemics of Non-Persistent
Viruses in Annual Crops by ELISA and
Use of the "Helper" Factor for
Resistance Breeding.**

B. Raccah, G. Loebenstein, T.P. Pirone

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Abstract

Progress was attained in three of four lines of research proposed in the grant, namely, in the purification, characterization and determination of the role of virus genome in coding for helper component, in the detection of PVY in aphids, and in the development of a monitoring system allowing the determination of the importance of aphid species in flight and those landing on the ability to vector PVY and CMV in peppers. We were unsuccessful, however, in our attempts to find PVY-infected pepper lines from which aphids are unable to transmit the virus because of lack of helper component. These unsuccessful attempts can be explained by our recent findings that helper component is coded by the virus genome rather than being produced by the host as a response to virus infection.

Non-persistent viruses as cucumber mosaic virus (CMV) and several potyviruses (e.g. potato virus Y (PVY), tobacco etch virus (TEV), bean yellow mosaic virus (BYMV) and watermelon mosaic virus (WMV)) are of major importance both epidemiologically and economically (Edwardson, 1974). The wide host range of these viruses and the brief times required for acquisition and transmission explain the difficulties in predicting diseases outbreaks and in their control.

This proposal was written at a time when two new findings had been reported:

1. The use of ELISA technique for the detection of viruses in plants (Clark and Adams, 1979) and in aphids (Gera et al. 1978), and the partial purification of the helper component which is necessary for aphid transmission of potyviruses (Govier et al. 1977). In view of these findings, the project was aimed at forecasting epidemics and purifying and characterizing the helper component with the eventual goal of attempting to select pepper lines in which a potyvirus infection did not induce the formation of helper component and from which, therefore, secondary spread of virus could not occur. The purpose of forecasting epidemics is to provide data on the time and location of epidemics making the choice of preventive measures and control possible. Purification of the helper component (HC) should make a better understanding of the mechanism of the non-persistent virus transmission by aphids possible. Also, if HC production was a plant response to potyvirus infection, selection of lines in which HC was not produced might be feasible.

In order to attain these objectives, research has been conducted in four parallel lines:

1. Purification of HC, determination of its mode of action, production of specific anti-HC sera and determination if HC is virus coded or is a plant response to infection.

2. Selection of pepper lines which might not contain HC when infected with PVY.
3. Development of a monitoring set-up for the collection of data needed for predicting diseases outbreaks.
4. Adaptation of the ELISA technique for the detection of PVY in aphids.

Progress has been attained in three of the four lines of research, namely, in the purification, characterization and determination of the role of virus in the coding of HC, in the detection of PVY in aphids, and in the development of a monitoring system allowing to determine the importance of aphid species in flight and those landing in the ability to vector PVY and CMV in peppers.

However, we were unsuccessful in our attempts to find PVY-infected pepper lines wherefrom aphids will not transmit the virus because of lack of helper. These unsuccessful attempts can now be explained by the recent findings of the colleagues in the U.S. where HC has been found to be coded by the virus rather produced as a host response to infection.

The findings of the present project greatly contributed to the understanding of the nature of HC, and the apparent role of the virus in its production. Also, it has made clear that lack of HC production in host as a characteristic for breeding is unlikely to be useful.

On the other hand, the monitoring for epidemiological data, provided valuable information on the role of various aphid species in CMV and PVY spread, throughout the pepper growing season. It was also found that though these two viruses can be detected by ELISA in aphids, the technique is not sensitive and reliable enough to be used in epidemiological studies.

Final report details:

Preface:

The laboratory in Lexington spent most of the research efforts toward the purification and characterization of the helper component and determination

whether it was virus or plant coded. (A.) The laboratory at Bet-Dagan, Israel, dealt with three topics: B. Development of a monitoring set-up to be used for collection of the data needed for the design of a forecasting system. C. Adapting the ELISA technique to enable its use in rapid determination of vectors among aphids trapped. D. Selection of peppers lines which do not contain the HC when infected with PVY. Each of these four topics will be reported here separately either in written text or by the attachment of the relevant publications deriving from this work.

A. Purification and Characterization of the Helper Component.

There were four major lines of investigation during the grant period and supported in part by BARD funds. These, and a summary of the findings were:

1.) Characterization of HC and its identification as a viral gene product.

Antiserum to HC partially purified from plants precipitates a unique subset of products of the cell-free translation of potyvirus RNA, and antisera to HC of two different potyviruses (TVMV and PVY) precipitate specific translation products of the homologous, but not the heterologous, RNA. These findings are in agreement with aphid assay data which show that PVY-HC and TVMV-HC antisera specifically inactivate homologous, but not heterologous, HC. Analyses of translation products of TVMV RNA with antisera to other potyvirus-coded proteins have enabled us to identify five cistrons and to tentatively locate the HC gene at the 5'-end of TVMV RNA.

2.) Role of the virion and the helper component in regulating aphid transmission of potyviruses.

Although the presence of HC appears to be an absolute requirement for aphid transmission, its presence does not guarantee transmission; we have demonstrated that there are potyvirus isolates which are intrinsically poorly- or non-transmissible. We have accumulated a spectrum of potyviruses which range from highly- to non-transmissible and are currently attempting to determine which properties of the virions affect their transmissibility.

3.) Mode of action of the helper component.

Extracts of the anterior alimentary canal of aphids allowed to acquire PVY in the presence of HC or of a comparable protein fraction from healthy plants were examined by electron microscopy. Particles of PVY were readily detected in aphids fed on a PVY-HC mixture but seldom or never detected in aphids fed on the PVY-healthy plant protein mixture. The results suggest that HC may have a protective effect on the virus.

4.) Purification of HC.

Using a combination of HPLC, immunoabsorption, and affinity chromatography steps, we have been able to purify HC, virtually to homogeneity, from TMV- and PVY-infected tobacco plants. Each source yields a single major band in SDS-PAGE, the molecular weights of which are 53 kd for TMV-HC and 58 kd for PVY-HC. This finding is consistent with in vitro translation results. While direct proof that these represent the HC protein awaits either isolation and assay from a non-denaturing system or inactivation of HC activity by antisera produced to protein isolated from these bands, it seems reasonable to assume that we have purified HC in hand.

With the exception of topic 4, the research on which is currently being completed as indicated, manuscripts have been prepared and have either been published or are in press. Copies of these are appended and may be referred to for complete details.

B. Monitoring for vector population

Parts of these studies have been reported at the Oxford 1981 international meeting on plant virus disease epidemiology (Racchah and Loebenstein, 1981). Additional data are reported in a recent review (Racchah, 1982). However, the majority of the findings are reported herein, and await for their final publication the completion of aphid identification in the British Museum. A general

introduction to the importance of monitoring vectors for virus epidemiology studies is presented in the attached review (Raccah, 1982). The present report reflects studies of the last three years in which monitoring of cucumber mosaic virus (CMV) and potato virus Y (PVY) in peppers was carried out in experimental plots at Bet-Dagan, Israel. The monitoring system was planned to record flying, inoculating and landing species. Data obtained in these three years were compared for vectorial potential and for the nature on viruses introduced.

The time of virus spread and the identity of the vector(s) responsible for spread could be thus determined. The prospect of these findings in forecasting non-persistent viruses epidemics in peppers is discussed.

Experimental procedure

Location: in 1980 the experimental plot was at the Bet-Dagan Farm. In 1981 and 1982 the plot was 3 km to the south of the former, in the Volcani Center Campus.

Description of the experimental plot: The pepper (Capsicum annuum cv. Weindale) was sown on beds, four rows on each bed. The 1980 plot was sown in late April, while those of 1981 and 1982 were sown in mid March. Agrotechnical procedures were those commonly used in commercial pepper plots in Israel and they are described elsewhere (Lobenstein et al. 1970). Plot size was 4 dunam ($=4000m^2$).

Abundance of aphids in flight: The abundance of aphids in flight was determined using suction traps (Taylor and Palmer, 1972). Two types were used: One sampling air at 1.7m and the other sampling at ground level. The traps were operated daily for 15 minutes. In 1980 and in 1981 the sampling was made in the morning hours, a time in the day when most aphid activity was recorded (Fig. 1). In 1982, the traps were operated three times each day for 15 minutes each time, in order to record differences in flight activity for each species.

Transmission studies: Aphids caught by suction were each caged on individual test plants, immediately after being caught in the field. Several hours thereafter, the aphids were removed, identified alive when possible and/or kept in alcohol (70%) for final identification at the British Museum by Dr. V. F. Eastop. Each aphid caught was designated with the same serial number as the test plant on which it was confined. The test plants were sprayed after the removal of the aphids and kept in a growth chamber for symptom appearance. The identity of the virus present in the test plants was determined by ELISA.

Virus diagnosis: Virus infection in the field and on test plants was recorded visually. The identity of the virus was mainly determined by ELISA with anti CMV and PVY sera. The general ELISA procedure of Clark and Adams (1977) was used. During the 1981 season, and in part also in 1982, a symptomless form of CMV was present in peppers which could only be recognized by ELISA. Test plants were kept for 30 days before being determined as non-infected. In the field, the first sampling for infection was made along with the thinning of pepper seedlings. Thereafter, periodically, sections of rows were indexed for virus presence.

Determination of landing rate: In 1980, bait plants were sprayed with an aerosol sticky compound in an attempt to record the aphids landing on the crop. This technique failed to represent the landing as more aphids were recorded on non-sprayed plants than on sprayed ones. Also, the sticky compound had a phytotoxic effect on the bait plants leaves thus altering their color. For landing in yellow pans, a plastic vessel of yellow color was used. The vessel was half filled with water in which Nicotine sulphate was added to stop aphids' escape. Following the description of the green tile landing trap (Irwin, 1980), this trap was used in the 1982 experiment along with brief time recording of aphids landing on designated pepper plants.

Time of flying and landing: The composition of aphid species in flight at different times of day was recorded by operating the suction trap at the morning, noon and afternoon hours. No trapping was made at night, as in preliminary tests, no flight activity was recorded. In order to monitor landing, a trap was designed and built in Bet-Dagan that collect aphids landing in yellow or green tiles traps at 90 minutes intervals throughout the day. This trap was operated in the 1982 season.

Results:

Composition of aphid species in flight:

The composition of aphids species in flight was determined for the three seasons of 1980, 1981 and 1982 (Table 1). As seen, 19 aphid species occurred in all three years. In 1980 only 22 species were recorded, that is attributed to the low aphid population in 1980 and to the late April operation of the suction traps. In 1981, 39 species were recorded and in 1982, 33 species have been identified to date. Additional species might be added when the identification is completed. The most abundant species found both in 1981 and in 1982 is the spirea aphid, A. citricola, explained by the large presence of citrus groves in the coastal plain of Israel where this species reproduce. Other species known to colonize graminaceae, leguminoase, solanaceae and compositae were also frequent. Periodical increase of incidence in flight was recorded for certain species immediately after grazing or harvesting.

Vectorial potential:

The species of aphids found to transmit PVY or CMV in all three years is given in Table 2. The total number of vector species for CMV and PVY is given in Table 3. As seen there, 1980 was exceptional in the number of vector species for PVY. CMV was more likely to be transmitted in the two following years. The differences in transmission can be partly attributed to the location

of the experimental plot: In 1980 it was where additional solanaceae were grown, in 1981 and 1982 the plots were adjacent to gladioli and other ornamentals known to be infected with CMV. The low number of vectors recorded in 1980 did not allow comparison of vectorial efficiency with the subsequent years.

A comparison of transmission efficiency for different aphid species caught by suction is presented in Table 4. As seen there, most aphid species transmitted CMV at about the same rate in the two years. However, for two important aphid vector species, namely, M. persicae and M. euphorbiae, the transmission in 1981 was higher than in 1982. This difference cannot be simply explained by fluctuation in transmission capacity as these two species are known to be efficient vectors for CMV in laboratory conditions. One possibility might be the difference in availability of virus-source-plants in the two years. However, no evidence was collected in this study to support this assumption.

For the majority of aphid species, flight activity was recorded during morning hours (Table 5), declining from noon to afternoon hours. This was true either for aphids caught by suction or for aphids landing in yellow traps. However, for one species, T. trifolii, the incidence at the morning and noon hours was similar. No capture was recorded during night hours. Based on these findings it was appropriate to collect aphids for transmission in the early hours of the day. Vectors for CMV appeared at different times in the season for 4 aphid species compared in Fig. 2. This indicates that A. pisum might contribute to infection early in the season while other species might do so later.

Landing rate:

Inoculation of the crop is caused by aphids in flight that land and probe on the foliage or by aphids colonizing the crop. Therefore, this parameter should be recorded for aphids in flight in order to ascertain whether a flying species will eventually land.

Aphid species which colonize the crop or which stay on it for long periods may be recorded by counting the aphids found on the vegetation. However, for vectoring non-persistent viruses, other species that do not colonize or stay can still inoculate during a brief landing and probing. These species might not be recorded by visual inspection of vegetation, unless counts are made at frequent intervals. Irwin (1980) designed a trap that is, for species landing on soybeans, supposed to respond to this necessity by using a green tile trap whose reflective spectrophotometry is most similar to that of the soybean leaves. In our study, we compared the green tile capture to that observed during brief counts of aphids on pepper vegetation. The data in Table 6, indicate that the tile used in Bet-Dagan, did not contain the same composition of species as that observed on the vegetation, although its reflective spectrophotometry was very similar to peppers leaves. Thus, better traps for landing are required in peppers (other tiles which match not only in color but also in catch).

The data presented in Table 6 are not complete, as there are additional species recorded landing in the green tile and on plants in May 1982, which are still under examination in the British Museum in London. These data, when available, will allow final analysis of the vectorial importance making the completion of Table 8 possible.

The use of the conventional yellow pans (Taylor and Palmer, 1972) is described in the attached review (Raccah, 1982). This trap is apparently preferential for A. citricola as its presence in the yellow trap outnumbered any other species, even more than was found by suction in flight (Table 1 in the attached review). This bias limits the use of the yellow traps for predicting vector presence.

Infection in the field:

The study undertaken in this project emphasized the determination of vectors in flight, as these are mainly responsible for primary infection. In peppers early infections are the most important contributors to the loss in quantity

and quality of yield. The infection in the field was assessed twice, once in early April during the thinning and the second in the first half of May. The infection in early April was negligible. The infection in May is compared in Table 7 with the incidence of vectors in each of the three seasons. As seen there, there was a low incidence of vectors for CMV in 1980 and so was the infection in the field. In subsequent years, the infection of CMV was more pronounced, and that was also reflected in occurrence of vectors. A similar picture was seen for PVY. Later on, when the secondary infection became predominant, the comparisons of the vectorial efficiency of aphids in flight with disease spread in the field was not valid.

Discussion and evaluation of the data obtained by monitoring:

The incidence of aphids in flight does not necessarily represent the abundance of vectors (Fig. 3 and Fig. 1 in the attached review). As seen in these figures, both in 1981 and in 1982 the proportion of vectors caught changed during the season, emphasizing the importance of assessing their infectivity for the entire period.

The use of bait plants provided complementary information on the time and identity of the "infection pressure" (Fig. 4 and Fig. 2 in the attached review). There was a good agreement both for the time and for the incidence of viruses in the two seasons.

The relative importance of the aphid species found in Bet-Dagan in 1981 and 1982 are compared in Table 8. As seen there, 4 species and one genus of aphids are responsible for 3/4 or more of the total infection, in both years. Although the relative importance of one or two species may vary from season to another, the overall importance of all four remained almost the same. If these findings will be found consistent in future years, this will allow to concentrate on the abundance, vectorial potential, and source of these species making the forecasting simpler. Also, prevention measures could be carried out

if the hosts wherefrom these species came will be available.

In conclusion, this epidemiological study, made it possible to establish the importance of A. citricola as potentially the most important vector for CMV and PVY in peppers. It also, showed that the end of April and the beginning of May is the period when the primary infection is maximal. At later dates, though aphids are still abundant, primary infection is reduced.

The source of A. citricola in Israel is the large plantations of citrus. This crop has a flushing period in March or the beginning of April, thus the increase in flight can be predicted and preparations can be made for taking appropriate measures. Also, as the major flight time for most species was found to be in the morning, some appropriate arrangements in pepper nurseries, like cover-up of the plastic protection or screenhouses, might reduce infection.

From the general viewpoint, it is apparent that suction traps, bait plants and landing determinations can provide the necessary data for making a reliable monitoring set-up possible. Additional set-ups in various regions will make large scale forecasting primary spread possible.

C. Detection of non-persistent viruses in aphids by ELISA and by ISEM

1.) Detection by ELISA

Introduction

Transmission studies are still the most reliable way to determine which aphid is a vector. However, these studies need at least 5-14 days for symptoms appearance in test plants, a duration that limits possible use in epidemiology. Alternative means have therefore been sought for detecting viruses in aphids, these means were mainly based either on electron microscopy or on its combination with serology and/or helper (Zoeten, 1968, Roberts and Harrison, 1979, Lopez-Abella et al., 1981). The use of EM made it possible to see particles in a cross section of stylets of aphids which acquired tobacco etch virus (Taylor and Robertson, 1974) and on grids on which were placed excised stylets of aphids

which acquired virus from a concentrated preparation (Mernaugh and Pirone, 1981). However, in these tests the incidence of cases where virus was found was relatively low. In 1977 the enzyme-linked immunosorbent assay (ELISA) technique was used first for detection of low concentrations of viruses in hosts. Following that, it was also successfully used for the detection of CMV in aphids (Gera et al. 1978). In the present project, it was proposed to use this technique for the detection of potyviruses by ELISA, and if applicable to use it for epidemiological studies. In the present section, the detection of PVY in aphids is described, and its limitation for use in epidemiology is discussed. All references cited in this section are included in the attached publication (Carlebach et al. 1982).

Materials and methods:

Materials used and methods adopted are described at length elsewhere (Gera et al. 1978; Carlebach et al. 1982). However, in general the general ELISA procedure described by Clark and Adams (1977) was maintained, with modifications in the composition of the extraction buffer where required, and in the duration of the incubation of the substrate.

Results:

Detection of CMV: Detection of CMV in Aphis gossypii was repeated using the Gera et al. (1978) serum, and confirmed. Moreover, using ELISA, CMV was also detected in M. persicae and in A. pisum. Also, CMV was detected in alates which were fed on an infected leaf (Racchah et al. 1981). However, poor detection if any was recorded for alates of A. gossypii, which were allowed to fly when caged together with a CMV infected cucumber. Therefore, experiments with suction trapped aphids in the field were discontinued.

New sera prepared in rabbits immunized with preparations of purified CMV in a similar way to that reported by Gera et al. (1978), did not yield the same quality of IgG, and with these sera it was more difficult to detect CMV in single aphids.

Detection of PVY: Potato virus Y was more difficult to detect in aphids.

Two main difficulties arose: 1. High non-specific reaction in virus-free aphids. 2. Low specific reaction in viruliferous aphids. The reduction of the non specific reaction was obtained:

i.) by further purifying the anti-PVY IgG by passing it through a protein-A column. ii.) by changing the composition of the extraction buffer (Racchah et al. 1981). iii.) by comparing sera deriving from several immunized rabbits and from different bleedings of the same rabbit. iv.) by adjustment of the conjugate concentration to that yielding the lowest non-specific reaction rather than that yielding the highest specific reaction (Carlebach et al. 1982).

Of these four procedures, we have obtained a reduction in background mainly by using a 0.2M phosphate buffer pH 6 containing 1mM Diethyl-di-thio-carbamic acid (Racchah et al. 1981) and by reducing the concentration of the conjugate (Carlebach et al. 1982), which allowed the use of the regular Clark and Adams buffer. Using the improved system, PVY could be detected in at least 50% of aphids extracts (Carlebach et al. 1982). However, an attempt to correlate the detection with transmission capacity from the same infected leaf has shown that the detection by ELISA does not reflect the rate of transmission obtained.

Discussion:

In this study, it was shown that ELISA can be used for detection of non-persistent viruses in aphids. However, the detection does not correlate with transmission capacity and therefore cannot be used as yet for epidemiology

studies. The obvious reason for this is that the quantity of virus needed for transmission is much lower than that needed for detection. Calculations show that the volume of sap taken in by a probing aphid is so minute that detection is only possible if virus is taken from an area of extremely high virus concentration. Other viruses which are carried within the internal system of the aphid such as in the circulative-persistent viruses, where apparently virus accumulates, the use of ELISA for detection of field collected aphid-vectors is feasible (Tamada and Harrison, 1981). As it stands, it seems that ELISA has to undergo a major modification and improvement before it can serve for epidemiological studies of non-persistent viruses.

2). The use of immunospecific electron microscopy for detection of viruses.

Roberts and Harrison (1979) made use of the ISEM (Derrick, 1973) for the detection of PLRV, a persistent virus in its aphid vector. Detection of CMV and PVY in aphids by ISEM was included in our attempts to develop sensitive techniques for detection of viruses in vectors. In the early stages of the work, the conditions for extraction or adsorption were established for viruses in plants (Cohen et al. 1982 - see attached preprint). Entire aphids which were fed on infected plants were tested for the presence of particles using the optimal detection conditioners. However, in no case were particles found in these preparations. Later, excised stylets from viruliferous aphids were also tested, but no positive detection was recorded. It seems that the number of particles involved in non-persistent virus transmission are also below the threshold needed for detection by the ISEM.

D. Screening for non-production of helper component (HC) in PVY infected pepper lines.

The aim of this study was to look for pepper lines which do not produce HC when infected with PVY. This characteristic, if found could add to the

means for reduction of spread of this virus. Obviously this characteristic is not expected to prevent primary infections, but if present would prevent secondary spread, as transmission from plant to plant would not be possible.

The pool of pepper lines present in the Dept. of Plant Genetics at the Volcani Center allowed access to a wide range of sources. The study was also based on the fact that, parallelly at Lexington, the USA partners were purifying and producing anti-HC serum, thus allowing direct determination of HC presence in the tested plants. However, during the course of the study, it became evident that none of the lines from which transmission was low were lacking HC. At the same time, from the studies at Lexington, it became evident that HC is coded by the virus rather than by the host. Therefore, this line of research was later abandoned.

Materials and Methods:

Pepper seedlings from each of the cultivars (see Table 9) were germinated on wet filter paper, at 30°C. Germination occurred after 6 days. The seedlings were then transplanted to plastic pots, 4 seedlings per pot. The seedlings were kept in the growth chamber at 22°C and 60% relative humidity.

Virus inoculation:

Pepper seedlings were inoculated with PVY 14 days after transplanting. The conventional procedure of mechanical inoculation was maintained. The seedlings were dusted with carborundum, and then rubbed with an extract from a PVY infected tobacto leaf, prepared in 1% KH_2PO_4 in a mortar. Each infected pepper plant was regarded as a line, and served as a source plant for aphid transmission.

Aphid transmission:

A clone of Myzus persicae served in all the experiments. The aphids were raised on Chinese cabbage (Brassica pekinensis). Aphids were fasted for 2 hr,

fed on service lines for 5 min, and transferred for an inoculation feeding to 12 seedlings of peppers (Capsicum annuum cv. Weindale), that served as a test plant. Two aphids were confined on each test plant. Inoculation feeding was overnight; thereafter, the test plants were sprayed with an aphicide, and kept for symptom development at 22°C. Plants that did not show symptoms after 30 days were regarded non infected and were discarded. Generally symptoms appeared after 6-10 days.

Experimental procedure:

Arbitrarily, all the mechanically inoculated lines from which transmission was above 20% were regarded as highly-transmissible and therefore, HC was considered present. Pepper lines from which transmission was 20% or lower were maintained for repeated transmission tests. Only lines from which transmission was < 20% in three consecutive transmission tests were kept, all the other were discarded.

Helper purification:

HC was purified according to the techniques developed at Lexington (Pirone and Thornbury, 1983). For our studies, HC was partly purified from PVY infected tobacco plants (Nicotiana tabacum cv. Burley 21) in the following procedure: One cycle of high centrifugation, then the HC was sedimented from the supernatant by two cycles of PEG, and the resulting HC was resuspended in 0.1M phosphate buffer pH 6.0. This HC preparation was used to test whether HC would improve transmission from the poorly transmitted pepper lines.

Results

As seen in Table 9, all cultivars of peppers served as sources for lines. A total of 619 lines were tested. Of these, 27 were found to be low-transmitted by aphids (20% or less). These lines were kept only if the low-transmissibility was maintained for three consecutive transmission tests.

All the 27 pepper lines defined as "low-transmissible" were maintained and propagated by a cut-back and use of cutting for vegetative reproduction. Six to eight months after the first tests, a new series of tests was made to examine the persistence of the transmissibility. 24 of the lines changed and were transmitted at a higher rate than at the beginning. Three pepper lines that preserved low transmissibility were tested for lack of helper by a sequential feeding of the aphids, first on partially purified HC then on the pepper line to be tested. All three lines remained low transmissible, suggesting that the reason for the low transmissibility is probably not lack of helper.

At this stage of the research, our colleagues at Lexington found that the helper is apparently virus coded rather than plant coded (Thornbury and Pirone, 1983). This finding together with our failure to find pepper lines from which transmission will not occur when infected with PVY, suggested that breeding for lack of helper is not likely to succeed, therefore, this line of research was discontinued.

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EVALUATION OF THE RESEARCH ACHIEVEMENT WITH RESPECT TO THE ORIGINAL RESEARCH PROPOSAL.

A. Forecasting epidemics of non-persistent viruses in annual crops.

The original objective in this topic required the development of a monitoring system for the determination of the aphid species involved in the virus spread and their relative importance during the season when infection takes place. An additional objective was to evaluate the possibility of replacing the transmission tests for determination of vectors with the faster ELISA technique.

Valuable data were collected using the monitoring system that was developed in Bet-Dagan, Israel: Five to six aphid species were found responsible for more than 75% of the infection caused by aphids in flight. Of these, the most important was Aphis citricola an aphid species which does not colonize peppers. The relative importance of each vector throughout the season was established, suggesting the possible role in primary infections deriving from external sources. Bait plants were used in the field to verify the time of the infection pressure as determined by live-trapping of aphids.

Modifications in the extraction buffer and in conjugate concentration made it possible to detect PVY in at least 50% of extracts of groups of aphids. However, transmission tests were still far more reliable than ELISA for vector determination, therefore, ELISA is probably not adequate yet for use in epidemiology studies.

These findings have an immediate implication on the time and nature of means for virus prevention in peppers in Israel. Moreover, the data and their interpretation is of general importance in the epidemiology of non-persistent viruses carried by colonizing and non-colonizing aphid species to annual crops.

B. Helper component (HC).

The ultimate objective of this research was to screen pepper lines to attempt to find lines which did not produce HC and which thus could not serve as sources

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of virus for further spread by aphids. At the time the proposal was written it was thought that production of helper was likely to be a plant response to infection rather than the result of translation of a cistron in the viral RNA. Two parallel lines of research were proposed: screening of pepper lines for lack of HC production (in Israel) and purification, characterization and production of antisera to HC to obtain basic information on HC and to provide biochemical and serological tools for detection of HC in peppers (in the U.S.).

Attempts to find pepper lines which did not produce HC when infected with PVY were unsuccessful. The reason for this was provided by the discovery that the HC protein is coded for by the virus genome. Thus it is very likely that, unless certain lines have the capacity to suppress translation of this specific part of the viral RNA, HC will be produced whenever translation of the viral message occurs.

Although achievement of the specifically proposed objective was not achieved, our research has been fruitful in that it has opened up new possibilities for the development of control strategies. The identification of a specific protein with HC activity and the production of HC purified virtually to homogeneity will allow studies on the specific chemical nature of HC and on the specific mode of action of HC. It is our firm belief that the information derived from these studies will suggest methods by which the action of HC can be nullified and hence spread of potyviruses controlled.

Description and results of cooperation

There was a considerable amount of close cooperation during the course of the grant period, particularly with regard to the helper component and ELISA studies. Professor Pirone spent two weeks at the Volcani in the spring of 1980, evaluating the ELISA technique with aphids for use in his studies on the mode of action of helper component. Dr. Raccah spent several weeks at the University of Kentucky learning our latest HC purification techniques and doing joint experiments on the ability of HC to promote aphid transmission of potyviruses from plants. We were in frequent contact by mail and sometimes by telephone to appraise each lab of the most recent findings relative to our joint research. We participated in two international meetings together and spent much time discussing the progress and future direction of our research.

APPENDIX I

Table 1. Aphid species trapped by suction and tested for CMV and PVY transmission to peppers.

	1980	1981	1982*
<i>Acyrtosiphon pisum</i>	31	12	33
<i>Acyrtosiphon malvae</i>	4	1	
<i>Anoecia</i> sp.		1	
<i>Aphis citricola</i>	7	373	471
<i>Aphis craccivora</i>	3	32	15**
<i>Aphis fabae</i>	8	12	6**
<i>Aphis gossypii</i>	4	25	17**
<i>Aphis nerii</i>		13	17**
<i>Aphis</i> spp.	19	30	363**
<i>Aploneura</i>			6
<i>Aulocarthum solani</i>			2
<i>Brachicaudus amygdalinus</i>		6	15
<i>Brachicaudus helichrysi</i>	6	26	41
<i>Brevicorynae brassicae</i>		19	188
<i>Capitophorus elaeagni</i>	7	16	75
<i>Capitophorus-inulae</i>		1	
<i>Dysaphis apiifolia</i>		7	
<i>Dysaphis</i> sp.		1	10**
<i>Fordini</i>			9
<i>Geoica</i> sp.	2		2
<i>Hayhurstia atriplicis</i>		3	
<i>Hyalopterus</i> spp.	6	4	2
<i>Hyperomyzus lactucae</i>		21	43
<i>Lipaphis erysimi</i>	3	13	26
<i>Macrosiphum euphorbiae</i>	9	61	132
<i>Melanaphis</i> spp.		1	
<i>Metopolophium dirhodum</i>	5	24	59
<i>Myzocallis</i> spp.		1	
<i>Myzus persicae</i>	3	32	78
<i>Pemphigus innunis</i>		1	
<i>Rhopalosiphum maidis</i>		10	16
<i>Rhopalosiphum padi</i>	4	34	158
<i>Rhopalosiphum rufiabdominalis</i>	2	2	12
<i>Saltusaphis scirpus</i>	1	2	3
<i>Schizaphis rotundiventris</i>		1	
<i>Schizaphis graminum</i>	3	3	5
<i>Sitobium avenae</i>		5	34
<i>Sitobium fragariae</i>		1	2
<i>Sminturodes betae</i>		1	2
<i>Semiaphis</i>			1
<i>Tetraneura</i>		1	
<i>Therioaphis trifolii</i>	2	88	99
<i>Uroleucon sonchi</i>		53	51
Unidentified	2	5	34
TOTAL	131	942	2030

* Aphids trapped in spring 1982 are only partly identified

Table 2: Aphid species found to vector CMV or PVY to peppers

Aphid species	Total trapped	Number transmitting		Virus transmitted in:				
		CMV (C)	PVY (P)	1980 C	1980 P	1981 C	1981 P	1982 C
<i>Acyrtosiphum pisum</i>	77	8	5	+	+	+	0	+
<i>Aphis citricola</i>	851	82	23	+	+	+	+	+
<i>A. craccivora</i>	50	1	1	0	+	+	0	0
<i>A. gossypii</i>	46	3	5	+	+	+	+	0
<i>A. nerii</i>	30	5	0	NT		+	0	+
<i>Aphis</i> spp. ¹	412	28	3	0	0	+	+	+
<i>Brachicaudus helichrysi</i>	73	4	1	0	+	+	0	+
<i>Brevicoryne brassicae</i>	203	12	0	0	0	+	0	+
<i>Capitophorus elaeagni</i>	96	2	2	0	+	0	0	+
<i>Hyperomyzus lactucae</i>	65	4	1	0	+	+	0	+
<i>Lipaphis erysimi</i>	42	2	0	0	0	0	0	+
<i>Macrosiphum euphorbiae</i>	202	18	7	+	0	+	+	+
<i>Metopolophium dirhodum</i>	88	3	1	0	0	+	+	+
<i>Myzus persicae</i>	113	9	7	0	+	+	+	+
<i>Rhopalosiphum maidis</i>	26	1	0	NT		+	0	0
<i>R. padi</i>	196	7	1	0	+	+	0	+
<i>R. rufiabdominalis</i>	16	0	1	0	+	0	0	0
<i>Sitobium avenae</i>	41	2	1	+	+	0	0	+
<i>Therioaphis trifolii</i>	193	7	3	+	+	+	0	+
<i>Uroleucon sonchi</i>	104	1	4	NT		+	+	+

1. *Aphis* spp. is still under identification, therefore number of species in this genus will be amended.

Table 3. Number of aphid species trapped by suction and tested for CMV and PVY transmission in 1980, 1981 and 1982

	<u>1980</u>	<u>1981</u>	<u>1982</u>
Total trapped	131	942	2030
Number of species	20	39	33
Number found vectors for:			
CMV	5	16	15
PVY	13	7	0

Table 4. Efficiency of transmission of CMV to peppers by aphids caught by suction in Bet-Dagan in the spring of 1981 and 1982

1981 Aphid species	No. tested	Percent transmission	1982 Aphid species	No. tested	Percent transmission
A. nerii	13	23.1	A. nerii	17	17.6
M. persicae	32	21.9	A. pisum	33	15.2
A. pisum	12	16.7	A. citricola	471	8.0
M. euphorbiae	61	16.4	L. erysimi	26	7.7
A. citricola	373	11.3	M. euphorbiae	132	6.0
H. lactucae	21	14.3	B. brassicae	188	5.8
R. maidis	10	10.0	R. padi	158	3.8
A. gossypii	25	8.0	M. dirhodum	59	3.4
B. helichrysi	26	7.7	T. trifolii	99	3.0
B. brassicae	19	5.3	M. persicae	78	2.6
M. dirhodum	24	4.2	B. helichrysi	41	4.9
T. trifolii	88	3.4	S. avenae	34	2.9
A. craccivora	32	3.1	C. elaeagni	75	2.7
R. paveli	34	2.9			
U. sonchi	53	1.9	H. lactucae	43	2.3
Aphis spp.	30	13.3	Aphis spp.	363	6.6

1. Vectorial efficiency was calculated only for species that were caught at least 10 times throughout the season.

2. Percent transmission $\frac{\text{no. infected test plants}}{\text{total no. test plants}}$ for each species.

The aphids trapped in 1982 were identified alive, final identification is still in process in the British Museum by Dr. V. F. Eastop.

*Aphis species number are to be changed when the identification of Aphis spp. will be completed.

Table 5. The capture of aphid species during daytimes in suction and yellow traps, April-May 1982. Bet-Dagan, Israel.

Aphid species	Suction trap			Yellow trap			
	6-9	11-13	14-16	6-9	9-12	12-15	15-18
<i>Aphis citricola</i>	205	14	1	10	2	0	0
<i>Aphis</i> spp.	110	20	9	3	5	5	2
<i>Brevicorynae brassicae</i>	51	3	0	0	0	0	0
<i>Rhopalosiphum padi</i>	90	15	11	0	0	0	0
<i>Macrosiphum euphorbiae</i>	80	8	5	7	1	1	0
<i>Therioaphis trifolii</i>	39	35	7	4	2	3	0
<i>Myzus persicae</i>	44	3	3	5	0	1	3
<i>Capitophorus elaeagni</i>	38	4	9	6	2	4	0
<i>Hyperomyzus lactucae</i>	20	3	2	0	0	0	0
<i>Metopolophium dirhodum</i>	34	3	5	0	0	0	1
<i>Brachicaudus helichrysi</i>	15	2	3	6	1	0	0
<i>Sitobium avenae</i>	19	4	4	0	0	1	0
<i>Acyrtosiphum pisum</i>	18	8	2	0	0	1	0
<i>Lipaphis erysimi</i>	11	1	2	0	0	0	0
<i>Aphis nerii</i>	7	0	1	1	2	0	0
<i>Uroleucon sonchi</i>	34	1	3	0	3	0	0
<i>Rhopalosiphum maidis</i>	9	8	2	0	0	0	0
<i>Aphis craccivora</i>	8	3	3	0	0	0	0
<i>Aphis gossypii</i>	3	1	1	1	1	1	0
<i>Brachicaudus amygdalinus</i>	7	3	0	0	0	0	2
<i>Rhopalosiphum rufiabdominalis</i>	7	1	0	5	1	1	0
<i>Disaphis</i> sp.	3	2	1	5	1	1	0
<i>Aphis fabae</i>	3	1	0	1	1	0	0
<i>Schizaphis graminum</i>	2	0	0	0	0	0	0
<i>Aploneura</i> sp.	3	0	0	1	0	0	0
<i>Fordini</i>	5	1	0	0	0	0	0
<i>Sitobium fragariae</i>	1	0	1	0	0	0	0
<i>Aulocarthum solani</i>	2	0	0	0	0	0	0
<i>Hyalopterus</i> sp.	0	0	1	0	0	0	0
<i>Geioica</i> sp.	3	0	0	0	0	0	0
<i>Saltusaphis scirpus</i>	1	1	0	0	0	0	0
<i>Semiaphis</i> spp.	1	0	0	0	0	0	0
Total	871	146	78	50	21	14	6
Percent of total	79.5	13.3	7.1	55	23	15.4	6.6

This table will be ammended when final identification of aphid species will be completed.

Table 6. Comparison of the landing rate in green tiles and on pepper plants for aphid species in April 1982 in Bet-Dagan, Israel.

<u>Aphid species</u>	Percent of total population	
	<u>Peppers</u>	<u>Green tiles</u>
<i>Acyrtosiphum pisum</i>	1	3.3
<i>A. citricola</i>	2.2	0
<i>A. gossypii</i>	2.5	0
<i>A. nerii</i>	2.0	0
<i>Aphis</i> spp.	11.1	13.3
<i>Brachicaudus amygdalinus</i>	0.5	3.3
<i>B. helichrysi</i>	2.2	20.0
<i>Brevicoryne brassicae</i>	0.5	0
<i>Hyperomyzus lactucae</i>	1.7	0
<i>Lipaphis erysimi</i>	0	3.3
<i>Macrosiphum euphorbiae</i>	30.4	3.3
<i>Metopolophium dirhodum</i>	1.1	3.3
<i>Myzus persicae</i>	41.8	10.0
<i>Rhopalosiphum padi</i>	0	10.0
<i>Sitobium avenae</i>	0.5	3.3
<i>Therioaphis trifolii</i>	0	3.3
<i>Capitophorus elaeagni</i>	0	10.0
<i>Uroleucon sonchi</i>	2.5	13.3
Total trapped	350	30

Table 7. Comparison of incidence of CMV- and PVY-infected plants in samples taken from the experimental plots in May 1980, 1981 and 1982 and the percentage of infective vectors in suction trap capture, at Bet-Dagan, Israel.

YEAR	Incidence of infection in samples				Incidence of vectors in traps	
	Number of samples	CMV Number (%) infected	PVY Number (%) infected		Number tested	Percentage of vectors CMV PVY
1980	92	4 (4.3)	49 (53.3)		456	3.5 10.7
1981	300	54 (18.0)	23 (7.7)		1459	11.3 5.7
1982	92	34 (36.9)	3 (3.2)		2030	6.0 0

Some plants and some vectors carried both viruses.

The higher capture of aphids in 1982 does not derive from higher abundance of aphids but from more frequent operation of the suction traps.

Table 8. Abundance, transmission efficiency and relative importance as vectors of CMV and PVY of the aphids caught in a suction trap in a pepper field at Bet Dagan, Israel, in 1981 and 1982.

Aphid species ¹	Number of aphid tested (percent of the total)				Number transmitting/ Percent transmitters in species				Percent of the total transmission				
	1981	(%)	1982	(%)	1981 CMV	(%)	PVY	(%)	1982 CMV	(%)	1981 CMV	PVY	1982 CMV
Aphis spp.	100	10.6	418	20.2	10	10.0	6	6.0	27	6.4	12.5	12.8	24.5
Aphis citricola	373	39.5	471	22.8	42	11.3	23	6.2	38	8.1	52.5	48.9	34.5
Macrosiphum euphorbiae	61	6.4	132	6.4	10	16.4	6	9.8	8	6.1	12.5	12.8	7.4
Myzus persicae	32	3.4	78	3.8	7	22.0	2	6.3	2	1.5	8.8	4.2	1.8
Rhopalosiphum padi	34	3.6	158	7.6	1	2.9	0	0	6	3.8	1.2	0	5.4
Subtotal	600	63.5	1257	60.8	70	11.66	37	6.1	81	6.44	87.5	78.7	73.5
Other vector species ²	236	25.0	598	28.9	10	4.2	10	4.2	29	4.8	12.5	21.3	26.4
Other non-vector species	109	11.5	211	10.3	0	0	0	0	0	0	0	0	0
TOTAL	945	100.0	2066	100.0	80	8.4	47	5.0	110	5.3	100.0	100.0	100.0

¹See table 2 for the identity of additional species.

²In 1981 aphids were caught in 15 minutes once a day in the morning hours; in 1981 the trapping represent 3 15 min periods at the morning, noon and afternoon hours. See text for details on the experimental procedure.

Table 9. Relative aphid transmissibility of PVY from pepper lines derived from 11 cultivars.

Pepper cultivars	Number of lines tested	Transmissibility from source lines (number of lines)	
		High (30-100%)	Low (0-20%)
Yolo wonder A	81	79	2
Yolo wonder B	64	61	3
Zaov Naharia	66	63	3
Kalinkov	46	45	1
California wonder	78	73	5
Keiston	84	76	8
Weindale	32	31	1
Sweet spice	56	52	4
Maor	67	67	0
Midway	20	20	0
Zohar	25	22	0
Total	619	589	27

¹ A line is defined as an individual pepper plant derived from each cultivar. Source plants were mechanically inoculated with PVY.

Figure 1. Total number of aphids caught in yellow trap at different times of the day throughout the spring of 1982 at Bet-Dagan, Israel.

Figure 2. Total number of Brevicorynae brassicae, Macrosiphum euphorbiae, Acyrthosiphum pisum and Aphis citricola (bottom of each section) and the number of vector for each species (top of section), caught by suction traps over a pepper field in Bet-Dagan, Israel, March-May, 1982.

Figure 3. Total number of aphids (top) and number of vectors (bottom) caught by suction over a pepper field in Bet-Dagan, Israel, March-May, 1982.

Figure 4. Rate of CMV infection (in percent) in peppers bait plants (top) and the incidence of aphid vectors caught by suction (bottom). Bet-Dagan, Israel, March-May, 1982.