The intent was to identify regions of the pepper genome that control response to CMV. However, preliminary phenotypic data suggest some hypothesis about inheritance of resistance derived from this source. About 5% of the families, each derived from a single second generation plant, consisted of plants with the desired characteristics - slight or no apparent symptoms. This is consistent with our hypothesis that resistance is conferred by at least two recessive genes, possibly with cooperative interactions. Several variables exist related to symptom expression which should be clarified in the fourth generation plants. We will also be evaluating these and related populations with the more severe isolates of CMV. The *C. frutescens* sources of resistance are freely available to any interested workers.

RAPD Analysis

Because of the difficulty in transferring resistance to CMV in peppers, we are exploring several alternative approaches to finding the keys to unlock the resistance puzzle. This was the motivation for turning to gene mapping to assist in the transfer of resistance. Restriction Fragment Length Polymorphism (RFLP) is a widely used method to detect genetic differences that can be used to select a desirable characteristic and is the base of most of out efforts in pepper. Recently a map of the tomato genome with 1,000 markers has become available to us from Dr. S.D. Tanksley, Cornell University. Pepper and tomato are related closely enough so this map is very useful in filling gaps in the pepper map. More recently an alternative method that avoids the use of radioactivity and cloned probes has become available that relies on Polymerase Chain Reaction (PCR) to generate characteristic DNA fragments that can be used similarly to fragments generated by cutting DNA with restriction enzymes. This method is based on analysis of RAPD fragments(random amplification of polymorphic DNA). A set of RAPD fragments are being evaluated to determine if this method will offer a useful alternative to RFLP markers in pepper.

Two genes that give resistance to the common strain of tobacco mosaic virus (TMV) in the cross between *C. chinense CA4 x C. annuum* RNaky have already been mapped using RFLP markers, and preliminary results from collaborative work with Dr. B. Staskawicz, U.C. Berkeley maps *Xanthomonas* resistance to chromosome 7 using RAPD data. These results suggest that our mapping procedures are working well and may thus be extended to traits that are more complex genetically, such as CMV tolerance. Our next target among viral resistance genes is recessive resistance to potyviruses found in *C. chinense*. The second generation population has been screened for resistance to pepper mottle, one of the three important potyviruses in the U.S., and tissue will be harvested in July, 1992 for DNA extraction and analysis. Genetics of recessive resistance to tobacco etch virus, pepper mottle virus and potato virus Y is another area of major effort in our program which is not covered by the Pepper Commission contract.

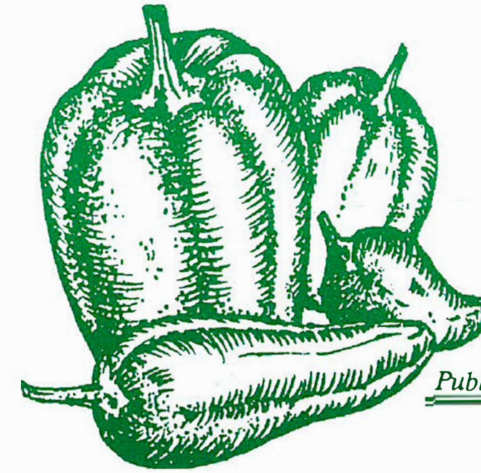
Tomato Spotted Wilt Virus

In addition to CMV and the potyviruses, tomato spotted wilt virus (TSWV) is the third virus that threatens pepper production that is under study in our program at Cornell. While TSMV occurs only sporadically in California, there is concern because of the devastating effects of infection when it does occur. Again, the first problem was to develop a reliable inoculation procedure because transmission of this virus in nature occurs through the activity of thrips, and it is very difficult to transmit most isolates of this virus without the insect. This has been accomplished with a very, very low rate (<1%) frequency of plants that escape infection. We have continued working with the *C. chinense x C. frutescens* families described in earlier reports to determine the nature of the response to TSWV and have extracted DNA from the populations to locate the regions of the genome that control the necrotic responses that when combined appear to create a localizing resistance. We have screened F_3 families from this cross which are segregating approximately 50% lethal necrotic and 50% highly resistant plants. The plants that survive appear to have a very high level of resistance that withstands repeated high concentration inoculations with the virus.

California Pepper Commission Financial Report

Fiscal Year: May 1, 1991 through April 30, 1992

Account Name	
INCOME:	
Carry-over	$60,103
Assessment Income, 1991-92	138,293
(Based on combined rate of $.50/ton)	
Interest	4,154
Total Income	**$ 202,550**
EXPENDITURES:	
Attorney's Fees	1,426
Management Services	25,200
Audits	1,671
Office Supplies	1,701
Telephone	559
Postage	696
Travel & Mileage	759
Meetings	937
Insurance, Taxes & Bonds	487
Marketing Branch	14,631
Production Research	106,837
Total Expenses	**$ 154,904**
Carry-over Reserve to 1992-93	47,646
Total Expenses & Reserve	**$ 202,550**



Pepper News

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July 1992

– ANNUAL REPORT ISSUE –

Moving Ahead

The California Pepper Commission is continuing to work to solve the problems of the pepper industry through research. Last year the research budget was over $85,000, this year that amount was raised to $106,000. Research now constitutes over 68% of the Commission's budget.

This issue gives an overview of the research reports for 1991-92. There are three project summaries contained in these pages. Their purpose is to give you the main details or recommendations of the reports. Some of you will find that you want to know more about the research - if so, the complete technical reports are available, free of charge, from the Commission office. Just call (209-591-3925) or write to the address on the masthead, requesting a complete technical report.

One unusual report is from Cornell. Last year we reported that their project had landed a large USDA grant. The Commission is still funding some of Dr. Kyle's work at Cornell, but is benefiting from the larger pool of funds from USDA. Dr. Kyle's report gives an overview of the larger scope of her work on Cucumber Mosaic and Tomato Spotted Wilt viruses on peppers.

Viruses and Fungal Pathogens in California Pepper Production

Bryce Falk/Bob Webster, UC Davis 916-752-0302

Last year's objectives were:

- 1. To continue to monitor California pepper disease incidence, and to collect representative isolates of the major viruses with emphasis on cucumber mosaic virus (CMV), the potyviruses [potato virus Y (PVY), tobacco etch virus (TEV), and pepper mottle virus PeMV)], as well as representative isolates of Phytophthora capsici and Verticilluim dahliae.
- 2. To obtain, evaluate and incorporate sources of disease resistance for viruses and Phytophthora into useful breeding lines of various pepper types.
- 3. To assess and develop accurate and effective virus inoculation and evaluation methods.

Good progress has been made on all of the above objectives. The results are summarized below.

Objective 1. This past year we again monitored virus incidence in peppers throughout selected pepper growing regions of California. This was done by three methods: 1) collecting selected samples in some locations; 2) analyzing samples sent to us from cooperators; and 3) specific collections made in our field plots. In general, pepper virus incidence was unpredictable and sporadic from location to location. In addition, peppers in some areas were affected by viruses other than those of interest to our program. For example, beet curly top virus was associated with two severely affected fields in Stanislaus County, alfalfa mosaic virus was recovered from peppers in several locations, especially early in the year, and tomato spotted wilt virus was recovered from two samples from the Gilroy area.

One field of relatively severe virus infection was noted by cooperators in the La Mesa area of Gilroy. We visited this field and collected samples for analysis. The general level of infection was high. Over 70% of the 100 random plants showed some virus infection symptoms. Most common were symptoms resembling CMV infection. Twenty five plants with varying degrees of virus symptoms were randomly recorded for their visual symptoms and the 5-6 pair of leaves from the top were sampled for ELISA. The most frequently detected virus was CMV. PeMV was the close second. TEV was detected only on 2 samples. Most severe plant symptoms were associated with mixed infections of CMV and PeMV.

The above results support results from previous years, that CMV is the predominant virus in Gilroy. However, PeMV always seems to be associated with severe virus outbreaks in Gilroy and other parts of California.

Field Screening in Spring 1991.

Field plots were placed in four locations. Plants used in some of these trials included many parental lines, some promising selections (for CMV resistance in our greenhouse studies) and some of the 'F1' progenies of these selections. In addition, all locations had a range of selected standard pepper lines including:

Calwonder 300	Del Ray Bell
Yolo Y	PI 159236
Verdel	PI 152225
VR2	CM334
VR4	Perennial 6

The primary objective of the trials was to assess virus incidence and severity in the range of standard pepper genotypes. Secondarily, we wanted to obtain some data on the field performance of our early material. We also planned to collect any CMV, PVY, PeMV and TEV isolates that infected the resistant/tolerant material.

Objective 2.

Several new Capsicum accessions were obtained this past year. This includes Serrano Vera Cruz materials from Spain which we will use in our potyvirus typing work; approximately 190 accessions for CMV screening from the plant introduction station in Georgia, and material from Harris Moran which behaved well towards CMV in some pepper field trials. Some of these have been tested in our program already. Eight lines from Georgia, identified by them as CMV resistant, were susceptible to CMV 144-I and showed some potential for CMV C. Osir, from Harris Moran was identified as having some field tolerance to CMV, but it also is susceptible to CMV 144-I but shows some potential to CMV C. Any promising material identified from further efforts will be incorporated into our program.

Our breeding and screening efforts have focused on CMV, and some on Phytophthora capsici. The focus was on Perennial lines, Perennial derivatives, CM 334, Pere. x CM 334, and various segregating populations of this cross. Perennial showed good promise in selecting against CMV 144-I, while CM 334 had mild resistance to some CMV isolates but good resistance to Phytophthora capsici infection. Now there are some lines selected against CMV 144-I over 3 generations and a total of five generations.

Some of the most promising specific lines have been inoculated with CMV 144-I and CMV C separately. Many of them are from those lines that did not show any symptoms or positive ELISA values in the previous generation after repeated inoculation. There are some single pod-to-row lines (self pollinated) selected from a large 'F2' population of a cross between Perennial x CM 334, and which was challenged with CMV 144-I. The usefulness of ELISA became very apparent in assessing the resistant/susceptible reaction of those individuals that did not show symptoms. However, high ELISA titer values were not always associated with good symptoms, but this is not totally unexpected (see results for objective 3).

It is our hope that a line(s) resistant to CMV-C would be available in the next round of selection. A line resistant to 144-I is also expected soon. The CMV 144-I isolate, being one of the most virulent, and it is assumed that a plant resistant to this isolate would be resistant to most of the other isolates as well. However, we still need to learn more about CMV variability.

We are planning to cross this CMV 144-I resistant plant with commercial varieties having good agronomic features and resistance to PeMV, TEV and PVY before handing over this multiple virus resistant seeds to the California Pepper Commission.

Work on Phytophthora capsici resistance:

During October 1990, all available pepper germplasm was screened against a California isolate of Phytophthora capsici C-8, A-1 which is reported to produce severe symptoms on susceptible plants. This isolate literally killed all susceptible young seedlings. Tolerant plants showed slow decline. Resistant plants were not showing any effect of the fungus.

The line from Mexico, Criollo de Morales (CM334) was the only line that showed complete resistance. Few other lines including Perennial selections showed partial resistance. Hence the decision was made to incorporate CM 334 into Perennial material before developing a plant resistant to CMV so that this plant also could have resistance to P. capsici.

Also crosses were made between C-8 P. capsici resistant CM 334 and partially resistant Perennial derivatives, completely susceptible commercial bells and anaheim pepper types. One month old seedlings were screened in the greenhouse and scored one week after all the susceptible Yolo-Y plants were killed. We used a standardized mycelial suspension inoculation procedure developed by Webster (Unpublished). Given below are the initial results:

Plant	# Resistant	# Susceptible
CM 334 (P. capsici Res.)	98	00
Conquistador	00	25
Jupiter	00	15
5A 3 (A Perennial Sele.)	31	07
(Jup. x CM 334) F1	38	01
(Jup. x CM 334) F2	195	72
(Conq. x CM 334) F2	331	131
(5A 3 x 10A1) F2	200	10

Relatively larger 'F2' populations from crosses between P. capsici resistant and susceptible patents have been raised and will be screened soon. Any speculation on the genetics of inheritance of resistance will be delayed until more data are collected.

there is an ongoing effort to locate sources of resistance to viral diseases and characterize the genetics of resistance to facilitate transfer to commercial varieties. Both natural and engineered genes are considered, and the intent of the program is to provide genetic resources and streamlined selection methods for pepper breeding programs.

One of the major objectives of this program is to evaluate the leading sources of CMV resistance available in order to develop recommendations for commercial breeding efforts. This virus is considered to be the most important and regularly recurring constraint to production in the state. One criterion in the selection of sources of resistance to be employed in a breeding program involves the range of viral isolates that are affected. A number of strains of CMV are known, but distinct strains are not well defined on pepper. The first question to be answered was whether sources of resistance in widespread use were genetically similar or different, and if different, to characterize the isolate-specificity. An important component of this question is to understand the nature of resistance.

The genetic material that was evaluated in the present study included three lines reported to have some resistance to CMV. First, *C. annuum* 'Perennial', an Indian variety used widely by breeders in Europe, Asia and N. America. Two *C. frutescens* lines collected in Mexico were found to have some resistance to CMV. Finally we tested one line inbred from each accession designated BG 2814-6 and BG 2816- 1, and the hybrid between these lines. We developed standard inoculation procedures that resulted in uniform infection of the susceptible check 'NuMex RNaky', other commercial pepper varieties and susceptible check species. Plants were evaluated over several months visually until seed was harvested to monitor symptom development. Some lines that do not develop strong symptoms may harbor the virus, so tissue was taken from plants at regular intervals and analyzed to determine whether the virus was present and if so at what level. These tests showed that symptom intensity is roughly correlated with the amount of virus present in the tissue.

Six viral isolates were selected, three from California (two mild strains and one severe), and three additional isolates from the Cornell program which varied significantly with respect to symptom type and intensity on susceptible plant genotypes including pepper and squash. These six isolates were used to inoculate sets of plants representing the four sources of resistance, 'Perennial', BG 2814-6, BG 2816-1 and the hybrid (BG 2814-6 x BG 2816-1). All four genotypes generally failed to develop clearly discernible symptoms or significant levels of virus in plant tissue with the milder isolates evaluated in the study. Susceptible genotypes uniformly developed characteristic symptoms for each isolate. Seasonal effects were noted with more severe reactions generally observed during the winter months. Two of the genotypes, Perennial and BG 2814 have good resistance to CMV symptom development according to our tests, and based on differences in reactions to the strains, these two sources do not have the same genes for resistance. Our results suggest that neither Perennial nor 14-6 confers absolute resistance to the virus spreading throughout the plant under all conditions; however, the amount of virus present in tissue of these lines is much less than in susceptible hosts, such as current varieties, and effects of the virus are much less severe, or absent completely. We are continuing to critically examine our detection systems to check control thresholds at low virus concentrations, and to improve detection of the various virus isolates involved in this study.

Current information suggests that the optimal strategy using existing sources should involve combining genes from Perennial and *C. frutescens* 14-6 to obtain the highest level of resistance to the widest array of CMV isolates. Based on results from this study, we are developing populations to test whether it will be possible to identify individuals that contain genes from both parents that show levels of resistance that are superior to either parent alone. There are ample genetic differences between each of these sources of resistance and commercial varieties to allow molecular markers unique to one genotype to be used as an easily selectable tag for genes from that genotype. There are several versions of the Perennial source available and we are currently working closely with collaborators at the Asian Vegetable Research and Development Center (AVRDC) in Taiwan to determine which Perennial line should be the focus of our future mapping work. The term mapping refers to the process of building ordered linkages between molecular markers and genes that are of importance to breeders. When the process is advanced, each chromosome of the plant is represented by a "map" that describes the information contained on that chromosome. It is then possible to select the region of the chromosome that contains the gene of interest, for example resistance, without the difficult whole plant level screening that is currently required, and has not to date yielded adequate results. There are good prospects to expand the mapping work in collaboration with the AVRDC and European workers to develop an international cooperative focused on the pepper genome. Cornell has been asked to assume leadership in the formation of the effort, and an inclusive effort has been made to contact groups interested in developing programs in this area.

Inheritance of Resistance

Another important question involves gathering information about how easily resistance to CMV can be transferred. The Perennial source is thought to contain several genes that are recessive and therefore have been difficult to transfer into adapted acceptable varieties. Only very preliminary information was available for the *C. frutescens* source that also suggested recessive inheritance. We have screened 69 third generation families from a cross between *C. frutescens* 14-6 and 'RNaky' to determine inheritance of resistance to our CMV-CA isolate, one of the milder in effect so seed could be produced on symptomatic and resistant plants for further confirmation of results. Dramatic differences in severity of CMV isolates was observed both within and between F_3 families. All screening was conducted during the spring, fall and winter seasons in temperature controlled greenhouses as consistent cooler conditions are critical in obtaining consistent symptom development with CMV.

California Pepper Commission

Objective 3.

Last year we planned to assess and develop accurate virus inoculation methods, with special reference to CMV. We feel that this is not a major problem, and we are confident that our inoculation methods are acceptable.

We have incorporated using ELISA with visual assessment of CMV resistance in our material. That is, plants are scored visually and then also assayed by ELISA. We cannot use ELISA as a quantitative measure of resistance under our current conditions. Greenhouse environmental conditions are too variable. ELISA is used to confirm the presence of CMV, and to ensure that no other viruses are present.

We also are still interested in comparing CMV isolates from California, and in developing methods which might allow us to rapidly discriminate and/or identify mild isolates from more severe ones. This past year we "typed" a number of California CMV isolates and compared them by various methods. We obtained antiserum to well- characterized New York CMV isolates from Dr. Dennis Gonsalves, Cornell University at Geneva. We used these antisera in comparative ELISAs with the 2 antisera we have made to California pepper CMV's.

The majority of California pepper CMV's belong to the CMV subgroup I. This supports the work done previously by Daniels and Campbell, and is similar to results from New York. The data also shows that the antisera we have made for California pepper CMV's are group I antisera, and these antisera do not react strongly with all group II isolates. We also know that from these data, ELISA absorbance values do not allow us to discriminate between severe isolates (eg. CMV 144-I) and milder isolates.

We also used a limited host range test to attempt to discriminate between severe and mild CMV's. Calwonder 300 peppers, Nicotiana tabacum Xanthi nc., N. glutinosa, small sugar pumpkin, and tomato plants were included in the study. In general, severe isolates such as CMV 144-I were more severe on all plants tested. However, this does not help us in rapidly and definitively discriminating between mild and more severe CMV isolates.

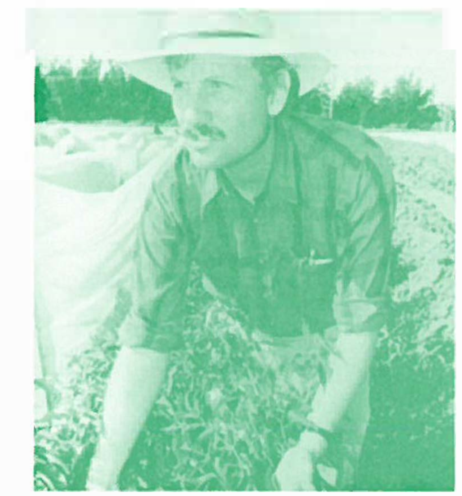
We also have done some collaborative work directed towards typing and classifying pepper potyviruses. Specifically we have cooperated with scientists in Spain who are working on pepper potyvirus resistance. Our collaborative efforts have demonstrated that what we call pepper mottle virus is clearly distinct from what they call severe potato virus Y. Our PVY's and theirs are similar, and are serologically detectable by using the antisera we have made as part of this project. This suggests that our sources of PVY resistance could benefit them, and that their best potyvirus resistant material is likely not to be resistant to our PeMV's.

We also have collaborated in a project to attempt to discriminate PVY and PeMV by the nucleotide sequences of their genomic RNA's. This is important because some virus taxonomists want to call PeMV a strain of PVY. Our collaborative efforts, with scientists in South Carolina, Florida and Beltsville, MD, show that PVY and PeMV can easily be differentiated at the molecular level. We do not know what these differences mean in regards to the host ranges of each of these viruses, however.

Developing Enhanced Pepper Germplasm Resistant to Verticillium Wilt

Paul Bosland, New Mexico State Univ. 505-646-5171

A verticillium wilt-resistant population is being developed from the accession P.I. 215699, by selection of highly resistant individuals through four cycles of screening and selfing. The fourth cycle of screening and selection will be done by June 1992. In the backcrossing program, a resistant individual from the second selfed- generation (S_2) of the original P.I. 215699 accession is the donor parent to bell, jalapeno, and New Mexican types. The additive and dominance variance effects are being determined for disease interaction phenotype score and percentage of resistant plants. Other sources of purported Verticillium wilt-resistant materials were also screened.



Dr. Paul Bosland

The experiments are performed under strict environmental control in soil temperature tanks. The soil temperature in the test pans is 25 ± 1 C. The inoculum level is 2000 microsclerotia per gram of soil. The seedlings are challenged 60-70 days. Individual plants are scored with an interaction phenotype scale, ranging from 1 to 9, where 1 = no aerial symptoms, and 9 = death. Also, shoot height is measured in centimeters.

P.I. 215699 continues to segregate for verticillium wilt resistance after three cycles of screening and selection. Fortunately, the percentage of resistant plants has increased from 38% to 75%. Currently, 28 S_3 highly resistant populations generated from 11 S_2 populations are being screened to select the population with the highest level of Verticillium wilt resistance.

The results of screening F_1 generations and their recurrent parents from the backcross breeding program to bell, jalapeno, and New Mexican types for Verticillium wilt resistance indicates Verticillium wilt resistance is a quantitative trait. Thus, the genetic inheritance for resistance is polygenic, controlled by many genes. To date, F_2's of bell, jalapeno, and New Mexican types have been produced and screened. Saved individuals with highest levels of Verticillium wilt resistance will be backcrossed, and appropriate horticultural traits will again be selected.

Additive and dominance variance effects are being determined for better understanding of which breeding method for Verticillium wilt is best. An F_1 between a resistant S_3 plant and a highly susceptible plant (B.G. 1168) has been accomplished. The F_1BC_1 and F_2 have been generated and an F_1BC_2 has been undertaken. The final experiment will have four replications for each generation P_1, P_2, F_1, F_2, F_1BC_1, and F_1BC_2. Means, variances, and standard deviations for interaction phenotype and percentage of resistant plant will be calculated. Populations means will be statistically compared by the 2-tailed t- test. The conformity of data to the additive-dominance model by Mather's A, B, C scaling test will be used. Estimates of additive (D), dominance (H), and environmental (E) variance will be obtained by the function $E = VF_1$, $H = 4[VBC_1 \; VBC_2 - VF_2) - VF_1]$ and $D = 2[VF_2 - 1/4 H + E)]$. Narrow- and broad-sense heritability and genetic advance of the trait will be calculated.

When other purported Verticillium wilt-resistant accessions were screened, some highly resistant individuals (class 1) from B.G. 1535 and B.G. 2815 were saved and their progenies are being screened. Results from the progeny test will be available by June 1992.

Previous investigation indicates that pepper plants surviving in Verticillium infested fields have some resistance to Verticillium wilt. Individual plants showing "field-resistance" were selected from pepper fields heavily infested with Verticillium wilt. These selections will be screened for Verticillium wilt resistance during the summer of 1992.

Development of Resistance to Cucumber Mosaic Virus and Tomato Spotted Wilt Virus in Commercial Pepper Varieties

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This project focuses on the important constraints to California pepper production caused by losses to viral diseases. The effort funded by the Commission in 1991-92 focuses on cucumber mosaic virus (CMV) and tomato spotted wilt virus (TSWV), a virus that currently causes only sporadic losses, but is present in the state and causes devastating losses when infection occurs. Other efforts in the group involve resistance to the potyviruses. At Cornell,