Maintaining and Improving a Collection of Pepper Viruses

Rodrigo A. Valverde, Associate Professor,
Plant Pathology, Louisiana State Ag. Center

The "Pepper Virus Bank" located in the Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center consists of a properly identified collection of pepper virus isolates from different locations in the United States and other countries of the Western Hemisphere. Viruses are stored as dried plant tissue in glass vials in a refrigerator. These virus strains are of practical use to pepper breeders breeding virus-resistant peppers. At the present, 202 pepper virus isolates from the U. S. and nine other countries are in storage. A computer file with a list of all stored viruses, together with the date, location and source has been generated.

California Pepper Commission Financial Report

Fiscal Year: May 1, 1996 through April 30, 1997

Account Name	*Amount*
INCOME	
Carry-over from 1995-96	$ 98,779
Assessment Income, 1996-97	182,644
Assessment Income, Prior Yrs.	2,741
Interest Income	5,773
Other Income	1,764
Total Income	$ 291,701
EXPENDITURES	
Management Services	28,800
Audits	1,700
Office Supplies	1,330
Telephone	401
Postage	711
Travel & Mileage	766
Meetings	531
Insurance	654
Marketing Branch, CDFA	5,109
Production Research	122,260
Total Expenditures	$ 162,262
Carry-over to 1997-98	$ 129,439
Total Expenses & Reserve	$ 291,701

California Pepper Commission
Producer Representatives

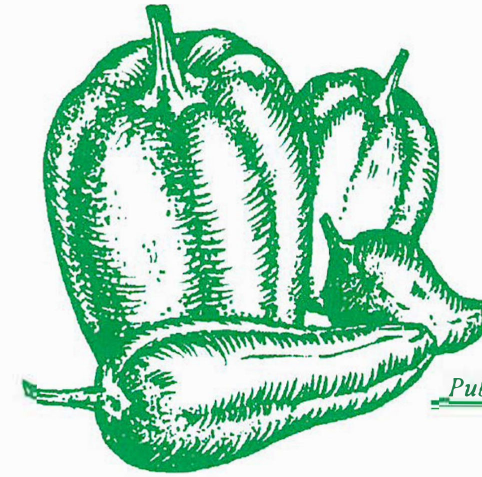
Members	Alternates
District 1	
Burt Silva King City 408/385-1428	**Marvin Borzini** King City 408/385-5441
District 2	
Frank Luenser Arroyo Grande 805/489-2508	**Chris Darway** Arroyo Grande 805/489-1817
District 3	
Mike Mantelli Gilroy 408/848-9228	**Fred Rohnert** Hollister 408/636-4898
District 4	
Bob Giampaoli LeGrand 209/389-4576	**Joe Marchini** LeGrand 209/389-4528
District 5	
Randy Johnston Lemoore 209/924-5339	**Richard Bradford** Coachella 619/398-6187

Public Representative

Don Nelson Mokelumne Hill 209/754-5605	Vacant

Handler Representatives

Bell Pepper Processors	
Tom Busenbark Modesto 209/538-5437	**Dave Veneman** Manteca 209/823-3181
Dehydrated Chili Processors	
Steve Banta King City 408/385-5406	**Paul Gniffke** Greenfield 408/674-5571
Other Pepper Processors	
Glen A. Fischer Saticoy 805/647-5266	Vacant
Fresh Market Pepper Handlers	
Vacant	**Tim Baloian** Fresno 209/485-9200
Pepper Seed Handlers	
Robert Heisey San Juan Bautista 408/623-4554	**Ken Owens** Woodland 916/666-0931



Pepper News

Published by the California Pepper Commission, 531-D North Alta Ave., Dinuba CA 93618

June, 1997

ANNUAL REPORT ISSUE

Commission Report

With the publishing of this issue, the Pepper Commission is beginning its ninth year of operation, and it is continuing its efforts to find solutions to the production problems of the California pepper industry. This annual newsletter contains a financial report for the past year, and reports of the Commission's production research projects that were funded during the 1996-97 fiscal year, as well as the Commission's membership roster as it functioned during that period.

Steve Banta served as Commission Chairman during the 1996-97 year and was re-elected for another one-year term at the April, 1997 meeting of the Commission. He will be backed by Vice-Chairman Bob Giampaoli of Le Grand, who is serving his third consecutive term in that position.

The financial report on Page 6 shows the Commission is still in excellent financial shape, with the income from marketed peppers increasing each of the past three seasons. Over the past five years, in an attempt to be conservative, the Commission has budgeted on the basis of receiving income from the equivalent of 270,000 tons of fresh peppers, which would bring in $135,000 at the $.50 per ton rate. However, the actual income during those years has ranged from the low of $149,772 in 1993-94 to this year's high of $182,644, which was the highest income ever recorded by the Commission. With the crop size continuing to increase, the Commission has been able to carry over those surpluses from year to year, thus making it somewhat easier to fund the needed research projects.

Insofar as the Commission's expenditures during the 1996-97 year were concerned, they were almost entirely within the budget and the Commission earned over $6,300 on its reserve funds. The Commissioners have always prided themselves on squeezing the last ounce of usefulness out of each dollar collected from the industry, and last year they were able to show that over 75% of the Commission's expenses went directly to research projects, with less than 25% spent on overhead items, which include the management, as well as the California Department of Food & Agriculture's monitoring of the Commission's activities.

During the 1996-97 year, the Commission funded eight research projects costing a total of $122,260, and the layman's reports for those projects are included with this report. Anyone wanting copies of the full, technical report of any of the projects can obtain a copy by calling or writing the Commission office.

Improved Methods in Screening Capsicums & Germplasm Evaluation for Verticillium Wilt Resistance

Paul W. Bosland, Professor, and Mary K. Riley, Research Assistant; New Mexico State University Department of Agronomy & Horticulture

Verticillium dahliae is a soil-borne fungus that causes the disease Verticillium wilt on peppers. In pepper fields infested with the fungus, yield losses range from 20 to 90%. Once the soil has been invaded by the fungus, the resting spores, or microsclerotia, can survive in the soil for up to 20 years, making crop rotation an unreliable method of control. Biological control, using other fungi or bacteria to attack the *Verticillium*, has not been successful, and soil fumigation and chemical applications are not economically practical for the grower. Therefore, production of resistant varieties is the best approach for controlling Verticillium wilt of peppers.

The Pepper Breeding Program at New Mexico State University developed a pepper line with a high level of resistance.

This resistance has been introduced to four commercial pod types: jalapeno, New Mexican, bell and pimiento. While these populations have been improved with respect to their levels of resistance, they have not attained the high level of resistance present in the original resistant source. In an effort to find peppers with even greater resistance we screened several hundred different pepper lines for resistance. Several lines from these tests have consistently exhibited high levels of resistance, and the introduction of this resistance to the commercial pod types may yield a stronger resistance in these populations.

In California, many fields are alternately planted with peppers and tomatoes. There is contradictory information in the literature about whether the Verticillium fungus strain that infects peppers will infect tomatoes. More conclusive evidence is necessary for growers with Verticillium infected fields to plan their field rotations. If there is no cross-infectivity then planting peppers following tomatoes is a viable planting scheme. If cross-infectivity exists, then the grower should opt to plant a different crop. Therefore, tests have been conducted to check for cross-infectivity. To date, no cross-infectivity has been found between peppers and tomatoes. However, there are two races of Verticillium that infect tomato and these tests have only included one race. Currently, the second tomato race is being screened for cross-infectivity.

The Pepper Breeding Program had been using a newly (1995) modified soil infestation method. However, most studies involving Verticillium wilt of tomato utilize a root-dip method of infecting the plants. To perform these pepper/tomato studies with the most precision, the root-dip method was employed and found to be reliable. This method also reduced the time needed for each experiment, thus enabling the testing process to move forward at a faster rate. Because the root-dip method is reliable and time-saving, the Pepper Breeding Program has adopted this method for all of the studies involving Verticillium wilt of pepper.

Epidemiology of Pepper Stip

Richard Smith & Bob Mullen, Farm Advisors in San Benito & San Joaquin Counties, & Tim Hartz, Vegetable Specialist, U.C. Davis

Pepper Stip is a problem on peppers in many parts of the world. It is a gray-brown to greenish spot that occurs primarily on the red fruit of bell and other types of peppers in the fall. The spots are typically 1/4 to 1/3 of an inch in diameter and extend from the surface to the interior of the pepper. There can be few (i.e., 2-3) to many (10 - 20) spots per fruit. The severity of pepper stip varies from field to field and from year to year. The problem occurs on bell, pimiento and other pepper types in San Benito, Santa Clara, Monterey, San Joaquin and other Counties. Growers and processors both suffer losses from fruit that possess this undesirable trait. The problem is most severe on open pollinated varieties that are used in the processing industry. Many hybrid varieties that are used for the fresh market are not affected by pepper stip. Pepper stip is thought to be linked to a calcium imbalance in the plant induced by high nitrogen or other environmental conditions.

Trials were established in three sites in the Central Coast and in one site in San Joaquin County to evaluate the effect of applications of calcium on the incidence of pepper stip. The four trials were established with two varieties, a susceptible variety, Grande Rio 66, and a resistant variety, King Arthur. At each test site the two varieties were established and 0, 393 and 786 lbs./acre of calcium in the form of 0, 1 and 2 tons of gypsum, respectively, were applied to the experimental plots prior to planting. In addition, during the season, 2.3 lbs./A of calcium as calcium nitrate was applied three times during fruit bulking.

The 1996 data indicated that the resistant variety, King Arthur had more calcium and less potassium in the leaf tissue than the susceptible variety, Grande Rio. Pre-plant applications of two tons of gypsum and foliar applications of calcium nitrate increase the levels of calcium in the leaf tissue but did not reduce the incidence of pepper stip in Grande Rio. King Arthur was free of pepper stip symptoms at all sites. High levels of nitrogen and low levels of light have been implicated in causing pepper stip symptoms in other areas and this observation is borne out in our examinations of this problem in California. This aspect of pepper nutrition needs further examination.

Strategies to Control Powdery Mildew

Richard Smith, Frank Laemmlen, Steve Koike & Krishna Subbarao, U C Cooperative Extension

Powdery mildew continues to be a serious threat to the pepper industry in California. In the past two years the disease has been under excellent control in most fields due to the awareness of the disease by growers and crop consultants, and the careful use of Bayleton. Powdery mildew infects the older leaves of the pepper plant first and subsequently causes the infected leaves to fall off. Losses can be from 50 to 90% if the disease is left unchecked. In 1992 a crisis exemption for the use of Bayleton was granted and it provided excellent protection. For 1997, Rally is being pursued under a "Section

as the target type parent the Rogers hybrid Verdel (bell); Cal-Compack 648 (Anaheim) provided by Paul Narewski, and a potyvirus-resistant jalapeno provided by Ken Owens, selected out of material bred by Dr. Ben Villalon, Texas A & M University, Weslaco, TX. We will continue to check the use of these varieties with the CPIF, and can add or switch parents at any time the Commission indicates.

Resistance to many plant viruses is governed by a single gene that is easy to transfer by cross-pollinating a resistant plant with a susceptible plant. In contrast, resistance to cucumber mosaic virus in pepper is controlled by several genes, each of which must be present in two copies in order to be fully expressed. This means that the transfer of resistance requires screening much larger populations to find the rare plant with the correct configuration of resistance genes. Because the genes are largely recessive, we can only screen alternating generations for resistance, hence the design of this breeding program.

We screen one generation for resistance here in Ithaca, and then send the seed that cannot be screened for resistance to California, to be screened for adaptability, type, etc. We started with the two leading sources of resistance to cucumber mosaic virus (CMR): *C. annuum* French Perennial, a Perennial line obtained from INRA, France that compared favorably with other versions of Perennial for stability and uniformity of resistance; and a wild pepper collected in Mexico, *C frutescens* BG2814-6.

By the term, resistance, we mean that plants that carry genes from these sources fail to develop symptoms after repeated mechanical inoculations beginning at the cotyledon stage when all check plants develop symptoms of the disease. Insofar as we have examined these plants, the virus titer appears to be substantially reduced relative to fully susceptible genotypes, but the virus is present at levels that are higher than uninoculated controls.

Our results indicate that we can backcross directly at the F_1 generation and recover resistance in the backcross F_2 generation, although inbreeding between backcrosses significantly increases the proportion of resistant individuals, thus improving the range of types from which to select. We are carrying forward both types of populations. In the greenhouse this spring, we currently are screening large BC_1F_2 progenies and have selected resistant plants for selfing and backcrossing.

We will soon be sending BC_2F_1 families to be grown in California during the summer of 1997, and will screen the BC_1F_3 progenies here to determine which BC_2 progenies will have the best resistance. This is similar to what was done during the last year when backcross populations derived from crosses made in the fall and winter, 1995, were selected for type and other disease resistances and selfed in the field in California last summer (1996) by Dr. Paul Gniffke, Universal Foods, Greenfield, CA; Bob Heisey, Asgrow/Seminis, San Juan Bautista, CA; and Dr. Ken Owens, Peto/Seminis, Woodland, CA. Seed harvested from these selections is freely available upon request. Based on these results we will begin pedigree work during this next year to more rapidly improve type and combine resistance.

In conjunction with the standard backcross-inbreeding approach for a recessive trait, we investigated the possibility of backcrossing the susceptible F_1 directly to the recurrent parent without inbreeding and selection for resistance. We have termed populations generated in this way blind backcrosses (BBC). Although blind backcrossing would increase the speed with which we could recover recurrent type, we were not certain we would be able to recover resistance without selection in the F_1 due to population size limitations.

In December, 1996, we screened F_3 families derived from all CMR individuals selected in straight F_2 or blind backcross F_2 generations during the last year and a half. Our goals with this screen were two-fold. First, this was a way to select superior F_2 individuals in order to focus our breeding program on pedigrees most likely to yield high levels of resistance after further backcrossing. Second, we were able to assess whether we were effectively selecting resistant material on the basis of F_2 phenotype in either the straight backcross program where populations are inbred at least to F_2 and selected before backcrossing vs. Blind backcrosses.

The bottom line from results of this study is that a resistant F_2 phenotype is not a good predictor of whether the F_3 progeny will be largely resistant. In other words, it is important to screen F_3 progeny in order to accurately identify resistant F_2 plants used for backcrossing, since in all backgrounds and types of pedigrees some phenotypically resistant F_2 individuals gave rise to less than 25% CMR progeny. This is not due to a high escpae frequency since we routinely include a number of seedling of two susceptible check genotypes in every screen, the respective recurrent parent and Anaheim, since Anaheim is very susceptible. We have virtually no escapes of checks (a couple of plants out of many hundreds over the last two years). Thus we hypothesize that the relatively low numbers of resistant individuals from resistant F_2 plants may be due to some incompletely penetrant tolerance to the disease (i.e., the tolerance may be exhibited in the F_2 but may fail in a genotypically similar plant), and that there is some dominance of resistance alleles (similar to that reported for Perennial, see Lapidot et al, 1997).

18" as a stop gap measure until we can obtain a full registration of a control material.

Sulfur provides some protection against powdery mildew, but only if it is applied in four to six applications as a preventative prior to the onset of the disease and at regular intervals thereafter. Multiple applications of sulfur prove to be problematic for growers due to the fact that the pepper canopy closes and does not allow for ground applications of sulfur later in the season. Sulfur also cannot be applied in temperatures over 90° F, and one case of sulfur phytotoxicity was documented in 1996 in the Coyote area south of San Jose. Clearly there is a need to obtain a registration for a material that can provide control of powdery mildew and that doesn't have the problems and uncertainties of sulfur.

San Benito County Trial: Powdery mildew progressed slowly but steadily after it was first detected on August 30th. Four to six applications of sulfur at one and three pounds per acre provided significant disease control over the season. Four applications of sulfur at five lbs. per acre also provided significant control of powdery mildew over the season. Six applications of Trilogy did not provide satisfactory control of powdery mildew. Two applications of Abound and Tilt provided significant season-long control of mildew. One application of Bayleton and Rally provided significant season-long mildew control. No phytotoxicity was observed with any treatments.

Santa Barbara County Trial: Pretreatment powdery mildew lesion counts averaged 11.5 lesions per leaf, with a range of 1-30 lesions per leaf, throughout the trial site. When leaf lesions were counted again after two fungicide treatments had been applied (Nov. 12th), lesion counts in all the treatments had declined. Lesion numbers in the control plots averaged 5.9 per leaf, with a range of 0 - 27 lesions. All fungicides applied to bell peppers for powdery mildew control suppressed the disease when compared to the water treated control. However, no particular fungicide provided significantly superior disease suppression when compared to other fungicides.

PGPR-induced Systemic Resistance in Pepper against Cucumber Mosaic Virus

John F. Murphy and Joseph W. Kloepper, Dept. of Plant Pathology, Auburn University

Cucumber mosaic virus (CMV) is a persistent threat to pepper production worldwide. This virus is difficult to manage due to its ubiquitous presence in nature and its ability to be transmitted by many different species of aphid. Management of CMV is particularly difficult when genetically resistant and commercially favorable varieties are available to growers. We have systematically evaluated an alternative approach to manage CMV infection in bell pepper. This involves treatment of plants with bacteria (referred to as plant growth-promoting rhizobacteria or PGPR) that colonize the plant's roots, and in so doing, induce the plant's own defense mechanisms.

Initial tests involved injection of PGPR into the stem of pepper plants followed 7-10 days later by inoculation with CMV. Treatment of pepper plants by several of the PGPR strains resulted in resistance against infection by CMV. These promising results encouraged us to further evaluate PGPR as inducers of resistance in pepper; however, application of the bacteria via stem injection is not practical, which led us to test different treatment application methods. PGPR were applied as soil drenches, as seed treatments (including four different types of seed treatments) and as combinations of seed treatments and soil drenches. Twenty strains of PGPR were evaluated with eight of the strains tested extensively using the different application methods.

Regardless of the approach used, with the exception of stem injection, PGPR treatment of pepper plants did not result in protection against infection by CMV. These findings are in contrast to observations in cucumber and tomato where PGPR treatment did induce resistance against CMV. Pepper may be recalcitrant to induction of resistance by PGPR against a systemically-infecting virus. We will continue to evaluate this approach as an alternative means to managing CMV in pepper.

Detection of Curly Top Virus & Screening for CTV Resistance

Dr. Robert L. Gilbertson, U.C. Davis Pathology

The overall objective of this project is to identify and characterize the type of curly top virus (CTV) that infects peppers in California and to develop an efficient method for screening peppers for curly top resistance that does not involve the use of the leafhopper vector. We have developed a number of tools that allow us to detect curly top virus in pepper tissues in as little as 5-6 hours. In a matter of days, we can determine the precise curly top strain infecting pepper.

These techniques are very reliable, and we are actively seeking growers who have suspected symptoms of curly top to send us plants (c/o Dr. R. L. Gilbertson, Department of Plant Pathology, UC Davis, 95616) for testing with these

methods. This will help us further establish the type of curly top that is most prevalent in peppers in California.

Based on our previous results, we have consistently found one CTV strain associated with pepper curly top. This is the so-called Worland strain of CTV, and it is different from the CTV that has been commonly found in sugar beet. This information indicated that this was the appropriate CTV strain to use to screen peppers for CTV resistance. We next purified the genetic material of the virus (DNA) from infected plant tissue and cloned it so that we could reproduce large quantities of the viral DNA to infect plants.

Using a "gene gun" we were able to introduce this DNA into tobacco, pepper, and tomato plants and reproduce CTV symptoms. Thus, we now have infectious cloned DNA of the pepper CTV. A double copy of this DNA was prepared, confirmed to still be infectious, and it is presently being transferred into the bacterium, *Agrobacterium tumefaciens*. This bacteria possesses the unique property of being able to direct DNA from the bacterial cell and incorporate it into plant cells. When this is done with the CTV DNA, the viral DNA escapes and proceeds to infect the plant in a manner that is indistinguishable from when the leafhopper introduces the virus. This technique is referred to as agroinoculation. Once we get the agroinoculation technique working, we will evaluate the best procedure for infecting peppers and then begin to screen pepper germplasm for resistance to CTV infection. The materials identified with CTV resistance can then be used in a breeding program to generate CTV-resistant peppers.

Verticillium Wilt -- A Potential Threat to the Pepper Industry

Krishna Subbarao, Associate Plant Pathologist/CE Specialist, Plant Pathology, UC Davis

Verticillium wilt occurs on a wide variety of crops and affects such diverse crops as forage, fruit and fiber crops, forest trees, legumes, ornamentals and several vegetables. The disease is caused by two species of *Verticillium: V. Dahliae* and *V. albo-atrum*. *V. Dahliae* is widely distributed in the agricultural soils of California and is a much more destructive pathogen than *V. albo-atrum*. *V. Dahliae* survives in the soil through resting structures called microsclerotia which are known to survive for up to 13 years. It therefore become a major problem in infested fields. In recent years, a sudden increase in the incidence of Verticillium wilt on peppers has been noticed by growers in coastal California. The symptoms of the disease on pepper appear when the fruit load is heavy and include, a sudden yellowing and progressive drying of leaves, and defoliation, exposing the fruits to sunburn. When the disease is severe, the entire plant may die. Cutting the stems of diseased plants reveals a brownish discoloration of the vascular tissue.

This project was initiated to better understand the biology of Verticillium wilt so as to develop long-term strategies for its management in pepper and to complement the ongoing program of breeding for wilt resistance in pepper. The objectives last year included a) assess the severity of Verticillium wilt on pepper in coastal California and collect *V. Dahliae* isolates for further study; b) determine the natural populations of *V. Dahliae* microsclerotia in pepper fields; c) determine the temperature optima for the pepper, tomato and potato isolates of *V. Dahliae*, and d) determine the host range of *V. Dahliae* isolates from pepper, tomato and potato on each other.

Depending on the area of the four fields evaluated, wilt incidence ranged from 0 - 97%. Within each of the fields evaluated, wilt incidence was significantly higher near the edge of the field than in the middle, suggesting that the inoculum was introduced and its concentration was higher near the edges. In two fields, even though wilt incidence was higher near the edges, the disease was fairly uniform throughout the filed suggesting that the inoculum in these fields was introduced much earlier than the other fields and was higher. The average numbers of microsclerotia in the fields evaluated this year were 97, 54, 35, and 6 per gram of soil. Regardless of the origin of the isolates, maximum growth in culture occurred at 25° C. even though the growth of the tomato isolate at this temperature was significantly higher. The pepper isolates infected both pepper and tomato while the tomato isolates were pathogenic only to tomato. Results obtained this year suggest that Verticillium wilt has the potential to be a major production constraint on peppers. In the coming year, we will conduct additional surveys of affected fields, determine the virulence of pepper isolates of the fungus, clarify the host range of pepper isolates on other vegetables, and screen a few promising breeding lines.

Development of Improved Sources of Resistance & Selection Strategies for Resistance to Pepper Viral Diseases

Dr. Molly Kyle, Assistant Professor, Dept. of Plant Breeding & Biometry, Cornell University

We have just completed the third year of a pepper breeding program to produce resistant versions of the three major California types: bell, Anaheim, and jalapeno. We are using