

55616 was not screened because we wanted first to see the results from PI 555614. If PI 555614 does approach a higher level than 75%, then PI 555616 will be screened.

Previous observations indicated that pepper plants surviving in *Verticillium* infested fields had some level of resistance to *Verticillium* wilt (not escapes). Individual plants were selected from a *Verticillium* infested field in New Mexico. The plants selected from the field were selfed for three generations. Each generation was screened for *Verticillium* wilt resistance. Individuals highly resistant to *Verticillium* wilt from generations 92C1588 S₂-1, S₂-3 and 92C1591 S₂-2 were saved, and they will be used to start a genotypic recurrent selection program.



Commission Report

Continued from page 1

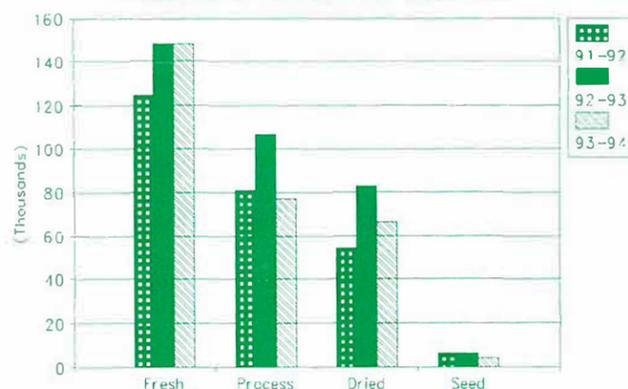
Research Work

The mainstay of the Pepper Commission is research work. The assessments that California pepper growers and handlers pay into the Commission go primarily for funding research work on the major viruses. The Commission funded three projects in the 93-94 year, one each at UC Davis, New Mexico State University and Cornell University. A summary report from each of these projects is contained in this newsletter. This is an appropriate time to mention that the full text and charts for these reports can be obtained by writing the Commission office at the address on the masthead. There is no charge for the full technical publication.

Another question which often arises relates to the promotional activities of the Commission. The answer is simple — there are none. The Commission cannot engage in any promotion or legislative activities and will not be able to unless the industry changes the law with an industry vote.

Pepper Assessments

Production in tons as if for fresh mkt.



1993-94 Crop

Figuring out the direction of the industry is an interesting exercise. If you look at the figure above, a three year comparison is given showing the number tons of peppers produced for

California Pepper Commission Financial Report

Fiscal Year: May 1, 1993 through April 30, 1994

Account Name	Amount
INCOME:	
Carry-over from 1992-93	\$77,231
Assessment Income, 1993-94 (Based on combined rate of \$.50/ton)	147,991
Prior Year Assessments	5,187
Interest	3,803
Total Income	\$ 234,212
EXPENDITURES:	
Management Services	25,200
Audits	1,300
Office Supplies	1,535
Telephone	378
Postage	586
Travel & Mileage	358
Meetings	276
Insurance, Taxes & Bonds	405
Marketing Branch	15,737
Production Research	101,460
Outside Research	1,360
Total Expenses	\$ 148,595
Carry-over Reserve to 1994-95	85,617
Total Expenses & Reserve	\$ 234,212

the four destinations tracked by the Commission. Overall the crop is 85% of last year's, but is 110% of the 91-92 season. Different areas grow and shrink for various reasons which are not always clearly apparent. If the trend continues, it appears that the fresh market is growing while the others change from year to year.

Budget

Looking at the budget, the expenditures were on target with research expenditures running about the same as last year. This amount, \$102,820, went to finance the three projects reported in this newsletter and the work performed by Richard Smith. The remaining 30.8% financed everything else — meetings, management, newsletters, marketing branch, office supplies, insurance, postage, etc. The Pepper Commission has a fairly small budget and the Commissioners work hard to stretch your research dollars.

The Pepper Commission welcomes your comments and suggestions. Please feel free to contact one of the Commissioners listed or the Commission office at 209-591-3925.



Pepper News

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May 1994

— ANNUAL REPORT ISSUE —

Commission Report

Welcome to another edition of the annual report of the Pepper Commission. Work for the pepper industry is continuing to progress and several significant things happened this year.

Powdery mildew remains a significant problem in many pepper growing regions. A Section 18 for Bayleton 50% Dry Flowable formulation was again assembled and submitted with updated information to the California Department of Pesticide Regulation. This information was reviewed by CDPR and the Federal EPA and has resulted in the granting of a renewal for the product for the 1994 season. If you plan on using Bayleton, please get the complete label and usage requirements from your County Ag. Commissioner and carefully follow all directions.

The major reason some growers will not use Bayleton is the plant back restriction of 12 months for many of the crops traditionally used in rotation with peppers. The problem is not the product, but the lack of certain information for the label. Richard Smith set out to remedy this by doing the required research and submitting the information to the IR-4 program. The final result should be a label change to allow a shorter time period for the plant-back restriction. Currently the IR-4 request has been submitted to EPA for review.

This was the year that the Pepper Commission celebrated its fifth anniversary, which also meant that it was time for a renewal vote. Ballots were mailed in February to all industry members and the results totaled by CDEA. The voting is done by producers and handlers separately, and both groups must approve the program for it to continue another five years. The results of this year's renewal ballot were: 35 handlers voted with 74% of those voting in favor of continuation, and 77 producers voted with 71% voting to continue the program. As a result, Secretary Henry Voss approved the Commission to remain in effect for another five year period.

Continued on page 6

Viruses and Fungal Pathogens in California Pepper Production

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

We have now worked on pepper viruses and evaluation/enhancement of resistance to California pepper viruses for 4 years. This research has been successful on several fronts. We have developed a good deal of information and knowledge about California pepper viruses, developed specific diagnostic methods and reagents, evaluated pepper virus pathogenicity, evaluated various germplasm sources for virus resistance, developed specific inoculum conditions, and enhanced germplasm lines for CMV resistance. Below is a summary of results from last year.

Objective 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 *Verticillium dahliae*.

Over the past 4 years we have visited several hundred different pepper fields, some several times in a given year. In general, virus incidence has been unpredictable. Spatial and geographic incidence has been variable in all years, and mixed infections have been very common.

In 1993 virus incidence was widespread during the latter part of the growing season, with some isolated fields affected early in the season. The early season fields generally had a higher incidence of CMV, while late season infections had plants infected by the complex of pepper viruses (PeMV, TEV, PVY and CMV). Mixed infections were again more common than were single infections, and overall PeMV was the most common pepper virus in California. We collected only two specific isolates for further study. One of these is a CMV from

the early infections in the San Joaquin Valley, while the second is explained below.

In addition, we detected potato leafroll and beet western yellows luteoviruses in selected samples collected during the spring from Stanislaus county. Several plants showed uniform overall light chlorotic symptoms. Chlorosis was largely interveinal and leaves had an upright posture. Such symptoms are typical for luteovirus infections of many hosts. We did not have time to characterize these viruses, or to in fact determine if they caused the field symptoms. We have proposed to look further into this during the next year. We also found limited plantings affected by beet curly top virus in early summer, primarily in Stanislaus and Merced counties. A few affected pepper samples from various locations were determined to have tomato spotted wilt virus, but no rapid diagnostic methods are available, nor have we characterized these viruses in detail.

Virus epidemics occurred late in the season in selected fields in Santa Maria. Some plants had PVY, and several had a virus which did not react in initial ELISA tests. We have subsequently transmitted this virus to various tobaccos and peppers. Recent SDS-immunodiffusion tests have shown that it is an isolate of PeMV. More detailed analyses of this virus are in progress in order to determine if it is a different PeMV.

Objective 2. *To assess the natural incidence of severe and mild virus isolates in differential cultivars and genotypes.*

In the 1993 growing season we established eight field trials including 25 pepper cultivars/lines in each. Field plantings began in early May and ran through June, depending upon location. Locations were at UC Davis, Elk Grove, Gilroy, Visalia, Porterville and two in Irvine. Plots were continuously observed and plants were evaluated for symptoms, and several plants from each plot were assayed by ELISA. In most locations virus incidence was low. However, in two locations there was good virus infection.

No potyviruses were detected in either location in the most potyvirus resistant material (i.e. PI159225 and PI159236), however, VR4 and VR2 plants were infected by both PeMV and TEV at the Visalia location. It is important to note that CMV pressure was high in Visalia. Under these conditions the original Perennial source line plants were CMV infected. However, our DPP breeding line materials remained CMV-free, supporting our laboratory studies on the resistance/tolerance in these advance materials.

Somewhat in addition to the above results, we also evaluated 39 hot chili breeding lines obtained from the AVRDC for their resistance/susceptibility to California viruses under natural inoculum conditions. Many plants in several of these lines were infected by potyviruses. As some of these are reported to be potyvirus resistant, our data suggest that they may not have acceptable levels of potyvirus resistance for California isolates/strains of these viruses. Adjacent to these plots were lines of the potyvirus-resistant PI lines, and they showed their expected levels of resistance. Only two plants of 156 plants

tested were CMV infected. Natural CMV incidence was low, so it is difficult to determine the significance of the reactions of the material to California CMV. However, limited greenhouse tests were done using 10 plants of each of these lines with CMV 144I as the challenging virus. None of the plants showed complete resistance under these admittedly severe challenging conditions, however, some showed the ability to outgrow symptoms over time. This is typical of the Perennial type of CMV resistance.

Objective 3. *To assess CMV variability, to continue efforts to enhance CMV resistance in Capsicum breeding lines, and to interact with efforts on genetically-engineered resistance to CMV.*

Our early focus for CMV resistant material was on the material made available to us from project cooperators (including CM 334 and Perennial resistance sources). Our efforts have primarily focused on these materials. Of all the material tested by us so far, the Perennial lines have provided the most promising results. However, the Perennial CMV resistance is complicated and difficult to work with. When this is coupled with CMV variability, screening for resistance is even more difficult.

Our previous work, including comparisons of our materials from several generations of selected material in side-by-side tests has shown that the level of CMV resistance has increased in our selections as opposed to the original Perennial bulk, Perennial 4 and 6 lines. This is also supported by field data where our DPP lines have remained CMV-free while original Perennial material are occasionally CMV-infected. However, we have been unable to fix the resistance, or predict its behavior. A percentage of plants continue to be susceptible in all generations of material. Admittedly, these materials were challenged under stringent conditions using severe CMV's.

Under our test conditions some DPP lines have performed better than others. Almost all of them have shown high field tolerance over two seasons and many locations. In addition, they show good resistance to *P. capsici* infection and show improved plant architecture. Although some individuals from the DPP lines as well as from Perennial often show symptoms upon inoculation when plants are very young (i.e. cotyledon or 2-4 leaf stage), plants often recover and appear to grow out of the infection.

It is becoming increasingly clear that the mode of resistance to CMV in Perennial is complicated. As reported by the French group, Perennial has only partial resistance to their isolates of CMV. To quote the view held by them, "The analysis of the different results so far obtained with Perennial, i.e. tolerance to infection, inhibition of virus multiplication and lower specific infectivity of purified virus shows that this pepper line possesses several different mechanisms of partial resistance." Similarly, the latest test of the Perennial HDV (French source) at AVRDC shows that this material has good field tolerance, but is not considered as a CMV resistant line. We now believe that the varying degree of symptom expression and highly variable results under different test conditions may be associated with the complex resistance mechanisms of Perennial. This type of

size 236 since it is much easier to return to type. We have extended our studies to include TEV. Avelar is susceptible to the TEV isolates we have screened to date, while the *C. chinense* lines are resistant to the TEV-E, PVY-N, and susceptible to TEV-Mex 21. Again, we have not been able to demonstrate a difference between resistance in 236 and 225. We do see differences in symptom intensity in susceptible interactions of the *C. chinense* lines, but we have not identified an isolate that clearly causes disease in 159236 and does not in 152225. Thus we have no evidence to confirm the widely held opinion that the allele from 225 is "stronger" resistance. We have worked out procedures to isolate and infect protoplasts or individual leaf cells in culture with CMV, PeMV and TEV (Murphy and Kyle in press). We have shown that while CMV replicates in all protoplasts, protoplasts isolated from 236 and 225 are resistant to PeMV and TEV and we are currently working on PVY. This suggests that the *C. chinense* sources offer an extremely stable type of resistance. We are also working with two sources of dominant potyvirus resistance from Criollo do Morellos and PI 1591236.

Objective 4: Confirm results that suggest that dual infections of CMV + PeMV alter resistance to PeMV and extend these analyses to TEV.

After establishing that the mechanism of potyvirus resistance conditioned by the alleles from Avelar and *C. chinense* was quite different, we investigated the effect of dual infection with CMV on the stability of resistance from these two sources. These interactions occur routinely in commercial fields as CMV is ubiquitous most places where peppers are cultivated, and resistance to the virus is unavailable commercially. In Avelar, co-infection with the more virulent isolates of CMV can allow PeMV to move systemically and accumulate to significant titers. These studies suggest dual infection with CMV may account for the observation that PeMV has been recovered from PeMV-resistant varieties. Thus, before the conclusion that new strains account for the breakdown of resistance, the possibility that dual infection has overcome resistance should be considered since the implication for management strategies differ substantially. This breakdown of potyvirus resistance during dual infection with CMV did not occur in 236 or 225. We have not identified TEV isolates to which Avelar is resistant so we have not pursued the dual infection studies with TEV + CMV in Avelar.

Developing Enhanced Pepper Germplasm Resistant to Verticillium Wilt

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

An attempt to increase the level of Verticillium wilt resistance above 75% in PI 215699 was undertaken. Four highly resistant S₃ sib plants were intercrossed and the F₁ generations screened. Analysis of this experiment indicated that the maxi-

mum level of Verticillium wilt resistance in PI 215699 is 75%. In the backcross program, a Verticillium wilt resistant individual from the first selfed-generation of PI 215699 was crossed to Bell-, jalapeño-, and New Mexican-pod type peppers. The F₂BC₂ for the bell and jalapeño was screened. The F₂BC₁ for New Mexican-pod type has been produced and it will be screened. The first selfed generation of PI 555614, a novel Verticillium wilt resistant material, was screened and the highly resistant individuals were saved. Individual plants selected for "field-resistance" were screened and the highly resistant individuals were saved. A genotypic recurrent selection program is the best method to introduce Verticillium wilt resistance into pepper cultivars.

All Verticillium wilt experiments were performed under strict environmental control in soil-temperature tanks. Soil temperature was maintained at 25°C. The inoculum level was 2000 microsclerotia per gram of soil. The seedlings were scored at 60-70 days post-emergence, with an interaction phenotype scale, ranging from 1 to 9, where 1 = no aerial symptoms, and 9 = death.

After four cycles of screening and selection, PI 215699 continues to segregate for Verticillium wilt. The highest level of resistance to Verticillium wilt reached was 70-75%. To test for higher levels of resistance, four highly resistant sib plants from the S₃ generation of PI 215699 were intercrossed. Results from the screening of the F₁'s showed a segregation for Verticillium wilt resistance. This indicated that additive gene action was still present and dominance gene action was not present in any of the four lines. Unfortunately, progress in increasing the level of resistance was not significant. Consequently, it appears that the maximum level of Verticillium wilt resistance in PI 215699 is 75%. However, other root diseases, e.g. Fusarium wilt in radish, are successfully controlled in commercial plantings at a level of 50% resistance. Therefore, a 75% resistance level for Verticillium wilt of pepper may be satisfactory in commercial production.

F₂BC₂'s of bells and jalapeños were screened. In addition, F₂BC₁ seed from the New Mexican-pod type were screened. Presently, none of the lines have adequate horticultural traits for each pod type. However, resistance in approaching the 75% level. The segregation ratios for Verticillium wilt resistance in different *Capsicum* backgrounds continue to indicate that Verticillium wilt resistance is a quantitative trait. These findings indicate that the most efficient breeding method to use is the genotypic recurrent selection method.

When novel Verticillium wilt resistant accessions were screened, some highly resistant individuals from PI 555614 and PI 555616 were saved and their progeny were screened. The resistance to Verticillium wilt was increased from 4.42% to 21.78% in PI 555614. The resistant plants of PI 555614 from the second cycle of selection were screened, resistant individuals were saved, and a seed increase was made. These resistant plants could be used to introduce Verticillium wilt resistance into bell-, jalapeño-, and New Mexican-pod types. Inheritance studies could also be performed. At this time, PI

resistance may not be what was originally desired, but it may still be of value to the California pepper industry.

Objective 4: To evaluate the effects of specific mixed infections on virus resistance in peppers. Experiments to assess the effects of single and multiple virus infections on three pepper genotypes were completed. Previous field data have clearly showed that mixed infections are very common, and suggest to us that multiple virus resistance will be required to control pepper viruses in California. Therefore, we conducted specific greenhouse studies to compare effects of single and mixed infections on specific pepper cultivars/breeding lines. We assessed plant effects (symptoms), as well as specific ELISA titer for each virus in infected plants. The later was done in part to attempt to determine if potyvirus resistance was affected by co-infection of potyvirus resistant (tolerant) genotypes with a potyvirus and CMV, and similarly to assess mixed infections on one of our breeding lines which exhibits some CMV tolerance (resistance).

Plants inoculated with individual viruses alone, of simultaneously together, or first one virus followed 4-7 days later with the second virus. Symptom severity in general was greater in all cases for mixed infections. We also have noted this previously in field studies. CMV plus PeMV gave the most severe symptoms, followed by CMV plus TEV, and in some cases TEV plus PeMV. All of these combinations are common in the field in California peppers.

In general Del Rey Bell showed good tolerance (ELISA titer) to all potyviruses but not to CMV (both CMVC and CMV 144I) while CW300 was susceptible to both types of viruses and showed corresponding high ELISA titers. These data also showed that while potyvirus resistant (tolerant) Del Rey Bell was susceptible to both CMV's, in mixed infections the potyvirus ELISA titer was low and essentially unchanged compared to infections by only single potyviruses. These data suggest the CMV does not "break" the currently used potyvirus resistance in Del Rey Bell, and results were similar whether viruses were co-inoculated or first infected by one virus and followed a few days later by the second virus. This is encouraging for current potyvirus resistance strategies.

We were not able to unequivocally answer the reciprocal question as to whether potyvirus infections affected CMV resistance. In these experiments plants from our breeding line, MV5, exhibited high variability in CMV ELISA titer whether plants were single infected with CMV, or whether they were infected by CMV plus a potyvirus. This is somewhat in agreement to what we have observed in other analysis of the Perennial material. Additional analyses of CMV resistance and effects of potyviruses on such resistance will require better sources of fixed CMV resistance.

(Note: The Talk report contains many pages of charts which support the findings related in this summary. Complete reports are available upon request.)

CA Pepper Commission

1993-94

Producer Representatives

Members		Alternates
	District 1	
Burt Silva King City 408/385-1428		VACANT
	District 2	
Frank Luenser Arroyo Grande 805/489-2508		Chris Darway Arroyo Grande 805/489-1817
	District 3	
Mike Mondelli Gilroy 408/847-1337		Tom Obata Gilroy 408/842-9809
	District 4	
Joe Marchini Le Grand 209/389-4528		Bob Giampaoli Le Grand 209/389-4576
	District 5	
Randy Johnston Lemoore 209/924-5339		Carl Lindgren Irvine 714/551-4103

Public Representatives

David Ferguson Fresno 209/435-6034	Ken McCorkle Fresno 209/441-5017
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Handler Representatives

Members		Alternates
	Bell Pepper Processors	
Don Nelson Calif. Veg. Conc. Modesto 209/538-5429		George Stuit Eckert Manteca 209/823-3181
	Dehydrated Chili Processors	
Paul Gniffke Universal Foods Greenfield 209/667-2777		Steve Banta Cal-Compak Foods Santa Maria 805/925-1908
	Other Pepper Processors	
Jerry Schwab Vlasic Foods Gilroy 408/848-4116		Glen A. Fischer Saticoy Foods Saticoy 805/647-5266
	Fresh Market Pepper Handlers	
Chuck Filice Denice & Filice Hollister 408/637-7491		Fred Podesta Jr. Podesta Farms Linden 209/887-3701
	Pepper Seed Handlers	
Ken Owens Petoseed Co. Woodland 916/666-0931		Robert Heisey Asgrow Seed Co. San Juan Bautista 408/623-4554



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

Molly Kyle, Cornell Univ, Ithaca, NY 607-255-8147

Our proposal for 1993 was intended to begin the process of synthesizing and integrating the various strategies we had developed with previous funding to focus on creating the best sources of resistance. We had been carefully examining the effects of dual infections on expression of resistance and found these effects to be profound; therefore, as part of assessing the suitability of a source of resistance we also proposed to continue these investigations. Specifically, we focused this proposal on resistance to cucumber mosaic virus (CMV), resistance to potyviruses and on the effects of the interaction between these viruses on stability of resistance.

Objective 1: Determine whether selected *C. frutescens* 14-6 and Perennial contain different genes for resistance to CMV. Identify molecular markers for these sources to assist in selection to the polygenic recessive resistance.

Our results demonstrate that *C. frutescens* and Perennial contain most if not all the same genes for resistance to CMV. We have several lines of evidence that are consistent with this assertion. Initially, both parental lines we had obtained (*C. frutescens* from Drs. E. Loaiza-Figueroa and R. Provvidenti and Perennial for Dr. R. Heisey) were not homozygous so in the original F₁'s between these genotypes we observed variability in reaction to CMV. We have gone through 2 generations of screening with the most severe isolate we have (CMV Asgrow V27) and self-pollination of selected resistant plants, and when these selected lines are crossed to produce the F₁, all plants are uniformly resistant to CMV V27. When selected each line is crossed with a susceptible check, the offspring are uniformly susceptible, confirming that each source of resistance is essentially fully recessive under our extremely severe screen conditions. Similar results were obtained when Perennial obtained from Dr. A. Palloix from doubled haploid material was crossed with *C. frutescens*. We will be testing *C. frutescens* x Perennial F₂'s for transgressive segregation, but our results to date do not suggest there are unique CMV resistance genes in *C. frutescens*.

We have screened F₃ families of our mapping population involving *C. frutescens* with CMV and are in the process of entering data to determine regions of the pepper genome that contribute significantly to CMV resistance. This will allow us to identify linked molecular markers that could expedite selection of resistant genotypes, or the combining of resistance to CMV with other desirable resistance, horticultural features, etc. French researchers led by Dr. A. Palloix and Israeli researchers led by Dr. I. Paran are currently mapping the resistance in Perennial which will allow confirmation of the genetic results.

We have continued our genome mapping efforts in Capsicum as part of a related project, and have demonstrated the RFLP and RAPD's reveal sufficient polymorphism to be useful for the fingerprinting of varieties (Prince et al. submitted).

Objective 2: Continue work to transfer and express the defective CMV replicase gene using two regeneration strategies and two transformation methods.

We continued our efforts to optimize regeneration of pepper in order to determine the best strategies for transformation, but in our hands, regeneration was difficult at best and we have not demonstrated transformation. Our collaborators at AVRDC led by Dr. George Kuo have been pursuing a novel strategy for the last 4 years, and now report that they have worked out a relatively efficient system for pepper transformation and have tested the system using a GUS reporter gene to demonstrate stable transformation of pepper. We believe that the most efficient way to approach this objective in view of their success is to cooperate with them to provide them with the construct. If we can obtain stable support for our pepper research, we could send a member of our group to AVRDC with the construct which has not been generally released yet by the Cornell Research Foundation. It is possible, if we can continue our work in peppers, that we could engineer a monogenic dominant source of absolute resistance to this virus in the next 12-24 months. In order to test this strategy, we have produced a number of families from tomatoes transformed with this gene and will be inoculating these in the next 2 weeks or so to determine whether we have engineered resistance in tomato. If we have, then there is reason to be optimistic that this strategy is general. Since we began this work, it has been shown in a number of host-virus systems that expression of viral genes involved in replication in the plant can confer resistance to the virus, so in general this appears to be a promising strategy that could offer some key advantages over coat protein-mediated protection.

Objective 3: Confirm the allelic relationship between resistance in PI 159236 and PI 152225 for resistance to pepper mottle virus (PeMV) and tobacco etch virus (TEV). Confirm that the Avelar allele is entirely distinct and determine linkage if any, with the *C. chinense* allele(s).

In potyviruses, we have identified two distinct loci involved in resistance, one from the variety Avelar, which is the basis for potyvirus resistance in a number of widely used varieties, and the other from *C. chinense* PI 159236 (236) and 152225 (225). Complementation for susceptibility to pepper mottle virus is observed in the cross between Avelar and the *C. chinense* PIs confirming mechanistic studies that suggest that these are entirely different genes and we have demonstrated that there is no close linkage observed between these genes in modified test cross populations from Avelar x *C. chinense* crosses.

Our results confirm that for pepper mottle virus resistance in 236 and 225 is allelic, and currently, we have no definitive evidence that clearly distinguishes these alleles. If 236 and 225 have equivalent potyvirus resistance, breeders should empha-