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.

1992-93 Crop

The Pepper Commission sets a budget at the beginning of April for the next year's work. This budget always involves some guesswork regarding the size of the crop. So the Commissioners were rather surprised when the assessment income came in 27% higher than expected. Part of the surprise came because the prior two years had been almost identical and right where the Commissioners had estimated.

There was considerable curiosity concerning where the increase had come from. The accompanying chart on figure 1 shows the difference between 91-92 and 92-93. The eye deceives you a bit on this chart so let me give you the percentage change in each category; fresh 19%, process 31%, dried 52% and seed 29%. I might also mention that the chart shows the tonnage of peppers as if they were all produced for fresh market, not the actual tonnage produced. This equalizes for the production of peppers, not product.

Budget

Looking at the budget, the expenditures were on target. The financial report shows 68.1% expended for research. This amount, $101,869, went to finance the three projects reported in this newsletter and the work performed by Richard Smith. The remaining 31.9% financed everything else – meetings, management, newsletters, marketing branch, office supplies, insurance, postage, etc. The Pepper Commission has a fairly small budget and these are reasonable percentages with a budget of this size.

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.

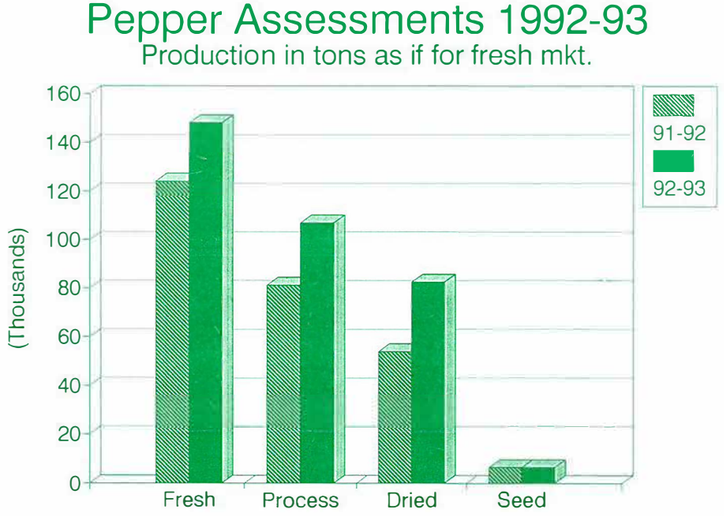
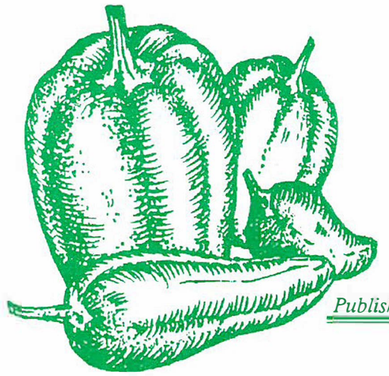


Figure 1 - Assessments

California Pepper Commission Financial Report

Fiscal Year: May 1, 1992 through April 30, 1993

Account Name	*Amount*
INCOME:	
Carry-over from 1991-92	$48,011
Assessment Income, 1992-93	174,623
(Based on combined rate of $.50/ton)	
Interest	4,229
Total Income	**$ 226,863**
EXPENDITURES:	
Attorney's Fees	0
Management Services	25,200
Audits	1,932
Office Supplies	1,380
Telephone	435
Postage	886
Travel & Mileage	953
Meetings	636
Insurance, Taxes & Bonds	405
Marketing Branch	15,809
Production Research	101,000
Outside Research	869
Total Expenses	**$ 149,505**
Carry-over Reserve to 1993-94	77,358
Total Expenses & Reserve	**$ 226,863**



Pepper News

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June 1993

– ANNUAL REPORT ISSUE –

Commission Report

Many times I find that the people who receive this newsletter are not aware of the activities of the California Pepper Commission – sometimes they are not aware it even exists. It does in fact exist and is at work for the pepper industry. Let me start with two activities which do not show up in the research report summaries, but which are important none the less.

Last year many regions of the state were hit with an outbreak of powdery mildew. As many of you know, the resulting defoliation of the plants and sunburn of the fruit was severe. Fortunately, in many areas this problem hit late in the season and did not cause the damage it could have if it happened earlier. But the potential is there for this year for more severe economic damage.

Through the diligent efforts of Richard Smith, Farm Advisor in San Benito county, with assistance from the Pepper Commission, a Section 18 for Bayleton 50% Dry Flowable was obtained in 1992 and has been renewed for 1993. The highlights of the emergency exemption are as follows:

- Location: Monterey, San Benito, Santa Clara, Kern, San Luis Obispo, Santa Barbara and Ventura counties
- Target problem: Powdery Mildew (Oidiopsis taurica)
- Dosage: Apply 2 to 5 ounces of product per acre
- Dilution rate: Apply using a minimum of 20 gallons of water per acre by ground and a minimum of 10 gallons water per acre by air
- PHI and Reentry: 24 hours/when dry
- Effective: April 19 - November 1, 1993
- Other: Applications may only be made upon the written recommendation of a licensed Pest Control Advisor

There are several pages of other directions which you need to have in your possession before applying this material. Please be careful to follow all instructions on the Section 18 when using this product.

One of the major problems with using Bayleton on peppers is the plant back restriction. Many of the crops used in rotation with peppers cannot be planted into a field treated

Continued on page 5

Viruses and Fungal Pathogens in California Pepper Production

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

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)].

The activities related to objective onc, i.e. monitoring the pepper pathogen incidence in the growing regions of California, were continued. As before, we used three methods to acquire samples, and our efforts were concentrated solely on pepper viruses.

- We visited fields and collected appropriate samples.
- We received samples for our cooperators.
- Specific collections were made from our field plots.

During this reporting period we visited at least 32 different field sites, and many fields were visited several times. The incidence of natural virus infections was low at almost all locations. We observed symptoms similar to alfalfa mosaic virus (AMV) on plants in some fields early in the season. We do not routinely test for AMV, but we were able to reproduce these symptoms on greenhouse inoculated plants. This was almost always associated with the presence of alfalfa fields close by the pepper fields.

During mid to late September, many fields were infested with aphids. Some locations (Merced, Davis and Woodland) showed relatively high levels of aphid-colonized pepper plants. Pepper samples received after September showed a higher level of virus infection, and most plants were found to be infected with potyviruses.

When compared to the 1991 season, however, virus incidence in peppers during 1992 was generally very low. We did save virus isolates for comparison with other virus isolates collected previously, and we initiated some preliminary analysis of CMV's.

Objective 2: To obtain, evaluate and incorporate sources of disease resistance for viruses into useful breeding lines of various pepper types.

This was one of the main thrusts of our program during the past year. Of the 56 lines that were carried over from last year, 8 lines were recognized as good breeding lines that showed at least some resistance to both CMV isolates, CMV C and 144 I. They were released to the representatives of the California Pepper Commission in late 1992. We also released some "F1" crosses involving some of these breeding lines and the two commercial varieties, Verdel and Jupiter.

All of these breeding lines, along with their appropriate progenitors, were tested simultaneously against CMV C and 144 I during Winter 1992. The results show the advanced generation selections exhibit better resistance to both CMV isolates than do the original parents. This also shows that our DPP (Dept. of Plant Pathology) lines hold well against both CMV isolates. Some plants showed rough leaf surfaces, but no viral symptoms. Hence there were not 100% healthy plants in many lines.

In addition to our tests, seeds of these lines also were distributed to interested cooperators last year. So far, we have received only one report on the behavior of the DPP lines, as tested by cooperators under their conditions. This report stated that our breeding lines showed excellent resistance to CMV 144 I.

We have also raised 5 different "F1" plants from the crosses involving DPP lines and Jupiter and Verdel and the "F2" seeds were planted in a commercial nursery to raise "F3" families. Selection for CMV resistance and agronomic features will be done on plants in these families. We have crossed some of the DPP lines with the *C. frutescens* lines BG 2814-6 and BG 22816-1, obtained from Dr. Kyle at Cornell, and with the paprika type 91-S, and the Anaheim chili variety "Sonora". The "F1" seed should be ready in approximately another 2 months.

Evaluation of other sources of virus resistance

We also evaluated various other sources of *Capsicum* germplasm for their potential resistance to California pepper viruses. Further testing of Serrano Vera Cruz received from Spain against 3 potyviruses showed that it is resistant to our isolates of PeMV (111.1) and PVY (111.5) but not to TEV (111.2).

A set of pepper lines that contained 173 PI accessions from Georgia Plant Introduction Center was tested against 144 I and CMV C. None of the accessions showed resistance to both CMV C and 144 I. Some lines showed delayed symptom development to isolate CMV c, but good systemic symptoms developed later on.

Two other lines of this species, BG 2814-6 and BG 2816-1 with resistance to some Cornell isolates of CMV were received during late September 1992 from Dr. Kyle. We have increased the seeds of these lines and they have been planted along with our best DPP breeding lines for side-by-side evaluation against CMV C, 116 and 144 I isolates of CMV.

We also have received a set of pepper lines from Asian Vegetable Crop Development Center (AVDRC) from Dr. Jean Poulus. Some of these lines are claimed to be resistant to CMV. A field trial will be conducted this Spring at Armstrong Field, UC Davis, to test their merits under California conditions.

Objective 3: To assess the natural incidence of severe and mild virus isolates in differential cultivars and genotypes.

In addition to observing fields for natural infection, we also used an 18 genotype indicator plant set that was comprised of various pepper varieties and lines. With collaboration of Farm Advisors and cooperating growers, we planted these plots along with their commercial plantings. The primary objective was to use these lines as a screen to pickup genetic variants within the virus gene pool that occur in California, since they have varying degrees of resistance to viruses and particularly to CMV. The secondary objective was that we wanted to know how well our breeding lines would perform in the field. If there are any virus isolates that could infect our late generation breeding lines, we wanted to know how they compared to 144 I, our most virulent CMV isolate.

As planned samples were collected pre-maturity, mid-maturity and for some locations late maturity, all plants were assessed for visual symptoms. During the early growth, there were very few to no symptoms of viral infection in all locations, even on susceptible indicator varieties such as Cal Wonder 300 and Yolo Y. During this period we did not note significant aphid activity.

The trial at Fresno had 4 plants which showed symptoms similar to AMV and we were able to reproduce these symptoms on CW 300 in the greenhouse. This site was flanked by a large alfalfa field. In many locations there were a few plants showing leaf puckering, yellowing and stunting, but did not test positive to any of the 4 test viruses.

During late September, both trials at Armstrong Field at UC Davis developed viral symptoms on susceptible Jupiter, Verdel and Yolo-Y after a heavy aphid infestation. This was also true for plants at Woodland, Elk Grove, Merced and Fresno locations. In general this late spread of viruses could be considered mild and spotty.

As such field screening in 1992 spring season gave us unsatisfactory data, we have modified the pepper genotype set marked for 1993 spring season so as to include lines better suited to detect different potyviruses as well. We intend to cover around 10 similar "hot spots" and the Farm Advisors have already been contacted.

Objective 4: To assess CMV variability, and to interact with efforts on genetically-engineered resistance to CMV.

Several CMV isolates were analyzed by plant host reaction, as well as be virion capsid protein analysis by SDS-

1. MS (Murashige and Skoog) salts and vitamins supplemented with indole-3-acetic acid (IAA, 1 mg/l) and 6-benzylaminopurine (BA, 2 mg/l)

2. MS salts and vitamins supplemented with IAA (4 mg/l) and BA (11 mg/l)

3. MS salts and vitamins supplemented with IAA (0.1 mg/l) and zeatin (1 mg/l)

All cultivars developed callus in each of the three media. However, callus that developed on medium 2 tended to turn brown and shoot development typically did not occur. A white to light green callus developed for each cultivar on media 1 and 3. Although shoot development often occurred on media 1 and 3, the extent to which the shoots developed was limited and this was especially true concerning medium 3.With information from other sources, it became clear that IAA and BA (within a range of concentrations) were preferred for pepper shoot induction *in vitro*. We observed that shoot induction was possible using medium 1 but development was limited. However, when we increased the IAA and BA concentrations to 2 mg/l and 4 mg/l, respectively, we obtained rather extensive shoot development with RNaky and Capastrano and to a lesser degree with Delray Bell. These shoots were leafy shoots and did not show much internodal growth. We have transferred these shoots to an elongation medium developed in Roger Beachy's program.

We extended our regeneration attempts to several other pepper lines including Avelar, PI 159236, PI 152225 and Early Calwonder. We continued to use shoot inducing medium containing IAA (2 mg/l) and BA (4 mg/l) and examined other variables such as different concentrations of vitamins, especially thiamine. The best callus and shoot formation occurred with samples maintained in the light with no apparent difference between those with 3M Filter Tape and those with parafilm.

We have recently initiated pepper regeneration using another approach which appears to be far more promising. In this procedure, shortly after pepper plants germinate they are removed from the medium, the roots are trimmed and the hypocotyl is cut several millimeters below the apex. The cut hypocotyl is then placed top-down into the shoot induction medium. Upon early stages of shoot development at the cut hypocotyl surface, the plant is removed from the medium and the roots are placed into elongation medium. Once the shoots elongate 1-4 cm, they are excised and placed in rooting medium. We currently have shoots which have and have not been treated with *Agrobacterium* elongating.

Objective 2: *Agrobacterium*-mediated transformation with constructs derived from the CMV RNA 2 (the replicase gene), the antisense sequence of CMV RNA 2, and vector alone (not containing CMV- related sequences).

We have initiated *Agrobacterium*-mediated transformation experiments using the Valero-Montero & Ochoa-Alejo approach on two pepper lines that we have found to be most consistent during regeneration experiments, RNaky and Capastrano. To date the "normal" stages of regeneration have occurred on media containing kanamycin with the Capastrano transformed hypocotyls, while those of RNaky have not shown any signs that regeneration has been initiated. We will extend this approach to include all of the pepper lines described previously in this report.

Having anticipated difficulty with objective 1 in pepper, we initiated similar experiments with tomato to generate transformed plants to evaluate for levels of resistance conferred by replicase constructs. We currently have kanamycin-resistant plantlets that we will be evaluating for expression of the construct in the next week or so. Work continues on a similar construct form CMV serogroup II by N. Banerjee and M. Zaitlin.

Objective 3: Characterization of transgenic lines for response to inoculation with various CMV strains (virion and RNA).

In this and other studies, we have been working with 6 CMV isolates selected in our program and at Asgrow Seed Co. based upon geographical and biological variability. In an effort to evaluate the extent to which the replicase construct from CMV-FNY confers resistance to pepper CMV isolates, we inoculated tobacco transformed with the replicase construct. In every case, the plants were completely resistant to the isolates. No systemic symptoms were observed and no virus was detectable. It appears from our studies and others that most all CMV isolates from pepper belong to the serogroup that is protected by the construct we already have. This result in tobacco suggests that, unless there is some host-specific effect in *Capsicum*, a possibility we consider unlikely, that the construct we are currently using in transformation experiments should protect pepper from all CMV field isolates, at least in California and New York.

Commission News

Continued from page 1

with Bayleton for 12 months. To get this restriction reconsidered and try for a regular labeling of Bayleton on peppers, Richard Smith is being financed by the Commission to do the data gathering work on an IR-4 project. This project fills in the missing information on minor crops that want to use a chemical where the company has not gathered all the required data. This work is progressing well along with Richard's other trial work.

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 presently funds three projects, one each at UC Davis, New Mexico State University and Cornell University. An annual report from each of these projects is contained in this

PAGE, and PCR analysis of viral nucleic acid. In general, the isolates varied tremendously in their pathogenicity on various hosts and their severity on one host does not predict the severity on another.

We have amplified specific cDNA regions of the CMV 144 I and CMV C genomes directly from tissues of infected plants. We also have cloned these amplified regions and determined their nucleotide sequences. Both are CMV subgroup I isolates, but differences were noted in the specific nucleotide sequences. It would be nice if the differences could be related to their pathogenicities, but this will be difficult to determine. We are proposing to continue with this effort and include more CMV's in our studies.

Objective 5: To evaluate the effects of specific mixed infections on virus resistance in peppers.

Examination of field samples has previously revealed to us that there is a frequent occurrence of multiple virus infections. There was a good correlation of physical distortion of affected plants (symptom severity) and the presence of two or more viruses. Hence we started experiments that would, at least in part, address some of these issues.

We conducted the experiment in the spring of '91 and repeated the experiment in the winter of '92 using a modified design. One week after inoculation it was evident that in some of the plants, inoculated leaves had partial necrosis or the leaves were abscised. Also, we observed that some plants which showed very clear leaf symptoms of the virus infection, did not show positive ELISA titer values. The observations support that combinations of viruses create more physical deformity in plants than when viruses act singly.

Developing Enhanced Pepper Germplasm Resistant to Verticillium Wilt and the Inheritance of Resistance

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

A verticillium wilt-resistant population is being developed from the accession P.I. 215699, by selecting the highly resistant individuals through four cycles of screening and selfing. The fourth cycle of screening and selection is completed. In the backcross program, a resistant individual from the first selfed- generation of P.I. 215699 was the donor parent to bell, jalapeno, and New Mexican types. To date, the F_2BC_1 for bell and jalapeno types have been screened and the F_2BC_2 produced. The inheritance for Verticillium wilt resistance has been determined. The first selfed-generation of novel Verticillium wilt resistant materials has been screened and the resistant individuals saved. Progeny from individual plants selected for "field resistance" were also screened and the resistant individuals saved.

All Verticillium wilt experiments were performed under strict environmental control in soil temperature tanks. The soil temperature was maintained at 25 ± 1 C. The inoculum level was 2000 microsclerotia per gram of soil. The seedlings were scored 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, P.I. 215699 continues to segregate for Verticillium wilt. The percentage of resistant plants has increased from 38% to approximately 70%. The 70% level may be a plateau for this population. Therefore, four highly resistant individuals from the S_3 are being used to produce an diallel-cross model IV. We expect to increase the percent of resistant plants above 70% by combining resistant alleles in the cross. The evaluation of general and specific combining ability will be done. Results from this experiment will be available by July 1993.

To date, F_2BC_1's of bell and jalapeno types have been produced and screened. Bell pepper and jalapeno individuals with the highest level of Verticillium wilt resistance were saved and backcrossed to their respective recurrent parent. Appropriate horticultural traits will be selected in the next three selfing generations. F_2 seed from the New Mexican type was screened and F_2BC_1 seed will be screened during 1993.

The segregation ratios for Verticillium wilt resistance in P.I. 215699 suggests that Verticillium wilt resistance is a quantitative trait. Additive and dominance variance effects were studied. The F_1, F_2, F_1BC_r and F_1BC_s using the resistant parent (P_r) and the susceptible parent (P_s), P.I. 215699 and B.G. 1688 respectively, were accomplished. The experiment consisted of four replications with 30 plants per replication for each generation. The seedlings were individually scored 70 days after sowing for the interaction phenotype. Means, variances, and standard deviations for the percent of resistant plants (IP = 1) were calculated. A joint 3-factor scaling test (dominance effects) revealed that the data did not fit a simple additive-dominance model. Epistasis was suspected to be present and a joint 6-factor model was therefore tested. These results indicate that additive and epistasis effects were involved in the genetic control of Verticillium wilt resistance in P.I. 215699. These results indicate that progress in increasing resistance from the F_2 to the F_3 generation will result in an increase of 14% in resistant plants, if the selection pressure is 5%. In addition, these findings indicate that the most efficient breeding method is the genotypic recurrent selection method.

When novel Verticillium wilt-resistant accessions were screened, some highly resistant individuals (class 1) from P.I. 555614 and P.I. 555616 were saved and their progeny screened. The resistance to Verticillium wilt was increased form 4.42% to 21.78% in P.I. 555614 and from 7.27% to 13.18% in P.I. 555616, respectively. The purported resistant accessions, P.I. 439376 and B.G. 2729 did not have resistant individuals under our test conditions.

Previous investigation indicates that pepper plants surviving in Verticillium infested fields have some resistance to Verticillium wilt (not true "escapes"). Individual plants were selected from heavily infested fields with Verticillium wilt. The progeny of these selected plants were screened for Verticillium wilt resistance during the summer of 1992. Selfed individuals highly resistant to Verticillium wilt (class 1) were saved and their progeny will be screened during 1993.

Transformation of Capsicum using a Cucumber Mosaic Virus Replicase Gene: Integration of Transgenic and Conventional Strategies for Breeding Resistance

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

We are approaching the objective of developing useful sources of CMV resistance for *Capsicum* breeding in three ways: 1) Combining resistance/tolerance genes from several different sources including *C. frutescens* accessions identified by Loaiza-Figueroa and Provvidenti and *C. annum* Perennial in commercial *C. annum* backgrounds to develop donor lines with the highest levels of resistance. 2) Identifying RFLP and RAPD markers for these genes to be used in marker-assisted selection strategies where necessary. This objective has been expanded to include the Perennial source and we are currently working out the details of a collaborative approach using doubled haploid populations developed at INRA and probes developed at Cornell. 3) Most recently, we have initiated a collaboration with Dr. M. Zaitlin to use a cloned, truncated CMV replicase gene that has conferred extreme resistance to CMV in transgenic tobacco, to transform pepper. This report will describe the progress we have made to date in developing a regeneration and transformation system that is essential for transgenic approaches and the strategies we have targeted based on results to date.

Objective 1: *In vitro* regeneration of pepper. We have tested a number of published and unpublished procedures to regenerate normal plantlets from hypocotyls and cotyledons using identical genotypes where possible. The following describes the procedures we have tried and those we are currently using with results to date.

We have thus far examined six commercially available pepper lines for their regenerative abilities: RNaky (susceptible), Delray Bell (potyvirus resistance but susceptible to CMV), Capastrano, TAM Jalapeno, Early Jalapeno and Yolo B (provided by Jon Watterson at Petoseed). Our initial attempts at pepper regeneration followed three published protocols.