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Project No. 89-X3 - Continued Investigations on Almond Brownline and a New Project on a Carmel Problem

Project Leaders:

Dr. W. D. Gubler
Cooperative Extension
Department of Plant Pathology
University of California
Davis, CA 95616
(916) 752-0304 or 752-0301

Dr. Jerry K. Uyemoto
USDA/ARS
Department of Plant Pathology
University of California
Davis, CA 95616
(916) 752-0309 or 752-0301

Cooperating Personnel: Joe Connell, Janine Hasey, Wes Asai, Wilbur Reil

Objectives:

Almond brown line - (1) Orchard plot. June bud Peerless and Price onto Marianna and Lovell rootstocks planted in 1988. Force bud growth and monitor for disease. (2) Maintain and read indicator trees grafted in 1988. ABL inoculum was grafted onto apparently healthy Peerless/Marianna 2624 trees, on Elberta peach, and Bing cherry trees. Also, chip-buds of peach yellowleaf roll inoculum were grafted onto Peerless/Marianna. (3) Conduct analysis of diseased tissues for double stranded ribonucleic acid (dsRNA), viroid, content. Examine protein preparations for presence of unique bands in gels.

Carmel disease - (4) Resurvey diseased orchards. (5) Produce Carmel/Marianna 2624 trees using Carmel buds collected from healthy orchard trees grown on Marianna 2624. In late summer, graft inoculate budling shoots with diseased inoculum chip buds. (6) Graft inoculate Elberta peach and Bing cherry trees. (7) As #3 above.

Interpretive Summary:

Almond brown-line (ABL). In total we have established 22 replicated units, i.e. budling trees of Peerless or Price almond on Marianna 2624 and Lovell rootstocks, in the diseased orchard site. Scion buds for these trees originated from healthy appearing 5th-leafed trees of each cultivar growing on M.2624. All budling shoots appeared healthy in their first year of growth. Among the Peerless/M.2624 indicator trees grafted with bud or bark patches or budchips of ABL and healthy scions or rootstock or peach yellow leafroll (PYLR) infected Loadel peach, only one of three indicator trees with PYLR inoculations developed disease symptoms. Hybridization assays with an oligo probe specific for PYLR were positive for extracts prepared from leaves of the almond and of the peach shoots that developed from the grafted inoculum buds. Extracts of either ABL or Carmel disease (below) failed to reveal any unique banding patterns in agarose or acrylamide gels. Carmel disease. Resurveys of diseased Carmel orchards in Yolo and Stanislaus counties showed zero and 3% new diseased trees, repectively. Last year, the incidence was 20% at each site. In Butte County, a first-leaf orchard contained 14% (Carmel) and 4% (Butte) diseased trees. Fifty buds each from diseased and healthy Carmel and Butte trees were t-budded onto separate M.2624 rootstocks. These trees will be pruned next spring to force growth of the scion bud.

Experimental Procedures and Results

Almond brownline (ABL)

Orchard surveys. Yuba County (Live Oak). A 100 acre site planted in 1985 contain Peerless, Price, and Carmel on Marianna 2624. It was first surveyed in 1987 and incidence of ALB was 15% for each cultivar. Many ABL trees were pulled in that year and replanted the following spring. Resurveys in 1988 and 1989 showed a decline in disease to 4.5 and zero %, respectively. (Note. Several of the diseased trees diagnosed in 1988 were identified previously). In this orchard and because of the spatial pattern of diseased trees was suggestive of a soil-borne nature, a replanting trial comparing Lovell peach and two sources of Marianna 2624 (from a nursery in Oregon and FSPMS in California) rootstocks was initiated in 1988. Once established, the rootstocks were budded with healthy Peerless or Price scion buds and budling trees were produced. There are 22 replicated units available for future observations.

San Joaquin County (Ripon). A first-leaf planting of Carmel and Price/Marianna 2624 was surveyed in November 1989 and 106 of 361 (29%) Carmel trees exhibited ABL symptoms. Price trees appeared healthy.

Yolo County (Davis). Another first-leaf planting of Peerless/Marianna 2624 contained 5% (14/300) diseased trees.

Pathogenicity tests. On July 5, 1988, 18 indicator trees of Peerless/Marianna 2624 were graft-inoculated with diseased or healthy Carmel and Peerless. In addition similar transmissions were attempted on peach and cherry trees. Inocula consisted of bud chips and bark patches from diseased scion and rootstock or healthy Carmel and Peerless scion buds. The inoculum tissues were placed into the scion or rootstock portion of almond and scion portions of peach and cherry trees. Grafted tissues were examined a month later and essentially all had callosed and united with the host tree. However, all indicator trees after a year's incubation remain healthy; trees read August 28, 1989.

Because of an apparent failure to graft-transmit ABL to almond indicators, 90 more almond/Marianna 2624 trees were inoculated (September 28 and October 4, 1989) with diseased tissues using 3 buds/tree. These trees will be examined in 1990.

Graft inoculations with peach yellow leaf roll (PYLR). On July 5 and August 8, 1988, two and one Peerless/Marianna 2624 indicators, respectively, were grafted with five bud chips of PYLR. The latter inoculated tree developed ABL symptoms in 1989. Dot-blot hybridization assays using a nucleic acid probe specific for the PYLR agent were positive for leaf extracts of diseased, but not healthy, Peerless almond. In view of these results, three trees each of Peerless/Marianna 2624 and Peerless/Nemaguard were grafted (August 30, 1989) with 10 PYLR buds per tree. About nine weeks later, all three of the Marianna rooted trees were in a severe stage of defoliation. Close examination of the unions revealed a shallow brown line (1-2 mm deep) that disappeared with deeper bark slices. In contrast, inoculated Peerless/Nemaguard and control trees were fully canopied with clean unions.

In late September, more PYLR inoculations were made onto 30 almond/Marianna 2624 trees. These trees will be read in 1990.

Laboratory tests. Dot-blot assays. With the exception of a single positive reaction with the PYLR infected almond extract (see above), several other preparations of ABL tissues collected from orchard trees were negative.

Acrylamide gel assays. To date, analyses have been negative for the detection of double-stranded RNA (dsRNA) or viroids in extracts of diseased and healthy tissues. Control preparations of cucumber mosaic virus and citrus exocortis viroid were included.

Almond stem grooving (ASG)

Orchard surveys. Yolo County (Esparto). In 1988, a fourth-leaf Marianna 2624 orchard contained 107 (23%) and 3 (< 1%) ASG trees of Carmel and Price, respectively. During the winter season, all but two ASG Carmel trees were removed and tree sites replanted in early 1989. Surveys in 1989 revealed no new ASG trees. The two diseased trees produced fewer flowers and set only a few fruits, which were misshappened and gummy.

Stanislaus County (Modesto). Another young orchard established last year possessed 94 (19%) diseased Carmels and 6 (1%) diseased Price trees. All diseased trees were removed and tree sites replanted in 1989. Resurvey in 1989 produced 16 new ASG trees, i.e., 15 Carmel, of which 2 were replants, and one Price.

Butte County (Chico). A first-leaf planting contained diseased Carmel (86/597, 14%) and Butte (22/572, 4%). In mid-September, 50 buds each of diseased and healthy Carmel and Butte were T-budded onto Marianna 2624. The rootstocks will be pruned next spring to force growth of the scion buds.

Laboratory analyses. No dsRNA or viroids were detected in extracts prepared from diseased and healthy Carmel or Butte tissues.

Discussion.

Almond brown line: a brief background and research summary. During 1987, two orchards in Butte County and one in Yolo County were found to contain declining Peerless/Marianna 2624 trees. Canopy symptoms consisted of light-green to yellowed, drooped leaves with stunted growth (Figure 1). The unions of symptomatic trees showed deep pits and/or a dark brown line (Figure 2), hence its name ABL. In orchard C in Butte County, comprised of Peerless, Price, and Carmel in alternate rows (all on Marianna 2624 and planted in 1984 and 1985), the disease was present only in Peerless, where over half of first-leaf replants (38 of 63 trees) exhibited ABL symptoms. Our initial impression was that this may be a "Peerless" problem. However, additional surveys indicated otherwise, i.e., Price and Carmel were susceptible too.

Furthermore, greenhouse and nursery row trials have demonstrated that it was possible to propagate diseased budling trees (see 1988 annual report).

For these trials, Peerless scion buds and Marianna 2624 rootstocks were derived from foundation trees at FSPMS, University of California, Davis. These results suggest that at least one tree in the foundation block, either the scion or rootstock source trees, is infected. And, this fact coupled with finding ABL in first-leaf trees plus an apparent lack of tree to tree spread suggests that ABL is being introduced into orchards through diseased plant stocks.

Our soil baiting studies also demonstrated that ABL was not soil-borne (see 1988 annual report).

The etiology of ABL is still uncertain. However, preliminary graft-transmission data indicate a possible involvement of a mycoplasma disease called peach yellow leaf roll. This will be investigated further.

Almond stem grooving (heretofore referred to as the Carmel problem). Leaf symptoms consist of drooped, curled leaf edges followed by early defoliation (beginning in September)(Figure 3). Affected trees display unions with a faint brown line in the bark and corresponding shallow grooves in the woody cylinder (Figure 4). Observations of two diseased trees (5th leaf) showed that they failed to produce edible nuts.

The disease is found primarily in the Carmel cultivar. Other than that very little is known about disease epidemiology. This is due in part to lack of diseased specimens to work with. Essentially all ASG trees identified last year were removed from two known diseased orchards. With the first-leaf trees, we replanted several of them into 5-gal containers in our lathhouse and these died. This year, we asked for cooperation from two growers (with first- and second-leaf orchards) to save several specimen trees on site. One agreed to cooperate fully and another had consented to leave 3 trees each of diseased Carmel and Butte. We will conduct graft-transmission studies next year.

Figure 1. Almond brownline (ABL) in a third-leaf almond/Marianna 2624 tree (foreground) and healthy tree (background).

Figure 2. Exposed unions from healthy (left), and ABL (all others) Peerless/Marianna 2624 trees. Note deep pits (center specimens) and necrotic line (far right) at the unions.

Figure 3. Almond stem grooving (ASG) in a first-leaf almond/Marianna 2624 tree (foreground) and a healthy one (background)

Figure 4. Examples of ASG union symptoms on Butte (left), Carmel (center) and healthy Carmel (right). Note shallow grooves on diseased unions.