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Project No. 88-X2 - Marianna Brown Line and Virus Survey

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Objectives: Brown Line - (1) Maintain and read graft inoculated indicator trees. In 1987, diseased scion and suckers were chip budded into Peerless on both Marianna 2624 and Lovell. (2) Continue to analyze soils for potential nematode vector populations. (3) Attempt trapping causal agent from soils using healthy rooted cuttings of Marianna 2624. (4) Attempt mechanical transmission of the causal agent. (5) Characterize the causal agent, if it can be transmitted to herbaceous hosts. (6) Establish an orchard plot which will evaluate Lovell and Marianna 2624 rootstocks with respect to the spread and incidence of almond brown line disease. Prunus Ringspot and Prune Dwarf Viruses - (1) Survey a number of young almond orchards (first to third leaf) for prunus ringspot virus and prune dwarf virus using the 'Elisa' serological technique as an indicator. (2) Determine the diagnostic value of the 'Elisa' serological technique by comparing it to bioassays using Shiro-fugen cherry as the virus indicator.

Interpretive Summary:

Almond brownline (ABL). Because of spatial distribution of diseased trees and presences of mild to severely affected trees in the orchards, it appeared that ABL may be a soil related disease. To investigate that possibility, in 1987 several soil samples were collected from diseased trees sites and then divided and treated as follows: fresh (untreated), 30-day air dry, and autoclaved. Into each soil lot, healthy rooted cuttings of Marianna 2624 (which were T-budded with Peerless almond; both components derived from foundation trees at FPMS) were transplanted and the Peerless buds were forced to grow. In early June 1988, 11 of 55 budling shoots exhibited symptoms of bronzed leaves (table 1). Only symptomatic shoots contained a brown line in the bark tissues and corresponding fine pits in the woody cylinder. These symptoms occurred at the union. Healthy shoots showed smooth unions. These results suggest that ABL is not a soil-borne disease, that one component (either the scion or rootstock) of the budling trees was already infected, and that the pathogen was unevenly distributed in the source tree. Presently, we suspect that the diseased component was the Peerless buds. More experiments are underway to confirm the above findings and to identify the causal agent.

TABLE 1

Almond brownline: a summary of soil baiting experiments using Marianna 2624 rooted cuttings budded with cv Peerless almond

Orchards (treatments)	Peerless buds that produced shoots with leaves: green	or	bronzed	Peerless buds that remained dormant	or	died
R (fresh soil)	4		6	3		2
(30-day dried soil)	12		1	0		11
(autoclaved soil)	3		3	0		0
Others:						
6 orchards (fresh soil)	18		1	1		10*
2 " (autoclaved soil)	6		0	1		7
	43		11	5		30

*One shoot grew 60 cm and died.

Prunus necrotic ringspot (PNRSV) and prune dwarf viruses. Young almond plantings (first to fifth leaf trees) were visited in Tehama to Kern counties. During April 1988, five to 10 trees per orchard were sampled and leaf tissues tested by ELISA (enzyme-linked immunosorbent assay). Some sampled trees exhibited symptoms of calico, which is caused by PNRSV. In June-July, budsticks from ELISA tested trees in Butte and Tehama counties were collected and buds grafted into Shirofugen flowering cherry, an indicator host for both viruses.

In total, 360 leaf extracts were tested and 73 (=20%) were positive for only PNRSV. For the most part, sero- and bio-assays were in agreement. These results suggest that virus-infected trees are being used to establish almond orchards.

Experimental Procedures and Results

1987 initiated experiment: soil transmission trials.

As outlined in the 1987 annual report, several soil samples from diseased orchard tree sites were collected and treated as follows: untreated, 30-day air dried, and autoclaved. Rooted Marianna 2624 cuttings were then established in these treated soils and budded with Peerless almond. Source of rootstock and scion buds was FSPMS. Plants were evaluated in June 1988.

Our findings are detailed in the current interpretative summary. The data suggest that almond brown line (ABL) is unlikely to be soil-borne.

1988 soil-transmission trials.

To partially duplicate the soil baiting experiment of 1987, more soil samples were collected (in March 1988), screened for nematodes, and baited with rooted Marianna 2624 cuttings. These were later T-budded with Peerless buds (source was a healthy appearing Peerless tree on Marianna 2624 root). All budling shoots appeared normal during the December 1988 reading. Further evalulations will be done in 1989. Nematode counts varied, but 14 of 15 samples contained one potential virus vector, Xiphinema americanum. Its population counts ranged from 5 to 750 per liter soil.

Pathogenicity tests.

Recent results suggest that ABL may be bud perpetuated. However, proof of its transmissibility to healthy indicator trees is still lacking. During 1988, we produced or received healthy appearing Peerless/Marianna 2624 trees. These were pressed into service and grafted as follows:

1. ABL trees and healthy trees were collected and bark patches from each grafted into indicator trees. The inoculations consisted of placing bark patches from diseased scion to the healthy scion portion of the trees or to the rootstock portion of the trees. Also, tissues from diseased rootstock were grafted onto the healthy scion portion of the indicator tree.

   In addition to almond to almond inoculations, diseased and healthy almond sources were grafted onto peach and cherry trees. This was done to evaluate potential pathogenicity and record symptoms in other Prunus spp. Any observable response in these hosts may provide information on the probable causal agent of ABL.

2. To determine if the ABL factor is latent (symptomless) in other almond cultivars, budsticks from several healthy appearing almond cultivars, e.g. Peerless with 3 source trees; Price, 2 trees; Texas (Mission), 2 trees; and Nonpareil, 2 trees, were collected. Thirty buds from each collection were grafted onto three Peerless/Marianna indicator trees. One of the Peerless collection was previously used to produce the diseased budling shoots obtained in the 1987-88 soil baiting experiment (see interpretive summary).

3. To determine distribution of the ABL factor in a source tree, single and multiple inoculum buds derived from diseased and healthy budling trees (those produced in the soil-baiting experiment) were grafted onto individual healthy appearing Peerless/Marianna trees. A number of uninoculated trees will serve as controls.

   All test (indicator) trees will be evaluated in 1989.

Orchard survey: orchard R, 100 acre in size, planted in 1985.

This orchard contains three cultivars, Peerless, Price, and Carmel on Marianna 2624. Last year, two areas within the orchard were surveyed and incidence of ABL was found to be similar for all cultivars, i.e. 15% each. Resurveys in 1988 showed an overall incidence of 15.9%. The data suggest that little or no spread of ABL occurred in 1987.

Rootstock plot: orchard R.

On March 14, 1988, two sources of Marianna 2624 (from FSPMS and Oregon Rootstock, Inc.) and Lovell peach rootstocks were transplanted into diseased trees sites in a randomized fashion. In total, 50 units of each source and kind were used. These will be June budded in 1989 and scion buds forced to grow. Resulting trees will be inspected for symptoms.

Survey for Prunus necrotic ringspot (PNRSV) and prune dwarf viruses (PDV).

Shoots with succulent leaves were collected in March-April, 1988, from orchard trees in the counties of Butte (9 orchards sampled), Kern (9), Merced (2), and Stanislaus (4). Orchard trees ranged in age from first- to sixth-leaf stage of growth. Leaf tissues were crushed in carbonate buffer, extracts strained through cheesecloth, and the liquid fraction spotted in duplicate wells in microtiter plates. ELISA tests were directed toward the detection of PNRSV and PDV. In June-July, selected orchards were revisited, budsticks collected, and dormant buds grafted onto Shirofugen flowering cherry tree, a sensitive indicator for PNRSV and PDV. The ELISA results are indicated in table 2. Only PNRSV was found among 360 samples tested. Its incidence was 20%. Note that newly established orchards contained a high incidence of infected trees.

Discussion

Although preliminary results indicated that ABL is not soil-borne, we have repeated the soil-baiting experiments and are proceeding with the rootstock trials. Data generated on these projects should prove invaluable toward our understanding the epidemiology and control of ABL. In addition pathogenicity and host range tests are underway. Other areas of endeavor and not mentioned above involve laboratory assays and include preparing tissue extracts for double-stranded ribonucleic acid (dsRNA), viroid, and

protein banding pattern analyses. It is hoped that a unique pattern is found in diseased but not healthy extracts. If this is realized then rapid indexing and detection of diseased trees should be possible.

Addendem project

Carmel problem

In September and October, 1988, site visits were made to two orchards located in Yolo and Stanislaus counties. Both sites contained the almond cultivars Carmel and Price on Marianna 2624. Carmel was the principal cv of concern. The affected trees exhibited apparently normal shoot growth (this is in contrast to shortened growth associated with ABL), but trees showed early defoliation (in early September). The union area showed a light brown color in the bark tissues and shallow pits and grooves in the woody cylinder. Each orchard was surveyed and disease incidence ranged from 19 and 23% among the Carmel trees (Table 3).

In orchard J, the September survey revealed nine "definitely" affected Carmel trees and several as "probables". Two diseased trees were removed and seven were left. In mid-October, a resurvey showed a dramatic increase in the number of defoliating trees, i.e. total count rose to 107 Carmel trees. Among the seven trees identified six weeks earlier, four had died.

Table 2. ELISA results for almond orchard surveys

Orchard no.	leaf stage	no. PNRSV/total tested
2	first	20/30
7	second	20/110
11	third	27/160
2	fourth	2/25
1	fifth	1/20
1	sixth	3/15
24		73/360 (20%)

No Prune dwarf virus was detected.

Table 3. Incidence of the "Carmel Problem" in young trees

Orchards (yr planted)		No.diseased trees (%): Carmel	Price
J	(1985)	107 (23)	3 (1)
D	(1988)	94 (19)	6 (1)