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Project No. 87-V1 - Almond Diseases
Brown line and stem pitting on Marianna

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Objectives: (1) Determine incidence and distribution of declining
young trees associated with union aberrations. (2)
Determine the nature of the causal agent(s).

Interpretive Summary:

A union brown line symptom has been observed on young almond trees (one- to three-leaf) growing on Marianna 2624 roots. Disease incidence in three orchards ranged from 18% (69 diseased trees/379 trees), 15% (168/1120), and 3% (1/30). The disease markedly affected tree growth in a third-leaf orchard (see results, table 3). Affected trees were diagnosed in the counties of Butte, Colusa, San Joaquin, and Sutter.

Based on the range of symptomatic trees, viz. from mild (involving one to a few limbs) to severe (entire tree declining) symptoms and clustering of diseased trees in one orchard, union brown line appears to be a soil borne disease. Experimentation to verify this is in progress. For example, soil samples were "baited" with rooted healthy cuttings of Marianna 2624, then June budded to Peerless, a disease indicator. To establish the biotic nature of this disease, healthy trees of Peerless on Marianna and Lovell (in addition to nursery rooted Mariannas) have been graft inoculated with chip buds of diseased Marianna suckers and fruiting budsticks. It will require a year or more incubation before these tests are completed. In other limited tests, virus indexings by ELISA, Shiro-fugen, and nematode analyses have not demonstrated a causal relationship.

EXPERIMENTAL PROCEDURES:

Orchard history.

Five orchards (designated M, B, TC, H, P) in Butte County, two in Colusa County (NC, GH), and an orchard each in Sutter and San Joaquin counties were examined. Although these orchards contained declining trees, only six showed definite brown line symptoms. At this time it may be noteworthy to describe the history and conditions at each site to gain an understanding of the disease complexes present.

Orchard M, planted in 1982 to Carmel and Price. Tree rows are oriented in an east to west direction with the west half on Lovell and east on Marianna 2624 rootstocks. Only the Carmel/Marianna trees show decline; Price trees and Carmel/Lovell appear healthy. In September, five rows of Carmel/Marianna were examined for no. of replants (53 found), symptomatic (19), and healthy trees (101). For symptomatic trees, the canopy consisted of sparse foliage and small, wilted leaves. The union of one tree showed brown line. The woody cylinder of other trees showed numerous pits that were confined to the union zone.

Orchard B, also planted in 1982 to Carmel and Monterey/Marianna 2624, showed a similar decline symptom as observed in orchard M. However, affected trees were free of brown line. Also, grooves at the union tended to be long extending down the root portion. During second leaf (1983), some Monterey trees were yellowed and Carmel trees developed bud failure, these were removed and replanted. At fourth leaf, orchard doing well. However, at 5th leaf, Carmel trees began to die and approx. 125 trees were replaced. Monterey trees again developed a yellow cast but made full recovery by the fall season.

Orchard NC, planted in 1984 to Carmel, Price, and Peerless/Marianna 2624. Only Carmel trees were declining. Two dead trees showed a brown discoloration of the rootstock cambium up to the union with the scion portion appearing healthy. We failed to isolate Phytophthora from canker margins and pear fruit baiting of soils.

In three orchards (planted 1984 and 1985 and located in Butte and Colusa counties), only Peerless/Marianna 2624 was affected with brown line. All Price and Carmel trees appeared healthy. Orchard TC, two rows of Peerless trees contained 70 brown lined trees of which 38 were first leaf replants (milk carton wrap at tree base). Among the 55 healthy Peerless trees, 25 were first leaf replants. Orchard H is an older orchard, but due to wet conditions of 1982-83 several trees/Lovell roots were lost, Peerless/Marianna 2624 were used as replants in 1984. Several of these showed brown line. Orchard GH established 1985 to Carmel, Price, and Peerless. A year later, several Peerless trees (only) had to be replanted. In 1987, examination of five such replants showed that four contained brown line.

Orchard P planted 1987 to Mission, Price, and Carmel/Marianna 2624. An examination of 10 trees of each variety revealed that one Carmel was brown lined.

In San Joaquin County, several replants (variety not determined) in one orchard contained brown line.

Perhaps the orchard site of most promise for field plot work is that located in Sutter County (orchard R). It is 100 acres in size, established in 1985 to Carmel, Price, and Peerless/Marianna 2624. All three varieties show brown line with an avg. incidence of 15% (see results table 1). Among the varieties symptomated trees range from mild (involving one to a few limbs; suggestive of recent infection) to severe (entire tree involved) forms of decline. In these, necrotic tissues at the union vary from scattered patches to a contiguous line, i.e. corresponding to mild and severe canopy decline, respectively. Affected areas in the orchard appear circular in pattern, which is indicative of a soil borne origin.

Soil transmission and nematode analysis.

Soil samples were taken from orchards M, B, TC, H, NC, GH, and R and processed in the following manner:

1) Soil samples were placed in 5 inch pots and transplanted with rooted cuttings of Marianna 2624 and cucumber seedlings. A portion of soils from orchards M and B was autoclaved and plants transplanted into them. Soils from orchard R were left untreated, air dried for 30 days, or autoclaved; all received transplants.

The cucumber plants were permitted to grow for 30 days, uprooted, and roots extracted before rubbing onto leaves of cucumber and Chenopodium quinoa. No virus transmissions were realized.

The Marianna cuttings were budded with Peerless during the summer. These will be examined periodically by removing bark tissues at the scion/root junction.

2) Soil samples were processed for extraction of nematodes, specifically the virus vector, Xiphinema americanum (see results table 2).

ELISA and Shiro-fugen tests.

In Butte County, orchards M, B, TC, and P were sampled. Leaf tissue collections of scion and roostock from orchard M (11 Carmel on Marianna and Lovell roots; 3 Marianna), B (5 Carmel, 5 Monterey, 2 Marianna), and TC (3 Peerless, 2 Marianna) were extracted and ELISA for prune dwarf (PDV), necrotic ringspot (NRSV), and tomato ringspot (TmRSV) viruses. Only one Carmel/Lovell tree was positive for NRSV.

From orchard P, budwood was taken from 10 trees each of Mission, Carmel, and Price and grafted onto Shiro-fugen. Grafts were read after 4-6 wk. Nine of 10 Price trees indexed positive; Mission and Carmel trees were negative.

Graft and sap inoculations.

Prunus tomentosa indicator. Budwood of disease Peerless and Marianna were chip budded (3/tree) into three P. tomentosa per collection. These were taken from orchards TC and GH.

Peerless/Marianna or Lovell or nursery Marianna plant indicators. Diseased and healthy budwood of Carmel, Price, Monterey, and Marianna were collected from orchards M, B, TC, and R and chip budded into Peerless/Marianna and Peerless/Lovell trees and nursery Marianna plants.

All graft inoculated trees and plants will be observed, viz. for growth and symptoms at scion/root union. Nursery Marianna plants were June budded with Peerless, a disease indicator.

Trunk measurements.

At orchard R, trunk diameters (max. and min. @ 3 inches above union) were taken for several trees of each variety diagnosed as healthy, mild (where one or more limbs are rosetted, but shoot growth elsewhere is evident), or severe (all limbs rosetted, no evidence of shoot growth). The two measurements per tree were avg and statistically analyzed (see results table 3).

RESULTS:

Table 1. Incidence of Almond Brown-Line Disease

<u>Orchard</u>	<u>Cultivars:</u>			<u>Site avg (%)</u>
	<u>Camel</u>	<u>Price</u>	<u>Peerless</u>	
R (south central)	64/320 (20)*	27/160 (17)	12/160 (7.5)	16.1
R (north east corner)	24/240 (10)	12/120 (10)	29/120 (24)	13.5
T C	0/127 (0)	0/127 (0)	69/125 (55)	18.2
P**	1/ 10 (10)	0/ 10 (0)	0/ 10 (0)	3.3

*No. trees with brown-line/total trees (%).

**All Shiro-fugen indexed; only nine Price trees were positive for an ilarvirus.

Table 2. Analyses of orchard soils for Xiphinema americanum

<u>County</u>	<u>Orchard</u>	<u>Variety/root</u>	<u>#Xiphinema/liter soil</u>
Butte	M	Carmel/Marianna	990
		" / "	40
		" /Lovell	20
		" / "	45
	B	" /Marianna	24
		" / "	0
	TC	Peerless/Marianna	55
		" / "	135
	H	" / "	10
		" / "	10
Colusa	GH	" / "	0
		" / "	0
	NC	Carmel/ "	45
		" / "	0

Table 3.

Almond brown-line: effect of disease on trunk diameter

<u>Tree condition</u>	<u>Carmel mean*</u>	<u>N</u>	<u>Price mean</u>	<u>N</u>	<u>Peerless mean</u>	<u>N</u>
Healthy	6.5a	30	6.6a	31	6.2a	30
Mild symptom	4.7b	11	4.4b	15	4.6b	11
Severe "	3.8c	15	4.0c	15	3.5c	19

*Figures represent means in cm of stem diam @ 7.6 cm above union. Letters within each column indicate significant differences @ p=0.05.

DISCUSSION

The union symptoms described for diseased almond trees (almond brown line, ABL) are indistinguishable for those described for prune brown line (PBL). However, PBL was clearly demonstrated to be caused by TmRSV, which is vectored by X. americanum sensu lato. Our attempts to recover TmRSV or like nepoviruses failed.

The various combinations of graft inoculations, viz. diseased chip buds (of scion or sucker) into healthy Peerless or Marianna, should upon test completion reveal whether ABL is 1) caused by a biotic agent and 2) determine which portion of the tree is susceptible and resistant. Results of soil baiting experiments should also shed light on the soil borne nature of ABL. Presently there doesn't appear to be a positive correlation of ABL incidence and presences or absences of a known nematode virus vector.

All in all it is for certain that ABL can drastically affect tree growth and development. And as more and more acreage become contaminated with Phytophthora through surface water irrigation practices, use of Marianna 2624 (which is Phytophthora tolerant) may increase. It's likely that the causal agent of ABL is not limited to the Sacramento Valley.

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**ALMOND BROWN-LINE, A NEWLY RECOGNIZED DISORDER OF TREES ON MARIANNA 2624
ROOTSTOCK**

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A plum rootstock, Marianna 2624 (Prunus cerasifera X P. munsoniana) is currently used for certain almond scions. Its advantages include being slightly dwarfing, and somewhat resistant to Phytophthora spp., oak root fungus (Armillaria mellea), and root knot nematode (Meloidogyne spp.). Also, it survives well under heavy, wet soil conditions. A drawback of Marianna 2624 is its full or partial incompatibility with several almond scions, Nonpareil, Drake, and Monterey to name a few. Recently, another problem has been observed in new almond plantings, a disorder called almond brown-line (ABL).

Symptoms. To date, ABL has developed on first- to third-leaf almond trees with the varieties Carmel, Peerless, and Price. On recently infected trees, canopy symptoms consist of one or more limbs with little or no current-season shoot growth. Leaves on such limbs are tightly clustered giving rise to a rosette appearance. With advanced infections, the canopy contains smaller-sized leaves that are drooped and yellowed. All limbs lack shoot growth and trees die. The graft unions of symptomatic trees display a light to dark

brown line in the bark tissues and corresponding pits impregnated with necrotic tissues in the woody cylinder. The latter symptom is especially evident on severely affected trees.

The incidence of disease in three young orchards was 15% (168 ABL trees/1120 trees) (Yuba County), 18% (69/379), and 3% (1/30) (both Butte County). Diseased trees have been observed in Colusa and San Joaquin counties also.

Future work. In some preliminary work, we failed to recover tomato ringspot virus (TmRSV), incitant of prune brown-line disease (see below). However, the spatial pattern of diseased trees and tree symptoms ranging from mild to severe at one location are suggestive of a soil-borne cause. The etiology of ABL is being addressed and efforts toward that goal are underway. Currently in progress are experiments involving 1) graft inoculations onto healthy trees of Peerless/Marianna 2624; 2) baiting of orchard soils with rooted cuttings of healthy Marianna 2624; and 3) attempts at sap transmission to herbaceous host plants. Extensive nematode analysis are planned. Hopefully, meaningful data will be realized in the coming months.

Remarks on prune brown-line (PBL) disease. The brown-line symptom at the unions of prune and plum scions grafted onto peach (Lovell, Nemaguard) or plum (Myrobalan) rootstocks are indistinguishable from those described for ABL. However, research data developed by others have clearly demonstrated that the incitant of PBL is TmRSV, a virus vectored by a nematode (Xiphinema californicum). Also, Marianna 2624 was found to be resistant to TmRSV and this rootstock is used commercially on diseased sites. So it appears that for ABL a pathogen other than TmRSV is likely involved.

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CONTROL OF NEEDLE CAST OF WHITE FIR

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Needle cast of white fir, Abies concolor, is caused by the fungus, Lirula abietus-concoloris. The disease occurs at low endemic levels in natural fir stands in the Sierra-Nevadas and generally causes only minor defoliation or damage. However in Christmas tree plantations located within the native range of white fir, the disease can cause severe damage in some years. In 1985 some white fir plantations in El Dorado County were heavily infected and growers suffered appreciable economic losses.

Infection occurs only on young tender needles that appear in early summer (June). Rainfall at this time is essential for the fungus to release spores and infect the young needles. No visible evidence of infection appears until the following summer (July) when the needles turn brown in color but remain

attached. In the fall and winter, the fungus produces hysterothecia in the infected needles. The spores are mature and ready for dispersal by late spring. Water in the form of rain or sprinkler irrigation is necessary for spore release and infection; the disease is serious only in years when rains coincide with the flush of new growth.

Since infection occurs only during a short period in the late spring or early summer, it is possible to protect new growth with suitable fungicides. The trial reported here was undertaken to select the most efficacious fungicides for disease control.

The experimental area was located at the Harris Tree Farm Pollock Pines, California. A single application of each of 9 fungicides was made on May 12, 1986, to 5 trees selected at random for each fungicide. The trees ranged from 5 to 10 feet tall and all bore a flush of new growth. To insure infection the experimental area was overhead irrigated for 12 hours per day from June 2 to June 6, 1986, following the fungicide applications. The trees were evaluated on June 29, 1987, by counting the number of infected and healthy needles on three shoots selected at random on each tree and calculating the percent infected needles. A second evaluation was made on August 30 by estimating the proportion of the 1986 foliage infected in the crown of each tree. The results are presented in Table 1.

Table 1. Effect of fungicides on needle cast of white fir.

Product	Fungicide	Formulation	Amt. of product per 100 gal.	Mean % 1986 Needles Infected		
				Three branches	Entire tree	Compo- site*
Benlate	benomyl	50 W	0.5 lb.	3.4	9.0	6.2
Fore	mancozeb	80 W	1.5 lb	6.3	10.4	8.4
Spotless	diniconazole	12.5 W	0.4 lb	9.6	7.5	8.6
Bravo	chlorothalonil	40.4 F	16.0 fl oz	12.4	13.2	12.8
---	prochloraz-Mn	50 W	0.25 lb	26.3	30.0	28.2
Rubigan	fenarimol	50 W	0.25 lb	26.3	32.0	29.2
(Control)	---	---	---	20.6	41.3	31.0
Fungaflor	imazalil	75 SP	0.25 lb	39.3	38.0	38.7
Funginex	triforine	18.2 E	8.0 fl oz	55.4	41.0	48.2
Bayleton	triadimefon	50 W	0.25 lb	34.6	32.0	33.3

*Average of the percent on three branches and entire tree.

Four fungicides: benomyl, mancozeb, diniconazole and chlorothalonil, protected the young needles from infection. Both benomyl and diniconazole are systemic. Under very rainy conditions they would probably provide better protection than the contact fungicides, chlorothalonil and mancozeb. None of the products evaluated in this trial mention needle cast of fir on the product label. However, chlorothalonil (Bravo, Daconil) and mancozeb (Fore, Dithane M45, Manzate 200) are labeled for use on conifers to control other needle diseases and it would be proper to use these fungicides to control needle cast of white fir.

Almost no infection occurred in the plantation outside the sprinkled area. Trees with infection levels of 10% or less probably would be marketable, whereas higher levels of infection or infections for 2 or more consecutive years would seriously reduce the value of the tree.

Some trees in the sprinkled area appeared to be resistant to infection. It is possible that a source of resistant white firs could be developed and it would then not be necessary to rely on fungicides.

REMINDER:

CONFERENCE ON THE CONTROL OF SOIL-BORNE PATHOGENS

Conference on the Control of Soil-Borne Pathogens will be held in Reno, Nevada, on March 10-11, 1988. Contact Dr. Gubler (telephone: 916 - 752-0304) for topic suggestions, comments, etc.

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