Project Number: 92-BJ1

Almond Research Results---1992-1993

Title: Investigating the possible involvement of mycoplasmalike organisms (MLOs) and bud-failure (BF) in Carmel almond

Project Leaders: Jerry Uyemoto, USDA-ARS and Bruce Kirkpatrick

Background. Historically, BF may be caused by infections with Prunus necrotic ringspot virus (PNRSV) (aka infectious BF) or because of "bad" genetics (= noninfectious BF). It seemed reasonable to conclude that other "causes" may also be involved. So, in partial response to concerns expressed by Farm Advisor Joe Connell about the apparent increased incidence of BF in young Carmel orchards, a pilot project to evaluate the effects of an antibiotic (i.e. tetracycline) and BF was initiated in March 1989. This effort was to determine if MLOs, organisms sensitive to the antibiotic, occurred in BF trees.

In a Carmel planting (esta. ca. 1983) near Chico, three groups of three trees with ratings of BF^{-1}, BF^{+1} and BF^{+2} were matched. Then, tetracycline solutions of 1 and 2 gm A.I. or a water control were infused into the trees. All nuts from treated trees were hand harvested and destroyed. A year later, the numbers of dead buds (= BF) on one- and 2-yr-old wood were counted and percentages calculated (Table 1). Two of the replicates showed an apparent response to the antibiotic. However, one replicate did poorly.

In April 1990, we proceeded to retreat the trees using the higher concentration of tetracycline only and five pairs of BF trees. The nuts from the treated trees were again destroyed and yields were nearly double that harvested in 1989. However, in April 1991, we were unable to score for numbers of dead buds because during 1990 the vegetative growth was lacking due to the heavy nut set that year.

Despite these mixed results, the project was expanded to include other Carmel orchards (see below).

Objectives:

Develop an objective rating system for BF.

Continue tetracycline treatments and compare BF incidence to nontreated controls.

Collect BF and healthy Carmel tissues and analyze for MLOs by PCR (polymerase chain reaction).

Graft-inoculate BF collections onto Fay Elberta peach, a sensitive MLO indicator.

Graft-inoculate Peerless/Nemaguard trees with PYLR and evaluate for symptoms.

Procedures:

Antibiotic treatments. Carmel orchards in Chico, Hughson and Cortez were rated visually for BF severities and five and seven pairs of trees were matched per site. One tree of each pair was infused with tetracycline at 2 gm A.I. All treated trees were harvested separately and nuts destroyed.

The Chico plot was a continuation of the project described above (see Background). The trees were treated in April 1991. In July 1991, abundant shoot growth was evident. At that time, a plastic ribbon was tied 12-15" below the shoot tips of 30 current season shoots per tree. During March 1992, live and dead buds per foot of shoot, i.e. above the ribbon tie, were counted.

At Hughson (orchard esta. 1982), treatments began in May 1990 and trees scored for BF a year later by counting numbers of strikes, i.e. symptomatic shoots and not numbers of affected buds. Again encouraging results were obtained (see Table 2, Hughson 1991). The same trees were retreated in May 1991 and during the summer the existing BF shoots on treated and control trees were pruned. All trees were reread in March 1992.

At Cortez, there were 7 pairs of trees. Treatments were made in May 1991, existing BF shoots pruned in the summer and trees scored for "new" symptomatic shoots in March 1992.

MLO assays. In late July 1992, fruit peduncle collections were made in BF orchards located in Butte, Kern, Merced, San Joaquin and Stanislaus counties. The tissues were extracted for total DNA and subjected to PCR amplification using specific primers for MLOs. Also, budwood from BF and healthy Carmel trees were T-budded into Fay Elberta peach (26 trees grafted) and Carmel almond (12 trees grafted) trees. Inocula consisted of 5 buds per indicator tree. These grafted indicator trees will be read in August 1993.

Pathogenicity test. Three Peerless almond/Nemaguard peach trees were grafted in 1989 with peach yellow leaf roll-MLO (PYLR-MLO) inoculum. These trees were observed for symptoms and nuts harvested in 1992.

Results:

In the Chico plot, where buds per foot of shoot were recorded,

it was more convenient to count the numbers of live buds rather than dead ones. Overall, bud survival among treated trees was twice that of untreated controls (Table 3). However, when the numbers of BF shoots were measured in the other orchards, one orchard showed some benefits with treatment while the second did not (Table 2).

In total, PCR analyses were performed on 99 collections of BF and healthy Carmel trees and 56 of BF, trees with thin canopy or healthy of Nonpareil, Mission, Merced and Peerless trees. Seven collections of Carmel and six of the mixed varieties proved positive for MLOs. It is interesting to note that 10 of the positives were BF collections and three others were of trees exhibiting yellowed or "see thru" canopies. All 36 collections of healthy trees (included in the totals above) were negative by PCR. At least one positive collection was taken from each county.

The grafted indicator trees will be examined in late summer 1993.

On the PYLR-inoculated Peerless/peach trees, disease symptoms consisted of light-green to yellow color, small leaves borne on stunted current-season shoots. Affected trees remain off-colored throughout the growing season and later, chronically-infected trees developed sparse canopies. In addition, fruit set and kernel development were greatly impacted (Table 4). The averages in numbers of fruit produced per tree, weight of 100 dried kernels and calculated yields per tree were 587, 15.5 g and 0.09 kg, respectively. All shelled kernels were shrivelled. In contrast, on healthy trees the averages were 1743 (range 1130 to 2099) fruit produced per tree, 130.2 g (ranging from 126.0 to 135.0) for weight of 100 dried kernels and 2.26 kg (ranging from 1.53 to 2.62) nut yield per tree. All shelled kernels appeared sound.

Discussion:

Due to seasonal differences in vegetative growth caused, in part, by tree age and by wide variations in the amount of fruit set each year, it was not possible to measure bud death or survival on an annual basis. Even though an objective procedure in scoring BF does not currently exist, the visual rating method in current use is easy and convenient to use.

Infusion of tetracycline into BF trees caused a favorable tree response initially. However, repeated treatments produced, at best, erratic results. If MLOs were involved a more consistent response was expected. Also, PCR analyses showed that only 11% (13 PCR positives among 119 BF or weak trees) of the collection were positive for MLOs. Again, this indicated that an MLO was not present in the bulk of the BF trees tested in our study. However, the wide-spread nature of MLO-infected almond trees, i.e. down the San Joaquin Valley, was still surprising. This was especially so,

because based on the occurrence of naturally infected peach trees (a sensitive MLO indicator host) the southernmost boundary was acknowledged to be Merced County. Until now, natural MLO-infected almond trees have been detected only in San Joaquin and Stanislaus counties.

Lastly, one commercial orchard located north of Modesto contained Butte almond trees infected with an MLO; diseased trees produced all shrivelled kernels. Experimentally, we proved that Peerless almond trees graft-inoculated with PYLR-MLO caused a similar kernel condition. This may have implications in a commercial orchard setting, whereby a lower than expected percentage in the final "crack out" for a given harvest may be indicative of the numbers of PYLR-infected trees in the orchard.

Table 1. Response of Carmel trees with bud-failure to tetracycline

Visual rating 1989	Tree positions	Treatments	Amount (ml) not absorbed into tree	% Dead buds on: 2-yr & 1-yr wood	
BF^{+2}	13T10	2 gm AI	0,0,0,0	81	13
	3T1	1 gm AI	0,0,0,0	91	21
	3T4	dist. H20	0,0,0,0	86	61
BF^{+1}	5T16	2 gm AI	0,90,208,368	89	33
	9T17	1 gm AI	0,0,0,258	78	11
	11T15	dist. H20	0,0,0,0	84	19
BF^{-1}	3T7	2 gm AI	0,0,0,462	85	13
	5T2	1 gm AI	0,0,0,0	87	17
	5T12	dist. H20	0,0,0,0	91	26

Treatment on 3/13/89; 4 bags/tree with 950 mls each; dead buds counted 4/90.

Table 2. Effect of tetracycline treatment on almond bud-failure

Number of Replicates	Hughson orchard: Read 5/3/91[1] Treated	Control	Read 3/25/92[2] Treated	Control	Cortez orchard: Read 3/25/92[2] Treated	Control
1	18	72	85	58	36	14
2	35	308	83	63	42	62
3	91	115	59	136	50	92
4	101	170	128	134	41	46
5	194	288	76	54	27	88
6	-	-	-	-	36	55
7	-	-	-	-	33	50
Averages:	88	191	86	89	38	58

[1]Trees infused with 2 gm AI tetracycline on 5/18/90; figures represents numbers of shoots with BF.

[2]Trees infused on 5/6/91 (Hughson) and 5/15/91 (Cortez); During summer of 1991, all obvious BF shoots were pruned off.

Table 3. Response of bud-failure trees to repeated infusion of tetracycline[1]

Number of replicates	%Live buds/ft of shoot Treated	Control
1	24.2	2.6
2	27.9	13.4
3	32.6	15.0
4	28.8	22.9
5	29.6	15.6
Averages:	28.8	13.6

[1]The third (annual) treatment was 4/24/91; buds on previous year's growth were counted on 3/30/92.

Table 4. Yields of Peerless almonds[1]

Tree status	No. in-shell nuts	Wt. (g) of 100 kernels[2]	Total Wt. (kg) kernels/tree[3]
PYLR-MLO	560	17.34	0.10
"	710	14.55	0.10
"	491	14.55	0.07
Healthy	1,980	132.00	2.61
"	1,760	126.00	2.22
"	1,748	133.00	2.32
"	2,099	125.00	2.62
"	1,130	135.00	1.53

[1]PYLR-MLO infected trees harvested on 8/25/92; remainder on 9/15 and 16.

[2]Shelled kernels were dried overnight in a heated forced-air oven.

[3]Values were calculated by multiplying wt. of 100 kernels x (no. in-shelled nuts divided by 100) and further divided by 100 = kg.

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Dear Susan:

Please find enclosed two copies of our results on the project entitled, "Investigating the possible involvement of mycoplasmalike organisms (MLOs) and bud-failure (BF) in Carmel almond". A delay in completing this report was due to the presence of an inhibitor(s) in the almond preparations. Fortunately, this problem was successfully resolved and we proceeded to analyze the samples by polymerase chain reaction (PCR) using primer sets specific for plant MLOs. Briefly, results of the PCR assays indicated that although MLO infections were present in all counties sampled, only 11% of the trees with bud-failure were positive for MLO.

I hope this report is helpful to you. Thank you for your support.

Sincerely yours,

Jerry Uyemoto
Research Plant Pathologist

pc: Bruce Kirkpatrick