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ALMOND BOARD OF CALIFORNIA
ANNUAL REPORT FOR 1989

Project No. 89-B1 - Moisture Stress and High Temperature Effects on Amino Acid Metabolism in BF affected almond Trees

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<u>Objectives</u>: To establish the effects of moisture stress on the amino acid patterns of nonaffected and BF-affected almond plants

a. to determine the seasonal pattern of bud growth and symptom development in normal vs. BF Nonpareil, normal vs. stressed Nonpareil BF and Milow comparisons

b. to relate BF symptoms and moisture stress level in individual trees

c. to establish the pattern of amino acid metabolism in relation to BF symptom development and moisture stress patterns.

<u>Interpretive summary.</u> Last year we reported that certain variations in the normal amino acid cycles involving arginine and proline were associated with BF symptom development. In 1989, we extended the study (a) to confirm if we could reproduce the same sequence of events correlating internal biochemical changes and the development of BF symptoms, and (b) to determine if moisture stress could produce the same physiological and biochemical patterns.

A bud forcing bioassay was used to monitor the time sequence of internal symptom development at two week intervals from June to October. The results on bud development were similar to previous work although somewhat at variance in certain aspects. Buds on non BF trees were able to sprout equally well through July and August but the rate decreased thereafter apparently due to beginning of "rest" induction. On the other hand, BF trees began to show

inhibition from early July with increasing severity after September. Exposing BF Nonpareil trees to stress under our conditions in 1989 had no effect until after mid-July but decreased spouting during the remainder of July and August.

Bud fresh weight was also found to measure bud development changes. These suggested that a transition occurred during late June possibly involving a shift from bud-scale formation to "summer dormancy" patterns during July and August followed by a shift to the "resting" condition about early September. The effect of BF in our material this year can be interpreted to be to delay the progress of this pattern about 10 days to 2 weeks.

In buds on BF Nonpareil trees subjected to stress, the fresh weight of buds was significantly less than that coming from the irrigated trees. Moisture stress was most severe under non-irrigated conditions, and the most severe BF symptoms also occurred under such treatment. Substantial tree-to-tree differences in moisture stress were also found within each treatment. Under irrigated conditions increasing tree water stress was associated with increasing tree BF symptoms, but under non-irrigated conditions increases in tree water stress were associated with decreasing BF symptoms. This may indicate that intermediate levels of water stress are the most damaging by inducing higher BF. We were unable to measure differences in "dormancy" levels prior to development of bud damage as we had reported earlier. The relatively mild summer temperatures this year may have influenced results.

<u>Biochemical studies</u>. Samples of buds were collected weekly from June through early October and have been stored for analysis of their amino acid content. This second phase of the investigation is to include the simultaneous analysis of approximately 39 amino acids and nitrogenous compounds in these samples. Analysis is started in January and will not be completed until later this spring.

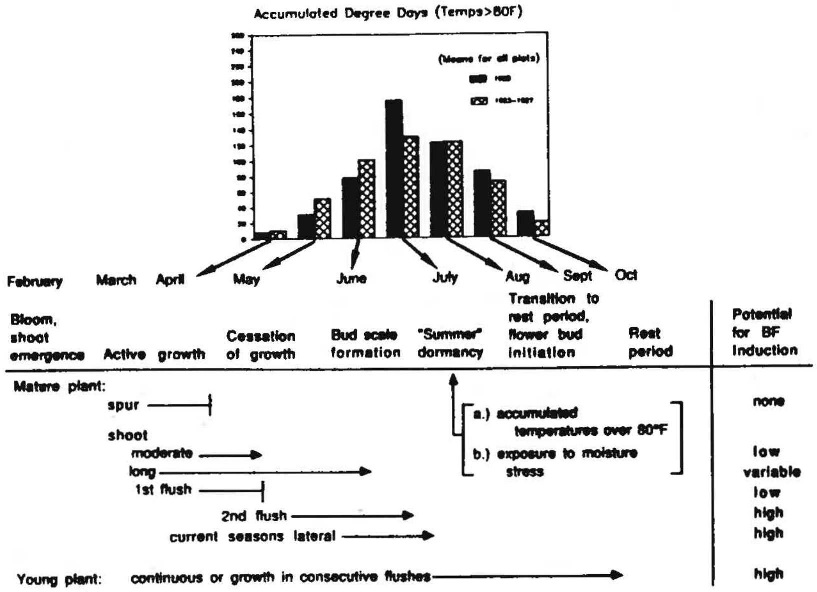
ANNUAL SUMMARY OF REPORT

PART I. SEASONAL PATTERNS

The seasonal cycle of vegetative growth of almond shoots has consecutive stages of development:

a). rapid growth in spring,
b). cessation of growth and bud scale formation,
c). "summer dormancy",
d). induction of "rest"
e). reduction of "rest" by chilling.

Figure 1 shows the relationship between this pattern and temperature sequences.



Seasonal patterns of BF-failure

BF symptoms arise as a result of damage to vegetative buds on current seasons shoots during summer. We have shown repeatedly that the initiation of this injury is a function of accumulated exposure to high temperatures. Orchard observations and some experiments also show that exposure to moisture stress can likewise be a contributing factor to symptom expression. We have produced evidence in prior studies that the visual initiation of BF symptoms was preceded by lack of dormancy in BF shoots. A similar pattern occurred in shoots from BF trees exposed either to heat or moisture stress.

The amino acid data from 1986 studies showed that the trends in growth activity of the shoots was correlated to changes in the concentration of total amino acids. Also certain amino acids or "families" of related amino acid appeared to be "markers" of specific changes associated with growth and dormancy.

Materials and Methods

Plant materials used in the 1990 experiments were as follows:

1. Nonpareil:normal vs. BF trees. Source-clones of Nonpareil were as follows: (a). Source FPMS 3-8-2-70 which have been shown to have a low BF potential in RVT and other trials and (b) "Clement" source which had been identified in studies at West Side Field Station (1986 Annual Rpt) as having a high potential for BF. Buds were

collected from 3 year old Nonpareil trees planted at the WEO orchard, Winters. Symptoms had begun to develop in trees in spring 1989.

2. Nonpareil BF: stressed vs. non-stressed. The source of these buds were from 20 year old trees at the WEO and UCD orchards of FPMS 3-8-1-63 which were part of an older experiment (Kester and Asay, 1978) and had been the trees used in prior similar experiments. These trees had been showing BF symptoms for many years and which were particularly severe in spring 1989. Half of the trees at the WEO and UCD blocks were given normal irrigation during 1989 and the other half were stressed for water after May. Unfortunately, as described in part II, many of the irrigated trees also showed stress.

3. Milow: stressed vs. non-stressed. This variety was growing adjoining the Nonpareil trees in the WEO and UCD blocks to provide cross-pollination. They had been grafted on to Nonpareil BF trees. Milow has never shown BF and none of these trees had symptoms. The irrigation treatment was the same as for the Nonpareil BF trees.

<u>Bud forcing bioassay</u>. A bud forcing bioassay was developed in previous years to determine the ability of bud explants to sprout when placed into a growth chamber. Sprouting tests started every two weeks consecutively during the season was used to characterize the pattern of growth and dormancy. Shoots were collected from representative trees, brought into the lab, cut into single node explants and inserted into a petri dish through a pliofilm cover. Nodal explants were arranged consecutively from base to tip after the surface of the upper cut was sealed with parafin wax. The unit was put into a growth chamber under lights and observations recorded for bud sprouting 3x per week for three weeks.

<u>BF symptoms</u>. Damage to individual buds could be determined by cutting into the buds and observing for characteristic necrotic spots at the growing points inside the buds. Examination was made of the non-forced buds at the end of a 3 week trial and from shoot collections made at the end of the season.

Results

1. Bud forcing patterns

<u>Normal vs. BF</u>. Figure 2A shows the seasonal pattern for bud sprouting (% of buds grownd at the end of 3 weeks) for all of the samples of the normal vs. BF trees, comparison 1. These results show that the buds on the normal trees retained their relative sprouting capacity throughout the season with some increase with time to a maximum in early September followed by a decrease. The latter may represent either initiation of rest period or some shift to flower buds.

The buds on the BF plants, on the other hand, showed consistently reduced sprouting capacity from early July through the rest of the season with a sharp decrease beginning early August. This trend evidently represents an increasing expression of BF symptom development throughout the summer with actual damage taking place from the end of August. This loss of sprouting was due to actual damage to be buds, which became evident by inspection made during September.

Figure 2B presents the same results but in a different manner by using the rate of sprouting (as expressed by the days required to produce 50% sprouting). Although buds on the normal trees showed some inhibition in June and early July, growth activity was consistently uniform through July and August. From September sprouting inhibition increased suggesting the induction of "rest". In contrast the buds from the BF trees showed inhibition from the earliest samples in June through July and August with sharp increase in bud sprouting potential in September and August when the primary symptoms developed.

Stressed vs. nonstressed. Figure 3A compares the seasonal bud sprouting pattern for stressed and nonstressed Nonpareil. Bud sprouting was nearly 100 per cent in June and early July bud showed a consistent decline through July and August leveling off in September and later. This decline occurred earlier in the summer than it did in the younger trees of Figure 2 and may reflect more severe damage and possibly more stress even in the irrigated trees.

The buds on the nonirrigated trees followed the same pattern as the irrigated through early July but showed a sharp decline. There was an indication of possible recovery by September. However, it is not certain from this data whether the differences between the two curves was actually significant. (We have not yet analyzed this particular data statistically).

Figure 3B gives a clearer effect of the stress by expressing the data as rate of sprouting. Here the two treatments were identical through mid-July with the "wet" treatment showing increasing inhibition through the rest of the season. However, the "dry" treatment resulted in consistently greater inhibition from mid July through mid September. The decreased inhibition may reflect the occurrence of 3 to 4 inches of rain that occurred in mid-September.

Bud growth

Sampling of buds for the amino acid studies required obtaining fresh weights of bud samples at weekly intervals throughout the year. This procedure provided an additional measurement of changes taking place in the buds during the season.

Normal vs. BF. Figure 4A and B show the trends in fresh bud weight from shoots on comparable normal Nonpareil trees growing at Winters and at Davis. Shoots on these moderately vigorous young trees consisted of 15 to 25 nodes. Such shoots show gradients in bud development in that the basal buds are laid down first, go into dormancy first and often are more resistant to BF injury. Consequently, buds were collected both from the basal part and from the terminal part of the shoot and are to be analyzed separately.

Tip buds (Fig. 4A) show a distinct trend of increasing bud size from June through September. The first important point about these graphs is that the data for Winters and Davis is essentially identical. The second point has to do with seasonal trends. Prior studies have shown that during June there is a transition involving bud scale formation. The reduction in bud weight at late June may be an artifact of the sampling but prior observations suggest it may represent a specific physiological point. From mid September there is a flattening of the curve suggesting the initiation of the "rest period". The basal buds (Figure 4B) are about the same size as the tip buds at the start of the summer and show a similar but much less marked growth pattern for the rest of the summer. Again the Winters and Davis data is essentially the same except that there were some fluctuations in size at Davis.

Comparing buds from normal and BF plants, important differences are shown (Fig. 4C). First, the apical buds on normal plants were consistently larger in size throughout the entire season except in June. Secondly. this difference reflects in part a difference in the "timing" of development. Prior work suggests a reduction in bud weight during the transition between the "budscale" phase in June and the induction of "summer dormancy" and active bud enlargement during July and August. THus the buds from BF shoots show a delay of about 2 weeks in the start of the "summer dormancy" phase (see June 27) as well as in the induction of "winter dormancy or rest" (compare early September and early October. Basal buds (Figure 4D) are also larger from the normal plant.

Stressed vs. nonstressed. Nonpareil BF. Figure 5A compares the bud growth of the irrigated Nonpareil BF trees at Winters and at Davis. Because the shoot growth was shorter and buds more uniform on these larger trees, bud collections were made only from the center of the shoot. The patterns at Winters and Davis were almost identical with a few more fluctuations in the pattern at Davis. However, there was an increase from a minimum in late June and an increase to a leveling off point at in mid-October. Similarly the data for the same trees without irrigation (Figure 5B) showed essentially the same data except for more fluctuations at Davis. These irregularities may reflect some problems with irrigation scheduling during the early July period.

Comparisons of irrigated vs. non-irrigated trees showed that the bud size in the irrigated plots (Fig. 5C) were consistently higher throughout the entire season with the bud size leveling off at early October. A similar comparison occurred at Davis (Fig. 5D). The fact that bud size was identical during the July period indicates that an irrigation artifact had occurred.

Stressed vs. nonstressed. Milow. Bud growth of Milow (nonBF) at Davis and Winters were essentially identical in both the irrigated (Figure 6A) and nonirrigated (Fig. 6B) plots with the same greater fluctuations occurring in the Davis area. In all cases there is an indication of leveling off in bud size suggesting the initiation of "winter dormancy".

Comparing the wet vs. dry at Winters, the buds on irrigated trees were consistently larger than those of the dry trees (Fig. 6C). There appears to be a distinct leveling off in the September period. At Davis, the same trend aoccurred although the difference was disappeared by September. Again there was a distinct leveling off during September (Fig. 6D).

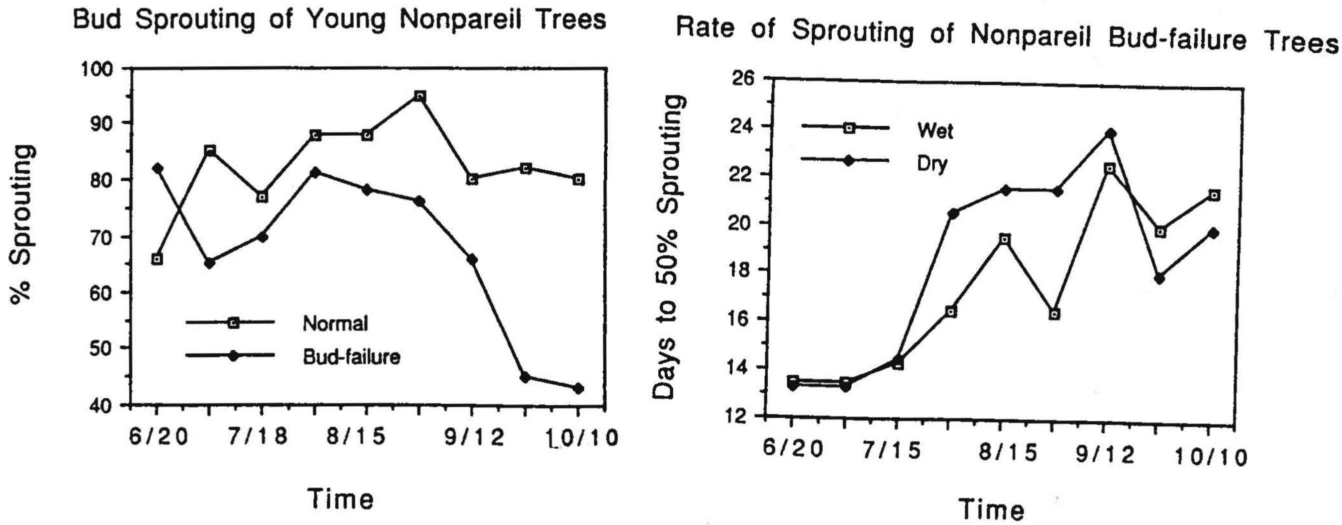


Figure 2. Bud sprouting patterns of Nonpareil (normal) compared to Nonpareil BF (Clement source) during 1989 at Winters, California. Three year old trees. Left. Percent sprouting. Right. Rate of sprouting as shown by days to produce 50% bud emergence.

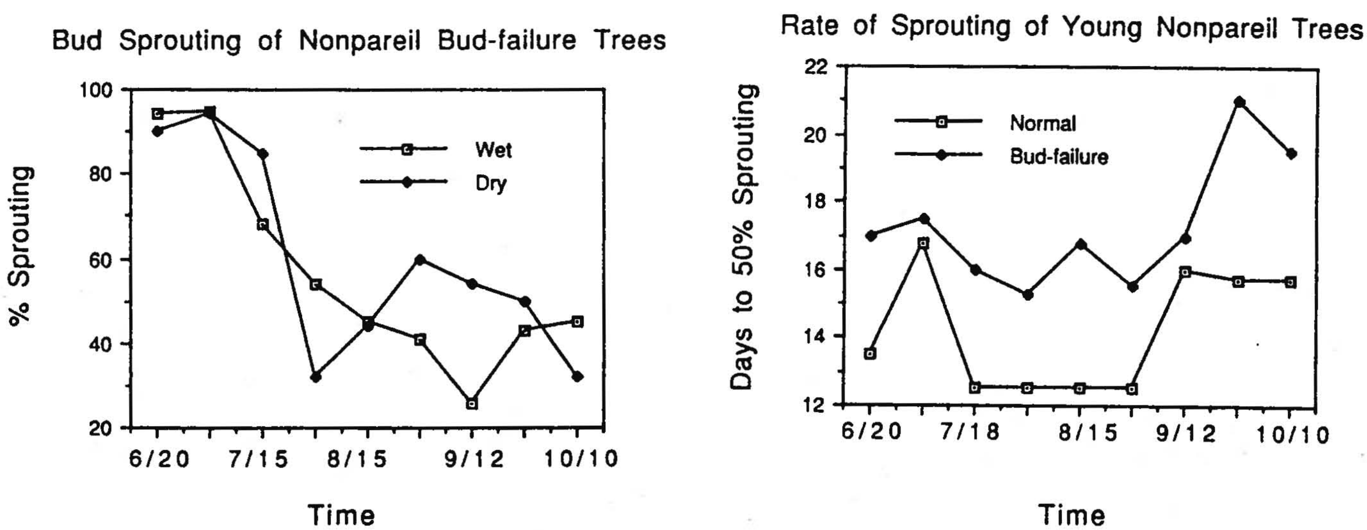


Figure 3. Bud sprouting patterns of Nonpareil BF during 1989 in mature trees comparing nonirrigated (dry) and irrigated (wet). Left. Percent spouting. Right. Rate of sprouting as shown by days to produce 50% bud emergence.

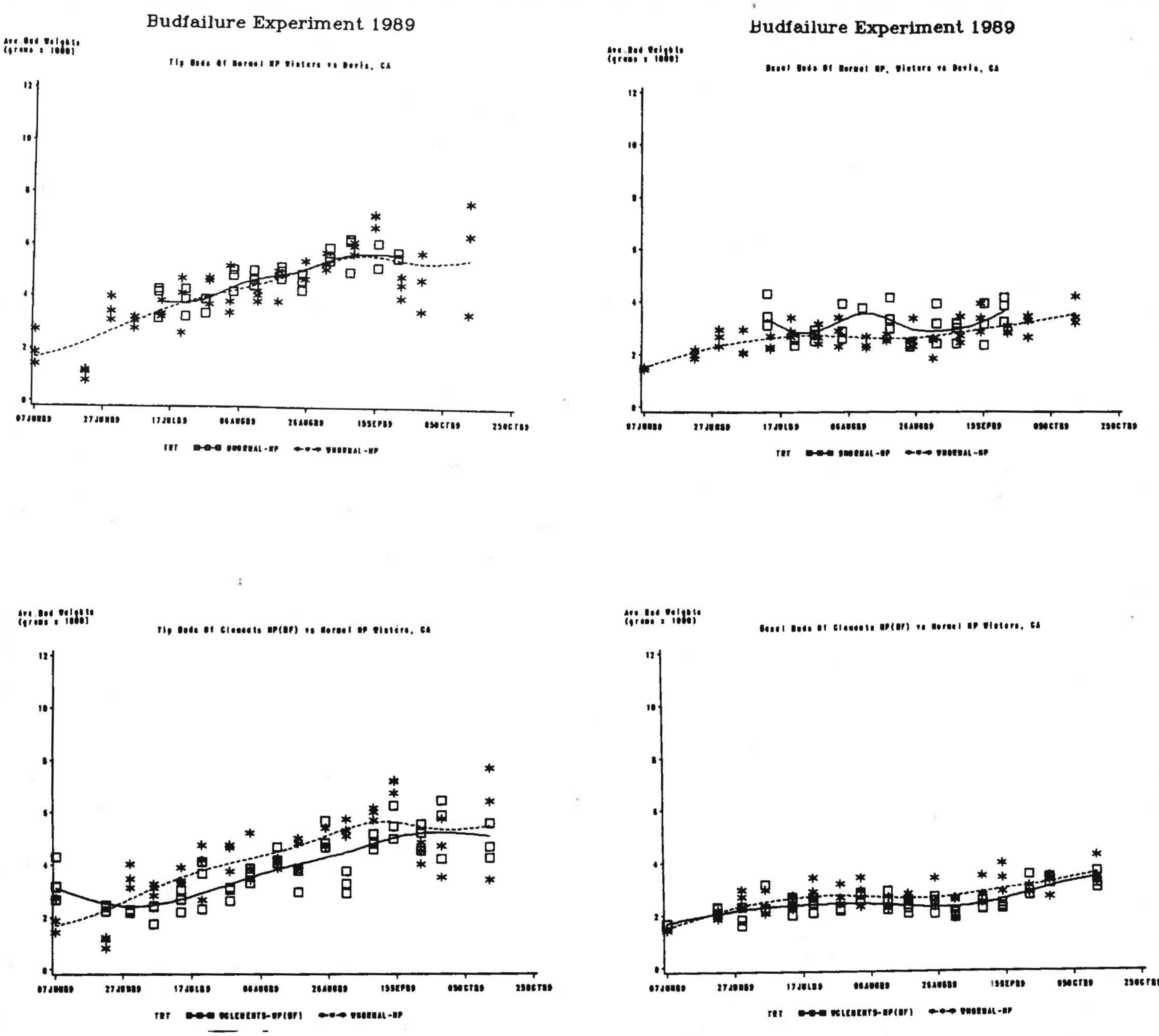


Figure 4. Bud growth patterns as shown by average fresh weight at measured at weekly intervals during the summer of 1989 for normal trees at Davis (solid line) and Winters (dashed line).

<u>Upper left</u>. Apical buds.
<u>Upper right</u>. Basal buds.
<u>Lower left</u>. Compares bud weight pattern of normal (dashed line) and BF buds (solid line): apical buds.
<u>Lower right</u>. Same comparison for basal buds.

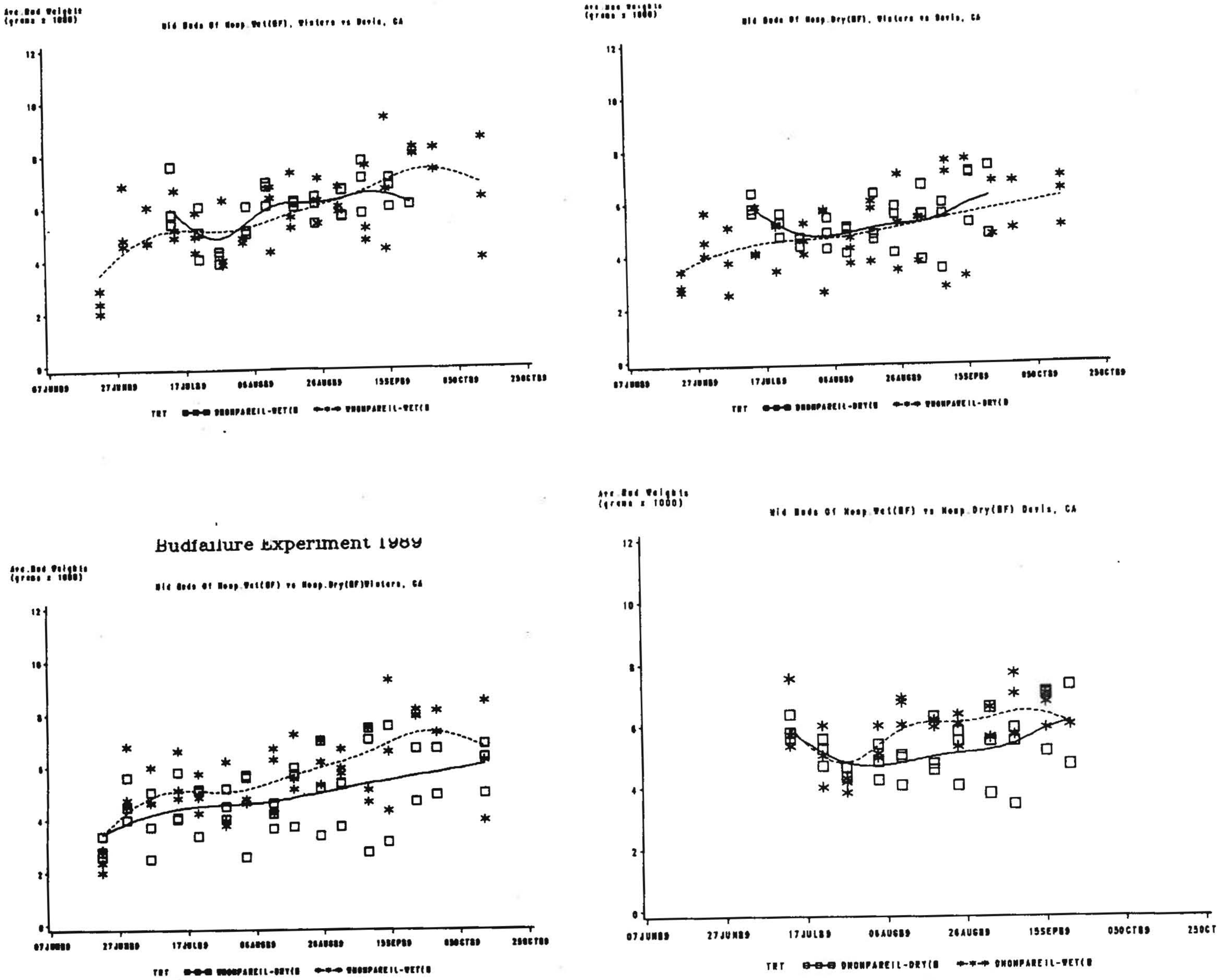


Figure 5. Bud growth patterns in Nonpareil BF trees as shown by average fresh weight as measured at weekly intervals during the summer of 1989 for BF trees.

<u>Upper left</u>. Compares buds at Winters (dashed line) and Davis (solid line) from irrigated trees.
<u>Upper right</u>. Same comparison for nonirrigated trees.
<u>Lower left</u>. Compares buds from irrigated (dashed line) with nonirrigated (solid line) at Winters.
<u>Lower right</u>. Compares buds at Davis.

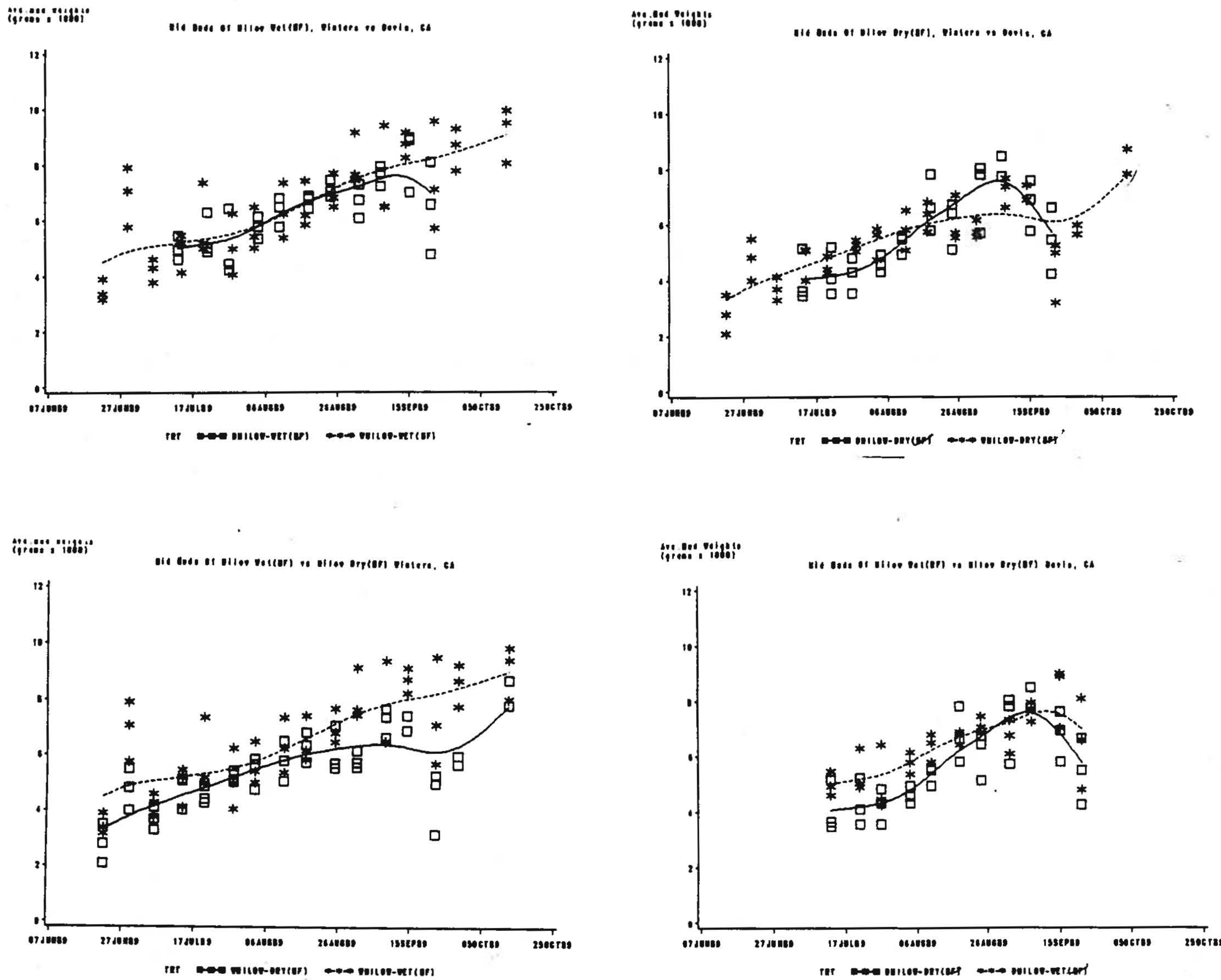


Figure 6. Bud growth patterns in Milow as shown by average fresh weight as measured at weekly intervals during 1989.

<u>Upper left</u>. Compares Winters (dashed line) and Davis (solid line). Irrigated orchard.
<u>Upper right</u>. Compares Winters (dashed line) and Davis (solid line). nonirrigated.
<u>Lower left</u>. Compares irrigated (dashed line) and nonirrigated (solid line) at Winters.
<u>Lower right</u>. Compares irrigated (dashed line) and nonirrigated (solid line) at Davis.