Project No. 87-K14-Tree and Crop Research
Bud Failure

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Objectives: The following have been long-range objectives: (1) Develop a mathematical model for describing variability and development of bud failure (BF) potential that can be used to predict future BF expression within varieties and sources. (2) Develop selection procedures and apply them to the development of low BF potential propagation sources with protocols for maintenance, multiplication and distribution. (3) Determine the inheritance pattern for BF potential in different progeny of commercial almond, wild almond and almond-peach parentage and identify low BF potential germplasm and varieties. (4) Determine the seasonal pattern and physiological basis for the expression of symptoms and the effect of environmental conditions on them. (5) Establish inherent differences in BF potential by cell and tissue cultures with emphasis on the role of proline (or other compounds) as a marker or causal agent in the expression of bud failure. (6) Develop a shoot tip culture system as a method of maintaining, multiplying and distribution of source-clones of predetermined BF potential.

Interpretive Summary: Observations continued on 6 Carmel orchards (Kern, Fresno, Cos.) which were the basis of the BF development model. (A paper by Fenton, Kester and Kuniyuki describing this model is being published in Phytopathology). Increased incidence of BF affected trees occurred this spring indicating that 1986 was a higher than normal inductive year. The reasons for high incidence of BF trees in spring 1987 are not completely obvious from the 1986 temperature patterns but the long dry fall may have been a factor, subjecting orchards to greater stress. Likewise there was greater than usual shoot growth occurring during the 1986 season as a result of the reduced crop loads on the tree. Vigorous shoot growth has been shown to be one of the conditions conducive to higher BF-potential.

We have extended the model to analyze the relationships among the extent of BF development within the tree, the age of initiation of symptoms, and the proportion of the framework affected. Early appearance of BF usually means that all subsequent parts of the tree are affected and any extensive pruning back will only enhance its reappearance although it may be delayed.

Further observations were made on the progeny trees planted in 1973 from 15 Nonpareil source orchards. These also showed increased incidence of BF affected branches this spring. We are further defining the difference between SOURCE ORCHARD SELECTION (use of an entire orchard for budwood collection) as compared to SOURCE-CLONE SELECTION (single tree used as a

source of budwood followed by vegetative progeny testing in a high temperature area). As reported earlier, we have a series of apparently low BF-potential source clones of Nonpareil which are available for propagation. Visual inspection of the source trees is unreliable for the detection or measurement of BF-potential. Visual inspection of the progeny trees however, can provide a test for BF potential of the source. In addition to the vegetative propagation tests, BF inheritance studies underway now and previously have indicated that some types of seedling progeny tests involving crosses to selected parents may also provide a method of measuring BF-potential.

Concern continues to be felt for the selection of low BF-potential sources of Carmel.

During the past two years, special research has been conducted to study the relationship of specific biochemical substances, notably amino acids and one, in particular, proline, to BF. The concentration of some 45 compounds have been compared in leaves and buds from normal and BF affected plants collected throughout the year. Increased levels of proline have been found to occur in the leaves and buds of BF plants during September and later in both 1985 and 1986 which coincides with the time that visible evidence of bud necrosis was beginning to appear. Particular attention is being focused on the period prior to that time.

Shoot tip cultures have been established from normal (low BF-potential) and affected (high BF-potential sources) Nonpareil trees. Some differences between the low BF and high BF-sources have occurred during their establishment. We are looking at this procedure as a way to maintain and multiply source material without change of BF-potential.

Additional cases of Mission affected by Nonproductive Syndrome (NPS) have surfaced this year. Examination of orchard-progeny relationships indicate that this occurrence is a continuation of the problem which we researched previously, which involved the low frequency incidence of mutants in commercial budwood source orchards. A comprehensive summary of the present and previous research on this problem is in progress.

Particular effort is being made to complete the analysis of previously developed data and information on both the BF and the NPS problem and to prepare research papers and publications on the different aspects of these problems.

Objective 1, 2, and 3. See Interpretive summary.

Objective 3. SEASONAL PATTERN AND PHYSIOLOGICAL BASIS FOR SYMPTOM EXPRESSION.

Amino acid analyses of leaves and buds collected in summer and fall 1985 (Durzan and Ventimiglia) showed a correlation between higher BF-potential, BF symptom development and increased proline levels. The sharp increase in proline in late summer and early fall coincided with the time when leaves deteriorated and abscised and the bud develops a rest period. The pattern suggested an hypothesis in which toxic substances which were released during leaf senescence to the bud and surrounding tissue caused the necrosis. Excessive proline production or another correlated substance(s) could be the agent of the necrosis or at least a marker for disruption of the normal processes of development.

In other experiments, proline content of cells in culture from high BF-potential (affected) sources was greater than in cells from low BF-potential sources although exceptions occurred (Fenton, et al., in press).

Since then, two series of studies have been carried out with the following purposes:

1. To study the seasonal pattern of free amino acids and related compounds that are detected by the methods used in high BF-potential (affected) and low BF-potential (normal) source clones (with Don Durzan and Frank Ventimiglia).

2. To compare proline concentrations between normal and BF affected sources at different times of the year and at different locations. The object was to extend the baseline information of proline concentration as an indicator of BF-potential level.

Procedures:

Amino acid analyses of leaves and buds of BF-affected and non-affected Nonpareil were carried out during 1986. Forty five different substances were analyzed simultaneously in both leaves and buds. Collections were made weekly, biweekly, or monthly from June through the following February.

1. The source-clones used were from Nonpareil and included source-clones 3-8-1-63 (high BF-potential) and 3-8-2-72 (low BF-potential). The BF affected trees (3-8-1-63) were growing at the WEO Orchard, Winters in a separate block (Field 8) with Milow, a non BF-affected pollinizer. The normal (3-8-2-70) samples came from a single tree in a variety collection nearby (Field 7). The trees were more than ten years old and had standard pruning and care. Three replications were collected at each collection date. In addition, chlorophyll content was measured, as was tetrozolium, a staining test which had been used by Fenton to measure viability of living cells.

Shoots were collected from August through November to monitor the development of BF symptoms and to measure the growth and dormancy status. (a) In one series, individual buds were dissected upon collection and the presence of flower buds, vegetative buds and evidence of necrosis. (b) In a second series, single node cuttings in petri dishes were placed in a lighted growth chamber to allow buds to sprout. The percentage of vegetative buds growing at two weeks was used as an "index of sprouting".

2. For the second proline study buds and leaves of normal and BF affected source clones at Davis or WEO, Winters were collected at monthly intervals from various sources and locations. Ten replications were used at each sampling. Proline was analyzed by the method of Bates, et al. ( ).

RESULTS

I. GROWTH AND BUD DEVELOPMENT PATTERNS IN NORMAL NONPAREIL

The seasonal changes in total soluble amino acids (Figure 1), proline concentration (Figure 2) and proline as a percentage of total amino acids (Figure 3) provide baseline patterns for growth and bud development cycles in the plant as well as correlations to significant periods of the seasonal growth cycle. Figure 4 shows the changes in growth potential in buds on single node shoots with time from July through November. Table 1 compares the bud morphology of shoots (a) collected for initial examination and (b) those used in forcing. In general, longer shoots were used for examination and the shorter shoots for forcing. Approximately half of the buds were flower buds.

Stage 1. ACTIVE PERIOD OF SPRING GROWTH.

Active growth began with the emergence of new shoots in mid-February and the elongation of shoots during March and April. When collections were started April 1, the shoots had attained about 8-10 inches in length. The succulent apical shoot tip used for analysis extended down the shoot several inches to where leaves were about half full size, avoiding the basal part of the shoot. By the first of May, shoot growth had ceased and the leaves had attained full size and were mature.

Concentration of total amino acids (Fig. 1) in the leaves was high during this period as was proline (Fig. 2). The percentage proline of total free amino acids was about 25% (Fig. 3).

Stage 2. DEVELOPMENT OF BUDS AND BUD SCALES.

From May to early June, the growing points in the axils of the leaves harden into buds with well developed bud scales (Hellali, 1978). At the start of this period, shoot growth stop and leaves complete their expansion and become mature. Amino acid concentration dropped to a lower level as did

both percentage and total amount of proline, indicating less metabolic activity.

Stage 3. QUIESCENT BUD STAGE or SUMMER DORMANCY.

After initiation of the bud scales the vegetative buds are dormant but quiescent through the remainder of the summer and do not grow unless forced by pruning, removal from the plant or pinching (June, July, August). Earlier studies (Hellali, 1978) had shown that buds undergo a growth period as shown by increase in size during late June and early July, a period correlated to increased levels of growth promoting substances. This growth period is apparently shown by the increase in total amino acids during late June and early July. Other earlier studies (Kester and Liu, 1980 Report and later) have shown that the shoot growth potential (as shown by forcing treatments) remains high during June, July and early August, with a tendency to decrease during the summer. During August, about 40% of the buds initiated into flower buds although these cannot be identified morphologically until mid September. From early September there was a sharp decrease in shoot growth potential to a low level through the remainder of October (Figure 4).

Both amino acid concentration (Figure 1) and total proline (Figure 2) in the leaves gradually decreased from early July to the end of August. Consequently, percentage proline remained constant (Figure 3).

Stage 4. DECLINE IN GROWTH POTENTIAL AND INITIATION OF REST IN BUDS.

In September the forcing potential of vegetative buds decreased sharply and remained at a low level through November. Flower buds could be distinguished from September.

Both soluble amino acids and proline continued to decrease in the leaves until the end of August. The percentage of proline of total amino acids showed a sharp increase in later August increasing to a peak in late September and early October. At this point there was a sharp reduction associated with the deterioration and abscission of the leaves.

The pattern of growth potential in the buds now begins to show important correlations to proline. Total amino acids increased during late September, which is accounted for primarily by the increase in both proline percentage and concentration. There was a single low point in mid October (which could be an error in measurement) but the concentration leveled off until mid-November. It is difficult to avoid reaching the conclusion that changes in proline concentration is closely associated with the pattern of rest induction, maintenance and subsequent emergence. During this period, proline concentration fluctuates around a value of 20 to 30 per cent of all the soluble amino acids.

Stage 5. EMERGENCE FROM REST.

Even though the buds appeared dormant, there is evidence that the vegetative buds were coming out of their rest period during November (Table

1). Other studies conducted previously had indicated that in most years "end-of-rest" for almond occurs around mid-December. However, independent studies in 1986 by Weinbaum (unpublished) also had shown that "end-of-rest" occurred in early November during that season.

From mid-November through mid-January, total amino acids showed gradual increase reaching a very high level in mid-February at the time of shoot emergence and flowering occurred. The pattern appears to correlate closely with the increasing growth potential of the buds after the rest period is over and temperatures begin to increase. During the early part of the winter, proline concentration increases along with the amino acid concentration but the percentage of proline begins to decrease, suggesting a change in the role of proline during this growth period.

II. DEVIATIONS FROM NORMAL PATTERN SHOWN IN THE BF-AFFECTED PLANT

In general, the amino acid and proline patterns in the BF-affected plant showed a similar pattern to that of the normal plant but there were certain differences (Figures 6,7,8). During STAGE 1 (active growth) the slight differences in pattern may reflect differences in timing of growth as well as amount and rate. Shoots on the BF plant emerge from the few surviving buds and tend to grow vigorously and later in the spring. The differences shown by amino acid patterns and proline concentrations in the BF-affected and non-affected shoots and leaves may be accounted for by shifts in the pattern of growth rather than representing basic differences in amino acid or proline metabolism. The initiation of STAGE 2, therefore, may have been delayed in the BF plant. However, this difference in timing may be significant in the overall bud-failure development pattern, however growth during high temperature periods early in the growing season has been suggested as contributing to change in BF-potential.

During STAGE 3 (summer dormancy or quiescence), the rise in amino acid activity in late June was less, as compared to that in the normal plant, and was followed by a gradual decline during June, July and August with sharp fluctuations which could relate to temperature variation. Since the percentage proline was not different between the normal and the BF plant, the total proline concentration in the leaves was low and in these collections less than in the normal. (This pattern is not completely consistent with other data, particularly from 1987, in which the proline concentration was higher in the BF leaves during this period. See later.)

In STAGE 4, important differences between the normal and the BF occur. The bud sprouting potential on the BF shoots remained high during August and early September and although following the pattern shown by the normal was consistently high. The pattern suggests a delay in rest induction during this period. Evidence for bud necrosis buds in the BF shoots began to appear late August and September 1 (Fig. 5). The percentage increased through mid- September and then fluctuated with sampling until it increased to near one hundred percentage by December. The final bud-failure percentage was nearly 100 per cent in most of the trees at leafing out but specific counts were not made. Likewise amino acid concentration in the leaves tended to be higher with considerable fluctuation during September

and October. There was a sharp increase in both percentage or proline and total proline concentration during the same period. The subsequent sharp decline apparently is a response to the deterioration of the leaves which occurred earlier in the BF plant than the normal.

The free amino acid concentration in the vegetative buds of the BF plant during September and October followed the same general pattern as the normal plant except to increase more sharply than in the normal. The parallel increase in proline, in both percentage and concentration, was 2 to 3 times that of the normal. Increase proline appears to have come at the same time or after the appearance of the symptoms except that there was a higher concentration in the buds on the BF plant in August. During Stage 4, the proline accounts for 40 to 50% of the total free amino acid concentration.

In STAGE 5, the buds on the BF plant showed a sharp increase in proline concentration beginning as early as October being much higher than in the buds of the normal plant, including the time of emergence of the shoots in February.

III. SPECIFIC AMINO ACID METABOLIC PATTERNS

Simultaneous analysis of 45 amino acids in buds detected 17 specific kinds. Differences were quantitative rather than qualitative. Proline was the most important amino acid but others were glycine, alanine, asparagine, glutamic acid, threonine and valine (Fig. 9). Figure 8 shows differences in alanine in which a fluctuating pattern occurs with both the normal and BF samples with time with that of the BF material possibly being delayed in sequence. When alanine values of the BF and normal samples are plotted against each other (Fig. 10 right) a cyclic pattern is shown during the critical September to October period.

A further report will be made as the data on the remainder of the amino acids is analyzed.

IV. COMPARATIVE PROLINE ANALYSES AND CONTINUATION PATTERN

Proline concentration was measured in leaves and buds at consecutive monthly intervals from September 1986 through September 1987 (Tables 2 and 3). When the average monthly values for all Winters samplings were compared, there was significantly greater concentration of proline in the tissues of the high BF-potential Nonpareil source than those of the low BF-potential source. The overall range of samples from normal and BF samples at Davis were not found to be significantly different from each other. However, inspection of the Davis data shows that lower proline concentrations occurred during the period of rapid growth in the spring. This suggests differences might be in timing of shoot growth rather than in inherent differences of tissues. When comparisons are made at other times of the year, as in the quiescent period during June through August, there was consistently greater proline in the BF tissue.

Overall proline concentration was greater in tissues collected from Winters than Davis. Increased BF expression and BF-potential development at Winters, as compared to Davis, has been associated with the higher average summer temperatures and accumulated degree days that exist at Winters (Kester and Asay, 1977). Flower buds were found not only to have a higher proline concentration of proline than leaf buds but the buds from BF plants also produced more. Flower buds are more resistant to the factor producing necrosis.

When proline values are connected in a time sequence, a second but less detailed seasonal pattern for proline is produced in the leaves and buds (both flower and shoot) or normal and BF affected Nonpareil at both Winters and Davis (Fig. 11) that extends to the end of summer 1987. The pattern shown is similar to that produced in the 1986 study. However, in 1987 the proline concentration of leaves during summer 1987 is significantly higher than in 1986.

V. COMPARISONS OF PROLINE CONCENTRATION IN DIFFERENT NONPAREIL SOURCES.

1. Leaves

Proline concentration was compared in leaves of six separate Nonpareil source-clones growing at the FSPMS Foundation orchard at Davis (Table 4). In addition, collections were made from six separate source trees at Winters with different degrees of BF expression.

Leaves collected in August from 4 of the 5 source-clones growing in the Davis Foundation Orchard (FSPMS) showed lower proline concentrations than the BF source-clone 3-8-1-63. The 5th source - one that has tested as low BF-potential showed a proline concentration near to that of 3-8-1-63. Leaves collected at Winters showed consistently higher proline concentration than at Davis and a correlation with BF symptoms in the source tree.

Samples collected one month later (late September) showed similar trends but the concentration of the normal source trees increased to near the BF sources obscuring the differences found with the other samples.

2. Shoot tip cultures.

Proline concentration was tested in leaf samples collected from shoot cultures. The first series of cultures were started March 87 from trees in Irrigation block, Davis. Experiment was started September 22, 1987.

Nonpareil 3-8-1-63	High BF-potential	1102 + 343 μg/g proline
Nonpareil 3-8-2-70	Low BF-potential	584 + 235 μg/g proline

The second series of cultures originating from scion source trees in FSPMS block, UCD.

July 28, 1987	Nonpareil 3-8-1-63	High	3206 + 2308 μg/g
	Nonpareil 3-8-2-70	Low	2139 + 1212 μg/g
Sept. 22, 1987	Nonpareil 3-8-1-63	High	716 + 235 μg/g
	Nonpareil 3-8-2-63	Low	1029 + 355 μg/g

3. Callus cultures.

One series was started directly from shoots, started June 1985, transferred monthly. Experiment June 1987. The results were as follows:

Nonpareil 3-8-1-63 High BF-potential in source plant:

"Fluffy", fast growing callus - 416 + 96 μm/g
"Watery", slower growing callus - 181 + 54 μm/g

Nonpareil 3-8-2-70 Low BF-potential in source plant:

slower growing callus - 156 + 54 μm/g

A second series of callus was started in 1985 from a suspension culture which itself was started in 1982. Thereafter the callus was maintained for an additional year with monthly transfers before testing at the same time as the cultures of the previous culture. The results were as follows:

Nonpareil 3-8-2-70 Low BF-potential in source plant:

Vigorous growing callus
Proline concentration = 140 + 15 μm/g.

SUMMARY AND CONCLUSION

At present perhaps the most important outcome of the study is to establish a basic pattern correlating proline and free amino acids to growth and development. These can serve as a basic model of the natural seasonal cycle in almond. There is a clear indication that proline plays a major role in the natural growth cycle particularly in the processes of bud maturity and development of the rest period. It has been associated widely in physiological research in resistance and tolerance to stress. An increase in proline concentration has frequently been reported to occur in plant tissue in response to exposure to stress. Thus proline could serve as a physiological marker for changes of physiological activity in the plant irrespective of the relationship to BF.

BF apparently acts to disrupt the normally functioning of the plant to resist stress and thus deviations of the proline pattern might be expected to occur. Two possibilities exist (at least). One is that the disruption of the normal stress reaction and normal developmental pattern in the buds in the fall results in the overproduction of proline (or associated substances) that itself causes the necrosis as high concentrations of proline produced in the bud are released to the subtending bud as a consequence to the leaf deterioration and abscission. Or does the presence of a BF factor stimulate the production of proline, a process which does not result in the actual development of resistance of the tissues in the buds?

At present, only correlative relationships are shown. Tests to measure the direct toxicity of proline, other substances or extracts, could provide

some indication of causal effect and establish whether proline (or other substances) has a toxicity (causal) or a protective (effect) role.

The previous paragraphs have described possible relationships of proline to the induction of or protection from the development of necrosis that produces the BF symptoms. Another relationship is the difference in inherent proline concentration of the cells under nonsymptomatic conditions. Comparisons made on leaves, buds, cells and callus show that in most cases, high BF-potential sources have an inherently higher proline concentration than the lower BF-potential. These differences are not always consistent, however, and the seasonal study shows that differences in timing of seasonal patterns alone can have major effects on proline concentration. However, the comparisons made so far do suggest that there may be an association between of BF-potential of particular sources and their inherent proline level. To show that this possibility could have practical use, standardization of material and procedures are needed following which a wide range of genetic materials and sources under different environmental and management conditions should be tested.

VI. TISSUE CULTURE AND MICROPROPAGATION OF NORMAL AND BF SOURCES OF NONPAREIL

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I. Micropropagation

New shoot tip culture explants were started about the beginning of 1986 with new material collected every two weeks from normal and BF Nonpareil sources. These explants were established into culture, and then transferred to new test tubes for multiplication.

A. Early season explanting

Material established in January-February initially started well but then began to deteriorate. Shoot tip explants from BF trees were more adversely affected than those from normal sources. Shoots from BF plants also differed because they produced more callus. Eventually, however, all explants of the first culture series died.

B. Young shoot explants

Success in explanting and continuous subsequent culture resulted when explants were taken from actively growing young shoots collected in March and April. Once established, these lines of shoot tips have been growing well and vigorously from both normal and BF sources providing that the plants were transferred frequently, at least every three weeks.

Most shoots tend to grow more from the basal lateral shoots whereas the terminal shoots tended to be inhibited and sometime stopped growing. Comparison has been made during consecutive transfers from (a) the basal lateral shoots and (b) continuous culture of the terminal shoots. Culture from terminal shoots multiplies at a greater rate than from the lateral

shoots but terminal shoots eventually slow down. Eventually one can divide the cultured multiplied shoots into single shoots which can be rooted and transplanted to the field.

BF-potential was increased in consecutive propagations in the greenhouse and in the field by growing plants periods at high (80° or more) temperatures and maintained or increased at a lower rate at lower (70°F) temperatures (Kester, Hellali and Asay, 1976). Thus the possibility exists that BF-potential could be controlled by growing in culture. However some method of monitoring the change is needed. Proline concentration could be useful in this regard if correlation could be shown.

II. Tissue culture

Tissue culture lines established by Lou Fenton from normal and BF Nonpareil sources (Fenton, et al., I, II, in press) have been maintained as both cell suspension or as reconstituted callus. In addition new tissue culture lines were established directly from normal and BF sources in 1976. Significant differences between tissue lines have been described in growth rate, and response to temperature. At the present time, the older callus lines from the BF source have tended to have declined in growth capacity (a shift from what had occurred earlier) and the Callus lines from the normal source had increased in growth capacity. In contrast, the callus lines directly from BF tissues had shown high growth capacity since the beginning of their development and the callus lines from normal have continued to show much reduced growth capacity.

Recently observations have been made that these growth capacities are associated with the production of two kinds of cells. One is a "fluffy type" with actively growing cells; the other is a "watery type" which is slow growing. Callus masses vary in their capacity to produce these two types of cells such that continued culture involved some selection towards one or the other type. There is some correlation between these two types of cells and the BF-potential of the source of the material as well as changes during continued culture which may involve selection towards one or the other type.

Studies on callus and cell cultures have found that proline levels of the BF-affected sources were higher in general than that of the non-affected sources (Fenton, Kester and Liu, in press). These differences were not always consistent and variation could be related to the growth status and age of the cultures. Nevertheless, there is a concept that BF cells have an inherently higher potential for proline production as if they were in a constant state of stress. Similarly higher proline levels have been reported to be higher in other cases of plants susceptible or resistant to stress factors such as drought tolerance, virus infection or nematode resistance.

Table 1. Bud development patterns in normal (3-8-2-70) and BF (3-8-1-63) sources of Nonpareil almond. Winters, CA, 1986.

Source		Total No. of shoots	Ave. No. of Nodes	Ave. No. of Buds	Ave. % Flower buds	Ave. % of vegetative buds with BF
			A. Shoot morphology experiment			
3-8-1-63	BF	63	20.6	29.4	43	39
3-8-2-70	N	52	20.0	27.8	57	0
Milow	N	5	18.0	18.4	60	0
			B. Forcing Experiment			
3-8-1-65	BF		9.9	15.2	41	-
3-8-2-70	N	39	11.2	18.7	33	-

Table 2. Proline concentration of tissue samples from low BF-potential and high BF-potential sources of Nonpareil at Winters, CA.

Date	Proline concentration			Prob. for sig. (1)
	Nonpareil		Milow	
	normal	BF	normal	
	A. Vegetative buds			
September 16	174 ± 68	207 ± 54	418 ± 63	
October 14	253 ± 70	533 ± 100	701 ± 208	
November 11	459 ± 127	1044 ± 161	602 ± 116	
December 15	684 ± 162	962 ± 106	529 ± 118	
January 19	1213 ± 271	1509 ± 238	413 ± 106	
February 24	8395 ± 1120	9620 ± 1115	9620 ± 1000	
	B. Flower buds			
October 14	489 ± 74	758 ± 108	1508 ± 253	
November 11	834 ± 183	1408 ± 293	1761 ± 260	
December 15	1274 ± 218	1296 ± 184	1485 ± 329	
January 19	2221 ± 490	3119 ± 512	1552 ± 238	
February 24	2170 ± 725	3225 ± 560	1780 ± 445	
April 1	1068 ± 139	2248 ± 270	751 ± 95	
April 28	371 ± 73	752 ± 49	1208 ± 120	
	C. Leaves			
September 16, 1986 - April 1	620 ± 95	2248 ± 279	750 ± 95	
April 28	616 ± 90	829 ± 135	348 ± 79	
June 1	318 ± 14	411 ± 26	220 ± 10	
June 29	123 ± 30	339 ± 49	462 ± 178	
July 28	246 ± 78	335 ± 90	258 ± 78	
August 24	228 ± 56	365 ± 75	308 ± 68	
September 28	384 ± 69	384 ± 51	384 ± 53	

	T value	Probability
normal Nonpareil vs. BF Nonpareil	2.8	.010
normal Nonpareil vs. Milow	(1)	(1)
BF Nonpareil vs. Milow	(1)	(1)

(1) Calculations not completed.

Table 3. Proline concentration of low BF-potential and high BF-potential sources at Davis CA (FSMPS) during 1986-87 season.

Date	Proline normal nmoles/gr	Proline BF nmoles/gr	Prob. for diff. (1)	BF/N Dav. %	BF/N WEO %	WEO/Dav (2) BF %	WEO/Dav (2) N %
			A. Leaves (1986)				
June 16	279	317		113	-	-	-
July 14	518	699		120	-	-	-
August 13	306	374		122	-	-	-
September 15	600	671		118	-	-	-
October 13	313	281		90	-	-	-
			B. Vegetative buds (1986-87)				
August 13	278	275		99	-	-	-
September 15	370	900		118	120	42	40
October 13	247	168		92	210	190	81
November 10	692	806		116	227	130	66
December 15	415	477		115	141	202	165
January 13	627	850		136	124	178	193
February 24	7438	6970		93	115	138	113
			C. Leaves (1987)				
April 1, 1987	2985	6970		48	362	46	21
April 22	1178	568		116	135	146	52
May 26	270	303		129	129	131	118
June 22	199	256		129	136	180	170
August 25	169	281		167	136	129	135
September 15	225	254		113	100	151	171

normal Nonpareil vs. BF Nonpareil
t value = 0.815
prob. = (low)
no statistical significance due to collections on February 22 and April 22.

(1) Calculations not completed.
(2) Compares Table 2 and Table 3.

Figure Legends

Fig. 1. Seasonal pattern of total soluble amino acids in leaves and buds of a mature Nonpareil almond tree at Winters, CAlif. Lower: leaves: upper: buds. Micromoles/gram fresh weight. 1986.

Fig. 2. Seasonal pattern of proline in leaves and buds of a mature tree of Nonpareil almond at Winters, CA. Lower: upper, lower: buds. Nanomoles/gram fresh weight. 1986.

Fig. 3. Seasonal pattern of proline in percent of total amino acids in leaves and buds of a mature tree of Nonpareil almond at Winters, CA. Lower leaves: leaves; upper: buds. 1986.

Fig. 4. Change in sprouting potential of vegetative buds through summer and fall in single node shoot cuttings collected at Winters, CA. Fall 1986.

Fig. 5. Percentage of necrotic (BF) buds observed on samples of shoots collected from BF trees. Fall, 1986.

Fig. 6. Comparison of seasonal pattern in total amino acids (micromoles/gram fresh weight) in high BF-potential (3-8-1-63) and low BF-potential sources (3-8-2-70) in Nonpareil almond. Winters, CA. 1986. micromoles/gram fresh weight.

Fig. 7. Comparison of seasonal pattern of proline (nanomoles/gram fresh weight) in high BF-potential (3-8-1-63) and low BF-potential sources (3-8-2-70) in Nonpareil almond. Winters, CA. 1986.

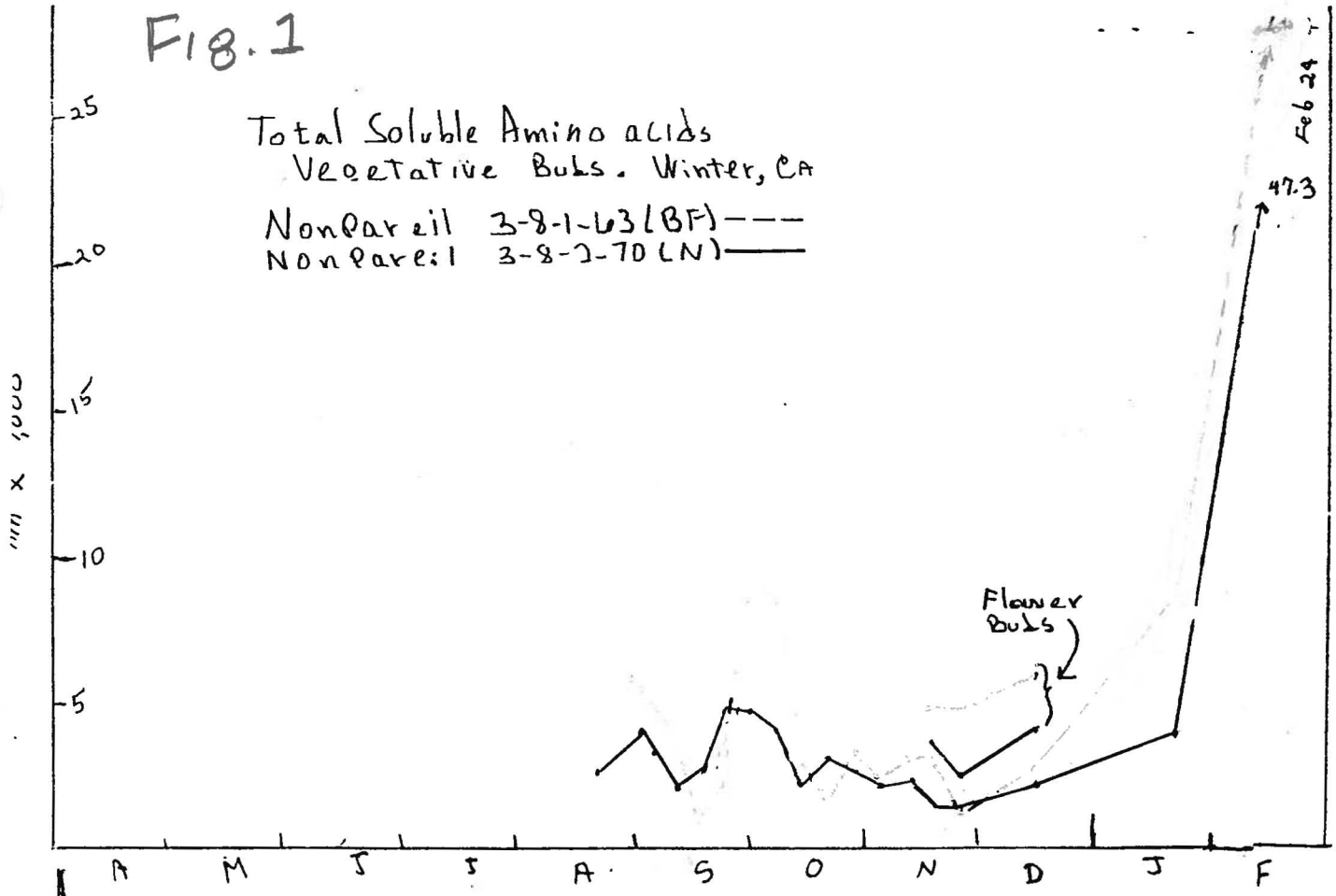
Fig. 8. Comparison of seasonal patterns of proline (% of total amino acids) in high BF-potential (3-8-1-63) and low BF-potential sources (3-8-2-70) in Nonpareil almond. Winters, CA. 1986.

Fig. 9. Comparison of different amino acid families found in buds of normal and BF source plants during fall 1987.

Fig. 10. Comparison of alanine found in buds of normal and BF source plants during fall 1987. Left. Concentration plotted against time. Right. Concentration of alanine in BF buds plotted against concentration of normal buds.

Fig. 11. Comparison of proline concentrations obtained from samples of leaves and buds through 1986 and 1987 at Winters, CA.

Fig. 1


Total Soluble Amino acids
Vegetative Buls. Winter, CA
NonPareil 3-8-1-63 (BF) ---
NonPareil 3-8-2-70 (N) ——
mm x 1000
25
20
15
10
5
Feb 24
47.3
Flower Buls
A M J J A S O N D J F

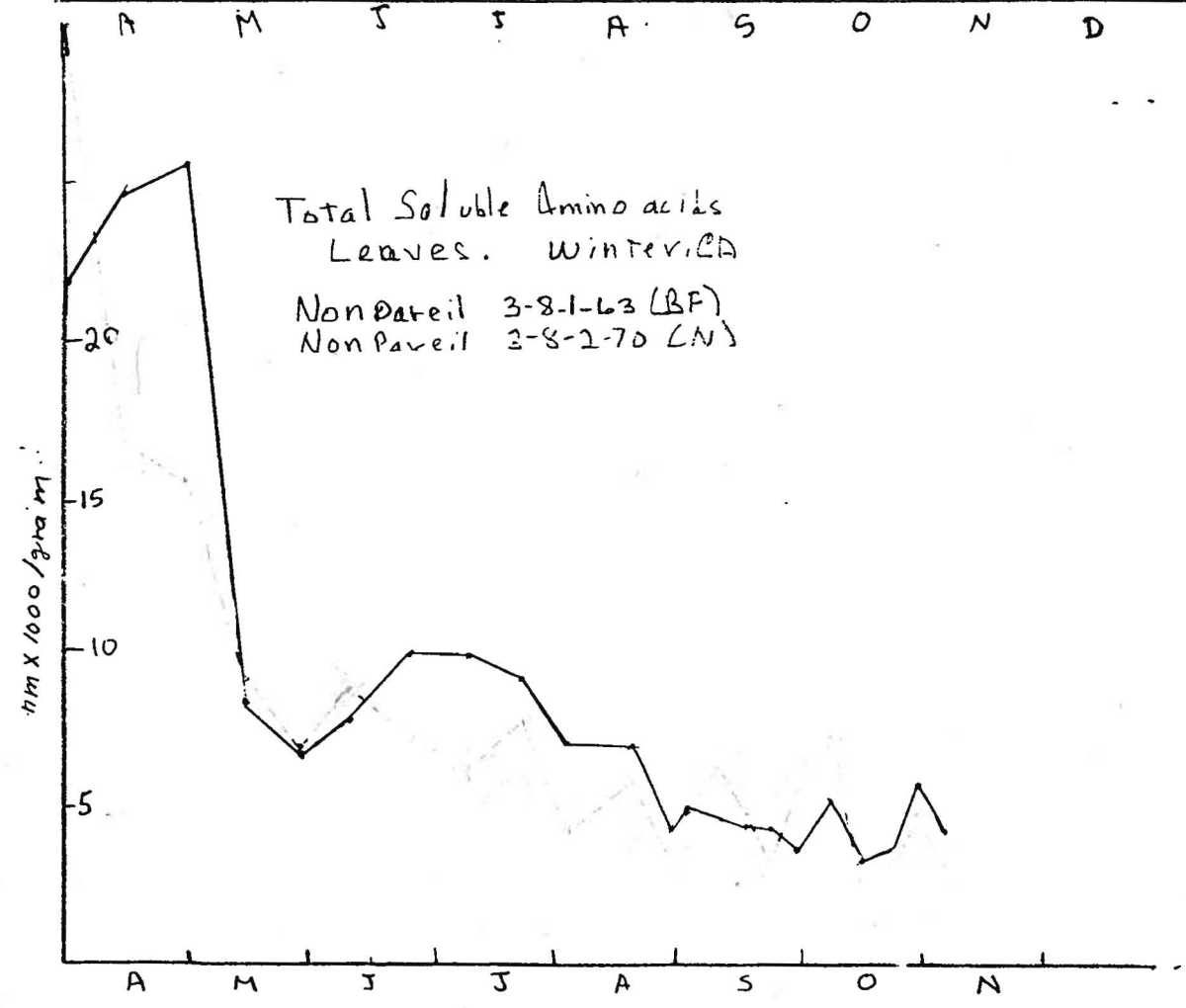
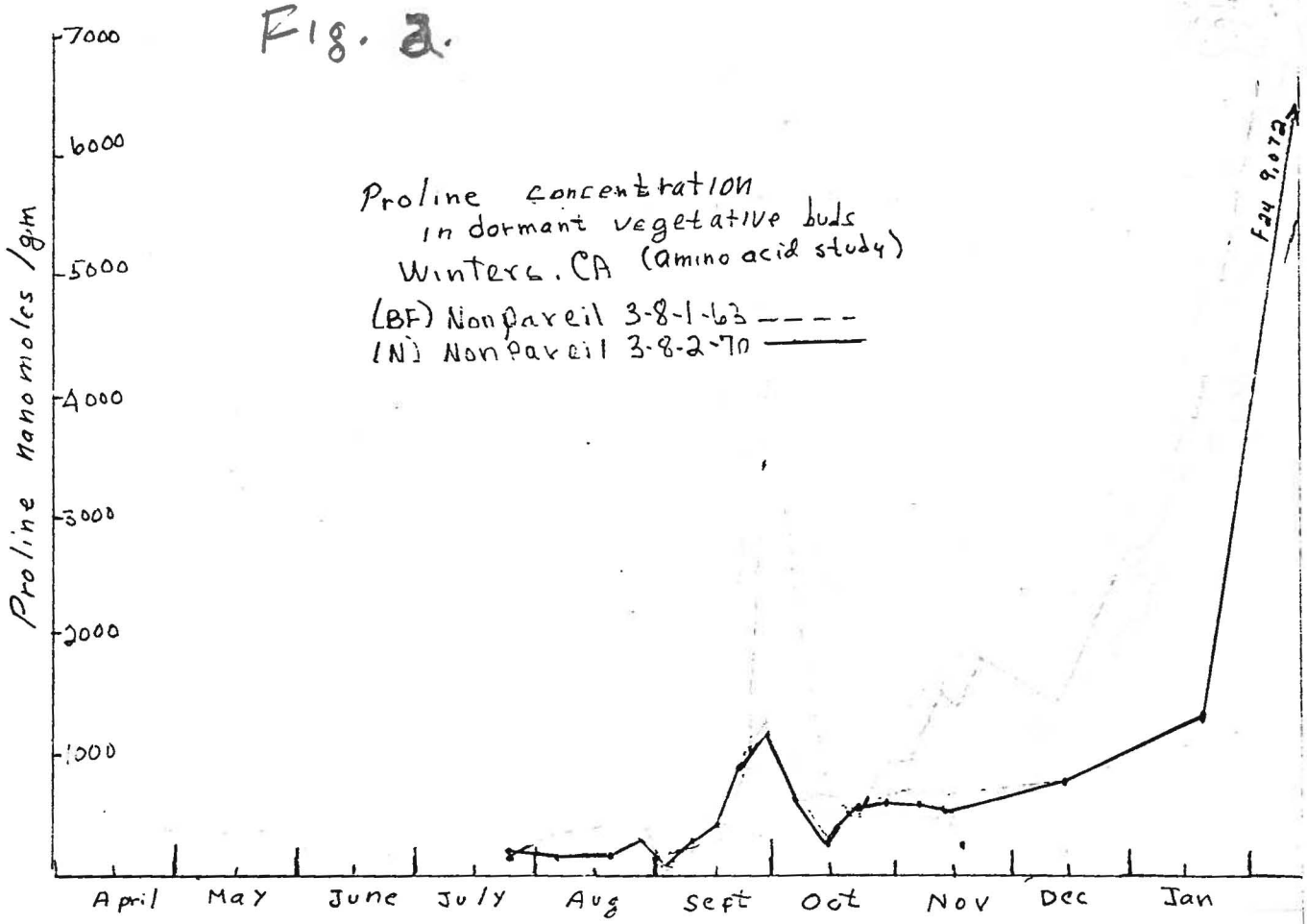

Total Soluble Amino acids
Leaves. Winter, CA
NonPareil 3-8-1-63 (BF)
NonPareil 3-8-2-70 (N)
mm x 1000/gram
20
15
10
5
A M J J A S O N

Fig. 2.


Proline concentration
in dormant vegetative buds
Winters, CA (amino acid study)
(BF) Nonpareil 3-8-1-63 - - - -
(N) Nonpareil 3-8-2-70 ——
Feb 9, 72
Proline nanomoles /gm
7000
6000
5000
4000
3000
2000
1000
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan

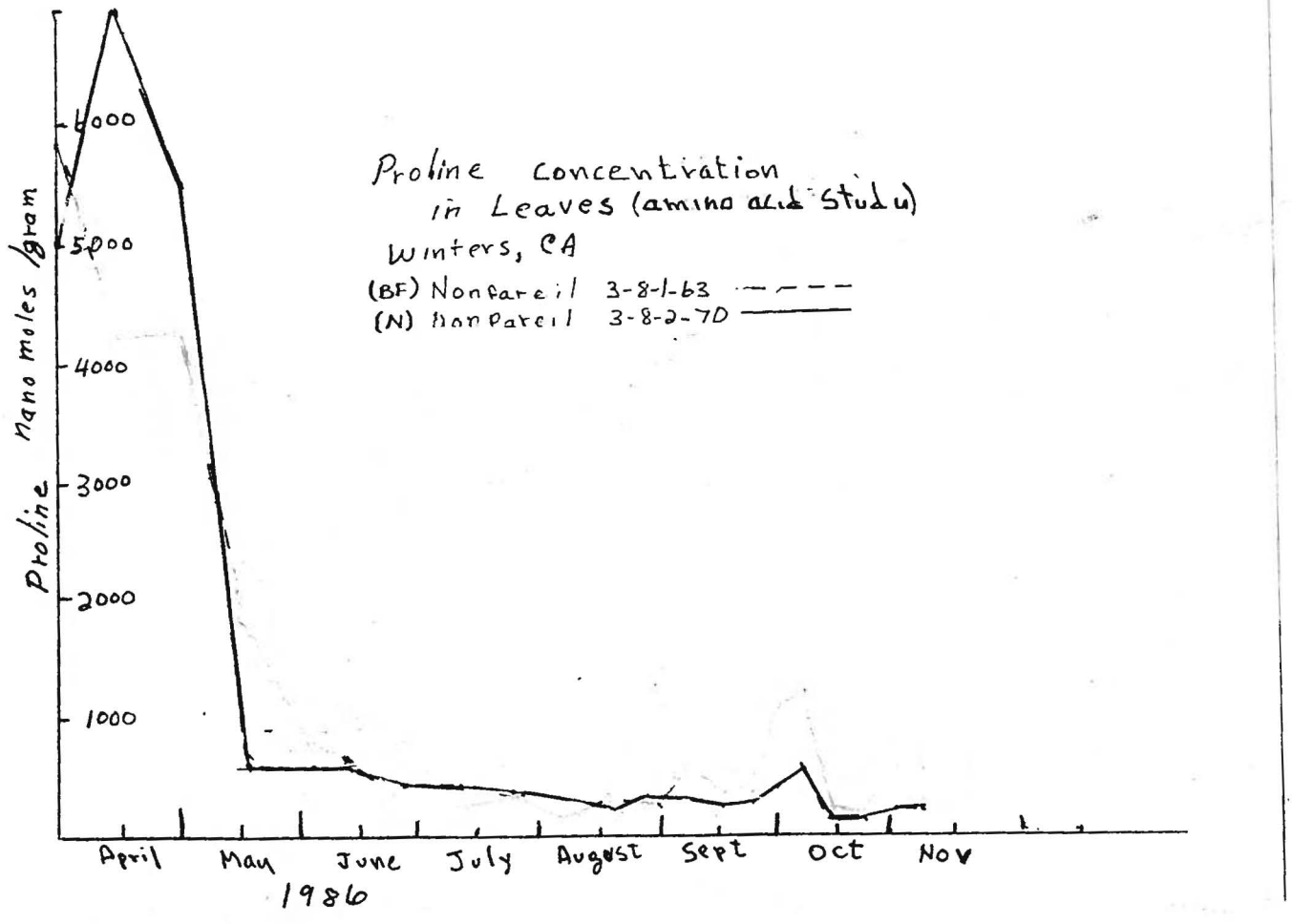
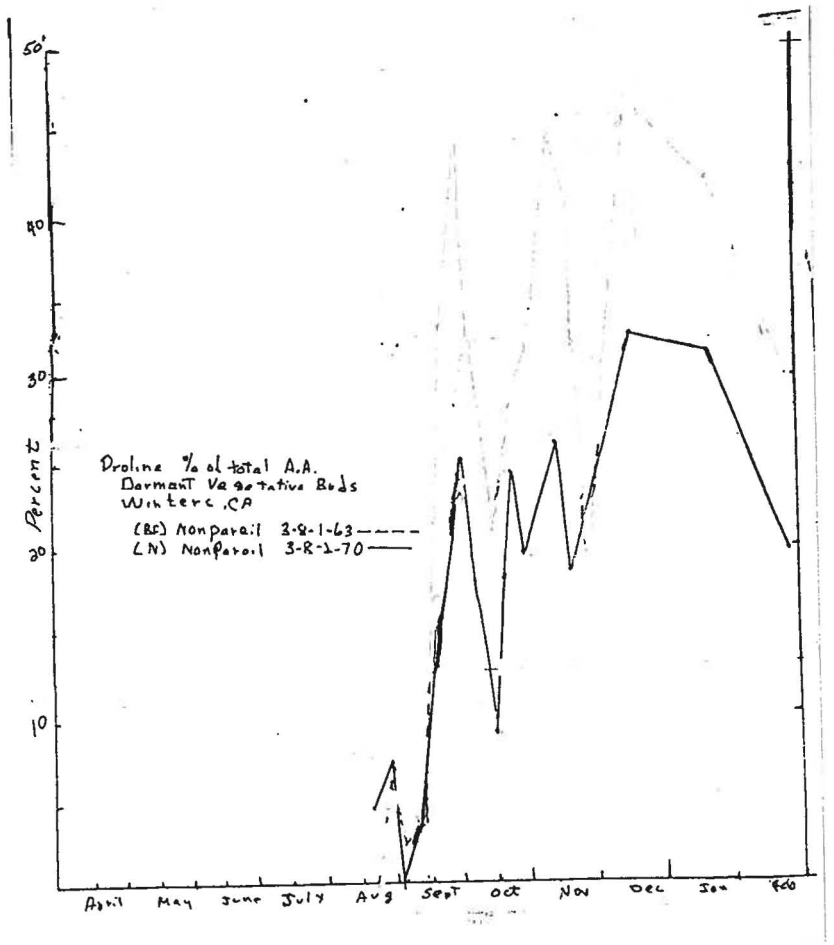

Proline concentration
in Leaves (amino acid study)
Winters, CA
(BF) Nonpareil 3-8-1-63 - - - -
(N) Nonpareil 3-8-2-70 ——
Proline nano moles /gram
6000
5000
4000
3000
2000
1000
April
May
June
July
August
Sept
Oct
Nov
1986

Fig. 3


Proline % of total A.A.
Dormant Vegetative Buds
Winters, CA
(BF) Nonpareil 3-8-1-63
(LN) Nonpareil 3-R-1-70
Percent
50
40
30
20
10
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Feb

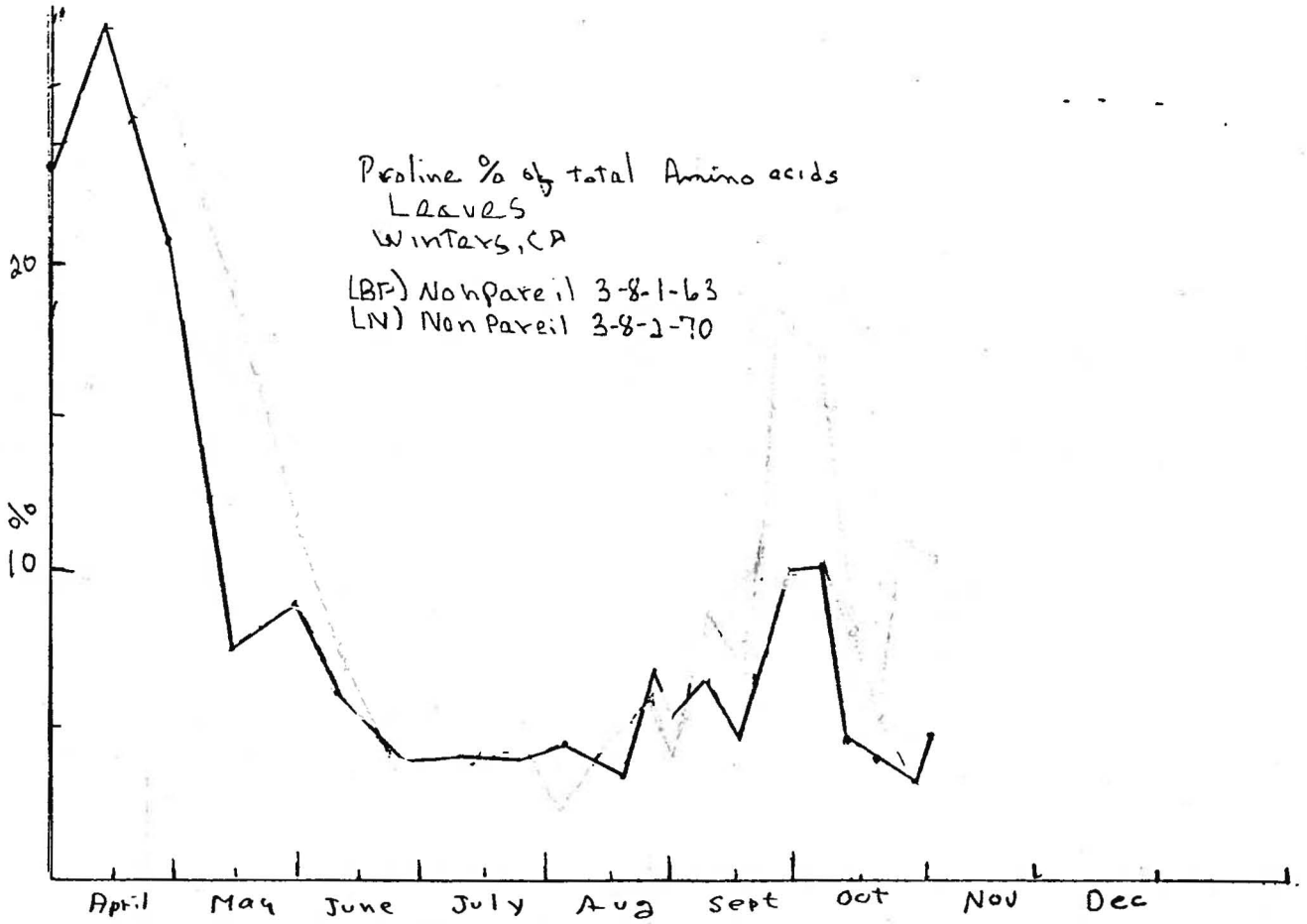

Proline % of total Amino acids
Leaves
Winters, CA
(BF) Nonpareil 3-8-1-63
(LN) Nonpareil 3-8-2-70
20
%
10
April
May
June
July
Aug
Sept
Oct
Nov
Dec

Fig. 5.

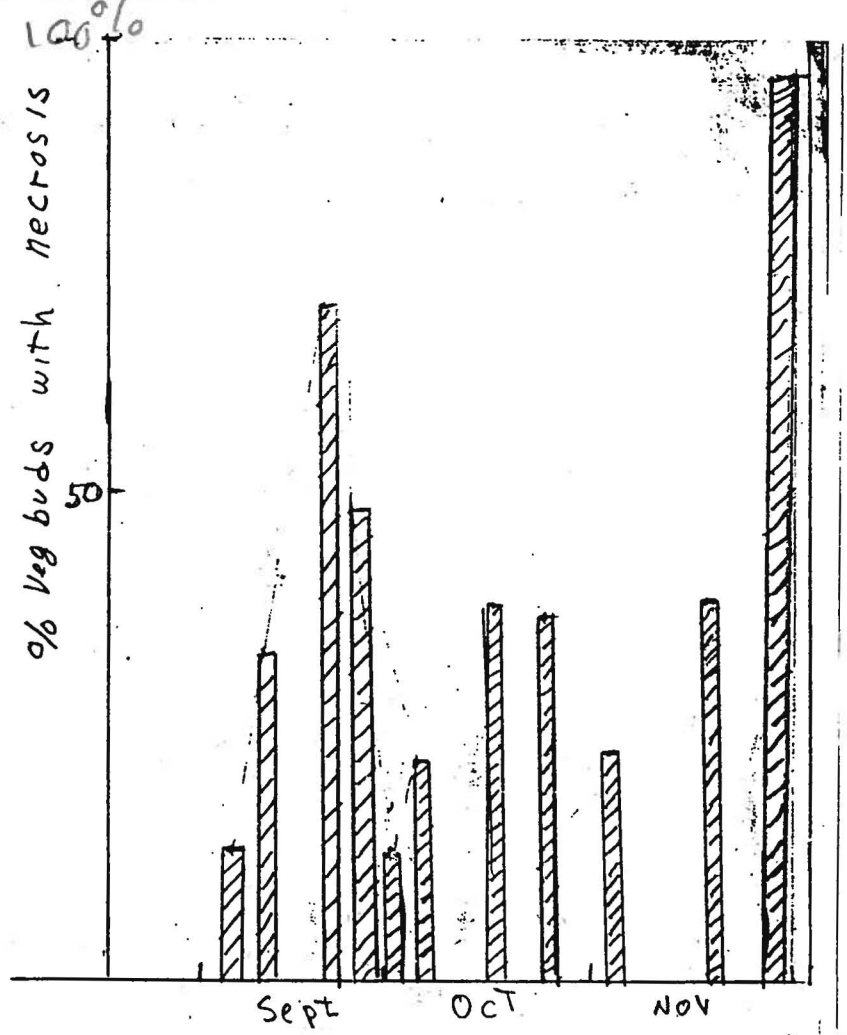

100%
% veg buds with necrosis
50
Sept
Oct
Nov

Fig. 4.

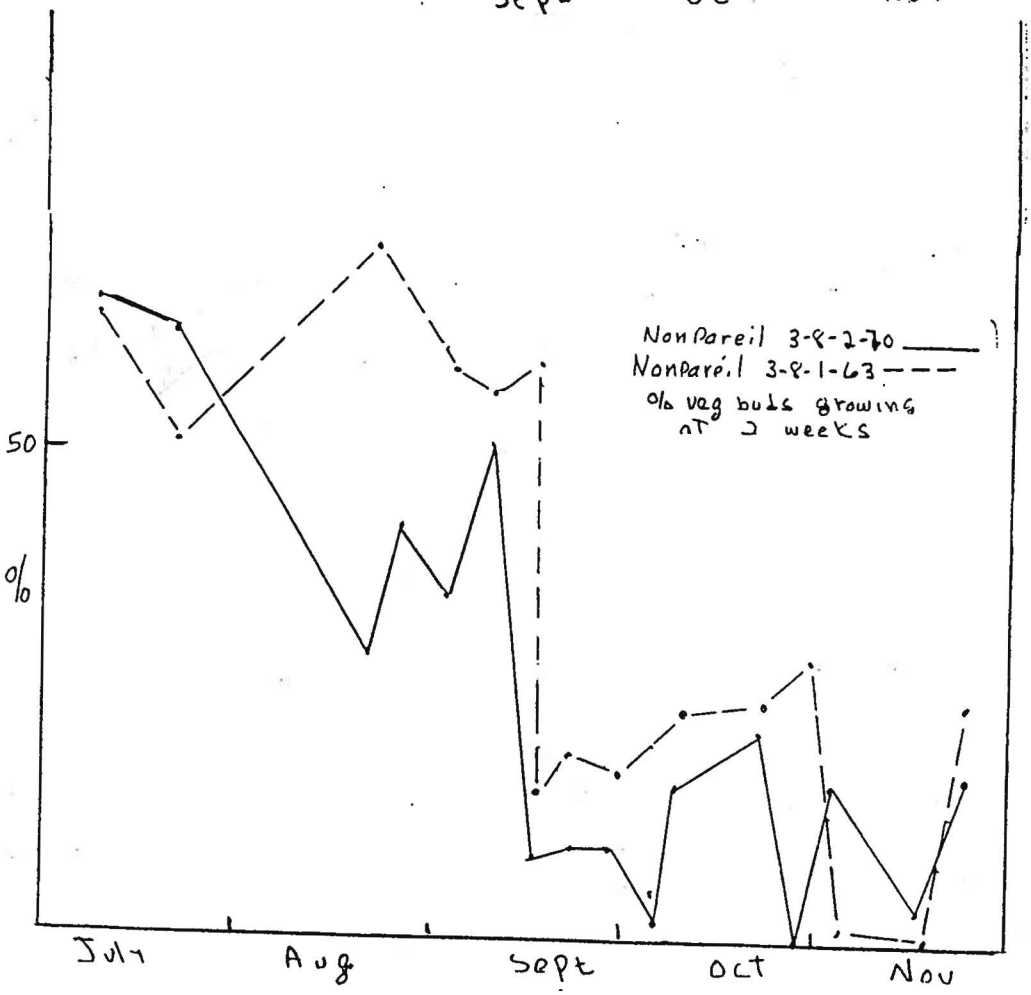
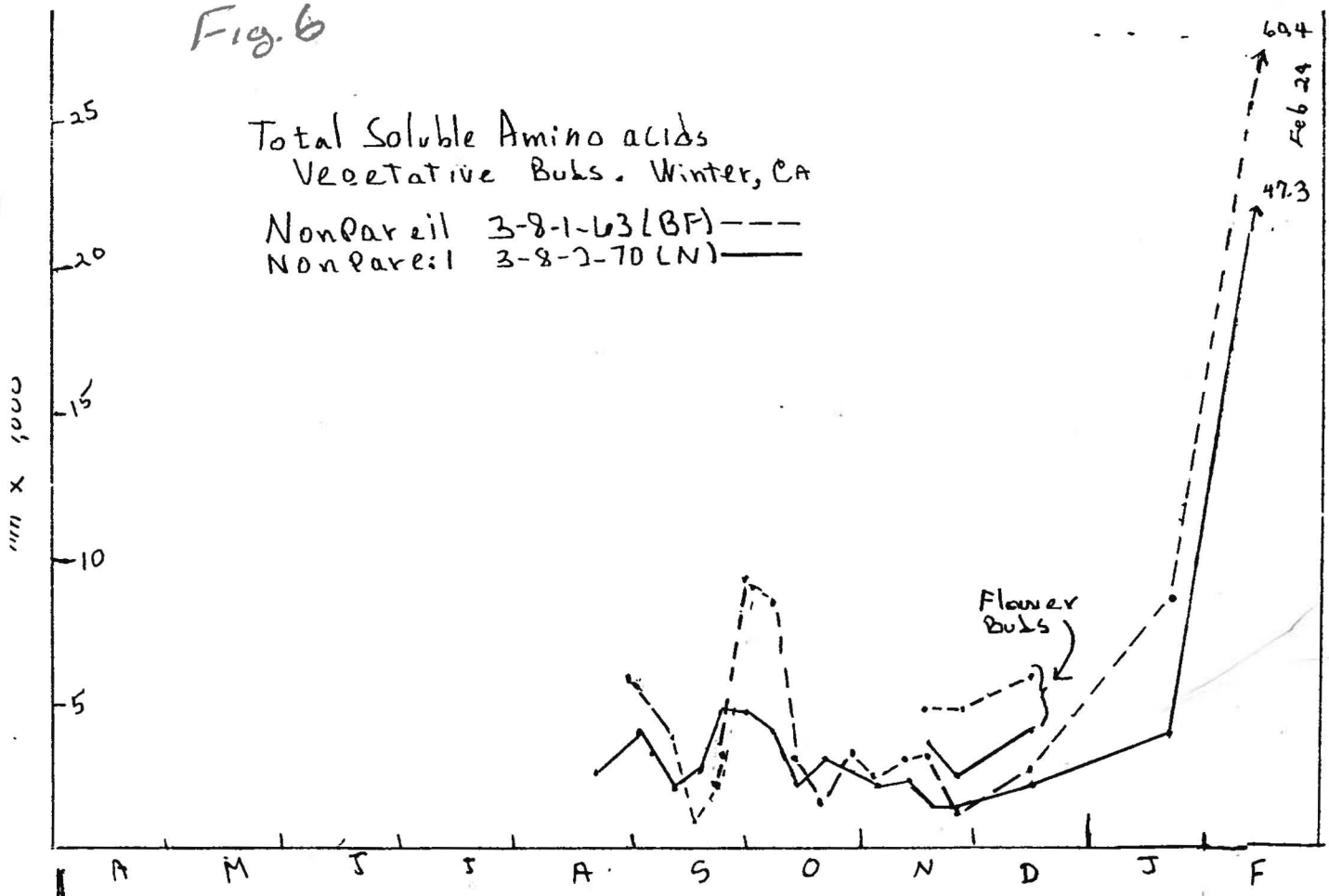

Nonpareil 3-8-2-70
Nonpareil 3-8-1-63
% veg buds growing at 3 weeks
50
%
July
Aug
Sept
Oct
Nov

Fig. 6


Total Soluble Amino acids
Vegetative Buds. Winter, CA
NonPareil 3-8-1-63 (BF)
NonPareil 3-8-2-70 (N)
Flower Buds
604
Feb 29
47.3
25
20
15
10
5
A M J J A S O N D J F

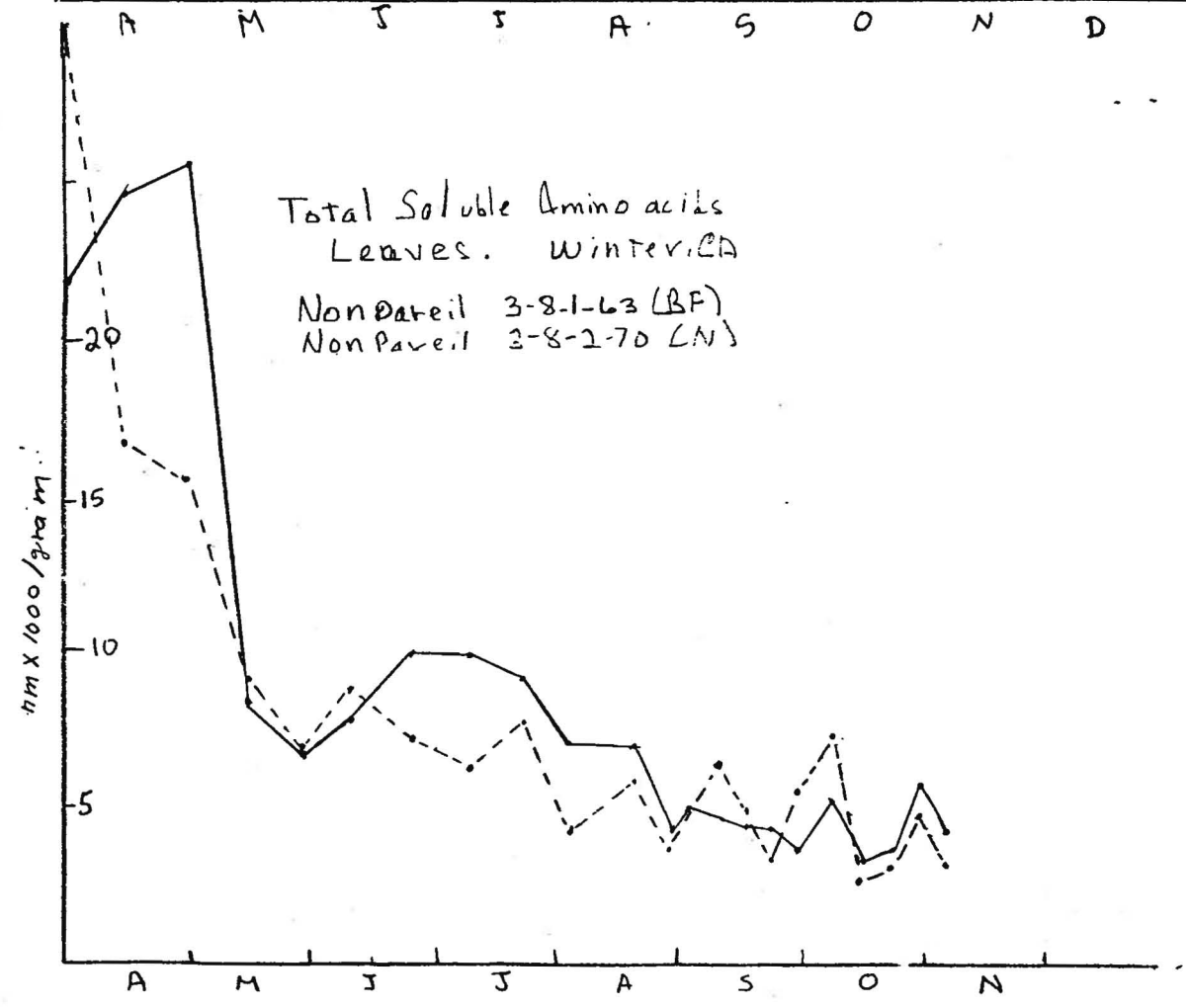
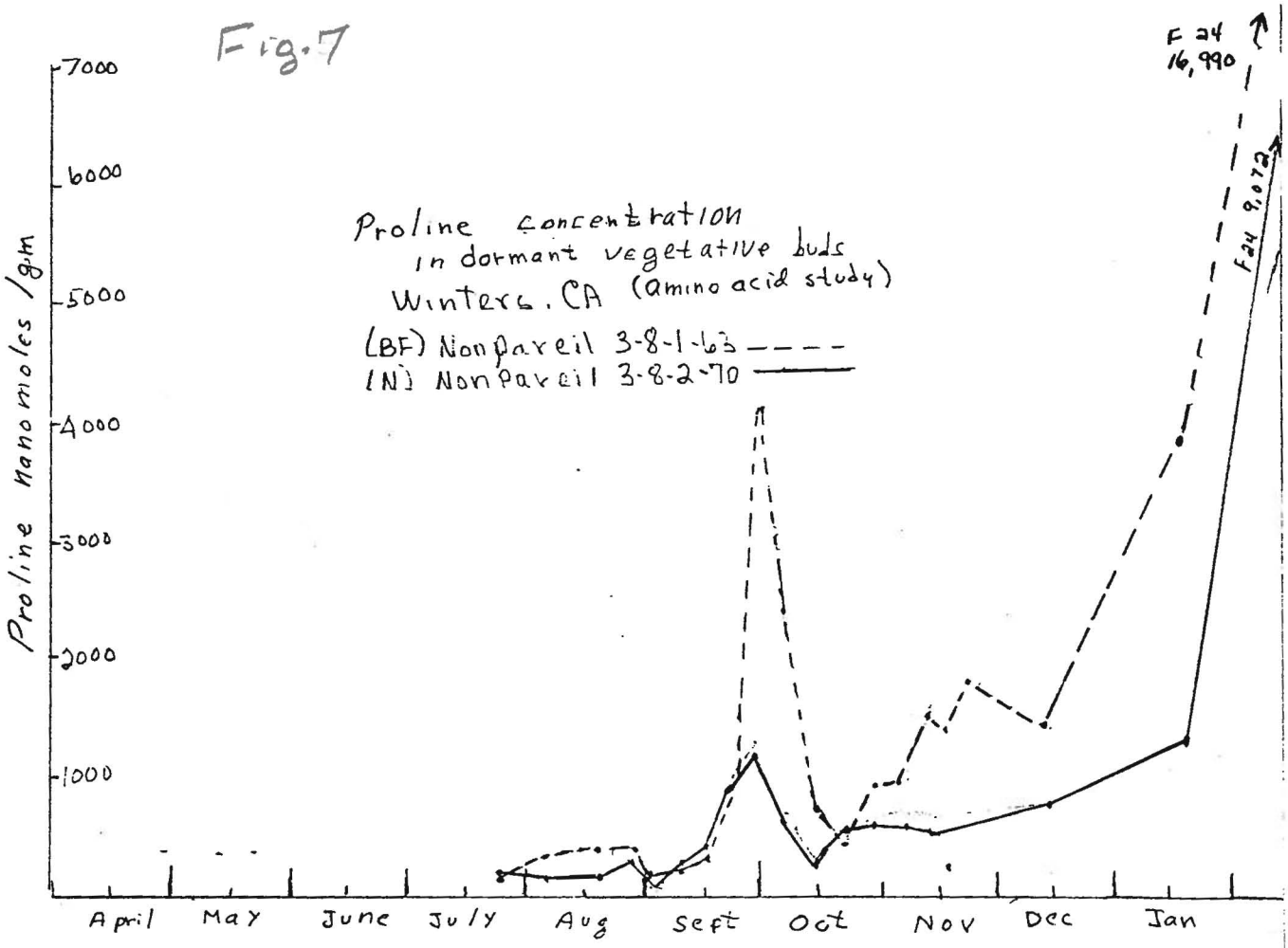

Total Soluble Amino acids
Leaves. Winter, CA
NonPareil 3-8-1-63 (BF)
NonPareil 3-8-2-70 (N)
20
15
10
5
A M J J A S O N

Fig. 7


Proline nanomoles /g.m
7000
6000
5000
4000
3000
2000
1000
F 24 16,990
F24 9,072
Proline concentration in dormant vegetative buds Winters, CA (amino acid study)
(BF) Nonpareil 3-8-1-63
(N) Nonpareil 3-8-2-70
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan

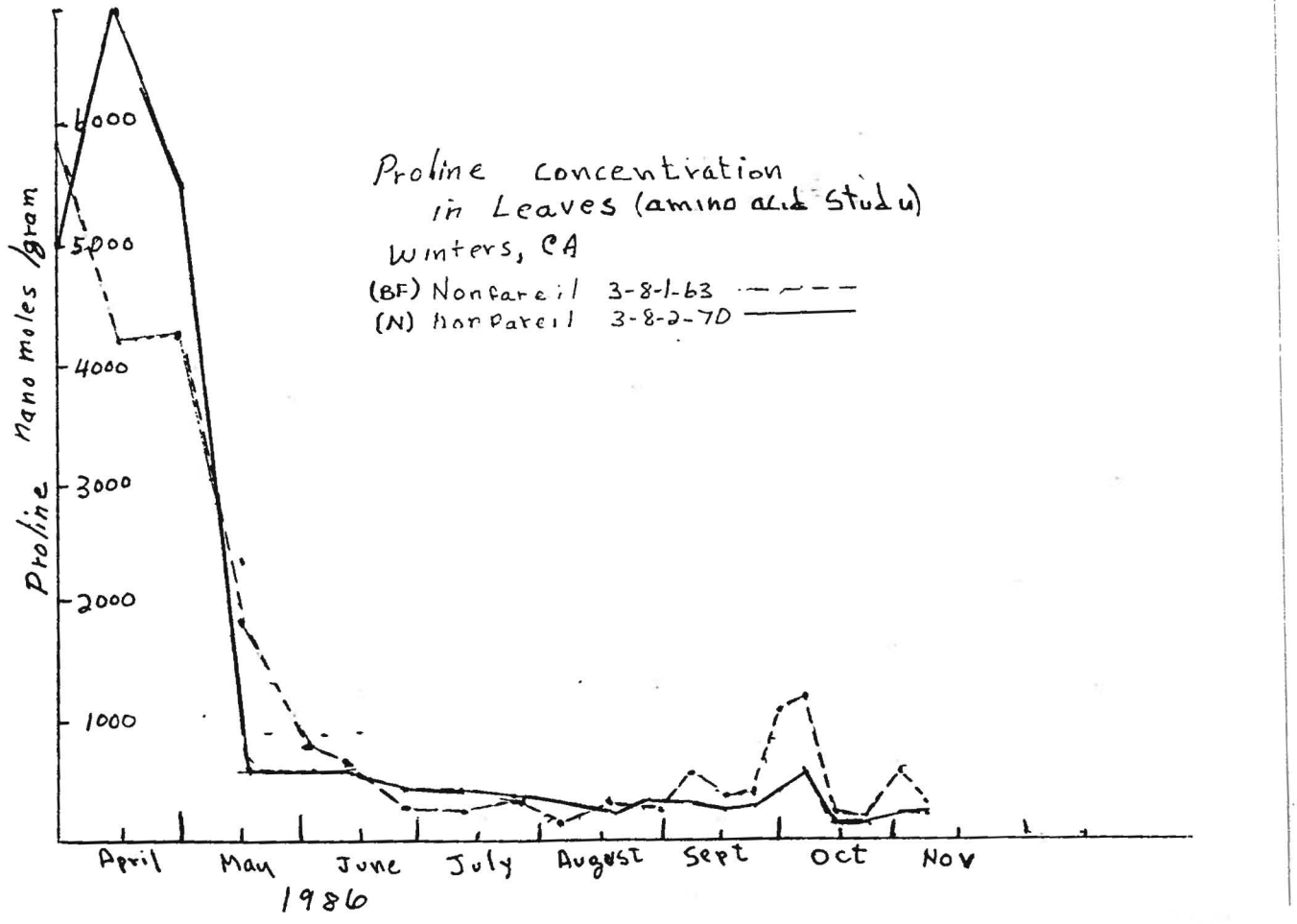
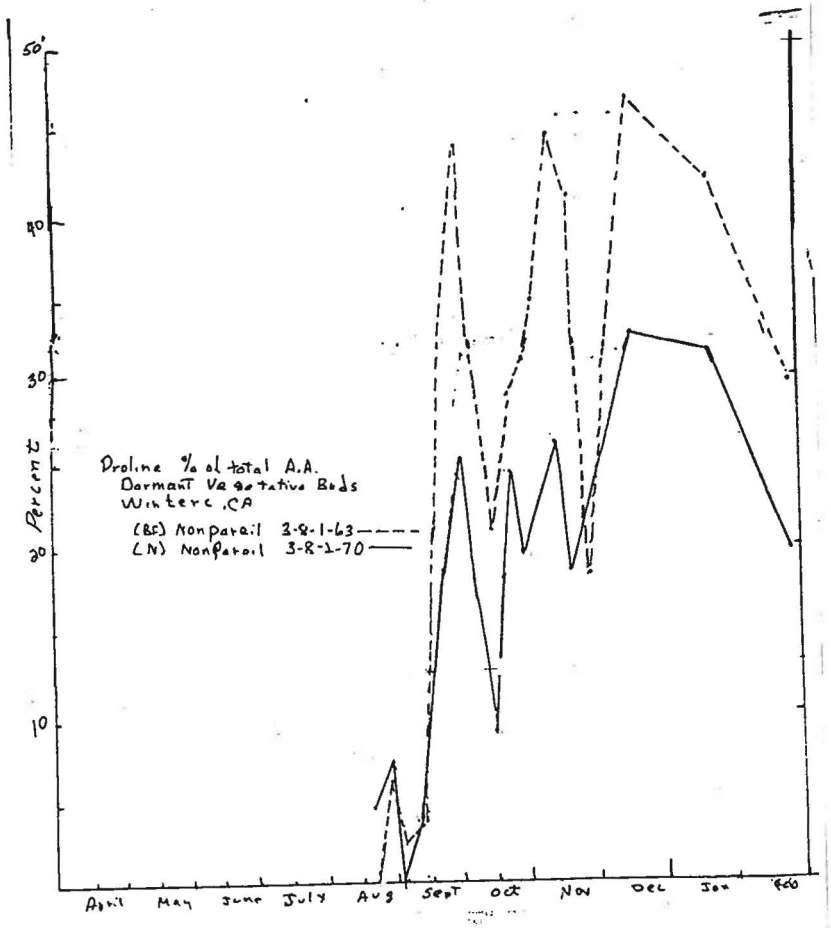
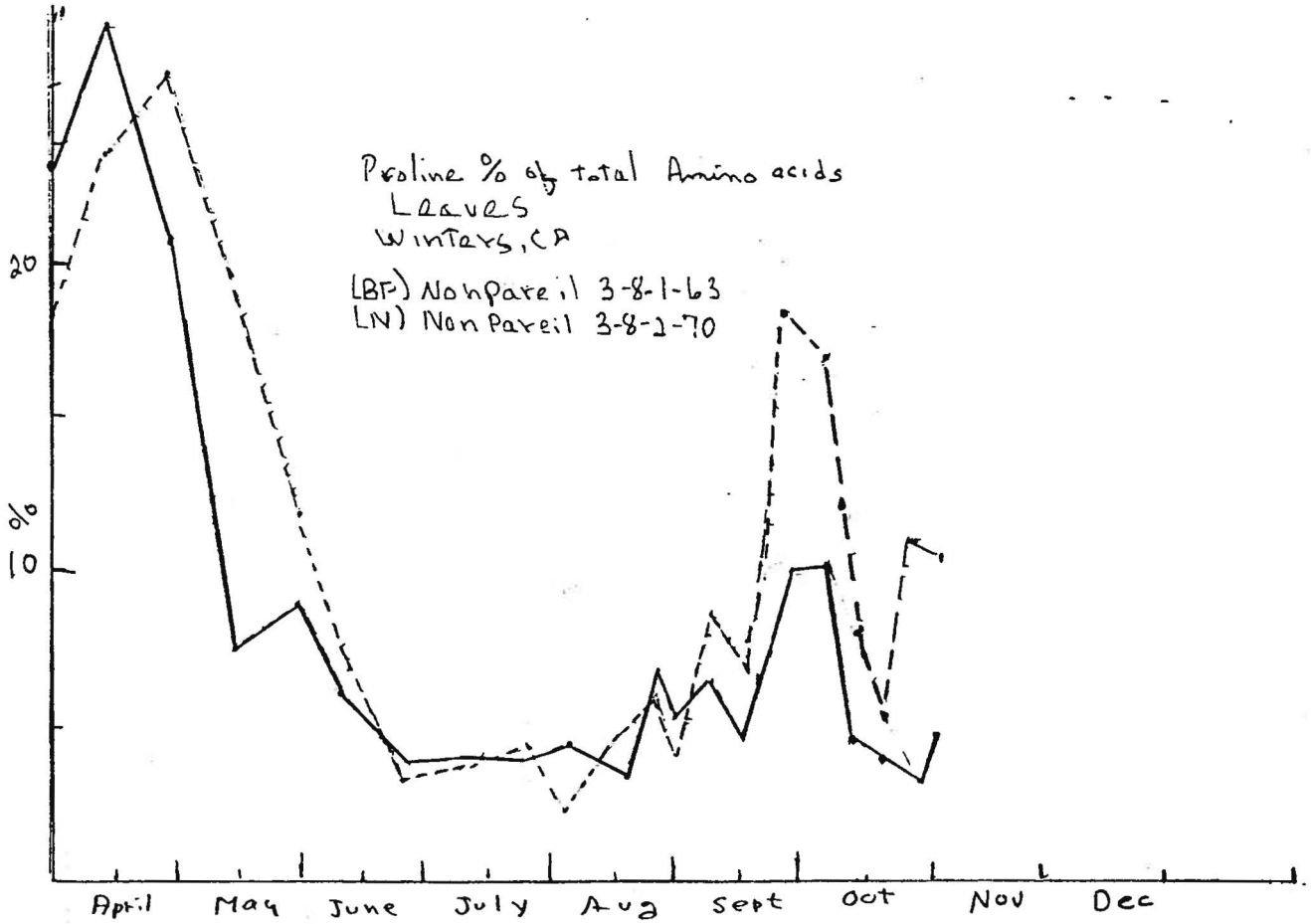
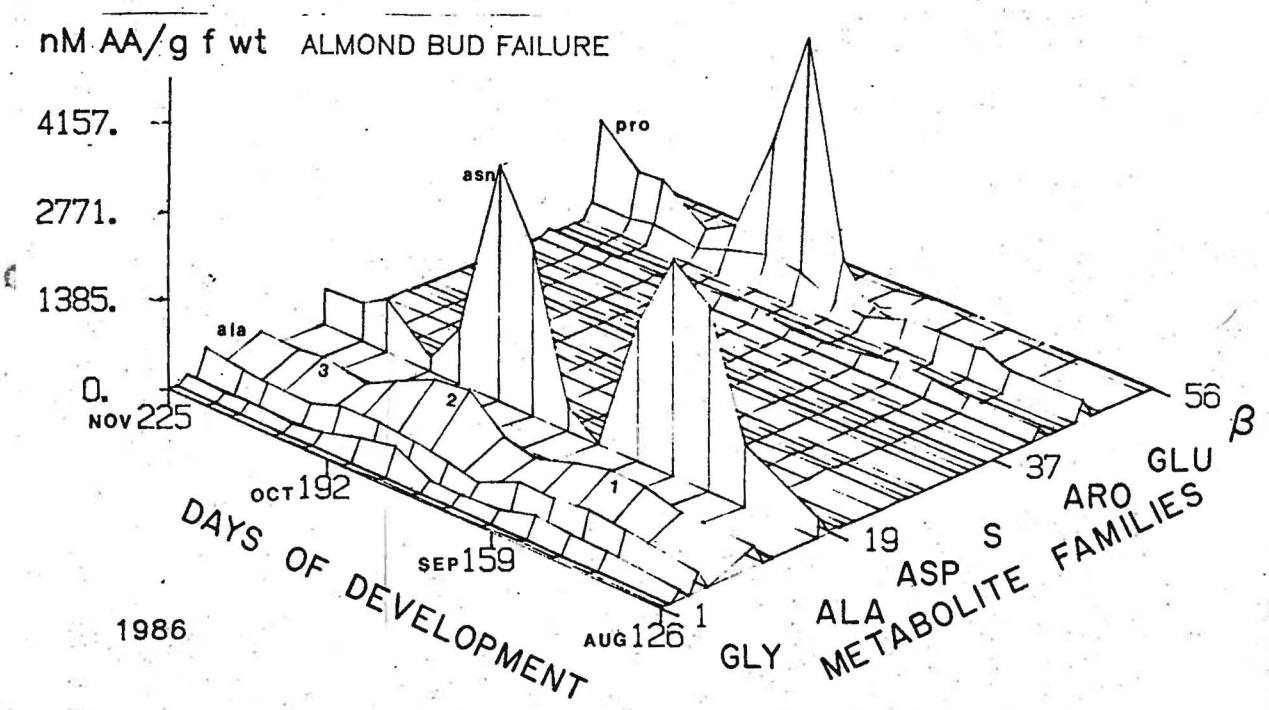

Proline nano moles /gram
5000
4000
3000
2000
1000
Proline concentration in Leaves (amino acid study)
Winters, CA
(BF) Nonpareil 3-8-1-63
(N) Nonpareil 3-8-2-70
April
May
June
July
August
Sept
Oct
Nov
1986

Fig. 8


Percent
50
40
30
20
10
Proline % of total A.A.
Dormant Vegetative Buds
Winters, CA
(BF) Nonpareil 3-8-1-63
(N) Nonpareil 3-8-2-70
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Feb


Proline % of total Amino acids
Leaves
Winters, CA
(BF) Nonpareil 3-8-1-63
(N) Nonpareil 3-8-2-70
%
20
10
April
May
June
July
Aug
Sept
Oct
Nov
Dec

L-18.9


nM AA/g f wt ALMOND BUD FAILURE
4157.
2771.
1385.
0.
pro
asn
ala
3
2
1
NOV 225
OCT 192
SEP 159
AUG 126
DAYS OF DEVELOPMENT
1986
1
19
37
56
β
ALA
GLY
ASP
S
ARO
GLU
METABOLITE FAMILIES

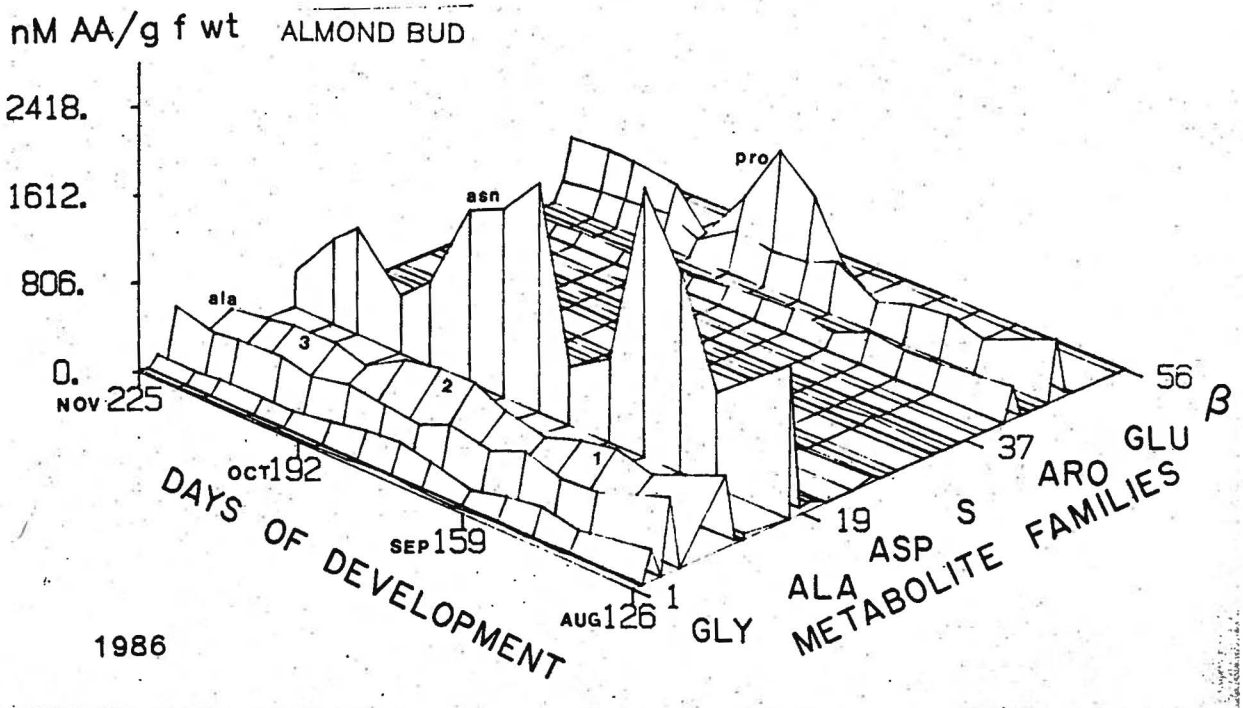

nM AA/g f wt ALMOND BUD
2418.
1612.
806.
0.
pro
asn
ala
3
2
1
NOV 225
OCT 192
SEP 159
AUG 126
DAYS OF DEVELOPMENT
1986
1
19
37
56
β
ALA
GLY
ASP
S
ARO
GLU
METABOLITE FAMILIES

Fig. 10

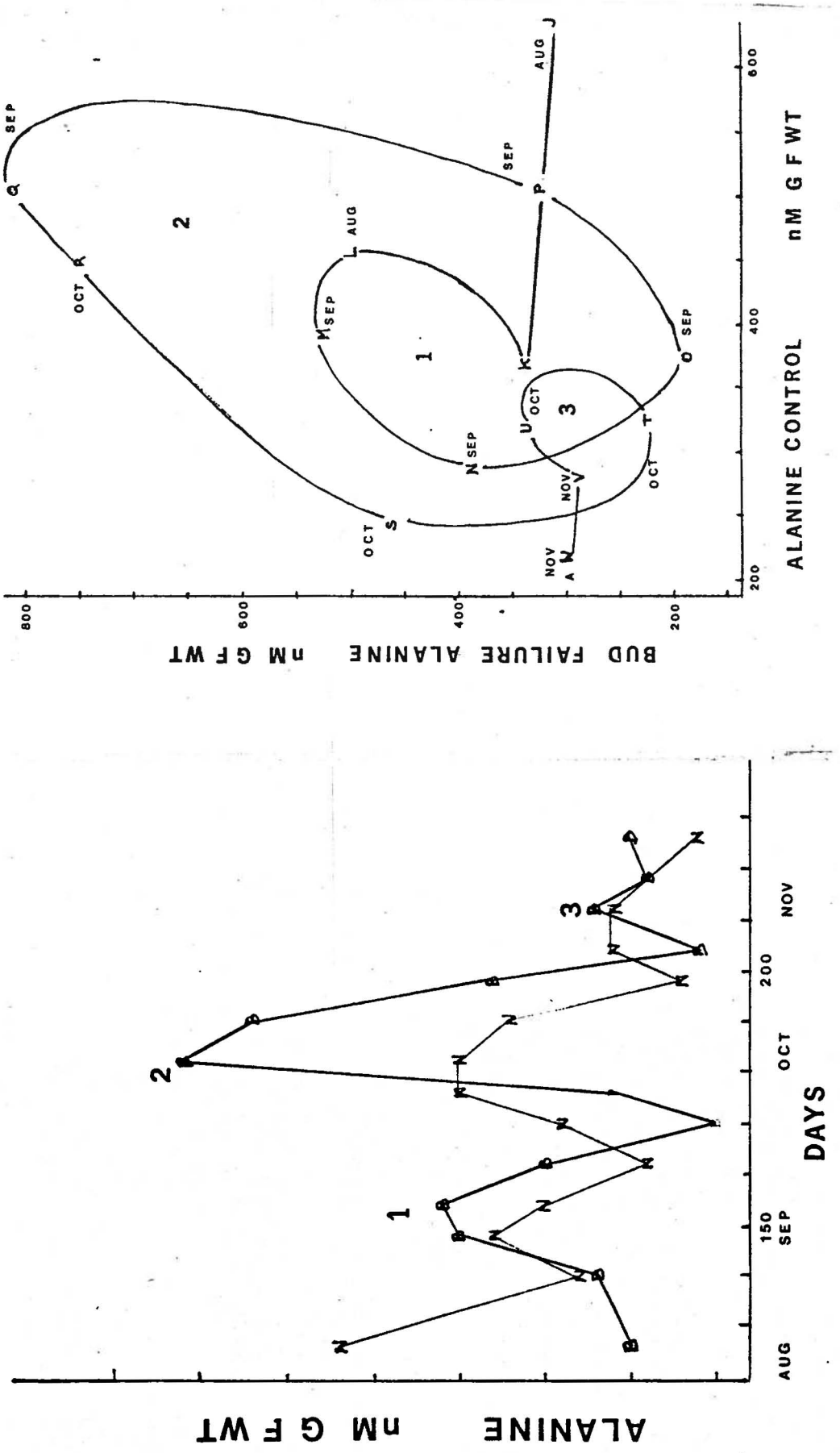
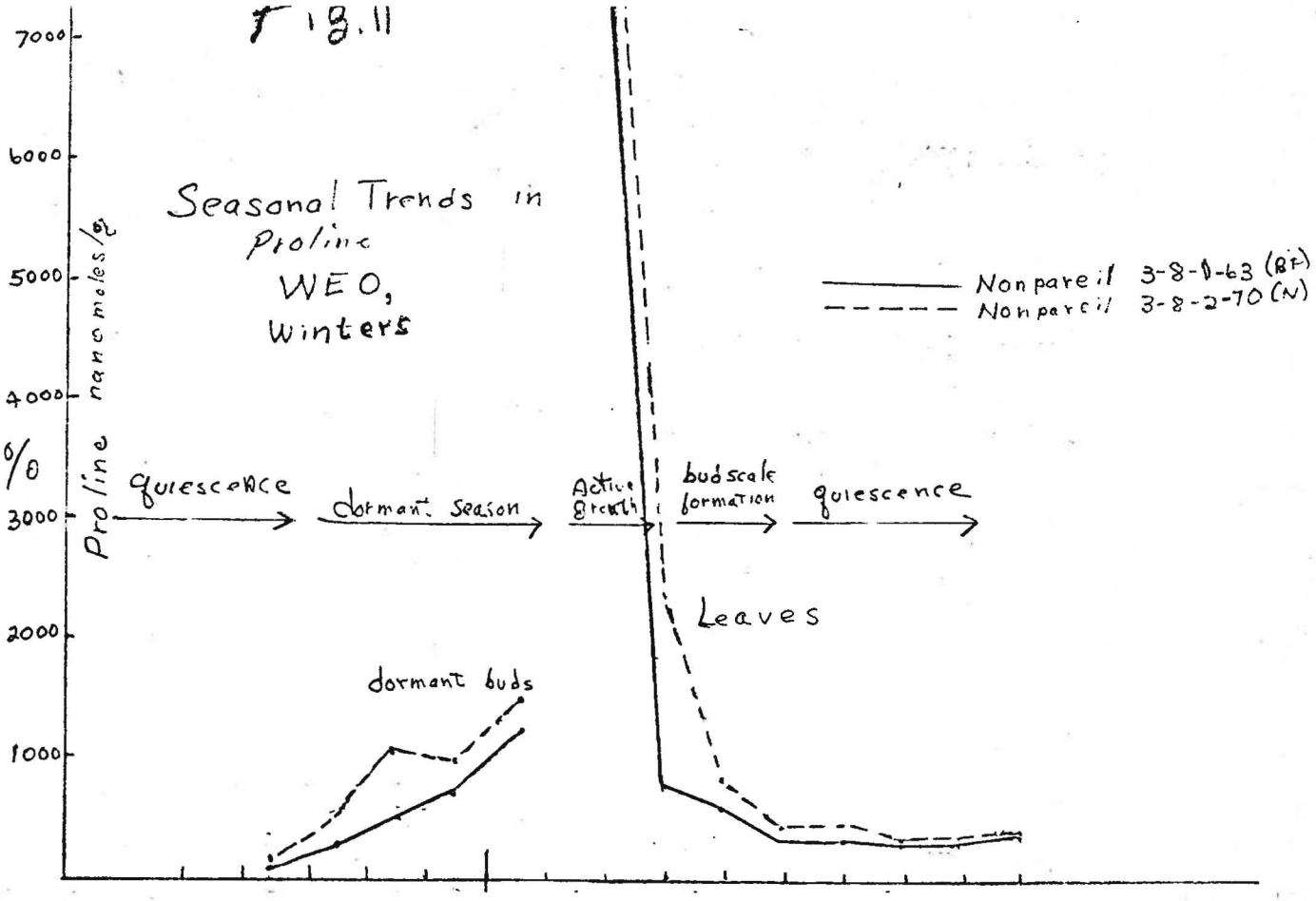
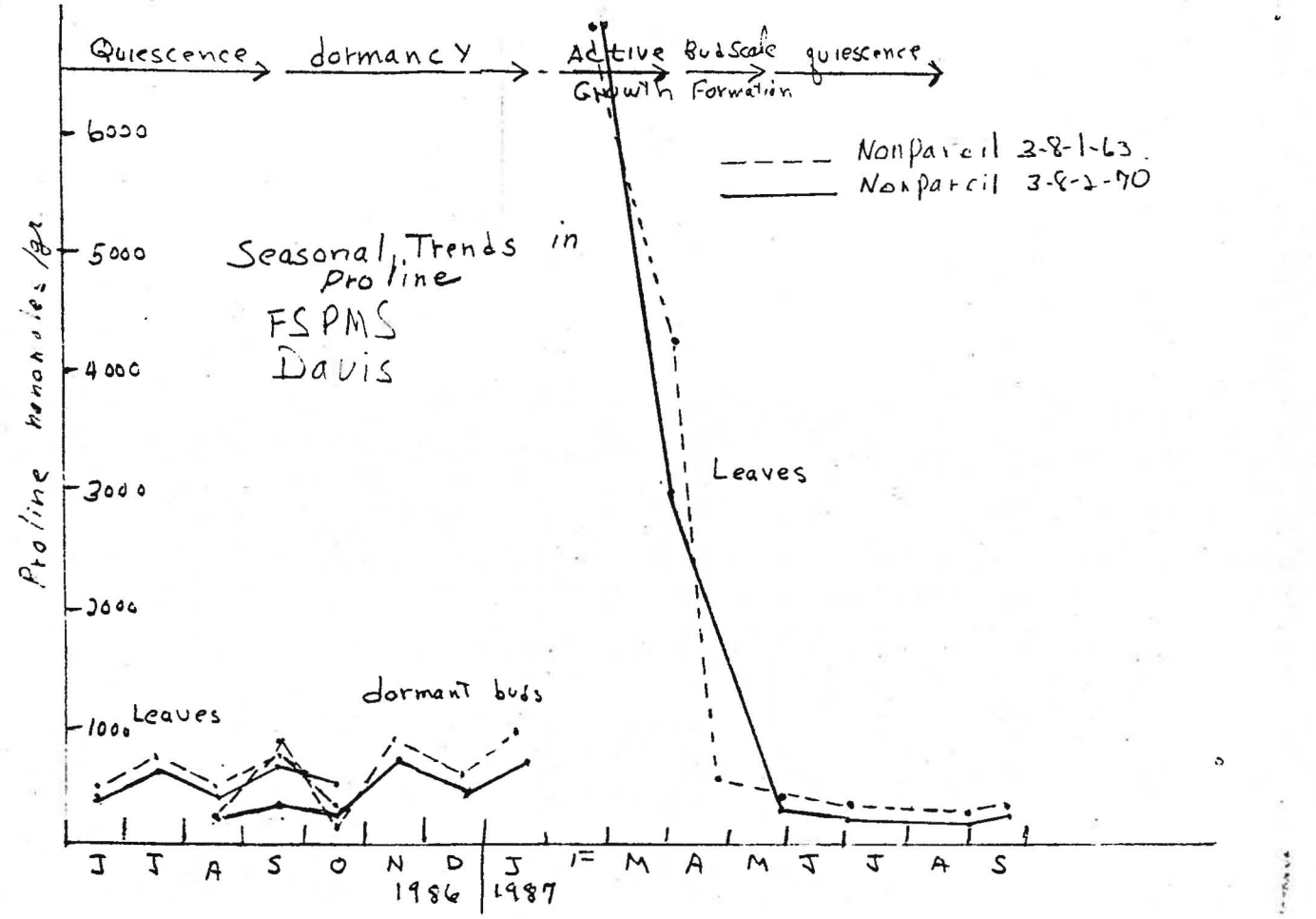

BUD FAILURE ALANINE nM G F WT
800
600
400
200
ALANINE CONTROL nM G F WT
600
400
200
AUG
SEP
OCT
NOV
J
P
Q
R
S
K
L
M
N
O
T
U
V
W
A
1
2
3
ALANINE nM G F WT
AUG
150
SEP
OCT
200
NOV
DAYS

Fig. 11


Seasonal Trends in Proline
WEO, Winters
Nonpareil 3-8-1-63 (RF)
Nonpareil 3-8-2-70 (N)
µ/g Proline nanomoles/g
7000
6000
5000
4000
3000
2000
1000
quiescence
dormant season
Active Growth
bud scale formation
quiescence
Leaves
dormant buds


Quiescence
dormancy
Active Growth
Bud Scale Formation
quiescence
Nonpareil 3-8-1-63
Nonpareil 3-8-2-70
Seasonal Trends in Proline
FSPMS
Davis
Proline nanomoles/g
6000
5000
4000
3000
2000
1000
Leaves
dormant buds
Leaves
J J A S O N D J F M A M J J A S
1986
1987