## ALMOND BOARD OF CALIFORNIA ANNUAL REPORT FOR 1990

Project No. 90-K17 - Selection, Maintenance and Monitoring for low BF-potential and Genetically True-to-type Propagation Sources for Almond.

Project Leaders: Dr. Dale E. Kester Dr. Thomas M. Gradziel Dept. of Pomology University of California Davis, CA 95616

cooperating Personnel: FSPMS (S. Nelson-Kluk, Nursery Advisory Committee (R. Wooley), M. Viveros, W. Micke, M. Freeman, K. Pelletreau, M. Conrad, K. Shackel, D. Durzan, F. Vertimiglia,

Objectives: (1). to conduct indexing (virus) and vegetative progeny tests (trueness-to-type), absence of nonproductive genetic disorders, level of BF-potential) of selected source clones of commercial varieties of almond;

- (2) to conduct parallel seedling progeny tests to characterize inherited BF-potential of same source-clones;
- (3). to provide material for parallel studies to measure BFpotential, including shoot-tip culture.

#### INTERPRETIVE SUMMARY

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The objectives of investigations on earlier phases of this project were to understand the basic biology of the BF phenomenon and its mode of expression. That research culminated in a series of models which define BF in terms of (a). seasonal patterns of expression, (b) variation within single varieties, and (c) genetic variation among various varieties. We have identified and tested in RVT plots selections of various "source-clones" of Nonpareil, Mission and others.

During the past three years we have begun to apply these models directly the selection and control of BF in nursery practice and orchard management with emphasis on the apparently increasing problem in Carmel. These recent efforts function both to verify the earlier models and to provide solutions to current BF problems.

In these recent efforts, we have begun to work closely with the commercial nursery industry to identify and test new clonal selections under commercial conditions and to evaluate BF variability, particularly in Carmel. A series of new orchard plantings to replace older test plots at UCD and at WEO, Winters, area being established to provide material to investigate more

precisely the effects of vigor and stress (Management experiment) and pruning maintenance (stabilization experiment) on the rate of development of BF in young orchards.

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In addition to the vegetative progeny test of specific nursery and tree sources, we are conducting parallel breeding tests using crosses to peach and to Nonpareil BF to compare the same clonal selections being grown in orchard tests. All of these tests are designed to apply the earlier models to the prediction of BF potential in individual sources and varieties.

We also badly need internal biochemical markers which can monitor changes in BF potential in nonexpressing trees. A series of studies to determine the internal physiological differences between the normal and the BF plant has involved seasonal studies on bud dormancy and bud failing patterns. The lack of a "heat dormancy" stage during mid summer in BF plant has been suggested by bud-forcing data and a confirming test is projected for next summer. Parallel studies on amino acid patterns have shown characteristic patterns in the normal plant associated with the hypothesized "heat dormancy" and its relative absence in the BF plant suggesting a mechanism for characterizing expression of BF.

#### PART 1. CLONAL SELECTION

Clonal selection utilizes single trees within a vegetatively propagated variety as a source for future propagation. The single source tree along with its vegetative progeny have been referred to as a clone, source-clone, or clonal selection. This procedure has been used to establish "virus-free" sources of varieties in "clean stock", or virus control, programs utilizing Registration and certification procedures. Such clones must also be "true-to-type" (actually "true-to-variety"), that is, representative of the variety in question.

Additional criteria are needed if the concept is to be extended to control genetic disorders, as noninfectious budfailure. The potential for expression of these traits cannot be indexed by transmission to nonaffected stocks but require other methods for characterization.

Prior research has provided theoretical models to apply clonal selection concepts to control BF (Fenton, et. al.1988, Kester et. al. , in preparation), has identified specific sourceclones with low BF potential of Nonpareil (as well as other varieties) and has tested their yield and horticultural potential in RVT plots (Kester, et al., annual reports). Similar research has been carried out with nonproductive syndrome (NPS or "Bull Mission") (Kester, 1988).

The sequence to select, maintain, and distribute sourceclones to control genetic disorders include the following steps: (a) selection of single trees that are true-to-type, tested free

- of harmful viruses, have a known pedigree and whose vegetative progeny have been test planted for yield and expression of latent disorders, including bud-failure,
- (b). maintenance of source-trees under conditions to stabilize their genetic potential and to prevent infection by virus diseases, and
- (c) distribution through a limited number of consecutive vegetative generations.

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Procedure. The selection process began in May 1988 by meeting with commercial nurseries and the Foundation Seed and Plant Materials Service (FSPMS) of UCD. It was decided to develop clonal selections of Carmel, Fritz, Butte, Price, Monterey, and Ruby from commercial sources. Additional sources of Mission, Nonpareil, Padre, and Sonora were also identified for further evaluation.

Twenty five trees from each source were propagated in the summer of 1988 by Burchell Nursery, Modesto. These trees were dug and then planted either at the Paramount Orchards, Wasco, CA or at an orchard in western Fresno Co. At the same time, budwood was provided to the FPMS at Davis for virus indexing and for inclusion into the Foundation nursery pending transplanting to the Foundation orchard when and if the virus tests were successful. Two seasons are required to finish the indexing tests.

Additional selections were made in the summer 1989, progeny tests established and virus tests begun.

Results. Results to date of the selection and virus tests are given in Table 1. varieties marked with an \* include sources which are free of the range of viruses being tested. Those with a \*\* have passed an initial virus screen with completion of indexing scheduled for summer 1991.

No BF-affected trees were found within the group of 5 clones (85 trees) of Carmel at the end of the initial year (Table 2). On the other hand 1 to 10 per cent of the trees provided by four commercial nurseries showed some BF in the spring of the second growing season. BF also appeared in trees planted originating from some of the same nurseries in other commercial orchards of the area.

Discussion. Nineteen ninety was the second consecutive year that BF affected trees appeared in second leaf trees of Carmel. This finding indicated that the incidences of BF trees in young orchards in 1988 could be part of an ongoing trend that required more action to track the source of BF within this cultivar. orchard experiments described subsequently in this report were initiated in response to this need. Close cooperation with the

commercial nurseries followed.

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PART II. ANALYSIS OF VARIABILITY WITHIN CARMEL.

A. Progeny tests of Carmel budwood sources.

Procedures: Eleven commercial nurseries are cooperating in this study. Ten to 20 individual trees of the bud-wood source block were sampled from each nursery, 2 to 5 budsticks collected from each tree, and 5 to 10 buds propagated from each stick, keeping the sequence from base to tip in order. Each budded tree in the nursery has been labeled with a unique number which will maintain the record of the exact source of each budded tree from source to orchard. All records have been logged into computer files.

The trees will be dug, graded for size and planted in a commercial orchard in Kern Co. for a long term test. Once the location of the individual numbered tree in the orchard has been identified and the log of accumulated information of each trees has been verified, all tags will be removed. Evaluation for BF will begin in spring 1992 and entered into the computer files. Preliminary BF development patterns are expected to appear at this time but the trends will need to be followed for a number of years.

Results: Two thousand eight hundred Carmel trees will be planted in the test. There was considerable variability in nursery procedure and philosophy of budwood selection. Seven sources were commercial orchards, and the remaining four scion orchards or nursery increase blocks.

Arrangements are being made with Paramount Orchards, Bakersfield, CA to plant the test trees during January or February. In addition, arrangements are being made to include Nonpareil selections of different nursery sources as pollinators.

B. Pedigree analysis of Carmel

Information on the origin and history of individual budwood sources has been provided by the 11 individual nurseries who provided the trees for the bud-wood source study (Part A). This information includes the original Carmel tree, each source block and the intervening generations of orchard trees in between. Dates of planting and use have been completely verified. This study will not be completed until after the first of the year 1991.

PART III. MANAGEMENT FACTORS IN BF CONTROL

A. Orchard Management Experiment.

The BF model predicts that the rate of development of BF symptoms in a young orchard is related to the BF-potential of the source, the vigor of growth and the moisture stress in the young orchard. Observations have supported the view that both stress and vigor increase the incidence of BF symptoms. The object of this experiment is to quantify these factors under experimental conditions.

Procedures. Source trees of Nonpareil and Carmel have been identified that have three levels of BF-potential: very low (no symptoms), medium (symptoms just beginning to develop within tree or adjoining trees), and high (severe symptoms present).

Trees have been propagated in summer 1990 in a commercial nursery (Burchell Nursery, Modesto). Trees will be dug in January 1991 and planted at close spacing at WEO, Winters, California. An irrigation system will be installed and stress and vigor will be controlled by nitrogen and water inputs. First observations for BF symptoms will be made in spring 1992 but observations and measurements may be undertaken prior to that time.

Results. The source and numbers of trees propagated are shown in Table 3.

B. Budwood source management experiment (Stabilization).

The concept of bud-failure is that BF-potential changes progressively during the season and consecutively from one seasonal cycle to another. This is why BF tends to increase with consecutive generations of propagated trees from the same source. In actuality this means that the BF appears at younger ages with consecutive propagations.

It follows that, if one can prevent consecutive growth flushes and renew annual growth from basal buds at the same location of the tree, one might stabilize the BF potential at near the initial level by pruning to a hedge row by continuous dehorning. Such a procedure is not practical in almond production but is the basis for maintaining budwood orchards in nursery operations. The procedure is used in virus control programs.

This study will utilize the same three budwood sources described in IIA (Orchard management study) and test the effect of hedgerow management on BF symptom production. Observations on both the trees and their progeny may be required. Plots will be established in a hot summer location (Winters, CA) and a cool summer location (Davis, CA). Trees of experiment IIA which will be pruned in a normal fashion will be available for comparison.

Results. Trees have been propagated as part of experiment IIA although some additional trees of Nonpareil (low BF potential) will need to be obtained from another commercial nursery.

#### IV. Seedling progeny tests

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Previous breeding experiments have been the basis of this

test. In almond x 40A-17 peach progeny severe BF and Roughbark seedlings have been recovered with young populations where the proportion of trees affected is correlated to the BF-potential of the almond variety. In almond x Almond BF progeny populations the rate of BF development in the seedling progeny was found proportional to the BF potential of the almond parent.

Results. Crosses of Almond x 40A-17 peach were made in spring 1989. Seedling progeny were planted in the orchard at close spacing in March 1990. These trees have grown through the 1990 season and will be ready for BF observations in the spring 1991.

Crosses of Almond x Almond BF were made in both 1989 and 1990. Seedlings have been planted in the orchard in March 1990 and more in November 1990. Some trees can be evaluated in spring 1991 with more in 1992.

V. Seasonal studies.

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In this part of the report, the record is brought up to date on studies related to the seasonal cycle of growth and development in relation to bud-failure expression. We believe that understanding this process is the key to understanding the BF phenomenon and its control.

We can define the following stages during the seasonal cycle of growth:

- (a) emergence of buds from rest (January),
- (b) shoot emergence and flowering (February),
- (c) rapid growth, laying down new nodes and leaf expansion (March, April),
- (d) slowing down of growth and maturation of shoots and leaves (May),
- *(e)* bud scale formation (June), and
- (f) bud dormancy (July through rest of fall and winter until growth resumes in spring).

We have found evidence that in the normal plant the 6 months period of bud dormancy includes a period of heat induced dormancy in mid summer (late June, July, August), gradual induction of "rest" (late September, October) and "rest" (late October, November, December).

Earlier research (Hellali, et al. 1973, 1978,1979), originally defined the morphology, anatomy and physiological changes associated with bud and shoot development in both normal and BF plants. Subsequent studies begun in 1978 utilized a bud forcing technique (petri dish test) to monitor the pattern of bud failure (sprouting percentages) and changes in bud dormancy (sprouting rates). The onset of bud failure in nonstressed, affected trees began about September although some variation occurred from year to year. In moisture stressed trees, the time of failure occurred in early July shortly after severe water stresses developed. This timing of failure indicated that the

potential for Bf symptom development was present in the buds by late spring and early summer but the time that symptoms actually developed occurred later and was affected by external factors.

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The pattern of the rates of bud forcing against time suggested that a period of dormancy was induced in the buds on the normal trees with the onset of high temperatures in June and July. Evidence for this pattern was absent in shoots from BF trees (Figure 1).

In 1986 a major study on the seasonal amino acid patterns of normal and BF shoots (Durzan and Kester project) was included in the investigations. Differences between normal and BF shoots were shown primarily by amino acids of the urea cycle (glutamic acid family) characterized by arginine and proline patterns. Asynchrony was shown between the normal and BF leaves and buds in the patterns of arginine, citrulline, ornithine and proline in the late spring (April, May and June) and again later in the summer (August, September). A sequence of arginine > ornithine > citrulline > proline in the normal plant was shown in the buds beginning in late August through fall paralleling the gradual decrease in growth and induction of rest. The appearance of this sequence was delayed in the BF affected plant (beginning in September) followed by an erratic pattern of arginine and proline production resulting in very large increases in proline concentration in the fall, paralleling the expression of BF symptoms.

Procedures. In 1989, studies (with Dr. Ken Shackel and Dr. Don Durzan) in amino acid patterns and bud forcing were repeated with the original objective to determine how the additional factor of moisture stress affected amino acid patterns (primarily arginine and proline) of normal and BF buds.

Two modifications from the 1986 tests were used. (a) Samples for both bud forcing and amino acid analysis were collected from two source-clones, one with no history of BF production and the other from a source which was just beginning to show BF. These trees had been propagated two years previously in a new planting at WEO, Winters. samples for chemical analysis were taken from both the basal and apical end of the stem. (b), Bud forcing was carried out under controlled environmental conditions at a constant temperature of 71oF. Results of the forcing tests were given in the 1989 report. Because of discrepancies in the bud forcing patterns in 1989 with previous studies, we repeated the bud forcing study in 1990.

Amino acid analyses were carried out in 1989 in bud samples collected every two weeks beginning in early June through mid october.

A reanalysis of the 1986 amino acid data was also carried out preparatory toward presentation of the results at the International Horticultural Congress at Florence, Italy in summer 1990.

#### RESULTS

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### 1. Bud-forcing

The patterns from bud-forcing studies in 1990 are shown in Fig. 2. The bud sprouting percentages from normal sources were consistently high throughout the season with a temporary decrease in November, suggesting some effect of rest. The high percentage reflects the presence of mostly vegetative buds (as compared to flower buds) and the responsiveness of vegetative buds to the physical and environmental conditions of the test.

In contrast, the sprouting percentage of the buds from the BF plant remained high only during July, decreasing sharply during August to a fluctuating average of about 5 and 25 per cent for the remainder of the test. Variation was related to differences among individual shoots collected. Visibly necrotic buds began to appear in samples beginning in Sepember. Many nodes bore two or three buds. On normal shoots, central buds were invariably vegetative whereas the outer buds were flower buds. In the BF affected plants, the center bud invariably failed. The outside buds, on the other hand, were not only vegetative but usually sprouted, showing resistance to the BF condition.

Rate of sprouting is shown as days to 50% aermination. The buds from the normal plants sprouted at essentially a constant rate through the summer and fall but began to decrease in mid-October, apparently marking the beginning of the rest. The buds from the BF plant sprouted at a higher rate than those from the normal plant in June and early July but the rate began to increase sharply, increasing through July and August paralleling the decrease in percentage. The pattern shows an initial high sprouting potential (similar to that observed in 1979 and 1986) which changed to increasing bud damage, as shown first by decreasing sprouting rate.

#### 2. Amino acid studies

Leaves. 1986 data: The group of about 25 amino acids observed were divided into five families, representing amino acids which are connected in sequential steps of specific chemical reactions (Figure 3).

a. ALANINE FAMILY (alanine, valine, leucine, ). Alanine is a first amino acid product of photosynthesis. It was one of the main ingredients of the soluble amino acid pool in the leaves during spring and summer and showed a characteristic pattern in the leaves of the normal plant not shown by the leaves of the BF plant. This high level decreased as the leaves matured during May and early June and bud scales formed. The concentration then increased to a peak in early July, coinciding with the apparent induction of heat induced dormancy, subsequently followed by a

continuous decrease through the remainder of the summer and fall. The pattern in the leaves of the BF plant was initially the same as that of the normal but continued to decrease through the remainder of the season. The difference between the normal and the BF can be seen most strikingly by plotting the normal/BF ratios against time (Figure 3, right). The same patterns are shown (although less consistently) by other amino acids of this family.

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b. ASPARTIC ACID FAMILY (aspartic acid, asparagine, threonine, isoleucine, lysine). Asparagine is the dominant amino acid of this family and is the most important translocation form of nitrogen early in the season. The concentration was higher in leaves of the normal as compared to BF. Other amino acids of the family tended to follow the alanine pattern but in all cases the normal/BF ratio was positive in the summer.

c. GLYCINE FAMILY (ethanolamine, serine, glycine, galactamine, sarcosamine, AAB). The basic patterns of the amino acids of this family tended to follow that shown by alanine.

d. AROMATIC AND CYCLIC AMINO ACIDS (cysteine, tyrosine, phenolamine, histidine, etc.). These compounds were at lower concentrations and tended to follow the pattern of alanine (not shown).

e. GLUTAMIC ACID FAMILY (arginine, ornithine, citrulline, proline). These amino acids showed high levels early in the season with low levels later on. Arginine showed a high normal/BF ratio early in the season with a second peak later in the summer. Proline relationships were cyclic with an increase followed by a reversal in the spring with the same pattern later in the summer, indicating a major increase in proline in the BF plant coincident with the expression of symptoms.

Buds: 1986. Bud collection began only in mid August such that a major part of the season was not mapped.

a. ASPARAGINE. THis amino acid family was the dominant compound during the bud development period with concentration tending to follow the fluctuation patterns of bud activity shown in the forcing tests.

b. GLUTAMIC ACID FAMILY. (Figure 4). The pattern sequence in the normal plant beginning in mid August showed the following arginine > ornithine > citrulline > proline which coincided with the gradual induction of rest period and are sequences of the UREA cycle. In the BF plant, in contrast, the initiation of the sequence was delayed until early September coinciding with the initiation of symptoms. There was then a series of arginine > proline cycles ending with concentrations of proline in the BF buds 3x or more that of the normal, It is evident that the

pattern was a reflection of the necrosis shown in the symptom expression.

#### Buds: 1989.

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The analyses were made on collections made at two week intervals resulting in somewhat erratic patterns. The period extended from early June through October. Apical and basal bud samplings showed the same patterns except that the concentrations were less in the basal buds. Nevertheless the patterns of the normal and the BF followed closely to the basic patterns shown in 1986.

Amino acid concentrations in the normal plant showed what may be considered to be a characteristic pattern. This shows a decreasing concentration through June and early July followed by an increase in July followed in turn by a further decrease through the season.

Buds in the BF plants showed a gradually decreasing pattern of amino acid concentration which was about the same level as in the normal. However, the rise in soluble amino acid concentration in the early summer was consistently absent.

This difference in pattern indicates the absence of a key physiological activity which may be associated with the inability of the BF affected plant either to generate a protective function during the high stress conditions of early summer or a basic shift in the metabolic pattern that results in toxic conditions within the bud tissue that leads to necrosis of the sensitive bud tissue.

# DISCUSSION

Two periods in the seasonal development pattern appear to be important in the BF syndrome. One is in the early part of summer and may involve disruption of the transition from active growth to "high temperature dormancy". The second is in the fall and leads to necrosis in the buds. The amino acid patterns may serve as internal markers for physiological changes in leaves and buds in both the normal and BF plant.

Further characterization of the protein and amino acid systems in normal and BF plants during the critical induction periods of mid summer and under stress conditions should be carried out utilizing some of the modern tools of molecular biology.

Bud forcing studies in 1979 and 1986 were conducted at uncontrolled ambiant laboratory temperatures and produced evidence of heat induced dormancy in mid-summer. Studies in 1989 and 1990 were carried out in controlled growth chambers at 71oF. No evidence of a heat dormancy period was observed although excellent patterns of BF symptoms developed. To clear up the difference, an additional comparison should be made on the bud forcing responses at critical stages of development under

contrasting conditions utilizing both temperature regimes (e.g., 700 and 860F).

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Table 1. List of clones of almond varieties undergoing progeny and virus indexing tests

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- 1). Progeny tests involve planting of nursery trees at Paramount Orchards, Bakersfield, CA.
- (2). Short index is a test for Prunus Necrotic Ring spot Virus either by ELISA test or by SHIROFUGEN INDEXING
- (3) Long index involves transmission tests to 5 different hosts. Requires 2 years to complete. Identifies most major viruses in Prunus.
- (4) Long index to be completed by end of summer 1991. Trees will be planted into the FPMS Foundation orchard
- (5) Source not eligible for Registration status unless heat treated to remove viruses
- (6) Source is eligible for Registration status when variety verification complete
- (7) Source has previously met Registration status and has been available commercially
- (8) Source is currently undergoing thermotherapy (heat treatment) to remove viruses

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# Table 2. Percentages of BF affected trees in one-year old Carmel trees

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\* number refers to separate nursery sources \*\* 5 separate clones represented. See Table 1.

> Table 3. Sources of Carmel and Nonpareil with different BF-potential being used for MANAGEMENT and STABILIZATION experiments.

# A. Nonpareil



Figure 1. Responses of seasonal bud-forcing tests with normal and BF almond. Upper: Sprouting rate percentages in normal Nonpareil tree growing at WInters, california, with no BF. 1979. (solid line). Nonpareil tree in normal tree at Davis, California (moderate temperature) in 1979 (dashed line). Nonpareil tree in normal tree at Winters, CA in 1986 (dotted line). Middle. Same material but tree with BF symptoms Bottom. Data in top and bottom graphs expressed as BF/Normal ratios.

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ORCHYR 1986 197 1979 ters i s **Winters** 



Figure 2. Bud-forcing studies in 1990. Upper left. BF (Clement) (solid line) and Normal (dashed line) Sprouting percentage. Lower left. Rate of sprouting of same material. Upper right. Milow and Nonpareil at Davis. Sprouting percentages. Lower right. Same but rate of sprouting.

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#### **ALANINE** FAMILY

Figure 3. Seasonal patterns of representative amino acids. 1886. Left. concentration in leaves of normal and bf trees. Right. Ratio of Normal/BF concentrations.



ASPARTIC ACID FAMILY



ASPARTIC ACID FAMILY (continued)



GLYCINE FAMILY









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Figure 4. Season patterns of amino acid in buds of normal and BF affected plants. 1986.







ALANINE FAMILY

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GLUTAMIC ACID FAMILY

GLYCINE FAMILY



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 $1, 7$ 

 $1.6$ 

 $1, 5$ 

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 $0.9$  $\overline{z}$  $\circ$  8 NANOMOLES/GRAM FREST  $0,7$  $0.6$  $0.5$  $0.4$  $0.3$  $0.2$  $0.1$ 120CT 25AUG 14SEP 28SEP  $28JUL$ 11AUG **7JUN 29JUN**  $14.8$ O BF(CLEMENTS) + NORMAL

1989 AMINO ACID ANALYSES

L-ISOLEUCINE (TIP BUDS, WINTERS)

1989 AMINO ACID ANALYSES

ETHANOLAMINE (TIP BUOS, MINTERS)



L-SERINE (TIP BUDS, WINTERS)

NANOMOLES/GRAM FRESH WEIGHT

 $160$ 

150

 $140$ 

130

120

110

100

90

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