Project Title: Synthetic Growth and Maturity Regulators

Tree Physiology - Project 76-J3

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Objectives: (1) To investigate the systemic nature of Orthene and Monitor, insecticides which control Navel Orangeworm, (2) analyze different almond varieties for their gum content as an approach to study the bases of stick-tights and mummies, (3) movement of sucrose from leaves to developing nuts on the same and adjacent spurs, (4) study the growth of individual cells of the hull and compare it with that of the intact nuts, (5) examine the effect of oil sprays on the ease of knocking the nut at harvest and for removing mummies during the dormant season.

Interpretive Summary: Under laboratory conditions where radioactive Monitor and Orthene were applied to leaves of young seedlings and detached nuts and discs thereof, analyses revealed the following:

1. Both insecticides are metabolized by leaves as indicated by the evolution of radioactive carbon dioxide. A fair portion of that applied was recovered in the surface rinse of leaves 5 days after application. Extracts of plant parts contained no evidence of residual insecticides but contained some unidentified substances.

2. With hulls, radioactivity was detected in the epidermal layer and in the region adjacent to the shell. Where discs treated with the insecticides were placed on agar, the latter also became radioactive in 1 day. No detectable amount of radioactive carbon dioxide was trapped with barium chloride nor residue discerned in the surface rinse of the hulls. Hull extracts after 24 hr contained some of the original insecticide and also unidentified metabolite(s).

Thus Monitor and Orthene penetrated hulls readily but not the leaves under our conditions of experimentation. Why the difference exists between the hull and leaves is yet unknown. Perhaps it may be due to the smooth waxy surface of the leaves resisting penetration in contrast to the thin-walled epidermal hairs on the surface of the hulls. These hairs or trichomes are known to be biologically active in other species. These results indicate that these insecticides are taken up by almond tissues and translocated readily, especially in the fruit so they should provide protection to the nuts from Navel Orangeworm.

A short experiment in which radioactive cane sugar (sucrose) was applied to leaves of bearing spurs revealed that radioactivity moved into kernels even when their increase in dry weight was no longer detectible. This may be due to the simultaneous utilization of materials deposited in the kernel earlier and their replenishment by fresh photosynthetic products from nearby leaves. This would indicate that the longer the nuts are left on the tree, the yield should be better.

Analyses of almond hulls from 18 varieties sampled 4 to 5 times during the season revealed considerable variation among them. Thompson, Walton, Westyn and Peerless contained relatively high amounts of gums whereas Almendro, Marcona and La Prima were low. Analyses of the hydrolyzed gum products from Nonpareil, La Prima and Walton indicated that their composition with respect to the sugars which make up the polymers was essentially the same.

Lastly, nuts treated with 1% summer oil tended to retain their moisture longer than those dipped or sprayed with water or water and an adjuvant. The amount of nuts shaken from Drake trees sprayed twice with ¼% oil

was greater than that from a single treatment or none. Whether the moisture content increased the nut weight or the tissue about the abscission zone was softened thereby facilitating their removal is not known.

In our departmental orchards located in Davis and Winters, where the trees are irrigated until mid-August by basins or furrows, the problem of sticktights and mummies is seemingly minimal. Comparing their observations with what we see in commercial orchards, the following questions are raised:

1. Does early shut-off of irrigation water prevent abscission layer from forming or if the layer does form, could the lack of water prevent the meristematic cells from enlarging? In many species, this final swelling of cells in the abscission zone disrupts the vascular bundles of leaves and fruits resulting in their separation from the stem and pedicels, respectively.

2. Could the high pressure of water from sprinklers cause injury to the spurs and even the hairs on the hull thus causing gum to exude?

3. What part does the extended period of high humidity from sprinkler irrigation contribute to fungal infection?

4. How much does the debris (nuts and prunings left on the orchard floor) contribute to the Navel Orangeworm problem? Generally nuts on the orchard floor were infested with worms to a greater degree than those plucked from the tree.

In the due course of answering the above questions, certain cultural practices will undoubtedly be modified and greater stress be placed on orchard sanitation.

Experimental procedures.

A. Insecticide research.

1. Seedling studies. Known amounts of ^{14}C-Orthene and ^{14}C-Monitor were applied to fully expanded mature leaves on open-pollinated Nonpareil seedlings growing in growth chambers (see attached scheme). These plants, 12 to 18" tall, were placed in glass containers equipped with a 2-hole lid through which hoses were inserted to circulate the atmosphere constantly. These hoses were connected to a circulating pump and carbon dioxide traps in series. Periodically these traps were changed and the radioactivity trapped within was counted. Five days after the experiment was begun, the seedlings were removed and the treated leaf washed to obtain an estimate of the surface residue. On removing the soil, the plants were partially dried and then placed in a plant press. When the plants were dry, they were taken into a dark room and a x-ray film placed firmly against the dry material. After three to five weeks exposure of the photographic plate, the film was developed. The plant was divided into several parts and each part combusted in a special furnace. The carbon dioxide resulting from the combustion was collected and the radioactivity of the contents counted.

2. The same experiment was conducted with seedlings which had been girdled.

3. Fruit studies. Radioactive Orthene and Monitor were applied to discs obtained from the sides of Nonpareil and Mission nuts at differing stages of maturity or to intact nuts (see attached scheme). To allay the possibility

of microbial decomposition of the insecticides, the nuts were surface sterilized with Clorox. The results were then compared with those from untreated nonsterile nuts.

B. Gum analysis. Nuts samples were collected from shell hardening period to maturity when the hulls were still partly green. Hulls were chipped into a beaker and an equal weight of water added. The mixture was then blended and the slurry centrifuged. The liquid phase was frozen until analyzed. Analysis consisted of mixing 50 ml of the juice sample with 400 ml of 95% ethanol after acidifying with 2½ ml of 9% hydrochloric acid. On standing overnight, the precipitated gums and mucilages were centrifuged. The supernatant liquid was discarded and the residue refluxed with 40 ml of 2½% sulphuric acid for 6 hours. The reducing capacity of the final solution was determined with an Anthrone reagent or Fehling solutions. Portion of the gum was hydrolyzed with trifluoroacetic acid and the resulting sugars reduced with borohydride and acetylated with acetic anhydride. The amounts and kinds of sugar acetylated were determined by gas chromatography.

C. Sucrose movement. Branches with nuts in stages 5 or 6 were brought into the laboratory and their cut ends placed in water. Radioactive sucrose was applied to leaves of bearing spurs. Few days later, the different parts of the shoot and nuts were dried and pulverized. Portion of the dry matter was combusted and the radioactivity of the resulting carbon dioxide counted as above.

D. Cell diameter of the hull tissue. Fruit samples collected on March 11, April 5, 19, 25, May 4 and 26 were placed in an alcoholic formalin:acetic acid solution for preservation. Ten micron sections imbedded in paraplast were

prepared and stained with safranin-fast green. The number of cells extending over a 0.30 mm micrometer field was counted in different parts of the hull.

E. Effect of oil sprays on the ease of knocking. After collecting a small initial sample of nuts with hulls attached, trees of La Prima, Drake, Ballicc, Milow and Merced were sprayed with 1% oil, 0.16% spray adjuvant (Amway product) and water. Subsequently, the same trees were knocked with a mallet and the weights of the nuts which fell were recorded. Nut samples from unsprayed trees were also collected and dipped in 1% oil and water to determine drying time needed to attain original weight of the nuts. In early November, a commercial cooperator sprayed Drake trees with ½% oil, once and twice, just prior to knocking.

Results and Discussion.

1. Studies on Orthene and Monitor. These materials were found to penetrate the leaves but part of the dosage was recoverable as radioactive carbon dioxide (Fig. 1), and some in the original form in the rinse water. The remainder was found to be in the plant, primarily in the young leaves (Table 1). Extracts of the treated leaves, when chromatographed on silica gel plates, revealed that much of the radioactivity was no longer the original insecticide. The metabolite(s) tended to remain at the origin of the chromatogram, unlike Orthene and Monitor. With girdled seedlings, nearly all the material was recovered in the rinse. The autoradiograph showed that very little penetrated the leaf.

With nuts and hull discs, the rinse contained only traces of radioactivity. The layers of discs taken from the hull were highly radioactive, as were the agar plates on which the discs were placed. No set pattern of radioactive

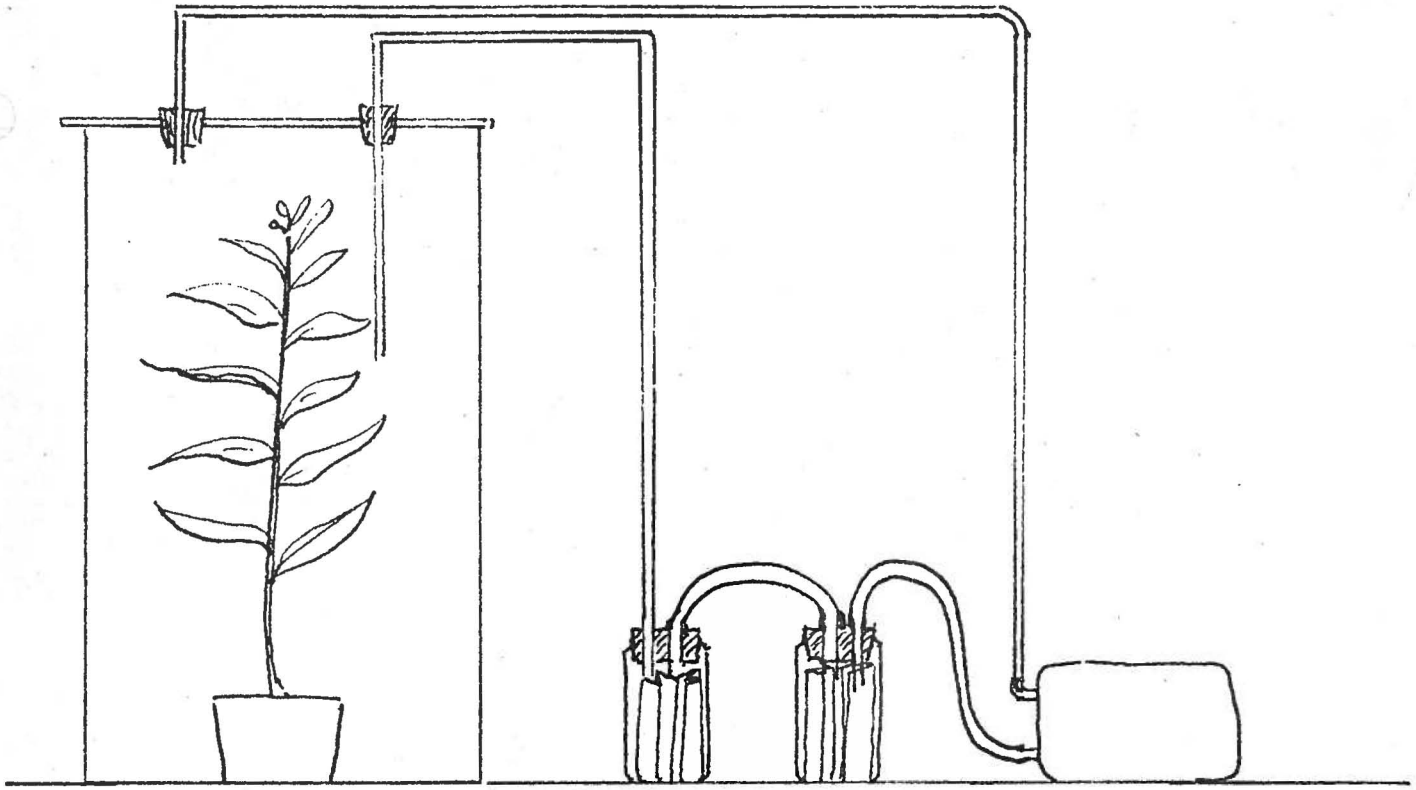
intensity was found in the different layers (Table 2). Chromatography of disc extracts revealed that a proportion of the radioactive insecticides was not metabolized.

2. Gum analyses. Preliminary studies showed that Merced and Nonpareil gums hydrolyzed to sugar-like substances in 6 hours but that by the end of 3 hr, differences could be determined between varieties (Fig. 2). When a given amount of gum obtained from Walton, Nonpareil and La Prima was hydrolyzed and analyzed by gas chromatography, the tracings showed that Nonpareil was relatively richer in some compounds than were Walton and La Prima. However, the makeup of the gum did not differ greatly (Fig. 3).

3. Sucrose uptake and transport. Analyses of kernel obtained from spurs on which the leaves were treated with radioactive sucrose revealed that the embryo were radioactive even when the experiment was conducted late in the season.

4. Oil treatment and water retention. Trials showed that nuts sprayed with or dipped in 1% oil retained moisture longer than those treated with water or water with an adjuvant added. Drake trees sprayed with ¼% oil yielded almost twice the number of nuts than those sprayed once or none at all.

5. Cell and fruit size study. In 1976, individual cell size of the hull ceased to increase after May 5. This was reflected in the nut growth curve.



ALMOND SEEDLING IN GLASS CONTAINER

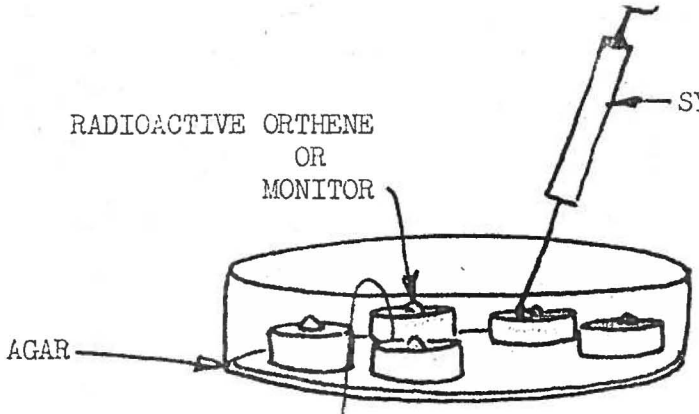
CARBON DIOXIDE TRAPS (FOLDED FILTER PAPER SAT'D WITH BARIUM HYDROXIDE)

CIRCULATING PUMP

CARBON DIOXIDE TRAPS WERE CHANGED AT SHORT INTERVALS THE FIRST DAY AND THEN DAILY THEREAFTER. AFTER FIVE DAYS THE SEEDLING WAS TAKEN OUT, THE TREATED LEAF RINSED TO REMOVE SURFACE RESIDUE AND THE ENTIRE PLANT DRIED IN A PLANT PRESS.

UPON DRYING, AN X-RAY FILM WAS TIGHTLY PRESSED AGAINST THE SEEDLING AND STORED FOR THREE TÔ FIVE WEEKS. AFTER THE FILM WAS PROCESSED, THE SEEDLINGS WAS DIVIDED INTO DIFFERENT PARTS AND AN ALIQUOT OF EACH COMBUSTED IN A FURNACE. THE COMBUSTION PRODUCTS INCLUDING CARBON DIOXIDE WAS COLLECTED IN A SCINTILLATION VIAL AND THE RADIOACTIVITY OF THE CONTENTS DETERMINED IN A COUNTER.

THE REMAINING PARTS OF THE SEEDLING, INCLUDING THE TREATED LEAF, WERE EXTRACTED WITH A SOLVENT AND A PORTION OF IT WAS CHROMATOGRAPHED.



DISCS TAKEN FROM NONPAREIL HULL AT DIFFERENT STAGES OF MATURITY

24 HOURS LATER THE SURFACE IS RINSED AND THE RINSE SOLUTION TESTED FOR RADIOACTIVITY

DISC IS SLICED INTO THREE LAYERS

THE DISCS AND AGAR PLATES WERE FREEZE-DRIED ON LYOPHILIZER

EACH DISC LAYER AND AGAR ARE COMBUSTED IN A SPECIAL FURNACE TO CONVERT RESIDUES TO CARBON DIOXIDE, PART OF WHICH IS RADIOACTIVE

THE AMOUNT OF RADIOACTIVE CARBON DIOXIDE IS DETERMINED IN A SCINTILLATION COUNTER

DISCS EXTRACTED WITH ACETONE AND METHANOL THE EXTRACTS ARE THEN CO-CHROMATOGRAPHED WITH OR WITHOUT THE ORIGINAL RADIOACTIVE ORTHENE OR MONITOR.

THE CHROMATOGRAMS ARE SCANNED UNDER A GEIGER COUNTER WITH AUTOMATIC RECORDER ATTACHED. THE TRACINGS REVEALS WHETHER THE EXTRACTS STILL CONTAIN THE ORIGINAL ORTHENE AND MONITOR AS AN INTACT MOLECULE OR NOT.

INTACT NUTS OF NONPAREIL AND MISSION WERE LIKEWISE TREATED. DISCS WERE EXCISED 24 HOURS LATER AND ANALYZED AS ABOVE. PART OF THE NUTS AND DISCS WERE SURFACE STERILIZED TO INHIBIT MICROBIAL BREAKDOWN OF THE INSECTICIDES.

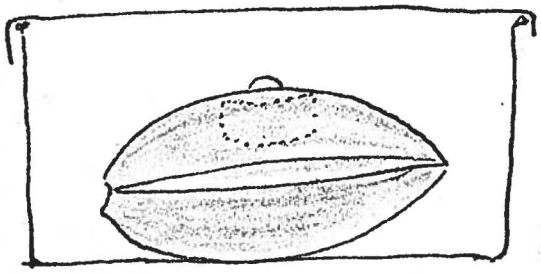


Table 1. Distribution of radioactivity in almond seedlings five days after treatment with ^{14}C-Monitor.

Fraction	Percent recoverable radioactivity	CPM per mg dry matter	Percent radioactivi on dry weight basis
Rinse	0.98	--	--
Young leaves	12.82	31.1	9.6
Leaves and stems above the treated leaf	12.20	18.7	5.8
Leaves and stem adjacent to treated leaf	13.77	12.9	4.0
Treated leaf	24.78	230.0	70.9
Leaves and stems just below treated leaf	9.42	9.8	3.0
Leaves and stem just above the soil	17.12	8.4	2.6
Roots	8.24	5.5	1.7
Carbon dioxide	0.67	--	--

Table 2. Distribution of radioactivity in different layers of almond hull 24 hr after treatment with radioactive Monitor.

Layers	Percent of recoverable radioactivity		Percentage distribution of radioactivity by dry weight basis	
Top	38.5	37.9	31.1	30.8
Middle	44.0	44.7	37.8	13.6
Bottom	14.3	16.9	20.4	22.1
Agar	3.2	0.5	10.7	3.5

Counts were derived from hull discs which were pretreated with 1% Clorox prior to application (left column) and non-treated (right column)

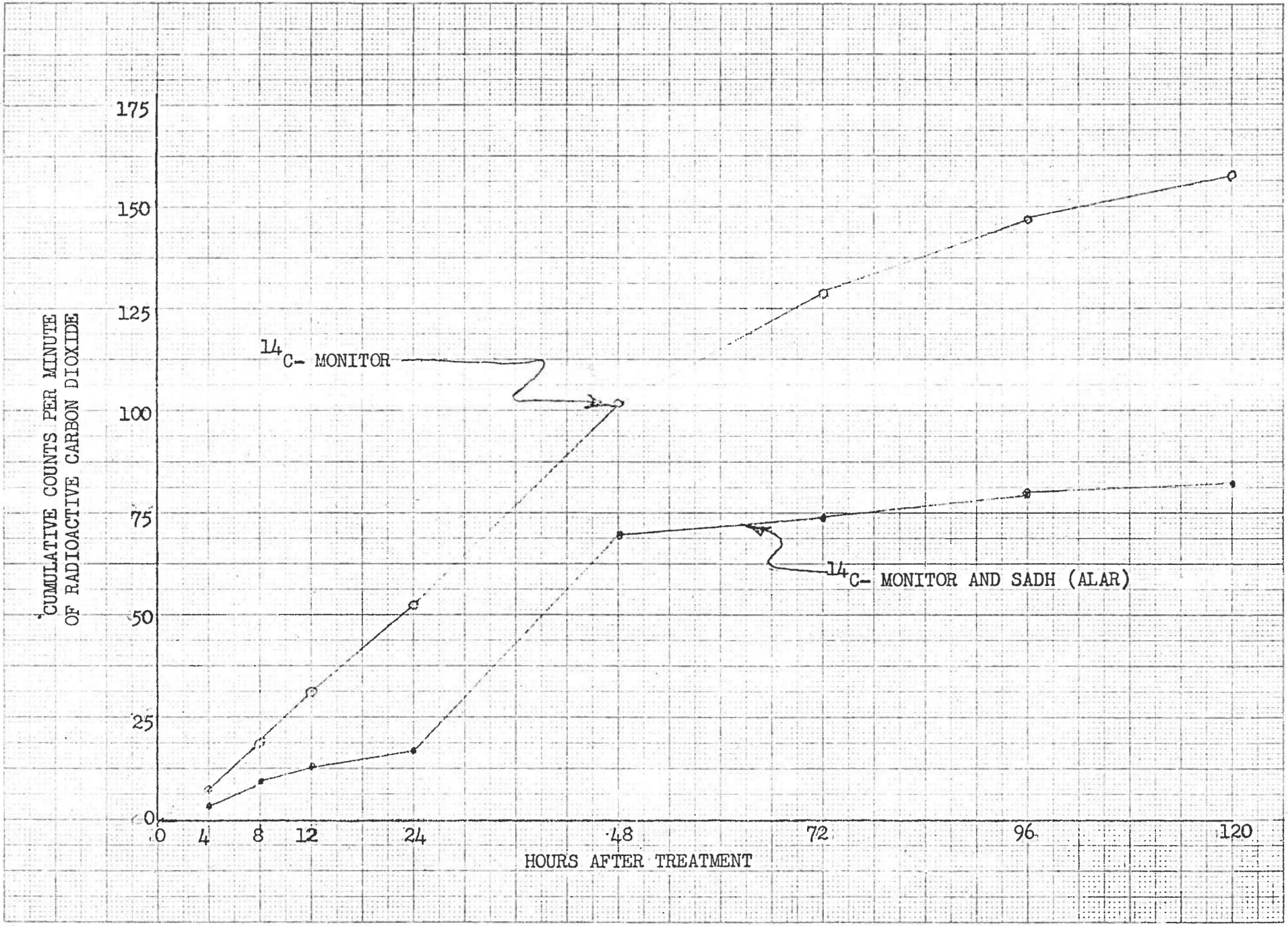

175
150
125
100
75
50
25
0
CUMULATIVE COUNTS PER MINUTE OF RADIOACTIVE CARBON DIOXIDE
14C- MONITOR
14C- MONITOR AND SADH (ALAR)
0
4
8
12
24
48
72
96
120
HOURS AFTER TREATMENT

Table 3. Gum and mucilage contents of 18 almond cultivars as estimated by the amount of acid hydrolyzable reducing substances.

Cultivar	Mg reducing substances per 50 ml 'slurry' Sampling Dates	
	July 16	August 4
1. Almendro	40	20
2. Davey	40	-
3. Drake	42	76
4. IXL	80	68
5. Kutsch	55	82
6. La Maria	38	68
7. La Prima	38	50
8. Long IXL	54	87
9. Marcona	28	30
10. Merced	85	85
11. Mission	95	50
12. Ne Plus Ultra	47	53
13. Nonpareil	55	57
14. Peerless	134	88
15. Tardy Nonpareil	57	66
16. Thompson	74	193
17. Walton	88	93
18. Weststyen	55	188