alone. Further trials will be required to determine the maximum levels of spray treatments which may be safely used and the levels which induce maximum cold resistance.

Effects of treatments upon subsequent growth. In all cases where spray treatments were applied, the resumption of normal growth was delayed. The plants receiving the high-level treatments did not resume growth for 3 months, and all new shoots originated from the axils of leaves other than those produced by the last growth flush. In most cases several shoots developed from two or more axils, and the resulting growth was poor for several weeks until one shoot gained dominance and developed into a new central stem. Plants receiving lower level spray treatments did not resume growth as quickly as control plants not subjected to cold treatments, but all resumed growth within 10 weeks. Two of the cold-treated plants sprayed with 10 ppm Dalapon showed no evidence of any damage, either to leaves or terminal meristems, and these resumed normal growth in late April. Unsprayed plants subjected to cold treatments developed new shoots in April from axils of the older leaves. Such shoots grew more rapidly on these plants than on those which had received the higher level spray treatments, suggesting that no treatment at all was superior to treatment with relatively high levels of these materials.

Summary and Conclusions

Ten ppm Dalapon and 500 ppm maleic hydrazide sprays increased the cold resistance of leaves of small lychee trees. These treatments retarded growth, but apparently caused no permanent damage. Sprays of 100 ppm Dalapon and 1000 ppm maleic hydrazide were toxic and appeared to cause more injury to the plants than the cold treatments used in this experiment. Further trials will be necessary before the value of these materials for increasing cold resistance can be adequately determined.

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INVESTIGATIONS ON GROWTH SUBSTANCES IN PEACH BUDS

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In plants, periods of growth by elongation alternate with periods in which little growth takes place. The phase of outward inactivity in the growth cycle has been termed "dormancy". A number of plants, particularly those adapted to growing in a temperate climate, will not resume active growth in the spring unless they have been exposed to a sufficient amount of cold during the winter. Many Floridians have observed this phenomena in trying to get temperate-climate plants to thrive under local climatic conditions. For example, certain varieties of apple, apricot, blackberry, blueberry, peach, plum, etc., will not grow and fruit well in this state. The major barrier to growing many of these plants in parts of Florida is that winters are frequently too mild to provide sufficient chilling to terminate bud dormancy.

For many years, empirical research has sought for chemicals and types of treatment to break or induce dormancy. A good many treatments have been found which can alter dormancy, but only in the last few years has there begun to evolve the semblance of a physiological understanding of dormancy and how it can intelligently be controlled.

Most present concepts of the physiological mechanisms controlling dormancy embrace the idea that the rest of buds is intimately associated with either a super or suboptimal

growth hormone content or with naturally occurring growth inhibitors. A few of the milestones in the development of these concepts are as follows: Boysen Jensen (4) first suggested that dormancy was due to a lack of auxin. To add strength to this idea, Zimmerman (19) could ascertain auxin only in sprouting buds, whereas Avery, *et al* (1) could find no auxin in dormant buds. Moreover, Bennett and Skoog (2) found that "free" auxin gradually increased as bud rest diminished. Also, they were able to hasten the termination of bud dormancy with the application of auxin.

Conversely, there are observations that cannot be explained solely on the basis of a suboptimal auxin concentration. Guthrie (7) observed that under some circumstances auxin treatment could actually stimulate sprouting of potatoes, whereas Eggert (5) showed that rest was terminated when the auxin content of apple buds dropped below 0.25 ug. Also, Gouwentak (6) found little or no forcing action when auxin was applied to dormant buds of several plant species.

The most recent milestone in the development of an understanding of dormancy was laid by Hemberg in his work on potato and *Fraxinus* buds (8) (9) (10) (11) (12) (13). Since there was a definite relationship between the growth inhibitor content of these buds and dormancy, he proposed that periods of outward inactivity of buds was caused by inhibitors. Following this extensive investigation, others (3) (14) (17) have reported that inhibitors are present in much greater quantities in dormant than in non-dormant buds. Also, Hendershott (14) has found a close correlation between the growth of peach buds on excised twigs and the disappearance of inhibitors.

Since the theories advanced to explain dormancy generally assume that growth-controlling substances are involved and because investigations on the natural regulators actually present in plants during the different phases of growth are rare, a study was initiated to determine the qualitative and quantitative changes in growth substances of peach buds at different stages in the yearly growth cycle. The present investigation was primarily concerned with the development of a method to separate the growth substances in actively growing buds.

Experimental Approach and Results

Dormant and non-dormant buds of *Prunus persica* var. Okinawa were randomly collected from two-year-old trees and quick-frozen immediately, using an acetone and dry-ice mixture. Samples of dormant buds were comprised of apical meristems plus bud scales, and samples of non-dormant buds were comprised of apical meristems plus the young unfolded leaves and the first 4-8 mm. of stem. One gram samples of the buds were pulverized while in the frozen state before trans-

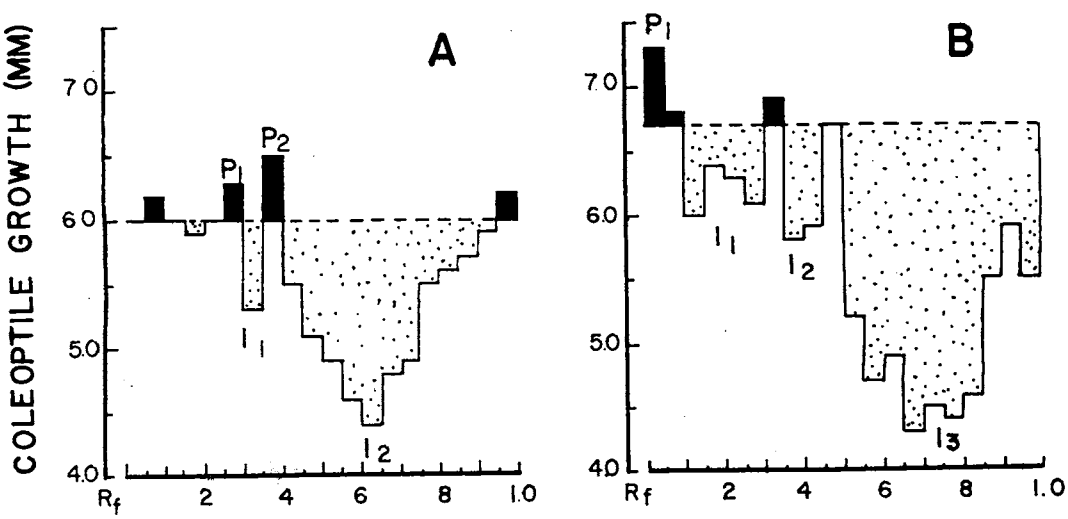

Figure 1 — Histograms representing the growth activity on chromatograms of methanol extracts from (A) actively growing peach buds and (B) dormant vegetative buds.

ferring to 50 ml. of 95 per cent methanol which was kept at 0-4°C during the extraction periods. The extraction was repeated 3 times at 30-minute intervals with 50 ml. of methanol each time. The combined extracts were evaporated to dryness under reduced pressure and the residue washed four times with 5 ml. portions of n-hexane. The washed residue was dissolved in absolute methanol, transferred to Whatman 3 MM chromatographic paper and developed in iso-propanol (80): ammonia (1): water (19) at room temperatures. After the solvent had ascended 26-30 cm. the chromatograms were dried and assayed biologically with the sensitized *Avena* coleoptile test (16).

When a comparison was made of the chromatograms of methanolic extracts from non-dormant and dormant peach buds, a striking difference was found in the growth inhibitors present. As can be seen in Fig. 1, dormant buds seem to contain both a greater number and a larger quantity of growth inhibitors. In contrast to the inhibitors, there was no marked change in the quantity of growth promoters. However, it was true that the growth promoters shifted positions on the chromatogram, thus denoting a change in the type of promoter.

It should be pointed out that there was a great deal of variability from test to test as to the apparent position and quantity of growth substances on the chromatograms, even with aliquots of the same extract treated identically. This indicated two main possibilities. Either the bio-assay had so much inherent variability that under present conditions it could not be quantitatively used with any reliability, or the extracts were such a complicated mixture of growth substances that comparable chromatograms could not be obtained. Using chromatograms of known compounds, an analysis of the bio-assay indicated that there was variability in the test, but that it could be used with precision under the conditions outlined by Nitsch (16). These tests also showed that any activity greater than 0.2 mm. can be regarded as significant.

Additional evidence indicated that the source of difficulty was due to a complexity of substances. When various solvents, i.e. methanol, ethyl acetate, ethyl ether, and water, were used as the extracting medium, the number, position and intensity of the zones of activity on the chromatograms varied greatly. Therefore, attention was turned to methods of separating further this mixture of growth substances.

Bud samples from non-dormant, vegetative peach buds were treated the same as outlined in the above method until the dried residue of the methanolic extract was obtained. Then, using a small amount of 95 per cent methanol, the residue was transferred directly to a silicic acid column which was prepared and developed in a manner similar to that described by Powell (18) for the separation of indole compounds. Briefly, silicic acid (100 mesh) was washed of fine particles with water and then oven-dried at 100°C to a constant weight. The dried silicic acid (16 gms.) was mixed with water (10.46 ml.) before it was suspended in a small amount of petroleum ether and used to prepare a column with a diameter of 2.0 cm.

After the plant sample was added to the top of the column, it was fractionated with the following solvent systems at a flow rate of 1-2 mls. per minute: (A) 200 ml. of petroleum ether and n-butanol (sat. with H_2O), 99.8:0.2; (B) 200 ml. of petroleum ether and n-butanol (sat. with H_2O), 97:3; (C) 200 ml. of petroleum ether and n-butanol (sat. with H_2O), 25:75; (D) n-butanol (sat. with H_2O); and (E) water. The solvent mixtures (e.g. solvent A, etc.) were collected separately and concentrated to dryness under reduced pressure. The residues were dissolved in 95 per cent methanol and spotted on Whatman 3 MM paper strips. The development and treatment of the paper chromatograms were identical to the method previously described.

When chromatograms of the various fractions were analyzed with the *Avena* coleoptile test, it was evident that at least seven significant growth promoters and six prominent growth inhibitors were present in the methanolic extracts from non-dormant peach buds. Histograms of the growth activity on the chromatograms can be seen in Fig. 2A, B, C, D, and E. When the graphs were studied collectively, the active materials were located within the following R_F ranges: growth promotors at 0.0-0.05, 0.0-0.45, 0.0-0.5, 0.05-0.15, 0.1-0.35, 0.45-0.55, and 0.9-0.95; and growth inhibitors at 0.05-0.1, 0.15-0.55, 0.45-0.8, 0.6-0.75, 0.6-0.8, and 0.85-1.0. As can be noted from these values, a considerable degree

of overlapping of active zones would be expected in a chromatogram of the crude extract.

When the activity found in the five fractions were arithmetically balanced, a histogram

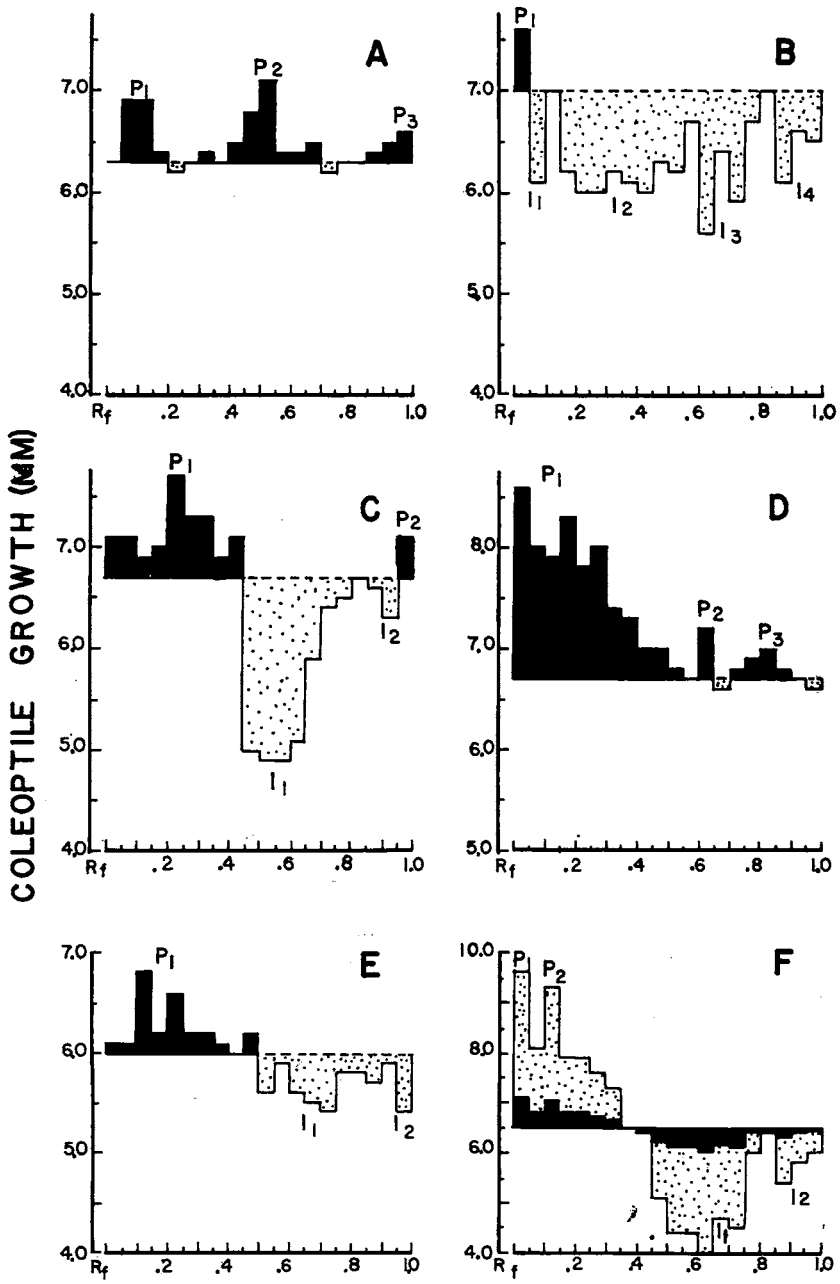

Figure 2 — Histograms representing the growth activity on paper chromatograms of column-fractionated portions from a methanol extract of non-dormant peach buds. Each solvent system was concentrated and rechromatographed. The solvent systems for the silicic acid column were (A) 200 ml. of petroleum ether and n-butanol (sat. with H_2O), 99.8:2; (B) 200 ml. of petroleum ether and n-butanol (sat. with H_2O), 97:3; (C) 200 ml. of petroleum ether and n-butanol (sat. with H_2O), 25:75; (D) n-butanol (sat. with H_2O; and (E) H_2O. (F) Dotted area represents the arithmetical total of activity of the five fractions and black area the total activity divided by five.

was obtained such as portrayed in Fig. 2F. The solid area plus the dotted area represents the calculated total activity and the solid area alone represents the activity divided by the number of fractions. Since the growth substances may not give a linear response of promotion or inhibition in the bio-assay and because secondary reaction may exist between promoters and inhibitors, a true picture would not be reflected either by the solid area or the dotted area plus solid area. However, the true values would be expected to fall some place within the dotted area. Even with these reservations in mind, a striking similarity can be seen between Fig. 2F (a compilation graph of five fractions obtained from a methanol extract of peach buds) and Fig. 1A (a chromatogram of a methanol extract of the same type of material). Therefore, it would seem reasonable to assume that further separation of the growth substances must be accomplished before definite conclusions can be reached as to the qualitative or quantitative changes occurring in the growth substances preceding, during or following dormancy.

Preliminary investigations on the nature and properties of these substances have yielded the following results: (a) None of the zones of activity on the chromatograms gave a positive reaction with Erlich's reagent (1 per cent solution of p-dimethylaminobenzaldehyde in IN HCl) or Gordon-Weber's reagent (0.01 M $FeCl_3$ in 5 per cent $HClO_4$). (b) Zones of activity I_2 and I_3 of fraction B (see Fig. 2B) yielded highly fluorescent compounds under ultra-violet light when treated with alcoholic $AlCl_3$. I_2 fluoresced a brilliant greenish-white and I_3 a brilliant whitish-yellow.

From a comparison between the data of Hendershott and Walker (15) and the above data, it would seem reasonable to assume that I_2 of fraction B is a flavonone and possibly naringenin.

Discussion

These results contribute experimental evidence to support the idea that several growth-controlling substances may be involved in bud dormancy. If this is the case, a great deal of caution will need to be taken in the search for naturally occurring compounds that play a role in controlling dormancy. For instance, growth activity found on paper chromatograms of crude or partial purified extracts may be the net activity resulting from several substances. Furthermore, it could well be that certain of these growth substances have no real part in controlling the growth of buds, but are artifacts arising from the disruption of cellular organization, chemical changes during extraction and fractionation, or extrapolation of bio-assay activity to bud growth. Nevertheless, it is necessary to separate the growth substances and to determine the chemical identity, qualitative and quantitative relation of the compounds to the periodicity of growth, and physiological effect of each before a better understanding of the biochemical mechanisms controlling dormancy can be reached.

Summary

1—Solvent fractionation, column and paper chromatography, and the sensitized *Avena* straight-growth bio-assay disclosed no less than seven growth promoters and six growth inhibitors in methanolic extracts of non-dormant peach buds.

2—Comparisons between the activity found by bio-assay on paper chromatograms of crude extracts from buds in different stages of growth and development may not be valid from either a qualitative or a quantitative standpoint since any segment of the chromatogram may contain several substances exhibiting activity.

3—Preliminary data are given on the response of the growth-promoting and inhibiting substances to indole and flavonone reagents.

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THE EFFECTS OF DIFFERENTIAL LEVELS OF NITROGEN, POTASSIUM AND MAGNESIUM ON THE GROWTH OF LYCHEES

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The lychee (Litchi chinensis, Sonn), a member of the Sapindacea family, was introduced into Florida during the latter part of the nineteenth century, but commercial production of this fruit in the state dates from World War II. Since that time more than 13,500 commercial lychee trees have been planted which are capable of producing an estimated million pounds of fruit.

Under Florida conditions the lychee is an erratic bearer and suffers from leaf tip necrosis. These two factors are among the major deterrents to the growth of the industry. This experiment was initiated on the belief that these factors are at least partially the result of nutritional deficiencies. No results of critical nutritional research has been found in the literature.

MATERIALS AND METHODS

Air-layered lychee trees from Palmer's Nursery, Osprey, Florida, were planted on April 13, 1957, in polyethylene containers (26-quart-size) filled with pure quartz sand. The containers were attached to a mechanical device so that the trees were subirrigated with nutrient solutions four times daily. Treatments consisted of three levels each of nitrogen (30, 80 and 210 parts per million), potassium (8, 32 and 180 ppm) and magnesium (12, 24 and 54 ppm), in a 3x3x3 factorial experiment, confounded in blocks of nine treatments and replicated four times. The other elements were provided in the solutions in the following elemental concentrations expressed as ppm: P 10, Ca 160-180, Mn 0.5, Zn 0.05, Fe 0.5, B. 0.25, Cu 0.01 and Mo 0.001.

TABLE 1. INTERACTION OF NITROGEN AND POTASSIUM ON INCREASE IN CALIPER GROWTH IN MILLIMETERS OF LYCHEE TREES GROWN IN SAND CULTURE, 1958

Levels	Nitrogen Levels in Substrate			Potassium
	N-1, 30 ppm	N-2, 80 ppm	N-3, 210 ppm	Level Means
K-1, 8 ppm	78.66	39.00	8.69	42.12
K-2, 32 ppm	83.58	32.14	14.45	43.39
K-3, 180 ppm	21.48	12.89	2.98	12.45
Nitrogen level means	61.24	28.01	8.71	
L.S.D.			0.05	0.01
Between nitrogen and potassium level means			12.17	16.16
Between means within table			20.98	27.86