7. Martin, R. E. and J. B. Crist. 1970. Elements of bark structure and terminology. *Wood & Fiber* 2:269-279.
8. Pokorny, F. A. 1966. Pine bark as organic amendment in the production of container plants. *Ga. Agr. Res.* 7:8-9.
9. _____ and J. Gugino. 1967. Comparison of potting media for herbaceous pot plants. *Ga. Agr. Res.* 9:3-4.
10. _____ and R. A. McCreery. 1967. Water relations in potting mixes for ornamental plants. *Ga. Agron. Abst.* 10:17.
11. Rasmussen, H. P. and G. R. Hooper. 1974. Electron optics: Principles, techniques, and application in horticulture. *HortScience* 9:425-433.
12. Sproull, R. C. 1967. Fiber, chemical, and agricultural products from southern pine bark. *For. Prod. J.* 19:38-44.
13. _____ and G. A. Pierce. 1963. Bark utilization. I - Soil amendment. *Tech. Assoc. Pulp & Paper Ind.* 46(8):175A-178A.
14. White, M. S., G. Ifju, and J. A. Johnson. 1974. The role of extractives in the hydrophobic behavior of loblolly pine rhytidome. *Wood & Fiber* 5:353-363.

J. Amer. Soc. Hort. Sci. 103(3):408–413. 1978.

Rootstock and Seasonal Influences on Carbohydrate Levels and Cold Hardiness of 'Redhaven' Peach[1]

R. E. C. Layne and G. M. Ward[2]
Agriculture Canada, Research Station, Harrow, Ontario, NOR IGO, Canada

Additional index words. Prunus persica

Abstract. **Flower buds and apical shoots of 'Redhaven' peach (*Prunus persica* (L.) Batsch) were shown to be slightly more cold hardy in the autumn and winter when propagated on seedlings of Siberian C than on those of Harrow Blood. Apical shoots consistently had higher levels of total carbohydrates, reducing sugars, and other carbohydrate fractions on Siberian C than on Harrow Blood seedlings from winter to spring. The cold hardiness of flower buds was closely correlated with the hardiness of apical shoots. In addition, both flower bud and shoot hardiness were closely correlated with total sugars, sucrose, and reducing sugars in the shoots from autumn to spring. However, hardiness of flower buds and apical shoots was not correlated with total carbohydrates or starch. The TI_{50}, a new method of expressing the hardiness of apical shoots was an objective index of cold hardiness and somewhat analogous to the T_{50} method for expressing hardiness of flower buds.**

Cold injury is a major limitation to peach culture in Canada (8) and the Northern U.S. (7). Therefore, any enhancement of cold hardiness of peach scion cultivars by whatever means is potentially very important.

Recently, peach seedling rootstocks obtained from different seed parents have been found to differ significantly in some of the effects they exert on peach scion cultivars, including such important characters as nutrient uptake (8), growth (10), time of leaf fall (11), cold hardiness in fall and winter (11), disease response (9) and winter survival (8, 13).

Certain carbohydrate fractions in peach flower buds and shoots, particularly sugars, have been found to be correlated with cold hardiness (1, 3, 4, 5, 6). According to Levitt (12), sugars may increase the freezing tolerance of plant cells in 2 ways – by the osmotic effect and by the metabolic effect. In the former case, sugars may increase cell avoidance of freeze-induced dehydration while in the latter they may be metabolized to produce unknown protective changes. A decrease in starch and a corresponding increase in sugars has long been associated with cold acclimation in woody plants (3, 12). Cold acclimation is an active process which requires an energy and carbon source. Carbohydrates play an important and ubiquitous role in this process. Their effects are both direct and indirect and are consequently important (12).

It is not known whether or not peach seedling rootstocks influence the carbohydrate metabolism of peach cultivars. If this occurred, it may provide an additional explanation, apart from the recently recognized rootstock effect on early defoliation and dormancy (11), for the promotion of increased scion hardiness by certain peach seedling rootstocks. The study reported here was undertaken to test this possibility, and also to observe the seasonal pattern of carbohydrate metabolism in peach in Southwestern Ontario.

Materials and Methods

This study was conducted beginning at leaf fall in Oct. 1973 and ending just before bloom in late April 1974. Samples were collected from a 5-year old replicated experimental orchard of 'Redhaven' propagated on each of 4 peach seedling rootstocks (10). The study was conducted on only 2 of the 4 seedling stocks – Siberian C and Harrow Blood, which induced early and late defoliation, respectively (11). Each scion-rootstock combination was in 3-tree plots that were replicated 5 times in a randomized complete block design.

The first samples were collected on Oct. 24 after the second fall frost had occurred. At this time trees on Siberian C seedlings were 90% defoliated while those on Harrow Blood seedlings were only 10% defoliated. Samples were also collected during the dormant period in Dec., Jan., and Feb., and the last samples were collected on April 29 when the flower buds were in the prepink to early pink stage of development.

Terminal shoots were collected about 1.5 to 2.0 m above the ground from the periphery of 3 trees per plot. On each sample date 9 shoots per plot were used for cold hardiness tests and 6 for chemical analysis. The shoots were trimmed to a uniform length of 30 cm measured from the apex.

The controlled freezing tests were conducted in a manner previously described using a liquid nitrogen freezing chamber (11). Flower bud hardiness was expressed as the estimated stress temperatures (T_{50}) required to kill 50% of the flower buds (11). Tissue hardiness was assessed based on continuity and intensity of tissue browning in the pholem, cambium and xylem regions of the apical 15 cm portions of each shoot. Ratings were made on a scale from 1 to 4 where 1 represented none to very little injury and 4 represented severe injury likely to cause death (11). A mean tissue injury rating of 2.5 was one

[1]Received for publication September 19, 1977.

[2]Research Scientists in Pomology and Chemistry, respectively. Dr. G. M. Ward is now retired. The technical assistance of H. O. Jackson and G. F. Zajacz is gratefully acknowledged.

in which 50% of the length of the region examined was severely injured and likely to die. The temp at which such injury occurred was estimated by plotting mean tissue injury ratings on the Y-axis and stress temp on the X-axis. The temp at which the resulting curves crossed the line with a tissue injury rating of 2.5 was defined as the TI_{50} (Fig. 1).

Shoots for carbohydrate analysis were collected at the same time as those for cold hardiness determinations, with the exception of Jan. 16, 1974, when only samples for hardiness tests were collected. Each sample was a composite of 6 shoots per plot. Samples were prepared for carbohydrate analysis as soon as possible after the shoots were collected. Flower buds on each shoot were removed and analyzed separately. Shoots were cut into small segments then ground in a Stein laboratory mill. The buds were sufficiently small that they were not further subdivided. The prepared tissue was extracted by refluxing in 80% ethanol and analyzed for reducing sugars, sucrose, and starch using methods outlined by Ward and Johnston (14). The moisture content of each sample was also determined.

Analyses of variance were conducted on the T_{50} and TI_{50} data for each collection date using rootstocks as treatments and standard errors about each mean were calculated and plotted (Fig. 2). The mean daily temp from Oct. 24, 1973 to April 29, 1974 were obtained from official weather records at the Harrow Research Station (Fig. 2). The weather station was located about 2 km from the experimental orchard and was at a similar elevation.

Analyses of variance were conducted on the chemical data for flower buds and for apical shoots using rootstocks as treatments and standard errors about the means were calculated and plotted (Fig. 2). Correlation analyses were conducted on the hardiness data for buds and shoots and chemical data for shoots for each scion/rootstock combination throughout the over-wintering period (Oct. 24 to April 29). In addition, the data were pooled over rootstocks and a separate analysis of variance was conducted for each carbohydrate fraction on each sample date using the data for flower buds and stems as the treatments to show seasonal trends. Again, appropriate standard errors about the means were calculated and plotted (Fig. 3). Correlation analyses of the chemical and hardiness data pooled over rootstocks was done for flower buds and also for stems for the overwintering period.

Results

Seasonal variations in air temp. During the sample period from Oct. 24, 1973 to April 29, 1974, mean daily temp at or below 0°C occurred 3 times in Nov., 19 in Dec., 17 in Jan., 21 in Feb., 9 in March and once in April. In each month rather wide fluctuations in mean daily temp were recorded. In Nov. the range was 13°C, while in Dec., Jan., Feb., March and April it was 19°, 16°, 18°, 23°, and 20°, respectively. Despite the wide fluctuations in mean daily temp a general cooling trend from Oct. to Dec. and a warming trend from Feb. to April was evident (Fig. 2).

Seasonal variations in hardiness. Flower buds were more cold sensitive in April than in Oct. and were least cold sensitive in Dec., Jan. and Feb. (Fig. 2). The trend towards an increase in hardiness from fall to winter and a decrease of hardiness from winter to spring followed the general cooling and warming pattern (Fig. 2). A similar pattern was observed for the hardiness of apical shoots as that described for flower buds (Fig. 2). In addition the TI_{50} values for apical shoots were similar to the T_{50} values for flower buds.

Rootstock effects on cold hardiness. On 2 of the 5 sample dates, Oct. 24 and Feb. 18, flower buds on rootstock seedlings of Siberian C were significantly (P = 5%) more cold hardy than on rootstock seedlings of Harrow Blood (Fig. 2). Apical shoots were slightly more hardy on Siberian C than on Harrow Blood seedlings in Jan. and Feb., but were not different in Oct., Dec. and April (Fig. 2).

Moisture content. Peach flower buds had a lower moisture content than apical shoots throughout the overwintering period (Fig. 3). Whereas the moisture content of flower buds was highest in spring just before bloom, lowest in winter and intermediate at leaf fall, the moisture content of peach shoots changed little from fall to spring.

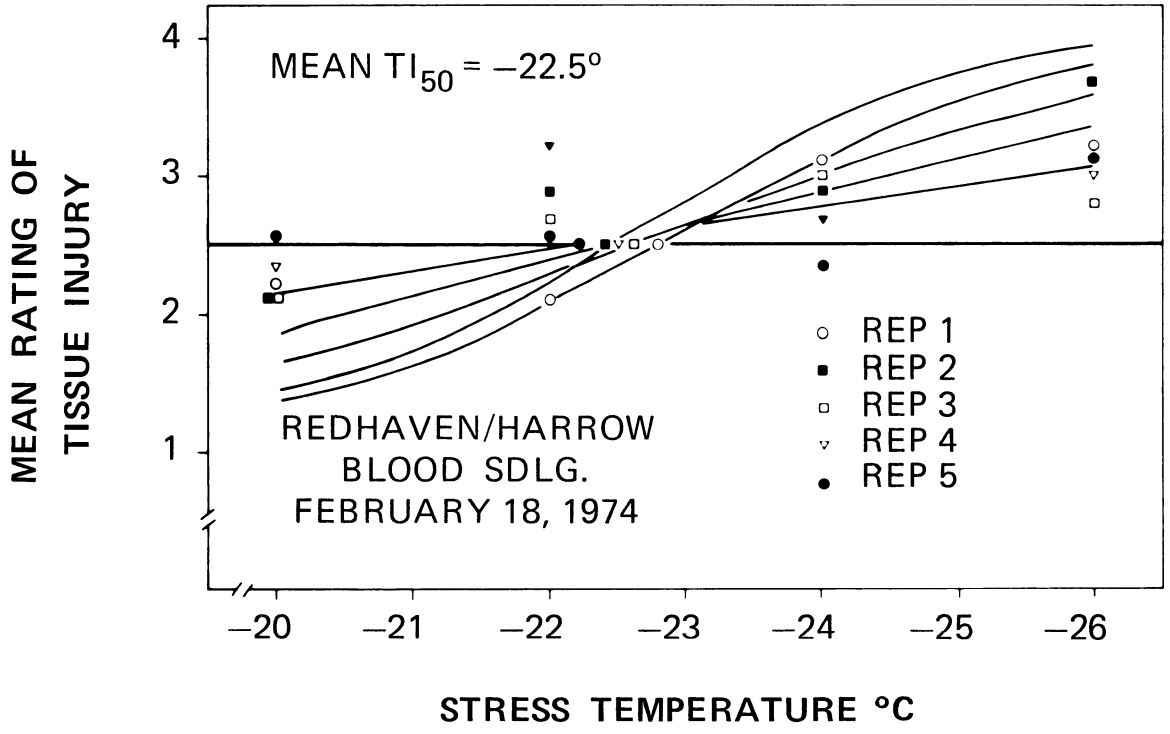

Fig. 1. Graphical method for determining TI_{50}, the temperature at which 50% of the shoot tissue is severely injured and possibly killed.

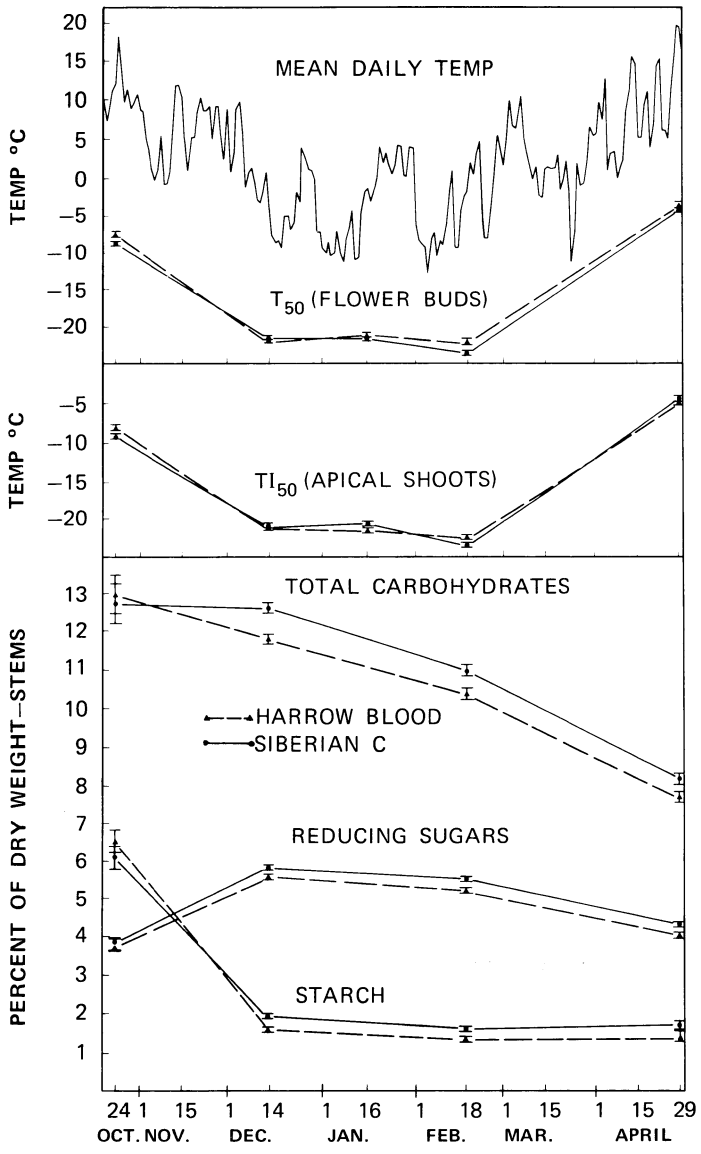

Fig. 2. Seasonal and rootstock effects on cold hardiness of peach flower buds and apical shoots and on levels of total carbohydrates, reducing sugars and starch.

Total carbohydrates. With the exception of the autumn when carbohydrate levels in flower buds and apical shoots were similar, the levels in flower buds were higher than in apical shoots for the remainder of the overwintering period (Fig. 3). Carbohydrate levels in shoots decreased progressively from fall to spring, while in flower buds they increased moderately from fall to winter, remained fairly constant during the winter, then increased sharply from winter to spring.

Total sugars. From fall to spring the level of total sugars in the flower buds was higher than in the shoots (Fig. 3). The pattern for total sugars in the flower buds was similar to that for total carbohydrates. However, total sugars in the apical shoots increased from fall to early winter then decreased progressively from early winter to spring.

Reducing sugars. Throughout the overwintering period the levels of reducing sugars in the flower buds were higher in the apical shoots (Fig. 3). Similar patterns were evident until mid Feb. when there was a sharp rise in the sugar levels in flower buds accompanied by decreasing levels in the shoots.

Sucrose. From Oct. to Dec. similar levels of sucrose were found in flower buds and apical shoots (Fig. 3). From Dec. to April the levels in the shoots declined steadily, while in the flower buds they increased in Feb. then decreased slightly in April.

Starch. In the autumn starch levels were higher in the shoots than the flower buds (Fig. 3). In the winter the levels were not different but in the spring they were slightly higher in flower buds than in apical shoots.

Rootstock effects on carbohydrate levels. There were no statistically significant rootstock effects on carbohydrate fractions in the flower buds, therefore the data are not presented. However, certain carbohydrate fractions in the stems, including total carbohydrates, reducing sugars and starch, were influenced by rootstocks (Fig. 2). Apical shoots had higher levels of total carbohydrates, reducing sugars and starch from winter to spring on Siberian C than on Harrow Blood seedling rootstocks. In the fall, there was a trend for higher levels of total carbohydrates and starch on Harrow Blood than on Siberian C seedlings but the differences were not statistically significant (Fig. 2).

Inter-relationships of cold hardiness and chemical characteristics. **Flower buds.** The cold hardiness of flower buds was negatively correlated with reducing sugars and moisture content in the buds but was not correlated with total carbohydrates, total sugars, sucrose or starch levels (Table 1).

Apical shoots. The cold hardiness of apical shoots was positively correlated with total sugars, reducing sugars and sucrose in the shoots (Table 1); and was negatively correlated with moisture content, but was not correlated with levels of total carbohydrates or starch. The cold hardiness of peach flower buds was positively correlated with the hardiness of peach shoots during overwintering ($r = +0.998$, $P = \leqslant 1\%$).

Rootstock effects. In general, the associations found for carbohydrate fractions in peach flower buds and shoots with hardiness were similar whether the rootstocks were Siberian C or Harrow Blood seedlings (Table 2). With each rootstock the highest correlations for carbohydrate fractions in the shoots with bud and shoot hardiness were obtained with total sugars, reducing sugars and sucrose. With neither rootstock were starch levels in the shoots correlated with bud or shoot hardiness. Total carbohydrates in the shoots were significantly correlated with bud hardiness when the rootstocks were Siberian C seedlings, but were not correlated with bud hardiness when they were Harrow Blood seedlings. The associations of carbohydrate fractions in the shoots with flower bud and shoot hardiness were generally closer with Harrow Blood than Siberian C rootstock seedlings. In addition, flower bud and shoot hardiness were very closely correlated whether the rootstocks were Siberian C ($r = +.998$, $P = \leqslant 1\%$) or Harrow Blood ($r = +.996$, $P = \leqslant 1\%$).

Discussion

The general hardiness pattern that we observed (Fig. 2) for peach flower buds and apical shoots during overwintering was similar to that previously reported (1, 3, 4, 5, 6). In this study wer were able to make a more direct comparison of the relative cold hardiness of peach buds and apical shoots than was previously possible. The TI_{50} for peach shoots was sufficiently analogous to the T_{50} for peach flower buds to make possible a direct comparison of the relative hardiness of these tissues (Fig. 2). The TI_{50} was an objective means of expressing the cold hardiness of peach shoots. It appeared to have many of the advantages already recognized for the T_{50} which is now the standard method of expressing the hardiness of peach flower buds (10).

We found, as other have (1, 3, 4, 5, 6), that cold hardiness of peach was closely correlated with certain carbohydrate fractions in the shoots and flower buds. Total sugars, reducing sugars and sucrose were the carbohydrate fractions in peach shoots that were most closely associated with shoot hardiness,

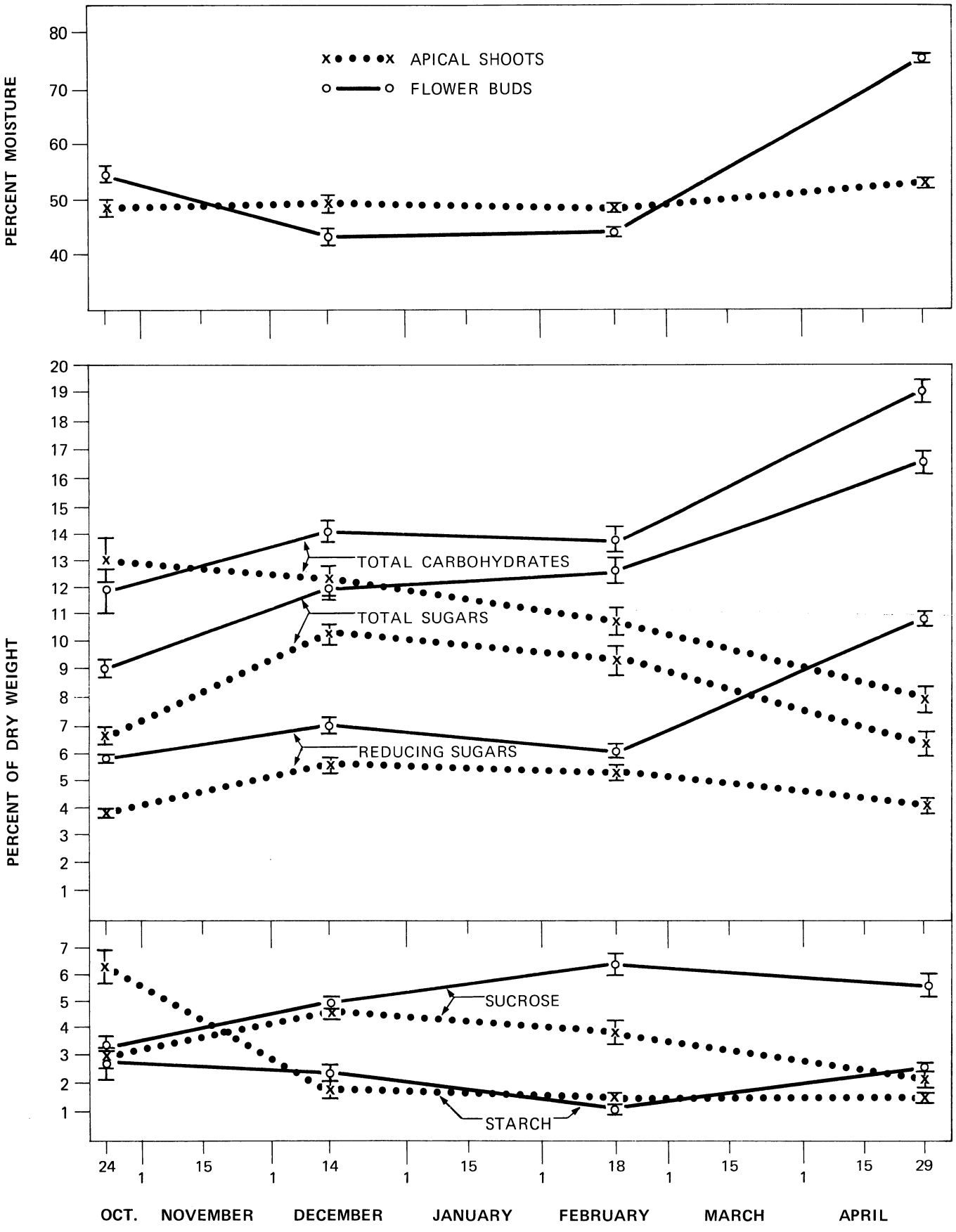

Fig. 3. Seasonal effects on moisture content and carbohydrate levels of peach flower buds and apical shoots.

Table 1. Correlation of cold hardiness of 'Redhaven' flower buds and apical shoots with corresponding chemical characteristics of these plant parts.

Chemical characteristics flower buds and shoots	Correlation coefficient	
	Flower buds cold hardiness	Apical shoots cold hardiness
Total carbohydrates	−.408[z]	+.381
Total sugars	−.264	+.915**
Reducing sugars	−.573**	+.890**
Sucrose	+.306	+.882**
Starch	−.385	−.403
Moisture content	−.875**	−.586**

[z]Each correlation coefficient is based on 20 pairs.

Table 2. Correlation of cold hardiness of 'Redhaven' flower buds and apical shoots on Siberian C and Harrow Blood seedling rootstocks with the chemical characteristics of apical shoots.

Chemical characteristics of apical shoots	Correlation coefficient			
	'Redhaven'/ Siberian C		'Redhaven'/ Harrow Blood	
	Bud cold hardiness	Shoot cold hardiness	Bud cold hardiness	Shoot cold hardiness
Total carbohydrates	+.447[z]*	+.442	+.323	+.317
Total sugars	+.869**	+.855**	+.937**	+.930**
Reducing sugars	+.853**	+.848**	+.918**	+.913**
Sucrose	+.861**	+.849**	+.896**	+.893**
Starch	−.374	−.368	−.427	−.427
Moisture content	−.531*	−.537*	−.603**	−.610**

[z]Each correlation coefficient is based on 20 pairs.

but reducing sugars in the flower buds were the only carbohydrate fraction that was closely associated with bud hardiness (Table 1). In fact, flower bud hardiness was more closely correlated with carbohydrate fractions especially sugars in peach shoots than those in the flower buds (Tables 1 and 2).

The flower buds usually accumulated higher levels of each carbohydrate fraction except starch than the shoots (Fig. 3). Both flower buds and shoots exhibited similar patterns in accumulation of sugars in the fall and early winter which paralleled the cold acclimation of peach buds and shoots (Fig. 2). During this period as sugars increased starch decreased – a relationship that appears to be quite general in temperate woody plants (12). As the winter progressed, and especially with the approach of spring, the levels of reducing sugars and sucrose increased substantially in flower buds as they decreased in the apical shoots (Fig. 3). The increased levels in the flower buds at this time were undoubtedly associated with growth and development which was very active compared with absence of similar processes in the shoots. The flower buds were undoubtedly metabolizing their own reserves during this period and withdrawing carbohydrates from the shoots to meet their requirements. Thus, in the late winter and spring, the carbohydrate fractions in the flower buds were apparently more closely related with morphogenesis than with cold hardiness. Meanwhile in the stems as total carbohydrates and sugars decreased so did the cold hardiness of the stems. Elmanova (4) found that carbohydrate metabolism of peach shoots was correlated with air temperature while that of flower buds was associated with morphogenesis.

Moisture content of peach flower buds appeared to be more closely but negatively associated with bud hardiness than carbohydrate metabolism (Fig. 3, Table 1). Moisture content of peach shoots was also negatively related to their cold hardiness during overwintering, but the hardiness relationship for shoots was not as close as for flower buds, nor was it as close as that between shoot hardiness and sugar accumulation in the shoots. Other workers have also found an inverse relationship between moisture content and cold hardiness in some plants (2, 12). The relationship in peach may be associated with the water content of flower buds and shoot xylem and their capacity to "deep supercool" (2). Presumably, the flower buds and stems "deep supercool" to lower temp when the moisture content of these tissues is also reduced.

A small rootstock effect on hardiness of 'Redhaven' flower buds and stems was demonstrated (Fig. 2) which could be quite important as critical temp are reached (11). The effect of Siberian C rootstock on flower bud and shoot hardiness was greatest during leaf fall and in Feb. (Fig. 2). In previous work, significant and larger rootstock effects on hardiness of peach flower buds and shoots were also detected in the autumn (11), but we did not observe such rootstock effects on hardiness of peach flower buds or shoots in the spring. Either such effects are not manifest in late spring just before bloom, or if present are sufficiently small that they are not readily detectable. They may also be suppressed by other factors such as moisture content (Fig. 3).

A possible mechanism for the nature of the rootstock effect on scion hardiness was the demonstrated rootstock effect on carbohydrate metabolism of peach shoots (Fig. 2). While the basic pattern of carbohydrate metabolism was the same, regardless of rootstocks, the main difference was that 'Redhaven' shoots had higher levels of each carbohydrate fraction from Dec. to April on rootstock seedlings of Siberian C than they did on those of Harrow Blood. Sugars are thought to play a cryoprotective role in the hardiness of plant cells (12). We obtained very close, positive, and highly significant correlations for the cold hardiness of peach flower bud and shoots during overwintering with certain carbohydrate fractions in the stems including total sugars, sucrose and reducing sugars (Table 2). The hardiness of peach buds and shoots, on the other hand, was not generally correlated with total carbohydrates or starch (Tables 1 and 2). Thus, rootstock seedlings of Siberian C compared with those of Harrow Blood, appeared to have a greater influence in promoting the cold hardiness of 'Redhaven' shoots because they also promoted greater accumulation or slower depletion of cryoprotective materials (e.g. sugars) in the shoots during overwintering.

Literature Cited

1. Bosi, A. and A. Zocca. 1971. Variations in carbohydrate contents and cold resistance in peach buds. (In Italian) *Riv. Ortoflorofrutt. It.* 55:118-123.
2. Burke, M. J., Gusta, L. V., Quamme, H. A., Weiser, C. J. and P. H. Li. 1976. Freezing and injury in plants. *Annu. Rev. Plant Physiol.* 27:507-528.
3. Dowler, W. M. and F. D. King. 1966. Seasonal changes in starch and soluble sugar content of dormant peach tissues. *Proc. Amer. Soc. Hort. Sci.* 89:80-84.
4. Elmanova, T. S. 1974. Changes in the accumulation and transformation of carbohydrates in the reproductive buds and one-year-old shoots of peaches. (In Russian). *Trudy Gos. Niki, Bot. Soda.* 64: 17-28.
5. Lasheen, A. M., Chaplin, C. E. and R. N. Harmon. 1970. Biochemical comparison of fruit buds of five peach cultivars of varying degrees of cold hardiness. *J. Amer. Soc. Hort. Sci.* 95:177-181.
6. ————, and C. E. Chaplin. 1971. Biochemical comparison of seasonal variations in three peach cultivars differing in cold hardiness. *J. Amer. Soc. Hort. Sci.* 96:154-159.
7. Layne, R. E. C. 1974. Breeding peach rootstocks for Canada and the Northern United States. *HortScience* 9:364-366.
8. ————, 1976. Peach rootstock breeding, rootstock hardiness and rootstock influence on scion cultivars. *Riv. Ortoflorofrutt. It.* 60:113-119.

9. _____, 1976. Influence of peach seedling rootstocks on perennial canker of peach. *HortScience* 11:509-511.
10. _____, G. M. Weaver, H. O. Jackson, and F. D. Stroud. 1976. Influence of peach seedling rootstocks on growth, yield and survival of peach scion cultivars. *J. Amer. Soc. Hort. Sci.* 101:568-572.
11. _____, H. O. Jackson, and F. D. Stroud. 1977. Influence of peach seedling rootstocks on defoliation and cold hardiness of peach cultivars. *J. Amer. Soc. Hort. Sci.* 102:89-92.
12. Levitt, J. 1972. Responses of plants to environmental stresses. Academic Press Inc., New York p. 111-167.
13. Ormrod, D. P. and R. E. C. Layne. 1977. Scion and rootstock influence on winter survival of peach trees. *Fruit Var. J.* 31:30-33.
14. Ward, G. M. and F. B. Johnston. 1962. Chemical methods of plant analysis. *Canada Dept. Agr. Publ.* 1064.

J. Amer. Soc. Hort. Sci. 103(3):413–416. 1978.

Influence of Borate and Pentaerythritol Concentrations on Germination and Tube Growth of *Lilium longiflorum* Pollen[1]

David B. Dickinson[2]
Department of Horticulture, University of Illinois, Urbana, IL 61801

Additional index words. gibberellins, indoleacetic acid, Amo 1618, Easter lily

Abstract. **Rapid tube growth occurred when lily (*Lilium longiflorum* Thunb. cv. Ace) pollen germinated in a liquid medium containing 0.29 M pentaerythritol. Growth was reduced by increasing or decreasing the pentaerythritol concn. There was a hyperbolic relationship between concn of H_3BO_3 in the medium and stimulation in rate of pollen tube growth, with 30 μM H_3BO_3 causing one-half maximal stimulation of growth. Neither early pollen tube growth nor percent germination was stimulated by the hormones indoleacetic acid and gibberellic acid, nor was there any effect by 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate (Amo 1618), an inhibitor of gibberellin biosynthesis.**

The requirements for pollen germinating in a defined medium include calcium, borate, and an osmoticum (4, 10, 15, 24, 25). There is information concerning the role of calcium (10), but the role of borate is unknown (20, 22) and the relation between pollen tube growth and osmotic concn of the culture medium is not well understood. Earlier work in this area utilized, as osmotic agents, sugars which were readily absorbed and metabolized (11, 16, 23). Gibberellin and auxins are also reported to stimulate germinating pollen (3, 5, 17, 21, 26).

The present work was undertaken to determine how germination of 'Ace' lily pollen is affected by the addition to the culture medium of boric acid, GA_3, IAA, Amo 1618, and pentaerythritol. Pentaerythritol is an osmoticum which is absorbed slowly and not metabolized by germinating lily pollen (11).

Materials and Methods

Glass distilled water was used for preparation of all reagents. GA_3 (grade III) was from Sigma Chemical Company, St. Louis, Missouri. IAA was from the Eastman Co., Rochester, N.Y. and Amo 1618 (B grade) from Calbiochem (San Diego, California).

Pollen of 'Ace' lily was used in all experiments. Procedures for collecting, handling, and germinating the pollen were reported earlier (10, 11). Each pollen sample (4.0 mg) was incubated with 1.0 ml culture medium in a 10 ml Erlenmeyer flask. Flasks were incubated on a metabolic shaker at 30°C. The standard culture medium contained 0.29 M pentaerythritol or 0.29 M glucose and the following salts: 300 μg/ml (1.27 mM) $Ca(NO_3)_2 \cdot 4\ H_2O$, 100 μg/ml (0.99 mM KNO_3, 10 μg/ml (0.162 mM) H_3BO_3. The pH was 5.2. In several experiments the concn of pentaerythritol and borate were altered as described under Results. There were duplicate pollen samples per treatment for each experiment. Small portions of germinating pollen were removed at intervals from each flask. Germination percentages and tube lengths were determined photographically on duplicate microscope fields as reported earlier (9), and average values were calculated for each flask. The difference between duplicate flasks is indicated by the vertical lines which accompany each point and bar in the figures. The number of pollen grains counted per flask is given in Fig. 4, and these values are typical for the experiments reported here.

Leakage of pollen carbohydrates was determined on samples of pentaerythritol medium after removal of pollen. At the times indicated, samples were withdrawn and placed on chilled microscope slides. These samples were used to determine percentage germination and tube length. The microscope slides were stored briefly on ice and photographed as soon as possible. The remainder of each pollen sample was immediately poured into a chilled 15-ml glass centrifuge tube (round bottom, Corex brand) and pollen was sedimented by 5 min centrifugation at 8,000 *g* and 0°C. The clarified culture medium was withdrawn carefully using Pasteur pipets with orifices which were reduced by flaming so that pollen grains could not enter. Total carbohydrate was determined in duplicate on each sample using the anthrone procedure described earlier (10).

Calculations for the reciprocal plot of the pollen growth data (Fig. 1B) are similar to calculations done earlier for the activator saturation curve of an enzyme (14). In both cases the basal value was subtracted from the activated values before calculation of reciprocal values for a double reciprocal plot.

Results

The effects of various borate concn on lily pollen germination and tube growth in glucose medium are presented in Fig. 1. Pollen tubes were quite short (100 μm) after 3 hr incubation without added borate, and progressive increases in tube length occurred when borate was increased from 0.5 to 20 μg/ml (Fig. 1A). The data of Fig. 1A gave a straight line in a double reciprocal plot (Fig. 1B), indicating a hyperbolic relation between borate concn and enhancement of tube length at 3 hr. Only the lowest borate concn did not fit the line. The asymptote for the curve in Fig. 1A was 1,330 μm as calculated

[1]Received for publication November 25, 1977. This research was supported by NSF grant PCM74-19113 and the Illinois Agricultural Experiment Station.

[2]Technical assistance from Ms. Doreen Weise is acknowledged. Dr. Jack Paxton is thanked for reviewing the manuscript.