The persistence of pistillate flower induction by ethrel as indicated by the position and number of staminate leaf axils in Table 2 varies with cultivar. The developmental sex patterns of 'Marketer' and 'Wisconsin' treated with 500 ppm of ethrel show that in 'Wisconsin' the effect of ethrel is dissipated early in plant development while it persists in 'Marketer' (Fig. 2). This could reflect in these 2 cultivars differences in potential female tendency, flower inhibition, or production of multiple pistillate flowers per leaf axil. 'Wisconsin' has a tendency to produce multiple pistillate flowers per leaf axil which is enhanced by ethrel.

The variation in persistence of the ethrel effect indicates that in some cases multiple spraying will be necessary for hybrid seed production or that hybrid seed can only be produced during certain early stages in plant development.

We conclude that the differential response of monoecious cultivars to sex conversion by ethrel is affected by the genetic system which controls female tendency. Cultivar responses such as inhibition of flowering and persistence of sex conversion should be considered in developing the use of ethrel to produce monoecious hybrids in cucumbers.

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Biochemical Comparison of Seasonal Variations in Three Peach Cultivars Differing in Cold Hardiness[1]

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Abstract. Leaves, shoots and flower buds of 3 peach cultivars differing in cold hardiness were compared biochemically throughout the year. The analyses included starch, reducing and total sugars, total protein, and total and individual amino acids. (a) Starch in leaves and shoots was low in early spring, but increased to peak concentrations in fall. Flower buds were devoid of starch. (b) Reducing and total sugars in leaves and shoots were high in early spring and decreased to a minimum in fall, but increased to a maximum in the shoots during winter. In flower buds reducing and total sugars were relatively high during winter and increased to peak concentrations in early spring. (c) Protein in leaves was high in spring but decreased to a minimum in summer, then steadily increased to a peak concentration in fall. A similar but less pronounced trend occurred in shoots. In flower buds a steady increase in protein occurred during dormancy and reached a maximum in early spring. (d) Total free amino acids in leaves was high in the spring, but decreased rapidly to a minimum in the fall. In shoots the level was relatively high in the spring, decreased in early summer, but increased to a maximum in late summer, then gradually leveled off during the fall and winter. In flower buds the level was relatively high in winter, but increased rapidly in early spring.

Some correlation existed between the levels of the biochemical constituents and the degree of hardiness in the 3 peach cultivars.

Lasheen et al. (11) working with peach flower buds found some relationship between the degree of cultivar hardiness and their biochemical make-up. The present work was undertaken to determine if such a relationship exists between the degree of hardiness in peach cultivars and the biochemical make-up of leaves, shoots and flower buds throughout the year. Such information might shed some light on the seasonal variation among peach cultivars and its effect on their cold hardiness.

Several studies (8, 10, 19, 21) have shown correlation between cold hardiness and seasonal variations of amino acid content in peach and other species. A similar relationship also exists between cold hardiness and certain amino acids (1, 2, 4, 8, 10, 12). Literature reported by Holubowicz and Boe (10) indicate that water soluble protein was directly related to cold hardiness. Some relationship was also reported (5, 6, 8, 14, 16, 19) to exist between seasonal changes in sugars and starch content in various organs of peach trees and cold hardiness.

Materials and Methods

The 3 peach cultivars used in this study were 'J. H. Hale,' the most tender, 'Redskin,' intermediate, and 'Lizzie,' the most hardy. Three 6-year-old trees of each cultivar were selected for uniformity and each individual tree was used as a replication in a randomized complete block design. The selection of these cultivars was based on (a) data obtained by Mowry (13), (b) unpublished performance data on peach cultivars grown for several winters in Kentucky, and (c) on artificial freezing tests (3). Samples were collected on the 15th day of each month with leaves collected from April through October 1968, shoots from

[1]Received for publication July 23, 1970. The investigation reported in this paper (No. 70-10-97) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the director.

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[3]The authors wish to acknowledge the technical assistance of Miss Z. M. McClure and Mr. E. M. Krzywy in the chemical analyses.

April 1968, through March 1969, and flower buds from January through April 1969. One hundred leaves and 50 unbranched shoots representing current season's growth were collected at eye level around the periphery of each tree. The leaves and shoots were immediately frozen at -20° F; flower buds were dissected from the frozen shoots. Leaves, shoots and flower buds were then lyophilized and ground to pass 60 mesh sieve for chemical analyses of total and reducing sugar, starch, protein, and total and individual amino acids. The methods used in these analyses have been described previously (11).

Results and Discussion

The analytical data of leaves, shoots and flower buds are discussed below under the following headings: reducing sugar, total sugar, protein, free amino acids and individual free amino acids.

Standard errors of means of the 3 peach cultivars compared biochemically for these constituents in leaves, shoots and flower buds as shown in Figs. 1-6 appear in Table 1.

Reducing Sugar

Leaves. The results presented in Fig. 1 show moderate levels of reducing sugar in the 3 cultivars occurring in April which rapidly increased to peak levels in May. This trend apparently coincided with active photosynthesis in the young and rapidly expanding leaves during that time. After reaching peak levels a sharp and progressive decrease in reducing sugar content took place throughout the summer and continued until leaf fall in October. The decrease was probably due to increased translocation throughout this period, and in part to less active photosynthesis in older leaves.

Shoots. In comparison with leaves, almost an identical but less pronounced pattern of reducing sugar levels occurred in shoots from April until October. Shoots seemed to act as channels through which soluble sugars were translocated and, as such, reflected the same pattern displayed by leaves. The lower levels of reducing sugar in the shoots are to a large extent due to a dilution effect since sugar analysis was made on the shoots rather than on the bark alone. Higher reducing sugar content in peach bark than wood was reported by Donoho and Walker (5). Also, soluble sugars were found by Dowler and King (6) to be higher in bark than in twigs of dormant peach tissue. Following the low levels in October, the concentration of reducing sugar steadily increased to a second high level in February then decreased again. The increase in reducing sugar during the period from October to February coincided with the period of greatest hardiness in peaches. This may suggest carbohydrate transformation taking place despite low temperatures normally prevailing at that time. It may also suggest that translocation of reducing sugar may also be taking place.

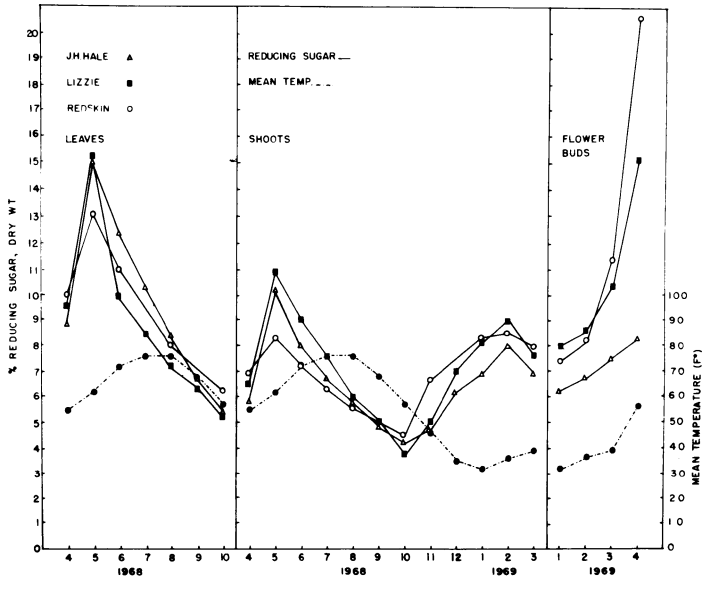

Fig. 1. Seasonal variation of reducing sugar in leaves, shoots and flower buds of 3 peach cultivars.

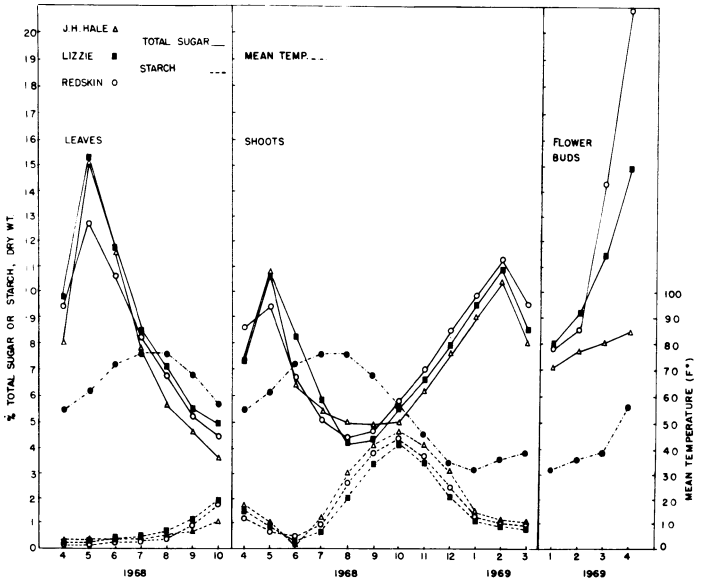

Fig. 2. Seasonal variation of total sugar and starch in leaves, shoots and flower buds of 3 peach cultivars.

Flower Buds. Approximately the same levels of reducing sugar were found in flower buds as in shoots during January and February confirming the suggestion that active translocation of soluble sugars from shoots to buds occurs despite low temperatures. After February, reducing sugar levels rapidly increased reaching a maximum in April when the flower buds were rapidly developing. A similar pattern was reported for 'Elberta' peach flower buds (8).

In conclusion, reducing sugar concentrations in leaves appear to differ between the 3 peach cultivars throughout the growing season with the least hardy cultivar 'J. H. Hale' showing the least concentration in the fall. In shoots the same relationship seems to exist during the winter which became more evident in the spring.

Total Sugar

Generally, throughout the year, total sugar concentrations in leaves, shoots and flower buds (Fig. 2) were only slightly higher than those of reducing sugar, and the periods of peak concentrations coincided indicating that the bulk of soluble sugars in these tissues occurs in the reduced form. This is in agreement with the findings of El-Mansy and Walker (8). The data also suggest that sugar translocation involves reducing as well as non-reducing sugars. The data of Swanson (18) and Vernon and Aronoff (20) indicate, however, that in 'Concord' grapes and soybean, respectively, sucrose was the main sugar translocated.

In conclusion, differences in total sugar content of leaves, shoots, and flower buds of the 3 peach cultivars were not pronounced. However, the least hardy of the 3 cultivars, 'J. H. Hale,' still showed less total sugar during the winter months in the shoots and in flower buds, especially in the spring. This further supports the view that soluble sugars play an active role in winter protection by decreasing the freezing point depression.

Starch

Leaves. Figure 2 shows that in early spring minimum concentrations of starch were found in leaves. A slight increase in starch content took place during June and July with appreciably more increase in August with peak concentrations occurring in October. This increase in starch content coincides

with fruit harvest in August and presumably a decrease in the rates of carbohydrate transformation and translocation. In contrast with starch content in shoots, the overall levels of starch in leaves was lower, indicating that the bulk of the photosynthate present in leaves was soluble sugars as reported by Vernon and Aronoff (20). It also indicates that starch storage in leaves was temporary and transient.

Shoots. In early spring moderate levels of starch were found in shoots. This was followed by a sharp decline in starch content, reaching a minimum in June, a period of active growth during which most of the available reserve food stored in shoots as well as from photosynthates from leaves were utilized. After June there was a steady increase in starch content reaching peak concentrations in October, indicating storage of reserve starch in shoots following a decrease in active growth and removal of fruit. Starch content then decreased progressively during the winter and leveled off in February and March. These results agree with those obtained by Ryugo and Davis (15) and by Dowler and King (6). Figure 2 further shows a quantitative relationship between starch and total sugar. An increase in total sugar always coincided with a decrease in starch. This indicates that a large portion of sugars in peach shoots is a product of starch transformation. This transformation did not appear to be temperature dependent since it took place in midsummer (a period of active growth) as well as in midwinter (a period of winter hardening in peach trees). This may offer further evidence that starch-sugar transformation is not solely controlled by temperature as suggested earlier by Dowler and King (6).

Flower buds. No starch was found in flower buds at any time from January to April. This confirms an earlier report by the authors (11). That dormant peach buds were fairly high in reducing and total sugars (Figs. 1-2), but were devoid of starch, strongly suggests that sugars were indeed translocated to these buds from other parts of the tree (8). It also suggests that not only sucrose (8) but both reducing sugar as well as sucrose were translocated.

In conclusion, starch content of leaves and shoots differed, but little, in the 3 cultivars. However, the data show that starch-sugar hydrolysis in midwinter for the least hardy cultivar, 'J. H. Hale,' was less than in others. This may be a reason for its susceptibility to freezing injury.

Protein

Leaves. Protein concentration was relatively high in leaves in early spring. High protein concentration may be due to active protein synthesis in young metabolically active leaves at that time. The concentration then dropped rapidly to a low level in May and slightly lower in June. This drop may be due to leaf expansion and the resulting dilution. The data of Hall (9) indicate this to be the case in expanding snap bean leaves when results were calculated on dry weight basis. After June, protein concentration leveled off until August when it began to increase gradually until October before leaf fall.

Shoots. A similar trend but with much less concentration of protein occurred in shoots. This may be due, however, to a dilution factor since analysis for protein was not made on bark alone but on shoots. Figure 3 shows a low level of protein in August which may be a result of hydrolysis of protein following harvest. Steward and Thompson (17) reported that asparagine and aspartic acid may be closely associated with protein breakdown. Figure 5 shows a significant increase in aspartic acid in shoots following harvest in August, a possible indication of protein hydrolysis.

Flower buds. Despite the low temperatures normally prevailing during the winter much higher concentration of protein was found in flower buds in January, February and March. The concentration of protein then dropped rapidly during bud swelling in April. This relative decrease in protein is apparently due to a dilution factor as a result of accelerated growth rate.

In conclusion, little variation in protein content of leaves and shoots existed among the 3 cultivars with 'J. H. Hale,' the least hardy cultivar showing the lowest protein content especially during the winter. Flower buds, however, showed larger variations among the cultivars in protein content, especially during the winter with 'J. H. Hale' again showing the least protein. In a tender cultivar such as 'J. H. Hale' with a lower protein content less water is hydrated than in a hardy cultivar. This would somewhat decrease the volume of 'free water' and in turn increase the freezing point depression.

Free Amino Acids

Leaves. Figure 3 shows free amino acid content in leaves follows a trend similar to that of protein, being very high in April then rapidly dropping to a low (about ¼) in May. The high protein content in April was possibly due to an active metabolic activity in young leaves at that time. As the leaves expanded the content dropped, possibly as a result of dilution as well as translocation of free amino acids from the leaves.

Shoots. Relatively high free amino acid content was found in shoots in April (Fig. 3) coinciding with blossom time. Bollard (2) also found considerable increase in certain amino acids and amides in tracheal sap in the shoots of several woody species of *Rosaceae* at blossom time. Following April, the content rapidly decreased, reaching a relatively low level in June probably because of removal through translocation from the shoots to the developing fruits. Concurrently seeds increase in protein (and fats) requiring amino acids. Following this low level the content rapidly increased again, reaching a peak in August after fruit harvest. This increase in August may be attributed to the degeneration of the cytoplasmic contents in vessel elements of the xylem. Bollard (1) reported that some or all of the N present in tracheal sap of apple shoots may well result from hydrolysis of storage proteins. The content decreased again during September and October. This low level was maintained steadily throughout the winter months, coinciding with an increase in protein content in the shoots during the same period (Fig. 3). This may suggest that translocation of free amino acids in shoots was curtailed during the winter.

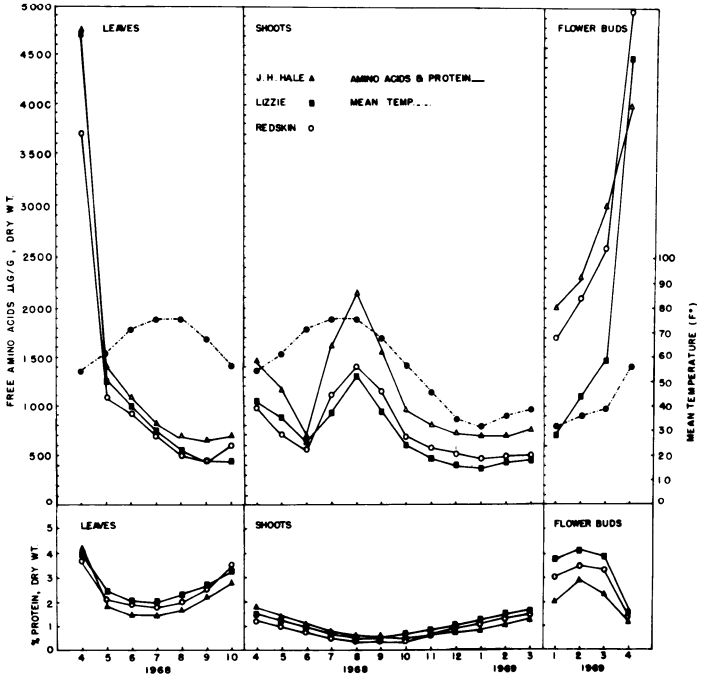

Fig. 3. Seasonal variation of free amino acids and protein in leaves, shoots and flower buds of 3 peach cultivars.

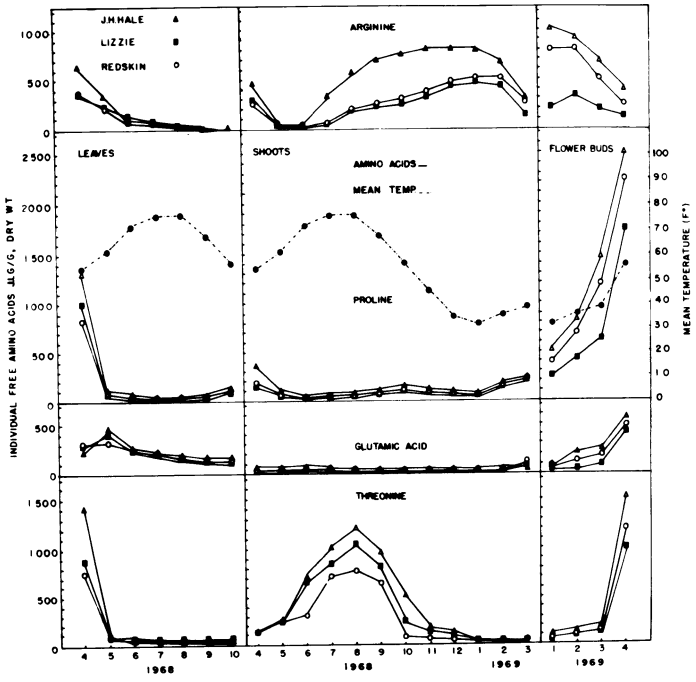

Fig. 4. Seasonal variation of arginine, proline, glutamic acid and threonine in leaves, shoots and flower buds of 3 peach cultivars.

Flower buds. Figure 3 shows relatively high free amino acid content in flower buds in January which steadily increased during February and March, reaching a peak concentration in April against a dilution gradiant caused by bud swelling at that time. This peak concentration in April coincided with peak concentration in leaves and relatively high level in shoots during the same time possibly indicating translocation of free amino acids from leaves to the young developing flower buds. Figure 3 further shows that during the same period protein content in flower buds followed an opposite trend, probably as a result of bud swelling. However, for amino acids this dilution factor was apparently overcome and its effect completely masked and reversed, possibly by an increased rate of translocation of amino acids to flower buds or for hydrolysis of storage proteins.

In conclusion, free amino acid content differed, at certain times significantly, between leaves, shoots and flower buds of the 3 cultivars. 'J. H. Hale,' the most tender cultivar, showed higher free amino acid content than the 2 other cultivars. This was particularly true with flower buds during the winter, which confirms earlier (11) conclusions that a tender peach cultivar would synthesize less protein from the available free amino acid pool than a hardy one. This would result in higher free amino acid and lower protein content in a tender cultivar than in a hardy one.

Individual Amino Acids

Leaves. The 18 amino acids reported (11) to exist in peach flower buds were all found in measurable quantities in leaves, shoots and fruit buds of the 3 peach cultivars studied. In leaves (Figs. 4, 5 and 6) most of these acids follow approximately the same trend exhibited by total free amino acids having peak concentrations in April when active growth was taking place followed by a rapid or a gradual decrease in concentration during the growing season. A secondary peak for aspartic acid was found in July and August which may indicate protein hydrolysis following fruit harvest. Leucine, isoleucine, methionine, ornithine, lysine, phenylalanine and tyrosine were found in trace amounts with no apparent change in their concentrations throughout the growing season.

Shoots. All amino acid concentrations in shoots with the exception of arginine were low during the winter. Only 7 of the peak concentrations of amino acids occuring in leaves in April also occurred in shoots, but at much lower concentrations (Figs.

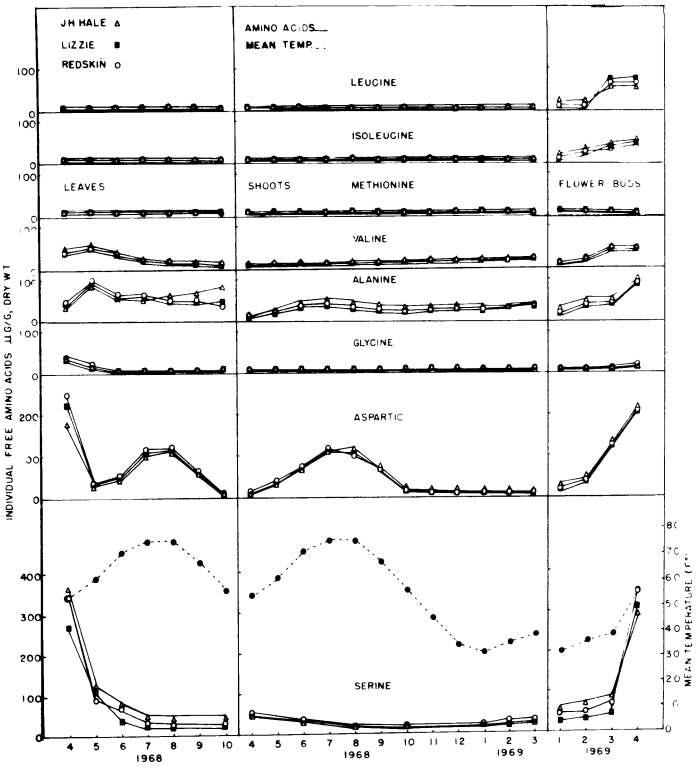

Fig. 5. Seasonal variation of leucine, isoleucine, methionine, valine, alanine, glycine, aspartic acid and serine in leaves, shoots and flower buds of 3 peach cultivars.

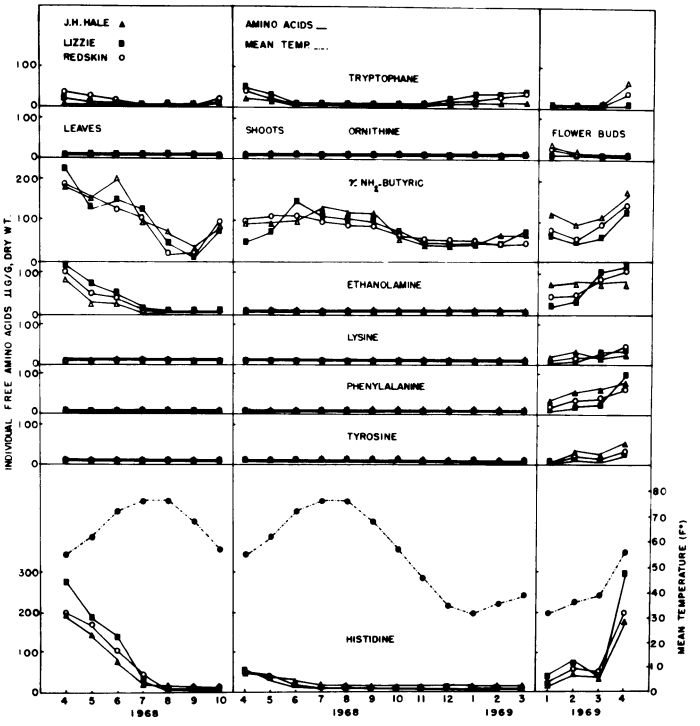

Fig. 6. Seasonal variation of tryptophane, ornithine, γ-NH_2-butyric, ethanolamine, lysine, phenylalanine, tyrosine and histidine in leaves, shoots and flower buds of 3 peach cultivars.

4, 5 and 6). A much higher peak concentration of arginine was found during the winter. This may be of some significance since it is the only free amino acid to occur at such relatively high concentrations in shoots during the winter. The data of Bollard (1) also show a striking increase in arginine in tracheal sap of apple shoots toward the end of the season. Taylor (19) working with 25-year-old peach trees reported peak concentrations of

free amino acid N and arginine in tree tissues before growth commenced and after growth ceased (woody tissues only), while a minimum was found at the end of the growing season. His results suggest that arginine is a more important constituent of the storage N of mature peach trees than are the amides. May and Taylor (12) introduced labeled arginine and asparagine in young dormant peach trees and recovered the radioactivity in soluble sugar plus acid and protein amino acid fractions, indicating that both compounds were metabolized by the trees. Their results also indicate that there was a turnover of arginine in the dormant tissues. On the other hand, high concentrations of threonine, aspartic acid and γ -NH_2 butyric were found in shoots during July and August. This is the period of fruit ripening and harvest, which may possibly indicate a reduction or cessation of translocation of these acids to the ripening fruit. More work is needed to verify such a possibility.

Flower buds. The concentrations of all free amino acids with the exception of arginine and to some extent proline were low in flower buds in January. But with the exception of arginine the concentrations gradually increased to peak concentrations in April (Figs. 4, 5, 6). These peak concentrations coincided with the active growth of flower buds during that time. More fluctuations in the concentrations of these acids than is shown in these figures may have taken place. El-Mansy and Walker (8) were able to show higher maximum values when they determined amino acids biweekly in 'Elberta' peach flower buds. The fact that only arginine gradually decreased to a minimum in April indicates that it may be actively involved in the growth process in peach flower buds during April through transformation to other amino acids. Similar transformation in Jerusalem artichoke was reported (7) to involve glutamic acid, alanine, aspartic acid and proline. These 4 acids showed increases in their concentrations beginning in February and reaching peak concentrations in April. Also in 'Elberta' peach flower buds (8) increases in glutamic acid and aspartic acid were shown to occur after completion of the rest period following an interval of elevated temperature in February. On the other hand, the accumulation of only arginine during the winter confirms the reported (11) correlation it had with cold hardiness in peach flower buds. Concentrations of arginine in the flower buds of the 3 peach cultivars differed significantly (highly significant F value of 1345 and L.S.D. value of 0.205) during the winter. This accumulation was highest in the tender cv. J. H. Hale. Proline was reported (11) to accumulate in peach flower buds in January (1968) 3 times higher than arginine. Figure 4, however, shows arginine to be twice as high as proline in peach flower buds in January (1969). Local weather records for January, 1968, show a mean daily temperature of 29.6° F with frequent temperature fluctuations. On the other hand, an average of 32.1° F was recorded for the same period in 1969 and with less temperature fluctuations. This probably means that under lower and more fluctuating temperatures during the hardening period, proline may accumulate in larger quantities than arginine. Whether this accumulation of either or both of these acids is a cause or a result of hardening is an open question.

Table 1. Standard errors of means of 3 peach cultivars (leaves, shoots and flower buds) compared biochemically as shown in Fig. 1-6.

Fraction and Part	S. E.	Fraction and Part	S. E.	Fraction and Part	S. E.
Reducing Sugar:		Leucine:		Tryptophane:	
Leaves	0.275	Leaves	0.333	Leaves	0.491
Shoots	0.282	Shoots	0.342	Shoots	0.735
Buds	0.081	Buds	1.11	Buds	0.630
Total Sugar:		Isoleucine:		Ornithine:	
Leaves	0.226	Leaves	0.260	Leaves	0.577
Shoots	0.307	Shoots	0.299	Shoots	0.597
Buds	0.142	Buds	0.758	Buds	0.700
Starch:		Methionine:		-NH_2-Butyric:	
Leaves	0.029	Leaves	0.554	Leaves	1.92
Shoots	0.093	Shoots	0.212	Shoots	1.42
Buds	---	Buds	0.423	Buds	1.50
Protein:		Valine:		Ethanolamine:	
Leaves	0.166	Leaves	0.752	Leaves	0.929
Shoots	0.004	Shoots	0.518	Shoots	0.700
Buds	0.068	Buds	0.536	Buds	1.750
Total Free Amino Acids:		Alanine:		Lysine:	
Leaves	48.32	Leaves	0.932	Leaves	0.554
Shoots	18.06	Shoots	0.518	Shoots	0.662
Buds	24.98	Buds	0.908	Buds	1.100
Arginine:		Glycine:		Phenylalanine:	
Leaves	6.06	Leaves	0.756	Leaves	0.261
Shoots	9.37	Shoots	0.277	Shoots	0.242
Buds	10.95	Buds	0.476	Buds	1.39
Proline:		Aspartic Acid:		Tyrosine:	
Leaves	5.15	Leaves	2.70	Leaves	0.563
Shoots	2.27	Shoots	1.39	Shoots	0.497
Buds	19.71	Buds	3.01	Buds	1.078
Glutamic Acid:		Serine:		Histidine:	
Leaves	6.37	Leaves	1.91	Leaves	1.530
Shoots	0.898	Shoots	0.989	Shoots	0.770
Buds	4.23	Buds	2.56	Buds	2.14
Threonine:					
Leaves	5.04				
Shoots	7.81				
Buds	5.77				

Measurable biochemical variations in leaves, shoots and flower buds exist among peach cultivars of varying degrees of cold hardiness. These differences involving sugars, protein, total free amino acids and certain individual amino acids appear significant only at certain times throughout the year, mostly during the winter and early spring.

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Embryo Sac Development in Relation to Virus Infection of Four Red Raspberry Cultivars[1,2]

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Abstract. In a factorial experiment each of 4 red raspberry cultivars was treated with each of 4 viruses. Embryo sacs were examined in treated and control flowers collected at anthesis and 4 days later. A numerical index was devised to quantify stages of embryo sac development, and thus facilitate statistical analysis. Effects on embryo sac development of cultivar differences, viruses, and year difference were observed. The virus effects on embryo sac development as such were not considered severe enough to account for failure of fruit-set or yield reduction. Embryo sacs of the 'Sumner' cultivar were retarded in development compared to the other cultivars.

Developmental studies of the embryo sac have explained the unreliable cropping behavior in sweet cherry (6) and led to practical recommendations for commercial sweet cherry orchards (9). Embryo sac studies have also been used to correlate temperature with poor fruit set in bean (13) and to serve as a basis for tracing the possible cause of poor fruit set in apricot (8).

Studies correlating pollen abortion and retarded embryo sac development in radiation-induced mutants of apple (1, 2) and sweet cherry (7) indicated that retarded embryo sac development might be expected in red raspberries. A correlation probably exists between pollen abortion and retarded embryo sac development in virus free 'Sumner', possibly explaining crumbliness (failure of fruit-set) in one naturally occurring somatic mutant of this cultivar (5). Increased pollen abortion induced by virus has already been established in red raspberry (10).

Virus interference with cell division necessary for fruit set in tomato has been found (3), and a similar interference with drupelet set in red raspberry has been suggested (4, 12, 15).

This study was undertaken to examine the effects of known viruses on red raspberry embryo sac development.

Materials and Methods

The material used in this study was from a replicated field trial involving 4 red raspberry cultivars deliberately infected with known viruses, established by Dr. J. A. Freeman at Abbotsford, British Columbia.

Flowers were collected from a factorial experiment involving 4 red raspberry cultivars: 'Willamette', 'Fairview', 'Newburgh', and 'Sumner'; and 4 viruses: black raspberry necrosis (BRNV), raspberry mosaic (RMV) (a combination of BRNV and *Rubus* yellow net viruses), tomato ringspot (TomRSV), and raspberry vein chlorosis (RVCV); and a virus free control (VF). There are

[1]Received for publication August 17, 1970. This work received financial support from the National Research Council of Canada under operating grant number A 2023.

[2]Technical assistance was provided by Miss E. Buckland and Mrs. L. Pui. J. A. Freeman of the Canada Department of Agriculture Research Station, Agassiz, B. C. generously allowed the collection of the plant materials.

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