Biochemical Comparison of Fruit Buds in Five Peach Cultivars of Varying Degrees of Cold Hardiness[1]

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Abstract. Fruit buds of 5 peach cultivars—'New', 'Daroga', 'Redskin', 'Mayflower', and 'Loring'—grown in Kentucky and exhibiting varying degrees of cold hardiness, were compared biochemically. Fruit bud analysis for total and reducing sugars, starch, total protein, and total and individual free amino acids indicate some correlation between the degree of hardiness and the biochemical make-up of these cultivars. Generally, a high sugar and protein content, and a low total free amino acids were associated with increase in hardiness. Specifically, significant correlation was found between hardiness and a high sugar and protein content when buds were frozen at −2½° F. Significant correlation was also found between 2 amino acids (arginine and γ-NH_2 butyric) and hardiness at both −2½° and −5°.

SEVERAL investigations, reported in reviews by Levitt (8, 9), Parker (19), and Tumanov (28), show the biochemical make-up to play a positive role in cold hardiness of plants. More specifically, soluble sugars, starch, amino acids and proteins have been found to play a more positive role in the many species studied. Li et al. (10, 11) reported changes in metabolites of Red-Osier dogwood during cold acclimation include a decrease in starch and amino acids and an increase in simple carbohydrates and proteins. El-Mansy and Walker (2) reported that sugars were relatively lower in peach buds during rest than after termination of the rest period. They also found that, in general, amino acids were relatively high in late summer and early fall. Riadnova (22) working with buds and shoots of peach, plum, cherry, and apricot showed a direct correlation between amounts of starch and sugar in winter and length of dormancy. Salcheva et al. (23) reported that large amounts of soluble sugars and free amino acids accumulate during hardening of winter wheat. High protein content in various species was associated with cold hardiness in those species (25, 26, 27).

Materials and Methods

The 5 peach cultivars used in this study were 'New',[5] 'Daroga', 'Redskin', 'Mayflower' and 'Loring', each represented by three 5-year old trees planted at the university experimental farm. Twenty representative, unbranched, current season's shoots approximately 18 inches long were collected at eye level around the periphery of each tree January 8, 1968. The shoots from each tree were divided into 3 samples having more or less equal numbers of fruit buds. The first 2 were stored at 45° F for 24 hr then artificially frozen. This was accomplished in an automated freezing chamber that lowered the temperature 3° per hour. Samples were removed at −2½° and −5°. They were then stored at 50° overnight and freeze injury to fruit buds was determined the next day by slicing the buds as described by Chaplin (1). The percentage of bud survival was then calculated for each of the 2 temperatures used. The third shoot sample was frozen immediately at −20° and, while frozen, the fruit buds were separated, lyophilized, and ground to pass 60 mesh for chemical analyses as follows:

1) Sugars were extracted from 200 mg samples by shaking for ½ hr with 0.2% benzoic acid followed by centrifugation. Quantitative analyses of both total and reducing sugars were made automatically according to a modification of the method of Hoffman (6) as follows: Aliquots of the sugar extracts and appropriate glucose standards were delivered from a sampler fitted with a 30/h, 1:1 ratio cam. The manifold assembly on a proportioning pump consisted of tygon manifold tubing with specified lumens and delivery rates. After emerging from the sampler an aliquot of each sample was diluted with water for determination of reducing sugar or with 0.05 N H_2SO_4 for total sugars, segmented with air and flowed into a water-jacketed coil then into a heating bath. After 10 min of incubation at 95° C in the heating bath a portion of the diluted aliquot was mixed with alkaline ferricyanide, segmented with air and again flowed for 10 min, to reach maximum color development, in the heating bath. Upon exit from the heating bath the reaction mixture was cooled in a water-jacketed mixing coil to prevent premature color fading before flowing into a colorimeter. The color was measured and automatically recorded at 420 mμ.

2) Starch was extracted from 1 g samples with perchloric acid according to the procedure reported by Hassid and Neufield (5). Quantitative analysis of starch using the anthrone reagent (15) was made automatically as follows: Aliquots of the starch extracts and appropriate glucose standards were delivered from a sampler as described above. The manifold assembly on the proportioning pump consisted of both tygon and acidflex tubing. After emerging from the sampler an aliquot of each sample was segmented with air, mixed with the anthrone reagent in 72% H_2SO_4, and flowed into a water-jacketed coil then into a heating bath. After 20 min of incubation at 95° C in the heating bath the reaction mixture was cooled in a water-jacketed coil before flowing into a colorimeter. The color was measured and automatically recorded at 619 mμ.

3) For protein analysis 100 mg samples were bleached by refluxing for 1 hr with pure methanol followed by centrifugation. The precipitate containing the protein was mixed with cold TCA to form a paste, then 10 ml cold TCA were added and samples stored in the cold overnight. After centrifugation the cold TCA was dis-

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[5]Introduced into Kentucky by E. H. New from Iowa and believed to be identical to 'Boone Co. Seedling.'

carded and the precipitate washed with cold TCA followed again by centrifugation and discarding of TCA. The centrifuge tubes containing the precipitate were inverted on paper towels to drip dry then the protein pellet was dissolved by mixing and shaking it for ½ hr with 10 ml 0.2 NaOH followed by centrifugation and filtering. Aliquots of the protein filterates and appropriate protein standards (prepared from bovine albumin fraction V) were delivered from a sampler as described above. Quantitative analysis of protein using the Folin reagent was made according to the method of Lowry et al. (12). This was done automatically following the Technicon[6] methodology.

4) Free amino acids were extracted from 1 g samples by shaking for ½ hr with 80% ethanol followed by centrifugation. This was repeated 4 more times. The combined ethanolic extract was cleared and the amino acids eluted according to a modification of the procedure reported by Plaisted (20) as follows: The extract was run through a dowex 50wx8, 200-400 resin bed 5 cm high. Flow rate was adjusted at 3 ml per minute with nitrogen pressure. Elution of the amino acids was accomplished with 15 ml 0.4 N NH_4OH in ethanol followed by 15 ml distilled water wash, 15 ml 4 N NH_4OH, and again 15 ml distilled water wash. The eluate was collected in a 125 ml Erlenmyer flask, frozen and lyophilized. The residue was taken up in 10 ml 0.1 N HCl and filtered. Quantitative analysis of amino acids in 0.5 aliquots was made automatically according to the method of Moore and Stein (13).

Results and Discussion

Dormant flower bud hardiness of peaches was found by Mowry (16) to be inherited on a quantitative basis with an undetermined number of genetic factors involved. Unpublished data on peach cultivar performance for several winters in Kentucky show that differences exist between cultivars in regard to bud hardiness. These differences were verified by artificial freezing tests (1). In light of these data and the data obtained by Mowry (17) the 5 peach cultivars studied were selected. Their performance in the artificial freezing test at −2½° F (Fig. 1) more or less agrees with their previous performance in the field and in the artificial freezing tests, the cultivar 'New' being the most hardy and 'Loring' the most tender. At −5° the per cent survival follows the same order with the exception of 'Daroga'. From these tests and from the statistical analysis in Table 1 the conclusion may be drawn that fruit buds of peach cultivars certainly differ in the degree of expression of cold hardiness. It may further indicate that the difference is largely genetic in nature, assuming some modification by the environment. This may well explain the discrepancy regarding 'Daroga'.

Sugars in peach buds were reported (2) to be relatively lower during the rest period than after its termination. Fig. 2 indicates that in January most soluble sugars in fruit buds exist in the reduced form. Only traces of nonreducing sugars were found, and in one cultivar, 'Mayflower', none was found. Fig. 2 further shows that more soluble sugars appear to accumulate in hardy cultivars than in tender ones. Differences in accumulation were not pronounced except between the very hardy 'New' and the very tender 'Loring' cultivars. Table 2 shows significant differences between varieties and total sugar

[6]Technicon Instrument Corporation, Chauncey, New York (flow diagram, protein lb., Lowery reaction.)

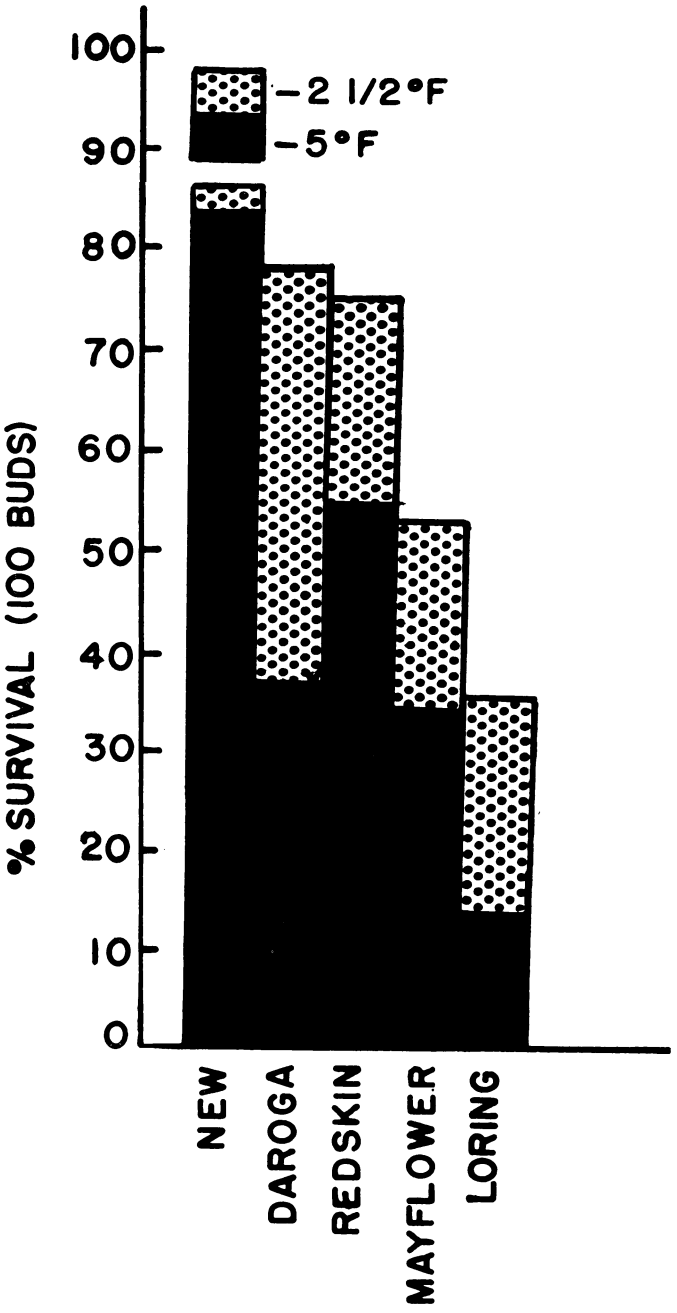


Fig. 1. Per cent survival of fruit buds (average of 3 trees) of 5 peach cultivars collected January 8, 1968 and frozen at −2½° and −5° F.

Table 1. Per cent survival of fruit buds of 5 peach cultivars frozen at −2½° and −5° F.

Variety	Temperature		
	−2½°	−5°	Mean
New	86.3	84.3	85.3
Daroga	77.0	37.7	57.3
Redskin	75.5	55.3	65.5
Mayflower	48.3	34.7	41.5
Loring	36.7	14.3	25.5
Mean	64.1	45.3	
L.S.D. for cultivars at the 1% level 3.53			
L.S.D. for temperature at the 1% level 5.03			
L.S.D. for interaction 11.25			

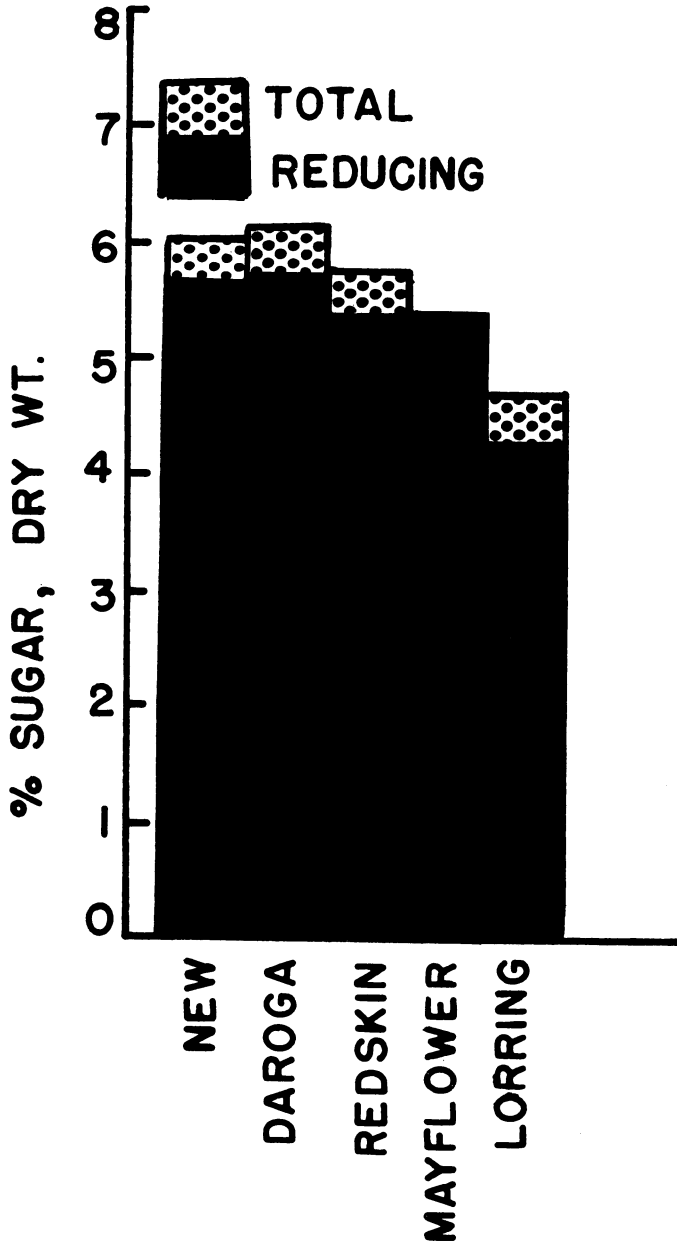


Fig. 2. Total and reducing sugar content (average of 3 trees) in fruit buds of 5 peach cultivars collected January 8, 1968.

content, but not reducing sugar content. The same table however, shows significant correlation between both total and reducing sugars, and bud survival at $-2\frac{1}{2}°$ F indicating a positive relationship between cold hardiness and sugar content at that temperature.

In no case was starch detected in fruit buds, a fact indicating complete hydrolysis of starch to soluble forms of carbohydrates. The complete absence of starch during this time agrees with reports dealing with other species (3, 18, 21, 24).

The significance of the role played by protein in winter hardiness of plants is not clear (8, 9, 19). Fig. 3, however, shows some positive relationship between the amounts of soluble protein in the 5 peach cultivars and the degree of hardiness, with more protein in hardy than in tender cultivars. This positive relationship appears obvious when comparing the very hardy 'New' with the very tender 'Loring'. The statistical analysis in Table 2 does not show significant differences in protein content between varieties, however, it does show a significant correlation between protein content and bud survival at $-2\frac{1}{2}°$ F indicating a positive relationship between cold hardiness and protein content at that temperature.

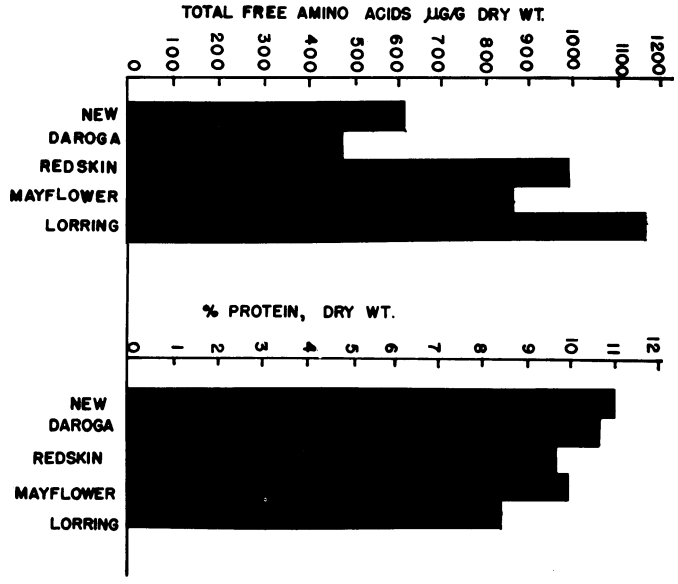


Fig. 3. Total free amino acid and protein content (average of 3 trees) in fruit buds of 5 peach cultivars collected January 8, 1968.

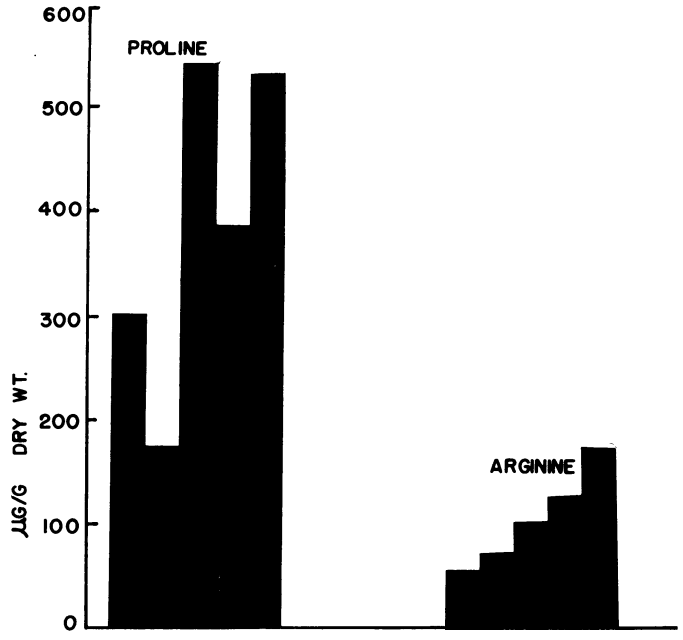
Table 2. Protein, sugars, total amino acids and certain amino acid content of fruit buds of 5 peach cultivars and their correlation with per cent bud survival at $-2\frac{1}{2}°$ and $-5°$ F.

Fraction[a]	Variety					L.S.D.[b]	Correlation coefficient	
	New	Daroga	Redskin	Mayflower	Loring		$-2\frac{1}{2}°$	$-5°$
Total sugar	6.1	6.2	5.8	5.5	4.8	1.3**	0.938**	0.740
Reducing suga	5.7	5.8	5.5	5.5	4.7	NS	0.811*	0.650
Protein	11.0	10.6	9.7	9.9	8.4	NS	0.860*	0.763
Total amino acids	611	470	980	860	1150	35.0**	−0.649	−0.504
Proline	500	180	539	391	531	17.0**	−0.198	−0.192
Arginine	59	80	100	130	170	17.0**	−0.977**	−0.862*
Glutamic acid	51	37	76	65	67	11.0**	−0.448	−0.177
γ-NH_2 butyric	48	69	75	80	85	12.0**	−0.839**	−0.917**
Alanine	31	29	38	35	59	4.3**	−0.794	−0.656
Valine	8	7	10	8	14	1.5**	−0.692	−0.574
Threonine	35	41	68	57	79	17.0**	−0.730	−0.671
Serine	24	14	28	20	31	5.4**	−0.374	−0.076

[a]Values for sugars and protein are percent, dry wt.; total and individual amino acids are ug/g, dry wt.
[b]At the 1% level except for sugars (F values not significant).
*Significant at the 5% level.
**Significant at the 1% level.

The reverse of the relationship exhibited with protein seems to exist regarding total free amino acids (Fig. 3), with less total free amino acids present in hardy than in tender cultivars. This may indicate that more active protein synthesis occurs, involving the amino acid pool, in hardy than in tender cultivars. The statistical analysis in Table 2 shows significant differences between varieties in total free amino acid content. The negative correlation shown between total free amino acids and bud survival at $-2\frac{1}{2}°$ and $-5°$ F is not significant, however, indicating little relationship between cold hardiness and total free amino acids at both temperatures.

In regard to individual amino acids, the situation was more or less similar, as is shown in Fig. 4 and 5 representing 8 amino acids. Eighteen free amino acids, in measurable but various quantities, were present in fruit buds of all 5 cultivars. These acids were aspartic, threonine, serine, glutamic, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ethanolamine, γ-NH_2 butyric, lysine, histidine and arginine. Traces of cystine and tryptophan were also present.



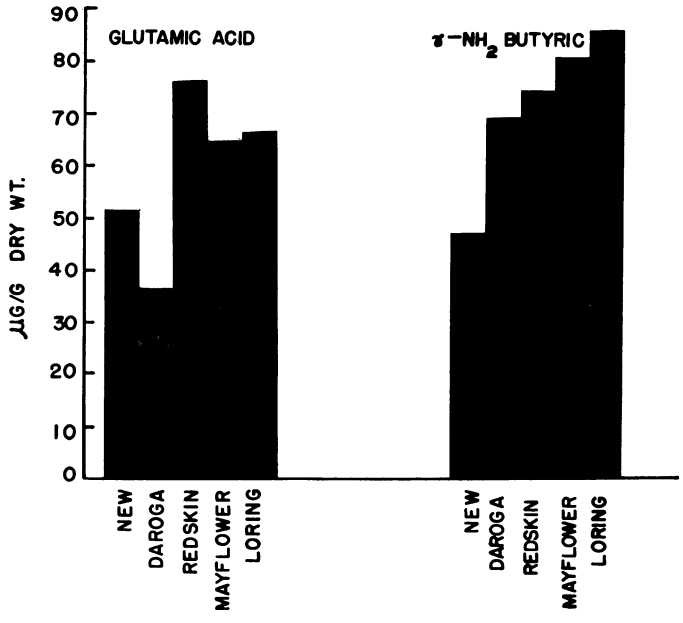


Fig. 4. Glutamic acid, γ-NH_2 butyric, proline and arginine content (average of 3 trees) in fruit buds of 5 peach cultivars collected January 8, 1968. Note the higher scale used for proline and arginine.

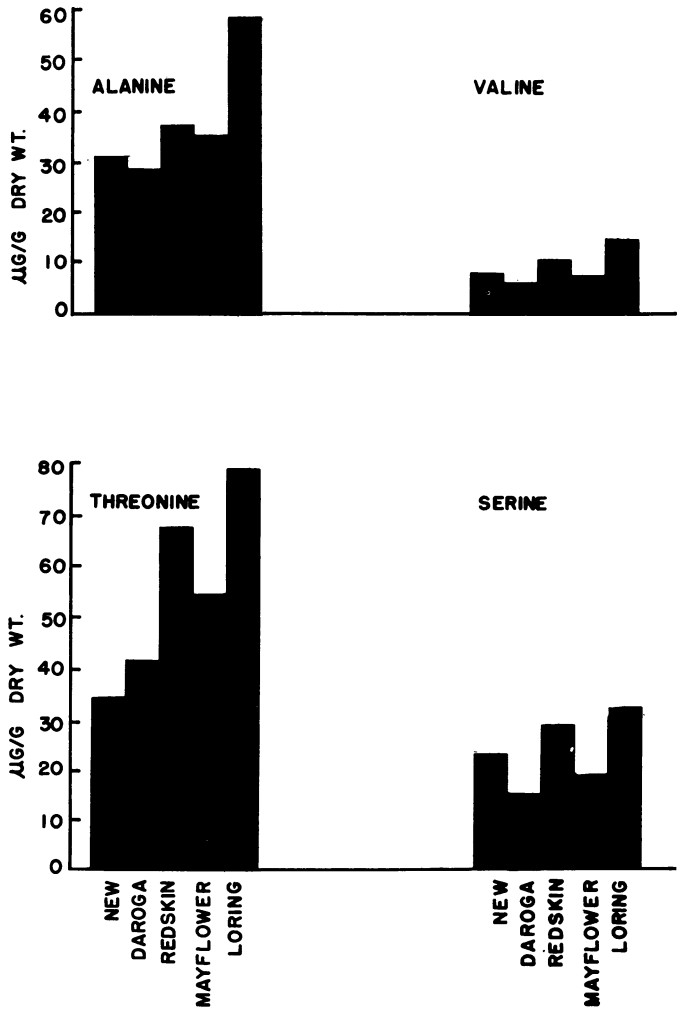


Fig. 5. Threonine, serine, alanine and valine content (average of 3 trees) in fruit buds of 5 peach cultivars collected January 8, 1968.

The statistical analysis in Table 2 shows significant differences between varieties and the 8 amino acids. The negative correlation shown between these acids and bud survival at $-2\frac{1}{2}°$ and $-5°$ F is significant only for arginine and γ-NH_2 butyric acid. This may indicate a relationship between cold hardiness and the content of these 2 amino acids. Although no significant correlation was found between proline and bud survival at $-2\frac{1}{2}°$ and $-5°$ F it is of interest to note the large accumulation of proline (Fig. 4) which is 3 times higher than the next highest amino acid arginine and accounts for about half the free amino acid pool in the fruit buds. This accumulation of proline in fruit buds takes place against a general decrease in total free amino acids during the period of hardiness (2, 7). Morel (14) reported other cases of proline accumulation associated with pathological conditions, such as presence of the potato leaf roll virus and the sugar beet yellows virus. Furthermore, he reported that during low temperature adaptation ("hardening") of cabbage 10 times more free proline was found in treated plants than in the non-hardened controls. The significant differences

between cultivars and their arginine and γ-NH_2 butyric acid content (Table 2), and the significant correlation between these 2 amino acids and bud survival is of particular interest. In Jerusalem artichoke (14) a very high arginine level was maintained all winter and only dropped in the spring. In light of these reports and the present findings it may be suggested that arginine, proline and γ-NH_2 butyric accumulate during low temperature acclimation of some species and probably play a key role in biochemical protection against freezing. Such a possibility deserves further investigation.

In general, the results of the biochemical analyses of flower buds in the 5 peach cultivars strongly suggest some relationship between the levels of protein, soluble carbohydrates, and certain free amino acids and the degree of hardiness exhibited by these cultivars. These results further indicate the possibilities offered by cultivars of known genetic background in the study of the nature of cold hardiness. As indicated earlier, cold hardiness in peaches is genetically controlled. By growing cultivars under controlled environments the different biochemical expressions controlled by the genetic make-up and their roles in hardiness would become more evident.

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