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EXPERIMENTS ON THE METABOLISM OF CAMELLIA FLOWERS IN STORAGE

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This paper concerns the factors which affect the life of the camellia flower. This flower, like many others, rapidly deteriorates after it has been cut from the plant when it is stored at room temperature. The deterioration involves discoloration of the petals, abscission of the petals from the floral axis, and subsequent wilting. In a separate paper, studies on empirical methods for increasing the life of the cut flower have been reported. It is obviously of importance, however, to know what processes are responsible for the normal deterioration of the cut flower. On the basis of such information, it might be possible in the long run to devise more rational methods for the preservation of the cut flower. This paper concerns then the metabolic processes of the camellia flower after excision from the plant.

Methods

Studies on respiration were carried out by means of the Warburg technique. Petals from the camellia flower either freshly cut from the plant or after various periods of storage were cut into small pieces (approx. 2 x 2 mm) and 400 mg of petals representing generally samples from 4 to 8 flowers were suspended in 2 cc of solution contained in each of a series of Warburg vessels. By this means, duplicate samples could be prepared which varied in their respiratory oxygen uptake by less than 5 percent. Oxygen uptake was determined by the direct Warburg method using vessels with a central KOH well. With this general procedure, the effects of age and of various substrata on the rate of respiration of the flower were studied. The effects of aging on chemical composition of the flower were also determined. In this portion of the investigation nitrogen content was determined by the micro Kjeldahl technique on samples harvested after various periods of storage and dried rapidly at 70° C. Carbohydrates were determined on water extracts of similarly dried samples. In all experiments in which flowers were stored for various lengths of time, storage was conducted by allowing the flowers to float on a water surface and were maintained in the dark at 26° C.

Results

The sucrose concentration in excised camellia flowers was found to be relatively low, approximately 0.5 mg/gram dry petals; this value was found to be constant from the time of excision of the flower until the end of storage life, approximately 6-11 days with the various varieties investigated. Content of reducing substances, including glucose, fructose, etc., on the contrary, was found to drop somewhat during storage from an initial level of 10.5% to a level of approximately 8% at the end of storage life as is shown in Figure 1. The results shown in Figure 1 were obtained with the variety Purity. Similar results were obtained with the varieties Daikagura and Pink Perfection. These results do not suggest that soluble carbohydrates are depleted extraordinarily rapidly during the storage of the camellia flower. Results to be reported later also show that the addition of carbohydrates in the form of sucrose to camellia flowers does not increase the rate of respiration either of freshly cut flowers or of flowers near the end of storage life.

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FIGURE 1
AGE EFFECTS ON REDUCING SUBSTANCES LEVEL IN PURITY.

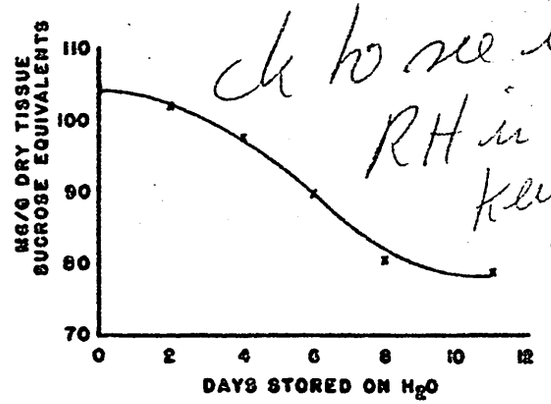

Table I presents data on changes in total nitrogen and in water soluble nitrogen during storage of flowers of the varieties Pink Perfection and Purity. It may be seen that the water soluble nitrogen (amino acids, amides, etc.), remains relatively constant throughout the storage life of the camellia flower. Protein nitrogen, as estimated by the difference between total nitrogen and water soluble nitrogen, also remains relatively constant. There seems to be no basis then for any supposition that depletion of protein nitrogen might be the effective factor in deterioration of the camellia flower in storage. It should be noted that the behavior of protein nitrogen in these excised flowers is quite different from the behavior of protein nitrogen in excised leaves. It has been found with a wide variety of species that leaves after excision rapidly lose their protein and that protein nitrogen may drop to half or less of the initial value within a period of one week after excision.

TABLE I

Protein and Non-Protein Nitrogen Content of Excised Camellia Flowers as a Function of Time of Storage. Flowers Floated on Water at 26° C.

Days After Excision	Variety Pink Perfection % of Dry Petals		Variety Purity % of Dry Petals	
	Protein N	Non-Protein N	Protein N	Non-Protein N
0	0.78	0.14	0.38	0.46
2	0.97	0.08	0.53	0.50
4	0.82	0.11	0.52	0.42
6	0.81	0.10	0.59	0.39
8	0.93	0.10	0.52	0.37
11	1.09	0.11		

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Course of Respiration with Time

Figure 2 gives data on the rate of respiration of samples of camellia petals taken at various times after excision of the flower. It may be seen from the data of Figure 2, which applies to the variety Yohei Haku, that respiration drops sharply and relatively continuously during storage, and that by the end of 6 days, the end of the storage life with this variety, respiration has dropped to approximately half to two-thirds of the initial value. Table II gives similar data with the variety Pink Perfection. Here again respiration drops sharply and continuously to a value of about half the initial value before complete deterioration of the cut flower sets in. These data show no suggestion of a climacteric rise in respiration during storage, such as has been found with many fruits. On the contrary, the behavior of respiration during the storage of the camellia flower is more similar to the steadily dropping respiration of many varieties of leaves after excision from the plant.

FIGURE 2
AGE EFFECTS ON RESPIRATION OF
Yohei-Haku

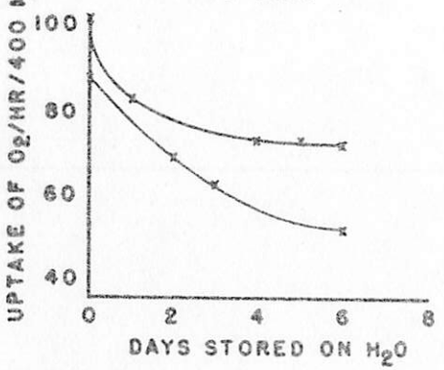
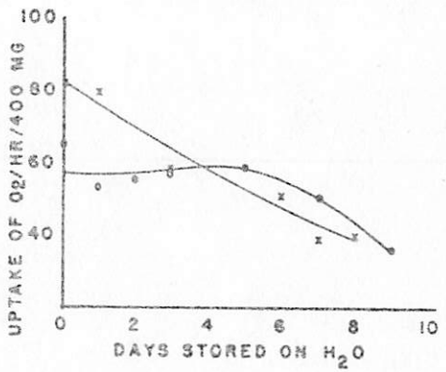


FIGURE 3
AGE EFFECTS ON RESPIRATION OF
DAIKAGURA.



Nature of the Limiting Factor in Respiration of Camellia Flowers

During the course of the experiments for which data are given in Figures 2 and 3, samples were supplied with additional sucrose. Such additional sucrose, given in a concentration of 1%, was without any effect on respiration either of the freshly cut flowers or of flowers at any period during their storage life. Available carbohydrate does not then seem to be the limiting factor in termination of the storage life of the camellia flower.

Organic acids do, however, exert a considerable effect on the rate of respiration of excised camellia flowers. Succinate in particular is highly effective in increasing the rate of respiration of all varieties tested, including Daikagura, Purity, Francine, Yohei Haku and Pink Perfection. The increase of respiration by succinate was found both with the freshly harvested flowers and with flowers at all times during storage up to the termination of storage life. Data on this point are given in Table II and III, which apply respectively to the varieties Pink Perfection and Yohei Haku. With the variety Pink Perfection organic acids other than succinate seem to be without effect on rate of respiration as is shown in Table II. Thus, citrate, alpha-ketoglutarate, fumarate and pyruvate were without any conspicuous effect on rate of respiration. With the variety Yohei Haku, on the contrary, citrate and alpha-ketoglutarate were also found to increase rate of respiration of the freshly excised flowers. With the flowers which had been in storage for 6 days and which were hence near the end of their storage life, alpha-ketoglutarate, succinate and fumarate all increased rate of respiration. It is noteworthy that pyruvate, which is known to be an intermediate in the respiration of the carbohydrates, was without any effect in increasing respiration with any of the species tested.

TABLE II

Effect of Added Organic Acids on Rate of Respiration of Camellia Flower Tissue. (Variety Pink Perfection.) Stored by Floating on Water at 26° C.

Added Substratum 5 mgs./cc Throughout	Uptake of O_2: mm³/400 mg. fresh wt./hr.					
	Days After Excision					
	0	1	4	6	7	8
None (control)	82	80	59	51	39	40
Citrate	86	85	52	55	38	41
Alpha-ketoglutarate	85	78	61	53	39	42
Succinate	96	85	71	59	42	46
Fumarate	80	73	61	52	40	43
Pyruvate	71	69	53	45	36	37

TABLE III

Effect of Added Organic Acids on Rate of Respiration of Camellia Flower Tissue. (Variety Yohei Haku.) Stored by Floating on Water at 26° C.

Added Substratum 5 mgs./cc Throughout	Uptake of O_2: mm³/400 mg. fresh wt./hr.			
	Days After Excision			
	0	2	3	6
None (control)	88	69	62	52
Citrate	105	81	71	56
Alpha-ketoglutarate	92	90	79	64
Succinate	100	90	83	71
Fumarate	85	74	76	71
Pyruvate	89	75	70	56

The metabolism of the camellia flower appears to be in a good measure at the expense of organic acids. This is confirmed by the data shown in Table IV, which applied to freshly excised flowers of the variety Purity. Table IV shows that the respiratory quotient, or ratio of CO_2 evolved to oxygen taken up, is considerably over 1; 1.14 to be exact, as would be expected for respiration involving oxidation of plant acids. In the presence of added succinate the respiratory quotient may go as high as 1.35. The respiration of the normal flower is therefore in part, at least, owing to the respiration of oxygen-rich substrata such as organic acids. Respiration at the expense of succinate thus apparently involves actual oxidation of succinate rather than catalytic effects of succinate on oxidation of pyruvate such as has been reported with other species.

These data indicate then an important role of organic acids in the respiration of the camellia flower. This view is confirmed by the fact that respiration may be inhibited by malonate, a substance which is known to specifically inhibit the oxidation

of succinic acid by succinic dehydrogenase. Table V gives data on this point. Malonate in a concentration of 5 mg/cc causes substantial inhibition of respiration. This inhibition may be reversed by addition of succinate, which is to be expected since succinate acts antagonistically to the inhibitor. Smaller reversal of the inhibition may also be brought about by alpha-ketoglutarate which is no doubt converted to succinate through oxidation. Fumerate on the other hand is without ability to cause reversal of malonate inhibition.

Although organic acids appear to be an important substratum for respiration of the camellia flower, still the storage life of such flowers is not increased by incorporation of succinate into the water in which the flowers are floated during their

TABLE IV

Respiratory Quotient of Camellia (var. Purity) Flower Tissues in Presence and Absence of Succinate

Added Substratum	Conc. mg/cc	O_2/400 mg/hr	Respiratory Quotient
None (control)	.....	95	1.14
Succinate	1.25	115	1.26
Succinate	2.50	117	1.35

storage. This is apparently due to a lack of penetration of succinate to the petals. The data given in Table VI shows that flowers floated on solutions containing succinate do not respire more rapidly after a period of storage than flowers floated on water alone. Flowers floated on succinate still maintain the ability to respond to added succinate just as do flowers floated on water alone. This fact that succinate cannot be supplied to the cut flower by floating the cut flower on solutions of succinate has thus far impeded attempts to extend the storage life of camellia flowers by use of this substance, which seems to be a suitable substratum for flower respiration.

The inhibitor sodium fluoride is known to affect the production of pyruvate from carbohydrate by inhibiting the enzyme enolase. It has been shown above that pyruvate is, however, not a limiting factor in rate of respiration of camellia flowers and the data given in Table VII show furthermore that sodium fluoride is not an inhibitor of respiration of camellia flowers. It would appear highly probable that the utilization of carbohydrate through its conversion to pyruvate is a minor contributor to the overall respiration of the camellia flowers.

TABLE V

Inhibition of Camellia (var. Daikagura) Flower Respiration by Malonate and Reversal of this Inhibition by Organic Acids

Inhibitor	Concentration	Substratum	Conc.	O_2 Uptake mm^3/400 mg./hr.
None	.....	None	.....	82
Malonate	2.5 mg/cc	None	.....	60
Malonate	2.5 mg/cc	Succinate	2.5 mg/cc	81
None	.....	None	.....	70
Malonate	5 mg/cc	None	.....	50
Malonate	5 mg/cc	Succinate	5.0 mg/cc	91
Malonate	5 mg/cc	Alpha-ketoglutarate	2.5 mg/cc	77
Malonate	5 mg/cc	Fumarate	5.0 mg/cc	55

TABLE VI

Effect of Additional Succinate on Respiration of Camellia Flowers (var. Francine) Stored With and Without Succinate

Storage Treatment	Substratum	O_2 mm^3/400 mg/hr
Water	Water	56
Water	Succinate 1.25 mg/cc	78
Water	Succinate 2.50 mg/cc	87
Succinate 1 mg/cc	Water	49
Succinate 1 mg/cc	Succinate 1.25 mg/cc	70
Succinate 1 mg/cc	Succinate 2.50 mg/cc	69

Nature of the Terminal Oxidase

Respiration of the camellia flower is inhibited by cyanide as is shown in Table VIII. Cyanide in a concentration of .01 molal inhibited respiration by approximately one-third. Respiration of the camellia flower is thus less sensitive to cyanide than is the respiration of many other species which have been investigated. Para-nitrophenol, a selective inhibitor of the enzyme polyphenoloxidase, is without effect on respiration of camellia flowers in concentrations which are known to inhibit this enzyme. It appears therefore unlikely that polyphenoloxidase is the terminal oxidase of the camellia flower. Similarly, atabrine, a substance known to inhibit flavoprotein enzymes, is without any effect in inhibition of the respiration of camellia flowers. It seems unlikely, therefore, that a flavoprotein represents the terminal oxidase of this tissue. It is most probable that respiration of camellia flowers may be mediated by a cytochrome oxidase system. However, definite evidence on this point has not as yet been obtained.

TABLE VII

Effect of Sodium Fluoride on Respiration of Camellia (var. Daikagura) Flower Tissue

Inhibitor	Conc.	Other Addenda	Conc.	O_2/400 mg/hr
None	.....	None	.....	70
NaF	0.1 mg/cc	None	.....	96
None	.....	Malonate	5 mg/cc	50
NaF	0.1 mg/cc	Malonate	5 mg/cc	66

TABLE VIII

Effect of Various Terminal Oxidase Inhibitors on Respiration of Camellia, (var. Purity)

Inhibitor	Conc.	O_2/400 mg/hr
None (control)	.....	95
KCN	0.01 M	62
KCN	0.10 M	37
p-Nitrophenol	0.01 mg/cc	96
Atabrine	0.10 mg/cc	98

Discussion

The deterioration of the excised camellia flower in storage is apparently due, morphologically speaking, to abscission of the petals from the floral axis. From a practical standpoint, this abscission may be delayed by placing the excised flowers in an atmosphere saturated with water vapor. Nevertheless the fact that the excised flower may be stored intact and without such abscission for several days indicates that progressive degeneration changes in the flower must take place during storage. The purpose of this present work has been the study of the metabolism of the excised flower with a view toward identification of the degenerative changes which result in the termination of storage life. It is known that when leaves are excised, rapid loss of protein ensues and that the loss of roughly half of the total initial protein frequently corresponds to the termination of storage life. With the excised camellia flower, however, such changes in protein content do not seem to take place. With the storage of many fruits, marked changes in respiratory rate accompany maturation and senescence. From this standpoint then it has been of interest to examine the respiratory behavior of camellia flowers. These flowers exhibit, after excision, a continuously dropping rate of respiration. Final death of the petals takes place when respiration has dropped to between one-half and two-thirds of the initial value. Interestingly enough the respiration of camellia flowers appears to be primarily at the expense of organic acids rather than of carbohydrates. Although the respiratory rate of camellia flower tissue taken from flowers near the end of storage life can be very considerably increased by the addition of organic acids, especially succinic acid, respiratory rate can still not be restored to the initial level. The addition of succinate to the solutions on which excised flowers are floated during storage has not resulted in any considerable increases in storage life. This is in part apparently because the added succinate does not penetrate the petal tissue. We do not, howaver, have any definite link implicating decreasing respiratory rate as the final cause of termination of storage life, reasonable as this relation may appear.