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Metabolism of Sucrose in Cut Roses I. Comparison of Sucrose Pulse and Continuous Sucrose Uptake[1]

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Abstract. Cut roses (*Rosa hybrida* L. cv. White Butterfly) pulsed with ^{14}C-sucrose were analyzed for changes in total and labeled sucrose, glucose, fructose and starch after chases of various periods in either water or sucrose solution. Changes of ^{14}C in an ethanol-insoluble fraction defined as the "cell wall fraction" were also studied. Changes in total amounts of each sugar did not correspond to changes in their respective labeled sugars, and these dissimilariteis in pattern indicated rapid turnover of each sugar in stems, and moderate turnover in leaves, with a general movement of ^{14}C out of both leaves and stems. In flower heads, there was an initially short, overall incorporation of labeled sugars, followed by a gradual increase of ^{14}C-glucose and ^{14}C-fructose, but not ^{14}C-sucrose. Starch turnover was appreciable in flower heads and in leaves, but not in stems. The leaves of roses held in the water chase showed the greatest turnover of starch. Incorporation of ^{14}C into that portion of the ethanol-insoluble fraction designated "cell wall fraction" was greater in flower heads of roses chased in sucrose than those chased in water, but the type of chase solution used had little effect on incorporation of ^{14}C into the "cell wall fraction" of leaf and stem tissue.

Depletion of available carbohydrates is an important factor influencing the vase life of cut flowers (1). Aging cut rose flowers held in water show a decrease of sugars with time (4, 5, 12, 13). Adding a carbohydrate source such as sucrose to the holding solution results in an extension of vase life, if growth of microorganisms is controlled (1, 6, 7). The sugar may perform multifunctional roles in delaying senescence; it acts as a respiratory substrate, as an osmoticum, or as an agent causing stomatal closure (1, 6). Other as yet unknown functions may also exist.

Since the use of floral preservatives containing sugar has become a widespread practice, it is desirable to gain a fuller understanding of translocation and metabolism of exogenously-fed sugars. The purpose of this research was to characterize differences in movements of various sucrose metabolites in cut rose flowers when held continuously in a sucrose solution or when transferred to distilled water.

Materials and Methods

'White Butterfly', a white hybrid tea rose, grown under accepted cultural conditions was obtained from a local greenhouse immediately after harvest. Stems were recut to a length of 37 cm and the foliage on the lower 10 cm of stem was removed. Eighteen roses prepared as above were allowed to take up collectively 10 ml of 1.6% sucrose containing 50 μ Ci of ^{14}C-U-sucrose. Nine of the roses were then transferred to an unlabeled 2% sucrose solution and the other 9 were transferred to distilled water.

At the termination of the pulse, and at 0.5 and 24 hr after beginning the chase, 3 roses were removed from chase solutions and separated into flower heads, leaves, and stems. Flower heads included petals and receptacles. After weighing, each cut rose portion of the three roses was homogenized with 80% hot ethanol in a Waring blender at maximum speed for 10 min. The extract was filtered through Whatman 541 filter paper and the residue was re-extracted twice. The final residue constituted the insoluble fraction. The combined ethanol-soluble fraction was evaporated to dryness and then redissolved in water to a volume of 50 ml.

Sugars in the soluble fraction were separated by TLC on cellulose MN300. The developing solvent consisted of 3 formic acid:6 tert-butanol:8 methylethyl-ketone:3 acetone:3 water. The chromatograms were developed twice, and sugars were located by co-chromatography with authentic sucrose, glucose and fructose. Authentic markers were visualized by aniline phthalate spray reagent (SGA Scientific, Bloomfield, NJ). For colorimetric sugar determinations, areas of the chromatogram corresponding to the markers were removed and suspended in water for 2 hr. Each suspension was centrifuged at 15,000 g for 1 hr, and sugar content in the supernatant determined. Reducing sugars were determined by the method of Somogyi (11) and sucrose was estimated by the thiobarbituric acid method (8). Starch was extracted from the insoluble fractions by cold perchloric acid and the amount determined by iodine reagent (9).

For determination of radioactivity, samples were transferred to an oxidation boat (Intertechnique) and oxidizer (Oxymat-Intertechnique). The $^{14}CO_2$ evolved was trapped in Oxifluor (New England Nuclear). Radioactivity of samples was determined using a scintillation counter (Beckman LSC-133).

In this investigation, only fresh wt of the experimental plant materials could be determined. Since it was felt that the results would be more meaningful on a dry wt basis, however, a duplicate group of roses were treated as in the experiment, except that unlabeled sucrose only was used. The fresh and dry wt of different portions of these roses were determined and the ratio of the two was calculated. Based on these ratios, the dry wt of the experimental materials were estimated.

Results and Discussion

Incorporation and distribution of ^{14}C into the ethanol-soluble fractions of all 3 rose portions, flower heads, leaves and stems, and into the ethanol-insoluble fractions of leaves and stems appeared to be unaffected as a result of the type of chase treatment (Fig. 1). Incorporation of ^{14}C into the ethanol-insoluble fraction of flower head tissue, however, was considerably greater when roses were given the sucrose chase treatment than when they were chased in water. Distributional patterns of ^{14}C in the ethanol-soluble fractions were similar to those previously described by Sacalis and Durkin (10), namely initially high ^{14}C content in leaves and stems decreased and initially low ^{14}C content in flower heads increased throughout the chase period. Incorporation of ^{14}C into the ethanol-insoluble fractions of all three rose portions increased during the chase, the greatest increase occurring in leaves.

Total sugar contents of all 3 rose portions were maintained when flowers were chased in a sucrose solution, but decreased when a water chase was used (Fig. 2). Total sugar content of leaves was greater than that of flower heads and stems.

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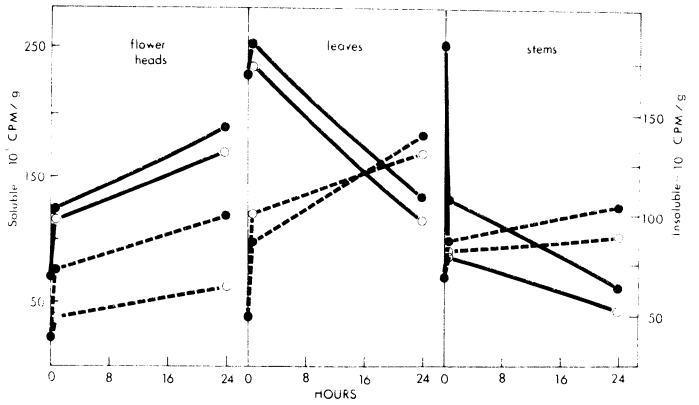

Fig. 1. Incorporation of ^{14}C into the ethanol-insoluble fraction (solid lines) and into the ethanol-insoluble fraction (dashed lines) from flower heads, leaves, and stems of cut roses pulsed 1 hr with ^{14}C-sucrose and chased with either 2% sucrose (closed circles) or with water (open circles).

Levels of sucrose, glucose and fructose decreased in all rose portions when flowers were chased in water, while placement in a sucrose chase resulted in maintained or increased levels of individual sugars (Fig. 3). The increases in sugar levels associated with the sucrose chase treatment were least prominent in glucose and fructose extracted from leaves and stems. Sucrose content of flower heads was much greater than that reported to be found in flower petals by Kaltaler and Steponkus (5), possibly due to the presence of sucrose in the receptacles, which were included as part of the flower heads in the present study.

Changes in levels of each of the individual sugars sucrose, glucose and fructose closely corresponded to changes of total sugars in the ethanol-soluble fraction during the chase period (Fig. 2 and 3).

Levels of ^{14}C-sucrose, ^{14}C-glucose and ^{14}C-fructose decreased in leaves and stems and with the exception of sucrose, increased in flower heads, (Fig. 4). ^{14}C-sucrose in flower heads remained constant. These changes in radioactivity of individual sugars appeared to be independent of the type of chase solution and closely resembled changes of ^{14}C in the ethanol-soluble fraction (Fig. 1 and 4).

Rapid incorporation of ^{14}C into glucose and fructose in stems, regardless of the type of chase treatment, suggests rapid sucrose inversion, but the site of inversion was not determined in this study. Relatively large quantities of ^{14}C glucose and ^{14}C-fructose in stem tissue early during the chase period may have been to some extent representative of carbohydrate translocation via phloem tissue, as has been reported to occur elsewhere (3). If this assumption is true, the data presented here would support the assertation that sucrose inversion is a requirement for movement past parenchyma cell membranes, as is reported to occur in sugarcane (2).

Initially large amounts of individual ^{14}C sugars found in stems during the chase period were not considered to have resulted from incomplete ^{14}C-sucrose removal from stem bases after pulsing because great care was taken to thoroughly wash stems before transfer to the chase. Furthermore, the rapid inversion in stems also supports the view that the sugars were irreverably taken up into the stem tissue. The possibility, however, of ^{14}C diffusing out of the tissue and into the chase solution should be studied to resolve this question.

Presence of labeled sugars other than ^{14}C-sucrose, ^{14}C-glucose and ^{14}C-fructose was also confirmed by thin layer chromatography, and although present in all portions, these other labeled sugars were most concentrated in the flower heads of the cut roses. The predominant unknown labeled sugar was found to have an Rf corresponding to that of authentic xylose.

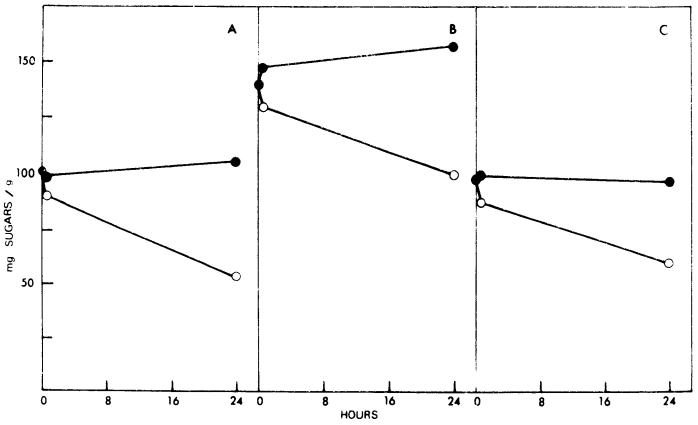

Fig. 2. Change in total sugar content in flower heads (A) leaves (B) and stems (C) of cut roses pulsed with ^{14}C-sucrose and chased with either 2% sucrose (closed circles), or with water (open circles).

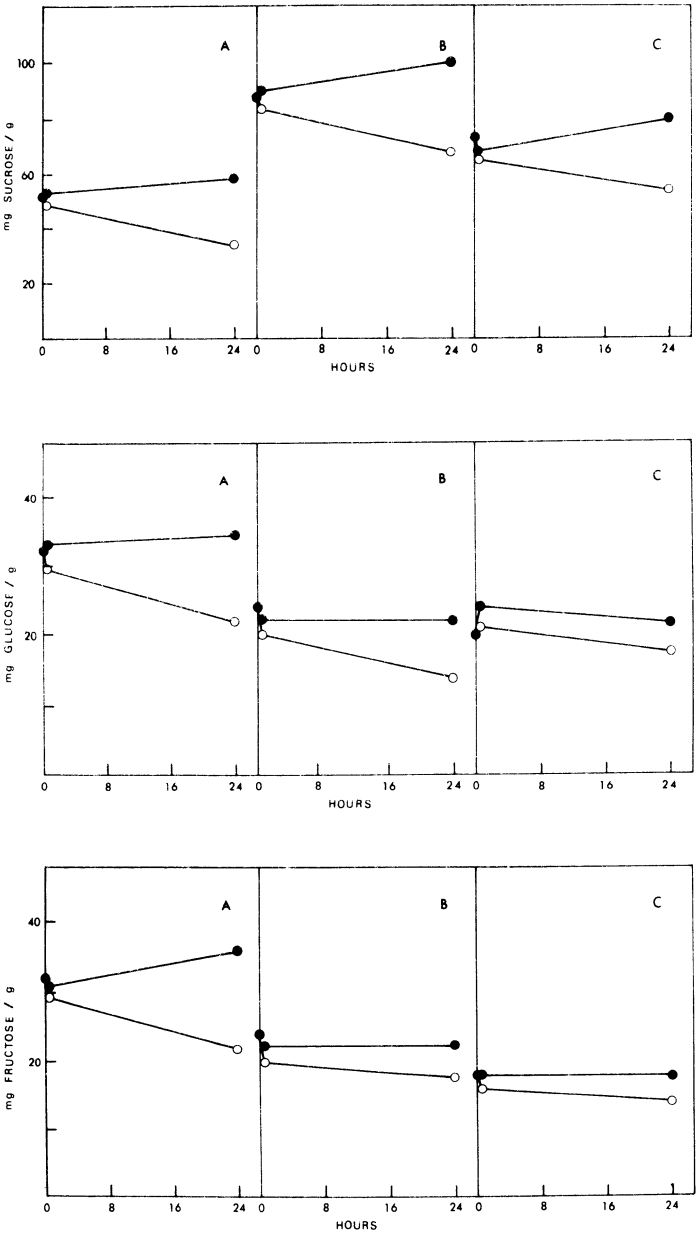

Fig. 3. Changes in total sucrose (top), glucose (center), and fructose (bottom) in flower heads (A), leaves (B), and stems (C) of cut roses pulsed with ^{14}C-sucrose and chased with either 2% sucrose (closed circles), or with water (open circles).

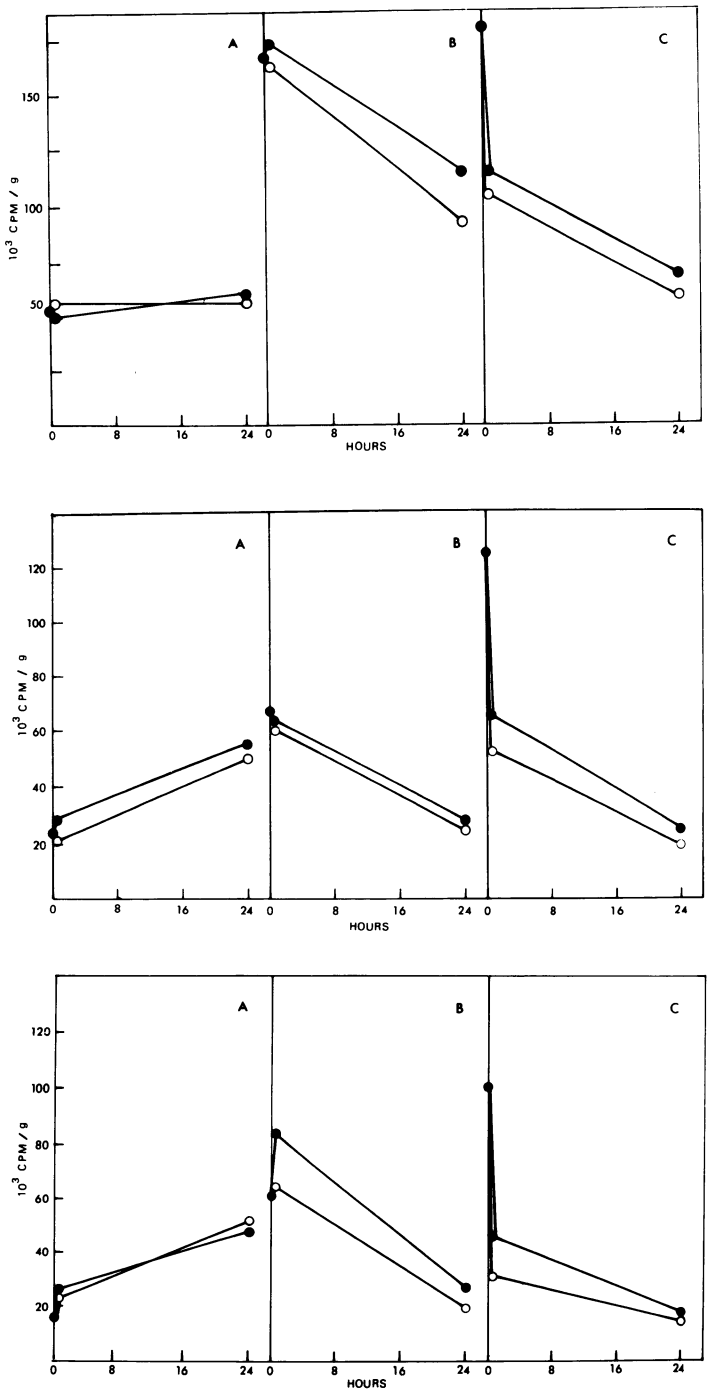

Fig. 4. Incorporation of ^{14}C into sucrose (top), glucose (center), and fructose (bottom) from flower heads (A), leaves (B) and stems (C) of cut roses pulsed with ^{14}C-sucrose and chased with either 2% sucrose (closed circles), or with water (open circles).

Xylose, as well as other pentoses and hexoses, has been found in rose petals by other investigators (13).

Total starch in all flower portions decreased when flowers were chased with water, but the sucrose chase treatment resulted in maintenance of starch levels (Fig. 5, top).

^{14}C-starch in leaves and stems of water-chased flowers increased rapidly and then decreased gradually, while ^{14}C-starch in the flower heads of these roses remained unchanged after a transitory early increase (Fig. 5, bottom). Roses chased with sucrose showed a general increase of ^{14}C-starch in all three rose portions.

Comparison of changes in total and labeled starch in leaf tissue revealed patterns suggesting a very rapid and early starch

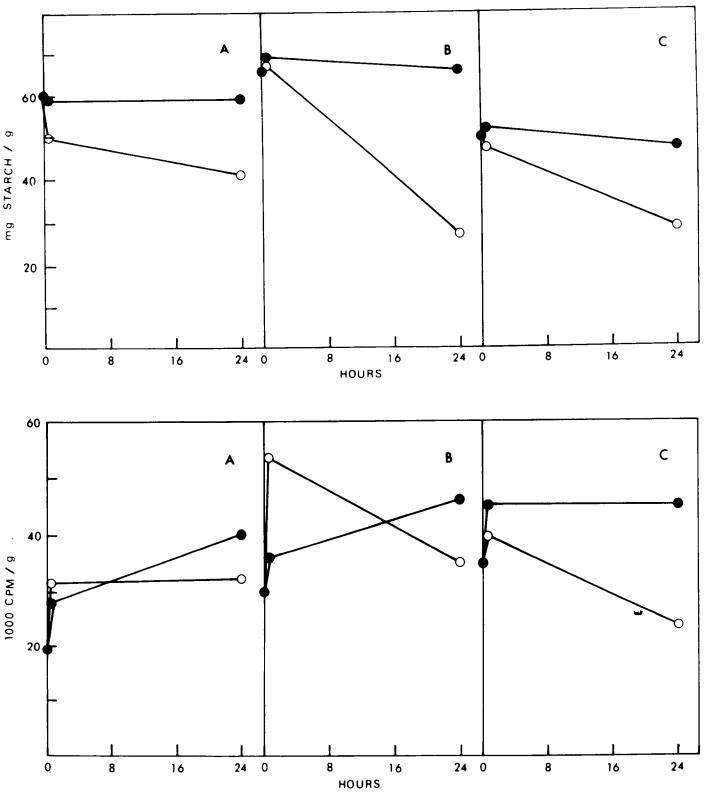

Fig. 5. Changes in total starch (top) and ^{14}C-starch (bottom) in flower heads (A), leaves (B), and stems (C) of cut roses pulsed with ^{14}C-sucrose and chased with either 2% sucrose (closed circles or water (open circles).

turnover followed by gradual depletion in leaves from roses chased in water, and a much slower turnover in sucrose-chased roses (Fig. 5). Starch turnover appeared to be somewhat lower in flower heads than in leaves, and since in stems, total and labeled starch patterns were almost identical, turnover of starch in stem tissue appeared to be very low.

It appears that rose leaves may function as carbohydrate recycling regions. This function is most evident when cut roses are held in water, possibly because the leaves are an important source of carbohydrates which are available for mobilization to the expanding flower. When cut roses are held in a sucrose solution, leaves appear to assume a less important role as a carbohydrate recycling region, and consequently, storage carbohydrate may accumulate in sugar-fed roses. Usefulness of such stored carbohydrate in roses held in water after receiving a short sugar

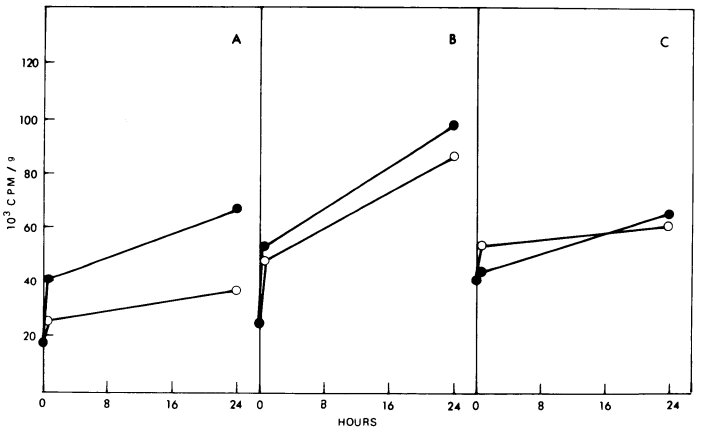

Fig. 6. Incorporation of ^{14}C into the "cell wall fraction" of flower heads (A), leaves (B), and stems (C) of cut roses pulsed with ^{14}C-sucrose and chased with either 2% sucrose (closed circles) or water (open circles).

pulse, is an area worthy of further consideration.

Subtraction of ^{14}C-starch from the ^{14}C-ethanol-insoluble fraction produced a radioactive quantity designated as that incorporated into the "cell wall fraction" (Fig. 6). In flower heads, leaves and stems, ^{14}C in the "cell wall fraction" increased during the chase period, with leaves showing the greatest rate of increase. Increase of ^{14}C in this same fraction in flower heads was greater with the sucrose chase than with the water chase, but in leaves and stems, ^{14}C incorporation appeared to be independent of the type of chase treatment.

Although the cut roses used in this study all received some external sugar source, the differences observed when roses remained in the sugar solution or were transferred to water reinforce the importance of carbohydrate depletion as a fundamental factor limiting cut rose vase life. The use of each kind of chase treatment resulted in only slight differences in ^{14}C uptake and movement of ethanol-soluble sugars, whereas such differences were most apparent in the ethanol-insoluble carbohydrate fractions. Rapid starch turnover in rose leaves suggests that the presence of leaf tissue on cut roses during and following short term preservative treatment is an important factor controlling vase life of roses when they are transferred to water.

Synthesis of that portion of ethanol-insoluble carbohydrate designated by the term "cell wall fraction" was, in flower heads, particularly dependent upon a constant uptake of sucrose. Transfer of the cut roses to a chase of plain water caused a reduction of one-half in ^{14}C incorporation into the "cell wall fraction." Although not actually characterized, the "cell wall fraction" was assumed to be largely representative of structural cellular material.

The data presented here suggest that carbohydrate movement and metabolism occurring during the short period examined in this work is a very complex phenomenon. To further elucidate the fate and role of exogenously-fed sugar in extending cut rose life, however, it will be necessary to determine such carbohydrate patterns over a much longer time span. In addition, it is also important to ascertain which regions of the stem are involved with the lateral movement of sucrose from the xylem tissue, as well as the manner by which sucrose moves from cell to cell.

Literature Cited

1. Aarts, J. F. Th. 1957. Over de houdbaarheid van snijbloemen. *Mededelingen van de Landbouwhogeschool, Wageningen.* 57:1-62.
2. Bowen, J. E. and J. E. Hunter. 1972. Sugar transport in immature internodal tissue of sugarcane. *Plant Physiol.* 49:789-793.
3. Ho, L. C. and R. Nichols. 1975. The role of phloem transport in the translocation of sucrose along the stem of carnation cut flowers. *Ann. Bot.* 39:439-446.
4. Kaltaler, R. E. L. and P. L. Steponkus. 1974. Uptake and metabolism of sucrose in cut roses. *J. Amer. Soc. Hort. Sci.* 99:490-493.
5. Kuc, R. W. 1964. Nitrogen and organic acid metabolism of aging 'Better Times' roses. PhD thesis, Purdue University, Lafayette, Indiana.
6. Marousky, F. J. 1969. Vascular blockage, water absorption, stomatal opening and respiration of cut 'Better Times' roses treated with 8-hydroxyquinoline citrate and sucrose. *J. Amer. Soc. Hort. Sci.* 94:223-226.
7. ———. 1972. Water relations, effects of floral preservatives on bud opening, and keeping quality of cut flowers. *HortScience* 7:114-116.
8. Percherson, F. 1962. Dosage colorimetrique du fructose et des fructofuranosides par l'acide thiobarbiturique. *Compt. Rend.* 255:2521-2522.
9. Putcher, G. W., C. S. Leavenworth, and H. B. Vickery. 1948. Determination of starch in plant tissues. *Anal. Chem.* 20:850-853.
10. Sacalis, J. N. and D. Durkin. 1972. Movement of ^{14}C in cut roses and carnations after uptake of ^{14}C-sucrose. *J. Amer. Soc. Hort. Sci.* 97:481-484.
11. Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* 195:19-23.
12. Stoltz, L. 1956. The keeping quality of cut flowers with special emphasis on 'Better Times' roses MS thesis, Ohio State University, Columbus, Ohio.
13. Weinstein, L. H. 1957. Senescence of roses. I. Chemical changes associated with senescence of cut 'Better Times' roses. *Contrib. Boyce Thompson Inst.* 19:33-48.

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A New Testing Approach for Breeding Peas Resistant to Common Root Rot Caused by *Aphanomyces euteiches* Drechs.[1]

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Abstract. **An "Environment Shift Technique" (EST), utilizing controlled environment chambers and a greenhouse bench, gave uniform and good separation of common root rot resistant from susceptible plants of pea (*Pisum sativum* L.). EST, based on environmental control during 2 phases of testing, reduced the resistance breakdown at an early stage of host development. Separation was based on time of plant death. Changes in the environment enabled the resistant host to overcome the heavy inoculation and live long enough to produce seeds. In EST vs. greenhouse testing, standard concentrations of inoculum produced different effects based on dry root weight and time of plant death. Utilization of EST in pedigree selection resulted in the development of Minn. 108, an *A. euteiches* resistant line of commercial type.**

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