Preparations of root meristems, differentiated zones and shoot meristems for cytophotometry (in situ nuclei) were made by fixing in glacial acetic acid - ethanol mixture (1:2), followed by hydrolysis at 60 °C in 1 N HCl for 10 min. After hydrolysis, materials were washed in distilled water, stained with Schiff's reagent for at least 30 min, and squashed in 45% glacial acetic acid.

For cytophotometric analysis, Reichert Zetopan with microphotometer was used. 2 wave-length method[12] was adopted and observations as obtained from 100 nuclei of each set are presented below.

The results clearly indicate that the content of DNA varies in different organs of an individual. This is significant, as no chromosome number variation is noted in the metaphase plates counted in the meristematic regions in any of the species. The constancy in chromosome number with DNA variability in meristematic region is possibly an indication of polynemy in metaphase present during organogenesis. A significant feature is that in all cases the shoot meristem has been noted to contain a less amount of DNA as compared to others. Unpublished results of the authors from this laboratory, show that, even while the DNA contents of other organs are measured, the shoot meristem shows the least amount. The low content of DNA in shoot meristem may be due to the fact that it represents the initial step during organogenesis. The differentiation of organs is initiated at this level. Therefore the basic amount of DNA necessary for maintaining genetic stability is present in this region wherefrom differential DNA amplification may play a role in initiation of organ differentiation.

A comparison of the data on in situ and extracted nuclei shows that, inspite of the similarity of relative values, there is an overall decrease in amount of DNA in nuclei analyzed after isolation. This is attributable to the limitation of extraction procedure which may result in leaching out of some amount of DNA. Moreover, the extraction technique results in isolation of nuclei representing a mixture of those lying at G_1, S and G_2 phases, which also contribute to their lowered DNA value.

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Effects of calcium and daminozide on ethylene production and softening of apple fruits

E.C. Lougheed, D.P. Murr and S.R. Miller[1]

Horticultural Science Department, University of Guelph, Guelph (Ontario, N1G 2W1, Canada), and Agriculture Canada Experimental Farm, Smithfield, Box 340, Trenton (Ontario, Canada), 28 April 1978

Summary. Ethylene production of McIntosh apples was reduced by both a postharvest treatment with $CaCl_2$ and a daminozide treatment applied in the field. The $CaCl_2$ treatment was more effective than daminozide in reducing the rate of softening during storage at 1 °C.

Fruit softening and its relationship to respiration and C_2H_4 production are fascinating and unsolved physiological puzzles. As well, the control of softening and fruit ripening are important practical problems. Calcium treatments reduce the respiration rate of fruits of apple[2] and avocados[3], retard senescence in some fruits[2] and reduce the rate of ripening in avocados[3] and tomatoes[4]. Yet an effect of calcium upon C_2H_4 production has been reported infrequently. Postharvest infiltration treatments with calcium have been noted to reduce C_2H_4 production during ripening of avocados[3], and orchard sprays of calcium reduced internal C_2H_4 content of apples[5]. Daminozide also reduces the respiration rate and C_2H_4 production of apple fruits and affects fruit firmness[6]. This paper describes a preliminary investigation of the effects of postharvest treatments of McIntosh apples with $CaCl_2$ after field treatments with daminozide.

Materials and methods. McIntosh apple trees were treated 37 days before harvest with 3 kg/ha of daminozide (wettable powder, 85% active ingredient). Fruit were harvested on 2 September 1976 in the preclimacteric stage as indicated by the pattern of respiration (CO_2 production) and C_2H_4 production. Before being placed in storage at 1 °C, 10-kg samples were dipped for 1 min in aqueous solutions of 6% $CaCl_2$+0.3% Keltrol, 6% $CaCl_2$ and 0.3% Keltrol (w/v). Samples were left dry as a control. The $CaCl_2$ was of 77% flake type and Keltrol (Ke), which is xanthan gum used to thicken and stabilize water-based solutions, was supplied as an 80-mesh powder. There were 3 replicates for each treatment and calcium treatments were imposed on the daminozide treatments in a factorial arrangement. Ethylene and CO_2 production were measured on 2-kg samples with an automatic sampling, flow-through system[7]. Firmness measurements were performed on pared flesh using an Effegi tester with 1.1-cm tip (10 fruit, 2 measurements per fruit). Analyses of variance were by standard procedures with differences among means for $CaCl_2$ treatments assessed using Duncan's Multiple Range Test at $p=0.05$ and the differences between daminozide treatments by the F-test at $p=0.05$.

Results and discussion. In the following discussion it has been accepted that Ca^{2+} is the effective ion as others have shown[8]. It is also accepted that Ca^{2+} has been taken up into the apple fruit following the pattern described by Betts and Bramlage[9].

The $CaCl_2$+Ke treatments consistently reduced C_2H_4 production (table 1). Although the effect upon CO_2 production was generally similar the differences were not always significant at $p=0.05$ (data not shown). Even the individual treatments of Ke and $CaCl_2$ reduced C_2H_4 production in

some cases. The effect of $CaCl_2$ alone might be attributed to uptake of Ca^{2+}, although not to the same level as with the thickener Ke[10], and that of Ke alone might be attributed to coating of the fruit thus reducing gas exchange[11]. Ethylene production was apparently more sensitive than CO_2 production to applications of $CaCl_2$ although this difference may be due only to more sensitive detection of differences for C_2H_4. The apparent reduction in both rate of C_2H_4 production and maximum C_2H_4 production by calcium may be not only a quantitative effect, but also a qualitative effect in that the peak of C_2H_4 production at 21 °C appeared to occur somewhat later in the treated fruit after 125 days of storage. However, neither the statistical analyses nor frequency of sampling permits rigorous comparison of individual calcium treatments over time. Daminozide reduced C_2H_4 production in many cases, and most often and to the greatest extent after 125 days of storage.

In general, fruit treated with $CaCl_2$ were firmer upon removal from storage and after 1 week at room temperature (table 2). The effect of $CaCl_2$ was not as apparent after 48 days as it was after 125 days of storage. Probably sufficient Ca^{2+} had not yet been taken up by the flesh to influence firmness. However, even after 48 days of storage there was a significant reduction in C_2H_4 production. This difference in effect could be interpreted that the reduction in softening was due to a decrease in rate of ripening as reflected in C_2H_4 production. There was no effect of daminozide on the firmness of the fruit even after 48 days of storage although there was a measureable effect at harvest-daminozide treated (8.12 kg) vs control (7.89 kg). These results are similar to other reports[12-14], in that the effect of daminozide in controlling the softening of apple fruits may be only until the fruit is mature and thereafter softening of daminozide-treated fruit may be more rapid than untreated fruit.

When considering the effect of calcium there is one further difficulty in interpretation. When calcium is applied as a field spray[15] or vacuum infiltrated into the intact fruit[3] levels of calcium tend to increase in all parts of the fruit. From the field spray this would occur through normal uptake, translocation and distribution, and from infiltration this would occur through the physical rapid uptake and subsequent translocation and distribution[8]. Thus, increased calcium levels would be expected in all parts of the fruit and if calcium does influence respiration rate and ethylene production the measured effect would be one induced in all areas of the fruit. This is especially true of tissue discs infiltrated with solutions containing calcium. However, when apples are dipped calcium penetrates initially only into the outer periphery of the fruit with the distance inwards becoming greater with time[9]. Thus, if the reduced respiration rate (and C_2H_4 production) is due to calcium application it must be assumed that the effect is only due to the lessened rate in the tissue 'invaded' by calcium. Therefore, in dipping studies of whole apple fruit the measured reduction in respiration rate and C_2H_4 production may be occurring only in part of the fruit and if the calcium level could be increased in all parts the reduction would be much greater. Furthermore, if the effect of calcium upon fruit firmness is direct the flesh in the periphery of the fruit should be firmer than that of the interior.

Table 1. Ethylene production (µl/kg h) of McIntosh apples at 21 °C after field treatment with daminozide, postharvest treatments with $CaCl_2$ and storage at 1 °C

Days at 1 °C	Days at 21 °C	$CaCl_2$* + Ke	$CaCl_2$	Ke	No treatment	Daminozide** −	Daminozide** +
48	1	54 a***	69 b	66 b	80 c	72 a	62 b
	2	72 a	92 ab	83 ab	100 b	109 a	82 a
	3	76 a	96 ab	88 ab	106 b	95 a	88 a
	4	82 a	100 ab	92 ab	108 b	99 a	92 a
	5	85 a	104 ab	95 ab	115 b	103 a	97 a
125	1	67 a	74 ab	82 b	81 b	87 b	65 b
	2	120 a	133 ab	139 ab	150 b	151 a	120 b
	3	125 a	144 b	152 bc	167 c	160 a	135 b
	4	127 a	145 b	148 b	161 b	160 a	131 b
	5	92 a	137 b	139 b	149 b	152 a	123 b

* $CaCl_2$ at 6% (w/v) of 77% commercial grade $CaCl_2$: Keltrol (Ke) at 0.3% w/v. ** Daminozide (3 kg/ha) applied in the field 37 days previous to harvest (2 September). *** Means within one row and set of treatments significantly different at $p=0.05$ if followed by different letters.

Table 2. Firmness (kg) of McIntosh apples after field treatment with daminozide and postharvest treatments with $CaCl_2$*

After Days at 1 °C	Days at 21 °C	CaCl + Ke	$CaCl_2$	Ke	No treatment	Daminozide −	Daminozide +
48 +	5	5.67 a	5.56 a	5.62 a	5.44 a	5.63 a	5.52 a
125 +	0	5.56 a	5.53 ab	5.35 ab	5.28 b	5.43 a	5.43 a
125 +	5	5.23 a	5.02 ab	4.92 bc	4.88 c	5.06 a	4.96 a

* For details see footnotes to table 1, means within one row and set of treatments significantly different at $p=0.05$ if followed by different letters.

1 Acknowledgment. This investigation was supported by the National Research Council, Canada, and the Ontario Ministry of Agriculture and Food. A gift of Keltrol from the Charles Tennant & Company, Weston, Ontario, is also acknowledged.

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