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# The Influence of 1-Methylcyclopropene on 'Cortland' and 'McIntosh' Apple Quality Following Long-term Storage

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***Abstract.*** **'Redcort Cortland' and 'Redmax' and 'Summerland McIntosh' apples *(Malus ×domestica* Borkh.) were treated with 900 nL·L$^{-1}$ of 1-methylcyclopropene (1-MCP) for 24 hours at 20 °C before storage and were kept at 3 °C in either a controlled atmosphere (CA) of 2 kPa $O_2$ and <2.5 kPa $CO_2$ or in an air (RA) environment for up to 9 months. After 4.5 months, half of the fruit were treated with a second 900 nL·L$^{-1}$ 1-MCP application in air at 3 °C for 24 hours and then returned to RA or CA storage. At harvest and following removal at 3, 6, and 9 months and a 7-day shelf life at 20 °C, fruit firmness, titratable acidity (TA) and soluble solids content (SSC) were measured, while internal ethylene concentrations (IEC) in the apple core were quantified after 1 day at 20 °C. Upon storage removal and following a 21-day shelf life at 20 °C, disorder incidence was evaluated. 1-MCP-treated apples, particularly those held in CA-storage, were more firm and had lower IEC than untreated fruit. Higher TA levels were maintained with 1-MCP in all three strains from both storages, while SSC was not affected. Following the 6- and/or 9-month removals, 1-MCP suppressed superficial scald development in all strains and reduced core browning and senescent breakdown in RA-stored 'Redmax' and 'Summerland' and senescent breakdown in RA-stored 'Redcort'. 1-MCP generally maintained the quality of 'Cortland' and 'McIntosh' fruit held in CA and RA environments (particularly the former) to a higher degree than untreated apples over the 9-month storage period. A second midstorage application of 1-MCP at 3 °C did not improve poststorage fruit quality above a single, prestorage treatment.**

Ethylene plays an important role in fruit ripening and senescence (Lelièvre et al., 1997; Watkins, 2002). Historically, the negative effects of ethylene in stored fruit have been mitigated through a combination of refrigerated and controlled atmosphere storages (Kader, 1986), and recently, by the use of the commercial formulation of aminovinylethoxyglycine (i.e., ReTain) (Shafer et al., 1995). In 2002, 1-MCP, a competitive inhibitor of ethylene action, was registered for application to edible products and labeled in the U.S. for use on apples (AgroFresh, 2002).

1-MCP is thought to function by complexing to a metal in the ethylene receptor, which blocks ethylene binding and results in no ethylene-induced physiological effects (Sisler and Serek, 1997).

In apples, 1-MCP suppresses ethylene generation, maintains firmness and acidity levels and reduces superficial scald and other disorders during extended storage periods. 1-MCP can, however, suppress the generation of aroma and flavor compounds and may promote internal browning incidence (Watkins and Miller, 2003). In addition, the benefits conferred by 1-MCP appear to be cultivar dependent, with cultivars like 'McIntosh' often showing variable 1-MCP responses (Beaudry and Watkins, 2001; Watkins et al., 2000).

Evaluations of apple fruit responses to 1-MCP are largely limited to fruit stored for ≤6 months (DeEll et al., 2002; Fan et al., 1999a; Lurie et al., 2002; Rupasinghe et al., 2000; Zanella, 2003) and many reports have not evaluated the influence of 1-MCP on a broad range of disorders during long-term storage, with the exception of superficial scald and/or rot (DeEll et al., 2002; Duany and Joyce, 2002). These studies could be more relevant for industrial application if the 1-MCP effects were evaluated on longer storage terms similar to those commonly seen in commercial storage operations (e.g., 6 to 9 months).

Repeated applications of 1-MCP have shown some quality benefits for RA-stored fruit (Mir et al., 2001). Although several applications during a storage season are largely impractical due to product expense, a second, midstorage 1-MCP treatment may be warranted if the maintenance of fruit quality was substantial.

Hence, the general objective of this work was to ascertain if a prestorage 1-MCP treatment promoted a higher level of fruit quality and less disorder incidence compared with untreated fruit during a 9-month CA and RA storage period, and if a second, midstorage 1-MCP application was more effective in maintaining fruit quality than a single treatment.

## Materials and Methods

During the 2001 growing season, 'RedCort Cortland', 'RedMax', and 'Summerland McIntosh' apples were harvested on 10- to 15-year-old trees at four different grower sites in the Annapolis Valley, Nova Scotia. Each grower was designated as an experimental replicate. On the day of harvest, 1-MCP (EthylBloc, BioTechnologies for Horticulture, Walterboro, S.C., 0.14% active ingredient) was applied at 900 nL·L$^{-1}$ for 24 h at 20 °C by mixing 3.2 g of the dry chemical with 22 mL of $H_2O$-based buffer (pH 8.6) in an enclosed 2 m$^3$ static air chamber. The fruit were then kept for 9 months at 3 °C in either an atmosphere of 2 kPa $O_2$ and < 2.5 kPa $CO_2$, or in air. Following 4.5 months of CA and RA storage, half of the fruit samples were exposed to a second 1-MCP treatment in air at 3 °C for 24 h and were then returned to their designated storage environment.

Fruit quality, including internal ethylene concentration (IEC), was assessed at harvest and after removal at 3, 6, and 9 months of storage plus 7 d at 20 °C. Fruit firmness (N) was measured on individual fruit in a 10-apple sample from each grower with the fruit quality tester (Geo-Met Instruments, New Minas, N.S.), having the time limit window set at >0.1 and <1.0 s (DeLong et al., 2000). From the juice collected during firmness testing, soluble solids content (SSC) and titratable acidity (TA) were measured with a hand-held refractometer (Atago Co., Tokyo) and by titration of 2 mL apple juice with 0.1 mol·L$^{-1}$ sodium hydroxide to an endpoint of pH 8.1, respectively. The latter was expressed as mg equivalents of malic acid per 100 mL juice (DeEll and Prange, 1998).

Fruit disorder incidence was evaluated on a 50-apple sample from each grower and expressed as a percentage of the total sample following 21 d at 20 °C after each storage removal period and included bitter pit ('Redcort Cortland' only), core browning, moldy core, rotted fruit, senescent breakdown, superficial scald, vascular breakdown and watercore. Disorders that averaged <5% incidence in one or more treatments were not presented.

IEC was measured at 20 °C immediately following harvest on a 40-apple sample (10 from each grower) and on a 20-apple sample (5 from each grower) for each treatment following removal of fruit from CA and RA storage. CA-stored apples were acclimated in RA for 24 h and then samples from both storage environments were kept at 20 °C for at least 2 to 3 h before measurement. A 1-mL gas sample was withdrawn by a syringe from each apple core and injected into a gas chromatogram (GC) (Carle Instruments, Inc., Anaheim, Calif.) equipped with a 1.9-m × 3.2-mm (o.d.) stainless-steel column packed with activated alumina, with helium carrier flow at 50 mL·min$^{-1}$ and a flame ionization detector. Quantitation was performed by comparison of the GC response of the sample to that of a certified standard (DeEll and Prange, 1998).

Individual growers were designated as experimental replications in this study. The at-harvest (0 storage months) data presented in the

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firmness (Fig. 1A–F) and ethylene (Fig. 2A–F) graphs are derived from a 10-apple sample from each grower and shows the average firmness and IEC of the fruit before the first 1-MCP treatment. For stored apples, a separate CA and RA cabinet (each containing all treatments) in each of the two storage rooms was designated as a block (1 room = 1 block). The fixed factors (1-MCP treatment, storage duration and storage environment) were arranged as an incomplete factorial using a split-plot experimental design with storage environment as the main plot and 1-MCP treatment as the subplot. The effects of 1-MCP and storage duration were analyzed for the CA and RA storage environments separately. Treatment means were separated by the Waller Duncan k ratio *t* test, where k = 100 approximates the 5% alpha level. Analysis of variance and mean separation were performed with SAS software using PROC GLM (SAS Institute, 1994).

## Results and Discussion

The second 1-MCP treatment, applied at 3 °C and following 4.5 months of storage, did not affect fruit quality or disorder incidence any more than the first prestorage application for the three strains tested; therefore, it was dropped from the data presentation. The second treatment was designed to reflect the most probable action of a storage operator who may consider a second 1-MCP application if it did not require raising the store room temperature to 20 °C in the midwinter to improve product efficacy.

The lack of response with the second 1-MCP treatment may have been influenced by the 3 °C environment in which the product was applied. Other work, however, has shown that prestorage 1-MCP application at 0 °C does maintain higher fruit firmness and quality over extended storage periods (DeEll et al., 2002; Duany and Joyce, 2002; Pre-Aymard et al., 2003). Thus, prestorage application temperatures appear not to be critical for product efficacy. However, application temperatures may be important after the fruit has been stored for a period of weeks or months. Mir et al. (2001) found that multiple 1-MCP applications during extended RA storage conferred firmness benefits to 'Redchief Delicious' when applied at 5, 10, 15, or 20 °C, but not at 0 °C. They postulate that the affinity of 1-MCP for ethylene binding sites decreases as temperatures decline, which may be overcome by higher dosages. It is possible that during cold storage (particularly CA storage), plant metabolism slows to a point where there is little synthesis of additional ethylene binding sites, making a midstorage 1-MCP treatment ineffective. Another possibility is that the midstorage 1-MCP treatment at 3 °C was not effectively absorbed by the fruit, although Mir et al. (2001) did find product efficacy when applied to 'Redchief Delicious' at 5 °C during storage. The assumption that 1-MCP can be applied to apples at 0 °C or other low temperatures during a storage period because product efficacy was observed when applied at 0 °C before storage (Duany and Joyce, 2002; Pre-Aymard et al., 2003) needs to be more thoroughly tested.

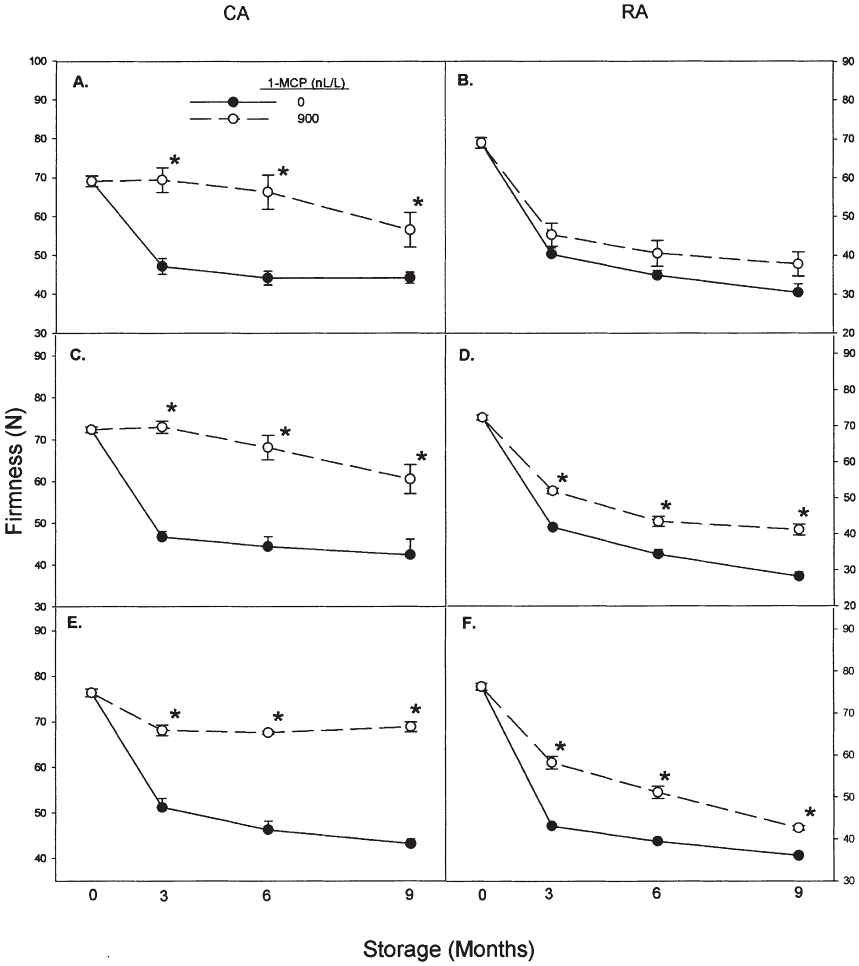

Fig. 1. Firmness (N) measured at harvest (0) and following 3, 6, and 9 months of storage plus 7 d at 20 °C for 'Summerland' (**A** and **B**), 'Redmax' (**C** and **D**), and 'Redcort' (**E** and **F**) fruit held in air (RA) and controlled atmosphere (CA). Treatment means ± standard errors are comprised of 40 observations. Statistical differences ($P \leq 0.05$) between means at each removal period are indicated by the asterisk, and were determined by SAS's LSMeans procedure (SAS Institute, 1994).

Comparison of fruit removed at 3, 6, and 9 months of storage plus a 1 or 7-d shelf life shows that 1-MCP-treated apples were generally more firm (Fig. 1A–F), particularly those that were kept in CA-storage. 1-MCP-treated 'Summerland' fruit stored in RA, however, were not firmer than those untreated at the same removal periods (Fig. 1B). The 'Summerland' at-harvest ethylene data indicate that 20% of the fruit measured were postclimacteric (ethylene >1%; data not shown). At this physiological state, these apples would likely have more ethylene binding sites than preclimacteric fruit, which would be expected to reduce 1-MCP efficacy (Watkins et al., 2000) and result in a loss of firmness. More of the 'at harvest' 'Redmax' and 'Redcort' apples were in the preclimacteric stage of maturity (90% with ethylene of <1%; data not shown), which may explain the more pronounced 1-MCP firmness response for the fruit in RA (Fig. 1D and F). For each strain, CA-stored apples were 7 to over 26 N more firm than those held in RA when similar treatment combinations of storage duration and 1-MCP were compared (Fig. 1A–F). Hence, the combination of CA and 1-MCP additively maintained a higher level of fruit firmness over the 9-month storage period.

Although TA declined in RA fruit in particular over the storage duration, the 1-MCP treatment maintained higher TA levels than untreated apples in all three strains in both storage atmospheres. Soluble solids content, which ranged from 11.25% to 14.5%, was not affected by 1-MCP (data not shown). Similar TA and SSC trends have been previously reported (Fan et al., 1999b; Saftner et al., 2003; Zanella, 2003), although in some cases, 1-MCP has had no effect on fruit acidity (Mir et al., 2001; Watkins et al., 2000).

The results of this study generally corroborate previous findings that 1-MCP is an effective inhibitor of ethylene action and subsequently, fruit ripening (Fan et al., 1999b; Rupashinghe et al., 2000; Saftner et al., 2003). Interestingly, the ethylene data show that autocatalytic ethylene generation was suppressed in treated fruit over the 9-month CA storage (Fig. 2A, C, and E), while the capacity of 1-MCP to suppress autocatalytic ethylene generation was lost between 3 and 6 months in the RA environment (Fig. 2B, D, and F), even following a second application at 4.5 months (data not shown). Increases in ethylene evolution over longer-term RA storage of 1-MCP-treated apples have been reported previously (Fan et al., 1999a; Watkins et al., 2000), although to a lesser extent than observed in the present study. The increase in ethylene production in RA-stored fruit corresponds to the loss of firmness retention (Fig. 1B, D, and F), but not to an increase in disorder expression (Tables 1–3). Where disorder incidence was high in untreated fruit, 1-MCP usually reduced its severity, regardless of the environment in which the fruit were stored (Tables 1–3).

In general, disorder incidence was less severe in CA- versus RA-stored apples irrespective of 1-MCP effects (Tables 1–3). Core browning, senescent breakdown and superficial scald were the most commonly observed disorders in the 'McIntosh' (Tables 2 and 3) and 'Cortland' (Table 1) strains. 1-MCP suppressed the expression of 'Redcort' superficial scald in both RA and CA compared with untreated apples following storage removal at 6 and 9 months. Interestingly, scald suppression after the 9-month removal was more pronounced in the treated RA compared with the treated CA-stored fruit. Although 1-MCP strongly inhibited scald expression in 'Redcort' apples kept for 6 months in both storage environments, it appeared to lose its scald-reduction efficacy between 6 and 9 months in the CA environment (Table 1). 'Cortland' fruit are highly susceptible to superficial scald in Nova Scotia and CA storage has not consistently reduced scald development, particularly if the apples are not treated with DPA (DeEll and Prange, 1998).

Compared with untreated fruit, 1-MCP markedly reduced RA-stored 'Summerland' core browning and superficial scald expression at 6 and 9 months (Table 3) as well as core browning at 6 months of storage for RA-stored 'Redmax McIntosh' (Table 2). 1-MCP also reduced senescent breakdown following the 6- and 9-month and 9-month removals in RA-stored 'Redmax' (Table 2) and 'Summerland' (Table 3) fruit, respectively. Rot was not present in CA-stored fruit and was generally low in RA-stored, 1-MCP-treated 'Redcort' (Table 1) and 'Redmax' (Table 2) apples, ranging from 0.5% to 5%. Although rot increased in nontreated apples over the 9-month storage, 1-MCP and control mean differences were not significant after the 9-month removal (Tables 1 and 2). Our findings basically agree with previous reports that 1-MCP reduces the severity of core browning, senescent breakdown and superficial scald (DeEll et al., 2002; Duany and Joyce, 2002; Fan et al., 1999a; Rupasinghe et al., 2000; Watkins et al., 2000).

In summary, 1-MCP maintained the quality of 'Cortland' and 'McIntosh' fruit kept in CA and RA environments to a higher degree than nontreated apples over a 9-month storage period. 1-MCP consistently suppressed the expression of superficial scald, core browning and senescent breakdown in the three strains tested. Overall, the combination of CA storage and 1-MCP treatment resulted in apples with a high degree of fruit quality retention. A second, midstorage application of 1-MCP did not, however, improve fruit quality above a single, prestorage treatment during the long-term storage period.

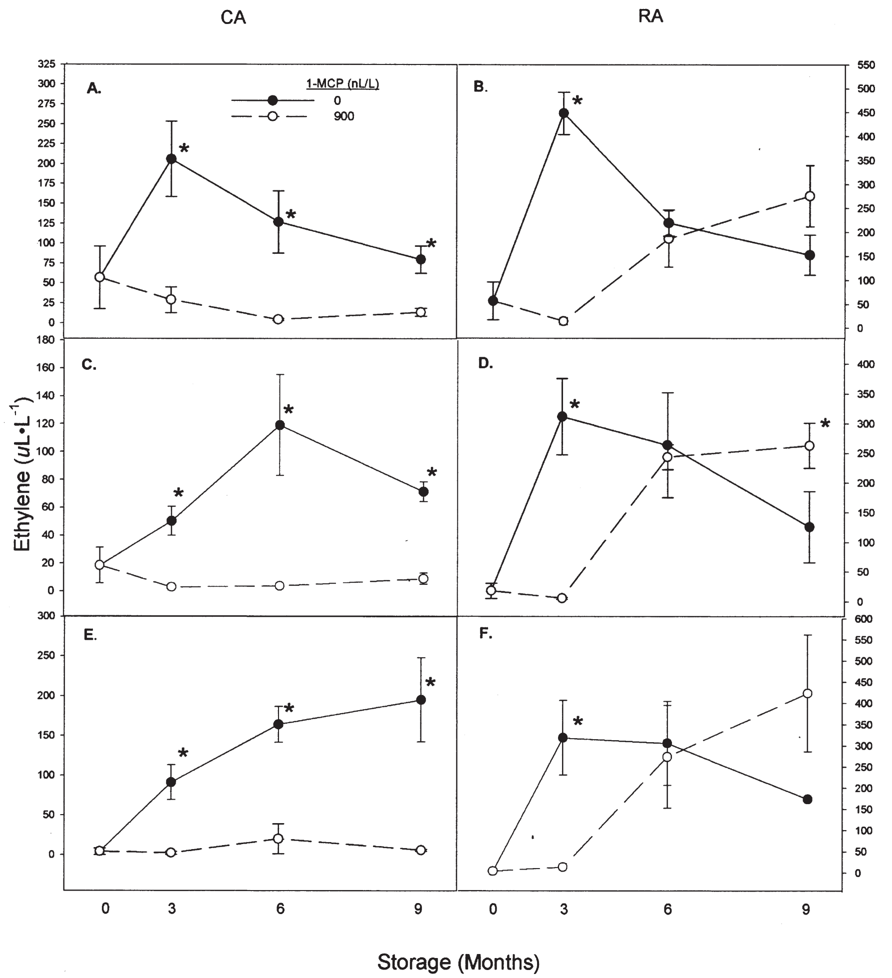

Fig. 2. Internal ethylene concentration (µL·L−1) measured at harvest (0) and following 3, 6, and 9 months of storage plus 1 d at 20 °C for 'Summerland' (**A** and **B**), 'Redmax' (**C** and **D**), and 'Redcort' (**E** and **F**) fruit held in air (RA) and controlled atmosphere (CA). Treatment means±standard errors are comprised of 20 observations. Statistical differences ($P \leq 0.05$) between means at each removal period are indicated by the asterisk, and were determined by SAS's LSMeans procedure (SAS Institute, 1994).

Table 1. Percent disorder incidence in 'Redcort Cortland' apples following 0 and 1 (900 nL·L−1)[z] 1-MCP application and 3, 6, and 9 months of air (RA) and controlled atmosphere (CA) storage.

| | | RA | | | CA | |
|---|---|---|---|---|---|---|
| 1-MCP concn (nL·L−1) | Storage (months) | Superficial scald[y] (%) | Rots (%) | Senescent breakdown (%) | Superficial scald (%) | Senescent breakdown (%) |
| 0 | 3 | 7 b | 0.6 b | 3 b | 10.0 c | 2 ab |
| 0 | 6 | 58 a | 3 b | 14 a | 67 a | 6 ab |
| 0 | 9 | 67 a | 10 a | 9 ab | 68 a | 8 a |
| 900 | 3 | 3 b | 3 b | 0 b | 4 c | 0 b |
| 900 | 6 | 0.5 b | 3 b | 2 b | 2 c | 1 ab |
| 900 | 9 | 5 b | 5 ab | 0.5 b | 33 b | 2 ab |

[z] Total amount of 1-MCP applied per experiment.

[y] Disorder incidence was assessed following 21 d at 20 °C after each storage removal. All disorders are given as percentage of total fruit. Means with different letters within disorder columns are significantly different as determined by the Waller Duncan k ratio *t* test, where k = 100 approximates the 5% alpha level.

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Table 2. Percent disorder incidence in ‘Redmax McIntosh’ apples following 0 and 1 (900 nL·L$^{-1}$)[z] 1-MCP application and 3, 6, and 9 months of air (RA) and controlled atmosphere (CA) storage.

| | | RA | | | | CA |
|---|---|---|---|---|---|---|
| 1-MCP concn (nL·L$^{-1}$) | Storage (months) | Superficial scald[y] (%) | Rots (%) | Senescent breakdown (%) | Core browning (%) | Senescent breakdown (%) |
| 0 | 3 | 0 b | 0.5 b | 5 c | 2 b | 2 b |
| 0 | 6 | 10 a | 5 ab | 48 b | 40 a | 3 b |
| 0 | 9 | 6 a | 12 a | 99 a | 26 a | 25 a |
| 900 | 3 | 0 b | 0.6 b | 2 c | 0 b | 0.5 b |
| 900 | 6 | 0 b | 3 b | 0.4 c | 2 b | 0.6 b |
| 900 | 9 | 0 b | 4 ab | 11 c | 25 a | 15 a |

[z] Total amount of 1-MCP applied per experiment.

[y] Disorder incidence was assessed following 21 d at 20 °C after each storage removal. All disorders are given as percentage of total fruit. Superficial scald in ‘Redmax McIntosh’ appeared as visible, liver-colored areas within the hypodermis. Means with different letters within disorder columns are significantly different as determined by the Waller Duncan k ratio *t* test, where k = 100 approximates the 5% alpha level.

Table 3. Percent disorder incidence in ‘Summerland McIntosh’ apples following 0 and 1 (900 nL·L$^{-1}$)[z] 1-MCP application and 3, 6, and 9 months of air (RA) storage.

| 1-MCP concn (nL·L$^{-1}$) | Storage (months) | Superficial scald[y] (%) | Senescent breakdown (%) | Core browning (%) |
|---|---|---|---|---|
| 0 | 3 | 0 b | 1 b | 17 b |
| 0 | 6 | 57 a | 2 b | 81 a |
| 0 | 9 | 82 a | 26 a | 75 a |
| 900 | 3 | 0 b | 0 b | 0.5 b |
| 900 | 6 | 15 b | 0 b | 11 b |
| 900 | 9 | 26 b | 4 b | 12 b |

[z] Total amount of 1-MCP applied per experiment.

[y] Disorder incidence was assessed following 21 d at 20 °C after each storage removal. All disorders are given as percentage of total fruit. Superficial scald in ‘Summerland McIntosh’ appeared as visible, liver-colored areas within the hypodermis. Means with different letters within disorder columns are significantly different as determined by the Waller Duncan k ratio *t* test, where k = 100 approximates the 5% alpha level.

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