Effect of High-Carbon Dioxide Atmospheres on Infestations of Apple Maggot (Diptera: Tephritidae) in Apples

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ABSTRACT Short-term storage regimens containing elevated atmospheres of carbon dioxide (CO_2) were evaluated for their ability to disinfest newly harvested 'McIntosh' apples of apple maggot, *Rhagoletis pomonella* (Walsh). Infested fruits containing newly laid eggs were either placed directly into the high-CO_2 atmosphere at 10°C to expose this life stage, or else held first for 7 d at room temperature, to allow development to the neonate larval stage. Treatment combinations consisted of three different CO_2 levels (10.6, 14.9, and 19.0% CO_2) and two periods of exposure (7 and 14 d). Apple maggot eggs subjected to the treatments always exhibited some survival, which was lower for the 14-d than the 7-d exposure periods. In contrast, newly hatched larvae were less able to survive the treatments. The 7-d exposure allowed low levels of survival of this life stage, but virtually none survived the 14-d exposure period. To determine the age at which eggs become more susceptible to high-CO_2 atmospheres, infested fruits containing eggs three or 5 d old were submitted to a 14-d exposure to 19.0% CO_2. Survival of 3-d old eggs was similar to that of eggs exposed at an age of 1 d or less, but this dropped to near zero for 5-d old eggs, indicating an increase in susceptibility sometime during the 3–5-d age range. Fruits exposed to 19.0% CO_2 for 14 d were significantly firmer than untreated fruits. No apparent browning, internal breakdown or other fruit defects were detected in any of the treatments.

KEY WORDS *Rhagoletis pomonella*, apple maggot, export, storage, carbon dioxide atmospheres

APPLE MAGGOT, *Rhagoletis pomonella* (Walsh), is a native pest of North America and is found in most of the apple producing areas of the midwestern and northeastern United States and Canada, including all of New York State. Cultivated hosts include apple [*Malus domestica* (Borkhausen)], hawthorn (*Crataegus* spp.) and crabapple (*Malus* spp.). Hawthorn and juneberry (*Amelanchier* spp.) are considered the native hosts of apple maggot; it has one generation per year (Dean and Chapman 1973). Apple maggot geographic distribution in the western United States is restricted to certain portions of California, Oregon, Idaho, Utah, Colorado, and Washington. It is not known to occur outside of these areas, nor in any of the principal export destinations for New York apples, such as Brazil and Mexico.

Together with all apple growers east of the Mississippi River, New York apple growers historically have had to contend with apple maggot as one of the major insect pests affecting this crop. The adult, a fly, deposits its eggs just beneath the skin of the developing fruit during the summer, and the larvae emerge and tunnel throughout the flesh while feeding on the tissue, which renders the fruit unmarketable. Most commercial growers rely on regular (two to four per season) pesticide applications during the summer to prevent apple maggot infestation above a nominal, commercially acceptable level (Agnello et al. 2001). To prevent establishment by accidental introduction of this pest in the largely unaffected western apple growing areas, states have instituted plant quarantine regulations such as those of the California Department of Food and Agriculture, which traditionally has permitted the importation of eastern-grown apples only after a prolonged period of storage under various conditions— 40 d of cold storage conditions at 0°C, or 90 d of controlled atmosphere conditions at 3°C—to ensure destruction of any apple maggot eggs or larvae present in the fruit (Anonymous 1959, 1960). To prevent the spread of apple maggot in apples exported from high-risk areas, other measures have been established as an alternative to these storage regimens, and are used on a limited basis by states such as California and Arizona, as well as importing countries such as Brazil. The most common measure requires a systems approach that consists of a monitoring protocol in each orchard designated for export production, with recommended insecticide sprays applied preventively if any apple

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maggot adults are trapped, followed by a final inspection of the fruit to verify the efficacy of the spray program (Purdue Research Foundation 2000). Although this protocol has been used successfully for some U.S. apple exports during the past few years, the operating procedures, record-keeping, compliance oversight and supervisory efforts required are often overly burdensome for many growers to accommodate, and few have availed themselves of this particular marketing option.

The use of controlled atmospheres has become standard in the storage of apples to retard ripening and reduce spoilage (Smock 1958, Smock and Blanpied 1972). Controlled atmospheres also have been shown to be an effective alternative or supplement to chemical treatment for preventing and controlling some arthropod infestations in fruits (Morgan and Gaunce 1975, Lidster et al. 1981, Gaunce et al. 1982, Potter et al. 1994, Mitcham et al. 1997). The time required to kill insects with controlled atmospheres is affected by specifics of the pest's life stage, the relative humidity, temperature, and the composition of the atmosphere. Considerable information has been published on the time to kill various insect species under a wide range of conditions. Most have found an increased mortality effect associated with elevated carbon dioxide (CO_2) and reduced oxygen (O_2) concentrations compared with those in air. Benschoter (1987) reported that the mortality of eggs and larvae of Caribbean fruit fly [*Anastrepha suspensa* (Loew)], a species closely related to apple maggot, could be 100% when specimens held in laboratory diet medium were exposed to high-concentration CO_2 atmospheres at temperatures between 10–15.6°C for relatively short periods of time (10 d or less). Similar findings have been reported in tests with mites (Lidster et al. 1981, Whiting and van den Heuvel 1995), San Jose scale, *Quadraspidiotus perniciosus* (Comstock) (Gaunce et al. 1982), thrips (Potter et al. 1994, Mitcham et al. 1997), and tortricid apple pests including codling moth, *Cydia pomonella* (L.) (Soderstrom et al. 1990) and light brown apple moth, *Epiphyas postvittana* (Walker) (Whiting et al. 1991). A direct relationship has been reported in several studies between arthropod pest mortality and the temperature at which the high-CO_2 treatment is administered (Harein and Press 1968; Marzke et al. 1970; Morgan and Gaunce 1975; Soderstrom et al. 1986, 1990; Whiting et al. 1991, 1999; Whiting and van den Heuvel 1995; Yahia and Ortega-Zaleta 2000). Other studies have focused more on treatments of elevated temperature and low O_2 without high CO_2 levels [e.g., Lay-Yee et al. 1997 for *E. postvittana* and the mirid, *Nysius huttoni* (White)] or without noting an effect of elevating the CO_2 [Shellie et al. 1997, on Mexican fruit fly, *A. ludens* (Loew)].

Little information exists for apple maggot in this regard. Other species of fruit flies are generally pests of tropical and subtropical fruits that are most often subjected to high-heat treatments that can be damaging to apples. However, an effective controlled atmosphere regimen consisting of exposure for a short period (<14 d) at a moderately higher temperature than normal storage conditions could be useful as a treatment administered preshipping or even while in transit for disinfestation of New York apples exported to other markets. Here we present results of experiments on the destruction of apple maggots infesting harvested apples using atmospheres containing elevated levels of carbon dioxide at 10°C.

Materials and Methods

Insects. Apple maggot adults were taken from a laboratory colony that had been maintained without introduction of wild flies for >80 generations at the New York State Agricultural Experiment Station in Geneva. Oviposition cages were 38 by 38 by 38 cm, consisting of a white painted wood (1.9 by 3.9 by 38 cm) frame, with a solid plywood bottom and sides made of aluminum window screening (1.5-mm mesh). Each cage had two shelves (9 cm deep), located across from each other, 20 cm above the base on opposite sides of the cage, upon which were placed individual 8.0-cm plastic cup lids containing eight small sugar cubes, 0.5 g each of vitamins (Vitamin Diet Fortification Mixture, ICN Biomedicals, Costa Mesa, CA) and apple maggot diet mix (4:1 N-Z-Amine casein hydrolysate:salt mixture No. 2 USP XII, ICN Biomedicals), plus a 50-ml Erlenmeyer flask filled with distilled water and capped by a plastic cap with a hole through which a 9.5-cm piece of dental wick (Mohawk Dental Supply, East Syracuse, NY) was inserted. All insects were reared in a walk-in growth chamber at 23 (±2)°C and 50 (±5)% RH with a photoperiod of 16:8 (L:D) h.

Fruit Infestation. Replicates of 30 recently harvested 'McIntosh' apples being held in cold storage (4.4°C) were allowed to warm to room temperature (22°C) and infested by placing them for 24 h at 22 (±2)°C and 50 (±5)% RH in the bottom of an oviposition cage containing 25 female and 15 male apple maggot flies. After the infestation period, the number of eggs deposited in each fruit was determined by examining the fruit surface for oviposition punctures under a dissecting microscope (0.7–3.0× magnification range). Because the potential number of eggs deposited per puncture is variable, punctures (n = 300) in a number of similarly infested apples were dissected to determine the mean number to be 1.033 (range, 0–7) eggs per puncture. A mean infestation level of 10–15 punctures per apple was considered to be an adequate minimum number for the purposes of this study.

The incubation period of apple maggot eggs has been reported by various researchers to last 2–10 d, averaging 4.6–6.8 d, depending on temperature (Dean and Chapman 1973). Three replicates of three apples each, exposed to apple maggots taken from the previously described colony, were dissected daily to determine the hatching progress of eggs in the fruit held at 22°C for 3–7 d after exposure. This procedure indicated mean cumulative percent hatch to be: 3 d, 7.2%; 4 d, 61.6%; 5 d, 83.8%; 6 d, 90.1%, and 7 d, 94.6% (n = 470 eggs). From this result, we concluded that apple maggots infesting the fruit in this study would be

primarily in the larval stage by 7 d after exposure to the adults. To test susceptibility of either the egg or neonate larval stage to the controlled atmosphere treatments, batches of 25 newly infested fruits were either placed immediately into the exposure chambers or else held first at 22°C and 50% RH for 7 d to allow sufficient time for most of the eggs to hatch before exposure to the high-CO_2 atmospheres.

Controlled Atmosphere Treatments. Exposure chambers consisted of an opaque 18.9-liter (5-gal) plastic bucket with a tight-sealing lid, and fitted with inlet and outlet ports through which the candidate gas mixture was passed at a constant, low rate (50 ml/min), using clear laboratory tubing (Tygon R-3603, 4.8-mm i.d., Norton Performance Plastics, Wayne, NJ). Gas was allowed to escape from the outlet port through a bubble tube to maintain the proper gas mixture in the chamber. The atmospheres tested were supplied from previously mixed calibrated gas cylinders prepared at the Cornell Univ. Orchard Storage Laboratory in Ithaca. Gases of the desired composition were obtained using a pressure mixing system, and concentrations were verified by gas chromatography (gas partitioner, model 1200; Fisher, Springfield, NJ).

The atmospheres tested contained mean concentrations of 10.6% (range, 9.7–12.6), 14.9% (range, 14.6–16.2), or 19.0% (range, 17.6–20.4) CO_2, 2.2% (range, 1.4–4.8) O_2, with the balance nitrogen (N_2). The entire bucket was placed in a darkened environmental chamber (model I-60LLVL, 1.30-m^3 cap, Percival, Boone, IA) kept at a constant temperature of 10 (±0.5)°C and 80 (±5)% RH for the duration of the treatment, which was either seven or 14 d. Temperature was controlled by a continuous-running condensing unit with dual setpoints wired to an electronic controller, and RH was monitored every 2–3 d during the treatment period using a temperature-compensated pen-type thermo-hygrometer with an accuracy of ± 5%.

Three replicates of 25 apples infested with either eggs or larvae of apple maggot were exposed to each high-CO_2 atmosphere for each treatment period (n = 13,171 total punctures). After the exposure period, the fruits were transferred to racks over pans of water and held at 22°C and 68% RH, where they were checked every 1–2 d for 6 wk, to collect any surviving apple maggot larvae exiting the fruit to pupate. The extra five infested apples in each replicate were placed on a separate rack at 22°C and 68% RH immediately after exposure to the flies, as a control for larval survival in untreated fruits. After the 6-wk larval emergence period, the fruits were also inspected for the presence of any larvae that might have pupated without emerging and dropping into the water.

Egg Age and CO_2 Susceptibility. It became apparent during these studies that apple maggot eggs were more difficult to kill with the high-CO_2 atmosphere treatments than were the larvae. An additional study was therefore conducted to supplement the information on efficacy of the highest (19.0%) CO_2 mixture against eggs of different ages. Mortality rates were already being determined for eggs that were a maximum of 1 and 7 d in age before exposure to the gas treatment, so these additional tests were conducted to determine the progression in susceptibility of apple maggot eggs in the fruit as they approached hatch. Batches of 30 apples were exposed for 24 h as previously described to allow oviposition of apple maggot eggs, and 25 fruits were then held in ambient storage at 22°C and 50% RH for either three or 5 d before being placed in the exposure chambers for treatment for 14 d at 10°C with the mixture of 19.0% CO_2, 2.2% O_2, and 78.8% N_2. After the exposure period, they were then transferred to racks over pans of water as before to collect any emerging larvae, and the five remaining fruits were again placed on racks immediately after infestation to serve as controls. Three replicates of apples were exposed to the 19.0% CO_2 mixture after a pretreatment holding period of either three or 5 d (n = 4,750 total punctures).

Because exposure to CO_2 can result in various types of fruit injury, depending on factors such as concentration of the gas, temperature and duration of the exposure, and fruit variety (Wilkinson and Fidler 1973, Bramlage et al. 1977, Watkins et al. 1997), fruits exposed to the 19.0% CO_2 treatment were evaluated for flesh firmness and visible internal breakdown. A batch of 30 recently harvested McIntosh fruits was exposed for 14 d at 10°C and 80% RH to an atmosphere containing 19.0% CO_2, 2.2% O_2, and 78.8% N_2. Another 20 fruits were held in ambient atmosphere for 14 d at 22°C and 50% RH for purposes of comparison. Flesh firmness was measured at three evenly spaced positions along the equatorial plane of each fruit using an EPT-1 pressure tester (Lake City Technical Products, Lake City, BC, Canada) fitted with an 11.1-mm-diameter head. Fruits were also assessed visually by cutting each one at least three times to inspect for any visible internal disorders.

Percent survival in all trials was determined by comparing the number of larvae collected with the total number of oviposition punctures counted in all fruits of each treatment replicate, assuming one egg per puncture. Mean percent survival values calculated for each replicate were subjected to arcsine square-root transformation, and a weighted analysis of variance (ANOVA) was used to determine treatment effects. Weights were based on the number of eggs or larvae in each replicate. Linear contrasts were used to assess the influence of different CO_2 concentrations (Stata Press 1999). Robust regression analysis was performed on arcsine-square root transformed survival means from the egg age study (Stata Press 1999). Fruit pressure readings were subjected to ANOVA followed by a least significant difference (LSD) test to determine treatment differences (Abacus Concepts 1991).

Results and Discussion

The ANOVA showed significant main effects of CO_2 treatment (F = 49.7; df = 3, 56; $P \approx 0.00$), life stage exposed (F = 13.0; df = 1, 56; $P < 0.001$), and duration of exposure (F = 15.9; df = 1, 56; $P < 0.001$) with no significant interaction terms. Larvae were more sus-

Table 1. Mean ± SEM survival of apple maggot eggs and larvae in 'McIntosh' apples held at 10°C under high-CO_2 atmospheres for two exposure periods

Treatment	Period of exposure					
	7 d			14 d		
	n		% survival[a]	*n*		% survival[a]
	Punctures	Emerged larvae		Punctures	Emerged larvae	
			Eggs			
Control	439	346	78.8 ± 24.5	602	449	74.6 ± 21.9
10.6% CO_2	853	339	39.7 ± 16.7	866	35	4.0 ± 4.3
14.9% CO_2	647	153	23.6 ± 5.4	735	22	3.0 ± 1.6
19.0% CO_2	666	255	38.3 ± 21.9	930	125	13.4 ± 19.0
			Larvae			
Control	504	370	73.4 ± 22.3	831	476	57.3 ± 20.2
10.6% CO_2	773	63	8.2 ± 10.9	1543	1	0.0 ± 0.0
14.9% CO_2	906	24	2.7 ± 3.4	966	0	0.0 ± 0.0
19.0% CO_2	748	145	19.4 ± 10.7	1159	0	0.0 ± 0.0

[a] Weighted average based on number of observations in each treatment-versus-control comparison.

ceptible than eggs, and a 14-d exposure was more effective than a 7-d exposure. The linear contrasts showed there was no effect of the different CO_2 levels. The survival rates of apple maggot eggs subjected to the various treatments indicate that this life stage is difficult to control completely with CO_2 treatment (Table 1). Exposure periods of 14 d under the different CO_2 regimens resulted in variable survival levels that were not directly related to CO_2 concentration, but even the numerically lowest survival noted (14.9% CO_2 concentration) was not close enough to 0% to be considered commercially acceptable. The mean survival of 3.0% in this treatment represents 22 larvae deriving from 735 punctures. In contrast, however, newly hatched maggot larvae (in the fruit held for 7 d before being exposed to the gas treatments) were more easily killed by the treatments. No survivors were obtained from apples held under the 19.0 or 14.9% CO_2 conditions for 14 d, and the actual (non-weighted) value of 0.06% mean survival in the 10.6% CO_2 treatment represents a single maggot out of 1,543 punctures that was able to survive and exit the fruit. Evidently, once the insect has passed beyond the egg stage, it is considerably more susceptible to the effects of the gases in the fruit's environment.

This pest obviously exhibited high variability in its response to the treatments tested in this study. Possible sources of the observed variation could have been either biological or methodological. Work involving insects from laboratory colonies always carries an implied risk of unspecified inbreeding effects, particularly when there has been no periodic introduction of wild genes. Hallman (1994) showed that rearing conditions affected subsequent fruit fly response to disinfestation procedures, but in that case, while high rearing temperatures increased the flies' tolerance to high-temperature treatments, their ability to survive cold storage was not affected. It is not known whether any selection for temperature tolerance was operating in our study, but our rearing and treatment temperatures were selected to be as close as possible to the conditions the insects would experience in normal development and fruit handling situations, so this potentially confounding effect should have been unlikely. Nonetheless, a great degree of genetic variability can be present even in colonies isolated for > 80 generations, so it is unknown why, for instance, the 14.9% CO_2 mixture resulted in lower response variability than that seen in the other treatments. Possibly, a larger number of treatment replicates might have reduced the magnitude of this irregularity.

Incomplete uniformity in our treatment conditions, particularly regarding the oxygen concentrations used, also could have influenced the results attained in this study. Investigators working with arthropod pests in several taxonomic groups (Harein and Press 1968, Marzke et al. 1970, Lidster et al. 1981, Soderstrom et al. 1986, Whiting et al. 1991, Whiting and van den Heuvel 1995, Shellie et al. 1997, Yahia and Ortega-Zaleta 2000) have noted either significantly increased mortality effects of high-CO_2 atmospheres when the O_2 concentration is lowered from 2–5% to 1%, or a more significant effect of the low-O_2 than the high-CO_2 atmosphere component. Inasmuch as the variation in our O_2 concentrations was greater than an optimal ±10%, the otherwise relatively small O_2 differences in the treatment atmoshperes could have had a relatively large impact on insect response. However, although technically the range of O_2 concentrations in the mixtures used overall was 1.4–4.8%, in all but two replicates (which had 4.8% O_2 against the egg stage for 7 d exposure at 19.0% CO_2, one of the least effective treatments), the range was 1.4–2.7%, which is much closer to the nominal target concentration of 2%. A greater degree of control over this aspect of the treatment conditions would have improved our ability to interpret the potential effects of the CO_2 component.

Results of the trials assessing CO_2 effects on eggs of different ages show a nonlinear increase in mortality as the eggs approach hatch (Fig. 1). The regression analysis revealed a significant influence of the CO_2 treatment and egg age on survival (overall $F = 24.13$; df = 3, 20; $P = 0.00$). The main effect of CO_2 was marginally significant ($P < 0.058$), age significantly

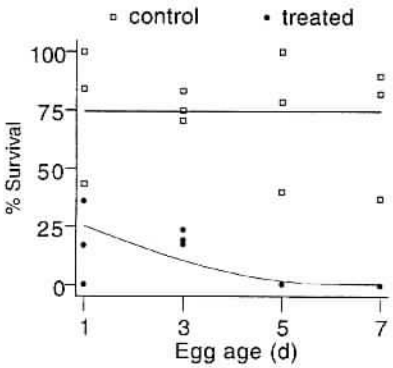

Fig. 1. Relationship between apple maggot egg age and survival of 14-d exposure to 19.0% CO_2 atmosphere at 10°C. [arcsine $\sqrt{s} = b_0 - b_1\ (trt) - b_2\ (trt * d)$, where s = proportion surviving, b_0 = (1.04, SEM = 0.147), b_1 = (0.417, SEM = 0.207), and b_2 = (0.098, SEM = 0.045). *trt* = treatment: 0 or 1; 0, control; 1, treated; d = egg age (days); SEM = standard error of mean. Robust regression does not provide a residual mean-square error.]

interacted with CO_2 treatment ($P = 0.042$), and there was no main age effect. Comparable levels of apple maggot survival (≈20%) were seen in fruits held for one or 3 d before the 14-d exposure to 19.0% CO_2, but in those held first for 5 d, survival dropped nearly to zero. This indicates that, at some point between three and 5 d after oviposition, the apple maggots reach a developmental stage that is optimally susceptible to the treatment effects, whether as a neonate larva or an egg that is very close to hatching.

Pressure readings in fruits exposed to the 19.0% CO_2 atmosphere for 14 d were significantly higher [mean ± SEM: 1.46 ± 0.15 kg/cm^2 (20.70 ± 2.08 psi)] than in those held under ambient conditions [1.23 ± 0.10 kg/cm^2 (17.56 ± 1.40 psi)] for the same period ($P < 0.0001$; df = 1, 29; $F = 111.87$; Fisher protected LSD). Inspection of the tissue revealed no apparent browning, internal breakdown or other defects in the treated fruits.

The use of CO_2 pretreatments to delay softening of apples during storage is common for certain varieties, such as 'Golden Delicious', which is often held first for 10 d under 15–17% CO_2 and 4–5% O_2 before being placed in controlled atmosphere storage (Couey and Olsen 1975). However, similar treatment of McIntosh has the potential to cause tissue damage. Bramlage et al. (1977) found that 12% CO_2 treatment for 14 d at the beginning of controlled atmosphere storage resulted in firmer fruit after 4–6 mo, but this was often accompanied by external or internal damage. In the same study, 10% CO_2 for 1–3 wk before controlled atmosphere storage did not produce fruit injury, but 20% resulted in external damage to over 60% of the fruit. Gaunce et al. (1982) reported improved firmness retention and no change in soluble solids in several varieties of apples exposed to 100% CO_2 at 22°C for 3 d; however, significant internal flesh browning occurred in treated 'Spartan' and McIntosh fruits, although not Golden Delicious. In contrast, Bond and Herne (1983) found no adverse effects on apples held in 75% CO_2 at 12°C for up to 14 d. In long-term studies using controlled atmospheres having elevated levels of CO_2, Watkins et al. (1997) detected greater external injury of 'Empire' apples exposed to 5% than 2% CO_2 for up to 20 wk, but found no differences in flesh firmness or titratable acidity.

The treatments evaulated in the current study were intended for fruit destined to be consumed relatively soon afterward, so long-term damage effects would not be anticipated under the most likely marketing situations. Although Benschoter (1987) obtained high mortality of *A. suspensa* life stages using CO_2 concentrations in the range of 20–80%, work on apples associates concentrations above 20% with the most severe incidence of "brownheart" or internal CO_2 injury, presumably owing to unacceptable buildup of alcohol and acetaldehyde (Wilkinson and Fidler 1973, Gaunce et al. 1982). It is apparent that several factors, including apple variety, maturity (harvest date), growing season and possibly production region, could potentially interact with the treatment duration, temperature, and CO_2 concentration, to produce undesirable effects in the fruit.

The operational significance of our findings can be explored within the context of apple maggot management programs as currently practiced in commercial New York plantings. Whether growers are monitoring apple maggot flight on their farms using visual traps (Agnello et al. 1990, 1999) or following a calendar-based preventive pesticide spray program, most tend to make their final apple maggot sprays no later than the middle of August, owing to the period of residual efficacy and the preharvest intervals of the organophosphate materials most commonly used for this purpose. When this is the case, the earliest apple varieties could be harvested as early as the beginning of September, although the bulk of the commonly exported New York apples, such as McIntosh and Empire, do not begin to be harvested for another 7–14 d, depending on the season.

Although apple maggot flies can be captured on traps long after the estimated period of residual effectiveness of the last spray (i.e., late into September), adult females active late in the season in apple orchards apparently do not oviposit in fruit, even though most of them have mature eggs in their ovaries (W. H. Reissig, N.Y. State Agriculture Experiment Station, Geneva, personal communication). Nonetheless, growers wishing to safeguard their fruit against the possibility, however small, of a late preharvest infestation, are naturally concerned about minimizing the period between the end of residual chemical protection and fruit harvest. If the apples are going directly into controlled atmosphere storage, any apple maggot eggs that may be present inside the fruit would be killed by the extended low-temperature, low-O_2 conditions of that storage regimen. However, if they are to be shipped to another destination for fresh-market distribution, the lag time involved in the shipping and handling process could increase the chances of larval presence in the fruit. The results of these studies

suggest the option of implementing a short-term, high-CO_2 storage or shipping environment for disinfesting any such fruits of apple maggots that may be present as eggs or neonate larvae. In view of our finding that ≈5-d-old insects (late stage eggs) can be completely killed by a 14-d treatment with 19.0% CO_2 at 10°C, it should be possible to optimize the amount of holding time required to achieve control without first allowing the insects to actually hatch into maggots within the fruit.

Further refinements of this principle are conceivable, such as working backwards from the 14-d exposure period in which all the apple maggots in the fruits were killed, to determine the shortest duration required to maintain 100% mortality of all the insects in the fruits. Additional efforts should be made to investigate various combinations of preexposure holding intervals, CO_2 and O_2 atmospheres and exposure periods, to determine a suitable protocol for commercial use. Because of the unacceptability of exported apples having even an extremely low level apple maggot infestation, a more effective storage regimen could be found that not only ensures that no insects will survive and emerge in export destinations, but also maintains the fruit quality so that it can be consumed fresh. It is possible that a 14-d or shorter exposure period at a lower O_2 concentration would result in a practical preshipping treatment for disinfestation of New York apples. Such a regimen at 38°F did not reduce McIntosh apple quality during long-term storage (Lidster et al. 1981), but other varieties may differ in this regard. Finally, any treatment elected should be tested on newly harvested apples to assess potential effects on fruit quality and short-term market storage and handling.

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