HORTSCIENCE 27(10):1094-1096. 1992.

Controlled-atmosphere Effects on Blueberry Maggot and Lowbush Blueberry Fruit

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Additional index words. Vaccinium angustifolium, Rhagoletis mendax, carbon dioxide, oxygen, temperature

Abstract. **Larvae of the blueberry maggot *(Rhagoletis mendax* Curran) were raised on apples *(Malus domestics* Borkh. cv. Idared), and exposed larvae were treated 48 hours with CO_2 concentrations ranging from 0% to 100% at O_2 concentrations of 2%, 5%, or 20% (0% for the 100% CO_2) at 5 or 21C. Blueberry maggot survival was reduced to 10% when the larvae were subjected to CO_2 concentrations > 45% at 21C. Fumigation was more effective at 21C than at 5C. Oxygen at 2% or 5% did not reduce larval survival when compared with treatments containing 20% O_2. In a separate experiment, six commercial shipments, each consisting of four hundred eighty 0.5-liter containers of infested lowbush blueberries *(Vaccinium angustlfolium* Ait.), were placed in a large fiberglass tank and fumigated with 54% CO_2 at 21C. The blueberries were sampled for quality and larval survival after 24 and 48 hours of CO_2 treatment. Atter 48 hours, 9% of the blueberry maggots in infested blueberries survived fumigation with 54% CO_2, while 68% of maggots survived in air. The quality of fumigated lowbush blueberries was not adversely affected by fumigation with 54% CO_2 for up to 48 hours, as indicated by marketable berries, berry weight, split berries, shriveled berries, epicuticular wax (bloom), firmness, soluble solids and titratable acid concentrations, off-flavors, and skin browning.**

The blueberry maggot infests highbush *(Vaccinium corymbosum* L.) and lowbush blueberries in the northeastern United States and the Maritime Provinces of Canada (Guibord et al., 1985; Neilson and Wood, 1985; Vincent and Lareau, 1989). The possible presence of live larvae in the fruit can restrict the movement of fresh fruit across provincial, state, and national boundaries. The larvae also cause fruit-quality deterioration because they consume the internal tissue of infested berries. In Canada and the United States, there is no registered quarantine treatment for this insect.

The purpose of this study was to determine whether CO_2 fumigation could be an effective disinfestation treatment. Carbon dioxide has already been registered in Canada and the United States as a fumigant to kill insects in stored grain (Liquid Carbonic, 1985), and it is also known to have a negative effect on other insects present on stored fresh fruit (Benschoter, 1987; Bond and Herne, 1983; Lidster et al., 1981; Morgan and Gaunce, 1975; Soderstrom et al., 1990). Research at the Agriculture Canada Food Production and Inspection Branch Fumigation Station in Montreal suggested that a CO_2 concentration of at least 44% at 21 to 22C for 24 h was sufficient to kill virtually all of the larvae of *R. mendax* in infested lowbush blueberries (D. Hedley, personal communication). Treatment effects on fruit quality were not assayed in that study. Carbon dioxide is known to extend the shelf life of various berry fruits, including blueberries, at concentrations up to 20% (Kader, 1989). Concentrations > 25% cause skin browning and off-flavors in the fruit of highbush and rabbiteye *(V. ashei* Reade) blueberries (Kader, 1989). However, we found no reports on the effect of short exposures of > 25% CO_2 on quality of lowbush blueberries.

Since the effect of CO_2 on insect survival may be influenced by O_2 concentration and temperature, a preliminary experiment examined all of these factors.

Blueberry maggot larvae were raised in the laboratory using 'Idared' apples. The apples were placed for 1 day in rearing cages containing mating adult flies. The cages were located in a controlled-environment room kept at 22.5C, 75% relative humidity, and 16-h daylength, with 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ radiation from cool-white fluorescent lights. After the apples were stung, they were kept in the same conditions for 21 to 40 days to allow the eggs to develop into larvae. The larvae, which were mainly second and third instar, were removed by hand from the apples before being used in experiments. The exposed larvae (maggots) were fumigated at 5 or 21C using a confounded factorial design of all combinations of 5% or 20% O_2 and 0%, 5%, 10%, 20%, 40%, or 60% CO_2 plus a 100% CO_2 treatment. Each treatment was applied to eight replicates of 10 larvae each for 48 h. The gas mixtures were humidified to saturation by bubbling through water and were passed through 0.5-liter containers at a flow of 1.5 $liters \cdot min^{-1}$. Flushing was stopped when the desired concentration was achieved. The O_2 and CO_2 concentrations in the containers were analyzed daily with a Varian 3400 gas chromatography equipped with a CRT 1 column (Alltech Assoc., Mandel Scientific, Guelph, Ont.) and a thermal conductivity detector. Larval survival was evaluated after 48 h of fumigation. Any larva that responded to a needle probe was considered alive (W. Neilson, personal communication).

To determine survival probabilities, the counts of living and dead larvae were analyzed as binary data. Using Genstat 5 (Payne,

Table 1. Effect of CO, concentration on larval survival after 48 h of exposure at 21C. Each larval survival value is the mean of the 2% and 5% O_2 treatments.

	1989		1991	
CO_2 (%)	Survivors/total	% (± SE)	Survivors/total	% (± SE)
0 (air)	57/60	95 (2.8)	47/50	94 (3.3)
25	---	---	31/50	62 (6.5)
45	14/60	23 (5.0)	8/50	16 (5.1)
65	7/60	12 (4.0)	6/50	12 (4.5)
80	10/59	17 (4.5)	5/50	10 (4.2)
90	13/60	22 (4.9)	---	---
95	12/59	20 (4.8)	---	---
100	21/58	36 (5.8)	17/50	34 (6.4)

Accumulated analysis of deviance

	1989		1991	
Source of change	df	Mean deviance	df	Mean deviance
Rep	5	6.18^{NS}	4	1.41^{NS}
Treatment[z]	2	149.5***	2	44.5***
CO_2[y]	4	4.05^{NS}	3	15.7***
O_2	1	0.97^{NS}	1	5.38*
$CO_2 \times O_2$	4	1.38^{NS}	3	0.69
Residual	67	1.60	46	1.27

[z]Differences among 0% CO_2, 100% CO_2, and mean of intermediate CO_2 concentrations.
[y]Differences among intermediate CO_2 concentrations.
NS,*,***Nonsignificant or significant at $P = 0.05$ or 0.001, respectively.

Received for publication 12 Nov. 1991. Accepted for publication 13 Apr. 1992. Contribution no. 2095, Kentville Research Station. We thank P. Harrison, A. Knowlton, and H. Lightfoot (technicians), K. McRae (statistician), C. Forney (postharvest physiologist), and M. Hardman (entomologist). R. Weatherhead graciously provided blueberries and a fumigation chamber. Partial support was provided by Liquid Carbonic. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regu. lations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 2. Effect of 54% CO_2 and exposure time at 21C on percent blueberry maggot survival in commercially packed blueberries.

Treatment	Exposure time (h) 24 Survivors/total	24 % (± SE)	48 Survivors/total	48 % (± SE)
Control (air)	51/54	94.4 (3.12)	48/70	68.8 (5.55)
CO_2 fumigation	31/66	47.0 (6.14)	8/89	9.0 (3.02)
Accumulated analysis of deviance				
Source of change	df			Mean deviance
Rep	5			4.148^{NS}
Treatment	1			75.54***
Time	1			44.14***
Trt × time	1			0.09^{NS}
Residual	11			2.33

^{NS,***}Nonsignificant or significant at $P = 0.001$, respectively.

Table 3. Effect of 54% CO_2 fumigation and exposure time at 21C on quality of commercial blueberries

Treatment	Duration (h)	Marketable berries (%)	Berry firmness[z]
Control (air)	24	77.6	3.2
	48	68.4	2.9
CO_2 fumigation	24	80.7	3.1
	48	75.1	3.3
SEM (n = 4, df = 9)		3.60	0.12
Significant effects		Time[y]	Trt × time[x]

[z]0 = Softest; 5 = firmest.
[y]Significant at $P = 0.07$.
[x]Significant at $P = 0.05$.

1987), an analysis of deviance was conducted using the generalized linear regression procedure (Dobson, 1990) to determine the factorial effects of O_2 and CO_2 levels and temperature on larval survival.

Survival of exposed larvae in the preliminary experiment was not affected significantly by reducing the O_2 concentration from 20% to 5% (data not shown). Larval survival decreased with increasing CO_2, and it was lower at 21C than at 5C, confirming previous observations on the effect of temperature during fumigation (Soderstrom et al., 1986).

Based on these results, two experiments were conducted (1989 and 1991) at 21C for 48 h. Atmospheres were composed of 2% or 5% O_2 with 45%, 65%, 80%, 90%, or 95% and 25%, 45%, 65%, or 80% CO_2 in the first and second experiments, respectively. In addition, a 100% CO_2 and an ambient air treatment (0% CO_2) were included in both experiments. There were five larvae in each experimental unit, with six and five replications in the first and second experiments, respectively.

There was no significant interaction between CO_2 and O_2 in either year (Table 1). Hence, the results can be explained in terms of CO_2 and O_2 individually. There was no significant difference in survival between 2% and 5% O_2, in 1989, and in 1991 there was a higher survival (31%) at 2% than at 5% (19%). Larval survival decreased with increasing CO_2 concentration from 94% to 95% alive at 0% CO_2, to a low of 12% alive (1989) and 10% alive (1991) at 65% and 80% CO_2, respectively (Table 1). All CO_2 concentrations between 45% and 95% were equally effective in reducing the number of surviving larvae in both 1989 and 1991, when compared with higher or lower CO_2 concentrations (Table 1). The 100% CO_2 treatment was less effective than treatments with 45% to 95% CO_2.

Six commercial-scale shipments, each consisting of four hundred eighty 0.5-liter containers of naturally infested lowbush blueberries were fumigated separately in 1989 at 21C in a large fiberglass tank, 1.2 × 2.4 × 1.2 m (width × length × height). Nonfumigated (air control) containers were kept adjacent to the fumigation chamber. The sealed tank was flushed with 100% CO_2 until a stable concentration of 54% was achieved (measured as above). The addition of CO_2 lowered the O_2 concentration to 7.9%. Using a resealable opening in the container top, up to 12 containers of blueberries from each shipment were sampled randomly for larval survival after 24 and 48 h. The chamber atmosphere was monitored after resealing, and CO_2 was added, if necessary. The control and fumigated blueberry samples were allowed to sit for an additional 24 h at 21C, and then mashed in a 17.5% (w/v) sugar solution with a sieve that did not crush the larvae (W. Neilson, personal communication). All larvae that floated to the surface were collected for survival evaluation. The effects of storage duration and 54% CO_2 fumigation on larval survival were analyzed using an analysis of deviance as described above.

Half-liter samples of fumigated and nonfumigated blueberries from five of the shipments were evaluated immediately after 24 and 48 h of CO_2 fumigation for marketable berries (%), weight of 100 berries, split berries (%), and shriveled berries (%). Epicutitular wax on the berries (bloom) was rated on a scale where 0 = no bloom and 5 = maximum bloom. The firmness of 25 berries per sample was determined by hand-rolling, using a scale where 0 = berry rupture on touch, 2.5 = berry surface depressed on touch, 5 = berry firm, not yielding to touch (Sanford et al., 1990). Juice was pressed from the berries; its soluble solids concentration (SSC) was measured using a hand-held refractometer (Atago, Japan) and titratable acid concentration (TA; milligrams malic acid per 100 ml blueberry juice) by titrating a 2-ml sample against 0.1 N NaOH to a phenolphthalein endpoint using a semiautomatic Multi-Dosimat E-415 Titrator (Metrohm AG, Herisau, Switzerland). We subjectively examined berries for off-flavors and skin browning. The blueberry quality results were tested by analysis of variance, computed with Genstat 5 (Payne, 1987).

Larval survival in the commercial shipments of lowbush blueberries fumigated with 54% CO_2 was influenced by fumigation duration (Table 2), resulting in about five times the larval survival after 24 h as after 48 h. The survival rate after 48 h was lower than the survival rate observed with the exposed larvae (Table 1).

Fumigated blueberries in the commercial fumigation experiment were slightly firmer than air control berries after 48 h (Table 3). Similar firmness benefits have been observed with 3 days of fumigation at 97% CO_2 at 21C for various apple cultivars when fumigated immediately after harvest (Gaunce et al., 1982). There were more marketable berries after CO_2 fumigation than in the air control (Table 3), which may be due to improved firmness and possible fungal growth reduction. With 54% CO_2, the O_2 concentration dropped to 7.9%, a concentration known to reduce respiration and extend storage life in highbush and rabbiteye blueberries (Kader, 1989). Carbon dioxide fumigation did not affect other quality attributes, e.g., split berries (%), shriveled berries (%), berry weight (grams), SSC, TA, or epicuticular wax (bloom rating). There were no obvious off-flavors or skin browning.

The fumigation of exposed larvae and larval-infested blueberries failed to confirm the results of an earlier experiment (D. Hedley, personal communication) that suggested a concentration of ≥ 44% CO_2 for 24 h was sufficient to kill virtually all larvae. Our study has shown that CO_2 concentrations between 45% and 95% reduced larval survival in blueberry down to 9% if applied at 21C for at least 48 h. However, complete kill was not achieved. This study also showed that lowbush blueberries were not adversely affected by 54% CO_2 for up to 48 h.

Reducing O_2 to as low as 2% did not reduce larval survival, confirming previous observations that O_2 is not as important as CO_2 in killing insects (Bond and Herne, 1983; Delate et al., 1990; Soderstrom et al., 1990). Reducing O_2 below 2% may enhance CO_2 lethality but may cause off-flavors in the fruit

if kept for many days (Kader, 1989). Since the 100% CO_2 treatment, which reduced O_2 concentration to <1%, was less effective in this study than other concentrations >45%, an O_2 concentration <2% is not likely to be more lethal to the larvae. This study lends support to the frequently reported conclusion that an atmosphere with between 40% and 60% CO_2 in air is the most effective combination for killing many insect species (Bell, 1984; Delate et al., 1990; Jay, 1984; Soderstrom et al., 1990). If quarantine regulations, such as the U.S. Dept. of Agriculture probit 9 requirement (Baker, 1939), require a lower survival level, the CO_2 fumigation time would have to be increased, but the relatively high temperature would probably produce unacceptable losses in quality of the blueberries. In our tests, CO_2 did not provide sufficient lethality of blueberry maggot for it to be used as a quarantine treatment.

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