EFFECT OF HIGH CO₂ AND TEMPERATURE ON BOTRYTIS STORAGE ROT AND QUALITY OF KIWIFRUIT

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SUMMARY

High CO₂ (60% CO₂, 20% O₂) at 30° or 40°C for 1, 3 or 5 days was tested *in vitro* and *in vivo* for the control of botrytis storage rot in kiwifruit (*Actinidia deliciosa* cv 'Hayward'). Spore germination and growth of *Botrytis cinerea* Pers. were completely inhibited in culture by high CO₂ at 40°C and partially suppressed at 30°C. Kiwifruit were inoculated with *B. cinerea* spores, exposed to high CO₂ at 30°C or 40°C, and stored in air at 0°C for up to 12 weeks after treatment. High CO₂ at 40°C reduced disease incidence from 85% (air at 20°C) to about 50%, but exposure to CO₂ at 40°C for longer than 1 day adversely affected fruit ripening.

Keywords: *Actinidia deliciosa*, *Botrytis cinerea*, botrytis storage rot, carbon dioxide, disease control, firmness, kiwifruit, respiration, temperature.

INTRODUCTION

Recently, as a result of imposed restrictions and bans on the use of chemical disinfestation treatments such as ethylene dibromide, interest in the development of pesticide-free treatments such as controlled atmospheres, heat and other non-chemical treatments has been revived (Armstrong 1992; Carpenter *et al.* 1993).

At the Levin Research Centre, a major programme is in progress to investigate the use of high CO₂ and temperature for pest and disease disinfestation of horticultural crops. We have found that high CO₂ and low O₂ can give effective control of disease (Brash *et al.* 1992, 1993) and insects (Carpenter 1993). O'Connor (1993) also reported that leafrollers are susceptible to high CO₂ (60%) and temperature (40°C).

This paper reports the results of tests using high CO₂ and temperature to control botrytis storage rot in kiwifruit. Our approach of using high CO₂ (up to 60%) and high temperature (up to 40°C) for short periods was expected to enhance disease control. Our aim was to determine whether treatments that will kill insects will also provide disease control without being detrimental to fruit quality.

MATERIALS AND METHODS

Botrytis cinerea cultures were established on malt extract agar (MEA) from a single spore isolated from a diseased fruit. Spore suspensions were made by washing spores from 10- to 14-day-old cultures with water. Suspensions were used for *in vitro* studies and to inoculate fruit in the disease studies. Fruit used for physiology and ripening studies were not inoculated with the pathogen.

Kiwifruit (*Actinidia deliciosa* cv. Hayward) used in experiments were harvested (Brix = 6.2) with pedicels intact, from vines growing at the Levin Research Centre.

In vitro tests

For the spore germination study, four 20 µl drops of *B. cinerea* spore suspensions (10⁵ spores/ml) were placed at four different sites on a MEA plate. When the droplets had dried (30 min), four plates per treatment were placed in containers (described below). For the mycelial growth study, plugs (6 mm diam.) were cut from a 3- to 4-day-old culture of *B. cinerea* with a cork borer. They were transferred onto MEA plates (1 plug to a plate) with the mycelium surface downwards, in the centre of each plate.

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Four plates per treatment were placed together with spore germination plates into 100 litre capacity metal containers. The lids were sealed, and humidified gas (60% CO₂, 20% O₂ balance N₂) was then fed into each container which was first flushed with the gas mixture to establish the correct atmosphere. The flow rate was then maintained at 200 ml/min. The containers were located in 30° or 40°C rooms. Air at 20°C was used as the control treatment to compare the combined effect of high CO₂ and temperature on disease control. Three timings (1, 3 and 5 days) for each treatment were used to give a total of nine treatments with two replications of each. After treatment, plates were incubated in air at 20°C for 24 h before they were examined under a microscope to determine percentage spore germination. Diameter of *B. cinerea* colonies was also measured at this time.

***In vivo* test**

In the laboratory, the pedicels of kiwifruit were removed and each picking wound was immediately inoculated with approximately 10,000 spores in a 30 µl water droplet. After the spore droplets had dried (1.5 h), 72 fruit (in two plastic trays) for each treatment were placed in a 100 litre capacity metal container. The same nine treatments as the *in vitro* tests, each in a separate container, were tested. Container lids were sealed and humidified gas mixes were fed into each container at a flow rate of 200 ml/min. The experiment was repeated three times for each treatment.

After treatment, fruit were packed into commercial trays with polyliners and stored at 0°C and 95% RH for up to 12 weeks. Individual fruit were examined for botrytis storage rot at weekly intervals beginning 6 weeks after treatment. Data were analysed with analysis of variance using the GLM procedure of the SAS package.

Fruit quality assessments

During 9 weeks cool storage, soluble solids and firmness were measured every 3 weeks on fruit exposed to the same treatments described above. There were 10 fruit/treatment. Slices were cut from each end of the fruit and soluble solids concentration (°B) in the juice was measured using an Atago refractometer (Atago Co. Ltd, Tokyo, Japan). Firmness was measured on two peeled sides of each fruit, with a mechanically driven penetrometer (Chatillon Co. Ltd., New York), with a 7.9 mm diameter plunger. After 9 weeks, fruit were placed in a room at 20°C. Respiration rate was measured on 10 individual fruit after 1, 3, 5 and 7 days at 20°C, as described by Cheah *et al.* (1992b).

RESULTS

***In vitro* tests**

Germination of *B. cinerea* spores was almost completely inhibited in 60% CO₂ at 30°C and was completely inhibited at 40°C after 1, 3 or 5 days treatment (Table 1). Microscopic examination revealed that spores had shrunk and ruptured. In the air treatment at 20°C, almost all spores germinated. Mycelial growth of *B. cinerea* was completely inhibited in high CO₂ at 30°C or 40°C, whilst in air at 20°C the fungus grew normally (Table 1).

***In vivo* tests**

Holding fruit in 60% CO₂ at 40°C for 1 day increased ($P < 0.05$) the incidence of rot over that of the air control at 20°C (Fig. 1A). After 3 days, the incidence of rot in the high CO₂ treatment was similar to that in the air control. After 5 days, the cumulative disease level had decreased ($P < 0.05$) from 85% in the air control to about 50%. There was an increase in disease incidence in high CO₂ at 30°C after 3 days and after 5 days of exposure to this temperature, the rot incidence was similar to that of the air control treatment.

Fruit quality assessment

Fruit ripening was adversely affected by 60% CO₂ at 40°C (Fig. 1B,C,D). Although soluble solids reached acceptable levels after 1 day of treatment and 9 weeks cool storage, 3 and 5 days CO₂ treatment prevented adequate solids accumulating (Fig. 1B). Fruit treated for 5 days did not soften normally (Fig. 1C), and the flesh underlying the skin became leathery. Respiration rates during 7 days at 20°C were markedly higher following the 5 day treatment (Fig. 1D).

TABLE 1: Spore germination and colony diameters for *Botrytis cinerea* exposed to different controlled atmosphere treatments for different periods.

Temperature and controlled atmosphere	Period of exposure (Days)	Mean % germination ¹		Mean colony diam. (mm) ¹	
20°C Air	1	98	a ²	50	a
	3	98	a	62	b
	5	100	a	80	c
30°C 60% CO ₂ 20% O ₂	1	2	b	0	d
	3	1	b	0	d
	5	1	b	0	d
40°C 60% CO ₂ 20% O ₂	1	0	b	0	d
	3	0	b	0	d
	5	0	b	0	d

¹Germination or growth assessed after the treatment and 24 h in air at 20°C.

²Any two means in a column followed by the same letter are not different (P<0.05).

Figure 1: A. Cumulative incidence of botrytis rot after 12 weeks storage at 0°C on kiwifruit previously exposed to different controlled atmosphere conditions. B. Soluble solids in kiwifruit exposed to 60% CO₂ for different periods. C. Firmness of kiwifruit during storage at 0°C after exposure to 60% CO₂ for different periods. D. Respiration rates for kiwifruit during 7 days at 20°C following treatment with 60% CO₂ for different periods, and 9 weeks cool storage. LSD (P<0.05) indicated in A. Bars in B, C and D indicate standard errors of means.

DISCUSSION

High CO₂ at 30° or 40°C markedly suppressed *B. cinerea* spore germination and mycelial growth. The early studies of Brown (1922) and Brooks *et al.* (1932) have already demonstrated the inhibitory effect of high CO₂ atmospheres on spore germination and mycelial growth of *B. cinerea* and other fungi. Wells and Uota (1970) found that high CO₂ at 20% gave 50% inhibition of *B. cinerea* mycelium growth and 45% CO₂ gave total inhibition at 20°C. Imolehin and Grogan (1980) showed that germination and growth of *Sclerotinia* spp. were also completely suppressed in 20% CO₂. Sommer (1985) found that the suppressive effect of CO₂ on pathogens was by the reduction in the rate of various metabolic functions, resulting in lowered respiration.

When high CO₂ with high temperature was tested on kiwifruit, we found that 40°C reduced disease incidence. The higher disease incidence in fruit treated at 30°C suggests control of disease is mainly attributable to the temperature effect rather than to the high CO₂. Our previous work which showed that dipping kiwifruit in water at 46° to 48°C effectively controlled botrytis storage rot (Cheah *et al.* 1992a, 1992b, 1993) further supports the assumption that high temperature was a major mechanism of control. Our results also indicate that prolonged exposure to high CO₂ can disrupt fruit metabolism and lead to increased disease incidence, as reported by Tonini *et al.* (1989) and Brigati *et al.* (1989). Sixty percent CO₂ at 0°C prevents normal fruit ripening (Irving 1992). From the present results, we cannot rule out the possibility that prolonged exposure to high temperature may also adversely affect fruit ripening.

A short exposure to high CO₂ at 40°C may have potential for the control of botrytis storage rot, as natural infection in commercial cool-stored fruit has never reached the 85% recorded here (Fig. 1A). The percentage control which we obtained may be commercially acceptable if the natural incidence of the disease is low. However, further tests should be carried out to study effects of high CO₂ at 40°C for short periods (less than 1 day) on disease control and fruit physiology.

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