Suppression of *Sclerotinia sclerotiorum* and Watery Soft Rot of Celery by Controlled Atmosphere Storage

ANDRES A. REYES, Research Scientist, Agriculture Canada Research Station, Vineland Station, Ontario, Canada L0R 2E0

ABSTRACT

Reyes, A. A. 1988. Suppression of *Sclerotinia sclerotiorum* and watery soft rot of celery by controlled atmosphere storage. Plant Disease 72: 790-792.

At 1 C, the growth in vitro of *Sclerotinia sclerotiorum* on celery extract agar was most suppressed in a storage atmosphere containing 7.5% CO + 1.5% O_2, but only slightly suppressed in 4% CO_2 + 1.5% O_2 or in 1.5% O_2 alone, compared with normal air. Watery soft rot caused by this fungus was severe on celery (*Apium graveolens* var. *dulce*) stored in normal air for 2 wk at 8 C. A comparable severity took 10 wk to develop at 1 C. At 8 C the suppression of this disease was greatest in atmospheres of 7.5–30% CO + 1.5% O_2, but only slightly reduced in 4–16% CO_2 + 1.5% O_2 or in 1.5–6% O_2 alone.

It was reported previously (5,6) that *Sclerotinia sclerotiorum* (Lib.) de Bary and *Botrytis cinerea* Pers. ex Fr. were the pathogenic fungi most frequently associated with decayed celery (*Apium graveolens* L. var. *dulce* (Miller) Pers.) in storage. Capellini et al (1) have observed that these pathogens caused serious damage to shipments of celery to the New York market from 1972 to 1985. Reyes and Smith (5) showed that diseases caused by these fungi were suppressed by controlled atmosphere storage at 0–1 C, but only one level of each controlled atmosphere (7.5% CO/1.5% O_2, 1.5% O_2, 4% CO_2/1.5% O_2) was tested. Also, the suppressive effect on fungal growth was reported only for *B. cinerea*. I now report the effect of various levels of controlled atmsopheres on the growth in vitro of *S. sclerotiorum*, the comparative development of watery soft rot caused by this fungus on celery in normal air at 1 and 8 C, and the effect of various levels of each controlled atmosphere at 8 C on disease development.

Accepted for publication 26 April 1988.

© 1988 Department of Agriculture, Government of Canada

MATERIALS AND METHODS

Ascospore inoculum of *S. sclerotiorum* (Isolate 55) was prepared as follows. Sclerotia were collected from 28-day-old cultures on autoclaved celery stalks maintained at 24 C, air-dried at 21 C for 7 days, and seeded in glass jars (100 × 80 mm) containing 150 g of nonautoclaved greenhouse soil (sand/loam/peat, 1/3/1, 35% moisture, v/w). The jars were held in the dark at 5 C for 28 days, and then under continuous fluorescent light (12 $\mu mol/m^2/s$) at 14 C. Apothecia were produced in approximately 28 days. Ascospores were vacuum-collected from apothecia with the aid of an inverted 50-mm funnel connected to a 250-mm filtering flask containing 80 ml of distilled water. This ascospore suspension was adjusted by dilution to 8×10^6 ascospores/ml following hemacytometer counts.

The mycelial inoculum of *S. sclerotiorum* was prepared by excising mycelial plugs (8 mm diameter) from the margins of 5-day-old cultures on potato-dextrose agar (100 × 15 mm plates, 2.5%, w/v) maintained at 20 C.

Ten levels of controlled atmospheres (mixtures of O_2, CO_2, and CO) were prepared as compressed gases by the Canadian Air Ltd. (Montreal, Quebec) in 6 m^3 cylinders. These mixtures were as follows: 1) 1.5% O_2, 2) 3% O_2, 3) 6% O_2, 4) 4% CO_2 + 1.5% O_2, 5) 8% CO_2 + 1.5% O_2, 6) 16% CO_2 + 1.5% O_2, 7) 7.5% CO + 1.5% O_2, 8) 15% CO + 1.5% O_2, 9) 22.5% CO + 1.5% O_2, and 10) 30% CO + 1.5% O_2. The remainder of each gas mixture was N_2. The control (normal air) contained 21% O_2.

To determine the effect of controlled atmospheres on mycelial growth of *S. sclerotiorum*, a mycelial plug of the fungus was placed aseptically in the center of each of 80 plates of celery extract agar (30 g of celery boiled for 25 min in 100 ml of distilled water and filtered, 2% agar). Similarly, 1 ml of ascospore inoculum was placed aseptically in each of 80 plates of 2.5% water agar.

Twenty plates containing mycelial plugs and 20 plates with ascospores were placed in each of four low density polyethylene (LDPE) bags (80 × 55 cm, 65 μ thick, Novacor Chemicals Ltd., Alberta). The permeability coefficients ($cm^3 \cdot cm \cdot s^{-1} \cdot Pa^{-5} \cdot cm^{-2}$) of the LDPE bags for O_2, CO_2, CO, and N_2, respectively, are 2.16×10^{-13}, 9.45×10^{-13}, 1.11×10^{-13}, and 7.27×10^{-14}. The bags were sealed with duct tapes and emptied of air by applying a vacuum.

Each bag was connected to a cylinder of 1) 1.5% O_2, 2) 4% CO_2 + 1.5% O_2, 3) 7.5% CO + 1.5% O_2, or 4) normal air (control). Then each bag was filled with 40 L of the appropriate gas mixture (10

L/min flow rate, TSA-15/2-stage regulator, Union Carbide Canada Ltd., Toronto) and maintained at 1 C. To minimize the interference from any CO_2 and ethylene respired by celery or *S. sclerotiorum* in the bags, used gas in each bag was removed by applying a vacuum and each bag was refilled with a fresh supply daily.

The 20 plates with mycelial plugs were removed from each bag every 5 days, up to day 20, to measure diameters of colonies. The plates were returned after each measurement. Starting at day 3 and ending at day 9, five replicate plates containing ascospores were removed from each bag every 2 days to determine the percentage of spore germination and germ tube length of 500 germinated spores. A spore was considered germinated when the length of the germ tube was half the diameter of the spore. These plates were not returned to the bags.

To compare the development of watery soft rot at 1 and 8 C storage, 10 25-cm lengths of detached petioles of equal age of freshly harvested cv. Utah 52-70 field celery were rinsed under running tap water and placed in a plastic tray (54 × 25 × 12 cm) lined with moistened paper towels. Each petiole was aseptically pricked (2 mm deep) at the middle with a 1-mm-diameter chrome wire and then inoculated by placing a mycelial plug (8 mm diameter) on the wound. Each of 10 trays of celery was then sealed in a LDPE bag and the bags were emptied of air by applying a vacuum. Each bag was supplied with 40 L of normal fresh air. Five replicated bags were maintained at 1 C and the other five replicated bags at 8 C. Removal of used air and refilling with fresh air for each bag were done daily.

Starting at day 3 and terminating at day 21, the petioles at 8 C were removed from each bag every 2–4 days to rate disease lesions for severity. Similarly, petioles at 1 C were rated for length of lesion each week for 10 weeks. Ratings of disease were based on a scale of 0–10, where 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 represented lengths of 0, 1–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, and 91–100 mm, respectively. The sum of the ratings was divided by the number of observations per replicate to obtain the severity index.

To study the effect of various levels of gas mixtures on watery soft rot, petioles of freshly harvested Utah 52-70 field celery were prepared and inoculated with mycelial plugs (8 mm diameter) as above. Five replicate bags of celery for each treatment were given a daily fresh supply (40 L) of the appropriate gas mixtures listed in Table 1, were maintained at 8 C, and indexed after 2 wk for disease severity as above.

All above experiments were performed at least twice, the results were averaged, then statistically analyzed (SEM, *t* test, and regression analysis).

RESULTS AND DISCUSSION

Mycelia of *S. sclerotiorum* on celery agar at 1 C grew rapidly in all controlled atmospheres between days 5 and 20, except in the 7.5% CO + 1.5% O_2 atmosphere where growth was very slow (Fig. 1A). Growth in 1.5% O_2 was significantly different (P = 0.05) from that in normal air. Growth in 4% CO_2 + 1.5% O_2 was significantly less than in 1.5% O_2 (P = 0.05).

Germination of ascospores and germ tube length of *S. sclerotiorum* also were affected by the various atmospheres between days 3 and 9 (Fig. 1B,C). Spore germination and germ tube length were reduced the most in 7.5% CO + 1.5% O_2 (P = 0.05). With 4% CO_2 + 1.5% O_2, germination of ascospores was suppressed more than in 1.5% O_2 (P = 0.05).

Watery soft rot of celery in normal air developed more rapidly at 8 C than at 1 C (Fig. 2). Disease was severe on celery stored in normal air for 2 wk at 8 C, while a comparable severity took 10 wk to develop at 1 C.

Watery soft rot was more severe 2 wk after inoculation at 8 C in normal air (P = 0.05) than in other atmospheres (Table 1). Disease severities in 7.5–30% CO + 1.5% O_2 were about 10 times less (P = 0.05) than those in 1.5–6% O_2 alone or in 4–16% CO_2 + 1.5% O_2. Although not statistically significant (r = 0.28, P = 0.05) (Table 1), disease severities tended to increase in the atmospheres of increasing O_2 concentrations, but this trend was reversed in the atmospheres containing increasing CO_2 concentrations (r = 0.58, P = 0.05). Low disease severities occurred in all CO atmospheres (7.5–30%) combined with 1.5% O_2 (Table 1). Here, however, disease severities did not differ from each other probably because disease ratings measured were very low; they did not change with increase of CO concentration.

This report shows that controlled atmospheres containing 1.5% O_2, with or without 7.5% CO or 4% CO_2, suppressed the growth of *S. sclerotiorum* in vitro as well as watery soft rot of celery in storage (Fig. 1, Table 1). But increasing the concentration of O_2 alone, from 1.5 to 6%, or the concentration of CO_2 in 1.5% O_2 from 4 to 16%, changed only slightly

Table 1. Disease index of watery soft rot caused by *Sclerotinia sclerotiorum* on celery maintained in various atmospheres at 8 C for 2 wk

Atmosphere[x]	Disease index[y]
1.5% O_2	6.6 e[z]
3.0% O_2	7.2 de
6.0% O_2	8.0 bc
4.0% CO_2 + 1.5% O_2	8.6 b
8.0% CO_2 + 1.5% O_2	7.4 cd
16.0% CO_2 + 1.5% O_2	6.8 de
7.5% CO + 1.5% O_2	0.8 f
15.0% CO + 1.5% O_2	0.8 f
22.5% CO + 1.5% O_2	0.6 f
30.0% CO + 1.5% O_2	0.6 f
Normal air check	9.6 a

[x] Remainder of each gas mixture was N_2.

[y] Disease indices were based on a scale of 0–10, where 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 represented lengths of 0, 1–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, and 91–100 mm, respectively.

[z] Within each group, values followed by the same letter are not significantly different (*t* test, P=0.05). Regression equation for O_2: Y = 76.75 + 3.88 X, r^2 = 0.28 (NS). Regression equation for CO_2 + O_2: Y = 110.29 − 1.63 X, r^2 = 0.58* (P = 0.05). Regression equation for CO + O_2: Y = 11.11 − 0.03 X, r^2 = 0.02 (NS). Normal air data were omitted from regression analysis.

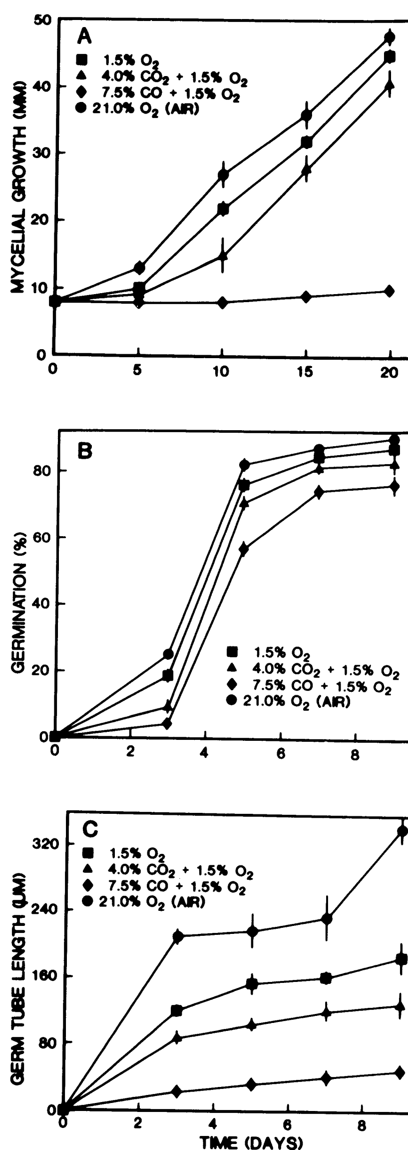

Fig. 1. Effects of controlled atmospheres on *Sclerotinia sclerotiorum*: **(A)** mycelial growth, **(B)** ascospore germination, and **(C)** ascospore germ tube length. The remainder of each gas mixture was N_2. Vertical lines indicate standard error of the means.

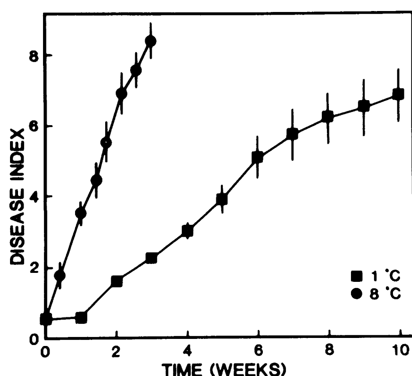

Fig. 2. Comparative development of watery soft rot caused by *Sclerotinia sclerotiorum* on celery in normal air at 1 and 8 C. Disease indices were based on a scale of 0–10, where 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 represented lengths of 0, 1–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, and 91–100 mm, respectively. Vertical lines indicate standard error of the means.

the suppressive effect on the celery disease at 8 C compared with the control. The most suppressive gas mixtures were those containing 7.5–30% CO + 1.5% O_2. However, the evidence (Table 1) shows that it was not beneficial to increase the concentration of CO in 1.5% O_2 from 7.5% up to 30%. It will be more economically practical if concentrations of CO lower than 7.5% in 1.5% O_2 suppress watery soft rot of celery in storage. This is being determined. Reyes and Smith (6) noticed that CO as low as 2.5% in 1.5% O_2 was effective in prolonging the shelf life of celery in storage.

El-Goorani and Sommer (2) reported that 9% CO suppressed *Monilinia fructicola* (Wint.) Honey, *Penicillium expansum* Link ex Thom, *P. italicum* Wehmer, *P. digitatum* Sacc., and *S. sclerotiorum*, as well as some diseases caused by these fungi. El-Kazzaz et al (3) and Kader et al (4) showed that 5–10% CO added to 2–4% O_2 during storage of strawberries and tomatoes suppressed decay caused by *B. cinerea*. Therefore, there is potential for CO to be used for watery soft rot control on celery in the storage because CO does not cause undesirable effects on celery quality (5). On the other hand, CO should be handled with care because it is highly poisonous to humans.

ACKNOWLEDGMENTS

I thank Ann M. Curwin and Diane M. B. Beaulieu-Aruvee for technical help. I am also grateful to the Horticultural Products Laboratory of the Ontario Ministry of Agriculture and Food at Vineland Station for the use of its cold storage facility.

LITERATURE CITED

1. Cappellini, R. A., Ceponis, M. J., and Lightner, G. W. 1987. Disorders in celery and carrot shipments to the New York market, 1972-1985. Plant Dis. 71:1054-1057.
2. El-Goorani, M. A., and Sommer, N. F. 1979. Suppression of postharvest plant pathogenic fungi by carbon monoxide. Phytopathology 69:834-838.
3. El-Kazzaz, M. K., Sommer, N. F., and Fortlage, R. J. 1983. Effect of different atmospheres on postharvest decay and quality of fresh strawberries. Phytopathology 73:282-285.
4. Kader, A. A., Chastagner, G. A., Morris, L. L., and Ogawa, J. M. 1978. Effects of carbon monoxide on decay, physiological responses, ripening, and composition of tomato fruits. J. Am. Soc. Hortic. Sci. 103:665-670.
5. Reyes, A. A., and Smith, R. B. 1986. Controlled atmosphere effects on the pathogenicity of fungi on celery and on the growth of *Botrytis cinerea*. HortScience 21:1167-1169.
6. Reyes, A. A., and Smith, R. B. 1987. Effect of oxygen, carbon dioxide, and carbon monoxide on celery in storage. HortScience 22:270-271.