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POSTHARVEST FUMIGATION OF CHINESE YA PEAR WITH CARBONYL SULFIDE FOR THE CONTROL OF BLACK SPOT DISEASE

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ABSTRACT

The black spot disease caused by *Alternaria alternata* (Fr.) is the main postharvest disease on Chinese Ya pear. Here we report the effect of temperature and exposure time on fungitoxicity of carbonyl sulfide fumigation against *A. alternata* and the response of Chinese Ya pear to the postharvest fumigation. Test results *in vitro* showed that *A. alternata* was susceptible to carbonyl sulfide fumigation at all the tested temperatures. The toxicity potency of carbonyl sulfide increased linearly and the LC99 decreased from 2,457.6 $mg \cdot l^{-1}$ to 174.5 $mg \cdot l^{-1}$ with temperature increasing from 4°C to 25°C. Exposure time extended from 4 h to 8 h at 25°C decreased linearly the concentration of LC99 from 199.2 to 104 $mg \cdot l^{-1}$. In confirmation and phytotoxicity tests, carbonyl sulfide fumigation of Chinese Ya pear artificially infected with *A. alternata*, with a schedule of 200 $mg \cdot l^{-1}$ dosage and 4 h exposure time at 25°C, inhibited completely the further growth of the fungus in the fruit pulp. However, when dosage was higher than 90 $mg \cdot l^{-1}$, the surface injury was not acceptable by market, although the fruit pulp quality parameters were not significantly changed.

Key words: Carbonyl sulfide, fumigation, black spot disease, Chinese Ya pear

INTRODUCTION

Black spot disease caused by *Alternaria alternata* (Fr.) Keissler is one of the most harmful diseases in Chinese Ya pears (*Pyrus pyrifolia*), particularly during storage stage (Zhang et al., 2003). Current control measures in the field mainly include cultivating resistant pear, placing the fruit in a special paper bag during growth, and spraying fungicides (He et al., 1995; Tetsuo et al., 1999; Terakami et al., 2007). These measures can effectively decrease the infection of Ya pear in the orchards, but cannot prevent infection after harvest. Moreover, some importing countries may consider *A. alternata* as a pest of quarantine importance, which directly influences the exportation of this fruit. Therefore, development of an economic and effective postharvest disinfection measure becomes necessity.

Carbonyl sulfide (COS) is a potential new fumigant that is present in nature (Fields and White, 2002). Laboratory and field studies have shown that COS is effective against a wide range of pests at all life stages, without any adverse effects on grains and stored products (Desmarchelier, 1994; Zettler et al, 1997; Weller, 1999; Xianchang et al., 1999). Quality studies on lemon, nectarine, papaya, and avocado indicated that COS fumigation did not cause significant skin or flesh injury at reasonable concentrations (<80 $mg \cdot l^{-1}$) and exposure times (1–24 h) (Chen and Paull, 1998; Weller et al., 1998; Aung et al., 2001). However, little is known about the fungitoxicity of COS against fungal plant pathogens. We report herein the effects of COS fumigation against *A. alternata* and the tolerance of Chinese Ya pears to COS fumigation.

MATERIALS AND METHODS

Fumigation of *A. alternata* in vitro

A.alternata L-3 was isolated from Ya pears infected with black spot disease in several orchards of Hebei Province, China. COS was purchased from Yanglilai Company (Beijing, China) as a compressed gas with 99% purity. COS fumigation tests with an exposure time of 4 h were performed separately at 4°C, 10°C, 15°C, 20°C, and 25°C to investigate the influence of treatment temperature on COS fungitoxicity. Fumigation with different exposure times of 3, 4, 5, 6, and 8 h at 25°C were also conducted. Three replicates were fumigated at each temperature and exposure time.

Fumigation of infected pears

Chinese Ya pears were purchased from a local orchard that did not receive any fungicide spray for 1 month before harvest. After storing at 25°C for 1 d, the fruit were surface-disinfested with 70% ethanol for 30 s. Eight small wounds (2 mm in depth and 5 mm in diameter) were made on each pear using a sterile pin. The wounded pear was inoculated with *A. alternata* by covering the fruit with small pieces of sterile filter paper that have been dipped in the previously prepared fungi suspension.

Fumigation containers were modified from 6-l vacuum containers. Eight Ya pears were placed in each container with about 40% load by volume. At the end of fumigation, the containers were quickly unsealed and forcibly aerated for 12 h at ambient temperature before the pears were again removed to store at 25°C for 7 d for efficacy evaluation. After 7 d storage at 25°C, the fungal spots on the infected pears were counted and the disease incidence rate was calculated.

COS phytotoxcity tests

Healthy Ya pears were fumigated at 25°C to evaluate phytotoxicity. Different dosages of 30, 60, 90 and 120 $mg \cdot l^{-1}$ COS were applied respectively with a same exposure time of 4 h. The possible phytotoxic response(surface injury) and the effect on fruit quality parameters (weight loss, firmness, soluble solids, total acidity) were examined after 7 d of storage at 25°C. Surface injury was classified as none (0), very slight (≤5%), slight (5%–15%), moderate (15%–25%), severe (25%–50%), and very severe (>50%). Fruits with moderate, severe, and very severe surface injury were considered to be unmarketable.

Data analysis

Probit analysis was performed by PoloPlus (Leora Software 2003, USA), and the slope, LC50 value, and LC99 value of each test were calculated. Mean comparisons were performed using Duncan's multiple range test. All analyses were performed with SPSS software package v.11.0 for Windows.

RESULTS AND DISCUSSION

COS fungitoxicity against *A. alternate* in vitro

The influence of temperature on efficacy of COS fumigation was distinctive. The LC50 values decreased by about 21% as the temperature increased every 5 degrees from 4°C to 25°C, while the LC99 values decreased by about 24%. However, when temperature was at 10°C, the 99% inhibition rate required a concentration of 1,807.8 $mg \cdot l^{-1}$ with a 95% confidence limit from 1,503.9 to 2,311.2 $mg \cdot l^{-1}$, the upper limit of which almost equals to pure COS gas, indicating complete inhibition (100%) could only be achievable when temperatures were higher than 15°C (Table 1).

Table 1. Probit analysis of COS fungitoxicity against *A. alternata* at different temperatures

Temperature (°C)	Slope[a]	Duncan test (0.95 CI)	Hetero.	LC50 ($mg \cdot l^{-1}$) (0.95 CI)	LC99 ($mg \cdot l^{-1}$) (0.95 CI)
4	3.12 ± 0.25	c	3.45	442.7 (302.1–563.1)	2,457.6 (1,584.7–6,476.2)
10	3.28 ± 0.27	c	0.75	353.0 (309.0–393.6)	1,807.8 (1,503.9–2,311.2)
15	5.19 ± 0.36	b	1.78	294.1 (255.8–330.4)	825.1 (672.7–1,141.0)
20	4.94 ± 0.29	b	3.18	116.0 (102.9–129.6)	343.0 (274.3–488.2)
25	6.12 ± 0.32	a	4.22	72.7 (62.0–82.6)	174.5 (146.6–228.3)

[a]Mean ± SE, CI means confidence interval

Parallelism comparisons of the probit regression lines showed that the probit regression lines were parallel between 4°C and 10°C, and also between 15°C and 20°C, which indicated that COS fumigation had the same fungitoxical potency within these two temperature ranges. Therefore, despite the linear decline of LC50 and LC99 values among all the temperatures tested, the temperature influence on fungitoxicity could be divided into 3 temperature groups. They were cold condition (<10°C), where the fungitoxicity of COS was low; cool condition (10°C–20°C), where the fungitoxicity of COS was medium; and warm condition (above 20°C), where the fungitoxicity of COS was high.

Exposure time also influenced COS fungitoxicity. The results demonstrated that COS dosage required to achieve a certain inhibition rate decreased when the exposure time increased, but not in a directly proportional manner. The LC99 value decreased from 199.2 to 104.0 $mg \cdot l^{-1}$ at a percentage of nearly 50%, and the slope increased from 6.4 to 12.0 when

Parallelism comparisons of the probit regression lines relative to different exposure times were also conducted. The probit regression lines of 3, 4, and 5 h exposure time seemed parallel (Table 2). The fact that the fiducial limits for all the inhibition levels with exposure time from 3 to 6 h overlapped at a large range further showed that COS fungitoxicity within 6 h exposure did not have substantial difference.

As described by Haber's (1924) rule, for a specific response level, the product of concentration (C) and exposure time (t) is constant (i.e., $Ct = k$), a famous relationship that has provided a good guide for methyl bromide fumigation. However, when calculating the CT products at a specific inhibition level in the tests with exposure time from 3 to 8 h (Table 2), we found the CT products increased linearly along with the extension of exposure time, which does not satisfy the relationship Ct=K.

Table 2. Probit analysis of COS fungitoxicity against *A. alternata* during different exposure times at 25°C

Time (h)	Slope[a]	Duncan test (0.95 CI)	Hetero.	LC50 ($mg \cdot l^{-1}$) (0.95 CI)	LC99 ($mg \cdot l^{-1}$) (0.95 CI)	$C_{LC50}t$ value ($g \cdot h \cdot m^{-3}$)	$C_{LC99}t$ value ($g \cdot h \cdot m^{-3}$)
3	6.37 ± 0.51	C	0.39	86.5 (81.6–92.1)	199.2 (173.6–239.5)	257.8	597.6
4	6.12 ± 0.32	C	4.22	72.7 (62.0–82.6)	174.5 (146.6–228.3)	291.0	697.6
5	6.30 ± 0.72	C	3.10	70.1 (58.6–84.6)	143.1 (105.4–226.0)	350.5	715.5
6	8.64 ± 0.74	B	2.82	68.6 (60.0–76.2)	127.4 (106.4–188.2)	411.4	764.4
8	12.01 ± 0.99	A	1.10	66.6 (63.2–70.3)	104.0 (94.0–122.0)	532.7	832.0

[a]Mean ± SE.

A more conventional form of $C^n t = k$ was further applied, where n represents the toxicity index. The values for n of 1.459 or $C^{1.459}t=7063$ was found to be comprehensively describing the relationship between the COS concentration and exposure time at an inhibition rate of 99% in all the tested exposure times. In this relationship, COS concentration played a more important role.

Efficacy of COS fumigation for infected pears

The infected pears were fumigated at 25°C with 4 h exposure time and different dosages of 30, 60, 90, 120, 160, and 200 $mg \cdot l^{-1}$. Concentrations of COS were measured at 0.5 h after introduction of the gas, and at 0.5 h before aeration. The initial concentrations of COS were nearly 1.5 times of the applied dosages because of the almost 40% load factor, which further demonstrated the correct dosing. COS concentration decreased during exposure, and the final concentrations were almost equal to the applied dosages, indicating about 35% adsorption. The incidence of black spot disease on the infected pears after COS fumigation revealed that the efficacy of COS fumigation is dose dependent: a dose of 200 $mg \cdot l^{-1}$ resulted in 99% inhibition, which was comparable to the LD99 value (174.5 $mg \cdot l^{-1}$) in the *in vitro* test (Fig. 1F).

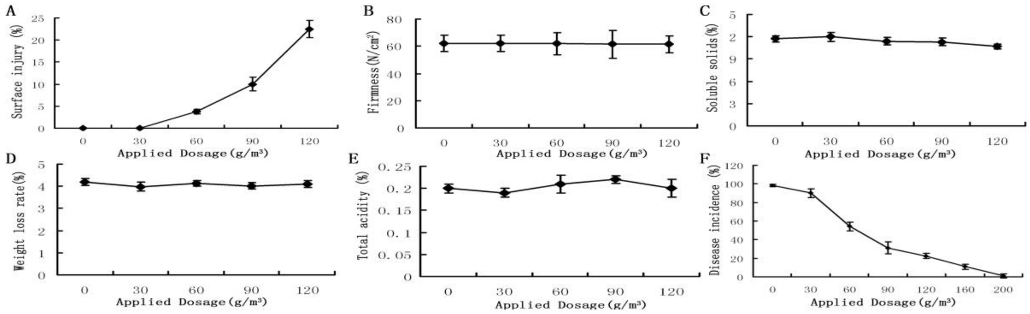

Fig. 1- Effect of COS fumigation on pear black spot disease and quality of Ya pears. (A) The surface injury of pears fumigated with COS (B) The firmness of pears fumigated with COS. (C) The soluble solids of pears fumigated with COS. (D) The weight loss rate of pears fumigated with COS. (E) The total acidity of pears fumigated with COS. (F) The disease incidence of infected pears. All data were averaged from 3 replicates after 7 d of storage. Error bars indicate SE.

Quality index of ya pears after COS fumigation

In our experiments, although there were no significant changes in internal quality parameters (Fig. 1B-E), large surface injuries occurred when the dose was higher than 90 $mg \cdot l^{-1}$, indicating it is unacceptable for the control of black spot disease in Chinese Ya pears with COS fumigation.

Different kinds of fruits differ in their response to COS fumigation, for example, avocado and mango could only tolerate 45 and 23 $mg \cdot l^{-1}$ COS for less than 4 h fumigation (Weller, 1999). On the contrary, lemons could tolerate COS fumigation with a dosage of 70 $mg\ l^{-1}$ and exposure time of 8 h (Weller, 1999). Therefore, COS can be considered as a postharvest disinfestation or disinfection measure on some kinds of fruits.

In conclusion, although COS could control *A. alternata* at reasonable temperatures (>15°C) and exposure times (4–8 h), the obvious surface injuries occurred with dosages higher than 90 $mg \cdot l^{-1}$ in 4-h exposures restricted its application as an effective fumigant for control of the black spot disease in Chinese Ya pears.

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