Studies on the Control of the Canker of Satsuma Orange with Special Reference to the Sterilization of Infected Fruit

II. Fumigation Test by Ethylene Oxide and Propylene Oxide*

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Introduction

In an effort to work out a feasible disinfection method for the fruit of canker infected Satsuma orange, studies were made on the direct bactericidal effect of ethylene oxide and propylene oxide gases on the causal bacteria of citrus canker and the possible fumigation injuries of Satsuma orange fruit were also investigated.

Materials and Methods

A. Test on bactericidal effect

1. Preparation of bacterial samples

Sterilized filter paper disks (6 mm diameter) were dipped into concentrated water suspension of *Xanthomonas citri* PQ-1. Disks thus inoculated were transferred to dry filter paper and excessive moisture was discarded. Thirty each of these disks were divided into aseptic test tubes having 3 small holes on the side wall. The test tubes were loosely plugged with cotton and subjected to the treatment.

2. Apparatus and operation

As is shown in Figure 1, glass jars of ca. 28 liter volume having two stopcocks at the top were used and the fumigation was carried out in normal atmospheric pressure. One stopcock was connected with a water manometer and the other with a suction pump. After reducing the inside pressure to a calculated level, the suction pump was replaced by a bomb of the fumigants and the predetermined dosage of the fumigant was discharged into jar in gaseous from, thus regaining the inside pressure toward the normal atmospheric level. After ascertaining the uniformity of gas concentration by the interferometer-type analyser, the rubber stopper at the top was opened and the bacterial sample was quickly suspended into jar with a thread by tightly squeezing the stopper.

3. Dosage, exposure time and temperature

Dosage: 32.6, 65.2, 97.8, 130.4, 163.0 mg/*l* (2, 4, 6, 8, 10 lbs/1,000 cubic feet)

Exposure time: 2, 3, 4 hours

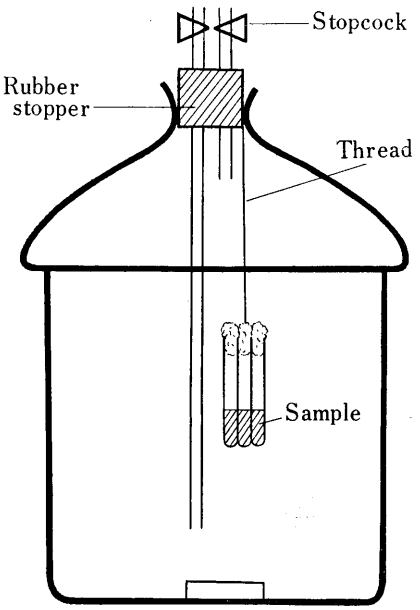

Figure. 1 Fumigation apparatus employed

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Temperature: 20°C

4. Determination of bactericidal effect

Fumigated disks were exposed to the aeration for 30 min. and then plated on potato semi-synthetic agar media. Five days after incubation at 25°C, the effect of the treatment was determined by reading the number of the disks from which developed an exudate of the growing causal bacteria.

Each treatment was replicated twice.

B. Test on chemical injuries on Satsuma orange

Five fruit each of Satsuma orange (*Gitrus unshu* MARC. *praecox* TANAKA) were wrapped in a small gauze bag, suspended with a thread from the top rubber stopper and fumigated by the dosage, exposure time and temperature of Test A. Fruits thus fumigated were stored at 20°C for one week and examined for the appearance of ill effect, if any, on the outlook as well as the taste and flavor of the fruit.

Experimental Results

A. Bactericidal effect

(1) Ethylene oxide

The results obtained with ethylene oxide are given in Table 1. All the treatment of 2 hour exposure time including the highest dosage of 163 mg/*l* were ineffective. Complete bactericidal effect was obtained at the dosage higher than 130.4 mg/*l* in 3 hour exposure series and 97 mg/*l* in 4 hour exposure series.

(2) Propylene oxide

Complete steriltzation was not obtained with this fumigant even at the highest dosage level of 163 mg/*l* -4 hrs.

B. Chemical injuries

Table. 1 Effect of gaseous ethylene oxide on *X. citri*

Dosage (mg/*l*)	Percent of sterilized disks* Exposure time (hrs)		
	2	3	4
32.6	0	0	0
65.2	0	0	6.7
97.8	0	6.7	100
130.4	0	100	100
163.0	73.3	100	100

* Average percentage of two replication each using 30 disks

(1) Ethylene oxide

In all the exposure time series, brownish to dark brown, somewhat sunken patches of irregular shape appeared within 3 days on the skin of the fruit that had been fumigated at the dosage level of more than 65.2 mg/*l*. These patches gradually expanded and, in a week or so, covered all over the fruit. Fruit thus affected readily succumbed to the secondary infection by anthracnose and other microorganisms and decayed very quickly.

Another type of injury was also noted particularly among the fruits treated with the higher dosage level of 130.4 to 163.0 mg/*l*. This was represented by the stoppage of after-ripening, gradual hardening and overall darkening of the fruit. No growth of the secondary microoganisms was found on the fruit of this type after more than two weeks.

The lowest toxicity level of 32.6 mg/*l* - 2 hrs also produced slight external injury and remained green one week after fumigation when all the untreated fruits attained mature coloration.

Appreciable deterioration in taste and flavor was noted even with the lowest dosage level which was represented by the tendency to reduce refreshing sourness and to impart a peculiar astringent taste. Dosages of more than 65.2 mg/*l* totally destroyed the taste and flavor and rendered the fruit unedible.

(2) Propylene oxide

Injuries by this fumigant were slightly milder than those by ethylene oxide. Dark sunken blotches appeared from the dosage level of 65.2 mg/*l* - 2 hrs becoming definitely severer at the dosage of more than 97.8 mg/*l*.

Discussion

The fungicidal efficacy of ethylene oxide has been reported by various workers including E. M. MRAK (1954), R. WHELTON et al. (1964), G.A. ZENTMEYER et al. (1949), F. LANZA (1950), H. DARPOUX et al. (1952), D.S. MACLACHLAN et al. (1953) and E. BETTO (1960). Data presented by these workers, however, cannot be adequately correlated with each other because of the difference in the fungi dealt with and the wide variation among the key conditions of the fumigation

treatment.

The writers in the present studies obtained complete sterilization of *Xanthomonas citri* by the fumigation of 97.8 mg/*l*－4 hrs or 130.4 mg/*l*－3 hrs at 20°C.

Ethylene oxide is commonly used for unsulfured dried fruit of figs, prunes, dates and natural raisins to prevent spoilage by yeasts and molds (R. E. deONG, 1956). Little information, however, is available on the promising application of this fumigant to the treatment of fresh fruit. R. E. deONG (1956) states that a limited use has been made of this fumigant in fumigating fresh fruit including blackberries and blueberries, using 2 lbs/1,000 cubic feet (32.6 mg/*l*) at 75°F. The treated blueberries, however, were rendered slightly inferior to the untreated berries. Bananas were severely injured (OSBURN and LIPP, 1935). LINDGREN and SINCLAIR (1951) report serious injury to avocado and oranges.

In the present studies, appreciable deterioration in taste and flavor and slight, but not altogether negligible, external injury as well as the retarding of after-ripening was noted even with the minimum dosage-exposure level tested, i. e. 32.6 mg/*l*－2 hrs. The treatment at the completely bactericidal dosage-expoure level, i. e. 130.4 mg/*l*－3 hrs or 97.8 mg/*l*－4 hrs, totally destroyed the fruit and rendered them worthless.

Propylene oxide did not give 100% sterilization even at the maximum dosage-exposure level of 163.0 mg/*l* 4 hrs, whereas severe fruit injuries resulted at the dosage of more than 97.8 mg/*l*.

These resuts evidently show that there is little hope for the application of both ethylene oxide and propylene oxide to the fumigation of cankered Satsuma orange.

Summary

(1) Fumigation effect on *Xanthomonas citri* of ethylene oxide and propylene oxide gases and the possible deterioration of the treated Satsuma orange were studied.

(2) *Xanthomonas citri* was completely killed by ethylene oxide at the dosage-exposure level of 97.8 mg/*l*－4 hrs or 130.4 mg/*l*－3 hrs.

(3) Propylene oxide was not effecitve within the dosage-exposure range of up to 163.0 mg/*l*－4 hrs.

(4) Satsuma orange, when fumigated with ethylene oxide, showed appreciable deterioration in taste and flavor, slight external injury and retarding of after-ripening at the lowest dosage-exposure level of 32.6 mg/*l*－2 hrs and was completely destroyed at the dosage of more than 65.2 mg/*l*.

(5) There seem to be little hope for the application of these two fumigants to the sterilization of canker-infected Satsuma orange.

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摘　要

温州ミカン潰瘍病の防除に関する研究

II　エチレンオキサイドおよびプロピレンオキサイドの殺菌力ならびに温州ミカンに対する薬害

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潰瘍病罹病果の殺菌試験の一環として，諸外国において穀類，食品，家具などの殺虫用，乾果の醱酵や腐敗防止などの殺菌くん蒸剤として用いられているエチレンオキサイドとプロピレンオキサイドをとりあげ，潰瘍病菌に対する殺菌力ならびに温州ミカンに対する薬害を検討した。

殺菌効果の検定には内容積約 28 *l* のガラス製くん蒸びんを用い，管壁に小孔をあけた試験管に予め病原細菌の濃厚浮遊液に浸漬し，接種した濾紙円板を入れ，くん蒸びん内につり下げ，所定濃度および時間で常圧くん蒸（温度 20°C）したのち，バレイショ半合成培地上で5日間（温度 25°C）培養し，病原菌の生死を検定した。

果実に対する薬害は早生温州を各処理区5個ずつ，前記の方法に準じてくん蒸したのち，定温室（20°C）に1週間放置し，果実の外観，味覚への影響を調べた。

試験結果を要約すると次のとおりである。

（1）潰瘍病菌はエチレンオキサイドの 97.8 mg/*l*—4 hrs または 130.4 mg/*l*—3 hrs/20°C のくん蒸により 100％殺菌することができたが，プロピレンオキサイドでは薬量 163.0 mg/*l*, 4時間までのくん蒸では完全殺菌に至らなかった。

（2）早生温州ミカンはエチレンオキサイドの 32.6 mg/*l*—2 hrs, プロピレンオキサイドの 65.2 mg/*l*—2 hrs のくん蒸によって，薬斑の発生，後熟（着色）の抑制，味覚の変化が認められ，エチレンオキサイドの 65.2 mg/*l*, プロピレンオキサイドの 97.8 mg/*l* 以上のくん蒸では完全に変質腐敗した。

（3）殺菌限界と薬害との関係から，両薬剤は潰瘍病罹病果の殺菌には利用できないと考えられる。