

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

I. Screening of Effective Fungicides*

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Introduction

The export of Satsuma orange to the United States dates back to 1910's. In 1917, the United States promulgated Notice of Quarantine No. 28 (Citrus Fruit Quarantine) and thus placed an embargo on the fruit of foreign origin as a precautionary action to prevent the introduction of citrus canker into the United States. By virtue of an unusually high resistance to this disease, Satsuma orange was exempted from the embargo and the average per annum of about 170,000 cases had been exported during the prewar period.

In 1947, however, the Citrus Fruit Quarantine was revised and Satsuma orange was subjected to the embargo on the grounds that the citrus canker had been from time to time intercepted on the shipment brought into U.S. ports of entry by way of passenger baggage or stored goods of vessels and airplanes.

In view of the inevitable trend of international trade liberalization, the expansion of overseas trade market is becoming increasingly important for the future status of Japanese citrus industry. The removal of U.S. embargo is no doubt a key to this problem. Repeated efforts made by Japan to have the ban removed has so far failed because it has been technically infeasible to establish a perfect sterilization method of infected fruits as is requested by the United States.

The writers, in their approach to help solve this pending problem, undertook a screening of effective fungicides which might be adequately applicable to the treatment of cankered fruit of Satsuma orange and the more effective control of this disease in the citrus orchards.

Acknowledgement

The writers wish to express their hearty thanks to Dr. H. Ishikura and Mr. Iwakiri of the Plant Protection Section of Agricultural Administration Bureau, Dr. H. Mukoo and Dr. T. Misato of the National Institute of Agricultural Sciences for rendering them helpful suggestions in the course of this investigation.

Materials and Methods

1. Fungicides employed

Fungicides employed in this test, their concentration and the duration of dipping treatment are shown in Table 1.

It is a prerequisite to the fungicides applicable to the treatment of fresh fruit that they possess high fungicidal action with no residual toxicity to man and livestock. This must also be coupled with no fear of affecting the marketability of treated fruit. In case of antibiotic chemicals, special considerations must further be given to the possible induction of resistant strains of human pathogenic bacteria by the residual dosage. Such conditions, however, are hardly to be met at the present time. Consequently, many of the fungicides that are apparently unusable for the fresh fruit were included in this test with a view to assay-

* This report was presented at the summer meeting of the Kanto Division of Japan Phytopathological Society held in Tokyo on June 16, 1962.

(Accepted for publication March, 1963)

Table 1. Fungicides used, their concentration and the duration of dipping

Fungicide and ingredient	Concentration	Duration of dipping
<i>Antibiotics</i>		
a) Medicinal antibiotics		
Aureomycin	10, 25, 50, 100, 200 ppm	1, 5 min.
Terramycin	25, 50, 100, 200	do
Acromycin	do	do
Neomycin	do	do
Kanamycin	50, 100, 200	do
Streptomycin	do	do
Dihydrostreptomycin	25, 50, 100	do
Chloramphenicol	do	do
b) Test formulation		
Acromycin 1% emulsion-Takeda (TA-403)	25, 50, 100 ppm	1, 5 min.
Aureomycin 1% emulsion-Takeda (TA-402)	do	do
Aureomycin 1% solution (Ihara)	do	do
Aureomycin 1% emulsion (Ihara)	do	do
<i>Copper Fungicide</i>		
Sankyo-KB 90 (Basic copper sulfate: Cu 48%)	1/800, 1/400, 1/200	5 min.
Copper wettable (Basic copper sulfate: Cu 20%)	do	do
Doitsu-Bordeaux (Basic copper chloride: Cu 44%)	do	do
TF 58 B (Basic copper silicate: Cu 23%)	do	do
BorsinT (Copper hydrate complex: Cu 23%)	do	do
Copper oxinate 20% Wettable	do	do
<i>Copper-Mercury Fungicide</i>		
Fumiron-Bordeaux { Basic copper sulfate: Cu 20% Phenylmercuric iodine: Hg 1%	do	do
Shin-Do-Mix { Basic copper sulfate: Cu 20% Phenylmercuric acetate: Hg 0.9%	do	do
Sankyo Mercuric Bordeaux { Basic copper sulfate: Cu 20% Phenylmercuric chloride: Hg 0.45%	do	do
Mer-Bordeaux { Basic copper sulfate: Cu 20% PMF+PMA: Hg 1% Basic zinc sulfate: Zn 5%	do	do
<i>Mercury Fungicide</i>		
Fumiron Tablet (Phenylmercuric-p-tolunene-sulfoanilide + Ethylmercuric phosphate + Ethylmercuric urea: Hg 2.5%)	1/2000, 1/1000, 1/500	5 min
Takeda-Mer (Phenylmercuric dinaphthylmethane disulfonate: Hg 4%)	do	do
Meran Tablet (Phenylmercuric acetate + Tollylmercuric-p-toluenesulfoanilide: Hg 3.2%)	do	do
PI Tablet (Phenylmercuric iodine: Hg 2.5%)	do	do
Hexa-Mercury Emulsion (2-oxy-2-phenylmercury-oxy-3, 5, 6, 3', 5', 6'-hexachloro-diphenylmethane: Hg 1.5%)	do	do
<i>Antibiotic-Copper-Mercury Preparation</i>		
TX-1 A Wettable { Dihydrostreptomycin 7.2% Basic copper sulfate: Cu 20% PMA: Hg 1%	1/800, 1/400, 1/200	do
TX-1 B Wettable { Dihydrostreptomycin 7.2% Basic copper sulfate: Cu 20%	do	do

Fungicide and ingredient	Concentration	Duration of dipping
TX-2 A Wettable { Aureomycin 5.2% Basic copper sulfate: Cu 20% PMA: Hg 1%	1/800, 1/400, 1/200	5 min.
TX-2 B Wettable { Aureomycin 5.2% Basic copper sulfate: Cu 20%	do	do
Aureo-Copper-Mercury Wettable	do	do
Aureo-Copper Wettable A { Aureomycin 5% Basic copper sulfate: Cu 20% PMA: Hg 1%	do	do
Aureo-Copper Wettable B { Aureomycin 5% Basic copper sulfate: Cu 20%	do	do
CM Copper-Mercury Wettable { Aureomycin 2% Basic copper sulfate: Cu 20%	do	do
{ Chloramphenicol 5% Basic copper sulfate: Cu 20%	do	do
8-Hydroxyquinoline sulfate 98%	625, 1250, 2500 5, 000, 10,000 ppm	1, 5 min.
<i>Dithane Stainless</i> (2-Ammonium ethylene bisdithiocarbamate 20%)	250, 500, 1,000 ppm	5 min.
<i>Invert Soaps</i> Septol (Benzalconium chloride 10%)	1/400, 1/200, 1/100	do
Hyamine-T (Benzetonium chloride 10%)	do	do
393 Emulsion (Octadecyl thiocyanate 20%)	250, 500, 1000 ppm	do

ing the effect of their application to the citrus orchards.

All the medicinal antibiotics and 8-hydroxyquinoline sulfate that do not contain surface-active agent were used with 0.1% polyoxyethylene-alkylether.

2. Determination of bactericidal effect

Skin of Satsuma orange was inoculated with pure cultured *Xanthomonas citri* PQ-1 and dipped into various fungicide preparations. The methods in detail are described as follows.

(1) Preparation of skin disks

Fresh fruit of Satsuma orange was surface-sterilized for two minutes with 0.1% mercuric chloride-80% alcohol solution, thoroughly washed and then peeled off. Disks of 6 mm diameter were punched out of the peeled skin with cork borer.

(2) Inoculation of skin disks

X. citri PQ-1 cultured in the standard potato decoction for one week at 25°C was used as inoculum. Disks were immersed into the inoculum suspension under low pressure provided by a suction pump. After the immersion, disks were transferred onto

sterilized filter paper and excessive moisture was discarded. Disks thus inoculated were immediately used for dipping treatment.

(3) Treatment

Forty disks per each treatment were dipped into 500 ml of respective fungicide preparation at 20°C. One half of the disks thus treated were thrown into sterilized water, gently rinsed and, after discarding excessive moisture, used for plating. The other half of the treated disks were, without rinsing, plated on the potato dextrose agar and incubated at 25°C. The effect of the treatment was determined after 72 hours by reading the number of disks from which developed an exudate of the growing causal bacteria. All treatments were replicated twice.

Experimental Results

Antibiotics

(1) Medicinal antibiotics

Good suppression of bacterial growth was obtained with three antibiotics belonging to the tetracycline

group but all the rest was found to be ineffective. As is shown in Figure 1, these three tetracyclines were highly effective against *X. citri*. The most effective of the three was acromycin, with which no growth was observed by the treatment of 50 ppm for 1 min. or 25 ppm for 5 min. When the disks were rinsed after treatment, the bacterial growth was suppressed at 100 ppm for 1 min. or 50 ppm for 5 min. Terramycin and aureomycin gave nearly the same inhibitory effect by suppressing the growth at 50 ppm for 5 min. In case of one minute dipping, however, aureomycin showed 100% inhibition at 100 ppm with no influence of rinsing, whereas terramycin was found to be more susceptible to the effect of rinsing.

(2) Emulsified antibiotic formulations

Four samples of antibiotic formulation were furnished for testing purposes. These formulations were specifically prepared to increase the permeation of antibiotics into the skin of Satsuma orange. As is shown

in Figure 2, all these formulations gave 100% inhibition at 100 ppm for 1 min. or 5 min. thus indicating more or less decrease in the effect when compared to the corresponding pure medicinal antibiotics. Among the three aureomycin formulations, aureomycin emulsion Takeda (TA-402) was relatively more effective than the other two, whereas aureomycin solution-Ihara is relatively superior in its freedom from the influence of rinsing. A remarkable decrease in the effect was observed with aureomycin emulsion-Ihara when disks were washed after dipping. Thus, there seems to be wide room for improvement in the mode of emulsification of these antibiotics.

Copper and Copper-Mercury Fungicides

Of the ten samples of fungicide furnished for this test, five were found to be more or less effective. Results obtained with these five fungicides are illustrated in Figure 3. It is to be noted that all these fungicides contain more than 20% of copper in the

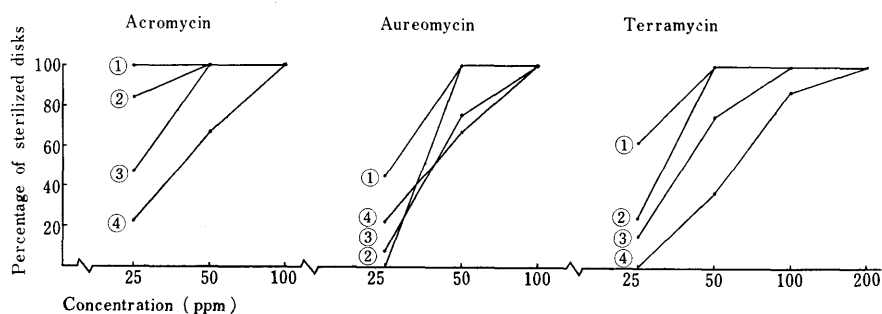


Figure 1. Effect of the antibiotics of tetracycline group
① 5 min. ② 5 min., washed ③ 1 min. ④ 1 min., washed

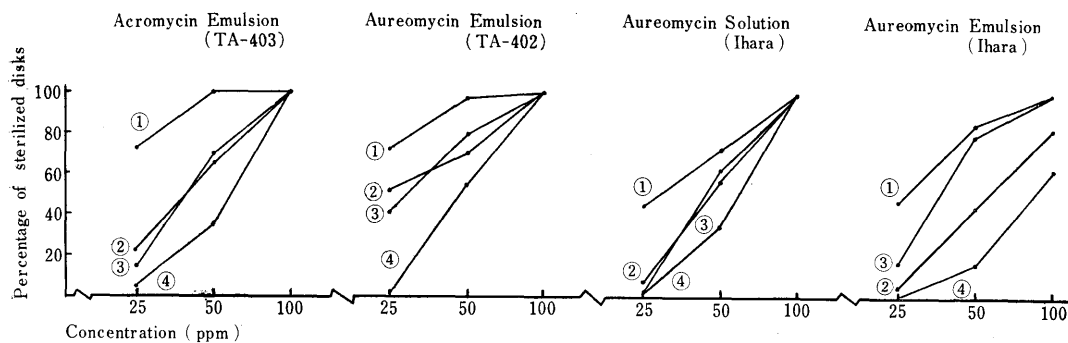


Figure 2. Effect of acromycin and aureomycin formulations
① 5 min. ② 5 min., washed ③ 1 min. ④ 1 min., washed

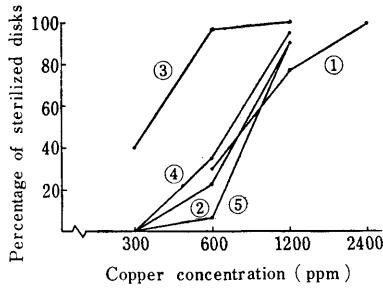


Figure 3. Effect of copper-containing fungicides
 ① Sankyo-KB 90 ② Borsin ③ Mer-Bordeaux 100
 ④ Fumiron-Bordeaux ⑤ Shin-Doh-Mix

form of basic copper sulfate as an active ingredient. Complete suppression was obtained with Sankyo-KB90 and Mer-Bordeaux at 1/200 dilution for 5 min. However, the effect in terms of copper content of these fungicides is not significantly different with only an exception of Mer-Bordeaux. Copper in the form of copper sulfate seems to be largely responsible for the inhibitory action of these fungicides.

Antibiotic-Copper-Mercury Formulations

The effect of three antibiotics when incorporated into copper or copper-mercury fungicides is shown in

Figure 4. These formulations were specifically prepared to obtain more powerful spray fungicides for the field control of citrus canker.

Dihydrostreptomycin, whereas it was not effective up to the concentration of 200 ppm when used singly, revealed a remarkable inhibitory action when combined with copper-mercury fungicide (See TX-1 A and TX-1 B). This synergetic effect was also noted with chloramphenicol to a lesser extent (See CM Copper-Mercury Wettable). Little change in the effect, however, was observed with aureomycin whether used singly or combined with copper-mercury fungicides.

8-Hydroxyquinoline sulfate, Dithane Stainless and Invert Soap

As is shown in Figure 5, 8-hydroxyquinoline sulfate completely suppressed the growth of *X. citri* by the treatment of 2,500 ppm for 1 min. or 1,250 ppm for 5 min. nearly free from the influence of rinsing. Complete suppression by Dithane Stainless was obtained at 1,000 ppm for 5 min. when rinsed and at 500 ppm for 5 min. when not rinsed after dipping. In terms of the concentration of active ingredient, the effect of Dithane Stainless is next to that of tetracycline group and is higher than the effect of basic copper sulfate

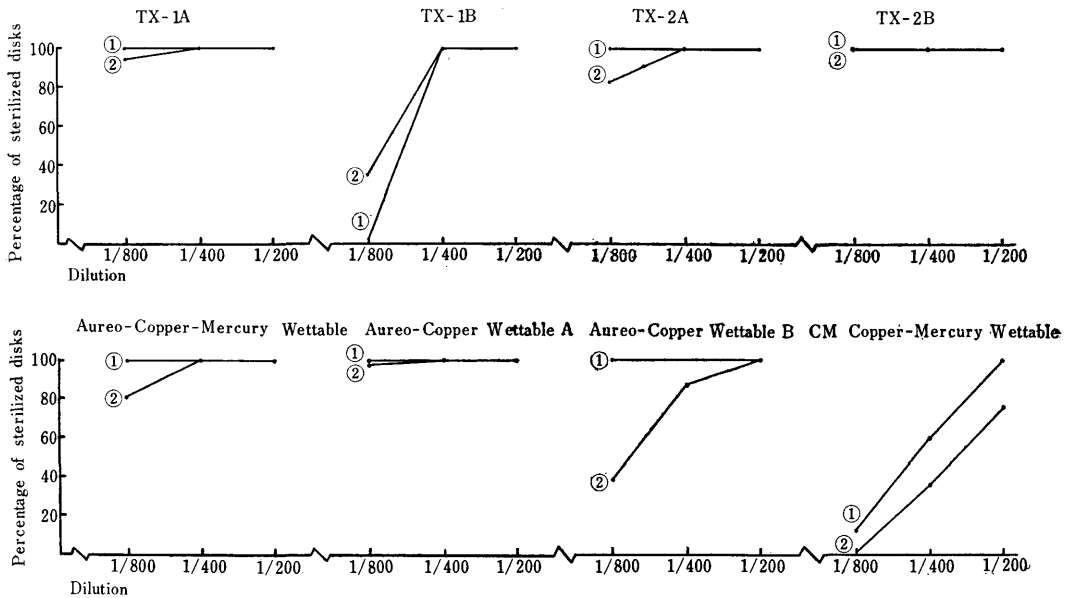


Figure 4. Effect of antibiotic-copper-mercury formulations
 ① 5 min. ② 5 min., washed

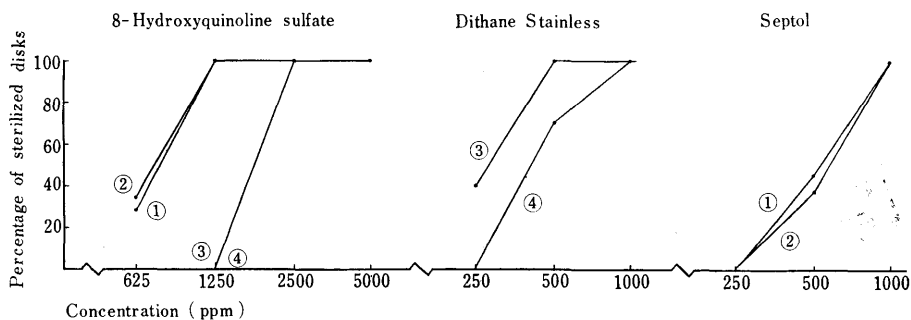


Figure 5. Effect of 8-hydroxyquinoline sulfate, Dithane Stainless and Septol

① 5 min. ② 5 min., washed ③ 1 min. ④ 1 min., washed

of copper fungicides. Of the two invert soaps tested, Hyamine-T was not effective, whereas Septol gave 100% inhibition at 1,000 ppm for 5 min. with no influence of rinsing observed.

Organic Mercury Fungicides

None of the five organic mercury fungicides was effective irrespective of the form or content of the active ingredient.

From the results obtained in this test, the fungicides employed can be divided as follows in terms of their inhibitory effect against *X. citri*.

Highly effective: Acromycin, aureomycin, terramycin, Dithane Stainless, Acromycin and Aureomycin Formulations (four samples), Dihydrostreptomycin-incorporated Copper and Copper-Mercury Fungicides (two samples), Aureomycin-incorporated Copper and Copper-Mercury Fungicides (five samples)

Effective: CM Copper-Mercury Wettable, Mer-Bordeaux, Borsin, Fumiron-Bordeaux, Sankyo-KB 90, Shin-Do-Mix, Septol, 8-hydroxyquinoline sulfate

Ineffective: Kanamycin, streptomycin, dihydrostreptomycin, chloramphenicol, neomycin, Doitsu-Bordeaux, TF 58 B, Sankyo-Mercuric Bordeaux, organic mercury fungicides (Fumiron Tablet, Takeda-Mer, Meran Tablet, Hexa-Mercury Emulsion), Copper wettable, Copper Oxinate, 393 Emulsion, Hyamine-T

Discussion

Some of the basic requirements for the disinfectants

applicable to the treatment of canker-infected fruit of Satsuma orange are that their bactericidal effect should be high enough to ensure complete sterilization by a shortest possible treatment, that there should be no residual toxicity to man and livestock and that they should not affect the marketability of treated fruit. As to the use of antibiotics, further considerations must be given to the possible induction of resistant strains of human pathogenic bacteria by the dose of antibiotic residues.

The writers in the present studies confirmed a remarkable inhibitory effect of three tetracyclines, i.e. aureomycin, acromycin and terramycin, against the causal bacteria of citrus canker, *Xanthomonas citri* (HASSE) DOWSON. These antibiotics are so far known to be least liable to induce resistant bacteria and thus considered to meet the requirements mentioned. Among the three tetracyclines, aureomycin has been used in the U. S. A. for the preservation of chicken within the maximum concentration of 7 ppm, whereas in Canada its application to raw fish at the concentration of 5 ppm has been authorized. A limited use of aureomycin is also authorized in Japan by incorporating it into ice at the concentration of 5 ppm for preserving raw fish. In addition, the fact that the skin of Satsuma orange is not for human consumption suggests that the tetracycline group is one and only antibiotics that would deserve a promise toward the application to fresh fruit of Satsuma orange.

Quick inhibitory action of these tetracyclines was indicated by 100% suppression of *X. citri* attained by the treatment of 100 ppm for 1 min. when not rinsed

after dipping. This may be an indication that these antibiotics would at least ensure complete surface sterilization of Satsuma orange contaminated with citrus canker bacteria by the treatment of very short duration provided that the contaminated fruit be thoroughly covered by chemicals either by dipping or spraying.

Of the three tetracyclines, acromycin is the most effective, while aureomycin is least susceptible to the effect of rinsing. As far as surface sterilization of harvested fruit is concerned, either one of these antibiotics may be applied at the concentration range of 50-100 ppm.

More or less decrease in the effect of tetracyclines was noted when they were formulated into liquid preparations, thus indicating wide room for improvement in the mode of emulsification of these antibiotics.

8-Hydroxyquinoline sulfate has been used in the United States for the disinfection of imported citrus seeds. In Japan, it has a limited use for the chemotherapy of silver leaf disease of apple caused by *Stereum purpureum* PERS. In the present studies, this compound was also found very effective against *X. citri* almost free from the rinsing effect. With no toxicity to man and livestock, this is considered to be one of the promising fungicides that may be applicable to the disinfection of Unshu orange.

Dithane Stainless showed an excellent inhibition, which, in terms of the content of active ingredient, follows antibiotics of tetracycline group. Because of its strong sulphureous odor, however, further test is needed to determine its value as a fruit disinfectant.

Of the two invert soaps tested, Septol was found to be effective at 1,000 ppm for 5 min. but the treatment of 1 min. duration was totally ineffective.

Among the copper, organic mercury and copper-mercury fungicides tested, good suppression was obtained with only those fungicides that contain basic copper sulfate as an active ingredient. Apparently, this may serve as a partial explanation for the satisfactory control of citrus canker by spraying Bordeaux mixture that has been traditionally practised throughout Satsuma orange growing areas.

Since the effect in terms of copper content is not significantly different among these fungicides, the copper in the form of basic copper sulfate seems to be largely responsible for the inhibitory action of these fungicides.

A limited screening of antibiotic-copper-mercury combinations revealed a positive synergism of dihydrostreptomycin when incorporated into copper or copper-mercury fungicides. A lesser degree of synergistic action was also observed with chloramphenicol in combination with basic copper sulfate. Results thus obtained seem to well justify more extensive screening of antibiotic-copper-mercury combinations for the search of more effective spray fungicides.

Summary

In the present report, the writers deal with the results of a screening test of effective fungicides for their possible application to the disinfection of Satsuma orange infected with citrus canker, *Xanthomonas citri* (HASSE) DOWSON. A number of copper, mercury and copper-mercury fungicides, that are apparently unusable for the fresh fruit treatment, were also tested for the purpose of assaying the effect of their application to the field control of this disease.

Of the eight antibiotics tested, only three antibiotics belonging to the tetracycline group were found to be remarkably effective against *X. citri* by the dipping treatment of 50-100 ppm for 1-5 min. and thus are considered to be applicable to surface sterilization, if not to the disinfection of cankered tissue, of Satsuma orange.

More or less decrease in the effect was observed with one acromycin and three aureomycin emulsified formulations specifically prepared to facilitate the permeation of antibiotics into skin tissue of Satsuma orange. There seems to be wide room for improvement in making liquid formulations of these antibiotics.

8-Hydroxyquinoline sulfate gave complete suppression at 1,250 ppm for 5 min. or 2,500 ppm for 1 min. With its stable inhibitory action, this compound is also promising toward its application to the fresh fruit treatment.

Dithane Stainless also showed an excellent inhibition.

Because of its sulphureous odor, further test is required to determine its value as a fruit disinfectant.

Among copper, mercury and copper-mercury fungicides employed, inhibitory effect was obtained with only those fungicides that contain basic copper sulfate as an active ingredient. These fungicides were effective at 1/200-1/400 dilution for 5 min. However, the effect in terms of copper concentration was not much different among these fungicides, thus indicating that copper in the form of basic copper sulfate is mainly responsible for the inhibitory action of these fungicides.

A limited screening of antibiotic-copper-mercury formulations showed a remarkable synergism of dihydrostreptomycin, thus justifying the need of more extensive screening of these antibiotic-copper-mercury combinations.

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摘 要

温州ミカン潰瘍病の防除に関する研究

I 病原菌に対する有効薬剤の検索

横浜植物防疫所調査課 永田利美・小畑琢志

温州ミカンの潰瘍病罹病果に対する適確な殺菌方法と本病の圃場における薬剤防除法を究明して無病果の生産をはかり、対米輸出解禁の資料とするため、本病病原菌 *Xanthomonas citri* (HASSE) DOWSON に対する有効薬剤の検索を試みた。

供試薬剤としては抗生物質 8 種、その製剤 (試作品) 4 種、銅剤 6 種、散布用水銀剤 5 種、銅水銀剤 4 種、抗生物質含有銅剤および銅水銀剤 8 種、逆性石鹼 2 種、硫酸オキシキノリン、ダイセステンレス、ロダン乳剤を用いた。

効果の検定は温州ミカン果皮のディスクに培養病原菌を接種し、これを各薬剤の所定濃度および時間で浸漬

し、ただちに標準ばれいしよ寒天平板に移し、25°C 72 時間、培養後、病原菌の発育を認めた果皮ディスクを数えて効果の比較を行なった。

実験結果は抗生物質のなかではテトラサイクリン系の 3 種、すなわちアクロマイシン、オーレオマイシン、テラマイシンがすぐれた効果を示し、他はいずれも無効であった。3 種のテトラサイクリンは濃度 50~100 ppm、浸漬時間 1~5 分の処理で、病原菌の発育を 100% 抑制し、果実表面に附着している潰瘍病細菌の殺菌に対しては充分実用に供することができると考えられる。

農薬用として試作されたアクロマイシンおよびオーレオマイシン乳剤は医薬用純品に比べて効果の劣る傾向が

認められ、これら抗生物質の製剤方法には改良工夫の余地のあることが示された。

硫酸オキシキノリンは 1,250 ppm-5 分または 2,500 ppm-1 分の浸漬により 100% の阻止効果を示し、浸漬後の水洗に対しても効果は安定していたので、本剤も果実の表面殺菌には利用できると思われる。

ダイセンステンレスの効果は有効成分濃度からみると非常にすぐれているが、本剤には特有の硫黄臭が強いので、果実処理よりは、むしろ圃場への利用を検討すべきであろう。

逆性石鹼のうちセプトールは 1,000 ppm-5 分間の浸漬で有効であったが、1 分間浸漬では全く効果が認められなかった。

圃場散布の効果を推定する意味で供試した銅剤、散布

用水銀剤および銅水銀剤のうち、有機水銀剤には効果が認められなかった。また銅剤、銅水銀剤を通じ、有効であったのは、いずれも有効成分として塩基性硫酸銅等を銅濃度で 20% 以上含有するもので、200~400 倍の範囲 (Cu として 1,000 ppm 以上) で特に効果が顕著であった。

オーレオマイシン、ジヒドロストレプトマイシン、クロラムフェニコールと銅剤、銅水銀剤との混合剤は、単用では効果を認めなかった。ジヒドロストレプトマイシンが混合によって著効を発揮し、クロラムフェニコールもある程度の相乗効果が認められたが、オーレオマイシンの効果は単用の場合と差異を認めなかった。今後の圃場防除剤としては、各種の抗生物質と銅剤または銅水銀剤との混用をさらに検討することが望ましい。