Retention, Tenacity and Effect of Insecticides in the Fungicidal Control of Apple Bitter Rot

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——사과 炭疽病 防除藥劑의 持續性 및 展着劑・殺蟲劑 混用의 効果——

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Summary

1. Retention of fungicides and effect of the mixing of spreaders and insecticides with fungicides in control of apple bitter rot were evaluated by "the assay of inhibition zones with apple exocarp disks."

2. The effectiveness of chemicals was reduced gradually as the time after treatment increased. Of all the chemicals tested, Difolatan retained approximately 60 percent of the original activity even after 15 days. Difolatan had the highest followed by Tuzet, Phaltan, Bordeaux mixture, and Delan, in that order.

3. The fungicidal acivity of Tuzet decreased with increasing application of simulated rain. The wash-off of Tuzet was reduced by adding spreaders. Dry skim milk and soybean extract were better than commercial chemicals such as Lino No. 1,2 and Tween 20.

4. The mixing of insecticides such as EPN, Folithion, Parathion and Lebaycid with Phaltan resulted in no significant differences in fungicidal effect even after 12 days of storage at room temperature.

INTRODUCTION

Apple bitter rot caused by *Glomerella cingulata* (St.) Spauld et Schroter is known to be one of the most important limiting factors in apple production in Korea. In the major apple growing district of Taegu, for example, there is about 5 to 10 percent loss of apple production due to the bitter rot annually. In the severe infestation of 1961 it was estimated that 40 to 50 percent of the crop was lost[9].

Many chemicals are commonly sprayed more than ten times throughout the year for effective control of bitter rot as well as other diseases and insect pests in Korea. As new chemicals are developed year after year investigations on control of the diseases and on the phytotoxcity to apple plants have been of great help to famers in choosing which chemicals to use.

In previous works, however, the emphasis in most of the experiments has been on the difference between the new chemicals and Bordeaux mixture for control of apple bitter rot[9)13]. Farmers also need to know how long the effect of sprayed chemicals will be retained, or how much of the chemicals will be washed off by rain during the wet season, and whether mixing fungicides with insecticides will be effective in the control of apple bitter rot.

The purpose of the present study is to investigate:

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(1) the retention of the several fungicides used in control of bitter rot, in order to regulate the spray intervals properly, (2) the effectiveness of several spreaders which are added to minimize the wash-off of chemicals which are sprayed in the wet season, (3) the effect of combining the fungicides with insecticides to reduce the labor of spraying.

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REVIEW OF LITERATURE

As extensive investigations have been made on the control of apple bitter rot in Korea[9, 13] and on the principles of fungicidal action [5], only literature closely related to the present paper is reviewed here.

Yano [18] reported that Tuzet retained its effectiveness for two weeks when sprayed at 1:3000 under "normal weather" for control of grape ripe rct, whereas spraying must be more frequent at lower concentration. In a similar study [3] Karathane persisted only a short time in the control of apple powdery mildew and thus should be applied in small amounts at frequent intervals, not exceeding two weeks. Mitchel et al. [12] reported that Dodine residues on apple fruit and foliage were greatly reduced even within ten days after application, depending upon the spray time, the amount of chemicals and variation in spray concentration; when the residues on fruit surfaces exceeded $0.75\mu g/cm^2$, infection apparently did not occur and lesions did not develop.

The amount of residues varies with different species of plants and types of chemicals. Rich [14] found that smooth celery leaves retained more Zineb deposit from a spray liquid than did hairy bean leaves, but that both types of foliage retained about the same amount of Bordeaux mixture deposit from a spray liquid. It has been shown by Wilson et al [17] that after ten days of weathering, the amount of tribasic copper sulfate retained on the foliage was 2.5 times as great on eggplant as on pepper.

It has generally been assumed that the best chemicals are not readily washed by rain and are stable to light and will not hydrolyze. Somer et al. [16] found that most of the particulate fungicides so far evaluated in weathering test adheres poorly when compared to Bordeaux mixture. Ishii et al. [6] examined the effect of washing the chemicals applied for the control of grape ripe rot with a simulated rain. The antibiotic potency of antimycin in a given simulated rain was analyzed by Leben [8] with leaf disk bioassay using *Glomerella cingulata* as the assay organism. He also found that with two inches of rain mixing with spreaders had a marked effect in control of tomato early blight.

Heuberger [4] devised the first quantitative bioassay of fungicide tenacity with tenacity coefficients. Similarly, wash-off curves were plotted by Chapman et al [2] and Keil et al. [7] from mortality counts of spores placed on differentially washed slides which had the same initial deposit of chemical. They showed that the protective value in the field on apple scab is satisfactorily predicted by the slope of the wash-off curve. Burchfield et al [1] found that effectiveness of Bordeaux formulations exposed to laboratory rain showed close agreement with copper analysis of deposits on banana leaves.

MATERIALS AND METHODS

The measurement of fungicidal effect

The result of preliminary experiment indicated that Difolatan which was sprayed on apples had marked fungicidal effect even after six months. Therefore, apples used were bagged during the spray period in the field. Because of negligible differences among the varieties of apples for the experiment, Ralls Genet was used throughout the experiment.

As the assay organism, *Glomerella cingulata* was isolated from diseased apple fruit and was grown on potato dextrose agar (PDA). The fungicidal effect was determined with a little modification of the leaf disk bioassay of Leben et al.[8] Twenty ml amounts of acidified PDA (pH 4.0) were allowed to harden in a series of petri plates and then flooded with a conidial suspension of 80,000 conidia/plate so as to grow uniformly on the whole surface of the agar plates.

Apples were surface-disinfected with 1% solution of NaOCl for 3-4 min., rinsed with sterile distilled water, and dried (2-4 hr.) prior to the chemical treatment in the expriment. The chemicals tested were sprayed with an atomizer as uniformly as possible on the apple surface until run-off was about to begin, dried (3-4hr.) and then exocarp disks were cut at random with a cork-borer 12mm in diameter.

The disks thus made were placed, sprayed side downward, on the surface of the agar, so that the chemicals could be brought into contact with conidia. The plates were incubated for 48 hr. at 26±2°C and the average diameters of inhibition zones were determined. Unless otherwise stated, three disks from each treatment were placed on each plate with four replications. The experiment was repeated three different times.

The fungicides tested

The fungicides listed below were sprayed on the surface of apples, dried, and then disks were taken from the exocarp immediately after treatment and 3, 9, 12 and 15 days later for the bioassay. The fungicides were: Bordeaux mixture, 2.6-2.6-100; Difolatan 80% 1:800 (N-tetrachloroethylthio 4-cyclohexene-1, 2-dicarboximide); Tuzet, 1:1400 (methylarasine-bisdimethyldithiocarbamate+Ziram+Thiram): Phaltan 50% 1:450 (N-trichloro- methylthio - phthalimide); Delan 1:500 (2,3-dicyano- 1,4-dithioanthraquraquinone).

The insecticides tested

Phaltan 50% 1:450 was mixed with the insecticides described below in the ratio of 1:1000. The bioassays were made with each suspensions right after treatment and 2, 6, 9, and 12 days later. The insecticides were: EPN 45% (O-ethyl-o-p-nitrophenylphosphonothioate); Folithion 50% (Dimethyl methylnitrophenylphosphonothioate); Lebaycid 95% (0,0-dimethyl 0-3-methyl-4 - methylthiophenylthiophosphate); Parathion 50% (Diethyl para-nitrophenyl thiophosphate).

The spreaders tested

Tuzet 1:1400 suspension was mixed with each of the following spreaders per 18 l base, and then tenacity of the fungicide was determined at a given simulated rain. The spreaders were: 4ml of Lino No. 1 and 2 (20,10% of Alkylphenolpolyethyleglycoether) and Tween 20, respectively: 10g of dry skim milk: 18ml of soybean extract made from 200g of ground soybean soaked for 24 hr. with 600ml of water.

RESULTS

Retention of fungicides

Apples treated with chemicals were kept in the greenhouse, in the open air of the roof and on laboratory desks with diffused sunlight in the beginning of October. The experimental results were found to be similar for the three cases. Therefore, averages of the three experimental results are presented in this paper. Two disks for each treatment were placed on one plate with three replications in each experiment.

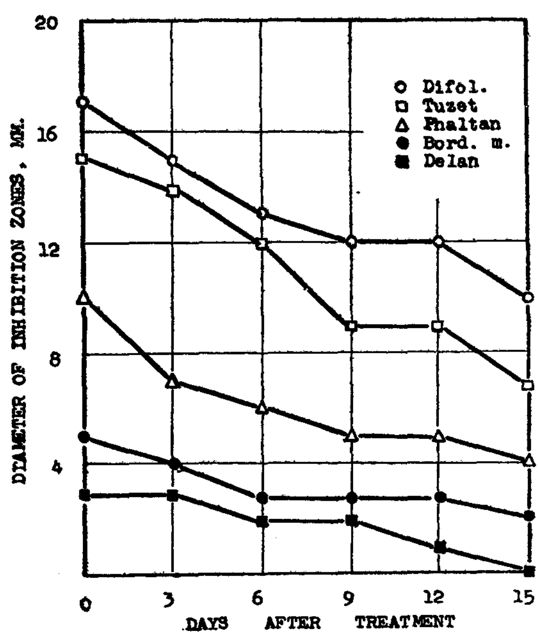

Fig. 1. Inhibition zones produced by apple exocarp disks treated with several fungicides at 0 to 15 days after treatment.

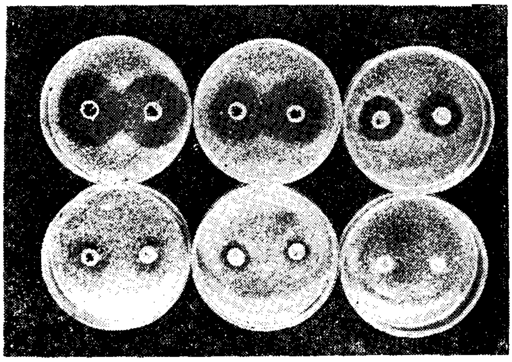

Fig. 2. Inhibition zones produced by apple exocarp treated with several fungicides on potato dextrose agar flooded with the assay fungus. From upper left. Difolatan, Tuzet, Phaltan From lower left: Bordeaux mixture, Delan, Control.

All the chemicals tested on the apple fruit surface on the same day showed broader inhibition zones than they did later with the same fruit. The effectiveness of chemicals was reduced gradually as the time after treatment increased. Of all the chemicals tested, Difolatan showed marked ability to retain fungicidal activity, with approximately 60 per cent of the original activity remaining even after 15 days. The others except Delan were approximately 40 percent as effective. The retention of the fungicides testd was highest for Difolatan, followed by Tuzet, Phaltan, Bordeaux mixture and Delan, in that order (Fig. 1, 2). The inhibition zones formed by concentrated mixture of 5.3-2.6-100 showed no difference in width from 2.6—2.6—100 and the zones were obscure.

Differences in retention of fungicidal activity due to the chemicals and to days after treatment were statistically significant at the 1% level.

Effect of spreaders

Simulated rain was produced by forcing tap water through a gun sprayer at 20 lb. pressure by a vacuum pump. The gun sprayer was directed at an angle of 45 degrees and at 60cm distance from the apples. Apples to be tested were placed on an electrically driven turntable which made 20 revolutions per minute (Fig. 3). Two treatments were used of approximately 20 and 40mm. of water, respectively.

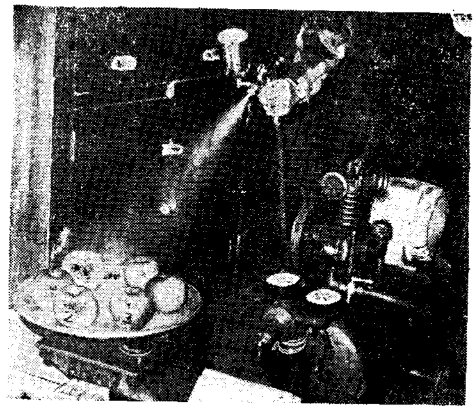

Fig. 3. Production of the simulated rain (details in the text)

The spreaders used had little initial effect on the fungicidal activity of Tuzet but the more the simulated rain, the greater was the decrease in fungicidal activity (Fig. 4). In the control, the fungicidal effect at 20mm of simulated rain was decreased to half that at zero, and the effectiveness of the chemical could hardly be detected at 40mm. The effect of commercial spreaders, such as Lino No. 1, 2 and Tween 20 was similar: one third of the original activity remained at 40mm. However, the fungicidal activity of Tuzet was retained much more effectively with dry skim milk or soybean

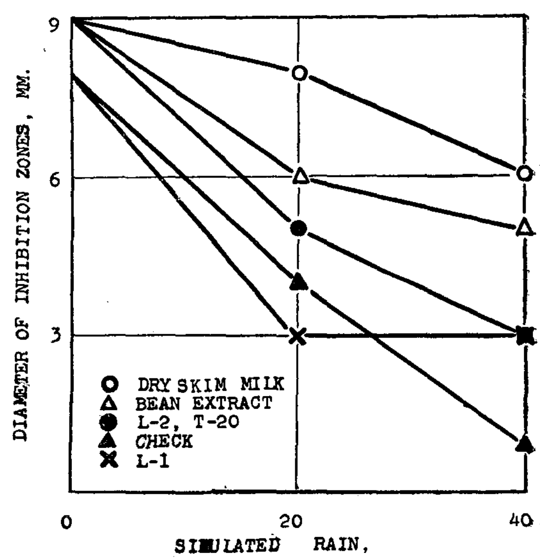

Fig. 4. Fungicidal activity of Tuzet with dry skim milk, soybean extract, Lino No. 1.2 or Tween 20 after apples were washed with various amounts of simulated rain.

extract than with the commercial spreaders regardless of the amounts of simulated rain (Fig. 4).

The differences of fungicidal activity remaining due to the spreaders tested and to the amounts of simulated rain were statistically significant at the 5 and 1% levels, respectively.

Effet of insecticides

When Phaltan 50% 1: 450 was kept as long as 12 days at room temperature, loss of the fungicidal activity was negligible. In addition, the mixing of insecticides such as EPN, Folithion, Parathion, or Lebaycid with Phaltan resulted in no significant differences in the fungicidal effect even after 12 days of storage at room temperature. It is, therefore, concluded that under the condition of these tests the insecticides had little if any effect on the fungicidal activity of Phaltan. The effect of the fungicidal preparation on the insecticidal properties of the various insecticides was not tested.

DISCUSSION

It would be desirable, of course, to correlate laboratory tests of fungicidal action with actual field performance. However, field trials require much cost, labor and time. In addition, it is extremely difficult to evaluate field results because of the influence of environmental factors and variability in host surface. In laboratory evaluation several factors can be controlled uniformly, results can be compared with previous findings readily and it is possible to screen many chemicals within a limited time.

The usual laboratory assay methods use weathering tests that measure inhibition percentage of spore germination due to the chemical on slide and the spore germination after shaking the chemicals on slide in water. According to McCallan[11] published reports on the correlation of slide germination tests with field results on protectant fungicides are rare. Since direct or indirect or interaction may occur between fungicides and host surface, there is need for reconsideration of these assay methods. In the present paper, the fungicidal effect was evaluated by "the inhibition zones with exocarp fruit disks," which was modified from the leaf disk assay of Leben[8] and was similar to the detached leaf technique of Schmidt.[15]

The effects of the several fungicides used in this study were more or less correlated with experimental results in the field for 5 years (1962-1966) by the Institute of Plant Environment, Office of Rural Development[13]. This suggests that in the future this assay method can be applied for the selection of chemicals not only in controlling apple bitter rot but also for similar diseases.

In an actual field situation, enough of a protectant material must be retained on the plant surface for the pathogen to take up an inhibitory dose. Rich[14] examining the behavior of Zineb and Bordeaux mixture found that with Bordeaux miture the larger the initial deposit, the smaller the percentage lost, whereas with dried Zineb particles the opposite was true. Martin[10] showed from an example of sulfur particles that the smaller the particles of chemical, the greater the tenacity. Results of the present study indicated that the organic fungicides generally had longer retention than Bordeaux mixture. In the preliminary experiment, Difolatan had marked fungicidal effect even six months after treatment, showing a good contrast with Bordeaux mixture. Although the retention mechanism was not examined in the present study, it should be investigated in order to regulate the range of spray intervals for practical use.

It is known that the most important factors leading to the reduction of the residues of sprayed chemicals are wash-off of residues by rain and dew[10]. Since epidemics of apple bitter rot occur particularly in the wet season, it seems inevitable that the residues of chemicals will be washed by rain. It is remarkable that, of all the spreaders tested, the dry skim milk and the wet soybean extract gave significantly higher retention of fungicidal effects than the commercial spreaders such as Lino No. 1,2 and Tween 20. However, these results must be checked against field performance, considering the interaction between fungicides and host under the influence of enviromental factors. In addition, these experiments were limited to biological assay, and should be accompanied by chemical analysis in order to be more reliable.

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摘　　要

1. "果皮圓板沮止帶法"으로 사과 炭疽病 防除藥劑의 持續性, 展着劑 및 殺蟲劑混用의 效果를 檢定하였다.
2. 殺菌劑를 뿌린 後 時日이 경과할 수록 殺菌力은 줄었다. 供試 藥劑 中에서 Difolatan의 藥効가 가장 컸으며 15日 후에도 60%의 殺菌力이 있었고 그 다음은 Tuzet, Phaltan, 보르도액, Delan의 順位였다.
3. 人工降雨量이 많을수록 Tuzet의 藥効는 줄었으나 展着劑를 添加함으로써 藥劑의 流失을 輕減할 수 있었다. 市販 商品인 리노 1,2號 및 Tween 20 보다도 脫脂粉乳 및 콩抽出液의 効果가 현저히 좋았다.
4. Phaltan을 單用하는 것보다 EPN, Folithion, Parathion 및 Lebaycid를 섞어서 室溫에 12日間 두어도 어느 것이나 殺菌効果에는 현저한 차이가 없었다.