

Effects of Fungicides on Sporulation of Apple Leaf Spot *Alternaria mali* Roberts

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사과 斑點落葉病菌의 孢子形成에 對한 殺菌劑의 效果

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Abstract

Regardless of the kind of the apple orchards whether received various fungicides application or not, Rovral exhibited the most efficient inhibitory effect on the sporulation of *Alternaria mali* with the spotted leaf disks at the recommended dose $0.5\mu\text{g/ml}$ as well as the lower or the higher doses.

When applied at the recommended doses $0.7\mu\text{g}$, $0.75\mu\text{g}$, and $0.7\mu\text{g/ml}$ of Antracol, Dithane M-45, and Dikar, respectively, these fungicides showed complete inhibitory effects on the sporulation. At the lower levels of application, they allowed the sporulation of 10,000 to 25,000 conidia/ml.

Polyoxin and Difolatan, when applied on the leaf disks from the orchard that had received the fungicides application, exhibited low effects allowing the sporulation of around 20,000 conidia/ml. On the leaf disks from the orchard that had received no fungicide application, however, both fungicides showed relatively high inhibitory effects of the sporulation.

The inhibitory effects of Captan, Dakonil, and Spat on the sporulation were lower than those of the aforementioned fungicides. Bayleton was the least effective among the fungicides used in the present investigation. Benlate showed promoting effect on the sporulation of the apple leaf spot fungus rather than inhibiting.

INTRODUCTION

Since several years ago, apple orchard trees all over the country including Daegu region have been suffering from the serious leaf spots a few months earlier than healthy leaves.

As early as 1924, Roberts⁷⁾ identified the fungus involved in the peculiar spotting of apple leaves in the United States as *Alternaria mali* Roberts. This fungus was unable to originate the leaf spots

but enlarge it only through the point of tissue killed by *Physarospora cydoniae* or chemical application. The leaf spot fungus currently causing the epidemic in Korea is different from that reported in the United States but it is similar to the type of *A. mali* identified by Sawamura^{9,10)} in Japan in view of its ability to originate the spotting without any predisposition of apple leaf^{6,9,14)}

As this fungus can infect not only apple leaf but its fruit also from the early period of growth in the spring, it has become one of the greatest thr-

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eats to production of apple in Korea.

The results of tests with several fungicides on the epidemic by Sawamura¹⁰⁾ in 1969 and by Lee⁶⁾ in 1972 indicated that Polyoxin and Difolatan were the most effective for control of the pathogenic fungus. Since then many other fungicides have been introduced for the disease control while no further test of these fungicides has been reported up to this date. Therefore, it is considered necessary to reevaluate the effects of various fungicides including those introduced recently. The present investigation aimed to clarify the effects of such fungicides as are being used by apple growers on the sporulation of the apple leaf spot fungus in Korea.

Authors extend their sincere gratitude to professors Kwang-Yeon LEE and Kyu-Rae KIM for their encouragement and the part of the experimental materials provided for the present investigation.

MATERIALS and METHODS

Preparation of spotted leaf disks:

At one apple orchard located in the midst of apple orchards area where various fungicides have been applied for several years and at another apple orchard located in an isolated area where no chemicals have been applied since it was establish-

ed nine years ago, fourth or fifth leaves from the top of similar branches with *Alternaria* leaf spots were collected separately by the orchard and carried to the author's laboratory for the tests. The leaves were washed more than five times in tap water and dried at room temperature.

Using a leaf cutter of 2.5mm diameter specially prepared with a metal tube, the leaf disks were cut out at edge of the leaf spots so as to include healthy and spotted tissues approximately half and half. Enough number of the leaf disks were completely mixed separately by the orchard, soaked in 0.5% sodium hypochlorite for five minutes, and then rinsed in sterile distilled water before use for tests of the fungicides.

Application of fungicides:

Each of the eleven fungicides (Table 1) was added to 1.5% water agar before solidification after autoclaving so as to make the five levels of concentration, namely, standard recommended dose to apple growers, one-third, one-ninth, three times and nine times concentrations in the active ingredients of the recommended dose, and then poured in petri dishes of nine centimeters diameter. Prior to plating, 36 leaf disks were dipped in each concentration of fungicides solution and then 12 leaf disks per petri dish were placed in three replication so as to have the underside leaf surface contacted

Table 1. Fungicides tested for their effects on the sporulation of *Alternaria mali* with the spotted apple leaf disks.

| No. | Fungicide | Active ingredient | Formulation |
|-----|--------------|--|-------------|
| 1. | Antracol | Zinc propylene bis dithio carbamate | Wp 70 |
| 2. | Bayleton | 1-(4-chlorophenoxy)-2,3-dimethyl-1-(1H-1,2,4-triazole)-1-yl-2-butanone | Wp 76 |
| 3. | Benlate | Methyl-1-(butyl carbamoyl)-2-benzimidazole carbamate | Wp 50 |
| 4. | Captan | N-trichloro methyl thio-4-cyclohexene-1,2-dicarboximide | Wp 50 |
| 5. | Dakonil | Tetrachloro isophthalo nitrile | Wp 75 |
| 6. | Difolatan | N-tetrachloro ethyl thiotetra hydro phthalimide | Wp 80 |
| 7. | Dikar | Zinc complex manganese ethylene bis dithio carbamate 66%+2-(1methyl heptyl)-4,6-dinitro crotonate 4.68% | Wp 70.68 |
| 8. | Dithane M-45 | Zinc complex manganese ethylene bis dithio carbamate | Wp 75 |
| 9. | Polyoxin | Polyoxin-ALB | Wp 10 |
| 10. | Rovral | 1-isopropyl carbamoyl-3-(3,5-dichloro phenyl) hydantoin | Wp 50 |
| 11. | Spat | N-(A-fluorophenyl)-2,3-dichloro makimide | Wp 75 |

with the amended water agar plate. The leaf disks thus treated were kept in an incubator at about 27° under 12 hours alternate light and darkness. The light source was a standard white fluorescent tube of 1,200lux at about 35cm distance

Evaluation of fungicidal effect on sporulation:

In seven days of incubation, the culture of the fungal sporulation on the spotted leaf disks were fixed by pipetting ten milliliters of 0.5% HgCl₂ solution per petri dish. After complete aegatation until all the conidia formed on the leaf disks were detached, they were counted by using the standard neubauer haemocytometer under an optical microscope with substage illumination.

RESULTS

The average number of *Alternaria mali* conidia sporulated on the 12 leaf disks per petri dish of the control plot in three replication was consistently around 30,000/ml regardless of the apple orchards where the spotted leaf disks were prepared from.

Among the fungicides used in the present tests, Rovral exhibited the excellent inhibitory effect on the sporulation with the leaf disks from both orchards whether various fungicides were applied or not. No sporulation was shown by the application of the recommended dose 0.5µg/ml. At one-third concentration 0.17µg/ml, around 3,000 conidia were produced and even at the lowest concentration 0.06µg/ml, this fungicide could suppress the sporulation down to approximately 5,000 conidia.(Fig. 1).

Antracol was effective next to Rovral in inhibition of the sporulation. At the recommended dose 0.7µg/ml or the higher levels, this fungicide allowed no sporulation at all. At the lower levels of the dose, however, this chemical allowed the sporulation of 10,000 to 20,000 conidia which were three to four times higher than with Rovral.

Dithane M-45 and Dikar exhibited very similar inhibitory effects on the sporulation with the leaf disks from both orchards. At the recommended or at the higher doses, the sporulation was completely inhibited but the number of conidia produced was

about 15,000 and 25,000 conidia at the lower and at the lowest doses, respectively. These fungicides were slightly inferior to Antracol for inhibition of the sporulation.

Polyoxin could not efficiently inhibit the sporulation at the recommended dose 0.1µg/ml when applied to the leaf disks from the orchard where various fungicides have been applied for several years. Even at the nine times high concentration 0.9µg/ml, this fungicide failed to suppress the sporulation allowing around 13,000 conidia. When applied to the leaf disks from the orchard where no fungicide has been applied, the effect of this fungicide on inhibition of the sporulation was very high except at oneninth concentration of the recommended dose that allowed the sporulation of about 10,000 conidia. Although around 5,000 conidia were produced at one-third concentration 0.03µg/ml, the sporulation was almost completely inhibited at the recommended dose or at the higher levels.

The effect of Difolatan was similar pattern to that of Polyoxin though the sporulation was lower than that of the control plot. This fungicide was not so effective when it was applied on the leaf disks from the orchard where received the various fungicides application. However, the inhibitory effect of this chemical on the sporulation with the leaf disks from the orchard where received no fungicide application was still very high except at the one-ninth concentration 0.09/ml that allowed the sporulation of about 20,000 conidia. Approximately 5,000 conidia were produced at the recommended 0.8µg/ml and at its onethird concentration 0.27µg/ml. Even at the nine times high concentration 7.2µg/ml, this fungicide could not completely suppress some sporulation.

When applied on the leaf disks from the orchard where received various fungicides application, Spat allowed the sporulation of 15,000 to 20,000 conidia at all the five levels including the recommended dose 0.75µg/ml. This fungicide when applied on the leaf disks from the orchard where received no fungicide application, however, could reduce the sporulation down to less than 15,000 conidia at the recommended dose and to about 10,000 conidia at

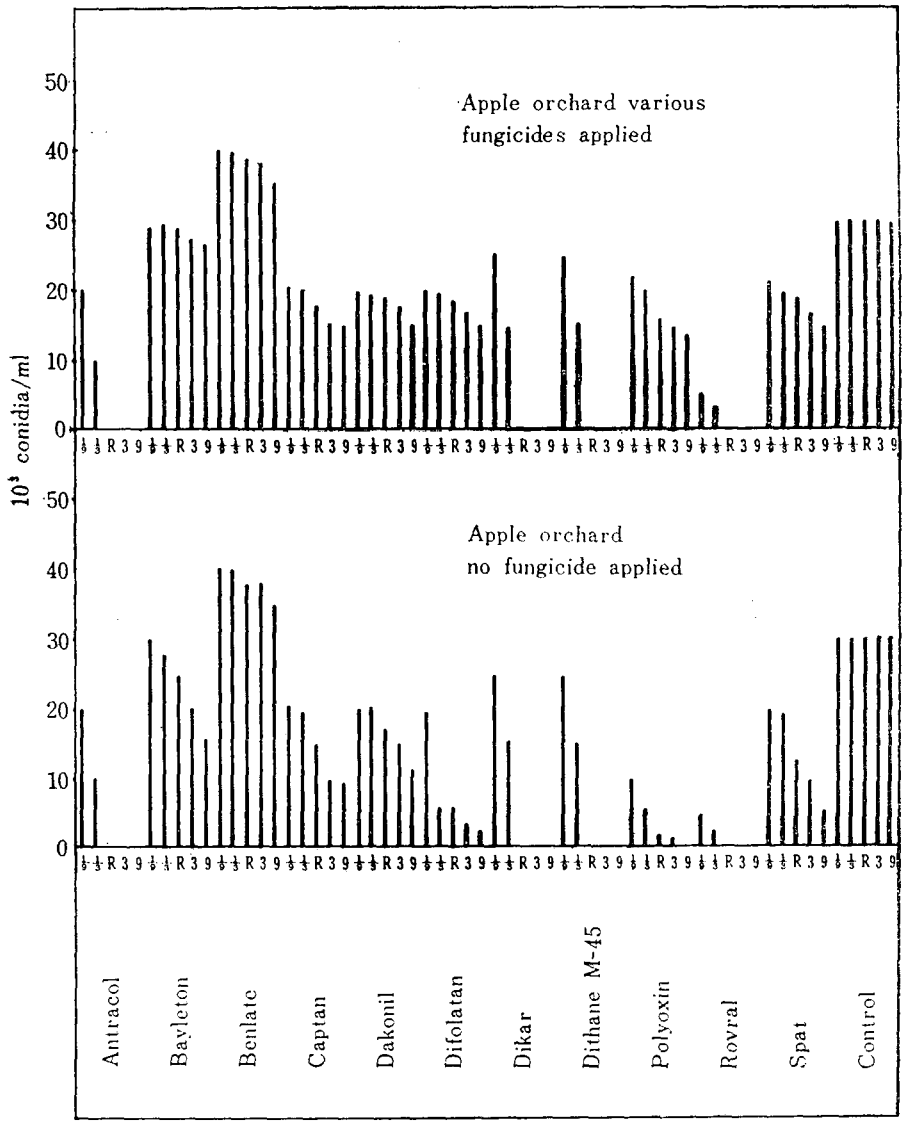


Fig. 1. The effects of eleven fungicides on the sporulation of *Alternaria mali* on the spotted leaf disks prepared from one apple orchard with various fungicides application for several years and another apple orchard with no fungicide application. The five levels of approximate doses in the active ingredients were as following: R: recommended dose to apple growers, 1/3: one-third strength, 1/9: one-ninth strength, 3: triple strength, 9: nine times strength of the recommended doses.

| Fungicides | Approximate doses in active ingredients ($\mu\text{g/ml}$) | | | | |
|--------------|--|------|------|------|------|
| | 1/9 | 1/3 | R | 3 | 9 |
| Antracol | 0.08 | 0.23 | 0.70 | 2.10 | 6.30 |
| Bayleton | 0.08 | 0.25 | 0.76 | 2.28 | 6.84 |
| Benlate | 0.06 | 0.17 | 0.50 | 1.50 | 4.50 |
| Captan | 0.06 | 0.17 | 0.50 | 1.50 | 4.50 |
| Dakonil | 0.08 | 0.25 | 0.75 | 2.25 | 6.75 |
| Difolatan | 0.09 | 0.27 | 0.80 | 2.40 | 7.20 |
| Dikar | 0.08 | 0.24 | 0.71 | 2.13 | 6.39 |
| Dithane M-45 | 0.08 | 0.25 | 0.75 | 2.25 | 6.75 |
| Polyoxin | 0.01 | 0.03 | 0.10 | 0.30 | 0.90 |
| Rovral | 0.06 | 0.17 | 0.50 | 1.50 | 4.50 |
| Spat | 0.08 | 0.25 | 0.75 | 2.25 | 6.75 |

its triple strength $2.25\mu\text{g/ml}$, but could not completely suppress the sporulation even at the nine times dose $6.75\mu\text{g/ml}$.

Captan and Dakonil showed about similar effects on the sporulation as Spat. Of the former two fungicides, Captan was a little more effective than Dakonil only when the higher doses than the recommended were applied on the leaf disks from the orchard where received no fungicide application. In this case the number of conidia was around 10,000.

When applied on the leaf disks from the orchard where received various fungicides application, Bayleton showed no inhibitory effect on the sporulation allowing to produce nearly 30,000 conidia which is not different from the sporulation at the control plot. The recommended dose $0.76\mu\text{g/ml}$ of this fungicide showed a trend of inhibitory effect on the sporulation allowing around 25,000 conidia when applied on the leaf disks from the orchard where received no fungicide application. Even at the higher $2.28\mu\text{g/ml}$ and the highest $6.8\mu\text{g/ml}$, the inhibitory effect was not marked allowing the sporulation of about 20,000 and 15,000 conidia, respectively.

Benlate had no inhibitory effect on the sporulation of the leaf spot fungus regardless of the orchards whether various fungicides applied or not. This fungicide promoted the sporulation rather than inhibited it at all the five levels of the chemical application.

DISCUSSION

Although Rovral exhibited the most efficient inhibitory effect on the sporulation of apple leaf spot *Alternaria mali*, this fungicide has been introduced recently in Korea and released for use by apple growers in 1980 so that it has not enough opportunity yet to know whether it induces tolerance of the fungus or not. It may be required to detect the possibility in an early time(5,8).

Antracol, Dithane M-45, and Dikar seemed to be effective and relatively stable fungicides to suppress the sporulation on the spotted leaf disks from both apple orchards (3, 4, 11). Nevertheless, special attention should be paid on the fact that the

lower doses than the recommended were not effective. Since all of the three fungicides are zinc containing compounds, this element must be playing the major role for inhibition of the fungal sporulation. Even if Dikar consists of Dithane M-45 plus 2-(1-methyl heptyl)-4, 6-dinitro crotonate, this additional chemicals seemed not to exhibit any prominent effect on the sporulation.

In contrast to the results reported that Polyoxin and Difolatan were the most effective for inhibition of *A. mali* sporulation (6, 7, 11, 12), both fungicides showed no satisfactory effects for inhibition of the fungal sporulation in the present investigation. This discrepancy may be due to development of the fungal tolerance to these fungicides in the light of the present results that the fungus showed its abundant sporulation on the spotted leaf disks from the orchard where received the fungicides application while it showed markedly reduced sporulation on the spotted leaf disks from the orchard where received no fungicide application(1, 2, 13).

Captan, Dakonil, and Spat seems to have considerable effects on inhibition of the sporulation(11, 12). However, so long as there are more effective fungicides as mentioned above, these fungicides are skeptical for recommendation provided that the similar effects are to be exhibited in the field conditions. Bayleton was little effective while Benlate was promoting the sporulation rather than inhibiting it.

The results of this present investigation may not be applicable as they are for control of the fungal sporulation at apple grower orchards because the spotted leaf disks in the semivivo condition must be in different physiological or biochemical conditions from the vital apple leaves with the *Alternaria* leaf spots hanging on alive apple trees. Before practical application of the results obtained in the laboratory, it is required to conduct further tests of the fungicides on the fungal sporulation at controlled field conditions of apple orchards.

摘 要

各種 殺菌劑를 多年間 使用한 사과園과 全然 使用하지 않았는 사과園에서 採取한 斑點落葉病 罹病葉 圓盤

에 各殺菌劑를 處理한 後 *Alternaria mali*의 分生孢子 形成을 測定한 結果는 다음과 같다.

殺菌劑 使用如否와는 關係없이 兩사과園의 罹病葉圓盤上에서 共히 Rovral은 그 勸獎濃度 0.5 μ g/ml 및 보다 低濃度와 高濃度에서 가장 높은 孢子形成抑制效果를 보였다.

Antracol, Dithane M-45, 및 Dikar는 各各 그 勸獎濃度 0.7 μ g, 0.75 μ g, 및 0.7 μ g/ml 그리고 보다 高濃度에서 兩사과園의 罹病葉圓盤上의 孢子形成을 完全히 抑制하였으나, 보다 低濃度에서는 10,000~25,000/ml의 孢자를 形成시켰다.

各種 殺菌劑를 使用한 사과園의 罹病葉圓盤上에서 Polyoxin 및 Difolatan은 孢子形成抑制效果가 낮아서 對照區의 30,000/ml에 比하여 20,000/ml의 孢자를 形成시켰으나, 殺菌劑를 使用하지 않았는 사과園의 罹病葉圓盤上에서는 比較的 높은 孢子形成抑制效果를 보였다.

Captan, Dakonil, 및 Spat의 孢子形成抑制效果는 上記한 殺菌劑보다 낮았으며, Bayleton은 사과斑點落葉病菌의 孢子形成抑制效果가 거의 없었고, Benlate는 오히려 그 孢子形成을 促進시켰다.

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