ASSAY OF ERADICANT CHEMICALS ON ASCOSPORES OF *VENTURIA INAEQUALIS*

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This is to certify that the

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Assay of eradicant chemicals on ascospores of *Venturia inaequalis*

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ASSAY OF ERADICANT CHEMICALS ON ASCOSPORES OF *VENTURIA INAEQUALIS*

By

Nicholas G. Vredeveld

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VENTURIA INAEQUALIS

ABSTRACT

Apple scab, a disease of apples, is caused by *Venturia inaequalis*. Control proceedures for this prevalent disease have utilized protectant sprays. The inconsistent results and phytotoxicity of these sprays, namely lime-sulfur and Bordeaux, led experimenters to reduce the primary inoculum potential by eradicant ground sprays on overwintering leaves. This would allow better control by milder foliage sprays. Elgetol, a dinitro produce, has been proven effective. The present experiment was carried out to test the properties of other chemicals for eradicant ground spraying and to test a laboratory method of assay.

Tests showed that DN 289 was superior to the other materials tested. Aerocyanamid dust showed some promise while the sodium cyanamide salt, the mercury compounds, and Actidione were of less value according to this test. The results of spraying leaves in screens and dipping leaves in solutions showed correlation with the simulated ground spraying application.

These results corroborate the experiments of others showing the effectiveness of dinitro compounds for orchard floor sprays. Cyanamid dust may have considerable merit as a floor application, judging from one experiment, but further work is needed to determine its real effectiveness. It is possible that it would need special moisture conditions following application to merit orchard use.

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Nicholas G. Vredeveld

Botany and Plant Pathology

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INTRODUCTION

Apple scab, a disease caused by <u>Venturia inaequalis</u>, is consistently the worst pest of apple orchards in the northern states. Infection by this disease is enhanced by cool, wet springs common in Michigan and Wisconsin. Recommended spray proceedure in the early part of the 1930-40 decade was five sprays of lime-sulfur applied according to the changing bud stages through the primary spore discharge period. In most years this method held the disease in check; but in years when critical rainfall did not coincide with timely applications, the disease was not well controlled. Coupled with inconsistent control, the use of lime-sulfur caused much spray damage on the fruit. With the increased stress on good fruit quality and realization that lime-sulfur has been reducing yields, it is desirous to use chemicals which are less phytotoxic.

The primary inoculum of apple scab is the ascospores. These ascospores are produced in perithecia which develop from the mycelium overwintering on dead apple leaves under the tree. The dosage of the primary inoculum and suitable rainfall-temperature relationship are the major factors in determining the intensity of the disease development (21). Keitt et al (19) showed that the ascospore quantity can be lowered by applying a dormant spray to the dead leaves on the ground. He found that Elgetol, the sodium salt of dinitro-ortho-cresol, if applied at 0.5% concentration and using 500-600 gallons of spray per acre could reduce the primary inoculum by more than 90%. Such a reduction would allow the use of mild, less toxic fungicides to prevent primary infection by the few ascospores which do escape the ground spray. This eradicant system has been recommended in several states.

There are several drawbacks to existing ground spraying techniques. The large amount of water required per acre is limiting in some areas. The need for special equipment which can be used only once each year is another. The fact that a large boom is difficult to maneuver in the low branched trees of Michigan orchards is a hindrance and the method is uncertain of success where nearby adjacent areas are unsprayed.

The published data dealing with ascospore eradication have been obtained from orchard results. Under such conditions an entire season is expended for each set of observations. In a situation where the primary inoculum is subject to dissemination by wind, considerable distances are necessary between the treated and the check plots, and between differently treated plots. It is nearly impossible to find isolated orchards with similar environments which are subject to the same disease-influencing factors.

These considerations lead us to consider a laboratory approach for evaluating other chemicals as possible agents to be used against scab. It would be exceptionally desirable to have an acceptable laboratory method of assay which would correlate with field results. Other cheaper and possibly better eradicant fungicides that might replace Elgetol require evaluation for scab control. This study attempted to compare several possible materials using laboratory techniques.

REVIEW OF THE LITERATURE

Apple scab occurs throughout the world wherever apples are grown with the exception of a few arid irrigated regions. The causal fungus has been known since 1819 when Fries described the conidial stage in Sweden. In 1894 Aderhold (2) in Germany found the overwintering stage of the fungus and thus completed the knowledge of the life cycle. Clinton (7), a worker in Illinois, in 1901 confirmed the findings of Aderhold by isolation and inoculation methods. Realizing that part of the control measures could be aimed at the overwintering stage, Clinton recommended the practice of sanitation consisting of picking up or plowing under the leaves from the previous season.

Concerning the protective measures which were used against the disease, it can be said that Bordeaux was the early mainstay of the industry. Keitt and Palmiter (23) are of the opinion that the success which was obtained with Bordeaux was responsible for the lack of complimentary control measures. In 1909, lime-sulfur was introduced as an apple scab spray because it caused less russeting of the fruit than Bordeaux. Keitt (21), however, noting the occasional failure of these protectant fungicides to control scab by following existing spraying schedules, began a study of the epidemiology of the disease.

By observing the relationship between time, amount of ascospore discharge in the spring, the weather conditions (particularly rainfall and temperature), the severity of the disease, and after considerable search for overwintering conidia, he concluded that the ascospores comprised the only important primary inoculum, and that the quantity of this inoculum was directly related to the amount of scab and its control. Spray recommendations at that time were aimed at control on the fruit. Leaf infection, which can be initiated over a longer period of time than fruit infection, built up the inoculum potential to a dangerously high level for the following spring. Besides the inability to reduce the amount of overwintering fungus, the available protectant fungicides were too toxic to the plant, causing objectionable visible and innate host injury. Concurrently rising standards of fruit quality made the use of milder materials mandatory for scab control during the growing season. Milder materials need more frequent applications--an added expense--or complimentary inoculum reduction for adequate disease control.

Keitt turned his attention toward a method of combatting the scab organism directly with attempts at eradication. It was noted already that an old practice of turning under the old leaves even when it was feasible did not materially reduce the threat of scab infection. Also, a so-called "clean up" spray of Bordeaux or copper sulfate applied after harvest did not cause a reduction in ascospore development (23). The two most vulnerable points in the disease cycle for eradication were considered to be: (1) the post harvest period before the infected leaves had fallen and when the tree could withstand a more drastic treatment than would be possible during the growing season, and (2) during the dormant period when the mycelium is confined to the dead leaves on the orchard floor.

For the post harvest foliage sprays Keitt (23), during the period of 1924 to 1935, tried a number of materials considered to have fungicidal value. Sprayed leaves were removed from the tree and placed in cloth mesh bags. These were laid on the ground and held intact at the corners by spikes. After overwintering on the ground they were brought into the laboratory and inspected for the number of perithecia that developed per unit area of leaf surface. Sprays consisting of certain arsenites mixed with Bordeaux proved most efficient in inhibiting the perithecial formation. The main disadvantage of such mixtures was their toxicity to the tree. Keitt finally abandoned this method in favor of attacking the overwintering inoculum in the dead leaves on the ground.

The mycelium in these leaves and the concurrent ascigerous stage offered the second vulnerable phase for eradicant sprays. The ascigerous stage of apple scab fungus was studied by Keitt and Wilson in 1926 (21). They gathered leaves off the trees at various times during the fall and placed them in different environments. They noted that the earlier the leaves fell in the autumn the earlier the ascospores would mature and be ready for discharge in the spring. The fungus required a period of cold weather after which it initiated its perithecia. The perithecia developed most rapidly when placed at 20°C. and kept alternately wet and dry.

The awareness of the importance of perithecial development for the continued existence of the fungus also suggested to others the second method of eradicating the fungus--that of applying a spray to the overwintered leaves on the ground. In 1924 Miss Curtis (9) in New Zeeland tested the effects of spraying the leaves on the ground in the spring with lime-sulfur at the strength used on tree foliage. A definite reduction in foliage infection the following season occured where she had made three applications. One application was not significant. She also noticed that applications immediately after a rain were most effective in retarding subsequent infection in the tree.

In 1935 Weismann working in Germany treated leaves in the same way with 5% Obstbaumkarbolineum. Eighty-five percent of the ripe perithecia were killed. Keitt and Palmiter in 1937 applied a solution of ammonium sulfate consisting of one pound of the material in one gallon of water to overwintering leaves. Investigation showed that the mature ascospores were killed and that further development was prevented.

In 1939 Keitt (17) tried a group of chemicals which were useful as weed killers in an attempt to find an efficient eradicant material. The compounds were sprayed on leaves which were placed in cloth mesh bags and allowed to remain in the field until the checks were discharging. The treated leaves were then brought into the laboratory. Those which bore the greatest number of perithecia were selected and a representative ½ inch disk was cut from each. The piece of leaf was placed on the inside surface of a petri dish cover with the dorsal surface orientated toward the water agar which was in the bottom. The spores which were discharged from the fruiting bodies were counted and the results recorded as the number of spores remaining per unit area of the leaf as an indication of the effectiveness of the eradicant spray material. Of a large number of compounds they found Elgetol, a product consisting of 34% sodium dinitro-ortho-cresol, to be superior.

A second part of the experiment involved the use of Elgetol in an actual orchard test. A ten acre sod orchard was sprayed with a 1% concentration. The closest unsprayed orchard was 0.3 mile distant. The application was made at 450 gallons per acre by using double nozzle guns at 400 pounds pressure. The result as determined by the discharge of ascospores per unit leaf area showed about a 96% inhibition. Tabulation of the amount of primary infection on the spur leaves in both sprayed and non-sprayed orchards indicated a 98% inhibition of ascosporic infection.

Other investigators working concurrently with and since the time of Keitt's experiments have run similar tests to compare the effectiveness of ground sprays. Kadow and Hopperstead (14) in 1937 sprayed an orchard floor with sodium-dinitro-ortho-cresylate at 0.5% concentration and a mixture of 100 pounds of sodium nitrate plus 4 pounds of calcium arsenite in 100 gallons of water. They determined the effectiveness of the sprays by the amount of primary infection on the trees. A good deal of variation was found both from season to season and between varieties of apples. Their conclusion was that the treatment was especially worthwhile when the amount of primary inoculum was high, at which time this treatment aided a good foliage program of mild sulfur sprays to materially reduce the extent of infection. Similar mild sulfur foliage sprays applied to non-ground treated portions of the orchard were less successful.

Carpenter (5) compared Elgetol and Lignasan (ethyl mercuri phosphate) in both *in vitro* and *in vivo* studies. Varying concentrations of the fungicides were added to media in which monosporically-derived cultures of *V. inaequalis*, *Coccomyces hiemalis*, and *Sclerotinia fructicola* were placed. In all cases *C. hiemalis* was most susceptible; *V. inaequalis*, second; and *S. fructicola*, most resistant. Lignasan was more toxic than Elgetol. However, when the same chemicals were applied to the scab fungus in the leaf, Elgetol was found to be more efficient. Elgetol killed the mature ascospores within three hours after application and prevented any further development of immature spores. When the leaves were kept constantly wet, the killing time was extended to six hours. This result was explained as a dilution of the fungicide. However, Palmiter (29) found that if Elgetol was applied to dry leaves at 0.5% using 500 gallons per acre, it dried before leaf penetration was complete. Rain or dew further aided penetration and a more complete kill of ascospores was accomplished.

In 1937 Henrick (12) in Tasmania sprayed urea and lime-sulfur on overwintered leaves infected with scab. He found no ascospores to be ejected from treated leaves into his spore trap boxes, but large numbers of spores were discharged from the untreated leaves with each wetting. In 1942 Sharville (32) began a project in which a ground spray of Elgetol was used in early spring in conjunction with a summer program in order to determine an acceptable summer spray schedule. Sulfur replaced lime-sulfur as the main material in the scab spray program and a reduction from 9.1% scab in the control orchards to 0.5% scab in ground treated orchards was demonstrated. The last found published work on ground spraying with Elgetol for apple scab was performed by Goldworthy et al (11) in 1949. They also experimented with possible summer spray programs to compliment a ground spray in the early spring.

Their results show a good scab control by using several mercurial sprays at pre-blossom and calyx in conjunction with the ground treatment.

Calcium cyanamid, a fertilizer and weed killer and one of the chemicals tested in these experiments, has been shown by Crowther and Richardson (8) to be toxic to seeds of higher plants. Bauer and Huber (4) used it as a dust application on the ground to prevent the emergence of the apothecia of Sclerotinia fructicola (Wint) Rehm. This dust was more efficient if it was relatively dry after the application. Calcium cyanamide dust is also being currently studied by Fulton for eradicating the apothecia of S. vaccinium from the overwintering mummies of blueberries.

Among other chemicals available for these tests was Actidione, an antibiotic known to be toxic to cherry leaf spot (C. hiemalis)(30). Thanos (34) showed toxicity to spores of various common pathogenic fungi at rates from 0.5 p.p.m. to 100 p.p.m. Leben and Keitt (26), in experiments with V. inaequalis in culture, inhibited growth of the fungus with an antibiotic at a concentration of 1:8,000,000. The material was a fractionated ethanol extraction of an unidentified Streptomyces culture.

METHODS AND MATERIALS

Leaves were collected on December 12 from beneath one unsprayed Mc Intosh apple tree that was heavily infected with apple scab during the previous season. The leaves were laid out between layers of ½ inch mesh hardware cloth with the dorsal side of the leaf facing upward. This permitted exposure of the leaves while at the same time holding them in place. These screens were placed on the dirt floor beneath a greenhouse bench in a cool room, kept about 50° to 60° F. at night. It has been shown by Wilson (35) that perithecia will mature most quickly if placed at a temperature of 20°C. (68°F.) and if subjected to alternating wet and dry periods. Therefore, the leaves were wet thoroughly with tap water from a garden hose at least every other day during the months of January, February, and March. The water quickly drained through the screens into the soil allowing good air circulation around the leaves causing them to dry out completely before the next wetting.

With these apparently optimum conditions, the perithecia developed faster than those outdoors and were ready for chemical spray tests on February 27. Examination of the perithecia at that time showed that many of the ascospores had differentiated, and about 5% of these had developed the cross septa.

On screens 1, 2, 3, and 4 chemicals were applied while screen number 5 remained as a check. The treatments given to the different series of leaves were as follows:

(1) A prepared dust (Aerocyanamid) containing 57% calcium cyanamide at the rate of 200 pounds per acre.

(2) A commercial preparation containing 65% sodium cyanamide in a spray at 0.35% concentration equivalent to 600 gallons per acre.

(3) DN 289 at 0.5% concentration at 600 gallons per acre.

(4) Coromerc at 0.06% concentration at 600 gallons per acre.

The fluid quantity of spray striking the leaves was determined by measuring the amount of spray issuing from the nozzle of a knapsack sprayer at 20 pounds pressure per square inch per minute. This calculated to 10 seconds for 50 cc. of spray. Thus, each square foot was sprayed for 10 seconds. These sprayed leaves in the screens were then replaced to their previous location on the ground and allowed to dry. The following day the usual treatment of watering was resumed to enhance perithecial development.

By the middle of March the perithecia in the control leaves were developed and many ascospores were mature and discharging. At this time watering was discontinued in order to inhibit excessive development and discharge. Leaves were then removed for laboratory testing.

A second experiment was performed in which application was made by dipping the leaves into the spray mixture. For this method leaves were gathered from the orchard several times during the last of April and first of May after many of the ascospores were mature. Applying chemicals to these leaves containing mature spores would indicate the ability of a material to inhibit ascospore discharge or ascospore germination after discharge.

The chemical solution was not sprayed on the leaves at the rate of 600 gallons per acre, the amount necessary to thoroughly wet the leaves; but they were wetted by being dipped into the chemical which was prepared to the same concentration as the spray. The leaves were dried at room temperature for a day before treating to assure equal moisture content and allow for similar absorption of chemical. The leaves were dipped into the solution with agitation for about 10 seconds. The excess solution was drained off and they were set out on paper toweling to dry.

Even though leaves which have weathered throughout the winter are very absorbent and wet easily, it was desirous to know if a wetting agent would increase the efficiency of a compound. A series of leaves were dipped in solutions with and without the addition of a wetting agent. Triton B 1956 at the rate of 2 drops per liter was the wetting agent used. The chemicals used in the dipping experiment were:

(1) Sodium cyanamide at 0.35% concentration.

(2) Coromerc at 0.06% concentration.

(3) DN 289 at 0.5% concentration.

(4) Actidione at 2,6,8,10 p.p.m.

(5) Calcium cyanamide was dusted on individual leaves by blowing an amount over the surface of the leaf until it was lightly covered.

In the third experiment actual conditions in an orchard were simulated. Five plots of one square yard each were marked out under a tree which had been infected the previous season. The ground was covered with a grass sod, onto which the leaves had fallen and lodged. Applying spray on the leaves here would duplicate normal orchard conditions, where the effect of sod and leaves situated in compact layers would be encountered. The time of spraying was determined by the amount of material it took to wet visibly the surface of the leaves and to approximate 600 gallons per acre. This spraying was carried out the last of April on a sunny afternoon when the leaves were relatively dry. The materials were applied with a small compressed air sprayer with a pressure of about 20 pounds per square inch. The chemicals which were used were:

(1) Sodium cyanamide at 18 pounds per acre with 600 gallons of water.

(2) Sodium cyanamide at 36 pounds in 600 gallons per acre.

(3) DN 289 at 0.5% concentration.

(4) Tag at 0.37% concentration.

(5) Coromerc at 0.06% concentration.

After spraying, the leaves were allowed to dry for several hours before the first sample was collected. A few leaves from the surface layer were then gathered at random from each plot. These were brought into the laboratory for discharge and germination counts. A second collection was made after a simulated rain. It was thought possible that the toxic action of the chemical occurs only when the ostiole of the perithecia was open. A rain subsequent to application would cause the ostiole to open and permit more fungicide to enter. Since no rainfall occured in the three day interval, the leaves and surrounding ground were sprayed with water at 20 pounds pressure and in relatively small drops several days after chemical treatment to simulate the effect of rainfall. After drying, the second sample of leaves was collected.

In all the experiments the effects of the treatments were determined by the discharge and germination of ascospores on agar plates. Petri dishes were used as the discharge chamber. Plain agar was prepared at 2% concentration and poured into the bottom plates. The apple leaves which were dry were then wetted and washed clean by running water to remove at least some of the remaining chemical and dirt. A piece of paper toweling was soaked and placed on the inner surface of the Petri dish cover. The wet leaf was placed on the toweling so that the side which was up during development of the perithecia was facing down toward the agar in the lower plate. Thus, the ostiole of the perithecia would be directed toward the agar.

With the perithecia wet, the mature ascospores were expelled from the ostiole down on to the agar. The ascospores of the check leaves were germinated in 24 hours and they were used as a basis of comparison. Consequently, the readings were taken 24 hours after the plates were prepared. For each leaf a count of 100 random spores was made and the percent of those which were germinated was recorded.

In an effort to reduce the amount of time required for petri plate examination and for clearer observation, several other methods were tried. One was to use a thin layer of distilled water on the bottom of the plate in order to get a more random distribution of spores. Several difficulties arose. If the layer was too thin, it would tend to dry out. If it was too thick, a good distribution would occur but movement of the dish during counting would interfere with the attempt to avoid counting the same spore twice. Also, the reflection of light by the meniscus of water which formed on the margin of the spore increased the difficulty of observing the smaller germ tubes from the spore.

Another attempt was made to use glycerin since it was clear but more viscous than water. Again, there was a more even distribution obtainable. However, the glycerin would vaporize and collect on the leaf and paper toweling situated on the upper surface of the dish, increasing the leaf weight and causing it to drop into the glycerin the end of 24 hours. Scotch tape was applied to hold the leaf up, but the tape did not hold well. The glycerin appeared to inhibit the germination of the spores, so it was discarded.

In order to facilitate a random count of the spores on a petri plate, the spores were counted throughout the entire area of the distribution pattern. One hundred spores were counted per plate. Mechanical counters were used, one for germinating spores, another for non-germinating spores. As the mechanical stage would not hold the petri plates, the plates were moved over the microscope stage by hand and as accurately as possible. When the distribution pattern on the agar was small, the agar was cut out and placed on a glass slide which was inserted in the mechanical stage for more accurate manipulation.

TABLE I

Materials Used and Manufacturer

	TRADE NAME	CHEMICAL COMPOSITION	COMPANY
1.	Actidione	Cycloheximide	Upjohn Drug Co.
2.	Aerocyanamid dust	Calcium Cyanamide	American Cyanamide Co.
3.	Coromerc	N-phenyl mercuri ethylene diamine	Pittsburg Plate Glass Co.
4.	DN 289	Sodium dinitro-ortho-sec-butyl phenol triethanal-amine	Dow Chemical Co.
5.	Aero Sodium Cyanamid	Mono sodium cyanamide	American Cyanamide Co.
6.	Tag	Phenyl mercuri acetate	California Spray Co.

RESULTS

The observed results of the experiments performed on the perfect stage of V. inaequalis are presented in the following pages. Within each test the various chemicals will be viewed for their efficiency and their practical use in ground spraying treatments of apple orchards.

The results of spraying the leaves before spore maturity are presented in table II. This table shows a comparison of the percentage germination of ascospores. The DN 289 treatment resulted in a characteristic reaction of plasmolysis of the spores, both mature and immature and also the undifferentiated material within the ascus. The asci of these perithecia were so disorganized that they did not discharge any spores down on to the agar surface. Calcium cyanamide materially inhibited spore germination. If one compares the inhibition of calcium cyanamide in table II with that in table III where the chemical was applied to mature ascospores, it is seen that this compound has a greater effect in preventing the development of the immature ascospore than it does in preventing germination of the mature spore. It appears that the solution of sodium cyanamide is more effective if applied on immature ascospores than on those that were mature.

TABLE II

Results obtained by spraying materials on leaves in screens containing immature ascospores.

Chemicals	Percentage Germination a	b	c
Calcium Cyanamide	0	5	3
Sodium Cyanamide	4	20	18
DN 289	P	P	P
Coromerc	12	54	56
Check	36	92	89

P = no ejection of spores and visible plasmolysis

TABLE III

Results obtained by dipping leaves containing mature ascospores into solution.

Chemical	Percentage Germination a	b	c	d	e
Check	100	89	92	96	80
Sodium Cyanamide	69	56	50	60	
* Calcium Cyanamide	24	17	20	12	
Coromerc	62	51	65	69	
DN 289	P	P	P	P	
Actidione 2 p.p.m.	47	54	27	37	25
Actidione 6 p.p.m.			45	77	55
Actidione 8 p.p.m.					62
Actidione 10 p.p.m.					53

P = no spores ejected and visible plasmolysis

* This was applied by dusting it on wet leaves.

The results of the dipping process are presented in table III. DN 289 again completely inhibited discharge. As the time lapse between the dipping of the leaf and the rewetting of it for discharge of the ascospores was about two hours, it appears that the speed with which DN 289 acts is very fast. Sodium cyanamide inhibited the germination of less than 50% of the spores. Calcium cyanamide showed better inhibition within the limits of this experiment, but all the dust may not have reacted before being washed from the leaves. Coromerc was more effective than sodium cyanamide. The Actidione series was apparently ineffective and somewhat erratic in its response.

In the fourth experiment the same chemicals used in Experiment 2 were used with a spreader added. The results given in table V may be compared with those in table III. The solutions to which the spreader was added gave no more inhibition than that obtained without the spreader.

Experiment number III attempted to simulate orchard conditions. The data are shown in table IV. As shown in this table, sodium cyanamide used on the ground at 36 pounds per acre, gave fairly good results. DN 289 again gave a high inhibition. In series a and c the number germinated for the DN 289 treatment were the only spores which were found on the agar. Tag and Coromerc, closely related compounds, gave comparable results.

TABLE IV

Results obtained by spraying leaves on the orchard floor.

Chemical	Percentage Germination before rain a	b	c	after rain d	e
Check	82	91	78	65	85
Sodium Cyanamide 18	11	13	16	20	22
Sodium Cyanamide 36	3	1	7	20	60
DN 289	6*	P	3*	P	P
Tag	14	19	13	14	16
Coromerc	17	13	16	18	27

P = no spores ejected and visible plasmolysis

* Actual number discharged--not percentage

TABLE V

Results obtained by dipping leaves into solutions with spreader (Triton B 1956) added compared with those without spreader.

Chemical	Percentage Germination a	b	c	d
Actidione 2 p.p.m.	47	54	27	37
Actidione 2 p.p.m. and spreader	41	65	23	44
Actidione 6 p.p.m.			45	77
Actidione 6 p.p.m. and spreader			24	68
Coromerc	62	51	65	69
Coromerc and spreader	67	71	62	60

DISCUSSION

The techniques of these experiments differ from those of other workers in that the percentage germination of discharged spores was observed instead of the number of ascospores which were discharged on to the agar. It would appear that the number of spores discharged from a unit area of leaf surface was not an exact criterion of the chemical effect on the fungus. Many of the spores of treated leaves discharged but did not germinate, while those of the untreated leaves were exceptionally viable. The results, therefore, of this experiment are based on the percentage of germination of discharged spores. This is not the entire answer, however, since DN 289, the most effective chemical, completely inhibited discharge. Therefore, in future work it would appear desirable to determine the degree of inhibition of discharge as well as the inhibition of germination.

The use of the chemicals, other than DN 289, as ground sprays would need the justification of orchard tests. Sodium cyanamide in this experiment did not give outstanding results, and it has been found by others to cause injury to the tree by absorption through the roots when applied to the ground.

SUMMARY

Apple scab, a disease of apples, is caused by <u>Venturia inaequalis</u>. Control proceedures for this prevalent disease have utilized protectant sprays. The inconsistent results and phytotoxicity of these sprays, namely lime-sulfur and Bordeaux, led experimenters to reduce the primary inoculum potential by eradicant ground sprays on overwintering leaves. This would allow better control by milder foliage sprays. Elgetol, a di nitro product, has been proven effective. The present experiment was carried out to test the properties of other chemicals for eradicant ground spraying and to test a laboratory method of assay.

Tests showed that DN 289 was superior to the other materials tested. Aerocyanamid dust showed some promise while the sodium cyanamide salt, the mercury compounds, and Actidione were of less value according to this test. The results of spraying leaves in screens and dipping leaves in solutions showed correlation with the simulated ground spraying application.

These results corroborate the experiments of others showing the effectiveness of dinitro compounds for orchard floor sprays. Cyanamid dust may have considerable merit as a floor application, judging from one experiment,

but further work is needed to determine its real effectiveness. It is possible that it would need special moisture conditions following application to merit orchard use.

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