EVALUATION OF CULTIVAR RESISTANCE TO ALFALFA DOWNY MILDEW AND CHEMICAL CONTROL IN GROWTH CHAMBER TRIALS

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TABLE OF CONTENTS

LIST OF TABLES

FIGURE

CHAPTER I

INTRODUCTION

Downy mildew of alfalfa (Medicago sativa L.), known as lucerne in some countries, is caused by the Phycomycete, Peronospora trifoliorum de Bary. The causal fungus attacks plants during cool, wet, or humid climate conditions, particularly in spring and fall, and it is an intercellular obligate plant parasite (81). Upper leaves and shoots of young seedlings are usually attacked first, resulting in shortened internodes. Infected leaves become light green to yellow, twisted, rolled or curled down, and then the leaves collapse (14). Grayish or pale violet downy growth of conidiophores and conidia on lower leaflet surface is often visible to the unaided eye (56). Dormant resting spores are produced in diseased tissue. Conidia are spread by wind and splashing rain. Seedlings may be killed when conditions are favorable to the disease (65).

The most practical means to control the disease is to develop and use resistant cultivars (47, 62, 65). Resistance differs markedly among cultivars (55, 71) and tends to be greater in improved cultivars developed in the northern United States while those developed in the central states of the U.S.A. are less resistant (61, 62, 107). The

disease is sporadic in Oklahoma. Consequently, cultivars developed in Oklahoma in field tests typically include a relatively high proportion of susceptible plants because susceptible breeding material is often not recognized and discarded. Therefore, it was necessary to develop a controlled environment chamber method to screen alfalfa plants for resistance to P. trifoliorum. Such a test was developed by Stuteville (114) at Kansas State University and this test served as a model for a similar test in Oklahoma.

Foliar or soil treatment with fungicides for control of alfalfa downy mildew in laboratory field trials has not been reported (3). Recently, a pre-sowing seed treatment with thiram, phenthiuram-molybdate, or phenthiuram for control of alfalfa downy mildew decreased infection and increased yield in the U.S.S.R. (33). Effective control of the disease in crops other than alfalfa has been obtained with applications of various fungicides.

The objectives of this study were to evaluate resistance of certain cultivars and breeding lines to P. trifoliorum in a growth chamber and to evaluate chemical control of the disease in a growth chamber. Seedlings of 16 cultivars and two breeding lines were used in these experiments. One highly-susceptible cultivar, Kanza, was used as a control cultivar in chemical tests and a highly-resistant cultivar, Saranac, was also used as a control cultivar in the resistance tests.

CHAPTER II

LITERATURE REVIEW

Peronospora trifoliorum de Bary on alfalfa was first described by de Bary in 1863 (30, 43) and, according to Jones, the disease was first reported in the U.S.A. in 1917 (118). This fungus was also called *P. aestivatis* Sydow (43, 55, 115) and *P. viciae* (Bert) de Bary (55, 68) on alfalfa. No biological races of the fungus on alfalfa have been distinguished (7, 53, 55, 70).

Downy mildew (DM) is usually less important than many other diseases of alfalfa in the world. It is widespread and can cause yield losses when infection is heavy but is not severe, common, or very harmful in South Africa (54), Australia (119), and Canada (5).

From early 1929 until 1960, losses from alfalfa DM were usually minor in many states of the U.S.A. (57, 62, 70, 75, 80, 86, 87, 103, 111, 120, 121); however, by 1950 the disease was severe in Iowa and Virginia (22, 41). During 1962 and 1965, yield of infected plants was markedly reduced or lost in New York and Utah (8, 99). In Minnesota and Nevada, a severe mildew epiphytotic was observed in 1972 and 1976 (71, 116).

In Oklahoma, epiphytotics of alfalfa DM have occurred

on Cimarron and Cody cultivars in Payne County for the years 1977-1981 (4). From May until June of 1977, it was observed in the counties of Wagoner and Kay (19). In April of 1978, it appeared in Coal and Payne counties showing a 100% prevalence and 10% severity (20). In April of 1979, a survey of alfalfa in Texas County by Williams and Conway revealed the presence of DM in Cimarron, Dawson, Resistador, and Kanza cultivars (21), and by June it was quite prevalent (122). Cimarron and Kanza were very susceptible. In the spring and late fall of 1980 and in the spring of 1981, alfalfa DM recurred (123).

A number of sources of resistance to downy mildew of alfalfa have been identified. Jones and Torrie (69) observed wide differences among classes from several cultivars and strains, and found that percentages of infected plants in cultivars ranged from 12 in Hardistan and Ladak to 60 in Hardigan. Smith (111) reported that the percentages of infected seedlings of four cultivars and 17 strains for DM resistance sown in the field ranged from 4.5% to 32.1%. Hanson and Smith (62) reported that of six cultivars and six synthetics of alfalfa tested for resistance to DM, Narragansett, Umta, Minn. Syn. M, and Utah Syn. J-2 were the most resistant, and Buffalo, Ranger, Nebr. Syn. 27, and Kans. Syn. KS-6 were the most susceptible. Hanson et al. (61) also reported the higher level of resistance to DM by Narragansett as compared to the susceptibility of Buffalo under field conditions. Thyr et al. (116) noticed that

cultivars Pacer, Thor, and WL 307 were the most resistant and AS 49R, Nevada Syn XX, and Washoe were the most susceptible. Thirty-six cultivars under a severe mildew epiphytotic were compared and were noted to have a range in resistant plants from more than 90% in Saranac and Narragansett to about 55% in Ranger and Vernal by Barnes and Frosheiser (71). New York DM data in 1962 by Nittler et al. (95) showed a range of 26%-61% plants resistant in ten cultivars. Buffalo was the most susceptible and Alfa was the most resistant. Caliverde cultivar was also shown as resistant in either the greenhouse (112) or in the field by several workers (8, 60, 71).

Berkenkamp and Folkins (5) found that 10 cultivars grown in the first year and 25 cultivars grown in the second year had highly significant resistance to P. trifoliorum. The data suggested that saponins may be a factor in resistance. HS Ranger (high saponin line) was very resistant and LS Ranger (low saponin line) was highly susceptible. Pedersen and Barnes (102) have postulated that resistance to alfalfa DM was conditioned by one tetrasomically inherited, incompletely dominant gene (Dm). Therefore, resistance was due to a dosage effect, with the multiplex genotype (dmdmdmdm) being most susceptible and the quadriplex genotype (DmDmDmDm) being homozygous for resistance.

Stuteville (114) reported that four cultivars evaluated at seedling stage in the laboratory generally agreed with the field infection rankings by other workers (62, 71, 95).

The cultivar Kanza was the most susceptible and Saranac was the most resistant.

Peronosporaceous fungi on alfalfa, vegetables, and garden flowers have been controlled by non-systemic and systemic fungicides, antibiotics, and oil mixtures. In tobacco DM (P. tabacina), McLean et al. (82-84) and Wolf (124) reported that benzol vapors and other organic substances were successfully controlled in early 1937 to 1939. Later, paradichlorobenzene vapor (15, 84, 104), zineb dust (16, 49, 92, 117) and zineb spray (66, 97, 117), ferbam (52), streptomycin sulfate (58), maneb (97), oil sprays plus copper (17), and cottonseed oil with zineb, thiram, and dichlone (106) provided excellent control of the disease. Recently, CGA 48988 (Ridomil) and CGA 38140 completely controlled tobacco DM (67, 96).

In broccoli DM (P. parasitica), streptomycin application resulted in better and more economic control of the disease (1). Copper-zinc plus streptomycin, maneb plus streptomycin (93), agrimycin (90, 92), copper and maneb (49, 89), Spergon SL (89, 92), polyram (63), Bordeaux mixture (48), tribasic copper with copper sulfate and maneb (76-79), and American cynamide 28720 (91) were the most effective for control of the disease. Recently, Ridomil (CGA 48988) (50W) was also the best treatment (100).

In cabbage DM (P. parasitica Fr.), spore germination and systemic growth of the fungus were inhibited by streptomycin (85), maneb (29) and Spergon (9, 28, 34-42). Recently,

Ridomil (CGA 48988) provided complete control (50, 51).

For control of onion DM (P. destructor), Dithane Z 78-sulfate dust (93), Rosin lime sulfate (125), and maneb plus $ZnSO_4$, zineb plus $ZnSO_4$(94), and zineb spray (31, 32) were recommended.

In lettuce DM (Bremia lactucae), zineb (75 WP) and maneb (80 WP) (113) and a granular formulation of Fongarid (24) were effective in control of the disease. Recently, Ridomil (CGA 48988) in the greenhouse and high humidity chamber effectively controlled as a protectant (25) and as a curative spray (23, 26, 27).

In sorghum DM (Peronosclerospora sorghi) Ridomil (CGA 48988) (25 WP) as a seed treatment completely controlled both systemic infection and local lesions (2, 45, 46).

For control of honeydew melon and cantaloupe DM (Pseudoperonospora cubensis), Bravo and zinc ion-maneb complex (80 WP) (59) and maneb plus zineb (44) gave good control of the disease.

Sugar beet DM (Peronospora farinosa) was effectively controlled by a maneb spray (11), and opium poppy DM (P. arborescens) was reduced by zineb and ferbam sprays (72). Copper oxychloride and zineb (25 WP) were the best control agents against rose DM (P. sparsa) (6), and maneb (80 WP) and zineb (75 WP) excellently controlled spinach DM (P. effusa) and cucumber DM (P. cubensis) (113). Recently, metalaxyl (CGA 48988) (Ridomil) as a seed treatment and foliar spray completely controlled sugar cane DM (Peronosclerospora

sacchari) (74), pea DM (Peronospora viciae and P. pisi) (64, 109), maize DM (Sclerophthora rayssioe var. zeae) (73), sunflower DM (Plasmopara halstedii) (108), and cucurbits DM (Pseudoperonospora cubensis) (98, 105). For control of snapdragon DM (Peronospora antirrhini), metalaxyl plus maneb was an excellent treatment in nursery beds, and metalaxyl + zineb or metalaxyl alone gave good results in flower trials (101). Other new systemic fungicides, SN 66752 and SN 41703, exhibited systemic antifungal activity against cucumber DM (18).

According to Patel (99), in early 1925, conidial germination of alfalfa DM fungus (Peronospora trifoliorum) was inhibited by formaldehyde, acetaldehyde, benzaldehyde, butyraldehyde, furfuraldehyde, toluol, and ethyl alcohol in the germinating chamber; and in 1981, Dokudovskaya (33) observed thiram, phenthiuram-molybdate or phenthiuram as pre-sowing seed treatment decreased infection of alfalfa DM in the U.S.S.R.

CHAPTER III

MATERIALS AND METHODS

Evaluation of Cultivar Resistance to Alfalfa Downy Mildew in Growth Chamber Trials

Sixteen cultivars and 2 synthetic cultivars were used for testing cultivar resistance against Peronospora trifoliorum. Seed lots of Agate, Arc, Caliverde, Kanza, Ranger, Saranac, and Vernal were obtained from Dr. D. L. Stuteville, Kansas State University; and OK1 Syn 1, OK 3 Syn 1, Riley, Team and WL 318 were obtained from Dr. J. L. Caddel, Department of Agronomy, Oklahoma State University. Seeds of other cultivars: Aztec II, Buffalo, Cherokee, Cimarron, Cody, and Liberty were obtained from Dr. G. L. Barnes, Department of Plant Pathology, Oklahoma State University.

All alfalfa cultivars were randomly seeded 1/2 inch deep in steam-sterilized masonry sand with vermiculite and perlite in a 3:1:1 ratio, to which was added a little Osmocote fertilizer (7.6g) in a flat (21.5" x 15.8"). Fifty seeds were planted per row. After seeding, the soil was watered until saturated and the flats were placed in a growth chamber maintained at 20°C and with approximately 2,000 feet candle of incandescent and fluorescent lighting.

The plants were watered daily until the fifth day, and then the seedlings were inoculated in the cotyledon stage of growth. No water was provided prior to inoculation.

Mass cultures of P. trifoliorum as inoculum were originally collected from more than 50 mildewed plants in an alfalfa field at the Plant Pathology Farm, Stillwater, Oklahoma. The fungus is an obligate parasite that requires living plants for sporangial production. The sporangia are weak and survive only a few hours in the laboratory, but mildewed plants kept in a deep freeze usually yield viable sporangia for a few weeks (114).

To prepare inoculum, infected shoots were placed in distilled water in a jar, then the jar was closed and vigorously shaken until the sporangia were dislodged. The spore suspension was passed through a tea strainer to remove plant material. The sporangial concentration was estimated by hemacytometer counts, and the suspension was adjusted to contain at least 90,000 sporangia/ml. The inoculum was sprayed with a small plastic atomizer between the cotyledons of seedlings to runoff. Each flat needed 25 ml (or more) inoculum. Sporangia would settle rapidly; therefore, it was necessary to shake the inoculum often while inoculating. Free water formed readily on the cotyledons of plants under moist conditions.

Inoculated plants were kept in 100% relative humidity condition and a dark period of at least 12 hours after inoculation to produce sporangia and to permit germination

and infection (114). For these requirements, the inoculated plants were put in an aluminum bun pan (18" x 26") and covered with an opaque plastic donut cover. The sides of the plastic cover set over the flat and inside the pan. Moisture in the planting medium was usually sufficient to maintain adequate humidity to produce sporangia; when it was not, water was placed in the pan.

Optimum temperature for the fungus germination, infection, and sporulation is near 20°C but 16°-24°C is also satisfactory (114).

Table I shows the schedule used. Sporangia were obtained from rogued susceptible plants for inoculum for the next set. This method was replicated eight times during September and November of 1980 and during January and April of 1981.

Evaluation of Chemical Control Against Alfalfa Downy Mildew in Growth Chamber Trials

This experiment was concerned with the protective and curative effects of Kocide 101 [copper hydroxide: $Cu(OH)_2$], CGA 48988 (Ridomil) [metalaxyl: n-(2,6-dimethylphenyl)-N-(methoxy-acetyl)-alanine methyl ester], SN 41703 (Previcur) [prothiocarb: S-ethyl-N-(3-dimethylaminopropyl)-thiol carbamate-HCl], and Streptomycin (streptomycin sulfate) against alfalfa downy mildew caused by P. trifoliorum. These fungicides were applied to foliage and their disease

TABLE I

SCHEDULE FOR EVALUATING RESISTANCE AGAINST
P. TRIFOLIORUM

Days	Methods
Day 1	Plant seeds 1/2" deep in flats of steam-sterilized masonry sand with vermiculite and perlite (3:1:1), and add 7.6 grams Osmocote fertilizer per flat. Place in growth chamber and add distilled water as needed.
Day 2-5	Add distilled water as needed.
Day 5	Inoculate, cover, and turn lights off for 12 hours. Do not water plants just before inoculating.
Day 6	Remove plastic cover, turn lights on, and rogue late-emerging plants.
Day 7-11	Add distilled water as needed and rogue late-emerging plants.
Day 11	Recover plants and turn lights off to induce sporulation. Do not water plants just before covering because the fungus will not sporulate in free water.
Day 12	Uncover and evaluate infection rates.

control effects and their effect on sporulation were recorded.

Seeds of a highly-susceptible alfalfa cultivar, Kanza, were sown 1/2" deep in steam-sterilized masonry sand mixed with vermiculite and perlite in a 3:3:1 ratio, to which was added 7.6 grams Osmocote fertilizer, in flats (21.5" x 15.8"). Fifty seeds were planted in each row in the flats. After seeding, the soil was watered until saturated, and the flats were placed in a growth chamber maintained at 20°C, with 100% relative humidity, and at approximately 2,000 feet candle intensity lighting for at least a 12 hour photoperiod. The plants were watered daily until the fourth day, but not just before the chemicals were applied.

Protectant treatments of CGA 48988 (60 mg/100 ml), Kocide 101 (360 mg/100 ml), SN 41703 (120 mg/100 ml), and Streptomycin (60 mg/100 ml) involved spraying plants at the second cotyledon stage up to one day before inoculation by atomizing each row in different flats with 25-30 ml of sporangial suspension. All fungicides were used approximately the same recommended levels in 100 ml distilled water. The procedures and materials to prepare inoculum were the same as the methods as previously stated in evaluating cultivar resistance to alfalfa downy mildew in the growth chamber.

Curative sprays were applied at one, three, and five days after inoculation with the same rates of fungicides per 100 milliliter of distilled water as protecting sprays.

Control or untreated plants were not sprayed with the fungicides. The following schedule of methods were replicated four times in the growth chamber on April 1981 (Table II). Sporulation and symptoms on the leaflets were assessed nine days after inoculation.

TABLE II

SCHEDULE OF EVALUATING CHEMICAL CONTROL AGAINST ALFALFA DOWNY MILDEW IN GROWTH CHAMBER

Days	Methods
Day 1	Plant seeds 1/2" deep in a flat of steam-sterilized masonry sand with vermiculite and perlite (3:1:1), and add 7.6 grams Osmocote fertilizer per flat. Place in growth chamber and add distilled water as needed.
Day 2-4	Add distilled water as needed.
Day 4	Spray fungicides. Do not water just before spraying.
Day 5	Inoculate, cover, and turn lights off for 12 hours. Do not water just before inoculation.
Day 6	Remove plastic, cover, turn lights on, and rogue late-emerging plants. Spray fungicides after inoculated. Do not water just before applying.
Day 7-8	Add distilled water as needed.
Day 8	Repeat the same schedule as Day 6.
Day 9-10	Add distilled water as needed.
Day 10	Repeat the same schedule as Day 6.
Day 11-13	Add distilled water as needed.
Day 13	Re-cover plants and turn lights off to induce sporulation. Do not water just before covering because the fungus will not sporulate in free water.
Day 14	Uncover and evaluate.

CHAPTER IV

RESULTS AND DISCUSSION

Evaluation of Resistance

Eighteen alfalfa cultivars, including the two synthetics evaluated at the seedling stage in the growth chamber, were rated for their reactions to resistance against Peronospora trifoliorum. Ratings were made in September and November of 1980 in which 11 and six cultivars, respectively, were randomly replicated five times. Estimates of the percentages of leaf area affected in the flat were recorded as ratings from 1 to 100, depending upon the number of resistant plants. The higher the number, the greater the resistance. The same method was used to rate other tests for downy mildew in January and April of 1981, in which six and five cultivars were replicated eight times.

Although no flat was rated lower than 10 or higher than 60, highly significant differences in resistance between alfalfa cultivars were found among tests shown in Table III and IV. No cultivars were completely free of symptoms, although some symptom-free plants were found in each cultivar.

All cultivars and synthetics varied markedly in percentages of resistant plants. Comparison of the cultivars

TABLE III

PERCENTAGE OF SEEDLINGS IN ELEVEN CULTIVARS RESISTANT TO *P. TRIFOLIORUM* IN GROWTH CHAMBER[1]

Cultivars	Percentage of Resistant Seedlings Average Score[2]	
	September/1980	November/1980
	[3]	
Saranac	47.00 a	51.00 a
Arc	40.13 b	40.75 b
Riley	37.00 c	--
WL 318	35.88 c	--
Liberty	35.50 c	--
Vernal	24.88 d	23.625 c
Ranger	20.25 e	20.125 d
Cody	15.13 f	--
Buffalo	13.50 g	--
Cimarron	12.13 gh	13.50 e
Kanza	11.75 h	12.375 e

[1]Data were averaged for eight replicates.

[2]Based on 1% to 20% = susceptible, 21% to 40% = intermediate, and above 40% = resistant.

[3]Cultivars followed by the same letter are not significantly different at .05 level by Duncan's multiple range test.

TABLE IV

PERCENTAGE OF SEEDLINGS IN EIGHT CULTIVARS AND TWO SYNTHETICS RESISTANT TO *P. TRIFOLIORUM* IN GROWTH CHAMBER[1]

Cultivars	Percentage of Resistant Seedlings Average Score[2]	
	January/1981	April/1981
	[3]	
Saranac	48.375 a	49.125 a
Aztec II	--	41.25 b
Caliverde	--	40.50 b
Arc	39.125 b	--
OK 1 Syn 1	37.750 c	--
Agate	--	31.75 c
OK 3 Syn 1	30.50 d	--
Cherokee	--	30.25 c
Team	30.125 d	--
Kanza	12.25 e	11.00 d

[1]Data were averaged for eight replicates.

[2]Based on 1% to 20% = susceptible, 21% to 40% = intermediate, and above 40% = resistant.

[3]Cultivars followed by the same letter are not significantly different at .05 level by Duncan's multiple range test.

indicated that Arc, Aztec II, Caliverda and Saranac appeared to be most resistant. Saranac was the most resistant. Buffalo, Cimarron, Cody and Kanza were most susceptible. Kanza was the most susceptible (Figure 1). The remaining cultivars fell into the intermediate group as moderately resistant or moderately susceptible on their symptoms.

The mildew rankings were in agreement with those of other workers under artificial infection (112, 114) and under natural infection conditions (5, 8, 12, 13, 60, 62, 71, 95, 107, 110, 116). However, severity of disease was much greater with the screening test of seedlings. Cultivars Saranac and Caliverde appeared as resistant under greenhouse (112, 114) and natural conditions (8, 60, 110) as in our results, and Agate, Ranger, Riley, Team and Vernal were intermediate, similar to other reports (5, 8, 12, 13, 71, 95, 114, 116). However, Ranger was observed to be the most susceptible by Hanson and Smith in Wisconsin (62). Buffalo and Kanza were the most susceptible according to several worker's results (62, 95, 107, 114); however, Smith (110) reported that Buffalo fell into the intermediate group. Resistant cultivars Arc and Aztec II, and susceptible cultivars Cimarron and Cody in the field mentioned by Barnes (4), agreed with the mildew characteristics in growth chambers. Cherokee, Liberty, OK 1 Syn 1, OK 3 Syn 1 and WL 318 had not been previously tested for resistance against P. trifoliorum; however, these cultivers appeared as resistant or moderately resistant against many insect pests

Figure 1. Severe Mildewed Symptoms on Susceptible Cultivar "Kanza"

and certain other plant diseases (12, 13).

The results with some cultivars were slightly different from the other worker's results. We believe that it may be caused by different environmental conditions or different test methods. We also believe that the screening technique of seedlings is a good tool for evaluating resistance to alfalfa downy mildew.

Evaluation of Chemical Control

In the results shown in Table V and VI, highly signigicant differences to control between fungicides were found among the treatments. Alfalfa downy mildew on the seedling-susceptible cultivar, Kanza, was completely controlled by a new systemic fungicide, Ridomil (CGA 48988) (2E) at 60 mg/100ml in a growth chamber. No sporulation or infection of downy mildew developed on either protectant teatment one day before inoculation, and curative sprays one, three and five days after inoculation (Table VI). No chlorotic symptoms appeared on any leaflets.

Streptomycin at 60 mg/100 ml was excellent in foliar treatment for controlling the disease one day after inoculation, and it was also superior in controlling the disease three days after inoculation. The other applications one day before or five days after inoculation were more effective than SN 41703 and Kocide 101 in the same length of time.

SN 41703 (120 mg/100 ml) and Kocide 101 (360 mg/100 ml)

TABLE V

EFFECTIVENESS OF FUNGICIDES FOR CONTROL OF P. TRIFOLIORUM ON SEEDLINGS OF SUSCEPTIBLE CULTIVAR "KANZA"

Treatments	Mean Number of Infected Leaf Lesions			
	SN 41703 (120mg/100ml)	Kocide 101 (360mg/100ml)	Streptomycin (60mg/100ml)	CGA 48988 (60mg/100ml)
Sprayed One Day Before Inoculation	8 c[1]	4 d	2.75 c	0
Sprayed One Day After Inoculation	2 e	2 d	0 d	0
Sprayed Three Days After Inoculation	6 d	8 c	1 d	0
Sprayed Five Days After Inoculation	16 b	12 b	5 b	0
Unsprayed Control	85 a	88 a	86 a	63

Foliar spray method used.

[1]Fungicide methods followed by the same letter are not significantly different at .05 level by Duncan's multiple range test.

TABLE VI

EFFECTIVENESS OF FUNGICIDES APPLIED AFTER INOCULATION FOR CONTROL OF P. TRIFOLIORUM

Treatments	Mean Number of Sporangium per Leaf Lesion			
	SN 41703 (120mg/100ml)	Kocide 101 (360mg/100ml)	Streptomycin (60mg/100ml)	CGA 48988 (60mg/100ml)
Sprayed One Day Before Inoculation	9,750 b[1]	6,000 b	6,000 b	0
Sprayed One Day After Inoculation	2,000 c	2,000 c	0 d	0
Sprayed Three Days After Inoculation	2,000 c	2,000 c	1,000 d	0
Sprayed Five Days After Inoculatio	9,750 b	8,000 b	4,000 c	0
Unsprayed Control	69,000 a	64,000 a	67,000 a	59,000

Foliar spray method used.

[1]Fungicide methods followed by the same letter are not significantly different at .05 level by Duncan's multiple range test.

were moderately effective one and three days after inoculation. They did not effectively control the disease compared to metalaxyl. The least control was obtained when the SN 41703 treatment was used five days after inoculation.

Kocide 101 was phytotoxic to many leaflets; however, the other fungicides were not phytotoxic to seedlings. Some completely controlled leaflets were difficult to check as to their symptoms (whether they still were infected or the fungus had already been killed) because the symptoms looked the same (chlorotic). Therefore, sporulation and infection were checked with microscopes.

On unsprayed controls in CGA 48988 applied flats, sporulation and symptoms appeared less than on the other fungicide treatments. It may be caused by the volatile activity of metalaxyl. Singh and Dickinson (109) reported that up to 25 mg/ml concentration of metalaxyl reduced sporulation more than on lower concentration. Therefore, the reduction in sporulation by volatiles may be proportional to concentration.

Streptomycin effectively controlled and inhibited broccoli and cabbage downy mildew (Peronospora parasitica), and tobacco blue mold (P. tabacina) as reported by several workers (1, 32, 58). However, it was moderately effective, it did not provide greater control than other combinations against broccoli downy mildew (88, 91) and tobacco blue mold (97).

Gabrielson and Getzin (50, 51) reported that SN 41703

as a soil treatment did not effectively control cabbage downy mildew as in our experiment. However, Cohen (18) noticed that SN 41703 had very good systemic antifungal activity against cucumber downy mildew (Pseudoperonospora cubensis), as soil drench.

For controlling downy mildews, Kocide 101 treatment was not used. However, fixed copper compounds or other copper containing fungicides related to Kocide 101 effectively controlled broccoli and rose downy mildew (Peronospora sparsa) (6, 77, 78, 79, 91), but Paulus et al. (100) reported a copper containing fungicide as a foliar spray was moderately effective against broccoli downy mildew as in our experiment results.

Although systemic fungicides have had little success against downy mildew in the past, seed, foliar and soil application of metalaxyl (Ridomil) recently has been found to be a good control for downy mildews incited by species of Peronospora (50, 51, 678, 96, 100, 101, 109), Peronosclerospora (2, 45, 74), Bremia lactucae (23-27), Plasmopara halstedii (108), Pseudoperonospora cubensis (98, 105) and Sclerosphthora raysiae (73). With a foliar spray, Singh and Dickinson (109) found sporangia on pea leaflets six and two days before inoculation. Our results were different because of a different host, different length of time or different date of dosage. Quite recently, Bruck et al. (10) reported new evidence of metalaxyl (Ridomil 2EC) resistant isolates on tobacco blue mold. The isolates

were from presoil treatment of metalaxyl collected in nature. The condidial suspension (2000 spore/ml) were atomized onto 4-leaf stage potted plants treated 24 hours prior to inoculation with metalaxyl ranging from 0 to 200 µg/ml. Three out of 14 isolates tested were able to form lesions, sporulate, and reinfect plants up to 100 µg/ml. They used less number of sporangia of inoculum, less dosage, and older leaf stage of plants than our experiments. This data suggested that metalaxyl resistant isolates may exist in nature.

In our chemical experiments in growth chamber, CGA 48988 (Ridomil) provided outstanding control of alfalfa downy mildew as either a protective or curative spray application. An antibiotic, Streptomycin, as a foliar spray was also superior in controlling alfalfa downy mildew.

CHAPTER V

SUMMARY

1. Downy mildew (Peronospora trifoliorum) on alfalfa was collected in alfalfa fields in Payne County, Stillwater, Oklahoma.

2. The fungus was inoculated onto 18 cultivars five days after seeding in growth chambers at 20°C with 100% relative humidity and in twelve hours of darkness.

3. Inoculum contained 90,000 sporangia per milliliter distilled water.

4. All cultivars were evaluated one week after inoculating for their reaction to the pathogen.

5. The average number of resistant plants ranged from 11.0% for Kanza to 51.0% for Saranac.

   a. Arc, Aztec II, Caliverde and Saranac were resistant. Saranac was the most resistant.

   b. Buffalo, Cimarron, Cody and Kanza were susceptible. Kanza was the most susceptible.

   c. The remaining cultivars, Agate, Cherokee, Liberty, OK 1 Syn 1, OK 3 Syn 1, Ranger, Riley, Team, Vernal and WL 318 fell into the intermediate group.

6. Ridomil (CGA 48988) (60 mg/100 ml), Kocide 101 (360 mg/100 ml), SN 41703 (120 mg/100 ml) and Streptomycin (60 mg/100 ml) were evaluated for control of the disease in growth chamber.

a. The fungicide as a protectant and curative spray was sprayed at cotyledon stage one day before and one, three, and five days after inoculating.

b. Sporulation and symptoms of the pathogen in leaflets were evaluated nine days after inoculating.

c. CGA 48988 was extremely effective in controlling the disease as either a protective or curative spray; Streptomycin was also completely effective one day after inoculating and was superior to SN 41703 and Kocid 101.

d. SN 41703 and Kocide 101 were moderately effective one day after inoculating, but they were ineffective one day, three days, and five days before inoculating.

e. CGA 48988 reduced sporulation on unsprayed control plants because of volatile activity.

f. Kocide 101 was phytotoxic to the leaflets.

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