THE EFFECT OF CERTAIN DISEASES AND INSECTS ON SEED SETTING IN ALFALFA

by

Peter Pankiw B.S.A.

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INTRODUCTION

A problem of great importance to the alfalfa growing industry in Canada and the U.S.A. is the production of alfalfa seed. Production is limited to certain areas in the Western U.S.A. and the three Prairie Provinces of Canada. Scarcity of alfalfa seed and the consequent high price have limited the forage acreage of alfalfa, (9),(35).

Genetic factors are known to affect the seed yield of individual alfalfa plants. Variations in seed yields from year to year have been attributed to such factors as weather, insects and disease. The investigations reported here were begun in 1949 to provide additional information concerning the factors affecting seed production in alfalfa.

REVIEW OF LITERATURE

Factors affecting seed setting in alfalfa have been a subject of investigation ever since the crop attained economic importance. Increasing attention in recent years has been prompted by the continual decrease in alfalfa seed yields in the major seed producing areas. Genetic and environmental factors have been dealt with extensively. Numerous investigations have been concerned with insects (beneficial and injurious) and diseases.

Genetic and environmental factors

Kirk (17) found a pronounced and progressive reduction in vigor of the alfalfa plants with each generation of selfing. The mean yield of seed for second generation selfed lines showed a marked decrease as compared with the seed yield of the corresponding open-pollinated strains. He indicated that controlled pollination with selection in self-fertilized lines which did not show decreases in yield may be utilized in a systematic breeding program.

Clarke and Fryer (6) found that many plants had a hereditary trait of producing a high percentage of sterile pollen under varied environmental conditions. Their investigations indicated that plants had to be tripped to set seed.

Bolton and Fryer (2) found that number of flowers per raceme and number of seeds per pod were heritable characters which affeeted seed yield. High temperatures were found to promote greater pollen growth and better seed set.

Grandfield (14) found that moderate air temperatures, low humidity and low soil moisture were conducive to the storage of high organic reserves, resulting in physiological conditions favourable to seed setting.

Tysdal (33) mentions that high soil moisture is not an inhibiting factor in seed production, unless lodging results.

Jones and Olson (16) found that cross pollination of hand tripped flowers markedly increased seed setting.

Silversides and Olson (26) showed that the mechanical devices they used for tripping alfalfa flowers were not effective in increasing seed set. The increased tripping of the alfalfa blossoms by these devices was offset by the damage caused to the plants.

Southworth (27) outlined a method of producing a high seed setting alfalfa which was both self-tripping and highly fertile. He crossed Medicago sativa with M. lupulina, a self-fertile seed setter. The variety Macsel originated from this cross.

Fryer (12) outlined a method of breeding for increased seed setting by maternal line selection involving a four-year cycle. Plants were scored in the second and third year for desirable vegetative characters and density of seed pods. Plants with a poor score were discarded. The seed obtained in the fourth year was used for another cycle. The variety Ferax was developed by this method.

Tysdal et al. (31) reviewing the literature on the different methods of alfalfa breeding stated that the principles of breeding alfalfa, with some modifications were essentially the same as those which have been established for corn.

Tysdal and Kiesselbach (32) suggested the use of selected

clones of high combining ability and high self-sterility for the production of hybrid alfalfa. The best double cross seed would be made available for commercial utilization. Natural intercrossing of selected clones would result in the formation of synthetic varieties.

Stevenson and Bolton (28) stated that self-tripping alfalfa plants have low forage yields and should not be used in a breeding program.

Bolton (1) indicated that there is a wide range in the combining ability of different alfalfa plants. He outlined a method of alfalfa breeding using disease resistant stock. Self-tripping plants would be discarded. The remaining plants would be rated for self- and cross-fertility. Progeny of both selfed and crossed seed would be grown, and in the selfed lines, plants with undesirable genes would be eliminated. Combining ability of each plant is determined from the progeny of the polycross seed. Progenies grown from the seed of plants crossed in all possible combinations would be used for the final selection.

Tysdal and Crandall (34) outlined a slightly different method. Clones selected for such desirable characters as high seed yield, high forage yield, bacterial wilt resistance, cold resistance and potato leaf hopper resistance, are allowed to intercross at random, polycross seed being obtained. Progenies are then tested for low self-fertility, high seed yield and high forage productivity.

Beneficial and injurious insects

Tysdal (30) mentions that Megachile and Nomia bees are the most efficient trippers of alfalfa, while honey bees are rather poor trippers. Weather conditions and insect visitations determine the seed set of alfalfa.

Lejeune and Olson (20) noted that bumble bees were efficient pollinators of alfalfa, tripping most of the blossoms visited. Honey bees, however tripped very few flowers.

Knowles (18) found a correlation between the presence and number of Megachile and alfalfa seed set. He states that temperature was found to be the most important factor influencing insect activity and tripping of alfalfa blossoms.

Bolton and Peck (2) indicated that, in Northern Saskatchewan, burning of alfalfa fields on which appreciable amounts of lygus bugs were present increased the seed yield.

Hare and Vansell (15) stated that honey bees are important trippers in alfalfa seed fields in the Delta tract of Utah.

Vansell and Todd (36) noted that honey bees would work alfalfa for pollen if competing sources (clovers, mustard, thistles) were absent. Otherwise they were more apt to collect only nectar from the alfalfa.

Carlson (4) found that the use of insecticides increased alfalfa seed yields where lygus bugs were a factor. He obtained a yield of 175 pounds per acre from treated plots compared to 66 pounds per acre from untreated plots. He found that genotypic differences were related to alfalfa seed production. Yields of clonal lines ranged from 37 to 441 pounds per acre.

Drake (8) noted that wild bees were more efficient pollinators of alfalfa than are honey bees. However honey bees were more numerous. He found that the efficiency of pollinating insects was doubled and sometimes trebled by improved cultural practices. Highest yields were obtained where the cutting of the first crop of hay was staggered at 8 to 10 day intervals

to extend the blooming period. A ten percent DDT dust was applied to kill injurious insects.

Wilson (37) found that one pound of DDT and eight ounces of chlordane per acre gave very good control of lygus bugs. Significant increases in yields of alfalfa seed were found in the insecticide treated plots. The insecticide did not affect the population of the pollinators.

McMahon (22) states excellent control of the common injurious insects of alfalfa is obtained in western Canada by the application of three quarters of a pound of DDT per acre, before flowering becomes general.

Diseases

McDonald (21) stated that bacterial wilt is found in Manitoba but so far has not resulted in significant crop losses. Pseudopeziza medicaginis is not very important. Black stem is prevalent in the Province especially in the seed producing areas.

Cherewick (5) reports that crown rot, a strain of Rhizoctonia solani, which attacks both alfalfa and sweet clover, inflicts a heavy loss on alfalfa in Manitoba. He further states that blossom drop in alfalfa, prevalent in Manitoba and frequently resulting in a complete failure of seed set, may be caused by a species of Alternaria.

Peterson and Melchers (24) found that Black stem causes destructive defoliation and discoloration of hay crops. The disease was found to be most severe during cool moist weather. Their investigations showed that the optimum temperature for growth of the pathogen in culture was 21 C with a range of 9 C to 30 C.Of 4 Medicago species tested, (M. sativa, M. lupulina, M. falcata and M. ruthenica) M. falcata was found to be the most resistant to Blackstem.

Koepper (19) tested several varieties and species of alfalfa

for resistance to Uromyces striatus. A plant selection of Ladak which was resistant to rust also exhibited high resistance to Blackstem.

Cormack (7) in studies on Ascochyta imperfecta Peck, stated that the pathogen was both seed and soil borne. Cool moist conditions were most favorable for disease development. New improved Ceresan and Arasan were most effective in seed treatment of alfalfa. The fungus in the soil disappeared two years after the soil was plowed. No blackstem was found in the soil of virgin prairies, virgin woods or fields with cereal crop rotations. The fungus persisted on dry stems and leaves for 5 years and on alfalfa seed for three years. He found that it lowered the forage yield. He reported cases where seed yields may have been decreased because of Black stem.

Reitz, (25) tested 10 varieties and species in both field and greenhouse for resistance to Black stem. Medicago ruthenica was found to be the most resistant. The commercial varieties, Ladak and Grimm were third and fourth in order of resistance.

Stevenson (28) stated that immunity to Black stem has not been found and it seemed probable that a high degree of resistance may be difficult to obtain.

Ellis (10) in preliminary spray tests with chemicals to control Ascochyta abelmoschi, Harter, on a variety of okra (Hibiscus esculentis L.) found that Fermate gave higher yields of marketable pods and lower defoliation ratings than Spergon, Lime sulfur, tribasic copper sulfate and Bordeaux mixture. However, even on the Fermate treated plots the disease was severe and control was not considered satisfactory.

Mitchell et al. (23) experimenting with sulfanilamide derivatives to eradicate leaf rust, found that sulfadiazine as a spray was the most effective. The minimum effective concentrations was 0.1 percent sulfadiazine. A concentration of 0.4 percent sulfadiazine was toxic to the wheat plants.

MATERIALS AND METHODS

1949

Various experiments were conducted, and observations made concerning factors affecting seed setting in alfalfa. Twenty-three lines were tested for seed and forage yield. A caging experiment was conducted in which bees were used as pollinators. The effect of Black stem on seed yield and total plant weight was determined. Observations were made on the numbers and efficiency of pollinating insects present and on the extent of damage caused by injurious insects.

Testing of lines for seed and forage yield

The lines used for this study originated from a nursery established in 1945 consisting of 25 lines each of 25 plants. Plants were rated for seed set in 1946 and 1947. In 1947 twenty-three superior seed yielding plants were selected. Two racemes of each of these plants were self-pollinated. Both open-pollinated and self-pollinated seed was harvested from these plants.

An individual plant nursery was established from this seed in 1948. The nursery was a split-plot randomized design with four replicates. Three plants of open-pollinated origin and three plants of self-pollinated origin of each line formed the main plots of each replicate. Spacing of plants was three feet by three feet.

Plants were harvested individually at maturity and total dry weight per plant taken. Plants were threshed and weight of clean seed was recorded.

Caging experiment with honey bees

Determination of the effectiveness of honey bees in pollination of alfalfa was one of the objectives of this experiment. Evaluation of lines as to selective pollination by honey bees was to be considered. For this purpose a cage, constructed of hardware cloth over a wooden frame, was placed across one replicate in such a position that it enclosed one open-pollinated and one self-pollinated progeny plant of each line. The size of the cage was 72 feet by 6 feet by 6 feet. This cage also provided space for a bee hive at each end.

Visitation by pollinators began about 7 days after the first sign of alfalfa bloom in the nursery. On this date (June 18) two colonies of bees were placed inside the cage. Each colony was provided with two entrances one to the outside and one to the inside of the cage.

One of the colonies dwindled and was removed on August 11. The second colony was normal and was retained till September 16, at which time harvesting operations commenced.

Effect of Black stem on seed yield and total plant weight

Black stem symptoms were first noted on some plants in the nursery at the beginning of June. The disease was prevalent throughout the nursery by the end of July. Individual plants were rated for amount of disease on a percentage basis. Plants were rated for Black stem on both stems and leaves on August 14-16 and on stems alone on September 7.

Two hundred plants of open-pollinated origin were used to determine correlation coefficients to show the effect of Black stem on seed yield and total plant weight. In calculating the correlation coefficients the percentage ratings of the amount of disease on each plant were transformed to degrees. ie. percentage (p) = $\sin^2 \phi$. (Fisher 11).

Pollinating insects

Observations were made on the relative populations and effectiveness of the insect pollinators in the nursery.

Injurious insects

Visual observations were made on the relative number of the different kinds of injurious insects. To evaluate the damage caused by clover seed chalcid, four racemes, from each of six plants taken at random were collected on August 18, and counts of damaged and undamaged seed were taken.

Analysis of data

Correlation coefficients and analysis of variance were determined according to Goulden (13), and transformation of frequencies according to Fisher (11).

1950

Experiments in 1950 dealt mainly with the use of fungicides in the control of Black stem. An experiment to determine the effect of DDT on insects and seed set was conducted. Observations were made on pollinating insects and their habitat.

Effect of fungicides on Black stem and seed yield

Three fungicide dusts were tested as to their effectiveness in controlling Black stem and increasing seed yield. Their active ingredients and composition are as follows:

Trade name	Percentage of Active ingfedients	Composition of active ingredients
Fermate	10	Ferric dimethyl dithio carbamate
Trox	7	Tri-basic copper sulphate
Mulsoid sulfur	95	sulfur

The Red River flood of 1950 necessitated the transfer of the experiment from the University of Manitoba field plots to Teulon, Manitoba, 45 miles N.N.W. of Winnipeg. A 4 acre field of a 3 year old stand of Grimm alfalfa, which had yielded 200 pounds of seed per acre in 1949, was selected. The effect of additional inoculum in the form of old alfalfa straw on the incidence of Black stem was also tested. A split-plot design was used with fungicide treatments comprising the main plots. Additional inoculum (10 pounds of alfalfa straw) was spread over one-half of each main plot. The experiment was replicated 4 times and occupied an area of 2 acres.

Heavy dew every night and occasional rains made weekly application of the fungicides necessary. Applications commenced on June 30th and ended August 16. Trox and sulfur were applied at the rate of 30 pounds and 10 pounds per acre respectively. The first application of Fermate at the rate of 30 pounds per acre resulted in slight burning of the leaves so the rate was subsequently reduced to 20 pounds per acre.

Samples of plants from the different treatments were taken on July 15, July 27, August 15, August 24, and September 6.

A 4 square rod sample of each sub-plot was harvested at maturity. The alfalfa was threshed and weight of cleaned seed recorded.

Effect of fungicides on insect populations

Ten sweeps with a 12 inch insect net were made on each treatment in all replicates on the following dates: July 3, July 5, July 25, August 2, August 10 and August 24. Counts were taken on weevils, alfalfa plant bugs, aphids and pollinators.

Effect of DDT on insect populations and alfalfa seed yield

To test the effect of DDT on insect populations and seed yield of alfalfa a simple randomized block design with 3 replications of 4 square rods each, was set up. One half of each replicate was left as a check.

The other half of each replicate was sprayed on July 5 with a DDT solution (2 pounds of 50% wettable DDT powder to 100 gallons of water) at the rate of three-quarters of a pound of DDT per acre. Five percent of the alfalfa was in bloom, but there was very little pollinating insect activity at this date.

Ten sweeps of each of the six plots were made with a 12 inch insect net on the following dates: July 5, July 7, July 10, July 14, July 25, August 2, August 10 and August 24. Counts were made of weevils, alfalfa plant bugs, aphids and pollinators. The weight of cleaned seed of each plot was recorded.

Study of wild bees

An attempt was made to find the nesting places of the wild bees that pollinate alfalfa. Observations were made on their activities in relation to the environment.

Greenhouse tests on susceptibility of certain alfalfa varieties to Black stem

One plant of Medicago falcata and two plants each of the varieties, Du Puits, Ferax, Ladak, Grimm and Wisconsin Synthetic A, were sprayed with a suspension of Black stem pycnidiospores obtained from old alfalfa straw. The sprayed plants were placed in a moisture chamber for three days. Comparisons of susceptibility were made two weeks after inoculation.

Effect of certain fungicides on the germination of Black stem spores and growth of mycelium on potato-dextrose agar medium

1. Fungicides: Streak cultures of pycnidiospores of Black stem were made on potato dextrose agar medium which contained the following percentages of active ingredients of different fungicides: check; 0.1% and 1.0% of Trox and Fermate: 0.5% and 5% of sulfur. Plates were incubated

at room temperature of 70 degrees Fahrenheit for seven days after which the petri plates were photographed, and comparisons made.

2. Sulfadiazine: Cultures of pycnidiospores and surface sterilized infected leaves were grown in potato dextrose agar medium, which contained the following percentages of sulfadiazine 0.0, 0.05, 0.1, 0.5, 1.0, and 5.0. The petri plates were incubated at a temperature of 70 degrees Fahrenheit for seven days. Observations of germination of spores and growth of mycelium were recorded at the end of this period.

Effect of Sulfadiazine on control of Black stem on alfalfa in the greenhouse.

One test was made to determine the effect of single doses of sulfadiazine at varying concentrations. The test consisted of 2 replicates with 7 treatments. Fourteen plants of a Grimm clone and 14 plants of a Du Puit clone were used. The concentrations used were: 0.0, 0.1, 0.2, 0.4, 0.5, 1.0, and 2.0 percent. Each plant was sprayed with 15 c.c. of one of the concentrations of sulfadiazine. The plants were sprayed 24 hours later with a suspension of Ascochyta imperfecta spores and placed in a moisture chamber for 3 days. Two weeks after inoculation observations on the control of Black stem disease and toxic effects on the plants were recorded.

The second test was made to determine the effect of a varying number of doses of a non-toxic concentration of sulfadiazine. The experiment consisted of 2 replicates of 4 treatments, using 8 plants of a Du Puit clone and 8 plants of a Grimm clone.

Treatments were 0, 1, 2, and 3 doses of 0.4% sulfadiazine with 6 day intervals between each dose. Twenty four hours after the last application of sulfadiazine, each plant was inoculated with a spore suspension of Ascochyta imperfecta and placed in a moisture chamber for three days. Observations were recorded two weeks after inoculation.

RESULTS

1949

Favorable weather and a long pollingting season resulted in a fairly high seed yield. The alfalfa blooming period commenced about June 15, and ended about September 15, The above normal temperatures and below normal rainfall in the spring and summer were favorable for an increase of the native bee populations.

Testing of lines for seed and forage yield

Visual observations of the nursery revealed a pronounced reduction in vigor and seed set in the self-pollinated progenies in comparison with the open-pollinated progenies. Considerable variation was noted between individual plants within lines in both vigor and seed set. Four lines were discarded from the test because of a large number of missing plants. Seed yield data of the remaining 19 lines are given in Table 1; and analysis of variance is given in Table 2

Table 1 -- Average seed yields of 19 lines in pounds per acre.

Line number	Average yield of open-pollinated progeny	Average yield of self-pollinated progeny	Average yield of line
16-1	460.7	132.5	296.6
10-2	347.5	136.0	241.7
3-2	332.5	121.2	226.9
15-3	327.2	161.7	244.5
7-3	309.5	252.5	281.0
5-2	298.0	152.7	225.4
14-1	297.5	116.5	206.7
2-2	281.0	101.5	191.2
13-1	276.5	175.0	225.8
12-3	261.5	176.2	218.9
7-4	258.5	188.5	223.5
4-5	253.5	176.7	215.1
5-4	247.0	168.7	207.9
14-2	241.0	73.7	157.4
5-1	225.2	180.7	203.0
12-2	199.2	78.2	138.7
16-2	185.0	120.7	152.9
10-1	172.5	137.7	155.1
8-3	170.2	131.7	150.9
Average	270.7	146.5	208.6
Necessary Diff. @ 5% level	59.0	59.0	41.7

Table 2 -- F values of seed yield data

	F value calculated	5 percent point	1 percent point	necessary difference at 5 percent point.
Lines	2.16	1.79	2.23	41.7
Progeny origin	74.82	4.02	7.12	14.4
Lines x progeny origin	1.51	1.79	2.23	

The F values show that there was a significant difference at the 5 percent level between the average seed yield of lines. They also show a highly significant difference at the 1 percent level between the self-pollinated progenies and the open-pollinated progenies. The interaction of lines by progeny origin did not exceed the 5 percent probability level.

The average seed yields of the open-pollinated progeny ranged from 170.2 pounds to 460.7 pounds per acre. This range is similar to that obtained by Carlson (4). The average seed yield of the self-pollinated progeny was 53.7 percent of the open-pollinated progeny, compared to 62 percent obtained by Tysdal et al. (31).

The average total plant weights in tons per acre are given in Table 3. The F values of the data in Table 3 are given in Table 4.

The F values indicate highly significant differences between lines, progeny origins, and interaction of lines by progeny origin.

Table 3. Average total plant weights (dry) in tons per acre.

Line No.	Open-pollinated progeny	Self-pollinated progeny	Average of line
16-1	3.68	2.19	2.94
7-3	3.39	2.83	3.11
12-3	3.38	3.07	3.22
3-2	3.22	2.51	2.76
15-3	3.06	3.06	3.06
14-1	3.00	1.90	2.45
5-2	2.95	2.08	2.51
13-1	2.85	2.19	2.52
10-2	2.83	2.93	2.88
8-3	2.77	2.86	2.81
2-2	2.71	1.72	2.22
12-2	2.70	2.07	2.39
16-2	2.39	2.14	2.39
7-4	2.38	1.74	2.06
14-2	2.37	1.64	2.00
5-1	2.34	2.21	2.27
5-4	2.22	2.07	2.14
10.1	1.91	1.64	1.78
4.5	1.83	1.82	1.83
Average	2.74	2.25	2.49
Necessary difference at 5% level	0.76	0.76	0.54

Table 4. F. values of plant weights

	F. value calculated	5 percent point	1 percent point	nec. diff at 5 %
Lines	4.6	1.79	2.29	0.54 tons
Progeny origin	54.8	4.02	7.12	0.04 tons
Lines by progeny origin	4.0	1.79	2.23	0.57 tons

In average total plant weights the self-pollinated progeny was 72% of the open-pollinated progeny. This percentage was only slightly higher than the 68% obtained by Tysdal et al. (31).

The results indicate that forage crop yields do not decrease as markedly as seed yields in the first generation of selfing.

Caging experiment with honey bees:

Two colonies of honey bees were placed inside the cage one week after alfalfa bloom was noted. Approximately one week elapsed before the honey bees became accustomed to their new surroundings. A large number of the honey bees inside the cage were observed to work the blossoms for nectar. A few (mostly young honey bees) were observed tripping the florets. One honey bee was observed to trip 13 florets before returning to the hive. Honey bees tripping alfalfa had some pollen in their pollen baskets. The bees using the outside entrance brought in a large supply of foreign pollen.

Visual observations indicated that with open-pollinated progeny the majority of the plants inside the cage had a lower seed set than their counter parts outside the cage. No difference in seed set was observed between caged and uncaged self-pollinated progeny.

The caged plants yielded 97.5% of the uncaged plants in the self-pollinated progeny, and 69.8% in the open-pollinated progeny. Average yields of alfalfa seed in pounds per acre were as follows:

No. of plants	Caged or uncaged	Progeny origin	Average yield
18	caged	self-pollinated	94.3
18	uncaged	self-pollinated	96.6
16	caged	open-pollinated	172.0
16	uncaged	open-pollinated	246.7

The possibility that shading reduced the seed yield must be considered. This is indicated by the fact that the average seed yields of the partially shaded uncaged plants in the caged replicate were considerably lower than the average yields of the unshaded plants in the other three replicates.

Indications that certain lines may be adapted to pollination by honey bees are exemplified by line 16-1. One caged plant of the open-pollinated progeny of line 16-1 yielded 580 pounds per acre in comparison with the average yield of 460.7 pounds per acre for the whole line.

Effect of Black stem on seed yield and total plant weight

Visual observations of the plants in the nursery showed variation in prevalence of disease on different lines and on plants within lines. It was not proven whether these differences were due to inherent resistance of the plants or to uneven infection. However, it was evident that the heavily infected plants showed a marked reduction in seed set and total plant weight. An obvious reduction in plant weight was due to the loss of most of the leaves in the more heavily diseased plants.

The correlation coefficients of disease, seed yield and forage yield are given in Table 5.

Table 5. Correlation coefficients of disease, seed yield and total plant weight:

Total correlation coefficients.

	Seed yield	Total plant weight
Disease	-0.34	-0.33
Seed yield		+0.61

Partial correlation coefficient with total plant weight constant.

	Seed yield
Disease	-0.24

All coefficients were significant at the 1% level.

The coefficients indicate that Black stem has a detrimental effect on seed yield and total plant weight of alfalfa.

Observations on pollinating insects in the nursery:

The pollinating insects found in the nursery were megachilids, bumble bees, honey bees and halicitids. The leaf cutter bees or megachilids were the most efficient pollinators. They tripped practically every flower visited at a very fast rate. Their population however, was small.

The bumble bees tripped about 80% of the flowers visited. Their rate of tripping was lower than that of the megachilids. The bumble bees were much more numerous than the megachilids.

The percentage of honey bees tripping alfalfa was very low. However, the honey bees comprised about 90% of the total population as determined by visual counts throughout the blooming period.

Some sweat bees or halictids were observed working the alfalfa florets, but very little tripping was noted. The bees appeared to be going over the blossoms tripped by other pollinators.

The weather conditions were favorable for the increase of pollinating insect populations. This and a long pollinating season resulted in a very good seed set.

Observations of injurious insects:

Some of the more important injurious insects present in the nursery were weevils, alfalfa plant bugs, leaf hoppers, aphids and clover seed chalcids.

Some weevil damage was noted in May, but with the advanced growth, the damage became negligible. Alfalfa plant bugs, leaf hoppers and aphids were observed in large numbers, but the damage done was not ascertained.

Extent of clover seed chalcid damage is shown in Table 6.

Table 6. Clover seed chalcid damage

Plant number	Total number of seed in 4 racemes	Number of seeds destroyed by chalcids		Percent destroyed
1	187	37		19.8
2	192	83		43.2
3	327	74		22.6
4	214	88		41.1
5	190	25		13.1
6	246	95		38.6
Total	1456	402	Average	27.6

About 50 percent of the affected seeds were empty shells from which the adults had emerged. The rest of the affected seeds contained either the larvae or the pupae of the clover seed chalcid.

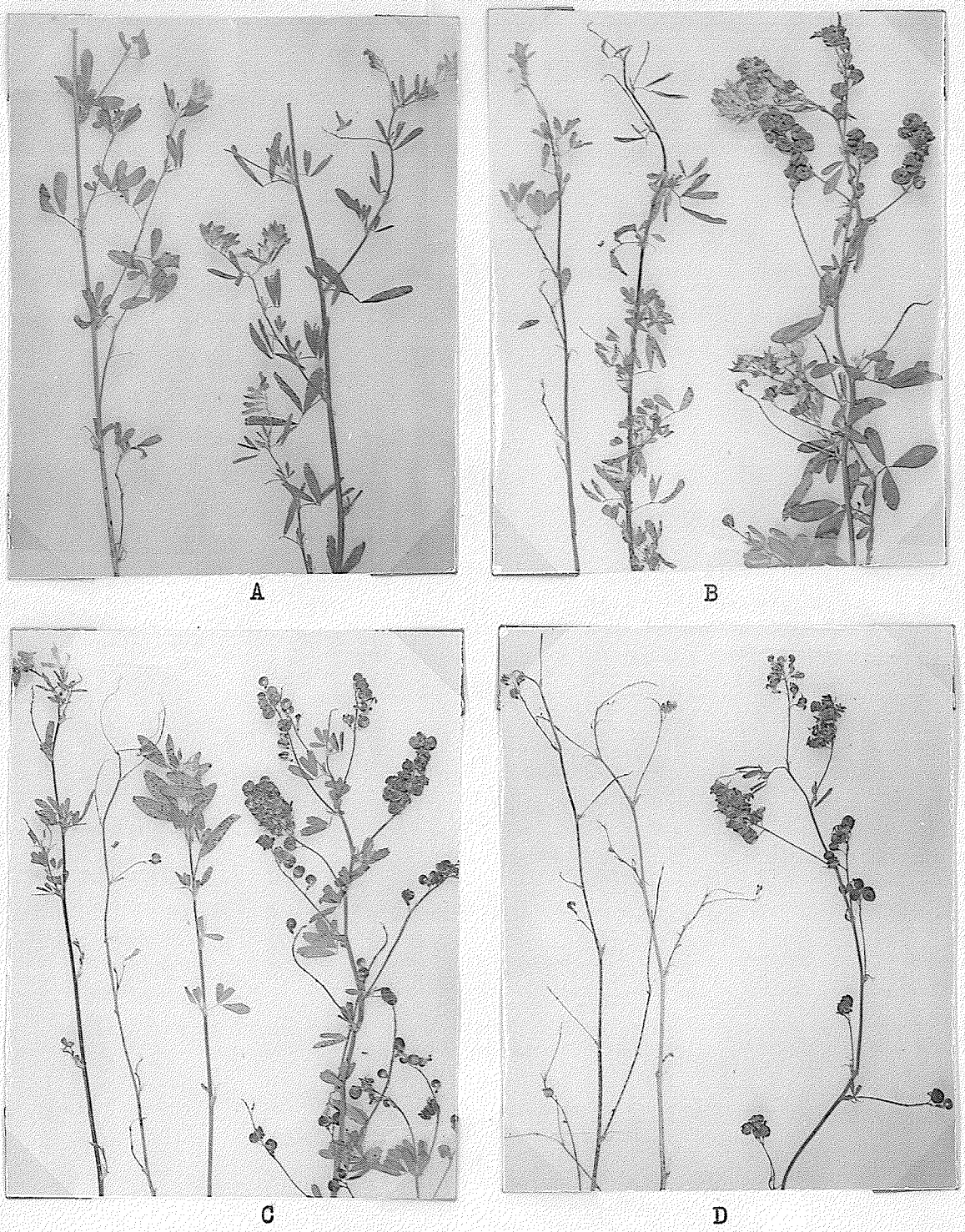
1950

Unfavorable weather and a short pollinating season resulted in a very low seed yield. The alfalfa blooming period began about July 1 and ended on August 24 when a ground frost occurred. Cool weather and heavy rainfall in the spring and summer delayed the increase of the native bee populations. The season was very favorable for the development of Black stem.

Black stem was prevalent throughout the whole field, some parts of the field being more seriously affected than others. Seed set was observed to be inversely proportional to the amount of disease. Severely infected plants were characterized by loss of leaves, stunting and destruction of first growth, with subsequent heavy infection of the second growth.

Figure 1 shows differences in amount of bloom and seed set at different dates. Sparse pod formation on the first-formed racemes was characteristic of plants showing the least disease.

Figure 2 shows stems taken at July 15 and September 6 from plants that were heavily diseased. On July 15, the leaves were almost completely covered with Black stem lesions. On September 6, the disease had caused a loss of leaves, stunting and eventual death of the first growth. The second growth was very heavily infected.



A B C D

Fig. 1. Amount of bloom and seed set on alfalfa plants at different dates. Stems on left are heavily diseased. Stems on right have less disease.

A. July 27 B. August 15 C. August 24 D. September 6

A B

Fig. 2. Stems from plants severely infected with Black stem

A. July 15

B. September 6. First growth on right; second growth on left.

Effect of Fungicides on Black stem and alfalfa seed yield.

Visual observations of the treated plots showed no apparent control of Black stem by the fungicides. However, seed pods were noted on the Fermate treated plots 3 days earlier than on the other plots. Artificially inoculated plots did not appear to have any more disease than the uninoculated. Results of the average yields of the treated plots and the analysis of variance of those yields are shown in Table 7.

Table 7. Effect of Fungicides on the seed yields of alfalfa

Treatment	Mean yield (pounds per acre)
Check	8.4
Fermate	14.4
Trox	7.7
Sulfur	8.7
Additional inoculum	10.0
No additional inoculum	8.7

Treatments	F value calculated	5 percent point	1 percent point	Necessary difference at 5%
Fungicide	4.21	3.86	6.99	4.8 pounds
Inoculum	.88			

The results in Figure 7 show that Fermate was the only fungicide which significantly increased the seed yields of the alfalfa plots.

Effect of fungicides on insect populations:

Total counts of the insect populations as obtained from sweeps at the 6 dates are shown in Table 8.

Table 8. Effect of fungicides on insect populations

Treatments	Weevils	Alfalfa plant bugs	Aphids	Pollinators
Check	59	298	500+	9
Fermate	35	51	500+	4
Sulfur	47	211	500+	5
Trox	45	305	500+	4

An analysis of variance was performed on the data in Table 8 using the square root transformation of frequencies (11). The analy-

sis showed a significant difference occurred only in the alfalfa plant bug populations. The results are presented in Table 9.

Table 9. Effect of fungicides on the alfalfa plant bug population.

Treatment	Average number of insects per date			
Check	6.1			
Fermate	2.5			
Sulfur	4.9			
Trox	5.9			
	F value calculated	5 percent point	1 percent point	Necessary diff at 5 percent
Treatments	7.5	3.25	5.42	1.24 insects

The correlation coefficient of seed yield with the alfalfa plant bug population was -0.96, a value which exceeds "r" at the 1 percent level of significance. This suggests that some of the increase in seed yield in the Fermate treated plots may have been due to the decrease in the alfalfa plant bug population.

Effect of DDT on insect populations and seed yield of alfalfa:

Total counts of sweeps taken on the check and treated plots are given in Table 10.

Table 10 shows several important facts. The DDT spray practically eliminated the weevils, alfalfa plant bugs and aphids, but had no apparent effect on the pollinators. The lethal effect of the DDT residue varied, being about 9 days for the aphids and about 28 days for the weevils and alfalfa plant bugs. The maximum population of the plant bugs and weevils was reached on July 7, when approximately 20 percent of the alfalfa was in bloom. The aphid population increased steadily throughout the season. Two life cycles of the weevils and alfalfa plant bugs are apparent.

Table 10. Insect populations of DDT treated and Check plots.

Dates	weevils		alfalfa plant bugs nymphs		adults		aphids		pollinators	
	check	DDT	check	DDT	check	DDT	check	DDT	check	DDT
Before spraying July 5	9	9	57	64	0	0	7	6	0	1
After spraying July 7	10	0	90	1	1	1	9	0	0	0
July 10	5	0	75	0	7	1	13	0	2	2
July 14	7	0	54	0	20	0	19	3	1	0
July 25	0	0	1	0	11	0	23	4	0	2
August 2	0	0	3	0	8	1	30	7	1	0
August 10	1	1	0	0	0	1	143	47	2	5
August 24	11	5	6	0	3	0	119	40	0	0

The average seed yields of the treated and check plots and the analysis of variance are given in Table 11.

Table 11. Effect of DDT on alfalfa seed yields.

Treatment	Yield (pounds per acre)			
Check	10.2			
DDT	18.2			
	F value calculated	5 percent point	1 percent point	necessary diff. at 5 percent
Treatments	58.60	18.61	98.45	4.6 pounds

The results in Table 11 indicate a significant increase in seed yield in the DDT treated plots. Although this increase of 8 pounds is not economically important, the reduction of the insect populations as shown in Table 10, may have given entirely different results had the season been normal for alfalfa seed set.

Observations on alfalfa pollinators:

The pollinating insects found in 1950 in the alfalfa field were similar to those found in 1949, viz. megachilids, bumble bees, honey bees and halictids. The populations however, differed tremendously. The number of honey bees was almost negligible, as the closest apiary was 2 miles distant. The cool wet spring had an adverse effect on the increase of the wild bee populations. Small numbers of wild bees were first observed on July 20. The pollination season was short. Appreciable numbers of bumble bees and leaf cutter bees were not observed after August 16.

Fifteen bumble bee nests and two leaf cutter bee nesting places were found within 300 yards of the alfalfa field . The popula-

tion of the bumble bee colonies varied from 6 to 35. Observations on one colony found in the middle of the alfalfa field, showed a relatively higher seed set in the vicinity of the nest than in the remainder of the field. Bumble bees were the most numerous of the pollinators present in the alfalfa field.

The population of the megachilids was small. Very few megachilids were taken in the sweeps. Observations on the activities of the megachilids on a warm sunny day indicated that an average period of 20 minutes was spent on each foraging trip, and 5 minutes was spent in the nest between trips.

Halictids were not observed tripping alfalfa flowers, but most of those taken in the sweeps had pollen on their legs.

Greenhouse inoculation of certain alfalfa varieties with Black stem:

A very good infection resulted from the inoculation (Fig. 5A-1). The varieties of alfalfa tested showed varying degrees of susceptibility to Black stem. Medicago falcata showed the least amount of disease. Ladak, Grimm, Ferax and Wisc.A. were moderately susceptible. Du Puits was very susceptible to Black stem. There was some variation between plants of the same variety. This suggests the possibility of selection of plants with greater resistance.

Effect of fungicides on Black stem spore germination and mycelial growth:

The effect of the fungicides used in field tests for control of Black stem are shown in Figure 3. The results show no spore germination or mycelial growth on Fermate treated plates, some spore germination and very little mycelial growth on Trox treated plates. The growth on

the sulfur treated plates was approximately equal to the check. The fact that Fermate completely inhibited growth of Black stem, indicates that part of the increased alfalfa seed yield may be attributed to the fungicidal properties of Fermate.

Observations on the test using different concentrations of sulfadiazine on streak spore and infected leaf cultures of Black stem, showed that the 1 percent and 5 percent concentrations had no growth whatsoever. The relative amount of mycelial growth on the other concentrations is shown in Figure 4. The amount of mycelial growth decreased with increase of concentration of sulfadiazine.

Effect of sulfadiazine on control of Black stem in the greenhouse:

Alfalfa plants inoculated with Black stem showed decreased disease with increased sulfadiazine concentrations. However, toxic effects were noted on the 0.5 percent, 1.0 percent and 2.0 percent concentrations. This resulted in blackening of stems and stipules, loss of leaves and general breakdown of the plant. (Figure 5A.). The 2 percent concentration resulted in the death of the plants. The plants treated with a 1 percent concentration or less recovered. (Figure 5B.)

Repeated doses of 0.4 percent sulfadiazine at 6 day intervals on plants inoculated with Black stem exhibited varying degrees of control. The check plants were heavily diseased. Plants with 3 doses showed no disease, while plants with 1 and 2 doses showed some disease symptoms. However, plants which received 2 and 3 doses of 0.4 percent of sulfadiazine, developed a prostrate habit with reduced vigor and slight yellowing of the leaves.

Fig. 3. Effect of fungicides on growth of Ascochyta imperfecta on potato-dextrose agar.

A C-1 Check

B S-1 0.5% Sulfur S-2 5% Sulfur

C T-1 0.1% Trox T-2 1% Trox

D F-1 0.1% Fermate F-2 1% Fermate

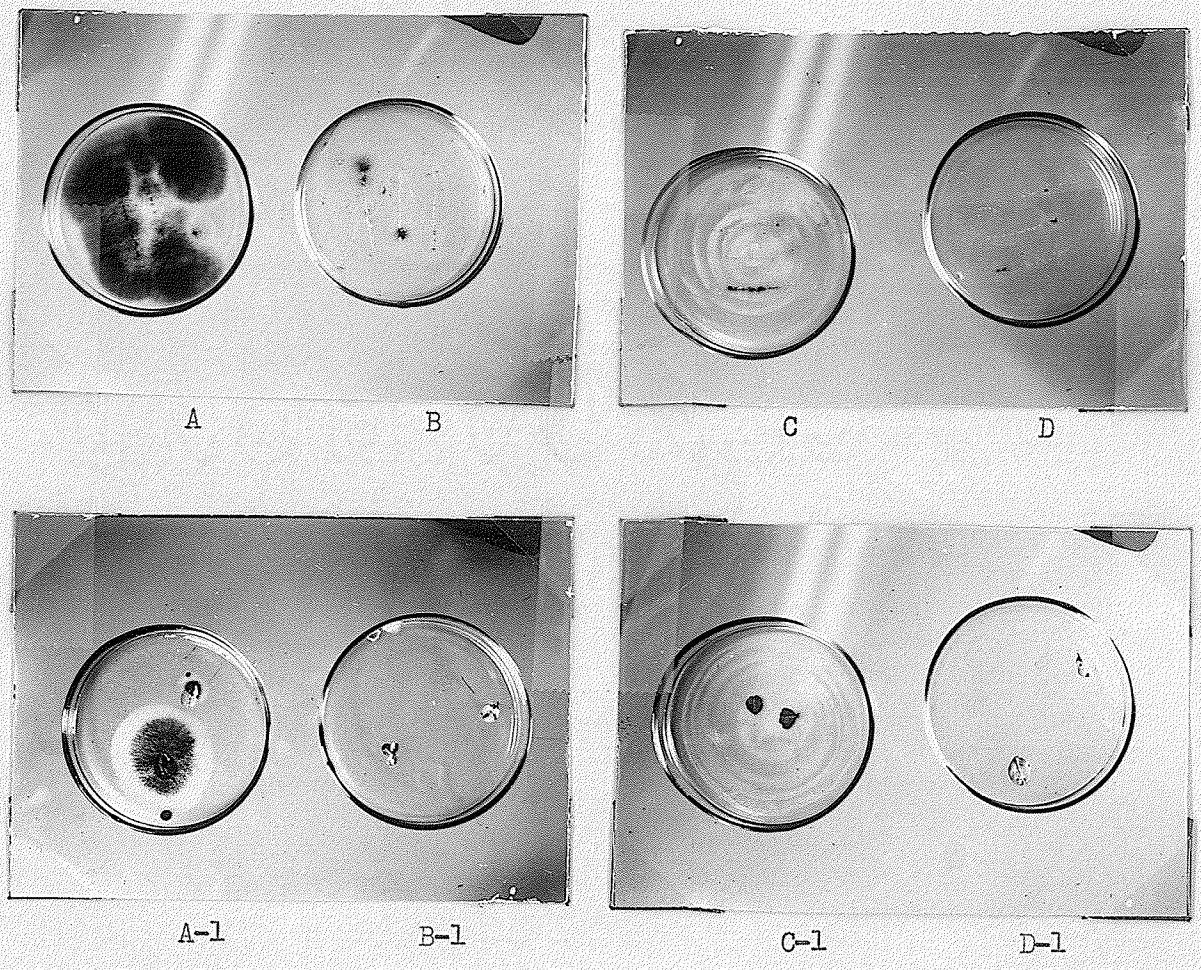


Fig. 4. Effect of sulfadiazine on growth of Ascochyta imperfecta on potato-dextrose agar.

A, B, C, D, spore cultures.

A-1, B-1, C-1, D-1, infected leaf cultures.

A, A-1, Check B, B-1, 0.05% Sulfadiazine

C, C-1, 0.1% Sulfadiazine D, D-1, 0.5% Sulfadiazine

A-1 A-2 A-3 A-4 B-1 B-2

Fig. 5. Effect of Sulfadiazine on Black stem disease and alfalfa plants.

A-1 Check A-2 0.5% A-3 1.0% A-4 2.0% Sulfadiazine 3 weeks after inoculation.

B-1 2.0% B-2 1.0% Sulfadiazine 6 weeks after inoculation

DISCUSSION

Several factors influencing seed set to a marked degree were in evidence during the course of the investigations. The weather was very important, both directly and indirectly. The weather had a pronounced effect on native bee populations, blooming period, pollinating conditions and spread of disease. Favorable weather conditions in 1949 resulted in a remarkably high seed yield. Cool weather and excessive moisture in 1950 resulted in a very low seed yield.

The variation in seed and forage yields of the different lines shows the influence of heritable characters. The results indicated that genotypes with improved seed and forage yields may be selected.

Increases in the population of wild bees depend to a large extent on the weather and therefore, the pollination of alfalfa by wild bees is uncertain. Although the plants inside the cage pollinated by honey bees only, produced less seed than the plants outside the cage open to all pollinators, the possibility that shading reduced the seed yield should be considered. It is therefore probable, that honey bees may be effective in supplementing the pollination of alfalfa by wild bees if competing sources of nectar and pollen, particularly the latter, are not present.

Black stem was shown to affect the seed and forage yield of alfalfa. Variation between plants in incidence of Black stem indicates that selection for resistance to this disease is possible. The fungicides used did not offer effective measures of control of the disease. Sulfadiazine offers a measure of control but, because of its toxic effects on alfalfa is not considered practical. Fermate, though it increased seed yields and inhibited mycelial growth on petri plate cultures

of Black stem, did not control the disease in the field satisfactorily. Sulfur and Trox were of little value in controlling the disease either in the field or on Petri plate cultures. The heavy dews and frequent rains during the summer may have lowered the potential value of the fungicides.

The use of a DDT spray in the early bloom stage resulted in a decided increase in the seed yield with a decrease of such injurious insects as weevils, alfalfa plant bugs and aphids. DDT did not affect the pollinators when spraying was done at the first bloom stage.

Clover seed chalcid damage was estimated at 27.6% on the basis of counts made in 1949. The results indicate that the clover seed chalcid can be a problem of economic importance in certain years.

SUMMARY

1. The effect of insects (beneficial and injurious), Black stem and genotypes, on seed setting in alfalfa was studied in 1949 - 50.

2. Favorable weather conditions resulted in a high average yield of alfalfa seed in 1949. The cool wet weather in 1950 resulted in a very low seed yield.

3. Nineteen lines, consisting of open and first generation self-pollinated progenies of single plants were tested for seed and forage yield. The self-pollinated progeny yields in percentage of the open-pollinated were 53% and 72% of seed and forage respectively. The range of seed yield of the open-pollinated progeny lines was 170.2 -460.7 pounds per acre.

4. The yield of caged plants with honey bees as pollinators was 97% of the comparable uncaged plants in the self-pollinated progeny, and 69.8% in the open-pollinated progeny.

5. The clover seed chalcid was found to have destroyed 27.6% of the total alfalfa seeds in 1949.

6. Three quarters of a pound of DDT per acre significantly increased seed yields and reduced the injurious insect population in 1950.

7. Black stem was found to have a detrimental effect on the seed yield and total plant weight of alfalfa.

8. The fungicide Fermate (ferric dimethyl dithio carbamate) was found to increase alfalfa seed yields, while Trox and Sulfur had no effect.

9. Sulfadiazine was found to be effective in controlling Black stem in the greenhouse, but because of its toxic effects on the alfalfa plants,was not considered a practical method of control.

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