The Effect of Captan, Demeton, and 4(2,4-DB) on Alfalfa Growth and Seed Production

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THE EFFECT OF CAPTAN, DEMETON, AND 4(2,4-DB) ON ALFALFA GROWTH AND SEED PRODUCTION

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INTRODUCTION

The widespread use of chemicals for the control of diseases, insects, and weeds in the production of alfalfa seed has caused concern with regards to what effect these materials may have on the inherent reproductive potential of the plants. It has been reported that captan (N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) has both a morphological and physiological effect on some plants. The action of this chemical with regards to alfalfa reproduction has not been reported. Demeton (0,0-diethyl 0(and S)-2-(ethylthio) ethylphosphorodithioate) is another chemical used in alfalfa seed production for which the direct effects on reproduction have not been studied. Adverse effects have been reported on a number of plant species with the use of organic phosphate insecticides. A selective herbicide, 4(2,4-DB), (2,4-dichlorophenoxy-batyric acid) shows promise of being satisfactory for the control of broad-leaf weeds in alfalfa. Work reported on studies of forage and seed yields is quite varied as to the effect of 4(2,4-DB) on alfalfa.

It is the object of this study to determine, in a preliminary way, the effects of captan, demeton, and 4(2, 4-DB) on the growth, pollen and ovule fertility , and seed and chaff yields of alfalfa.

REVIEW OF LITERATURE

Mechanism of Action of Growth Regulating Substances

Thimann (1956) found that the amount of an auxin required to produce a significant effect in different parts of a plant varied widely. For instance, the concentration of IAA (indoleactic acid) required to inhibit stem growth was about one million times that required to inhibit root growth. Even within the same tissue, different dosages cause different responses. Several weeks may be required for some of the more slowly induced effects to appear as shown by Ropp and Markley (1955). It was likely, therefore, that the variety of growth and toxic effects of growth regulating substances were the result of the many different concentrations of these substances that occurred at different intervals of time in the various tissues and organs of the treated plant. Woodford, Jolly and McCready (1958) reported that local concentrations will in turn be determined by the factors controlling the penetration, translocation, and the persistance of the compounds within the plant.

A common characteristic of all growth regulators, according to Thimann (1956), was that at low concentrations they stimulate or accelerate plant metabolism and growth while at high concentrations they were inhibitory. Thimann

(1956), also stated, that there was little justification for the viewpoint that the phytotoxicity of a chemical was due solely to the inhibitory rather than the stimulatory response. It seemed much more likely that the toxic effect of growth regulators, which often extend over many weeks in annual plants and longer in perennials, were made up of many different types of stimulatory as well as inhibitory effects.

Many possible mechanisms of toxic action have been suggested. Leopold (1955), listed the following; respiratory depletion, abnormal cellular proliferation, the production of toxic substances (lactones and amino solids), abnormal phosphatase activity, protein hydrolysis, and upset in potassium uptake and metabolism. He pointed out that any of these effects could result in death. In view of the extreme diversity of the responses that can be brought about by growth regulating substances it seemed unlikely that any one factor could account for all the observed effects. More general theories were required to explain the whole range of differences between the endogenous and synthetic hormones.

Woodford, Holly and McCready (1958), pointed out that the ability of a growth regulator to persist in an active form in plant tissues was likely to be of primary importance whatever the precise mechanism of action of that growth regulator may be. A simple and attractive explanation of why IAA is useless as a herbicide, while many synthetic growth regulators are highly effective, is that plants contain a mechanism for the inactivation of IAA when present in excess, but have no such safety mechanism for the control of synthetic growth regulators. They suggested that IAA control within the plant might be established by destruction, immobilization due to formation of association products, or counteraction by inhibitors.

Normal growth of plants is often considered to be controlled by a balance between growth promoting and growth inhibiting systems (Steward and Shantz, 1956; Thimann, 1956). Although, there have been reports of the chemical extraction of inhibitors, attempts to demonstrate the diffusion of inhibitory substances into the external solution have been tried unsuccessfully by Bennet-Clark and Kefford (1954), and Osborne (1958). This, they concluded, was caused by the balance between growth promoters and growth inhibitors. In a normal organism this balance is so accurately maintained that it is difficult to upset it sufficiently in favor of the inhibitor. However, situations have been reported where short period treatments with IAA have subsequently caused inhibition. Ball and Dyke (1956), using whole seedlings of <u>Avena</u> which had previously been subjected to a short period of immersion in IAA, found that there was a stimulation of growth at first, but this subsequently fell below that of the control plants. This suggested that it was possible, under certain conditions, to upset the balance sufficiently for the presence of the inhibitor to be detected by growth measurements. Similar pretreatment with 2,4-D (2,4-dichlorophenoxyacetic) resulted in growth stimulation but no subsequent growth inhibition.

In a more detailed experiment undertaken by Osborne (1958), who measured the growth responses of sections of etiolated pea internodes pretreated for a short period with IAA, 2,4-D, and 2,5-dichlorobenzoic acid, it was found that the growth of sections pretreated with sub-optimul concentrations of IAA for a few hours and then transferred to water, fell below that of the control sections kept in water all the time. In addition the sections did not subsequently respond fully to further application of other growth regulators. When, 2,4-D and 2,5-dichlorobenzoic acid were used for pretreatment instead of IAA, there was no subsequent inhibition of growth and the sections responded fully to further application of other growth regulators. The author suggested that treatment with IAA induced the formation of an inhibitory substance which accumulated within the tissue and upset the growth processes. The tissues were in consequence rendered less sensitive to further application of growth regulators. There was no evidence for the formation of such an inhibitor following treatment with 2,4-D and 5-dichlorobenzoic acid.

IAA differed from synthetic growth regulators in the following respects according to Woodford, Holly and McCready (1958), a. it was less persistent in plant tissues; b. it was capable of forming inactive association products within the plant; c. it may have induced the formation of inhibitors which counter balanced its stimulatory action. They also concluded that whatever the mechanisms be which growth regulating substances exert their effect, their potency depends on their ability to persist in plants and to exert their growth regulating effect over an extended period of time. In addition, the range of susceptibilities of different species may also be due in part to variations in their detoxication mechanisms.

Effects of Captan on Plants

Captan, used for the control of certain fungus diseases (Kittleson, 1952), has been reported to produce beneficial effects, other than fungicidal, in crop plants (Daines, 1953, 1957; Szkolnik, 1952). These effects were more noticeable in regions where the environmental conditions of temperature and light intensity were such that maximum growth and fruit production were not obtainable. In areas where high night temperatures prevail during part of the growing season, increased yield in citrus and vegetables has been obtained with the use of captan. Daines (1953), reported that in New Jersey and in other areas, apple production was increased and quality improved by the application of captan sprays during the growing season. However, these beneficial effects were not obtained in California with low night temperature and high light intensity which were conducive to better growth and higher yields.

Under certain conditions high concentrations of captan caused foliage injury to plants as reported by Daines, Lukens, Brennan, and Leone (1957). They found injury generally occurred when conditions were favorable for captan decomposition. With an increase in temperature, the phytotoxicity increased with the critical range occuring between 85° and 100° F. Shading plants immediately before or after spraying increased foliage injury approximately one and one half times over that of unshaded plants.

In decomposition studies by Miller (1957), and Daines, Lukens, Brennan, and Leone (1957), it was found that as captan decomposed the hydrogen ion concentration increased in the solution. The hydrolysis of the $-SCCl_3$ group giving off free chloride ions resulted in the formation of hydochloric acid. Miller (1957) showed using 0.01 N HCl on apple leaves that the foliage injury produced was identical to the injury caused by captan. Under conditions of low light intensity plants developed thin cuticles and cell fluids with increased hydrogen ion concentration. Under such conditions, resistance to penetration into the leaf may be decreased and injury from the acid would be increased.

Daines, Lukens, Brennan and Leone (1957) found evidence to indicate that photoxicity may result from captan within the cells. They showed that amino acids and derivatives possessing a sulfhydryl group (cysteine and glutathione) inactivated captan as a fungicide. The reaction with sulfhydryl groups in cells may provide an additional mechanism of toxic action whereby captan was decomposed with the production of hydrogen and chloride ions and hydrogen sulfide.

Lukens and Sisler (1958) proposed that captan was a metabolic inhibitor of the sulfhydryl enzymes by virtue of its cellular decomposition to the toxicant, thiosphosgene, which was thought to combine with free sulfhydryl, as well as amino, hydroxyl and, possibly, carboxyl groups in the cell and thus inhibit vital metabolic processes. It was reported by Hochstein and Cox (1956), that the fungicide inhibited the decarboxylation of pyruvate by yeast carboxylase preparation. McCallan, Miller, and Weed (1954) observed that captan, at a concentration which prevented the germination of conidia, also inhibited respiration.

Dugger, Humphreys and Calhous (1959) working with excise pea root tips concluded that captan was capable of inhibiting certain enzyme systems that have cocarboxylase as a coenzyme. This inhibition probably was caused by the reaction of the intercellular breakdown product of captan, thiophosgene, interfering with the coenzyme linkage necessary for decarboxylation. They observed that this general mechanism would account for the observations reported by Hochstein and Cox (1956), and McCallan, Miller and Weed (1954). They also proposed that any inhibition that causes a buildup of one intermediate product, such as pyruvate, might indirectly result in an increase in cellular products that would influence quality or yield.

For example, in citrus production a higher concentration of soluble solids has been found in fruit produced on trees sprayed with captan, and the keeping quality of apples in storage has also been improved. In vegetable and flower production, yields have been increased significantly by early application of captan.

Silber (1959) found that the growth of tomato, Red Kidney beans, corn, and peppermint plants in both nutrient and soil culture was inhibited by captan. In Hoagland's nutrient solution supplemented with one, three, and 10 ppm captan the plants grew less than control plants receiving none. Of the plants tested, tomato was the most sensitive to captan with both the fresh and dry weight being significantly lower at the three ppm level. In the soil culture of zero, 25, 100, 200, and 400 ppm captan, on a dry weight basis, tomato, Red Kidney beans, corn, cucumber, and cabbage plant growth was found to be inhibited by captan. Growth of tomato at 25 ppm and cabbage at 100 ppm was significantly lower, while corn and bean growth was not significantly below the 400 ppm level of captan. Some marginal necrosis of cotyledons and leaves of plants grown in soil containing a high concentration of captan was observed. This resulted in stunting the growth of these plants.

Effect of Demeton on Plants

In reviewing the literature no work was found that reported the effect of demeton on plants, but a number of papers were found for other organic phosphate insecticides. From reviewing these papers it was found that most of these insecticides seemed to affect the plants in the same manner. Haeskaylo and Ergle (1955) studied cotton plants grown in nutrient cultures containing zero, 10, 100, and 1000 ppm of Schradan (bis (dimethylamino) phosphoric anhydride). The Schradan, at concentrations of 10 and 100 ppm, stimulated vegetative growth; compared with the control on a dry weight basis, it showed 23 per cent and 12 per cent more growth respectively. The highest concentration was phytotoxic to cotton, reducing dry weight yield by 63 per cent. All three levels were found to be antagonistic in some degree to fruiting activity and resulted in reduced yields of seed cotton. These concentrations, however, had little effect on seed and fiber properties. Schradan was found capable of either inhibiting or accelerating the rate of growth of peas by Casida, Chapman, and Allen (1952). When applied to peas at a high concentration, it caused an initial decrease in growth rate followed by the appearance of chlorotic spots and a marginal leaf chlorsis leading finally to necrosis.

The effects of HETP (hexaethyl tetraphosphate), TEPP (tetraethyl pyrophosphate), and parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate) on cotton plants were reported by McIllrath (1950). He found that applications of commercial formulation of HETP and TEPP in concentrations of 30 to 50 times that recommended for insecticidal dosage resulted in malformation of leaves subsequently produced.

The leaf malformation closely resembled that induced by 2,4-D. Concentrations greater than 50 times that recommended for insecticidal control caused severe burning of the plant foliage resulting in death of the plant. Parathion application resulted in the production of leaves which were many lobed as contrasted to the three to five lobes of normal leaves.

Hall (1951) reported the effect of organic phosphate insecticides on tomato. He puts the effects into three classes: a. lethal, b. inhibitive, c. stimulatory. A concentration greater than 2000 ppm was found to be lethal with severe wilting within two hours and death within 24 hours. The first symptons were epinasty, almost immediate chlorosis, and wilting and drying of the growing point. Generally, concentrations of 800 to 2000 ppm produced temporary inhibition, and resulted in death or complete inhibition of the terminal growing point. Almost immediate stimulation in growth occurred with the application of 100 to 400 ppm TEPP but resulted in a decrease in total dry weight produced. Zimmerman and Hartzall (1947) also found when tomato plants were treated with TEPP and HETP at high concentrations that the plants were severly injured or killed. At low dosages the chemicals caused epinasty of the leaves. The same responses were reported with strawberry, smartweed, rose, and alsike clover.

When HETP was applied to carnations about the time the floral buds were differentiating, Hall (1951) observed that the abnormalities were confined primarily to the flower or flowering shoot. The flowering stalk became repeatedly branched so flowers were borne in a cluster. The other extreme was characterized by having the stamen and pistil excessively over developed and protruding from the calyx tube. The ovary was also prominent and contained matured seeds before the vegetative parts of the flower were developed.

Using respiratory measurements Hall (1951) found that 400 ppm of TEPP accelerated oxygen uptake by 62 per cent above the controls but 800 ppm inhibited oxygen uptake by 72 per cent. He also observed that potassium, phosphorous and calcium increased in plants treated with 400 ppm TEPP and concluded that the increased absorption was due to the increase in respiratory energy resulting from the TEPP treatment. Casida, Chapman, and Allen (1952) reported the respiration was inhibited in pea plants when treated with schradan.

Organic phosphate insecticides influenced the carbohydrate content of plants. Due to the liberation of free inorganic phosphate from the insecticide within the plant an increase in carbohydrates resulted according to Casida, Chapman and Allen (1952). Hall (1951) found that the carbohydrate content of tomato was decreased when TEPP was applied and this decrease was due to the hydrolysis of reserve carbohydrates and the accumulation of soluble sugars.

Effects of 4(2,4-DB) on Plants

The fundamental studies that led to the development of the phenoxybutyric acid herbicide, 4(2,4-DB), have been described in a review by Muir and Hansch (1955). Experiments by Shaw and Gentner (1957) on whole plants have confirmed the fact that species differ markedly in their relative susceptibility to acetic and butyric homologues of 2,4-dichlorophenoxy acids. Thus, merely by adding two methylene groups to the side chain of a herbicidal phenoxyacetic acid, it is possible to change its relative toxicity to different species. Shaw and Gentner (1957) believed the change in specificity was associated with the ability of the plant to convert the inactive butyric compound into the active phenoxyacetic acid. This slow internal conversion of the phenoxybutyric acid to the acetic acid homologue would result in a lower dose of active herbicide spread over a longer period of time.

Wain (1955) stated the hypothesis that the substituted straight-chain 4-phenoxybutyric acids were active as plant growth regulators only after B-oxidation to the corresponding phenoxyacetic acids within the plant. Any advantage that the 4-phenoxybutyric acids have over the phenoxyacetics as a selective herbicide is due to the relative phytotoxicity of the homogues, varying with different species. Very little is known concerning the location and behavior of the enzymes responsible for B-oxidation in the living plant. Woodford, Holly and McCready (1958) reported that some of the differences between the phytotoxicity of the butyric and acetic acids could be explained by differences in uptake, penetration, and translocation. Holly (1956) reported that within a period of a few hours after application, the phenoxybutyric acids showed contact phytotoxic effect on many species, noticeable primarily as lear scorch. This demonstrated that there was no undue delay in entry into the plant.

The experimental results obtained with the application of 4(2,4-DB) were quite varied as to the effect the herbicide has on alfalfa and other legumes. Yeo and Dunham (1956a) found that rates as high as five pounds per acre of 4(2,4-DB) for control of Canada Thistles in alsike clover, had no apparent effect on seed yield. No injury to alfalfa was reported by Dowler and Willard (1956), with rates up to two pounds per acre being applied, when alfalfa was in the two to four leaf stage. The rate gave nearly complete control of smartweed, lambsquarter, Canada Thistle, and red root pigweed that were present in the alfalfa stand.

Harmful effects of 4(2,4-DB) have been reported by a number of workers when the herbicide was applied to legumes. The herbicide at rates of 24, 30, and 36 ounces per acre gave slight initial depression on alfalfa growth, and some epinasty at the 36 ounce rate was still evident four weeks after treatment (LeBrocq and Beech, 1956). The alfalfa sprayed at 24 and 30 ounces per acre had recovered completely by this time. Elliott (1956) using rates from one to four pounds per acre found that 11 days after spraying, the alfalfa had suffered a reduction in leaf area and height, the latter due to curling of the stems. In addition the leaves had lost their bright green color. All treatments of 4(2,4-DB) caused a reduction in dry weight of the alfalfa that was substantial a month after spraying, but very small the following year. He concluded that there was no clear cut effect on dry weight produced by increasing the dosage of 4(2,4-DB). Peters (1958) also found that two pounds per acre reduced the dry weight yeild of alfalfa while the one pound per acre rate had no apparent effect. Deakins and Ormrod (1956) working with red and white clover found that 4(2,4-DB) at two pounds and four pounds per acre reduced the number of flowering heads, the vigor, and seed yield markedly. One pound per acre of 4(2,4-DB) reduced the vigor appreciably and seed yield by 16 per cent, but at all rates there was no delay of the date of flowering. A reduction in seed yield of legumes following the application of 4(2,4-DB) was also reported by Yeo and Dunham (1956b), and high rates caused the sweetclover blossoms to "strip off."

METHODS AND MATERIALS

Three separate experiments were conducted, one in the greenhouse and the other two on H-field of the Greenville Experiment Farm, North Logan, Utah. The methods and materials, and the results are given for each experiment separately. The rates of the chemical treatments are given in the weight or ppm of the active ingredient. The methods and procedures for computing the statistical analyses are those given in Ostle (1954). In the text, significant values are those which are significant at the .05 level of probability, while the highly significant values are those which are significant at the .01 level of probability.

Experiment A

Four clones (C-900, C-89, C-84, and U-55) of alfalfa were taken from field plots in November 1959, and planted in six inch pots in the greenhouse. Eight plants from each clone were used with each clone representing a single replication. The experimental design used was a randomized block with a two by four factorial arrangement of the treatments with four replications. The treatments consisted of foliage and soil applications of captan.

On December 20, four levels of captan (zero ppm, 937.5 ppm, 1875.0 ppm and 3750.0 ppm on a dry weight basis) were applied to the soil. The alfalfa plants were cut back to a stem height of two and one half inches before the soil application was made. The captan solution was allowed to diffuse into the soil two inches below the surface through a glass tube. Plants were watered equally every other day so that the captan would not be leached from the pots, also they were rotated on the greenhouse bench to reduce the environmental effects of light and temperature.

Three average stems on each plant were tagged and height measurements were made every seven days from December 28 through February 2 to check the rate of growth. Due to dormancy of some of the tagged stems, the three tallest stems were measured once a week from January 30 until the plants produced mature seeds.

Two levels, zero and 1200 ppm, of captan were used for foliage treatments and applications were made when the plant reached early bud stage. The spray was applied until the spray solution ran off the leaves so that all plants received presumably the same amount of captan per unit area of foliage present.

Pollen was taken from nontreated plants of clone 57 and crossed with all plants to check the fertility of the female gamete. To check the fertility of the male gamete, the pollen from the treated plants, including the controls, was crossed to clone 57. Self pollinations were also made on all plants. For each cross, four racemes of about ten flowers each were used giving approximately 40 crosses. All crosses were harvested when the pods turned brown. The number of pods per cross, seeds per pod, and weight per seed were determined. All aborted racemes, those which failed to develop, were counted to determine aborted racemes per plant. In addition to the racemes used in crossing, all other racemes were counted and the total racemes per plant, aborted racemes per total racemes produced, and flowers per raceme were determined. The plants were then harvested to measure the total vegetative growth produced on a dry weight basis.

Experiment B

This investigation was conducted in the field using a randomized block design with a two by two by three factorial arrangement of the treatments with four replications. Ranger alfalfa was seeded April 15, 1960, in five foot four inch rows, 24 inches wide and thinned to a four inch spacing after emergence. Two levels of captan were used. The zero level served as a check and the other treatment consisted of treating the seed and applying 1200 ppm of captan as a spray to the seedlings when they were three inches in height. Two levels of demeton were also used, zero and 5.75 ounces per acre. The 5.75 ounces per acre were applied as a spray when the alfalfa was in the bud stage. Zero, 6.7 pounds per acre, and 13.0 pounds per acre, which were heavier rates than recommended for weed control, were the three levels of 4(2,4-DB), applied as a spray when the plants were eight inches in height.

Four plants were tagged by marking on a measuring stick in one foot intervals and selecting the plants in the row that was closest to the mark. Starting June 16, height measurements were recorded of the tagged plants every seven days through July 21 and a final measurement was made August 15 when the plants had produced mature pods. The height measurements were used to determine the rate of growth. On July 21, using the same four tagged plants, the number of racemes with only flowers present and the number of racemes with pods were counted to determine maturity.

On July 27, a sample of 15 flowers was taken from each plot. From these 15 flowers 10 were randomly selected and tripped on a glass slide and the pollen stained with aceto carmine. The stained pollen grains were mixed and a sample taken and observed under the microscope. A count of the normal and abnormal pollen was then made.

Approximately, 100 flowers on 10 racemes were self-pollinated. These were harvested when the pods produced had turned brown. The number of pods per cross, seeds per pod, and weight per seed were determined. Ten racemes that had been pollinated naturally were selected at random and harvested to determine seeds per pod, pods per raceme, weight per seed, and flowers per raceme.

The whole plots were harvested on September 20. After allowing the plants to dry, they were weighed, threshed, and the seed yield determined on the basis of pounds per acre. The seed weight was subtracted from the total forage weight to determine the chaff yield in tons per acre.

Plots were hand weeded. Insecticides were applied as follows: dieldrin at four ounces per acre on June 29, parathion at six ounces per acre on July 14 and July 25, toxaphene at two pounds per acre on August 2, and toxaphene at three pounds per acre on August 11. The plots were irrigated on May 2, June 10, June 24, and July 14.

Experiment C

This experiment was also conducted of H-field. The experimental design used was a randomized block with six replications. Ranger alfalfa was seeded on April 15, 1960 in five foot four inch rows spaced 24 inches apart. The plants were thinned to four inch spacing within the row after emergence. The treatments consisted of three levels of 4(2,4-DB), zero, two pounds per acre, and 4.5 pounds per acre. The 4(2,4-DB) was not applied until the alfalfa had reached the five per cent bloom stage.

The same pollen samples, self pollinations, and raceme samples were taken as in Experiment B. Likewise, the weed and insect control, irrigation and harvesting of the whole plots were the same as for Experiment B.

EXPERIMENTAL RESULTS

Experiment A

Effect on vegetative parts

Clone C-89 showed yellowing of the leaf tips resulting from the soil application of captan. The amount of yellowing of the leaf tips increased with increasing concentration of captan, while the control plant was normal. The other clones, (C-900, C-84, and U-55) showed no yellowing of the leaf tips.

Effect on rate of growth

The mean squares for the rate of growth of the three tagged stems of the alfalfa plants are given in Table 1. The one significant effect of the captan treatments occurred during the January 25 to February 8 growth period. The foliar application of 1200 ppm of captan resulted in an increase in the growth as shown in Figure 1. Soil application of captan had no significant effect on growth.

The overall effect of captan during the five growth periods on the growth of the tagged stems is given in Table 2. Dates and foliar application of captan were both highly significant. The amount of growth during the first two growth periods was high, low in the third and fourth, and increased again in the fifth growth period as shown in

Table 1. Mean squares for the rate of growth of the tagged stems of alfalfa as affected by captan during five growth periods. Greenhouse, 1959-1960

Source of variation	Degrees freedom	Mean Squares				
		12/28-1/4	1/4-1/11	1/11-1/19	1/19-1/25	1/25-2/2
Blocks	3	70.06**	123.90	47.87*	16.88	3.95
Treatment	7	14.96	12.67	5.74	1.92	12.36
Captan in soil (A)	3	22.16	22.75	1.14	1.13	13.08
Captan on foliage (B)	1	28.32	13.26	13.52	5.96	29.45*
A x B	3	3.31	2.39	7.75	1.36	5.94
Error	21	8.32	16.43	14.07	3.55	6.02
Total	31					

*Significant at .05 level
**Significant at .01 level

Table 2. Mean squares for the tagged stems for the combined analysis for the rate of growth of alfalfa as affected by captan. Greenhouse, 1959-1960

Source of variation	Degrees freedom	Mean Squares Average of three tagged stems
Dates	4	394.64**
Blocks	3	162.35**
Blocks X Dates	12	24.99
Treatments	7	19.65
Captan in soil (A)	3	15.03
Captan on foliage (B)	1	84.10**
AXB	3	2.80
Treatments X Dates	28	6.93
A X Dates	12	11.31
B X Dates	4	1.60
AX B X Dates	12	4.32
Error	105	10.64
Total	159	

**Significant at .01 level

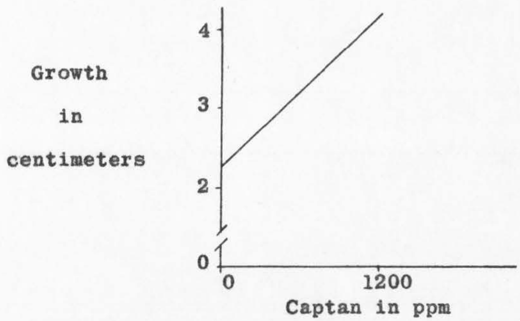


Figure 1. The effect of foliage application of captan on the growth of three tagged alfalfa stems from January 25 to February 2, greenhouse, 1959-1960

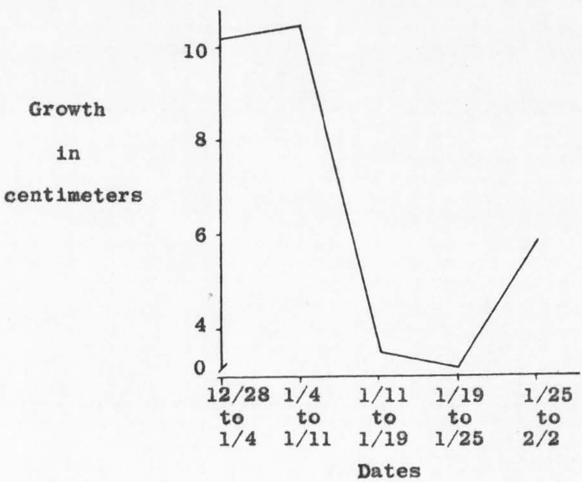


Figure 2. The mean growth of three tagged alfalfa stems. Greenhouse 1959-1960

Figure 2. An increase in growth resulted from foliar application of 1200 ppm captan during the five growth periods as shown in Figure 3. No significant effect on growth of the three tagged stems was produced by applying captan to the soil.

The mean squares for the rate of growth of the three tallest stems of the alfalfa plants are given in Table 3. In the growth period, from February 23 to March 8, both soil and foliar applications of captan had a significant effect on growth of the alfalfa. Both 937.5 and 3750.0 ppm captan increased growth, while the 1875.0 ppm decreased growth when applied to the soil as shown in Figure 4. The foliar application of 1200 ppm of captan resulted in a decrease in growth as shown in Figure 5. There was no significant effect produced by captan on the growth of the three tallest stems of the alfalfa plants during any of the other five growth periods.

The mean squares, in Table 4, for the combined analysis of the six growth periods of the three tallest stems, show that dates and captan applied to the soil were both highly significant. Growth decreased for the first four growth periods, with a slight increase in the fifth growth period, and dropped off again in the sixth as shown in Figure 6. The mean growth was increased at 937.5 and 3750.0 ppm captan applied to the soil, while 1875.0 ppm decreased the mean growth of the three tallest stems over the six growth periods as shown in Figure 7.

Table 3. Mean squares for rate of growth of the three tallest stems of alfalfa as affected by captan during six growth periods. Greenhouse, 1959-1960

Source of variation	Degrees freedom	Mean Squares					
		1/25-2/2	2/2-2/9	2/9-2/16	2/16-2/23	2/23-3/8	3/8-3/30
Blocks	3	20.48	3.67	17.53	3.09	24.52**	21.16*
Treatments	7	9.74	4.53	5.85	1.33	7.94*	4.58
Captan in soil (A)	3	19.40	6.62	8.45	1.98	9.25*	7.54
Captan on foliage (B)	1	1.32	1.71	10.58	1.40	12.25*	0.01
A X B	3	2.90	3.38	1.67	0.65	5.19	3.15
Error	21	7.95	3.95	6.58	2.44	2.82	5.36
Total	31						

*Significant at .05 level
**Significant at .01 level

Table 4. Mean squares for the combined analysis for rate of growth of alfalfa as affected by captan. Greenhouse, 1959-1960

Source of variation	Degrees freedom	Mean squares Average of three tallest stems
Dates	5	126.22**
Blocks	3	34.83
Blocks X Dates	15	11.10
Treatments	7	13.55*
Captan in soil (A)	3	23.26**
Captan on foliage (B)	1	9.99
A X B	3	5.03
Treatments X Dates	35	4.07
A X Dates	15	5.97
B X Dates	5	3.39
A X B X Dates	15	2.39
Error	126	5.17
Total	191	

*Significant at .05 level
**Significant at .01 level

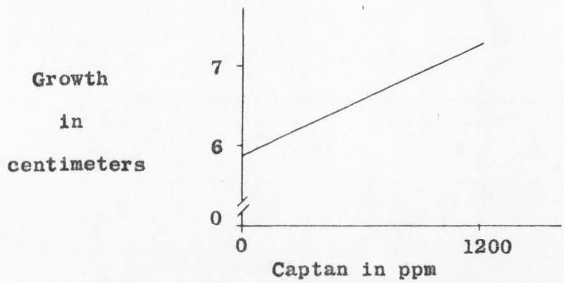


Figure 3. The effect of captan applied to the foliage on the mean growth of three tagged alfalfa stems during five growth periods from Dec. 28 to Feb. 2. Greenhouse, 1959-1960

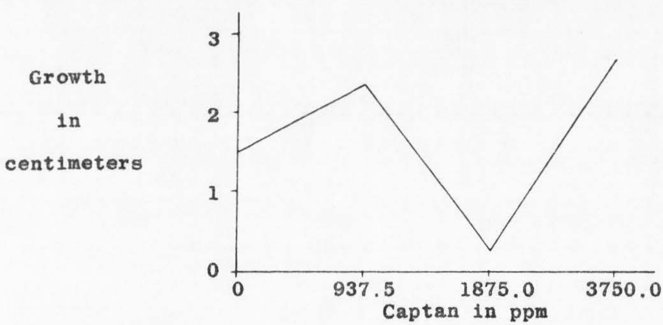


Figure 4. The effect of soil application of captan on the growth of three tallest alfalfa stems from Feb. 23 to March 8. Greenhouse, 1959-1960

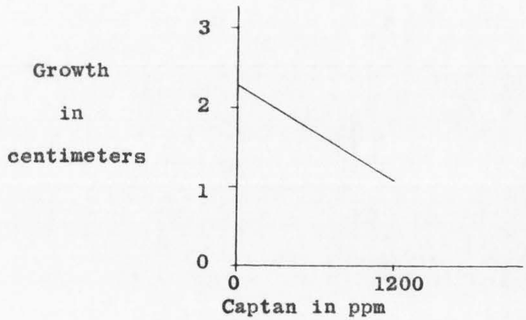


Figure 5. The effect of foliage application of captan on the growth of three tallest alfalfa stems from Feb. 23 to March 8. Greenhouse, 1959-1960

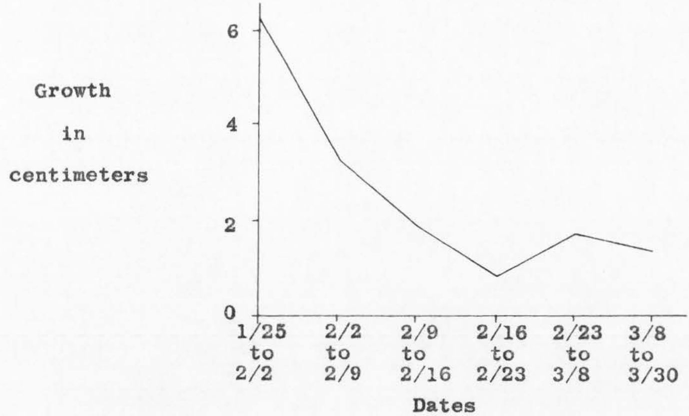


Figure 6. The mean growth of three tallest alfalfa stems. Greenhouse, 1959-1960

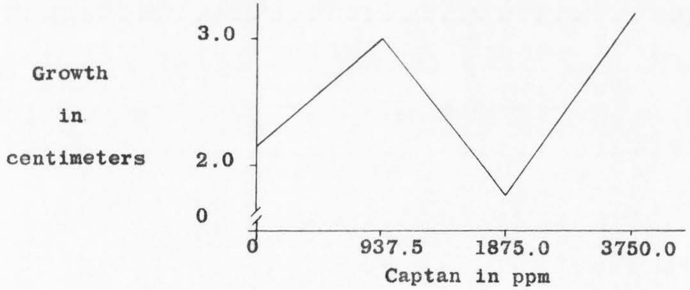


Figure 7. The effect of the application of captan to the soil on the growth of three tallest alfalfa stems during six growth periods from Jan. 25 to March 30. Greenhouse, 1959-1960

Effect on fertility and flower production

Captan had no significant effect on the fertility of either the female or male gametes as given in Table 5. The analysis of variance of the three different types of crosses made for pods per flower crossed in Table 5, showed that the pollen and ovules were not affected by captan treatments. The number of fertile ovules produced per flower was unaffected by captan treatments as shown by the mean squares for seeds per pod.

The effect of captan on weight per seed is given in Table 6. Seeds produced from each cross were not significantly different in weight showing that captan did not affect seed development.

Mean squares for racemes per plant, flowers per raceme, and total dry weight produced by the alfalfa plants are given in Table 7. None of these factors were found to be significantly affected by the captan treatments. The number of aborted racemes per plant was adjusted to the total number of racemes produced per plant using a covariance anaylsis and was non-significant as given in Table 8.

Experiment B

Effect on vegetative parts

Epinastic curling of the stems and leaves resulted from the application of 4(2,4-DB) to the alfalfa seedlings at both the 6.7 and 13.0 pounds per acre rates. The epinastic effect was more than doubled with the 13.0 pound

Table 5. Mean squares for the effect of captan on pods per flower crossed and seeds per pod on alfalfa. Greenhouse, 1959-1960

Source of variation	Degrees freedom	Mean Squares: Pods per flower crossed: Selfs	Pods per flower crossed: Crosses: Clone 57 male	Pods per flower crossed: Crosses: Clone 57 female	Seeds per pod: Selfs	Seeds per pod: Crosses: Clone 57 male	Seeds per pod: Crosses: Clone 57 female
Blocks	3	3,386.72**	1,03.56**	665.62*	2.31*	3.06**	11.83**
Treatments	7	160.20	236.43	95.36	0.08	0.29	0.37
Captan in soil (A)	3	172.48	280.80	89.69	0.10	0.38	0.30
Captan on foliage (B)	1	2.88	0.55	317.52	0.00	0.00	0.95
A X B	3	200.35	270.69	26.97	0.09	0.30	0.25
Error	21	156.35	112.30	139.02	0.09	0.38	0.61
Total	31						

*Significant at .05 level
**Significant at .01 level

Table 6. Mean squares for the weight per seed of alfalfa as effected by captan and different crosses. Greenhouse, 1959-1960

Source of variation	Degrees freedom	Mean Squares		
		Weight per seed		
			Crosses	
		Selfs	Clone 57 male	Clone 57 female
Blocks	3	0.80*	0.437**	0.0043
Treatments	7	0.26	0.054	0.0404
Captan in soil (A)	3	0.25	0.063	0.0397
Captan on foliage (B)	1	0.45	0.120	0.0080
A X B	3	0.23	0.022	0.0573
Error	21	0.19	0.042	0.0183
Total	31			

*Significant at .05 level
**Significant at .01 level

Table 7. Mean squares for the effect of captan on racemes per plant, flowers per raceme, and dry weight produced per plant on alfalfa. Greenhouse 1959-1960

Source of variation	Degree freedom	Mean Squares		
		Racemes per plant	Flowers per raceme	Dry weight per plant
Blocks	3	191.88	21.60**	8.07**
Treatment	7	22.55	2.26	0.30
Captan in soil (A)	3	22.88	2.63	0.39
Captan on foliage (B)	1	0.13	4.96	0.03
A X B	3	29.79	0.98	0.30
Error	21	63.06	2.31	0.59
Total	31			

**Significant at .01 level

Table 8. Covariance analysis for the effect of captan on aborted racemes per plant on alfalfa. Greenhouse, 1959-1960

Source of variation	Degrees Freedom	Σx^2	Σxy	Σy^2	Deviations from regression: Degrees freedom	$\Sigma dy,r^2$	Mean square
Blocks	3	575.63	-157.63	82.25			
Treatments	7	157.88	52.75	114.50			
Captan in soil (A)	3	68.63	28.12	90.25			
Captan on foliage (B)	1	0.13	1.12	10.12			
A X B	3	89.12	23.51	14.13			
Error	21	1324.37	100.38	534.88			
Error plus treatment	28	1482.25	153.13	649.38	20	527.27	26.36
		Among adjusted means			7	633.56	
Error plus A	24	1393.00	128.50	625.13	23	613.28	
		Among adjusted means			3	86.01	28.67
Error plus B	22	1324.50	101.50	545.00	21	537.22	
		Among adjusted means			1	9.95	9.95
Error plus A X B	24	1413.49	123.39	549.01	23	538.15	
		Among adjusted means			3	10.88	3.63

compared to the 6.7 pound rate. Five days after treatment with 4(2,4-DB), the plants receiving the lighter rate appeared to be normal, while normal growth of the plants receiving the 13.0 pound rate was delayed for about a month when new growth was becoming dominant.

Severe scorching of the leaves resulted from the 13.0 pound application of 4(2,4-DB) with some scorched leaves being present in the plots receiving the 6.7 pound rate. The scorched leaves turned white and were completely dry within two days. Other leaves present on the 4(2,4-DB) treated plants lost their bright green color as compared to the controls.

Effect on rate of growth

The effect of captan, demeton, and 4(2,4-DB) on the rate of growth of alfalfa is given in Table 9. 4(2,4-DB) had a significant effect on growth during the June 16 to June 23, June 23 to June 30, July 8 to July 16, and July 16 to July 21 growth periods, with June 16 to June 23 and June 23 to June 30 being highly significant. The effect of 4(2,4-DB) on growth during these growth periods is shown in Figure 8. In all growth periods 6.7 pounds per acre caused a decrease in growth while the 13.0 pound rate of 4(2,4-DB) caused a decrease in growth in all but one growth period, July 16 to July 21, in which an increase in growth resulted. Captan and demeton had no significant effect on growth. The interaction of captan and 4(2,4-DB) caused a significant effect in the July 8 to July 16

Table 9. Mean squares for the rate of growth of alfalfa as affected by captan, demeton and 4(2,4-DB) at different growth periods. H-field, 1960

Source of variation	Degrees freedom	Mean Square					
		June 16 June 23	June 23 to June 30	June 30 to July 8	July 8 to July 16	July 16 to July 21	July 21 to Aug. 15
Blocks	3	0.46	0.57	0.39	0.48	3.99**	0.85
Treatments	11	1.44**	4.36**	0.34	0.72	1.27	1.61
Captan (A)	1	0.78	2.21	0.002	0.08	1.24	0.33
Demeton (B)	1	0.42	0.11	0.73	0.27	0.29	0.85
4(2,4-DB) (C)	2	5.67**	19.08**	1.04*	0.98	3.03*	2.50
A X B	1	0.11	0.59	0.09	0.19	0.99	3.01
A X C	2	0.14	1.86	0.01	1.73*	2.19	1.22
B X C	2	0.95	0.93	0.06	0.03	0.37	0.25
A X B X C	2	0.96	0.63	0.39	0.93	0.10	2.78
Error	33	0.41	0.84	0.25	0.44	0.89	1.26
Total	47						

*Significant at .05 level
**Significant at .01 level

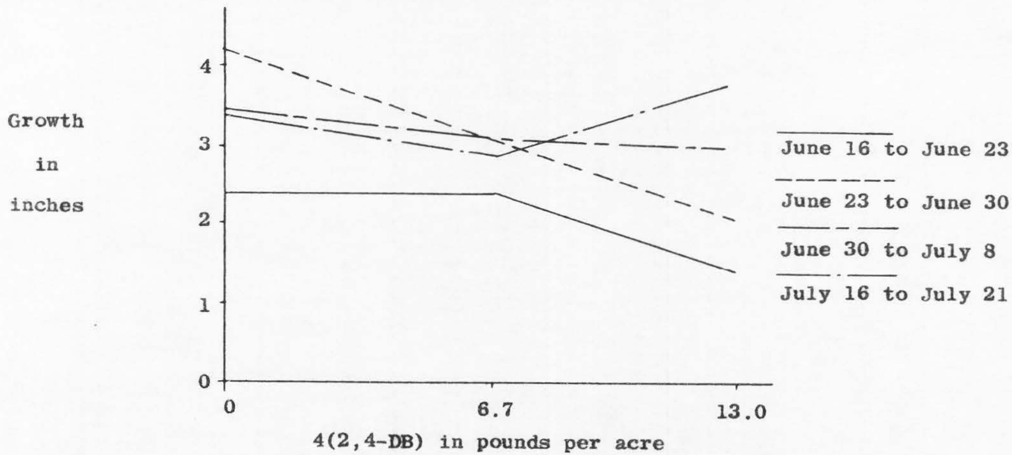


Figure 8. The effect of 4(2,4-DB) on the growth of alfalfa. H-field, 1960

growth period, and this interaction is shown in Figure 9. Captan at 1200 ppm increased growth with the application of 6.7 pounds per acre of 4(2,4-DB), but depressed growth at the zero and 13.0 pound rates of 4(2,4-DB).

The mean squares for the combined analysis of the six growth periods from June 16 to August 15 are given in Table 10. The main effects of dates and 4(2,4-DB), as well as, the interactions of captan, demeton, and 4(2,4-DB) and the interaction of 4(2,4-DB) and dates were all highly significant, while the interaction of captan and 4(2,4-DB) was significant in its effect on the growth of the alfalfa. The mean growth for each date is given in Figure 10, which shows that growth was low during the first growth period, increased in the second and third, was low in the fourth, increased again in the fifth, and decreased in the sixth growth period. The mean growth during the six growth periods decreased with increasing rates of 4(2,4-DB) as shown in Figure 11. Captan at 1200 ppm stimulated growth with 4(2,4-DB) applications of 13.0 and 6.7 pound rates, but depressed the growth slightly at the zero level as shown in Figure 12. Figure 13 shows the interaction of captan, demeton, and 4(2,4-DB) on the mean growth of the alfalfa during the six growth periods. Captan and demeton depressed the growth at the zero level of 4(2,4-DB), but stimulated growth at the 6.7 and 13.0 pound rates of 4(2,4-DB). With captan at the zero level, demeton stimulated growth at the zero level of 4(2,4-DB), had no effect at the 6.7 pound rate, and reduced the growth at the 13.0

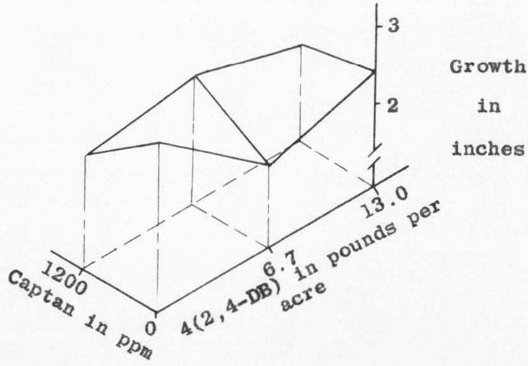


Figure 9. The interaction of 4(2,4-DB) and captan on the growth of alfalfa from July 8 to July 16, H-field, 1960

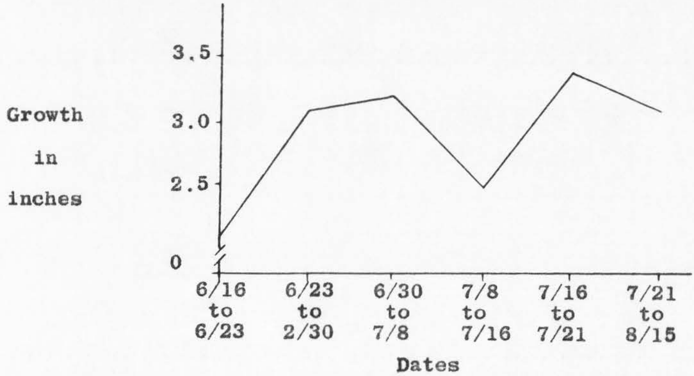


Figure 10. The mean growth of alfalfa at different growth periods. H-field, 1960

Table 10. Mean squares for the combined analysis for rate of growth of alfalfa as affected by captan, demeton, and 4(2,4-DB). H-field, 1960

Source of variation	Degrees freedom	Mean Squares Average of four plants
Dates	5	12.690**
Blocks	3	2.920*
Blocks X Date	15	0.765
Treatments	11	2.821**
Captan (A)	1	0.700
Demeton (B)	1	0.100
4(2,4-DB) (C)	2	8.015**
A X B	1	0.130
A X C	2	2.425*
B X C	2	0.685
A X B X C	2	4.055**
Treatments X Dates	55	1.385**
A X Dates	5	0.788
B X Dates	5	0.514
C X Dates	10	4.860**
A X B X Dates	5	0.968
A X C X Dates	10	0.949
B X C X Dates	10	0.379
A X B X C X Dates	10	0.274
Error	198	0.686
Total	287	

*Significant at .05 level
**Significant at .01 level

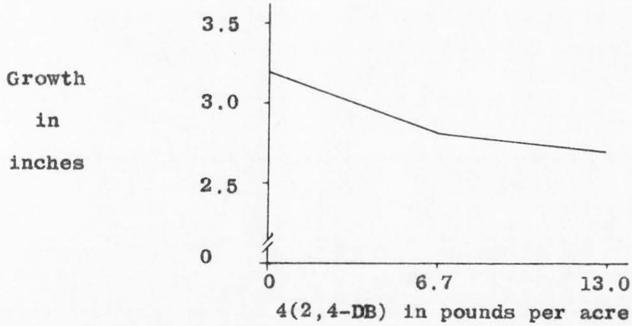


Figure 11. Effect of 4(2,4-DB) on the mean growth of alfalfa during the six growth periods from June 16 to Aug. 15. H-field, 1960

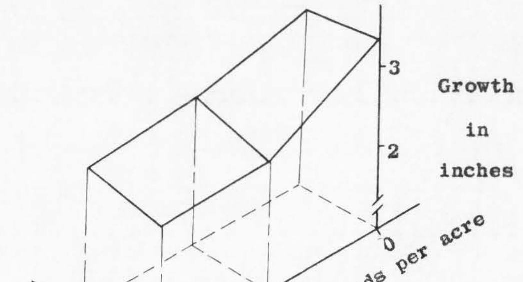
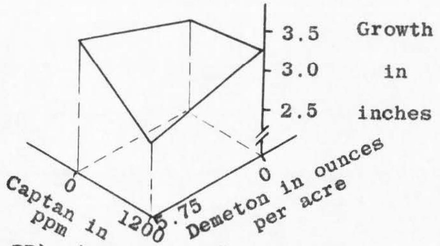
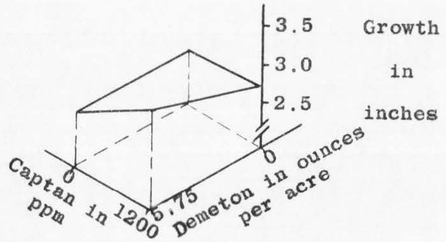


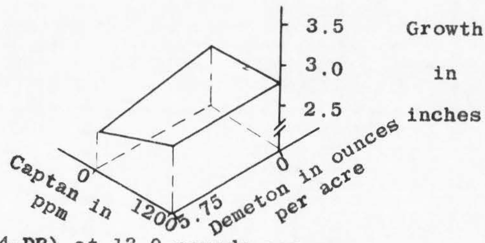
Figure 12. The interaction of captan and 4(2,4-DB) on the mean growth of alfalfa during the six growth periods from June 16 to Aug. 15. H-field, 1960



4(2,4-DB) at zero pounds per acre



4(2,4-DB) at 6.7 pounds per acre



4(2,4-DB) at 13.0 pounds per acre

Figure 13. Interaction of captan, demeton and 4(2,4-DB) on the mean growth of alfalfa during six growth periods from June 16 to Aug. 15. H-field, 1960

pound rate of 4(2,4-DB). At the zero level of both captan and demeton, 4(2,4-DB) decreased the mean growth of the alfalfa at both rates. The interaction of 4(2,4-DB) and dates given in Figure 14, shows that the 6.7 pound rate of 4(2,4-DB) decreased the growth of the alfalfa in all six growth periods. The 13.0 pound rate decreased the growth in the first four growth periods but an increase in growth resulted in the last two periods.

Effect on maturity

The mean squares for the number of racemes, with flowers only, per plant and the number of racemes with pods per plant for measuring maturity of the alfalfa plants are given in Table 11. 4(2,4-DB) significantly reduced the number of racemes with flowers only per plant at the 13.0 pound rate, and a slight increase in flowering racemes resulted from the 6.7 pounds rate as shown in Figure 15. Indicating that the heavier rate of 4(2,4-DB) delayed flowering and the lower rate had little or no effect on maturity. The significant interaction of captan and demeton on racemes with pods per plant is given in Figure 16. Plants with the greatest number of racemes with pods were those plants receiving no captan or demeton. At zero level of demeton, 1200 ppm of captan greatly reduced the number of racemes with pods while with the application of 5.75 ounces of demeton per acre with the captan increased the number of racemes with pods.

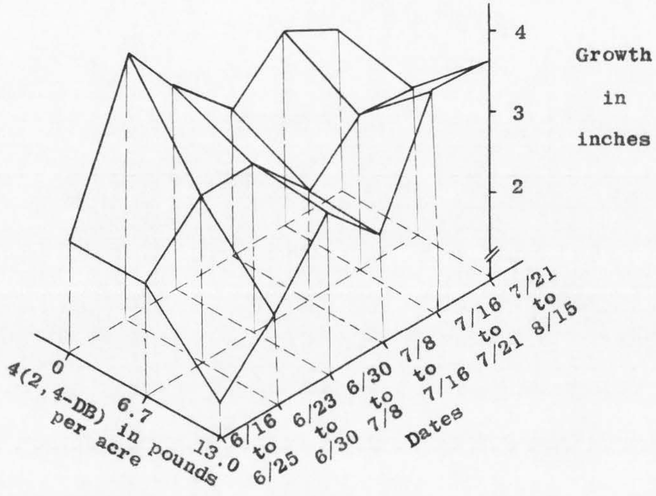


Figure 14. The interaction of 4(2,4-DB) and date on the mean growth of alfalfa during the six growth periods from June 16 to Aug. 15. H-field, 1960

Table 11. Mean squares showing the effects of captan, demeton, and 4(2,4-DB) on racemes with flowers only per plant and racemes with pods per plant of alfalfa take July 21. H-field, 1960

Source of variation	Degrees freedom	Mean Squares	
		Racemes with flowers only per plant	Racemes with pods per plant
Blocks	3	35.35	1.09
Treatments	11	36.77	3.38
Captan (A)	1	0.85	5.00
Demeton (B)	1	1.54	0.01
4(2,4-DB) (C)	2	96.77*	5.41
A X B	1	11.80	12.71*
A X C	2	59.16	0.48
B X C	2	34.97	1.41
A X B X C	2	1.26	2.42
Error	33	25.53	2.82
Total	47		

*Significant at .05 level

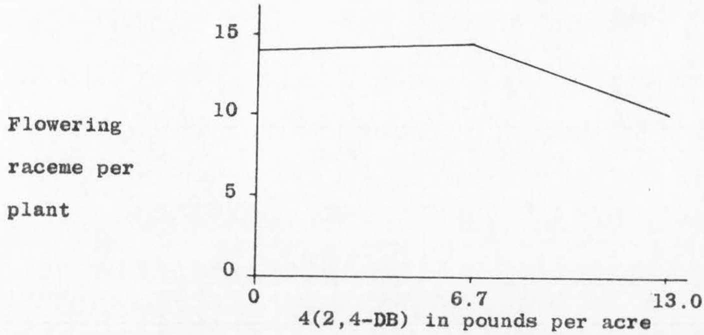


Figure 15. The effect of 4(2,4-DB) on the stages of flowering of alfalfa on July 21. H-field, 1960

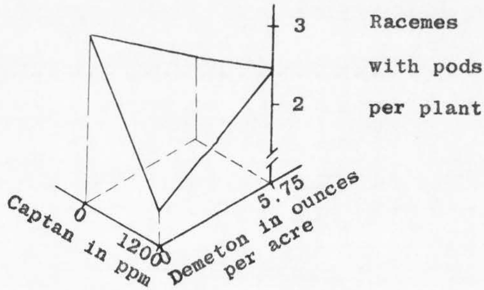


Figure 16. The interaction of captan and demeton on stage of pod development of alfalfa on July 21. H-field, 1960

Effect on fertility and flower production

The effect of captan, demeton, and 4(2,4-DB) on fertility of the pollen and ovules is given in Tables 12 and 13. The treatments had no effect on the development of normal pollen grains. From the self pollinations, 4(2,4-DB) had a highly significant effect on pods per flower crossed by increasing the self fertility of the flowers as shown in Figure 17. The captan, demeton, and 4(2,4-DB) interaction was significant for seeds per pod from the self pollinations while treatments had no significant effect on seeds per pod when natural cross pollination took place as given in Table 12. The captan, demeton, and 4(2,4-DB) interaction is shown in Figure 19. Demeton at the 5.75 ounce level increased the number of seeds per pod when 4(2,4-DB) was applied at the rate of 6.7 pounds per acre but had little effect at the 13.0 and zero pounds rates at the zero level of captan. When 1200 ppm of captan was applied, demeton increased the number of seeds per pod at the 13.0 pound rate, but decreased it at the other two rates of 4(2,4-DB). 4(2,4-DB) at the 6.7 pound rate reduced the number of seeds per pod when captan and demeton were both at the zero level, but with 1200 ppm of captan and the zero level of demeton the 13.0 pound rate had a depressing effect. 1200 ppm of captan increased the number of seeds per pod when both demeton and 4(2,4-DB) were at the zero levels.

Table 12. Mean squares showing the effect of captan, demeton, and 4(2,4-DB) on per cent normal pollen and self fertility of alfalfa. H-field, 1960

Source of variation	Degrees freedom	Mean Squares		
			Self pollinated	
		per cent normal pollen	Pods per flower crossed	Seeds per pod
Blocks	3	53.42	532.04**	0.913
Treatments	11	22.10	126.38*	0.233
Captan (A)	1	1.26	172.21	0.008
Demeton (B)	1	0.11	59.70	0.008
4(2,4-DB) (C)	2	102.06	376.42**	0.029
A X B	1	10.31	0.86	0.115
A X C	2	12.11	20.51	0.059
B X C	2	0.01	142.51	0.412
A X B X C	2	1.50	39.28	0.713*
Error	33	37.21	58.96	0.217
Total	47			

*Significant at .05 level
**Significant at .01 level

Table 13. Mean squares showing the effect of captan, demeton, and 4(2,4-DB) on cross fertility and flowers per raceme of alfalfa. H-field, 1960

Source of variation	Degree freedom	Mean Squares		
		Naturally crossed		Pollinated
		Seeds per pod	Pods per raceme	Flowers per raceme
Blocks	3	0.159	7.14*	3.56
Treatments	11	0.329	1.75	3.82
Captan (A)	1	0.008	0.23	0.73
Demeton (B)	1	0.002	0.01	1.05
4(2,4-DB) (C)	2	0.111	1.24	9.45*
A X B	1	1.070	1.72	0.06
A X C	2	0.667	1.92	6.66*
B X C	2	0.490	1.82	0.38
A X B X C	2	0.000	3.65	3.64
Error	33	0.299	1.76	1.86
Total	47			

*Significant at .05 level

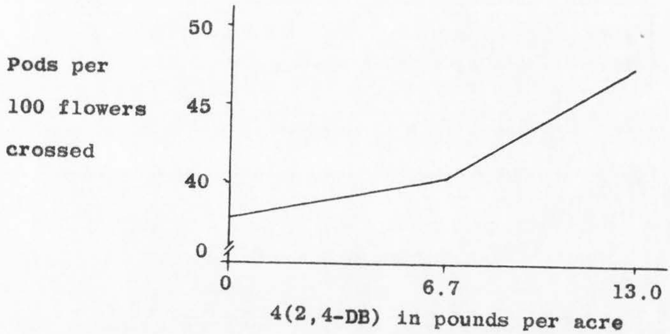


Figure 17. The effect of 4(2,4-DB) on pods per flower crossed of alfalfa which was self pollinated. H-field, 1960

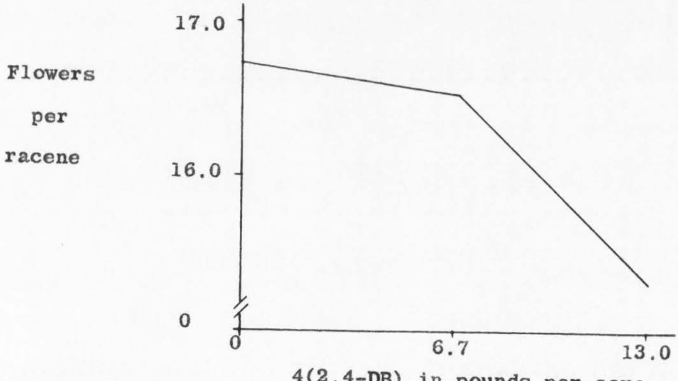
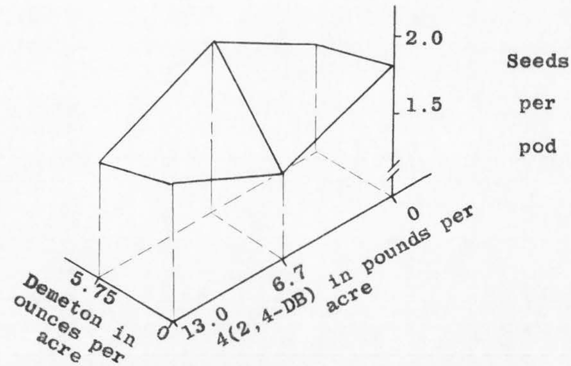
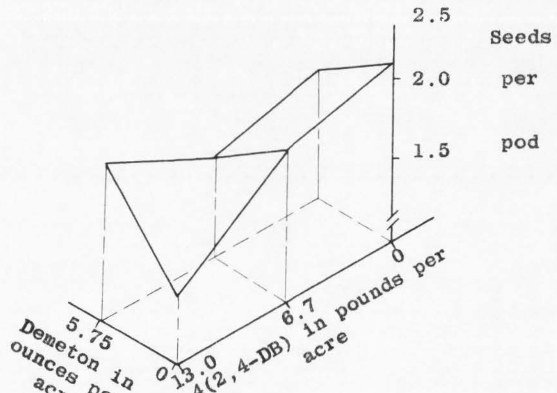


Figure 18. The effect of 4(2,4-DB) on flowers per raceme of alfalfa. H-field, 1960



Captan at zero ppm



Captan at 1200 ppm

Figure 19. The interaction of captan, demeton and 4(2,4-DB) on seeds per pod from self pollination of alfalfa. H-field, 1960

The mean squares for flowers per raceme are given in Table 13. Both 4(2.4-DB) and the interaction of captan and 4(2,4-DB) were significant. 4(2,4-DB) reduced the number of flowers per raceme at both the 6.7 and 13.0 pound rates, with the greatest reduction at the 13.0 pound level as shown in Figure 18. 1200 ppm of captan had no effect on flowers per raceme at the 13.0 pound rate of 4(2,4-DB) but greatly increased the number of flowers per raceme with 6.7 pounds per acre. A reduction in flowers per raceme resulted from 1200 ppm of captan at the zero level of 4(2,4-DB) as shown in Figure 20.

Captan, demeton, and 4(2,4-DB) had no significant effect on weight per seed produced from either the self-pollinations or the naturally cross pollinated flowers as given in Table 14.

Effect on seed and chaff yield

The mean squares for seed yield in pound per acre are given in Table 14. 4(2,4-DB) had a highly significant effect on the yield of alfalfa seed. The effect of demeton and the interaction of captan and 4(2,4-DB) were significant. Demeton at 5.75 ounces per acre reduced the seed yeild. Yield of seed as affected by the treatments is shown in Figure 22. Captan, demeton, and 4(2,4-DB) all reduced seed yield. The captan reduction was not significant. The interaction of captan and 4(2,4-DB) on seed yield is shown in Figure 21. Captan reduced the yield of seed at all levels of 4(2,4-DB) with the greatest reduction

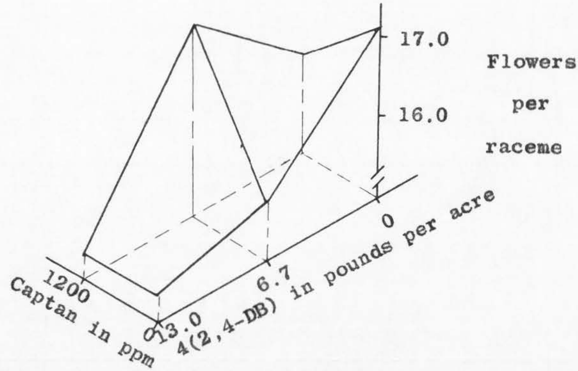


Figure 20. The interaction of captan and 4(2,4-DB) on flowers per raceme of alfalfa. H-field, 1960

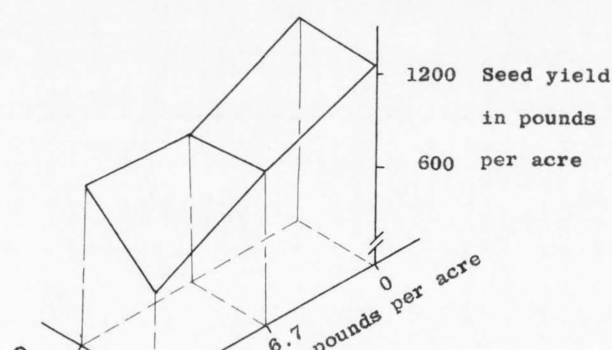


Figure 21. The interaction of captan and 4(2,4-DB) on alfalfa seed yield. H-field, 1960

Table 14. Mean squares showing the effect of captan, demeton and 4(2,4-DB) on weight per seed, and seed and chaff yield of alfalfa. H-field, 1960

Source of variation	Degrees freedom	Mean Squares: Pollinations: Selfed: Weight per seed	Crossed: Weight per seed	Yield of seed in pounds per acre	Yield of chaff in tons per acre
Blocks	3	0.213	0.103**	28,312.53	0.373*
Treatments	11	0.245	0.014	280,562.74**	1.061**
Captan (A)	1	0.000	0.026	84,756.00	0.100
Demeton (B)	1	0.025	0.025	170,766.00*	0.030
4(2,4-DB) (C)	2	0.001	0.009	1,093,204.15**	5.555**
A X B	1	0.061	0.000	114,172.60	0.060
A X C	2	0.034	0.023	144,117.70*	0.055
B X C	2	0.027	0.008	43,894.15	0.090
A X B X C	2	0.030	0.010	77,031.75	0.040
Error	33	0.261	0.014	39,490.02	0.105
Total	47				

*Significant at .05 level
**Significant at .01 level

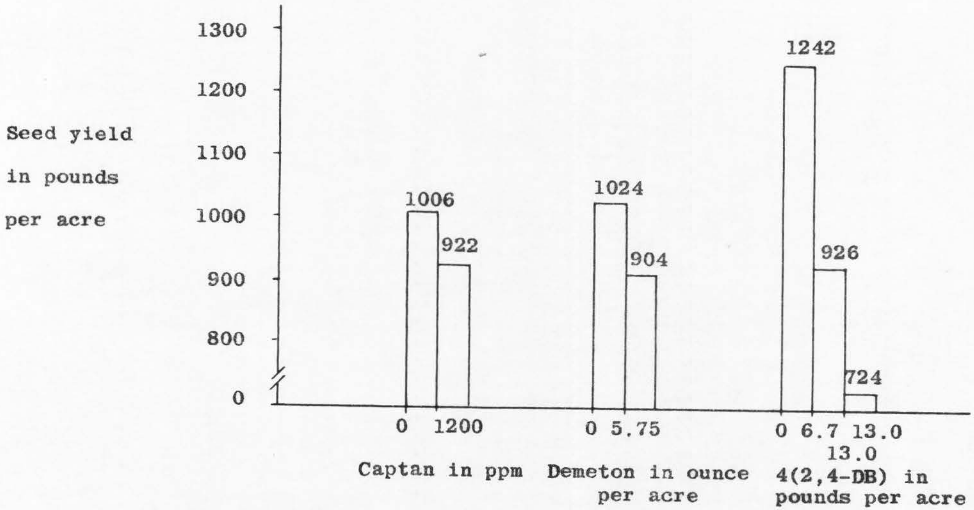


Figure 22. The effect of captan, demeton and 4(2,4-DB) on alfalfa seed yield. H-field, 1960

occurring with 1200 ppm of captan and 13.0 pounds per acre of 4(2,4-DB).

The mean squares for the effect of captan, demeton, and 4(2,4-DB) on the yield of chaff of alfalfa grown for seed is given in Table 14. A reduction of chaff yield resulted from all three chemicals, with 4(2,4-DB) being highly significant as shown in Figure 23. The other treatments were non-significant.

Experiment C

Effect on vegetative parts

Two days after the application of 4(2,4-DB) to the alfalfa plants, epinastic curling of the stems and leaves resulted, with the 4.5 pound per acre rate showing about twice the effect of the two pound per acre rate. Some epinastic curling was still present seven days after application of the 4(2,4-DB) on the plants which received the 4.5 pound rate. The 4.5 pound rate also resulted in scorching some of the leaves. The flowers that were present when the 4(2,4-DB) was applied dried up and produced no seed.

Effect on fertility and flower production

The effect of the 4(2,4-DB) on the fertility of the gametes is given in Tables 15 and 16. Pollen grain development was not significantly affected by the 4(2,4-DB) treatment as shown by the mean squares in Table 15. Pods

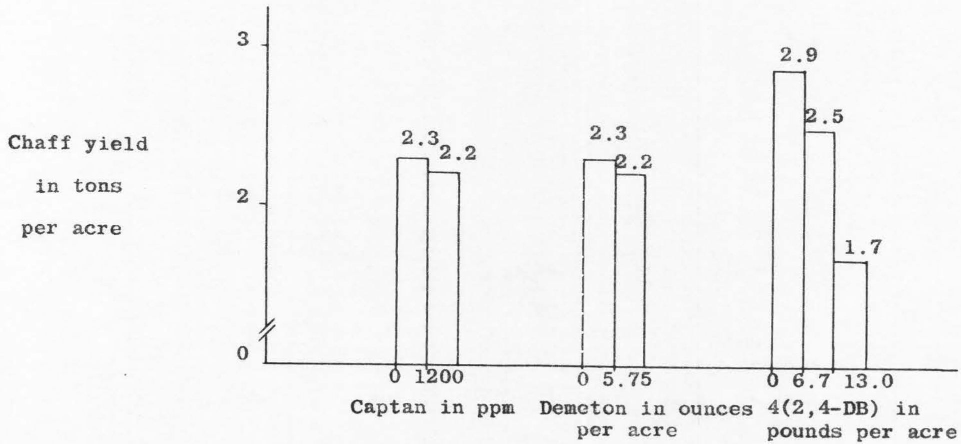


Figure 23. The effect of captan, demeton, and 4(2,4-DB) on alfalfa chaff yield. H-field, 1960

Table 15. Mean squares for the effect of 4(2,4-DB) on fertility of alfalfa. H-field, 1960

Source of variation	Degrees freedom	Mean Squares		
			Self pollinated	
		Per cent normal pollen	Pods per flower crossed	Seeds per pod
Blocks	5	22.96	106.84	0.17
Treatments	2	13.56	19.14	0.03
Error	10	28.04	222.35	0.07
Total	17			

Table 16. Mean squares for the effect of 4(2,4-DB) on fertility and flowers per raceme of alfalfa. H-field, 1960

Source of variation	Degrees freedom	Mean Squares		
		Naturally crossed pollinated		
		Seeds per pod	Pods per raceme	Flowers per raceme
Blocks	5	0.366	1.98	3.45
Treatments	2	0.052	0.42	1.23
Error	10	0.221	1.50	3.59
Total	17			

per flower crossed, and seeds per pod obtained from self pollination showed that the ovule development was not significantly affected. Mean squares, in Table 16, from data taken from naturally cross pollinated racemes show that the treatment was non-significant for seeds per pod, pods per raceme, and flowers per raceme.

Weight per seed produced from self pollination was not significant as given in Table 17. The weight per seed was significant for the seed produced from the naturally cross polinated flowers. 4(2,4-DB) at the two pound rate reduced the weight per seed more than the 4.5 pound per acre rate as shown in Figure 24.

Effect on seed and chaff yield

Alfalfa seed and chaff yield were both decreased by 4(2,4-DB) with the reduction in yield being highly significant in both cases. The mean squares for seed and chaff yields are given in Table 17. The yield of seed and chaff were both reduced more at the 4.5 pound per acre rate than at the two pound rate as shown in Figure 24.

Table 17. Mean square for the effect of 4(2,4-DB) on the weight per seed, and seed and chaff yield of alfalfa. H-field, 1960

Source of variation	Degrees freedom	Mean Squares			
		Pollinations			
		Selfed weight per seed	Crossed weight per seed	Yield of seed in pounds per acre	Yield of chaff in tons per acre
Blocks	5	0.046	0.046	35,916.34	0.104
Treatments	2	0.215	0.095*	729,769.95**	2.035**
Error	10	0.074	0.014	31,108.03	0.102
Total	17				

*Significant at .05 level
**Significant at .01 level

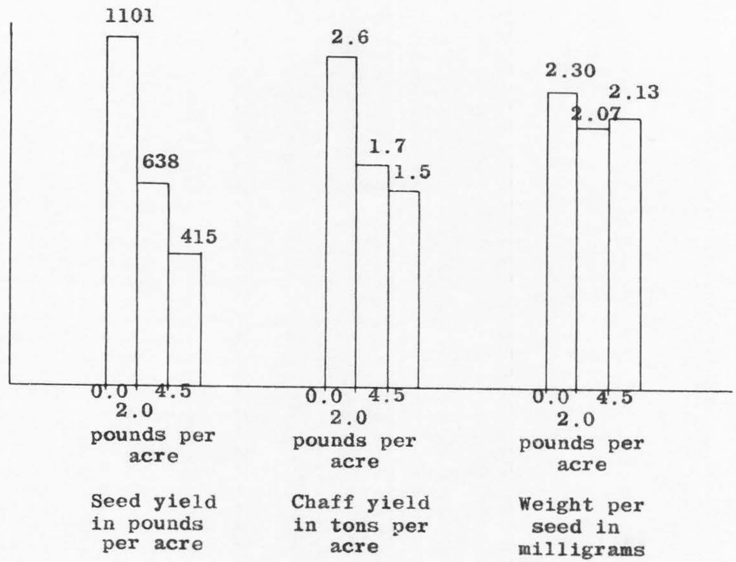


Figure 24. The effect of 4(2,4-DB) seed yield, chaff yield, and weight per seed of alfalfa. H-field, 1960

DISCUSSION

Effect of Captan on Alfalfa

Vegetative parts

In experiment A, captan caused the tips of the leaves of clone C-89 to turn yellow around the margin of the leaf. This marginal yellowing of the leaves was reported by Silber (1959), working with tomato plants that were grown in soil containing high concentrations of captan. The results showed that by increasing the concentration of captan in the soil that the amount of yellowing of the leaf margins will increase. Since clones, (C-900, C-84, and U-55) are not affected by captan, there is a difference in the susceptability of alfalfa clones to captan. The contact injury of captan resulting from decomposition and the formation of hydrochloric acid as found by Miller (1957), was not observed in either experiment A or experiment B.

Rate of growth

The results of foliar application of captan, in experiment A, as determined from the three tagged stems are questionable because in the growth period January 25 to February 2 an increase in growth resulted. In contrast, the combined analysis for the three tagged stems showed an overall effect of foliage application to be a decrease in growth. The significant effects of captan on growth for the three tallest stems in the growth period from February 25 to March 8 are probably due to environmental conditions in the greenhouse. Silber (1959), growing plants in nutrient solutions found that plants receiving one, three, and 10 ppm of captan grew less than the control plants. The results of experiment A, showed that captan applied to the soil at concentrations of 937.5 and 3750.0 ppm grew more than the control plants but plants receiving 1875.0 ppm of captan grew less than the controls. These results indicate that the effect of captan on the growth of alfalfa varies with the concentrations applied. When captan was applied to alfalfa seedlings, as in experiment B, an increase in growth resulted when captan interacted with 6.7 and 13.0 pounds per acre of 4(2,4-DB), but, it decreased the growth at the zero level of 4(2,4-DB). This indicates that captan may overcome some of the effects produced by 4(2,4-DB), perhaps blocking the B-oxidation of 4(2,4-DB) to 2,4-D by affecting an intermediate product produced in the plant.

Fertility and flower production

Under the conditions of experiment A, captan had no effect on fertility or flower production of alfalfa. The main effects of captan on fertility and flower production are non-significant in experiment B. The number of ovules developed increases when captan is applied with demeton at the 13.0 pound per acre level of 4(2,4-DB), but they are decreased by captan at the zero and 6.7 pound rates of 4(2,4-DB) as shown in Figure 19. Captan also interacts with the 6.7 pound rate of 4(2,4-DB) to increase the number of flowers per raceme. This interaction is shown in Figure 20. Captan delayed the maturity (Figure 16) of alfalfa when interacting with demeton, showing that captan and demeton acting together will delay the date of flowering.

Seed and chaff yield

McCallan, Miller, and Weed (1954), reported that vegetable and flower production have been significantly increased by early applications of captan. Daines (1953), found that the application of captan was beneficial in areas or under environmental conditions which are not conducive for maximum growth. The results of experiment B, show that alfalfa seed and chaff yields were reduced under the conditions of the experiment by the early application of captan, but this reduction in yield of seed and chaff was non-significant of the .05 level of probability. The significant interaction (Figure 21) of captan and 4(2,4-DB) shows that captan did reduce seed yield of alfalfa when interacting with 4(2,4-DB).

Effect of Demeton on Alfalfa

Hall (1951), and Haeskaylo and Ergle (1955), working with different organic phosphate insecticides concluded that the phytotoxicity of these insecticides depends on the concentrations applied to the plants. In general, low concentrations stimulated growth while higher concentrations were either lethal or have an inhibitory effect on the plant. In experiment B, in which demeton was included, 5.75 ounces per acre were applied. The 5.75 ounce rate of demeton significantly reduced the seed yield of alfalfa. From the combined anaylsis for the rate of growth, the interaction of captan, demeton, and 4(2,4-DB) show that demeton interacting with captan reduced the growth rate of alfalfa at the 6.7 pound rate of 4(2,4-DB) and had no effect at the 13.0 pound rate. An increase in the number of seeds per pod results when demeton interacts with 6.7 pounds per acre of 4(2,4-DB). When captan is present at 1200 ppm, demeton interacts with the 13.0 pound rate of 4(2,4-DB) to increase the number of seeds per pod.

Effect of 4(2,4-DB) on Alfalfa

Vegetative parts

The results of experiment B and experiment C show that the application of 4(2,4-DB) to alfalfa seedlings or to alfalfa in the early bloom stage, will have an adverse effect on the alfalfa, resulting in epinastic curling of the stems and leaves. These results support the finding of LeBrocq and Beech (1959). The recovery of the alfalfa plants treated, varied from a week for the lighter rates to over a month for the 13.0 pound rate when the plants again looked normal.

Rate of growth

Rate of growth of the alfalfa seedlings as affected by 4(2,4-DB) was only measured in experiment B. Both the 6.7 pound rate and the 13.0 pound rate decreased the rate of growth from June 16 to July 8. The 6.7 pounds per acre decreased the growth in the July 16 to July 21 growth period, while the growth increased at the 13.0 pound rate due to the recovery of the alfalfa plants from the effect of the treatment. The effect of 4(2,4-DB) over the six growth periods on total growth is a reduction in growth, with the 13.0 pound rate giving the greatest reduction. The highly significant interaction of 4(2,4-DB) and dates, Figure 14, is probably due to the recovery of alfalfa plants receiving the 13.0 pound rate of 4(2,4-DB) and to the low rate of growth during the July 8 to July 21 growth period. The slow rate of growth during this period was due to lack of soil moisture. The interaction of 4(2,4-DB) with captan and demeton on growth, in Figure 13, shows that captan and demeton interacted with 4(2,4-DB) to increase growth. This indicated that both captan and demeton may have reduced the effect of 4(2,4-DB) on growth on alfalfa. Since captan and demeton reduce growth without 4(2,4-DB) being present, 4(2,4-DB) appears to have an effect on the action of these chemicals on alfalfa.

Fertility and flower production

The results show that when 4(2,4-DB) was applied to alfalfa seedlings that the self fertility was increased as shown in Figure 17. When 4(2,4-DB) was applied to the alfalfa plants in the early bloom stage the treatments had no effect on self fertility, indicating that the time of application is important in affecting fertility. The number of seeds per pod or the number of developing ovules per flower was affected by 4(2,4-DB) interacting with captan and demeton. The interaction of captan, demeton, and 4(2,4-DB) indicated that 4(2,4-DB) decreased the number of seeds per pod. However, by adding demeton to the 6.7 pound level of 4(2,4-DB) and the addition of both captan and demeton to the 13.0 pound level of 4(2,4-DB) an increase in the number of seeds per pod resulted. Captan and demeton were able to overcome the effects of 4(2,4-DB) on seeds per pod probably by blocking the action of the chemical.

The results of experiment B, show that 4(2,4-DB) when applied to alfalfa seedlings affect the development of the racemes by reducing the number of flowers per raceme. In experiment C, the 4(2,4-DB) treatments did not affect the number of flowers per raceme, indicating that forial initiation had probably taken place before the treatments were applied.

The delay in maturity of the 4(2,4-DB) treated plots was probably due to the initial effects that the 4(2,4-DB) had on the alfalfa. These results do not support the work of Deakin and Ormrod (1956), working with red and white clover, where they found that two and four pounds per acre of 4(2,4-DB) did not delay the date of flowering.

Seed and chaff yield

The results, of experiment B and experiment C, for the effect of 4(2,4-DB), on seed and chaff yield support the work of Deakin and Ormrod (1956), Yeo and Dunham (1956b), and Elliott (1956), who found that 4(2,4-DB) reduced both seed and forage yields of legumes. The seed and chaff yields were reduced with increased rates of 4(2,4-DB) in both experiments.

SUMMARY AND CONCLUSIONS

Foliar and soil applications of captan were given to transplants of four different alfalfa clones in the greenhouse. Measurements were made to determine the effect of captan on rate of growth, and the fertility of the pollen and ovules. The results showed that different clones of alfalfa differ in their susceptibility to the action of captan when applied to the soil. Yellowing of the leaf tips resulted from treating one of the clones with captan.

In a field experiment, captan, demeton, and 4(2,4-DB) were applied to alfalfa seedlings. Data were taken for the effects of these chemicals on rate of growth, fertility of the pollen and ovules, and for seed and chaff yield. A significant reduction in seed yield resulted from the application of 5.75 ounces per acre of demeton. Factors causing this reduction in seed yield were not conclusive.

Epinastic curling of the stems and leaves, and scorching of the leaves of the seedlings resulted from the application of 6.7 and 13.0 pounds per acre of 4(2,4-DB). The time required for the alfalfa seedlings to recover from these initial effects was dependent on the dosage applied. 4(2,4-DB) reduced the rate of growth, flowers per raceme, and delayed the date of flowering of the alfalfa. A highly significant reduction in seed and chaff yield resulted from the use of 4(2,4-DB).

The results shown by the interactions of captan, demeton and 4(2,4-DB) indicated that the effects of these chemicals can be either additive or that one chemical can reduce the effect of another chemical. In some cases the interaction of the chemicals resulted in an increase in the factor being measured.

The results of another field experiment showed that 4(2,4-DB) when applied at rates of two and 4.5 pounds per acre to alfalfa in 5 per cent bloom caused epinastic curling of the stems and leaves, and scorching of the leaves. The flowers that were present at the time of application, dried up producing no seed. Seed and chaff yields were reduced as a result of the treatments of 4(2,4-DB).

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