QUALITY CHANGES IN FORAGE CROPS FOLLOWING APPLICATIONS OF 2,4-D

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE

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We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF SCIENCE IN AGRICULTURE.

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ABSTRACT

Quality Changes in Forage Crops Following Applications of 2,4-D

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A Thesis Submitted in Partial Fulfilment of
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Very few investigations have been made which report directly on the effect of the plant growth regulator 2,4-D on a great group of plants, principally grasses and legumes, the forages used by livestock. Under certain conditions such growth regulation substances may effect considerable changes in the chemical composition of higher plants. The fact that forage value may be deliberately altered by applying sub-herbicidal concentrations of growth regulators seems not to have been carefully studied. An investigation appeared warranted and is the subject of this research.

Under field conditions several sub-herbicidal

levels of 2,4-D were applied at several stages in the development of red and white clover, perennial ryegrass, Kentucky bluegrass, and timothy growing on Ladner clay and Alderwood loam. Appreciable increases in dry matter and nitrogen yield obtained in certain treatments. Other significant effects were noted in flowering and seed production. Confirmation of the field results was obtained in greenhouse studies on red clover and Olli barley. A search for the source of the increased nitrogen did not reach a definable end but work with the influence of treated plants on soil Rhizobia and Azotobacter suggest that 2,4-D applied to forages may in some instances modify the ecology of the soil flora.

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To my wife I must accord, not only patience and interest, but help in the management of the plots in the field.

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The assistance of Mr. Tom Willis, Superintendent of the Dominion Range Experiment Station, Kamloops, B. C., is recorded for sending me several hundred pounds of a special soil from the B. C. Interior.

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QUALITY CHANGES IN FORAGE CROPS FOLLOWING APPLICATIONS OF 2,4-D.

I THE INTRODUCTION

Since 1933, when Kogl, Haagen-Smit and Erxleben announced the isolation of auxin a (auxentriolic acid), many substances have been found to possess growth regulating properties, thousands of papers have been published about them, and dozens of uses with many species of plants have been described for them. However, very few papers report directly on their effects on a great group of plants, principally grasses and legumes, the forages used by livestock.

Under specified conditions growth regulating substances may effect considerable changes in the chemical composition of the plant. The fact that plants treated with such materials may have an altered nutritional value has been given scant attention. The fact too that forage value might be altered after applying low concentrations of growth regulators seems not to have been entertained. An investigation of the possibility appeared warranted and is the subject of this research.

A first step to the solution of the problem of modifying the nutritional value of forage using growth regulators was to define the nature and magnitude of the chemical changes and the circumstances under which they could be effected. The growth regulator used in our studies was 2,4-D (2,4-dichlorophenoxyacetic acid) because it is

inexpensive and widely used. Changes in nitrogen metabolism and the associated changes in carbohydrates were the subject of the most intensive studies. The special attention directed towards nitrogen metabolism arose as a result of the fact that, on occasion at least, the crude protein or Kjeldahl Nitrogen percentage of a forage could often be increased without loss of dry matter yield following light 2,4-D application. Since protein percentage is a major factor in determining forage quality, special attention in this direction seemed warranted.

Attempts to find reasons for the crude protein increases have led to studies with soil microorganisms known to be important in nitrogen fixation, notably the Rhizobia and Azotobacter.

Forage whose composition had been modified by 2,4-D was not in this series of studies, subjected to that which should ultimately follow, feeding trials with grazing animals.

II. THE REVIEW OF LITERATURE

There are several excellent reviews on the subject of plant growth regulators. (103), (109), (81), (36). The following review is therefore, in a sense, a summary account, in which special reference is made to studies which appear to bear rather directly on this research.

1. The background for modern studies on growth regulation in plants.

Julius Sachs, nearly a century ago, from a series of bold experiments, decided that there are special substances which regulate the growth of different plant organs. His "lead", however, was not pursued very effectively and growth and movement in plants, usually considered as distinctly different phenomena, were viewed as a result of external stimuli. Some support for Sachs' views were given by Ch. Darwin in his treatise (1881), entitled, "Power of Movement in Plants", wherein he noted that a labile substance in certain plants moves from lower parts to upper parts causing movement of leaves and bending of shoots. Prior to the work of Fitting (1907) little penetrating investigation was undertaken, although the works of Pfeffer (1890), Haberlandt (1905) and Oltmanns (1897) maintained interest in growth phenomena. Fitting shed

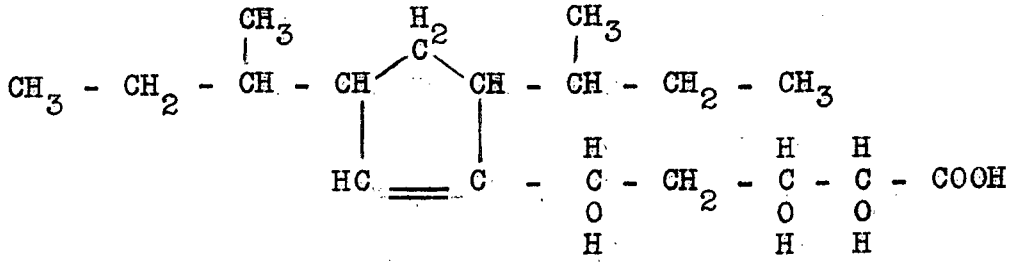
new light on the conduction of the "phototropic stimulus" in the coleoptiles of graminaceous plants. He found that the stimulus could be transmitted laterally as well as lengthwise and that it was not impeded by two incisions on opposite sides of a coleoptile up to 3/4 of the width. He found, too, that in a plant exposed to a sublethal temperature of 40°C an active principle was inhibited or inactivated. Modest success attended Fitting's efforts to chemically characterize a growth regulating substance in orchid flowers.

From the laboratory of F.A.F.C. Went, in the second decade of this century, came a series of investigations ascertaining threshold value and presentation time required for stimulation by light in etiolated coleoptiles of Avena sativa. The techniques developed became favorites for experimentation relative to the auxin hypothesis.

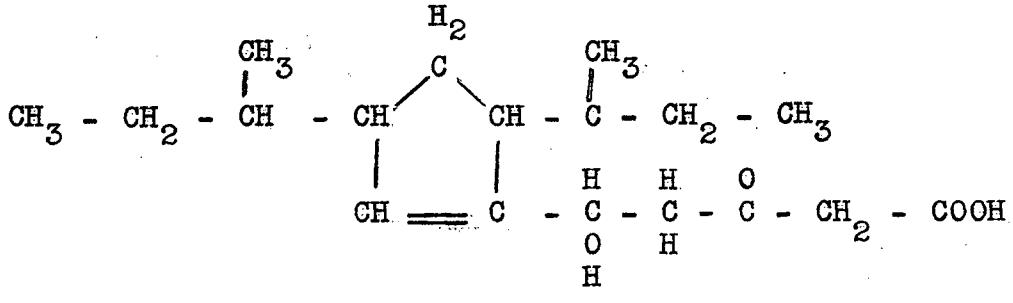
A truly decisive step came in 1926 when F.W. Went isolated in agar cubes a substance, termed then, auxin. The problem, says Weevers, soon became one for the analytical chemist. After a lengthy and difficult chemical research Kogl and his associates were able to prepare the auxin in crystalline form, but in amounts so small that it could not be fully characterized - although over 100,000 maize coleoptiles were used in the isolation. Then, from a convenient source, human urine, Kogl, Haagen-Smith and Erxleben isolated an active substance, later termed auxin-a,

in sufficient quantities to fully characterize it.

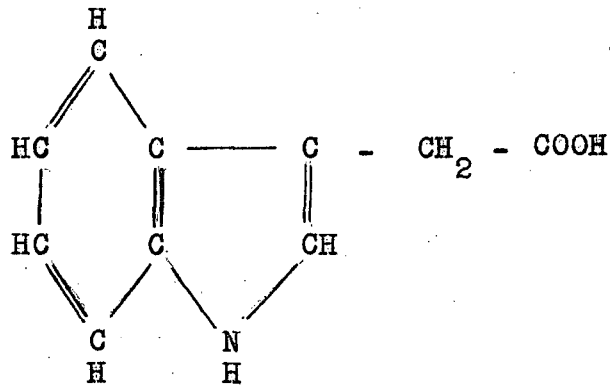
Auxin-a was shown to be acidic, to have a molecular formula of $C_{18}H_{32}O_5$ and a probable structural formula of



Other auxins were obtained later. From maize oil Kogl and his associates isolated the less active auxin-b in 1934, a substance with a molecular formula of $C_{18}H_{30}O_4$ and a probable structural formula of



In 1934, too, Kogl isolated hetero-auxin or indole acetic acid. In parallel work by Neilson, who isolated it, and by Thimann, who characterized it, the same substance was isolated from cultures of the fungus, Rhizopus suinus. It proved to have the formulae: $C_{10}H_9O_3N$ and



Isolation of auxin-a and auxin-b has not been repeated, for it soon became apparent that hetero-auxin, in nature, was the most common growth substance. There is no doubt to-day that it is of general importance as a natural plant growth substance.

Not long after the isolation of indole acetic acid in 1934, several groups of workers were able to show that a number of chemicals possessed growth regulating activity; some of them are synthetic and have not been demonstrated to occur naturally in plant tissues.

Bonner places these active substances, which to-day form a long list, in four categories:

a) indole derivatives other than indole acetic acid

b) naphthalene derivatives

c) phenoxyacetic acid derivatives

d) substituted benzoic acids

At present emphasis is being placed on the synthesis of homologs and analogs of the naturally occurring auxins, and on the role of auxins in intermediary metabolism.

2. Definition and structural requirements of growth regulators.

As has been noted, growth substance activity is shown by many quite different substances, some occurring in nature and some synthetic. Definition, however, of growth substance activity is not simple. Bonner (17), probably correctly, implies that all growth regulators are active in the pea curvature test, i.e. are effective in causing cell elongation. He points out, of course, that any given active compound reacts differently to the other "growth tests", such as the tomato petiole bending test, the etiolated Avena coleoptile curvature test, the Avena section test, the root initiation test, etc. The differences may sometimes be quite spectacular; for example, naphthalene acetic acid is only 2.5% as active as indoleacetic acid in the oat curvature test, but it is more effective than indoleacetic in the split pea curvature test. In part, the differences in response may be explained by differences in "transportability" of the different growth substances, by differences in dissociation constants and so forth.

To possess primary activity Koepfli, Thimann and Went (47) in 1938 postulated that the following minimum structural features must be possessed by a growth substance: (a) a ring system as a nucleus, (b) a double bond in the ring

system, (c) a side chain, (d) a carboxyl group on the side chain, or a group readily converted to a carboxyl group, at least one atom removed from the ring, and (e) a particular space relationship between the ring and the carboxyl group.

Subsequently Thimann (103) has suggested that the carboxyl group requirement should be broadened and stated merely as an acid group which is not too highly dissociated.

Veldstra (101), too, would extend the structural requirements for activity. He notes that the basal ring system must have a high surface activity and that the unsaturation of the ring is also necessary for activity. This unsaturation cannot be replaced by unsaturation on the side chain. Van Overbeek (94) finds, that in general increasing the length of the side chain decreases activity. Insofar as pH may modify the structure of auxins, in a sense, it too, is a "structural" requirement for penetration and activity of the auxins (Blondeau (15)).

Varied though the chemical nature and activity of the many "growth substances" may be, it appears that these compounds, variously, but synonymously, termed "growth substance", "growth regulator" and "auxin" have in common, the physiological characteristic of promoting elongation of cells and the structural characteristic of having a specific type of molecule.

Auxins, it is now clear, are but a class of phytohormone. Other kinds of organic compounds which regulate plant physiological processes are being isolated and characterized viz. the reproduction carotenoid hormones, cis and trans dimethyl crocetin, the wound hormone, traumatic acid, and others.

3. Forms of auxin in the plant.

Auxins and related compounds occur in the plant in a variety of forms: in a free molecular form, and in association with other molecules forming a complex (17). The group of free molecular forms contains acids such as indoleacetic acid, auxin-a, etc. and neutral forms such as indoleaceticaldehyde, auxin-a lactone, etc. The bound forms yield free auxin only after autolysis of the plant tissues.

Van Overbeek (36) states that free auxin has been isolated after enzymatic digestion of proteins isolated from seeds and from spinach leaves. He notes, however, that some bound forms may not contain auxin in a more or less free acid form, but may require a series of oxidations to make the conversion. Further to this he adds that the status of bound auxin is very much in doubt, but that a high level of free auxin is frequently associated with a high level of auxin activity.

4. Auxin physiology.

a) The fundamental roles.

From the point of view of applied physiology the

auxins play a multitude of roles and each growth regulating substance may play the roles with different intensities. It seems now that auxins exert their multiple effects as a result of controlling a few fundamental physiological processes.

J. Bonner (18) and associates in 1946 isolated an auxin protein with the properties of a phosphatase, an enzyme closely associated with the release of energy from phosphate bonds. From this and later work it appears that auxins regulate the energy "flowing" to a number of synthetic processes. In van Overbeek's words "auxin would be the foot on the throttle." These researches confirm and extend those of Thimann and associates (89), who, in 1939 were able to demonstrate that auxin treated oat coleoptiles showed increased respiration rates and that there was a linkage with the Krebs cycle.

Elongation remains as the most directly recordable general role of the auxins. They appear to make cell wall more plastic, which, in turn, results in increased diffusion pressure deficit inside the cell. The further result of this is for the cell to take up water and elongate. What, then, is usually referred to as the "growth effect" of auxins is really cell elongation.

b) <u>Auxin translocation.</u>

Auxins are transported "rapidly" inside the plant in at least three different ways:

1) through the phloem, 2) through parenchymatous tissue devoid of vascular bundles and 3) through the xylem transpiration stream. Weaver and de Rose (99) found they could pass upward through dead tissue but not downward. Oddly enough too, they found that stomata were not portals of entry for leaf sprays of the hormone. A number of investigations have shown that auxins are present constantly in all living plant tissues, but are most abundant in the meristems.

c) Auxins in gall and nodule formation

Of some physiological interest is the role of auxins in gall and nodule formation. Observations relevant to this phase are of special interest to our researches reported upon later. Crown galls caused by Phytomonas tumafaciens have a high auxin content. Auxins, however, do not produce the galls directly: it has been concluded (39) that the production of galls involves two phases, a) the bacteria change normal host cells to tumour cells and b) the auxin produced by the host induces the neoplastic cells to actual tumour formation. Auxin, too, plays an important role in nodule formation on legumes. Here again, the Rhizobium, although quite capable of producing auxin in vitro seems to change the cells of the host in such a way that they are "conditioned" to grow. Coincidentally with the increased auxin production in the developing nodule is a very exact chromosome doubling in

bacteroid cells (Wipf and Cooper (100)). It may be the effect ~~in~~ on the chromosomes is a direct one and mutagenic. (see Unrau (100)).

d) Auxins and enzymes.

In plants, as has been noted, auxins appear to function by regulating enzymatic processes especially those involved in energy releasing processes. The researches of Thimann et al (89) have demonstrated the probable relation of auxins to the energy yielding Krebs cycle, and Bonner et al (18) have further supported the argument by associating auxin directly with phosphatase activity.

Puzzling relationships of auxins to enzymes, however, remain. Euster (34) found for example that auxin retarded the action of isolated diastase, but enhanced the activity of the diastase adsorbed on charcoal. Berger and Avery (14) found that naphthaleneacetic acid inhibited glutamic acid and isocitric dehydrogenases, but that indoleacetic acid inhibited only the first named enzyme.

e) Auxins and vitamins.

In animals the relationships of hormones to vitamins and to enzymes have been the subject of intensive study. In plants, however, the relationships have not been accorded the same attention. Luecke, Hamner and Sell (53), however, have found that the auxin hormones, when applied to leaves of the bean, generally reduced the thiamine, riboflavin, nicotinic acid and pro-vitamin A content, but

that they increased the pantothenic acid content. 2,4-D, for example, reduced the carotenoids by as much as 1/3. Mitchell et al (61) found that auxin treated fruit after a period of storage contained 45% more ascorbic acid than the untreated fruits in storage. Hey and Hope (44) were able to relate the herbicidal effectiveness of auxins to vitamin K levels in plants - the plants with the high vitamin K contents succumbed easily to herbicidal auxin levels.

f) Auxins in mineral nutrition.

Auxins may significantly influence the mineral nutrition of plants. Brunstetter (23) found that leaves treated with 3-indoleacetic acid showed increases in K, Mg, Mn and B. Some increases were also recorded for P, Al, Fe, and Cu. Went (102) found some evidence that auxin, added to the nutrient solution, partially overcame zinc and boron deficiencies. Rhodes, Templeman and Thurston (75) found that increasing concentrations of methoxone reduced potassium uptake, but did not appreciably alter the uptake of Na, Mn, Ca, and Fe. They admitted to a number of possible interpretations of their data, admissions which seems to be characteristic of most of the studies in this field.

g) Auxins in nitrogen metabolism.

The relationships of auxins to nitrogen assimilation are being actively sought for and delimited. As

yet the fundamental body of thought seems to be largely in the making and some incoherence in the literature accordingly may be expected.

How intimately auxins relate to nitrogen metabolism and assimilation is problematical. It is, of course, obvious that they influence it indirectly through their well-known influence on carbohydrate metabolism. It is a frequent observation, following foliar applications of auxins to growing higher plants, to see the foliage green intensifying. These and other observations can often be associated with metabolic changes involving protein synthesis. In more detailed observations, Mitchell (65) found beans treated with auxin had higher nitrogen levels than untreated beans. Smith et al (84), Borthwick, Hamner and Parker (19) and Wort (106) found nitrogen to decrease in bean leaves, but to increase in other plant parts following auxin applications. Rasmussen (74) found 2,4-D applications to be associated with increases in total soluble nitrogen in dandelion leaves. Davis, (86), using bean seedlings treated with indoleacetic acid, found nitrogen moving from plant upper parts to lower. Gordon (38), studying rooting responses of Hibiscus cuttings to auxins, found soluble nitrogen highest at the base of the treated cuttings, but not on the controls. Sell (79)

and others found protein and amino acids accumulating in the stems of auxin treated bean plants and noted declines in aspartic acid but increases in lysine, valine, methionine and phenylalanine. Borthwick and associates (19) under somewhat different conditions found marked increases in protein in meristematic areas subjected to auxin influences. Recording the changes in nitrogenous constituents following auxin treatments seems, not yet, to have taken physiologists very far in relating these phytohormones to nitrogen assimilation.

Although good experimental evidence is yet forthcoming, Chibnall (29) has suggested a very probable hypothesis that the protein levels of leaves are determined by phytohormones. Whether or not auxins are the hormones involved cannot yet be stated. A somewhat similar viewpoint is to be found in the suggestion by Ali-Zade (4) that hormones, possibly auxins, control the nitrogen metabolism of legume root nodules.

If nitrogen metabolism is in fact, intimately related to the action of auxins, and, since auxins are so extensively used in agriculture, one might expect to find records of quality changes in crops. Casual references are, in fact, quite numerous, but few detailed studies are available. Deepening of the green colour of lawns commonly follows herbicidal applications. To mention a few other observations: Aberg, Hagsend and Vaartnou (1)

found 2,4-D sprays increased the nitrogen content of wheat, flax and peas. Erickson, Seeley and Klages (33), using 2,4-D in wheat, obtained increases in the protein of the grain of 4.6% (from 10.9% in the control to 15.5% in the treated plants) and voiced the view that such responses should be taken into consideration in the control of weeds in wheat. On the Great Plains, 2,4-D is used extensively to control annual weeds in grain crops and quality in the wheat crop is, in some measure, associated with its protein content. It is interesting to notethat Aitken, Meredith and Olson (2) found significant increases in the crude protein of wheat, barley and oats which had been sprayed for weed control. They suggested that there is a period during the growing period when applications of 2,4-D may cause changes in the quality of bread wheat and malting barley.

h) Auxins in carbohydrate metabolism.

One of the commonly recorded changes in chemical composition of higher plants following 2,4-D application is a rapid increase in the percentage sugars in leaves. Mitchell and his associates in a series of papers (62), (63), (66) have, for example, recorded that under certain treatments with indoleacetic acid that in leaves placed in darkness, the percentage sugar increased to twice the percentage in the controls within four hours. The source of sugars appear to be the starchy reserves which are depleted as the sugars increase. When, however,

the starch reserves are depleted, often in the field after two weeks, decreases in the sugars ensue. It is fairly generally believed that herbicidal levels of auxins heighten respiration activity to the point where the carbohydrate reserves are depleted and death through starvation is a result. Sell et al (79) found that as total sugars decreased total protein increased and suggested that protein synthesis was "fostered" at the expense of carbohydrates in the plant. In the experiments by Sell et al, crude fiber, too, decreased in kidney beans treated with 2,4-D. Most workers, however, found that crude fiber changes very little following auxin treatments (Aberg, Hagsand and Vaartnou (1)).

i) Auxin and soil micro-organisms

Patterns of auxin activity in the soil and in soil micro-organisms are not yet well developed.

Many soil bacteria are capable of producing auxin in vitro. Bacillus radiobacter, for example, which occurs in the soil and in legume roots, is a "free" producer of auxin. Rhizobium too, which is directly or indirectly responsible for legume root nodulation produces less (Wilson (104)). It probably follows from in vitro results such as the above, that many soil organisms produce auxin in the soil.

The addition of auxins to the soil, artificially, often effects considerable change in the ecology of the soil microflora. According to Worth and McCabe (107)

aerobic bacteria are usually "sensitive to" added 2,4-D on the soil, but that anaerobic organisms are much less sensitive. Marcelli (56), Smith et al (85) report, what appears to be a fairly common observation, that if 2,4-D is added artificially to soil - that the populations of micro-organisms reduce very rapidly in size at first, but that later the populations become larger than before 2,4-D treatment. The recovery of the microfloral populations from soils which were not leached, according to Newman and Norman (68) is due to the fairly rapid decomposition of 2,4-D by micro-organisms. Amounts of auxins required to considerably modify the plant ecology of the soil are low: Payne and Fults (72), for example, found that .075 lbs. of 2,4-D per acre of soil could prevent nodulation of common beans. Carlyle and Thorpe (27) also note that .5 p.p.m. 2,4-D in the soil will seriously interfere with nodulation and growth of beans, peas, red clover, and alfalfa.

Very small amounts of 2,4-D added to the soil or in cultures <u>in vitro</u> may "benefit" some organisms. Thus Ball (11) finds that the addition of very small amounts of indoleacetic acid in a glucose-tryptophane medium more than doubled the number of viable <u>Escherichia coli</u> cells in 24 hours. Anker (8) found that endogenous respiration of "starving" glycogen containing yeast cells was stimulated by heteroauxin. Stimulation of reproduction was noted in <u>Chlorella vulgaris</u> and <u>Coccomyxa simplex</u> by

Brannon and Bartsch (21) when growth substances were added to cultures *in vitro*.

No investigations were found which report upon the effects on soil microflora following applications of auxins to the foliage of higher plants.

j) Auxins in agricultural practice

Only a brief reference can be made to the many uses to which auxins have been placed.

Went and Thimann in 1934, soon after indole acetic acid was isolated and crystallized, discovered that this hormone promoted root formation in isolated stem sections of pea seedlings. This discovery has been followed by an immense amount of study with the result, that to-day, several growth regulating substances are widely used in the vegetative propagation of many kinds of plants, herbacious and woody. In somewhat similar fashion aerial buds may be initiated (102), (45) by applying auxin paste to cut stem surfaces. On the other hand, bud development may be suppressed by their use viz. naphthalene acetic acid is used to prevent bud development and initiation in potatoes in storage.

The role of auxins in flower formation is far from clear. That they are in some way involved is undoubted. Auxin-induced flower formation has only been established with certainty in the pineapple (95). In most plants there appear to be other agents which are rather directly associated with the auxins in flower formation (51).

Far from inducing flowering in some plants, auxin applications may delay maturity and the onset of flowering. (87), (99), (71)

Flowers of solanaceous plants last for an abnormally long time after being treated with growth regulating substances (40), (102) and in some species parthenocarpic development of fruits may ensue. To a limited extent this fact has been commercially exploited with the development of parthenocarpic fruit.

Abscission of many organs, fruits, flowers, petals, and leaves, is sometimes associated with a low auxin level. Abscission of this type may be ~~corrected~~ delayed by an auxin spray. Utilizing this information orchardists have economically controlled pre-harvest drop of tree fruit.

The effectiveness of certain hormone herbicides is too well known to more than mention it for the sake of completeness of the review. The persistence of 2,4-dichlorophenoxy acetic acid (2,4-D) and 4 chloro-2-methyl phenoxy acetate (Methoxone) is probably a prime factor in their effectiveness as herbicides for they accumulate in meristematic zones and effect an exaggerated respiration, which, since it is long continuing, is associated with starvation and eventual lethality.

III. THE REPORT ON CERTAIN FIELD, GREENHOUSE AND LABORATORY STUDIES

1. Field Trials, 1950-52

Trials were maintained over three growing seasons (1950-52) with a view to assessing, under field conditions, the effects of 2,4-D, at various times and concentrations, on the chemical composition of several forage grasses and legumes commonly grown in the Lower Fraser Valley.

a) Location and Materials:

The trial on the Alderwood gravelly loam (glacial till and outwash) of the U.B.C. farm in Point Grey was duplicated on Ladner silty clay (alluvium) close to the Fraser River North Arm at the foot of Blenheim St., Vancouver, B. C. The U.B.C. soil was low in fertility and subject to severe summer drought. The silty clay was productive and moist but possessed a pH level somewhat below that considered desirable.

The forage species used in the trials included two legumes, Red Clover (Trifolium pratense) and White Clover (T. repens) and three grasses, timothy (Phleum pratense), perennial ryegrass (Lolium perenne) and Kentucky Bluegrass (Poa pratensis). All seed used was "commercial".

Prior to seeding, the land at both locations received 800 lbs. of agricultural lime per acre.

The sodium salt of 2,4-dichlorophenoxyacetic acid was the growth regulator used, largely because it is readily available and commonly used.

b) Methods:

Ultimate plots for all treatments were 9' x 18', of which an area, 6' x 15' was harvested for yield. Each treatment was replicated five times but only four replicates were used for yield; one was used for studying seed production and viability. All seeding was in 10" rows using a one-spout "Planet Junior" "push seeder". Only the Kentucky Bluegrass seeding on the Ladner clay failed to produce a good stand in the spring of 1950; it was reseeded in August 1950. The plots were kept free of weeds by "push hoe" and "hand pulling" so that stands were, in all cases, "pure" stands.

The 1951 growing season started slowly, following snows in late March, but in May growth was very rapid and the first spraying was accomplished on May 12th. All spray applications were made with a low pressure knapsack sprayer and aquaeous solutions of the sodium 2,4-D were always used.

In one series of experiments, 2,4-D in different concentrations was employed. The range of concentrations on clovers was from .01 to 1 lb. and on grasses from .1 lb. to 8 lbs. parent acid per acre. In a second series of experiments, 2,4-D was applied at different times. Spraying in this series was undertaken in May and in June at 7-day intervals.

The plots on the Ladner clay were harvested on June 23rd and on the Alderwood gravelly loam on July 1st. Fresh weights were taken and 1-kilogram samples were taken for determining dry matter, crude protein and invert sugar.

Records were taken from time to time on flowering behavior and seed setting.

The chemical analyses were accomplished in the winter of 1951-52. Official A.O.A.C. methods were used. Analyses were in duplicate or triplicate.

In the summer of 1952 observations were made on all plots (including those of the "biennial" red clover*). Effects of 2,4-D which might "carryover" into the second year after treatment were sought for. In addition, a new series of treatments was superimposed on the 1951 U.B.C. treatments, the object of which was, primarily, to determine the effects of 2,4-D on flowering intensity and maturation.

c) Results:

Red clover - vid. Table I - the effects of 2,4-D concentration on red clover and Table II - the effects of time of 2,4-D application on red clover.

An examination of the data in Tables I and II will show that yield and protein levels of red clover, a biennial, tap-rooted legume, may be materially modified by 2,4-D applications.

*Many of the red clover plants persisted for a third year on the plots.

Marked variation in fertility occurred over short distances in the Alderwood soils. Nonetheless, significantincreases in mean yield over the control were recorded with all concentrations of 2,4-D (applied May 12th when the plants were yet vegetative) except with the highest concentration. With the control yielding, on the average 3805 lbs. per acre, the highest yielding concentration, giving 5435 lbs. per acre, was .05 lbs. 2,4-D per acre.

Ladner soil was more homogeneous and while again significant increases in mean yield over control were recorded for low concentrations of 2,4-D the increases were not so striking. Where the average yield of controls was 6400 lbs. per acre .1 lbs. 2,4-D per acre increased the yield to 7200 lbs. per acre. Higher concentrations of 2,4-D (viz. .5 and 1.0 lb. per acre) markedly depressed the red clover yields.

Crude protein percentage, as the tabled data will show, increased significantly. There was no indication from the harvest taken in a more or less optimum hay cutting time, weeks after the actual 2,4-D was applied, that this increase was at the expense of the sugars in the plant.

Acre yields of protein again were significantly increased under all applications of 2,4-D but with the higher concentrations the increases are small. Optimum concentration for protein yield per acre appears to be

around .1 lb. per acre for red clover. Again, the response on Alderwood soil was more marked than on Ladner soil.

2,4-D was applied on red clover on five occasions (representing five stages of development from early leaf to mid-flowering) at the rate of .1 lb. per acre. Very different responses were noted on the two soils. On the red clover grown on Alderwood soil, applications made early (May 12) were seemingly effective in elevating dry matter yields and protein per acre yields as were all applications made a later date. However, plot variability was so great that increases could not be regarded as significant. The early application on the Ladner clay grown red clover resulted in a significant increase in yield and protein per acre. Later applications were depressive. It would appear that the time of application and the stage of growth of the red clover plants determines to a very considerable extent the nature of the response to 2,4-D.

It is to be noted that in Tables I and II, and in some of the others to follow, that both Kjeldahl nitrogen and crude protein are reported. The reason for this is that the form of the nitrogen following 2,4-D applications is not known; it may be that non-protein nitrogen only is increased.

White clover - vid. Table III - the effects of 2,4-D concentration on white clover and Table IV - the effects

of time of 2,4-D application on white clover.

The effects of 2,4-D on white clover, a creeping rooted perennial legume, were not so marked as those on red clover, a tap-rooted biennial.

Possibly because the shallowness of the root system of white clover and the dryness of the season, the plot yields on Alderwood soil were visibly about as variable as one could hope to find, and yet visibly sharp qualitative differences could be accorded treatment. Undoubtedly the trials should be repeated under more favourable conditions.

Nonetheless it would appear that on Ladner clay grown white clover, intermediate concentrations of 2,4-D increased both yield and protein per acre. On Alderwood grown white clover, the reaction is not clear - but increases in yield and protein were obtained at the higher concentrations.

Consistently, too, the relatively low concentration of 2,4-D applied to Alderwood grown white clover, .1 lb. per acre, proved to be depressive of yield and protein per acre in the "time of application series". Protein percentage increases are recorded on the white clover grown on the Ladner clay. 2,4-D applied early (May 12) increases yield of dry matter, protein per acre and protein percentage. Late applications ~~were~~ are depressive.

Kentucky Bluegrass - vid. Table V - the effects of 2,4-D concentration on Kentucky Bluegrass and Table VI - the effects of time of 2,4-D application on Kentucky Bluegrass.

When spraying was started, the Kentucky Bluegrass was well developed, with panicles ~~well developed~~ fully formed and, on the Alderwood plots, past flowering. The stage of development was probably too advanced to influence chemical composition very much. The processes of maturity had not advanced so far in the plants growing on the Ladner soil.

Nonetheless, a significant increase in yield was noted on Alderwood grown Bluegrass when 4.0 lbs. 2,4-D per acre was applied. On Ladner clay-grown Bluegrass, however, increases in yields, protein per acre and protein percentage were obtained at several of the higher rates of application. Early applications appeared to be most effective in increasing yield and protein on both soils.

Perennial Ryegrass - vid. Table VII - the effects of 2,4-D concentration on perennial ryegrass and Table VIII - the effects of time of 2,4-D application on perennial ryegrass.

Maturation processes were well advanced in the perennial ryegrass before the plot treatments were made; especially was this the case in the grass on the upland soil. Nonetheless the growth regulator caused some recordable changes in yield of dry matter, on protein per acre, and percentage protein. All concentrations, for example, applied May 12th, increased yields and crude protein of perennial

ryegrass grown on Ladner clay but only the high concentrations increased them on Alderwood soil. In general, the early applications tended to increase yield and protein and the late applications to depress them. The most significant response was obtained with the earliest application on the grass grown on Ladner clay.

Timothy - vid. Table IX - the effects of 2,4-D concentration on timothy and Table X - the effects of time of 2,4-D application on timothy.

Timothy matured more slowly than Kentucky Bluegrass and perennial ryegrass. It was, therefore, noted with interest that the "low applications" of 2,4-D were quite effective in modifying the yield and chemical composition of this species. Both in Alderwood and Ladner soils all concentrations of 2,4-D applied early (May 12) increased dry matter yields and, as well, on the Ladner soils, crude protein per acre and protein percentage. Early applications of the auxin tended to increase dry matter yield, crude protein percentage and total protein per acre, but later application on grass grown on both soil types were depressive.

TABLE I: THE EFFECTS OF 2,4-D CONCENTRATION ON RED CLOVER

Na 2,4-D Concentrations lbs. p.a.	Dry Matter Yields				Crude Protein as % Dry Matter				Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein				Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
	lbs. p.a.	% of control	Deviation in lbs. from control +	Deviation in lbs. from control -	% dry matter	% of control	Deviation in % from control +	Deviation in % from control -		lbs. p.a.	% of control	Deviation in lbs. from control +	Deviation in lbs. from control -		
a) On Alderwood Soil															
0	3805	100.0	0	0	10.55	100.0	0	0	2.0	401	100.0	-	-	64.1	1.68
.01	4730	124.3	925		9.66	91.6		.89*	1.9	457	113.9	56		73.1	1.54
.05	5435	142.8	1630*		11.59	109.8	1.04*		1.9	630	157.1	229*		100.8	1.85
.1	4525	118.9	720		11.18	105.9	.63*		2.1	506	126.1	105		80.9	1.78
.2	4815	126.5	1010		14.23	134.8	3.68*		2.0	685	170.8	284*		109.6	2.27
.5	4735	124.4	930		13.31	126.1	2.76*		1.8	630	157.1	229*		100.8	2.12
1.0	3785	99.4		20	12.76	120.9	2.21*		1.9	483	120.4	82		77.2	2.04
b) On Ladner Soil															
0	6400	100.0	0	0	13.58	100.0	0	0	2.1	869	100.0	-	-	139.0	2.17
.01	6390	99.8		10	14.27	105.0	.69*		2.1	912	104.9	43		145.9	2.28
.05	6975	108.9	575		14.92	109.8	1.34*		2.0	1040	119.6	171*		166.4	2.38
.1	7200	112.5	800*		13.96	102.7	.38*		1.8	1005	115.6	136*		160.8	2.23
.2	6555	102.4	155		16.02	117.9	2.44*		2.1	1050	120.8	181*		168.0	2.56
.5	5985	93.5		415	15.91	117.1	2.33*		1.9	952	109.5	83*		152.3	2.54
1.0	5040	78.7		1360*	15.79	116.1	2.21*		1.9	852	98.0		17	136.3	2.52

M.S.D.
a)
1. - 1591 lbs.p.a.
2. - .14 %
3. - 212 lbs.p.a.

b)
1. - 608 lbs.p.a.
2. - .09 %
3. - 61 lbs.p.a.

TABLE II: THE EFFECT OF TIME OF 2,4-D APPLICATION ON RED CLOVER

Time of Application (.1 lbs.Na 2,4-D p.a.)	Dry Matter Yields				Crude Protein as % Dry Matter				Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein				Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
	lbs. p.a.	% of control	Deviation in lbs. from control +	Deviation in lbs. from control -	% dry matter	% of control	Deviation in % from control +	Deviation in % from control -		lbs. p.a.	% of control	Deviation in lbs. from control +	Deviation in lbs. from control -		
a) On Alderwood Soil															
0	3805	100.0	0	0	10.55	100.0	0	0	2.0	401	100.0	-	-	64.1	1.68
May 12, 1951	4525	118.9	720		11.18	105.9	.63*		2.1	506	126.1	105		80.9	1.78
" 19 "	4045	106.3	240		11.73	111.1	1.18*		1.8	474	118.2	73		75.8	1.87
" 26 "	4400	115.6	595		11.00	104.2	.45*		2.0	484	120.6	83		77.4	1.76
June 2, "	3930	103.2	125		12.25	116.1	1.70*		2.0	481	119.9	80		76.9	1.96
" 9 "	3360	88.3		445	11.92	112.9	1.37*		1.9	400	99.7		1	64.0	1.90
b) On Ladner Soil															
0	6400	100.0	0	0	13.58	100.0	0	0	2.1	869	100.0	-	-	139.0	2.17
May 12, 1951	7200	112.5	800*		13.96	102.7	.38*		1.8	1005	115.6	136*		160.8	2.23
" 19 "	6240	97.5		160	13.44	98.9		.14*	1.8	839	96.5		30	134.2	2.15
" 26 "	6145	96.0		255	11.78	86.7		1.80*	1.9	724	83.3		145*	115.8	1.88
June 2 "	5410	84.5		990*	11.41	84.0		2.17*	2.1	617	71.0		252*	98.7	1.82
" 9 "	5592	87.3		808*	13.62	100.2		.04	1.8	762	87.6		107*	121.9	2.18

M.S.D.
a)
1. - 1027 lbs.p.a.
2. - .14 %
3. - 143 lbs.p.a.

b)
1. - 601 lbs.p.a.
2. - .09 %
3. - 55 lbs.p.a.

TABLE III: THE EFFECTS OF 2,4-D CONCENTRATION ON WHITE CLOVER

Na 2,4-D Concentrations lbs. p.a.	Dry Matter Yields: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Crude Protein as % Dry Matter: % dry matter	% of control	Deviation in % from control: +	-	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	3940	100.0	0	0	14.09	100.0	0	0							
.01	3140	79.6		800*	13.94	98.9		.15*	1.7	555	100.0	-	-	88.8	2.25
.05	3235	82.1		705*	13.61	96.5		.48*	1.8	437	78.7		118*	69.9	2.23
.1	3265	82.8		675*	12.67	89.9		1.42*	1.9	440	79.2		115*	70.4	2.17
.2	4185	106.2	245		13.01	92.3		1.08*	1.7	414	74.5		171*	66.2	2.02
.5	4000	101.5	60		15.01	106.5	.92*		2.0	544	98.0		11	87.0	2.08
1.0	3045	77.2		895*	16.79	119.1	2.70*		1.8	600	108.1	45		96.0	2.40
									1.7	511	92.0		44	81.7	2.68
b) On Ladner Soil															
0	3930	100.0	0	0	16.80	100.0	0	0	1.8	660	100.0	-	-	105.6	2.68
.01	4055	103.1	125		14.49	86.4		2.31*	1.7	587	88.9		73*	93.9	2.31
.05	4335	110.3	405*		14.99	89.3		1.81*	1.9	650	98.4		10	104.0	2.39
.1	4295	109.2	365*		17.89	106.6	1.09*		1.7	768	116.3	108*		122.8	2.86
.2	4105	104.4	175		18.25	108.8	1.45*		1.8	749	113.4	89*		119.8	2.92
.5	3645	92.7		285*	18.17	108.3	1.37*		2.0	662	100.3	2		105.9	2.90
1.0	3255	82.8		675*	14.62	87.1		2.18*	1.8	476	72.1		184*	76.1	2.33

M.S.D.

a) 1. 567 lbs.p.a.
2. .09 %
3. 50 lbs.p.a.

b) 1. 248 lbs.p.a.
2. .12 %
3. 30 lbs.p.a.

TABLE IV: THE EFFECT OF TIME OF 2,4-D APPLICATION ON WHITE CLOVER

Time of Application (lbs.Na 2,4-D p.a.)	Dry Matter Yields: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Crude Protein as % Dry Matter: % dry matter	% of control	Deviation in % from control: +	-	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	3940	100.0	0	0	14.09	100.0	0	0	1.7	555	100.0	-	-	88.8	2.25
May 12, 1951	3265	82.8		675*	12.67	89.9		1.42*	1.7	414	74.5		141*	66.2	2.02
" 19, "	3360	85.2		580*	15.12	107.3	1.03*		1.6	508	91.5		47*	81.2	2.41
" 26, "	2735	69.4		1205*	15.44	109.5	1.35*		1.9	422	76.0		133*	67.5	2.47
June 2, "	3170	80.4		770*	14.30	101.4	.21*		1.8	453	81.6		102*	72.4	2.28
" 9, "	2425	61.5		1515*	12.61	89.49		1.48*	1.7	306	55.1		249*	48.9	2.01
b) On Ladner Soil															
0	3930	100.0	0	0	16.77	100.0	0	0	1.8	660	100.0	-	-	105.6	2.68
May 12, 1951	4295	109.2	365*		17.89	106.6	1.09*		1.7	768	116.3	108*		122.8	2.86
" 19, "	3870	98.4		60	16.91	100.7	.14*		1.9	654	99.0		6	104.6	2.70
" 26, "	3775	96.0		155*	16.72	99.7		.05	1.8	631	95.6		29*	100.9	2.67
June 2, "	3535	89.9		395*	16.81	100.2	.04		1.8	594	90.0		66*	95.0	2.68
" 9, "	3245	82.5		685*	16.37	97.6		.40*	1.7	531	80.4		129*	84.9	2.61

M.S.D.

a) 1. 399 lbs.p.a.
2. .09 %
3. 36 lbs.p.a.

b) 1. 129 lbs.p.a.
2. .12 %
3. 16 lbs.p.a.

TABLE V: THE EFFECTS OF 2,4-D CONCENTRATION ON KENTUCKY BLUEGRASS

Na 2,4-D Concentrations lbs. p.a.	Dry Matter Yields: lbs. p.a.	Dry Matter Yields: % of control	Dry Matter Yields: Deviation in lbs. from control +	Dry Matter Yields: Deviation in lbs. from control -	Crude Protein as % Dry Matter: % dry matter	Crude Protein as % Dry Matter: % of control	Crude Protein as % Dry Matter: Deviation in % from control +	Crude Protein as % Dry Matter: Deviation in % from control -	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	Acre Yields of Crude Protein: % of control	Acre Yields of Crude Protein: Deviation in lbs. from control +	Acre Yields of Crude Protein: Deviation in lbs. from control -	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	1729	100.0	0	0	2.70	100.0	0	0	1.4	46	100.0	-	-	7.3	.43
.1	2058	119.0	329		1.70	62.9		1.00*	1.5	35	76.0		11	5.6	.27
.5	2093	121.0	364		1.68	62.2		1.02*	1.4	35	76.0		11	5.6	.26
1.0	2198	127.1	469		1.67	62.2		1.03*	1.4	38	82.6		8	6.0	.26
2.0	2149	124.2	420		1.71	62.9		.99*	1.3	37	80.4		9	6.0	.27
4.0	2624	151.7	895*		2.33	86.2		.37*	1.5	61	132.6	15		9.7	.37
8.0	2212	127.9	483		2.84	105.1	.14*		1.5	63	136.9	17		10.1	.45
b) On Ladner Soil															
0	3052	100.0	0	0	5.44	100.0	0	0	1.5	166	100.0	-	-	26.5	.87
.1	2744	89.9		308	5.40	99.2		.04	1.5	148	89.1		18	23.6	.86
.5	2905	95.1		147	5.34	98.1		.10	1.4	155	93.3		11	24.8	.85
1.0	3283	107.5	231		6.50	119.4	1.06*		1.6	213	128.3	47		34.0	1.04
2.0	3465	113.5	413		6.82	125.3	1.38*		1.5	236	142.1	70		37.7	1.09
4.0	3332	109.1	280		5.72	105.1	.28*		1.4	190	114.4	24		30.4	.91
8.0	3101	101.6	49		5.53	101.6	.09		1.4	171	103.0	5		27.3	.88

M.S.D.
a)
1. - 489 lbs.p.a.
2. - .12 %
3. - 58 lbs.p.a.

b)
1. - 669 lbs.p.a.
2. - .25 %
3. - 167 lbs.p.a.

TABLE VI: THE EFFECT OF TIME OF 2,4-D APPLICATION ON KENTUCKY BLUEGRASS

Time of Application (1.0 lbs. Na 2,4-D p.a.)	Dry Matter Yields: lbs. p.a.	Dry Matter Yields: % of control	Dry Matter Yields: Deviation in lbs. from control +	Dry Matter Yields: Deviation in lbs. from control -	Crude Protein as % Dry Matter: % dry matter	Crude Protein as % Dry Matter: % of control	Crude Protein as % Dry Matter: Deviation in % from control +	Crude Protein as % Dry Matter: Deviation in % from control -	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	Acre Yields of Crude Protein: % of control	Acre Yields of Crude Protein: Deviation in lbs. from control +	Acre Yields of Crude Protein: Deviation in lbs. from control -	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	1729	100.0	0	0	2.70	100.0	0	0	1.4	46	100.0	-	-	7.3	.43
May 12, 1951	2198	127.1	469		1.67	62.2		1.03*	1.4	38	82.6		8	6.0	.26
" 19 "	1561	90.2		168	1.79	66.2		.91*	1.6	30	65.2		16	4.8	.28
" 26 "	1659	95.9		70	1.86	68.8		.84*	1.3	31	67.3		15	4.9	.29
June 2 "	1701	98.3		28	1.75	64.8		.95	1.5	30	65.2		16	4.8	.28
" 9 "	1659	95.9		70	1.93	71.4		.77*	1.5	32	69.5		14	4.9	.30
b) On Ladner Soil															
0	3052	100.0	0	0	5.44	100.0	0	0	1.5	166	100.0	-	-	26.5	.87
May 12, 1951	3283	107.5	231		6.50	119.4	1.06*		1.6	213	128.3	47		34.0	1.04
" 19 "	2709	88.7		343	5.29	97.2		.15	1.4	143	86.1		23	22.8	.84
" 26 "	3157	103.4	105		5.44	100.0	0	0	1.5	172	103.6	6		27.5	.87
June 2 "	2898	94.9		154	5.13	94.3		.31	1.4	149	89.7		17	23.8	.82
" 9 "	3024	99.0		28	5.17	95.0		.27	1.3	156	93.9		10	24.9	.82

M.S.D.
A)
1. - 222 lbs.P.A.
2. - .12 %
3. 26 lbs.p.a.

b)
1. - 850 lbs.p.a.
2. - .25 %
3. - 212 lbs.p.a.

TABLE VII: THE EFFECT OF 2,4-D CONCENTRATION ON PERENNIAL RYEGRASS

Na 2,4-D Concentrations lbs.p.a.	Dry Matter Yields: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Crude Protein as % Dry Matter: % dry matter	% of control	Deviation in % from control: +	-	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	10206	100.0	0	0	4.14	100.0	0	0	2.4	422	100.0	-	-	67.5	.66
.1	9636	94.4		570	4.34	104.8	.20*		2.6	418	99.0		6	66.8	.69
.5	8760	85.8		1440*	3.97	95.8		.17*	2.5	348	82.4		74	55.6	.63
1.0	9462	92.7		744	3.95	95.4		.19*	2.3	374	88.6		48	59.8	.63
2.0	10950	107.2	744		6.65	160.6	2.51*		2.3	728	172.5	306*		116.4	1.06
4.0	10698	104.7	792		4.90	118.3	.76*		2.4	524	124.1	102*		83.8	.78
8.0	9822	96.2		384	5.15	124.3	1.01*		2.3	509	120.6	87*		81.4	.82
b) On Ladner Soil															
0	10542	100.0	0	0	5.82	100.0	0	0	2.6	613	100.0	-	-	98.0	.93
.1	10854	102.9	312		5.27	90.5		.55	2.6	572	93.3		41	91.5	.84
.5	12174	115.4	1632*		6.32	108.5	.50*		2.8	769	125.4	156*		123.0	1.01
1.0	11100	105.2	558		6.46	110.9	.64*		2.5	717	116.9	104*		114.7	1.03
2.0	11412	108.2	870*		6.59	113.2	.77*		2.7	752	122.6	139*		120.3	1.05
4.0	10776	102.2	234		6.61	113.5	.79*		2.7	712	116.1	99*		113.9	1.05
8.0	10710	101.5	168		5.88	101.0	.06		2.5	630	102.7	17		100.8	.94

M.S.D.
a)
1. - 957 lbs.p.a.
2. - .09 %
3. - 85 lbs. p.a.

b)
1. - 628 lbs. p.a.
2. - .07 %
3. - 43 lbs. p.a.

TABLE VIII: THE EFFECT OF TIME OF 2,4-D APPLICATION ON PERENNIAL RYEGRASS

Time of Application (1.0 lbs. Na 2,4-D p.a.)	Dry Matter Yields: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Crude Protein as % Dry Matter: % dry matter	% of control	Deviation in % from control: +	-	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	10206	100.0	0	0	4.14	100.0	0	0	2.4	422	100.0	-	-	67.5	.66
May 12, 1951	9462	92.7		744	3.95	95.4		.19*	2.3	374	88.6		48	59.8	.63
" 19 "	10224	100.1	18		4.10	99.0		.04	2.4	419	99.1		3	67.0	.65
" 26 "	9012	88.3		1194*	4.63	111.8	.49*		2.2	417	98.9		5	66.7	.74
June 2 "	9744	95.4		462	4.17	100.7	.03		2.3	406	96.2		16	64.9	.67
" 9 "	10140	99.0		66	3.93	94.9		.21*	2.1	398	94.3		24	63.6	.62
b) On Ladner Soil															
0	10542	100.0	0	0	5.82	100.0	0	0	2.6	613	100.0	-	-	98.0	.93
May 12, 1951	11100	105.2	558*		6.46	110.9	.64*		2.5	717	116.9	104*		114.7	1.03
" 19 "	10746	101.9	204		5.81	100.0		.01	2.7	624	101.7	11		99.9	.93
" 26 "	10752	101.9	210		5.93	101.8	.11*		2.4	638	104.0	25		102.0	.95
June 2 "	10152	96.3		390	5.91	101.5	.09*		2.4	600	97.8		13	96.0	.94
" 9 "	9768	92.6		774*	6.37	109.4	.55*		2.6	612	99.8		1	97.9	1.01

M.S.D.
a)
1. - 988 lbs. p.a.
2. - .09 %
3. - 89 lbs. p.a.

b)
1. - 458 lbs.pa.
2. - .07 %
3. - 31 lbs. p.a.

TABLE IX: THE EFFECT OF 2,4-D CONCENTRATION ON TIMOTHY

Na 2,4-D Concentrations lbs.p.a.	Dry Matter Yields: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Crude Protein as % Dry Matter: % dry matter	% of control	Deviation in % from control: +	-	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	4140	100.0	0	0	4.59	100.0	0	0	2.1	190	100.0	-	-	30.4	.73
.1	4476	108.1	336		4.34	94.5		.25*	2.1	194	102.1	4		31.0	.69
.5	4260	102.8	120		4.26	92.8		.33*	2.3	181	95.2		9	28.9	.68
1.0	4620	111.5	480		4.67	101.7	.08		2.0	215	113.1	25		34.4	.74
2.0	4518	109.1	378		4.14	90.1		.45*	2.0	187	98.4		3	29.9	.66
4.0	4320	104.3	180		4.28	93.2		.31*	2.2	185	97.3		5	29.6	.68
8.0	4596	111.0	456		4.29	93.4		.30*	2.1	197	103.6	7		31.5	.68
b) On Ladner Soil															
0	4308	100.0	0	0	4.82	100.0	0	0	2.3	207	100.0	-	-	33.1	.77
.1	4740	110.0	432		4.87	101.0	.05		2.4	231	111.5	24		36.9	.77
.5	4992	115.8	684*		7.72	160.1	2.90*		2.1	385	185.9	178		61.6	1.23
1.0	5034	116.8	726*		7.78	161.4	2.96*		2.3	391	188.8	184		62.5	1.24
2.0	4836	112.2	528*		7.23	150.0	2.41*		2.3	350	169.0	143		56.0	1.15
4.0	4644	107.3	336		7.72	160.1	2.90*		2.1	358	172.9	151		57.2	1.23
8.0	4062	94.2		246	7.63	158.2	2.81*		2.1	310	149.7	103		49.6	1.22

M.S.D.
a)
1. - 548 lbs.p.a.
2. - .07 %
3. - 37 lbs.p.a.

b)
1. - 510 lbs.p.a.
2. - .07 %
3. - 35 lbs.p.a.

TABLE X: THE EFFECT OF TIME OF 2,4-D APPLICATION ON TIMOTHY

Time of Application (1.0 lbs. Na 2,4-D p.a.)	Dry Matter Yields: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Crude Protein as % Dry Matter: % dry matter	% of control	Deviation in % from control: +	-	Invert Sugar Percent in Dry Matter	Acre Yields of Crude Protein: lbs. p.a.	% of control	Deviation in lbs. from control: +	-	Kjeldahl N lbs.p.a.	Kjeldahl N % in Dry Matter
a) On Alderwood Soil															
0	4140	100.0	0	0	4.59	100.0	0	0	2.1	190	100.0	-	-	30.4	.73
May 12, 1951	4620	111.5	480		4.67	101.7	.08		2.0	215	113.1	25		34.4	.74
" 19 "	4134	99.8		6	4.24	92.0		.35*	1.9	175	92.1		15	28.0	.68
" 26 "	4404	106.3	264		4.29	93.4		.30*	2.0	189	99.4		1	30.2	.68
June 2 "	4056	97.9		84	4.24	92.0		.35*	1.8	172	90.5		18	27.5	.68
" 9 "	3528	85.2		612*	3.90	84.9		.69*	1.9	137	72.1		53	21.9	.62
b) On Ladner Soil															
0	4308	100.0	0	0	4.82	100.0	0	0	2.3	207	100.0	-	-	33.1	.77
May 12, 1951	5034	116.8	726*		7.78	161.4	2.96*		2.3	391	188.8	184		62.5	1.24
" 19 "	4608	106.9	300		6.83	141.7	2.01*		2.2	315	152.1	108		50.4	1.09
" 26 "	4662	108.2	354		6.98	144.8	2.16*		2.0	325	157.0	118		52.0	1.11
June 2 "	4236	98.3		72	5.73	118.8	.91*		1.9	243	117.3	36		38.8	.91
" 9 "	3966	92.0		342	4.72	97.9		.10	2.2	187	90.3		20	29.9	.75

M.S.D.
a)
1. - 608 lbs.p.a.
2. - .07 %
3. - 64 lbs.p.a.

b)
1. - 533 lbs.p.a.
2. - .07 %
3. - 37 lbs.p.a.

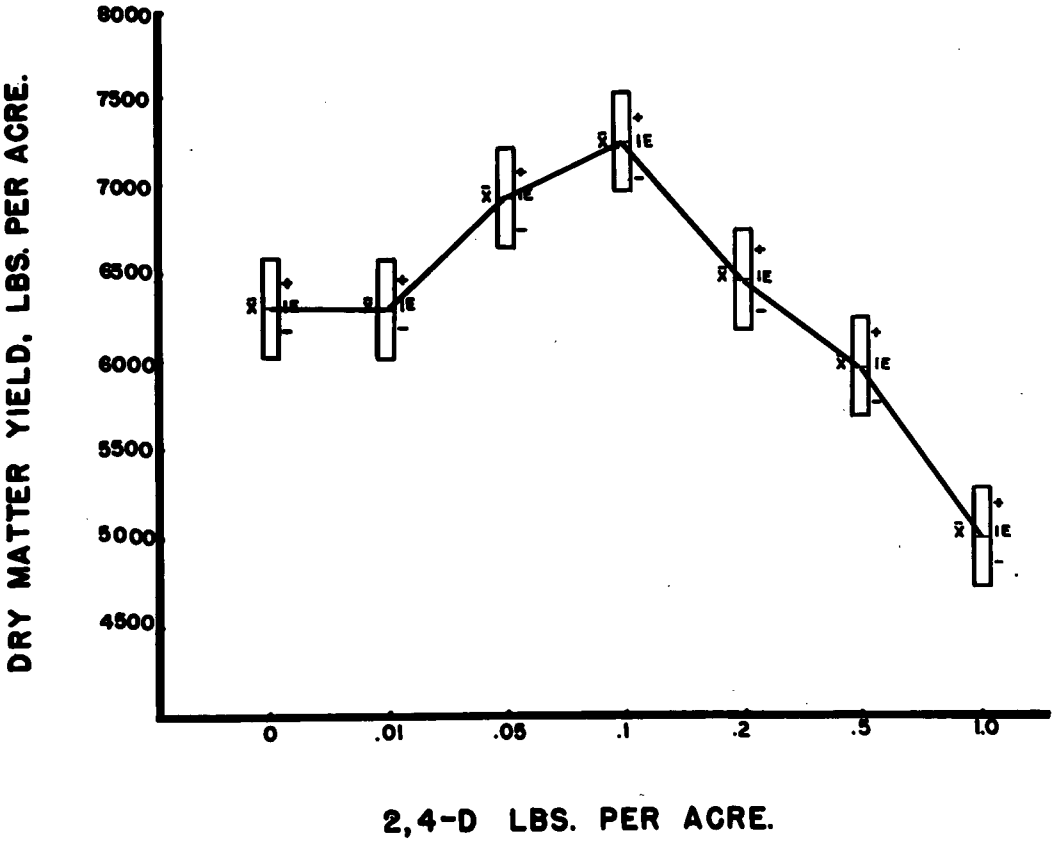


Figure 1. - Dry matter yields of red clover grown on Ladner clay and treated on May 12 with several concentrations of 2,4-D.

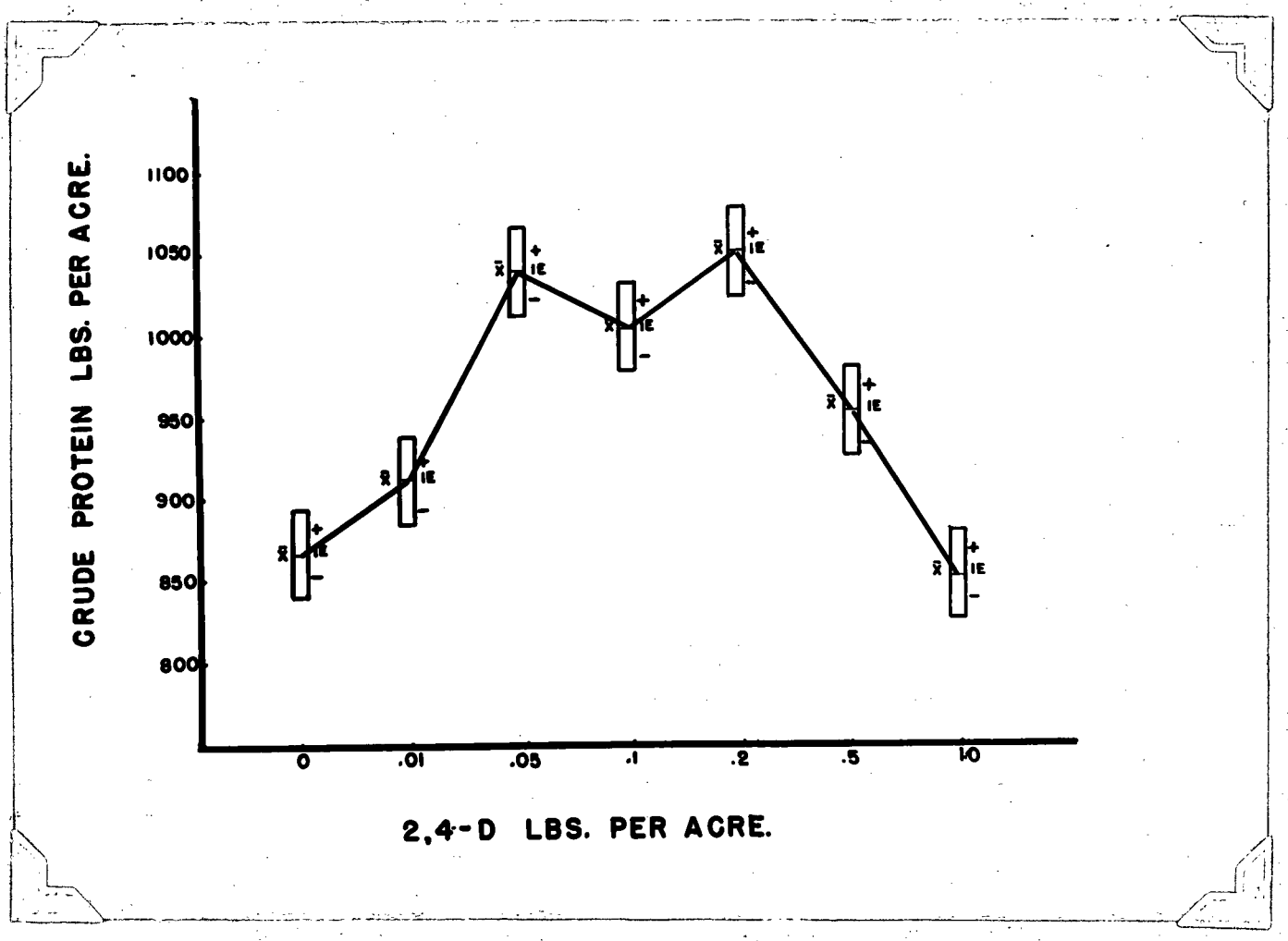


Figure 2. - Crude protein yield per acre in red clover grown on Ladner clay and treated May 12 with several concentrations of 2,4-D.

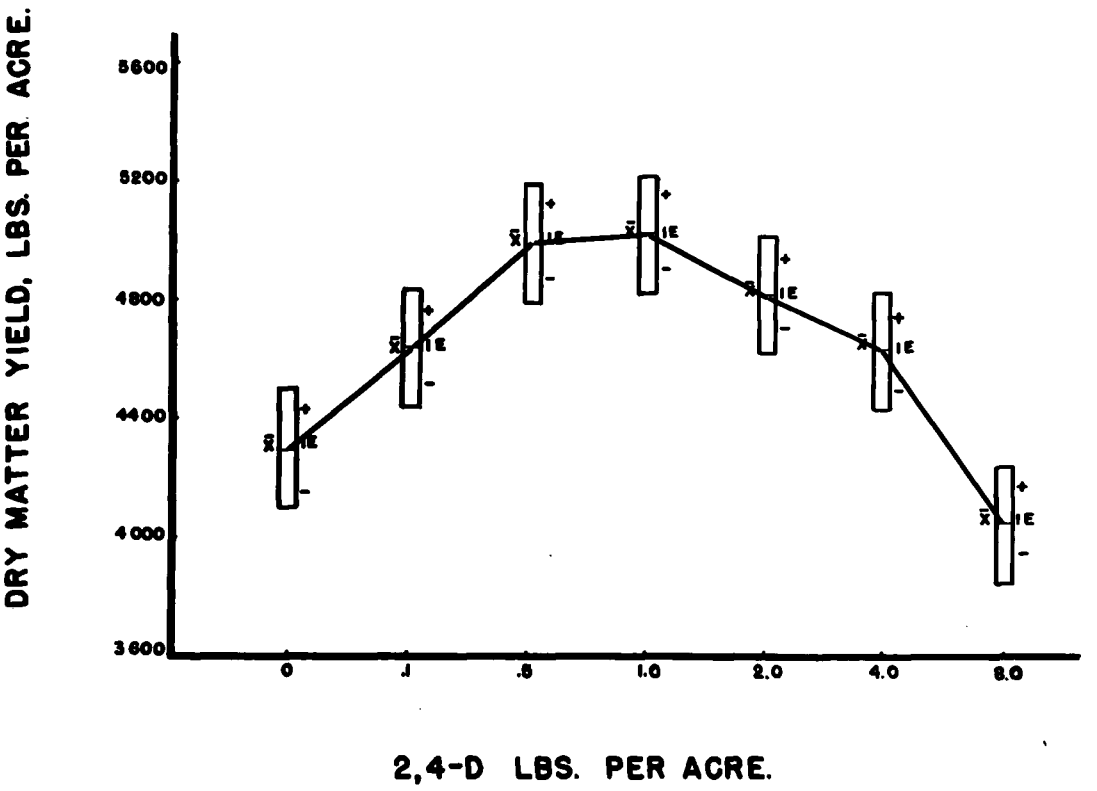


Figure 3. - Dry matter yields of timothy grass on Ladner clay and treated on May 12 with several concentrations of 2,4-D.

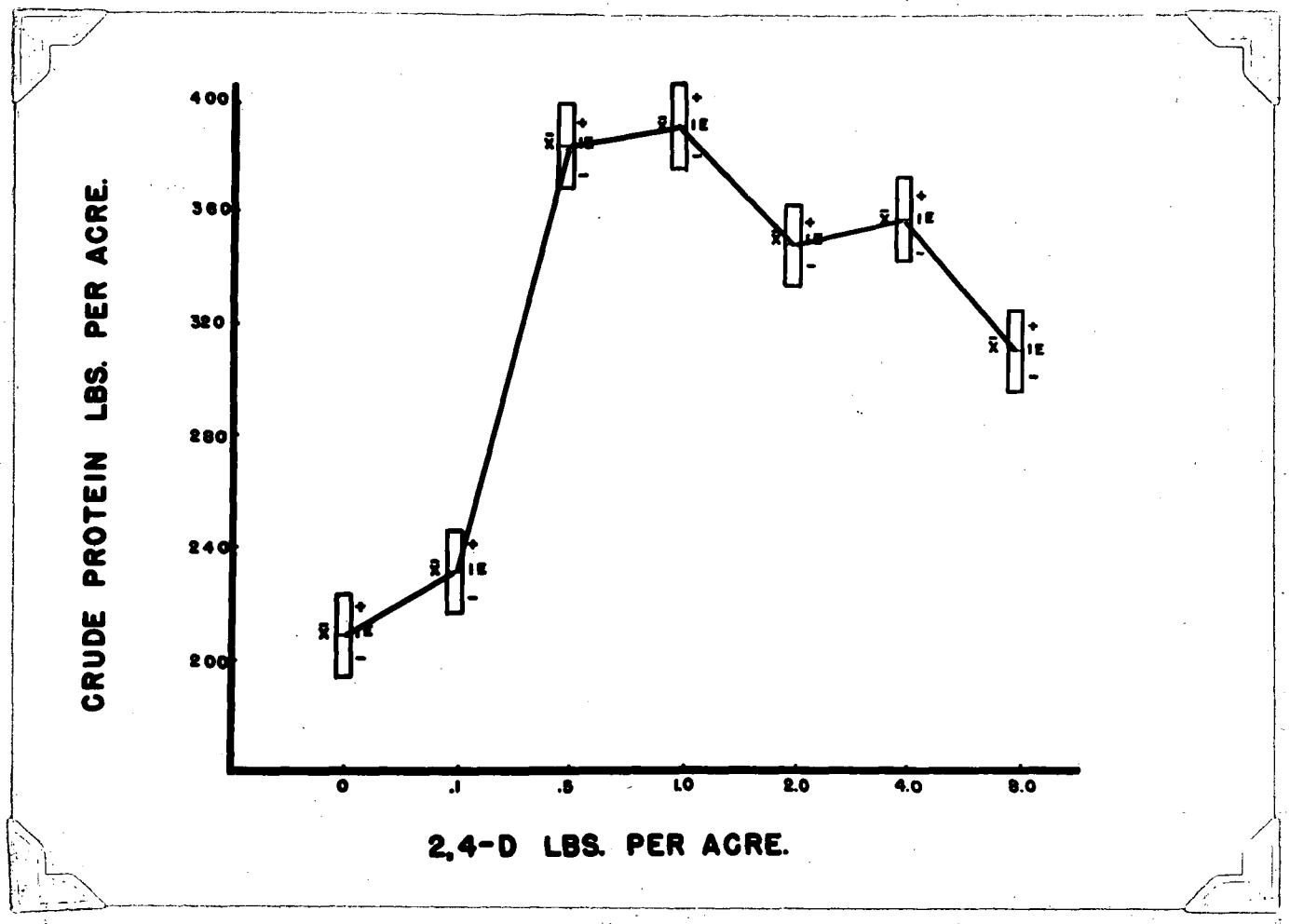


Figure 4. - Crude protein yield per acre in timothy grown on Ladner clay and treated on May 12 with several concentrations of 2,4-D.

Seed production of Red and White Clovers - vid. Table XI - seed production and quality in red clover treated with 2,4-D in different concentrations and Table XII- seed production and quality in red clover treated with .1 lb. 2,4-D at different stages of development.

Table XIII - seed production and quality in white clover treated with 2,4-D in different concentrations.

Table XIV - seed production and quality in white clover treated with .1 lb. 2,4-D at different stages of development.

The effects of 2,4-D on the seed produced in the plots of red and white clover, of earlier mention, are very similar. Seed size and weight were not materially influenced by the 2,4-D applications, but heavy concentrations and late applications reduced flowering and hence seed production. While seed size and weight, as has been noted, were not influenced, significant changes in hard seed percentage were recorded: in red clover the hard seed percentage was consistently increased but in white clover it was sometimes decreased. Again, however, with late applications the effects were not pronounced.

Seed production of the grasses - vid. Tables XV, XVI, XVII, XVIII, XIX and XX - the effects of concentration and time application of 2,4-D on seed from the Kentucky Bluegrass, perennial ryegrass and timothy plots.

In general, the seed quality of the grasses was not materially influenced by the applications of 2,4-D. There are, probably, a series of subtle interactions here which our data do not reveal - primarily for the reason that seed yield per plot was not taken. It is probable that, where seed set is light, ~~then~~ seeds which are produced are plump and viable and where seed set is high single, seed weights and viability may not be much different from those where seed set is low. In timothy, seed production effects were not noticeable, although timothy yield and protein modification was most noticeable. Again, early, light and heavy applications increased seed set; later applications did not, even when "heavy", produced changes

TABLE XI

SEED PRODUCTION AND QUALITY IN RED CLOVER TREATED WITH Na 2,4-D IN DIFFERENT CONCENTRATIONS

Na 2,4-D Concentration lbs.p.a.	On Alderwood Soil						On Ladner Soil			
	Germination		1000 Seeds Weight		Seeds from 50 Heads gms.	Estim. Flower Intensity 1-10	Germination		1000 Seeds Weight	
	Sprouted %	Hard Seed %	gms.	%			Sprouted %	Hard Seed %	gms.	%
0	72	25	1.560	100.0	4.372	10	73	23	1.490	100.0
.01	54	39	1.380	88.4	3.200	10	61	33	1.330	89.2
.05	46	44	1.085	69.5	3.162	10	53	40	1.140	76.5
.1	36	55	1.400	89.7	3.891	9	35	58	1.420	95.3
.2	35	55	1.425	91.3	4.201	10	38	51	1.330	89.2
.5	48	46	1.430	91.6	3.488	8	43	50	1.455	97.6
1.0	50	46	1.325	84.9	2.806	6	52	42	1.560	104.6

TABLE XII

SEED PRODUCTION AND QUALITY IN RED CLOVER TREATED AT DIFFERENT STAGES OF DEVELOPMENT WITH .1 LB. Na 2,4-D p.a.

Time of Application	On Alderwood Soil						On Ladner Soil			
	Germination		1000 Seeds Weight		Seeds from 50 Heads gms.	Estim. Flower Intensity 1-10	Germination		1000 Seeds Weight	
	Sprouted %	Hard Seed %	gms.	%			Sprouted %	Hard Seed %	gms.	%
0	72	25	1.560	100.0	4.372	10	73	23	1.490	100.0
May 12, 1951	36	55	1.400	89.7	3.891	9	35	58	1.420	95.3
" 19 "	52	39	1.335	85.5	2.259	9	47	43	1.295	86.9
" 26 "	58	37	1.590	101.9	2.579	8	75	23	1.590	106.7
June 2 "	54	39	1.685	108.0	2.716	6	58	36	1.630	109.3
" 9 "	62	34	1.945	124.6	2.899	5	57	38	1.915	128.5

TABLE XIII

SEED PRODUCTION AND QUALITY IN WHITE CLOVER TREATED WITH Na 2,4-D IN DIFFERENT CONCENTRATIONS

Na 2,4-D Concentration lbs.p.a.	On Alderwood Soil						On Ladner Soil			
	Germination		1000 Seeds Weight		Seeds from 50 Heads gms.	Estim. Flower Intensity 1-10	Germination		1000 Seeds Weight	
	Sprouted %	Hard Seed %	gms.	%			Sprouted %	Hard Seed %	gms.	%
0	25	70	.665	100.0	1.206	10	32	64	.685	100.0
.01	36	56	.720	108.2	.736	10	33	58	.670	97.8
.05	46	45	.670	100.7	.801	10	42	46	.640	93.4
.1	36	54	.625	93.9	.846	10	37	54	.565	82.4
.2	32	62	.660	99.2	1.139	10	39	57	.620	90.5
.5	29	64	.640	96.2	.970	9	17	77	.665	97.0
1.0	30	63	.610	91.7	.634	7	14	80	.620	90.5

TABLE XIV

SEED PRODUCTION AND QUALITY IN WHITE CLOVER TREATED AT DIFFERENT STAGES OF DEVELOPMENT WITH .1 LB. Na 2,4-D P.A.

Time of Application	On Alderwood Soil						On Ladner Soil			
	Germination		1000 Seeds Weight		Seeds from 50 Heads gms.	Estim. Flower Intensity 1-10	Germination		1000 Seeds Weight	
	Sprouted %	Hard Seed %	gms.	%			Sprouted %	Hard Seed %	gms.	%
0	25	70	.665	100.0	1.206	10	32	64	.685	100.0
May 12, 1951	36	54	.625	93.9	.846	10	37	54	.565	82.4
" 19 "	38	58	.705	106.0	.950	10	32	62	.650	94.8
" 26 "	39	54	.690	103.7	1.203	10	44	49	.645	98.5
June 2 "	42	51	.685	103.0	.903	7	37	55	.700	102.1
" 9 "	33	60	.660	99.2	1.260	6	33	61	.680	99.2

TABLE XV

SEED PRODUCTION AND QUALITY IN KENTUCKY BLUEGRASS TREATED WITH Na 2,4-D IN DIFFERENT CONCENTRATION

Na 2,4-D Concentration lbs.p.a.	On Alderwood Soil				On Ladner Soil		
	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %	Seeds from 50 Panicle gms.	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %
0	87.5	.365	100.0	1.609	85	.415	100.0
.1	82	.450	123.2	2.218	82	.410	98.7
.5	85.5	.385	105.4	2.570	83	.410	98.7
1.0	84.5	.435	119.1	1.763	86.5	.430	103.6
2.0	82	.425	116.4	1.814	81.5	.405	97.5
4.0	84	.450	123.2	1.801	85.5	.490	118.0
8.0	82.5	.440	120.5	2.345	85	.420	101.2

TABLE XVI

SEED PRODUCTION AND QUALITY IN KENTUCKY BLUEGRASS TREATED AT DIFFERENT STAGES OF DEVELOPMENT WITH 1.0 LBS. Na 2,4-D P.A.

Na 2,4-D Concentration lbs.p.a.	On Alderwood Soil				On Ladner Soil		
	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %	Seeds from 50 Panicle gms.	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %
0	87.5	.365	100.0	1.609	85	.415	100.0
May 12, 1951	84.5	.435	119.1	1.763	86.5	.430	103.6
" 19 "	83	.420	115.0	1.920	84	.410	98.7
" 26 "	87.5	.340	93.1	2.006	85.5	.385	92.7
June 2 "	82.5	.375	102.7	1.521	83	.380	91.5
" 9 "	80	.385	105.4	1.684	81	.405	97.5

TABLE XVII

SEED PRODUCTION AND QUALITY IN PERENNIAL RYEGRASS TREATED WITH Na 2,4-D IN DIFFERENT CONCENTRATIONS

Na 2,4-D Concentration lbs.p.a.	On Alderwood Soil				On Ladner Soil		
	Germination %	1000 Seeds Weights gms.	%	Seeds from 50 Head (panicle) gms.	Germination %	1000 Seeds Weight gms.	%
0	92	2.070	100.0	3.664	89.5	2.160	100.0
.1	87.5	2.150	103.8	6.431	91	2.120	98.1
.5	88.5	1.960	94.6	5.584	95.5	1.820	84.2
1.0	88.5	1.930	93.2	5.853	97.5	2.220	102.7
2.0	100	2.110	101.9	4.742	89.5	2.000	92.5
4.0	90	2.210	106.7	3.923	93	2.280	105.5
8.0	92.5	2.200	106.2	4.199	96.5	2.280	105.5

TABLE XVIII

SEED PRODUCTION AND QUALITY IN PERENNIAL RYEGRASS TREATED AT DIFFERENT STAGES OF DEVELOPMENT WITH 1.0 LBS. Na 2,4-D P.A.

Time of Application	On Alderwood Soil				On Ladner Soil		
	Germination %	1000 Seeds Weight gms.	%	Seeds from 50 Head (panicle) gms.	Germination %	1000 Seeds Weight gms.	%
0	92	2.070	100.0	3.664	89.5	2.160	100.0
May 12, 1951	88.5	1.930	93.2	5.853	97.5	2.220	102.7
" 19 "	89.5	2.070	100.0	3.288	89	2.210	102.3
" 26 "	89.5	1.800	86.9	3.980	100	1.810	83.7
June 2 "	100	1.890	91.3	2.432	93	1.930	89.3
" 9 "	92	1.940	95.1	2.921	90	1.920	88.8

TABLE XIX

SEED PRODUCTION AND QUALITY IN TIMOTHY
TREATED WITH Na 2,4-D IN DIFFERENT CONCENTRATION

Na 2,4-D Concentration lbs.p.a.	On Alderwood Soil				On Ladner Soil		
	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %	Seeds from 50 Heads gms.	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %
0	100.0	.515	100.0	6.191	100.0	.505	100.0
.1	98.5	.475	92.2	5.564	98	.465	92.0
.5	97.5	.440	85.4	7.289	99	.450	89.2
1.00	98	.415	80.5	8.198	99	.415	82.1
2.0	98.5	.415	80.5	8.476	98.5	.420	83.1
4.0	98.5	.445	86.4	7.596	99.5	.465	92.0
8.0	99.5	.460	89.3	8.800	99.5	.445	88.1

TABLE XX

SEED PRODUCTION AND QUALITY IN TIMOTHY
TREATED AT DIFFERENT STAGES OF DEVELOPMENT WITH
1.0 LBS. Na 2,4-D p.a.

Time of Application	On Alderwood Soil				On Ladner Soil		
	Germination %	1000 Seeds Weight gms.	1000 Seeds Weight %	Seeds from 50 Heads gms.	Germination	1000 Seeds Weight gms.	1000 Seeds Weight %
0	100.0	.515	100.0	6.191	100.0	.505	100.0
May 12,1951	98	.415	80.5	8.198	99	.415	82.1
" 19 "	98.5	.425	82.5	6.882	98	.405	80.1
" 26 "	100	.440	85.4	7.241	98	.395	78.2
June 2 "	98.5	.380	73.7	5.175	98	.390	77.2
" 9 "	98.5	.365	70.8	5.578	99	.380	75.2

IV. GREENHOUSE TRIALS

Objects: The purpose of this series of experiments, conducted in the greenhouse, were twofold. First of all, it was thought that an experiment should be set out, under the more uniform conditions under glass, which would serve as a check on the field trials. Also, since nitrogen uptake appeared to be an important factor in the field trials, it seemed that a study should be made of the effects of 2,4-D sprayed plants, on the free-living, nitrogen-fixing, soil bacteria such as Azotobacter vinelandii and A. chrococcum and symbiotic nitrogen-fixing bacteria such as the Rhizobia of the legumes.

Materials: One legume, red clover, and one cereal grass, barley var. Olli, were chosen as the test plants. Cultures of Azotobacter vinelandii and A. chrococcum were obtained from the U.B.C. Soil Bacteriology laboratory stocks. Alderwood soil came from the U.B.C. area, the muck soil from a half-bog in West Point Grey, the Ladner clay from the Fraser River alluvium at the foot of Blenheim Street in Vancouver and the Interior pedocal from the Dominion Range Station lands at Kamloops, B. C.

Methods: The Alderwood soil, pH 5.6, the muck soil, pH 4.2, and the Ladner soil, pH 4.8, some weeks before seeding were given an application of superphosphate, 50 mgs. per lb. of soil. The pH of all soil seeded to legumes was adjusted to Ca pH 6.0 by adding 175 mgs.

agricultural lime per lb. to Alderwood soil, 2.0 gms. per lb. to muck soil, and 700 mgs. per lb. to Ladner clay. Prior to seeding, legume seeds and soils were inoculated with the Rhizobium-containing "Nitragin". Somewhat more agricultural lime was added to the soils seeded to Olli barley; viz: Alderwood, 200 mgs. per lb., muck, 2.6 gms. per lb., and Ladner clay, 800 mgs. per lb. The lime was added on June 26th and on the date of planting, July 17th, the pH readings on the soil were as follows: Alderwood, 6.5, Ladner clay, 6.3, muck, 6.3 and Interior pedo§al, 6.3. Prior to planting the barley, one-half of the seeds were soaked for 24 hours in an Azotobacter vinelandii and A. chrococcum suspension made from the surface growth of cultures in Ashby's mannitol agar. Also 5 c.c. (20 million cells per c.c.) of the Azotobacter suspension were added to one-half of the pots of soil in which Olli barley was seeded.

In the red clover experiment, each treatment was accorded three pots, and 25 seeds were planted in each pot, but after the seedlings were well established they were thinned to 5 plants per pot. 2,4-D ranges were as follows: 0.0 lb., .01 lb., and .1 .b. per acre and applications were made by aqueous spray on July 23, 1952 when the plants were still strictly vegetative - 4 true leaves on the plants grown on Ladner and muck soils and 3 true leaves on the plants grown on Alderwood soil. Cuttings were made at 4

different times: the first was accomplished on August 7th, while the plants were still vegetative, the second on September 6th, just before the flowering of the plants on Alderwood soil and early flower for the plants on the Ladner and muck soils. Plants whose first cut was taken August 6th were again cut on October 6th when Alderwood-grown plants were vegetative, and Ladner and muck soil grown plants were pre-bud. The last cutting was made on plants which were in milk seed stage on Alderwood, and on plants which were mature on Ladner and muck soils. In the greenhouse, clover sprayed with only .1 lb. per acre of 2,4-D showed marked narrowing of leaves while in the field trials the morphological response was only noted after much heavier applications. Roots of all harvested plants were marked carefully and their nodules studied; they were classified as to white, green and pink and their lengths in m.m. recorded.

In the barley experiment the 2,4-D was applied with an atomizer on July 23, 1952 when the barley on all soils was in the 3rd leaf stage. The concentrations of 2,4-D used were 0, .1 and 1.0 lbs. per acre. Half of the barley was harvested at pre-boot stage, 15 days after spraying, and the other half at maturity, 57 days after spraying. Four pots were assigned to each treatment and 20 seeds were planted per pot. 10 seedlings only, however, were permitted to develop. One-half of the pots in each

treatment were taken for the early cutting treatment, the other half were allowed to develop directly to maturity.

a) Red Clover

Vid. Table XXI and Table XXIA. - Nodulation, crude protein percentage, dry matter, and total nitrogen, in red clover grown in the greenhouse, in 3 soils, treated with two concentrations of 2,4-D, and harvested at 4 different stages of development.

Few significant percentage protein changes were recorded. Depression of protein percentage occurred in plants from Alderwood and Ladner soils, but increases occurred in plants grown on the muck soils. However, the percentage composition table scarcely portrays the true picture, for the maturation process was altered by the 2,4-D, as well as the nitrogen uptake and total dry-matter yield. Accordingly, a somewhat better idea of the interactions isobtained by an examination of the photos, which show the marked differences in blooming time of the red clover under treatment, and of the Table XXIA which shows the dry matter elaboration and total nitrogen uptake. When these are appraised it is noted that again, the 2,4-D early applications frequently result in greater nitrogen uptake and greater yields.

No differences in nodulation were observed; the size, number and kind of nodules were much the same under all treatments. The 2,4-D concentrations of this experiment was not high enough, apparently, to interfere with nodule formation.

TABLE XXI
NODULATION AND CRUDE PROTEIN PRODUCTION IN RED CLOVER GROWN IN THE GREENHOUSE, ON THREE SOIL TYPES TREATED WITH TWO CONCENTRATIONS OF Na 2,4-D AND HARVESTED AT FOUR DIFFERENT STAGES OF DEVELOPMENT

Na 2,4-D Concentration lbs.p.a.	Alderwood Soil						Unclassified				Muck Soil		Ladner Soil					
	Crude Protein				Nodules		Crude Protein				Nodules		Crude Protein				Nodules	
	% of Dry Matter	% of Control	Deviation from Control +	Deviation from Control -	Number per Plant	Length Range in m.m.	% of Dry Matter	% of Control	Deviation from Control +	Deviation from Control -	Number per Plant	Length Range in m.m.	% of Dry Matter	% of Control	Deviation from Control +	Deviation from Control -	Number per Plant	Length Range in m.m.
	Harvested 15 Days										After Spraying							
0	23.80	100.0	0	0	3.1	¼-1½	23.86	100.0	0	0	78.9	¼-4	24.22	100.0	0	0	16.6	¼-2½
.01	24.93	104.7	1.13*		4.2	¼-2½	23.43	98.1		.43	100.7	¼-3	22.33	92.1		1.89*	11.9	¼-2
.1	23.94	100.5	.14		2.4	¼-1	23.06	96.6		.80	52.8	¼-2½	23.85	98.4		.37	6.9	¼-2
	Harvested 45 Days										After Spraying							
0	19.64	100.0	0	0	43.2	¼-5	16.21	100.0	0	0	69.7	¼-6	16.14	100.0	0	0	38.1	¼-5
.01	18.83	95.8		.81	36.3	¼-6	17.50	107.9	1.29*		56.7	¼-6	14.93	92.5		1.21*	29.3	¼-7
.1	20.85	106.1	1.21*		34.2	¼-5	18.10	111.6	1.89*		61.1	¼-6	14.49	89.7		1.65*	29.8	¼-7
	Harvested 75 Days										After Spraying							
0	19.82	100.0	0	0	42.0	¼-4	16.27	100.0	0	0	60.6	¼-5	15.81	100.0	0	0	63.9	¼-2
.01	17.48	88.1		2.34*	23.0	¼-5	20.05	123.2	3.78*		109.2	¼-5	15.70	99.3		.11	63.0	¼-5
.1	17.34	87.4		2.48*	34.3	¼-5	18.29	112.4	2.02*		83.0	¼-6	16.22	102.5	.41		75.0	¼-4
	Harvested 70 Days										(Second Cut) After Spraying							
0	18.75	100.0	0	0	36.2	¼-5	21.55	100.0	0	0	65.2	¼-5	18.56	100.0	0	0	60.2	¼-4
.01	17.69	94.3		1.06*	35.4	¼-5	22.55	104.6	1.00		84.0	¼-5	19.28	103.8	.72		62.8	¼-5
.1	19.56	104.3	.81		35.1	¼-5	21.64	100.4	.09		92.1	¼-5	19.22	103.5	.66		67.1	¼-5

M.S.D. (5% point) 1.0397%

TABLE XXIA.

Production of Red Clover Grown in the Greenhouse, on Three Soil Types, Treated with Two Concentrations of Na 2,4-D, and Harvested at Four Different Stages of Development.

2,4-D p.a.	Alderwood Soil		Unclassified Muck Soil		Ladner Soil	
	Dry Matter per 5 plants gms.	Crude Protein per 5 plants gms.	Dry Matter per 5 plants gms.	Crude Protein per 5 plants gms.	Dry Matter per 5 plants gms.	Crude Protein per 5 plants gms.
Harvested 15 Days After Spraying						
0	.794	.188	2.719	.647	2.257	.546
.01	.678	.169	3.014	.706	2.316	.517
.1	.666	.159	2.208	.509	1.380	.329
Harvested 45 Days After Spraying						
0	2.583	.507	8.115	1.315	8.610	1.389
.01	3.475	.654	9.219	1.613	8.415	1.256
.1	1.501	.312	9.102	1.647	8.420	1.220
Harvested 75 Days After Spraying						
0	2.736	.542	8.234	1.339	11.500	1.818
.01	3.731	.652	9.475	1.899	12.506	1.963
.1	2.770	.480	9.925	1.815	12.811	2.077
Harvested 70 Days (Second Cut) After Spraying						
0	2.454	.460	2.245	.483	3.405	.631
.01	2.339	.413	2.301	.518	4.082	.787
.1	2.356	.460	2.710	.586	3.462	.665

M. S. D. (5% point)

Dry Matter - .3109 gms.

Crude Protein .3232 gms.

Figure 5. - Red Clover grown in the greenhouse in pots of Alderwood gravelly loam and treated at the third true leaf stage as follows: 1. control, no 2,4-D, 2. .01 lb. 2,4-D per acre 3. .1 lb. 2,4-D per acre. Photo taken four weeks after treatment.

Figure 6. - Red clover grown in the greenhouse in pots of Ladner clay and treated at fourth true leaf stage as follows: 1. control, no 2,4-D, 2. .01 lb. 2,4-D per acre, 3. .1 lb. 2,4-D per acre. Photo taken four weeks after treatment.

b) Olli Barley

Vid. Table XXII: Yield of dry matter in Olli barley, grown in the greenhouse, in four soils, with and without Azotobacter vinelandii and A. chrococcum, with and without 2,4-D.

Vid. Table XXIIA - yield of crude protein in Olli barley, grown in the greenhouse, in four soils, with and without Azotobacter vinelandii and A. chrococcum, with and without 2,4-D.

Vid. Table XXIII - as for Table XXIIA preceding but the expression is in terms of percentage of protein.

To the eye, qualitatively appraising this greenhouse experiment, the results were striking. Much of the error variation of the field trials was avoided and the responses showed a welcome uniformity.

The quantitative appraisal, as recorded in the tables mentioned above, is also striking. Not only are the variables of 2,4-D levels and of Azotobacter inoculation interesting per se, but also their interactions with the different soils.

Azotobacter inoculation of the Alderwood soil increased the yield of the barley grown on the soil promptly after inoculation (pre boot stage). On all soils the influence of the inoculation was generally observed, in the increased yield of seed and of haulm, and in larger, taller plants.

2,4-D effects, *per se*, were most pronounced on the Alderwood soil. Apparently, as far as influence on yield is concerned, 2,4-D was somewhat more effective on the plants of coastal soils than was inoculation of soil with *Azotobacter*. *Azotobacter* and 2,4-D in combination did not give an additive response, or if so, not an appreciable one.

Curiously, in an Interior soil, well populated with *Azotobacter*, 2,4-D depressed yield.

Expressed as percentage composition protein, the effects of 2,4-D and *Azotobacter* are not striking. Some percentage increases are to be noted, especially on the muck soil and on the Ladner clay. However, far more revealing, are the effects expressed in terms of the nitrogen taken up. Quite generally with both treatment series alone and in combination, increased nitrogen uptake by the barley is noted. It is observed additionally that in the Interior alluvium, barley treated with 1.0 pounds 2,4-D per acre took up less nitrogen than the control.

Azotobacter populations - vid. Table XXIV: estimated Azotobacter per gram of dry soil following 2,4-D treatments of plants grown in the soil.

In an attempt to record the changes on the soil flora following 2,4-D application to plants, the populations of the free-living, nitrogen-fixing, *Azotobacter* were followed. The record is somewhat inconclusive for all but the Alderwood soil. Here the populations increased rapidly following 2,4-D

application until at 15 days after treatment, there were ca. 7x as many organisms in the soil supporting 2,4-D treated plants, as in the control soil. 72 days after spraying, the population differences in the soils were negligible. Possibly because of the infrequent sampling, but possibly for other inherent reasons, appreciable differences in Azotobacter populations were not recorded for the other soils

TABLE XXII
YIELD OF DRY MATTER IN OLLI BARLEY,
GROWN IN THE GREENHOUSE IN FOUR SOIL TYPES,
WITH AND WITHOUT AZOTOBACTER VINELANDII AND A. CHROCOCCUM,
WITH AND WITHOUT 2,4-D

Treatment		Pre Boot Stage	Mature 57 Days After Spraying		Length of Halm
Azotobacter	2,4-D lbs.p.a.	15 Days After Spraying gms.	Seed gms.	Halm Only gms.	57 Days After Spraying cm.
ALDERWOOD GRAVELLY LOAM (COASTAL)					
Without	0	2.111	1.967	2.320	35.0
"	.1	2.334	6.118*	4.298*	47.7*
"	1.0	2.456*	7.051*	4.486*	51.0*
With	0	4.289*	5.590*	3.780*	45.0*
"	.1	4.875*	6.130*	4.294*	48.7*
"	1.0	4.779*	5.401*	4.402*	51.5*
UNCLASSIFIED MUCK (COASTAL)					
Without	0	4.582	1.630	2.580	34.7
"	.1	4.784	5.518*	3.883*	53.0*
"	1.0	4.369	6.139*	3.841*	57.0*
With	0	4.991*	4.634*	3.726*	51.5*
"	.1	5.709*	5.737*	3.946*	55.7*
"	1.0	4.525	6.707*	3.781*	53.0*
LADNER CLAY (COASTAL)					
Without	0	4.090	6.369	4.100	52.2
"	.1	5.104*	7.637*	5.056*	57.7
"	1.0	5.854*	8.091*	5.636*	63.7*
With	0	4.251	6.521*	4.178	54.5
"	.1	4.247	6.711*	4.222	48.7
"	1.0	5.951*	7.513*	4.922*	54.7
SILTY PEDOCAL (ALLUVIUM FROM THE "DRY INTERIOR")					
Az. occurring naturally	0	6.784	6.896	5.762	66.7
	1.0	5.988*	4.687*	4.677*	59.5*

M.S.D. % Dry Matter (5% point)

a) Pre Boot Stage .3141 gms.
b) Seed .2364 "
c) Halm only .2627 "
d) Length (Halm) 5.9165 cm.

TABLE XXIIA

Yield of Crude Protein in Olli Barley Grown in the Greenhouse in Four Soil Types, with and without Azotobacter Vinelandii and A. Chrococcum, with and without 2,4-D

Treatment		Preboot Stage 15 days after spraying.	Mature 57 days after spraying	
Azotobacter	2,4-D lbs: p.a.	gms.	Seed gms.	Halm only gms.
Alderwood Gravelly Loam (Coastal)				
Without	0	.302	.148	.064
"	.1	.339	.458*	.121*
"	1.0	.299	.530*	.134*
With	0	.613*	.523*	.106
"	.1	.649*	.522*	.115*
"	1.0	.679*	.463*	.122*
Unclassified Muck (Coastal)				
Without	0	.397	.170	.064
"	.1	.453	.518*	.097
"	1.0	.411	.523*	.091
With	0	.469	.473*	.086
"	.1	.534*	.535*	.085
"	1.0	.434	.751*	.094
Ladner Clay (Coastal)				
Without	0	.339	.575	.101
"	.1	.416	.719*	.122
"	1.0	.567*	.676*	.137
With	0	.448	.628*	.104
"	.1	.434	.639*	.101
"	1.0	.671*	.691*	.155*
Silty Pedocal (Alluvium from the "Dry Interior")				
Az. Occurring Naturally	0	.765	.628	.202
	1.0	.674	.462*	.203

M.S.D. (5% point)

A.) Crude Protein Preboot Stage .112 gms.

B.) " " Seed .043 "

C.) " " Halm Only .038 "

TABLE XXIII

CRUDE PROTEIN IN OLLI BARLEY,
GROWN IN THE GREENHOUSE IN FOUR SOIL TYPES,
WITH AND WITHOUT *AZOTOBACTER VINELANDII* AND *A. CHROCOCCUM*,
WITH AND WITHOUT 2,4-D

Treatments		CRUDE PROTEIN								
		Leaf (Pre Boot) 15 Days after Spraying			Seed 57 Days after Spraying			Halm Only 57 Days after Spraying		
Azotobacter	2,4-D lbs.p.a.	% of dry matter	% of control	Deviation from control	% of dry matter	% of control	Deviation from control	% of dry matter	% of control	Deviation from control
ALDERWOOD GRAVELLY LOAM (COASTAL)										
Without	0	14.34	100.0	± 0	7.56	100.0	± 0	2.77	100.0	± 0
"	.1	14.55	101.4	+ .21	7.50	99.2	- .06	2.83	102.1	+ .06
"	1.0	12.17	84.8	-2.17*	7.53	99.6	- .03	3.00	108.3	+ .23*
With	0	14.31	99.7	- .03	9.36	123.8	+1.80*	2.83	102.1	+ .06
"	.1	13.33	92.9	-1.01*	8.52	112.6	+ .96*	2.70	97.4	- .07
"	1.0	14.21	99.0	- .13	8.58	113.4	+1.02*	2.78	100.3	+ .01
UNCLASSIFIED MUCK (COASTAL)										
Without	0	8.67	100.0	± 0	10.43	100.0	± 0	2.50	100.0	± 0
"	.1	9.48	109.3	+ .81*	9.40	90.1	-1.03*	2.52	100.8	+ .02
"	1.0	9.41	108.5	+ .74*	8.53	81.7	-1.90*	2.38	95.2	- .12
With	0	9.41	108.5	+ .74*	10.22	97.9	- .21⊛	2.33	93.2	- .17⊛
"	.1	9.36	107.9	+ .69*	9.34	89.5	-1.09*	2.17	86.8	- .33*
"	1.0	9.60	110.7	+ .93*	11.21	107.4	+ .78*	2.51	100.4	+ .01
LADNER CLAY (COASTAL)										
Without	0	8.31	100.0	± 0	9.04	100.0	± 0	2.48	100.0	± 0
"	.1	8.17	98.3	- .14	9.42	104.2	+ .38*	2.42	97.5	- .06
"	1.0	9.69	116.6	+1.38*	8.36	92.4	- .68*	2.44	98.3	- .04
With	0	10.56	127.0	+2.25*	9.64	106.6	+ .60*	2.51	101.2	+ .03
"	.1	10.22	122.9	+1.91*	9.53	105.4	+ .49*	2.40	96.7	- .08
"	1.0	11.28	135.7	+2.95*	9.21	101.8	+ .17⊛	3.15	127.0	+ .67*
SILTY PEDOCAL (ALLUVIUM FROM THE "DRY INTERIOR")										
Az. occurring	0	11.29	100.0	± 0	9.11	100.0	± 0	3.51	100.0	± 0
naturally	1.0	11.27	99.8	- .02	9.87	108.3	+ .76*	4.36	124.7	+ .85*

M.S.D. % Dry Matter (5% point)

a) Crude protein leaf .3593
b) " " seed .1823
c) " " halm .1483

TABLE XXIV

ESTIMATED *AZOTOBACTER* PER GRAM OF DRY SOIL FOLLOWING 2-4-D TREATMENTS OF PLANTS GROWN ON THE SOIL

TREATMENT Na-2-4-D lbs. p.a.	Number of *Azotobacter chroococcum* + *Azotobacter vinelandii* per Gram Soil in Millions					
	15 Days After Spraying				72 Days After Spraying	
	Dilutions 1:100,000		Dilutions 1:1,000,000		Dilutions 1:100,000	
	Number	Difference	Number	Difference	Number	Difference
Alderwood Gravelly Loam (Coastal)						
0	3.3	± 0	2.7	± 0	2.7	± 0
.1	21.7	+ 18.3*	18.3	+ 15.6*	2.3	- .4
1.0	12.9	+ 9.5*	14.0	+ 11.3*	3.3	+ .6
Unclassified Muck (Coastal)						
0	4.2	± 0	3.7	± 0	1.3	± 0
.1	3.5	- .67	3.7	± 0	2.0	+ .7
1.0	1.1	- 3.07*	1.7	- 2.0*	1.3	± 0
Ladner Clay (Coastal)						
0	8.8	± 0	9.3	± 0	2.3	± 0
.1	7.6	- 1.2	9.0	- .3	4.3	+ 2.0*
1.0	9.7	+ .9	9.0	- .3	2.3	± 0
Silty Pedocal (Alluvium from the "Dry Interior")						
0	.8	± 0	1.0	± 0	4.3	± 0
1.0	1.97	+ 1.14	1.7	+ .7	3.3	- 1.0

M.S.D. (Number of *Azotobacter* in millions per gram of soil)

(a) 15 days; dil. 1:100,000 1.6

(b) 15 days; dil. 1:1,000,000 2.3

(c) 72 days; dil. 1:100,000 2.0

Figure 7. - Olli barley grown on Alderwood gravelly loam in the greenhouse. Treatments as follows: 1. Control, no 2,4-D, no soil inoculation with Azotobacter. 2. Azotobacter inoculation of soil prior to planting. 3. 2,4-D applied at .1 lb. per acre at third leaf stage, and soil inoculated with Azotobacter prior to planting. Photo taken 5 weeks after 2,4-D treatment. Note immaturity and smaller size of the control. Note the uniformity of 2 and lack of it in 3.

Figure 8. - Olli barley grown in the greenhouse in muck soil. Treatments as follows: 1. Control, no 2,4-D and no soil inoculation. 2. Soil inoculated prior to seeding with Azotobacter and, 3. soil inoculated prior to seeding with Azotobacter and plants treated at third leaf with .1 lb. 2,4-D per acre. Photo taken 5 weeks after 2,4-D treatment. Note immaturity and smaller size of the control plants.

Figure 9. - Olli barley grown on Ladner soil in the greenhouse. Treatments as follows: 1. Control, no soil inoculation and no 2,4-D. 2. Azotobacter inoculation prior to seeding and, 3. Azotobacter inoculation prior to seeding and 2,4-D at .1 lb. per acre at third leaf stage. Photo taken 5 weeks after 2,4-D treatment. The control in this case matures at about the same time as the treated plants but is the lowest in yield. Plants of 3 are heaviest yielders of dry matter and crude protein.

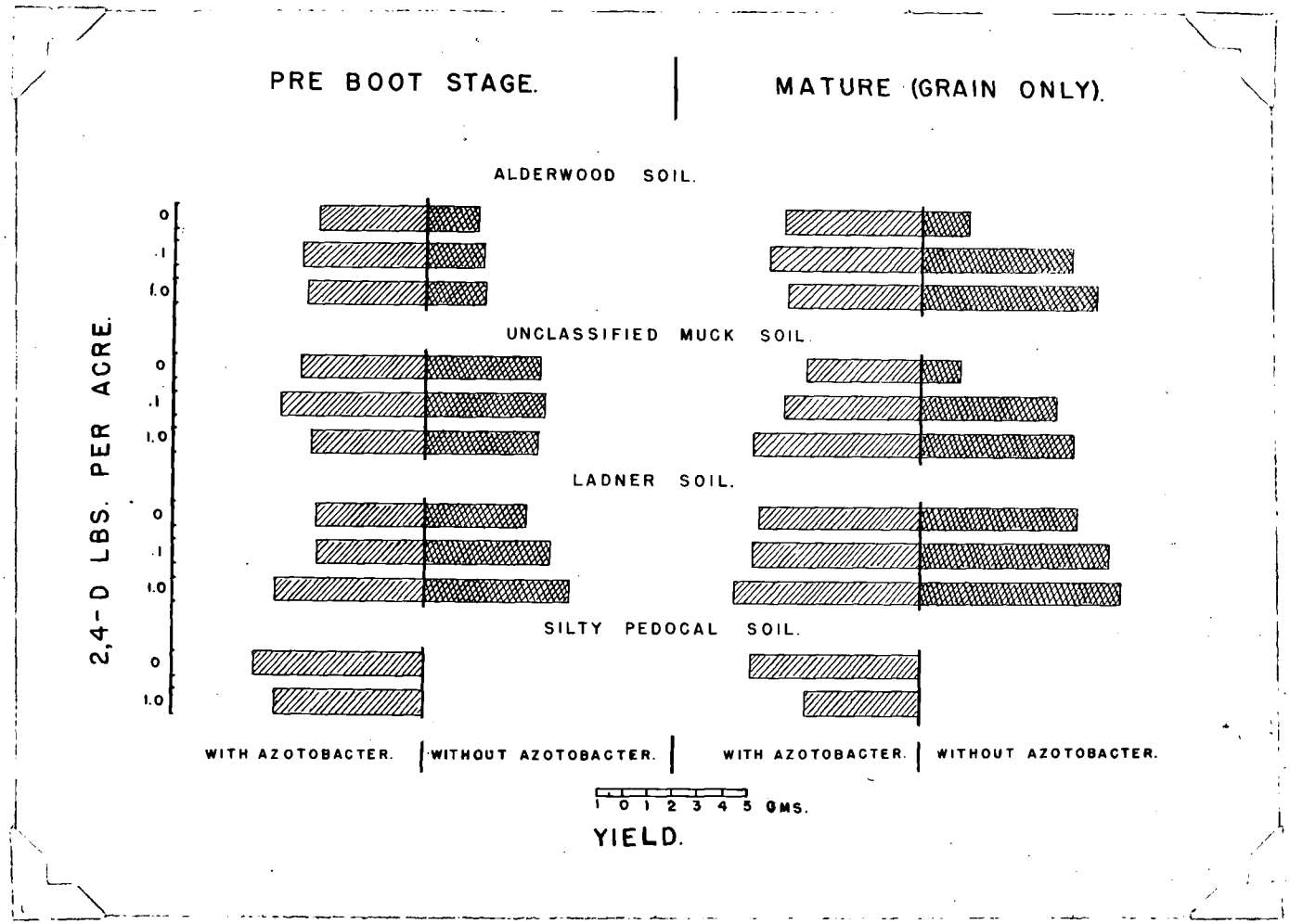


Figure 10. - A graphical summary of the responses, in terms of dry matter yield, greenhouse grown Olli barley, to four different soils, to soil inoculation with Azotobacter and to different concentrations of 2,4-D.

V. DISCUSSION

Many authors have pointed out that 2,4-D and other auxins are organic regulators of physiological processes and are not in themselves, like carbohydrates, energy yielding. Therefore, it becomes of some interest to determine the manner in which the 2,4-D in certain of our experiments increased dry matter yields and nitrogen uptake.

Several possible answers to the question are offered. In the first place, it may be asked if in our experiments the energy potential of the plant was increased through the application of 2,4-D.

Does auxin concentration limit the metabolic activity of the forage plant during the grand period of its growth? Does 2,4-D added artificially, at an early stage in development, meet this deficiency which, when wet, permits greater synthetic activity by the plant? Our experiments neither support nor detract from such a possible explanation but certainly 2,4-D acting in this way could increase the energy potential of a plant.

Other possibilities would permit auxin to act in such a manner that total plant energy is increased. Virtanen and others in extensive, well-known experiments, have established to their satisfaction, but not to that of many others, that a number of organic substances are excreted from the roots of plants in general, and in particular, from the

nodules of legumes. The excretion, they say, is to be noted at times when the plant is growing vigorously. If such plant excretions are a fact, then it seems possible that they might profoundly modify the flora and ~~fuana~~ fauna of the rhizosphere.

2,4-D itself might be excreted following its application to aerial portions and this might produce a heightened nitrogen f~~i~~ix ation in the soil and an increase in nitrogen uptake. Two items came to mind which might favour this view, viz. (a) that 2,4-D added to soils in non-herbicidal concentrations produces yield and nitrogen responses similar to those obtained when 2,4-D is applied to the aerial parts of forages, and (b) that Rhizobia and Azotobacter, nitrogen-fixing bacteria, are known to produce auxin in larger quantities than many bacteria. Sugars, too, might be the excreta, for 2,4-D, it is well known, increases the sugar levels in plants at the expense of reserve carbohydrates. Sugars, too, might modify the ecology of the soil organisms. In any event, the possibility exists that the increases in dry matter yield and nitrogen uptake by plants treated with sub-herbicidal levels of 2,4-D results from a modification of the ecology of the soil microflora. In an analagous way, antibiotics are known to modify the ecology of intestinal flora and f~~u~~au na of young animals and thereby increase their rate of gain.

Another possible answer to our question lies in the kind of substances which are found in the plant. Auxins could profoundly modify the synthetic processes in the plant

without materially altering the energy potential of the plant. It may be that structural compounds of the plant might be elaborated at the expense of reserve carbohydrates, i.e. cellulose, lignin, protein, etc., might increase at the expense of starches, fructosans, sugars, etc. Such a redirection of the synthetic function of the plant could possibly explain increased dry matter yields. Forage produced of such redirection would, of course, be of less value to the grazing animal. Calorimetric studies should quickly determine whether or not synthetic redirection is an answer. That redirection occurs is well brought out by van Overbeck when he related carbon-nitrogen ratios to auxin levels and to fruitfulness. But whether it explains dry matter and yield increases or not is, quite possibly, another aspect.

The topic seems to be worthy of pursuit. The vistas of usefulness which control of maturation processes, nitrogen uptake, and dry matter production appear to open up are intriguing.

VI. A SUMMARY STATEMENT OF OBSERVATIONS AND CONCLUSIONS

1. 2,4-D applied in sub-herbicidal concentrations to the forage plants of these experiments when they were young and actively growing resulted in an eventual increase in dry matter yield and nitrogen uptake. "Too little" 2,4-D gave no response, "too much" 2,4-D was depressive.

2. The initial effect of even the "low" concentrations was to depress yields but increased synthetic (?) activity followed.

3. As the plants matured, larger amounts of 2,4-D were required to produce eventual increases in ~~synthetic activity~~ nitrogen and dry matter and the point was reached at later stages of growth where such 2,4-D additions were simply depressive.

4. 2,4-D, it is apparent, in our experiment, impinged on the maturation processes of the forages but not in very predictable ways. Applied when the plants were young it seemed to hasten maturity; when applied later, to delay it. However, these effects ~~appeared~~ may to be related in some subtle way to the soil, the photoperiod and probably the C/N ratio of the plant.

5. To obtain "beneficial" responses related above, 5 to 10 times as much 2,4-D was required for grasses as for clovers.

6. Percentage composition was not a reliable guide to the effects of 2,4-D on the forage. Total yields of dry matter and protein are important factors in effect appraisal.

7. Kjeldahl nitrogen, expressed as crude protein, did not adequately indicate the effect of 2,4-D on the nitrogen metabolism of the plant. Non-protein nitrogen would be worthy of appraisal, but was not determined for our experiments.

8. In general, it appeared that plants growing in the relatively infertile Alderwood soil showed more marked responses when young to 2,4-D than those in the more fertile Ladner clay or muck soil. Maturation processes apparently proceeded more rapidly with season on the upland Alderwood soil so that plants were responsive for a shorter period to the sub-herbicidal concentrations of 2,4-D.

9. Not only did responses to 2,4-D differ with different soils but also with different forage species. This may have been due to different rates of maturation of the species.

10. Effects of 2,4-D on seed production were variable. High concentrations unquestionably reduced seed yield but not necessarily seed size. The responses were confounded somewhat with time of flowering. In general, it may be said that some responses are marked and worthy of further specific study. An interesting aside was the effect of 2,4-D on the percentage of hard seeds of red and white clover and the uniformity of development.

11. Time of application and concentration did not have much apparent effect on nodulation or kind of nodules in red clover.

12. Applications of 2,4-D made to aerial parts of the plant, Olli barley, under certain of the conditions, were associated with changes in the Azotobacter population of the soil, but the association may not be causal.

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