STUDIES ON VERTICILLIUM WILT OF FORAGE LEGUMES

by

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I- INTRODUCTION

Hay is the most important crop in Quebec. According to the annual report of the Quebec Bureau of Statistics for 1961, of a total cultivated area of 7,864,176 acres in Quebec, 3,458,000 acres were in hay. The value of the hay crop in 1961 was 55.8 percent of the value of all field crops in the province. Since alfalfa, birdsfoot trefoil, red, alsike, and ladino clovers are cominant in this hay crop, their agricultural importance is apparent. This, together with the fact that some of these forage legumes, mainly red clover and alfalfa, are the most common rotation crops, led to the choice of these plants for study.

Forage legumes are plagued with many diseases and environmental hazards. Damage caused by a single disease or environmental condition may, if severe enough, affect the yield of hay or seed. In Quebec, these factors act in such a manner that by the end of the second harvest year alfalfa, red clover, and birdsfoot trefoil fields have just a part of their potential crops remaining. This reduction in the life of these plants is generally attributed to "winter killing". "Winter killing" may actually be due to a number of factors such as: low temperature, root and systemic diseases, or other factors.

Among the important systemic diseases, Verticillium is one of the most destructive on alfalfa in Europe, but it is apparently not present in North America.

These factors, together with the fact that Verticillium albo-atrum (Reinke & Berthold) was found associated with wilt of red

clover at Macdonald College, Quebec, in 1938-1940 (42) emphasizes the importance of investigating this disease. We need to know if the causal organisms of wilt are present; why the disease is not present, or if it is present, why is it not important here; if Canadian strains of Verticillium are the same, or different, or more or less virulent than those of Europe; if Canadian legume varieties are as susceptible, or more resistant than European; and if our environment is less favorable to the growth of the pathogens and the development of the disease than in Europe.

The problem has been tackled by making a survey, looking for Verticillium spp. on forages in Quebec. The isolates obtained were studied in culture and their host range determined. The effects of host varieties and host age on susceptibility to wilt were investigated. The effects of air temperature and soil moisture on severity of disease development (symptoms) and on growth of alfalfa shoots and roots were studied.

II- REVIEW OF LITERATURE

Richter and Klinkowski (39), in Germany, were the first to report a species of Verticillium causing wilt of alfalfa. They identified the fungus as V. albo-atrum (R. & B.). No further mention of Verticillium wilt appeared in German reports dealing with alfalfa diseases until 1957 when Kiessig and Haller-Kiessig compared the symptoms induced on alfalfa by V. albo-atrum infection with those produced by Fusarium sp. and Corynebacterium insidiosum (28). Wagner (54) cultured more than 700 alfalfa root fragments on agar and found that nearly 7 percent were attacked by V. albo-atrum. Weltzien (55) made a survey on alfalfa in Germany and Switzerland from 1954 to 1956, and found that alfalfa plants were attacked from 5 to 100 percent, with an average of 50 percent. He concluded that almost total loss of the seed crop in parts of Germany was due to Verticillium and that it was not advisable to maintain an alfalfa crop for more than three years.

Various reports indicate that Verticillium is of some importance in Denmark. Troubles on alfalfa crops were reported in the country. The troubles were investigated and V. albo-atrum was isolated from wilted alfalfa plants in 1941 and 1944. Holme et al (19) reported that this fungus was found on Lolland-Falster Islands in 1943. Verticillium wilt of alfalfa has been reported from Denmark almost every year to the present. Stapel (47) mentioned that some years the disease is serious, destroying large areas in fields, or

even entire stands, in a week or two during hot weather.

According to an anonymous report, Verticillium was isolated from 3-year-old alfalfa in Holland, in 1950 (1). Van den Ende (51) mentioned that the disease was steadily increasing on alfalfa and also on flax. As reported by Kreitlow (29), Kort and van Rheenen in 1960, stated that the disease is destructive on alfalfa in the province of Zeeland where many farmers cannot maintain stands longer than two years.

Courtillot in 1961 reported that alfalfa is a host of V. albo-atrum in France (12). Kreitlow (29) quotes correspondence with Prof. Viennot-Bourgin, Paris, to indicate that Verticillium wilt is relatively wide-spread in France.

The microsclerotial form of V. albo-atrum, referred by many workers to V. dahliae Kleb, was reported for the first time from diseased roots of alfalfa from the Anconetano and Catonese areas in Italy, in 1957 (29). Since that time, it has not been possible to find other mention of this disease in reports.

A wilt of alfalfa caused by V. albo-atrum was first reported in Britain by Noble et al (34). From 1954 to 1956, alfalfa plants attacked by Verticillium wilt were found in various parts of England and Wales. V. albo-atrum was present in all wilted plants, except at two places where they were attacked by V. dahliae. After Isaac and Heale (26) the latter fungus is of little economic importance.

According to Isaac (24, 25), Verticillium wilt which was first reported by Noble in 1953, is now much more important, causing

serious losses in all alfalfa fields. Previously, it was possible in Britain to maintain alfalfa fields for at least four years, but new crops affected by Verticillium wilt frequently have no value at the end of the third harvest year. The incidence of Verticillium wilt in alfalfa fields is from 1 percent in the first harvest year to over 50 percent in the third. Thus, it is advisable not to keep alfalfa fields for a third crop. Among the varieties attacked by Verticillium wilt, Isaac (23) mentions the Dupuits variety which is a very important one in Quebec.

Apparently, Verticillium wilt of alfalfa has not been found in U.S.S.R. and the United States. According to Parker (36), Soloveva and Polyarkova in 1940 observed that alfalfa is resistant to Verticillium strains attacking cotton. Smith (46) inoculated, in both greenhouse and field, six alfalfa varieties with V. albo-atrum from cotton and found it to be non-pathogenic. V. albo-atrum has been mentioned on plants of alfalfa in Quebec, but this report is not documented (11).

Red clover has also been reported as a host of Verticillium. Böning (8), in a report on various diseases of horse-radish, mentioned that one of its pathogens, V. dahliae, also attacked clovers, but the species of clovers were not specified. Seymour (44) reported V. dichotemum Ell. and Ev. among the organisms found on T. pratense. Rudolph (40), in his monograph on V. albo-atrum, stated that it attacked the bur clover in California. Red clover is the only leguminous plant on which Verticillium wilt is reported with certainty in Canada

as far as the literature is concerned, and Sackston reported that V. albo-atrum, mycelial type, was recovered frequently from dead plants of red clover in spaced blocks at Macdonald College in 1938-1940 (42).

The first report of Verticillium wilt on sainfoin (Onobrychis sp.) was made in Germany by Richter and Klinkowski (39). The pathogen was identified as V. albo-atrum. But two years later, a microsclerotia-forming species of Verticillium, V. dahliae, was isolated at Cambridge, from wilting common and giant sainfoin plants. This infection seems to have made no further progress since Isaac (20) could not find it later.

Böning (8) reported that V. dahliae from horse-radish also attacked an unspecified species of lupine.

Verticillium wilt of leguminous crops probably occurs in other parts of the world where the disease has not yet been recognized or reported.

III- MATERIALS AND METHODS

Verticillium cultures

Three cultures of *Verticillium* were isolated from infected plants of clover and alfalfa collected in Quebec and at Ottawa. In addition to these, cultures of *V. albo-atrum* R. & B. and *V. dahliae* Kleb were obtained from Dr. Ivor Isaac, Swansea, Wales, and Dr. Mary Noble, Edinburgh, Scotland. Three isolates from other hosts were also used in pathogenicity tests. All the cultures were maintained on potato dextrose agar slants (2% dextrose, 2% agar).

Table 1 presents a list of the cultures and the hosts and localities from which they were isolated. Throughout the text, each of the isolates will be referred to by the number assigned to it in this list.

V. albo-atrum isolates 3 and 4, and 2 and 3 of *V. dahliae* were obtained from Ivor Isaac and *V. albo-atrum* 5 and 6 from Mary Noble. *V. dahliae* isolate no. 3 isolated from Italian clover (*Hedysarum coronarium*) was a biotin-requiring strain; no. 6 of *V. albo-atrum* was isolated from alfalfa seed. Cultures 1, 2 of *V. albo-atrum* and *V. dahliae* no. 1 were isolated from host stems and petioles by the writer. Mr. Alain Devaux of Macdonald College isolated cultures 7 and 8 of *V. albo-atrum* and *V. dahliae* no. 4 from the hosts indicated, during the summer of 1962.

Table 1 - Isolates of *Verticillium* studied

Isolate number	Host	Source	Specific name of the isolate
V. albo-atrum			
1	Ladino clover	Normandin, P.Q.	V. albo-atrum R. & B.
2	Alfalfa	"	" " " " "
3	Alfalfa	Wales	" " " " "
4	Alfalfa	"	" " " " "
5	Alfalfa	Scotland	" " " " "
6	Alfalfa seed	"	" " " " "
7	Potato	St. Germain (Kam.) P.Q.	" " " " "
8	Tomato	St. Hyacinthe, P.Q.	" " " " "
V. dahliae			
1	Red clover	Ottawa, Ont.	V. dahliae Kleb
2	Alfalfa	Wales	" " "
3	Italian clover	Wales	" " "
4	Sunflower	La Pocatiere, P.Q.	" " "

Plants inoculated

Seeds of the forage legumes studied were secured from Mr. R.W. Robertson, Genetics and Plant Breeding Institute, Central Experimental Farm, Ottawa, and from Dr. J. Bubar, Department of Agronomy, Macdonald College of McGill University. The species and varieties of the various hosts and the sources from which they were obtained are listed in Table 2.

Method of isolating fungi from stem, petiole and root tissue

Isaac (23) reported that Verticillium could be readily isolated from the lateral rootlets, the main root, the stem, and the petioles. Isolations from the lateral rootlets and the main root of all leguminous plants in the experiments at Macdonald College were not consistent, and were rarely free from contaminants. Small pieces of roots, stems, and petioles were surface-sterilized with sodium hypochlorite containing about 2% free chlorine and plated on 2% water-agar. Only very rarely was Verticillium isolated from roots, but it was obtained fairly consistently from stems, petioles, and sometimes leaves.

Survey specimens of alfalfa, birdsfoot trefoil, and clover plants to be sectioned for the isolation of fungi were first washed and brushed under running tap water. After being cleaned, they were cut into pieces about half an inch in length. These were then immersed momentarily in 95% ethyl alcohol to make them wettable. From

Table 2 - Host plants inoculated

Common name	Scientific name	Variety	Obtained from
Birdsfoot trefoil	Lotus corniculatus L.	Empire	R.W. Robertson
" "	" "	Viking	" "
Lupine	Lupinus albus L.	- - -	" "
Alfalfa	Medicago sativa L.	Dupuits	Dr. J. Bubar
"	" "	Ladak	R.W. Robertson
"	" "	Narraganset	" "
"	" "	Ranger	" "
"	" "	Vernal	Dr. J. Bubar
Sweet clover	Melilotus alba Desr.	Artic	R.W. Robertson
Sainfoin	Onobrychis viciifolia Scop.	Common	" "
Alsike clover	Trifolium hybridum L.	Common	" "
Red clover	Trifolium pratense L.	Altaswede	" "
" "	" "	Chesapeake	" "
" "	" "	Dollard	" "
" "	" "	Kenland	" "
" "	" "	Lakeland	" "
" "	" "	Lasalle	" "
" "	" "	Tetraploid	" "
Ladino clover	Trifolium repens L.	Pilgrim	" "
Tomato	Lycopersicon esculentum Mill	John Baer	Commercial seed

the alcohol they were put into a solution of Javex brand sodium hypochlorite which contained 2% available chlorine, for two minutes. From Javex the plant parts were transferred to Petri plates containing 15 ml. of potato dextrose agar.

Each plate comprised a sample of root, stem, and petiole of one plant. In general, each plate contained 2 root, 3 stem, and 2 petiole sections.

To protect the fungi from being overrun by bacteria the P.D.A. was acidified by two drops of 10% lactic acid to every petri dish. This acid was added before pouring the melted P.D.A. in the plate. Sometimes there was interference from bacteria even when lactic acid was added to the medium.

The plates were incubated at room temperature in a laboratory. They were wrapped in polyethylene bags, five Petri plates per bag, to protect them from drying out and contaminations. It was quite easy to see the fungi growing in the plates through the transparent bags. After two or three days, if there was evidence of fast-growing fungi like Rhizopus nigricans overrunning a slow growing species, hyphal tip transfers of the slow growing colonies were made to other plates. To keep track of the origin of each of the isolated fungi a code system was adopted. Also, after 10 days, if I was not able to identify a fungus in the original petri dish, it was transferred to another petri plate and to a test tube for identification and maintenance.

Aids in the identification of fungi

At the beginning, every species of fungus was presented to Dr. R.H. Estey, Mycologist at Macdonald College, for identification. All cultures of Fusarium were also sent to Dr. W.L. Gordon, Canada Department of Agriculture, Laboratory of Plant Pathology, Winnipeg, after I had identified them myself. Duplicate cultures of all fungi identified by the above mentioned specialists were maintained on test tube slants.

Among the most helpful of the published aids to identification of fungi were: "A manual of soil fungi" by J.C. Gilman (15) and the "Illustrated Genera of Imperfect Fungi" by H.L. Barnett (4).

For the identification of Fusarium spp., two publications were mostly used: "The Occurrence of Fusarium species in Canada" II by W.L. Gordon (16) and "Fusaria of Potatoes" by C.O. Sherbakoff (45).

Preparation of inoculum

Two kinds of inoculum were prepared. The first was made from cultures grown for 2 weeks on P.D.A. in test tubes. The material in the tube was added to about 250 ml. of distilled water and macerated in a Waring blender for 5 to 10 seconds; care was taken to include as little P.D.A. as possible. The resulting suspension contained spores, mycelium, and in some cases microsclerotia. It was diluted to 500 mls. before use. This kind of inoculum will be referred to in the test as "macerated fungus inoculum". The second

type of inoculum was made by adding sterile distilled water to a culture of the pathogen grown on a sand-soil-cornmeal medium, and gently shaking it. Such a suspension contained spores, some mycelial fragments, and a few soil particles. This was used to inoculate tomato.

Methods of inoculation

The root-dipping technique was found very effective in inducing wilt of forage legumes. In this method, roots of the plants to be inoculated were washed clean under running tap water, then were dipped in the macerated fungus inoculum for 30 minutes. Inoculated plants were planted in pasteurized greenhouse soil. The residual inoculum was diluted and poured on the soil near the treated plants in all tests, except the soil moisture experiments. A different method was used to inoculate tomato plants. The roots were injured by thrusting a stiff wire into the soil at four places, about one inch from the stem of plant. The plants were then inoculated by pouring onto the soil the suspension of spores and mycelium described above.

Facilities used

Most experiments were made in greenhouses. Experiments on the effects of temperature and relative humidity were performed in controlled-environment growth cabinets. The temperature and humidity in all experiments were recorded on hygrothermographs.

Method of rating intensity of disease symptoms

In those experiments where it was necessary to rate the intensity of disease symptoms, this was done on the F.A.O. scale, where "0" means no information, and where "1" is the best rating for any characteristic and "9" is the worst. The values assigned were as follows:

0 - no information.

1 - plant healthy.

2 - vein clearing.

3 - vein clearing; yellowing of leaves starting.

4 - yellowing of leaves and some vein clearing.

5 - almost all leaves yellow; none falling yet and some showing vein clearing.

6 - leaves yellow; starting to fall.

7 - leaves falling and remaining leaves yellow.

8 - all leaves fallen.

9 - plant dead.

IV- FIELD STUDIES

The disease survey

During the summer of 1962, many forage legume plots were examined in the course of survey trips through the Province of Quebec. To have an idea of the health condition of legume crops, samples were collected at all Federal Experimental Farms in the Province of Quebec, at the Central Experimental Farm at Ottawa, at two farms of the Provincial Government and at Macdonald College. The Federal Experimental Farms all had almost the same species and the same varieties of leguminous plants and they were conducting the same projects. Unfortunately, it was a very bad year to collect plants since, according to research workers of those stations, a lack of snow cover had caused the death of a good part of the forage legumes. This situation was remarked in almost all research stations with some variance and with the exception of the Experimental Station of Normandin where there was practically no winter killing.

During this survey, alfalfa, ladino and white clovers, and birdsfoot trefoil plants showing wilting, yellowing, and stunting were collected and brought at Macdonald for study. Samples of these four forage legumes were collected on the spaced plots at Normandin, red clover and alfalfa at the Experimental Farm at L'Assomption, at the Deschambault Provincial Farm and at Macdonald College, and at Ottawa, red clover plants were collected and alfalfa plants were obtained at La Pocatiere and at the Farm of the Dairy School at

St. Hyacinthe. During the same summer, forage legume fields were also examined on the Seed Farm at Macdonald College and some farms in the vicinity of Ste. Anne de Bellevue, i.e. Ste. Genevieve, Pointe-Cascade, Dorion and Vaudreuil.

Microorganisms collected

In the work here reported, many kinds of soil fungi were found associated with dead or sick leguminous plants. Fifteen genera of fungi belonging to three different classes were isolated from the 202 forage legume plants plated: Fungi Imperfecti were the most numerous followed by Phycomycetes and Ascomycetes. Bacteria were also present in many cases, but they were not identified.

Among the 327 colonies of fungi obtained, Fusarium species were isolated with the greatest frequency, i.e. 58% followed by Alternaria 14%, Rhizopus 7%, Sclerotinia 5%, Rhizoctonia 4%, and others 12%. Nematodes were also isolated from red clover plants collected at Macdonald College. They were identified by Dr. R.H. Estey as Rhabditis sp. which is free-living.

Classification of the isolates from forage legumes

Alternaria tenuis Nees ex Cda

From alfalfa at Macdonald College, L'Assomption, Caplan, Normandin, and La Pocatiere; from red clover at Macdonald College and L'Assomption; and ladino clover at Normandin.

Arthrobotrys oligospora Fr.

From alfalfa at Lennoxville.

Colletotrichum spp.

From alfalfa at L'Assomption; and red clover at Macdonald College.

Fusarium avenaceum (Fr.) Sacc.

From alfalfa at Macdonald College, L'Assomption, Normandin, La Pocatiere, St. Hyacinthe, and Caplan; from red clover at Macdonald College and L'Assomption; from ladino and white clovers at Normandin.

Fusarium culmorum (W.G. Sm.) Sacc.

From alfalfa at L'Assomption, Deschambault, and La Pocatiere; from red clover at L'Assomption.

Fusarium equiseti (Cda) Sacc.

From alfalfa at Macdonald College and L'Assomption; from red clover at L'Assomption.

Fusarium moniliforme Sheld. emend. Snyder & Hansen

From alfalfa at St. Hyacinthe.

Fusarium oxysporum Schlecht. emend. Snyder & Hansen

From alfalfa at St. Hyacinthe, Lennoxville, and L'Assomption; from red clover at Macdonald College, Lennoxville, and L'Assomption.

Fusarium poae (Pk.) Wr.

From alfalfa at Caplan; from red clover at l'Assomption.

Fusarium solani (Mart.) App. & Wr. emend. Snyder & Hansen

From alfalfa at Lennoxville; from red clover at Macdonald College and L'Assomption.

Gliocladium roseum (Link.) Thom.

From ladino clover at Normandin.

Helminthosporium sp.

From alfalfa at Macdonald College.

Hormodendrum sp.

From alfalfa at Macdonald College.

Phoma spp.

From alfalfa at Macdonald College and Lennoxville; from red clover at Macdonald College, L'Assomption, and Lennoxville.

Rhizoctonia spp.

From alfalfa at L'Assomption, Caplan, Normandin, and La Pocatiere; from red clover at Caplan.

Rhizopus spp.

From alfalfa at Lennoxville and La Pocatiere; from red clover at L'Assomption; from birdsfoot trefoil at Macdonald College.

Sclerotinia trifoliorum Erikss.

From alfalfa at Macdonald College, La Pocatiere, and Normandin; from red clover at Macdonald College and L'Assomption.

Stemphylium sp.

From alfalfa at Macdonald College.

Trichoderma viride Pers. ex Fr.

From alfalfa at L'Assomption; from red clover at Macdonald College and L'Assomption; from birdsfoot trefoil at Normandin.

Verticillium albo-atrum R. & B.

From alfalfa and ladino clover at Normandin.

Verticillium dahliae Kleb.

From red clover at Ottawa.

Zygorrynchus sp.

From red clover at L'Assomption.

Since the description of Verticillium albo-atrum by Reinke & Berthold, in 1879, and V. dahliae by Klebahn, in 1913, the relationship between these two species has been the subject of much discussion. Many authors do not differentiate the two species, calling them both V. albo-atrum. The results of their researches

sometimes contradict those of other workers who use the same name for the fungus they studied. Thus, Bewley (6) stated that he worked with V. albo-atrum, when the description of his isolates makes it clear that he was dealing with both V. albo-atrum and V. dahliae.

Our studies of isolates 1, and 2 of V. albo-atrum from ladino clover and alfalfa and 1 from red clover respectively have shown that two different species were present; number 1 of V. dahliae forming pseudo-sclerotia (PS type) and isolate of V. albo-atrum nos. 1 and 2 that remained entirely white (W type). The PS type formed pseudo-sclerotia which could be recognized in culture after a few days. Usually, they gave a black appearance to the undersurface of the culture. Pseudo-sclerotia developed either by monohyphal budding resulting in a nearly round body or by intermingling and budding of contiguous hyphae. In culture, this fungus grew fairly well at high temperature (30°C.) and slowly at 7.5°C. This agrees with previous reports by Ludbrook (30) and Isaac (21) who identified their cultures as V. dahliae. Since this isolate has shown all these characteristics, it has been identified as V. dahliae.

The W type remained white after prolonged culture and never formed any resting structures. Cultures of this type grew very rapidly and sporulated sparsely. Isolates of this type grew fairly well at low temperature and did not show much growth at 30°C.; isolate 1 did not grow at all at this temperature. The two isolates of W type show the same characteristics as isolates 5 and 6 of Mary Noble who identified them as V. albo-atrum.

Because of the conspicuous and consistent differences between these two types of cultures, we agree with those workers who assign them to the species V. dahliae and V. albo-atrum respectively.

V- CULTURE STUDIES: TEMPERATURE

There is considerable published work concerning the effect of temperature on the growth of Verticillium in culture from which it is clear that confusion exists in the nomenclature adopted for the strains of fungi used in various studies. Bewley (5) stated that the minimum, optimum, and maximum temperatures for growth in pure culture of his strains of V. albo-atrum were 4.4°C., 23.3°C., and 30°C. respectively, but he did not indicate whether the cultures used were of the V. dahliae or V. albo-atrum type. It is evident from his description of his collection of cultures that both types were included by him under the simple designation of V. albo-atrum, since he states: "the mycelial cells, which give rise to microsclerotia, become swollen and by a process akin to budding a bead-like aggregate is formed, the cells of which thicken and turn brown". Chaudhuri (10) gives 10°, 22.5° and 27°C. as the minimum, optimum, and maximum for the culture he used, which he described simply as V. albo-atrum. Ludbrook (30) and Isaac (21) state that V. albo-atrum shows no growth at 30°C., while cultures of V. dahliae, at this temperature, show some degree of development. Robinson et al (41) have also reported results which agree with those of the two last workers. Where the type of fungus used is described, cultures of V. albo-atrum type generally seem to produce little or no growth at 30°C., while those of V. dahliae type grow fairly well at 30°C., and in some cases at higher temperatures.

Tests were conducted on the first nine isolates of Verticillium described in Table 1 to observe the growth of these isolates in culture at different temperatures. The results of these studies were then correlated with the effects of temperature on disease intensity on inoculated plants.

All isolates were grown on "Bacto Potato Dextrose Agar Dehydrated" (Difco), in 9 cm. petri dishes, containing 15 mls. of medium. By means of a cork borer, 5 mms. in diameter, uniform disks were cut from plate cultures of the fungus and used as inoculum. One disk was placed in the center of each dish. Cultures used as a source of inoculum were of the same age, one week. Only the advancing margin of the fungus was used for inoculum. The cultures were incubated at 7.5°, 10°, 15°, 20°, 25°, 30°, and 35°C. At 35°C., an oven was used and a refregirator served as an incubator at 7.5°C. Five plates of each isolate were used at each temperature and each test repeated after an interval of 2 weeks. The diameter of the colonies was measured in millimeters 15 days after inoculation. This method of measuring growth was chosen because it was relative growth rate that I wished to determine. The results of measurements are given in Table 3.

All isolates grew well over the range 15° to 25°C. with the best growth at 25°C., except for isolates 1 and 3 of V. dahliae which had 2 peaks, one at 30° and the other at 20°C. At high temperatures, a marked difference was observed between V. albo-atrum and V. dahliae isolates. At 30°C. the V. dahliae isolates grew moderately

well, whereas all V. albo-atrum isolates were very slow growing. At 35°C. none of these isolates showed any growth. The isolates grew at 7.5°C. The minimum temperature for the growth of any of the isolates, with the exception of V. dahliae no. 3, appears to be just below 7.5°C.

Table 3 - The effect of temperature on growth of various isolates of Verticillium for 15 days

Isolate No.	Diameter in mm. after 15 days (1)						
	Temperature centigrade						
	7.5°	10°	15°	20°	25°	30°	35°
V. albo-atrum							
1	3	9	38	41	48	0	0
2	2	21	42	47	70	6	0
3	9	24	41	45	62	8	0
4	10	18	41	42	58	12	0
5	14	19	35	38	54	6	0
6	14	19	42	42	61	6	0
V. dahliae							
1	2	12	22	29	23	29	0
2	11	17	37	45	54	39	0
3	0	7	25	27	23	30	0

(1) Average of 10 plates in two experiments.

VI- GREENHOUSE STUDIES

Isolates of the two species of Verticillium collected on forage legumes during the survey were studied in greenhouse experiments. The pathogenicity of these isolates was first compared with others on various forage legume plants; this was followed by a study on the effect of age of alfalfa plants on their susceptibility to the respective isolates.

A) Pathogenicity tests

Tests on relative pathogenicity were carried out by the root-dip method for leguminous plants and by a special method described above for tomato plants. All the species of plants mentioned in Table 2 were used. Nine isolates were tested on all the species of leguminous plants, six (V.a 1, 2, 3, 4; V.d 1, 2) on tomato plants, and three others (V.a 7, 8; V.d 4) were used to inoculate the Vernal and Dupuits varieties of alfalfa. Twenty plants were inoculated in every case with the different isolates, with the exception of Lupinus albus for which only ten plants were inoculated with each isolate. Ten tomato plants were inoculated with each of the six isolates previously mentioned. In all the tests, two non-inoculated plants in each treatment were used as control. Greenhouse temperatures were in the range of 21° to 27°C.

In each case, when the symptoms of disease became evident in the plants inoculated with V. albo-atrum and V. dahliae, the symptoms

were recorded and after the plants were removed from the soil, they were plated on P.D.A.

The results of these tests are reported in the following tables, 4 to 12.

1) Alfalfa (Medicago sativa)

All alfalfa plants were inoculated when they were 46 days old and they had 4 to 11 trifoliate leaves.

From the data of Table 4, the conclusion may be drawn that the isolates of V. albo-atrum are more virulent to alfalfa than those of V. dahliae. Isolates of V. albo-atrum from Quebec are as virulent to this host as those from Wales; isolate 1 of V. dahliae seems to be more pathogenic than isolates 2 and 3. The Dupuits variety was generally attacked earlier, but no appreciable differences were noted in total number of plants attacked in each variety.

Isolates 7 and 8 of V. albo-atrum and 4 of V. dahliae from hosts other than legumes failed to attack the two varieties of alfalfa inoculated.

2) Red clover (Trifolium pratense)

When red clover plants were inoculated, they were 45 days old and they had 3 to 5 trifoliate leaves.

Two cultures of V. dahliae (nos. 2 and 3) were strongly pathogenic to all varieties of red clover. V. dahliae no. 1 which

Table 4 - Pathogenicity of *Verticillium* isolates on five varieties of alfalfa (*Medicago sativa*)

Isolate No.	Varieties tested														
	Dupuits			Vernal			Ladak			Ranger			Narragansett		
	Days after inoculation														
	20	30	40	20	30	40	20	30	40	20	30	40	20	30	40
V. albo-atrum															
1	20[1]	20	20	8	15	16	5	10	16	5	9	10	9	12	12
2	9	19	19	15	19	20	7	18	19	16	19	19	3	8	11
3	9	14	16	8	18	20	12	19	20	6	18	19	8	19	20
4	20	20	20	0	19	20	0	18	18	0	20	20	0	17	19
5	15	19	19	11	16	16	8	16	17	14	18	18	14	14	14
6	16	18	18	12	16	17	17	18	18	15	18	20	16	19	19
7	0	0	0	0	0	0	-[2]	-	-	-	-	-	-	-	-
8	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
V. dahliae															
1	0	0	2	0	0	2	7	8	8	1	1	1	6	10	11
2	0	0	0	0	0	1	3	4	4	0	1	2	0	2	2
3	3	5	5	2	3	3	1	1	1	0	1	2	4	4	4
4	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-

1) Number of plants infected out of 20 inoculated.

2) No plants inoculated.

Table 5 - Pathogenicity of *Verticillium* isolates on seven varieties of red clover (*Trifolium pratense*)

Isolate No.	Varieties tested																							
	Chesapeake			Kenland			Tetraploid			Dollard			Lakeland			Lasalle			Altaswede					
	Days after inoculation																							
	25	50	75	25	50	75	25	50	75	25	50	75	25	50	75	25	50	75	25	50	75			
V. albo-atrum																								
1	9[1]	12	13	11	14	15	0	4	7	0	1	1	0	1	1	0	1	2	0	1	2			
2	10	14	14	0	1	5	5	5	6	0	1	2	0	0	0	0	0	0	0	1	4			
3	0	0	7	0	1	1	3	8	9	0	0	0	0	0	4	0	0	2	0	0	3			
4	0	0	2	0	0	3	0	0	0	0	2	2	0	0	3	0	0	0	0	0	0			
5	0	6	6	0	2	7	0	3	4	0	4	4	0	0	0	0	0	0	0	6	6			
6	0	1	9	0	1	9	1	4	6	0	0	5	0	2	6	0	0	4	0	0	3			
V. dahliae																								
1	0	17	18	0	0	5	6	18	18	0	0	0	0	0	2	0	0	1	0	0	1			
2	0	9	14	0	6	19	5	14	15	0	11	14	0	17	17	0	12	17	0	11	13			
3	0	17	17	0	17	20	0	17	17	0	20	20	0	20	20	0	12	13	0	10	16			

1) Number of plants infected out of 20 plants inoculated.

was isolated from red clover plants is much less virulent than the two others. The isolates of V. albo-atrum were not very virulent on red clover, but isolates 1 and 2 from Quebec were more virulent than the V. albo-atrum isolates from England.

The varieties can be divided into two groups: those which are susceptible and those which have a certain resistance. The more susceptible varieties have their plants attacked earlier than others; some plants were attacked 25 days after inoculation. Chesapeake, Kenland, and Tetraploid red clover were susceptible to Verticillium spp., especially V. dahliae, and out of these three, the Chesapeake variety is the most susceptible.

3) Birdsfoot trefoil (Lotus corniculatus)

Lotus corniculatus plants were 39 days old and had 4 to 9 leaves when they were inoculated.

The results given in Table 6 indicate that isolates of V. dahliae do not attack birdsfoot trefoil. Some isolates of V. albo-atrum are pathogenic to this forage legume and isolates from Quebec, nos. 1 and 2, were more virulent than the others. The variety Viking appeared more susceptible than Empire.

Table 6 - Pathogenicity of *Verticillium* isolates on two varieties of birdsfoot trefoil (*Lotus corniculatus*)

Isolate No.	Varieties					
	Empire			Viking		
	Days after inoculation					
	20	40	60	20	40	60
V. albo-atrum						
1	0[1)]	13	15	0	17	18
2	0	9	9	0	11	14
3	0	0	1	0	0	5
4	0	0	0	1	1	7
5	0	0	0	0	0	2
6	2	5	7	4	8	15
V. dahliae						
1	0	0	0	0	0	0
2	0	0	0	0	0	1
3	0	0	0	0	0	0

1) Number of plants infected out of 20 plants inoculated.

4) Alsike clover (Trifolium hybridum)

Alsike clover plants were inoculated when they had 3 to 5 trifoliate leaves, and were 46 days old.

From the results of Table 7, it is impossible to conclude that the two species of Verticillium act differently, but some difference can be noticed between individual isolates. All isolates of V. albo-atrum, except 3 and 5 were fairly virulent on common alsike clover. Two isolates of V. dahliae, nos. 2 and 3, attacked alsike clover; isolate no. 1 was not very virulent since 4 plants were attacked. This clover is attacked quite late.

5) Ladino clover (Trifolium repens)

Ladino clover plants were inoculated at 39 days when they had 4 to 5 leaves.

The two species of Verticillium differed in their attack on ladino clover plants. The two V. albo-atrum isolates from plants collected at Normandin showed some ability to attack ladino clover. These two isolates were as virulent as the V. albo-atrum 5 and 6 from Mary Noble. Cultures of V. albo-atrum, 3 and 4 from Isaac, were not virulent to ladino clover. Isolate 2 of V. dahliae attacked 15 out of 20 plants; the two other isolates of V. dahliae, nos. 1 and 3 were less virulent.

Table 7 - Pathogenicity of *Verticillium* isolates on Common variety of alsike clover (*Trifolium hybridum*)

Isolate No.	Days after inoculation 30	60	90
V. albo-atrum			
1	4[1)]	5	12
2	3	5	8
3	0	1	3
4	4	5	8
5	1	1	4
6	0	14	15
V. dahliae			
1	0	0	4
2	1	1	13
3	0	0	15

1) Number of plants infected out of 20 plants inoculated.

Table 8 - Pathogenicity of *Verticillium* isolates on Pilgrim variety of ladino clover (*Trifolium repens*)

Isolate No.	Days after inoculation 20	40	60
V. albo-atrum			
1	0[1)]	6	8
2	0	5	12
3	0	0	2
4	0	1	3
5	0	4	4
6	0	12	14
V. dahliae			
1	0	2	8
2	0	10	15
3	0	3	6

1) Number of plants infected out of 20 plants inoculated.

6) Sweet clover (Melilotus alba)

Sweet clover plants were inoculated at the age of 40 days when they had 3 to 4 leaves.

From Table 9, the conclusion may be drawn that the isolates of V. albo-atrum and V. dahliae tested do not attack this leguminous plant. Two isolates, V. albo-atrum no. 6 and V. dahliae no. 3 were pathogenic to a few plants; isolate 3 of V. dahliae attacked some in the early stages and 6 of V. albo-atrum acted later.

7) Sainfoin (Onobrychis viciifolia)

Sainfoin plants were inoculated at the 3 to 6 leaf stage when they were 40 days old.

The two species of Verticillium have the same pathogenicity on sainfoin. The two isolates, nos. 1 and 2, collected in Quebec are less pathogenic than the isolates of V. albo-atrum from England. On the other hand, our V. dahliae no. 1 had a more drastic effect on this plant than the two isolates from England, since it attacked 20 plants out of 20. The isolate no. 6 of V. albo-atrum attacked this species of plant early whereas all the others have acted more slowly. From these results, it is logical to conclude that sainfoin is susceptible to the two species of Verticillium which we are dealing with.

Table 9 - Pathogenicity of *Verticillium* isolates on Artic variety of sweet clover (*Melilotus alba*)

Isolate No.	Days after inoculation 20	40	60
V. albo-atrum			
1	0[1)]	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	4	4
V. dahliae			
1	0	0	0
2	0	0	0
3	4	6	6

1) Number of plants infected out of 20 plants inoculated.

Table 10 - Pathogenicity of *Verticillium* isolates on Common variety of sainfoin (*Onobrychis viciifolia*)

Isolate No.	Days after inoculation 25	50	75
V. albo-atrum			
1	8[1)]	10	13
2	9	13	15
3	1	7	13
4	1	16	18
5	7	12	17
6	15	17	17
V. dahliae			
1	5	9	20
2	4	18	18
3	2	11	11

1) Number of plants infected out of 20 plants inoculated.

8) Lupine (Lupinus albus)

Lupine plants were inoculated when they had 5 to 6 leaves and they were 22 days old.

From Table 11, it can be concluded that all the isolates of V. albo-atrum and V. dahliae studied can attack Lupinus albus. All the isolates infected all the plants inoculated, except V. dahliae no. 2 with 9 out of 10, and differed only in the speed with which symptoms appeared.

9) Tomato (Lycopersicon esculentum)

Tomato plants were inoculated at 32 days old when they had 5 to 6 leaves. These plants were grown for the duration of the study in "Dixie cups" which had been previously coated with paraffin wax.

From the results given in Table 12, it can be concluded that the isolates of V. albo-atrum and V. dahliae from forage legumes are not very pathogenic to tomato plants. Good symptoms of Verticillium wilt were seen on just a few plants. The plant attacked by isolate 2 of V. albo-atrum was the only one to show vessel discoloration.

Table 11 - Pathogenicity of *Verticillium* isolates on *Lupinus albus*

Isolate No.	Days after inoculation 22	44	66
V. albo-atrum			
1	1[1)]	3	10
2	3	7	10
3	6	8	10
4	5	10	10
5	3	8	10
6	3	9	10
V. dahliae			
1	5	9	10
2	4	6	9
3	0	6	10

1) Number of plants infected out of 10 plants inoculated.

Table 12 - Pathogenicity of *Verticillium* isolates on John Baer variety of tomato (*Lycopersicon esculentum*)

Isolate No.	Number of plants infected out of 10 inoculated, 44 days after inoculation
V. albo-atrum	
1	4
2	1
3	3
5	3
V. dahliae	
1	2
2	1

B) Range of pathogenicity of various isolates from forage legumes

Isaac (24) tested isolates of V. albo-atrum and V. dahliae from alfalfa on plants of potato, tomato, sainfoin, and clover. His tests indicated that these isolates were not virulent to sainfoin and clover and that potato and tomato were susceptible to isolates of both V. albo-atrum and V. dahliae.

To investigate the range of pathogenicity of the isolates collected during the survey and also that of isolates obtained from Wales, the plants described in Table 2 were tested in the greenhouse. The results are shown in Table 13.

The data of Table 13 prove conclusively that the isolates of V. albo-atrum and V. dahliae which occur in our region are able to attack leguminous plants. Some of the forage legumes are attacked very heavily while others are less susceptible. These results are of obvious practical significance not only in Quebec, where forage crops are so important, but wherever they are grown in North America.

When the two species of pathogens are compared on the forage legumes as a group, it can be noted that V. albo-atrum is somewhat more virulent than V. dahliae; V. albo-atrum attacked 56.7% and V. dahliae 46.2% of the plants inoculated.

The two isolates of V. albo-atrum collected from alfalfa and ladino clover in Quebec, have proved highly virulent on alfalfa plants, exceeded only by the two isolates from Isaac and one from Noble.

Table 13 - Reaction of forage legumes to Verticillium spp. isolated from leguminous plants

Plants inoculated	Per cent of plants infected								
	V. albo-atrum isolates						V. dahliae isolates		
	1	2	3	4	5	6	1	2	3
Alfalfa [1]	74	88	95	97	84	92	24	9	15
Red clover	29	22	19	7	19	30	32	69	88
Birdsfoot trefoil	82	58	12	13	5	55	0	2	0
Alsike clover	60	40	15	40	20	75	20	65	75
Ladino clover	40	60	10	15	20	70	40	75	30
Sweet clover	0	0	0	0	0	20	0	0	30
Sainfoin	65	75	65	90	85	85	100	90	55
Lupinus albus	100	100	100	100	100	100	100	95	100
Tomato	40	10	30	-[2]	30	-	20	10	-

1) 100 alfalfa, 140 red clover, 40 birdsfoot trefoil, 20 alsike clover, 20 ladino clover, 20 sweet clover, 20 sainfoin, 10 Lupinus albus and 10 tomato plants were inoculated with each isolate. The controls invariably remained healthy.

2) (-) indicates that no plants were inoculated.

Isaac (23) reported that wilt of alfalfa caused by V. dahliae is of little importance. The results obtained here are in accordance with his.

The clovers tested proved to be susceptible to Verticillium species, and more so to V. dahliae than to V. albo-atrum. These results are not in agreement with those of Isaac (24) who stated that clover was resistant to the alfalfa pathogens, without mentioning the species of clover. Isolates 1 and 2 of V. albo-atrum are more pathogenic than all but no. 6 to red, alsike and ladino clovers. Isolates 2 and 3 of V. dahliae are more virulent than no. 1 to red and alsike clovers, but even no. 1 proved more pathogenic to red clover than did any isolate of V. albo-atrum. Ladino clover was susceptible to all three isolates of V. dahliae, particularly to no. 2

More plants of birdsfoot trefoil were attacked by isolates 1 and 2 of V. albo-atrum than by the four other isolates. From the results in Table 13, birdsfoot trefoil appears to be quite resistant to V. dahliae.

Isaac (24) reported that sainfoin was resistant to isolates of V. albo-atrum and V. dahliae from alfalfa and stated that it "may be encouraged as a fodder crop in those regions most severely affected by lucerne wilt". This does not agree with our results since all the isolates of both species attacked sainfoin. Isolate 1 of V. dahliae infected 100% of the plants inoculated.

Lupinus albus was very susceptible to all isolates of both species of Verticillium. The cultivation of this crop in rotation

with potato or other susceptible plants should be discouraged, because the lupine crop might increase the reservoir of inoculum.

The inability of the forage legume isolates to cause wilt of sweet clover was most surprising since all the other leguminous plants tested were susceptible to almost all isolates of V. albo-atrum and V. dahliae. This resistance in sweet clover plants may be due to the presence of coumaric acid or to other factors, although no such explanation was found in the literature.

The cultivation of sweet clover as a green manure crop in preference to other legumes might be advisable in fields were Verticillium wilt is a problem.

C) Symptoms of Verticillium wilt on various leguminous plants

The symptoms of Verticillium wilt on herbaceous plants have been described by Rudolph (40). Isaac (20, 24) has described symptoms on sainfoin and alfalfa respectively. Since the wilt symptoms incited in leguminous plants by V. albo-atrum and V. dahliae are identical they will not be distinguished. The symptoms of red, alsike, and ladino clovers will be described together, and those of other plants separately.

1) Symptoms on alfalfa

The symptoms are generally those of a typical hadromycotic disease. The initial symptom is clearing of veins of young terminal

leaflets, usually on branches from the lower stem. Epinasty and wilting are very pronounced during warmer periods of the day with some recovery as temperature drops towards evening, but gradual decline of the plant due to a loss of cell turgor continues until it dies. Usually the terminal leaves become pale yellow (Figure 1) and are easily detached before the basal ones, leading to a rapid defoliation (Figure 2). It sometimes happens that the petioles fall with the leaflets.

Isaac (23, 24) states that the lower part of infected alfalfa stems, even when not completely dead, are frequently covered by Verticillium conidiophores. These give a superficial grayish appearance, which, as the stem dies, turns black due to the formation, in the outer cortex and epidermis, of either the dark resting-mycelium of V. albo-atrum or the black microsclerotia of V. dahliae.

Since plants were removed from the soil as soon as there were significant symptoms, it was not possible to observe these symptoms during our tests, in the greenhouse. Cut stems and roots of infected plants show the dark-brown discoloration of the xylem characteristic of Verticillium diseases.

The fungus can be readily isolated from the stem, petioles, and leaflets of a diseased plant. Vascular colonization by the fungus seems complete since when petioles or stems are plated, the fungus grows over the whole surface.

Figure 1. Dupuits alfalfa: check plant healthy, yellow leaves on inoculated plant.

Figure 2. Wilting, yellowing, and defoliation of diseased alfalfa (Dupuits).

2) Symptoms on red, alsike, and ladino clovers

The symptoms of Verticillium wilt on red, alsike, and ladino clovers seemed to me all the same, so will be discussed together.

The symptoms on clover plants suffering from wilt differ from those of alfalfa. They start with a slight clearing of veins on the older leaves. The leaves become flaccid, then gradually dry out and the lamina shrivels (Figure 3). The color of leaves changes slowly from green to yellow green (Figure 3); as the leaves die they become brown (Figure 4). In many cases, one side of a leaf appears diseased, while the rest seems healthy (Figure 5). The petioles of affected leaves bend downwards and as the disease develops, they dry out and turn brown (Figure 6). In some cases, plants are dwarfed (Figure 7).

The fungus can be easily isolated from petioles and leaves of diseased plants.

3) Symptoms on birdsfoot trefoil

Symptoms of birdsfoot trefoil are very difficult to recognize. Young stems generally show the trouble first. These do not develop very well and bend downwards. The color of leaves changes slowly from green to yellow green and they are easily detached from the petiole which sometimes falls with them (Figure 8).

The fungus can be easily isolated from the aerial parts of the plant.

Figure 3. Yellowing and shrivelling of leaves of alsike clover (Common).

Figure 4. Chlorosis and browning of leaves of red clover (Chesapeake).

Figure 5. Yellowing of one side of a leaf of red clover (Chesapeake).

Figure 6. Shrivelling and discoloration of leaves of red clover (Chesapeake).

Figure 7. Stunted inoculated plant and healthy check of red clover (Chesapeake).

Figure 8. Yellowing of leaves and defoliation of birdsfoot trefoil (Viking).

4) Symptoms on sweet clover

Symptoms of sweet clover look very much the same as those of alfalfa and birdsfoot trefoil. As in the plants previously mentioned, the youngest stems show symptoms first. The leaves change color from green to yellow green and are easily detached from the petioles. The pathogens can be isolated from stems, petioles, and leaves.

5) Symptoms on sainfoin

The symptoms of sainfoin wilt induced by V. albo-atrum and V. dahliae have been previously described by Courtillot (12) and Isaac (20).

These symptoms are those of a hadromycotic disease and are first shown by the outer leaves of affected plants. The first symptom observed is a clearing of veins of the leaflets. This condition is followed by a change in their colour which passes from green to pale green. The leaflets, in turn, fold upwards along the midrib, then turn yellow and finally brown and dry out (Figure 9). Later the inner leaves develop similar symptoms. The fungus can be isolated easily from petioles and sometimes from leaves.

6) Symptoms on lupine

Young leaflets generally show the trouble first; they become flaccid and droop (Figure 10), occasionally with some degree of twisting and curling. This condition is soon followed by a diffuse chlorosis spreading from veins (Figure 11). The leaflets and petioles gradually collapse and dry out (Figure 12) and later they fall resulting in progressive defoliation. Usually the affected plant develops only to a limited extent and then dies.

The fungus can be readily reisolated from the above ground parts of a diseased plant. Vascular colonization by the fungus is complete.

Figure 9. Healthy check plant, and inoculated sainfoin (Common) showing chlorosis, curling, and browning of leaves.

Figure 10. Healthy check plant, and inoculated lupine with flaccid leaves.

Figure 11. Healthy check plant and inoculated lupine showing yellowing of leaves.

Figure 12. Healthy check plant and inoculated lupine showing shrivelling of leaves.

D. Relation of age of alfalfa plants to infection by isolates of V. albo-atrum and V. dahliae

From our preliminary inoculations in 45-day old plants, it was evident that these were suitable for the study of the relative pathogenicity of different isolates of Verticillium. Then it was decided to study the effect of age of alfalfa plants in relation to infection by isolates of Verticillium.

In the present experiment, plants started at 20°C. were inoculated at ages of 15, 30, 60, 75, 90, 108, and 130 days respectively to learn if age had some effect on the susceptibility of plants. First, plans were made to make inoculation at every 15 days starting with 15-day old plants. Because of an accidental shortage of plants we did not inoculate 45-day old plants and inoculated at 108 and 130 days of age, instead of 105, 120, and 135 as planned.

Plants of the varieties Dupuits and Vernal were inoculated with isolates 1, 2, 3, and 5 of V. albo-atrum and 1 and 3 of V. dahliae. Inoculation was performed by dipping roots for 30 minutes in the macerated fungus inoculum. Ten plants per treatment were grown, two per 4-inch pot, in a greenhouse where the thermostatic controls were set at 70 degrees F. Two non-inoculated plants were used as controls for each treatment.

The results of this experiment are presented in Tables 14 and 15 and are very interesting facts have arisen from them. The amount of infection varied with the age of plants at time of inoculation. The data indicate that infection is minimum when plants are inoculated

when very young (15 days) or old (108 days). Symptoms develop more slowly on plants inoculated when 15 or 108 and 130 days than on plants from 30 to 90 days old, although the symptoms on older plants were more severe than on plants inoculated when younger. There was practically no difference in the reaction of the two varieties.

The two isolates of V. albo-atrum collected in Quebec and the isolate of V. dahliae from Ottawa, were comparable in virulence, to isolates from Great Britain on alfalfa plants at all ages.

Table 14 - Number of plants infected by various Verticillium isolates on alfalfa plants (Dupuits) inoculated at different ages, at 20, 40, and 60 days after inoculation.

Isolate No.	Age of plants (in days) at time of inoculation																							
	15			30			60			75			90			108			130					
	Number of days after inoculation																							
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60			
V. albo-atrum																								
1	1[1]	3	5	9	10	10	8	10	10	6	9	10	10	10	10	2	9	9	5	10	10			
2	2	4	4	10	10	10	10	10	10	6	8	8	9	9	9	3	5	5	2	4	4			
3	0	2	7	3	9	10	2	3	8	0	0	9	5	5	9	0	7	9	1	4	5			
5	6	6	7	3	10	10	4	7	7	4	8	10	10	10	10	2	9	9	5	10	10			
V. dahliae																								
1	0	0	0	1	2	3	1	1	1	1	2	2	5	5	5	0	1	1	0	0	0			
2	0	0	0	0	0	7	2	3	3	4	4	4	3	4	5	0	3	5	1	1	1			

1) Number of plants infected out of 10 plants inoculated.

Table 15 - Number of plants infected by various Verticillium isolates on alfalfa plants (Vernal) inoculated at different ages, at 20, 40, and 60 days after inoculation.

Isolate No.	Age of plants (in days) at time of inoculation																							
	15			30			60			75			90			108			130					
	Number of days after inoculation																							
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60			
V. albo-atrum																								
1	1[1]	4	4	9	10	10	9	10	10	3	5	9	10	10	10	1	8	8	4	9	9			
2	5	9	9	9	10	10	9	10	10	9	10	10	10	10	10	0	6	9	6	10	10			
3	0	2	10	2	8	10	6	8	9	3	4	10	3	4	8	0	3	3	1	5	5			
5	6	7	7	4	9	10	9	9	9	4	8	10	5	8	10	2	7	10	2	6	8			
V. dahliae																								
1	0	0	0	0	1	1	1	1	1	2	2	2	4	4	4	1	2	3	1	2	2			
2	0	0	0	0	1	1	2	2	2	3	3	3	0	0	0	0	0	0	0	0	0			

1) Number of plants infected out of 10 plants inoculated.

VII- ENVIRONMENTAL FACTORS IN RELATION TO WILT INCIDENCE

The importance of environment in the development of soil-borne disease has been extensively explored by many investigators and this phase of research has been given increasing attention. As a consequence, there is well documented proof that environment often determines the geographic distribution, seasonal occurrence, and economic importance of a given disease. In studies of soil-borne diseases caused by the vascular parasites, the reactions to environmental influence have often been very striking. Much attention has been given to this phase of research in the study of Verticillium wilt of forage legumes.

A) Relation of the water content of the soil to the development of the disease

Soil moisture is an influential factor in the ecology of diseases caused by soil-borne organisms. This is sometimes a factor which determines the distribution of diseases with a restricted geographical range. From the literature, it is evident that no one set of moisture conditions can be optimum for Verticillium wilts. The general opinion is that the disease is endemic in areas of high soil moistures and sporadic where there is excessive rainfall in a season, but there is much controversy on the subject in the literature.

Workers on Verticillium wilts of different plants have arrived at varying conclusions on the effect of soil moisture. Bewley (5)

and Williams et al (57) showed that heavy watering increased the intensity of attack in tomato by V. albo-atrum and V. dahliae. Baker and Locke (3) were of the opinion that the epiphytotic of Verticillium wilt of annual crops in Southern California in 1944 was the result of extended cool, cloudy, and humid weather which perhaps maintained a uniform soil moisture. Again, wilt of horse-radish caused by V. dahliae was found by Blattny (7) to be severe in damp soil. Rudolph (40) and Harris (18) observed that damp soil increased the intensity of attack in raspberry and hop respectively. Caroselli (9) working with maple, Rada (38) with cotton, and Vanderwalle and Parmentier (53) with chicory, also found that the disease was most severe when the soil was damp. McKeen (31) believed that Verticillium wilt of various crop plants occurred infrequently in the Niagara Peninsula because soil moisture was low at the time when high summer temperature might have favoured the growth of the fungus. Isaac (22) found that V. dahliae, V. nigrescens, V. nubilum, and V. tricorpus induced external symptoms in anthirrinum only when the soil moisture was high.

On the other hand, contradictory results have been presented in other reports. For instance, Van der Meer (52) studying potato wilt found that damp soil reduced the incidence of disease caused by V. albo-atrum and V. dahliae. Bidwell and Childs (6) for maple trees, and Haenseler (17) and June (27) for stone-fruit trees, reported extensive injury after prolonged dry weather. Strong (49, 50) observed that heavy soaking of the soil beneath infected maple trees in the early stages of wilt resulted in the disappearance of symptoms and stimulated

good growth. Böning (8) working with horse-radish, found that infection by V. dahliae was not severe in damp soil. Ludbrook (30) in experiments with eggplants growing under greenhouse conditions maintained the soil at different percentages of the moisture-holding capacity by adding the required amount of water, and found that soil moistures between 45 and 95 percent of the soil capacity had little effect upon the incidence of Verticillium wilt. Nelson (32) working with peppermint, Schneider (43) working with V. albo-atrum in guayule, and Isaac (20, 22) with V. dahliae in sainfoin and V. albo-atrum in anthirrinum, observed that maximum wilt development occurs in soils of comparatively low moisture content.

The results cited show that there is no agreement on the relation of soil moisture to the development of Verticillium wilts. Some authors believe that the disease is more severe in wet soils and other disagree. Discrepancies in observations and experimental results are possibly the result of lack of evaluation of the effect of soil moisture on the host plant or misidentification of the pathogen.

The present investigation was conducted to determine whether variations in the soil water content affected the incidence of disease in alfalfa. The moisture-holding capacity of the greenhouse soil was determined following a method described by Piper (37). Soil was passed through a 0.5 mm. mesh sieve with round holes. After thorough mixing, the sieved soil was placed in a weighed (W_1) circular metallic box (4" x 1") with a perforated bottom which was lined inside with a Whatman No. 1 filter paper. Care was taken that the soil was

well packed in the container, by adding small quantities of soil and tapping it each time. The metallic box was then placed in a dish containing water which was gradually absorbed by the soil. As the water level in the dish fell, more water was added to keep the level constant. After about 13 hours, the metallic box was gently taken out and all the water adhering to the outer surface of the bottom was wiped off with a piece of filter paper. The container with the saturated soil was again weighed (W_2) and then transferred to an electric oven at 105°C. and kept there in such a position as to allow free access of air to the perforated bottom. A plate of calcium chloride was kept in the oven to absorb the moisture released from the soil. The metallic box with the soil was weighed from time to time until the weight became constant; it was cooled in a desiccator and weighed again, recording the weight as "weight of box and oven dry soil" (W_3). The amount of water absorbed by the filter paper was also determined. Five filter papers were weighed together and then saturated with water. Excess water was removed by placing them on a flat glass plate and gently rolling a glass rod over them. The filter papers were then weighed again to determine the water retained from which the average for one filter paper was calculated (W_4).

Finally, the water holding capacity as percentage of oven dry soil was determined as follows:

$$\frac{W_2 - W_3 - W_4}{W_3 - W_1} \times 100$$

Where W_1 is weight of empty metallic box

W_2 is weight of box and saturated soil

W_3 is weight of box and oven dry soil

W_4 is weight of water retained by one filter paper

The experiment was performed in three sets simultaneously and the average calculated.

The water-holding capacity in three replicate determinations was 57.96, 57.98, and 56.93 percent respectively. With an average value of 57.29 percent

Three moisture levels, i.e. 25, 35, and 45 percent were maintained on oven dry weight basis of soil and ranged well within moisture-holding capacity of the greenhouse soil. "Dixie cups" coated with paraffin wax, having 200 gms. of soil in each, were used. The 210 cups necessary for the experiment were first weighed and the mean was taken as the standard weight of each cup. Plants of Vernal and Dupuits varieties of alfalfa were inoculated at 40 days old by the root dipping method and transplanted, one per pot, in the "Dixie cups". This experiment was performed with 6 isolates and 1 control for each variety. Five replicates (5 cups) were maintained with each isolate and control. This experiment was performed at two different dates in a greenhouse where the thermostatic controls were set at 70°F. Each experiment was concluded 30 days after inoculation. The moisture levels were kept constant by weighing each cup every day and adding water to maintain the original weight. Each cup was placed at random on a table in the greenhouse (See appendix for set up of experiments).

Disease intensities were appraised three times during the study. The height of plants (shoots) was measured the day of inoculation and again when the experiment was terminated, 30 days later. The above-ground parts of the plants (shoots) and the below-ground parts (roots) were carefully washed, oven-dried, and the weights determined on a gram-atic balance.

1) Disease intensity

To determine disease development with time, the intensity of symptoms was appraised following a system described in "MATERIALS AND METHODS". Notes were taken every 10 days, till 30 days after inoculation. The results presented in Figures 13 and 14 are the average of two experiments.

From the results of Figures 13 and 14, it seems that dry soil (25%) favors the development of the disease caused by V. albo-atrum isolates and that soils with higher water content are favorable to V. dahliae isolates. The six graphs of these two figures show very well this differentiation between the two groups of isolates. Isolates nos. 1, 2, and 5 of V. albo-atrum gave drastic results; nos. 1 and 2 of V. dahliae are very mild pathogens, and no. 3 of V. albo-atrum seems to be an intermediate between the two groups at the three soil moisture levels.

V. albo-atrum isolates nos. 1, 2, and 5 have a more pronounced effect at 25% than 45% of soil moisture, on both varieties of alfalfa. Isolate no. 3 of V. albo-atrum is a mild isolate inducing

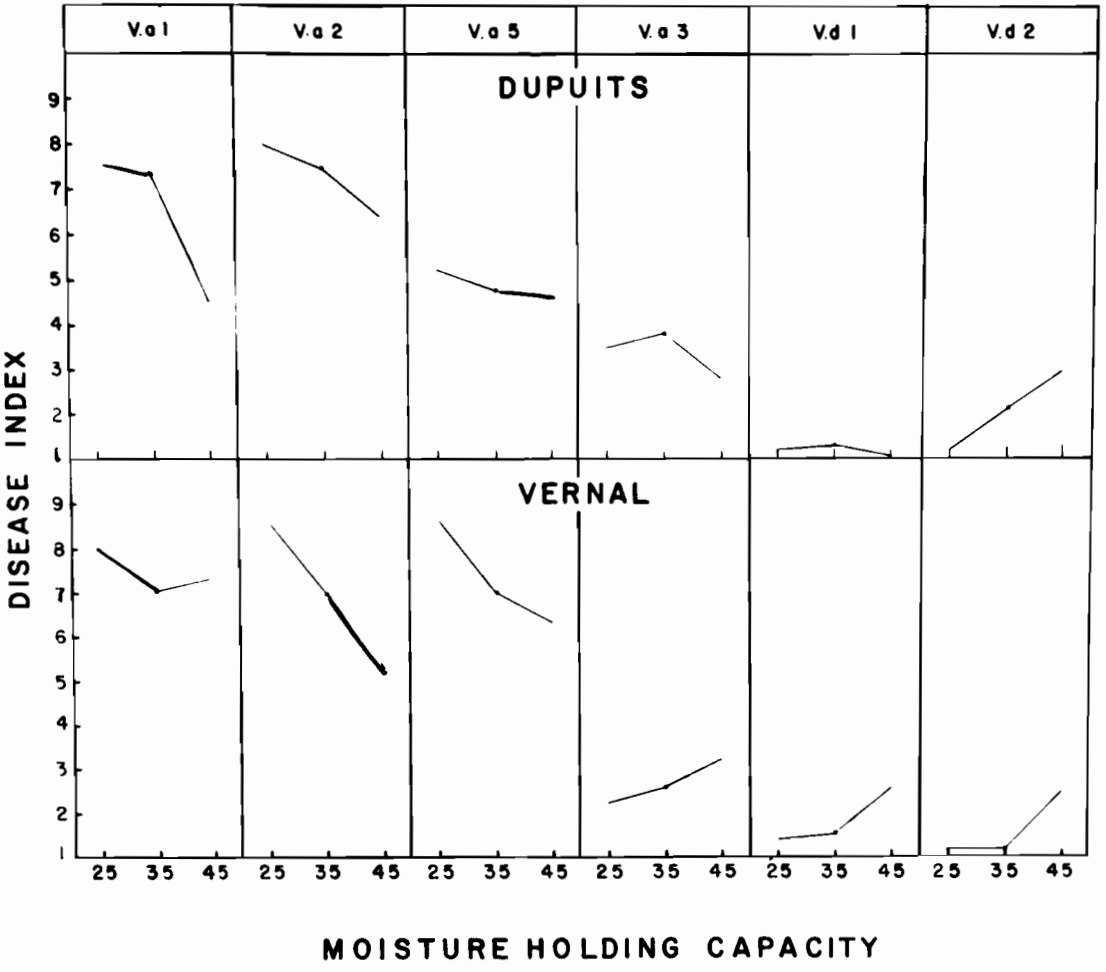

Figure 13. Effect of three soil moisture levels on disease index of two varieties of alfalfa inoculated with various isolates of Verticillium.

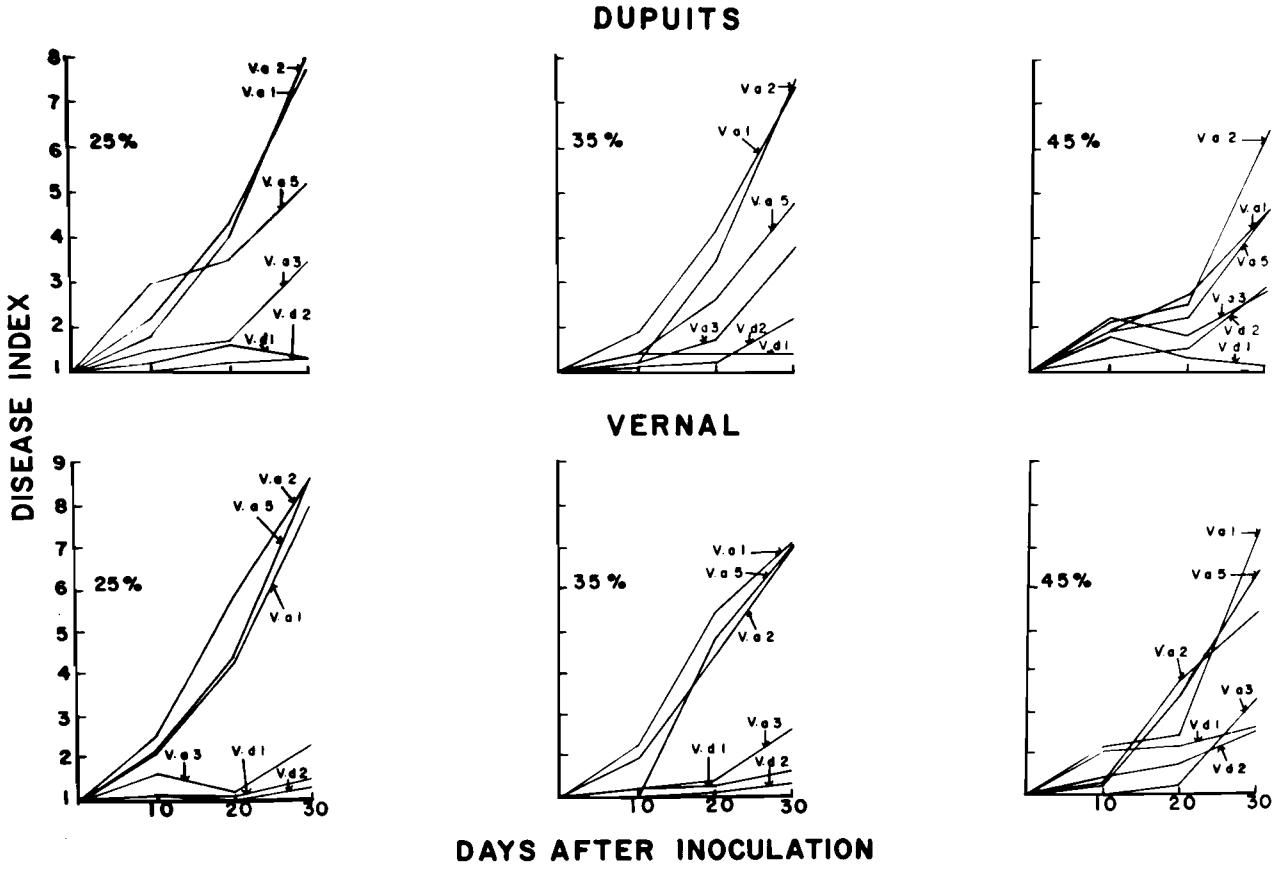

Figure 14. Effect of three soil moisture levels on disease index of two varieties of alfalfa inoculated with various isolates of Verticillium.

symptoms later than the two others. Isolates 1 and 2 of V. dahliae seem to be more pathogenic at 45% than at 25% soil moisture. V. dahliae 1 appeared avirulent on Dupuits but was more virulent than V. dahliae 2 on Vernal.

An interesting fact is that the disease index was higher 10 and 20 days after inoculation than after 30 days in some instances. This was observed with V. dahliae isolate no. 1 on Dupuits at 45%. V. albo-atrum isolate no. 3 on Dupuits at 45% soil moisture and on Vernal at 25% gave a lower disease index at 20 days than at 10, rising again to a maximum at 30 days. In all the other cases, the curve was at its maximum 30 days after inoculation.

2) Increase in length of shoots of alfalfa plants

The increase in length of alfalfa plants was determined by measuring their height from the soil level to the top of the plant, on the date of planting and again at the end of the experiment, 30 days later. The results are given in Figure 15.

The results are difficult to interpret and varietal difference are not consistant and sometimes the results between the two varieties are contradictory.

Uninoculated control plants of both varieties grew equally well at 35% and 45% soil moisture. The data of Figure 15 show clearly that isolates of V. albo-atrum inhibit growth much more than V. dahliae; V. albo-atrum isolates nos. 1 and 2, from Normandin, had the most

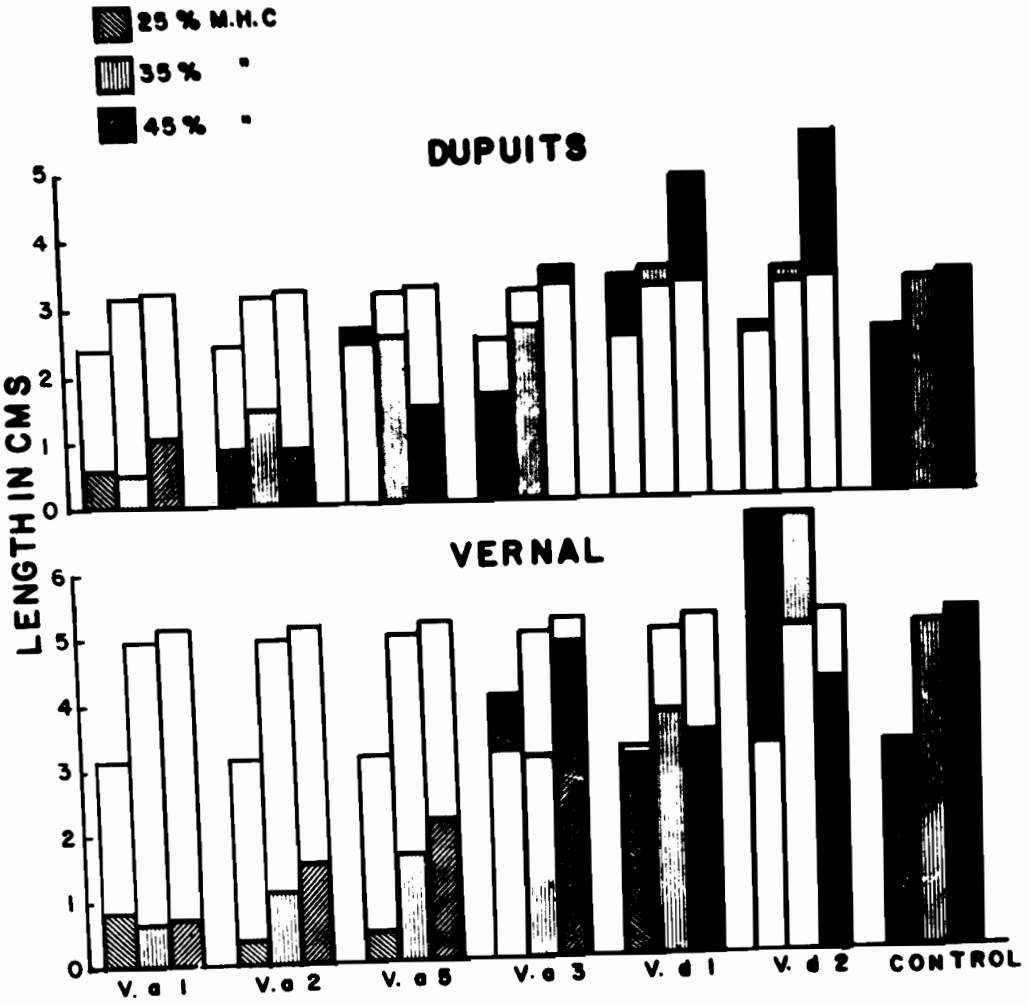

Figure 15. Effect of three soil moisture levels on the increase in length of shoots of two varieties of alfalfa inoculated with various isolates of Verticillium. Unshaded portion of bar in each case represents value for corresponding uninoculated control.

drastic effect.

V. albo-atrum isolates nos. 2 and 5 restricted growth of Dupuits most at 45% moisture-holding capacity; V. albo-atrum no. 1 was equally drastic at all moisture levels. On Vernal, V. albo-atrum 2 and 5 were as injurious at low as at high soil moisture levels, and V. albo-atrum no. 1 again was injurious at all levels.

An interesting fact here encountered is that in some series inoculated plants grew much taller than the corresponding uninoculated controls. It appears that in these cases where the Verticillium isolates were not virulent they exerted a stimulatory effect.

3) Dry weight of shoots of alfalfa plants

To determine the effect of soil moisture on the dry weight of shoots inoculated with various isolates, the following experiment was conducted. Thirty days after transplanting, all the shoots were severed from the roots by cutting them at soil level, they were dried to constant weight in a thermostatically controlled oven at 100°C., usually for about 24 hours. To absorb moisture, a plate of calcium chloride was put in the oven. The shoots were weighed with a gram-atic balance. The results of one experiment are presented in Figure 16.

The graphs in Figure 16 demonstrate that alfalfa plants of both varieties tend to attain maximum dry weight at 35% soil moisture. Isolates of V. albo-atrum, 1, 2, and 5, cause the greatest reduction in dry weight at all moisture levels, with the exception of isolate 5 at 25% soil moisture for the Dupuits variety.

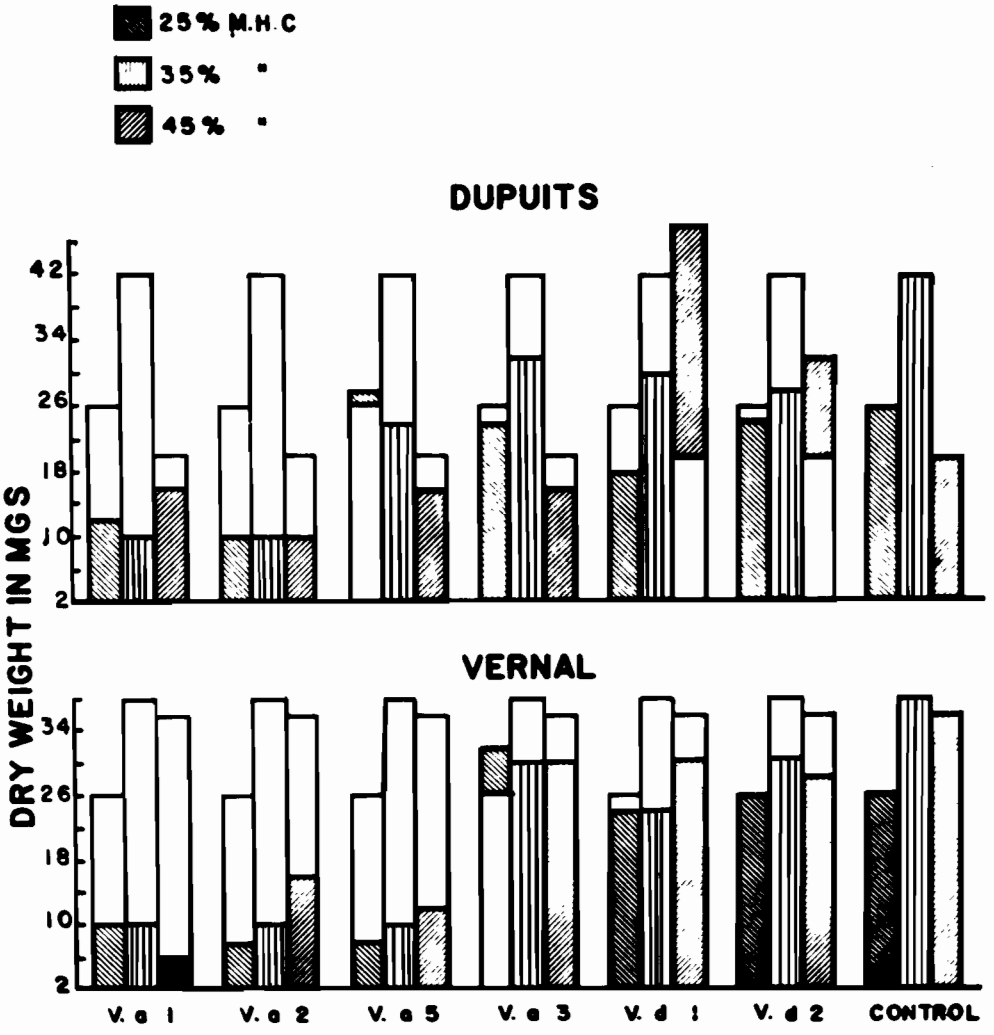

Figure 16. Effect of three soil moisture levels on the dry weight of shoots of two varieties of alfalfa inoculated with various isolates of Verticillium. Unshaded portion of bar in each case represents value for corresponding uninoculated control.

Dry weights of the two varieties were affected differently by the pathogens. The weight of Vernal plants was reduced more than that of Dupuits. This is in agreement with the data for disease intensity; the mean disease index was higher for Vernal than for the Dupuits variety.

On the Dupuits variety, all the isolates of V. albo-atrum and of V. dahliae caused a greater decrease in the dry weight of shoots at 35% than at the other soil moisture levels. Isolates 1 and 2 of V. dahliae seem to have stimulated the growth of Dupuits at 45% soil moisture. The Vernal variety also shows complex results: V. albo-atrum isolates 2, 3 and 5 and isolate 1 of V. dahliae were more injurious at 35% whereas isolate 1 of V. albo-atrum was more pathogenic at 45%. Isolate 2 of V. dahliae was equally injurious at 35% and 45% moisture-holding capacity, and caused no injury at 25%.

The bar graphs in Figure 16 give a good idea of the effects of the pathogens. The dry weights can be correlated with disease intensity, since the effect of the loss of leaves and the reduction of photosynthesis are measured by this method.

4) Dry weight of roots of alfalfa plants

The present investigation was conducted to determine the effect of water content on roots of inoculated plants. Plants were kept for 30 days after inoculation and then the shoot and the root were severed. The plants were removed from the "Dixie cups" by cutting the cups in two parts, then teasing the roots from the soil. The roots

were washed and rinsed in two changes of warm water, then were oven dried in the same way as the shoots. The results of one experiment are given in Figure 17.

It is difficult to give any conclusion from the above results since there are conflicting results. But it seems that when an isolate stops the development of the shoots, it also does the same for the roots. The control plants of Dupuits developed a slightly better root system at 35% than at 25% soil moisture; the Vernal control plants had better root system at 35% and 45% than at 25% soil moisture.

V. albo-atrum isolates 1 and 5 affected roots of Dupuits more at 35% soil moisture than at 25% and 45%. Isolate no. 2 did not show much difference in its action at 25% and 35% soil moisture; isolate no. 1 of V. dahliae reduced the weight of the root system most at 25% soil moisture. Inoculation with V. albo-atrum 5 and V. dahliae 2 isolates increased the dry weight of the root system above that of the control at 25% soil moisture, and isolates 1 and 2 of V. dahliae both increased it at 45%.

Vernal reacted differently than Dupuits. There is no difference in the weight of roots of the controls at 35% and 45% soil moisture. Isolates 1, 2, and 5 of V. albo-atrum interfered most with the development of root system, followed by isolate no. 1 of V. dahliae. Isolates nos. 1, and 3 of V. albo-atrum had most effect on root development at 45%, no. 2 at 35%, and both nos. 2 of V. dahliae and 5 of V. albo-atrum induced equal effects at 35% and 45%. The dry weight of roots was greater than that of the control when inoculated with V. dahliae

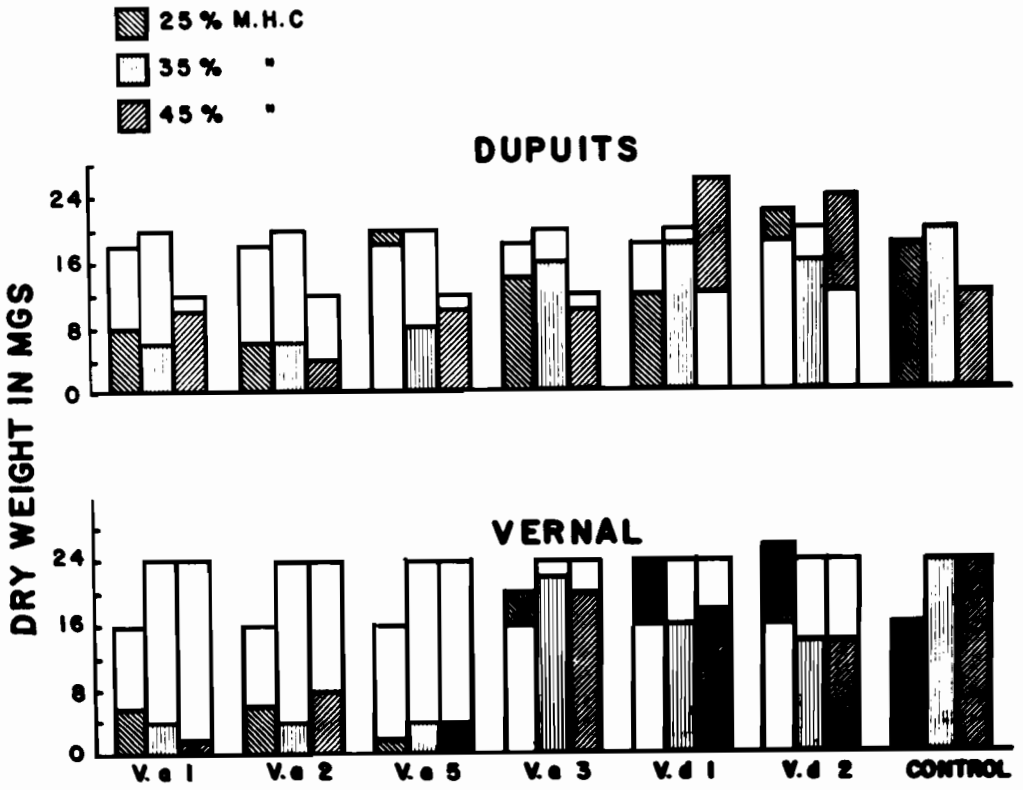

Figure 17. Effect of three soil moisture levels on the dry weight of roots of two varieties of alfalfa inoculated with various isolates of Verticillium. Unshaded portion of bar in each case represents value for corresponding uninoculated control.

no. 2 at 25% soil moisture. This isolate is the only one which had the same effect on both alfalfa varieties.

B) Relation of air temperature to the development of the disease

Workers on Verticillium wilts of plants have arrived at different conclusions on the effect of air temperature on the disease. Edson and Shapovalov (14) dealing with Verticillium wilt of potato suggested that there were two strains of the fungus, one found in the northern regions of the U.S.A. and the other in the southern area. The southern strain, which formed microsclerotia in culture, showed a better adaptation to higher temperatures and grew fairly well at 30°C., while the northern strain in which microsclerotia were practically absent adapted itself more readily to lower temperatures and did not grow at 30°C. Nielsen (33) working with V. albo-atrum of potato, noticed that infection is dependent upon early summer temperatures. In 1945, in Idaho, when the early growing season was cool, the disease appeared late and was of minor importance. In 1946, when the early summer was much warmer, the disease developed generally in epiphytotic proportions and the vines in numerous potato fields were dead by September 1. Bewley (5) stated that in greenhouses in England, the months of June, July, and August were unfavourable to the rapid progress of the disease on tomatoes, and he suggested that control could be effected by raising the temperature of the tomato-house above 15°C. He did not differentiate between his isolates and simply referred to his

organism as V. albo-atrum. Later, Williams (57) presented experimental evidence indicating that infection of a variety of tomato by V. albo-atrum was checked by warm conditions, and that by V. dahliae somewhat less so. Low temperatures were also found to favour infection of cucumbers by V. albo-atrum. Ludbrook (30) differentiating between the two types, reported that V. dahliae induced disease symptoms in eggplant at soil temperatures of 12°-30°C., but not at 32°C., whereas V. albo-atrum caused disease at 28°C. and below but not at 30°C., the optimum temperature in each case being between 19 and 23°C. Robinson et al (41), working with potato, found that V. dahliae (pseudosclerotial type) was most pathogenic at 24° and 28°C., whereas V. albo-atrum (dark mycelium) caused more disease at 16° and 20°C. Isaac (21) working with sainfoin and tomato found that the microsclerotial form of Verticillium was pathogenic to these two plants at 25° and 27°C., but at these temperatures the dark mycelium form did not induce wilt. Arndt (2) found that V. albo-atrum did not produce lesions on cotton seedlings at 24°C. and higher temperatures; and at lower temperatures produced small lesions on only a small percentage of the seedlings. Other workers, like Stepantsev (48) working with cotton and Osmun (35) with eggplant and Edgington et al (13) with tomato agree that V. dahliae is more effective in its pathogenicity at relatively high temperature than V. albo-atrum.

To determine the relation between temperature of air and the pathogenicity of the Verticillium isolates nos. 1, 2, 3, and 5 of V. albo-atrum and V. dahliae 1 and 2 on Dupuits and Vernal alfalfa

varieties, experiments were carried out in three growth chambers maintained at 15, 20, and 25°C. respectively. These temperatures are within the growth range of the isolates used.

Plants were inoculated when 40 days old by the root dipping method and transplanted one per Dixie cup.

There were five replicates (5 cups) for each isolate and the control. The whole experiment was repeated after an interval of several weeks. The cups were placed at random within the growth chambers. Observations were taken on disease intensity, length of plants, and dry weight of roots and shoots as described in the previous section. All the results presented are the average of two experiments and all the techniques used were the same as in the soil moisture studies.

1) Disease intensity

The results of Figures 18 and 19 show good uniformity in the behaviour of the two varieties. The two varieties reacted much alike to all the respective isolates. V. albo-atrum isolates gave higher disease indices than V. dahliae, and the index tended to be somewhat higher in some instances at 15°C. or 20°C. than at 25°C. The disease index was relatively higher at 25°C. than at 15° or 20°C. for both isolates of V. dahliae. Disease indices were slightly on Vernal than on Dupuits. Symptoms developed more rapidly on Vernal than on Dupuits.

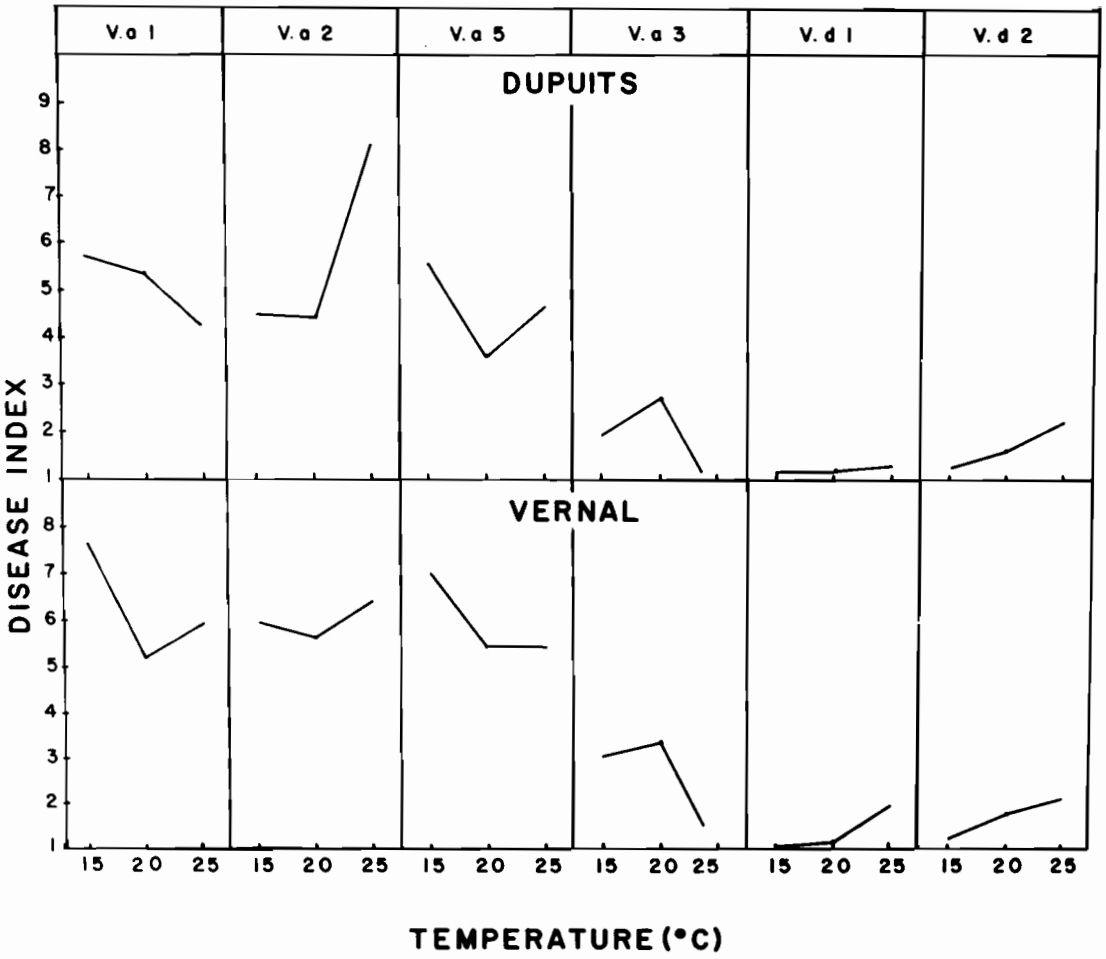

Figure 18. Effect of three temperatures on disease index of two varieties of alfalfa inoculated with various isolates of Verticillium.

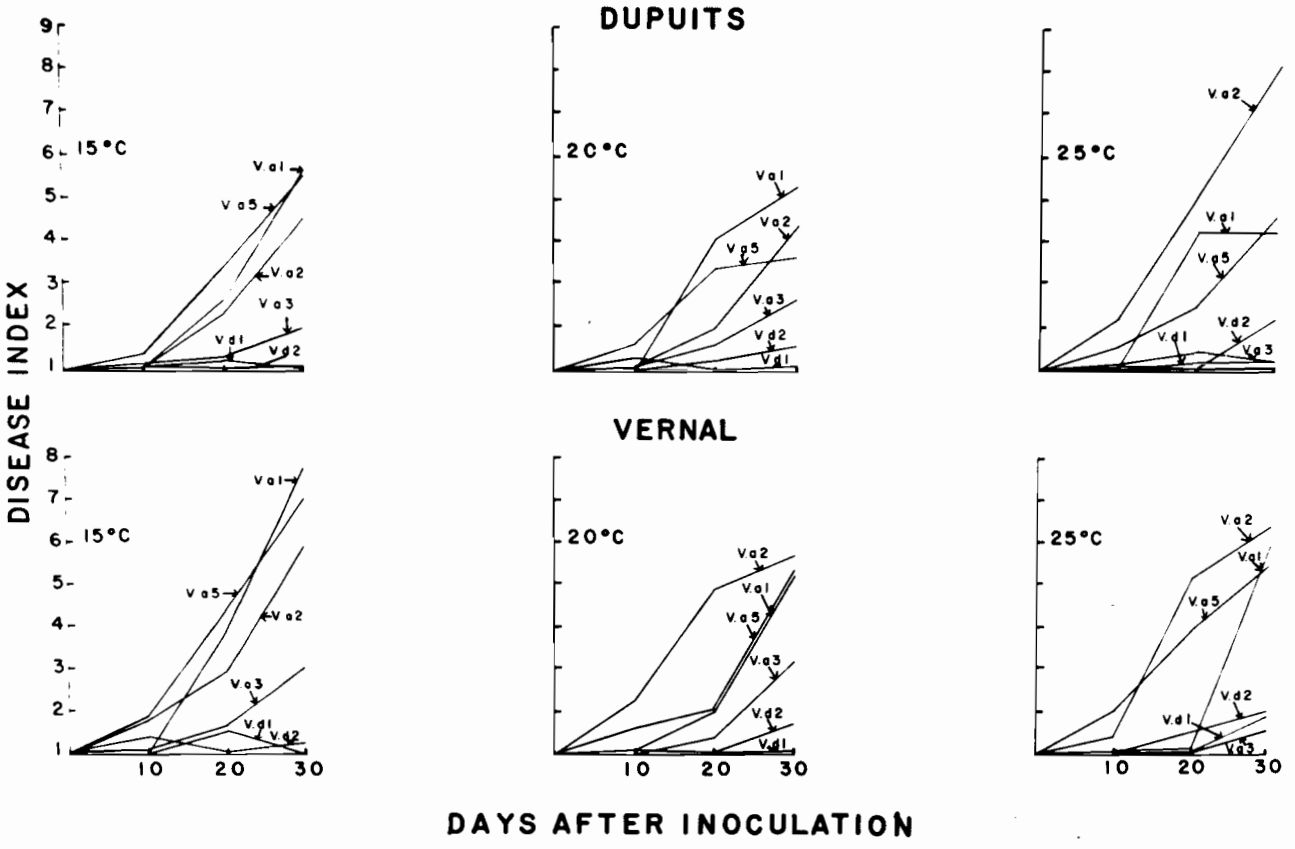

Figure 19. Effect of three temperatures on disease index of two varieties of alfalfa inoculated with various isolates of Verticillium.

2) Increase in length of shoots of alfalfa plants

As shown in Figure 20, the most suitable temperature for the increase in the length of plants is 20°C., regardless of variety and inoculation. Both varieties responded alike to the three temperatures. The isolates of V. albo-atrum had more effect on plant length than did those of V. dahliae. V. albo-atrum isolates nos. 1 and 2 were most injurious than nos. 5 and 3. V. dahliae isolates nos. 1 and 2 were alike in their effects.

Isolates of V. albo-atrum had their greatest effect on the length of inoculated plants at 20°C. Vernal was affected adversely by all isolates of V. albo-atrum at all temperatures. Dupuits was affected little or not at all by V. albo-atrum isolate no. 1 at 15°C. and by V. albo-atrum nos. 5 and 3 at 25°C. V. dahliae isolates 1 and 2 both reduced length of shoots of Dupuits slightly at 20°C., but increased length over that of uninoculated controls at 15° and 25°C., and increased the length of Vernal at all three temperatures.

3) Dry weight of shoots of alfalfa plants

The data given in Figure 21 demonstrate that the dry weight of shoots of Dupuits was decreased most at 20°C. by V. albo-atrum isolates 1 and 2 and was reduced by V. albo-atrum at 25°C. There was little effect at 15° and 25°C. with V. albo-atrum isolates 1 and 5, and an increase at all temperature with V. albo-atrum 3.

The dry weight of Vernal shoots was decreased by all four

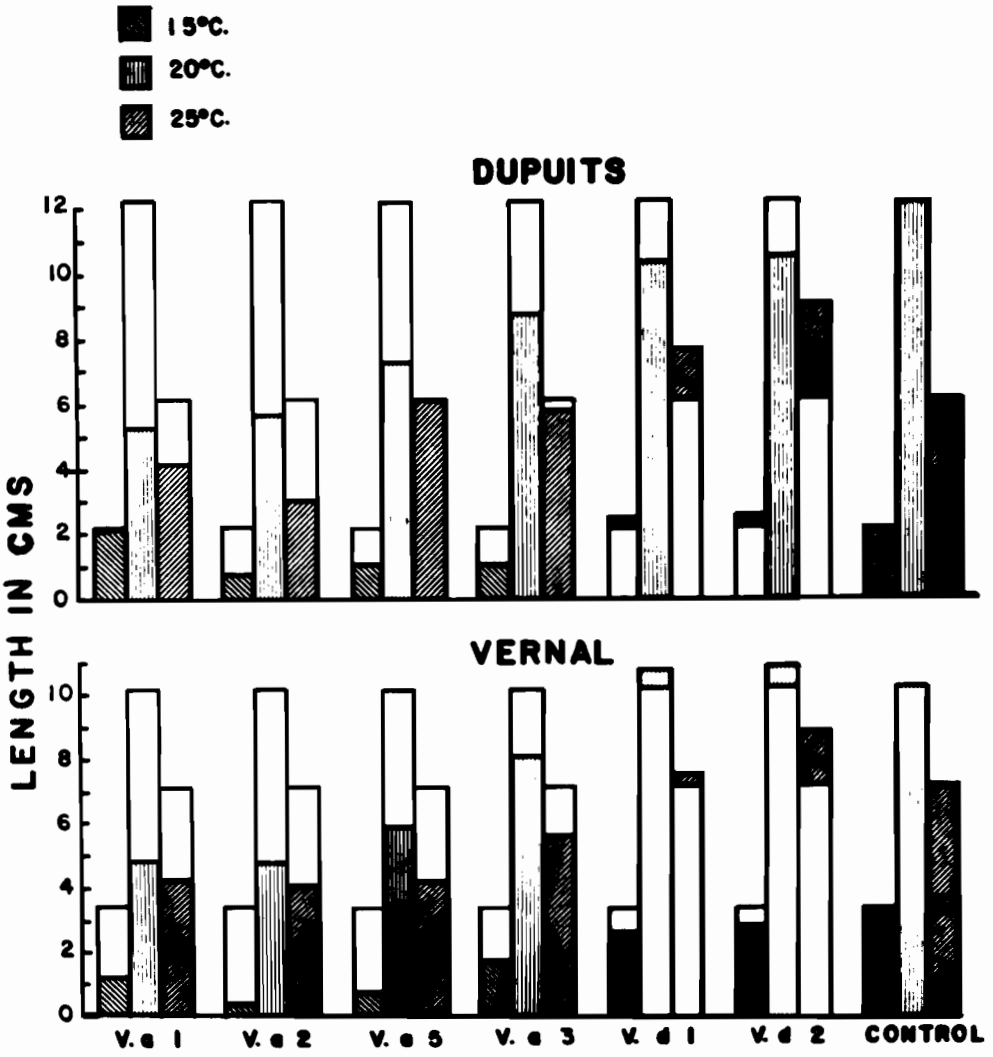

Figure 20. Effect of three temperatures on the increase in length of shoots of two varieties of alfalfa inoculated with various isolates of Verticillium. Unshaded portion of bar in each case represents value for corresponding uninoculated control.

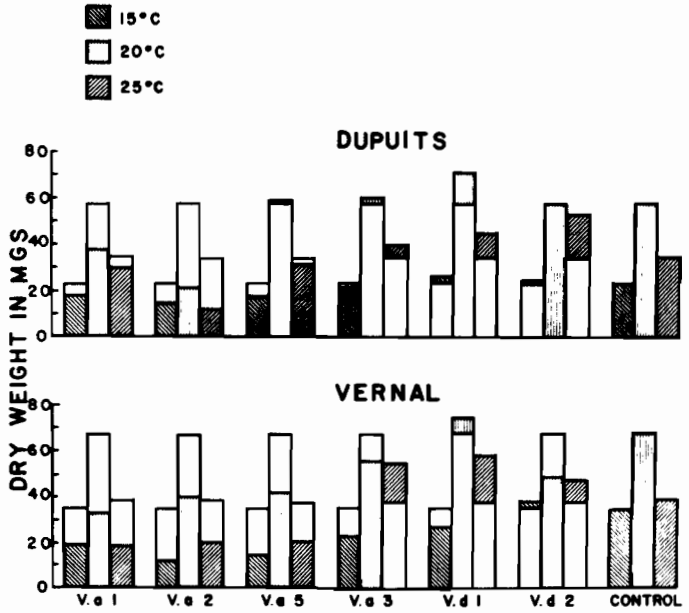

Figure 21. Effect of three temperatures on the dry weight of shoots of two varieties of alfalfa inoculated with various isolates of Verticillium. Unshaded portion of bar in each case represents value for corresponding uninoculated control.

isolates of V. albo-atrum at all temperatures, except 25°C., where V. albo-atrum 3 induced an increase. V. dahliae isolate 1 caused a slight decrease in shoot weight of Vernal at 15°C. and V. dahliae 2 at 20°C.; the weights were increased by inoculation at the other temperatures, and in all cases on Dupuits.

These results, like those for effects of soil moisture, indicate that disease intensity and the dry weights of shoots are two measures of disease effect which appear closely correlated.

4) Dry weight of roots of alfalfa plants

The results presented in Figure 22 show that 20°C. is the temperature which favors the development of the root system of alfalfa plants. These results also demonstrate that the isolates of V. albo-atrum affect the dry weight of roots more than do those of V. dahliae, and that isolates of V. albo-atrum are injurious at the three temperatures employed.

The dry weights of roots of both varieties were decreased by all isolates of V. albo-atrum at 20°C.; reductions were also induced at 15°C. by all isolates, except V. albo-atrum no. 3 on Dupuits, and small reductions at 25°C. except by V. albo-atrum no. 1 on Dupuits, and by V. albo-atrum no. 3 on Vernal where there were slight increases in weight.

V. dahliae isolate 1 reduced dry weight of Dupuits shoots at 15° and 20°C., and increased them slightly at 25°C.; V. dahliae 2 increased weight of Dupuits slightly at all temperatures. Weight of

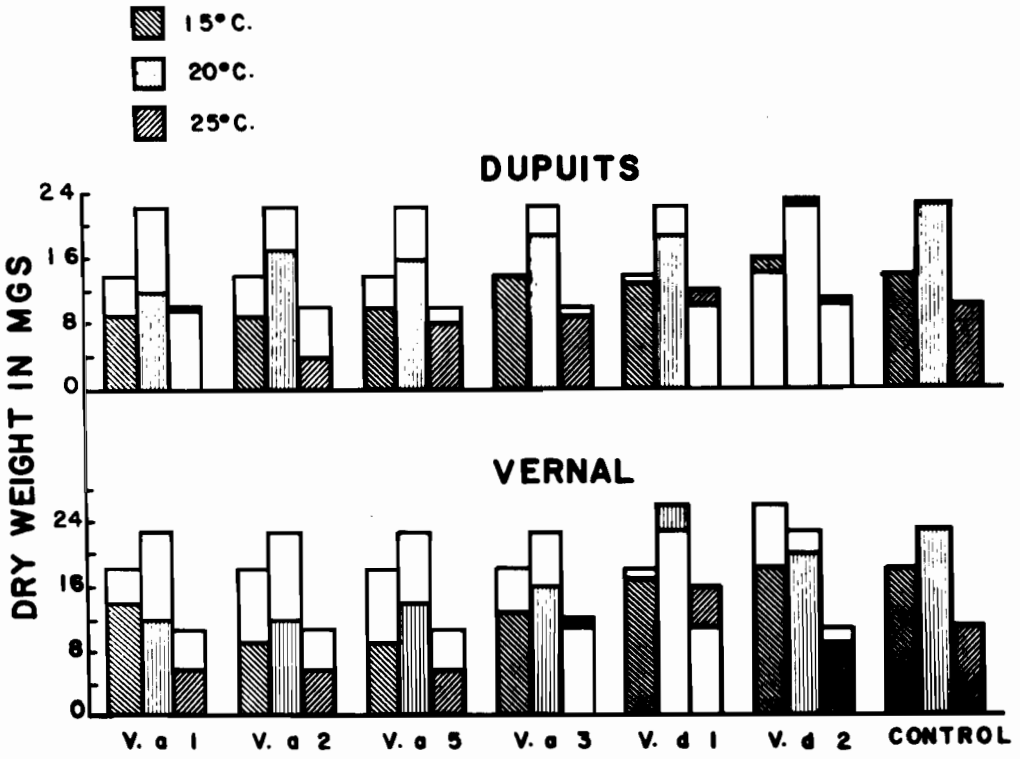

Figure 22. Effect of three temperatures on the dry weight of roots of two varieties of alfalfa inoculated with various isolates of Verticillium. Unshaded portion of bar in each case represents value for corresponding uninoculated control.

Vernal shoots was decreased slightly by V. dahliae 1 at 15°C., increased slightly at 20° and 25°C., and decreased slightly by V. dahliae 2 at all temperatures.

VIII- DISCUSSION AND CONCLUSIONS

Surveys on farm fields of forage legumes in the Province of Quebec have given us the chance to learn something about these crops. The presence of snow as a covering appears to be very important for the survival of leguminous plants. No winter-killing occurred in legume plots at Normandin, where the fall of snow was very abundant, and at Caplan plant survival was much better near fences where the snow had accumulated.

Plants which had been "winter-killed" were collected on all the farms visited during the first survey made in June, and cultures of Verticillium were not isolated. During the survey in the month of August, V. albo-atrum was isolated from plants of alfalfa and ladino clover collected at Normandin, and V. dahliae from red clover plants collected at the Central Experimental Farm, Ottawa.

Two conclusions may be drawn from the isolation of Verticillium in August and not in June, and from the occurrence of V. albo-atrum at Normandin, and V. dahliae at Ottawa. The finding of Verticillium isolates in August might have been expected, since it has been reported that the symptoms on alfalfa are more severe towards the end of the season (58). The occurrence of V. albo-atrum at Normandin and V. dahliae at Ottawa may be a result of the temperature relationships of the two species. The mean temperature during July at Ottawa was 68.6°F., and at Bagotville, in the region of Lake St. John, it was 63.8°F.

Cultures of Fusarium were the fungi most commonly isolated from the materials collected; F. culmorum and F. avenaceum were the species most frequently isolated. The role of Fusarium species in the root disease complex may be that of a weak pathogen capable of doing damage when the plant has been weakened by other factors, since these fungi were commonly isolated from dead or rotten plant material.

Interesting observations were made during the studies on the effect of temperatures on the mycelial growth of different isolates. The cultures of V. albo-atrum type generally grew better at lower temperatures than did cultures of V. dahliae. Isolates of V. dahliae grew well at 30°C. and those of V. albo-atrum slowly. These differences in temperature response are in accord with other reports (21, 30). A reduction of pseudosclerotial formation at 30°C. was noted with isolates of V. dahliae, nos. 1 and 3. This fact has been previously reported (41, 55) and Wilhelm (56) states that it weakens the reliability of species separation based on this character.

The presence of two growth peaks, at 30° and 20°C., with isolates 1 and 3 of V. dahliae is difficult to explain since usually a fungus has a minimum, a maximum, and an optimum temperature for growth. In the present case, the growth in diameter of the colony was measured; this kind of measurement is valid only to a limited extent because it does not consider the mass of mycelium. At 20° and 30°C. these strains of V. dahliae spread faster on the agar than at 25°C., but at 25°C. there was more aerial growth than at 20° and 30°C., a factor which was not measured in our studies. If dry weight of the colonies had been

measured, it is possible that there would have been only one growth peak.

In the pathogenicity trials both V. albo-atrum and V. dahliae isolates from forage legumes were strongly pathogenic to lupine and sainfoin, but isolates of V. albo-atrum were more pathogenic to alfalfa, birdsfoot trefoil, and tomato than were those of V. dahliae. The isolates of V. dahliae were more virulent to red, alsike, and ladino clovers than were those of V. albo-atrum. The results obtained with sweet clover are not very significant since only one isolate of each species was able to attack only a few plants. The isolates of V. albo-atrum, nos. 1 and 2, collected at Normandin were as pathogenic to birdsfoot trefoil, red, alsike, and ladino clovers and lupine as any other isolates, but they were less virulent to alfalfa than the European isolates 3, 4, and 5. The isolate 1 of V. dahliae from red clover collected at Ottawa was appreciably more pathogenic to alfalfa than the two European isolates of V. dahliae. These results do not agree with those obtained by Isaac (24) who tested isolates of V. albo-atrum and V. dahliae from alfalfa on clover (Dorset Marl, Merker, Double Cut American, Italian Broad Red, American Mammoth, and S 123), sainfoin (Common), and tomato (Kondine Red) and found that they were very pathogenic to tomato and could not attack sainfoin and clover. The varieties used in the tests at Macdonald College may have accounted for the different results. The varieties used for determining the host range of Verticillium isolates may be a very important factor.

The results obtained indicate that most forage legumes, with

the possible exception of sweet clover, may be attacked to a certain extent by isolates of Verticillium from any forage legume. The apparent inability of V. albo-atrum isolates 7 and 8 from potato and tomato, and V. dahliae no. 4 from sunflower, to attack alfalfa is encouraging. It explains the failures reported by various workers in their attempts to infect forage legumes with Verticillium isolates from other crops. If isolates from most other hosts are unable to infect forage legumes, then Verticillium wilt of these crops may be kept within limits by preventing or showing the spread of strains which attack them.

Symptoms obtained on inoculated plants are generally those of a true hadromycotic disease on alfalfa, birdsfoot trefoil, sainfoin, and lupine. The symptoms on clover differ from those on alfalfa and are variable, sometimes differing from plant to plant within the same treatment. The symptoms on alfalfa wilt can be confused with those caused by Corynebacterium insidiosum (McCulloch) Jensen, the causal organism of bacterial wilt of alfalfa, and those on alfalfa and clovers can be confused with those caused by Fusarium species or boron deficiency.

The study of the effect of age of alfalfa plants on the pathogenicity of different isolates has demonstrated that both Vernal and Dupuits are less susceptible when they are young, and relatively old. Both varieties were most heavily infected when 90-day old plants were inoculated. Both isolates of V. dahliae have failed to attack plants of the two varieties when they were 15 days old. It has

appeared that old plants which became infected showed much more pronounced yellowing of leaves than younger plants. It would have been interesting to continue the tests with still older plants, but none were available. This apparent resistance of older plants may be due to heavier lignification of older roots and consequent inability of Verticillium to penetrate them.

The three levels of soil moisture-holding capacity of greenhouse soil, 25%, 35%, and 45%, can be described as dry, normal, and wet soil respectively. The effects of moisture content are of interest since an increase or a decrease may favor or slow down the growth of the fungus. This is well shown by the two species of fungi: V. albo-atrum isolates induced more severe external symptoms when the soil moisture was low with the exception of isolate 3 on Vernal alfalfa, where the disease index was higher at 45% soil moisture. The symptoms induced by isolates of V. dahliae were more pronounced at 45% than at other soil moisture levels, except for isolate 1 on Dupuits where the disease index was lower at 45%.

Why did these two groups of Verticillium isolates react differently? The host-parasite balance may have been upset by the effect of these conditions on the fungus, or perhaps by a change in the metabolism of the host resulting in reduced resistance. This should be investigated further in future.

The effect of moisture content of soil on increase in length of plants and on the dry weight of shoots and roots was similar in most cases. The results show that almost all isolates affected the

dry weight of shoots and roots and the increase in length of plants of both varieties more at 35% and 45% than at 25%. The increase in length of Dupuits was affected by isolate 3 of V. albo-atrum most at 25%, and the dry weight of roots of the same variety was reduced by isolates nos. 2 of V. albo-atrum and 1 of V. dahliae most at 25%.

A most interesting finding is that when plants were not injured by Verticillium isolates, their dry weights or lengths were greater than that of the checks in some cases. In these instances, the avirulent isolates appeared to stimulate growth in some way.

In the experiments on soil moisture relationships, the data for disease intensity, shoot length, and dry weight of shoots and roots are not in very close agreement. One can study the effect of specific soil moisture levels on the development of the disease by a method of disease appraisal or by quantitative measurements, such as number of plants wilted out of a certain number of inoculated plants, as did Isaac (22) . Both kinds of data are interesting and useful, and the choice depends on the information desired. As far as we are concerned, the dry weight of shoots is very important since it is affected by the falling of petioles and leaves. Judging by our results, roots seem to be affected as much as shoots, since their weights follow almost the same pattern. The dry weight of shoots is greater than that of roots.

The results of our studies show that the effect of temperature on Verticillium wilt of alfalfa differs according to the species of Verticillium involved. Isolates of V. dahliae induce more external symptoms at 25°C.; isolates of V. albo-atrum are favored by lower

temperatures with the exception of isolate 2, which gave a higher disease index on alfalfa plants at 25°C. These results correspond with the reports of Williams (57), Ludbrook (30), Robinson (41), Stepantsev (48), Osmun (35), and Edgington (13), working respectively with tomato, eggplant, potato, cotton, eggplant and tomato, that V. dahliae caused more severe symptoms than V. albo-atrum at relatively high temperatures.

There seems to be a good correlation between the effect of temperature on the increase in length of plants, and on the dry weights of shoots and roots. The growth of Dupuits was affected most adversely at 20°C. by most isolates, except that isolate 1 of V. dahliae stimulated plant growth at 15° and 25°C., and isolate 2 of V. dahliae at all three temperatures. The variety Vernal reacted differently; most of the isolates affected it most adversely at 20°C., but isolate 1 of V. dahliae was more injurious at 15°C., and isolate V. dahliae 2 varied in its activity.

These results were unexpected since they do not show the distinction between temperature effects on the activity of the two species which has been reported in the literature. This discrepancy may be explained by the fact that the range of temperature was not wide enough to distinguish effects on the two groups of isolates. Our results for the effects of temperature on the severity of symptoms induced by the two species are in accordance with those of many other workers. Apparently no earlier investigators have determined the effect of Verticillium infection on the increase in length of plants, on the dry

weight of roots and shoots, and the influence of temperature in producing these effects.

In conclusion, this work has demonstrated that species of Verticillium capable of attacking forage legumes are present in the Province of Quebec. Studies in controlled environments should be extended to include wider ranges of temperature and soil moisture, and the results should be analysed statistically for greater reliability. Further studies are planned to find why Verticillium wilt of leguminous plants is not yet prevalent in North America, and particularly in Quebec.

IX- SUMMARY

Verticillium albo-atrum was isolated from diseased plants of alfalfa and ladino clover, and Verticillium dahliae was isolated from wilted red clover plants. This is the first time that Verticillium has been isolated from ladino clover.

The fungi were grown on P.D.A. at various temperatures. Isolates of V. albo-atrum grew much more slowly at 30°C. than the isolates of V. dahliae, but more rapidly at 7.5°C.

Alfalfa, red, alsike, ladino, and sweet clovers, sainfoin, lupine, birdsfoot trefoil, and tomato plants were inoculated. Isolates of V. albo-atrum and V. dahliae from forage legumes were strongly pathogenic to lupine and sainfoin. Isolates of V. albo-atrum were more pathogenic to alfalfa, birdsfoot trefoil, and tomato than were those of V. dahliae. Isolates of V. dahliae were more virulent on red, alsike, and ladino clovers than were the isolates of V. albo-atrum.

Young (15 days) and relatively old plants (108 and 130 days) were less susceptible to all isolates of Verticillium than were plants of intermediate age.

The symptoms of wilt were described on the various leguminous plants and in most cases were those of a typical hadromycotic disease. Those obtained on clovers were different in some respects.

Studies in controlled environments showed that a soil with low moisture content favors the development of symptoms on alfalfa plants inoculated with V. albo-atrum, and that high soil moisture favors

V. dahliae. The increase in length of plants, and the dry weight of shoots and roots, are affected most adversely when plants inoculated either species are maintained at 35% soil moisture.

A temperature of 15°C. is most favorable for the induction of symptoms on alfalfa by V. albo-atrum and 25°C. for V. dahliae. The most drastic reductions in the length of plants and the dry weight of shoots and roots occurred usually at 20°C. Some isolates of V. dahliae appear to have stimulated the growth of Vernal alfalfa at various temperatures.

It has been shown that strains of Verticillium capable of attacking leguminous plants occur in Quebec and that there is need for more research on this problem.

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XI - APPENDIX

Description of experimental design of soil moisture and temperature experiments

In these experiments, the two varieties (Dupuits and Vernal) and the seven inoculation treatments (six isolates of *Verticillium* and the uninoculated controls) were completely randomized in each of five replicates within each temperature or each soil moisture level. As the respective temperatures were maintained in different controlled-environment cabinets, it was not possible to randomize the temperature treatments. The respective soil moisture levels also were maintained as blocks, to facilitate bringing all the Dixie cups within each moisture level treatment to the same constant weight by adding water daily.

When the temperature and soil moisture experiments respectively were repeated, the same design was used but the individual inoculations and varieties were re-randomized. The randomization for the first temperature experiment is shown in Tables 1, 2, and 3, and for the first soil moisture experiment in Tables 16, 17, and 18. In the tables of randomization, "V" refers to Vernal and "D" to Dupuits. The numbers have the following meanings:

1- Control

2- Inoculated with isolate 1 *V. albo-atrum*

3- " " " 2 " "

4- " " " 1 *V. dahliae*

5- " " " 3 *V. albo-atrum*

6- Inoculated with isolate 2 *V. dahliae*

7- " " " 5 *V. albo-atrum*

Thus, in Table I the first number of the first line (2 V) means that at that position there was a dixie cup containing one plant of the variety Vernal inoculated with *V. albo-atrum* isolate 1.

Appendix Table I - Randomization at 15°C.

2 V	7 V	1 V	3 D	5 V
7 V	1 V	6 D	6 V	1 V
6 D	5 D	7 D	3 V	7 V
1 D	1 D	3 V	5 V	6 V
5 V	6 D	5 D	4 D	1 D
5 D	6 V	6 V	5 D	7 D
7 D	3 D	2 D	1 V	4 D
6 V	2 V	3 D	7 D	3 D
3 V	5 V	7 V	1 D	4 V
1 V	4 V	4 V	2 V	2 V
3 D	2 D	5 V	6 D	5 D
4 D	3 V	2 V	4 V	6 D
2 D	4 D	1 D	7 V	2 D
4 V	7 D	4 D	2 D	3 V

Appendix Table 2 - Randomization at 20°C.

7 D	6 D	6 V	2 D	7 V
7 V	4 D	3 D	5 V	1 D
6 V	3 D	7 D	7 D	2 D
2 V	6 V	4 V	6 D	1 V
5 D	7 V	6 D	4 D	4 V
3 V	2 V	5 D	7 V	6 V
2 D	1 D	5 V	6 V	5 V
1 D	5 V	2 V	2 V	4 D
4 V	1 V	1 D	1 D	3 V
5 V	3 V	7 V	3 V	3 D
1 V	5 D	2 D	3 D	2 V
6 D	7 D	4 D	1 V	5 D
4 D	4 V	3 V	4 V	6 D
3 D	2 D	1 V	5 D	7 D

Appendix Table 3 - Randomization at 25°C.

6 V	4 V	6 D	7 D	3 D
2 V	3 V	5 D	3 V	7 D
1 V	6 V	4 V	5 D	6 V
5 V	5 V	2 V	1 V	4 D
3 V	7 D	5 V	2 V	3 V
7 V	1 V	7 V	4 V	5 V
5 D	4 D	6 V	2 D	1 D
4 D	2 D	4 D	6 D	2 D
1 D	6 D	3 D	4 D	5 D
4 V	2 V	7 D	7 V	6 D
3 D	3 D	1 D	3 D	2 V
2 D	7 V	1 V	5 V	4 V
7 D	1 D	2 D	1 D	1 V
6 D	5 D	3 V	6 V	7 V

Appendix Table 4 - Disease indices on Dupuits alfalfa at 15°C.

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	1.2	1.0	1.0	1.2	1.0	1.0
"	2	1.0	1.2	1.2	1.8	1.4	1.4	1.4
Average		1.0	1.2	1.1	1.4	1.3	1.2	1.2
20	1	1.0	2.4	2.4	2.8	1.8	1.0	1.2
"	2	1.0	2.8	2.2	4.0	1.0	1.6	1.0
Average		1.0	2.6	2.3	3.4	1.4	1.3	1.1
30	1	1.0	5.6	6.4	6.4	1.6	1.0	1.4
"	2	1.0	5.8	2.6	4.8	2.2	1.2	1.0
Average		1.0	5.7	4.5	5.2	1.9	1.1	1.2

Appendix Table 5 - Disease indices on Dupuits alfalfa at 20°C.

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	1.0	1.2	2.2	1.2	1.2	1.0
"	2	1.0	1.0	1.0	1.0	1.0	1.4	1.0
Average		1.0	1.0	1.1	1.6	1.1	1.3	1.0
20	1	1.0	3.2	1.6	4.6	1.8	1.0	1.0
"	2	1.0	5.2	2.2	2.0	1.4	1.0	1.4
Average		1.0	4.1	1.9	3.3	1.6	1.0	1.2
30	1	1.0	4.2	4.6	4.6	4.4	1.2	1.0
"	2	1.0	6.6	4.2	2.6	1.0	1.0	2.0
Average		1.0	5.4	4.4	3.6	2.7	1.1	1.5

Appendix Table 6 - Disease indices on Dupuits alfalfa at 25°C.

Days after inocl.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	1.0	3.6	2.0	1.4	1.0	1.0
"	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Average		1.0	1.0	2.3	1.5	1.2	1.0	1.0
20	1	1.0	1.4	7.2	4.0	1.8	1.4	1.0
"	2	1.0	7.2	3.2	1.0	1.0	1.0	1.0
Average		1.0	4.3	5.2	2.5	1.4	1.2	1.0
30	1	1.0	1.2	9.0	4.4	1.4	1.4	3.4
"	2	1.0	7.4	7.4	5.0	1.0	1.0	1.0
Average		1.0	4.3	8.2	4.7	1.2	1.2	2.2

Appendix Table 7 - Disease indices on Vernal alfalfa at 15°C.

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	1.0	2.4	2.4	1.2	1.0	1.6
"	2	1.0	1.0	1.0	1.2	1.0	1.0	1.2
Average		1.0	1.0	1.7	1.8	1.1	1.0	1.4
20	1	1.0	2.0	3.0	3.8	2.0	1.2	1.2
"	2	1.0	5.6	2.8	5.0	1.4	1.8	1.0
Average		1.0	3.8	2.9	4.4	1.7	1.5	1.1
30	1	1.0	8.0	7.4	6.0	5.0	1.0	1.6
"	2	1.0	7.4	4.4	8.0	1.0	1.0	1.0
Average		1.0	7.7	5.9	7.0	3.0	1.0	1.3

Appendix Table 8 - Disease indices on Vernal alfalfa at 20°C.

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V. d 1	V.d 2
10	1	1.0	1.4	3.4	1.4	1.0	1.0	1.0
"	2	1.0	1.0	1.4	1.8	1.0	1.0	1.4
Average		1.0	1.2	2.4	1.6	1.0	1.0	1.2
20	1	1.0	2.8	6.8	3.6	1.8	1.0	1.2
"	2	1.0	1.2	3.0	1.6	1.0	1.0	1.0
Average		1.0	2.0	4.9	2.1	1.4	1.0	1.1
30	1	1.0	6.4	7.0	6.0	3.8	1.2	1.4
"	2	1.0	4.2	4.2	4.8	2.8	1.0	2.0
Average		1.0	5.3	5.6	5.4	3.3	1.1	1.7

Appendix Table 9 - Disease indices on Vernal alfalfa at 25°C.

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	1.0	1.8	2.6	1.0	1.0	1.0
"	2	1.0	1.2	1.0	1.4	1.0	1.0	1.0
Average		1.0	1.1	1.4	2.0	1.0	1.0	1.0
20	1	1.0	1.0	5.8	5.4	1.0	1.0	1.0
"	2	1.0	1.4	4.6	4.4	1.0	1.2	2.0
Average		1.0	1.2	5.2	4.9	1.0	1.1	1.5
30	1	1.0	3.0	6.4	6.0	2.0	1.0	1.4
"	2	1.0	8.8	6.4	4.8	1.0	2.6	2.6
Average		1.0	5.9	6.4	5.4	1.5	1.8	2.0

Appendix Table 10 - Increase in length of plants (in cms.) of Dupuits alfalfa at three temperatures

Temp. °C.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
15	1	2.26	1.82	0.18	0.5	0.78	2.48	2.02
"	2	2.14	2.32	1.48	2.26	1.7	2.68	3.24
Average		2.2	2.07	0.83	1.15	1.24	2.58	2.63
20	1	15.74	5.5	4.8	8.6	9.64	16.6	14.44
"	2	8.76	5.3	6.74	6.1	8.06	4.34	6.64
Average		12.25	5.4	5.77	7.35	8.85	10.47	10.54
25	1	4.48	6.9	2.22	5.08	4.68	8.24	9.98
"	2	8.04	1.66	3.84	7.08	7.14	7.36	8.24
Average		6.26	4.28	3.03	6.08	5.91	7.80	9.11

Appendix Table 11 - Increase in length of plants (in cms.) of Vernal alfalfa at three temperatures

Temp. °C.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
15	1	4.4	1.02	1.0	0.52	0.72	2.2	2.66
"	2	2.52	1.4	1.92	1.04	2.75	3.0	2.96
Average		3.46	1.21	1.46	0.78	1.74	2.60	2.81
20	1	13.42	4.04	4.86	5.9	10.56	13.68	14.92
"	2	7.3	5.62	4.82	5.9	5.52	8.08	6.98
Average		10.36	4.83	4.84	5.9	8.04	10.88	10.95
25	1	10.7	6.08	4.22	6.52	7.02	8.72	10.72
"	2	3.68	2.8	4.0	2.3	4.28	6.52	7.08
Average		7.19	4.44	4.11	4.41	5.65	7.62	8.90

Appendix Table 12 - Dry weight of shoots (in grams) of Dupuits alfalfa at three temperatures

Temp. °C.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
15	1	.03	.022	.01	.018	.03	.032	.034
"	2	.016	.012	.018	.016	.016	.022	.016
Average		.023	.019	.014	.017	.023	.027	.025
20	1	.08	.042	.024	.068	.08	.106	.066
"	2	.036	.032	.02	.05	.042	.038	.05
Average		.058	.037	.022	.059	.061	.072	.058
25	1	.04	.042	.01	.028	.036	.052	.046
"	2	.03	.018	.016	.036	.044	.038	.062
Average		.035	.030	.013	.032	.040	.045	.054

Appendix Table 13 - Dry weight of shoots (in grams) of Vernal alfalfa at three temperatures

Temp. °C.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
15	1	.048	.028	.012	.016	.034	.034	.056
"	2	.02	.01	.014	.014	.014	.02	.018
Average		.034	.019	.013	.015	.024	.027	.037
20	1	.092	.032	.038	.042	.07	.102	.048
"	2	.04	.034	.042	.042	.04	.048	.05
Average		.067	.033	.040	.042	.055	.075	.048
25	1	.046	.02	.02	.028	.076	.066	.064
"	2	.03	.016	.02	.014	.032	.048	.032
Average		.038	.018	.02	.021	.054	.057	.048

Appendix Table 14 - Dry weight of roots (in grams) of Dupuits alfalfa at three temperatures

Temp. °C.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
15	1	.022	.012	.01	.012	.02	.018	.024
"	2	.006	.006	.008	.008	.008	.008	.008
Average		.014	.009	.009	.010	.014	.013	.016
20	1	.038	.018	.024	.016	.024	.022	.022
"	2	.008	.006	.01	.016	.014	.016	.024
Average		.023	.012	.017	.016	.019	.019	.023
25	1	.014	.016	.002	.006	.008	.018	.008
"	2	.006	.004	.006	.01	.01	.006	.014
Average		.010	.011	.004	.008	.009	.012	.011

Appendix Table 15 - Dry weight of roots (in grams) of Vernal alfalfa at three temperatures

Temp. °C.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
15	1	.03	.022	.012	.01	.02	.026	.042
"	2	.006	.006	.006	.008	.006	.008	.010
Average		.018	.014	.009	.009	.013	.017	.026
20	1	.036	.01	.01	.014	.02	.03	.026
"	2	.01	.014	.014	.014	.012	.022	.014
Average		.023	.012	.012	.014	.016	.026	.020
25	1	.014	.006	.006	.006	.014	.024	.012
"	2	.008	.006	.006	.006	.01	.008	.006
Average		.011	.006	.006	.006	.012	.016	.009

Appendix Table 16 - Randomization at 25% soil moisture

6 V	3 D	3 V	4 D	2 D
7 D	5 V	7 V	3 V	3 D
3 V	5 D	6 D	1 D	7 D
4 V	2 D	2 D	7 V	4 D
5 V	7 D	3 D	5 V	4 V
4 D	6 V	4 V	3 D	6 D
2 V	3 V	5 D	7 D	6 V
6 D	7 V	1 D	4 V	5 V
3 D	4 D	4 D	6 D	1 D
1 V	1 D	5 V	1 V	2 V
2 D	2 V	1 V	5 D	5 D
7 V	6 D	7 D	2 D	7 V
1 D	1 V	6 V	6 V	3 V
5 D	4 V	2 V	2 V	1 V

Appendix Table 17 - Randomization at 35% soil moisture

5 V	2 V	1 V	5 D	2 V
3 D	6 D	3 D	7 D	5 V
4 V	2 D	5 V	4 V	4 D
1 V	4 D	3 V	2 D	4 V
4 D	7 D	1 D	5 V	3 V
2 V	5 D	4 D	3 D	6 V
1 D	5 V	7 V	1 D	7 D
7 V	3 V	6 V	7 V	5 D
5 D	1 V	5 D	3 V	2 D
2 D	7 V	2 V	4 D	3 D
3 V	6 V	6 D	2 V	7 V
6 D	4 V	2 D	6 D	6 D
7 D	1 D	7 D	1 V	1 D
6 V	3 D	4 V	6 V	1 V

Appendix Table 18 - Randomization at 45% soil moistume

2 V	1 V	4 V	4 D	5 D
7 V	3 V	1 V	5 V	1 D
1 D	7 D	7 D	2 V	7 V
6 D	4 D	5 D	7 D	4 V
6 V	6 V	1 D	1 V	1 V
7 D	6 D	3 V	4 V	7 D
5 V	2 D	3 D	1 D	5 V
4 D	2 V	6 V	5 D	2 V
5 D	4 V	6 D	6 V	4 D
3 V	7 V	2 D	2 D	2 D
2 D	5 D	7 V	6 D	3 V
4 V	3 D	5 V	3 D	6 V
3 D	5 V	2 V	3 V	3 D
1 V	1 D	4 D	7 V	6 D

Appendix Table 19 - Disease indices on Dupuits alfalfa at 25% soil moisture

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	3.4	2.4	2.0	1.6	1.0	1.0
"	2	1.0	1.0	1.2	4.0	1.4	1.4	1.0
Average		1.0	2.2	1.8	3.0	1.5	1.2	1.0
20	1	1.0	5.2	4.0	3.0	1.8	1.8	1.4
"	2	1.0	3.4	4.0	4.0	1.6	1.4	1.0
Average		1.0	4.3	4.0	3.5	1.7	1.6	1.2
30	1	1.0	8.6	7.4	4.4	4.0	1.0	1.4
"	2	1.0	6.8	8.6	6.0	3.0	1.6	1.2
Average		1.0	7.7	8.0	5.2	3.5	1.3	1.3

Appendix Table 20 - Disease indices on Dupuits alfalfa at 35% soil moisture

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	2.6	1.2	1.2	1.4	1.8	1.2
"	2	1.0	1.2	1.2	1.6	1.0	1.0	1.0
Average		1.0	1.9	1.2	1.4	1.2	1.4	1.1
20	1	1.0	4.8	2.8	1.0	1.2	1.8	1.2
"	2	1.0	3.6	4.2	4.2	2.2	1.0	1.2
Average		1.0	4.2	3.5	2.6	1.7	1.4	1.2
30	1	1.0	7.6	6.0	4.2	3.8	1.8	1.4
"	2	1.0	7.2	7.0	5.4	3.8	1.0	3.0
Average		1.0	7.4	7.5	4.8	3.8	1.4	2.2

Appendix Table 21 - Disease indices on Dupuits alfalfa at 45% soil moisture

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	2.4	2.6	2.0	2.8	1.2	1.6
"	2	1.0	1.4	1.6	1.8	1.6	2.4	1.0
Average		1.0	1.9	2.1	1.9	2.2	1.8	1.3
20	1	1.0	3.0	3.4	1.6	1.6	1.0	1.8
"	2	1.0	1.4	1.6	3.8	2.0	1.6	1.2
Average		1.0	2.2	2.5	2.7	1.8	1.3	1.5
30	1	1.0	5.2	5.6	3.8	1.8	1.0	2.4
"	2	1.0	4.0	7.2	5.4	3.8	1.2	3.4
Average		1.0	4.6	6.4	4.6	2.8	1.1	2.9

Appendix Table 22 - Disease indices on Vernal alfalfa at 25% soil moisture

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	1.8	2.6	3.0	1.8	1.0	1.0
"	2	1.0	2.6	2.4	1.2	1.4	1.2	1.2
Average		1.0	2.2	2.5	2.1	1.6	1.1	1.1
20	1	1.0	3.0	3.4	3.8	1.0	1.2	1.0
"	2	1.0	5.6	8.2	5.0	1.4	1.0	1.0
Average		1.0	4.3	5.8	4.4	1.2	1.1	1.0
30	1	1.0	8.2	8.2	8.6	3.2	1.0	1.0
"	2	1.0	7.8	9.0	8.6	1.4	2.0	1.6
Average		1.0	8.0	8.6	8.6	2.3	1.5	1.3

Appendix Table 23 - Disease indices on Vernal alfalfa at 35% soil moisture

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	3.0	1.0	1.0	1.0	1.4	1.0
"	2	1.0	1.4	2.8	1.0	1.4	1.0	1.0
Average		1.0	2.2	1.9	1.0	1.2	1.2	1.0
20	1	1.0	4.6	2.6	2.6	1.4	1.6	1.0
"	2	1.0	6.2	6.2	7.0	1.4	1.0	1.2
Average		1.0	5.4	4.4	4.8	1.4	1.3	1.1
30	1	1.0	8.4	7.2	6.4	4.2	2.2	1.0
"	2	1.0	5.8	6.8	7.6	1.0	1.0	1.6
Average		1.0	7.1	7.0	7.0	2.6	1.6	1.3

Appendix Table 24 - Disease indices on Vernal alfalfa at 45% soil moisture

Days after inoc.	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
10	1	1.0	2.0	1.2	1.2	1.0	3.0	1.8
"	2	1.0	2.2	1.4	1.2	1.0	1.0	1.0
Average		1.0	2.1	1.3	1.2	1.0	2.0	1.4
20	1	1.0	2.4	3.2	1.8	1.4	3.2	2.2
"	2	1.0	2.4	4.2	5.0	1.0	1.0	1.2
Average		1.0	2.4	3.7	3.4	1.2	2.1	1.7
30	1	1.0	7.4	3.8	6.2	2.2	3.2	2.0
"	2	1.0	7.4	7.0	6.6	4.0	2.0	3.0
Average		1.0	7.4	5.4	6.4	3.3	2.6	2.5

Appendix Table 25 - Increase in length of plants (in cms.) of Dupuits alfalfa at three soil moisture levels

Soil Moisture Level	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
25%	1	2.0	1.04	1.02	2.58	1.54	1.72	2.1
"	2	2.82	0.26	0.7	2.7	1.76	4.86	2.92
Average		2.46	0.65	0.86	2.64	1.65	3.29	2.51
35%	1	2.76	0.38	1.36	0.58	1.78	4.06	1.0
"	2	3.46	0.74	1.44	4.4	3.28	2.74	5.66
Average		3.11	0.56	1.40	2.49	2.63	3.40	3.33
45%	1	2.5	1.4	0.8	1.28	1.26	5.22	3.28
"	2	3.88	0.74	0.84	1.62	5.66	4.30	7.42
Average		3.19	1.07	0.82	1.45	3.46	4.76	5.35

Appendix Table 26 - Increase in length of plants (in cms.) of Vernal alfalfa at three soil moisture levels

Soil Moisture Level	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
25%	1	2.7	0.4	0.42	0.5	4.02	1.58	5.64
"	2	3.6	1.44	0.44	0.48	4.00	4.42	7.74
Average		3.15	0.92	0.43	0.49	4.01	3.00	6.69
35%	1	4.48	0.74	0.76	0.84	3.4	2.86	3.9
"	2	5.46	0.66	1.56	2.4	2.72	4.60	9.48
Average		4.97	0.69	1.16	1.62	3.06	3.73	6.69
45%	1	3.66	0.38	0.6	0.54	3.82	3.72	5.06
"	2	6.54	1.4	2.54	3.78	5.78	3.1	3.22
Average		5.10	0.71	1.57	2.16	4.80	3.41	4.14

Appendix Table 27 - Dry weight of shoots (in grams) of Dupuits alfalfa at three soil moisture levels

Soil Moisture Level	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
25%	1				No results			
"	2	.026	.012	.01	.028	.024	.018	.024
Average		.026	.012	.01	.028	.024	.018	.024
35%	1				No results			
"	2	.042	.01	.01	.024	.032	.04	.028
Average		.042	.01	.01	.024	.032	.04	.028
45%	1				No results			
"	2	.02	.016	.01	.016	.02	.048	.032
Average		.02	.016	.01	.016	.02	.048	.032

Appendix Table 28 - Dry weight of shoots (in grams) of Vernal alfalfa at three soil moisture levels

Soil Moisture Level	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
25%	1				No results			
"	2	.026	.01	.08	.008	.032	.024	.026
Average		.026	.01	.08	.008	.032	.024	.026
35%	1				No results			
"	2	.038	.01	.01	.042	.03	.024	.03
Average		.038	.01	.01	.042	.03	.024	.03
45%	1				No results			
"	2	.036	.006	.016	.012	.03	.03	.028
Average		.036	.006	.016	.012	.03	.03	.028

Appendix Table 29 - Dry weight of roots (in grams) of Dupuits alfalfa at three soil moisture levels

Soil Moisture Level	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
25%	1				No results			
"	2	.018	.008	.006	.02	.014	.012	.022
Average		.018	.008	.006	.02	.014	.012	.022
35%	1				No results			
"	2	.02	.006	.006	.008	.016	.018	.016
Average		.02	.006	.006	.008	.016	.018	.016
45%	1				No results			
"	2	.012	.01	.004	.01	.01	.026	.024
Average		.012	.01	.004	.01	.01	.026	.024

Appendix Table 30 - Dry weight of roots (in grams) of Vernal alfalfa at three soil moisture levels

Soil Moisture Level	Exp. No.	Inoculation treatments						
		Control	V.a 1	V.a 2	V.a 5	V.a 3	V.d 1	V.d 2
25%	1	No results						
"	2	.016	.006	.006	.002	.02	.024	.026
Average		.016	.006	.006	.002	.02	.024	.026
35%	1	No results						
"	2	.024	.004	.004	.004	.022	.016	.014
Average		.024	.004	.004	.004	.022	.016	.014
45%	1	No results						
"	2	.024	.002	.008	.004	.020	.018	.014
Average		.024	.002	.008	.004	.020	.018	.014