STUDIES ON DISEASES OF BIRDSFOOT TREFOIL WITH SPECIAL REFERENCE TO THE CAUSE OF WILT

by

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TABLE OF CONTENTS

I. INTRODUCTION

A. THE PROBLEM

Birdsfoot trefoil, *Lotus corniculatus* L., and closely related species are long-lived perennial legumes, which have a potential in agriculture for replacing clovers and alfalfas in locations which have proven to be generally unsuitable for the growth of most other legumes. However, the life of birdsfoot trefoil has been only two or three years in Quebec, instead of at least ten which is expected in the southeastern area of the United States (Drake 1958). The reduction in the life span of this plant in Quebec is generally attributed to "winter killing," but the cause of winter killing could be due to a number of factors such as: frost; *Sclerotinia trifoliorum* Erikss. (crown and root rot of red clover); or other factors.

In the Macdonald College experimental fields, a plot of *Lotus* was severely damaged during the winter of 1959 - 1960, and in the spring of 1960 a large number of plants recovered only to be stricken by a wilt in May and June. As a direct result of wilt, 10% of the plants were killed, while another 13% of the plants in the *Lotus* plot were severely attacked, but managed to survive.

A leaf spotting and stem lesion disease was observed in May of 1960 and again in 1961 in the college experimental fields. This disease was also found in the greenhouses throughout the year. It caused defoliation at the base of the stems, but did not attack the younger foliage.

B. NEED

The wilt disease in the college fields resulted in so much damage that it was imperative to isolate the cause of the wilt, to make field observations on its host range so that any source of resistance could be incorporated into breeding programs, and to conduct laboratory inoculation experiments to establish a broader range of susceptible species throughout the genus Lotus.

A study into the nature of association between disease and winter killing would contribute to the knowledge of winter hardiness of this plant.

The symptoms of the leaf spotting and stem lesion disease agreed with those caused by Stemphylium loti Graham, but it was necessary to identify the microorganisms which contributed to this disease besides S. loti. The host range of S. loti has been investigated by Ford (1960).

The other principal diseases of Lotus, including Sclerotium rolfsii Sacc., the cause of southern blight, Rhizoctonia solani Kuhn, a cause of root rot, Fusarium root rot, and Cercospora leaf spot have been studied extensively by Drake (1960), Drake (1961), Ford (1959) and Kreitlow et al. (1956) respectively. None of these diseases were observed on Lotus at Macdonald College in 1960 or 1961.

Physoderma potteri (Bartlett) Karling, the cause of Physoderma crown gall, has been reported on Lotus corniculatus in England, while L. uliginosus, growing in the same location, remained uninfected (Bartlett 1926). Hardison (1956) found L. uliginosus attacked along the Oregon coast, while L. corniculatus growing nearby, remained healthy. Physoderma

alfalfae (Pat. & Lagerh.) Karling, the cause of crown gall or wart of alfalfa, has been reported frequently throughout the United States and once in British Columbia (Cormack 1945). P. potteri and P. alfalfae may be identical organisms as there is little morphological difference, and their classification is based, in part, on the host they attack, but the host range of neither has been investigated. Studies into the morphology and host range would contribute towards the taxonomy of this group as well as make agriculturists aware of the potential dangers of these fungi.

C. DELIMITATIONS

The relationship between Sclerotinia trifoliorum and winter killing is not thoroughly established in the current investigations, because of the necessity for controlled field studies over a number of years.

The host range of S. trifoliorum within Lotus corniculatus and within the genus, has been tentatively determined, but further studies into the nature of resistance are required; however, before the host range or the association of the disease to winter killing can be established, it is necessary to determine the exact disease cycle of the fungus on Lotus.

Collections of fresh galls caused by Physoderma on Lotus uliginosus, L. corniculatus and Medicago sativa should be obtained from Europe and North America, and the host range and taxonomy should be investigated further.

D. DEFINITIONS

"Birdsfoot trefoil" refers exclusively in this thesis to *Lotus corniculatus* L. When other species are referred to, they are specified.

Stemphylium loti Graham is the cause of "Stemphylium leaf spot."

Physoderma potteri (Bartlett) Karling is the cause of "Physoderma crown gall" on *Lotus* spp. This name readily distinguishes the disease from bacterial or nematode galls. The literature may refer to the disease as "wart." The old name of the fungus was *Urophlyctis potteri* Bartlett.

Physoderma alfalfae (Pat. & Lagerh.) Karling is the cause of "Physoderma crown gall" of alfalfa. The literature may also refer to the disease as "alfalfa wart," "marbled gall," or simply "crown gall." The old name of the fungus was *Urophlyctis alfalfae* Pat. & Lagerh.

The term "resting spore" is used for the resting stage of *Physoderma* spp., to distinguish it from the epibiotic and endobiotic sporangia. The literature may refer to the resting spore as "resting sporangium." The resting spore gives rise to "zoospores" upon germination.

Sclerotinia trifoliorum Erikss. causes wilting and crown rot of *Lotus* spp. In this thesis it is referred to as "Sclerotinia wilt and crown rot." The literature refers to this disease as "crown and root rot," "crown rot," "root rot," or simply "rot."

"Winter killing" or "winter killed" refers to loss during the winter months and, by itself, does not specify the cause, which could be due to frost, *Sclerotinia trifoliorum*, or some other factor.

E. SYMPTOMS

1. Physoderma Crown Gall

Bartlett (1926) states that Physoderma potteri causes galls which appear on Lotus corniculatus most frequently about the crown at soil level, but sometimes they are found on the lateral stems. They have a more or less spherical or ovoidal form, and are rarely lobed. The largest galls are about 1 cm* in the largest diameter, but the average size is that of a fair size garden pea. Very small galls, about 1 mm in diameter, may appear on the underground stems, bearing some resemblance to root nodules. The colour of the galls, when freshly obtained, is pale brown, but when young, the galls are pale yellow. The gall bearing plants appear to be healthy and reach the same size as ordinary individuals, but they do not produce flowers. The cut surface of a gall has a white and brown mottled appearance and under a microscope discloses parenchyma tissue and numerous cavities of varying sizes mostly filled with golden brown resting spores.

Jones et al. (1920) described symptoms of Physoderma crown gall on alfalfa which do not differ greatly from Bartlett's (l.c.) description of the disease on birdsfoot trefoil; however, the galls are apparently more numerous on alfalfa and may occur as much as 10 in. above the crown. Line (1921) adds that infected alfalfa may appear yellowish and give signs of wilt during the summer.

2. Stemphylium Leaf Spot

Leaves, stems and pods of Lotus spp. may be attacked by Stemphylium

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* Abbreviations and symbols in this thesis conform to the rules outlined in: "Style Manual for Biological Journals," published by The American Institute of Biological Sciences, 1960.

loti. On the leaves appear small reddish-brown necrotic spots, about 1 to 3 mm in diameter, surrounded by a water soaked zone. Chlorosis may appear and result in the entire leaf yellowing, but in the field the leaf usually drops before the chlorosis becomes extensive. On the stems small reddish-brown spots and greatly elongated lesions may appear which are surrounded by a water soaked area. In severe cases the stem may be girdled, but this condition more frequently occurs in the greenhouse and seldom in the fields at Macdonald College.

Stemphylium loti attacks the older leaves around the base of the stem, while the younger leaves remain healthy. Infection may be found in May, but does not reoccur during summer months in the field. In the greenhouse, however, the disease can occur throughout the year.

3. Sclerotinia Wilt and Crown Rot

The first symptoms appear as wilting of one or more branches, which often progresses to the entire plant within a few days, when the weather conditions are warm and sunny. In cool weather the spread of the wilt is slowed down, and frequently the plant recovers and shows no further signs of the disease. At the base of branches killed by the fungus, it is possible to detect small to minute sclerotia, 1 to 3 mm in diameter. These sclerotia may appear several inches up the stem and below the epidermis, which readily peals off. In the crown of severely wilted plants it is possible to detect sclerotia ranging from 1 to 7 mm in diameter. The sclerotia first appear as whitish masses of hyphae soon after the first signs of wilt, but by the time a stem is completely dead, they are hard and black.

The disease is checked by cool weather, and plants which show severe or total wilt, may send out new shoots and thus apparently outgrow the fungus; however, a stem will not recover once it shows signs of wilt.

Wilt may first be seen in early May and will continue until the middle of June at Macdonald College. Wilt has also been observed in November. Plants which winter kill may also reveal many sclerotia in the old crown when examined early in the spring.

II. LITERATURE REVIEW

A. PHYSODERMA CROWN GALL

1. Historical

The occurrence of galls caused by Urophlyctis (Physoderma) on birdsfoot trefoil was reported by Bartlett (1926), who named the organism Urophlyctis potteri, because it differed morphologically from other species. He found that Lotus corniculatus was infected when grown in moist locations in northern England, while Lotus uliginosus, growing nearby, remained uninfected.

MacDonald (1944) reported Urophlyctis potteri as occurring on L. corniculatus in moist soils in New York. Hardison (1956) found one-year-old L. uliginosus attacked by this fungus along the Oregon coast, but L. corniculatus remained uninfected in the same location.

Hey (1946) claimed that Urophlyctis (Physoderma) alfalfae caused galls on L. corniculatus and clovers in Germany.

According to Karling (1950), Kirschner found galls caused by Physoderma trifolii Passerini on L. corniculatus and Trifolium spp.

Physoderma alfalfae has been recorded on Medicago sativa from Europe, North and South America (Karling 1950). Cormack (1945) reported P. alfalfae on alfalfa from British Columbia. P. trifolii has been recorded on clovers from Europe, India and Japan (Karling 1950), but this fungus forms warts on leaves, petioles and stems, rather than on crowns.

Two other species of Physoderma have been recorded in Eastern Canada. P. graminis (Busgen) de Wild. was reported from Ottawa on Agropyron repens (Childers 1948), and Urophlyctis (Physoderma) on Lathyrus japonicus from Quebec (Jacques 1945).

2. Classification of the Genus Physoderma

Karling (1950) placed Urophlyctis potteri and other species of this genus into Physoderma as he considered there was insufficient evidence to justify separating these two genera. He claimed that the genus Physoderma contained approximately fifty species parasitic on higher plants, and that many of these parasites are so slightly known that their validity is highly questionable. The principal difference between P. alfalfae, P. trifolii and P. potteri is in the morphology of the resting spore; P. trifolii has a resting spore with a smooth brown wall and a ring of pits, P. alfalfae differs by having a ring of haustoria around the resting spore. P. potteri has ridges on its resting spore and a ring of blunt haustoria (Karling 1950). In size, the resting spores of all three species are similar.

3. Resting Spore Germination

The only recorded attempt to germinate the resting spores of P. potteri was by Bartlett (1926), but he was unsuccessful.

Tisdale (1919) reported that temperature, moisture and fresh air were important for the germination of resting spores of Physoderma maydis Miyabe. After the spores had become dry, it was difficult to obtain germination. Fresh spores were found to germinate best when placed in a small watch glass, with either distilled or tap water, and then placed in a humidity chamber. The temperature was found to be best between

82 and 84 F, but germination would occur between 73 and 87 F.

Jones et al. (1920) found that galls of P. alfalfae deteriorated in transit, which often prevented germination. They found that germinating zoospores escaped in a mass of protoplasm from the resting spore by passage through pores in the wall.

Line (1921) reported that resting spores of P. alfalfae were aided in germination by very slight pressure, and that germination was better in rotted galls. He claimed that the zoospores escaped from cracks in the resting spore wall.

Voorhees (1933) found resting spores of P. maydis germinated equally well in a sealed or exposed container, but the absence of light inhibited germination, whereas sunlight was lethal. At the optimum pH of 7.4 there was 88% germination. After storage for three years the resting spores were no longer viable. Germination was found not to be inhibited by freezing for 30 days.

Sparrow (1957) determined that resting spores of Physoderma plurian-nulatum (Berkeley & Curtis) Karling germinated best at 46 F, whereas at 77 F there was no germination.

Hebert et al. (1958) reported germination of P. maydis occurred over a wide range of pH values from 3 to 10, and temperatures from 65 to 97 F. They found light and oxygen necessary.

4. Inoculation

Jones et al. (1920) reported little or no infection in most cases when alfalfa was inoculated with Physoderma alfalfae. Two-year-old alfalfa

failed to become infected when crushed galls were placed around the crowns, covered with sphagnum moss and kept thoroughly wet for 10 days; however, this method was successful on three seedling alfalfas, which were six inches high, infection showing three months later. They managed to infect 3 out of 9 plants when seed was mixed with spores; in this case infection showed after 9 months.

Line (1921) was able to infect alfalfa plants six-months-old and upwards, but he was unable to infect seedlings of alfalfa or other legumes. He observed that a very wet condition in the field, but not necessarily flooding, was necessary for natural infection.

B. STEMPHYLIUM LEAF SPOT

1. Historical

Stemphylium loti was first reported and described by Graham et al. (1951) who found the fungus caused leaf spots and stem lesions on birdsfoot trefoil. This disease has since been reported from: Pennsylvania (Kreitlow et al. 1953), New York (Roberts 1954, 1955), West Virginia (Elliott 1954), Iowa (Baxter 1955), New Jersey (Cappellini 1957), and Minnesota (Renfro et al. 1960). Kilpatrick (1956) stated that Stemphylium leaf spot could be found wherever trefoil was grown in the New England States.

2. Classification of the Fungus

Graham et al. (1951) reported that Stemphylium loti was similar to S. sarcinaeforme, which causes zonate leaf spots on red clover. Spore characteristics were found to be alike, but mycelial growth slightly different. In cross inoculation, the trefoil isolates failed to infect

red clover, and the red clover isolates failed to attack birdsfoot trefoil.

Dickson (1956) claimed that the morphology of this smooth-spored fungus on clovers and trefoils varied over a similar range, and that they were more likely specialized varieties of the same species, that is, S. sarcinaeforme.

3. Host Range

Graham (1953) found that Stemphylium loti failed to attack alfalfa, alsike, Ladino and red clovers. Lotus uliginosus showed slight resistance, but within the species L. corniculatus only variety Granger showed any signs of resistance.

Ford (1960) tested a large number of species of Lotus, and many types and varieties of L. corniculatus. Five introductions of L. corniculatus and several species of the genus showed resistance to Stemphylium leaf spot.

C. SCLEROTINIA WILT AND CROWN ROT

1. Historical

According to Güssow (1903) stands of red clover failed in England as early as 1804. It was noted that healthy autumn stands would be seriously reduced by the spring. Eriksson claimed that the relationship between clover failure and Sclerotinia trifoliorum was first established by Herman Hoffman in Germany in 1863 (Gilbert et al. 1917). The first report of the disease in North America was on red clover by Chester in 1890 (Wolf et al. 1919).

According to Frandsen (1946) Lotus corniculatus was first reported as a host of S. trifoliorum by Line in Denmark in 1916, and L. uliginosus by Lemacke in 1923.

Nilsson-Leissner et al. (1929) found L. corniculatus susceptible in Sweden. Eastam (1940) reported that 15% of the birdsfoot trefoil was wilted in infection trials of various leguminous fodders in British Columbia. MacDonald (1944) observed that S. trifoliorum infected birdsfoot trefoil in certain places in New York, particularly on moist soils of high organic content. He claimed that the disease might become serious enough to limit the use of the crop.

Pohjakallio (1947) reported the disease in Finland and that L. corniculatus was susceptible. Kauter (1948) found sweet trefoil (Lotus sp.) to be very susceptible in Switzerland. Hansen et al. (1954) stated that there were very virulent outbreaks of the disease on birdsfoot trefoil in Denmark.

Elliott (1954) recorded that Sclerotinia attacked L. corniculatus after a rather uniformly wet spring in West Virginia, while Roberts (1954) reported that there was only a trace of the disease in New York the same year. Drake (1958) has recorded the disease on birdsfoot trefoil in six southern States.

2. Host Range and Resistance

Hanson and Kreitlow (1953) stated that S. trifoliorum has a broad host range, which includes all important true clovers, alfalfa (Medicago sativa), black medic (M. lupulina), birdsfoot trefoil, sainfoin (Onobrychis viciaefolia), and many other legumes and nonlegumes, including numerous

weeds. They claimed that red clover (Trifolium pratense), crimson clover (T. incarnatum) and alsike clover (T. hybridum) were very susceptible, while white clover (T. repens) was generally considered to be less susceptible, but not immune.

Valleau et al. (1933) included Plantago lanceolata, Chrysanthemum cinerariaefolium, and Lactuca sp. as common nonleguminous hosts of the fungus in North America. They found that adapted varieties of red clover, when spring-sown, were undoubtably less injured by S. trifoliorum than unadapted American and foreign clovers. When autumn-sown, the adapted clovers seemed to survive slightly better than the unadapted, but the difference was slight.

Pape (1931) reported from five years of practical observations in the fields in Schleswig-Holstein, Germany, that red clover seemed to be the most susceptible to rot, closely followed by crimson clover. Kidney vetch, white clover, Swedish clover, yellow clover, alfalfa and birdsfoot trefoil were more resistant. He claimed that no varieties of clover were immune, however, American, French and Italian clovers were more susceptible than native red clovers.

Frandsen (1946) lists 85 hosts of the fungus which includes 13 genera of legumes. He found early-maturing varieties of clovers were more resistant in Denmark although other workers frequently claimed late-maturing varieties more resistant.

Vestad (1960) tested the resistance of diploids and corresponding autotetraploid clovers to S. trifoliorum and found the tetraploid more resistant.

3. Physiological Races

Nicolaisen et al. (1940) were unable to observe mycelial anastomoses and concluded that there was presumably no risk of the development of a new physiological race of the pathogen Sclerotinia trifoliorum. Wadham (1925), however, had previously reported that the fungus frequently anastomoses in culture. Frandsen (1946) was also able to find anastomoses in very young cultures between hyphae of different mycelia, and concluded that this afforded the opportunity for the development of new physiological races of different virulence, presenting difficulties in breeding for resistance.

Nilsson-Leissner (1942) observed physiological races when he was conducting breeding experiments for resistance to clover rot. He found Mercur, a variety of red clover, had a resistant reaction to rot in south and central Sweden, as well as in Germany, but was found highly susceptible in the north of Sweden and Denmark.

Frandsen (1946) concluded, from extensive studies in Denmark, that there was no evidence that S. trifoliorum comprises races peculiar to the host species, but the results of infection experiments showed that isolates of species often differ in virulence.

Pohjakallio (1947) reported that a strain of the fungus from Leipzig attacked L. corniculatus mildly, whilst another isolate from bean (Phaseolus vulgaris) was fairly pathogenic, causing 20 to 38% infection. Two other strains, from white and red clovers, were strongly pathogenic.

Held (1955) tested the pathogenic differences between a normal and a degenerate strain of S. trifoliorum. He found that a degenerate strain

produced no sclerotia in culture.

4. Disease Cycle

The literature reveals that there are two schools of thought concerning the stage in the life cycle of Sclerotinia trifoliorum which initiates infection. No speculation on the disease cycle, as it affects Lotus spp., could be found in the literature.

Wolf et al. (1919), Hino (1930), Pape (1931), Frandsen (1946), Kauter (1948) and R. G. Hanson (1951) claim that infection results directly from mycelium in the soil, and that ascospores play a secondary role, or are of no consequence at all in initiating infection.

Wadham (1925), Nilsson-Leissner et al. (1929), Valleau et al. (1933), Dillon-Weston (1950), Justham et al. (1950), Loveless (1951), E. W. Hanson and Kreitlow (1953) and Estey (1956) consider that ascospores play the primary role in producing heavy field infection.

Wolf et al. (1919) believed that the vegetative mycelium was responsible for the disease, because infection began on the stem at or near the ground level, and concluded that little disease resulted from natural ascospore infection.

Wadham (1925) stated that when leaves were inoculated with spores contained in pure water, they invariably failed to become infected unless the tissue of the leaves was damaged, or small amounts of nutrient solution were added to the water. He claimed, therefore, that saprophytic mycelium must first develop before the plant can be attacked. He concluded that mycelium originates on the decayed clover leaves on the ground, and spreads

slowly to the clover plant, but he did not disregard the possibility that sclerotia in the soil may occasionally give rise to mycelium.

Nilsson-Leissner et al. (1929) observed that under humid conditions in Germany and Sweden, the fungus can spread from the exterior of one plant to those in the vicinity, but they favored ascospores as being the primary means of infection.

Hino (1930) reported from Japan that the primary source of infection may be the mycelium arising directly from the sclerotia in the soil or mixed with the seed.

From observations in Germany, Pape (1931) noted that apothecia were completely absent in infected fields of the current year's crop in October and November of 1930, and only a few isolated ones occurred in two-year-old fields. He concluded that since the diseased new crops were situated a long way from the old fields, the ascospores must either have been conveyed for a considerable distance through the air, or the apothecial stage must be relatively unimportant in producing autumn infection.

Valleau et al. (1933) considered that direct infection from wind blown ascospores may be considered a satisfactory explanation for field infection. They observed that the nearly perfect infection in 1931 - 1932 plots could not be attributed to soil infection with a vegetative stage of the fungus or sclerotia, as legumes had not been grown on this field for many years, or to seed infection or direct infection from sclerotia carried in the seed, as these could hardly have been present in all lots in sufficient amounts to cause such extensive infection.

At the end of August, Frandsen (1946) infected a plot, consisting of pots laid in the ground, with seven strains of the fungus. He observed that early in October, the sclerotia in some pots began to produce apothecia, but during the latter part of September mycelial infection had already occurred. He noticed that mycelium crept along the surface of the soil in the humid atmosphere between plant rows, thereby contributing to the spread of the pathogen, which in some cases seemed to proceed from the dead stems and leaves on the ground to the living plants. He claimed from these observations, that the mycelial dissemination of S. trifoliorum, without the intervention of apothecia or ascospores, may be of great importance in dense plantings. R. G. Hanson (1951) supported Frandsen's hypothesis claiming that mycelium can initiate infection in the autumn in Pennsylvania.

Kauter (1948) reported that in Switzerland, while infection is spread to some extent by spores, it is propagated mainly by the sclerotia.

Dillon-Weston (1950) claimed there was no valid evidence that sclerotia germinated in the soil to form mycelia, and that the spread of the disease is brought about in this way in England. He observed the fungus in clover leaves after ascospore infection remained quiescent until the host resistance breaks down, or weather conditions are favorable for the onset of the aggressive phase of the disease.

Justam et al. (1950) also claim that ascospore infection is important in England for initiating the disease.

Loveless (1951) demonstrated that the fungus was able to grow from infected clover leaves into the crown, and subsequently cause death of the plant.

E. W. Hanson and Kreitlow (1953) claimed that the greatest reduction in stands occurs during the late winter and early spring in the United States. They concluded that ascospores are the main source of infection.

Estey (1956) reported, from two years' field results taken at Macdonald College, that the fungus was spread largely by spores, because the crown rot was scattered rather than in groups.

5. Ecology

Baudyš (1923) reported exceptionally heavy outbreaks of crown rot in Moravia. He claimed that factors favoring the disease included a wet autumn, the time of mowing the pasture, density of growth, and the previous cropping and fertilizer in the field. Esmarch (1925) found that heavy applications of nitrogen fertilizer favored the disease.

In his observations in Schleswig-Holstein, Pape (1931) found that severe outbreaks were followed by relatively mild winters, proceeded by a wet autumn, and that the disease flourished only under humid conditions.

Kauter (1948) claimed that in Switzerland the severity of attack depended upon a warm spell the previous November and December.

Justam et al. (1950) reported that heavy infections in March in England followed exceptionally dry Novembers. They claimed that heavy rains in the autumn were associated with retardation of spore discharge and decay of apothecia which occur in November and December in southwestern England. Dry weather, on the other hand, favored survival of the apothecia. Loveless (1951), however, found in eastern England, after 17 years' results, that spring infection was associated with a high rainfall in the preceding

December and January, and concluded that high rainfall was essential for ascospore infection. Loveless (l.c.) demonstrated that cutting or grazing clover stands in the autumn reduced infection the following spring and concluded that since there was less leaf surface, the chance of ascospore infection was reduced.

Kreitlow et al. (1951b) reported that crown infection of Ladino clover was greater at 60 F than at 80 F and was better at 90% R.H. than at 50% R.H.

Allison et al. (1951) determined that the optimum temperature for foliar infection of red clover with mycelium of the fungus was between 60 and 70 F.

Taurikut et al. (1951), in culture studies of S. trifoliorum, found that it did not display preference to minerals, and grew on a wide range of substrates regardless of the presence or absence of specific mineral or organic acid nutrients. They found that growth was best between 59 and 77 F, but it was able to grow from 32 to 86 F and growth was possible on media of pH 2.4 to 9.6.

Cappellini (1960) found the best temperature for mycelial growth was between 59 and 68 F.

6. Methods of Artificial Inoculation

According to Wolf et al. (1919), Rehm in 1872 suspended mature apothecia of Sclerotinia trifoliorum over healthy clover plants, and within six to eight days the mycelium was present within the leaf blades, and the plants subsequently collapsed. Wadham (1925), however, was not able to get infection of clover leaves with an ascospore suspension unless the

leaves were previously damaged.

Rudorf (1937) found that the best type of inoculum for large scale inoculation was to culture the fungus on small bread rolls, soaked in plum juice or clover leaf decoction; masses of sclerotia were produced and the rolls dried and ground up to powder, which was then sprayed in suspension onto the leaves of the test plants. Another method was to inoculate ripe tomato fruits with the fungus and to use the mycelial-permeated pulp as a spray on the test plants. Rudorf (l.c.) also found that clover plants were easily infected with conidia of the fungus.

Frandsen (1946) used mycelium for inoculation. He grew the fungus for eight days on clover leaf decoction, plus dextrose, and then transferred it to a glass cylinder and thoroughly shook it up with glass pellets so as to break up the hyphae. In this way a relatively homogenous mycelial emulsion was obtained, which was applied directly to clover plants by means of an atomizer. Frandsen (l.c.) tested seedlings by this method; he grew plants in wooden frames which were covered with a layer of damp peat moss, and when the plants reached the age of two or three months, they were inoculated, and the frames left in situ for another three or four weeks, by which time the disease was sufficiently advanced to enable the reaction of the seedlings to be assessed.

Carr et al. (1950) were able to infect seedlings of clover by dusting the leaves with a ground up inoculum of bran. They used Erlenmeyer flasks which were filled with moist bran and inoculated with S. trifoliorum, and after two weeks the contents were removed to shallow dishes and oven dried at 80 F for 48 hours, and finally ground up in a mortar. Inoculum was

with water. Tests were carried out in the greenhouse on a bench moist chamber. It was found that 10 to 15 days was sufficient for readings to be made.

R. G. Hanson (1951) used Kreitlow's dried grain inoculum method for inoculating plants, and was able to obtain a significant correlation between field and greenhouse inoculation when he was testing for resistance of red clover to S. trifoliorum.

Estey (1956) modified Kreitlow's (1951) method for studies on the pathogenicity of S. trifoliorum to red clover seedlings. He placed 180 g of a grain mixture, consisting of one part oats to two parts wheat, in 500 ml wide-mouth Erlenmeyer flasks. He added 4 g of dextrose, 1 g of calcium carbonate and 150 ml of tap water, and allowed the flasks to stand overnight to permit thorough moistening of the contents.

Drake (1960), in studies with Sclerotium rolfsii, inoculated birdsfoot trefoil in three different ways. In one group dried grain inoculum was placed around the base of each plant. In the second, macerated cultures of the fungus mycelium were sprayed on the plants. In the third group, a suspension of fungus sclerotia was placed around the base of the plants. It was determined that the dried grain inoculum produced 87% infection, the fungus mycelium 23% and the fungus sclerotia 20% infection.

7. Fusarium as a Secondary Invader

Young (1924) claimed that some species of Fusarium, which are weak parasites of clover, are able to infect clover, but require the cooperation of other deleterious agencies to induce fatal results.

Roberts (1954) reported loss of birdsfoot trefoil in New York due to Fusarium crown and root rot. Drake (1958) found root rot of birdsfoot trefoil, caused by Fusarium spp. in the southeastern United States.

Ford (1959) studied the host range of F. roseum within the genus Lotus and came to the conclusion that this fungus was a primary cause of blight of birdsfoot trefoil in New York during warm, humid weather.

Kainski (1960) isolated a number of species of Fusarium from root rotted birdsfoot trefoil in New York, and concluded after inoculation experiments, that several different species of Fusarium along with other fungi were the agents that caused root rots.

8. Biological Control

The control of plant disease by antagonistic microorganisms has been thoroughly reviewed by Gerrard et al. (1938), and by Wood et al. (1955). Trichoderma has possibly received more attention than any other antagonistic fungus. Weindling (1932 & 1934) showed that Rhizoctonia solani and other pathogenic fungi were inhibited and parasitized by Trichoderma lignorum. Allen et al. (1935) reported that seed decay and damping-off in cucumber, induced by Rhizoctonia and Pythium was appreciably reduced by inoculating the soil heavily with Trichoderma. Gregory et al. (1952) found that damping-off of alfalfa caused by Pythium was significantly reduced when Trichoderma was applied to the soil.

Hino et al. (1940) showed that sclerotia and mycelium of Sclerotinia trifoliorum was destroyed by Trichoderma. Wright (1954) found that Trichoderma viride, in addition to parasitizing hyphae of many fungi, also produced gliotoxin, which has a lethal effect in the soil on microorganisms.

Kainski (1960) claimed that some isolates of Trichoderma caused a destruction of roots and basal parts of stems of seedling Lotus corniculatus. He believed that this, and other fungi, predispose seedlings to infection.

Tribe (1957) found that sclerotia of Sclerotinia trifoliorum were destroyed by Coniothyrium minitans. He suggested that this antagonistic fungus might be used in the control of clover rot.

III. PHYSODERMA CROWN GALL

A. ORIGIN OF INOCULUM

Six galls of Physoderma potteri were obtained by mail from Dr. J. R. Hardison.* The galls arrived on November 15th, 1960, and were stored in a refrigerator until used.

B. GERMINATION OF RESTING SPORES

1. Methods

Resting spores were taken from a gall and placed in a watch glass with a small amount of distilled water. The watch glass was placed in a petri plate which had been partly filled with vermiculite. The vermiculite, when soaked, served to keep the atmosphere in the petri plate moist, and to steady the watch glass. The base of the watch glass rested on a small plastic disc of about 1 cm in diameter, which permitted light to pass through the petri plate and watch glass for microscopic examination. The temperature was kept between 70 and 80 F, and only normal basement laboratory light was provided for 16 hours daily. The spores were microscopically examined at frequent intervals.

2. Results

Approximately 50% of the resting spores observed appeared to germinate within two days. The resting spores split in one or more places and a mass of protoplasm oozed out. The actual splitting of the resting spore

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was not seen, and no movement of zoospores within the oozing protoplasm was observed.

C. INOCULATION OF PLANTS

1. Methods

Experiment (a)

Four types of two-month-old Lotus uliginosus (numbers: 73, 74, 75, & 76) were washed free of soil and repotted in fresh sterilized soil. After three weeks, four plants of each type were inoculated by placing crushed galls on moist cotton wool, and wrapping the cotton wool around the crowns. Inoculation was done in January, 1961, and the pots placed in the greenhouse, which was maintained at 65 to 75 F. The pots were kept thoroughly watered for six weeks with distilled water, and then for the remainder of the experiment with tap water. Four months after inoculation the plants were examined for signs of infection. Crown, stem and root tissues were fixed in F.A.A., embedded, sectioned and stained in safranin-fast green.

Experiment (b)

Successful infection in the first experiment would have provided fresh resting spores for subsequent inoculations, but this was not achieved, and thus the old galls obtained from Dr. Hardison, which had already been in a refrigerator for 7 months, had to be used again.

Inoculation was undertaken in the same manner as in the first experiment, except that the process was carried out on seven-week-old seedlings, and that the crown on one plant was severely scratched with a needle before inoculating, and one plant left uninjured. Thus, two plants of

the following species were inoculated: L. uliginosus (numbers 73 & 74); L. corniculatus (numbers 49 & 56); L. japonicus (number 72); L. siliquosis (number 84); L. hispidus (number 68); L. pedunculatus (number 67); Trifolium pratense variety Dollard (number 85); and Medicago sativa varieties Vernal (number 87), Dupuit (number 88), and Narragansett (number 89). One plant of each species was kept as a control. All plants were placed in a chamber, with artificial lighting only, for 12 hours daily, the temperature maintained between 65 and 75 F, and humidity 100% R.H. for two weeks, and then placed in the greenhouse for the remainder of the experiment.

Inoculation was carried out in July, 1961, and after $4\frac{1}{2}$ months, the plants were examined for signs of infection by sectioning under a stereoscopic microscope. Any suspicious growth was sectioned and examined under a compound microscope at high magnification.

2. Results

In both experiments, (a) and (b), no infection was detected although the inoculated plants were examined critically.

D. DISCUSSION

It can not be concluded that the resting spores actually germinated, or that they split as a result of imbibition by water. These results agree with Line (1921), who observed that zoospores of Physoderma alfalfae escaped through cracks, but Jones et al. (1920) reported that zoospores escaped through pores in the resting spore cell wall.

The complete failure to obtain infection would indicate that either the resting spores were no longer viable, and germination observed was in fact splitting by imbibition, or the methods employed for artificial inoculation were unsatisfactory.

It is difficult to believe that no infection would take place if 50% of the spores germinated readily at room temperature. During inoculation, the temperature was maintained between 65 and 75 F, while spores germinated in the laboratory between 70 and 80 F. Tisdale (1919) found germination of resting spores of Physoderma maydis occurred between 73 and 87 F, and Hebert et al. (1958) reported germination between 65 and 97 F. Sparrow (1957) found different results for P. pluriannulatum, the optimum temperature being 46 F and no germination occurring at 77 F.

Physoderma potteri has not been recorded in Canada, but since it has been reported from New York and Oregon, it is possible that crown gall could become a serious problem in moist, low lying areas.

Physoderma alfalfae has been reported throughout the United States and in British Columbia (Cormack 1945) and there is no evidence that this fungus will not attack birdsfoot trefoil; on the contrary, Hey (1946) reported P. alfalfae on birdsfoot trefoil in Germany, which would further indicate the potential menace of this fungus on Lotus in North America, as the crop becomes increasingly popular and widespread.

IV. STEMPHYLIUM LEAF SPOT

A. ISOLATION

1. Methods

During the springs of 1960 and 1961 a number of leaves with leaf spots, and stems with stem lesions were collected from the Lotus plot in the Macdonald College fields, and from the greenhouses.

The diseased plant parts were rinsed in Javex[1] solution, consisting of one part Javex to one part water, washed in sterile water and plated on potato dextrose agar containing 1:18,000 parts of rose bengal to inhibit the growth of bacteria.

2. Results

Isolates of Stemphylium obtained from the college fields and greenhouses compared morphologically with cultures obtained from Dr. C. R. Drake,[2] and identified by him as Stemphylium loti. Alternaria, Gliocladium, Botrytis, and three unidentified fungi were also repeatedly isolated from diseased leaves and stems of Lotus corniculatus.

B. ARTIFICIAL INOCULATION

1. Methods

Seven fungi, which were repeatedly isolated from diseased material, were selected for inoculation of L. corniculatus. The fungi were grown

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1 Javex, a commercial disinfectant and bleach containing 5.25% available chlorine, manufactured by Javex Company Limited, Montreal, P. Q.

2 Crops Research Division, U.S.D.A., Blacksburg, Virginia, U.S.A.

on malt extract broth for one week and then a mycelial and spore suspension was sprayed onto test plants. The inoculated plants were placed in an inside chamber, held at 100% R.H. for 48 hours, and then for seven additional days at high humidity. Artificial light alone was provided for 12 hours daily. After two weeks the plants were examined for symptoms typical of those found in the field and greenhouses.

2. Results

Only plants inoculated with isolates of Stemphylium loti showed leaf spots and stem lesions typical of the field and greenhouse symptoms.

C. DISCUSSION

The leaf spots and stem lesions found in the Macdonald College fields and greenhouses were determined to be caused by Stemphylium loti.

Stemphylium leaf spot results in defoliation of the older leaves in the spring, but the damage is slight, hence this disease may be considered of minor importance.

V. SCLEROTINIA WILT AND CROWN ROT

A. FIELD RESULTS

1. Isolation of *Sclerotinia trifoliorum*

a. Methods

A plant of *Lotus corniculatus* from the Macdonald College fields, which had been killed as a result of Sclerotinia wilt was removed from the soil and taken to the laboratory. The sclerotia from this plant were washed for three minutes in Javex solution consisting of one part Javex to one part water, then in 95% ethanol for one minute and rinsed thoroughly in sterilized water. Finally, the sclerotia were halved with a sterile scalpel and plated on malt extract agar containing 1:18,000 parts of rose bengal to inhibit the growth of bacteria. Within one week several plates showed colonies of *Sclerotinia trifoliorum* which were transferred to potato dextrose agar in petri plates. One such colony, which showed the greatest production of sclerotia, was transferred to several slants of potato dextrose agar, and material from this colony was used in all experimentation.

b. Results

Surface sterile sclerotia were germinated on nutrient agar with rose bengal as a bacterial inhibitor. Within one week, numerous fungal colonies of *Sclerotinia trifoliorum*, *Trichoderma* and *Penicillium* were observed.

S. trifoliorum was able to cause typical field symptoms of wilt and crown rot upon inoculation of birdsfoot trefoil, growing in pots in the greenhouse, whereas *Trichoderma* did not cause these symptoms.

2. Methods of Observing Field Injury

Three field plots of Lotus were observed during the spring, summer and autumn of 1960, and spring of 1961 for injury resulting from natural infection by Sclerotinia trifoliorum. Each plant in the three plots was examined periodically for the degree of wilt resulting from infection.

The field originally consisted of seven plots of Lotus prepared by Mrs. A. Chamberlain in the summer of 1959 for agronomic studies. The winter killing of 1959 - 1960 resulted in so much loss that only three plots were examined and analysed statistically. Plot 1. consisted of Lotus corniculatus largely originating from Turkey and Iran (Thesis numbers 1 - 15). Plots 5. consisted of European and commercial varieties of L. corniculatus (Thesis numbers 16 - 52). Plots 7. consisted of L. tenuis and related species (Thesis numbers 57 - 66). Plot 7. was observed in the spring of 1960 only, as the number of plants killed during that summer and the following winter made the plot incomplete. Each plot was randomly designed.*

A two year record of weather conditions, commencing in September 1959, and consisting of rainfall, snowfall and temperature readings, was obtained from the Horticulture Department of Macdonald College.

3. Field Observations

In 1960, the Lotus plots in the Macdonald College fields were examined in spring and early summer. Recordings of wilt were taken once in May and twice in June. In 1961, the first readings were made on May 6th, and

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* The field plan is filed with the Agronomy Department, Macdonald College, McGill University.

subsequently every five days until June 20th.

Observations in May of 1960 and 1961 revealed that the total number of plants wilted (Table I), 21.9% as against 18.3%, was not appreciably different. However, in June of the two years, 22.8% showed wilt in 1960 as against 7.6% in 1961. The difference in the degree of wilt, as illustrated by the total number of plants killed in Table I, shows that 46% of infected plants died in 1960 compared to only 6% in 1961.

Total moisture, as shown by snowfall and rainfall readings in Table I, reveal that in 1960 the total rainfall for the months of April and May was 8.31 inches, and snowfall the previous winter of 78.2 inches. In April and May of 1961, the total rainfall was 5.69 inches and snowfall the previous winter of 46.5 inches. Soil moisture readings were not made, but it was noted that the soil dried out more rapidly in the late spring of 1961, than it did the previous year.

The mean temperature for the month of May in 1960 was 61.8 F, whereas in 1961 it was 54.3 F (Table I). There was no appreciable difference between April temperatures of 1960 and 1961, and the June temperatures of the two years. In 1961, there was a cool period of about 15 days in the second half of May when the mean daily temperature (averaged over five day periods) did not exceed 54 F. There was no corresponding cool period during the spring of 1960 when the mean daily temperature (averaged over five day periods) ranged from 63 to 70 F.

Observations made on May 6th, 1961, revealed that 17 plants in the three plots, but primarily in plot 7 (the farthest east of the plots),

TABLE I

ECOLOGICAL FACTORS, AND DAMAGE CAUSED BY
SCLEROTINIA TRIFOLIORUM, IN 1960 AND 1961

	Mean daily temp. (F)	Monthly rainfall (inches)	Snowfall (inches)	No. of plants observed	No. of plants wilted	Percent wilt	No. of plants killed	No. of wilted plants killed
Winter 1959-1960			78.2					
April 1960	40.3	5.84						
May	61.8	2.47		1692	371[a]	21.9%	177	45.7%
June	66.5	2.69		1692	387[b]	22.8		
Winter 1960-1961			46.5					
April 1961	42.0	3.70						
May	54.3	1.99		1402	247[a]	18.3%	14	5.7%
June	64.0	4.44		1402	106[b]	7.6		

[a] Readings taken on May 26th, in 1960 and May 31st, in 1961.
[b] Readings taken on June 10th, in 1960 and 1961.

which were healthy the previous autumn, had been killed during the winter. In seven of the 17 winter killed plants there were sclerotia of Sclerotinia trifoliorum, indicating that the fungus probably played a part in winter killing.

The Lotus field was not examined for the presence of apothecia in the autumn of 1959, since this research project was not initiated until the spring of 1960.

In 1960 apothecia of S. trifoliorum were first seen on October 19th, and were kept under observation until the middle of November. Apparently as a result of frost, the leaves of birdsfoot trefoil were yellowing and dropping at approximately the same time as the apothecia were first observed. No plants showed wilt during this period of 1960 in the Lotus plots.

In 1961, apothecia were observed on November 3rd, in a mixed grass, clover and birdsfoot trefoil plot (but not in the Lotus plots which had been plowed). No foliar infection of birdsfoot trefoil was noted in this plot, but mycelium was seen growing naturally on the soil beneath the plants, and one plant of birdsfoot trefoil was showing symptoms of wilt, apparently as a direct result of infection from a mycelial mat which surrounded the crown.

The analysis of variance of the 1960 results shows a significant difference at the 5% level ($P = 0.05$) between types and varieties of birdsfoot trefoil to resistance to infection in plot 1. and plot 5. (Tables XIV and XVII in appendix). In plot 7 there was a significant

difference at the 1% level (P = 0.01) (Table IV following, and Table XX in appendix).

In the 1961 results, analysis of variance showed a significant difference at the 1% level in both plot 1. and plot 5. (Tables XV and XVII). Plot 7. readings were not analysed because of the small number of plants remaining in 1961.

The results of 1960 and 1961 were accumulated, which virtually amounted to a double opportunity for each plant to become infected, thus reducing the chance of escape from infection due to the absence of the pathogen. Any plant which showed wilt in 1960 and recovered, and was again infected in 1961, was recorded only once. Accumulation of the total number of plants of each type and variety in both plot 1. and plot 5. for the two seasons gave a significant difference at the 1% level (Tables II and III following, and Tables XVI and XIX in appendix).

B. ARTIFICIAL INOCULATION EXPERIMENTS

1. Preparation of Experimental Plants

Seeds of *Lotus* spp., alfalfa and red clover were planted in four inch pots. When the seedlings were at the first of second trifoliate-leaf-stage, each was transferred to an individual four inch pot. All plants were grown in the greenhouse under normal greenhouse conditions for three to five months before inoculation with *S. trifoliorum*.

2. Tests to Determine the Best Inoculum Preparation

In a preliminary experiment, the dried grain inoculum preparation of Kreitlow (1951a) was tested, but found to dry out before the fungus had

TABLE II

THE NUMBER OF PLANTS, ACCORDING TO TYPES AND VARIETIES OF LOTUS CORNICULATUS, WHICH SHOWED WILT DURING THE 1960 AND 1961 SEASONS IN PLOT 1.

Thesis no.	Origin of plants	Number of plants wilted	Duncan's multiple range test (b)				
6	Greece	62% (a)	c				
1	Sweden	55	c				
5	Argentine	47	c	d			
11	Turkey	31		d	e		
8	Turkey	29		d	e	f	
10	Switzerland	29		d	e	f	
7	Iran	28			e	f	
9	Turkey	28			e	f	
13	Iran	16			e	f	g
3	Iran	13			e	f	g
14	Iran	11				f	g
12	Turkey	11				f	g
2	Iran	3					g
4	Afghanistan	(h)					
15	Spain	(h)					

[a] Angular transformation of means.

[b] The vertical lines, represented by letters c - g, indicate groups of means within which it is not possible to demonstrate a significant difference at the 5% (P = 0.05) level.

[h] These plants were killed during the winter of 1959 - 1960.

TABLE III

THE NUMBER OF PLANTS, ACCORDING TO TYPES AND VARIETIES OF LOTUS CORNICULATUS, WHICH SHOWED WILT DURING THE 1960 AND 1961 SEASONS IN PLOT 5.

Thesis no.	Origin of plants	Number of plants wilted	Duncan's multiple range test (b)
49	Rouen, France	59% (a)	c
46	Viking (m)	58	c d
23	Fak Region, Hungary	52	c d e
50	Warsaw, Poland	51	c d e
19	Rožňava, C.S.R.	50	c d e f
20	Cascade (m)	49	c d e f
30	Sotsgorodok, U.S.S.R.	48	c d e f
52	Tana (m)	47	c d e f g
25	Malejov Region, C.S.R.	47	c d e f g
39	Mansfield (m)	47	c d e f g
17	Halle, Germany	46	c d e f g
44	Gelsvis, U.S.S.R.	46	c d e f g
28	Morshansk, U.S.S.R.	46	c d e f g
32	Moscow, U.S.S.R.	44	c d e f g h
35	Leningrad, U.S.S.R.	43	c d e f g h
29	Empire (m)	42	c d e f g h i
36	Granger (m)	42	c d e f g h i
22	Uppsala, Sweden	41	c d e f g h i
43	Viglas, C.S.R.	41	c d e f g h i
18	Tabor, C.S.R.	40	c d e f g h i
24	Copenhagen, Denmark	38	c d e f g h i
38	Dotnuva, U.S.S.R.	38	c d e f g h i j
45	Kirov, U.S.S.R.	38	c d e f g h i j
31	Trebitsch, C.S.R.	38	c d e f g h i j
27	Moscow, U.S.S.R.	37	c d e f g h i j
26	Italy	37	c d e f g h i j
33	U.S.S.R.	36	c d e f g h i j
47	Budapest, Hungary	34	d e f g h i j
16	Graz, Austria	34	d e f g h i j
34	Munich, Germany	34	e f g h i j
41	Prekule, U.S.S.R.	32	e f g h i j
51	Novgorod, U.S.S.R.	26	f g h i j k
40	Stavropol, U.S.S.R.	24	g h i j k
42	Yalta, U.S.S.R.	21	h i j k
37	New York, U.S.A.	19	i j k
21	Besançon, France	14	j k
48	Doonfoot, Scotland	4	k

a Angular transformation of means.

b The vertical lines, represented by letter c - k, indicate groups of means within which it is not possible to demonstrate a significant difference at the 5% (P = 0.05) level.

m Commercial varieties.

TABLE IV

THE NUMBER OF PLANTS, ACCORDING TO SPECIES, TYPES AND VARIETIES IN THE *LOTUS TENUIS* GROUP, WHICH SHOWED WILT DURING THE 1960 SEASON IN PLOT 7.

Thesis no.	Species, types and varieties	Origin	Number of plants wilted	Duncan's multiple range test (b)
61	*L. tenuis* Var. Los Banos	(e)	41% (a)	c
62	*L. tenuis* (f)	C.S.R.	38	c
66	*L. tenuis*	France	37	c
65	*L. tenuis*	Turkey	29	c d
63	*L. tenuis*	France	22	c d
59	*L. tenuis*	Greece	12	d
57	*L. krylovii* (g)	Sweden	9	d

[a] Angular transformation of means.
[b] The vertical lines, represented by letters c & d, indicate groups of means within which it is not possible to demonstrate a significant difference at the 5% (P = 0.05) level.
[e] Commercial variety.
[f] Synonymy: *L. tenuifolius*
[g] Synonymy: *L. corniculatus* var. *heterophylliarus*

thoroughly permeated the substrate. It was particularly difficult to remove the inoculum from wide-mouthed Erlenmeyer flasks in which it had been prepared. Furthermore, the inoculum was hard to grind up and it was difficult to separate the sclerotia and permeated substrate from the oat chaff and unpermeated grain.

Sclerotinia trifoliorum is principally a parasite of legumes, therefore, it was thought that an inoculum prepared from a legume substrate would be more pathogenic than one prepared from grain. Whole dry peas (of the type used to make pea soup) and whole dry red kidney beans were each used to prepare an inoculum in a manner similar to Kreitlow's (l.c.) grain method.

Red kidney beans did not prove very satisfactory, however. They were too large to handle easily, and the fungus took too long to permeate each bean thoroughly.

On the other hand, the pea inoculum showed promise. The fungus grew more rapidly than it did on grain, produced abundant sclerotia, and the soaked peas retained enough moisture to prevent drying out during the three week incubation period of the fungus. Pea inoculum could readily be removed from the flasks in which it was prepared, and was much easier to grind up than the grain inoculum. Finally, in the ground up pea inoculum the small particles of sclerotia and fungus-permeated peas were easily separated from the unpermeated peas which had become dust in the grinding.

This pea inoculum was then compared with the dried grain inoculum (consisting of two parts wheat to one part oats) to determine which was

most desirable and effective. At first, laboratory tests were carried out to determine the number of sclerotia produced, and secondly, pathogenicity tests were used to compare the two types of inoculum.

a. Laboratory tests

i. Methods

Inoculum was prepared in seven different ways to determine in which most sclerotia were produced: (1) Sixty grams of peas per flask.

(2) One hundred grams of peas per flask.

(3) One hundred and forty grams of peas per flask.

(4) Sixty grams of peas per flask and incubated in a dark cupboard at laboratory temperature.

(5) Sixty grams of peas and one gram of dextrose per flask.

(6) Sixty grams of Kreitlow's grain mixture per flask.

(7) Sixty grams of grain mixture and one gram of dextrose per flask.

Five flasks were prepared for each treatment. Each flask with substrate was soaked for 24 hours in tap water after which time the excess water was drained off. It was found that in the case of treatments nos. 6 and 7, with the grain mixture, it was necessary to add 10 ml of tap water after draining to prevent drying out.

With the exception of treatment no. 4, all flasks were incubated on a laboratory bench under normal basement laboratory light, but without sunlight. All flasks were incubated for three weeks at temperatures between 70 and 75 F.

Each flask was weighed empty, numbered, and again weighed with its contents after sterilization in the autoclave.

The criterion selected to determine the best method of preparing inoculum was based upon the weight of sclerotia produced (Held 1955).

The sclerotia from the pea inoculum were separated by placing the thoroughly dried inoculum from an individual flask in a Waring blender with 200 ml of tap water. The blender was allowed to operate for 30 seconds after which time the contents were screened through a 20-mesh sieve. The sclerotia retained by the sieve were oven dried and weighed.

The grain inoculum was treated in a Waring blender and the mixture screened through a 20-mesh sieve in the same manner as the pea inoculum; however, in addition to sclerotia, a large amount of oat chaff and wheat particles was also retained by the sieve. In order to separate the sclerotia, it was necessary to pass the mixture through a 10-mesh sieve which collected the wheat particles and much of the chaff, thus separating the sclerotia which were then dried and weighed.

ii. Results

The highest mean weight of sclerotia produced by preparing the inoculum in seven different ways, was in the dried pea preparation where 60 g of peas were inoculated, and incubated in the dark (Table V) at room temperature. There was no appreciable difference in the weight of sclerotia produced between the pea and grain preparations. The weight of sclerotia was greatly reduced when 100 g or 140 g of peas were used in a single 500 ml wide-mouth Erlenmeyer flask. The addition of 1 g of dextrose had no effect on sclerotial production.

TABLE V

WEIGHT OF SCLEROTIA PRODUCED BY SEVEN METHODS OF INOCULUM PREPARATION

Treatment				Mean weight of sclerotia per flask
Exp. no.	Type of inoculum	Weight of inoculum	Illumination	
4	Pea	60 g	dark	8.88 g ± 1.21*
6	Grain	60	light	6.18 0.30
7	Grain + dextrose	60	light	5.88 1.96
1	Pea	60	light	5.62 0.92
5	Pea + dextrose	60	light	5.48 1.13
2	Pea	100	light	2.96 1.06
3	Pea	140	light	2.13 0.48

* Standard deviation.

b. Pathogenicity tests

i. Methods

Five flasks, each containing 60 g of soaked peas, and five flasks, each containing 60 g of the grain mixture were prepared and incubated for three weeks in a dark cupboard. The dried inoculum was applied to 70 five-month-old plants of L. corniculatus.

The 70 plants were divided into groups of 10 (which constituted a replicate). Five plants in each replicate were inoculated with one gram of the dried pea inoculum, and five plants with one gram of the grain mixture. One additional plant in each replicate remained uninoculated and was kept as a control.

ii. Results

The number of plants killed by the dried pea inoculum was 54%, while with the dried grain inoculum, 34% were killed. Analysis of the results using Fisher's "t" test showed that the probability of dried pea inoculum being more effective was not significantly different.

Dried pea inoculum was found to cause more rapid wilt than the dried grain inoculum.

It was noted that the dried pea inoculum, placed around the crown of a plant, occasionally became permeated with bacteria a few days later, destroying the pathogen and thus some plants escaped infection. On the other hand, the dried grain inoculum, placed around the crown of a plant, frequently became covered with the fungus Trichoderma, which was also a cause for escape from infection.

3. Preparation of Dried Pea Inoculum and Inoculation Procedure

On the basis of the results obtained from the tests to determine the best inoculation preparation, a modification of Kreitlow's (1951a) dried grain technique was devised and used for the majority of laboratory pathogenicity studies with Sclerotinia trifoliorum. Sixty grams of whole dried peas were placed respectively in 500 ml wide-mouthed, Erlenmeyer flasks which were then filled with tap water. The flasks were plugged with plugging cotton and allowed to stand 24 hours to permit thorough moistening of the contents. They were then drained of all excess water and sterilized in an autoclave at 15 pounds pressure for half an hour. When the flasks were cooled to room temperature, they were inoculated with a sclerotium from one of the original cultures of S. trifoliorum and placed in a dark laboratory cupboard for three weeks by which time the mycelium had thoroughly covered the peas, and many black sclerotia were formed. The flasks were emptied and contents air dried in the laboratory for four to seven days. The dried inoculum, containing many sclerotia, was passed through a grain grinder to reduce the size of the particles. The inoculum was finally screened through a 20-mesh sieve. Inoculum retained by the sieve was placed in a screw-cap glass jar and refrigerated at 37 F for use, while that passing through the sieve, which consisted of powdered pea particles, was discarded.

In all experiments using dried pea inoculum, the following procedure was carried out.

Healthy three- to five-month-old seedlings growing in four inch pots were placed in an inside chamber with artificial lighting only. In each

experiment, or replicate of an experiment, the plants were of the same age and size.

The soil was carefully removed in order to expose the crown, and approximately half a level teaspoonful of dried inoculum was placed around the crown of each plant. The inoculated plants were then thoroughly watered. The chamber was kept at 60 to 65 F and between 70 and 80% R.H. The plants were supplied with artificial lighting for 12 hours each day.

Observations of the degree of wilt were made when the first symptoms of wilt appeared, and subsequently every two days. After 14 days in the chamber, the plants were removed to a greenhouse bench and final readings on the number of plants killed, were taken one week later.

4. Results of Crown Inoculation Experiments

a. Lotus corniculatus

Twenty-nine types and commercial varieties of Lotus corniculatus were tested for their reaction to crown inoculation with the fungus Sclerotinia trifoliorum. Five plants of each type or commercial variety were inoculated in each experiment, and the experiment was repeated twice. Analysis of variance was carried out on the number of plants wilted and the number of plants killed. In neither case was there significant difference between the most susceptible and most resistant types and varieties (Table VI following and Tables XXI and XXII in the appendix).

b. Lotus species and other Legumes

Seventeen species of Lotus, two varieties of alfalfa, and a single variety of red clover were tested for their reaction to crown inoculation with the fungus S. trifoliorum. Five plants of each species or variety

TABLE VI

SUSCEPTIBILITY OF *LOTUS CORNICULATUS* TO *SCLEROTINIA TRIFOLIORUM* IN CROWN INOCULATION EXPERIMENTS

Thesis no.	Origin of plants	Percent infection	Percent killing
44	Gelsvis, U.S.S.R.	100%	87%
27	Moscow, U.S.S.R.	80	75 (a)
42	Yalta, U.S.S.R.	93	64
26	Italy	80	63 (a)
4	Afghanistan	93	57
39	Mansfield (b)	87	54
14	Iran	87	54
9	Turkey	100	53
12	Turkey	80	50
31	Trebitsch, C.S.R.	100	50 (a)
2	Iran	100	47
25	Malejov Region, C.S.R.	87	46
56	Colyton, England	87	46
19	Rožňava, C.S.R.	74	45
11	Turkey	80	42
17	Halle, Germany	80	42
37	New York, U.S.A.	80	42
29	Empire (b)	67	40
49	Rouen, France	87	38
1	Sweden	87	38
48	Doonfoot, Scotland	80	38 (a)
52	Tana (b)	100	36
3	Iran	100	33
6	Greece	80	33
36	Granger (b)	80	33
46	Viking (b)	80	33
18	Tabor, C.S.R.	60	33
54	St. Andrews, Scotland	70	29 (a)
22	Uppsala, Sweden	67	0

[a] Two replicates only.
[b] Commercial varieties.

were inoculated in each experiment, and the experiment was repeated twice. Analysis of variance was carried out on the number of plants wilted and the number of plants killed. In both cases there was a significant difference at the 1% level (Tables XXIII and XXIV in the appendix). Duncan's multiple range test (Robinson 1959) was applied (Tables VII and VIII following).

5. Ascospore Inoculation

a. Methods

Apothecia of Sclerotinia trifoliorum were collected in early November, 1961, from the Macdonald College fields.

Sixteen plants of L. corniculatus, consisting of a number of different types and commercial varieties, were placed in an inside chamber and supplied with high humidity, and artificial lighting only for 12 hours daily.

A cotton thread was sewn through the stipe of each apothecium and tied in order to form a loop, and then attached to a bent paper clip which served as a two-way hook. Two pieces of string were placed above the plants in the chamber. Eighteen mature apothecia were hung evenly on the string about one foot above the plants.

The humidity of the chamber was maintained at 100% R.H. for three days. On the second day, a small drop of 75% ethanol was placed on the end of the stipe of each apothecium. The ethanol forced the discharge of the remaining ascospores. Glass slides were placed at intervals to show that ascospores were being discharged. This actually proved to be the case.

TABLE VII

SUSCEPTIBILITY OF *LOTUS* SPECIES AND OTHER LEGUMINOSAE TO *SCLEROTINIA TRIFOLIORUM* IN CROWN INOCULATION EXPERIMENTS (NUMBER OF PLANTS INFECTED)

Thesis no.	Species	Percent infection	Duncan's multiple range test (b)
81	*L. maroccanus*	90% (a)	c
58	*L. palustris*	90	c
84	*L. siliquosus*	90	c
73	*L. uliginosus*	90	c
74	*L. uliginosus*	81	c d
68	*L. hispidus*	72	c d e
75	*L. uliginosus*	72	c d e
60	*L. filicaulis*	64	c d e f
76	*L. uliginosus*	59	c d e f
85	*Trifolium pratense*	55	c d e f
70	*L. ornithopodioides*	51	c d e f
72	*L. japonicus*	47	d e f g
83	*L. peregrinus*	47	d e f g
71	*L. jacobaeus*	43	d e f g
67	*L. pedunculatus*	34	e f g h
78	*L. suaveolens*	26	f g h
89	*Medicago sativa*	9	g h
87	*M. sativa*	0	h
77	*L. parviflorus*	90	These results were not included in the analysis of variance as too few plants were tested.
80	*L. weilleri*	90	
79	*L. pusillus*	90	
69	*L. requienii*	68	
82	*L. coccineus*	63	

[a] Angular transformation of means.

[b] The vertical lines, represented by letters c - h, indicate groups within which it is not possible to demonstrate a significant difference at the 5% ($P = 0.05$) level.

TABLE VIII

SUSCEPTIBILITY OF *LOTUS* SPECIES AND OTHER LEGUMINOSAE TO *SCLEROTINIA TRIFOLIORUM* IN CROWN INOCULATION EXPERIMENTS (NUMBER OF PLANTS KILLED)

Thesis no.	Species	Percent killing	Duncan's multiple range test (b)
81	*L. maroccanus*	90% (a)	c
58	*L. palustris*	77	c d
68	*L. hispidus*	59	c d e
84	*L. siliquosus*	51	d e
60	*L. filicaulis*	47	d e f
72	*L. japonicus*	47	d e f
83	*L. perigrinus*	47	d e f
71	*L. jacobaeus*	43	d e f
73	*L. uliginosus*	39	d e f
85	*Trifolium pratense*	39	d e f
74	*L. uliginosus*	39	d e f
75	*L. uliginosus*	35	e f
70	*L. ornithopodioides*	34	e f
76	*L. uliginosus*	31	e f
67	*L. pedunculatus*	26	e f
78	*L. suaveolens*	22	e f
89	*Medicago sativa*	9	f
87	*M. sativa*	0	f
77	*L. parviflorus*	90	These results were not included in the analysis of variance as too few plants were tested.
79	*L. pusillus*	68	
80	*L. weilleri*	63	
82	*L. coccineus*	63	
69	*L. requienii*	58	

[a] Angular transformation of means.

[b] The vertical lines, represented by letters c - f, indicate groups within which it is not possible to demonstrate a significant difference at the 5% ($P = 0.05$) level.

At the end of three days, the spent apothecia were removed and a duplicate set of plants placed in the chamber to serve as control. All plants were kept in the chamber for an additional two weeks with humidity maintained at approximately 75% R.H. and artificial lighting for 12 hours each day. Observations were made every four days after inoculation.

b. Results

Ascospore infection of Lotus corniculatus was characterized by the presence of minute brown spots on the leaves, followed by yellowing, and soon after, by defoliation of all infected leaves. Symptoms on all types and commercial varieties such as in the number of leaves infected could not be compared as only a single plant of each was tested.

6. Mycelial Inoculation

a. Methods

Sclerotinia trifoliorum was grown on undiluted tomato juice, prepared from fresh tomatoes, in 250 ml Erlenmeyer flasks. Twenty-five ml of tomato juice was used in each flask. After six days of growth, the mycelial mats formed in the flasks were placed in a Waring blender and macerated. The undiluted mycelium suspension was sprayed onto test plants in an inside chamber, using a small hand-operated insect spray gun. Glass slides were placed throughout the chamber to test the effectiveness of cover of the spray.

The chamber was maintained at 100% R.H. for three days and then at high humidity for an additional four days. The temperature was held between 60 and 70 F. There was no natural light; artificial light was provided for 12 hours daily. One week after inoculation, the plants

were placed in the greenhouse under natural conditions. Observations were made every two days after inoculation.

Twelve species of Lotus, thirty-three types and varieties of L. corniculatus, and two commercial varieties of each of red clover and alfalfa were inoculated. Three plants of each species or type were inoculated while one additional plant of each was kept as an uninoculated control.

b. Results

Inoculation of the foliage of L. corniculatus using a mycelial suspension of S. trifoliorum resulted in identical symptoms to ascospore inoculation, that is, minute brown spots, yellowing of the leaf and defoliation. However, a total of nine plants, within six types of L. corniculatus, as recorded in Table IX, were killed. The fungus grew externally down the stems, from the leaves, and into the crown of these nine plants alone.

The species of Lotus tested showed a number of symptoms as recorded in Table IX. The symptoms on Trifolium pratense were severe necrosis, a little chlorosis followed by dying back of infected leaves; however, no clover plant was killed as a result of foliar inoculation.

C. FUSARIUM AS A SECONDARY INVADER

A mildly pathogenic strain of Fusarium, which had been isolated from a severely wilted birdsfoot trefoil plant in the Macdonald College fields, was tested to determine its influence on Sclerotinia wilt and crown rot,

TABLE IX

SYMPTOMS RESULTING FROM FOLIAR INOCULATION WITH *SCLEROTINIA TRIFOLIORUM*

Thesis no.	Species	Symptoms
85 86	*Trifolium pratense* *T. pratense*	Leaf necrosis and die-back of petioles
(a)	*Lotus corniculatus*	Leaf necrosis, die-back of petioles and death of plants
83 70	*L. perigrinus* *L. ornithopodioides*	Severe chlorosis, defoliation and death of plants
87 89 (b) 58 81 78 71 67	*Medicago sativa* *M. sativa* *Lotus corniculatus* *L. palustris* *L. maroccanus* *L. suaveolens* *L. jacobaeus* *L. pedunculatus*	Small necrotic spots, chlorosis and defoliation
74 75 76 72	*L. uliginosus* *L. uliginosus* *L. uliginosus* *L. japonicus*	Trace of chlorosis
68 84	*L. hispidus* *L. siliquosus*	No infection

[a] Thesis numbers: 2 (Iran), 3 (Iran), 4 (Afghanistan), 7 (Iran), 8 (Turkey), 27 (Moscow, U.S.S.R.).

[b] Thesis numbers: 1, 6, 11, 12, 17, 18, 19, 22, 23, 25, 26, 29, 31, 36, 37, 39, 40, 42, 43, 44, 46, 48, 49, 52, 53, 54, 56.

and its *in vitro* effect on *Sclerotinia trifoliorum*.

1. *Inoculation Tests (in vivo)*

a. *Methods*

The isolate of *Fusarium* was grown in 250 ml Erlenmeyer flasks each containing 50 ml of Czapek broth. Healthy *Lotus corniculatus* plants were inoculated with *S. trifoliorum* and, or, *Fusarium*, in one of the following methods of treatment: (1) *S. trifoliorum* alone.

(2) *Fusarium* alone.

(3) *Fusarium* and *S. trifoliorum* inoculated together on the same day.

(4) *S. trifoliorum* inoculated three days prior to *Fusarium*.

(5) *S. trifoliorum* inoculated six days prior to *Fusarium*.

(6) *Fusarium* inoculated three days prior to *S. trifoliorum*.

(7) Uninoculated control.

Birdsfoot trefoil was inoculated with *Fusarium* by pouring a macerated mycelium and spore suspension around the crown of each plant. *S. trifoliorum* was inoculated by using the dried pea inoculum. Each treatment consisted of five plants, and the whole experiment was repeated twice, thus consisting of three replicates.

b. *Results*

The isolate of *Fusarium* used did not have any importance as a secondary invader in Sclerotinia wilt and crown rot as seen in Table X. The isolate proved to be mildly pathogenic since it killed one plant out of 15 when inoculated alone, and in the greenhouse, was able to cause yellowing of leaves when inoculated into the soil of a pot containing birdsfoot trefoil.

TABLE X

THE EFFECT OF FUSARIUM AS A SECONDARY INVADER ON WILTED LOTUS CORNICULATUS

Treatment	Percent killing
S. trifoliorum alone	73% ± 19*
Fusarium alone	7 10
S. trifoliorum and Fusarium together	53 10
S. trifoliorum 3 days prior to Fusarium	73 19
S. trifoliorum 6 days prior to Fusarium	60 42
Fusarium 3 days prior to S. trifoliorum	87 28

* Standard deviation of mean percent of wilt of three replicates.

2. Laboratory Tests (in vitro)

a. Methods

Sclerotinia trifoliorum and the isolate of Fusarium were inoculated into a series of petri plates, approximately 5 cm apart, containing potato dextrose agar. After seven days the plates were observed for signs of inhibition of growth.

b. Results

The isolate of Fusarium inhibited the growth of S. trifoliorum when both organisms were inoculated 5 cm apart in a petri plate. The Fusarium colony grew in a uniform manner from the point of inoculation on the petri plate, while S. trifoliorum did not grow closer than 0.5 cm from the perimeter of the Fusarium colony on any of the six petri plates.

D. BIOLOGICAL CONTROL

Investigations into the biological control of soil-inhabiting phytopathogens has received much attention (Wood et al. 1955). One of the most promising organisms for this task is Trichoderma spp., which parasitizes a number of fungi as well as producing a toxin which inhibits the growth of many mircoorganisms (Wright 1954).

1. Inoculation Tests (in vivo)

a. Methods

An isolate of Trichoderma was obtained from a parasitized sclerotium of Sclerotinia trifoliorum and was inoculated into a series of fourteen, 250 ml Erlenmeyer flasks, each containing 50 ml of Czapek broth, on seven, three-day intervals.

The following treatments were performed on Lotus corniculatus using Trichoderma and, or, S. trifoliorum: (1) S. trifoliorum alone.

(2) Trichoderma alone.

(3) S. trifoliorum and Trichoderma inoculated together and on the same day.

(4) Trichoderma inoculated three days prior to S. trifoliorum.

(5) Trichoderma inoculated six days prior to S. trifoliorum.

(6) Trichoderma inoculated nine days prior to S. trifoliorum.

(7) S. trifoliorum inoculated three days prior to Trichoderma.

(8) S. trifoliorum inoculated six days prior to Trichoderma.

(9) S. trifoliorum inoculated nine days prior to Trichoderma.

(10) Uninoculated control.

Birdsfoot trefoil was inoculated with Trichoderma by using ten-day-old cultures which were macerated in a Waring blender, and the mycelial and spore suspension was applied to the area around the crown of each plant. S. trifoliorum was inoculated by using the dried pea inoculum. The experiment was so arranged that S. trifoliorum was inoculated on the same day in each treatment. Five plants were used for each treatment, and the experiment repeated once.

b. Results

Trichoderma caused a reduction in infection, as seen in Table XI, when applied to the soil around the crown of birdsfoot trefoil at the time of inoculation with S. trifoliorum, or when it was applied up to nine days prior to inoculation. Trichoderma has no effect on reduction of infection when applied three, six or nine days after inoculation with S. trifoliorum. Trichoderma failed to cause any disorder in birdsfoot trefoil when applied to the crown alone and the soil around the crown.

TABLE XI

THE EFFECT OF *TRICHODERMA* ON SCLEROTINIA WILT OF *LOTUS CORNICULATUS*

Treatment	Number of plants killed		
	Exp.1	Exp.2	Total
S. trifoliorum alone	2[a]	4	6[b]
Trichoderma alone	0	0	0
S. trifoliorum and *Trichoderma* together	1	0	1
Trichoderma 3 days prior to *S. trifoliorum*	2	0	2
Trichoderma 6 days prior to *S. trifoliorum*	0	1	1
Trichoderma 9 days prior to *S. trifoliorum*	3	0	3
S. trifoliorum 3 days prior to *Trichoderma*	4	3	7
S. trifoliorum 6 days prior to *Trichoderma*	5	3	8
S. trifoliorum 9 days prior to *Trichoderma*	3	2	5

[a] Each treatment contained five plants.
[b] A total of 10 plants.

2. Laboratory Tests (in vitro)

a. Methods

A large number of Actinomycetes were isolated from soil by using the phenol technique of Lawrence (1956), and then tentatively screened for antagonism by spraying a suspension of S. trifoliorum over the cultures of Actinomycetes growing in petri plates. Twelve Actinomycetes, which showed antagonism, were tested against an isolate of Fusarium (which was used in the previous experiments to test its importance as a secondary invader), Sclerotinia trifoliorum isolated from a wilted birdsfoot trefoil at Macdonald College, Sclerotium rolfsii, Rhizoctonia solani, and Stemphylium loti. These latter cultures were obtained from Dr. C. R. Drake. Three isolates of Penicillium and two isolates of Trichoderma were also tested for antagonism against these five fungi.

The antagonistic organism and the fungus in each case were plated on potato dextrose agar about 5 cm apart, and observed daily for signs of antagonism.

Antagonism was measured by visual inspection for a clear zone between the two approaching colonies, and microscopically for signs of killing of the hyphal tips of the fungus as it approached the antagonistic organism.

b. Results

In tests on the inhibition of five pathogenic fungi of birdsfoot trefoil by antibiotics from Actinomycetes, it was found that Sclerotinia trifoliorum was inhibited the most and Fusarium the least (Tables XII and XIII). S. trifoliorum was inhibited by 7 Actinomycetes whilst Fusarium was inhibited by a single one only. S. trifoliorum was readily inhibited

TABLE XII

THE EFFECT OF ACTINOMYCETES (*IN VITRO*) ON FIVE PHYTOPATHOGENS OF *LOTUS CORNICULATUS*

Phytopathogen	Actinomycete cultures											
	1	2	3	4	5	6	7	8	9	10	11	12
Fusarium	-	-	-	-	-	-	-	-	+	-	-	-
Sclerotium rolfsii	-	-	-	-	-	-	+	-	-	+	+	+
Rhizoctonia solani	-	-	-	-	+	-	-	-	-	+	+	+
Stemphylium loti	-	-	-	+	+	-	-	+	-	-	+	+
Sclerotinia trifoliorum	-	-	-	-	-	+	+	+	+	+	+	+

+ indicates antagonism of the Actinomycete on the phytopathogen.
- indicates no antagonism.

TABLE XIII

THE EFFECT OF ANTAGONISTIC FUNGI (*IN VITRO*) ON FIVE PHYTOPATHOGENS OF *LOTUS CORNICULATUS*

Phytopathogens	*Penicillium* cultures			*Trichoderma* cultures	
	1	2	3	1	2
Fusarium	++	++	++	-a	-a
Sclerotium rolfsii	++	++	++	++	++
Rhizoctonia solani	-	-	++	+b	+b
Stemphylium loti	+	+	+	+b	+b
Sclerotinia trifoliorum	+	+	+	+b	+b

\+ Indicates antagonism on the phytopathogen.
\- Indicates no antagonism.
++ Indicates mutual antagonism of the two organisms on one-another.
a *Fusarium* inhibited the growth of *Trichoderma*.
b Antagonism by parasitism.

by the three isolates of Penicillium and was parasitized by Trichoderma, while Fusarium proved to be antagonistic against Penicillium and Trichoderma. The pathogenic fungus Sclerotium rolfsii also produced an inhibitor (Table XIII).

E. DISCUSSION

Since Sclerotinia trifoliorum inoculated into the crown of birdsfoot trefoil in pots was able to reproduce the symptoms of wilt and crown rot observed in the Macdonald College fields in 1960 and 1961, it could be concluded that this fungus was the cause of the disease. The presence of black sclerotia in rotted crown tissue, and below the epidermis on the lower parts of the stems, can be considered as positive proof that S. trifoliorum is responsible for the wilt and crown rot. It cannot be a secondary invader, since this fungus is unable to compete successfully with other soil organisms, as shown by its ready inhibition in the studies on biological control. Furthermore, it cannot compete with Fusarium, and therefore the presence of sclerotia and Fusarium in wilted plants indicates that S. trifoliorum was the primary, and Fusarium the secondary invader. Fusarium spp. have been reported by Roberts (1954), Ford (1959) and Kainski (1960) as being a primary cause of crown and root rot of birdsfoot trefoil in New York State, but this was not found to be the case at Macdonald College. Every seriously wilted plant examined showed the presence of S. trifoliorum sclerotia.

S. trifoliorum in the Lotus plots probably originated from successive clover plantings in this section of the field over a number of years. Sclerotia remain viable in the soil for long periods (Handson and Kreitlow

1953) so that it is probable that the disease organism built up in intensity over a long period. This would also imply that the strain of S. trifoliorum originated on clover, since birdsfoot trefoil had not been grown there before.

The most controversal aspect of S. trifoliorum is the disease cycle, whether infection is from mycelium in the spring or ascospores in the autumn. Favoring the theory of direct crown infection by sclerotial mycelium in the spring, is the fact that the leaves of birdsfoot trefoil killed by frost, are dropping at the time when apothecia are being formed. This leaves little opportunity for foliar infection in the autumn and subsequent growth of the fungus down the stem and into the crown. Wadham (1925) claimed that the ascospores germinated on the fallen clover leaves and from there invaded the crowns of clover; this theory could also hold true for birdsfoot trefoil since the fungus is able to grow at near freezing temperatures and below the snow (Taurikut et al. 1951, and Loveless 1951). This would also account for Sclerotinia damage being a contributing factor to winter killing, as indicated by the presence of sclerotia in seven out of 17 plants killed during the winter of 1960 - 1961.

The spring temperature and rainfall readings do not give any clue to the disease cycle, since it can be argued that the fungus in the crown, has originated from foliar infection in the autumn, and remains quiescent until spring conditions are favorable for its growth.

The principal evidence in this thesis on the nature of the disease cycle is derived from the foliar infection experiments. Ascospore or mycelial inoculation resulted in rapid defoliation of most birdsfoot

trefoil types, and since natural defoliation occurs in the autumn, the fungus is contributing to a more rapid loss of leaves, giving the fungus little chance to grow down into the crown. Defoliation was not observed after foliar inoculation of red clover, however. Six types of birdsfoot trefoil were killed after foliar inoculation, as shown in Table IX, but five of these proved to be more resistant in the field, which indicates that their susceptibility to foliar infection could have been of little or no consequence in nature. Only one type (number 4 from Afghanistan), which was killed after foliar infection, was completely killed out during the winter 1959 - 1960, but this winter killing could have been as a result of frost, fungus, or other damage.

The killing of birdsfoot trefoil in foliar inoculation experiments (Table IX) could be attributed to the ideal infection conditions, which would not normally be found during November at Macdonald College.

A possible factor favoring the ascospore infection theory is that the greatest winter killing in 1960 - 1961 occurred in plot 7. Since this was on the eastern end of the field, it could be argued that the spores were blown from a previous center of high infection in plot 5, just to the west, by the prevailing westerly winds.

The disease cycle of S. trifoliorum on birdsfoot trefoil is not necessarily the same as on red clover. It is probable, furthermore, that the disease cycle would vary according to the regional climatic conditions. The differences of opinion of various workers would support this view. Moreover, it is possible that both stages in the life cycle of the fungus might play a part in initiating infection.

The effect of S. trifoliorum on winter killing could be entirely due to the fungus, or frost damage could predispose the plant to infection, or finally, the fungus could predispose the plant to frost damage. The healthy or frost-weakened plant may be invaded under the snow cover, when low temperatures could favor the competitive saprophytic ability of the pathogen over other organisms. The presence of sclerotia in seven of 17 winter killed plants in the spring of 1961 shows only that S. trifoliorum has some effect in the winter killing, but does not positively prove that the fungus alone, was responsible for the death of the plants.

Two years' field observations are barely sufficient to draw any conclusions on the ecology of a disease. However, the marked difference in the severity of Sclerotinia wilt and crown rot in 1960 and 1961 can probably be attributed to a difference in rainfall and snowfall affecting the soil moisture, or to the temperature. The total precipitation, as shown in Table I, was considerably higher in the early spring of 1960 and the May temperature was 5.5 degrees (Fahrenheit) higher. Sclerotinia wilt and crown rot first appears in early May and progresses until the summer heat starts about mid-June. The number of plants showing wilt in May of the two years was similar (21.9% in 1960 and 18.3% in 1961), but the difference was shown in degree of wilt (number of plants killed) and the amount of wilt in early June. The results indicate that soil moisture content and warm weather (60 - 65 F) are important factors favoring infection and disease development.

Inoculation experiments were carried out by three methods: direct crown inoculation using dried pea inoculum; inoculation of leaves using either ascospores or, thirdly, using mycelial suspension. It is not

possible to compare the three methods unless the disease cycle is first determined. Crown inoculation would be equivalent to spring infection from germination of sclerotia in the soil. Mycelial suspension inoculation of leaves would be equivalent to ascospore invasion, which some workers claim is the natural method of infecting clover in the autumn.

Inoculation of the crown of birdsfoot trefoil and other legumes using either Kreitlow's (1951a) dried grain method, or the dried pea method developed by the writer, proved to be a very effective way of obtaining infection. However, as nearly all birdsfoot trefoil plants inoculated became infected, it is an unsatisfactory method of determining resistance to infection since a plant which might resist infection in nature (a thick epidermis or cuticle), would thus succumb to artificial crown infection. Results in Table VI show that from 67 to 100% infection was obtained with types of Lotus corniculatus. The number of plants killed when inoculated by this method, gave an index of susceptibility, but this could not be correlated with the number of plants, in each type and variety of birdsfoot trefoil killed in the field. The correlation coefficients, although positive, did not prove to be significant (Tables XXV and XXVI in appendix).

It was found that pea inoculum was superior to dried grain principally because it was easier to handle, but also because fungus growth was more rapid and sclerotial formation occurred earlier on peas than on grain. Pea substrate did not have the same tendency to dry out as grain had. The weight of the sclerotia produced in each method was approximately equal (Table V), therefore dried pea and dried grain inoculum would probably have the same keeping qualities. Pea inoculum, prepared by incubating in the dark, contained more sclerotia than pea inoculum or grain inoculum

prepared by incubating in (laboratory) light, but this does not establish whether grain inoculum would have produced more or less sclerotia than pea inoculum if both had been incubated in the dark. The addition of one gram of dextrose did not aid in sclerotial production. Dried pea inoculum did not prove statistically to be more pathogenic than dried grain inoculum (54% as against 34% of inoculated plants killed), but it caused more rapid infection.

Resistance to foliar infection and resistance to crown infection was dissimilar for many species and types of Lotus. L. peregrinus, for instance, showed 47% infection by crown inoculation, but by foliar inoculation, defoliation was so rapid and complete that all inoculated plants died. L. hispidus showed 72% infection to crown inoculation, but failed to become infected when inoculated with a mycelial suspension. L. maroccanus showed complete infection and killing after crown inoculation, but only necrotic spots, chlorosis and defoliation by mycelial inoculation.

Owing to the large number of species, types and varieties tested for resistance, it was necessary to reduce the number of plants per replicate, and the number of replications. Five plants to a replicate for each species or type of Lotus is barely sufficient since one plant can affect the reading by 20 percent. Three replications alone gives little chance of obtaining significant differences. All plants were obtained from seed in order to survey the population; this again increases the need for replication, whereas, if the plants had been obtained from cuttings originating from one clone, the variation would be reduced.

According to Frandsen (1946), resistance of legumes to S. trifoliorum is due to the ability of the plant to outgrow the fungus. Two factors

determine whether the plant can outgrow the fungus: The first is the environmental factor, which is not likely to alter the resistance with closely related legumes. Hot (75 - 85 F), sunny weather with long day lengths is favorable to rapid growth of birdsfoot trefoil, but unfavorable to the fungus. The second factor is characteristic of each species, or variety of legume. Medicago sativa and Lotus corniculatus regenerate more rapidly than Trifolium pratense. Frandsen (1946) claimed that once Anthyllis vulneria was infected, it was doomed. Results in Tables VII and VIII show that Lotus uliginosus is readily infected, but the regenerative nature of this plant enables it to outgrow the attack; L. jacobaeus, on the other hand, shows some resistance to infection, but once infected, it dies, as there is no crown to give regeneration.

Within Lotus corniculatus there appears to be no differentiation in ability to outgrow the fungus once infected. However, according to field observations (Tables II and III) there appears to be a definite resistance to infection. The resistance to infection can be significantly correlated to growth type (Tables XXVII and XXVIII in appendix); the upright plants being more resistant than the prostrate. A scatter diagram (Figure 1. in appendix) of growth types plotted against susceptibility, showed that the more prostrate and resistant birdsfoot trefoil originated from "Outer" Europe, that is from Scotland, the Alps, the Crimea, the Caucasus, and Eastern Asia consisting of Iran and Turkey. The more upright and susceptible birdsfoot trefoil originated from Central Europe including France, the Low Countries, Germany, Austria, Sweden, and all of Eastern Europe, including Russia. The apparent exceptions on Figure 1. are numbers 37 (United States), 8 (Turkey), 26 (Italy) and 51 (Novgorod, U.S.S.R.).

The United States type (no. 37), an introduction, is more resistant, yet with fairly upright growth, because it is locally adapted to this region. Locally grown varieties of red clover are more resistant than unadapted varieties according to Pape (1931) and Valleau et al. (1933).

The type from Turkey (no. 8) shows a definite upright growth and is more susceptible which separates it from the other Turkish types. The Italian type (no. 26) again shows upright growth and susceptibility, but according to its location should be included in the Outer European group. On the other hand, the Russian type (no. 51) from Novgorod District near Leningrad, should be included in the Central European group according to its location; however, it is prostrate and resistant.

The problem arises in knowing the exact origin of seed which was obtained from Botanical Gardens. For example: Number 34 was obtained from the Botanic Garden in Munich, Germany. The seed could have been collected from the Alps, 40 miles to the south, in which case it would conform to the theory that prostrate and more resistant plants originate in Outer Europe (as the Alps are included in Outer Europe); on the other hand, it could have originated from the Bavarian plains, or elsewhere in Central Europe, in which case it would be an exception.

All commercial varieties fall within the Central European group.

There is no correlation between growth type and the ability of birdsfoot trefoil to outgrow attack.

Sclerotinia trifoliorum was readily inhibited by many Actinomycetes as well as by Trichoderma and Penicillium. Trichoderma parasitized

S. trifoliorum in culture, and in inoculation experiments it reduced infection. It would therefore appear that *Trichoderma* might reduce field infection if it were present in sufficient concentration in the soil. The need arises for determining a feasible method of applying *Trichoderma* to the soil in a large area such as a field.

VI. SUMMARY

1. *Physoderma potteri* was not successfully inoculated onto the legumes tested owing to the non-viability of the resting spores.

2. *Stemphylium loti* caused leaf spots and stem lesions of birdsfoot trefoil (*Lotus corniculatus*) during the spring in the Macdonald College fields, but in the greenhouses, infection occurred at any season.

3. *Sclerotinia trifoliorum* caused serious wilt, crown rot and killing of birdsfoot trefoil in the Macdonald College fields during the springs of 1960 and 1961.

4. Warm temperatures (mean daily of 60 - 65 F) and sufficient soil moisture were favorable for the development of Sclerotinia wilt and crown rot in the spring.

5. Within the species *Lotus corniculatus*, types and varieties showing a prostrate growth habit were more resistant to infection by *S. trifoliorum* than upright types and varieties. Growth habit had no effect upon the ability of the plant to outgrow infection once infected. No type or commercial variety of *L. corniculatus* showed a high degree of resistance.

6. Species of *Lotus*, red clover and alfalfa showed varying degrees of susceptibility to *S. trifoliorum*; some showing resistance by being able to resist infection while others showed resistance by being able to outgrow the fungus once infected.

7. In winter killing of birdsfoot trefoil, S. trifoliorum may be an important factor.

8. Infection of birdsfoot trefoil by S. trifoliorum probably occurs in the spring in Quebec, rather than in the autumn, because ascospore infection of leaves resulted in rapid defoliation. Furthermore, the more susceptible types of birdsfoot trefoil to foliar inoculation , were the most resistant in the field to natural infection.

9. Dried peas formed a more successful substrate than dried grain for preparing inoculum of S. trifoliorum. Pea inoculum was easier to handle and plants were more rapidly infected than was the case with grain inoculum.

10. S. trifoliorum is sufficiently pathogenic to kill invaded plants without the assistance of a secondary invader. Therefore, Fusarium is unimportant as a secondary pathogen as shown in the dual inoculation experiments.

11. S. trifoliorum is readily inhibited by a number of soil microorganisms. Trichoderma is a parasite of S. trifoliorum and was capable of reducing Sclerotinia wilt and crown rot in greenhouse experiments.

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APPENDIX

APPENDIX

SOURCE OF PLANTS USED IN EXPERIMENTATION

Lotus corniculatus Collections

Number in Thesis	Description
1.	Obtained through U.S.D.A.[a] P.I.193725[b]. Originated from Sweden.
2.	Obtained through U.S.D.A. P.I.230348. Originated from Iran.
3.	Obtained through U.S.D.A. P.I.227512. Originated from Iran.
4.	Obtained through U.S.D.A. P.I.223272. Originated from Afghanistan.
5.	Obtained through U.S.D.A. P.I.161878. Originated from Argentine.
6.	Obtained through U.S.D.A. P.I.229568. Originated from Greece.
7.	Obtained through U.S.D.A. P.I.228286. Originated from Iran.
8.	Obtained through U.S.D.A. P.I.206447. Originated from Turkey.
9.	Obtained through U.S.D.A. P.I.204586. Originated from Turkey.
10.	Obtained through U.S.D.A. P.I.234811. Originated from Switzerland.
11.	Obtained through U.S.D.A. P.I.204882. Originated from Turkey.
12.	Obtained through U.S.D.A. P.I.206896. Originated from Turkey.
13.	Obtained through U.S.D.A. P.I.227849. Originated from Iran.
14.	Obtained through U.S.D.A. P.I.230347. Originated from Iran.
15.	Obtained through U.S.D.A. P.I.214112. Originated from Spain.

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[a] Obtained from U.S.D.A. Plant Introduction Stations at Ames, Iowa or Geneva, N.Y.

[b] U.S.D.A. Plant Introduction numbers.

Number in Thesis	Description
16.	(5813)[c] Obtained from: Lot 7/57, Institut für Kulturpflanzen-forschung, Gatersleben, Krs. Aschersleben, E. Germany. Originated from: Botanischer Garten, Graz, Austria.
17.	(5814) Obtained from: Lot 3/57, Institut für Kulturpflanzen-forschung, Gatersleben, Krs. Aschersleben, E. Germany. Originated from: Samenhandlung Ganzen & Spiessbach, Halle, Germany.
18.	(5818) Obtained from: Lot 273/54, Institut für Kulturpflanzen-forschung, Gatersleben, Krs. Aschersleben, E. Germany. Originated from: Institut für Pflanzenzüchtung, Brno, C.S.R. Collected at Tabor, C.S.R.
19.	(5817) Obtained from: Lot 286/57, Institut für Kulturpflanzen-forschung, Gatersleben, Krs. Aschersleben, E. Germany. Originated from: Košice, C.S.R. Collected in the Rožňava Region, C.S.R.
20.	Variety Cascade, Certified Seed. Obtained through Ottawa 1893 - 6607.[d]
21.	(5831) Obtained through H. A. Senn,[e] from: Jardin Botanique, Besançon, France.
22.	(5874) var. sativus Hyl., obtained from: Hortus Botanicus Universitatis Uppsalensis, Uppsala, Sweden.
23.	(5847) Obtained through H. A. Senn, from: Plant Science Department, Faculty of Agriculture, University of Agriculture, Budapest, Hungary. Collected from the Fak Region, Hungary.

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[c] All numbers appearing in "brackets" are Macdonald College accession numbers.

[d] Forage Crops Division, Central Experimental Farm, Ottawa.

[e] Dr. H. A. Senn, Director, Plant Research Institute, Research Branch, Canada Department of Agriculture (now retired).

Number in Thesis	Description
24.	(5828) Obtained through H. A. Senn, from: Hortus Botanicus Hauniensis, Copenhagen, Denmark.
25.	(5816) Obtained from: Lot 272/54, Institut für Kulturpflanzen-forschung, Gatersleben, Krs. Aschersleben, E. Germany. Originated from: Institut für Pflanzenzüchtung, Brno, C.S.R. Collected from the Malejov Region, C.S.R.
26.	(5404) Obtained from: No. H.2404, National Institute of Agricultural Botany, Cambridge, England. Originated from: Italy.
27.	(56105) var. vulgaris Koch, obtained from: 27113, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: No. 287, Moscow District, U.S.S.R.
28.	(56103) var. vulgaris Koch, obtained from: 27276, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: No. 528, Morshansk, U.S.S.R.
29.	Variety Empire, Certified Seed bought commercially.
30.	(58155) Obtained through Ottawa 1893 - 6531, from: 30111, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Sotsgorodok (according to the best information available) 85 miles east of Kharkov, on the Ukraine - Russian border), U.S.S.R.
31.	(58125) Obtained from: Czechoslovakian Academy of Science, Institute for Plant Breeding, Brno, C.S.R. Collected from No. 269, Trebitsch, C.S.R.

Number in Thesis	Description
32.	(58153) Obtained through Ottawa 1893 - 6539, from: 15600, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Moscow District, U.S.S.R.
33.	(58151) Obtained through Ottawa 1893 - 6532, from: 25495, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: U.S.S.R. (exact location uncertain).
34.	(5852) var. hirsutus Koch, obtained from: Botanischer Garten, Munich, Germany.
35.	(58152) Obtained through Ottawa 1893 - 6538, from: 27592, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Leningrad District, U.S.S.R.
36.	Variety Granger, Certified Seed bought commercially.
37.	(5101) Seed collected by Mr. A. Charbonneau, Agronome, Joliette, P.Q. from an old stand near Ste. Melanie, P.Q. Seed used to establish this stand originated in New York State.
38.	(56106) var. vulgaris Koch, obtained from: 29772, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: No. 9, Dotnuva, Lithuania, U.S.S.R.
39.	Variety Mansfield, Certified Seed bought commercially.
40.	(5841) var. ciliatus Koch, obtained from: 16428, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Stavropol District, U.S.S.R.

Number in Thesis	Description
41.	(58158) Obtained through Ottawa 1893 - 6535, from: 30421, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Prekule (according to the best information available), Lithuania, U.S.S.R.
42.	(58134) Obtained from: Hortus Botanicus Nikitensis, Yalta, U.S.S.R.
43.	(58126) Obtained from: Československa adademie zemedelskych ved, Vyzkumny Ustav Krmivarsky, Brno, C.S.R. Collected from the Viglas Region, C.S.R.
44.	(58154) Obtained through Ottawa 1893 - 6534, from: 31562, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Gelsvis (exact location unknown but probably in the U.S.S.R.).
45.	(58156) Obtained through Ottawa 1893 - 6537, from: 32192, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Kirov District (about 450 miles NE of Moscow), U.S.S.R.
46.	Variety Viking, Certified Seed bought commercially.
47.	(5846) Obtained through H. A. Senn, from: Plant Science Department, Faculty of Agriculture, University of Agriculture, Budapest, Hungary.
48.	(5699) Originated from cuttings taken from plants on sand dunes at Doonfoot, Ayrshire, Scotland. Collected by I. V. Hunt, West of Scotland College of Agriculture Farm, Auchincruive, Ayrshire, Scotland.

Number in Thesis	Description
49.	(5869) Obtained from: Jardin Botanique, Rouen, France.
50.	(5840) Obtained from: Hortus Botanicus Universitatis Varsaviensis, Warsaw, Poland.
51.	(58157) Obtained through Ottawa 1893 - 6536, from: 28497, All-Union Research Institute of Plant Industry, Leningrad, U.S.S.R. Originated from: Novgorod District (about 100 miles south of Leningrad), U.S.S.R.
52.	Variety Tana, Certified Seed bought commercially.
53.	(6101) Originated from: Arthur's Seat, Edinburgh, Scotland. Collected by E. O. Callen.
54.	(6102) Originated from: Seacliffs beside Step Rock Pool, St. Andrews, Scotland. Collected by E. O. Callen.
55.	(6104) Originated from: South Downs at Heyshott, W. Sussex, England. Collected by E. O. Callen.
56.	(6105) Originated from: Southleigh, Near Colyton, Devon, England. Collected by D. J. S. Barr.
57.	(5872) var. heterophyllarius Pet.-Stib. obtained from: Hortus Botanicus Universitatis, Uppsalensis, Uppsala, Sweden. (Correctly named: Lotus krylovii Schischk, & Serg.).

Collection of Lotus species and other Leguminosae

58.	Lotus palustris Willd (58139) Obtained from: Soil Conservation Service, California. U.S.D.A. P.I.200346.
59.	L. tenuis Waldst. & Kit (5912) Originated from: Greece.

Number in Thesis	Description
60.	L. filicaulis Durieu (5429) Obtained from: P. Henson, U.S.D.A., Beltsville, Md. U.S.D.A. P.I.210439.
61.	L. tenuis Waldst. & Kit variety Los Banos, Certified Seed bought commercially.
62.	L. tenuifolius Burm. (58131) Obtained through H.A. Senn, from: Československa akademie zemedelskych ved, Vyzkumny Ustav Krmivarsky, Brno, C.S.R. (Correctly named: Lotus tenuis Waldst. & Kit).
63.	L. tenuis Waldst. & Kit (5911) Originated from: France.
64.	L. palustris Willd (5418) Obtained through Ottawa 1893 - 3375, from: Jerusalem, Israel.
65.	L. tenuis Waldst. & Kit (5910) Originated from: Turkey.
66.	L. tenuis Waldst. & Kit (5870) Obtained from: Jardin Botanique, Rouen, France.
67.	L. pedunculatus Cav. (56111) Obtained from: Jardin Botanique, Besançon, France.
68.	L. hispidus Desf. (56109) Obtained from: Botanic Garden, Melbourne, Australia.
69.	L. requienii Fisch. & Mey. (5807) Obtained from: Botanic Garden, Adelaide, South Australia.
70.	L. ornithopodioides L. (5635) Obtained through H. A. Senn, from: Royal Botanic Gardens, Kew, Surrey, England.
71.	L. jacobaeus L. (5424) Obtained through Ottawa 1893 - 4538, from: Botanic Garden, Lisbon, Portugal.

Number in Thesis	Description
72.	L. japonicus (Regel) Larsen (5501) Originated from a riverbank, Gifu, Japan. Collected by Professor Isawo Hirayoshi, Kyoto University, Kyoto, Japan.
73.	L. uliginosus Schkuhr. (5809) Obtained from: Botanic Garden, Adelaide, South Australia.
74.	L. uliginosus Schkuhr. (5821) Obtained from: Lot 10/57, Institut für Kulturpflanzenforschung, Gatersleben, Krs. Aschersleben, E. Germany. Originated from: Vogt, Rittergut Schlüsselburg-Neuhof, Holstein, Germany.
75.	L. uliginosus Schkuhr. (5889) Obtained from: Sectio Meliorationis Plantarum Instituti Experimentalis Agrarii, Pannoniae Austroccidentalis, Keszthely, Hungary.
76.	L. uliginosus Schkuhr. (58117) Obtained from: Botanischer Garten, Bremen, Germany.
77.	L. parviflorus Desf. (5426) Obtained through Ottawa. Originated from: Portugal.
78.	L. suaveolens Pers. (5513) Obtained through Ottawa.
79.	L. pusillus Viv. (5689) Obtained from: 14865, Commonwealth Plant Introduction Service, Australia. Originated from: Israel.
80.	L. weilleri Maire (56142) Obtained through Ottawa 1893 - 3836, from: Jardin Botanique, Paris, France.
81.	L. maroccanus Ball. (5506) Obtained from: K.2794, Dept. of Agronomy, University of California, Davis. Originated from: French Morocco.

Number in Thesis	Description
82.	L. coccineus Schlecht. (5806) Obtained from: Botanic Garden, Adelaide, South Australia.
83.	L. perigrinus L. (56140) Obtained from: Soil Conservation Service, California. U.S.D.A. P.I.200347.
84.	L. siliquosus L. (5829) Obtained through H. A. Senn, from: Botanischer Garten, Graz, Austria.
85.	Trifolium pratense L. variety Dollard, Certified Seed bought commercially.
86.	T. pratense L. variety Ulva, Certified Seed bought commercially.
87.	Medicago sativa L. variety Vernal, Certified Seed bought commercially.
88.	M. sativa L. variety Dupuit, Certified Seed bought commercially.
89.	M. sativa L. variety Narragansett, Certified Seed bought commercially.

ANALYSIS OF VARIANCE TABLES

TABLE XIV: Number of Plants Wilted, Plot 1, Field, 1960.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	12	9054	754	2.04*	1.88	2.41
Between Replicates	7	1308	187	0.51	2.12	2.87
Error	84	31086	370			
Total	103	41448				

TABLE XV: Number of Plants Wilted, Plot 1, Field, 1961.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	12	27859	2322	6.57**	1.88	2.41
Between Replicates	7	5648	807	2.28*	2.12	2.87
Error	84	29683	353			
Total	103	63190				

TABLE XVI: Number of Plants Wilted, Plot 1, Accumulation of Field Results for 1960 and 1961.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	12	30677	2556	9.10**	1.88	2.41
Between Replicates	7	3762	537	1.91	2.12	2.87
Error	84	23570	281			
Total	103	58009				

TABLE XVII: Number of Plants Wilted, Plot 5, Field, 1960.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	36	19380	538	1.53*	1.51	1.79
Between Replicates	5	16270	3254	9.24**	2.27	3.14
Error	180	63380	352			
Total	221	99030				

* Significant at P = 0.05
** Significant at P = 0.01

TABLE XVIII: Number of Plants Wilted, Plot 5, Field, 1961.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	36	26113	725	2.08**	1.51	1.79
Between Replicates	5	10336	2067	5.94**	2.27	3.14
Error	180	62605	348			
Total	221	99054				

TABLE XIX: Number of Plants Wilted, Plot 5, Accumulation of Field Results for 1960 and 1961.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	36	29529	820	2.99**	1.51	1.79
Between Replicates	5	29566	5913	21.60**	2.27	3.14
Error	180	49276	274			
Total	221	108371				

TABLE XX: Number of Plants Wilted, Plot 7, Field, 1960.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	6	4314	719	4.52**	2.66	4.01
Between Replicates	3	3166	1055	6.63**	3.16	5.09
Error	18	2863	159			
Total	27	10343				

TABLE XXI: Number of Plants Wilted, Lotus corniculatus in Inoculation Experiments.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	23	6371	277	0.82	1.75	2.22
Between Replicates	2	13089	6544	19.33**	3.20	5.10
Error	46	15572	338			
Total	71	35032				

* Significant at P = 0.05
** Significant at P = 0.01

TABLE XXII: Number of Plants Killed, Lotus corniculatus, in Inoculation Experiments.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	23	15571	677	1.68	1.75	2.22
Between Replicates	2	17660	8830	21.94**	3.20	5.10
Error	46	18522	403			
Total	71	51753				

TABLE XXIII: Number of Plants Wilted, Lotus spp. and other Leguminosae, in Inoculation Experiments.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	17	38218	2248	5.54**	1.94	2.55
Between Replicates	2	3501	1750	4.23*	3.28	5.29
Error	34	14059	415			
Total	53	55778				

TABLE XXIV: Number of Plants Killed, Lotus spp. and other Leguminosae, in Inoculation Experiments.

	D.F.	S.S.	M.S.	F.value	F.5%	F.1%
Between Collections	17	23236	1367	3.09**	1.94	2.55
Between Replicates	2	707	353	0.80	3.28	5.29
Error	34	15067	443			
Total	53	39010				

* Significant at P = 0.05
** Significant at P = 0.01

CORRELATION TABLES

TABLE XXV

CORRELATION OF FIELD RESULTS, PLOT 1,
TO ARTIFICIAL INOCULATION EXPERIMENTS

Thesis no.	Origin	Field 1960-61 Percent killing	Green-house Percent killing
4	Afghanistan	(a)	57%
14	Iran	67% (b)	54
7	Iran	20	53
12	Turkey	67	50
2	Iran	0	47
11	Turkey	0	42
1	Sweden	25	38
3	Iran	0	33
6	Greece	46	33

Correlation coefficient, $r = 0.381$
Therefore no significant correlation at the 5% ($P = 0.05$) level.

a Total winter killing in field, therefore not included in calculation of "r".

b The number of infected plants which were killed in 1960 and 1961.

TABLE XXVI

CORRELATION OF FIELD RESULTS, PLOT 5,
TO ARTIFICIAL INOCULATION EXPERIMENTS

Thesis no.	Origin	Field 1960-61 Percent killing	Green-house Percent killing
44	Gelsvis, U.S.S.R.	33% (b)	87%
42	Yalta, U.S.S.R.	50	64
39	Mansfield (a)	80	54
25	Malejov, C.S.R.	58	46
19	Rožňava, C.S.R.	50	45
17	Halle, Germany	33	42
37	New York, U.S.A.	67	42
29	Empire (a)	42	40
49	Rouen, France	0	38
52	Tana (a)	12	36
36	Granger (a)	38	33
46	Viking (a)	47	33
18	Tabor, C.S.R.	33	33
22	Uppsala, Sweden	20	0

Correlation coefficient, r = 0.317 Therefore no significant correlation at the 5% (P = 0.05) level.

a Commercial varieties.

b The number of infected plants which were killed in 1960 and 1961.

TABLE XXVII

CORRELATION OF FIELD RESULTS VS. GROWTH TYPE

Thesis no.	Origin (Plot 1)	Growth type (a)	No. of plants wilted
6	Greece	5	62% (b)
1	Sweden	4	55
5	Argentine	4	47
11	Turkey	1	31
8	Turkey	5	29
10	Switzerland (c)	-	29
7	Iran	1	28
9	Turkey	1	28
13	Iran	1	16
3	Iran	1	13
14	Iran	1	11
12	Turkey	2	11
2	Iran	2	3
4	Afghanistan	1	(d)
15	Spain	1	(d)

Correlation coefficient (Number of plants wilted to growth type), r = 0.697 Therefore significant at the 2% (P = 0.02) level.

a Growth types obtained from Mrs. A. Chamberlain (1961), 6 = the most upright, 1 = the most prostrate.

b Angular transformation of means.

c Not included in calculation of "r".

d Winter killed, not included in calculation of "r".

TABLE XXVIII

CORRELATION OF FIELD RESULTS VS. GROWTH TYPE

Thesis no.	Origin (Plot 5)	Growth type (a)	No. of plants wilted	No. of plants killed
49	Rouen, France	6	59% (b)	11% (c)
46	Viking (d)	5	58	36
23	Fak Region, Hungary	5	52	54
50	Warsaw, Poland	3	51	23
19	Rožňava, C.S.R.	5	50	30
20	Cascade (d)	5	49	38
30	Sotsgorodok, U.S.S.R.	5	48	28
52	Tana (d)	6	47	6
25	Malejov Region, C.S.R.	5	47	44
39	Mansfield (d)	5	47	35
17	Halle, Germany	4	46	24
44	Gelsvis, U.S.S.R.	2	46	28
28	Morshansk, U.S.S.R.	4	46	25
32	Moscow, U.S.S.R.	4	44	20
35	Leningrad, U.S.S.R.	6	43	31
29	Empire (d)	4	42	29
36	Granger (d)	5	42	21
22	Uppsala, Sweden	5	41	8
43	Viglas, C.S.R.	5	41	27
18	Tabor, C.S.R.	3	40	13
24	Copenhagen, Denmark	4	38	46
38	Dotnuva, U.S.S.R.	3	38	25
45	Kirov, U.S.S.R.	4	38	15
31	Trebitsch, C.S.R.	3	38	31
27	Moscow, U.S.S.R.	4	37	50
26	Italy	5	37	21
33	U.S.S.R.	4	36	23
47	Budapest, Hungary	- (e)	34	17
16	Graz, Austria	5	34	33
34	Munich, Germany	1	34	50
41	Prekule, U.S.S.R.	5	32	18
51	Novgorod, U.S.S.R.	1	26	11
40	Stavropol, U.S.S.R.	2	24	57
42	Yalta, U.S.S.R.	3	21	43
37	New York, U.S.A.	4	19	40
21	Besançon, France	2	14	75
48	Doonfoot, Scotland	1	4	0

Correlation coefficient (Number of plants wilted to growth type), $r = 0.634$. Therefore significant at the 1% ($P = 0.01$) level.
Correlation coefficient (Number of plants killed to growth type), $r = -0.153$. Therefore no correlation.

a Growth types obtained from Mrs. A. Chamberlain (1961). 6 = the most upright, 1 = the most prostrate.
b Angular transformation of means.
c The number of infected plants killed in 1960.
d Commercial varieties.
e Not included in calculation of "r".

FIGURE 1

SCATTER DIAGRAM SHOWING GROWTH TYPE PLOTTED AGAINST SUSCEPTIBILITY

(Three pages)

EXPLANATION OF FIGURE 1

ORIGIN OF PLANTS (p.xviii)

(a)	-	Commercial varieties
Arg	-	Argentine
Au	-	Austria
Cz	-	C.S.R.
Den	-	Denmark
Fr	-	France
Ger	-	Germany
Gre	-	Greece
H	-	Hungary
Ir	-	Iran
It	-	Italy
Pol	-	Poland
Ru	-	U.S.S.R.
Scot	-	Scotland
Sw	-	Sweden
T	-	Turkey
US	-	U.S.A.

THESIS NUMBERS (p.xix)

21	-	France, Besançon, northern Alps.
27	-	U.S.S.R., Moscow District.
28	-	U.S.S.R., Morshansk, about 200 miles south-east of Moscow.
30	-	U.S.S.R., Sotsgorodok, near Kharkov, about 85 miles east of Kharkov, on the Ukraine-Russian border.
32	-	U.S.S.R., Moscow District.
33	-	U.S.S.R., Location unknown.
34	-	Germany, Munich, about 40 miles north of the Bavarian Alps.
35	-	U.S.S.R., Leningrad District.
37	-	U.S.A., New York State.
38	-	U.S.S.R., Dotnuva, Lithuania.
40	-	U.S.S.R., Stavropol Region, north of Caucasus.
41	-	U.S.S.R., Prekule, Lithuania.
42	-	U.S.S.R., Yalta, Crimea.
44	-	U.S.S.R., Gelsvis, exact location unknown.
45	-	U.S.S.R., Kirov, District, about 450 miles north-east of Moscow.
49	-	France, Rouen, on the north coast of France.
51	-	U.S.S.R., Novgorod District, about 100 miles south of Leningrad.

See also the list describing the collection of Lotus corniculatus, pages i - vi in appendix.

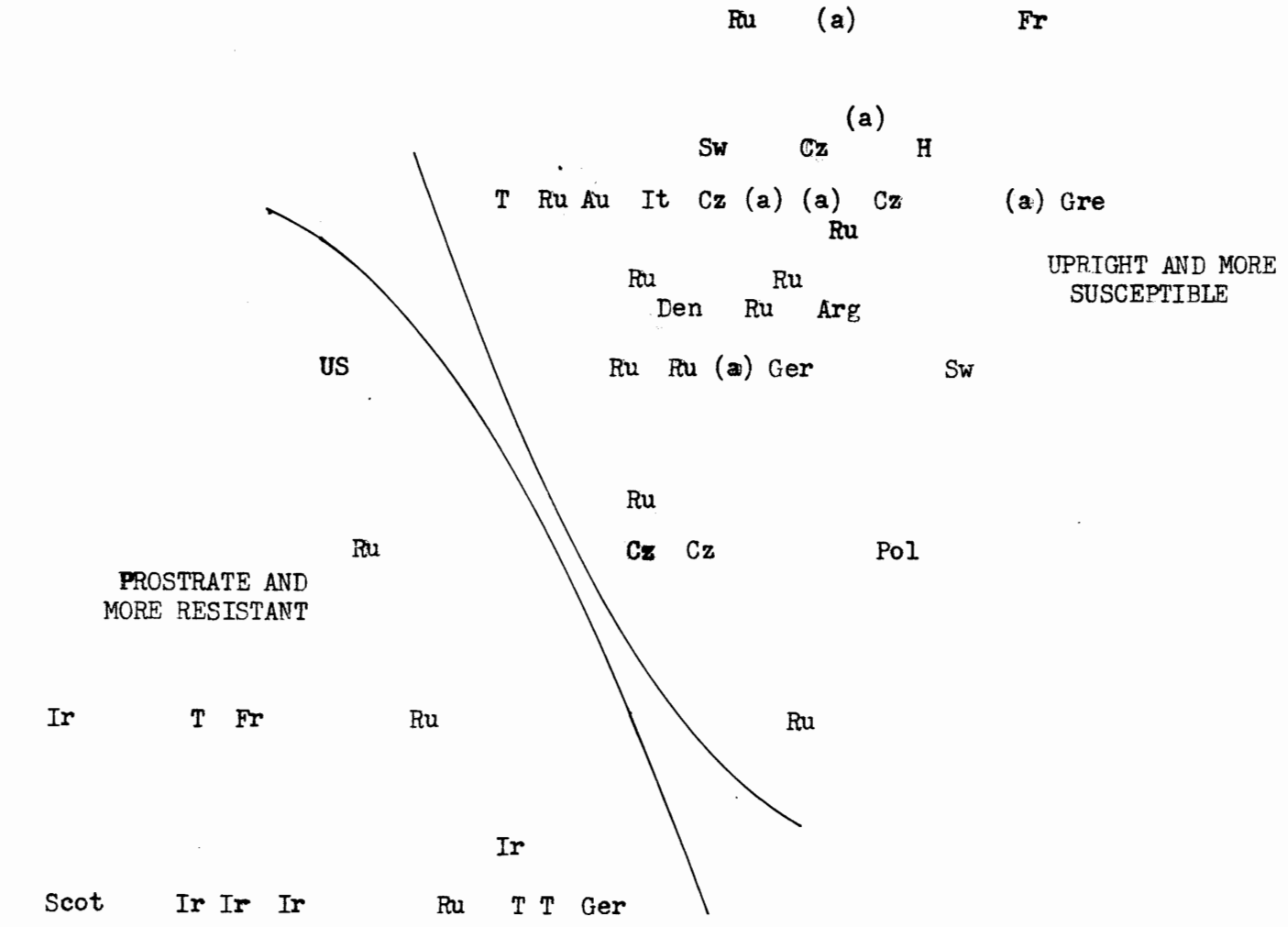

FIGURE 1 (ORIGIN OF PLANTS INDICATED)

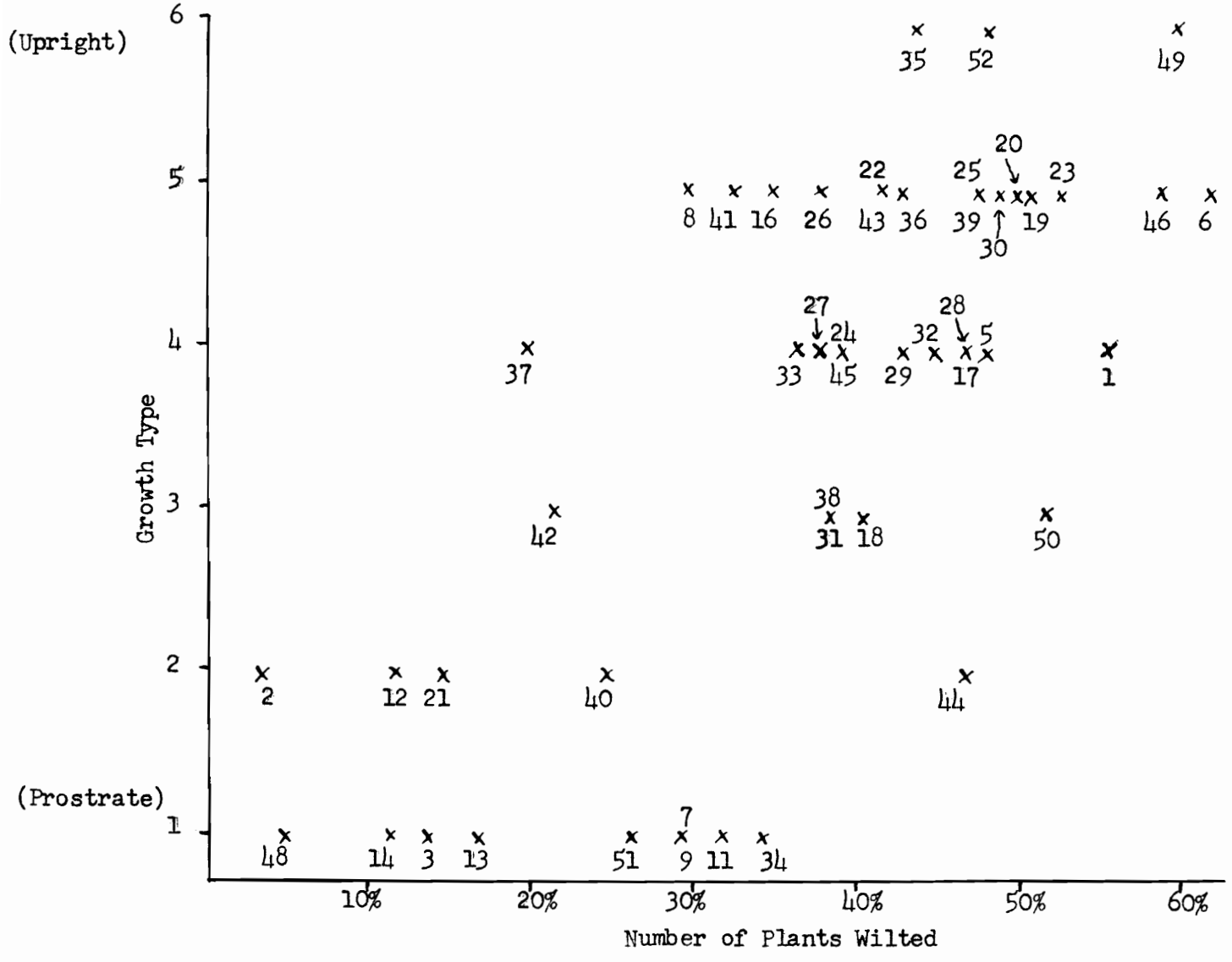

FIGURE 1: SCATTER DIAGRAM SHOWING GROWTH TYPE PLOTTED AGAINST SUSCEPTIBILITY (THESIS NUMBERS INDICATED)