STUDIES OF ROOT AND CROWN ROTS ON ALFALFA (*MEDICAGO SATIVA* L.)

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ABSTRACT

The objective of this study was to evaluate reactions of several alfalfa clones and their progeny to selected species of three genera of fungi causing root- and crown-rots in irrigated soils of the lower desert valleys of Arizona. The clones were from plants of the cultivars 'African' and 'Lahontan' which had survived for three summers under field conditions where the soil was heavily infested with *Rhizoctonia solani*, *Phymatotrichum omnivorum*, and *Fusarium* spp. Plants grown in a greenhouse with limited environmental control were inoculated with *R. solani* and *Fusarium* spp. grown on sterile oats. Reactions to the cotton root-rot pathogen, *Phymatotrichum omnivorum*, were evaluated by the degree of persistence of plants grown in naturally-infested field soils. Commercially-grown cultivars served as checks for each experiment. Results in greenhouse experiments were based on the degree of root discoloration and persistence of stand.

All of the plants were more susceptible to *R. solani* alone and to combinations of *R. solani* and *Fusarium* spp. than to *Fusarium* spp. alone. There was variation among and between polycross progeny and selfed progeny of selected alfalfa plants for disease classification and percentage survival. In some cases there was as much variation among progeny of the same clone as among progeny of different clones. Polycross progeny generally were more tolerant than selfed progeny.

INTRODUCTION

Alfalfa ranks third in importance in Arizona as a monetary crop and third in total acreage. It is planted on approximately 200,000 acres in the State. Alfalfa is utilized mainly as a hay and seed crop, as well as for soil building which usually fits into the system of crop rotation.

With today's intensive farming practices, alfalfa is being harvested more frequently. This causes stands of alfalfa to last only a year or two until renovating and reseeding are necessary. Stand decline has become one of the most serious problems in alfalfa production in southern Arizona.

There are several factors determining longevity of an alfalfa stand. Some of these are cultivaral tolerance or resistance to various pathogenic organisms, cultural practices such as fertilization, irrigation and harvest frequency.

Pathogens attacking alfalfa roots and crowns are a major factor contributing to stand decline. A number of fungi, bacteria, viruses, and nematodes have been found to cause diseases of alfalfa in the irrigated, lower desert valleys of Arizona.

Since various levels of resistance to insects and diseases have been observed in alfalfa, the main objective of this study was to evaluate selected alfalfas (individual plants or cultivars) for resistance or tolerance to fungi causing root- and crown-rots. Fungi considered to be the primary cause of root- and crown-rots were utilized in these studies. Several methods of inoculation were tried in an attempt to find the best means of evaluating and selecting alfalfas for resistance, and to study methods used by other workers.

REVIEW OF LITERATURE

Introduction

There have been many studies and reports on the occurrence, symptoms and classification of various pathogens associated with root- and crown-rots of alfalfa. Keener (P. D. Keener, 1948. Alfalfa Impr. Conf. Rep. 11:20-21) made the following statement concerning alfalfa diseases in Arizona:

> Infections of alfalfa by wilt pathogens are not "pure." That is, wilt is usually accompanied by such root rot fungi as *Phymatotrichum omnivorum*, *Rhizoctonia* sp., and *Fusarium* sp. These microorganisms along with the bacterium, *Corynebacterium insidiosum* compose an "alfalfa root disease complex" in the State.

More recently it was reported (Dennis et al., 1961) that fungi found in the root- and crown-rot complexes in Arizona included *Phymatotrichum omnivorum* and species of *Fusarium*, *Rhizoctonia*, *Stemphylium*, *Stagonospora*, and *Diplodia*.

Graumann and Hanson (1954) stated that alfalfa was susceptible to more than 75 diseases caused by fungi, bacteria, viruses, and nematodes. Many of these occurred sporadically and were rarely destructive; however, several were important in most major alfalfa-growing areas and were responsible for appreciable losses in forage production. They also stated that root- and crown-rots occurred in all regions where alfalfa was grown. The fungi causing these diseases were among the most destructive pathogens, because they caused injury at all stages of growth, from seedling to mature plants. Some of the pathogens were most active during cool, moist periods of winter or early spring; whereas, others were strictly warm-temperature fungi.

Lehman (W. F. Lehman, 1966. Alfalfa Impr. Conf. Rep. 20:75-78) reported that the root- and crown-rot complex of alfalfa, or an individual disease in it, was listed as the major disease problem by all workers in Arizona and California.

Selected species of three genera of fungi, which are most prevalent in the root- and crown-rot complex in Arizona, were chosen for this study.

Occurrence of Selected Species of Three Genera of Fungi Causing Root and Crown Rot

1. *Rhizoctonia* spp.

Smith (1943) probably gave the first description of Rhizoctonia root canker caused by *Rhizoctonia solani* Kuehn in alfalfa. This finding was made at the U. S. Yuma Field Station at Bard, California in May 1940; however, Smith believed that the disease had been present several years prior to this observation. During a survey shortly after this discovery, the disease was found in southern California and southwestern Arizona. Smith (O. F. Smith, 1940. Alfalfa Impr. Conf. Rep. 12:19-21) reported that *R. solani* was an important factor causing short-lived stands of alfalfa in the extreme southwestern United States. Jones and Smith (1953) described *R. solani* as one of the causal agents of root and crown rot of alfalfa. In the warmer portions of the Southwest, *R. solani* caused root canker of alfalfa, while *R. crocorum* DC. ex Fr. caused a root rot. This latter species was more prevalent in poorly-drained fields. Erwin (1956) and Houston et al. (1960) also reported that Rhizoctonia root canker, caused by R. solani, was responsible for great economic losses to growers in the irrigated desert regions of California, which included the Imperial, Palo Verde, and Coachella valleys. Since no method of control was known, it was probably the most serious disease in that area. Estimated stand losses as a result of this disease varied from 10 to more than 40%.

Houston et al. (1960) reported that certain strains of R. solani attacked alfalfa stems and caused a disease known as Rhizoctonia stem canker. This disease was most evident in one- and two-year-old stands and occurred mainly during the spring, early summer and fall.

Smith (1943) described Rhizoctonia root canker as seasonal in development and closely correlated with high temperatures. Lesions were characteristic of the disease and developed mainly in June, July, August, and September, when soil temperatures, at 7.62 cm below the surface, ranged from about 21 to 35 C. There was practically no disease development during winter months, when soil temperatures at 7.62 cm below the surface were as low as 5 to 10 C.

Crown-rot diseases have been observed in almost all irrigated fields of alfalfa in California (D. C. Erwin, 1954. Alfalfa Impr. Conf. Rep. 14:32; Erwin, D. C., 1954). Erwin studied three root- and crown-rot diseases. An isolate of R. solani from one of the diseased plants caused a stem and crown canker.

Erwin (1955) discovered a dark-crown necrosis on alfalfa in California. R. solani was one of the organisms found among isolates from diseased plants. A study of these isolates showed that R. solani was involved in dark-crown necrosis; also, some isolates of R. solani caused crown-bud necrosis and root canker.

Crall (1951) reported that root- and crown-rot caused by R. solani was one of the less important diseases of alfalfa in Iowa. However, later workers in Iowa (C. P. Wilsie, D. C. Norton and W. F. Wedin, 1965. Central Alfalfa Impr. Conf. 11:21, C. P. Wilsie and D. C. Norton, 1966. Central Alfalfa Impr. Conf. 12:29) reported that root- and crown-rot were common and R. solani was the organism most often isolated from diseased alfalfa.

Root diseases of alfalfa have been a major concern of growers in Canada (Chi and Childers, 1965; M. W. Cormack and E. J. Hawn, 1954. Alfalfa Impr. Conf. Rep. 14:30-31; Hawn and Cormack, 1952). Many fungi have been found in association with diseased crowns and roots. They were believed responsible for the deterioration and lack of persistence of stands in that area. Species of Fusarium and Rhizoctonia were found to incite wilt and root rot. These genera along with other organisms were also found consistently associated in rotting crown buds of alfalfa (Cormack and Hawn, 1951).

Crown-bud rot which caused heavy damage to alfalfa stands in the Lethbridge area in Canada and in southern Manitoba has been reported by Stevenson (T. M. Stevenson, 1952. Alfalfa Impr. Conf. Rep. 13:2-8). He confirmed that at least one species of Rhizoctonia and two of Fusarium were involved in this disease. In another study by Hawn and Cormack (1952) three isolates of crown-bud rot (R. solani, F. acuminatum Ell. and Ev., and F. avenaceum (Fr.) Sacc.) were described as a disease complex and proven pathogenic in greenhouse tests. *Rhizoctonia solani* was the most pathogenic of the three isolates.

In a survey of alfalfa fields in Canada, Hawn (1953) discovered that the annual increase in alfalfa crown-bud rot occurred during the early part of the growing season. This observation was confirmed in field plot work in 1952 which showed that 80.6% of the total increase occurred in May. Hawn (1957) also confirmed the role of *R. solani* in crown-bud rot of alfalfa by microscopic examination of artificially inoculated crown buds. A number of fungi associated with rotting of roots and crowns of alfalfa in Manitoba, Canada was studied by McDonald (1955). *Rhizoctonia solani* was the most virulent of the pathogens isolated.

Chi (D. C. Chi, 1966. Alfalfa Impr. Conf. Rep. 20:59-61) also found many fungi in association with diseased roots and crowns which were responsible for the deterioration of stands in Canada. *Rhizoctonia* sp. was a frequent isolate. All isolates of the *Rhizoctonia* sp. had the mycelium and basidiospore characteristics of *R. solani*. This organism was prevalent on seedlings, but was not often isolated from more mature plants later in their first year. The organism was again isolated frequently from plants more than one year old.

Twenty-one isolates of *R. solani* from various sources were tested on alfalfa and red clover (Chi and Childers, 1965). All of the isolates incited disease on these hosts, although their virulence varied greatly. Eleven other *R. solani* isolates from alfalfa were also tested. Five were pathogenic on seedlings and to both crown and stems of older alfalfa plants. Three affected only seedlings and three were non-pathogenic.

2. *Fusarium* spp.

The occurrence of a root-rot disease was discovered on alfalfa in New Mexico in 1945 (Staten and Leyendecker, 1949). It was found in a bacterial wilt study, when a large number of diseased plants were encountered that did not show typical bacterial wilt symptoms. Further study showed the fungus, *F. solani* (Mart.) Appel and Wr., was the cause of the root rot. Wilson (1961) also stated that *F. solani* was generally prevalent in the major alfalfa-growing areas of New Mexico. In New Mexico, the disease's prevalence and severity in reducing yield and stands were considered equal to or greater than bacterial wilt.

In California, *Stagonospora meliloti* (Lasch) Petr. is another fungus associated with the crown-rot complex of alfalfa (Erwin, 1952). Erwin studied the pathogenicity of isolates associated with this crown rot. In 37 experiments, *F. roseum* Lk. and *F. solani* were isolated from a crown rot that was characterized by dark, dry necrosis of the crown branches. None of these isolates caused root or crown rot when inoculated on plants growing in sterilized soil. *Rhizoctonia solani* and *Pythium* sp., isolated frequently during the winter from necrotic crown buds, were pathogenic to buds and seedlings. A *F. roseum* isolate was severely pathogenic to seedlings but not to buds or crowns. Stanford et al. (Agron. Abstr. 1956:72), also working in California, discovered that *F. oxysporum* Schlecht f. *medicaginis* (Weimer) Snyd. & Hans. produced a wilt disease in alfalfa which under certain conditions resulted in reduction of stands. When two- or three-month-old alfalfa plants were inoculated with this organism in the greenhouse, complete killing of very susceptible cultivars occurred.

Evidence of the occurrence of Fusarium spp. in root and crown rot was presented by Couch (H. B. Couch, 1958. Alfalfa Impr. Conf. Rep. 16: 40). He found that F. roseum and F. solani were the most common isolates from diseased alfalfa roots and crowns in Pennsylvania. Inoculations with isolates of F. solani caused severe crown canker, while some isolates of F. roseum incited a less severe root and crown canker.

Several species of Fusarium were isolated from weak alfalfa plants from nearly all locations tested in northeastern United States (E. S. Elliot, 1966. Alfalfa Impr. Conf. Rep. 20:63-64). The most frequent isolates were F. oxysporum, F. solani, F. moniliforme, and F. roseum. The isolates of Fusarium spp. exhibited a wide range of pathogenicity when used to inoculate alfalfa plants of different ages. Some isolates of F. oxysporum were highly pathogenic; however, a majority of the Fusarium spp. and most isolates within a species were obviously less virulent. Under field conditions, there were usually fewer seedlings lost from damping-off as compared to those lost under laboratory conditions. Although the roots of alfalfa appeared clean, species of Fusarium were usually present and easily isolated from them. More and more evidence showed that any stress factor, such as plant competition, insect attack, unfavorable nutrition, and many other factors which weaken plants, enhanced an invasion by the normally present Fusarium flora. Millar (R. L. Millar, 1966. Alfalfa Impr. Conf. Rep. 20:62) found that increased frequency of harvesting was associated with an increase in root rot and recovery of Fusarium spp. from the roots.

In Chi's (C. C. Chi, 1965. Alfalfa Impr. Conf. Rep. 20:59-61) study of root and crown rots of alfalfa in Canada, Fusarium spp. were isolated most often from diseased plants and comprised 58% of the total isolations from a survey over a four-year period. The following species were involved: *F. oxysporum*, *F. solani*, *F. acuminatum*, and *F. avenaceum*. Seasonal differences in abundance were noted in some of these species. *Fusarium solani* was abundant during summer and early fall, while *F. avenaceum* and *F. acuminatum* were common early in the growing season on both first- and second-year plants. *Fusarium solani* was more prevalent on two-year-old plants. *Fusarium* spp. were obtained from both cortical and vascular tissue.

Schmitthenner (1964) isolated species of *Fusarium* and *Rhizoctonia* from diseased seedlings of alfalfa. He tested these for their virulence and found the *Fusarium* spp. were avirulent or only slightly virulent; the *Rhizoctonia* sp. was moderately to highly virulent on alfalfa.

McGuire, Walters, and Slack (1958) obtained results from inoculations of alfalfa with both Fusarium wilt (*F. oxysporum* f. *vasinfectum*) and root-knot nematodes which showed that the number of Fusarium wilt-infected plants and the severity of the infection were directly correlated with severity of root-knot nematode infection. Thomason et al. (1959) also showed that nematodes increased the occurrence of wilted plants. Two and one half percent of the plants showed wilt when inoculated with *Fusarium* alone. When inoculated with both *Fusarium* and nematodes, 10% of the plants died, 10% had wilt symptoms and the rest of the plants showed no infection.

3. *Phymatotrichum omnivorum* (Shear) Duggar

Phymatotrichum root rot is known as cotton root rot, Texas root rot, Ozonium root rot, or just root rot. This disease is caused by *P. omnivorum*, a soil-inhabiting fungus that is indigenous and restricted to alkaline soils of southwestern United States and northern Mexico (Blank, 1953). This fungus attacks approximately 2,000 species of dicots and gymnosperms but is not known to cause disease in monocots. It forms mycelial strands on roots of higher plants; mycelial strands and sclerotia in the surrounding soils, and "spore mats" on the surface of damp, warm soil (Streets, 1937).

Streets (1953) described Texas root rot as one of the major plant diseases in the Southwest. It attacks most tap-rooted crop plants, including cotton, alfalfa, fruit trees, ornamental trees, and shrubs. He stated that the fungus occurred in our soils before "white man" arrived, and very probably before Indian tribes occupied the area. The natural occurrence of the fungus is in a very unpredictable manner in many soils. It is often severe in one field and absent from an adjoining field. It may spread as much as 1.83 to 4.88 m in irrigated fields during a year's time, although the greater distance of spread is not common. The fungus has been found as deep as 3.66 m.

Isolates of *P. omnivorum* were made by Hosford (R. M. Hosford, 1965. Cytology of the fungus, *Phymatotrichum omnivorum* (Shear) Duggar. Ph.D. Thesis. University of Arizona, Tucson) from roots of wilted cotton and alfalfa plants collected from locations in southern Arizona and southern California.

In California, although not widely distributed, Houston et al. (1960) found the fungus in the Palo Verde, Coachella, and Imperial valleys. In addition to alfalfa, it attacked all other broad-leaved crops which grew during the hot summer months in these lower desert valleys. The disease was not a problem during winter months; therefore, various susceptible winter crops can be grown on infested land. Nichols et al. (1955) also described the disease, its occurrence, and economic importance in California.

In Nevada, this root rot occurred only in the southern portion of the State where warm summer temperatures favored its development (Smith 1948). Smith reported that the fungus infected the roots, caused them to decay and eventually killed the plants. Damage varied from year to year. Development of the disease was favored by fairly moist soil, but it was unfavorably affected by either very dry or very wet soils. In relatively warm soil, the disease was most severe. The optimum for disease development occurred around 24 C.

Staten (1957) reported that the cotton root-rot fungus was one of the most important organisms which attacked alfalfa in Texas. This fungus was extremely destructive and injured plants at any stage of development from seedling to maturity. Brooks (1959) later reported it to be the most serious problem associated with alfalfa production in Wichita Valley of Texas.

Symptoms of Root and Crown Rot Caused by Selected Species of Three Genera of Fungi

1. Rhizoctonia spp.

Smith (1943) reported that Rhizoctonia root canker of alfalfa was characterized by dark, sunken areas, which sometimes had brownish borders. Diseased areas were usually circular, but in some cases they were oblong and extended part way around the root. The lesions generally occurred where young roots emerged from larger ones, as evidenced by the fact that the dead stub of a small root was often found near the center of each lesion. Lesions often developed inward toward the central region of the root, but the latter was not completely rotted off. There was very little spread of the disease up or down the root from the lesion. Lesions that developed in summer usually healed over during winter, and by mid-winter or early spring, there was often only a scar on the root to indicate the location of the diseased area from the previous summer season.

The organism causing Rhizoctonia root canker often attacked plants in the field forming a circular or irregular-shaped area of dead plants and in many cases, diseased plants were more prevalent in the wheel tracks made by farm implements (Houston, et al., 1962). This was probably due to crown injury to the plants in such areas. Since all infected plants did not die at the same time, a spotty appearance occurred in fields. Plants affected with this disease did not have predominant or identifying leaf symptoms. Plants were damaged most when cankers formed on the crowns. The Rhizoctonia stem canker fungus attacked the crown and stem tissues below the soil line. After infection the stem canker often extended 2.54 cm or more above the soil surface. Areas of the canker varied in color from tan to dark brown. Tissues invaded by the fungus died, causing yellowing and wilting of leaves and tips of stems.

As a crown rot, R. solani invades the crown and destroys new buds and shoots (Jones and Smith, 1953). The plants are so weakened that they are easily attacked by saprophytic or weakly parasitic soil fungi, which enhances destruction of crown tissue. R. crocorum causes a root rot which is characterized by spread of the fungus usually in all directions through the soil, killing plants as it progresses. The foliage turns yellow, wilts, and eventually dies. Infected roots have a mat of reddish-brown or violet fungal threads on the outside of the bark. The threads penetrate the roots, which then begin to decay and slough their bark.

The dark-crown necrosis on alfalfa described by Erwin (1955) produced wedge-shaped lesions on the crown, or longitudinal lesions on crown branches. Eventually the entire crown was affected and the plant died. Isolates of R. solani from these diseased plants gave different effects. Some strains were pathogenic, others were not. Certain strains were pathogenic to seedlings and produced stem and crown canker that resembled dark-crown necrosis. Other strains caused crown-bud necrosis.

An isolate of R. solani, which caused bud cankers of alfalfa, was found in the vessels of infected stems, and caused a discoloration of the crown tissue above and below the canker (Erwin, 1954). Hawn (1953, 1957) stained preparations of crown-bud tissue which revealed extensive penetration and ramification by mycelium of R. solani and other fungi in 15 days after inoculations under greenhouse conditions.

In further studies, sections of diseased tissue stained with Conant's quadruple stain showed identifiable mycelium and fruiting structures of the above-mentioned fungus. Mycelium of R. solani ramified the crown buds inter- and intra-cellularly.

Chi (C. C. Chi. 1966. Alfalfa Impr. Conf. Rep. 20:59-69) isolated Rhizoctonia sp. mainly from cortical tissues of alfalfa plants. Cultures of this organism were applied to seedlings which emerged but were badly stunted and malformed. The hypocotyls of many were swollen but had not elongated.

2. Fusarium spp.

The Fusarium root-rot fungus (F. solani) produced reddish-brown vascular discoloration in alfalfa that could be followed to the point of origin, which in most cases was from lateral roots (Staten and Leyendecker, 1949). Many infections remained localized in the crown area causing typical crown rot.

3. Phymatotrichum omnivorum

Streets (1953) found a loose network of fungal strands of P. omnivorum forming along the surface of plant roots. Soon, filaments from the strands wedged their way into the outer tissues of the root, killing them before invading the woody part. Simultaneously, the fungus spreads in all directions along the root. The fungus may grow independently from roots of one susceptible plant to another, spreading as it grows.

Stunting of the plants was about the only visible symptom shown by the foliage of plants infected with P. omnivorum (Houston et al., 1960). Infested areas appeared as circles of dead plants. Lesions on the taproots were yellow to brown, sunken, quite clearly defined, and irregular in shape.

Smith (1948), describing the symptoms of Phymatotrichum root rot, stated that the first above-ground sign of root infection was usually the wilting of the tops which always was preceded by root decay. One or two days after wilting, the infected plants died. P. omnivorum first infected the bark of the root and slowly penetrated into the woody cylinder until the root was rotted through. The disease in the field spread in all directions (Smith, 1948; Staten, 1957). It usually started from a small localized infection and spread in a well-defined, circular area, killing the plants. At the margin of this area was a zone of dead and dying plants beyond which were apparently healthy plants. The taproots of surviving plants generally were rotted off and the plants were kept alive by means of lateral roots above the decayed portion of the taproot. Affected roots usually broke off easily at or near the soil surface when the plants were pulled. Brownish, fuzzy strands of mycelium usually were seen on affected roots.

Alfalfa Resistance to Root and Crown Rot Caused by Selected Species of Three Genera of Fungi

1. Resistance to Rhizoctonia spp.

Smith (1945) inoculated California Common alfalfa with isolates of R. solani. Some of the plants did not exhibit or develop root canker lesions when inoculated with highly pathogenic strains. This was the earliest work that indicated a possible source of resistance to this fungus.

Erwin (1954, 1956) found no differences between alfalfa cultivars in level of resistance to root canker, stem canker and crown-bud necrosis. Later, Houston et al. (1960) stated that a search was being made by California workers for resistant plants to Rhizoctonia root canker. Progeny from selected plants, while not highly resistant, showed promise of being more vigorous and longer-lived than existing cultivars.

Matocha and Cowley (1965) tested 12 alfalfa cultivars for forage yield and persistence of stand in a Rhizoctonia root canker infested area in the Rio Grande Valley of Texas. The cultivars 'African,' 'Moapa,' and 'Sonora' demonstrated the greatest persistence of stand after two years of testing.

2. Resistance to *Fusarium* spp.

Wilson (1961) studied six cultivars and 53 strains of alfalfa for their reaction to *Fusarium* spp. Selections from New Mexico Common alfalfa were the most resistant to Fusarium wilt (*F. solani*).

In tests at University Park, N.M., Wilson and Melton (1962) found that strain 9-64 was more resistant to Fusarium wilt than New Mexico Common. There were other lines that had a lower disease index than New Mexico Common. Selections with resistance to bacterial wilt and Fusarium wilt (*F. solani*) were utilized to develop the cultivar 'Zia' (Wilson et al., 1959).

3. Resistance to *Phymatotrichum omnivorum*

Houston et al. (1960) mentioned that to date no report of resistance in alfalfa to *P. omnivorum* had been published.

In past tests at Iowa Park, Texas, the major local cultivar, 'Oklahoma Common,' showed more tolerance to this root-rot organism than any other cultivar tested (Brooks, 1959). In a study of nine new cultivars at Iowa Park in 1957-1958, all entries were susceptible to cotton root rot. 'Buffalo,' 'Du Puits,' and 'Williamsburg' had the lowest mortality and highest forage yield in this test.

Methods of Inoculation With Selected Species of Three Genera of Fungi and Their Disease Classification

1. *Rhizoctonia* spp.

Smith (1943, 1946) made pathogenicity tests by growing young alfalfa plants in soil infested with *R. solani*. Plants about three months old were used, for at this age they had roots large enough to test the ability of the fungus to cause root lesions. They were transplanted to galvanized iron cans, 17.78 cm in diameter and 30.48 cm deep. In all cases transplants were allowed to grow at soil temperatures between 16 to 20 C, which was unfavorable for disease development, for at least four weeks before soil temperatures were raised to 25 to 30 C, which favored disease development. The soil was infested with barley grain, previously sterilized, on which the fungus had grown for about two weeks. The grain inoculum was applied at 20 g dry weight to 7,000 g soil. The grain had been soaked with water prior to sterilization. The inoculum was added either by mixing with the soil just prior to transplanting or the transplants were allowed to grow about a month in non-infested soil. After this time lapse, five holes of about 0.64 cm in diameter and 20.32 to 25.40 cm deep were made in each can of soil, and the inoculum placed in the soil. Both methods were satisfactory. The plants were grown at optimum soil temperatures of 25 to 35 C for two months, then removed and examined for symptoms.

In studies of crown-bud rot of alfalfa, Hawn and Cormack (1952) used inoculum grown in soil-corn-meal medium. This was scattered among crowns and lightly covered with soil. The maximum infection developed after two weeks on three-month-old alfalfa plants.

Using pathogenic isolates of *R. solani*, Erwin (1954) inoculated various cultivars of alfalfa planted in soil which had never been planted to alfalfa. The different cultivars were planted in rows 10.16 to 15.24 cm apart in plots 3.05 m wide by 3.66 m long. Both check and test plots were replicated. In May, when plants were two months old, 21 g of ground-dried oat inoculum of a pathogenic *R. solani* isolate were distributed in a shallow furrow close to each row of plants except in the check plot. He took samples of 150 to 200 plants in July, sampling diagonally across plots examining and rating each plant for severity of root canker, stem canker, and crown-bud necrosis. No differences were found among cultivars. *Rhizoctonia solani* was reisolated from diseased plants.

McDonald (1955) prepared inoculum by mixing agar plates of several isolates of *Rhizoctonia*. Twenty-five ml of H_2O per plate were added and placed in a Waring Blender and mixed for three minutes. The suspension was mixed with silica sand to half fill the tumblers. Tests on mature plants were conducted at 16, 20, 24, and 28 C in a temperature tank. Plants were grown in steam-sterilized soil until 14 weeks old then transplanted to a sterile soil and sand-corn-meal inoculum in 1.89 liter crocks. Roots were dipped in a suspension of mycelium and/or spores of the fungus. Six weeks later the plants were examined for disease lesions.

To study post-emergence seedling root rot, it was necessary for the seedlings to develop beyond the damping-off stage before infection (Schmitthenner and Hilty, 1962). They were able to do this by placing the inoculum of Rhizoctonia sp. four centimeters below the seed at planting. This allowed sufficient time for the alfalfa plants to develop before inoculation occurred.

Chi and Childers (1965) studied the virulence of Rhizoctonia sp. on alfalfa and red clover. They prepared inoculum by growing the isolates for two weeks on an autoclaved corn-meal-sand medium. Seedlings to be tested were grown in white silica sand and in sterilized and nonsterilized field soil. The soil was steam sterilized for two hours at approximately 1 kg/cm^2 pressure for three successive days before use. The inoculations were made by mixing one part inoculum to 15 parts white sand or soil. Surface-disinfected seed of 'Ranger' alfalfa and 'Lakeland' red clover were planted in a mixture of three parts vermiculite to one part sand to produce seedlings. Two-week-old seedlings were transplanted to pots and placed on greenhouse benches. Plants in sand were kept moist with 0.5-normal Hoagland's solution while those in soil were watered with distilled water. Control plants were grown in the same mixtures without the pathogen, also controls in sand or soil were used without the corn-meal-sand mixture. Sixty-five days after transplanting, all plants were removed from the pots, washed and examined for the development of disease symptoms on the root and crown areas. Virulence of the *Rhizoctonia* strain was measured by disease severity and oven-dry weights of plants.

2. *Fusarium* spp.

Staten and Leyendecker (1949) used two methods to study the pathogenicity of their isolates of *F. solani*. One method utilized surface sterilized seed that were planted in autoclaved soil infested with the pathogen. The second method consisted of surface sterilizing healthy alfalfa plants with mercuric chloride and then planting them into pots containing artificially-infested soil which had been autoclaved. After the plants had become well established their crowns were split and inoculated with the organism. After 18 weeks as many as 80% of the plants from both methods exhibited symptoms like those that had been previously noted in the field. Plants showed stunted growth with coloring of leaves being light green to yellow. The fungus was easily reisolated from diseased roots and crowns.

Wilson (1961) used various methods of inoculation in testing alfalfa strains and cultivars for resistance to Fusarium wilt and Bacterial wilt. In preparing Fusarium inoculum, the organism was grown in petri dishes containing potato-dextrose agar. After a period of growth of the organism the entire contents of a dish with some distilled water were macerated in a Waring Blender. The material diluted with distilled water was kept in a sterilized glass container

For one method of inoculation with *Fusarium* sp., the root-ball method described by Wilson (1961) was used. The soil was knocked out of pots containing alfalfa plants, the roots and soil ball cut through, parallel to and about 5.08 cm below the soil surface. The lower portion of the root ball was returned to the pot and inoculum added. Then the top 5.08 cm of the soil ball containing the plants were replaced over the inoculum.

A second method, the split-crown method, utilized cotton balls which had been soaked in inoculum. The crowns of the plants were split with a knife and the cotton ball forced into the incision. In both of these greenhouse tests the top growth was removed prior to inoculation and later placed over the crowns. The pots were kept under high humidity until the disease organism had sufficient time to become established.

After a period of time, the plants were removed from the pots and scored for resistance. Classification was based on the amount of discoloration in the taproot. A longitudinal cut through the entire crown and root of each plant was made for classification. The plants were divided into seven classes based upon symptoms.

One field study used two inoculations on the same plants (Wilson, 1961). The first inoculation was accomplished by scraping stems near the crown and rubbing inoculum into the wound. A second inoculation was made by splitting stems near the crown and placing inoculum in the split. At the time of examination and classification the plants were dug, washed, and roots split longitudinally. A Fusarium wilt index was used to score each plant. The index ranged from 0 (no evidence of infection) to 7.5 (dead plant).

A second method of inoculation used in field studies was the split-crown method (Wilson, 1961; Wilson and Melton, 1962).

In a survey by Leach (1959), roots of alfalfa were collected and examined for vascular and cortical discoloration and insect injury. The disease symptoms were determined by splitting the roots longitudinally at several locations along their lengths. The amount of vascular discoloration and cortical discoloration or decay was rated.

Cralley (E. M. Cralley, 1952. Alfalfa Impr. Conf. Rep. 13:58-59) described a method of evaluating alfalfa breeding stock for resistance to root disease. Most of his work was done with Fusarium spp. isolated from diseased alfalfa plants. The plants were grown in sand and watered with a basic nutrient solution plus some trace elements. The solution was applied three times a week at the rate of 0.964 liter solution to each 11.355-liter jar. The inoculum was grown in a liquid medium. The fungus was allowed to grow about two days and then was applied to six-week-old plants. The inoculum was applied to roots which had been cut with a scalpel. Using one isolate, it was shown that a high level of N and P and a low level of K were very favorable for infection and subsequent death of the plant.

Schmitthenner (1957, 1964) described his methods for inoculation of plants with root-rot pathogens. Virulence of the pathogen was evaluated in the cotyledon stage by placing inoculum under the seed at planting time and at the unifoliolate stage by injecting a mycelium suspension around the base of ten-day-old plants. Such plants were usually beyond the damping-off stage. Plants from sterilized seed were grown in autoclaved quartz sand in 0.473 liter paper cups and watered with a complete mineral solution. Mineral nutrition was controlled and roots were removed easily from the sand culture. Each cup was infested by an injection of 50 ml of two- to three-day-old shake culture of fungi in liquid medium. These injections were made with a hypodermic syringe. The needle inoculations were faster, eliminated abnormal wounding of plants, and resulted in as severe disease as did transplanting in sand inoculum mixtures. These methods were used to demonstrate pathogenicity of certain isolates of Fusarium, Pythium, Rhizoctonia, and Phytophthora.

Fulton and Hanson (1960) conducted studies on root rots of red clover under greenhouse conditions at Wisconsin. Pure cultures of isolates were tested in white silica sand, steamed soil, fumigated soil, and five naturally-infested soils. Foliage of the plants was clipped at frequent intervals to weaken plants and increase their susceptibility to pathogens. The amount of clipping that plants could tolerate was largely influenced by their rate of growth, which was influenced by temperature, light, and other environmental factors. Twelve-week-old plants recovered less rapidly and developed more disease than eight-week-old plants when given similar clipping treatments. Clipping was a useful technique in predisposing red clover to attack by weak pathogens. High temperatures stimulated development of disease, and more disease developed in some soils than others. In this test the addition of pure cultures of isolates to naturally-infested field soil had little effect on disease severity on unclipped plants. When plants were weakened by clipping, some isolates increased the disease symptoms in some soils, some had no effect, and others reduced disease severity. The disease index used in this study was based on a scale of 0 to 8; 0 - no disease, and 8 - very severe, with dead plants not affecting the index.

Chi and Hanson (1961) studied the effect of balanced and unbalanced nutrient solutions on the development of red clover in sand culture with and without *Rhizobium* and with and without *F. oxysporum*, *F. solani*, or *F. roseum*. Less disease occurred under optimum conditions for the host. Six concentrations of Hoagland's solution were used. The solution ranged from 0.05 to 3.0 times normal; 0.5 was best for host development and least favorable for disease development. The pathogen grew over the entire range of solution concentration, but grew best at a concentration somewhat higher than the optimum for the host. Concentration of the nutrient solution seemed more important on the host than on the pathogen. Disease severity was highest at nutrient concentrations below optimum for the host. The nutrients N, P, and K were omitted from an otherwise balanced solution. This reduced plant development while the disease increased. When any of these nutrients (N, P, or K) were added, the disease decreased. Potassium caused the greatest decrease. Plants inoculated with nitrifying *Rhizobium* were more vigorous and had less disease than those uninoculated. If high infestation with high disease severity was wanted, the concentration would lack N, P, and K, and be below optimum for the host, with no *Rhizobium* inoculation.

Some methods used for wilts also may be used effectively for other diseases, such as root and crown rots. Armstrong and Armstrong (1965), in their study of *F. oxysporum* on alfalfa, grew plants in steamed sand in eight-liter glazed pots with nutrient solution added daily. The plants were inoculated by cutting the roots on one side and pouring a three-day-old liquid culture of the fungus around them. A temperature of approximately 28 C was maintained in a manually ventilated greenhouse.

3. Phymatotrichum omnivorum

Streets (1937) mentioned four types of inoculum used for the study of resistance of plants to P. omnivorum: (1) diseased roots fresh from the field, (2) pure cultures from diseased roots, (3) fresh conidia from spore mats, and (4) soil from root-rot-infested areas.

Butler (K. D. Butler, 1937. Studies on Phymatotrichum omnivorum with special reference to the watermelon, Citrullus vulgaris schrad. M.S. Thesis University of Arizona, Tucson) used both diseased roots direct from the field and pure culture in studying resistance of watermelons to P. omnivorum. In another test, roots of diseased cotton plants were placed in hills along with the watermelon seed at planting. A second test was conducted using a pure culture grown on steamed carrots. The inoculum was placed in holes in the center of the hills. Wilted and unwilted plants were removed from time to time. The roots were examined and isolations attempted.

Brooks (1959) conducted a test for resistance to P. omnivorum in alfalfa. The alfalfa cultivar test was planted on naturally-infested, Yohola, loamy, very fine sand following a crop of sweet corn. The cultivars were planted in single-row plots, 91.44 m long, 91.44 cm apart and replicated three times. The planting was made in late fall. The following spring all cultivars had good stands. Mortality from root rot occurred in all cultivars by mid-summer. Stand reduction was measured in linear meters of dead plants per row.

Infection studies (M. L. Milbrath, 1966. Histopathological studies of infection of root tissues by the fungus Phymatotrichum omnivorum (Shear) Duggar. M.S. Thesis. University of Arizona, Tucson) were conducted by exposing corn and cotton seedlings to a mycelial mat of P. omnivorum grown on agar. Histological sections were made of the roots to compare the infection process of this fungus on young monocot and dicot hosts.

MATERIALS AND METHODS

Parent Material

Selected plants from the 'African' and 'Lahontan' cultivars served as parent material for the study of the reaction of their propagules and progeny to the fungi studied. These fungi were *R. solani*, *P. omnivorum*, and *Fusarium* spp. The species of *Fusarium* were not identified.

The original plant selections were obtained from Dr. M. H. Schonhorst, alfalfa breeder, University of Arizona, Tucson. These parent plants had survived three summers in soils heavily contaminated with root- and crown-rot fungi at the University of Arizona's Mesa Branch Station where the primary pathogen was *P. omnivorum*. Cotton was interplanted among surviving alfalfa plants the last two years preceding selection to maintain a high level of infestation of this fungus in the test area. The African plants from Mesa were designated as Mesa African Root Rot Resistant (MAR) clones; the Lahontan selections were labeled the MLR clones.

The surviving plants after three years in the root-rot area were tagged for identification and cuttings were made from them as needed. The first propagules were established in pots in the greenhouse. These plants also were utilized for making more cuttings for field testing, greenhouse testing, and setting up polycross seed production blocks. Selfed seed was produced from potted plants in the greenhouse.

Other selections were made from plants growing in a root- and crown-rot-infested area at the U. S. Department of Agriculture Plant Materials Center, Tucson, Arizona. These plants, mainly of the cultivar African, had survived in this area for several years. The plants were treated similar to those from the Mesa Station. These alfalfa plants were designated as Root Rot Resistant-Plant Material Center (RRR-PMC) clones.

Some commercial cultivars were used as checks to compare their relative susceptibility to the root- and crown-rot fungi.

Propagule and Seed Production for Plant and Progeny Testing of Selected Plants

Propagules were obtained from the three plant sources described in the previous section.

Selfed seed was produced on some of these clones grown in pots in the greenhouse. Selfing was accomplished by rolling the raceme between the fingers to facilitate tripping and self pollination. This method has been described by Bolton (1962). The seed pods were collected at maturity and the seed was threshed and cleaned.

Methods of crossing also have been described by Bolton (1962). Individual selected plants were cross pollinated. The plants were selected for their vigor of growth in the greenhouse. Crossing was done in the greenhouse by using a paper boat (folded index cards cut to a point). The point of the boat was inserted between the keel and standard of the flower. This action tripped the flowers and the pollen was collected in the boat. The boat was then inserted into the flower of the other plant involved in the cross. This procedure was very effective in transfer of pollen. This was repeated until sufficient crosses were made. The first flower tripped was removed to avoid as many selfed seed as possible. In this method no emasculations were made. Amount of crossing depended on differential pollen tube growth and level of self-fertility.

Polycross seed blocks were set up in the field at the University of Arizona's Campbell Avenue Farm. The polycross method of testing alfalfa clones has been described by Tysdal et al. (1942). Clones originating from the same cultivar were kept in the same crossing block. A polycross of MLR's was established with 56 clones. Three replications were planted with blocks being randomized with two propagules per entry per replication. The MAR's also were established in a polycross seed production block. Forty-three clonal lines were established in plots and replicated four times. Two propagules per plot were spaced 30.48 cm apart in the row, and 66.20 cm between rows.

A smaller crossing block was planted to clones which produced seed designated as (PXS). There were 20 entries with two replications. Two propagules were planted per entry per plot. The entries consisted of some of the MAR selections and some PMC selections, which were available at the time of planting.

All polycross blocks were covered with plastic screen cages to insure isolation. Crossing was accomplished by introducing honey bees into the cages. The bees were provided by personnel of the U. S. Department of Agriculture Bee Research Laboratory in Tucson.

At maturity seed was harvested from each entry. This seed was used for producing plants for further testing.

Preparation of Inoculum and Soil Mixture

A Fusarium sp. was isolated from diseased alfalfa roots by the late Dr. P. D. Keener of the Plant Pathology Department at the University of Arizona, Tucson. Rhizoctonia solani was isolated by the author from diseased roots of alfalfa in greenhouse studies using naturally-infested, non-sterile soil.

Stock cultures were increased for use in inoculation studies in the greenhouse. The stock cultures and recent isolates were transferred to both slants and petri dishes containing potato-dextrose agar (PDA).

An oat medium was also prepared to increase cultures for inoculation. Jars (0.95 and 1.89 liter) were half filled with oats then distilled water was added to fill the jar to 3/4 full. A hole was punched in the lids of the jars and a cotton plug inserted. This made it possible to autoclave them without removing the lids thereby reducing contamination. It also facilitated inoculation of the oat medium without getting contamination. The oat medium was autoclaved three times, usually at one-week intervals at 120 C with approximately 1 kg/cm^2 pressure for 20 minutes or longer. Plugs cut with a cork borer from the mycelial growth on agar or portions of the stock culture including mycelial growth and agar were transferred to the oat medium. The culture grew until most of the oat medium was covered with mycelial growth. It then was used for inoculating soil mixtures in greenhouse benches.

Soil for the greenhouse experiments was prepared by mixing two parts loam with one part sand. This mixture was fumigated by pouring chloropicrin over the soil. This was covered with damp newspapers, then with a plastic sheet and a canvas tarpaulin to retain the fumes. This soil was allowed to remain undisturbed for three days before the covering was removed. After removal of the covering, the soil mixture was exposed to air for a day. The loam and sand mixture was moved with equipment which had been treated with 5% formaldehyde. All benches prepared for the soil mixture were sprayed with Captan fungicide prior to placing the soil mixture in them. The loam and sand mixture was then mixed at a rate of approximately 18 parts loam and sand to three parts peat moss and one part perlite. This mixture was placed in several of the benches which were to be used for the inoculation studies.

Methods of Inoculation and Material Tested

Greenhouse studies were conducted on four large benches which were partitioned into four parts each. These bench sections were given a letter designation for identification, e.g., Bench A, Bench B, Bench C, and Bench D composed the first large bench, as shown in Figure 1. Each designated bench, e.g. Bench A, was approximately 2.44 m by 60.96 cm and 25.40 cm high and was filled with about 0.31 m^3 of soil or soil mixture.

Greenhouse Experiment 1: Bench D was planted with propagules of selected plants on November 14, 1962. This investigation was conducted to determine the persistence of plants in the bench with no inoculation and to study plant spacing and salt accumulation. Sixteen entries were planted in the bench with six planting rates. Some of the rates were dependent on the amount of propagules available. Rows were 60.96 cm long with 6, 7, 10, 14, 15, and 20 plants per row. The rows were approximately 15.24 cm apart.

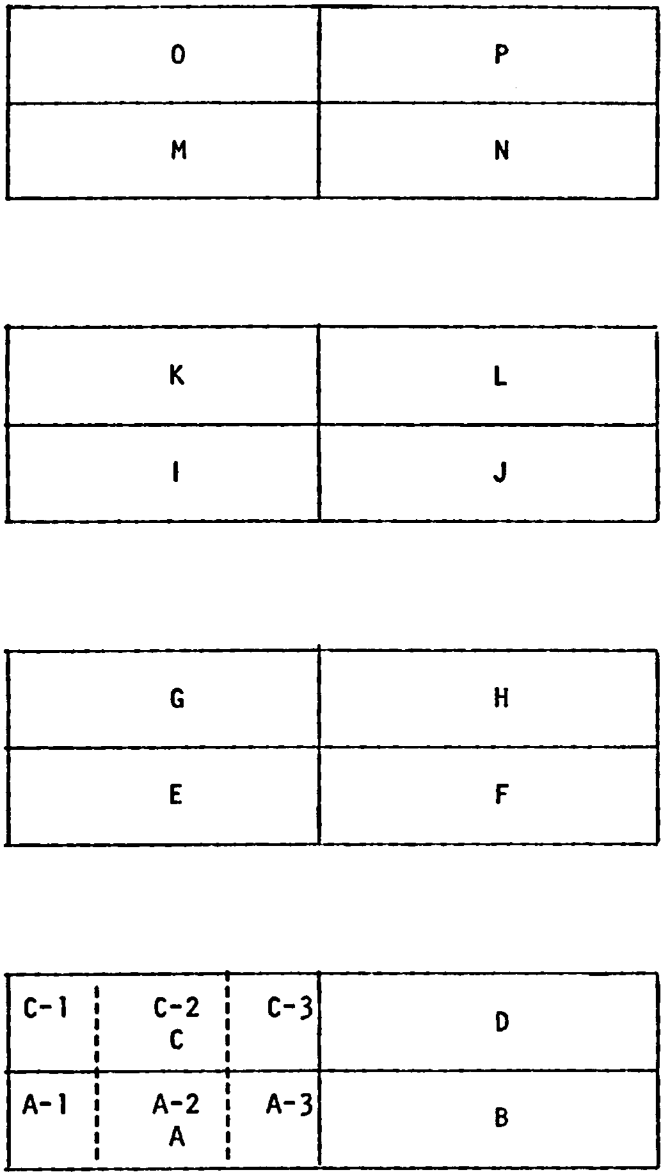

Fig. 1. Designation and bench arrangement used for alfalfa disease experiments in the greenhouse.

Benches M and N were filled with the soil mixture. Seed of nine cultivars or strains of alfalfa were planted in Bench M for later use in testing established seedlings. Six of the lines were planted at two rates in Bench M. Fifteen rows were planted with rows 15.24 cm apart. Bench N was treated the same as Bench M except two of the alfalfa lines were changed in this planting. The planting was made on November 28, 1962.

Greenhouse Experiment 2: Bench B containing the soil mixture was inoculated with 3.78 liters of Fusarium inoculum growing on oat medium. The inoculum was spread uniformly over the soil and covered to a depth of 2.54 cm on November 18, 1962. Certified seed of Hairy Peruvian alfalfa (Lot 6099A) was planted in 60.96 cm rows spaced 30.48 cm apart on February 16, 1963. This planting was made to determine the virulence of the Fusarium spp. being used. A reinoculation of Bench B was made August 15, 1963. Inoculum was placed in furrows between the rows of established plants.

Greenhouse Experiment 3: Bench J was inoculated with macerated roots and crowns of diseased alfalfa plants dug from a border-size plot (G-79) of Sonora alfalfa at the University of Arizona Mesa Branch Station. Macerated roots and crowns (18.93 liters) were mixed with the soil mixture. This experiment was planted on May 14, 1963 with selfed seed of selected clones. Certified Hairy Peruvian (Lot 6099A) was used as a check in all experiments except Greenhouse Experiments 1 and 2. Selfed seed of a selected plant, Caliverde "C", was used as a check in only part of the tests. Twenty-five seed were planted per row at 2.54 cm intervals 1.27 cm deep. Rows were spaced 15.24 cm apart. Selfed seed from 11 selected parent plants were planted along with the checks.

Greenhouse Experiment 4: Bench L was inoculated with macerated roots and crowns of Sonora alfalfa. Seedlings from Bench M were transplanted to Bench L on May 9, 1963. The root systems were injured by cutting off the roots about 5.08 cm below the crown. Thirteen seedlings from each of eight lines were planted in rows spaced 30.48 cm apart.

Greenhouse Experiment 5: This test was planted in Bench I and conducted similar to Experiment 3 and 4. Seedlings grown in Benches M and N were transplanted to Bench I. Six seedlings from eight lines were planted in rows that were spaced 30.48 cm apart. The soil was inoculated on May 14, 1963 and the seedlings were transplanted on May 16, 1963. Roots of these seedlings were not damaged by cutting or wounding.

Greenhouse Experiment 6: Bench E was filled with a mixture of approximately eight parts of soil from an area heavily infested with the cotton root-rot organism on the U. S. Department of Agriculture Plant Materials Center at Tucson with one part perlite and two parts peat moss. This bench was planted on June 21, 1963 with polycross seed of MLR plants and selfed seed of the MLR's and MAR's. Fifteen lines were planted with 25 seed of each line per row at 15.24 cm between rows.

Greenhouse Experiment 7: Bench F contained soil from the U. S. Department of Agriculture Plant Materials Center at Tucson with perlite at a rate of 10:1. The bench was planted on June 21, 1963 with polycross seed of MLR plants and selfed seed of the MLR's and MAR's. Fifteen lines were planted with 25 seed of each line per row at 15.24 cm between rows.

Greenhouse Experiment 8: Bench G was filled with a mixture of eight parts soil from the University of Arizona Mesa Branch Station root-rot area, with two parts peat moss and one part perlite. The bench was planted on June 21, 1963 with polycross seed of MLR plants and selfed seed of the MLR's and MAR's. Fifteen lines were planted with 25 seed of each line per row at 15.24 cm between rows.

Greenhouse Experiment 9: Bench H was filled with a mixture of 10 parts soil from the University of Arizona Mesa Branch Station root-rot area to one part perlite. The bench was planted on June 21, 1963 with polycross seed of MLR plants and selfed seed of the MLR's and MAR's. Fifteen lines were planted with 25 seed of each line per row at 15.24 cm between rows.

Greenhouse Experiment 10: Bench O was filled with a mixture of four parts soil from the root-rot area on the U. S. Department of Agriculture Plant Materials Center at Tucson to four parts soil from the University of Arizona Mesa Branch Station root-rot area to two parts peat moss and one part No. 2 perlite. The bench was planted on August 12, 1963 with polycross seed of MLR plants and selfed seed of the MLR's and MAR's. Fifteen lines were planted with 25 seed of each line per row at 15.24 cm between rows.

Greenhouse Experiment 11: Bench P contained a mixture of five parts soil from the University of Arizona Mesa Branch Station root-rot area to five parts soil from the root-rot area on the U. S. Department of Agriculture Plant Materials Center at Tucson to one part No. 2 perlite. The bench was planted on August 12, 1963 with polycross seed of MLR plants and selfed seed of the MLR's and MAR's. Fifteen lines were planted with 25 seed of each line per row at 15.24 cm between rows.

Greenhouse Experiment 12: Benches A and C were partitioned into three sections which were designated A-1, A-2, A-3, and C-1, C-2, C-3.

Bench A-1 contained a standard soil mixture to which 5.68 liters of R. solani oat culture were mixed. The inoculation of the soil was made on August 15, 1963 for all sections of A and C benches. Plants or seed were planted on September 19, 1963. Established seedlings from Bench M were planted in Bench A-1. These plants were selected for uniformity and their roots cut off 10.16 cm below the crown at the time of planting into the inoculated soil.

Bench A-2 was inoculated with 5.68 liters of Fusarium sp. oat culture and planted to established seedlings from Bench M. Four rows with six seedlings per row were planted. The plants were selected for uniformity and their roots cut off 10.16 cm below the crown at the time of planting into the inoculated soil.

Bench A-3 was inoculated with 3.78 liters of Fusarium sp. and 3.78 liters of R. solani oat culture. Established seedlings from Bench M were planted in four rows with six seedlings per row. The plants were selected for uniformity and their roots cut off 10.16 cm below the crown before planting into the inoculated soil.

Bench C-1 was prepared similar to A-1 with R. solani oat cultures. This was planted to four entries of 25 seed each.

Bench C-2 was inoculated with Fusarium sp. oat culture and planted to four entries of 25 seed.

Bench C-3 was inoculated with 3.78 liters *R. solani* oat culture and 3.78 liters of *Fusarium* sp. oat culture on August 15, 1963, and planted on September 19, 1963 with the same four entries as in C-1 and C-2.

Greenhouse Experiment 13: A second planting was made in Benches A and C after the first planting had been removed. The second planting was made on August 4, 1964. Bench A was not reinoculated as Bench C was with the same type of inoculum as used in the first planting. Both benches were planted with four entries. Two entries were the check cultivar Hairy Peruvian. One entry of Hairy Peruvian was untreated seed while the other was coated with a bacterial residue provided by Mr. Elton Mann, who at that time was with the Plant Pathology Department at the University of Arizona, Tucson.

Greenhouse Experiment 14: Benches I, J, and L were replanted after the first planting had been removed. The bench with soil mixture was covered with a canvas tarpaulin and treated with methyl bromide. The benches were exposed to the air for several days then replanted. Bench I was inoculated with 11.35 liters of *R. solani* inoculum and planted on September 19, 1964 with seed of five entries that were replicated three times. Bench J also was planted on September 19, 1964 with the same entries and replications as Bench I, but only inoculated with 11.35 liters of sterilized oats with no organism growing on the medium. Bench L was planted on September 21, 1964. No inoculum was added to Bench L. The same entries were used in Benches I and J.

Field Experiments

Field experiments were conducted in a root-rot infested area to determine the relative persistence of the progeny of MAR and MLR clones. The main organism in this area was P. omnivorum.

Field Experiment 1 was a progeny test of African plants which were selected for root-rot resistance. These were progeny of MAR clones from Field H Mesa Branch Station and some were progeny of RRR-PMC clones. Four replications of 50 seed were planted in rows 2.44 m long with a 30.48 cm alley and 30.48 cm between rows. Forty-three lines were included for progeny testing either of selfed seed, seed from the large polycross or small polycross blocks. Eleven other strains or cultivars also were in this test. This study was planted in Field H Border 16 Mesa Branch Station on December 30, 1963.

Field Experiment 2 was composed of progeny testing MLR plants. All of the seed was from a polycross block of the MLR clones. There were 30 entries for progeny testing. The test also included four cultivars. Four replications of 50 seed per entry were planted in rows 2.44 m long with 30.48 cm alleys at the ends of the rows and 30.48 cm between the rows. This test was planted on December 30, 1963 at the Mesa Branch Station in Field H Border 16.

Field Experiment 3 was a second test of polycross progeny of MAR and RRR-PMC clones. There were 30 MAR entries and two RRR-PMC entries. Two cultivars also were planted. This experiment was planted in a complete randomized block design with four replications in Field H Border 14 Mesa Branch Station on March 12, 1964. Fifty seed were planted per entry in rows 2.44 m long with 30.48 cm alleys at ends of the rows and 30.48 cm between the rows.

Field Experiment 4 was planted in Border 14, Field H, Mesa Branch Station on March 12, 1964. This was a progeny test of 46 MLR entries with four cultivars. Four replications in a randomized complete block design were planted with 50 seed per entry, similar to the other field experiments.

Evaluation and Classification

Greenhouse Experiments

Plants in Benches A through P except Benches M, N, B, and D were removed from the benches and scored for their resistance or susceptibility to a pure culture of organisms tested, those organisms present in root and crown inoculum or organisms present in naturally-infested soil. Percentage survival of entries was recorded. Classifications were based on discoloration of the root and crown. Discoloration was determined by cutting longitudinally through the entire root and crown of each plant.

Six classifications were used:

0 - No discoloration

1 - Slight discoloration

2 - Moderate discoloration

3 - Heavily discolored

4 - Discoloration extensive throughout root and crown

5 - Dead plant

A disease index was for the evaluation of tested lines. This showed the relative amount of resistance to each line to the organism in the greenhouse experiments.

Field Experiments

Experiments were evaluated according to ability of the alfalfa lines to persist in the root-rot areas in which the plantings were made. This evaluation was based on percentage survival.

RESULTS AND DISCUSSION

Greenhouse Experiment 1

Plants in Bench D were not dug for root examination. They were only observed for persistence in the bench under greenhouse conditions. All plants were alive at the time the last greenhouse experiment was concluded. The plants persisted over two years with no apparent ill effects. This would give an indication that a secondary pathogen could be used in studies in greenhouses where the plant deteriorates very slowly. Although this type of study would take several years, it could show whether an organism was pathogenic or non-pathogenic. One would also have to prevent contamination in the other benches.

Greenhouse Experiment 2

The *Fusarium* sp. isolates used in this experiment were not virulent. Plants were not injured in any way to make an avenue for infection. Plants in Bench B were not dug for root and crown examination, although periodically some plants were removed to make isolations. The plants persisted for over two years until the final greenhouse studies were concluded. The *Fusarium* sp. was reisolated easily on potato-dextrose agar. Thus, the fungus was present in the plant. Its presence could not be determined visually. There were no symptoms. Fulton and Hanson (1960) had a similar experience in their work with red clover. They found that the dry weight measurements indicated some stunting was caused by the *Fusarium* sp. used; also, weakening of the plant sometimes permitted the fungus to show its pathogenic nature. These results indicated that many of the so-called non-pathogenic or secondary pathogens may have effects on plants but present methods of testing or observation for disease symptoms are not adequate.

Greenhouse Experiment 3

Results showing various degrees of discoloration of roots and crowns of the entries in this test are shown in Table 1. The entries are listed in order of their disease index. One entry, MAR-38 S_1, was completely lost in the experiment. The disease index was low, this indicated that although macerated roots and crowns were a direct source of inoculum, the amount was probably small. Another possibility was either some resistance to the organisms or the parasites on the root and crown were not very virulent. Three entries of the check cultivar Hairy Peruvian were at various rankings among the entries. The open pollinated cultivars were usually more variable than plants from seed of a single clone. Sterilized oats could provide a better medium and greater distribution of the pathogen from the diseased roots and crowns if they were mixed together in the soil.

Greenhouse Experiment 4

In Experiment 4 (Table 2) transplants were placed in inoculated soil. The disease classification was taken for both roots and crowns. The disease index was determined by taking the average disease classification of the roots and crowns and dividing by the number of plants classified. Usually the crowns had a higher disease classification than the root. This indicated that the disease organism either attacked the

Table 1. Greenhouse Experiment 3. A comparison of disease classification of 12 S_1 populations and three check alfalfa cultivars; planted May 14, 1963 and classified January 31, 1964.

Entries	No. plants classified	Total disease classification a/	Disease index b/	Disease Index Rank
MAR-27 S_1	2	0	0.0	1
Cert. Hairy Peruvian	11	7	0.6	2
MAR-35 S_1	9	7	0.8	3
MAR-33 S_1	7	6	0.9	4
MAR-39 S_1	6	6	1.0	5-8
MAR-32 S_1	4	4	1.0	5-8
MAR-1 S_1	3	3	1.0	5-8
MAR-43 S_1	2	2	1.0	5-8
Cert. Hairy Peruvian	7	8	1.1	9
Caliverde "C" S_1	7	9	1.3	10-11
MAR-30 S_1	6	8	1.3	10-11
Cert. Hairy Peruvian	10	14	1.4	12
MAR-4 S_1	6	11	1.8	13
MAR-23 S_1	5	10	2.0	14
MAR-38 S_1	0	0	5.0	15

a/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

b/ Average of the disease classification for the entry.

Table 2. Greenhouse Experiment 4. A comparison of percentage survival and disease classification of eight cultivars; transplanted May 9, 1963 and classified January 27, 1964.

Entries	No. of plants		Survival		Total disease classification b/		Disease index c/	Disease index rank
	trans-planted	classi-fied	percentage	rank a/	root	crown		
Lahontan Lot 8-02-A	13	11	84.6	2	6	11	0.8	1
Cert. Hairy Peruvian	13	10	76.9	4	6	11	0.9	2
Cert. Chilean 21-5	13	13	100.0	1	12	17	1.1	3-6
Zia	13	11	84.6	3	10	14	1.1	3-6
Cert. Sonora	13	8	61.5	7	5	13	1.1	3-6
Lahontan Residue d/	13	7	53.8	8	5	10	1.1	3-6
Bam (PX)	13	10	76.9	5	14	17	1.6	7
Sirsa (Y-M)	13	9	69.2	6	13	25	2.1	8

a/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

b/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

c/ Average of the disease classification for the entry.

d/ Remnant seed of Lahontan which was originally planted in the root-rot area at the University of Arizona Mesa Branch Station and from which MLR selections were made.

crown first, thereby discoloring it first, or the crown provided a more optimum environment which allowed greater growth than in the root.

Greenhouse Experiment 5

Experiment 5 (Table 3) was comparable to Experiment 4. It was expected that the average disease index in Experiment 4 should have been higher than Experiment 5 since the roots in Experiment 4 were cut off to provide a path of infection while in Experiment 5 the attempt was made not to injure the roots. Secondly, the plants were spaced closer in Experiment 4 which created more competition between plants and therefore produced weaker plants than in Experiment 5. Using Spearman's coefficient of rank correlation, there was no correlation between the same entries in Experiments 4 and 5. A higher disease index in Experiment 5 could be due to greater exposure of the roots to the fungus because of less competition and more growth of the roots through the inoculated soil.

Greenhouse Experiment 6

Entries were ranked according to their disease index when grown in heavily infested soils from a root-rot area (Table 4). This ranking was used primarily because the author believed that disease-free plants, in the case of a classification of no discoloration, would be a better method of selecting than on the basis of percentage survival rank, where each individual plant was given a disease classification. There might be some escapes due to the lack of distribution of organisms or the lack of a path of infection into the plant, but these plants would be discovered in further tests. Soil from disease-infested fields was a very good source of inoculum. This gave the entire range of soil microflora

Table 3. Greenhouse Experiment 5. A comparison of percentage survival and disease classification of eight cultivars; transplanted May 14, 1963 and classified January 27, 1964.

Entries	No. of plants		Survival		Total disease classification [b]		Disease index [c]	Disease index rank
	transplanted	classified	percentage	rank [a]	root	crown		
Sirsa (Y-M)	6	6	100.0	1	5	8	1.1	1
P2 x M-56-11	6	6	100.0	2	7	7	1.2	2
Zia	6	5	83.3	6	5	7	1.2	3
Arlington Chilean	6	6	100.0	3	8	8	1.3	4-5
Cert. Chilean 21-5	6	4	66.6	8	11	9	1.3	4-5
Lahontan Lot 8-02-A	6	5	83.3	7	7	7	1.4	6
Cert. Sonora	6	6	100.0	4	11	9	1.7	7
Cert. Hairy Peruvian	6	6	100.0	5	10	11	1.8	8

[a] Ranked according to the number of mature plants at classification compared to the number of plants emerged.

[b] Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

[c] Average of the disease classification for the entry.

Table 4. Greenhouse Experiment 6. A comparison of percentage survival and disease classification of five S_1 and nine polycross populations with one check alfalfa cultivar; planted June 21, 1963 and classified January 31, 1964.

Entries	No. of plants		Survival		Total disease classification [c]	Disease index [d]	Disease index rank
	emerged [a]	classified	percentage	rank [b]			
MAR-15 S_1	18	6	33.3	15	3	0.5	1
MLR-1 (PX)	19	14	73.6	5	8	0.6	2
MLR-14 (PX)	17	10	58.8	8	7	0.7	3-4
Cert. Hairy Peruvian	15	6	40.0	14	4	0.7	3-4
MAR-30 S_1	17	15	88.2	3	12	0.8	5-6
MLR-56 (PX)	16	9	56.2	9	7	0.8	5-6
MLR-11 (PX)	17	16	94.1	1	14	0.9	7-9
MLR-18 (PX)	18	13	72.2	6	12	0.9	7-9
MLR-37 (PX)	17	9	52.9	10	8	0.9	7-9
MLR-3 (PX)	21	19	90.4	2	19	1.0	10-12
MAR-32 S_1	22	15	68.1	7	15	1.0	10-12
RRR-PMC-2 S_1	18	8	44.4	13	8	1.0	10-12
MAR-38 S_1	20	16	80.0	4	18	1.1	13
MLR-9 (PX)	14	7	50.0	12	9	1.3	14
MLR-6 (PX)	19	10	52.6	11	14	1.4	15

a/ 25 Seed planted per entry.

b/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

c/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

d/ Average of the disease classification for the entry.

found under actual field conditions, which would not be possible when using only a "pure culture" for inoculation of plants.

Rhizoctonia solani and *Fusarium* sp. were isolated from roots of plants from Bench E. Other fungi and bacteria were also isolated but these were mainly non-pathogenic. One isolate of *R. solani* was later used in Experiments 12 and 13.

Greenhouse Experiment 7

In Experiment 7 (Table 5) 13 PX progeny of MLR and MAR with two checks were tested in naturally-infested soil from the U. S. Department of Agriculture Plant Materials Center, Tucson. Conditions were similar to those in Experiment 6 except perlite replaced peat moss in the soil mixture. There was very little difference in the results from these two experiments. The average disease index of both experiments was about the same. This indicated that the addition or deletion of the peat moss did not increase the amount of infection. Again, the use of naturally-infested soil provided a good source of inoculum for disease study.

Rhizoctonia solani was identified as the major cause of damping-off. In isolates from older plants, *Fusarium* sp., plus some non-pathogenic organisms were found. This showed both the presence of fungi, and that the naturally-infested soil could be used as an inoculum.

Greenhouse Experiments 8 and 9

The rank of lines according to their disease index from Experiments 8 and 9 is shown in Tables 6 and 7, respectively. The average disease index was very low. The check cultivar had a similar ranking in both Experiments 8 and 9. Both were inoculated with naturally-infested

Table 5. Greenhouse Experiment 7. A comparison of percentage survival and disease classification of eight S_1 and nine polycross populations with one check alfalfa cultivar; planted June 21, 1963 and classified January 31, 1964.

Entries	No. of plants emerged [a]	No. of plants classified	Survival percentage	Survival rank [b]	Total disease classification [c]	Disease index [d]	Disease index rank
MLR-22 S_1	17	15	88.2	5	8	0.5	1
MLR-25 (PX)	21	16	76.1	8	11	0.7	2-3
MLR-35 S_1	20	11	55.0	14	8	0.7	2-3
MAR-45 S_1	19	18	94.7	2	15	0.8	4-6
Caliverde "C" S_1	10	6	60.0	11	5	0.8	4-6
MLR-44 (PX)	9	5	55.5	12	4	0.8	4-6
MAR-46 S_1	20	19	95.0	1	17	0.9	7-12
MLR-41 (PX)	15	14	93.3	3	12	0.9	7-12
MLR-29 (PX)	23	20	86.9	6	17	0.9	7-12
MLR-13 (PX)	15	11	73.3	9	10	0.9	7-12
Cert. Hairy Peruvian	10	7	70.0	10	6	0.9	7-12
MLR-7 (PX)	19	10	52.6	15	9	0.9	7-12
MLR-32 S_1	15	9	90.0	4	9	1.0	13
MAR-34[2] S_1	18	10	55.5	13	12	1.2	14
MAR-38 S_1	18	14	77.7	7	19	1.4	15

a/ 25 Seed planted per entry.

b/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

c/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

d/ Average of the disease classification for the entry.

Table 6. Greenhouse Experiment 8. A comparison of percentage survival and disease classification of six S_1 and eight polycross populations with one check alfalfa cultivar; planted June 21, 1963 and classified January 30, 1964.

Entries	No. of plants		Survival		Total disease classification c/	Disease index d/	Disease index rank
	emerged a/	classified	percentage	rank b/			
MLR-2 (PX)	25	11	44.0	12	3	0.3	1-2
MAR-1 S_1	21	9	42.8	13	3	0.3	1-2
MLR-37 (PX)	24	17	70.8	7	7	0.4	3-4
Cert. Hairy Peruvian	24	11	45.8	11	4	0.4	3-4
MLR-17 (PX)	23	23	100.0	1	17	0.7	5-6
MLR-6 (PX)	22	13	59.0	9	9	0.7	5-6
Root Rot PMC 4-8 S_1	18	18	100.0	3	14	0.8	7-11
MAR-45 S_1	23	23	100.0	2	18	0.8	7-11
MAR-33 S_1	23	22	95.6	4	18	0.8	7-11
MLR-4 (PX)	24	22	91.6	5	18	0.8	7-11
MAR-35 S_1	22	19	86.3	6	15	0.8	7-11
MLR-10 (PX)	21	14	66.6	8	13	0.9	12-15
MLR-44 (PX)	15	8	53.3	10	7	0.9	12-15
MLR-7 (PX)	21	9	42.8	14	8	0.9	12-15
MAR-24 S_1	20	7	35.0	15	6	0.9	12-15

a/ 25 Seed planted per entry.

b/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

c/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

d/ Average of the disease classification for the entry.

Table 7. Greenhouse Experiment 9. A comparison of percentage survival and disease classification of seven S_1 and seven polycross populations with one check alfalfa cultivar; planted June 21, 1963 and classified January 31, 1964.

Entries	No. of plants emerged a/	No. of plants classified	Survival percentage	Survival rank b/	Total disease classification c/	Disease index d/	Disease index rank
MLR-55 (PX)	17	10	58.8	9	5	0.5	1
MLR-7 S_1	11	9	81.8	4	6	0.7	2-3
Cert. Hairy Peruvian	19	10	52.6	14	7	0.7	2-3
RRR-PMC-1 S_1	13	12	92.3	1	9	0.8	4-9
MLR-3 (PX)	23	21	91.3	2	16	0.8	4-9
MLR-7 (PX)	15	10	66.6	7	8	0.8	4-9
MLR-12 (PX)	22	14	63.6	8	11	0.8	4-9
MAR-29 S_1	21	12	57.1	10	10	0.8	4-9
MAR-34^2	17	9	52.9	13	7	0.8	4-9
MLR-16 (PX)	18	14	77.7	5	13	0.9	10-13
MAR-23 S_1	20	14	70.0	6	13	0.9	10-13
MLR-56 (PX)	14	8	57.1	11	7	0.9	10-13
MAR-27 S_1	15	8	53.3	12	7	0.9	10-13
MLR-18 (PX)	23	20	86.9	3	19	1.0	14-15
MAR-2 S_1	14	4	28.5	15	4	1.0	14-15

a/ 25 Seed planted per entry.

b/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

c/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

d/ Average of the disease classification for the entry.

soil from the root-rot area at the University of Arizona Mesa Branch Experiment Station. The disease index obtained in Experiment 8 was lower than that obtained in Experiment 9. This could be due to the different entries used in each experiment. Also, the addition of peat moss might have some hindering effect on the organisms in this soil. If plants had been allowed to remain in the soil for longer periods of time, a better determination in the difference between lines probably could have been made.

Greenhouse Experiments 10 and 11

In Experiments 10 and 11 (Tables 8 and 9) a mixture of two soils from different root-rot areas was used for testing. Experiment 10 had peat moss while the soil mixture in Experiment 11 did not. The disease indices were low. This would normally occur because these plants had a shorter growing period, i.e., from time of planting to harvest. The shorter the time in the bench, usually the less infection and root and crown discoloration. This soil was the same as that used for Experiments 6, 7, 8, and 9. There was a longer lapse of time between collecting of the soil from the field and planting in Experiments 10 and 11, than in other experiments. Plants of check cultivar Hairy Peruvian gave the same disease index in both experiments. The mixture of naturally-infested soils provided the source of inoculum. The mixture of the two soils might have had a greater diversity or organisms and possibly an interaction between sources since they did not occur together under field conditions

Table 8. Greenhouse Experiment 10. A comparison of percentage survival and disease classification of six S_1 and eight polycross populations with one check alfalfa cultivar; planted August 13, 1963 and classified January 28, 1964.

Entries	No. of plants		Survival		Total disease classification c/	Disease index d/	Disease index rank
	emerged a/	classified	percentage	rank b/			
MLR-1 (PX)	21	10	50.0	14	5	0.5	1
MAR-46 S_1	20	17	85.0	7	10	0.6	2-3
MLR-45 (PX)	23	19	82.6	8	11	0.6	2-3
MLR-7 (PX)	22	12	54.5	13	8	0.7	4
MAR-23 S_1	25	23	92.0	3	19	0.8	5-7
MLR-52 (PX)	23	21	91.3	4	17	0.8	5-7
Cert. Hairy Peruvian	16	12	75.0	10	9	0.8	5-7
MAR-45 S_1	23	23	100.0	1	20	0.9	8-12
MAR-38 S_1	23	22	95.6	2	20	0.9	8-12
MLR-37 (PX)	20	18	90.0	5	16	0.9	8-12
Caliverde "C" S_1	16	10	90.0	6	9	0.9	8-12
MAR-34^2 S_1	23	16	69.5	11	14	0.9	8-12
MLR-18 (PX)	23	19	82.6	9	19	1.0	13-15
MLR-25 (PX)	25	17	68.0	12	17	1.0	13-15
MLR-36 (PX)	22	8	36.3	15	8	1.0	13-15

a/ 25 Seed planted per entry.

b/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

c/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

d/ Average of the disease classification for the entry.

Table 9. Greenhouse Experiment 11. A comparison of percentage survival and disease classification of eight S_1 and six polycross populations with one check alfalfa cultivar; planted August 13, 1963 and classified January 28, 1964.

Entries	No. of plants: emerged a/	No. of plants: classified	Survival: percentage	Survival: rank b/	Total disease classification c/	Disease index d/	Disease index rank
MAR-1 S_1	20	15	75.0	13	2	0.1	1
MLR-1 (PX)	22	22	100.0	1	7	0.3	2-3
MAR-32 S_1	20	20	100.0	2	6	0.3	2-3
MLR-7 S_1	17	17	100.0	3	6	0.4	4
MAR-15 S_1	22	19	86.3	10	10	0.5	55
MAR-29 S_1	25	20	80.0	11	11	0.6	6
MAR-45 S_1	25	24	96.0	5	16	0.7	7-9
MLR-43 (PX)	24	22	91.6	7	15	0.7	7-9
MAR-34[2] S_1	20	15	75.0	14	11	0.7	7-9
MLR-18 (PX)	21	21	100.0	4	16	0.8	10-12
MLR-37 (PX)	19	17	89.4	8	13	0.8	10-12
Cert. Hairy Peruvian	17	12	70.5	15	10	0.8	10-12
MAR-38 S_1	21	20	95.2	6	17	0.9	13-15
MLR-48 (PX)	25	22	88.0	9	19	0.9	13-15
MLR-49 (PX)	22	17	77.2	12	15	0.9	13-15

a/ 25 Seed planted per entry.

b/ Ranked according to the number of mature plants at classification compared to the number of plants emerged.

c/ Classification is based on a scale of 0 to 5: 0 = no discoloration of root and crown, and 5 = mature plant dead at classification. This is the total disease classification for all plants in the entry.

d/ Average of the disease classification for the entry.

Greenhouse Experiment 12

Bench A

Bench A, in this experiment, contained transplants from Benches M and N. Only one plant from each line from each section was given a disease classification. Reisolation of the inoculating organism was also made. The *Fusarium* sp. was easy to isolate; the *R. solani* was present on transplants but not common. The number of plants placed in Bench A and the number remaining at harvest are shown in Table 10. In Bench A Section 1, which was inoculated with an isolate of *R. solani* a comparison was made on the discoloration of the root bark of Sirsa (YM) and Sonora plants. Sirsa (YM) had a lighter-colored root bark than Sonora. The individual plants, chosen at random to make isolates, showed more interior root discoloration in the plant from Sonora than Sirsa (YM). There might be some correlation between exterior and interior root and crown discoloration but this phase was not investigated.

In Bench A Section 2, which was inoculated only with a *Fusarium* sp. isolate, the root bark discoloration was about the same for all entries. This might indicate that there was either little difference in resistance or the pathogen acted uniformly on all entries as far as the exterior of the root was concerned.

Section 3 of Bench A was inoculated with both *Fusarium* sp. and *R. solani*. In a comparison of exterior root discoloration between two entries, Hairy Peruvian showed a lighter discoloration than Sirsa (YM). On individual plants taken for interior root discoloration and isolation,

Table 10. Greenhouse Experiment 12 (Bench A). Plant survival and single plant classification of four alfalfa cultivars in three inoculations; transplanted September 19, 1963 and classified January 25, 1964.

Entries	Transplants per bench section	No. plants at harvest [a]			Single plant classification		
		Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3
Cert. Hairy Peruvian	6	6	6	5	1	0	1
Certified Sonora	6	6	6	5	3	2	0
Lahontan	6	6	5	4	1	2	1
Sirsa (YM)	6	6	5	6	1	1	4

[a] Sec 1 = *Rhizoctonia solani*
Sec 2 = *Fusarium* sp.
Sec 3 = *Rhizoctonia solani* and *Fusarium* sp.

Hairy Peruvian again had less discoloration than Sirsa (YM). Whether this would be true for a larger population, is not known.

Bench C

Three lines and one check cultivar were planted with three inoculations. These inoculations were Rhizoctonia sp. alone, Fusarium sp. alone, and a combination of the two. An analysis of variance was run to determine if differences occurred between lines or treatments (Table 11). Hairy Peruvian, MLR-7 (PX), MLR-29 (PX), MAR-46 S_1 were compared. The Fusarium treatment had no effect on emergence so this was used as the standard for normal emergence as well as for the pre- and post-emergence of the other two treatments. The error degrees of freedom was corrected for one value which was missing in the data. Duncan's multiple range test between the three treatment means is shown in Table 12. There were differences between the Rhizoctonia sp. inoculation, Rhizoctonia sp. and Fusarium sp. and the Fusarium sp. alone. There was a non-significant difference between Rhizoctonia sp. and the combination of Rhizoctonia sp. and Fusarium sp. The Fusarium sp. either was not virulent or acted as a secondary pathogen with little effect by itself or in combination with Rhizoctonia sp. The Fusarium sp. also appeared not to hinder the development or pathogenicity of the Rhizoctonia sp. used.

Greenhouse Experiment 13

This experiment was conducted in Benches A and C. Bench C was reinoculated with the same strain of inoculum as used in Experiment 12. Hairy Peruvian, Hairy Peruvian coated with a bacterial residue, MAR-46 S_1, and MAR-46 S_2 were used in this test. The analysis of variance was based

Table 11. Greenhouse Experiment 12. Analysis of variance for four alfalfa entries and three inoculations based on percentage survival.

Source	df	SS	MS	F
Inoculations	2	15,072	7,536	36.44**
Entries	3	525	175	0.85ns
Error a/	4	827	207	
Total	9	16,424	206.75	

** Significant at the 0.01 level

ns Not significant at the 0.05 level

a/ Corrected for missing data

Table 12. Greenhouse Experiment 12. Duncan's new multiple range test of mean percentage survival of four alfalfa entries when subjected to three inoculations. a/

Rhizoctonia + Fusarium	Rhizoctonia	Fusarium
22	28	100

a/ Means underscored by the same line are not significantly different at the 0.01 level.

on root and crown discoloration. There were no differences between lines as shown in Table 13. There was a difference in treatments, which is shown by Duncan's multiple range test in Table 14. These differences were for root discoloration at harvest. The amount of survival calculated for post- and pre-emergence damping-off also showed differences between treatments (Tables 15 and 16). The higher means show greater survival.

Greenhouse Experiment 14

The results of Experiment 14 are shown in Tables 17, 18, and 19. There were significant differences among entries (Table 17). Error (b) in Table 17 was corrected for six missing values. The entries were ranked according to their disease index (Table 18). The MAR-46 S_1 showed considerably more root and crown discoloration than any of the other entries. There were no differences between the inoculations (Table 19). This was probably due to ineffective soil sterilization by the methyl bromide treatment and meant the soil in the benches was already contaminated. This gave about the same effect as adding a pathogenic organism.

The (MLR-7 PX) S_1 had the best disease index ranking (Table 18). This entry was the selfed progeny of a selected plant from MLR-7 (PX). The plant was selected for its lack of discoloration in the root and crown. This indicated that this was an effective method of selecting plants for more disease resistance. The (MAR-38 x MAR-34^2) S_1 was selected like (MLR-7 PX) S_1. The entry from which this parent plant was selected was not in this experiment. The disease index of (MAR-38 x MAR-34^2) S_1 was slightly lower than the check, Hairy Peruvian.

Table 13. Greenhouse Experiment 13. Analysis of variance of six inoculations and four alfalfa entries based on disease classification.

Source	df	SS	MS	F
Inoculations	5	0.85	.17	8.94**
Entries	3	0.03	.01	.526ns
Error	15	0.28	.019	
Total	23	1.16		

** Significant at the 0.01 level

ns Not significant at the 0.05 level

Table 14. Greenhouse Experiment 13. Duncan's new multiple range test of mean disease classification of four alfalfa entries when subjected to six inoculations. [a/]

Fusarium	R + F	Inoc R	*Rhizoctonia*	Inoc F	Inoc R + F
0.25	0.4	0.4	0.5	0.5	0.8

a/ Means underscored by the same line are not significantly different at the 0.05 level

Table 15. Greenhouse Experiment 13. Analysis of variance of six inoculations and four alfalfa entries based on percentage survival.

Source	df	SS	MS	F
Inoculations	5	23,616.94	4,723.39	30.65**
Entries	3	125.89	41.96	0.27ns
Error	15	2,311.66	154.11	
Total	23	26,054.9		

** Significant at the 0.01 level

ns Not significant at the 0.05 level

Table 16. Greenhouse Experiment 13. Duncan's new multiple range test of mean percentage survival of four alfalfa entries when subjected to six inoculations. [a]

Inoc R + F	Inoc R	Inoc F	R + F	Fusarium	Rhizoctonia
10.9	11.3	25.7	47.9	82.9	87.3

[a] Means underscored by the same line are not significantly different at the 0.01 level

Table 17. Greenhouse Experiment 14. Analysis of variance of five alfalfa entries and three inoculations based on disease classification.

Source	df	SS	MS	F
Replications	2	2.37	1.87	2.27ns
Entries (Seed Lots)	4	47.22	11.80	14.29**
Error (a)	8	6.61	.83	
Inoculations	2	3.08	1.54	3.83*
Inoculations x Entries	8	3.00	.37	1.00ns
Error (b) a/	18	7.23	.40	
Total	42	69.50		

* Significant at the 0.05 level

** Significant at the 0.01 level

ns Not significant at the 0.05 level

a/ Corrected for missing data

Table 18. Greenhouse Experiment 14. Duncan's new multiple range test of mean disease classification of five alfalfa entries when subjected to three inoculations. [a/]

(MLR-7 PX) S_1	MLR-7 PX	(MAR-38 x MAR-34[2]) S_1	Hairy Peruvian	MAR-46 S_1
0.66	0.7	1.02	1.06	3.39

[a/] Means underscored by the same line are not significantly different at the 0.01 level

Table 19. Greenhouse Experiment 14. Duncan's new multiple range test of mean disease classification for three inoculations on five alfalfa entries. [a/]

No inoculation	Sterilized oats	Rhizoctonia inoculation
1.26	1.45	1.45

[a/] Means underscored by the same line are not significantly different at the 0.05 level

MAR-46 S_1's were the selfed progeny of a field-selected plant. It is possible that the parent plant could have been an escape or different pathogens were present in the bench than those that occurred in the root-rot area from which it was selected.

Field Experiment 1

Results of Field Experiment 1 are shown in Tables 20 and 21. This was a polycross progeny test of African selections. The analysis of a split plot design based on stand counts at three different stages of disease development in the field is shown in Table 20. The first stand count was taken April 29, 1964. This was before the pathogen had become active. The second stand count was taken on August 13, 1964, which was about the middle of the season for the main pathogen (*P. omnivorum*) in the test area. The final stand count was taken on October 3, 1964, when the pathogen was once again entering its dormant stage. The stand counts were significantly different (Table 20), which indicated the pathogen was killing plants during the period the stand counts were taken. This was especially noticeable between the first and second stand counts. Percentage survival at the last stand count is shown in the last column in Table 21. This could be a criterion used for selecting parent clones for a disease-resistant synthetic. Those clones whose progeny were above the mean in the test would be expected to perform the best. In a recurrent selection program, the surviving plants could be isolated and intercrossed. The seed planted from these crosses would be for another cycle of selection to increase the gene frequency for resistance against the pathogens causing root and crown rots.

Table 20. Field Experiment 1. Analysis of variance for percentage survival of polycross and S_1 progeny of 43 African clones with 10 check alfalfa cultivars.

Source	df	SS	MS	F
Stand Count	2	15280.74	7640.37	1214.30**
Replication	3	51.26	17.09	2.72ns
Error (a)	6	37.75	6.29	
Entries	52	783.59	15.07	3.97**
Entries x Stand	104	1164.60	11.20	2.95**
Error (b)	468	1775.74	3.80	
Total	635	19093.68		

** Significant at the 0.01 level

ns Not significant at the 0.05 level

Table 21. Field Experiment 1. Averages of stand count on three dates and percentage survival of polycross and S_1 progeny of 43 African clones with 10 check alfalfa cultivars.

Entries a/	Stand Count 1 4/29/64	Stand Count 2 8/13/64	Stand Count 3 10/3/64	% Survival b/
MAR-16 (PX) L	7.500	2.500	2.250	30.000
MAR-24 (PX) L	8.000	2.500	2.250	28.125
MAR-18 (PX) L	6.500	1.750	1.500	23.076
MAR-3 (PX) L	8.500	2.500	1.750	20.588
MAR-45 (PX) L	10.750	2.250	2.000	18.605
MAR-6 (PX) L	7.250	1.250	1.250	17.241
MAR-24 S_1	12.750	3.250	2.000	15.686
MAR-34 S_1	13.750	2.250	2.000	14.545
MAR-27 S_1	20.250	3.500	2.750	13.580
MAR-38 (PX) L	11.500	1.500	1.500	13.043
MAR-4 (PX) L	13.750	2.500	1.750	12.727
MAR-19 (PX) L	10.500	1.750	1.250	11.905
MAR-12 (PX) L	11.750	2.000	1.250	10.638
MAR-29 (PX) L	12.750	1.500	1.250	9.804
MAR-27 (PX) L	10.250	1.500	1.000	9.756
MAR-32 (PX) L	10.500	1.250	1.000	9.524
MAR-30 (PX) L	8.750	1.250	0.750	8.571
Zia (Ck)	6.500	0.500	0.500	7.692
RRR-PMC-4-8-S_1	10.000	1.000	0.750	7.500
MAR-34^2 S_1	14.000	1.750	1.000	7.143
MAR-22 (PX) L	11.000	1.500	0.750	6.818
MAR-13 (PX) L	11.250	1.250	0.750	6.667
Arlington Chilean (Ck)	11.750	2.250	0.750	6.383
MAR-26 (PX) S	8.500	1.000	0.500	5.882
RRR-PMC-4-8 (PX) S	12.750	1.000	0.750	5.882
Hairy Peruvian (Ck)	9.500	0.750	0.500	5.263
MAR-40 (PX) L	9.500	1.750	0.500	5.263
RRR-PMC-4-8 S_1	19.500	1.500	1.000	5.128
MAR-25 (PX) S	10.250	1.000	0.500	4.878
MAR-43 (PX) L	10.250	1.000	0.500	4.878
MAR-14 (PX) L	10.750	1.000	0.500	4.651
MAR-46 S_1	16.500	1.000	0.750	4.545
RRR-PMC-2 S_1	16.500	1.250	0.750	4.545
P-2 X M-56-11 (Ck)	17.250	1.000	0.750	4.348
MAR-23 (PX) L	12.000	0.750	0.500	4.167

Table 21 (continued).

Entries [a]	Stand Count 1 4/29/64	Stand Count 2 8/13/64	Stand Count 3 10/3/64	% Survival [b]
Chilean 21-5 (Ck)	12.250	1.500	0.500	4.082
MAR-30 S_1	14.000	0.750	0.500	3.571
MAR-10 (PX) L	9.000	1.250	0.250	2.778
MAR-9 (PX) S	9.250	1.500	0.250	2.703
Bam (Ck)	9.250	0.500	0.250	2.703
Y-M Sirsa (Ck)	9.500	0.500	0.250	2.632
MAR-31 (PX) L	10.500	1.250	0.250	2.381
MAR-2 (PX) L	10.750	0.500	0.250	2.326
MAR-33 (PX) L	10.750	0.500	0.250	2.326
MAR-35 (PX) L	11.000	0.750	0.250	2.273
M-56-11 X P-2 (Ck)	11.250	0.500	0.250	2.222
RRR-PMC-2 (PX) S	11.500	0.750	0.250	2.174
MAR-1 (PX) S	12.000	0.500	0.250	2.083
RRR-PMC-1 S_1	12.000	1.000	0.250	2.083
RRR-PMC-1 (PX) S	13.250	1.000	0.250	1.887
MAR-37 (PX) L	14.250	1.250	0.250	1.754
Lahontan (Ck)	14.500	0.250	0.250	1.724
Sonora (Ck)	10.250	0.000	0.000	0.000
Means	11.472	1.330	0.835	7.279

a/ (PX) L = Seed produced in the large polycross block.
(PX) S = Seed produced in the small polycross block.
S_1 = Selfed seed produced in the greenhouse.

b/ Ranked according to the percentage of plants at final count (Stand Count 3) compared to the number of plants at the original count (Stand Count 1).

Field Experiment 2

Results of Field Experiment 2 are shown in Tables 22 and 23. This was a progeny test of Lahontan clones. Stand counts of this field experiment were significantly different, as were those stand counts in Field Experiment 1. The stand counts were taken at the same time in both experiments. Percentage survival is shown in the last column in Table 23. For recurrent selection, those surviving plants could be intercrossed and the seed produced planted back in the root-rot area for another cycle of selection.

Field Experiment 3

The analysis of Field Experiment 3 (Table 24) was based on three stand counts. The first stant count was made on June 4, 1964 before the pathogen in the naturally-infested soil had killed any plants. The second stand count was made on August 26, 1964 which was about mid-season for the predominant pathogen (P. omnivorum). The third and final stand count was made on October 3, 1964 at the time when the pathogen entered its dormant period. There was a significant difference between stand counts (Table 24). The greatest reduction in number of plants occurred between the first and second stand counts. Percentage survival is shown in the last column in Table 25. Selection of clones for further testing could be based on this ranking. The parents of progeny that were above the mean in ranking could be combined for a synthetic. Surviving plants in the test could also be used for intercrossing and subsequently used for another generation of selection.

Table 22. Field Experiment 2. Analysis of variance for percentage survival of polycross progeny of 28 Lahontan clones with six check alfalfa cultivars.

Source	df	SS	MS	F
Stand Counts	2	7299.95	3649.97	95.74**
Replication	3	148.11	49.37	1.30ns
Error (a)	6	228.74	38.12	
Entries	33	517.58	15.68	2.65**
Entries x Stand	66	833.22	12.62	2.13**
Error (b)	297	1760.40	5.93	
Total	407	10788.00		

** Significant at the 0.01 level

ns Not significant at the 0.05 level

Table 23. Field Experiment 2. Average stand count on three dates and percentage survival of polycross progeny of 28 Lahontan clones with six check alfalfa cultivars.

Entries	Stand Count 1 4/29/64	Stand Count 2 8/13/64	Stand Count 3 10/3/64	% Survival a/
MLR-30	9.500	3.000	2.250	23.684
MLR-29	7.000	2.000	1.500	21.428
MLR-20	8.750	2.250	1.750	20.000
MLR-19	4.000	1.250	0.750	18.750
MLR-24	13.750	3.000	2.500	18.182
MLR-26	10.000	2.500	1.750	17.500
Hairy Peruvian (Ck)	6.250	2.000	1.000	16.000
MLR-17	10.000	2.250	1.500	15.000
MLR-6	10.250	2.000	1.500	14.634
MLR-16	10.500	2.750	1.500	14.286
MLR-33	7.000	1.250	1.000	14.285
MLR-7	13.000	2.000	1.500	11.538
MLR-32	9.500	1.500	1.000	10.526
MLR-10	12.500	2.000	1.250	10.000
MLR-8	10.250	1.250	1.000	9.756
MLR-34	10.500	1.500	1.000	9.524
Zia (Ck)	5.500	0.750	0.500	9.091
MLR-25	11.500	1.250	1.000	8.696
MLR-18	6.250	0.750	0.500	8.000
MLR-3	10.250	1.500	0.750	7.317
MLR-11	10.500	1.000	0.750	7.143
MLR-13	14.000	2.000	1.000	7.143
MLR-21	10.750	1.500	0.750	6.977
MLR-4	11.250	0.750	0.750	6.667
MLR-12	15.250	1.000	1.000	6.557
MLR-23	12.500	1.250	0.750	6.000
MLR-22	8.500	2.000	0.500	5.882
MLR-31	10.000	1.250	0.500	5.000
MLR-1 (Ck)	18.750	1.250	0.750	4.000
Bam (Ck)	6.750	0.750	0.250	3.704

Table 23 (continued).

Entries	Stand Count 1 4/29/64	Stand Count 2 8/13/64	Stand Count 3 10/3/64	% Survival a/
MLR-9	7.000	1.000	0.250	3.571
MLR-15	10.500	0.250	0.250	2.381
MLR-27	10.500	0.750	0.250	2.381
Lahontan (Ck)	15.000	1.000	0.000	0.000
Means	10.221	1.544	0.978	9.569

a/ Ranked according to the percentage of plants at final count (Stand Count 3) compared to the number of plants at the original count (Stand Count 1).

Table 24. Field Experiment 3. Analysis of variance for percentage survival of polycross progeny of 32 African clones with two check alfalfa cultivars.

Source	df	SS	MS	F
Stand Count	2	4679.37	2839.69	152.88**
Replications	3	33.02	11.01	0.59ns
Error (a)	6	111.45	18.58	
Entries	33	906.02	27.46	3.78**
Entries x Stand	66	499.13	7.56	1.04ns
Error (b)	297	2156.03	7.26	
Total	407	9385.02		

** Significant at the 0.01 level

ns Not significant at the 0.05 level

Table 25. Field Experiment 3. Average stand count on three dates and percentage survival of polycross progeny of 32 African clones with two check alfalfa cultivars.

Entries [a]	Stand Count 1 6/4/64	Stand Count 2 8/26/64	Stand Count 3 10/3/64	% Survival [b]
MAR-19 (PX) L	9.250	4.000	3.250	35.135
MAR-45 (PX) L	9.500	3.750	3.000	31.598
MAR-12 (PX) L	11.750	5.250	3.250	27.660
MAR-27 (PX) L	9.250	3.250	2.500	27.027
MAR-23 (PX) L	12.500	5.250	3.250	26.000
MAR-10 (PX) L	18.000	8.750	4.500	25.000
MAR-31 (PX) L	12.500	4.500	3.000	24.000
Hairy Peruvian (Ck)	6.250	3.500	1.500	24.000
MAR-43 (PX) L	5.250	2.500	1.250	23.809
MAR-16 (PX) L	8.750	2.250	2.000	22.857
MAR-25 (PX) S	8.000	3.750	1.750	21.875
MAR-2 (PX) L	13.750	5.500	3.000	21.818
MAR-18 (PX) L	12.000	3.000	2.500	20.833
MAR-14 (PX) L	8.500	2.750	1.750	20.588
MAR-30 (PX) L	9.750	3.750	2.000	20.512
MAR-3 (PX) L	6.750	4.000	1.250	18.518
MAR-38 (PX) L	11.000	3.500	2.000	18.182
MAR-29 (PX) L	10.000	2.750	1.750	17.500
MAR-37 (PX) L	11.750	4.500	2.000	17.021
MAR-24 (PX) L	9.500	4.500	1.500	15.789
MAR-9 (PX) S	8.000	2.750	1.250	15.625
MAR-22 (PX) L	13.000	4.000	2.000	15.385
MAR-6 (PX) L	7.500	1.500	1.000	13.333
MAR-40 (PX) L	11.500	2.500	1.500	13.043
MAR-32 (PX) L	8.250	1.500	1.000	12.121
MAR-35 (PX) L	14.750	5.750	1.750	11.864
MAR-33 (PX) L	14.250	2.750	1.500	10.526
MAR-1 (PX) S	10.000	1.750	1.000	10.000
MAR-4 (PX) L	9.500	1.250	0.750	7.895
RRR-PMC-2 (PX) S	12.750	3.000	1.000	7.843

Table 25 (continued).

Entries a/	Stand Count 1 6/4/64	Stand Count 2 8/26/64	Stand Count 3 10/3/64	% Survival b/
MAR-26 (PX) S	8.000	1.250	0.500	6.250
RRR-PMC-1 (PX) S	13.000	2.500	0.750	5.769
MAR-13 (PX) L	9.000	3.500	0.500	5.556
Sonora (Ck)	10.500	1.000	0.500	4.762
Means	10.412	3.412	1.824	17.518

a/ (PX) L = Seed produced in the large polycross block.
(PX) S = Seed produced in the small polycross block.

b/ Ranked according to the percentage of plants at final count (Stand Count 3) compared to the number of plants at the original count (Stand Count 1).

Spearman's coefficient of rank correlation was calculated for the rankings of common entries in Field Experiments 1 and 3. There was positive correlation between the two experiments at the 0.05 level. This indicated a similar reaction in these two field tests.

Field Experiment 4

Results of Field Experiment 4 are shown in Tables 26 and 27. The stand counts for this experiment were made on the same dates as Field Experiment 3. The stand counts were significantly different between the first and second counts, showing that the pathogen was most active during that period. Selection of parent clones of tested progeny again would be made on percentage survival shown in the last column in Table 27. A selection criterion for a synthetic would be those parent clones whose progeny had a percentage survival above the mean. Recurrent selection could be made by intercrossing surviving plants and planting the seed back in the root-rot area.

Spearman's coefficient of rank correlation was made between common entries in Field Experiments 2 and 4, which were both of Lahontan selections. There was no correlation between the two experiments. This might have been due to the fact that during the previous growing year, the border in which Field Experiments 1 and 2 were planted had the highly susceptible cultivar 'Bam' grown in it. All the Bam plants in the border, with very few exceptions, were either killed or stunted. This meant that the organism was well distributed over the entire border. The border with Field Experiments 3 and 4 had been planted the previous year to cotton. The cotton was planted between surviving alfalfa plants. The infected areas were large spots, but did not cover the entire border.

Table 26. Field Experiment 4. Analysis of variance for percentage survival of polycross progeny of 46 Lahontan clones with four check alfalfa cultivars.

Source	df	SS	MS	F
Stand Count	2	9169.81	4584.91	360.18**
Replication	3	1.03	0.34	0.33ns
Error (a)	6	76.38	12.73	
Entries	49	1008.91	20.59	3.78**
Entries x Stand	98	775.52	7.91	1.45*
Error (b)	441	2401.09	5.44	
Total	599	13432.74		

* Significant at the 0.05 level

** Significant at the 0.01 level

ns Not significant at the 0.05 level

Table 27. Field Experiment 4. Average stand count on three dates and percentage survival of polycross progeny of 46 Lahontan clones with four check alfalfa cultivars.

Entries	Stand Count 1 6/4/64	Stand Count 2 8/26/64	Stand Count 3 10/3/64	% Survival a/
MLR-37	10.500	3.250	2.750	26.190
MLR-33	12.000	3.000	2.750	22.917
MLR-17	11.250	2.750	2.500	22.222
MLR-39	11.750	3.250	2.500	21.277
MLR-32	7.250	4.000	1.500	20.689
MLR-18	10.000	3.750	2.000	20.000
Hairy Peruvian (Ck)	10.000	3.000	2.000	20.000
MLR-34	14.250	3.000	2.750	19.298
MLR-16	8.000	4.000	1.500	18.750
MLR-35	13.500	2.750	2.500	18.519
MLR-27	5.500	2.000	1.000	18.181
Lahontan (Ck)	14.250	5.500	2.500	17.544
MLR-47	8.750	3.000	1.500	17.142
MLR-13	6.750	1.250	1.000	14.814
MLR-42	10.750	1.500	1.500	13.953
MLR-49	10.750	2.500	1.500	13.953
MLR-41	14.500	3.000	2.000	13.793
MLR-55	12.750	2.250	1.750	13.725
MLR-56	11.250	3.000	1.500	13.333
MLR-40	13.250	3.250	1.750	13.208
MLR-21	7.750	1.500	1.000	12.903
MLR-19	6.000	2.250	0.750	12.500
MLR-25	9.500	1.750	1.000	10.526
MLR-6	7.750	3.000	0.750	9.677
MLR-46	10.750	2.250	1.000	9.320
MLR-15	8.250	1.750	0.750	9.091
MLR-36	11.000	1.750	1.000	9.091
MLR-31	12.500	3.250	1.000	8.000
MLR-52	9.500	1.000	0.750	7.895
MLR-10	6.500	3.250	0.500	7.692
Zia (Ck)	3.250	1.000	0.250	7.692
MLR-24	13.500	2.250	1.000	7.407
Bam (Ck)	13.500	3.250	1.000	7.407
MLR-22	8.250	1.500	0.500	6.061
MLR-4	12.500	0.750	0.750	6.000

Table 27 (continued).

Entries	Stand Count 1 6/4/64	Stand Count 2 8/26/64	Stand Count 3 10/3/64	% Survival a/
MLR-43	8.500	0.750	0.500	5.882
MLR-7	8.750	1.500	0.500	5.714
MLR-53	8.750	0.750	0.500	5.714
MLR-45	9.000	1.500	0.500	5.556
MLR-8	10.000	2.500	0.500	5.000
MLR-20	10.500	1.500	0.500	4.762
MLR-26	11.000	0.500	0.500	4.545
MLR-30	11.250	2.250	0.500	4.441
MLR-50	6.750	1.500	0.250	3.704
MLR-44	14.000	2.500	0.500	2.857
MLR-3	11.250	1.000	0.250	2.222
MLR-9	7.500	0.750	0.000	0.000
MLR-38	6.500	0.250	0.000	0.000
MLR-48	6.250	0.000	0.000	0.000
MLR-11	6.750	0.750	0.000	0.000
Means	9.885	2.170	1.115	11.279

a/ Ranked according to the percentage of plants at final count (Stand Count 3) compared to the number of plants at the original count (Stand Count 1).

SUMMARY

Testing of alfalfa for its reaction to *R. solani*, *P. omnivorum*, and *Fusarium* spp. was conducted on propagules and progeny of parent material selected from the cultivars African and Lahontan. Check cultivars were also used.

Several inoculation experiments were made in the greenhouse. Fungi were grown on an oat medium and mixed with a sterile soil mixture. Macerated diseased roots and crowns also were mixed with a sterile soil mixture. Naturally-infested soil from root-rot areas also was used as a source of inoculum. Established seedlings and seed of various polycrosses and selfed seed from parent material were planted with the various sources of inoculum to determine effectiveness of the inoculation, and to determine differences in level of resistance of individual entries in the test.

Methods of inoculation and resistance of lines were evaluated by percentage survival, root and crown discoloration, and visual observation of plant condition. Some inoculations of fungi were made to determine which organisms were the causal agents of the root and crown rots.

Four field experiments were conducted in a root-rot area at the University of Arizona's Mesa Branch Station. Polycross seed from parent clones, selfed seed, specific crosses, along with check cultivars were planted using randomized complete block designs. This was to evaluate parent material by progeny testing and to determine which lines had the highest survival percentage. The surviving plants in theses tests were saved to produce intercrossed seed to continue the program of recurrent selection for resistance to the root- and crown-rot complex of southern Arizona.

There were several observations made from the greenhouse and field experiments:

1. There was less effect on older plants when they were planted in inoculated soil than on younger plants or plants produced by direct seeding into inoculated soil.
2. The longer plants grew in inoculated soil the higher their disease index was, based on root and crown discoloration.
3. Inoculations made with diseased plant material were less severe on plants than inoculation with pure cultures grown on an oat medium. Naturally-infested soil also was a good source of inoculum for greenhouse experiments.
4. Plants persisted for two years in benches containing sterilized soil.
5. In inoculation studies, fungi were easily reisolated from plants inoculated with specific fungi.
6. There were differences between lines of alfalfa in susceptibility to the strains of pathogens used.
7. Field experiments were more valuable than greenhouse tests. They permitted testing much larger populations with much less effort.
8. In general, polycross progeny from resistant parent plants had a higher level of survival than the original or parent cultivars.

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