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TOLERANCE TO FLOODING AND RESISTANCE TO PHYTOPHTHORA MEGASPERMA IN ALFALFA (MEDICAGO SATIVA L.) CULTIVARS

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TOLERANCE TO FLOODING AND RESISTANCE TO PHYTOPHTHORA MEGASPERMA OF ALFALFA (MEDICAGO SATIVA L.) CULTIVARS

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This thesis is dedicated to my late father, my mother, my son Ngoni and my wife.

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ABSTRACT

TOLERANCE TO FLOODING AND RESISTANCE TO PHYTOPHTHORA MEGASPERMA OF ALFALFA (MEDICAGO SATIVA L.) CULTIVARS

By

Gladman Mapurisa Kundhlande

Alfalfa (*Medicago sativa* L.) is adapted to well-drained soils. Flood tolerance and *Phytophthora* root rot (PRR) resistance are essential for alfalfa production on imperfectly drained soils. The objective of this study was to determine the effects of flooding on stand, vigor, yield, ethanol accumulation in roots, PRR resistance, and root structure.

Flooding during the summer reduced plant population, vigor, and persistence. Under the cool moist conditions of spring, injured and thinned alfalfa stands produced tillers and adventitious roots. Recovery was not sustained during the dry months.

Alfalfa selections with well developed branching root systems with lateral roots close to the crown were the most flood-tolerant. The flood-tolerant selections MI 80-16 and MI 80-16P produced less undesirable ethanol than flood-intolerant cultivars. PRR-resistance and improved flood tolerance increased the yields and persistence of alfalfa growing under flooded conditions. Alfalfa selections resistant to PRR and flood tolerant may increase the adaptation of alfalfa on somewhat imperfectly drained soil not suitable for alfalfa production.

INTRODUCTION

Alfalfa is grown worldwide and under a wide range of environments. Alfalfa cultivars are classified into hardy, medium-hardy, and non-hardy cultivars based on their ability to survive severe, medium, or mild winter temperatures. Hardy cultivars have survived temperatures of $-27^{\circ}C$ in Alaska, while non-hardy cultivars have been grown in the Death Valley of California where temperatures can reach $60^{\circ}C$.

Alfalfa is best adapted to deep, well drained loam soils with porous subsoils and neutral pH. Alfalfa is very drought resistant. It becomes dormant during dry periods and breaks dormancy when moisture becomes available.

Despite its wide adaptability, alfalfa does not grow well under hot, wet, and humid conditions. Root, crown and foliar diseases reduce the productivity and persistence of the crop under such conditions. The temperate regions where most of the world's alfalfa is grown have less foliar diseases, but root and crown diseases have restricted alfalfa cultivation to well-drained soils. However, there are some alfalfa cultivars which are tolerant to saturated soil conditions and resistant to root, crown, and foliar diseases. These cultivars can be grown in those areas which experience periodic flooding.

Up until the 1960's, alfalfa breeding in the northern alfalfa region of U.S.A. concentrated on breeding for winter-hardiness, high yields, and resistance to foliar diseases. Most alfalfa cultivars on the market are medium-hardy to winter-hardy and are resistant to bacterial wilt (*Corynebacterium insidiosum* L.) and anthracnose (*Colletotrichum trifolii* L.). A relatively new disease, *Verticillium* wilt (*Verticillium arbo-atrum* L.) is causing some concern to farmers in certain parts of the region.

The current trend in alfalfa breeding is to breed for resistance to root diseases. *Phytophthora* root rot (PRR) caused by the organism *Phytophthora megasperma* f.s. *medicaginis* is the most serious disease affecting alfalfa growing under poorly-drained soil conditions. Resistance to PRR is the key factor to alfalfa cultivation under imperfectly drained conditions. The first PRR-resistant cultivar adapted to the northern alfalfa region was Agate released in 1973 (Frosheiser and Barnes, 1973b).

Phytophthora root rot is always associated with excessively wet conditions. The symptoms on diseased plants are usually the sum total of the effects of flooding and those of the pathogen. It is, therefore, often difficult to separate the two under field conditions. The objective of this research was to study the tolerance to flooding and the resistance to PRR of four alfalfa cultivars grown in Michigan and two selections of one of the cultivars.

LITERATURE REVIEW

Flooding and anaerobiosis

In the absence of convention currents, which are a result of rapid temperature and pressure fluctuations, the movement of gases is mainly by diffusion (Grable, 1966). Gaseous diffusion through the air-filled soil pore spaces is more than adequate for the respiratory activity of the soil flora and fauna. The rates of diffusion of oxygen and carbon dioxide in air are approximately 10,000 times greater than they are in water (Armstrong, 1975). Therefore, the rate of gaseous exchange between the soil organisms and the atmosphere may be dependent on the thickness of water films surrounding the organisms rather than their distance from the soil.

In flooded soils, water displaces air from the soil pore spaces. When the soil is fully flooded, anaerobic conditions approach the soil surface. Where the water table does not reach the soil surface, there is a zone of anaerobiosis above the water table due to capillary movement of water (Boggie, 1972). In freshly flooded soils, the respiration of aerobic organisms will reduce the oxygen concentration to zero within a few hours (Scott and Evans, 1955). Once the oxygen is depleted, diffusion cannot maintain aerobic conditions and anaerobiosis sets in. Without a lateral influx of oxygen-containing water, the part of the soil profile below the water table will be totally anaerobic (Armstrong and Boatman, 1967).

In a single growing season, crops experience flooding for a day or two each time there is a heavy rain storm. There may be several rain storms in one season. Depending on the period in the life cycle of the plant that the flooding occurs, the yield of the crop can be affected. One day's flooding just before and during blooming, reduced the total biomass of peas by more than one-third (Erickson and Van Doren, 1960).

Metabolic changes induced by anaerobiosis

The oxygen concentration in the soil has to be very low before soil conditions become anaerobic. The rate of oxygen uptake by plant roots when the oxygen partial pressure in the soil is reduced to 0.0095 atmospheres is 71% of its rate under air (Greenwood, 1968).

Anaerobiosis produces several changes in the soil. In mineral soils some nutrients are lost while others are released into the soil solution in amounts which might be toxic to plants (Armstrong, 1975). Nitrates are lost through denitrification. Manganese and ferrous ions rise to levels which may be toxic to crops. Sulphates are reduced to sulphides and accumulate as ferrous sulphide.

Anaerobic microbial metabolism produces hydrocarbons, fatty acids, carboxylic acids, aldehydes, ketones, alcohols, mercaptans, and heterocyclic compounds. Some of these organic products of anaerobiosis are plant growth promoters (Wang, Cheng, and Tung, 1967) while others are phytotoxic (Sanderson and Armstrong, 1978). Aliphatic monocarboxylic acids are particularly harmful.

In plant roots, the reduction of soil aeration is accompanied by an increase in glycolysis and an induction of alcohol dehydrogenase activity (Crawford, 1978). The reduction in aeration by flooding or cessation of aeration in water culture can give rise to an immediate increase in the ethanol concentration in plant roots. However, the concentration varies with plant species. Flood-intolerant species accumulate more ethanol than flood-tolerant species (Crawford and Baines, 1971; Crawford, 1982). Flood-tolerant birdsfoot trefoil (*Lotus corniculatus*) synthesised less ethanol than flood-intolerant alfalfa in a field experiment (Bartá, 1984).

Flood-tolerant species avoid the accelaration of glycolysis. These species switch from ethanol production to malate production (Crawford, 1978). The amounts of ethanol and malate in root exudates may be used as indicators of the capability of a plant to tolerate flooding.

Effects of flooding on alfalfa

Flooding for ten days or longer produces injury symptoms in alfalfa (Frosheiser and Barnes, 1973a). Most alfalfa crops in the northern alfalfa region of U.S.A. experience water saturated soil conditions for periods of ten days or longer during early spring, just after the snow has thawed. Over-irrigation and continuous rain cause waterlogged conditions during the summer and fall. Spring flooding does not, however, reduce yields of first-cut alfalfa (Lueschen et al., 1975). Root injury due to flooding manifests itself as moisture stress symptoms when soil moisture falls during the dry periods of the summer and fall. Alfalfa cultivars susceptible to flooding and Phytophthora root rot (PRR) show more severe drought symptoms and greater reductions in yield after the first harvest (Wahab and Chamblee, 1972).

Root injury is greater during the hot summer months than during the cool spring (Erwin et al., 1959). This is in agreement with the findings of Pulli and Tesar (1975) who found that late-seeded (May 19) alfalfa showed more PRR damage than a crop seeded earlier (April 27) with 50 mm irrigation on July 28 and August 15. The water does not have to pond on the soil surface to produce injury (Erwin, 1966).

The effects of a single period of flooding can still be evident in subsequent seasons. Flooding and PRR reduce the crop stand. This is more evident during the second summer of newly established alfalfa (Lehman et al., 1968). The irrigation of alfalfa to maintain soil moisture at a level above 50% of the available moisture holding capacity of the soil was beneficial during the first year, but during the second year, yields and stand counts declined, and the percentage of weeds increased (Wahab and Chamblee, 1972).

The amount and distribution of soil moisture affect root biomass and root distribution of crop plants. Mayaki et al., (1976) observed that 67% of the total root biomass of irrigated soybean was within 15 cm of the soil surface.

Severe water stress causes poor root development, but limited water stress coupled with low evapo-transpirational demand produced greater root growth in alfalfa than with irrigation (Jodari-Karimi et al., 1983). Extreme moisture regimes, therefore, restrict root development.

The growth of a strong root system during the year of seeding is essential for the persistence of an alfalfa crop. Flooding and disease cause permanent damage to the roots of young alfalfa plants. Alfalfa plants have a low capacity to recover from the effects of flooding during the year of seeding (Mittra and Stickler, 1961). Severe PRR results in the deterioration of the lower part of the tap root (Erwin, 1954). Generally, this occurs below 20 to 40 mm below the top of the root.

Alfalfa plants recover from flooding and PRR injury by producing adventitious roots above the point of injury. These adventitious roots are usually too shallow to supply enough water for optimum growth even during mild moisture stress periods. The tap root of alfalfa is a major food storage organ and once it is severed, the plant cannot withstand the rigors of periodic harvesting and grazing. Plants with damaged tap roots do not persist in pasture (Mittra and Stickler, 1961).

Resistance to PRR is a key factor to the persistence of alfalfa growing in a periodically flooded field. In two growing seasons, cultivars resistant to PRR and susceptible cultivars lost 50.5% and 60.8% of their original populations, respectively, (Bohl and Gray, 1983). The average plant population of resistant cultivars was 160% of susceptible cultivars. The resistant cultivars had a 36.8% higher yield during the year of seeding.

The Phytophthora root rot pathogen

Phytophthora root rot is caused by a fungus called *Phytophthora megasperma* f.s. *medicaginis* Drechs. (Erwin, 1954; Erwin, 1965; Kuan and Erwin, 1980a). The genus *Phytophthora* belongs to the class *Oomycetes*. The *Oomycetes* are found in most soils but thrive under waterlogged conditions. *P. megasperma* f.s. *medicaginis* always attacks alfalfa grown under flooded conditions if the organism is present.

Phytophthora root rot is more severe on a young alfalfa crop than on old stands (Leuchen et al., 1976). The root symptoms of *Phytophthora* root rot are: dull yellow to brown necrotic lesions on the tap roots and lateral roots; lesions that girdle the roots and kill the roots below the infection when the disease is in advanced stages; and fibrous roots produced above the girdled portion of the root (Erwin, 1954).

The predisposition of alfalfa and other plants to Phytophthora root rot by flooding

Flooding predisposes alfalfa to PRR. Soil saturation with water prior to inoculation with minced *Phytophthora megasperma* f.s. *medicaginis* mycelia produced more severe disease symptoms, lower yields and higher seedling mortality in alfalfa than no saturation prior to inoculation (Kuan and

Erwin, 1980b). The longer the period of saturation prior to inoculation, the more serious the effects of Phytophthora megasperma are.

The roots of plants grown in flooded soils prior to inoculation attracted more P. megasperma zoospores than roots of plants grown in unsaturated soils (Zentmyer, 1961; Allen, 1974; Kuan and Erwin, 1980b). The zoospores are attracted mostly to the junctions of the tap roots and the lateral roots.

Phytophthora zoospores are attracted to root exudates and to root extracts (Zentmyer, 1961). This suggests that the roots of plants grown under flooded conditions release some chemicals into the soil. Kuan and Erwin (1980b) observed that the exudates of alfalfa roots grown in saturated soils contained more amino acids, sugars and alcohol than the roots of plants grown under unsaturated conditions.

Ethanol accumulation in the cells of roots under anaerobic conditions disrupts the integrity of the cells. Ethanol and other aliphatic alcohols decreased the permeability of cell membranes to water (Kiyosawa, 1975). The resistance of membranes to water flow increased linearly with increasing alcohol concentration. Ethanol accumulation leads to membrane destruction by lipid solubilization (Kiyosawa, 1975; Crawfod, 1978) and the inactivation of mitochondrial enzyme activity and the increase of ethanol production.

Scanning electron microscopy has revealed that alfalfa roots from saturated soils developed fissures to which

Phytophthora megasperma zoospores are attracted (Kuan and Erwin, 1980b). Cell contents leak from the roots through the fissures. The zoospores are attracted by the ethanol (Allen and Newhook, 1973), moving up the concentration gradient. The exudates of soluble sugars facilitates the growth of soil pathogens which invade the roots (Crawford, 1978).

Flooding, therefore, trigers a chain reaction which starts with the acceleration of glycolysis and the accumulation of ethanol. The ethanol causes the disruption of cell membranes and consequently, cell contents leak from the root cells. The exudates escaping from the roots attracts soil pathogens which invade the root cells.

Phytophthora root rot resistance in alfalfa

The susceptibility of alfalfa to PRR is determined by a tetrasomic gene with incomplete dominance (Lu et al., 1973). Plants with a nulliplex condition exhibit high resistance to PRR. The simplex condition produced moderate resistance. Susceptibility increased progressively in the duplex, triplex, and quadruplex conditions. Alfalfa should have over 40 percent resistant plants if it is to survive injury and killing from PRR (Barnes, D. K., personal communication to Tesar, 1985).

There is no unanimous agreement in the literature as to how PRR resistance is exhibited in alfalfa. Marks and Mitchell (1971) observed that the penetration of epidermal cells of susceptible cutivars by *P. megasperma* f.s.

medicaginis hyphae was very rapid (less than 24 hours) while in resistant cultivars the hyphae failed to penetrate the cells and in some cases ended up growing along the cell walls. This suggests that resistance was expressed through the exclusion of the pathogen from the host cells.

The findings of Marks and Mitchell (1971) are not in full agreement with the observation of Miller and Maxwell (1984). Miller and Maxwell (1984) observed fungal hyphae in the epidermal and the outermost cortical root cell layers two hours after inoculation in both resistant and susceptible cultivars. Twelve hours after inoculation, there were intercellular and intracellular hyphae of P. megasperma f.s. medicaginis in the epidermis, cortex and the central stele of susceptible roots while in resistant alfalfa, the hyphae were usually intercellular and were restricted to the root epidermis and the outer half of the cortex. Root cells in contact with fungae hyphae were plasmolysed, especially in the resistant cultivars. The reaction suggests that the defense against the disease is through hypersensitivity of the infected cells.

MATERIALS AND METHODS

Cultivars

Four cultivars, Iroquois, Oneida, Vernal, and Saranac and two selections from Iroquois designated Michigan 80-16 (MI 80-16) and Michigan 80-16P (MI-80-16P), were used in the study. A brief description of each of the cultivars is given below.

Iroquois alfalfa was developed by the Department of Plant Breeding, New York State College of Agriculture and the Cornell University Agricultural Experiment Station, Cornell University (Murphy and Lowe, 1968). The recurrent parent in the breeding program was Narragansett. Iroquois is similar to Narragansett except that it is resistant to bacterial wilt. Varietal trials in various states including Michigan, New York and Wisconsin (personal communication, Tesar, 1985) have shown greater tolerance of Narragansett in trials between 1950 and 1970 and its progeny Iroquois between 1968 and 1980 than other cultivars to somewhat imperfectly drained soils. The resistance of Narragansett and of Iroquois to injury in somewhat imperfectly drained soils is due to the breeding involved in the development of Narragansett (Graber, 1953).

Narragansett was developed at Rhode Island Agricultural Experiment Station from hybrids of Don alfalfa between 1932 and 1946. Natural selection played an important role in its development when heavy winds and salt spray from a hurricane killed all the shoots. The plants which recovered and survived the subsequent winter were crossed, selfed, selected, and composited to produce Narragansett breeder's seed. The natural selection for salt tolerance imposed by the hurricane might have been responsible for the tolerance of Narragansett and its progeny Iroquois to somewhat poorly-drained soils.

Iroquois was the parent of MI 80-16. Twenty plants (Fig. 1) were selected for superior root branching from a ten-year old stand of Iroquois which had been subjected to occasional flooding in the periods of February-April, 1969-1979, even though the field was tiled with lines 15 m apart in 1955 (Tesar, 1970-1979). Originally, an 8,000 plant stand was established in 1969, and of these, only 400 plants remained in 1980. Breeder's seed of MI 80-16 was produced from the 20 plants in cages with pollination by bees in California. Iroquois and MI 80-16 have similar shoot and growth characteristics, bacterial wilt resistance (61%), Phytophthora root rot (PRR) resistance (1%), and Fusarium wilt resistance (22%) as determined in the official USDA disease resistance trials (Agricultural Experiment Station-University of Minnesota). Item Number AD-Mr-1953 (Formerly Minnesota Report 24). MI 80-16 has a more branching root system than Iroquois according to Nishikawa and Suzuki (1982).

Figure 1. The 20 ten-year old Iroquois plants selected for superior root branching and used for producing MI 80-16.

MI 80-16P was selected from MI 80-16 for PRR resistance. Ninety plants free from PRR were selected after flooding flats of about 1,000 two-week old seedlings of MI 80-16 for three weeks in the greenhouse in the winter of 1982. The soil was naturally infested with _Phytophthora megasperma_ f.s. _medicaginis_, and additional inoculant was applied to the flats. The selected plants were planted in pots and pollinated by honey bees in the greenhouse during the winter of 1983. The seed produced, as determined in the Minnesota PRR field test in 1983, had about 17% PRR resistance. A further selection for increased resistance to PRR was conducted in the fall of 1983 and by selecting 50 plants from a spring seeding of MI 80-16P. The plants were established in pots and hand pollinated in the greenhouse during the winter of 1984 to produce seed for MI 80-16P GH4.

Oneida was released by the New York Agricultural Experiment Station in the late 1970's. It is highly resistant to bacterial wilt (62%) and PRR (52%). Oneida was used in the study as a measure of progress in the selection for resistance to PRR in MI 80-16P.

Vernal alfalfa was developed at the University of Wisconsin and registered in 1956 (Garber, 1956). It is one of the oldest cultivars grown in the region. It is winter-hardy and resistant to bacterial wilt (42%) and _Fusarium_ wilt (32%). Vernal is susceptible to anthracnose and PRR

(5%) as determined in the Minnesota disease trials.

Saranac was developed at Cornell University and registered in 1966 (Murphy and Lowe, 1966). It was developed from the "Flamande-type" varieties. It is moderately winter-hardy, bacterial wilt (49%), and _Fusarium_ wilt (34%) resistant. It is susceptible to PRR (3%).

Precipitation and Sprinkler Irrrigation

Yearly precipitation and the amounts of water added with sprinkler irrigation are presented in the addenda for the experiments described below.

Experiment 1: East Lansing, Capac loam soil, effects of flooding on alfalfa stand, vigor and yield 1983-1984.

This experiment was established to study the response of the four cultivars and two selections growing in a field naturally infected with P. megasperma f.s. medicaginis to flooding. The experiment was a split-plot in a randomized complete block design in four replications. The flooded and the well-drained treatments were the main plots and the cultivars and selections were the subplots. The soil type was a Capac loam characterised by fine texture and moderately poor drainage. The flooded half of the experiment was located in a poorly drained section of the field because the tile drains are broken and not effective.

The experiment was established on June 10, 1983 in plots 0.91 x 7.62 m (3 x 25 ft). A five-row Carter precision drill with rows spaced 15 cm (6 inches) apart was used. The seed rate was 12 kg ha^{-1}. The seed was inoculated with appropriate Rhizobia before planting.

The alfalfa was fertilized according to recommendations based on soil tests and sprayed with appropriate insecticides and herbicides to control insects and weeds.

On June 11, 1983, one day after planting, about 25mm of irrigation water were applied to ensure the even germination of the crop. The plots were not irrigated for the rest of the 1983 growing season. A significant date during the 1983 season was June 28 when about 83 mm (3.3 inches) of rain fell during a rain storm (Appendix 1) when the poorly drained half of the experiment was flooded for four days following the storm.

The plots experienced normal spring rainfall during the spring of 1984. On July 10, two weeks after the first harvest of 1984, the flooding treatment was imposed on the more poorly-drained half of the experiment, so that soil was saturated at all times. The irrigation sprinklers were left on for periods ranging from four to eight hours as the need arose to keep the soil saturated. An average of 62 mm (2.4 inches) of water was applied daily for 14 days until July 24 (Appendices 2 and 3). The plots were allowed to drain and dry for 21 days before the ground was firm enough to support harvesting machinery.

A second irrigation regime was initiated two weeks after the second harvesting. An average of 48 mm of irrigation water was applied daily for nine days from August 31 to September 8. The plots were allowed to drain and dry for 23 days before harvesting.

Following the imposed flooding treatments, the alfalfa showed varying degrees of stunting and chlorosis (Fig. 2). The condition of the plants improved with time after flooding was terminated. A shoot color ind x, ranging from 1 to 10, was used to evaluate the condition of the crop following flooding. The most chlorotic and stunted plots were rated 1, while those showing normal green, lush growth were rated 10. The plots were evaluated on July 24 and 27

Figure 2. Injury to alfalfa after 14 days of flooding. The cultivars are (L to R) MI 80-16, Oneida, Saranac, and MI 80-16P.

and August 6, 1984, i.e. 1, 4, and 14 days after irrigation was terminated, respectively. The shoot color index was used as a measure of crop recovery following flooding.

The well-drained half of the experiment received about 75 mm of irrigation water at the beginning of each flooding regime. Additional water was supplied only if there were symptoms of moisture stress.

Eighteen days after heavy rain on June 28, the plant population of the plots was evaluated by counting the plants within 0.91 m x 0.915 m (3 ft x 3 ft) quadrats chosen at random from the 7.625 m (25 ft) plots. The results are expressed as plants per square meter.

On July 22, 1983, twenty-two days after the heavy rain, the plots were rated for vigor. A visual assessment of the plots, based on plant height, completeness of stand, and the degree of chlorosis of the plants was used as a measure of plot vigor. Each plot was rated on a scale ranging from 1 to 10.

Experiment 2: East Lansing, Capac loam soil, root branching characteristics, 1983.

Experiment 2 was established adjacent to Experiment 1 in a randomized complete block design in four replications. The date of planting, soil fertility, weed and insect control programs were the same as in Experiment 1, but the rate of seeding was 2.8 kg ha^{-1} (2.5 lb/acre), one fifth of the rate Experiment 1. The low rate was used for a lower plant population to facilitate root sampling and better root development of individual roots.

Root samples were taken on August 24 with a hydraulic root sampler. Isolated plants were selected. One root was sampled in a soil core 10 cm in diameter to a depth of 45 cm. Five roots were sampled from each plot. The cores were wrapped in burlap bags to prevent them from crumbling and stored at a temperature of $5^{\circ}C$.

The soil cores were soaked in a saturated solution of sodium hexametaphosphate overnight to disperse the soil aggregates from around the roots and facilitate root washing. The roots were washed in a manner that ensured very little loss of fibrous roots. The roots were stored at $5^{\circ}C$ and then evaluated for fibrous root density and the number of side roots. The fibrous root density was evaluated on a visual scale ranging from 1 to 10 (high). The number of well-developed secondary roots in the top 20 cm of the tap root was counted.

Experiment 3: East Lansing, Plant Science Greenhouse, root characteristics, 1983.

The objective of this experiment was to study the root characteristics of the four cultivars and two selections in the greenhouse. The design of this experiment was a randomized complete block design replicated five times. There were two plants in each treatment.

The plants were grown in the growth medium-Metromix 350 (Appendix 5) in perforated plastic pots 20 cm deep and 15 cm diameter. Five inoculated seeds were planted per pot on December 12. Two weeks after germination on December 15, the alfalfa was thinned to one vigorous plant per pot.

The plants were watered at planting and once every other day after germination until it drained through the holes at the bottom of the pots. Nutrient solution (Appendix 6) was added every week and appropriate insect control was maintained.

On March 7, 1984, when the plants were three months old, the tops were cut and removed at crown level and stored. The roots were removed and washed as in Experiment 2. The lengths of the tap roots were measured and the number of side roots were severed from the tap roots with a razor blade and weighed.

Experiment 4: East Lansing, Plant Science greenhouse, root characteristics, 1984-1985.

The objective of this experiment was to study the degree of root branching of the alfalfa cultivars and selections. The experiment was a randomized complete block design in ten replications. The alfalfa was grown in Super 45 rootrainers measuring 51 x 64 x 229 mm in the medium Metromix 350.

Five inoculated seeds were planted in each compartment on September 22, 1984. The alfalfa was thinned to one plant per compartment on October 10, sixteen days after germination.

Watering, fertilization, and pest control were the same as in experiment 3.

The tops were removed, and the roots were washed on January 5, 1985 when they were three months old. The degree of root branching was rated on a scale of 1 to 5: no lateral roots = 1; 1 to 3 lateral roots = 2; 4 to 7 lateral roots originating further than 3 cm from the crown = 3; 4 to 7 lateral roots originating closer than 3 cm to the crown = 4; and 8 lateral roots or more = 5. The distance in mm of the first lateral root from the crown was also measured.

Experiment 5: East Lansing, Plant Science greenhouse, resistance of alfalfa to PRR, 1985.

The objective of this experiment was to determine the level of resistance of the four cultivars and two selections to *Phytophthora megasperma* f.s. *medicaginis*.

Five inoculated seeds were planted in Metromix 350 in Ferinard rootrainers on January 15, 1985. Six trays, each with 96 cavities, 20 mm x 20 mm and 100 mm deep were used. The experiment was a completely randomized design with 24 cavities allocated to a cultivar per replication.

The plants were watered at planting and four times after germination on January 18. The plants were counted on January 29, 11 days after germination. The trays were flooded with water upto crown level for five days starting on February 1, drained, and then inoculated with a culture of *Phytophthora megasperma* f.s. *medicaginis*.

The culture P410, obtained from the Department of Botany and Plant Pathology was plated on V8 agar plates on January 16. The plates were incubated in a growth chamber at 30°C for 21 days. Eight culture plates were blended in 450 ml of water in a Waring blender set at low for 30 seconds. The agar and fungal mycelia were cut into small pieces. About 100 ml of the suspension was diluted to one liter and used to inoculate one replication. The growth medium was soaked four times with the suspension of mycelia. The plants were evaluated two weeks later on February 19.

The healthy resistant plants were separated from the diseased plants which had roots with red to brown lesions or rotted roots, and chlorotic or stunted tops (Fig. 3).

Experiment 6: Plant Science greenhouse, ethanol production on flooding, 1985.

The objective of this experiment was to determine the level of ethanol production as an indication of plant injury in roots of flooded plants of the four cultivars and two selections. The experimental design was a split plot in randomized blocks in five replications. The flooded and well-drained treatments were the main plots and the cultivars and selections the subplots.

The growth medium was a steam-sterilized sandy soil with a pH of 6.7 in galvanized steel stove pipes, 15 cm in diameter and 60 cm tall, and open at both ends. These pipes were placed in larger stove pipes, 20 cm in diameter and 60 cm tall and closed at the base. With this double

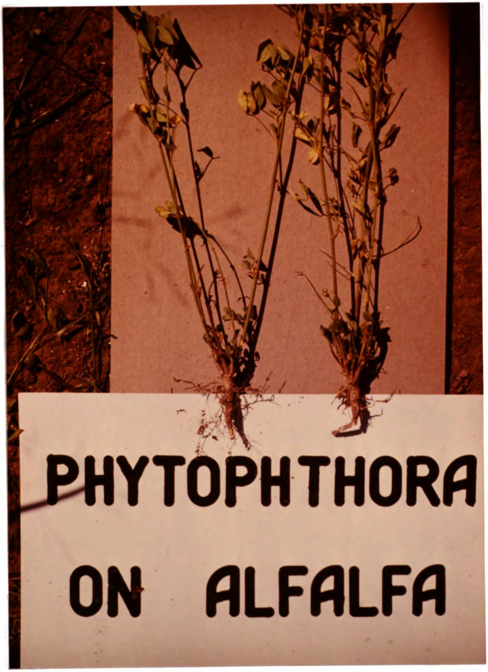

Figure 3. <u>Phytophthora</u> root rot injury in alfalfa

tube arrangements, the water table could be regulated by adding water to or removing water from the space between the two tubes.

The growth medium was watered well just before planting. Five inoculated seeds were planted 5 mm deep at the center of each pot; plants were thinned to one plant per pot three weeks after emergence. The plants were watered every other day starting at the first-trifoliate-leaf stage. Water accumulating in the outer pipe was removed using a suction pump. About 125 ml of nutrient solution was supplied to the plants every week.

The flooding treatment was imposed when the plants were three months old and flowering. Water was added to the outer stove pipe so that it infiltrated through the bottom of the inner stove pipe expelling all the air from the growth medium. The water table was maintained at a level of 1.5 cm above the soil surface for three days.

After flooding for three days, the root xylem exudate was collected from all the plants using transluscent latex tubing 5 cm long with internal diameters of 1.59, 3.18, and 4.76 mm to match stems of different thickness. The tops of plants were cut at crown level. Latex tubing of appropriate internal diameter was fitted over the root stumps so that they formed a water-tight joint. The root exudates were collected in the latex tubes after about one to two hours. The root stubs were cut just below the soil level such that they formed the bottom plugs of the tubes, the root exudates.

The latex tube containing the exudates were put in sterile vacutainer tubes and immediately frozen in dry ice.

The ethanol content of the root xylem exudates was determined by gas liquid chromatography. Two microliter samples of the exudates were injected into a Porapak Q column. The samples were analysed at an oven temperature of 150°C with gas flow rate of N_2 = 40 cc/min, H_2 = 50 cc/min, and air = 250 cc/min. The ethanol content of the xylem exudates was read from an intergrator print-out expressed in parts per million.

Experiment 7: East Lansing, Capac loam soil, ethanol production on flooding, 1984.

The objective of this experiment was to determine the level of ethanol production and _Phytophthora_ root rot (PRR) severity of cultivars and selections following flooding in the field. The experiment was a split plot in randomized complete block design in four replications. The treatments are as in Experiment 6.

Land preparation, soil fertilization, pest and weed control practices were similar to those used in Experiment 1. Inoculated alfalfa was planted at a rate of 5.6 kg ha^{-1} in two rows 30 cm apart in plots 0.60 x 7.63 m in size on August 1.

The plants were flooded daily for four days starting on October 1 when they were two months old. On the sixth day, the plants were sampled for ethanol. The sampling method used was the same as the one used in Experiment 6.

After two more weeks of flooding, 75 plants were dug from each plot, the roots washed, and then evaluated for PRR damage. Longitudinal sections of the roots were made using a razor blade, and the roots were scored for disease severity. Roots were scored from 1 to 5 as follows: healthy roots = 1; slight yellowing in the central cylinder = 2; a deep yellow central cylinder with slight lesions on root surface = 3; yellow central cylinder and part of the root rotted = 4; dead root = 5.

Statistical Procedure

The data on plot vigor, plant population, crop recovery, alfalfa yields, ethanol production in the roots, and PRR severity were analysed by factorial analysis of variance. The degree of flooding (main plots) and the cultivars and selections (subplots) were the factors. The analysis of variance for randomized complete blocks was used on the root characteristics data. Unless otherwise stated, the 5% level of significance was used in all the comparisons.

RESULTS AND DISCUSSION

Experiment 1. Field, Capac loam soil, flooding effect on stand, vigor, and yield.

Vigor

Flooding, due to a single rain storm of 83 mm on June 28, 1983, reduced alfalfa vigor under field conditions (Table 1). It was more severe in the poorly drained plots where the soil was saturated for up to four days. The well-drained plots did not show any significant varietal differences except for MI 80-16 which showed less vigor

Table 1. Plot vigor of alfalfa after flooding on a scale ranging from 1(low) to 10(high). Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in vigor#
	vigor		%
Oneida	8.67ab	6.67a	23
MI 80-16P	9.67ab	6.67a	31
MI 80-16	8.33b	5.00b	40
Vernal	10.00a	4.67b	53
Saranac	10.00a	4.67b	53
Iroquois	9.67ab	4.00b	59

LSD 0.1.35
CV% (between well-drained and flooded) = 6.1
CV% (among cultivars) = 7.9
Reduction in vigor from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

than Vernal and Saranac. MI 80-16P and Oneida were equal in vigor and significantly greater than the rest under flooded conditions. MI 80-16 was third in vigor although its superiority over Iroquois, Saranac, and Vernal was not statistically significant.

The degree to which flooding affected the cultivars and the two selections varied. MI 80-16, which was selected for tolerance to wet conditions had a 40% reduction in vigor. MI 80-16P, with 17% PRR resistance, had a 31% reduction in vigor. Oneida, with high (52%) PRR resistance had a reduction of only 23%. The three cultivars bred for well-drained conditions had higher vigor reductions: Iroquois-59, Vernal-53, and Saranac-53%.

Alfalfa cultivars with tolerance to wet conditions were more vigorous than cultivars bred for well-drained conditions. Resistance to PRR is particularly necessary in the first two months of seedling growth when the seedlings are particularly susceptible (Leuchen et al., 1976). Resistance to imperfectly drained conditions with PRR-susceptible Iroquois in a six-year Michigan variety trial was most likely responsible for the excellent performance of the cultivar (Tesar, 1981). Heavy rains do not induce PRR sysmptoms in alfalfa seedlings as long as the soil is well-drained and excess water rapidly drains out of the root zone, particularly on the surface (Frosheiser and Barnes, 1973a).

Plant population

Well-drained plots had higher seedling populations than poorly drained plots (P=0.10) (Table 2). Well-drained plots did not show any varietal differences (P=0.05) except for MI 80-16 which had a low number of 186 plants m^{-2}.

Table 2. Plant population following flooding. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in stand#
	—— plants per m^{-2} ——		%
Oneida	249ab	236a	5
MI 80-16P	260ab	189ab	27
MI 80-16	186b	136b	27
Saranac	304a	152b	50
Iroquois	302a	149b	51
Vernal	304a	137b	55

LASD 0.05 = 75
CV% (between well-drained and flooded) = 14.8
CV% (among cultivars) = 17.3
Reduction in stand from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

Under flooded conditions, Oneida had a significantly higher plant population (236) than all cultivars except MI 80-16P. Iroquois, Vernal, and Saranac had populations of 50% or more. The selections from Iroquois had reductions of 27% each. Oneida, because of high resistance to PRR, had only a 5% reduction. Bohl and Gray (1983), in a similar experiment found 50.5 and 60.8% losses in plant population in PRR-resistant and susceptible cultivars, respectively, after one year.

Forage yield

Seeding-year harvest, 1983

The alfalfa was harvested once in the year of seeding. For the sole 1983 harvest, well-drained plots had significantly higher yields than poorly drained plots (P=0.03) (Table 3). Under well-drained conditions,

Table 3. Forage yield of alfalfa for the first cut on August 18, 1983. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in yield#
	——— t ha^{-1} ———		%
MI 80-16P	1.61bc	0.96a	41
Oneida	1.53c	0.95a	38
MI 80-16	1.54c	0.68b	56
Vernal	1.89a	0.82ab	57
Saranac	1.84a	0.70b	62
Iroquois	1.79ab	0.62b	65

LSD 0.10 = 0.22
CV% (between well-drained and flooded) = 15.2
CV% (among cultivars) = 10.0
Reduction in yield from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.10).

Iroquois, Vernal, and Saranac yielded significantly more than MI 80-16, MI 80-16P, and Oneida. This was to be expected since these three cultivars were developed primarily for well-drained conditions.

Under poorly-drained conditions, MI 80-16P and Oneida had significantly greater yields than the rest. The seeding-year harvests were highly correlated to plant population (r=0.77) and plot vigor (r=0.93). The cultivars resistant to PRR had an average yield reduction of 39.5% while cultivars with low resistance to PRR had 60% yield reduction.

First full harvest year, 1984

Natural flooding during the spring of 1984 did not produce significant differences in yields between the well-drained and the poorly-drained plots (Table 4) in the first cutting. This agrees with the findings of Lueschen, et al., 1975 who reported that spring flooding does not reduce the yield of first-cut alfalfa. There were no significant differences between the yields of the cultivars and selections under well-drained conditions.

Under poorly-drained conditions, Oneida had higher yields than all the rest of the cultivars except MI 80-16P and MI 80-16. Iroquois, MI 80-16, Saranac, and Vernal had 7, 7, 11, and 14% reductions in yield while Oneida had a 5% increase and MI 80-16P no increase in yield. There was no correlation (r=0.05) between plant population and yield suggesting compensatory growth in the plants that survive

Table 4. Forage yield of alfalfa for cut 1 on June 25, 1984, under conditions of natural spring flooding. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in yield#
	t ha^{-2}		%
Oneida	7.47a	7.85a	+5.0
MI 80-16P	7.41a	7.42ab	0.0
MI 80-16	7.84a	7.39ab	7.0
Saranac	7.91a	6.83bc	14.0
Iroquois	7.21a	6.80bc	7.0
Vernal	7.28a	6.50c	11.0

LSD 0.05 = 0.72
CV% (between well-drained and flooded) = 1.9
CV% (among cultivars) = 5.1
Reduction in yield from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

and the recovery of the roots from flooding and PRR injury as suggested by Erwin (1954). Poorly-drained plots retained moisture longer than the well-drained plots. This, together with less root damage and better root recovery, could have accounted for the yield increase exhibited by Oneida.

Flooding during July reduced yields (P=0.002) of the second cutting on August 14 (Table 5). In the well-drained plots, only Iroquois and Vernal showed significant differences.

Table 5. Forage yield of alfalfa for the second cut on August 14, 1984. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in yield#
	t ha^{-1}		%
Oneida	4.50ab	3.30a	27
MI 80-16P	4.59ab	2.72b	41
MI 80-16	4.71ab	2.39bc	49
Vernal	4.94a	2.02cd	59
Iroquois	4.41b	1.87d	58
Saranac	4.75ab	1.82d	62

LSD 0.05 = 0.49
CV% (between well-drained and flooded) = 3.7
CV% (among cultivars) = 6.9
Reduction in yield from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

Under flooded conditions, Oneida was significantly higher in yield than MI 80-16P and MI 80-16, which, in turn, were significantly higher than Vernal, Iroquois and Saranac. Flooding-tolerant and PRR-resistant MI 80-16P and Oneida had an average of 34% reduction in yield; flooding-intolerant and PRR-susceptible Iroquois, Vernal, and Saranac had an average of 60% reduction. The effects of flooding on alfalfa were more severe during the summer than during the spring in agreement with other research (Erwin, et al., 1959; Pulli and Tesar, 1975; Lehman, et al.,1968).

The third forage harvest on October 2 also showed significant differences (P=0.01) between the two main water treatments (Table 6). The well-drained plots showed no varietal differences. Under flooded conditions, Oneida had a significantly higher yield than all the cultivars except MI 80-16P which was significantly higher in yield than Vernal, Saranac, and Iroquois but not higher in yield than MI 80-16. Oneida and MI 80-16P had an average yield reduction of 45%; MI 80-16 was 63%; and Iroquois, Saranac, and Vernal had an average 73% yield reduction.

Table 6. Forage yield of alfalfa for the third cut on October 2, 1984. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in yield
	—— t ha^{-1} ——		%
Oneida	2.94a	1.78a	39
MI 80-16P	2.99a	1.49ab	50
MI 80-16	3.19a	1.18bc	63
Vernal	2.67a	0.91cd	66
Iroquois	2.82a	0.69d	76
Saranac	2.95a	0.70d	76

LSD 0.05 = 0.52
CV% (between well-drained and flooded) = 10.0
CV% (among cultivars) = 12.6
Reduction in yield from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

Total forage yield for 1984 (Table 7) and the total two-year yield for 1983 and 1984 (Table 8) showed the superiority of PRR-resistant and flood-tolerant cultivars under flooded conditions. In the two seasons, cultivars resistant to PRR had 25% higher yield than cultivars susceptible to PRR. MI 80-16, which is flood-tolerant, had a 15% higher yield than flood-intolerant Iroquois, Saranac, and Vernal.

Table 7. Forage yield of alfalfa for 1984 in the year after seeding. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in yield#
	t ha^{-1}		%
Oneida	15.23abc	12.93a	15
MI 80-16P	14.98abc	11.62b	22
MI 80-16	15.75a	10.96b	30
Vernal	14.82abc	9.43c	37
Iroquois	14.44c	9.36c	35
Saranac	15.60ab	9.35c	40

LSD 0.05 = 1.08
CV% (between well-drained and flooded) = 1.3
CV% (among cultivars) = 4.4
Reduction in yield from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

Table 8. Total forage yield of alfalfa for 1983 and 1984 combined. Means of three replications.

Cultivar or selection	Well-drained	Flooded	Reduction in yield#
	t ha^{-1}		%
Oneida	16.76a	13.88a	17
MI 80-16P	16.60a	12.58ab	24
MI 80-16	17.27a	11.64b	33
Vernal	16.78a	10.25c	39
Saranac	17.44a	10.05c	42
Iroquois	16.23a	9.98c	88

LSD 0.05 = 0.49
CV% (between well-drained and flooded) = 3.7
CV% (among cultivars) = 6.9
Reduction in yield from well-drained.
Means within a column followed by the same letter are not significantly different (P=0.05).

Crop recovery after flooding

The degree of chlorosis 1, 4, and 14 days after flooding was terminated was used as an indicator of injury and plot recovery from flooding injury (Table 9). Oneida, MI 80-16P, and MI 80-16 were significantly less chlorotic than iroquois, Vernal, and Saranac. All cultivars and selections showed signs of recovery four days after irrigation was terminated. Oneida MI 80-16P and MI 80-16 were significantly better than the rest. MI 80-16 did not recover from chlorosis as well as MI 80-16P and Oneida.

Table 9. Degree of chlorosis of alfalfa on 24 and 27 July and 6 August, 1984, following termination of flooding on 24 July on a scale ranging from 1 (very chlorotic) to 10 (normal green). Means of three replications.

Cultivar or selection	Well-drained plots	Days following flooding 1	4	14
Oneida	10.0	6.7a	8.0a	9.0a
MI 80-16P	10.0	6.3a	7.7a	8.0b
MI 80-16	10.0	6.0a	6.3b	7.3b
Vernal	10.0	3.0b	4.7c	6.0c
Iroquois	10.0	3.7b	4.7c	4.3d
Saranac	10.0	3.7b	4.3c	3.7d
LSD 0.05		1.2	0.7	0.9
CV% (well-drained and flooded)		6.4	2.6	4.4
CV% (among cultivars)		8.0	4.5	5.7

Means within a column followed by the same letter are not significantly different (P=0.05).

After 14 days, Oneida, MI 80-16P, MI 80-16 and Vernal were still improving while recovery in the Iroquois and Saranac plots had stopped and plants were deteriorating. The roots of Saranac and Iroquois probably could not absorb enough water to sustain plant growth when the soil started drying up and, consequently, plant stands became thinner following flooding. The degree of chlorosis was highly correlated (r=0.95) (P=0.001) with the yield of the subsequent harvest.

Experiment 2. Field, Capac loam soil, root branching characteristics, 1983.

The average number of secondary roots in the top 200 mm of 20 tap roots in the field ranged from a high of 15.5 (MI 80-16P) to a low of 10.6 (Vernal) (Table 10). MI 80-16P, Oneida, MI 80-16, and Iroquois were not significantly different. It is significant that all of these had a genetic background tracing to Narragansett which was a synthetic cultivar based on clones which survived a hurricane with salt in the water. Oneida

Table 10. The number of branch roots in the top 200 mm of the tap root and the fibrous root score of 20 plants in field on a scale of 1 to 10 (highest).

Cultivar or selections	Number of branch roots	Fibrous root score
MI 80-16P	15.5a	5.38a
Oneida	14.6a	5.25a
MI 80-16	13.1ab	5.65a
Iroquois	13.2ab	5.23a
Saranac	11.ob	5.65a
Vernal	10.6b	5.15a
LSD 0.05	3.4	1.38
CV%	17.2	17.0

Means within a column followed by the same letter are not significantly different (P=0.05).

and MI 80-16P were, however, significantly higher than

Vernal and Saranac. There were no significant differences in the fibrous root scores although MI 80-16, Saranac, and MI 80-16P had the highest scores. This is in agreement with the findings of Nishikawa and Suzuki (1982) that MI 80-16 and Saranac had a more branching root system than Iroquois.

Experiment 3. Greenhouse, root characteristics, 1983.

The cultivars and selections did not show any significant differences in tap root length, total root weight, tap root weight, and weight of branch roots of plants grown in the greenhouse (Table 11). Iroquois had the highest number of branch roots although it was only significantly higher than Vernal.

Experiment 4. Greenhouse, root characteristics, 1984 to 1985.

MI 80-16 and MI 80-16P had lateral roots emerging closer to the soil surface than the other cultivars (Table 12). The differences were not statistically significant, but it is important to note that oxygen can diffuse from the aerial plant parts into flooded roots down to a depth of about 20 mm (Greenwood, 1968). MI 80-16 and MI 80-16P had the largest number of secondary roots, and their first secondary roots were closer to the crown, but these differences were not significantly different from Saranac and Iroquois. It is important to note that the root branching scores follow the same pattern as yields under

Table 11. Root characteristics of alfalfa plants grown in the greenhouse, 1983. Means of ten plants.

Cultivar or selection	Branch roots	Weight: Total root	Weight: Tap root	Weight: Branch roots	Tap root length
	Number	g	g	g	mm
Iroquois	29.0a	8.63a	3.76a	4.37a	402ab
MI 80-16	25.1ab	7.76a	3.14a	5.14a	332b
Oneida	23.2ab	6.35a	2.82a	3.53a	375ab
MI 80-16P	22.8ab	6.91a	3.32a	3.59a	400ab
Saranac	22.6ab	6.70a	3.28a	3.42a	415a
Vernal	21.7b	7.44a	3.46a	3.98a	354ab
LSD 0.05	7.1	2.68	1.12	2.15	76
CV%	21.1	27.9	25.7	39.9	15.2

Means within a column followed by the same letter are not significantly different (P=0.05).

Table 12. Root branching characteristics of plants grown in rootrainers in the greenhouse. Means of three replications.

Cultivar or selection	Distance of first branch root from crown (mm)	Root branching score#
MI 80-16P	13.7a	4.7a
MI 80-16	13.7a	4.7a
Iroquois	20.8a	3.2c
Oneida	20.1a	4.4ab
Vernal	20.2a	4.0abc
Saranac	18.2a	3.7bc
LSD 0.05	7.2	0.9
CV%	11.5	9.7

Means within a column followed by the same letter are not significantly different (P=0.05).
Scoring: 1 is low, 5 is high.

flooded conditions, i.e., a high degree root of branching is associated with high yield, except where a cultivar is highly resistant to PRR (Tables 3, 5, and 6).

Experiment 5. Greenhouse, resistance of alfalfa to PRR, 1985.

The resistance of the cultivars and selections to PRR under flooded conditions in the greenhouse is presented in Table 13. Oneida had the highest percentage resistance, MI 80-16P was second and Iroquois had the least. This is consistent with the resistance ratings of Oneida (52%) and Iroquois (5%) in the official USDA-Minnesota

Table 13. Resistance of an average of 400 alfalfa plants to Phytophthora megasperma f.s. medicaginis under flooded conditions in the greenhouse.

Cultivar or selection	Resistance
	%
Oneida	55
MI 80-16P	22
Vernal	11
Saranac	6
MI 80-16	5
Iroquois	3

disease resistance trials (Barnes and Martin, 1984) and the 17% resistance in MI 80-16P in results reported to Tesar (personal communication from Barnes, Minnesota, fall 1983).

The increase in resistance from 5% in MI 80-16 (Iroquois progeny) to 17% in the progeny MI 80-16P as a result of one generation of selection in the Michigan State University Plant Science Greenhouse in the spring of 1981 is significant. It indicates, further, the efficacy of selection for PRR-resistant plants under flooded conditions of one-month old seedlings in the greenhouse.

Root branching, which was the criterion in the selection of MI 80-16 from Iroquois, (Fig. 1) did not produce an appreciable improvement in the resistance to PRR. This shows that although there appears to be some relationship between root branching and tolerance to flooded conditions, higher root branching is not associated with resistance to PRR.

Experiment 6. Greenhouse, ethanol production in flooded roots, 1985.

Flooding induced the accumulation of ethanol in the roots of all cultivars and selections grown under conditions of flooding in the greenhouse (Table 14). The ethanol concentration of the root exudates increased with time after the onset of flooding. MI 80-16P and MI 80-16 had

Table 14. Ethanol concentration of alfalfa root exudates following one or two days of flooding in the greenhouse. Means of five replications.

Cultivars or selections	Well-drained pots	Days of flooding 1	2
	ppm		
MI 80-16P	0	616a	1459a
MI 80-16	0	953b	1525a
Saranac	0	760ab	1756bc
Oneida	0	915b	1819c
Vernal	0	945b	1920c
Iroquois	0	1272c	1920c
LSD 0.05		210	278
CV% (well-drained and flooded)		16.2	10.9
CV% (among cultivars)		32.3	12.4

Means within a column followed by the same letter are not significantly different (P=0.05).

significantly lower ethanol conenctrations than Iroquois, Vernal, and Oneida after two days of flooding in the greenhouse. There were no significant differences between MI 80-16 and Saranac.

The results in the greenhouse and field showed that the selection of MI 80-16 from Iroquois for tolerance to flooding was, as hypothesised, accompanied by the reduction in ethanol accumulation in flooded roots. Although there were differences between cultivars, there was considerable variation among cultivars. It is likely that a large part of this variation might be attributable to imprecise technique since the CVs were high, especially in the one-day tests. Further determinations on ethanol content would not likely be determined after two or more days of flooding. Based on exploratory research, it was not possible to get any exudate from roots flooded for a period of four days, probably because this period of flooding caused leaf wilting and root deterioration.

Experiment 7. Field, Capac loam soil, ethanol production and PRR severity after flooding, 1984.

Under field conditions, MI 80-16P desirably accumulated less ethanol after flooding than the parent Iroquois (Table 15). Flood-intolerant Saranac accumulated the highest amount of ethanol, significantly higher than the flood-tolerant MI 80-16P. This is in agreement with the findings of Nishikawa and Suzuki (1982) and D. C. Erwin

Table 15. Ethanol concentration of alfalfa root exudates after four days of flooding in the field on October 6, 1984. Means of four replications.

Cultivars or selections	Well-drained	Flooded
	ppm	
MI 80-16P	2	258a
MI 80-16	0	354ab
Vernal	0	362ab
Iroquois	1	370b
Oneida	0	386b
Saranac	0	405b
LSD 0.05		107
CV% (well drained and flooded)		21.6
CV% (among cultivars)		25.5

Means within a column followed by the same letter are not significantly different (P=0.05).

(personal communication to Tesar, 1985) who found that MI 80-16 accumulated less ethanol after flooding than 18 other alfalfa cultivars; only one other cultivar had a low ethanol concentration similar to MI 80-16.

The ethanol concentration was higher in the roots of plants grown in the greenhouse than those sampled from the field (TAbles 14 and 15). This is probably due to higher temperatures within the greenhouse (25-30°C) than in the field (5-17°C).

Oneida, MI 80-16P, and MI 80-16 had the lowest PRR score, i.e., the highest resistance to PRR of the alfalfa entries under flooded conditions (Table 16). Iroquois, Vernal, and Saranac exhibited significantly higher susceptibility. These results once again stress that root injury is lowest when a cultivar has both tolerance to flooding and resistance to PRR.

Table 16. PRR# of alfalfa plants flooded in the field. Means of 25 plants replicated four times.

Cultivar or selection	Well-drained plots	Flooded plots
Oneida	1.0	2.0a
MI 80-16P	1.0	2.1ab
MI 80-16	1.0	2.2b
Iroquois	1.0	2.4c
Vernal	1.0	2.4c
Saranac	1.0	2.5c

LSD 0.05 = 0.11
CV% (between well-drained and flooded = 2.5
CV% (among cultivars) = 6.4
Scoring: 1 = healthy; 5 = severely diseased
Means within a column followed by the same letter are not significantly different (P=0.05).

If the greater root branching characteristic and low ethanol content of MI 80-16P are to be reflected in a commercial cultivar likely to be more resistant than present cultivars to somewhat imperfectly drained soils, greater resistance than the present 17% will be required. It is likely that a resistance of over 30% (personal communication of Tesar with D. K. Barnes, 1985) will be necessary.

SUMMARY AND CONCLUSIONS

1. Flooding in the field reduced the vigor of seeding-year alfalfa by 27% in cultivars resistant to PRR, 40% in those tolerant to flooding, and 52% in those adapted to well-drained soils.
2. Flooding in the field for four days reduced the population of three-week-old alfalfa by 16% in cultivars resistant to PRR, 27% in those tolerant to flooding, and 52% in those adapated to well-drained soils.
3. There was a high correlation between the yield of seeding-year alfalfa and vigor (r=0.93) and plant population (r=0.77) (P=0.001).
4. The effects of flooding injury in the year of establishment were not reflected in the yields of the first harvest in the second year.
5. Alfalfa yield losses were more pronounced in the seeding-year than in the first full harvest year. Seeding-year yield reductions were 40% for PRR-resistant, 56% for flood-tolerant, and 61% for flood-intolerant cultivars. The corresponding yield losses for the second year were 20, 23, and 40%, respectively.
6. Flooding during the summer and fall injured alfalfa more than spring flooding.

7. PRR-resistant selections recovered more rapidly than flood-tolerant and flood-intolerant cultivars after flooding was terminated. Flood-intolerant cultivars started wilting even when the soils were still moist.
8. MI 80-16P and MI 80-16 showed more root branching than the other cultivars or selections. Total root weights were not different.
9. Flooding induced less undesirable ethanol accumulation in MI 80-16 and MI 80-16P, selected to be tolerant, than in flood-intolerant cultivars.

APPENDICES

APPENDIX 1

MSU Crops Field Laboratory 1983 Rainfall (mm).

Date	April	May	June	July	Aug.	Sept.	Oct.	Nov.
1	-	10.4	0.5	12.1	0.5	-	-	-
2	-	29.5	-	-	-	-	-	2.0
3	-	2.8	-	-	-	-	-	9.1
4	20.1	0.5	16.5	-	-	-	-	-
5	1.0	-	0.5	-	-	-	9.1	-
6	3.3	-	5.6	-	-	22.1	-	-
7	7.9	1.5	-	-	-	-	-	-
8	-	23.9	-	-	-	-	-	-
9	-	-	-	-	-	-	8.9	-
10	10.2	-	0.5	-	-	-	-	-
11	0.5	-	-	-	25.4	5.1	-	7.1
12	-	-	-	-	3.1	-	-	-
13	5.1	-	-	-	-	-	17.0	-
14	22.4	-	-	-	-	-	15.2	-
15	2.8	2.0	-	-	-	-	-	-
16	-	-	4.6	-	-	13.7	-	15.2
17	3.1	-	-	-	-	-	-	1.3
18	1.3	-	-	9.7	1.3	-	-	-
19	-	-	-	1.3	-	45.0	-	6.9
20	-	21.1	-	-	-	-	-	4.6
21	-	-	-	-	-	16.8	-	3.6
22	-	-	-	20.3	20.8	1.3	-	-
23	-	25.4	-	-	-	3.1	19.3	13.7
24	-	-	-	-	-	-	1.8	18.3
25	-	1.0	-	3.8	-	-	-	-
26	-	8.1	-	-	-	-	4.3	-
27	-	-	8.1	-	-	-	-	3.8
28	9.7	-	83.7	-	-	-	-	17.8
29	16.0	9.9	4.1	6.6	-	-	-	0.8
30	1.5	1.8	-	13.5	-	-	-	-
31	-	4.1	-	1.8	11.4	-	-	-
Total	104.9	142.0	128.3	69.1	62.5	107.1	75.6	104.2
Cumulative	104.9	246.9	375.2	444.3	506.8	613.9	689.5	793.7

APPENDIX 2.

MSU Crops Field Laboratory 1984 Rainfall (mm).

Date	April	May	June	July	Aug.	Sept.	Oct.	Nov.
1	-	-	-	-	-	2.3	-	0.5
2	-	-	-	-	-	-	-	25.9
3	-	2.3	-	-	-	-	-	-
4	0.8	-	-	-	23.4	3.3	-	-
5	9.9	0.8	0.3	-	-	3.3	-	-
6	5.6	3.8	-	4.8	-	-	-	-
7	-	-	1.0	-	-	3.6	17.3	-
8	-	-	-	-	57.4	3.1	11.2	-
9	-	2.5	-	-	4.1	-	1.2	-
10	-	-	-	5.1	5.1	8.9	-	16.5
11	-	0.5	-	25.9	-	11.4	-	-
12	-	-	-	4.8	-	1.0	-	-
13	8.9	5.6	-	-	-	16.3	-	-
14	1.5	10.3	-	-	-	6.9	12.5	-
15	11.4	-	-	3.8	-	-	0.8	-
16	19.1	-	-	-	-	-	0.5	-
17	5.8	-	-	-	-	-	2.5	-
18	8.6	0.8	-	-	-	-	-	-
19	1.5	2.0	-	-	-	-	13.2	-
20	-	1.0	-	-	-	-	-	-
21	-	-	-	0.9	-	-	33.5	-
22	-	9.7	-	-	-	-	-	-
23	10.9	41.7	-	-	-	-	-	-
24	2.0	-	2.0	2.5	-	5.1	-	-
25	-	2.8	-	-	-	0.3	-	-
26	1.5	29.5	-	-	-	22.1	3.3	-
27	-	-	1.0	-	-	-	0.8	-
28	-	-	-	-	0.5	-	-	-
29	-	22.9	-	-	4.1	2.5	-	-
30	3.6	1.8	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-
Totals	91.1	137.9	4.3	47.8	94.6	90.1	96.8	42.9
Cummulative	91.1	229.0	233.3	281.1	375.7	465.8	562.6	605.5

APPENDIX 3

Irrigation regime on Experiment 1, July 1984.

Date	Water applied (mm)
July 10	38
11	-
12	51
13	76
14	76
15	-
16	102
17	76
18	102
19	51
20	25
21	90
22	107
23	76
Total	870
Daily average	62

APPENDIX 4

Irrigation regime on Experiment 1, August/September 1984

Date		Water applied (mm)
August	31	89
September	1	-
	2	76
	3	51
	4	44
	5	57
	6	51
	7	-
	8	64
Total		432
Daily average		48

APPENDIX 5

Metromix 350 ingredients

Canadian sphagnum peat moss
Domestic horticultural vermiculite
Processed bark
Washed granite sand

APPENDIX 6.

Nutrient solution used in greenhouse experiments.

Macronutrients:

Compound	g per 10 l water
Calcium nitrate	5.93
Potassium nitrate	3.07
Potassium phosphate (mono)	1.27
Potassium chloride	6.03
Ammonium phosphate (mono)	2.43
Ammonium chloride	2.43
Magnesium sulphate	5.50

Micronutrients:

Compound	g per 1 l water
Boric acid	0.14
Manganese chloride	0.36
Zinc chloride	0.10
Cupric chloride	0.05
Ammonium molybdate	0.06

The micronutrient stock solution was used at the rate of one ml per liter.

One ml of 0.62% ferric citrate solution was added per liter of nutrient solution.

APPENDIX 7.

Irrigation regime on Experiment 7, October 1984.

Date	Water applied (mm)
October 1	51
2	32
3	-
4	76
5	-
6	-
7	-
8	-
9	38
10	-
11	38
12	-
13	-
14	-
15	-
16	25
17	-
18	-
19	-
20	-
21	-
23	-
24	-
25	25
Total	285
Daily average	11

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