STUDIES ON ISOLATES OF PHYTOPHTHORA MEGASPERMA FROM ALFALFA

by

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ABSTRACT

Pathogenicity studies were conducted with isolates of Phytophthora megasperma recovered from tap roots of infected alfalfa from the following locations in Arizona: Benson, Buckeye, Gilbert, Laveen, Many Farms, Parker, Safford, Snowflake, Tucson, and Yuma. Alfalfa entries tested included those with Phytophthora resistance such as Germaine AS-13, University of Minnesota experimental Mn-PC_3, University of California experimentals UC-66 and UC-47, University of Arizona experimental Hayden PX, Waterman-Loomis 451 and Lahontan. Hayden, a major non-dormant variety planted in Arizona, was used as a susceptible check.

There were significant differences in pathogenicity among the isolates. The isolate from Many Farms was significantly more pathogenic to all entries tested than the other isolates. The Safford isolate was significantly more pathogenic than the isolates from Yuma, Gilbert, and Tucson in seedling damping-off of Certified Hayden, Lahontan and Hayden PX. The isolates from Yuma and Gilbert were the least pathogenic to all entries tested. Isolates pathogenic to seedlings were equally pathogenic to older plants.

Inoculum consisting of mycelium or mycelium and oospores was used in the studies. Both types of inoculum were similarly pathogenic. There was no association between number of oospores produced and pathogenicity of the various isolates.

INTRODUCTION

Alfalfa is grown on approximately 20 percent of the irrigated crop land in Arizona. Approximately 1.2 million tons of hay were produced in 1973. At lower elevations, where non-dormant varieties are grown, the major problem is stand and yield decline. In a state-wide survey conducted by Hine, Gray and Schonhorst (18), Phytophthora megasperma, causal agent of root rot disease, was shown to be a major contributing cause of this decline. This disease had not previously been described from Arizona. The disease was first reported from California in 1954 (9), and is known to occur throughout the alfalfa producing areas in the United States (3, 8, 12, 19) as well as in Canada (4), Australia (25), and Brazil (17).

Control of the disease is primarily based upon the development of resistant varieties. Presently The University of California (21), The University of Minnesota (13) and The University of Arizona (15) are attempting to find resistance in varieties adopted to their areas.

In developing resistant alfalfa varieties for Arizona, it was important to determine if significant differences in pathogenicity occurred among isolates of P. megasperma from varying locations in the state. Although differences have not been reported for this species (11), P. megasperma var. sojae, closely related to P. megasperma, has been reported to contain pathogenic races (24).

This study is concerned with the biology of a number of isolates of P. megasperma from widely separated geographical areas in Arizona.

LITERATURE REVIEW

Phytophthora root rot of alfalfa was described in 1954 (9). The causal organism was identified as P. cryptogea, but reclassified in 1965 (10) as P. megasperma.

Although Erwin (11) reported in 1966 that most alfalfa varieties were susceptible to P. megasperma, he found that Lahontan, a semi-dormant variety, and the non-dormant variety, California Common 49, were less susceptible. In 1969, Lehman, Erwin and Stanford (21), University of California, Davis, California, released UC 38 and UC 47 as Phytophthora tolerant germ-plasms. Currently, The University of Minnesota (13) and The University of Arizona (16) are both developing alfalfa varieties with resistance to P. megasperma.

Variation of pathogenicity in P. megasperma was investigated by Erwin (11) in 1966. Erwin noticed that some isolates lost pathogenicity over an extended period of time, which he attributed to attenuation in culture. He also noticed differences in pathogenicity between single spore isolates from the same parent. Schmitthenner (28) also indicated that certain single spore isolates from California differed in their virulence to alfalfa. Chi and Childers (5) compared several isolates from Canada and found them to be very similar in their pathogenicity. Frosheiser (12), in comparing pathogenicity and morphology of isolates from California and Minnesota, found them to be similar. Marks and

Mitchell (23) found isolates from alfalfa plants and from soil to be similar in pathogenicity.

Differences in pathogenicity among isolates of a specific fungus have been known for years. Isolates with these differences have commonly been referred to as races. The determination of races within a given fungal species is often accomplished through the use of differential varieties of the host plant. Such a system was used by Rodenhiser and Hotton (27) with Tilletia tritici on wheat, Atlow et al. (2) with Cercospora sojina on soybeans, Sundheim (29) with Aphanomyces euteiches, and Reyes (26) with Plasmodiophora brassicae on cabbage. Several members of the genus Phytophthora have been shown to have races by the use of differential varieties. Phytophthora infestans (20), P. parasitica var. nicotiannae (1, 22), P. phaseoli (30), P. fragariae (6), and P. megasperma var. sojae (24) have all been shown to contain races by this method.

MATERIALS AND METHODS

General Techniques

Isolates

Isolates used in this study were obtained from diseased tap roots of alfalfa plants collected near Tucson, Yuma, Buckeye, Parker, Laveen, Gilbert, Snowflake, Many Farms, Safford, and Benson, Arizona. The name of the isolate refers to the geographical area from which it was recovered from alfalfa. Cultures were maintained on a selective antibiotic medium (7). Isolates were transferred approximately every month.

Media

Inoculum was produced on a V-8 juice medium and Potato Dextrose Broth (PDB) medium. The V-8 juice medium consisted of 100 ml of V-8 juice, 2.0 g calcium carbonate in a total volume of one liter of distilled water. One hundred sixty ml or 80 ml of the media was placed in 32-ounce or 16-ounce prescription bottles and sterilized. After the medium cooled, an agar plug (5 mm diameter) containing the fungus was aseptically placed in each bottle. After three days, each bottle was shaken to fragment the fungal hyphae for increased growth and placed in a horizontal position on a bench in the laboratory. Laboratory temperatures ranged from 20-25^{o} C.

The PDB medium consisted of 500 g fresh potato and 44 g dextrose in a total volume of 3500 ml. One hundred ml of the medium was poured into a 250 ml Erlenmeyer flask. A single piece of Whatman Number One filter paper was secured over the mouth of the flask with a rubber band. After autoclaving and cooling, two agar plugs (5 mm diameter) were aseptically placed in each flask. Flasks were shaken daily for five seconds to fragment the hyphae. Five days after inoculation, flasks were placed on a mechanical shaker for another five days to facilitate growth.

Soil

For the flat and four-inch pot studies, soil consisted of a 1:1 (V/V) mixture of river bottom sand and sandy loam. For the six-inch pot studies, field soil was collected from an alfalfa field at the Campbell Avenue University Farm. All soil was pasteurized in a Soil King soil sterilizer.

Varieties

A number of experimental and commercial alfalfas were used to determine if differences in virulence existed among the ten isolates of P. megasperma. They included AS-13, UC-47, Mn-PC3, UC-66, WL451, Lahontan, Certified Hayden and Hayden PX. Alfalfa varieties and selections used in experiments were chosen to demonstrate the increased Phytophthora resistance of Hayden PX over Hayden from which PX was selected. Lahontan was used as a comparison because of known high natural resistance. Other varieties were selected for determination of level of resistance.

Greenhouse temperatures were maintained at 24-27° C. Growth chambers were maintained on a 12-hour light cycle at 24° C and a 12-hour dark cycle at 18° C. All experiments were watered daily to insure optimum conditions for fungal growth and infection.

Variation in Pathogenicity of Isolates of P. megasperma

A number of studies were conducted to investigate differences in pathogenicity between geographically separated isolates of Phytophthora megasperma to Certified Hayden, Hayden PX, and Lahontan, a semi-dormant variety with a known high level of resistance. Isolates tested were selected on the basis of their geographical separation and represented Tucson, Many Farms, Safford, Gilbert, and Yuma.

Studies were conducted in metal flats 54 x 38 cm containing approximately 14.7 kg of a 1:1 mixture of sand and loam. Each isolate was tested against all three varieties.

Inoculum was grown in V-8 juice medium for three weeks under the previously described conditions. Mycelial mats from five 32-ounce prescription bottles were blended in three liters of distilled water. One liter of the inoculum was applied to the soil in each flat.

The soil and inoculum were mixed, placed in the flat, and seeded. The flats were then randomized. The flats were sprinkled daily, and data collected three weeks after seeding by counting the number of surviving seedlings. All tests were replicated at least three times and repeated twice.

A second experiment was conducted in 1:1 sand-soil mixture in four-inch pots with five isolates: Safford, Gilbert, Tucson, Many Farms, and Yuma. The entries were UC-47, Lahontan, Certified Hayden and Hayden PX. Approximately 584 gm of soil was placed in each pot. Inoculum was grown in PDB medium and contained no oospores. The inoculum consisted of 45 g of mycelium blended in one liter of distilled water. Inoculum was hand mixed into the soil at the rate of one liter per 14.7 kg of soil or .003 g mycelium/g soil. Thirty seeds were planted in each pot and there were five replications of each variety. The pots were randomized on the greenhouse bench and watered daily. Counts of surviving plants were made three weeks after seeding.

An experiment was conducted to determine the effect of ten isolates (Benson, Buckeye, Gilbert, Laveen, Many Farms, Parker, Safford, Snowflake, Tucson, and Yuma) on the initiation of root rot in month-old plants of Certified Hayden, Hayden PX and Lahontan. Infested field soil was sterilized in a Soil King soil sterilizer. Approximately 1500 gm of soil was placed in each of 55 plastic pots (12 cm diameter) and three alfalfa entries, Lahontan, Certified Hayden and Hayden PX planted, with 25 seeds of each entry.

Plants were thinned after one month to ten per pot and inoculated with two-week-old mycelial mats. Mats from five 32-ounce prescription bottles were blended in three liters of distilled water and 50 ml poured onto the soil surface.

After three months, roots were rated according to disease severity with the following system.

Rating system:

1. Disease free.
2. One to two lesions on tap root or lateral roots less than 2 mm in diameter.
3. Two or more lesions on tap root or lateral roots more than 2 mm in diameter.
4. Tap root rotted within a few cm of the crown.
5. Tap root destroyed.

A study, similar to the preceding study, was conducted to determine the effect of three isolates (Many Farms, Tucson, and Yuma) on the initiation of root rot in month-old plants of Hayden PX, Lahontan, AS-13, Mn-PC3, UC-66, and Waterman-Loomis 451. The same procedures were followed in this study as in the preceding study.

Effects of Inoculum Levels on Disease Incidence

To determine the role of mycelium in pathogenicity, three different levels of inoculum of the Tucson isolate were tested against the alfalfa entries Lahontan, Certified Hayden, and Hayden PX. Inoculum levels of .001, .002, and .003 g mycelium/g soil were blended into a 1:1 sand-soil mixture and placed in four-inch pots. Inoculum (free of oospores) was grown in PDB medium. Thirty seeds were planted in each pot with five replications of each variety. Pots were randomized on the greenhouse bench, watered daily, and counts of surviving seedlings made after three weeks.

Loss of Pathogenicity by Continuous Culturing

To investigate the possibility that the Gilbert isolate had lost pathogenicity with continuous culturing, the fungus was reisolated from a diseased Hayden PX seedling. This reisolation of the Gilbert isolate was designated Gilbert-Recovered (G-R). Pathogenicity of the recovered isolate was determined by inoculating the two isolates against Certified Hayden, Lahontan, Hayden PX, and UC-47. Thirty seeds were planted in each pot with five replications of each variety. Counts of surviving seedlings were made three weeks after planting.

Oospore Production and Size

Variation in number and size of oospores was studied with isolates from Buckeye, Gilbert, Many Farms, Safford, Tucson, and Yuma. Mycelial mats were produced in V-8 juice medium in 16-ounce prescription bottles. After two weeks, each mat was blended in 50 ml of distilled water in a Servall Omni-Mixer. Oospore counts were made from two one-tenth ml subsamples from each 50 ml sample. One hundred oospores of each isolate were measured with an ocular micrometer to determine the average oospore diameter of the isolate.

RESULTS

Variation in Pathogenicity of Isolates of P. megasperma

When data from the flat studies were subjected to an analysis of variance, it was found that three statistically different pathogenic populations existed among the isolates from Yuma, Gilbert, Tucson, Safford, and Many Farms (Table 1). The isolates from Yuma, Gilbert, and Tucson were the least pathogenic. Hayden PX had a survival percentage of 94.7, 95.0 and 93.3, respectively, when tested against the Yuma, Tucson, and Gilbert isolates. The isolate from Safford was statistically more pathogenic to all entries than the above three isolates. Certified Hayden, Hayden PX, and Lahontan had a survival percentage of 36.7, 68.7 and 56.0 against the Safford isolate. Certified Hayden, Hayden PX and Lahontan had a survival percentage of 11.7, 27.0 and 26.7, respectively, when tested against the isolate from Many Farms.

In the pot studies, pathogenic variation among isolates from Yuma, Gilbert, Tucson, Safford, and Many Farms to Certified Hayden, Hayden PX, Lahontan and UC-47 are shown in Table 2. Results were similar to Table 1. The isolate from Yuma was the least pathogenic. Lahontan, Hayden PX, Certified Hayden and UC-47 had a survival percentage of 37.0, 62.0, 41.0 and 90.0, respectively, when tested against the Yuma isolate. The isolate from Safford was slightly more pathogenic on Hayden PX and Certified Hayden which had a survival percentage of 34.0 and 7.0,

Table 1. Pathogenicity of Five Arizona Isolates of *Phytophthora megasperma* on Seedling Survival of Three Alfalfa Entries When Mycelium and Oospores Were Used as Inoculum.

Isolates	Alfalfa Entries (% survival)		
	Certified Hayden	Hayden PX	Lahontan
Yuma	99.0a*	94.7a	97.0a
Tucson	68.0b	95.0a	82.3a
Gilbert	67.0b	93.3a	88.7a
Safford	36.7c	68.7b	56.0b
Many Farms	11.7d	27.0c	26.7c

* Values are expressed as a percentage of the control. Values represent the means of three replications, each consisting of placing two rows (100 seed/row) of each entry in infested soil in flats (54 x 38 cm) in the greenhouse. Means with the same letter are not significantly different at .05 level of probability. Data were taken three weeks after seeding.

Table 2. Effect of Five Arizona Isolates of Phytophthora megasperma on Seedling Survival of Four Alfalfa Entries When Mycelium was Used as Inoculum.

Isolates	Alfalfa Entries (% survival)			
	Certified Hayden	Hayden PX	Lahontan	UC-47
Yuma	41.0*	62.0	37.0	90.0
Gilbert	23.0	55.0	27.0	23.0
Tucson	11.0	53.0	55.0	46.0
Safford	7.0	34.0	38.0	38.0
Many Farms	6.0	12.0	10.0	32.0

* Values are expressed as a percentage of the control. Values represent the means of five replications, consisting of placing 30 seeds of an entry in a four-inch pot in infested soil in the greenhouse. Data were taken three weeks after seeding.

respectively. The isolate from Many Farms was the most pathogenic to Lahontan, Certified Hayden and Hayden PX which had a survival percentage of 6.0, 12.0 and 10.0, respectively.

Other pot studies were designed to determine variation in pathogenicity of isolates from Gilbert, Yuma, Safford, Laveen, Buckeye, Many Farms, Benson, Parker, Tucson, and Snowflake, to one-month-old plants of Lahontan, Certified Hayden and Hayden PX (Table 3). The isolate from Many Farms was significantly more pathogenic than all other isolates to the three alfalfa entries. Certified Hayden, Hayden PX and Lahontan had mean root ratings of 3.7, 2.6 and 1.9, respectively, when tested against the Many Farms isolate. The isolate from Many Farms caused extensive damage to the root system of the susceptible Certified Hayden, but fewer lesions were observed on the resistant Hayden PX and Lahontan entries. Exposure to the other isolates, excluding Many Farms, resulted in a range of mean root ratings to Certified Hayden, Hayden PX and Lahontan entries of 1.6-2.7, 1.1-2.6, and 1.0-2.2, respectively.

Another study was designed to determine the effect of the isolates from Tucson, Yuma, and Many Farms on the initiation of root rot in older plants of Hayden PX, Lahontan, Germaine AS-13, Waterman-Loomis 451, Mn-PC3 and UC-66 (Table 4). All entries had a mean root rating of 1.2, 2.8 and 3.7 when tested against the isolates from Yuma, Tucson and Many Farms, respectively. The Yuma isolate initiated very few lesions on any of the entries. Lahontan, Waterman-Loomis 451, Mn-PC3, UC-66, AS-13 and Hayden PX had mean root ratings of 1.2, 1.2, 1.2, 1.3, 1.5 and 1.5, respectively, when tested against the isolate from Yuma. The isolate from Tucson caused a greater number of root lesions than the Yuma isolate.

Table 3. Effect of Ten Arizona Isolates of Phytophthora megasperma on the Initiation of Root Rot in Three Alfalfa Entries.

Isolates	Alfalfa Entries		
	Certified Hayden	Hayden PX	Lahontan
Parker	1.6*	1.2	1.5
Buckeye	1.7	1.2	1.7
Gilbert	1.7	1.1	1.2
Safford	1.8	1.4	1.3
Yuma	1.8	1.3	1.0
Laveen	1.8	1.6	1.6
Benson	1.8	1.3	1.3
Snowflake	1.9	1.7	2.0
Tucson	2.3	1.3	2.2
Many Farms	3.7	2.6	1.9
CHECK	1.0	1.0	1.0

* Values represent the mean root rating of five replications on a scale of 1 to 5. An increase in mean root rating indicates an increase in disease severity. Replications consisted of ten plants in a six-inch pot and inoculated one month after seeding. Data were taken four months after seeding.

Table 4. Susceptibility of Six Alfalfa Entries to Three Isolates of *Phytophthora megasperma*.

Isolates	Alfalfa Entries					
	Mn-PC3	UC-66	AS-13	WL-451	Lahontan	Hayden PX
Yuma	1.2*	1.3	1.5	1.2	1.2	1.5
Tucson	1.7	3.2	4.1	1.4	2.5	3.0
Many Farms	2.7	3.9	5.0	3.0	3.7	4.5
Check	1.0	1.0	1.0	1.0	1.0	1.0

* Values represent the mean root rating of five replications on a scale of 1 to 5. An increase in mean root rating indicates an increase in disease severity. Replications consisted of ten plants in a six-inch pot and inoculated one month after seeding. Data were taken four months after seeding.

Waterman-Loomis 451, Mn-PC3, Lahontan, Hayden PX, UC-66 and AS-13 had mean root ratings of 1.4, 1.7, 2.5, 3.0, 3.2 and 4.1, respectively, when tested against the Tucson isolate. The isolate from Many Farms was the most pathogenic of all the isolates to all the entries. The Many Farms isolate caused extensive damage to the root systems of all entries. Mn-PC3, Waterman-Loomis 451, Lahontan, UC-66, Hayden PX and AS-13 had mean root ratings of 2.7, 3.0, 3.7, 3.9, 4.5 and 5.0, respectively. The Phytophthora resistant Mn-PC3 from Minnesota was the most resistant entry with a mean root rating of 1.8 when exposed to the three isolates. Lahontan and Hayden PX had a similar degree of resistance with mean root ratings of 2.4 and 2.9, respectively. AS-13 was the most susceptible entry in this study with a mean root rating of 3.5 when tested against the three isolates.

Effects of Inoculum Levels on Disease Incidence

Effects of inoculum levels (mycelium, free of oospores) of the Tucson isolate on the alfalfa entries Certified Hayden, Hayden PX, Lahontan and UC-47 demonstrated that increasing inoculum from .002 to .003 g mycelium/g soil did not increase damping-off of Lahontan and Hayden PX (Table 5). Hayden PX and Lahontan had a survival percentage of 63.0 and 62.0 and 66.0 and 57.0, respectively, when tested against an inoculum level of .002 and .003 g mycelium/g soil. There was a correlation between seedling survival and inoculum level in the variety, Certified Hayden. At .001, .002 and .003 g mycelium/g soil, there was 56.0, 31.0 and 15.0 percent survival, respectively.

Table 5. Effects of Varying Inoculum Levels of the Tucson Isolate of Phytophthora megasperma on Seedling Survival in Three Alfalfa Entries.

Inoculum Levels* (g mycelium/g soil)	Alfalfa Entries (% survival)		
	Certified Hayden	Hayden PX	Lahontan
.001	56.0**	82.0	89.0
.002	31.0	63.0	66.0
.003	15.0	62.0	57.0

*Inoculum, consisting of mycelium, free of oospores was grown in PDB.

**Values are expressed as a percentage of the control. Values represent the means of five replications, consisting of placing 30 seeds of an entry in a four-inch pot in infested soil in the greenhouse. Data were taken three weeks after seeding.

Loss of Pathogenicity by Continuous Culturing

The isolate from Gilbert was virulent in initial studies. After approximately six months in culture, pathogenicity was reduced. A study designed to determine if the pathogenicity of the Gilbert isolate could be restored after passage through a resistant Hayden PX seedling. Certified Hayden, Hayden PX, Lahontan and UC-47 were tested against the parent isolate, Gilbert, and the sub-isolate, Gilbert-Recovered. Certified Hayden, Hayden PX, Lahontan and UC-47 had a survival percentage of 56.0, 98.0, 65.0 and 68.0, respectively, when tested against the Gilbert isolate and a survival percentage of 2.0, 37.0, 33.0 and 19.0, respectively, when tested against the Gilbert-Recovered isolate (Table 6).

Oospore Production and Size

A study was conducted to determine variation among six isolates (Safford, Buckeye, Many Farms, Yuma, Tucson and Gilbert) of oospore diameter and production. There was no correlation between production of oospores in culture and pathogenicity. The most pathogenic isolate, Many Farms, produced no oospores in a V-8 juice medium (Table 7). Yuma, the least pathogenic isolate, produced 2,307 oospores/ml. Safford produced the most oospores, 12,865/ml. Oospore diameter of the isolates ranged from 17.5 microns to 42.5 microns. The isolate from Gilbert had the smallest average diameter of 23.5 microns. The isolate from Yuma had the largest average diameter of 30.9 microns.

Table 6. Restoration of Pathogenicity of the Gilbert Isolate After Passage Through a Resistant Hayden PX Seedling.

Isolates	Alfalfa Entries (% survival)			
	Certified Hayden	Hayden PX	Lahontan	UC-47
Gilbert	56.0*	98.0	65.0	68.0
Gilbert-Recovered	2.0	37.0	33.0	19.0

* Values are expressed as a percentage of the control. Values represent the means of five replications, consisting of placing 30 seeds of an entry in a four-inch pot in infested soil in the greenhouse. Data were taken three weeks after seeding.

Table 7. Variation in Oospore Size (in Microns) and Production in Six Geographically Separated Isolates of Phytophthora megasperma.

Isolates	Oospores/ml*	Range	Average Diameter**
Safford	12,865	17.5-37.5	26.99
Buckeye	5,621	17.5-42.5	30.54
Yuma	2,307	20.5-40.0	30.94
Gilbert	2,210	20.5-40.0	23.48
Tucson	0	-	-
Many Farms	0	-	-

* Two-week-old mycelial mats were blended in 50 ml of distilled water in a Servall Omni-Mixer. Oospore counts were made from two one-tenth ml sub-samples from each 50 ml sample.

** One-hundred oospores of each isolate were measured.

DISCUSSION AND CONCLUSIONS

Isolates from widely separated geographical locations in Arizona were statistically different in pathogenicity. When isolates from Gilbert, Many Farms, Safford, Tucson and Yuma were tested against seedlings of Certified Hayden, they were separated into four pathogenic populations. When tested against seedlings of Hayden PX and Lahontan, they were separated into three pathogenic populations. It is interesting to note that the susceptible entry, Certified Hayden, indicated the presence of more pathogenic populations than the resistant entries, Hayden PX and Lahontan.

Seedling damping-off was the most sensitive method of dividing isolates into statistically different pathogenic populations. This probably occurs because seedlings are more susceptible than older plants to Phytophthora megasperma (14).

Studies to determine the effect of three isolates (Many Farms, Tucson and Yuma) on month-old plants of Mn-PC3, Waterman-Loomis 451, AS-13, UC-66, Hayden PX and Lahontan also indicated the presence of three distinct pathogenic populations. Each isolate was statistically different from the other two in ability to initiate root rot. A similar study with ten isolates (Benson, Buckeye, Gilbert, Laveen, Many Farms, Parker, Safford, Snowflake, Tucson and Yuma) on month-old plants of Certified Hayden, Hayden PX and Lahontan indicated the presence of two distinct pathogenic populations. One consisted of the isolates from

Benson, Buckeye, Gilbert, Laveen, Parker, Safford, Snowflake, Tucson and Yuma. The other consisted of the isolate from Many Farms.

The Many Farms isolate has been consistently pathogenic in both seedling damping-off and initiation of root rot in older plants. After being in culture a year longer than the Many Farms isolate, the Tucson isolate has become erratic in pathogenic behavior. The Tucson isolate, initially, was highly pathogenic to Certified Hayden, Hayden PX and Lahontan. Yet a year later, pathogenicity was greatly reduced in studies on these same entries (Table 8).

The isolates from Gilbert, Yuma and Safford were isolated approximately two years ago. The Yuma and Safford isolates were shown to be highly virulent to Certified Hayden and to a lesser extent to Hayden PX (15). The Gilbert isolate was much less virulent than the Yuma and Safford isolates. Two years after initial isolation, the Yuma isolate demonstrated reduced virulence, while the Safford isolate showed a smaller decrease in virulence over the same period. Pathogenicity of the Gilbert isolate has remained consistently low from the initial isolation until the present. This loss of pathogenicity of an isolate after extended periods of culturing on artificial media agrees with work done by Erwin (11) and observations made by Gray et al. (16).

Increased pathogenicity of isolates after passage through resistant juvenile plants has been reported to occur in Phytophthora infestans (14). Erwin, however, was not able to increase pathogenicity in isolates of P. megasperma from alfalfa.

Table 8. The Effect of Continuous Culturing on Pathogenicity of Isolates of Phytophthora megasperma from Tucson and Buckeye.

Date*	Isolate	Alfalfa Entries (% survival)	
		Certified Hayden	Hayden PX
March 1973	Tucson	28.0**	60.0
November 1973	Tucson	68.0	95.0
March 1973	Buckeye	15.0	62.0
November 1973	Buckeye	10.0	49.0

* The date indicates the month and year a study was conducted with the isolates and alfalfa entries indicated.

** Values are expressed as a percentage of the check. Values represent the means of three replications, each consisting of placing two rows (100 seed/row) of each entry in infested soil in flats (54 x 38 cm) in the greenhouse. Data were taken three weeks after seeding.

Mycelium alone was as effective as mycelium and oospores in causing seedling death. This indicates that oospores are not required for infection in studies of this type. This observation is reinforced by the study of variation in oospore production and size in six isolates of the fungus from Gilbert, Buckeye, Many Farms, Safford, Tucson and Yuma. The most pathogenic isolate, Many Farms, produced no oospores in liquid media. Whereas the isolate from Yuma, the least virulent, produced over 2,000 oospores/ml.

The Hayden PX selection from Certified Hayden is more resistant to P. megasperma than the parent variety. Hayden PX has resistance similar to Lahontan. Resistance obtained in seedling screening tests of Hayden PX (15) did not break down when one-month-old Hayden PX plants were tested.

Although statistically different pathogenic populations of P. megasperma were shown to exist, the differences in virulence were not great. Presently, there are no resistant varieties available for commercial use in Arizona. The eventual release of resistant varieties will probably portend the development of strains of Phytophthora megasperma with greater variation in pathogenicity than described for the isolates in this study.

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