THE CAUSE OF EARLY DYING OF POTATO [1/]

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SUMMARY

Verticillium albo-atrum (microsclerotial type =MS= V. dahlae) caused early dying of potato in laboratory and greenhouse experiments. This organism was isolated from 100% of wilted stems incubated on moist filter paper but from only 20% of wilted stems, when placed on the most common medium ethanol streptomycin agar. Early dying was not caused or increased by a Colletotrichum sp., suspected of contributing to early dying, a Fusarium sp. Rhizoctonia solani, an Erwinia sp. a Pseudomonas sp., and several unidentified imperfect fungal isolates when they were introduced either individually or in combination.

INTRODUCTION

Growers, processors, and packers in the Northwest have built a thriving potato industry with an estimated annual value exceeding 500 million dollars based on the variety, Russet Burbank (10). Unfortunately, this variety is susceptible to an early dying disease (10, 12, 13) which causes an annual loss to the Northwest potato industry of up to 31 million dollars (10). "Early dying" describes an unthrifty growth and premature senescence of potato plants (4, 6, 21).

The principal causes of early dying are not clearly understood. In 1879, Reinke and Berthold first reported a species of Verticillium caused wilt in potato. Other reports establising Verticillium spp. as a cause of wilt have since been published by Orton (16), Pethybridge (18), Neilson (15), Folsom, Syman, and Westin (3), Keyworth (7), McLean (13), Robinson, Larson and Walker (19), Guthrie (4), and Isaac (6).

Colletotrichum spp. are generally considered minor pathogens of potato (20), but recently they have been reported to cause wilt in potatoes (1, 9, 17). This organism has been associated with Rhizoctonia solani, Macrophomia phaseoli (5), Fusarium spp. (2), and Verticillium dahliae (1). Colletotrichum spp. are readily isolated from diseased potato stems; however, there is no convincing evidence that they cause wilt or early dying. Information on the possible disease potential or economic importance of Colletotrichum spp., on potatoes in Washington is incomplete.

The objective of this study was to determine the cause of early dying in potato.

MATERIALS AND METHODS

Over 100 Russet Burbank potato plants with symptoms of early dying were collected in June 1978 from a field near Paterson, Washington where early dying is a recurring problem. Portions of roots and excised vascular tissues (.4 to .8 inches) from each plant were washed for 1 hr in running tap water and surface-disinfected in 95% ethanol and 10% Clorox ^{R} (5.25% sodium hypochlorite, 1 part Clorox:9parts water) for 10 min. The disinfected root tissues

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were then rinsed in a large volume of sterile water and transferred to ethanol-streptomycin agar (14), Difco ^{R} cornmeal agar, Difco potato dextrose agar (PDA), Difco water agar, and a selective medium for Fusarium (8). Plates were incubated at 70°F for 4 days. Hyphal tips of fungi appearing on the various media were transferred to fresh PDA plates and incubated 7 days for later identification. Unidentified bacteria were also streaked on PDA plates for isolation of single colonies which were subsequently maintained on PDA slants at 70°F.

Naturally infested soil was collected from the field near Paterson, Wa. and from a field at the Roza Unit of the Irrigated Agriculture Research and Extension Center near Prosser, Wa. Portions of soils were steam pasteurized 14 hrs at a pressure of 5 psi for later inoculation with suspected pathogens. A greenhouse mixture (containing 8:2:1:1 by volume of sand, soil, peat, and vermiculite, respectively) was similarly pasteurized. Unpasteurized field soil was used for controls. The three field soils and greenhouse mix were fertilized with ammonium sulfate, treble super phosphate, potassium sulfate, and Zn-Mn-S, equivalent of 300 N, 100 P, 500 K and 10 lb Zn/A, respectively.

Inocula of a Colletotrichum sp., a Fusarium sp., Rizoctonia solani, several unidentified imperfect fungi, and an Erwinia sp. and a Pseudomonas sp., isolated from potato roots and vascular tissue, were individually increased on PDA. The Verticillium albo-atrum (MS) isolate was increased on ethanol-streptomycin agar. Plates of inocula were incubated for 2 weeks at 70°F.

The contents of 5 culture plates of each isolate were added to 700 ml of sterile tap water and triturated in a sterile food blender for 5 min. The inoculum suspensions for each isolate were added to a new polyethylene bag and mixed into a volume of pasteurized soil equivalent to six 6-inch greenhouse pots. The food blender was disinfected between inocula by soaking in 50% Clorox (1 part Clorox:1 part water) for 5 min and rinsing in sterile tap water. Inoculum for each isolate averaged 4.2×10^4 propagules per gram of dry soil as determined by plating diluations on PDA.

Certified healthy tubers of Russet Burbank potato were surface disinfected by washing in nonsterile tap water, soaking for 5 min in a 5% formaldehyde solution (1 part 37% formaldehyde:19 parts water), rinsing in tap water and, finally, soaking for 15 min in 15% Clorox (1 part Clorox:5.7 parts water). Tuber eyes (1.2-inch diameter scoops) were removed with a flamed melon ball scoop. The tuber eyes were planted in a steam-pasteurized greenhouse soil mixture and held in a 60°F greenhouse. Additional tubers were tested for internal contamination by V. albo-atrum (MS) or other vascular pathogens by plating small sections excised aseptically from the stem end on PDA. Seedlings of Black Beauty eggplant were grown in flats of the pasteurized greenhouse soil mix in a 60°F greenhouse for later use as an index host.

Using disinfected polyethylene gloves three plants of each host were transplanted into each 6-inch clay pot containing inoculated soil. Six pots of potato and eggplant were exposed to each inoculum. Greenhouse pots were washed with tap water and either disinfected by steam pasteurization overnight at 5 psi or by 20% Clorox (1 part:4 part water) for 15 min. Before use benches were washed with tap water and disinfected with 20% Clorox. During all pathogenicity tests the greenhouse was maintained on a day-night temperature regime of 80°F and 70°F, and a relative humidity of 60 to 100%.

Water bath soil temperature control tanks were used in the greenhouse to study the effect of different soil temperatures on disease development. Two soil temperature regimes were involved: 1) a constant 70°F, and 2) an initial 55°F with a 5°F weekly rise up to a constant 70°F. Two-week-old cultures of prospective pathogens were mixed into pasteurized field soil singly and in combinations. Three potato plants derived from excised tuber eyes were transplanted into 10-inch diameter plastic pots containing inoculated soil. Three pots of each isolate were placed in each of the two soil temperature regimes for 10 weeks. Plants growing in non-pasteurized field soil and in non-inoculated pasteurized field soil served as controls.

Soil and stem inoculation methods were compared on eggplant using two-week-old cultures of the prospective pathogens. Three eggplant seedlings were transplanted into five 2-inch clay pots each containing inoculated soil. For stem inoculation, a Q-tip^{R} (Chesebrough Ponds Inc., Greenwich, Cn. 063830) was dipped into an inoculum suspension of each prospective pathogen and fastened with paper masking tape to a wound in the stem of each 3-week-old eggplant growing in pasteurized soil. Five pots with 3 seedlings were inoculated with each inoculum. Disease data were recorded after 8 week's growth in the greenhouse.

The effect of irrigation frequency on early dying was studied in soil collected from fields near Paterson and Prosser, Wa. The soils were steam pasteurized and inoculated with prospective pathogens. Irrigation treatments were: 1) watered daily, 2) watered at 2-day intervals, and 3) watered only when plants began to wilt. Each treatment involved 3 plants per pot replicated 5 times for each combination of soil, irrigation treatment, and pathogen. The plants were held in the greenhouse for 8 weeks.

Numbers of wilted plants in each pot were recorded for each experiment. Re-isolations were made from wilted stems. Clorox disinfected stem segments, .4 to .8 inches in length, were placed on ethanol-streptomycin agar media and on moist filter paper (11) in petri dishes for re-isolation of pathogens.

RESULTS

Isolation of Pathogens

From 100 early dying plants collected near Paterson, Wa., 95% yielded isolates identified as Fusarium spp. and R. solani, 85% yielded Colletotrichum spp. and Erwinia spp., 75% yielded Pseudomonas spp., and only 20% yielded V. albo-atrum (MS). Occurrence of other unidentified fungi was less frequent. Colletotrichum spp. and V. albo-atrum (MS) were isolated most frequently when ethanol-streptomycin agar was used. Erwinia spp. and Pseudomonas spp. occurred most often on PDA, Fusarium spp. predominated on the Fusarium medium and R. solani occurred on all media. V. albo-atrum (MS) was isolated more frequently from excised vascular root tissues than from intact root segments.

The method used for isolation was found to be very important. V. albo-atrum (MS) was isolated from only 20% of wilted potato stems on ethanol-streptomycin agar or PDA but from 100% of stems of moist filter paper (11).

Determining Pathogenicity of Isolates

Effect of soil temperature. -- In steam-pasteurized soil, only pots containing V. albo-atrum (MS), alone or in combination with other organisms, caused early dying or wilt of potato (Table 1 and Fig. 1). Disease symptoms occurred sooner and in more plants in a soil held at a constant high temperature than in a soil subjected first to cool and then to a high temperature. V. albo-atrum was consistently re-isolated from wilted plants using the moist filter technique.

Effect of method of inoculation and watering frequency -- Soil and stem inoculation was equally effective in causing wilt of eggplants (Table 2). Incidence of wilt decreased as the watering frequency decreased in both soil types (Table 3). Again only V. albo-atrum (MS), alone or in combination with other organisms, caused wilt in eggplants (Table 2) or potatoes (Table 3).

Organisms used as soil inoculum, singly or in combinations, were re-isolated from wilted stems (Tables 2 and 3). Again the moist filter paper technique was more effective for isolation of V. albo-atrum (MS) than plating on ethanol-streptomycin agar on PDA.

DISCUSSION

The cause of early dying disease of potato has not been clearly understood. V. albo-atrum (4, 6, 15, 16, 18) and Colletotrichum spp. (1, 9, 17) were generally believed to be the principal causes. We found wilting was induced by V. albo-atrum (MS) and not by Colletotrichum spp., Erwinia spp., Fusarium spp., Pseudomonas spp., R. solani, or any of the unidentified imperfect fungi isolated from potato plants expressing early dying.

The method of stem sampling and the media used for isolation of the pathogen are important considerations in determining the cause of early dying. In this study Colletotrichum spp. were isolated much more frequently than V. albo-atrum from early dying stem tissues collected near the soil surface and then plated on ethanol-streptomycin agar, the most commonly used medium. However, Colletotrichum spp. were isolated from only 10% of wilted stems and V. albo-atrum (MS) from 100% of such stems when sections were collected 12 inches or more above the soil surface and placed on moist filter paper (11). On filter paper V. albo-atrum (MS), grew from stem lenticels in 3 or 4 days, while the growth of contaminants and competitors such as Colletotrichum spp. and Fusarium spp. were retarded since no medium other than stem tissue was present to support them. Our results continue to indicate that V. albo-atrum (MS) is the principal causal organism of the early dying disease (4, 6, 15, 16, 18).

Although Colletotrichum spp. frequented potato stems readily near the soil line of wilted potato plants, it did not induce wilt nor intensify wilt either alone or in combination with other organisms. We conclude, therefore, that Colletotrichum spp. are not important in the etiology of early dying disease.

High temperature and high irrigation frequency have been reported to increase Verticillium wilt (13, 15, 19). Our greenhouse results support those conclusions and our observations of field of potatoes with early dying in the Columbia Basin in Washington State (Easton, unpublished data).

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Table 1. Pathogenicity of potential early dying organisms when tested in two temperature regimes.

Inoculum[1]	% Wilted Potato Plants[2]	
	Constant 70°F	55°F to 70°F[3]
C	0 a[4]	0 a
E	0 a	0 a
F	0 a	0 a
P	0 a	0 a
V	100 c	88.8 bc
C+E	0 a	0 a
C+F	0 a	0 a
C+P	0 a	0 a
C+V	88.8 bc	77.7 b
E+F	0 a	0 a
E+P	0 a	0 a
E+V	88.8 bc	77.7 b
F+P	0 a	0 a
F+V	88.8 bc	77.7 b
P+V	88.8 bc	77.7 b
C+E+F	0 a	0 a
C+E+F+V	88.8 bc	77.7 b
UN-F	0 a	0 a
NFS (Control)	88.8 bc	77.7 b
PS (Control)	0 a	0 a

[1] Organisms used as inoculum: C=Colletotrichum sp., E=Erwinia sp., F=Fusarium sp., P=Pseudomonas sp., V=Verticillium albo-atrum (MS), UN-F=unidentified fungi, NFS=natural wilt-infested field soil, PS=steam-pasteurized field soil.

[2] Percent wilted from a total of 9 potato plants per treatment.

[3] 55°F at start with weekly 5°F rise to 70°F.

[4] Means followed by the same letter are not significantly different according to Duncan's Multiple Range test at P=.05.

Table 2. Effect of inoculation method on the pathogenicity of potential early dying organisms.

Inoculum[1]	% Wilted eggplants[2]	
	Soil inoculation	Stem inoculation
C	0 a[3]	0 a
E	0 a	0 a
F	0 a	0 a
P	0 a	0 a
V	86.6 d	80.0 c
C+E	0 a	0 a
C+F	0 a	0 a
C+P	0 a	0 a
C+V	80.0 c	80.0 c
E+F	0 a	0 a
E+P	0 a	0 a
E+V	80.0 c	73.3 b
F+P	0 a	0 a
F+V	73.3 b	73.3 b
P+V	73.3 b	73.3 b
C+E+F	0 a	0 a
C+E+F+V	80.0 c	80.0 c
UN-F	0 a	0 a
NFS (Control)	73.3 b	--
PS (Control)	0 a	0 a

[1] Organisms used as inoculum: C=Colletotrichum sp., E=Erwinia sp., F=Fusarium sp., P=Pseudomonas sp., V=Verticillium albo-atrum (MS), UN-F=unidentified fungi, NFS=natural wilt-infested field soil, PS=steam-pasteurized field soil.

[2] Percent wilted from a total of 15 plants per treatment.

[3] Means followed by the same letter are not significantly different according to Duncan's Multiple Range test at P=.05.

Table 3. Effect of watering frequency on pathogenicity of potential early dying organisms.

Isolate[1] or treatment	% Wilted potato plants[2]					
	Columbia River Farms Soil[3]			Roza field soil[3]		
	W	M	D	W	M	D
C	0 a[4]	0 a	0 a	0 a	0 a	0 a
R	0 a	0 a	0 a	0 a	0 a	0 a
V	100 f	93.3 ef	86.6 def	93.3 ef	80.0 cde	73.3 bcd
C+R	0 a	0 a	0 a	0 a	0 a	0 a
C+V	93.3 ef	80.0 cde	73.3 bcd	93.3 ef	73.3 bcd	66.6 bc
C+R+V	93.3 ef	86.6 def	80.0 cde	86.6 def	66.6 bc	60.0 b
NFS (control)	86.6 def	80.0 cde	66.6 bc	80.0 cde	73.3 bcd	66.6 bc
PS (control)	0 a	0 a	0 a	0 a	0 a	0 a

[1] Isolates used as inoculum: C=Colletotrichum sp., R=Rhizoctonia solani, V=Verticillium albo-atrum (MS), NFS=Natural wilted infested field soil, PS=Steam pasteurized field soil.
[2] Percent wilted from a total of 15 plants per treatment.
[3] Watering frequency treatments of soils were: W=daily (wet), M=every other day (medium), and D=at wilting point (dry).
[4] Horizontal means followed by the same letter are not significantly different according to Duncan's Multiple Range test at P=.05.

Figure 1. Early dying symptoms when potato plants grown in: left to right: PS= steam pasteurized field soil, C+V soil inoculated with a Colletotrichum sp. + V. albo-atrum (MS), C=Colletotrichum sp., and V=V. albo-atrum (MS).