EFFECT OF PSEUDOMONAS FLUORESCENS ON POTATO PLANT GROWTH AND CONTROL OF VERTICILLIUM DAHLIAE [1]

by
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Abstract

Pseudomonas fluorescens strain M-4 was studied for its potential to inhibit V. dahliae and promote growth of potato plants. In greenhouse tests, the fresh weights of shoots and roots were significantly greater in soil infested with V. dahliae at 10^4 prop/g when seedpieces were treated with M-4. However, M-4 had no effect on tuber weights.

In field trials a liquid suspension of M-4 was coated on seedpieces before handplanting, or M-4 was sprayed into furrows in which seedpieces were machine planted. Both methods resulted in root populations of M-4 comparable to those obtained in greenhouse tests with about 10^4 cfu/g of root. Treatments with M-4 did not increase yields, percent U.S. No. 1 tubers, or suppress Verticillium wilt.

Introduction

Verticillium wilt, caused by Verticillium dahliae, is a serious disease of potatoes in the Pacific Northwest. The ability of V. dahliae to persist in soil for years makes this disease difficult to control (8). Soil fumigation has been effective in controlling V. dahliae but provides only annual control and is expensive. Long rotations with nonsusceptible crops to reduce V. dahliae are usually neither economical or effective (2).

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Mention of a product used in these studies does not constitute a recommendation of the product by Washington State University over other products.

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Wadi (10) found over 150 bacterial isolates from potato roots that were antagonistic in vitro to V. dahliae. Some strains of Pseudomonas, Cellulomonas, and Streptomyces spp. were shown to colonize potato roots, reduce the number of propagules of V. dahliae in potato rhizospheres, and increase plant growth and tuber production in greenhouse tests.

We evaluated the effect of one of Wadi's isolate, P. fluorescens biotype C, strain M-4 (10), on potato plant growth and control of V. dahliae. The influence of method and rate of application of P. fluorescens, inoculum density of V. dahliae, and cropping history of the soil on plant growth and incidence of Verticillium wilt was also studied.

Materials and Methods

Greenhouse Studies. Cropped and uncropped (desert) soils were obtained in and near a center pivot irrigation circle, near Paterson, Wa. in September 1982. This Quincy loamy sand circle had been previously cropped to potatoes, corn and wheat. A portion of each soil was steam pasteurized 8 hr at 77 C so the effect of bacterial treatments in the absence of V. dahliae could be tested. Soil to fill each steam pasteurized pot (15 cm dia) was mixed in a polyethylene bag with N:P:K:Zn added equivalent to 252:84:420:8 lb/A, respectively. For treatments with no V. dahliae, 50 ml of tap water was mixed into the fertilized soil. For treatments with V. dahliae, 50 ml of the appropriate inoculum concentrations to provide either 10^3 or 10^4 prop/g of soil was added to the mix. Eight replicate pots were prepared for each treatment. Pots were placed in the greenhouse on the bottom of an overturned steam pasteurized saucer to prevent contamination from the bench.

P. fluorescens strain M-4, a mutant resistant to rifampicin and streptomycin sulfate each at 100 µg/ml, was transferred from a lyopholized culture and tested for in vitro antagonism to V. dahliae (10). Dust formulations containing 0, 10^4 and 10^8 cfu/g of dust were prepared as described by Kloepper (5) and stored at 4C.

Certified seed tubers of Russet Burbank were removed from cold (4C) storage six days before planting and held at 24 C. Tuber eyes were removed with a sterile 3-cm-dia melon ball scoop. Seedpieces were dusted in a polyethylene bag with the appropriate inoculum equivalent to 15g dust/kg of potato seed. The treatments were arranged in factorial completely randomized design. Three seedpieces were planted per pot and the eight pots in each treatment were randomly located on greenhouse benches. Greenhouse day/night temperatures were maintained at 26/21 C. The experiment was repeated twice and data combined for final statistical analysis.

Heights of plants in each pot were measured six weeks after planting. A scale of 0 to 10 was used to estimate the percentage of wilted foliage seven weeks after planting where 0 = all foliage healthy, 2 = 20%, 4 = 40%, 6 = 60%, 8 = 80%, 10 = 100% foliage wilted or dead.

Plants were harvested eight weeks after planting by cutting at the soil line and determining fresh shoot weight. To determine populations of M-4 on root surfaces, roots were shaken to remove all but tightly adhering soil. Root samples (0.5 g) were agitated 15 min in 49 ml of sterile distilled water which was then used to make serial dilutions. One aliquot (0.10 ml) of each serial dilution was spread on plates of King's medium B containing rifampicin, streptomycin sulfate, and cycloheximide each at 100 µg/ml, nystatin and Botran 75 W® each at 20 µg/ml, and Benlate 50 W® at 30 µg/ml. Plates were incubated at 23 C and bacteria counted after two days.

Populations of V. dahliae in the rhizosphere were obtained from 0.5 g soil samples diluted as above. Samples (0.3 ml) of each dilution were spread in triplicate on plates of ethanol-streptomycin medium (1). Plates were incubated at 23 C and propagules of V. dahliae were counted after 15 days.

Fresh weights of roots and tubers were determined following washing in tap water and air drying for one hr. Potato stems were dried in a forced air dryer for three days at 31 C and ground through a 60-mesh screen by a Wiley mill. propagules of V. dahliae in dried stems were estimated from serial dilutions in sterile distilled water plated on ethanol-streptomycin medium and counted as for soil samples.

Populations of P. fluorescens and V. dahliae were transformed to Log_{10} (x + 1) prior to statistical analysis.

Field Studies. A test was conducted in a field of Warden silt loam soil near Prosser, Wa. under sprinkler irrigation. In April 1984, the field was chisel plowed 45 cm deep, two ways on the diagonal. Ammonium sulfate was applied at 300 lb of N/A, broadcast on the soil surface prior to plowing 30 cm deep. A liquid suspension of P. fluorescens was prepared by adding the bacteria to 3% methylcellulose to give a concentration of 10^8 cfu/ml. P. fluorescens and the untreated control treatments were applied by two methods in plots 12 ft wide (4 rows) by 20 ft long in a randomized complete block design with six replications. For the bacterization treatment, seedpieces of Russet Burbank were shaken with the P. fluorescens inoculum at a rate of 20 ml per 27 seedpieces, sufficient to hand plant one row on April 24, 1984. The second method involved spraying bacterial inoculum at 25 gal/A, into the furrows as potatoes were machine-planted on April 25, 1984. Inoculum for this second method consisted of a 2 L suspension of M-4 in 3% methylcellulose added to 15 L of water. Control plots for each method of application of P. fluorescens were planted with untreated potato seed in the same manner.

Potato root and soil samples were collected five weeks after planting and at seven-week intervals thereafter. Populations of V. dahliae and P. fluorescens were determined as previously described. The incidence of Verticillium wilt was recorded as a percentage of the total number of plants per 20 ft-row showing wilt in late Sept. Stem infection by V. dahliae was determined from ten 25-cm-long stems/plot, cut 5 cm above the soil line. Samples were collected in Oct., dried, ground, and plated as in greenhouse tests.

Potatoes were harvested October 17, 1984 from a single row in each plot. Total weight and percent of U.S. No. 1 tubers were recorded.

Results

Greenhouse Studies. Populations of M-4 were significantly greater for both treatments (10^4 or 10^8 cfu/g) on the roots of plants grown in the pasteurized soil than on roots in soil infested with V. dahliae (Table 1). In soil infested with V. dahliae at either 10^3 or 10^4 cfu/g soil, populations of M-4 on roots were significantly higher when seedpieces were treated at 10^8 than at 10^4 cfu/g.

Shoot fresh weight was significantly greater in pasteurized soil than in soil infested with V. dahliae, regardless of whether seedpieces were inoculated with none, 10^4 or 10^8 cfu of M-4/g dust (Fig. 1). In soil with V. dahliae at 10^3 prop/g, there was again no growth response associated with bacterization of seedpieces. However, in soil with V. dahliae at 10^4 prop/g, weights of shoots from seedpieces treated with either rate of M-4 were significantly greater than those from the untreated check.

Shoot fresh weight was significantly greater in cropped than in uncropped soil when both were infested with V. dahliae (Table 2). In both soils, fresh weights of plants grown from seedpieces treated with either rate of M-4 were greater than plants grown from untreated seedpieces. Plants treated with M-4 were significantly taller than check plants, but treatment with M-4 had no effect on tuber weight (Table 3).

In pasteurized soil, fresh weights of potato roots from seedpieces not treated with M-4 were significantly greater than in V. dahliae-infested soil (Fig. 2). In the soil inoculated with none or 10^3 V. dahliae prop/g, root weights were not increased by inoculation of seedpieces with either rate of M-4. In soil with V. dahliae at 10^4 prop/g, root weights of plants from seedpieces treated with M-4 at either 10^4 or 10^8 cfu/g were significantly greater than those from the untreated control.

Populations of V. dahliae in the rhizosphere of plants treated with either rate of M-4 did not differ from the untreated control (Fig. 3). However, the number of propagules of V. dahliae in dried stems at 10^8 cfu of M-4/g was significantly less than at 10^4 cfu/g, and both were significantly less than the untreated control.

Verticillium wilt symptoms were more severe in uncropped than cropped soil (Table 4). Plants treated with either rate of M-4 had a significantly lower mean wilt rating than the untreated control.

Field studies. Coating seedpieces with M-4 at 10^8 cfu/ml resulted in significantly higher populations (10^4 cfu/g) on roots than did spraying the bacteria into the open furrow (10^2 cfu/g) five weeks after planting at the first sampling date in June (Fig. 4).

Twelve weeks after planting (July), populations on roots in the furrow spray treatments were near 10^4 cfu/g and did not change significantly between July and September, 19 weeks after planting. In contrast, populations of P. fluorescens on roots of potatoes from dusted seedpieces had decreased to 10^2 cfu/g, 19 weeks after planting.

There were no significant differences in populations of V. dahliae in the rhizosphere at any sampling date whether P. fluorescens was introduced on the seedpieces or sprayed in the furrow (Table 5). Wilt was not severe under 1984 field conditions and symptoms were not evident until September. Treatments of P. fluorescens did not significantly affect the incidence of wilt or the number of propagules of V. dahliae in dried, ground stems (Table 6). Neither total yield nor tuber quality were significantly increased by treatments of P. fluorescens (Table 7).

Discussion

P. fluorescens strain M-4 does not appear to promote plant growth in pathogen-free soil. In greenhouse tests in pasteurized soil, the fresh weight of potato shoots and roots of plants from seedpieces dusted with M-4 were not different than the untreated control, even though M-4 colonized the roots in pasteurized soil better than in V. dahliae-infested soil. This agrees with other reports comparing growth responses in sterile or pathogen-free natural soil (4,9).

In field soil with low populations of V. dahliae (less than 10^3 prop/g), P. fluorescens had no effect on yields or the incidence of wilt. Likewise, in the greenhouse, in soil with V. dahliae at 10^3 prop/g, P. fluorescens had no effect on plant growth. However in glasshouse tests in soil with V. dahliae at 10^4 prop/g, both root and shoot fresh weights and height of plants from seed pieces treated with P. fluorescens were significantly greater than untreated plants. Although plant growth was better, tuber weight was not affected.

Variability or poor colonization (3) of P. fluorescens would not explain the inconsistencies in plant growth response in this work. Both dust and liquid treatments of P. fluorescens in glasshouse and field tests resulted in populations of 10^3 to 10^5 cfu/g of root, depending on the initial inoculum density of P. fluorescens. Application method and cropping history of the soil did not influence populations significantly.

The mechanisms by which P. fluorescens suppressed Verticillium wilt at high inoculum densities of V. dahliae are not known. It is unlikely P. fluorescens killed propagules of V. dahliae in the soil (10). Populations of V. dahliae in the rhizosphere of M-4-treated plants were not less than untreated plants in either glasshouse or field tests.

P. fluorescens may prevent or delay penetration by V. dahliae, since the number of propagules of V. dahliae in dried, ground stems of plants treated with M-4 was significantly less than in untreated plants.

Roots of plants not treated with P. fluorescens but grown in soil with V. dahliae at 10^4 prop/g were discolored and generally deteriorated, consistent with other reports of the adverse effect of V. dahliae on root health (6,7). In contrast, roots of M-4-treated plants appeared healthy with fresh weights comparable to roots of plants grown in pasteurized soil.

P. fluorescens appears to protect roots from extensive colonization by V. dahliae in soil heavily infested with the pathogen. However, infection was not prevented and plants treated with P. fluorescens developed wilt symptoms. It is not known whether treatments of P. fluorescens could delay early dying of potatoes enough to produce a yield response under field conditions. Growth responses in greenhouse tests occurred only in soil with an inoculum density of V. dahliae of 10^4 prop/g of soil, a density exceeding observed field populations of 10^2 to 10^3 prop/g. Bacterization with P. fluorescens has had no effect on tuber yields in field soil with the low inoculum densities of V. dahliae (10). Optimum cultural practices such as crop rotation, fertilization, and irrigation suppress Verticillium wilt in Russet Burbank potatoes most years. Therefore, bacterization following good cultural practices apparently will not give additional control and increase yield.

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Figure 1. Effect of initial populations of Pseudomonas fluorescens and Verticillium dahliae on fresh weight of potato shoots.

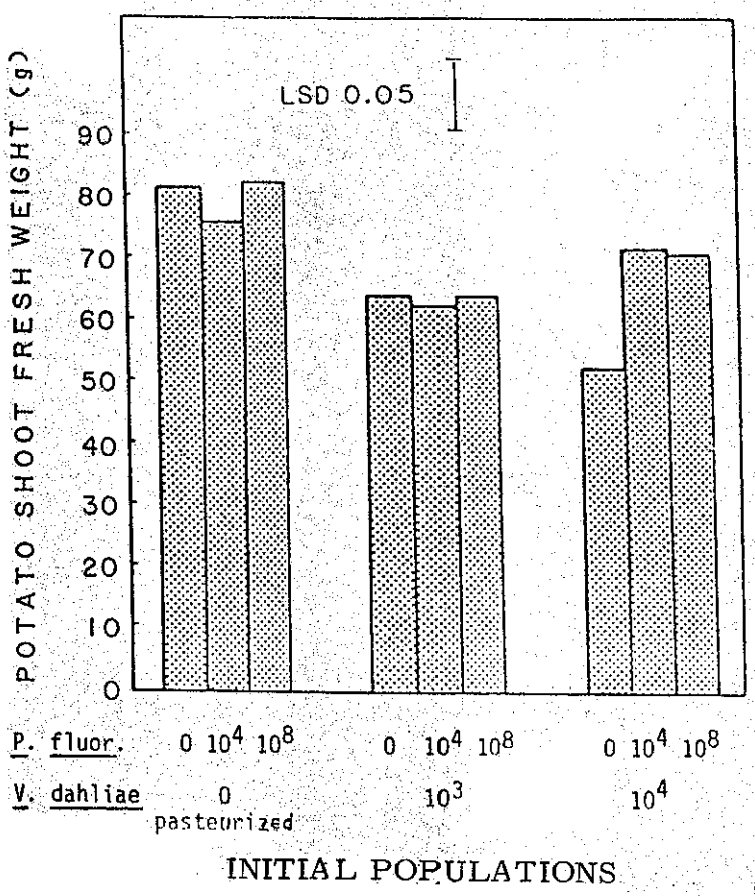

Table 1. Populations of Pseudomonas fluorescens M-4 on roots of potato plants grown in Verticillium dahliae-infested or pasteurized soil after bacterization of potato seedpieces.

Population of V. dahliae in soil	Log population of P. fluorescens 0	10^{4w}	10^{8w}
0	0	5.01[x]b[z]	5.29 a
10^{3y}	0	3.29 e	4.22 c
10^{4y}	0	3.89 d	4.36 c

w cfu/g of P. fluorescens dusted on seedpieces at planting.
x Log_{10} (cfu + 1)/g root.
y V. dahliae prop/g soil.
z Mean separation by protected LSD = 0.27, P = 0.05.

Table 2. Influence of previous potato cropping and initial populations of Pseudomonas fluorescens on shoot fresh weight of potatoes grown in Verticillium dahliae-infested soil.

V. dahliae-infested soil	Fresh shoot weight (g) 0	10^{4x}	10^{8x}	Mean
Cropped	68.0	77.3	70.5	71.9 a[y]
Uncropped	49.8	57.7	63.1	56.9 b
Mean	58.9 b[z]	67.5 a	66.8 a	

x cfu/g of P. fluorescens dusted on seedpieces at planting.
y Mean separation by Fisher's F test.
z Mean separation by protected LSD = 7.2, P = 0.05.

Table 3. Effect of initial populations of Pseudomonas fluorescens on potato plant height and tuber weight.

Initial population of P. fluorescens[x]	Plant height (cm)	Tuber weight (g)
0	46.9 c[y]	51.60 a[z]
10^4	51.3 b	48.17 a
10^8	54.5 a	52.10 a

x cfu/g dusted on seedpieces at planting.
y Mean separation by protected LSD = 3.0, P = 0.05.
z Mean separation by protected LSD = 4.11, P = 0.05.

Figure 2. Effect of initial populations of Pseudomonas fluorescens and Verticillium dahliae on fresh weight of potato roots.

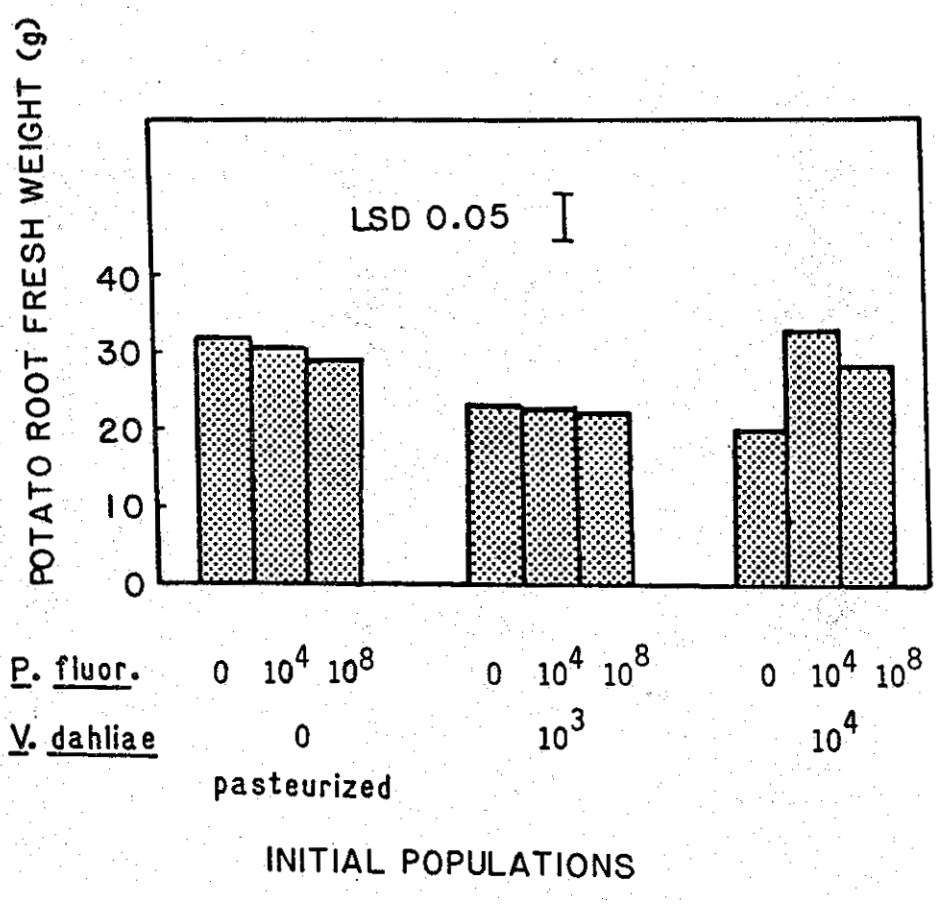

Figure 3. Effect of initial populations of Pseudomonas fluorescens on rhizosphere and stem populations of Verticillium dahliae. Different letters denote significant differences, P = 0.05.

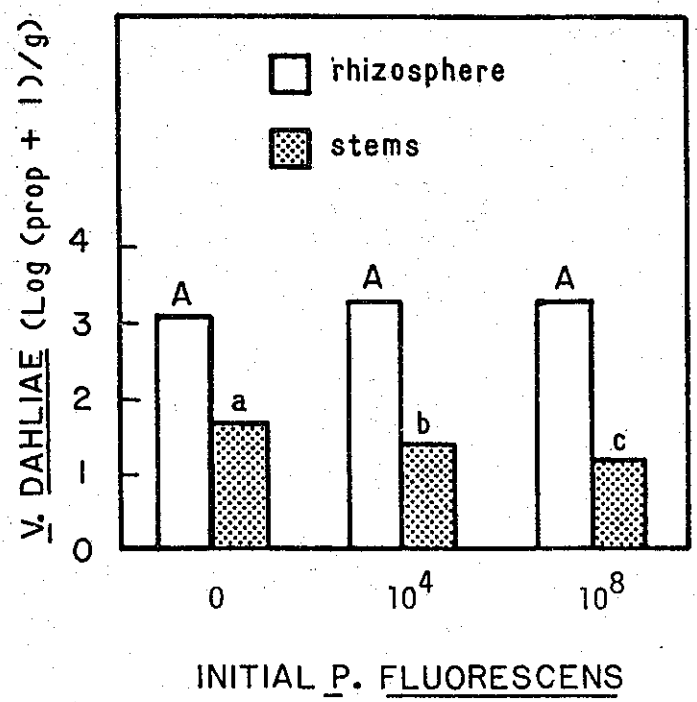

Table 4. Effect of initial populations of Pseudomonas fluorescens and cropping history on the index of Verticllium wilt.

	Index of Verticillium wilt[x]			
Soil	0	10^{4w}	10^{8w}	Mean
Cropped	3.8	2.8	2.7	3.0 b[y]
Uncropped	8.2	6.9	6.3	7.2 a
Mean	6.0 a[z]	4.9 b	4.5 b	

w cfu/g dusted on seedpieces at planting.
x Ratings based on % foliage and wilted, 0 = no wilt, 10 = 100% wilted.
y Mean separation by Fisher's F test.
z Mean separation by protected LSD = 0.52, P = 0.05.

Figure 4. Effect of method of application of Pseudomonas fluorescens on root populations throughout the growing season. Different letters denote significant differences, P = 0.05.

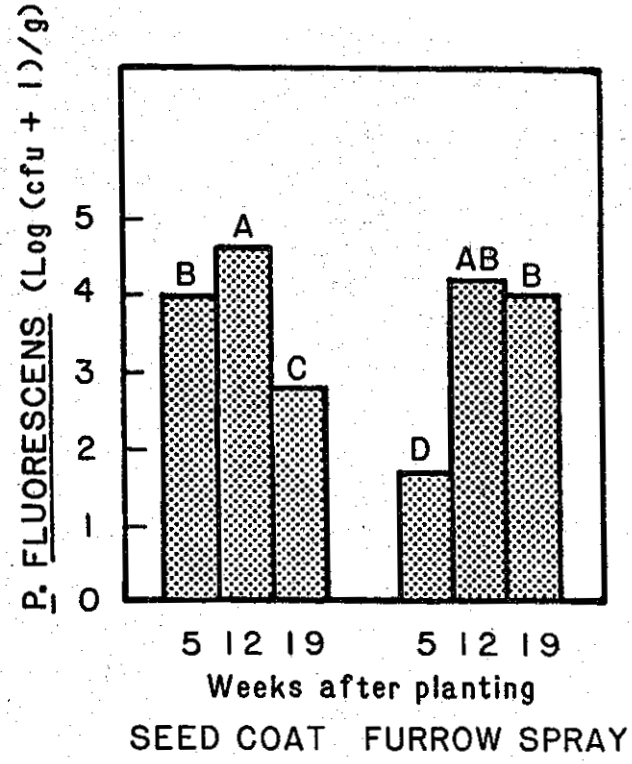

Table 5. Effect of method of application of Pseudomonas fluorescens (M-4) on field populations of Verticillium dahliae in the rhizosphere.

	Log population of V. dahliae[y]		
Application method	June	July	Sept
Seedpiece M-4-treated	0.76[z]	2.12[z]	1.48[z]
Seedpiece untreated	1.23	2.84	2.02
Furrow spray M-4	1.14	1.79	1.23
Furrow spray untreated	0	2.28	1.45

[y] Log_{10} (prop + 1)/g soil
[z] Column means not significantly different according to Duncan's Multiple Range Test, P = 0.05.

Table 6. Effect of method of application of Pseudomonas fluorescens (M-4) on incidence of Verticillium wilt and stem infection.

Application Method	% wilted plants[w]	V. dahliae[x]
Seedpiece M-4-treated	35[y]	2.2[z]
Seedpiece untreated	52	2.3
Furrow spray M-4	22	2.0
Furrow spray untreated	23	2.5

[w] Percent of total plants in a 20 ft single row showing wilt symptoms.
[x] Mean Log_{10} (V. dahliae prop + 1)/g in dried, ground tissue of 10 stems/plot.
[y] Differences not significant according to Duncan's Multiple Range Test, P = 0.05.
[z] Differences not significant according to Duncan's Multiple Range Test, P = 0.05.

Table 7. Effect of method of application of Pseudomonas fluorescens (M-4) on potato yield and tuber quality.

Application method	Yield (cwt/A)[w]	% U.S. No. 1 tubers[x]
Seedpiece M-4-treated	919 a[y]	54[z]
Seedpiece untreated	861 ab	53
Furrow spray M-4	833 ab	57
Furrow spray untreated	806 b	57

[w] Calculated from the weight of tubers from single row 20 ft long.
[x] Percent by weight of U.S. No. 1 tubers.
[y] Different letters denote significant differences according to Duncan's Multiple Range Test, P = 0.05.
[z] Differences not significant according to Duncan's Multiple Range Test, P = 0.05.