Entomology and Applied Science Letters
Volume 5, Issue 2, Page No: 55-61

Available Online at: www.easletters.com

ISSN No: 2349-2864

Pythium-Induced Root Rot of Potato and Its Control

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ABSTRACT

In recent years, the frequency of Pythium leak of potato, caused by a soil pathogen Pythium ultimum, increased in many regions of Russia. This soilborn pathogen infects tubers in the field and storage facilities and also affects plant roots causing their rot. In spite of many publications about Pythium leak of tubers, data on the influence of this pathogen on the growth and development of potato plants are absent. In this study performed under greenhouse conditions, the effect of a pre-planting soil infection with P. ultimum on some morphometric traits of potato plants (cvs. Lorh and BP-808) was investigated, and the efficiency of a protective pre-planting treatment of soil with the Uniform (azoxystrobin + mefenoxam) fungicide was evaluated. The most negative influence of the pathogen presence in the soil was observed for plants of the middle-late cv. Lorh. For both cultivars (Lorh and BP-808), the pathogen caused reduction of the plant height (by 35 and 23%), root length (by 47 and 35%), and total yield (35 and 26%); in the case of cv. Lorh, a decrease in the germination rate and the number of stems per a plant were also registered (6 and 10%, respectively). A pre-planting soil application of the Uniform fungicide not only prevented the above-described negative influence of P. ultimum, but also provided a significant positive effect on the development of plants. In the case of such treatment, plants of cv. Lorh demonstrated increase in their height, number of stems, root length, and the total yield (24, 10, 29, and 29%, respectively) comparing to the control; for plants of the middle-early cv. BP-808, pre-planting Uniform application resulted in a significant increase in the root length and total yield (10 and 11%, respectively). Thus, the Uniform fungicide may be considered as an efficient tool to control Pythium-induced rot.

Keywords: Pythium Ultimum, Potato, Pythium-Induced Rot, Uniform Fungicide, Root System Development

HOW TO CITE THIS ARTICLE: Kuznetsova M.A., Rogozhin A.N., Smetanina T.I., Demidova V.N., Denisenkov I.A., Statsyuk N.V., Pythium-Induced Root Rot of Potato and Its Control, Entomol Appl Sci Lett, 2018, 5 (2):55-61.

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Received: 09/01/2018
Accepted: 26/04/2018

INTRODUCTION

Pythium genus, which includes plant pathogenic microorganisms, belongs to the division *Oomycota*, class *Oomycetes*, order *Pythiales*, and family *Pythiaceae* (http://www.indexfungorum.org). *Pythium* species have a fine and colorless non-septate mycelium, which diameter reached 7 µm, and chlamydospores. At the anamorphous stage, they form zoosporangia, in which biflagellate spores are generated. At the teleomorphic stage the pathogen prodices spherical oogonia with elongated antheridia. Fertilization results in the formation of single smooth oospores [1, 2] The main sources of infection are zoospores and oospores; the last ones may survive in the soil up to 10 years [3].

To date, more than 150 plant pathogenic *Pythium* species are known around the world. In the case of Russia, a tendency to the increase of a *Pythium* fraction in fungal coenoses causing root rots of various agricultural crops (red beet, wheat, barley, pea, sunflower, maize, potato, cucumber, etc.) is observed in recent years [2, 4-8].

Infection of wheat and barley with pathogenic *Pythium* spp. causes reduced seed germination, delayed growth and development of plants, loss of fine roots and root hairs, and, therefore, yield losses. An intensive development of a *Pythium*-induced root rot is observed in the case of a cold and wet spring, when the development of plant root system is delayed, and, due to air deficiency in over-wet soil, the dumping-off of peripheral roots occurs. Inhabiting dead root parts, the parasite produces toxic substances killing adjacent live cells; in that way the pathogen moves along plant roots. Under conditions favorable for the pathogen development, potato yield losses caused by the *Pythium*-induced root rot may reach 5-9% (http://aspria-seeds.com/pythium-spp/).

During a routine quality assessment of potato tubers sent to the All-Russian Research Institute of Phytopathology from different regions of Russia, we observed an increased frequency of a leak rot of tubers caused by *Pythium* spp.; this phenomenon was observed mainly in the years characterized by a hot summer. In the most cases, tubers are infected with *Pythium ultimum* Trow. (syn. *P. debaryanum*), *P. aphanidermatum,* and some other species [9]. Infected tubers are characterized by wet black lesions on their surface with cankers formed under these lesions. The cover tissue of cankers becomes stretched and then breaks resulting in excretion of a specific fluid. A tuber in section has a grey affected area with a dark border.

On exposure to air, affected tissues turn brown, then black and may smell alcoholic. Infected tubers become soften, and their internal part often is completely rotten, so only tissues located beyond the vascular ring remains relatively intact. *Pythium*-induced root rot may be the reason of tuber decay in storage facilities, but its identification may be difficult because of the presence of secondary infections. At the same time, a well-visible dark border between healthy and infected tissues represents a typical sign of this infection [10].

Pythium species survive in the soil and penetrate into tubers only through mechanical injuries, especially in the case of a hot summer. Infected seed tubers cause dumping-off of plants in a field. At the same time, there are no published data on the effect of the soil infection with *Pythium* spp. on the growth and development of potato plants during a vegetation season.

The purpose of this study was the investigation of the effect of pre-planting soil inoculation with *Pythium ultimum* on the growth and development of potato plants and also the determination of efficiency of a pre-planting soil application of the Uniform fungicide (azoxystrobin + mephenoxam) to control the *Pythium*-induced root rot.

MATERIALS AND METHODS

The study was carried out in the March–June of 2017 under greenhouse conditions using an artificial infection background. Soil inoculation was carried out using a *Pythium ultimum* strain isolated from a potato tuber in Moscow region in 2014. Potato cultivars used in the study included middle-late Lorh and middle-early BP-808 cultivars bred by domestic and foreign breeders, respectively. Before planting, tubers of the BP-808 cultivar were undergone to a 3-week vernalization; to the date of planting, they had sprouts and roots reaching 3 cm in lengh. The sod-podzol soil used in the experiment was characterized by a humus content equal to 4.3%. The content of mineral compounds (mg/100 g of soil) was the following: P_2O_5 – 1062; K_2O – 503; Mg – 2.17; and CaO – 10.1.

For each cultivar, the experiment included the following variants (see Table 1):

Variant 1: Soil inoculation with *Pythium ultimum.*

Variant 2: Soil inoculation with *Pythium ultimum* + treatment with the Uniform preparation.

Variant 3: Control (soil inoculation with distilled water).

Each variant was arranged in six replications (pots); each pot was used to plant three tubers. Thus, each variant included 18 planted tubers.

Table 1. Scheme of the experiment

Variant no.	Description		
1	Soil (36 kg)	+ suspension of *Pythium ultimum* (1 L)	+ Distilled water (1 L)
2	Soil (36 kg)	+ suspension of *Pythium ultimum* (1 L)	+ working s olution of the Uniform preparation (1 L)
3	Soil (36 kg)	+ Distilled water (2 L)	

In the March of 2017, three 36-kg soil samples were prepared from the soil collected in the preceding autumn. One sample (control) was treated with 2 L of distilled water, while two others were combined in a one tray (3 m^2) and treated with the suspension of *Pythium ultimum* (2 L;

see Table 1). To obtain inoculum, *Pythium ultimum* strain was cultured in Petri plates with potato-glucose agar for 12 days. Then 10 Petri plates were washed with distilled water (20 mL per a plate). The obtained inoculum (spore–mycelium suspension) was filtered through a two-layer gauze to remove agar medium residues, and the volume of the resulting filtrate was adjusted to 2 L using distilled water; the final concentration of the inoculum was 1.6×10^6 spores/mL. After addition of the inoculum, soil was mixed to provide a uniform distribution of the pathogen in the whole volume. Then soil was divided into two equal parts, which were transferred onto 1.5-m^2 trays. One sample was supplemented with 1 L of the Uniform preparation (at a dosage of 1.5 L/ha), while the second one was supplemented with 1 L of distilled water to provide the same humidity level of all samples.

Pots of each variant were filled with the corresponding soil and placed in separate trays to prevent possible cross-contamination with *Pythium ultimum* or Uniform between variants during watering. Potato tubers were planted into pots at a depth of 10 cm.

To provoke stress conditions, the watering of plants was carried out one time a week for the first month and one time per two weeks for the rest of the season. The volume of watering provided a full saturation of soil. The weight of each pot with soil was 3 kg; in the case of a full saturation with water, it reached 4 kg.

In the beginning of the vegetation season, the day/night air temperature in a greenhouse box was 14–16/9–12°C; during the active plant growth stage, it was maintained at the level of 24–28/16–18°C. The planting date was March 9; the date of registration of the disease development level was April 10; the harvesting date was June 16.

For the first registration of the disease development level, three pots from each variant were taken. The pots were submerged to 10-L buckets with water to prevent the damage of roots during their taking out of the soil. After the removal of soil, the following plant development indices were assessed: stem height (cm), stem number per a plant, root length (cm), and level of affection of roots and stolons (scores, see Table 2). During harvesting, the total tuber weight was additionally registered.

Table 2. Scale for determination of the level of affection of potato roots and stolons with *Pythium*-induced rot

Manifestations	Scores
Stolons	
Healthy stolons	0
Some surface cankers are observed	1
Deep cankers affecting the whole circumference of stolons, but not causing a plant death	2
The end of a stolon and its young forming tuber are dead	3
Roots	
No affection	0
Weak affection	1
Strong affection	2

The statistical treatment of data was carried out according to Dospekhov (1985) at a 95% confidence interval using Microsoft Excel 2003 and Statistica 6.0 software packages [11].

RESULTS

The performed study showed that pre-planting soil inoculation with *Pythium ultimum* negatively influenced the germination, growth, and development of potato plants. The maximum negative effect was observed for the Lorh cultivar planted without any preliminary sprouting (Table 3, Figs. 1–4). Shoot appearance was delayed by 5 days. Plants were characterized by a significantly lower height and number of stems, and had shorter roots (Table 3, Figs. 1–4, 5a, 6a). Obviously, prolonged germination of tubers of the Lorh cultivar caused protraction of the “susceptible” phase of development. As a result, first roots were actively affected by the pathogen causing reliable inhibition of a plant development. In contrast to other experimental variants, roots of plants almost did not have root hairs (Fig. 7).

Table 3. Effect of soil inoculation with *Pythium ultimum* on the germination and biometric parameters of potato plants (cv. Lorh) and protecting efficiency of the Uniform fungicide

Morphometric parameters and indices of plant affection with a pathogen	Variants			$HCP_{0,95}$
	1 (*Pythium*)	2 (*Pythium* +Uniform)	3 (Control)	
Germination rate	17 ± 0.7	18 ± 0	18 ± 0	0.5
% of the control	–6%	0%		3%

Stem height, cm	26.4 ± 0.6	50.4 ± 1.3	40.6 ± 1.7	2.2
% of the control	–35%	+24%		5%
Stem number	2.6 ± 0.6	3.2 ± 0.5	2.9 ± 0.2	0.29
% of the control	–10%	+10%		10%
Root length, cm	7 ± 0.9	17 ± 0.9	13.2 ± 1.4	1.5
% of the control	–47%	+29%		12%
Yield, g/pot	287 ± 1.8	570 ± 2.7	443 ± 1.1	100.8
% of the control	–35%	+29%		24%
Stolon affection, scores	3	0	0	-
Root affection, scores	2	0	0	-

Table 4. Effect of soil inoculation with *Pythium ultimum* on the germination and biometric parameters of potato plants (cv. BP-808) and protecting efficiency of the Uniform fungicide

Morphometric parameters and indices of plant affection with a pathogen	Variants			$HCP_{0,95}$
	1 (*Pythium*)	2 (*Pythium* +Uniform)	3 (Control)	
Germination rate	18 ±0	18 ±0	18 ±0	
% of the control	0%	0%		
Stem height, cm	38,8 ±1,8	51,6 ±1	50,4 ±1,4	2,6
% of the control	-23%	+2%		5%
Stem number	3,6 ±0,5	3,6 ±0,5	3,6 ±0,5	0,2
% of the control	0%	0%		
Root length, cm	11,1 ±1,2	18,9 ±0,4	17,2 ±1,1	1,2
% of the control	-35%	+10%		7
Yield, g/pot	467 ±0,9	705 ±1	635 ±1	27
% of the control	-26%	+11%		4
Stolon affection, scores	2	0	0	-
Root affection, scores	2	0	0	-

Germinated tubers of the BP-808 cultivar developed more actively and were less infected with *Pythium ultimum* in the beginning of a vegetation season (Table 4, Figs. 1–4, 5b). At the same time, pathogen presence in the soil and influence of abiotic factors (temperature and soil humidity drops) observed during a vegetation season, facilitated infection of the root system. In spite of a sparing harvesting method, we observed a large number of injured stolons and roots (watery appearance, presence of necroses and brown constrictions) in plants grown in soil inoculated by *Pythium ultimum.*

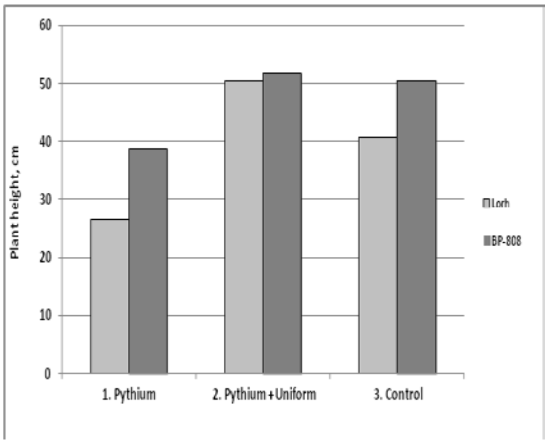

Fig. 1. Influence of the *Pythium ultimum* presence in soil on the height of potato plants (cvs. Lorh and BP-808) and efficiency of a pre-planting protective treatment of soil with the Uniform fungicide.

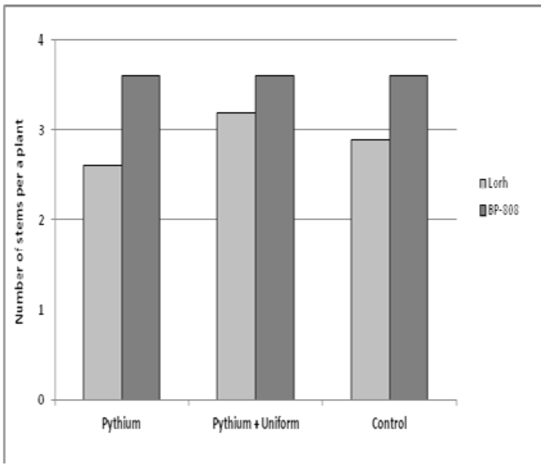

Fig. 2. Influence of the *Pythium ultimum* presence in soil on the number of stems in potato plants (cvs. Lorh and BP-808) and efficiency of a pre-planting protective treatment of soil with the Uniform fungicide.

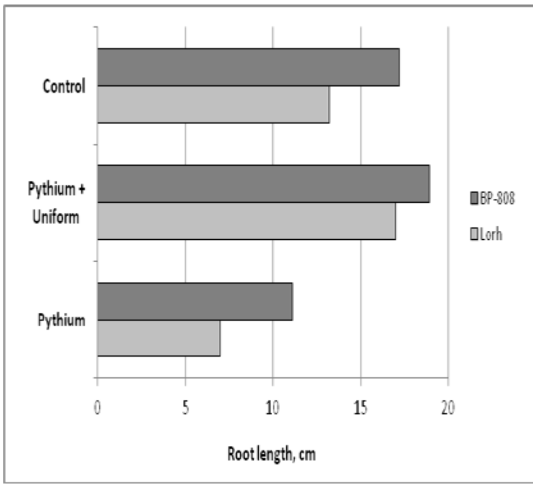

Fig. 3. Influence of the *Pythium ultimum* presence in soil on the length of potato roots (cvs. Lorh and BP-808) and efficiency of a pre-planting protective treatment of soil with the Uniform fungicide.

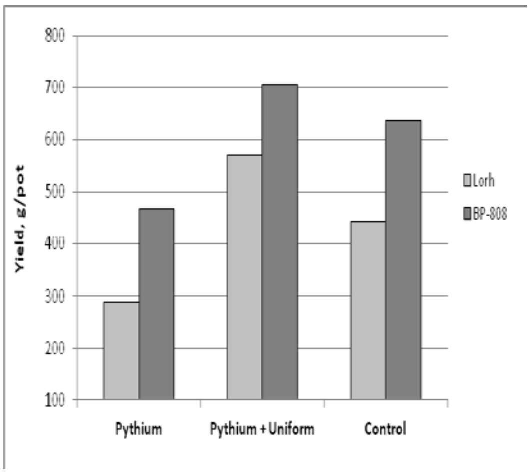

Fig. 4. Influence of the *Pythium ultimum* presence in soil on the potato yield (cvs. Lorh and BP-808) and efficiency of a pre-planting protective treatment of soil with the Uniform fungicide.

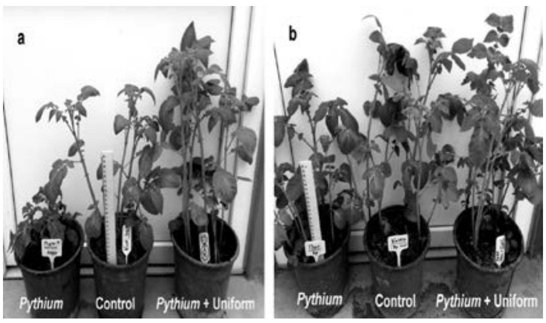

Fig. 5. Effect of *Pythium ultimum* and the treatment with the Uniform fungicide on the development of potato plants of (a) cv. Lorh and (b) cv. BP-808.

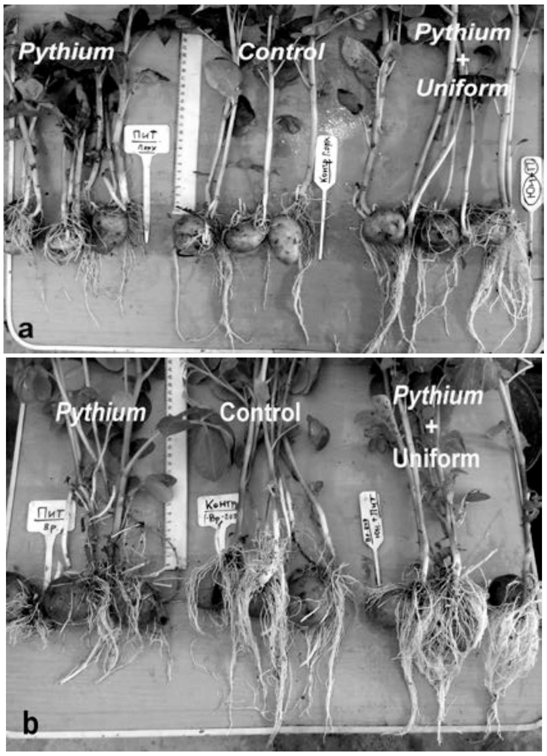

Fig. 6. Effect of *Pythium ultimum* and the treatment with the Uniform fungicide on the root development in potato plants of (a) cv. Lorh and (b) cv. BP-808.

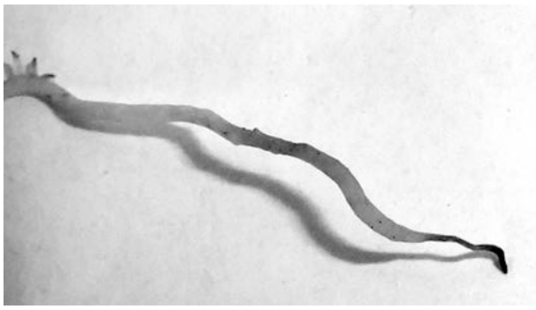

Fig. 7. Visual manifestations of a potato root infection (cv. Lorh) with *Pythium ultimum.*

DISCUSSION

Pythium ultimum is known as a pathogen causing the damping off and root rot diseases of a wide range of plant species. At the same time, in the case of potato it is considered mainly as a tuber disease developing in a post-harvest period due to infection of tubers via harvesting wounds [12, 13]. As far as we know, there are no publications describing the exact effect of this soil-born pathogen on the potato plant development during vegetation season. From this point of view, information obtained in this study is new and valuable. As we showed, *P. ultimum* presence in soil results in a very strong affection of roots: their number and length were significantly reduced comparing to the control. In addition, a significant affection of stolons was also observed, as well as reduction in the number of stems and their height. As a result, infected plants did not receive the required amount of water and soil nutrients, while reduced volume of the haulm limited the synthesis of necessary organic compounds. All these factors determined a final yield reduction. A comparison of the effect of *P. ultimum* presence on the development of plants from germinated and non-germinated tubers showed that pre-panting germination of tubers, which accelerates plant development, is able to reduce in some extent the consequences of the infection and, therefore, may be used as an additional tool to control the Pythium leak of potato.

In the second part of our study we evaluated the effect of the soil application of the Uniform fungicide to control Pythium leak. A common way to suppress the development of *P. ultimum* on various agricultural crops is in-furrow application of mefenoxam- or fludioxonil-based fungicides, such as Ridomil Gold, Ridomil Gold MZ, Ultra Flourish, or Flouronil [12, 14]. At the same time, resistance of *P. ultimum* to metalaxyl and mefenoxam has been reported from several locations in the US and Canada [12]; therefore, the effect of fungicides based on different active substances may be more reliable. In our case, the fungicide tested included azoxystrobin and mefenoxam. This fungicide not only prevented the negative influence of *P. ultimum*, but also increased the root length, total yield and also (in the case of the middle-late cv. Lorh) plant height and number of stems. Therefore, the Uniform fungicide may be considered as an efficient tool to control *Pythium*-induced rot.

The obtained results open some new possible directions for future studies. First, there are some publications about a successful use of *Brassica* green manures or antagonistic microorganisms to control Pythium leak [15, 16] and also about a combined application of fungicides and antagonistic *Pseudomonas fluorescens* strain [17]. It would be probably promising to test the efficiency of the Uniform fungicide in combination with some bacteria producing antifungal metabolites. In addition, the evaluation of the possibility of *P. ultimum* to develop resistance to this fungicide would be useful for the further successful control of this disease.

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