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POPULATION DYNAMICS AND CHARACTERIZATION OF *CLOSTRIDIUM MACERANS* ON HOST PLANT OF FLAX

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The *Clostridium (Bacillus) macerans* is the most common bacteria in the soil that under certain conditions turns to pathogen for flax (*Linum usitatissimum* L) causing serious disease and reducing yield of the plant. A detailed understanding of flax and pathogens interactions would underpin crop improvement for fibre and seed production. The aim of this study was to clarify possibilities of development of the bacteriosis in dependence of genotypes of flax and environmental conditions. Experimental material for the study consisted of 25 flax genotypes. The evaluation was carried out of field trials over the period of time from 2015 to 2016 and of seeds phytosanitary qualities from 2014 to 2016 in the Research Centre of Priekuli, part of Vilani in Latgale. Progress of the disease was estimated every week. For the each estimation the index of severity of disease and the area under the disease progressive curve (AUDPC) were calculated. The highest and statistically significant pathogen's AUDPCs were observed in the beginning of flowering and in full flowering stages of flax. There was found that the *Clostridium macerans* population statistically significantly be changed depending from flax genotypes both in field trails and in the seeds. The most resistant genotypes were 'S13/5-7/5-93', 'S64-17-93'and 'Ruda 1'. The correlation was found that most genotypes of flax have susceptibility to diseases in arid years.

Key words: *Clostridium macerans*, AUDPC, disease severity index, flax.

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INTRODUCTION

Bacteriosis caused by *Clostridium macerans* Schardinger (syn. *Bacillus macerans* Schardinger, 1905) is the saprophytic bacteria of soil, widespread in nature and constantly accompany flax (*Linum usitatissimum* L) (Лошакова et al. 2000). The bacterial diseases reported to infect flax crop are very few (Jaczewskii 1928, Hetegurova, 1957). Detailed of phytopathological works about flax of the causal agent *Clostridium maceran* were studied more in Russia (Лошакова et al. 2000) and Belarus (Миренков et al. 2003).

Disease could cause death of the plant and plants thinning in sowing field, but in flax later development phases it could reduce yield of stem by 40% and of seeds - from 18 to 20% (Лошакова

et al. 2000) and in the epiphytotic years even more (Easson & Molloy 2000). The bacteria enter the plant from the soil through the plant's root cover and spread along the vascular bundles in plant's stem and preserved in soil as well in seeds. The bacteria are moving sticks with round tips with sizes 4-5 x 0.8-1 μm, gram-negative rods, spore are oval (Лошакова et al. 2000).

Insufficient moisture and high temperature, excess phosphorus and boron deficiency contribute to development of the disease (bacteriosis) of flax (Лошакова et al. 2000). Similar influence on the bacteriosis of flax have dark-coloured, humus, excessively compacted or re-emerged soils in which organic substances slowly decompose and insoluble compounds predominate - in this case bacteria do not receive adequate nutrition and pass to a parasitic lifestyle causing flax disease (Миренков et al. 2003). However, there is still a lacking in a thorough understanding of bacterial disease epidemiology and how bacteria make transition from one life style to other during an infection process (Mansfield et al. 2012, Nomura et al. 2005, Underwood et al. 2007, Vidaver & Lambrecht 2004). Species of *Bacillus* are common inhabitants among the resident microflora of inner tissues of various species of plants, including cotton, grape, peas, spruce, and sweet corn, where they play an important role in plant protection and growth promotion (Bell et al. 1995, Shishido et al. 1999, Berg et al. 2005).

The area of research in plant-bacterial interactions has been well studied. Several mechanisms have been proposed to understand pathogenicity of bacterial pathogens in plants (Ahlemeyer & Eichenlaub 2001, Burger & Eichenlaub 2003).

However, scientists are still facing big challenges such as proper annotation of bacterial genomes and functions of all genes. Normally, a higher population of bacteria, ~106 CFU (colony forming units/millilitre) is required to cause diseases in plants (Freeman & Beattie 2008). Compared to fungi and viruses, plant pathogenic bacteria cause relatively less damage and economic cost throughout the world (Kennedy & Alcorn 1980, Vidhyasekaran 2002, Glazebrook 2005, Freeman & Beattie 2008).

The *Clostridium macerans* affects plants in all phases of their development, but manifests itself in different ways. The bacteriosis causes two types of infections death of the root tip and death of the growth point of the stem. With the most dangerous form of disease the growth site dies at the shoots and the knotty of the roots arises. Less dangerous is the partial damage of seedlings and flax seedlings, associated with the infection of seeds. They do not germinate or give seedlings, which, rotting, become transparent, and then brown. With a later bacteriosis (in the budding or flowering phases), which is often associated with arid conditions, the death of the tops of the stem is noted, the upper leaves become blush or yellow, the lower part of the plant remains green and powerful (Лошакова et al. 2000).

Progress of disease in plants is usually observed several times during pathogen epidemics. Extent of disease is assessed at each observation using scales that are based on disease incidence, severity, or a combination of both. To combine these repeated observations into a single value, Van der Plank (chapter 12 of literature citation 13) proposed calculating the area under the disease progress curve (AUDPC) (Simko & Piepho 2012) for summarizing and comparing plant disease epidemics.

The presented study tries to clarify the possibilities of development of the *Clostridium macerans* in dependence on genotypes of flax and environmental conditions.

MATERIAL AND METHODS

The research was conducted at the Research Centre of Priekuli, part of Vilani in Latgale. Plants were grown in random block plots 1 m^2 with a distance between rows 10 cm, 1700 flax seeds per 1 m^2 were sown by hand with sowing depth 1.5 - 2 cm. Prior to that sowing seeds' germination tests were performed for all used genotypes. Seeds

were sown between 1st and 2nd decades of May. Flax was grown in humi-podzolic gley soil. The main agrochemical parameters of the arable soil layer were following: humus content – 6.5%, pH_{KCl} – 6.4 - 7.0, available P_2O_5 – 130-145 mg kg^{-1} and available K_2O – 118-124 mg kg^{-1} soil (by results of State Plant Protection Service).

The analyses of seed microflora and infected parts of plants were done following the method of Лошакова et al. (2000). The flax seeds were analysed at the 10 seeds from each sample with three replicates over the period of time from 2014 to 2016. In field trails were assessed for 30 plants of flax from each sample at the 1 m^2 plots every 7th day till flax pulling over the period of time from 2015 to 2016. Experimental material for the study consisted of 25 flax genotypes. For determination of internal pathogen infection the seeds were disinfected with 96% ethyl alcohol for 1 min then rinsed in distilled water and dried on sterile filter paper. The seeds and infected parts of plants were sown on agar in the Petri plates. After 5–7 days of incubation at a temperature of 30 °C the plates were inspected for infection on the plants. The presence of bacteria was conducted using a light microscopy.

The disease incidence was estimated by visual symptoms. Per cent of affected plants was estimated and disease severity was recorded following a five-point scale (Лошакова et al. 2000):
0 – healthy;
1 – weakly affected;
2 – moderately affected;
3 – heavily affected;
4 – very heavily affected or dead plants.

Disease severity index was calculated by applying formula:

$$I = \frac{\sum(ab) \times 100}{A \cdot S}$$

Where I – disease severity index %, a – number of infected plants, b – degree of infection, A – total number of plant samples (healthy and infected), S – the highest degree if infection (Лошакова et al. 2000).To combine these repeated observations into a single value the area under the disease progress curve (AUDPC) have been calculated by applying formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Where y_i is an assessment of a disease (percentage) at the i^{th} observation, t_i is time (in days) at the i^{th} observation, and n is the total number of observations (Van der Plank 1963, Simko & Piepho 2012).

Agro-meteorological condition was determined by ADCON installed meteorological stations which are connected to the computer program Dacom Plant Plus. The facility provided information in direct nearby field trials. The amount of precipitation in 2016 growing period was by 45% higher and in 2015 by 6% lower in comparison to the long-term average of 311 mm. According to the air temperature in 2015 average air temperature was 13.26 °C and in 2016, it was 14.00 °C, respectively, while the long-term average result is 13.00 °C.

MS-Excel software was used for data statistical analysis (Arhipova & Bāliņa 2006). Used data analysis tools Descriptive Statistics, ANOVA and correlation. The ANOVA analyse was used Fisher's protected least significant difference ($LSD_{0.05}$) for the average AUDPC pathogens at the genotypes of flax during ontogenesis and by years in field trails and disease severity index for seeds of flax. The correlation coefficient was used to analyse the effect of significant and not significant relationship between sum precipitation and severity index of seed for each genotype by years.

RESULTS AND DISCUSSION

The population of *Clostridium maceran* was estimated using the AUDPC of the flax during ontogenesis. (Fig.1.). Result was showed that

bacteriosis AUDPC not significant different between BBCH compared both growing period. Highest and statically significant pathogen AUDPC was observed in beginning of flowering and full flowering stages (BBCH-63 - BBCH-65).

The first symptoms of bacteriosis were observed on flax in BBCH-32 and latest in BBCH-75. In the field trials was observed the same type of infection as in dying point of stalk growth and it was a nodularity of the roots. In this case, the upper part of the plant is getting brighter, revolves, turns yellow then becomes copper-red colour and dry out. The recovery of infected plants was observed during the growing season.

Correlation between temperature and precipitation with average AUDPC of *Clostridium macerans* at the plants are low and not statistically significant during ontogenesis. It hat seems that the weather conditions' in summary did not significantly influence the spread of pathogens, but the spread of pathogens was dependent more from plant genotypes.

The bacteriosis were statistically significantly different in dependence on flax genotypes (showed in Table1.). Evaluation of pathogen and plant relationships showed that the genotype 'Rezekne' has resistance during ontogenesis but susceptible on the seeds. The results of the research of diverse flax genotypes show that bacteriosis on flax is influenced not only by the physiological condition of the plant, but also by the genetic interaction between the plant and the pathogen. Similar results were obtained by Родионов (2011) and Toropova et al. (2014) that provide information mainly about different genotypes response, but few characteristics about of pathogen development reasons.

By the average results of diseases severity index (%) there was found statistically significant difference between genotypes of the seeds (Table1.). The genetic diversity of flax genotypes and lower disease index is more valuable with less susceptibility. There were founded three resistant genotypes ('S13/5-7/5-93', 'S64-17-93', 'Ruda 1'), but in field trials they were susceptible. According to soil agrochemical indicators have not significantly different by years which suggested about complex factors influencing on the growth and development of pathogens and flax. Observation shows that infected seed on the roots appear brick red spots. By pathogen development of the roots the end dies off. On the

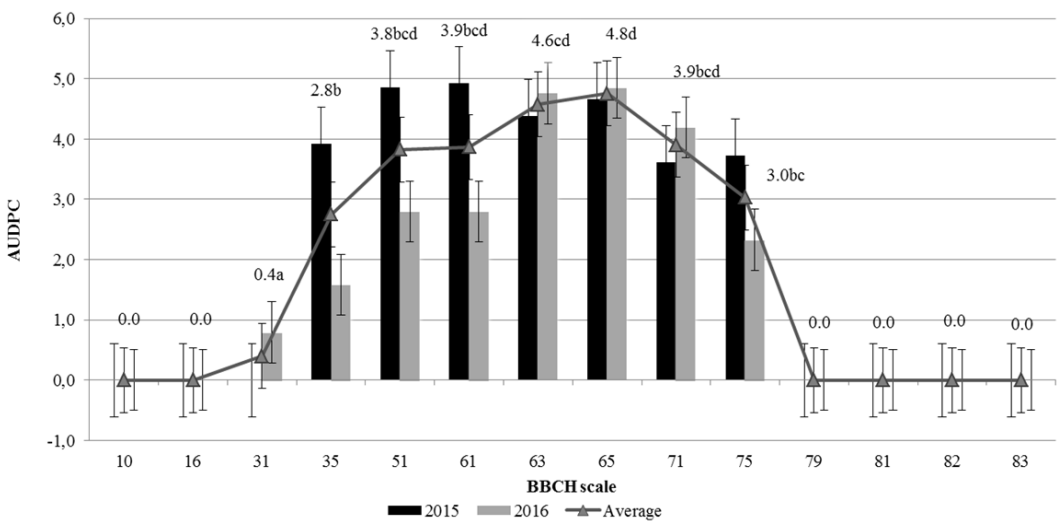

Fig.1. The AUDPC of *Clostridium macerans* as pathogen on the genotypes of flax during ontogenesis. [abcd] - AUDPC followed by the same letters in each column are not statistically significant by $LSD_{0.05}$ (1.69).

Table 1. The *Clostridium macerans* population severity of the flax genotypes of plants and seeds by years

Genotypes	Average AUDPC of the plants	Average diseases severity index (%) of the seeds
S13/5-7/5-93	18.66[a]	0.00
S32/4-8-93	72.68[b]	1.56 [abc]
S53/8-3-93	78.22[c]	8.00 [cd]
S64-17-93	22.14[ab]	0.00
T11-6/2-15-94	21.97 [ab]	0.22 [ab]
T11-13/3-1-94	30.78 [abc]	3.33 [abc]
T25/5-33/12-8-94	36.19 [abc]	1.78 [abc]
T29-36/10-5-94	52.46 [abc]	5.56 [cd]
T29-36/7-1-94	47.81 [abc]	0.89 [ab]
T31-40-94	43.17 [abc]	3.33 [abc]
T36-26/4-8-94	27.96 [abc]	0.89 [ab]
K47-17/11-1-95	22.12 [ab]	3.11 [abc]
K47-17/11-6-95	5.83[a]	4.67 [bcd]
L2-14/6-97	23.30 [ab]	1.33 [abc]
L11-11/10-97	17.48 [a]	3.56 [abc]
L11-11/11-97	23.35 [a]	1.78 [abc]
L19-6/15-97	9.34 [a]	1.11 [ab]
L23-26/3-97	30.35 [abc]	2.00 [abc]
L26-47/1-97	22.20 [ab]	4.00 [abcd]
Altgauzen	3.51 [a]	1.56 [abc]
Rezeknes	0.00	3.11 [abc]
Rota 1	10.49 [a]	2.22 [abc]
Rota 2	5.84 [a]	4.44 [abcd]
Ruda 1	8.16 [a]	0.00
ST Vega 2	43.76 [abc]	0.44 [ab]
$LSD_{0.05}$	53.35	4.24

[abcd] Means followed by the same letters in each column are not statistically significant.

subfamily knee, streaks are formed in the form of strokes and on the cotyledons of the shed with brick red bordering.

The pathogen spread, evaluated by disease severity index, varied on the field in range from 0.67% to 5% for the infected plants and in range from 0.67% to 16.67% for seeds. This suggests that the seeds are more susceptible to the spread of the pathogen that is one of the factors that affect the flax seed germination in field.

Positive and significant relationships were found between disease index and sum of precipitation (r=+9.7*) for flax genotype 'T31-40-94'. Negative and significant relationships were found between disease index and sum of precipitation for flax genotypes 'T29-36/10-5-94'(r=-9.7*), 'T29-36/7-1-94'(r= -9.5*), 'K47-17/11-1-95'(r= -9.7*) and 'L2-14/6-97'(r= -9.9**) (Fig.2.).

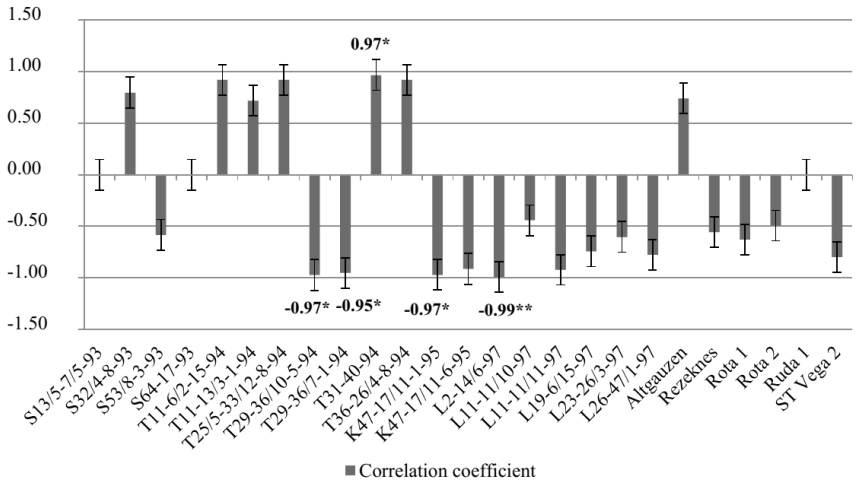

Fig.2. Correlation coefficient between disease severity index of *Clostridium macerans* and sum of precipitation (mm). * − correlation significant at p≤0.05, ** − correlation significant at p≤0.01

The genotypes with higher diseases index have positive correlation results in years with higher precipitation level and negative, when drier years occur. According to Лошакова et al. (2000) seeds are more affected when harvesting flax in rainy weather. The results by Toropova et al. (2014) showed that flax seeds bacteriosis was noted in the most humid and warm years, but in the subsequently more arid years the transfer of bacteria to flax seeds was not detected. However, the present study shows that the seeds infection is dependent also from genotype - from 25 genotypes of flax 60% of them has tendency to higher susceptibility of diseases in arid years, but 28% - in most humid years. The specificity of these plant-pathogen combinations is unclear and need to be analysed wider, taking into account the specific characteristics of the soil.

CONCLUSIONS

The infection of *Clostridium macerans* population could be significantly changed in dependence of the flax ontogenesis, genotypes and the environmental conditions. The most resistant genotypes were 'S13/5-7/5-93', 'S64-17-93' and 'Ruda 1'. The study proved that for the genotypes of flax the precipitation significantly influenced the spread of pathogens by years. However, the study does not provide biological reasons - why certain plant-pathogen combinations interact in a specific way while others do not. To gain more mechanistic understanding the specific studies are required.

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