

The Influence of *Tilletia* spp. Inoculum Source and Environmental Conditions on the Frequency of Infected Wheat Spikes

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SUMMARY

The influence of inoculum source on the incidence of common bunt, caused by fungi from the genus *Tilletia*, was estimated based on the frequency of bunt infected wheat spikes in our agroecological conditions. The cultivar Novosadska rana 5 was sown in a random split plot design with four replicates at Rimski Šančevi on three sowing dates in 1999/2000 and 2000/2001. The following variables were evaluated: I – control, II – soilborne inoculum (4 g teliospores/1 l soil), III – seedborne inoculum (2 g teliospores/1 kg seeds), IV – seedborne inoculum + soilborne inoculum (4 g teliospores/1 l soil + 2 g teliospores/1 kg seeds). Correlation and regression analysis were used to evaluate the effect of temperature and precipitation on the frequency of infected spikes.

The frequency of bunt infected spikes depended on the source of *Tilletia* spp. inoculum, and difference in infection frequencies between variables II and III, as well as III and IV, were determined for the assessed infection parameters.

When teliospores are the only source of inoculum in soil, 60 days after sowing ($r > +0.52$) is a critical period in which temperature influences the development of infection. The highest number of plants was infected in the first, while less were infected in the second ten days (decade) after sowing ($r > 0.41$), when temperature was the optimal 5.0-6.0°C.

The initial 60 days after sowing were also critical for disease development when teliospores on seeds were the only source of inoculum ($r > +0.50$). The highest number of plants was infected in the third and fewer in the fifth decade after sowing ($r > 0.41$), when temperature was the optimal 5.0-6.0°C.

When infection was caused by teliospores on seeds and in soil, the critical period lasted 120 days after sowing ($r > 0.42$), with a maximum frequency of infection found at the optimal temperatures for the period of 4.0-5.0°C.

Keywords: *Tilletia* spp.; Soil; Seed; Temperature; Rainfall

INTRODUCTION

Common bunt had a severe impact on organic wheat seed production during the 1990s, when chemical treatments of seed were largely discontinued. It had been considered eradicated in Serbia after the Second World War, but in the last decade of the 20th century it became widespread again, causing significant damage (Stojanović et al., 1993; Jevtić et al., 1997).

Common bunt is caused by two fungi, *Tilletia tritici* (Bjerk.) Wint. (syn. *T. caries* (DC) Tul) and *T. laevis* Kuhn (syn. *T. levis*, *T. foetida* (Wallr.) Liro, *T. foetens* (Berk. & Curt.) Schoert.) distinctive by the shape of their teliospores and regional distribution. Hybridization of these two species has been often reported. As different species, races and hybrids are present in Serbia, this article will be referring to them collectively as *Tilletia* spp.

Tilletia spp. survive as seedborne and soilborne inocula. It had been considered for a long time that *T. tritici* teliospores found on seed surface were the main source of inoculum. However, after developing during harvest and being deposited in fallow soil to remain there throughout the dry summer, they can germinate and infect wheat sown in the autumn (Purdy and Kendrick, 1957; Hoffmann, 1982; Williams, 1987; Yarham and Mckeown, 1989). This may also happen in cases of a hybridization between *T. tritici* and the soil pathogen *T. controversa*, the causal agent of dwarf bunt (Kendrick, 1964; Yarham, 1993).

Soilborne teliospores had not been considered an important source of inoculum until some commonly used fungicides for seed treatment failed to provide sufficient efficacy. Fungicides with carboxin or pentachloronitrobenzene as their active ingredients have been efficient in reducing seedborne but not soilborne inocula.

Fisher and Colton (1957) and Line (1993) (cited by Wilcoxson and Saari, 1996) reported that soilborne inocula of common bunt were viable in soil for different periods of time, especially during periods of low humidity and production of wheat year by year. These conditions are specific for Mediterranean agricultural areas (Parlak, 1986).

Purdy and Kendrick (1957) reported that wheat infection by soilborne teliospores was more severe when soil temperatures were 5-10°C. Disease incidence is reduced at temperatures higher than 15°C. They also reported that after soil inoculation with teliospore race

T-5 seven days before sawing, the incidence of common bunt increased at 15-20°C.

Johnsson (1992) found that a common bunt infection of winter wheat in field experiments performed over the period 1940-1988 was correlated with climate data. A positive correlation between the frequency of infected spikes caused by seedborne teliospores and temperature was found only for the period of 1-11 days after sawing. Temperature after germination of wheat, precipitation and duration of snow cover were not found to affect the frequency of infected spikes. The attack was strongest when the mean temperature during the critical period of days 1-11 after sawing was 6-7°C. When infection was caused by soilborne inoculum, both in the laboratory and the field, the frequency of infected spikes and environmental conditions strongly correlated over a period of one month after sawing.

Very few data are available on the effect of sawing dates when teliospores in soil are the only source of inoculum. The influence of temperature and precipitation on common bunt incidence in our agricultural conditions has not been examined in detail.

This work aimed to determine which source of inoculum and environmental conditions are more decisive on the outcome.

MATERIAL AND METHODS

Field experiments

Field experiments were carried out at Rimski Šančevi in the autumns of 1999 and 2000. Cv Novosadska rana 5, reported as highly susceptible to common bunt (Jevtić et al., 1997), was sown in a randomized split plot design on three sawing dates. The randomized split plot design was used in order to examine the influence of inoculum source and sawing dates on the frequency of infected spikes with the smallest possible experimental error. Four variables were examined in field experiments: **I** – control; **II** – soilborne inoculum; **III** – seedborne inoculum; **IV** – soilborne inoculum + seedborne inoculum.

Each variable had 4 replicates for each sawing date. Each plot had 1 m², or 6 rows that were 1 m long. The plots examined in the first experimental year were 100 m away from those examined in the second year. Soil at the locality of Rimski Šančevi was carbonated gleyed chernozem.

Noninfested seeds were used as the control variable. In variable III, seeds were infested with teliospores by mixing 1 kg of seeds with 2 g of teliospores according to a method described by Stojanović et al. (1997a, 1997b).

A mixture of soil and teliospores was prepared for variables II and IV. For each sowing date, 1 l of soil was prepared per each variable by mixing two parts of soil with one part of humus and a small amount of sand and homogenizing them with 4 g of teliospores. Each 1 l amount of mixture was divided into 24 parts. The application of 4 g of teliospores to 1 l of soil is equal to 2 g of teliospores to 1 kg of seeds as concentration of about 60.000 teliospores is achieved in both cases (EPPO standards, 1997).

Each plot was sown in rows with 120 seeds/m², or 20 seeds per row, to a depth of 5 cm. The mixture of soil and teliospores was incorporated in rows prior to sowing and the seeds sown on top. The sowing dates for the vegetation period 1999/2000 were: November 2 (optimal), January 12, and February 4 (late), and for 2000/2001: October 30 (optimal), December 6 (mid late), and December 22 (late).

Wild plants and weeds were removed during vegetation. Plants were not treated with insecticides, herbicides or fungicides in order to avoid any effect on the experiment.

Spikes were sampled during harvest from all six rows in each plot. The samples contained spikes from a maximum of 5 plants per row or 30 spikes per 1 m².

Analysis of the influence of inoculum source on the frequency of bunt infected spikes

A field experiment was set up to examine the influence of inoculum source on the frequency of bunt infected spikes. Common bunt frequency on spikes was monitored using the formula 1.

The analysis of variance was applied to detect significant or very significant differences between means of the examined variables, sowing dates and their interactions at 0.05 and 0.01 confidence levels. T-test was used to compare data by groups or individually for all sources of inoculum between years. An analysis of regression

was used to determine dependence of the frequency of bunt infected spikes on sowing dates.

Analysis of the influence of environmental conditions on the frequency of infected spikes after exposure to different *Tilletia* spp. inoculum sources

An analysis of the influence of environmental conditions on the frequency of infected spikes after exposure to different inoculum sources was performed using the analyses of correlation and regression. In order to determine correlation between temperature and precipitation and the examined parameter of infection, sums of temperatures and precipitation were calculated for the following periods after sowing (days): 1-5, 1-8, 1-11, 1-17, 1-20, 1-30, 1-60, 1-120 and 1-170. In regression analysis, sums of temperature and precipitation were calculated for the following decades after sowing: 1-10, 11-20, 21-30, 31-40, 41-50 and 51-60. Correlation and regression analyses were performed using Statistics for Windows software.

Statistical methods, including the analysis of variance, regression analysis and correlation analysis, were performed in order to analyse the examined parameters using M-Stat, Statistics for Windows and Excel (Microsoft, 1998).

RESULTS

Influence of *Tilletia* spp. inoculum source on the frequency of infected spikes in 2000

The frequency of infected spikes depended on the source of inoculum of *Tilletia* spp. (variables II, III and IV) in 2000. The highest percentage of infected spikes was found in variable IV (Figure 1). When seed-borne teliospores were the source of inoculum (variable II), significantly and very significantly fewer infected spikes were found, compared to infection caused by teliospores from soil and seed (variable IV) at 1% and 5% confidence levels for LSD_{0.01}=15.33 and LSD_{0.05}=11.41. Between variables II and III, as well as between III and

Formula 1.

$$\text{frequency of infected spikes} = \sum \left\{ \frac{\text{number of spikes with at least 1 infected grain}}{\text{number of examined spikes}} \times \frac{1}{6} \right\} \times \frac{1}{4}$$

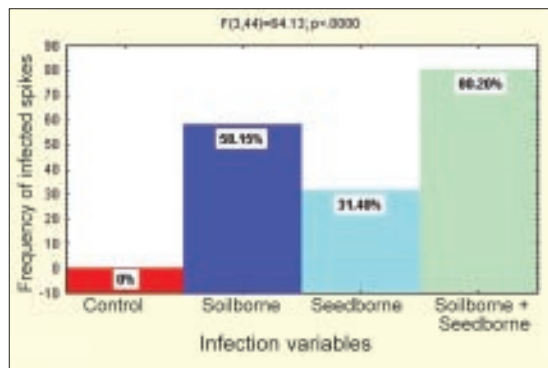


Figure 1. Influence of *Tilletia* spp. inoculum source on the frequency of infected spikes in 2000

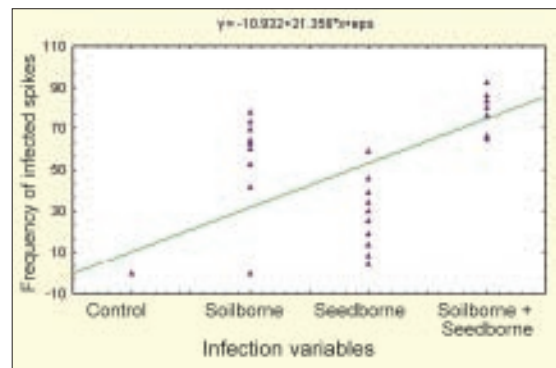


Figure 2. Dependence of the frequency of infected spikes on inoculum source in 2000 (y = frequency of infected spikes, x = source of inoculum of *Tilletia* spp.)

IV, significant differences in the frequency of infected spikes were determined as LSD values.

Sawing dates influenced significantly and very significantly the frequency of infected spikes at confidence levels 0.05 and 0.01 for $LSD_{0.05}=9.879$ and $LSD_{0.01}=13.27$. The highest frequency of infected spikes was found for the third sawing date (45.68%) and the least one for the optimal (39.82%).

When interactions between the source of inoculum and sawing dates were analysed, the highest frequency of infected spikes was found on the second sawing date for variable IV (Table 1). Significant and very significant differences in infected spikes frequency were not detected between variable IV on all sawing dates and variable II on the third sawing date at confidence levels 0.01 and 0.05 for $LSD_{0.01}=26.54$ and $LSD_{0.05}=19.76$. The lowest frequency of infected spikes was found for both seedborne and soilborne inocula on the second sawing date.

The frequency of infected spikes depended on the source of inoculum of *Tilletia* spp. (discrimination coefficient 0.73) in 2000 at confidence level $p < 0.05$. The regression equation $y = -10.932 + 21.358 * x + eps$, presents

a dependence of the frequency of infected spikes on inoculum source (Figure 2).

Influence of *Tilletia* spp. inoculum source on the frequency of infected spikes in 2001

The frequency of infested spikes depended on the source of inoculum (variables II, III and IV) in 2001 (Figure 3). Significant and very significant differences in the frequency of infected spikes were determined between variables II and III, as well as III and IV at confidence level 0.01 and 0.05 for $LSD_{0.01}=9.626$ and $LSD_{0.05}=11.12$.

Sawing dates had significant and very significant impact on the frequency of infected spikes in 2001 at confidence levels 1% and 5% for $LSD_{0.01}=9.622$ and $LSD_{0.05}=12.93$. The highest frequency of infected spikes was determined on the first sawing date (42,73%). There were no significant differences between late sawing dates (second and third) for the referred LSD values.

The frequency of infected spikes was lower when the sawing dates were in late autumn and winter for all ex-

Table 1. Frequency of infected spikes depending on inoculum sources and sawing dates (A – optimal, B and C – late) in 2000

| Source of inoculum | Frequency of infected spikes (%) | | |
|-----------------------------------|----------------------------------|--------------|-------------|
| | Sawing date | | |
| | A | B | C |
| I Control | 0.00 e E | 0.00 e E | 0.00 e E |
| II Soilborne inoculum | 61.45 abc BC | 43.03 bcd CD | 69.97 ab AB |
| III Seedborne inoculum | 15.60 de E | 37.98 cd D | 40.63 cd D |
| IV Seedborne + soilborne inoculum | 82.12 a AB | 86.47 a A | 72.01 a AB |

$LSD_{0.05}=19.76$; $LSD_{0.01}=26.54$

Values in columns followed by different letter differ significantly; small letters (P=0.05), capital letters (P=0.01)

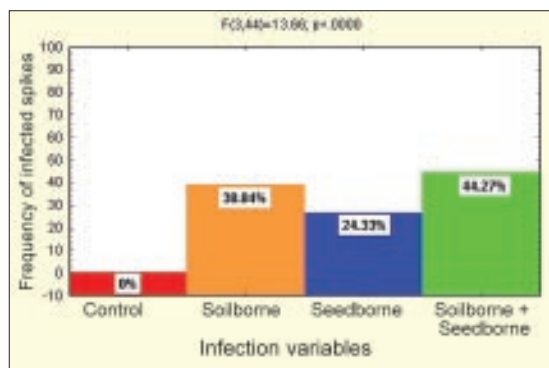


Figure 3. Influence of inoculum source on the frequency of infected spikes in 2001

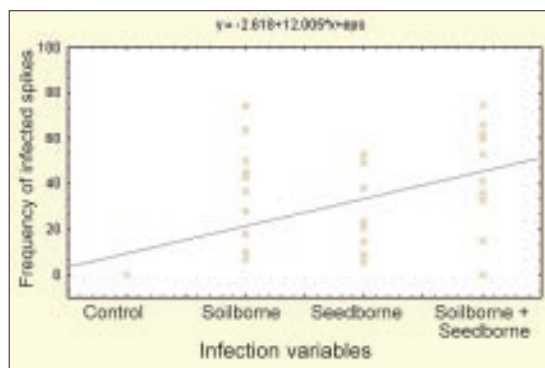


Figure 4. Dependence of the frequency of infected spikes on inoculum source in 2001 (y = frequency of infected spikes, x = sources of inoculum)

amined sources of inoculum (Table 2). There were no significant or very significant differences between the examined sources of inoculum on the first sowing date for confidence levels 0.01 and 0.05 for $LSD_{0.01}=25.85$ and $LSD_{0.05}=19.24$. Significant and very significant differences were found between variables II and III, and variables III and IV on the second and third sowing dates for the mentioned LSD values.

Frequency of the infected spikes strongly correlated with the sources of inoculum ($r=0.53$), as well as sowing dates ($r=0.46$). The dependence of the frequency of infected spikes on inoculum source can be presented with a regression equation $y = -2.618 + 12.005 * x + \text{eps}$, (Figure 4).

Influence of temperature on the frequency of infected spikes caused by different sources of inoculum

The influence of temperature on the frequency of infected spikes caused by soilborne teliospores (variable II) was found significant in the period of 1-60 days after sawing ($r>+0.41$) (Figure 5; Table 3). The range of tem-

peratures that affected frequency of the infected spikes in that period was 1.9- 6.6°C (Table 5). The highest frequency of infected spikes was found when temperatures during that period were 4.0-5.0°C. Soil temperatures in the first and fourth decade after sawing highly affected the frequency of infected spikes ($r>+0.43$) (Table 4) when the temperature range was 1.1- 7.5°C, and the optimal temperature was 4.0-5.0°C. Infected spikes were found in statistically significant numbers for the second sowing date in the first year, and for the third sowing date in the second year, when the crop was 30 and 11 days under snow cover, respectively.

When the infection was caused by teliospores from seed surface, the frequency of infected spikes depended on temperature in the periods of days 1-60 and 1-120 ($r>+0.43$) (Figure 5; Table 3). Infection was possible within a temperature range of 2.5- 8.6°C, while the optimal temperature was 6.0-7.0°C (Table 5). Temperature highly influenced the frequency of infected spikes in the third and fifth decades after sawing (Table 4). During the third decade, temperature was in negative correlation with the frequency of infected

Table 2. Frequency of infected spikes depending on inoculum sources and sowing dates (A – optimal, B – mid-late and C – late) in 2001

| Source of inoculum | Frequency of infected spikes (%) | | |
|--|----------------------------------|--------------|---------------|
| | Sawing date | | |
| | A | B | C |
| I Control | 0.00 d E | 0.00 d E | 0.00 d E |
| II Soilborne inoculum | 60.52 a A | 31.54 bc BC | 22.45 bcd CD |
| III Seedborne inoculum | 47.23 ab AB | 15.89 cd CDE | 9.86 cd DE |
| IV Seedborne + soilborne inoculum | 61.06 a A | 45.35 ab AB | 26.41 bcd BCD |

$LSD_{0.05}=19.24$; $LSD_{0.01}=25.85$

Values in columns followed by different letter differ significantly; small letters ($P=0.05$), capital letters ($P=0.01$)

spikes ($r=-0.66$), and in positive ($r=+0.41$) during the fifth. Soil temperatures during these decades ranged $-0,9-8,5^{\circ}\text{C}$, the optimal being $6.0-7.0^{\circ}\text{C}$.

When infection was caused by a mixture of seedborne and soilborne inocula the critical time period was 1-120 days after sowing ($r=+0.42$) (Figure 5; Table 3). Temperature range in this period was $2.0-12.3^{\circ}\text{C}$ (Table

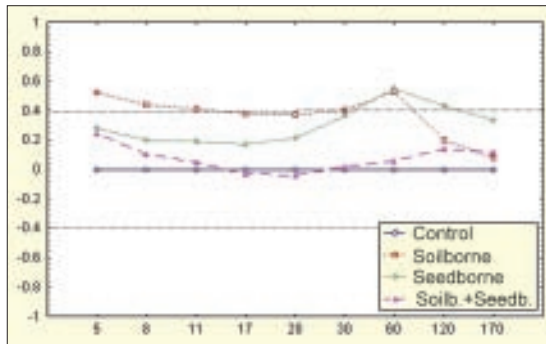


Figure 5. Correlation between the frequency of infected spikes and temperature (values higher or lower than ± 0.4 present significant correlations; x = different periods after sowing, y = correlation coefficient)

5). The highest frequency of infected spikes was found for the temperature range $3.0-4.0^{\circ}\text{C}$. Temperatures in the fourth decade mostly influenced the frequency of infected spikes ($r=+0.34$) because they were closest to the significant value of $+0.41$ (Table 4).

Influence of precipitation on the frequency of infected spikes caused by different inoculum sources

When infection was caused only by soilborne inoculum the influence of precipitation was significant only in the time period of 1-120 and 1-170 days after sowing ($r=-0.51$) (Figure 6; Table 6). Optimal precipitation was within a range of $103.7-190.1 \text{ l/m}^2$ (Tables 7 and 9).

The frequency of infected spikes in seedborne inoculum variable depended on precipitation in almost all periods before and after sowing ($r<-0.51$) (Figure 6; Table 6). No significant correlation was detected only for the periods of 5-1 day before sowing and 1-5 days after sowing. Negative correlation in these periods show that a reduction in precipitation increases frequency of infect-

Table 3. Correlation coefficients between the frequency of infected spikes and temperature presented for different sources of inoculum and period of days after sowing

| Period of days | Correlation coefficients | | |
|----------------|--------------------------|--------------------|------------------------------|
| | Soilborne inoculum | Seedborne inoculum | Seedborne+soilborne inoculum |
| 1-5 | 0.52* | 0.28 | 0.24 |
| 1-8 | 0.44* | 0.20 | 0.10 |
| 1-11 | 0.41* | 0.19 | 0.05 |
| 1-17 | 0.38 | 0.17 | -0.03 |
| 1-20 | 0.37 | 0.21 | -0.04 |
| 1-30 | 0.40 | 0.36 | 0.02 |
| 1-60 | 0.53* | 0.55* | 0.18 |
| 1-120 | 0.20 | 0.43* | 0.42* |
| 1-170 | 0.08 | 0.33 | 0.38 |

Values marked with * present significant correlation ($b \geq \pm 0.40$)

Table 4. Dependence of the frequency of infected spikes on temperature presented for different sources of inoculum and decades after sowing

| Decades | Regression equations between frequency of infected spikes and temperature | | |
|---------|---|-----------------------|------------------------------|
| | Soilborne inoculum | Seedborne inoculum | Seedborne+soilborne inoculum |
| 1-10 | $y=37.56-(0.42)^2x^*$ | $y=23.94-(0.19)^2x$ | $y=60.40+(0.06)^2x$ |
| 11-20 | $y=42.87-(0.30)^2x$ | $y=25.02+(0.20)^2x$ | $y=66.34-(0.20)^2x$ |
| 21-30 | $y=41.05+(0.30)^2x$ | $y=15.18-(0.66)^2x^*$ | $y=59.39+(0.23)^2x$ |
| 31-40 | $y=43.00+(0.41)^2x^*$ | $y=11.04-(0.26)^2x$ | $y=61.36+(0.34)^2x$ |
| 41-50 | $y=39.23+(0.20)^2x$ | $y=18.49+(0.41)^2x$ | $y=63.55+(0.04)^2x$ |
| 51-60 | $y=46.90+(0.06)^2x$ | $y=23.76-(0.21)^2x$ | $y=61.97+(0.01)^2x$ |

Regression equation marked with * represent significant regression ($b \geq \pm 0.40$) (y = frequency of infected spikes; x = temperature, values in brackets are discrimination coefficients)

Table 5. Frequency of infected spikes presented for different sources of inoculum and sowing dates

| Year/ sowing date | Frequency of infected spikes (%) | | | Sum of temperatures (°C) calculated for periods of days after sowing | | | | | | | |
|-------------------------|----------------------------------|-----------------------|--------------------------------------|---|------|------|------|------|------|------|-------|
| | Soilborne inoculum | Seedborne inoculum | Seedborne + soilborne inoculum | 1-5 | 1-8 | 1-11 | 1-17 | 1-20 | 1-30 | 1-60 | 1-120 |
| 2000/A | 61.45 | 15.6 | 82.12 | 10.0 | 9.2 | 8.4 | 7.0 | 6.3 | 4.5 | 3.1 | 2.0 |
| 2000/B | 43.01 | 37.98 | 86.45 | -1.0 | -1.0 | -1.2 | -1.7 | -1.3 | 0.3 | 1.9 | 7.7 |
| 2000/C | 69.97 | 40.63 | 72.01 | 3.7 | 3.7 | 3.8 | 3.4 | 3.1 | 3.2 | 5.0 | 12.3 |
| 2001/A | 62.55 | 47.23 | 61.06 | 13.1 | 12.5 | 12.1 | 11.0 | 11.1 | 10.3 | 6.6 | 4.7 |
| 2001/B | 31.54 | 15.89 | 48.85 | 3.4 | 4.3 | 5.0 | 3.7 | 2.7 | 2.8 | 2.3 | 4.7 |
| 2001/C | 22.45 | 9.86 | 26.39 | -1.8 | 1.1 | 1.2 | 2.1 | 2.5 | 1.7 | 2.5 | 5.5 |

ed spikes. When precipitation was 190.1 l/m² or 192.2 l/m², the frequency of infected spikes was 15.6 or 15.8, while precipitation lower than 103.7 and 117.9 l/m² resulted in the frequency of infected spikes of 40.63% and 47.23% (presented for the period of 1-120 days after sowing) (Tables 8 and 10). Critical decades in which the frequency of infected spikes depended on precipitations were the first one before sowing and the first, second, third and fourth after sowing ($r < -0.45$) (Table 7). Precipitation in these decades ranged 0.1-48.5 l/m², the optimal being around 5.2 l/m².

Frequency of the infected spikes also negatively correlated with precipitation when infection was caused both by seedborne and soilborne inocula and this correlation was detected for the periods of 8-1 and 5-1 days before sowing and 1-11, 1-120 and 1-170 days after sowing ($r < -0.41$) (Figure 6; Table 6). With lower precipitation (96.8 l/m²), the frequency of infected spikes was

86.45, while higher precipitation (250.1 l/m²) resulted in 26.39% infected spikes (shown for period of days 1-120 after sowing) (Tables 8 and 11). Precipitation

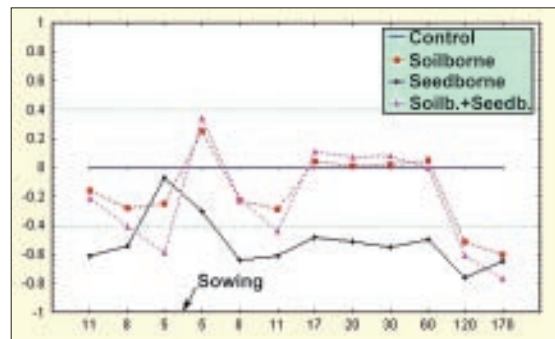


Figure 6. Correlation between the frequency of infected spikes and precipitation (values higher or lower than ± 0.4 present significant correlation; x = different periods after sowing, y = correlation coefficient)

Table 6. Correlation coefficients between frequency of infected spikes and precipitation presented for different sources of inoculum and periods of days after sowing

| Periods of days | Correlation coefficients | | | |
|-----------------|--------------------------|--------------------|--------------------|---------------------------------|
| | Control | Soilborne inoculum | Seedborne inoculum | Seedborne+soilborne inoculum |
| 11-1 | 0.00 | -0.16 | -0.61* | -0.21 |
| 8-1 | 0.00 | -0.28 | -0.54* | -0.41* |
| 5-1 | 0.00 | -0.25 | -0.07 | -0.59* |
| 1-5 | 0.00 | 0.25 | -0.30 | 0.34 |
| 1-8 | 0.00 | -0.23 | -0.64* | -0.22 |
| 1-11 | 0.00 | -0.29 | -0.61* | -0.44* |
| 1-17 | 0.00 | 0.04 | -0.48* | 0.11 |
| 1-20 | 0.00 | 0.01 | -0.51* | 0.07 |
| 1-30 | 0.00 | 0.02 | -0.55* | 0.08 |
| 1-60 | 0.00 | 0.05 | -0.50* | -0.00 |
| 1-120 | 0.00 | -0.51* | -0.76* | -0.61* |
| 1-170 | 0.00 | -0.51* | -0.65* | -0.77* |

Values marked with * present significant correlation ($b > \pm 0.40$)

Table 7. Dependence of the frequency of infected spikes on precipitation presented for different sources of inoculum and decades after sawing

| Decades | Regression equations between frequency of infected spikes and precipitation | | |
|---------|---|-----------------------|------------------------------|
| | Soilborne inoculum | Seedborne inoculum | Seedborne+soilborne inoculum |
| 10-1 | $y=50.64-(0.11)^2x$ | $y=36.03-(0.55)^2x^*$ | $y=65.59-(0.16)^2x$ |
| 1-10 | $y=55.48-(0.22)^2x$ | $y=39.74-(0.49)^2x^*$ | $y=75.17-(0.38)^2x$ |
| 11-20 | $y=59.32-(0.29)^2x$ | $y=47.18-(0.67)^2x^*$ | $y=63.86-(0.04)^2x$ |
| 21-30 | $y=47.35+(0.05)^2x$ | $y=35.67-(0.45)^2x^*$ | $y=58.21+(0.16)^2x$ |
| 31-40 | $y=47.64+(0.03)^2x$ | $y=40.64-(0.59)^2x^*$ | $y=62.05+(0.01)^2x$ |
| 41-50 | $y=43.69+(0.30)^2x$ | $y=30.97+(0.25)^2x$ | $y=58.87+(0.31)^2x$ |
| 51-60 | $y=42.70+(0.16)^2x$ | $y=31.00-(0.11)^2x$ | $y=41.85+(0.51)^2x^*$ |

Regression equation marked with * represent significant regression ($b \geq \pm 0.40$) (y = frequency of infected spikes; x = precipitation, values in brackets are discrimination coefficients)

Table 8. Frequency of infected spikes presented for different sources of inoculum and sawing dates (optimal – A, and late – B and C) and precipitation during periods of days for which strong correlation was found

| Year/ sawing date | Frequency of infected spikes (%) | | | Precipitation sums (l/m ²) calculated for periods of days after sawing | | | | | | | | |
|-------------------------|----------------------------------|--------------------|--------------------------------|--|------|------|------|------|------|-------|-------|-------|
| | Soilborne inoculum | Seedborne inoculum | Seedborne + soilborne inoculum | 11-1 | 1-5 | 1-8 | 1-11 | 1-17 | 1-20 | 1-30 | 1-60 | 1-120 |
| 2000/A | 61.45 | 15.6 | 82.12 | 175 | 27.7 | 27.7 | 29.3 | 87.5 | 99.2 | 125.4 | 141.1 | 190.1 |
| 2000/B | 43.01 | 37.98 | 86.45 | 0.1 | 0.0 | 5.2 | 5.6 | 15.1 | 15.6 | 21.2 | 40.3 | 96.8 |
| 2000/C | 69.97 | 40.63 | 72.01 | 0.5 | 0.0 | 5.6 | 5.6 | 7.0 | 7.3 | 24.4 | 51.0 | 103.7 |
| 2001/A | 62.55 | 47.23 | 61.06 | 2.6 | 1.8 | 1.8 | 17.1 | 20.2 | 20.5 | 23.4 | 71.4 | 117.9 |
| 2001/B | 31.54 | 15.89 | 48.85 | 2.9 | 5.9 | 5.9 | 15.6 | 20.3 | 20.6 | 55.3 | 91.8 | 192.2 |
| 2001/C | 22.45 | 9.86 | 26.39 | 14.7 | 27.6 | 27.6 | 33.6 | 37.6 | 48.0 | 51.2 | 74.0 | 250.1 |

during the sixth decade after sawing was critical for the frequency of infected spikes ($r=+0.51$) (Table 7). Optimal precipitation in the sixth decade ranged 18.4-26.2 l/m².

Overall influence of temperature and precipitation on the frequency of infected spikes when infection was caused by different sources of inoculum

The results can be presented together for temperature and precipitation because environmental conditions influenced the frequency of infected spikes over the period of 1-120 days after sawing for variables III and IV (Tables 10 and 11) and in the first 60 days after sawing (in relation to temperature) and first 120 days after sawing (in relation to precipitations) for variable II (Table 9).

DISCUSSION

The two-year field experiments at the locality Rimski Šančevi show that soilborne teliospores can be an important source of inoculum, which is in line with reports from Purdy and Kendrick (1962), Hoffman (1982), Williams (1987), Yarham and McKeown (1989), Line (1993) (cited by Wilcoxson and Saari, 1996). Only Parlak (1986) reported on which source of inoculum was more important.

In most experiments, examination has focused on one parameter alone – the frequency of infected spikes. Frequency was normally evaluated by collecting a certain number of spikes, for example 200 spikes from 5 m long rows, which were sawn with 10 g of wheat (Gaudet et al., 1989). The frequency of infected spikes in our experiment was evaluated as a number of infected spikes per tiller. Potential statistical error at sampling was reduced that way as almost all plants were included in evaluation (only 8.41 of the potential 20 plants germinated in 2000, and 5.62 plants in 2001, so that 5 evaluated plants per row largely reduced the statistical error).

Table 9. Influence of temperature and precipitation on the frequency of infected spikes when infection was caused by soilborne teliospores in the first 60 days (temperature) and 120 days (precipitation) after sawing

| Temperature (°C) | Frequency of infected spikes (%) | Precipitation (l/m ²) | Duration of snow cover (days) |
|------------------|----------------------------------|-----------------------------------|-------------------------------|
| 1-2 | 43.01 b | 96.8 # | 30 |
| 2-3 | 22.45 d | 250.1 | 0 |
| 2-3 | 31.54 c | 192.2 # | 11 |
| 3-4 | 61.45 a | 190.1 | 0 |
| 4-5 | 69.97 a | 103.7 | 0 |
| 6-7 | 62.55 a | 117.9 | 0 |

snow

Values in column followed by different letter differ significantly (P=0.05)

Table 10. Influence of temperature and precipitation on the frequency of infected spikes when infection was caused by seedborne teliospores in the first 120 days after sawing

| Temperature (°C) | Frequency of infected spikes (%) | Precipitation (l/m ²) | Duration of snow cover (days) |
|------------------|----------------------------------|-----------------------------------|-------------------------------|
| 1-2 | 37.98 ab | 96.8 # | 30 |
| 2-3 | 9.86 d | 250.1 | 0 |
| 2-3 | 15.89 cd | 192.2 # | 11 |
| 3-4 | 15.6 cd | 190.1 | 0 |
| 4-5 | 40.63 a | 103.7 | 0 |
| 6-7 | 47.23 a | 117.9 | 0 |

snow

Values in column followed by different letter differ significantly (P=0.05)

Table 11. Influence of temperature and precipitation on the frequency of infected spikes when infection was caused by seedborne and soilborne teliospores in the first 120 days after sawing

| Temperature (°C) | Frequency of infected spikes (%) | Precipitation (l/m ²) | Duration of snow cover (days) |
|------------------|----------------------------------|-----------------------------------|-------------------------------|
| 1-2 | 82.12 a | 190.1 # | 30 |
| 4-5 | 61.06 b | 117.9 | 0 |
| 4-5 | 48.85 bc | 192.2 # | 11 |
| 5-6 | 26.39 d | 250.1 | 0 |
| 7-8 | 86.45 a | 96.8 | 0 |
| 12-13 | 72.01 a | 103.7 | 0 |

snow

Values in column followed by different letter differ significantly (P=0.05)

The experiments showed that the frequency of infected spikes was higher when infection was caused by soilborne inoculum in our agroecological conditions and that soilborne inoculum is more important than seedborne. When infection was caused by teliospores from soil the frequency of infected spikes was 58.15% in 2000 and 38.84% in 2001, while infection caused by seedborne inoculum resulted in 31.40% infected spikes in 2000 and 24.33% in 2001. Plants infected with soilborne teliospores had 71.78% infected spikes in 2000 and 43.21% in 2001.

Parlak (1986) reports that seedborne inoculum is a more significant source of inoculum than soilborne. Cv Heines VIII was sown in soil which was inoculated with teliospores one year before the trial and the whole experimental design was different from ours. In order to determine what is more significant as a source of inoculum, seeds were sown very close to teliospores to allow contact with the same quantity of inoculum as it was in case with seedborne inoculum. Johnsson (1990) reported a prolonged vitality of teliospores in soil in an experiment that was set up in a similar manner.

Analyses of environmental conditions reveal more correlations. Johnsson (1992) examined a correlation between temperature and frequency of infected spikes, and found it significant during the first month after sawing when infection was caused by teliospores from soil in different locations in Sweden. A strong correlation ($r > 0.40$) in our environmental conditions lasts during the first 60 days after sawing, when teliospores from soil are the only source of inoculum. Differences in the duration of these periods after sawing that correlation was determined for can be explained by different climatic conditions in Sweden and Serbia and also by different populations of *Tilletia* spp. in the two countries.

Johnsson (1992) reports a strong correlation between the frequency of infected spikes and temperature within the first 11 days after sawing when infection was caused by teliospores from seeds. The results of our experiments show that this period lasts 120 days after sawing in our environmental conditions. Johnsson (1992) does not report on the duration of the critical period for the influence of temperature on infection for either seedborne or soilborne inoculum. Temperature had influence on the frequency of infected spikes under our environmental conditions during the first days after sawing.

Johnsson (1992) did not perform regression analyses, so there are no data showing in which decades the plants were infected with common bunt. In our study, most of the plants were infected during the first two decades when soilborne teliospores were the only source of inoculum, although favorable conditions for infection lasted throughout the first two months after sawing. Teliospores from seeds caused infection during the third and fifth decade after sawing. Infection with both seedborne and soilborne inocula occurred during the fourth and sixth decade. Since germination of wheat seeds takes place during the first 11 days after sawing it can be presumed that most of the plants were infected after germination. Most authors (Wiese, 1987; Wilcoxson and Saari, 1996) specify that teliospores of *T. tritici* and *T. laevis* infect wheat during germination. Data from these experiments demonstrate that a new race is predominant in Serbia or a hybrid between *T. tritici* and *T. controversa* that can cause infection after germination.

The first critical decade has negative values of discrimination coefficients both under conditions of infection with seedborne and soilborne teliospore inocula. Negative values of discrimination coefficient indi-

cate that a temperature increase has led to a decrease in common bunt incidence. This may be due to the fact that, during exposure to lower temperatures, germination of wheat was slower and the fungi much faster after penetration in reaching apical meristem tissue.

Johnsson (1992) reports that precipitation does not affect the frequency of infected spikes, while in our environmental conditions this varies for the different variables. Teliospores from seed were not stimulated by high precipitation to cause infection, which is evident from the negative values of correlation coefficient.

Correlation curves of temperature and precipitation for the infection caused by soilborne inoculum have the same shape as those for infection caused by soilborne and seedborne inoculum (variable IV). To presume that soilborne teliospores are a more important source of inoculum even in variable IV, is supported not only by the fact that the frequency of infected spikes is similar or very close for the two variables (II and IV), but also by the similar shapes of their curves, while with seedborne inoculum (variable III) it differed and was similar to the control.

Johnsson (1992) reports that temperature the range of 5-6°C is optimal to obtain the highest frequency of infected spikes. Data from our experiments were in line with data reported by Johnsson (1992). The range of optimal temperature was 5-6°C when infection was caused by soilborne inoculum in our environmental conditions. There are differences in relation to data reported by Purdy and Kendrick (1963). Higher temperatures were relevant in their report to infection with soilborne inoculum because, in their view, teliospores had already germinated during wheat sawing. In our experiments soil was inoculated with teliospores during the sawing of seeds.

Temperature range of 6-7°C was optimal for the infection caused by seedborne inoculum (Johnsson, 1992) and that is in line with our data. There is similarity with data presented by Purdy and Kendrick (1959), showing that, with teliospores and seeds germinating at same time, the optimal temperature range is 5-10°C. Johnsson (1992) does not give data for infection caused by seedborne and soilborne inoculum. The temperature range in that case was 4-5°C in our environmental conditions.

Our experiments also demonstrated that higher precipitation was required at lower temperatures for the infection, and vice versa. The fact that with decreasing temperature a quantity of heat is created can ex-

plain the high infection level even when temperatures were low (1-2°C).

Milošević et al. (1998) reported that the earliest sowing dates support faster germination of winter wheat, which enables plants to avoid fungi infection, and recommended wheat sowing in optimal dates. These experiments show that even on optimal sowing dates the frequency of infection can be high, which is probably the cause of an extended period critical for infection incidence (minimum 60 after sowing).

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Uticaj izvora inokuluma *Tilletia* spp. i uslova sredine na učestalost zaraženih klasova

REZIME

Uticaj izvora inokuluma na pojavu glavnice pšenice koju prouzrokuju gljive roda *Tilletia* u našim agroekološkim uslovima praćen je, između ostalih, ispitivanjem parametra infekcije: stepen zaraženosti klasova. Sorta Novosadska rana 5 je sejana po split plot metodu u tri roka setve sa 4 ponavljanja na lokalitetu Rimski Šančevi tokom 1999/2000. i 2000/2001. godine. Ispitivane su sledeće varijante: I – apsolutna kontrola, II – zemlja infestirana teleutosporama (4 g teleutospora/1 l zemlje), III – seme infestirano teleutosporama (2 g teleutospora/1 kg semena) i IV – zemlja infestirana teleutosporama + seme infestirano teleutosporama (4 g teleutospora/1 l zemlje + 2 g teleutospora/1 kg semena). Izvršena je korelaciona i regresiona analiza ispitivanog parametara infekcije u odnosu na temperaturu i padavine.

Stepen zaraženosti klasova je zavisio od izvora inokuluma *Tilletia* spp., a uočene su razlike u nivou infekcije između varijanti II i III, kao i III i IV. Kasniji rokovi setve (drugi i treći) su uticali značajno na ispitivani parametar infekcije, ali između njih nisu utvrđene značajne razlike.

Kritični period u kom temperature utiču na ostvarivanje infekcije u uslovima kad su teleutospore u zemlji jedini izvor inokuluma traje 60 dana nakon setve ($r > +0,52$). Najveći broj biljaka je zaražen u prvoj i nešto manje u drugoj dekadi posle setve ($r > +0,41$), pri čemu je optimalna temperatura u tom periodu iznosila 5,0-6,0°C.

Prvih 60 dana nakon setve je, takođe, kritičan period za ostvarivanje infekcije u uslovima kad su teleutospore na semenu jedini izvor inokuluma ($r > +0,50$). Najveći broj biljaka je zaražen u trećoj i nešto manje u petoj dekadi posle setve ($r < -0,45$), pri čemu je optimalna temperatura u tim dekadama iznosila 6,0-7,0°C.

U uslovima kad infekciju pšenice ostvaruju teleutospore prisutne na semenu i u zemlji kritičan period traje 120 dana posle setve ($r > +0,42$), pri čemu je maksimalna infekcija ostvarena pri optimalnoj temperaturi 4,0-5,0°C.

Ključne reči: *Tilletia* spp.; zemlja; seme; temperatura; padavine