Proceedings of the 7th Congress on Plant Protection

Доклады 7-ого Конгресса по защите растений

Plant Protection Society of Serbia
Общество по защите растений Сербии

International Organization for Biological Control
-East Palearctic Regional Section (IOBC-EPRS)
-West Palearctic Regional Section (IOBC-WPRS)

Международная организация по биологической борьбе
- Восточно палеарктическая региональная секция (МОББ-ВПРС)
- Западно палеарктическая региональная секция (МОББ-ЗПРС)

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(November 24-28, 2014, Zlatibor, Serbia)

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„Интегрированная защита растений - научно обоснованный шаг к устойчивому развитию сельского хозяйства, лесоводства и пейзажной архитектуры“
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по поводу 60-летия ОЗРС

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PREFACE

The Plant Protection Society of Serbia (PPSS) and two regional sections of the International Organization for Biological and Integrated Control (IOBC-EPRS and IOBC-WPRS), on the occasion of the 60th anniversary of the PPSS organized VII Congress on Plant Protection with a motto: *"Integrated Plant Protection – a Knowledge-Based Step towards Sustainable Agriculture, Forestry and Landscape Architecture"* (November 24-28, 2014, Zlatibor, Serbia). The Congress enabled exchange of up-to-date scientific and technical information on plant protection in Agriculture, Forestry and Landscaping among researchers, teachers, experts in extension and public services and the business community, and promoted international cooperation. The Congress focused on basic knowledge and management practices established in plant protection, as well as on the development of alternative and innovative approaches. In addition, biological control as an important tool for the control of the harmful organisms with a minimal risk for ecosystems was discussed. A total of 209 contributions was presented - 8 keynote presentations, 28 oral presentations and 173 poster presentations - prepared by 467 authors from 26 countries. The Congress Proceedings comprise 65 contributions - 5 keynote presentations and 60 oral and poster presentations in six sessions, prepared by the authors from 18 countries (Algeria, Austria, Bosnia-Herzegovina, France, Georgia, Hungary, Italy, Kazakhstan, Montenegro, Poland, Russia, Rwanda, Serbia, Slovenia, Switzerland, Turkey, Uganda, USA). All contributions were reviewed by members of the Scientific Committee and other reviewers selected and invited by the editors of this publication.

Belgrade, November 2015 Editors

ПРЕДИСЛОВИЕ

Общество по защите растений Сербии (ОЗРС), Международная организация по биологической борьбе с вредными животными и растениями - Восточно палеарктическая региональная секция (МОББ-ВПРС) и Международная организациая по биологической борьбе и интегрированной системе защиты растений - Западно-палеарктическая региональная секция (МОББ-ЗПРС), по поводу 60-летия ОЗРС организировали VII Конгресс по защите растений, под девизом: *"Интегрированная защита растений - научно обоснованный шаг к устойчивому развитию сельского хозяйства, лесоводства и пейзажной архитектуры"* (24-28 ноября 2014 года, Златибор, Сербия). Цель Конгресса была обеспечение континуитета взаимообмена научно-техническими информациями, отвечающими современным требованиям защиты растений в сельском хозяйстве, лесоводстве и пейзажной архитектуре, которые представляют интерес для ученых, исследователей, преподавателей, экспертов-советников в области сельского хозяйства, лесоводства и пейзажной архитектуры, специалистов государственных и коммунальных служб, деловых кругов и средств массовой информации. Целью Конгресса является и продолжение содействия развитию и популяризации международного сотрудничества. Конгресс был концентрирован на основные знания и практический менаджмент в защите растений, а также на развитие алтернативних и новых подходов. Биологическая защита каторая представляет значительный способ для безопасной борьбы с вредними организмими была тоже рассмотривана. На конгрессе представлено 209 презентаций - 8 докладов по приглашению, 28 устных и 173 постер презентаций - которые подготовило 467 авторов из 26 стран. Сборник имеет 65 докладов - 5 докладов по приглашению и 60 устных и постер презентаций, распределенных в шести секциях. Авторы докладов приехали из 18 стран (Алжир, Австрия, Босния-Герцеговина, Франция, Грузия, Венгрия, Италия, Казахстан, Черногория, Польша, Россия, Руанда, Сербия, Словения, Швейцария, Турция, Уганда, США). Рецензенты всех опубликованных докладов в сборнике – члены Научного совета и другие рецензенты, выбранные редакторам этого издания.

Белград, Ноября 2015 — Редакторы

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OCCURRENCE OF GRASS BUNT IN VOJVODINA AND ITS INFLUENCE ON WHEAT SEED QUALITY CONTROL

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ABSTRACT

Causal agents of grass bunt, which infect wild grass hosts in *Bromus, Festuca, Ventenata* and *Vulpia,* are *Tilletia* species whose teliospores could be found in wheat seed samples too. Among them, *T. bromi* is morphologically similar to those of quarantined *T. contraversa,* and misidentification of this species could lead to commodity rejection. As a result, monitoring the presence of teliospores of quarantined species in seed samples, and identification of *Tilletia* species on the basis of morphological characteristics is difficult and in some cases impossible. In order to investigate the presence of grass bunt in seed samples of wheat in Vojvodina, teliospore extraction was carried out by using the size-selective sieving wash method and the OEPP/EPPO diagnostic protocol for *Tilletia indica* (2007). The analysis of 151 samples of basic, certified and commercial non-processed seed of wheat revealed that 127 samples were contaminated with *T. caries*, while 12 samples were contaminated with teliospores of *Tilletia* species which had morphological characteristics that correspond to *T. bromi* complex or quarantined *T. contraversa*. These teliospores displayed prominent gelatinous sheath with conspicuous depth of reticulations. Molecular identification of grass bunt teliospores was not possible in this study, because contamination level was too low (1 teliospore per 10 seeds). However, knowing that teliospores of *T. bromi* often occur in wheat seed samples in very low numbers there is a need for standardization of molecular techniques for the identification of a single teliospore of *Tillletia* species in order to make plant protection more efficient and reliable.

Key words: grass bunt, *Tilletia bromi, Tilletia contraversa,* seed testing

INTRODUCTION

The genus *Tilletia* includes approximately 100 known species that infect grass hosts (Pimentel et al., 2000). Economically important pathogens of wheat (*Triticum aestivum* L.) are *Tilletia caries* (DC.) Tul. & C. Tul. and *T. foetida* (Wallr.) Liro causal agents of common bunt, as well as *T. contraversa* J.G. Kühn causal agent of dwarf bunt (Goates, 2012). In addition, there are *Tilletia* species whose teliospores could also be found in samples of wheat seed, although it is known that wheat is not a host. Representatives of *T. bromi* (Brockm.) Brockm complex (*T. bromi-tectorum* and *T. guyotiana*), are causal agents of grass bunt, and infect wild grass hosts in *Bromus, Festuca, Ventenata* and *Vulpia*. The presence of teliospores of *T.bromi* in wheat seed samples could make quarantine inspection very difficult (Peterson et al., 2009) because of morphological similarity of teliospores. As a result, investigation of *T. bromi* has become increasingly important.

Identification of *Tilletia* species using light microscopy is almost impossible because of overlapping of morphological characteristics of teliospores (Mathre, 1996; Peterson et al.,2009). According to Goates (1996) sheath thickness of *T. contraversa* is 1.5 to 5 μm, and depth of reticulations is 1.5 to 3 μm. Boyd and Carris (1998) reported that depth of reticulations and sheath thickness of *T. bromi* is 1 to 3 μm, thus teliospores with sheath thickness between 1 and 3 μm could be identified as *T. bromi* as well as *T. controversa*. Although identification of *Tilletia* species is done using areolae diameter, it is not discriminative enough. Goates (1996) reported that diameter of areola for *T. contraversa* is 3 to 5 μm, while Boyd and Carris (1998) reported areolae diameter 2 – 5 - (7) μm for *T. bromi*. Trione and Krygier (1977) noted that average sheath thickness and depth of reticulations of *T. bromi-tectorum* and *T. guyotiana* are 1.54 μm and 2.52 μm, respectively, which was in accordance with results reported by Boyd and Carris (1998).

Common and dwarf bunt have been successfully controlled by using combination of fungicides and resistant cultivars, however, European agriculture has been moving toward organic production and low-input farming systems (Matanguihan et al., 2011). As consequence, the need for monitoring the presence of *Tilletia* species, varieties and races in wheat production areas is growing (Jevtic, 1998). In addition, *T. contraversa* is still quarantined species in number of countries including Republic of Serbia. As a result, investigation of presence of *Tilletia* species in wheat production areas in Vojvodina, with special reference to the presence of grass bunt in seed samples of wheat is conducted as part of the program of establishment and implementation of international standards for phytosanitary field.

MATERIAL AND METHODS

Autumn-sown wheat samples were collected in cooperation with the regional phytosanitary laboratory Agroinstitut-Sombor, during the 2007-2008. In total, 151 non-processed seed samples of 1 to 2 kg were sampled from trucks. Seed samples were stored at 15°C and relative humidity of< 60% (Elias et al., 2007). In order to investigate the presence of grass bunt in seed samples of wheat in Vojvodina, teliospore extraction were carried out by using the size-selective sieving wash method described by Peterson et al. (2000) and modified OEPP/EPPO diagnostic protocol for *Tilletia indica* (2007). Teliospores were extracted from 50 g subsamples of each grain sample using 10 μm nylon mesh. Extracted teliospores were suspended with 100 μl (or more) of 15% glycerol, depending on the pellet volume (OEPP/EPPO, 2007). If teliospores were not found in the first 50 g subsample, two further 50 g subsamples were examined in order to determine the presence of *Tilletia* species with confidence level of 99% (OEPP/EPPO, 2007). Identification of extracted teliospores was processed using light microscopy at 630× magnification on the bases of morphological characteristics of *Tilletia* species reported by Goates (1996) and Boyd and Carris (1998). Contamination level was determined by calculating total number of teliospores per 50 g seed subsample, with the use of the Breed Method.

RESULTS

The analysis of 151 samples of basic, certified and commercial non-processed seed of wheat revealed that 12 samples were contaminated with teliospores of *Tilletia* species morphologically similar to quarantined *T. contraversa* and *T. bromi*. These teliospores displayed prominent gelatinous sheath with conspicuous depth of reticulations, which made them different from the most dominant *T. caries*. Diameter of grass bunt teliospores was 18 – 22 μm, and that characteristic was not used for making distinction between *T. contraversa* and *T. bromi* since teliospore diameters of those species are 16.8 - 32 μm and 18 – 29 μm, respectively. Taking into account that areolae diameter ranged from 2 to 4 μm, only teliospores with areole diameter lesser than 3 μm were assumed to belong to *T. bromi* (2 – 7 μm) since areolae diameter of *T. controversa* is 3 - 5 μm. Sheath thickness of newly found teliospores ranged from 1.5 to 3.5 μm, which overlaps with sheath thickness of *T. contraversa* (1.5 – 5 μm) and *T. bromi* (1 - 3μm). Morphological characteristics of newly found teliospores of *Tilletia* spp. are presented in Table 1 and Figure 1.

Teliospores of grass bunt were found in seed samples which were contaminated with less than 100 teliospores per 50 g subsample, that is, one teliospore per 10 seeds, thus it was not possible to make identification using molecular technique regarding difficulties in production of mycelal mats for DNA extraction.

Table 1. Morphological characteristics of teliospores of *Tilletia* spp.

Teliospore	Areolae diameter		Sheath thickness		Identification
	T. contravesa 3 - 5μm	*T. bromi* 2 – 5- (7) μm	*T. contraversa* 1.5 – 5 μm	*T. bromi* 1 – 2.53 (3) μm	
a	-	2.67 – 2.83	1.95 – 3.39	-	Non- identified
b	-	2.73 – 3.80	1.56 – 2.56	1.56 – 2.56	*T. bromi*
c	3.55 – 3.77	3.55 – 3.77	2.49 – 2.92	2.49 – 2.92	*T. bromi / T. contraversa*

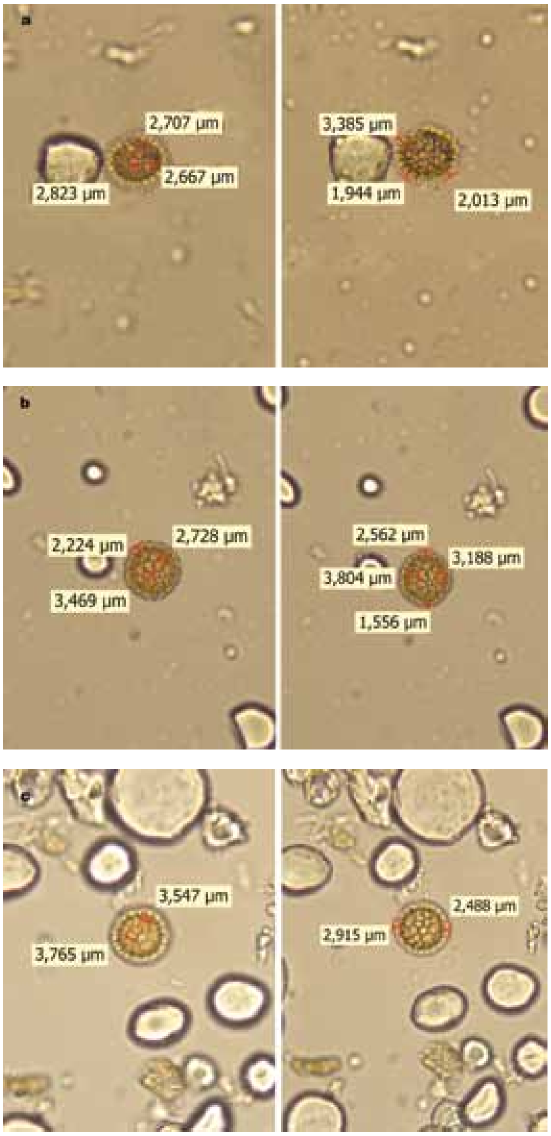

Figure 1. Teliospores of *Tilletia* spp. with prominent gelatinous sheath

a – Non-identified teliopsore
b – Teliospore identified as *T. bromi*
c – Teliospore that could be identified as either *T. bromi* or *T. contraversa*

DISCUSSION

Occurrence of teliospores of *Tilletia* spp. causal agents of grass bunt in wheat seed samples has been reported by many authors (Peterson et al., 2000; Pimentel et al., 2000; Trione and Krygier, 1977). The presence of teliospores of *T. bromi* in wheat seed samples could be a problem for commodity inspection due to teliospore similarity with *T. contraversa*. As a result, seed testing for the presence of *Tilletia* species is almost impossible when it is based only on morphological characteristics of teliospores (Goates, 1996, Mathre, 1996; Peterson et al., 2009). In this study, areolae diameter and sheath thickness were not discriminative enough for making distinction between *T. conraversa* and *T. bromi* because of overlapping of morphological characteristics. Majority of teliospores could be identified as either *T. contraversa* or *T.bromi*.

In addition, there is a rising question about measurement uncertainty, especially in cases when measured values reach the limit separating one species from the other. Areole diameter of teliospore marked as ***a*** (Fig 1) was measured to be 2.83 µm which is very close to the limit of 3 µm used to discriminate *T. bromi* from *T. contraversa*. As a result, teliospore marked as ***a*** could not be identified as neither *T. bromi* nor *T. contraversa*.

Inability to identify single teliospore in seed samples using morphological characteristics could make not only quarantine decisions difficult, but also decisions about intervention in organic agriculture where determination of exact teliospore number is of crucial importance. For example, prescribed teliospore thresholds for intervention in organic agriculture vary in different countries and it is 20 common bunt teliospore/seed in Germany, 10 teliospores/seed in Austria and Switzerland and one teliospore/seed in the United Kingdom (Matanguihan et al., 2011). Contamination limits for *T. contraversa* in Austria, Switzerland and Scotland are the same as the previously mentioned (Micheloni et al., 2007). However, in Denmark, intervention is recommended at the first detection of teliospores (Matanguihan, 2011).

In order to overcome the problem related to identification of *Tilletia* species many different molecular techniques were used. Initially, genomic fingerprinting techniques were good enough to make distinction between wheat and grass bunt fungi, but not precise enough in distinguishing *T. contraversa* from *T. caries*. Usually, they were processed by using molecular markers such as RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) and PCR-RFLP (restriction fragment length polymorphism) of ITS rDNA region. (Boyd et al., 1998, Pimentel et al., 1998; Shi et al., 1996). Later, species specific primers were selected for making distinction between *T. contraversa* and *T. caries*, however it has not be confirmed that those primers could make distinction between *T. contraversa* and *T. bromi* (Yuan et al., 2009; Liu et al., 2009; Gao et al., 2010; Gao et al., 2011). Finally, rep-PCR fingerprinting technique, with REP, ERIC and BOX primers, succeeded in making distinction not only between *T. contraversa* and *T. caries* but also between *T. contraversa* and *T. bromi* (McDonald et al., 2000). Župunski et al. (2011) confirmed results obtained by McDonald et al. (2000), and reported coefficients of similarities between *Tilletia* species which were in accordance to those reported by McDonald et al. (2000).

In this study, molecular identification of grass bunt teliospores was not possible since contamination level was too low (1 teliospore per 10 seeds). Župunski et al. (2011) succeeded in application of rep-PCR fingerprinting for *Tilletia* species identification only when contamination level was 1 teliospore per 1 seed or higher. Molecular techniques such as real-time PCR and multiplex real-time PCR techniques was successful in quantifying bunt contamination levels down to less than one spore per seed, but they were not successful in distinguishing *T. caries, T. contraversa* and *T. bromi* (McNeil et al.,2004; Tan et al.,2009). McDonald et al. (1999) also tried to make identification of various *Tilletia* species using single ungerminated teliospore but test sensitivity varied for the different *Tilletia* species and different teliospore lots. Knowing that teliospores of *T. bromi* often occur in wheat seed samples in very low numbers and that all molecular techniques which were successful in distinguishing *Tilletia* species were established using large number of teliospores (Gao et al., 2010, 2011; Kellerer et al., 2006; Liu et al., 2009; McDonald et al., 2000; Yuan et al., 2009), there is a growing need for validation of these methods on wheat seed samples with low levels of contamination. Standardization of molecular techniques for the identification of a single ungerminated teliospore of *Tillletia* species will contribute not only in testing for the presence of quarantined species but also in investigations where extraction of DNA from germinated teliospores is the great obstacle.

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