LARGE-SCALE OCCURRENCE OF *RHIZOPUS* HEAD ROT ON SUNFLOWER AS A CONSEQUENCE OF COTTON BOLLWORM (*HELICOVERPA ARMIGERA*) ATTACK IN THE VOJVODINA PROVINCE IN 2003

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Abstract

In 2003, *Rhizopus* head rot occurred on a large scale in the Vojvodina Province (northern part of Serbia and Montenegro), especially in the regions of northern Banat and Bačka. The disease was due to the attack of the cotton bollworm (*Helicoverpa armigera* Hbn). The damage caused by the fungus from the genus *Rhizopus* was up to 50% reduction of seed yield. Cotton bollworm caterpillars primarily damaged the generative organs of sunflower. Oil content was drastically reduced to 27.79% in the infected plants compared to 41.61% in the healthy plants. Seed quality was greatly impaired. Protein content in seed was 14.59% in the infected plants and 16.47% in the healthy plants. The differences increased as the growing season progressed. Mycological tests showed that the agent of head rot was the fungus *Rhizopus stolonifer* Ehrenb. Fr. (syn. *R. nigricans* Ehrenb).

Introduction

The 2003 outbreak of *Rhizopus* head rot is the most intensive occurrence of a disease caused by a fungus from the genus *Rhizopus* ever registered in this region. Up to 55% infected plants were counted in some fields. Disease symptoms were manifested differently, from 3 to 5 cm spots on the surface of infected heads to heads totally pervaded by the fungus. First symptoms were fairly similar to those caused by other fungal agents of head rot. Mostly those were wet brownish spots on the reverse side of the head. The spots eventually grew larger, the mycelium penetrated the parenchymatous tissue more and more, and the inside of the head turned soft. In cases when humidity was sufficiently high, a white film would cover the spots. When cut, the inside of the head was shown to be filled with a mass of black sporangia which resembled black pepper grains when looked at with a naked eye. This was a fairly reliable diagnostic sign for determining the agent of head rot. In cases of intensive infection, heads would rot through and fall off. In 2003, many infected heads became mummified in the extremely dry and hot weather. The presence of black sporangia was the best diagnostic sign for distinguishing *Rhizopus* head rot from white head rot (*Sclerotinia sclerotiorum* [Lib] de Bary) and gray mold (*Botrytis cinerea* Pers.) (Maširević and Gulya, 1992).

Materials and Methods

This study targeted monitoring the intensity of occurrence of *Rhizopus* head rot in sunflower commercial plots. Disease intensity was assessed in several spots per field, checking diagonally 100 plants per spot. Since it had been previously observed that head rot incidence was associated with the occurrence of cotton bollworm, fluctuations of the insect imagoes in the fields were monitored too. The insect numbers were monitored by placing trap lamps in several locations around the Vojvodina Province. Flight activity of the insect imago was monitored in the period 2000-2003. Infected plants were divided into categories "high damage," "medium damage," "low damage," and "healthy heads." The first category included heads with up to 20% of surface covered with spots. Medium damage included from 20 to 50% of damage on the surface. High damage described heads completely pervaded with the fungus' mycelium. Oil content was determined by NMR, per replication. Proteins were determined by Kjeldahl's method.

Mycological tests included the measurements of dimensions of sporangia, sporangiophores and sporangiospores. The tested material was observed for eventual forming of chlamydospores which could be used for determination of *Rhizopus* species. The fungus was isolated on potato dextrose agar and cultivated at 20C.

Results and Discussion

The cotton bollworm occurred on a large scale during the 2003 growing season which was characterized by extremely high temperatures. The situation was similar in the neighboring countries Hungary and Romania (Szeoke and Vasas, 2003). In the Vojvodina Province, air temperature was 2.1C higher relative to the long-term average. The average temperature over 30C occurred during the so-called "tropical days." Such weather conditions combined with abundant food encouraged the proliferation of the cotton bollworm. Flight behavior of the imago was changed accordingly in comparison with the previous years (Sekulić et al., 2003, 2004).

Botanically, cotton bollworm hosts belong to a wide spectrum of families which include many cultivated and weed plants. Sunflower is one of them. The pest attacks plant leaves which can lead to complete defoliation. Eggs are oviposited in the youngest plant parts. In the sunflower head, eggs are most frequently found in the petals and sepals of the ray and disk flowers. A total number of oviposited eggs in the heads of a double-cropped sunflower ranged from 38 to 178 in the first third of August. The first generation of the newly hatched caterpillars feeds on leaves. Subsequent generations attack head primordia and fully developed heads which suffer the most extensive damage. Caterpillars also feed on flowers and seeds at different stages of development and thus they directly reduce plant yield (Jasnić et al., 2004). Furthermore, by drilling holes in the head, caterpillars encourage *Rhizopus* invasion which results in the rotting and total destruction of sunflower heads.

In the location of Ada in 2003, *Helicoverpa armigera* caterpillars occurred on a large scale in a field planted late, on 20 May. *Rhizopus* head rot occurred subsequently. In some parts of the field, the intensity of infection of the head exceeded 70%. Seed yield of the healthy plants was 2.18 t/ha and the yield of the infected plants only 1.01 t/ha. Slightly infected plants yielded 1.99 t/ha (Table 1).

Table 1. Effect of *Rhizopus* head rot on sunflower seed yield (t/ha) in 2003.

Healthy plants	Low damage	Medium damage	High damage
2.18	1.99	1.96	1.01

On infected heads, 15 to 57 feeding sites were found which differed in shape and size. Table 2 shows that large differences in oil content existed between the healthy and infected heads. It was interesting to note that the different extents of damage did not result in significant differences in oil content. It is important to mention that the largest number of caterpillars was found near field borders, 10.1 per plant on the average. One hundred and two hundred meters inside the field, the respective numbers were 4.8 and 3 individuals per plant. *Rhizopus* head rot infection followed the same spacial pattern.

Table 2. Effect of *Rhizopus* head rot on sunflower oil content (%) in 2003.

Healthy plants	Low damage	Medium damage	High damage
41.69	27.79	28.85	29.97

Rhizopus head rot also affected sunflower protein content. At the time of the first sampling (28 August), the contents in intensively infected and medium infected plants were 14.59% and 15.23%, respectively. The contents in slightly infected and healthy plants were 16.24% and 16.47%, respectively. At the time of the second sampling (10 September), the protein contents remained in the same proportions but the actual figures for the intensively infected and healthy plants were 14.98% and 18.19%, respectively (Table 3).

Table 3. Effect of *Rhizopus* head rot on protein content (%) in sunflower seed in 2003.

Infection intensity	Protein content (%)	
	1st date (28 August)	2nd date (10 September)
High damage	14.59	14.98
Medium damage	15.23	17.00
Low damage	16.24	17.20
Healthy plants	16.47	18.19

Microscopic viewing and fungal cultivation on nutritive medium indicated that the causal agent of the disease was the fungus *Rhizopus stolonifer* (syn. *R. nigricans*).

Since there is no recent literature on the harmfulness of *Rhizopus* head rot in oil sunflower, the objective of this paper was to assess its harmfulness under the current conditions of production. The most recent data on *Rhizopus* head rot harmfulness pertain to confectionery sunflower (Shtienberg, 1997). According to that report, it was important to mitigate the aggravation of quality of confectionery sunflower while the fungus' effect on yield performance was less important. The situation seems to be reversed with oil sunflower.

Correlations were established between the number of and extent of damage by cotton bollworm on one hand and the number of plants infected by *Rhizopus* head rot on the other.

Relation of infestation with sunflower moth *Homoeosoma electellum* Hulst. larve to the incidence of *Rhizopus* rot in sunflower seed head has been reported by Klisiewicz (1979) in the USA.

Control Measures. Intensity of *Rhizopus* head rot may be reduced by controlling the cotton bollworm. Integrated pest management should be employed in the control of this pest. All available measures should be used but primarily agro technical and chemical ones. The most effective way is to grow resistant or tolerant sunflower hybrids. Since this is a new disease in our country, a way to achieve full disease resistance is still unknown. Inoculation methods have been worked out for the testing of breeding material (Yang and Thomas, 1981). Among agro technical measures, deep plowing in the fall and early sowing have been shown to be most effective. Between-row cultivation and weed control in and around the fields, i.e., measures of regular crop hygiene, reduce pest pressure and through it the intensity of *Rhizopus* head rot. Crop rotation is less important for this disease.

Conclusions

Extremely high temperatures in the course of spring and summer of 2003, 2.1C higher than the long-term average, and a large number of tropical days brought about a heavy proliferation and large-scale occurrence of the cotton bollworm (*Helicoverpa armigera* Hbn) in the Vojvodina Province. *Helicoverpa armigera* caterpillars attacked sunflowers, primarily their generative organs and leaves. The attacks were of varying intensity. The percentage of damaged plants ranged from 80% to 100%, and the number of caterpillars per plant from 2 to 17. Caterpillars fed on the upper and lower surfaces of sunflower heads, causing damage which facilitated a heavy invasion of the fungus *Rhizopus*. The number of damage sites on the infested heads ranged from 15 to 57. These sites differed in size. A correlation was found between the number of damaged heads and the number of plants infected by *Rhizopus* head rot. Intensively infected plants yielded 1.01 t/ha of seed while healthy plants yielded 2.18 t/ha.

Significant differences were also found in oil content. Healthy plants had a 41.61% oil contnet, while infected plants had from 27.79% to 29.97% oil.

The fungus *Rhizopus stolonifer* was found to be the causal agent of head rot in the Vojvodina Province.

A way to control *Rhizopus* head rot is to reduce the numbers of the cotton bollworm. This can be achieved by deep plowing in the fall, early sowing of threatened crops, between-row cultivation and weed control in and around the fields. Because of overlapping generations and a clandestine way of life, it is difficult to devise a cost effective and ecologically acceptable method of chemical control of *Helicoverpa armigera* caterpillars.

Fungicide application is fairly difficult under the local conditions and sometimes completely unfeasible.

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