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The Distribution of *Alternaria* sp. on *Brassica napus* and *Sinapis* sp. from the South Region of Romania

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Abstract

Alternaria sp. is a diverse group of pathogenic and saprophytic fungi often found in conjunction. The aim of this study was to estimate the distribution of six *Alternaria* sp. present in infected seeds samples taken directly from eight fields from the Calarasi County.

Five samples of *Brassica napus* and five samples of *Sinapis* sp. seeds were taken directly from the growing fields in June, just prior to harvest. Three replicates for each sample were performed. The seeds have not been disinfected prior to incubation in order to determine the distribution of the alternaria species on their surface. Five seeds were incubated in Petri dishes (ø 10 cm) on potato dextrose agar for each replicate. The fungi presence on seeds was evaluated after incubation for 10 days, at 22 ºC. A few semi-permanent slides were performed from each colony and were examined under a microscope (x40), field by field, until a total of 50 conidia of *Alternaria brassicae, Alternaria brassicicola, Alternaria radicinum, Alternaria dauci, Alternaria solani* and *Alternaria tenuis* were identified.

The distribution of *Alternaria* sp. was compared with the Chi2 or Fisher test ($p<0.05$ was considered significant). The analysis of the data obtained after the microscopic analysis of samples showed that the *Alternaria brassicicola* (~63.92%) and *A. brassicae* (13.44%) represent the most frequent species identified on *Brassica napus*. The *Alternaria brassicicola* (~53.76%) and *A. brassicae* (19.6%) are also the most frequent species of *Alternaria* found on *Sinapis* sp. seeds. The overall distribution of all *Alternaria* sp. identified on *Brassica napus* and *Sinapis* sp. seeds was different ($p<0.05$). These results demonstrated that the mixed infections with the *Alternaria* species are common events on investigated agricultural areas and support the variable affinity of *Alternaria* sp. for the host.

In conclusion our results indicated a significant dispersion of several members of *Alternaria* sp. on seeds from investigated area.

Keywords: *Alternaria, Brassica napus, Sinapis* sp, South Romania

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Introduction

Alternaria sp. is a cosmopolitan and diverse group of pathogenic and saprophytic fungi, widespread distributed in soil and organic matter [1].

Alternaria sp. have a variable affinity for host although often they are found in conjunction. For example *A. brassicicola* is the dominant invasive species of the vegetable Brassicas whereas *A. brassicae* is hosed mainly by oleiferous crucifers [2]. The majority of species that form this group can cause symptoms in different plants (grains, broccoli, carrots, potatoes and cauliflowers, tomatoes) with an important economic impact [3]. Thus, *Alternaria sp*. can infect siliquae and infected seeds represent an important source for pathogen transmission [4].

Data regarding the distribution of *Alternaria sp*. are useful for understanding epidemiology, disease prevention or chemical protection [5]. In addition some metabolites from *Alternaria sp*. are of interest for medical research (e.g. from *A. brassicicola* has been isolated Depudecin, an inhibitor of histone deacetylase and an antitumor agent) [6, 7], agriculture (e.g. from *A. brassicae* were isolated abscisic acid, plant growth regulation agent, and the phytotoxin Destruxin B, which has antitumor, antiviral, insecticidal, cytotoxic, immunosuppressant, and antiproliferative effects) [8, 9] or industry (isolation of cellulase from *A. brassicicola*).

Brassica napus is a plant that has seeds rich in oil and proteins, reason for which has various uses (e.g. forage, oil or biodisel production). Large fluctuations in surface and rapeseed production were recorded in the recent decades in Romania. In Romania, the rape area was 46,859 ha in the Calarasi County (2013) and represented 43.8% of the country' surfaces, with a production of 140,096 tonnes (2013) [10].

Sinapis sp. are phylogenetically close to the *Brassica* species and includes important aromatic and medicinal weed species [11] which were also used as experimental plants in research, citological, molecular [12] and physiological studies. Some hybrids derived from these species have important economical properties (e.g. are resistant to some diseases [13], high temperatures and drought stress [14]).

The data regarding disease prevalence and severity and the species of fungi present in the seed in the different agricultural areas from Romania are very scarce. The conditions for growing fields nearby Danube, including those from Calarasi, are permisive for the *Alternaria sp.* development and the epidemics may occurrs when wind and rainfall are frequent.

Aim

The aim of this study was to estimate the distribution of six *Alternaria* sp. present in the infected seed samples taken directly from ten growing fields affected by alternaria diseases from Calarasi county.

Materials and Methods

Five samples of *Brassica napus* and five samples of *Sinapis* sp. seeds were taken directly from growing fields affected by alternaria diseases in June (week 23-29), just prior to harvest. The growing fields with *Brassica napus* are located around Unirea, Dichiseni, Fundulea, Coslogeni, Dragalina (Calarasi County) whereas the growing fields with *Sinapis* sp. were located in Roseti, Coslogeni (Calarasi County), Frasinet, Alexandria, Smardioasa (Teleorman). The distribution of growing fields is shown in Figure 1.

Figure 1. *The Map of Growing Fields from Calarasi and Teleorman County Used to Harvest Brassica napus and Sinapis sp. Seeds*

From each investigated growing fields we collected 0.5 kg of the seeds. Three replicates for each sample were performed. For each replicate 5 seeds from the same cultivars were incubated in Petri dishes (ø 10 cm) on PDA culture environment (potato- dextrose- agar), sterilized at 121ºC / 20 min and then were incubated for 10 days, at 22 ºC. After incubation, the *Alternaria* species present in each of the colonies were examined under a microscope (x40), field by field, on semi-permanent slides until 50 *Alternaria* species were identified. The presence and absence of *Alternaria brassicae, Alternaria brassicicola, Alternaria radicinum, Alternaria dauci, Alternaria solani* and *Alternaria tenuis* was recorded and the percent of each fungus per sample was computed. The Petri dishes were stored at 5ºC until they were used.

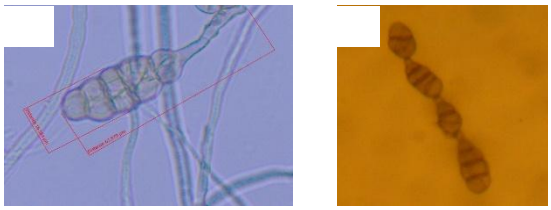
The distribution of *Alternaria* sp. was compared with the Chi2 or Fisher test with StatsDirect software (version 2.8.0). The p values <0.05 have been considered statistically significant.

Results

Samples from ten growing fields, with signs of alternaria diseases from Calarasi, were investigated. We analyzed the in vitro colonies formed by pathogens surrounding of all 150 seeds used in this experiment (5 seeds/plate x 3 replicates x 5 growing fields). These colonies were white-gray and brown and black on the reverse side. A total of 7500 *Alternaria sp.* individuals were

identified and were counted (50 / colony). The images obtained from seeds cultivated on PDA medium are shown in Figure 2.

Figure 2. *Mycelium of A) Alternaria dauci and of B) Alternaria brassicicola (Light Microscope, 40x)*



The main results obtained in this study are shown in Table 1. The overall distribution of all *Alternaria* sp. identified on the colonies formed surrounding all 75 *Brassica napus* seeds ($p<0.001$) and of all 75 *Sinapis* sp. seeds was different ($p<0.001$). The most common *Alternaria* species identified in all 150 colonies investigated is *Alternaria brassicicola* (average 58.84%, 29.42 conidia /colony). The analysis of the data obtained after the microscopic analysis of the samples also showed that the *Alternaria brassicicola* (~63.92%) and *A. brassicae* (13.44%) represent the most frequent species identified on in vitro colonies formed surrounding of 75 *Brassica napus* seeds. The *Alternaria brassicicola* (~53.76%) and *A. brassicae* (19.6%) are also the most frequent species of *Alternaria* found on 75 seeds of *Sinapis* sp.

Table 1. *Distribution of Alternaria Species Identified on Seeds from Brassica napus and Sinapis sp. Colected from South Region of Romania*

Seeds	Fungus distribution	*A. brassicicola*	*A. brassicae*	*A. solani*	*A. radicinum*	*A. tenuis*	*A. dauci*
Brassica napus	average/ colony	31.96	6.72	4.04	3.68	3.12	0.48
	interval /colony	17-38	0-16	0-8	0-9	0-9	0-6
Sinapis sp.	average/ colony	26.88	9,8	4.12	5.96	2.8	0.44
	interval /colony	15-34	6-16	0-11	0-12	0-9	0-6
All seeds	average/ coloni	29.42	8.26	4.08	4.82	2.96	0.46
	%/colony	58.84	16.52	8.16	9.64	5.92	0.92

The distribution of *Alternaria sp.*, which infect the 5 seeds taken from the same field and seeded in the same plate, was also compared. In 12 of the 15 (80%) plates the distribution of the six *Alternaria* species between seeds from the same plate is different ($p<0.05$). These seeds are from all growing fields. The highest differences ($p<0.001$) are detected for a plate in which was seeded with seeds harvested from Unirea. The distribution of the *Alternaria* species in 9 of the 15 (60%) plates with the *Sinapis* seeds is different ($p<0.05$). The

highest differences ($p<0.001$) are detected for two plates with seeds recolted from Coslogeni (Calarasi County) and Alexandria (Teleorman).

The *Alternaria* species showed significant differences when the analysis was restricted to the colonies formed surrounding the *Sinapis* seeds harvested from the growing fields from Teleorman (45 colonies, $p <0.0001$) or the Calarasi ($p = 0.0003$) county.

No other significant differences are identified for samples from these fields.

Discussion

Alternaria sp. species cause symptoms on leaves, stem and siliquae and reduction in the oil content of rapeseed cultivars [15]. This study was undertaken because the infections with pathogenic *Alternaria sp.* are common and have serious consequences. In addition, the information regarding disease prevalence, seed infection and the transmission of these funguses in the Romanian growing fields are lacunar.

We observed a great dispersion of several members of this group of fungi in the investigated area. The analysis of the data obtained after the microscopic analysis reveals that for all 150 colonies investigated the most common *Alternaria* fungus detected were *A. brassicicola* (on average 29.42 conidia /colony) and *A. brassicae* (8.26 conidia /colony) whereas the most rare were *A. dauci* (0.46 conidia /colony).

Also the *Alternaria brassicicola* (~63.92%) and the *A. brassicae* (13.44%) represent the most frequent species identified on in vitro colonies formed surrounding of 75 *Brassica napus* seeds. The *Alternaria brassicicola* (~53.76%) and *A. brassicae* (19.6%) are also the most frequent species of *Alternaria* found on 75 *Sinapis* sp. seeds.

In 21 of the 30 (70%) plates the distribution of the six *Alternaria* species between seeds from the same plate is different ($p<0.05$). This result indicated that seeds from the same field are infected in different ways by these pathogens.

Various species of pathogens are infected and disturb the growing, development and production of *Brassica* sp. and *Sinapis* sp. From them *Alternaria* sp., and especially *A. brassicae* and *A. brassicicola*, are considered to be the most widespread pathogens and to have with the greatest economic impact on *Brassica sp*. and *Sinapis* sp. The *Alternaria* species are often found in conjunction on infected plants. However, *A. brassicae* produces more severe infections and is more frequently identified in plants with *Alternaria* leaf spot disease than in *A. brassicicola* [16]. Also it was reported that for mustard, cabbage and cauliflower the infections with *Alternaria brassicae* (26.5%) or *A. brassicicola* (26.5%) were more rare compared to coinfections (50.9%) [17], whereas in seeds of *Brassica oleracea* from Victoria *A. brassicicola* was detected (26 of 44 samples) but not *A. brassicae* [18]. *A. brassicicola* were detected in all colonies formed *in vitro* surrounding booth type of seeds. Also our results are partially in concordance with these data and 100% of the *Sinapis*

sp. and 96% of the tested *Brassica* seeds are concomitant infected by *A. brassicae* and *A. brassicicola.* We can speculate than these differences are the consequence of factors favoring the dispersion of these funguses or of the affinity pathogen for the host.

Mixed infections with *Alternaria* species have diverse consequences. The most common types of *Alternaria* can affect the plants cells (e.g. induces apoptosis or can directly damage host cells) and seeds [19] and can produce different toxic chemical componunds (e.g. secondary metabolites, proteins) that facilitates their necrotrophic lifestyle [20] and also can predispose to some human ilenes (e.g. the association between mold-derived allergens and asthma). The presence of different *Alternaria* sp. on the same field may determine confusing symptomsmes on foliar parts of the plant and it becomes difficult to establish the species of the pathogen causing *Alternaria* leaf spot disease.

We can speculate that different factors (like rain and wind) may represent an important source of infection and of dispersion of this fungus in the investigated fields from Calarasi and Teleorman. The monthly amount of rainfall during harvesting in this region was between 76-125 mm [21]. The presence of several *Alternaria* sp. on each investigated seeds collected from the neighboring geographical areas indicates a clear need for field surveys for these common pathogens, for improving the methods used for the control of the seed health and quality and for preventing the potential impact of toxic metabolites. It is also necessary to extend this study on a high number of growing fields and to investigate a higher number of *Alternaria* sp. for a better understanding of the impact of the pathogenic species of *Alternaria* in Romania.

Conclusions

These results demonstrated that the mixed infections with *Alternaria* species are common events on investigated agricultural areas and support the variable affinity of *Alternaria* sp. for host.

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