

## MORPHOLOGICAL CHARACTERISTICS OF THE CAUSAL AGENT OF BROWN SPOT DISEASE IN NATURAL CONDITIONS

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### ABSTRACT

*Alternaria alternata* is a variable species which morphological characteristics highly depend on the effect of environmental factors. Measurements are usually made on pure culture of the fungus, but the aim of these investigations was to give data on morphological characteristic of the species in naturally infected tobacco leaves.

Microscopic measurements revealed that in this case the hyphae of *A. alternata* are thinner, while conidiophores are wider and longer compared to those in pure culture. It was also determined that the conidial size is larger and the number of transverse and longitudinal septa is higher. Oblique septa are also present. The chains are longer and consist of 3-7 conidia.

**Key words:** *Alternaria alternata*, tobacco

## МОРФОЛОШКИ КАРАКТЕРИСТИКИ НА ПРЕДИЗВИКУВАЧОТ НА БОЛЕСТА КАФЕНА ДАМКАВОСТ ВО ПРИРОДНИ УСЛОВИ

*Alternaria alternata* е доста варијабилен вид, чии морфолошки карактеристики можат да зависат од влијанието на еколошките фактори. Најчесто, мерењата се вршат во чиста култура од габата. Но, целта на овие истражувања беше да се дадат податоци за нејзините морфолошки карактеристики во природни услови, односно во инфицирани тутунски листови.

При микроскопските мерења беше утврдено дека во овој случај таа има потенки хифи, а конидиофорите се пошироки и подолги отколку во чиста култура. Исто така и конидиите се со поголеми димензии, со поголем број напречни и надолжни септи, а имаат и коси септи. Формираат подолги низи, со 3-7 конидии.

**Клучни зборови:** *Alternaria alternata*, тутун

## INTRODUCTION

*Alternaria alternata* is the most frequently mentioned and studied among all *Alternaria* species. It is a saprophyte feeding on dead plants, stored products, soil, skin and textile, an epiphyte on leaves and a parasite on living plants. *A. alternata* is mostly a pathogen of weakness which in suitable moments attacks the susceptible and stressed plants. In some cases, however, it can attack the healthy and vigorous plants too (Rotem, 1994).

Almost all *Alternaria* infected cultures can be also attacked by *A. alternata*. Thus, plants of the Solanaceae family, beside with *A. solani*, can be infected with *A. alternata* on tomato (Grogan et al., 1975), peppers (Halfon-Meiri and Rylski, 1983) and potato (Cakarevic and Boskovic, 1994).

*A. alternata* has been recorded on at least 115 host-plants in 43 families. 74% of the publications refer to 14 host-plants, of which tobacco is the most frequently mentioned (in 13% of all publications) (Rotem, 1994).

*A. alternata* is the causal agent of brown spot disease and it attacks all types of tobacco. The intensity of attack depends on climate conditions, susceptibility of the varieties and many other factors during tobacco production (Gveroska and Taskoski, 2006, 2007).

Typical for this disease is the occurrence of brown spots on leaf surface. As the infection spreads, their centers become brown and concentric rings appear around them. With maturation of leaves a yellowish chlorotic zone is formed around the spots. In periods with high humidity the sporulation is abundant and organs of the pathogen appear in the middle of the spots, forming black powdery cover.

The increased chlorotic zone and total infected area results in reduced humidity, alkaloids and reducing sugars in the leaf (Main, 1971). The increase of disease intensity results

in higher nicotine (21 - 31%), lower reducing sugars (1%) and higher filling capacity (51%) compared to the healthy tobacco (Karunakara, 1998).

According to Spurr and Main (1974), the necrosis includes gradual complexication among the oxidized phenols, amino acids and proteins. Beside its direct impact on leaf quality and yield, the pathogen also affects the smoking properties of leaf, reflected in less pronounced aroma and "sweet" taste, and strongly expressed "empty" taste.

Tobacco plant is a suitable substrate for development of this pathogen during the vegetation period and later, in the period of curing and processing. The fungus was isolated from molds typical for storehouses, but it was also found in cigarettes (Kantor et al, 1979).

*A. alternata* consists of species which release specific toxins with high pathogenicity to certain host-plants. For this reason, Otani and Khmoto (1992) and Rotem (1994) suggested that they should be marked as specialized forms of *A. alternata*. For example, the species marked *A. alternata* f. *sp. tabaci* releases the AT toxin, which is pathogenic to tobacco.

According to Kodama et al. (1990), the purified AT toxin of 0.2 µg/ml inhibits the root development in tobacco seedlings and that of 1-2 µg/ml provokes necrosis on each leaf. All isolates to which this toxin was added, including the spores of the non-pathogenic ones, cause brown spot disease on the inoculated plants. The results show that AT toxin is a distinguishing factor in the pathosystem of the genus *Nicotiana* – *A. alternata*.

The aim of investigations was to give data about morphological characteristics of *A. alternata* on infected tobacco leaves in natural conditions.

## MATERIAL AND METHODS

Infested leaves were sampled from 3 tobacco varieties, with severe attack of brown spot disease, i.e. the sporulation was abundant and spots were already covered with black powder.

The fungus morphology was investigated by microscopic measurement of hyphae width and the size (l/w) of conidiophores and conidia.

The material was collected from leaf spots using a sterile needle and 5 microscopic

preparations were made for each variety.

Measurements were made on 100 randomly selected samples using ocular

micrometer, previously calibrated, according to the method of Ziberoski (1998). Transverse, longitudinal and oblique septa were also counted.

## RESULTS AND DISCUSSION

Hyphae of the fungus are septate and they are branching laterally, under different angles. In preparations they are less prominent

compared to conidia and conidiophores. They are transparent to light brown, 2.35 - 4.80  $\mu\text{m}$  wide, or 3.87  $\mu\text{m}$  in average (Table 1).

Table 1. Hyphal width of *A. alternata* ( $\mu\text{m}$ )

| Variety | Width       |         |
|---------|-------------|---------|
|         | range       | average |
| MV 1    | 2,35 - 4,00 | 3,93    |
| O 110   | 3,20 - 4,00 | 3,64    |
| P 23    | 3,20 - 4,80 | 4,06    |
| Average | 2,35 - 4,80 | 3,87    |

The width of conidiophores ranges from 2.00  $\mu\text{m}$  to 6.00  $\mu\text{m}$ , or 3.80 - 4.51  $\mu\text{m}$  in average (Table 2). Their length ranges from 16.00  $\mu\text{m}$  to 56  $\mu\text{m}$ , or 32.80 - 45.33  $\mu\text{m}$  in average.

The lowest size of conidiophores from the spots of infected leaves was recorded in

MV1 variety.

Thus, it can be stated that the size of conidiophores in natural conditions is (2,00 x 6,00) $\mu\text{m}$  x (16.00 - 56.00)  $\mu\text{m}$ , or in average 4.19 x 37.95 (Table 2).

Table 2. Dimensions of conidiophores of *A. alternata* ( $\mu\text{m}$ )

| Variety | width      |         | length      |         | width x length |
|---------|------------|---------|-------------|---------|----------------|
|         | from - to  | average | from - to   | average |                |
| MV 1    | 2,00-6,00  | 3,80    | 16,00-56,00 | 32,80   | 3,80 x 32,80   |
| O 110   | 3,20 -6,00 | 4,27    | 32,00-47,00 | 45,33   | 4,27 x 45,33   |
| P 23    | 3,20 -6,00 | 4,51    | 16,00-52,00 | 35,71   | 4,51 x 35,71   |
| Average | 2,00- 6,00 | 4,19    | 16,00-56,00 | 37,95   | 4,19 x 37,95   |

Conidia are usually single or they can form a chain consisting of higher number of conidia (3-8) (Photo 1).

Photo 1. Conidia of *Alternaria alternata* in chain

Data presented in Table 3 reveal that conidia width ranged from 10.00  $\mu\text{m}$  to 24.00  $\mu\text{m}$ , and the average range was 16.00 - 19.01  $\mu\text{m}$ .

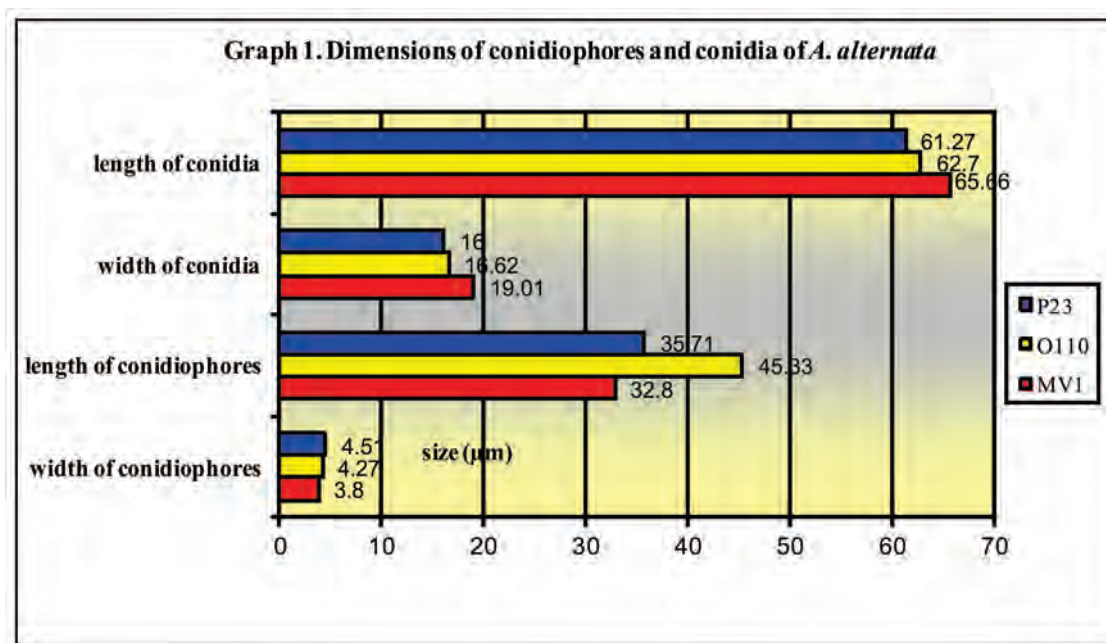
The conidia length ranged from 44.00  $\mu\text{m}$  to 96  $\mu\text{m}$ , and the average values are similar - 61.27 to 65.66  $\mu\text{m}$ .

Table 3. The size of *A. alternata* conidia ( $\mu\text{m}$ )

| Variety | width        |         | length       |         | width x length |
|---------|--------------|---------|--------------|---------|----------------|
|         | from - to    | average | from - to    | average |                |
| MV 1    | 15,20- 24,00 | 19,01   | 50,00-92,00  | 65,66   | 19,01 x 65,66  |
| O 110   | 10,00 -24,00 | 16,62   | 44,00 -96,00 | 62,70   | 16,62 x 62,70  |
| P 23    | 12,00 -20,00 | 16,00   | 52,00 -74,00 | 61,27   | 16,00 x 61,27  |
| Average | 10,00 -24,00 | 17,21   | 44,00 -96,00 | 63,21   | 17,21 x 63,21  |

According to the data presented, conidial size varies in a wide range from 10.00 - 24.00 x 44.00 - 96.00  $\mu\text{m}$ , with average values of 17.21 x 63.21  $\mu\text{m}$ .

The largest conidia were observed on the infected leaves of MV1 variety. (Table 3, Figure 1).



Conidiophores and conidia of *Alternaria alternata* in natural conditions are presented in Photo 2, 3 and 4.

Conidia have 3-8 transverse septa and 1-6 longitudinal septa. They also have up to four

oblique septa (Table 4).

Major percentage of conidia have 5-6 transverse and 2-3 longitudinal septa, and 1-2 oblique septa (Table 5, Fig.2).

Table 4. Number of septa in conidia

| Variety | Transverse septa | Longitudinal septa | Oblique septa |
|---------|------------------|--------------------|---------------|
| MV 1    | 3 - 8            | 1 - 6              | 0 - 4         |
| O 110   | 5 - 8            | 1 - 6              | 0 - 2         |
| P 23    | 1 - 7            | 1 - 5              | 1 - 3         |
| Average | 3 - 8            | 1 - 6              | 0 - 4         |

Table 5. Percentage of conidia with different number of septa

| a) Transverse septa |                 |      |       |       |       |       |
|---------------------|-----------------|------|-------|-------|-------|-------|
| Variety             | Number of septa |      |       |       |       |       |
|                     | 3               | 4    | 5     | 6     | 7     | 8     |
| conidia %           |                 |      |       |       |       |       |
| MV 1                | 20,00           | 0,00 | 46,66 | 13,33 | 6,66  | 26,66 |
| O 110               | 0,00            | 0,00 | 21,42 | 64,28 | 0,00  | 14,28 |
| P 23                | 8,33            | 8,33 | 25,00 | 33,33 | 25,00 | 0,00  |
| Average             | 9,44            | 9,44 | 31,03 | 36,98 | 10,55 | 13,65 |

b) Longitudinal septa

| Variety | Number of septa |       |       |      |      |       |
|---------|-----------------|-------|-------|------|------|-------|
|         | 1               | 2     | 3     | 4    | 5    | 6     |
|         | conidia %       |       |       |      |      |       |
| MV 1    | 13,33           | 20,00 | 26,66 | 6,66 | 0,00 | 26,66 |
| O 110   | 21,43           | 21,43 | 35,71 | 7,14 | 0,00 | 14,28 |
| P 23    | 16,66           | 41,66 | 25,00 | 8,33 | 8,33 | 0,00  |
| Average | 17,14           | 27,70 | 29,12 | 7,40 | 2,78 | 13,64 |

c) Oblique septa

| Variety | Number of septa |       |       |      |       |
|---------|-----------------|-------|-------|------|-------|
|         | 0               | 1     | 2     | 3    | 4     |
|         | conidia %       |       |       |      |       |
| MV 1    | 26,66           | 33,33 | 26,66 | 0,00 | 13,33 |
| O 110   | 35,71           | 57,14 | 7,14  | 0,00 | 0,00  |
| P 23    | 0,00            | 33,33 | 58,33 | 8,33 | 0,00  |
| Average | 20,79           | 41,26 | 30,71 | 2,78 | 4,44  |

Graph 2. Percentage of conidia with various number of septae

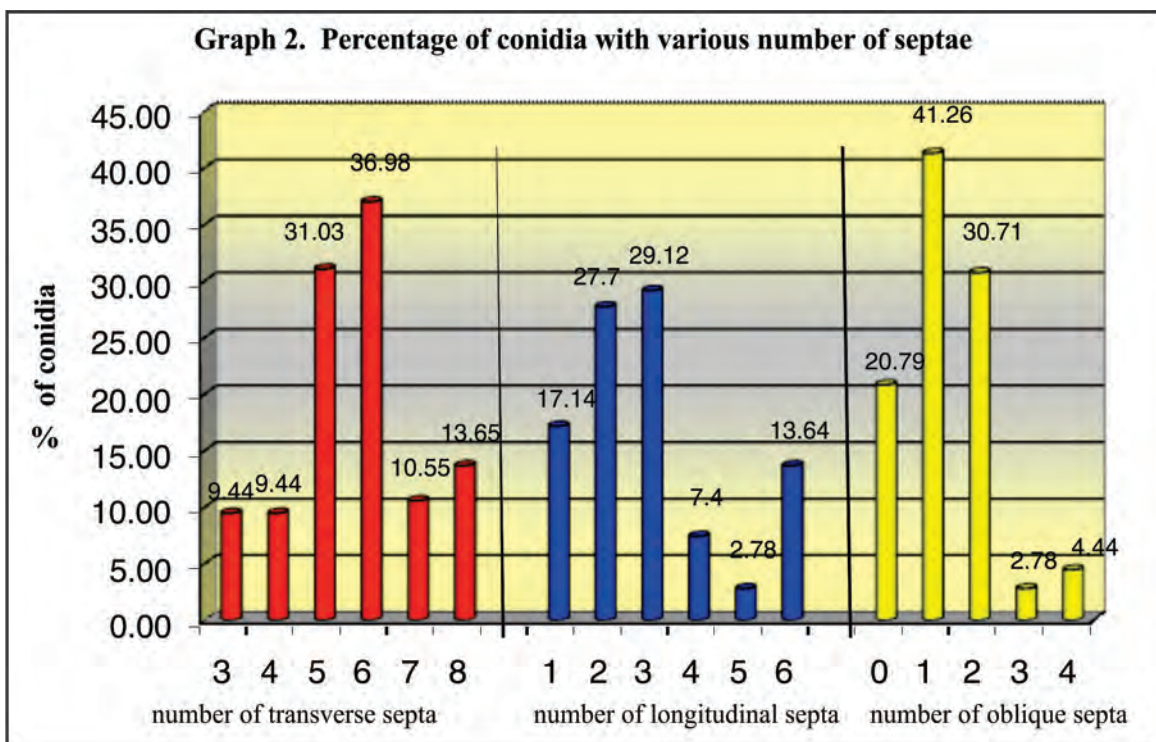




Photo 2. Conidiophores and conidia of *A. alternata* on infected leaves

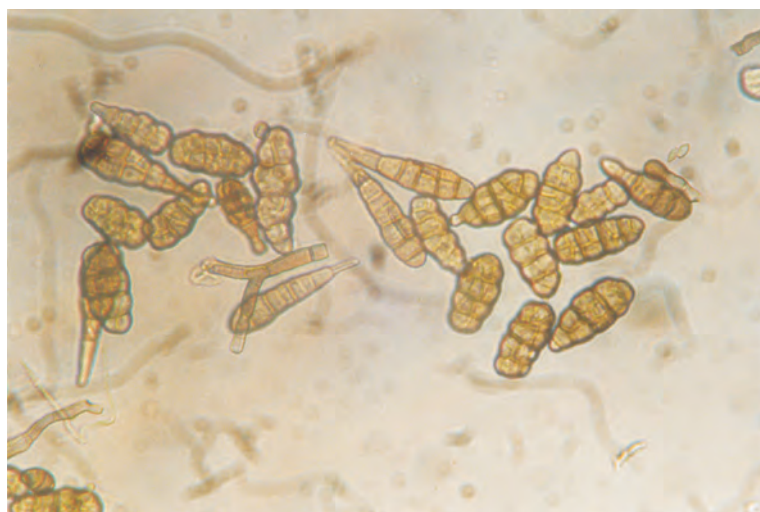


Photo 3. Conidia of *A. alternata* on infected leaves

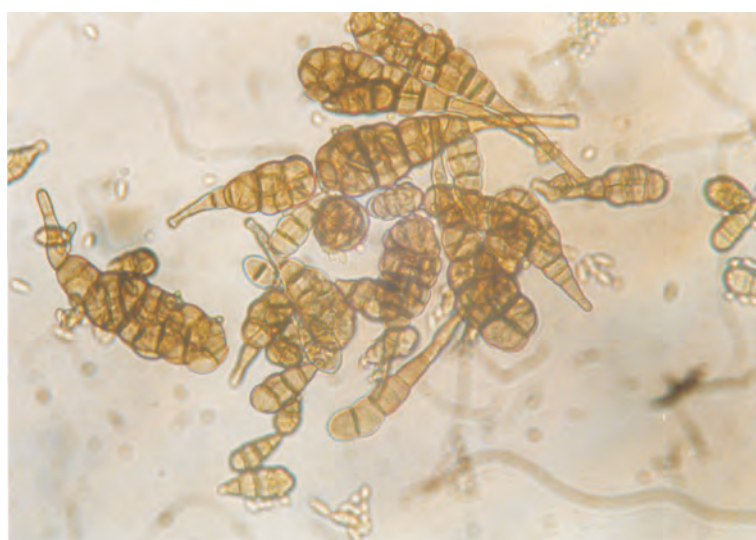


Photo 4. Conidia of *A. alternata* on infected leaves

Morphological data for *A. alternata* on naturally infected plant material are in compliance with the results of Rotem (1994) and Misaghi (1978).

Compared to hyphae of the pure culture (Gveroska and Taskoski, 2009), it can be noted that hyphae of the fungus are thinner and conidiophores are wider and longer. The conidia are single, but they can also form chains containing of higher number of conidia with transverse, longitudinal or oblique septa. According to

Misaghi et al. (1978), environmental factors have a significant effect on conidial size.

Su and Sun (1981) determined polymorph conidia in infected tobacco leaves. They had golden brown bodies with light brown to transparent beaks, long chains (10 conidia or more) which are sometimes branched, with transverse, longitudinal and oblique septa. They are also larger compared to the ones from the culture grown on media.

## CONCLUSIONS

➤ In naturally infected tobacco leaves, *Alternaria alternata* created hyphae which were 2,35 - 4,80  $\mu\text{m}$  wide (3,87  $\mu\text{m}$  in average).

➤ Conidiophore width ranged 2,00 - 6,00  $\mu\text{m}$  and length 16,00 - 56,00  $\mu\text{m}$ . Their average size was 4,19  $\mu\text{m}$  x 37,95  $\mu\text{m}$ .

➤ Conidial width ranged between 10,00 and 24,00  $\mu\text{m}$  and length between 44,00 and 96,00  $\mu\text{m}$ . Their average size was 17,21 x 63,21  $\mu\text{m}$ .

➤ Infected tobacco leaves of MV1 variety had the lowest size of conidiophores and the largest size of conidia.

➤ Number of septa per conidium ranged as follows: 3-8 transversal, 1-6 longitudinal and 0-8 oblique. The highest number of conidia have 5-6 transversal, 2-3 longitudinal and 1-2 oblique septa.

➤ *Alternaria alternata* in naturally infected tobacco leaves has thinner hyphae and conidiophores are wider and longer compared to those in pure culture. Also, the conidial size is larger and they have higher number of transverse, longitudinal and oblique septa. They form longer chains, consisting of 3-7 conidia.

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