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Cent. Eur. J. Biol. • 7(6) • 2012 • 1076-1083
DOI: 10.2478/s11535-012-0095-3

Central European Journal of **Biology**

# *In vitro* growth of some species of *Ascochyta* Lib.

**Research Article**

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**Received 23 April 2012; Accepted 28 August 2012**

**Abstract:** Fungi from the genus Ascochyta are generally facultative saprotrophs, which cause diseases in both monocots and dicots. Over 1 000 species belonging to this genus have been identified, 18 of which infect monocot plants from the family *Poaceae*. This study analyses the effects of temperature and light on the growth of selected fungi which infect monocots (*A. agrostidis, A. avenae, A. brachypodii, A. desmazieri, A. digraphidis, A. ducis-aprutii, A. festucae, A. graminea, A. hordei, A. hordei var. americana, A. hordei var. europea, A. hordei var. hordei, A. melicae, A. phleina, A. skagwayensis, A. sorghi, A. stipae, A. zeicola*), grown on three types of media; Potato Dextrose Agar (PDA), Coon's agar (CN) and oatmeal agar (OMA). The fastest growth among the analyzed fungi at low temperatures was found in *Ascochyta melicae*, while at high temperatures it was *A. zeicola*. The fastest *in vitro* growth (average of all fungi) was observed on CN medium at 20ºC (3.4 mm/day), while the lowest on OM medium at 5ºC (1.0 mm/day). Radial mycelial growth in dark and the light conditions varied. On average, all isolates grew faster in the dark (3.1 mm/day) than in the light (1.9 mm/day). The greatest effect on the production of pycnidia was found for the isolates. Variation in growth and production of pycnidia depended on temperature, medium and lighting for fungi from the genus *Ascochyta* infecting monocots. Such variation indicates a potential occurrence of these fungi in different environments.

**Keywords:** *Growth of fungi • Monocotyledonous plant pathogen*



## 1. Introduction

Most species of fungi from the genus *Ascochyta* are facultative saprotrophs. These organisms are known to cause diseases in a variety of mono- and dicot plants. In 1977, over 1 000 fungal species from the genus *Ascochyta* were described worldwide, though a large proportion of these species were later reclassified as synonyms. To date, no comprehensive description or classification of the species in this genus has been developed. *Ascochyta* fungi infect plants of the family *Poaceae*, with 18 plant species known to be diseased by members of this genus [1,2]. In the case of these host plants, the fungus is considered a "weak" pathogen (they become activated when the functioning of the immune system is disturbed or inhibited). Still, the fungi from the genus *Ascochyta* have the potential to infest commercially grown cereals, particularly wheat and barley, and grasses [3-6]. Thus, it is important to identify the environmental requirements of these pathogens in order to provide better management strategies for plant protection. Determining optimal growth and sporulation conditions of these fungi may facilitate the identification of conditions that may lead to plant infestation.

## 2. Experimental Procedures

The experiments were conducted in vegetation chambers at constant temperatures of 5, 10, 15, 20 or 25ºC. Additionally, the development of fungi was investigated in chambers kept at a temperature of 20ºC with a 12-h light cycle using Osram L36W/76 artificial light and Philips TLD 36W/108 fluorescent lamps (180 W/m$^2$). Analyzed isolates of fungi from the genus

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Ascochyta were obtained from the plant pathogen collection at the Department of Phytopathology, the Poznań University of Life Sciences, Poland and Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre. Fungi were isolated from cereal and grass plants.

Polystyrene Petri dishes were covered with 5mm discs of PDA media overgrown with the respective fungal isolates. The 24-h radial growth increment was calculated by subtracting ½ of the colony's diameter at day 2 from ½ of the colony's diameter at day 7. Fungal development was analyzed on a standard PDA (Potato Dextrose Agar, Sigma P2182) medium, and on two media recommended for the growth of pycnidia-forming fungi, OMA (oatmeal agar Sigma-O3506) [7,8], and CN (Coon's agar) [9,10]. The optimal temperature was determined by the fastest *in vitro* radial growth for the same isolate grown on different media. Observations of pycnidium production were taken in the course of the mycelial growth measurements.

Statistical analyses were performed using the software package Statistica version 8.0. Analyses of variance (ANOVA) were conducted and homogeneous groups were identified with the use of the Newman–Keuls test. A two-way ANOVA was performed, with temperature (T) and culture medium (M) as factors (Table 2). Another two-way ANOVA was conducted (Table 3), with fungal isolate (S) and light condition (L) as factors.

Regression curves of fungal growth within the range of temperatures from 5 to 25ºC (temperature points at every 5 degrees), where $f(T)=\beta_0+\beta_1 T+\beta_2 T^2$ were plotted. Next, The maximum of each function was calculated when $\Delta>0$ ($\Delta=\beta_1^2- 4\cdot\beta_2\cdot\beta_0$). There are two results of the function expressed by the following formulas:

$$T_1=\frac{-\beta_1-\sqrt{\Delta}}{2\beta_2}; \quad T_2=\frac{-\beta_1-\sqrt{\Delta}}{2\beta_2},$$

when $\Delta=0$, this is a one result expressed by the formula:

$$T_1=\frac{-\beta_1}{2\beta_2}, \text{ where } \Delta<0,$$

where there are no results (Table 1). When the resulting function did not have a maximum or the maximum was found outside the analyzed temperature range, the optimal growing temperature for the fungi was not determined. A Principal Component Analysis (PCA) was conducted and the location of points was presented in a scatterplot representing growth of individual isolates (Figures 1,2).

## 3. Results

The optimal temperature for fungal growth depended on the culture medium (Table 1). The coefficient of determination ($R^2$) exceeded 0.5 in most cases. Optimal temperature varied, although these differences were smaller than the variation in growth found among isolates. Differences were also found between optimal growth temperatures within a species grown on different media. The species *A. avenae* (isolate 1982), was characterized by optimal growth temperatures ranging from 18.2 to 21.9ºC, while another isolate (C811.84a) had a much lower optimal temperatures of 14.6 to 15.3ºC. A slightly lower variation was observed within *A. brachypodii*: from 15.8 to 16.2ºC, from 14.9 to 15.5ºC and from 19.1 to 21.7ºC for isolates 1155, 1523a/2 and 1968a, respectively. The lowest optimal temperature for fungal growth was 14.1 to 14.9ºC observed in *A. melicae* (2010), while the highest was found for *A. zeicola* at 24.2ºC. There was a significant effect of temperature, medium and the interaction between temperature and medium on fungi growth (temperature (T): df=4, F=1970.02, P=0.000, medium (M): df=2, F=28.95, P=0.000, for interaction C*I: df=8, F=11.13, P=0.000). The fastest growth of all the *Ascochyta* fungi was recorded on Coon's medium (CN), whereas differences in growth between Potato Dextrose Agar (PDA) and oatmeal agar (OMA) were non-significant. The average growth on OMA was 2.1 mm/day. There were significant differences in fungal growth among all the analyzed temperatures, with radial growth slowing down with decreasing temperature: 3.2 mm/day (20ºC), 2.9 mm/day (15ºC), 2.4 mm/day (25ºC), 1.3 mm/day (10ºC) and 1.1 mm/day (5ºC). An interaction was found between the analyzed factors, which makes it possible to state the best temperature-medium combination. The best temperature/medium combination for fungal growth was 20ºC using Coon's agar (3.4 mm/day), whereas the worst combination was 5ºC and oatmeal agar (1.0 mm/day) as well as Coon's agar (1.1 mm/day). When fungal growth was assessed on PDA medium in different lighting scenarios, the growth rate of fungi in the dark was markedly higher (3.1 mm/day) than the growth rate under 12 h of light (1.9 mm/day, ANOVA for strains (S): df=25, F=25.447, P=0.000, for light (L): df=1, F=62.774, P=0.000, for interaction C*I: df=25, F=7.985, P=0.000, Table 3).

Reaction of species and isolates to different light conditions varied greatly. For *A. brachypodii* (1523a/2), *A. hordei* var. *europea* (C817.84) and *A. phleina* (C517.74), growth in the dark and in the light was very similar. In turn, the following isolates responded with considerably slower growth under light: *A. brachypodii*

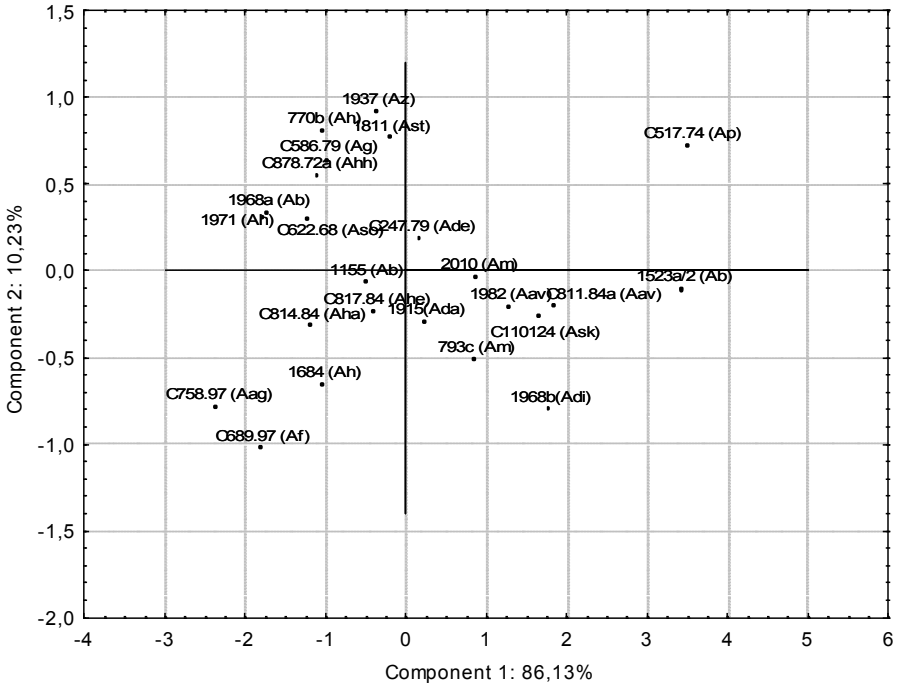


**Figure 1.** Distribution of points representing growth of fungi from the genus Ascochyta at different temperatures and on different media in the system of two principal components.
*A. agrostidis*- C758.97 (Aag), *A. avenae*- 1982 (Aav), C811.84a (Aav), *A. brachypodii*- 1155 (Ab),1523a/2 (Ab), 1968a (Ab), *A. desmazieri*- C247.79 (Ade), *A. digraphidis*- 1968b(Adi), *A. ducis-aprutii*- 1915(Ada), *A. festucae*- C689.97 (Af), *A. graminea*- C586.79 (Ag), *A. hordei*- 1684 (Ah), 1971 (Ah), 770b (Ah), *A. hordei* var. *americana*- C814.84 (Aha), *A. hordei* var. *europea*- C817.84 (Ahe), *A. hordei* var. *hordei*- C878.72a (Ahh), *A. melicae*- 2010 (Am), 793c (Am), *A. phleina*- C517.74 (Ap), *A. skagwayensis*- C110124 (Ask), *A. sorghi*- C622.68 (Aso), *A. stipae*- 1811 (Ast), *A. zeicola*- 1937 (Az)

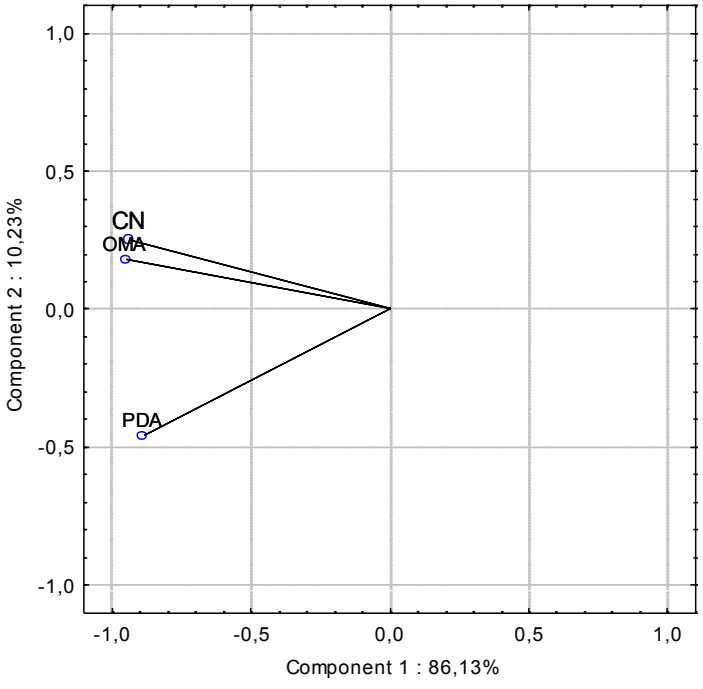


**Figure 2.** Distribution of straights representing different media in the system of two principal components.

| Species | Isolate | Temperature [°C] | | | adjusted $R^2$ | | |
|---|---|---|---|---|---|---|---|
| | | CN | OMA | PDA | CN | OMA | PDA |
| *A. agrostidis* | C758.97 | –* | –* | –* | – | – | – |
| *A. avenae* | 1982 | 20.7 | 21.9 | 18.2 | 0.87 | 0.77 | 0.61 |
| *A. avenae* | C811.84a | 14.6 | 15.3 | 14.9 | 0.44 | 0.65 | 0.67 |
| *A. brachypodii* | 1155 | 16.2 | 15.8 | 16.0 | 0.70 | 0.84 | 0.72 |
| *A. brachypodii* | 1523a/2 | 15.4 | 14.9 | 15.5 | 0.58 | 0.60 | 0.50 |
| *A. brachypodii* | 1968a | 21.7 | 19.1 | 20.2 | 0.79 | 0.85 | 0.67 |
| *A. desmazieri* | C247.79 | –* | –* | –* | – | – | – |
| *A. digraphidis* | 1968b | 20.1 | 16.0 | 16.2 | 0.5 | 0.69 | 0.62 |
| *A. ducis-aprutii* | 1915 | 15.3 | 15.2 | 14.9 | 0.6 | 0.68 | 0.64 |
| *A. festucae* | C689.97 | –* | –* | –* | – | – | – |
| *A. graminea* | C586.79 | 23.2 | 19.5 | 18.8 | 0.79 | 0.68 | 0.42 |
| *A. hordei* | 1684 | –* | –* | –* | – | – | – |
| *A. hordei* | 1971 | 16.9 | 16.1 | 17.1 | 0.61 | 0.64 | 0.78 |
| *A. hordei* | 770b | –* | –* | 16.5 | – | – | 0.24 |
| *A. hordei var. americana* | C814.84 | 19.3 | 16.6 | 22.7 | 0.72 | 0.74 | 0.71 |
| *A. hordei var. europea* | C817.84 | –* | –* | –* | – | – | – |
| *A. hordei var. hordei* | C878.72a | 20.8 | 18.4 | –* | 0.84 | 0.57 | – |
| *A. melicae* | 2010 | 14.5 | 14.9 | 14.1 | 0.72 | 0.68 | 0.49 |
| *A. melicae* | 793c | 17.0 | 15.4 | 15.7 | 0.46 | 0.69 | 0.67 |
| *A. phleina* | C517.74 | –* | –* | –* | – | – | – |
| *A. skagwayensis* | C110124 | 16.7 | 15.8 | 15.6 | 0.70 | 0.63 | 0.73 |
| *A. sorghi* | C622.68 | 23.1 | 18.1 | 20.8 | 0.85 | 0.81 | 0.74 |
| *A. stipae* | 1811 | 15.3 | 15.2 | 14.9 | 0.45 | 0.35 | 0.25 |
| *A. zeicola* | 1937 | –* | 24.2 | –* | – | 0.80 | – |

**Table 1.** Optimal temperature for the fastest growth of *Ascochyta* spp. isolates and R2 for the model.

**optimal temperature for the fastest fungal growth lies outside the analyzed area, or the function has no maximum*
*CN - Coon's agar; OMA - oatmeal agar; PDA - Potato Dextrose Agar*

(1155), (1968a), *A. digraphidis* (1968b), *A. ducis-aprutii* (1915) and *A. hordei* (1684), (1971), (770b). The fastest growing fungi were *A. agrostidis* (C758.97), *A. festucae* (C689.97), *A. graminea* (C586.79), *A. hordei* (1971), *A. hordei* var. *americana* (C814.84), *A. melicae* (2010), *A. sorghi* (C622.68) and *A. zeicola* (1937). They each grew at a rate of more than 3.0 mm/day. The slowest growth was recorded for *A. phleina* (C517.74), which grew at a rate of 0.4 mm/day. Observations of pycnidium production conducted at different temperatures, on different mediums and at different lighting regimes show that a given fungal isolate plays a decisive role in pycnidium formation or its absence (Table 4). Fungi in which pycnidium generation was observed included *A. brachypodii* (1523a/2), *A. hordei* (1684), *A. hordei* var. *europea* (C817.84), *A. hordei* var. *hordei* (C878.72a), *A. skagwayensis* (C110124) and *A. zeicola* (1937). In these isolates, the production of pycnidia was observed only under certain growth conditions. Pycnidium formation was promoted by alternating light conditions and culture on oatmeal agar. A Principal Component Analysis was performed to obtain homogeneous information. The many factors influencing the growth of Ascochyta were taken into consideration. Interdependencies determining the growth of individual isolates at different conditions are presented in Figure 1. A close location of points representing individual isolates reflects a similar growth rate of these isolates at different temperatures and on different media. The reaction of isolates within *A. hordei* varied, as shown by the high scatter of points representing these isolates. It is difficult to isolate distinct groups on the graph, but considerable separation from the other isolates can be observed in the

| Medium | Temperature [°C] | | | | | |
|---|---|---|---|---|---|---|
| | 5°C | 10°C | 15°C | 20°C | 25°C | Mean |
| CN | 1.1 | 1.3 | 3.0 | 3.4 | 2.6 | 2.3 |
| OMA | 1.0 | 1.4 | 2.8 | 3.0 | 2.3 | 2.1 |
| PDA | 1.2 | 1.3 | 2.8 | 3.1 | 2.2 | 2.1 |
| $LSD_{N\text{-}K\ 0.05}$ | | | 0.12 | | | 0.13 |
| Mean | 1.1 | 1.3 | 2.9 | 3.2 | 2.4 | |
| $LSD_{N\text{-}K\ 0.05}$ | | | 0.19 | | | |

**Table 2.** Radial growth of *Ascochyta* spp. fungi on different media at different temperatures.

*CN - Coon's agar; OMA - oatmeal agar; PDA - Potato Dextrose Agar*

| Species | Isolate | Fungal growth [mm/day] | | Mean |
|---|---|---|---|---|
| | | in the dark | 12 h darkness/12 h light | |
| *A. agrostidis* | C758.97 | 4.1 | 3.2 | 3.6 |
| *A. avenae* | 1982 | 3.1 | 1.6 | 2.4 |
| *A. avenae* | C811.84a | 2.9 | 1.5 | 2.2 |
| *A. brachypodii* | 1155 | 4.3 | 0.9 | 2.6 |
| *A. brachypodii* | 1523a/2 | 1.1 | 1.1 | 1.1 |
| *A. brachypodii* | 1968a | 3.5 | 1.4 | 2.5 |
| *A. desmazieri* | C247.79 | 2.4 | 1.7 | 2.0 |
| *A. digraphidis* | 1968b | 3.7 | 1.4 | 2.5 |
| *A. ducis-aprutii* | 1915 | 3.1 | 0.4 | 1.8 |
| *A. festucae* | C689.97 | 4.4 | 2.4 | 3.4 |
| *A. graminea* | C586.79 | 3.6 | 3.3 | 3.4 |
| *A. hordei* | 1684 | 3.6 | 2.1 | 2.8 |
| *A. hordei* | 1971 | 4.1 | 2.0 | 3.1 |
| *A. hordei* | 770b | 3.3 | 1.9 | 2.6 |
| *A. hordei var. americana* | C814.84 | 3.7 | 2.3 | 3.0 |
| *A. hordei var. europea* | C817.84 | 1.8 | 1.9 | 1.8 |
| *A. hordei var. hordei* | C878.72a | 2.8 | 1.5 | 2.1 |
| *A. melicae* | 2010 | 3.9 | 2.1 | 3.0 |
| *A. melicae* | 793c | 3.7 | 1.6 | 2.6 |
| *A. phleina* | C517.74 | 0.5 | 0.3 | 0.4 |
| *A. skagwayensis* | C110124 | 3.3 | 2.4 | 2.8 |
| *A. sorghi* | C622.68 | 3.6 | 2.4 | 3.0 |
| *A. stipae* | 1811 | 3.1 | 2.1 | 2.6 |
| *A. zeicola* | 1937 | 3.3 | 3.0 | 3.1 |
| | $LSD_{N\text{-}K\ 0.05}$ | | 0.06 | 0.11 |
| mean | | 3.1 | 1.9 | |

**Table 3.** Radial growth of *Ascochyta* spp. fungi on PDA medium at 20°C in the dark and in the light (12 h light/12 h darkness).

| Species | Isolate | Presence of pycnidia | | | | | |
|---|---|---|---|---|---|---|---|
| | | CN/OMA/PDA medium at temperatures | | | | | PDA medium/light |
| | | 5°C | 10°C | 15°C | 20°C | 25°C | 20°C |
| *A. agrostidis* | C758.97 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. avenae* | 1982 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. avenae* | C811.84a | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. brachypodii* | 1155 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. brachypodii* | 1523a/2 | –/–/– | –/–/– | –/–/– | –/–/+ | +/+/– | + |
| *A. brachypodii* | 1968a | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. desmazieri* | C247.79 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. digraphidis* | 1968b | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. ducis-aprutii* | 1915 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. festucae* | C689.97 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. graminea* | C586.79 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. hordei* | 1684 | –/+/– | –/+/– | –/+/– | –/+/+ | +/+/– | + |
| *A. hordei* | 1971 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. hordei* | 770b | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. hordei var. americana* | C814.84 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. hordei var. europea* | C817.84 | –/+/– | +/+/+ | –/+/– | +/+/– | +/+/– | – |
| *A. hordei var. hordei* | C878.72a | –/–/– | –/–/– | –/–/– | –/–/– | –/+/– | – |
| *A. melicae* | 2010 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. melicae* | 793c | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. phleina* | C517.74 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. skagwayensis* | C110124 | –/–/– | –/+/– | –/+/– | +/+/+ | +/+/– | + |
| *A. sorghi* | C622.68 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. stipae* | 1811 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | – |
| *A. zeicola* | 1937 | –/–/– | –/–/– | –/–/– | –/–/– | –/–/– | + |

**Table 4.** Production of pycnidia of *Ascochyta* spp. under different growth conditions (medium, temperature, light).

*+ observed pycnidium production, - no pycnidium production observed*

case of *A. phleina* - C517.74, which was characterized by the slowest growth. The points illustrating *A. brachypodii* - 1523a/2, *A. hordei* - 1684, *A. agrostidis* - C758.97, *A. festucae* - C689.97 are also located at slight distances from the other isolates. The presented graph reflects a considerable part of the total variation of the investigated system, as it is as much as 96.33%.

A dependence between media in the system of two principal components is presented in Figure 2. The location of straight lines in relation to one another shows a high positive correlation between fungal growth on oatmeal agar (OMA) and Coon's agar (CN). The correlation between growth on Potato Dextrose Agar (PDA) and oatmeal agar (OMA), and between growth on Potato Dextrose Agar (PDA) and Coon's agar (CN) is much smaller.

## 4. Discussion

Temperature is one of the basic environmental parameters influencing fungal growth. Growth of most

fungi is observed within a temperature range of 5-30ºC. Frequently, the pathogen and the host plant have similar temperature requirements. For instance, *A. zeicola* has the highest optimal growth temperature (24.2ºC) of all the analyzed fungi, and its occurrence was recorded on *Saccharum officinarum* and *Zea mays*, which are thermophilic plants. *Ascochyta stipae* (synonim *A. antarctica*) and *A. ducis-aprutii* were isolated from plants growing in Antarctica [2], and lower temperature optimums of approx. 15ºC were found for these species. The capacity to produce pycnidia was observed under different temperature and lighting conditions, but only in scarce species/isolates. Roger and Tivoli [11] found the formation of pycnidia and perithecia at 20ºC, and under light conditions in the related species *Mycosphaerella pinodes* (anamorph of *Ascochyta pinodes*). They did not observe sporulation at lower temperatures or in the dark. As in our study, Roger and Tivoli [11] found a high variation in the production of fruit bodies in individual isolates and a high suitability of oatmeal agar for pycnidium production. Zhao *et al.* [12] investigated the effect of temperature on the growth of *A. rhei* on PDA, and found the fastest growth to occur at 22ºC. It was noted that spot disease of rhubarb (*Rheum rhaponticum*) is observed only in hot summers [12]. The relationship between optimal temperatures for *in vitro* fungal growth and the occurrence of optimal temperatures in rhubarb plantations suggests that we may expect a similar dependence in the case of other plant species. *Didymella rabiei* (anamorph of *Ascochyta rabiei*) shows different growth rates at 15ºC and 25ºC, depending on the place of origin and cultivation method adopted for the host plants. Some isolates of common origin exhibit the same growth rates at both temperatures. Our results suggest growth rates consistent with those of other isolates, with faster growth recorded at 25ºC than at 15ºC [13]. *Didymella rabiei* is a pathogen of chickpea, but its occurrence was reported also on other plants, including wheat [14].

In recent years, fungi from the genus *Ascochyta* were identified as causes of wheat leaf necrosis in a variety of locations globally [15,16]. At the same time, a lack of reports on the subject and a very limited number of confirmations by the same authors on the occurrence of these pathogens in successive years indicate a very specific pathogen-plant-environment dependence. It seems that a specific system of temperature and humidity as well as the large-scale cultivation of a susceptible cultivar is important for the appearance of disease. Under these optimal conditions, the area of necrosis on wheat leaves may reach as much as 45% (artificial inoculation [16]). In New Zealand, the occurrence of *A. hordei* was reported on barley, *A. sorghi* on brome grass and wheat, *A. avenae* on oat and *A. tritici* on wheat [4].

Boerema *et al.* [5] identified *A. avenae, A. desmazieresii, A. tritici* and *Didymella exitialis* as causes of leaf spot in barley, wheat and rye grasses, while additionally mentioning *A. hordei* var. *hordei* as the agent of leaf spot in barley. It is reported that the role of these fungi in the incidence of spot diseases in cereals has been increasing, particularly in humid years. Since fungal growth depends on both the type of medium and temperature, these fungal species may have different nutrient requirements. In practice, it may mean that plant infestation will depend on the species, cultivar, and even age or nutrient status of the plant. In terms of the effect of light on fungal growth, none of the isolates grew faster in the light than in the dark. On the other hand, it is commonly known that light is required for sporulation in most fungi. However, our results also showed that several isolates were able to produce pycnidia both in the dark and in the light with the exception of *A. zeicola* (1937), which sporulated only in the light. A faster growth of fungi in the dark or a markedly superior growth of some fungi in the dark rather than in the light may predispose them to occurring on the lower leaves of plants where there is greater shading. It needs to be stressed that differing responses to light is a trait specific to isolates rather than species, For example, *A. brachypodii* (1155, 1968a) grows much better in the dark than in the light, as opposed to *A. brachypodii* (1523a/2), which grows at similar rates in the dark and in the light.

A high positive correlation of fungal growth on OMA and CN media, and at the same time a much weaker correlation of growth on both these media with the growth rate on PDA medium indicate a similar growth trend for the analyzed fungi on both these media. Additionally, the formation of pycnidia on these media confirms a particular suitability of OMA and CN media for culture of pycnidium-forming fungi, including *Ascochyta* sp.

## Acknowledgements

The studies were conducted thanks to the co-financing of the Ministry of Science and Higher Education within the framework of project no. N N310 086136.

## References


[1] Punithalingam E., Graminicolous Ascochyta species. Mycological Papers No. 142, Commonwealth Mycological Institute, Kew, England, 1979

[2] Melnik V.A., Braun U., Hagedor G., Key to the fungi of the genus Ascochyta Lib. (Coelomycetes), Parey Buchverlag Berlin, 2000

[3] Mathre D.E., Compendium of barley diseases. APS Press, St. Paul., 1997

[4] Braithwaite M., Alexander B.J.R., Adams R.L.M., Nationwide survey of pests and diseases of cereal and grass seed crops in New Zealand. 2. Fungi and bacteria., Proc. 51st N.Z. Plant Protection Conf. (11-13 August 1998), The New Zealand Plant Protection Society Inc., Hamilton, 1998, 51-59

[5] Boerema G.H.R., Pieters R., Hamers M.E.C., Check-list for scientific names of common parasitic fungi. Supplement Series 2b (additions and corrections): Fungi on field crops: Cereals and grasses, Eur. J. Plant. Pathol., 1992, 98, 1–32

[6] Bockus W.W., Bowden R.L., Hunger R.M., Morrill W.L., Murray T.D., Smiley R.W., (eds) Compendium of Wheat Diseases and Pests: Third Edition. APS Press, St. Paul, MN, 2010

[7] Boerema G.H., Dorenbosch M.M.J., The Phoma and Ascochyta species described by Wollenweber and Hochapfel in their study on fruit rotting, Stud. Mycol., 1973, 3, 18-19 and 38-39

[8] Chilvers M.I., Rogers J.D., Dugan F.M., Stewart J.E., Chen W.D., Peever T.L., Didymella pisi sp nov., the teleomorph of Ascochyta pisi, Mycol. Res., 2009, 113, 391–400

[9] Phan H.T.T., Ford R., Taylor P.W.J., Population structure of Ascochyta rabiei in Australia based on STMS fingerprints, Fungal Divers., 2003, 13, 111–129

[10] Gorfu D., Sangchote S., Fungi associated with field pea seeds from Ethiopia and seed transmission of Ascochyta pinodes, Seed Sci. Technol., 2005, 33, 387-396

[11] Roger C., Tivoli B., Effect of culture medium, light and temperature on sexual and asexual reproduction of four strains of Mycosphaerella pinodes, Mycol. Res., 1996,100, 304–306

[12] Zhao Y.B.W., Grout X.Xu., Effects of temperature on germination and hyphal growth from conidia of Ramularia rhei and Ascochyta rhei, causing spot disease of rhubarb (Rheum rhapondicum), Plant Pathol., 2006, 55, 664–670

[13] Ozkilinc H., Frenkel O., Abbo S., Shtienberg D., Sherman A., Ophir R., A comparative study of Turkish and Israeli populations of Didymella rabiei, the ascochyta pathogen of chickpea, Plant Pathol., 2010, 59, 492–503

[14] Trapero-Casas A., Kaiser W.J., Alternative host and plant tissue for the survival, sporulation and spread of the Ascochyta blight pathogen in chickpea, Eur. J. Plant Pathol., 2009, 125, 573–587

[15] Głazek M., Sikora H., Mączyńska A., Krzyzińska B., Epidemic occurrence of Ascochyta graminicola on winter wheat in 2001 [Epidemiczne występowanie Ascochyta graminicola na pszenicy ozimej w 2001 roku ], Prog. Plant Prot./Post. Ochr. Roslin, 2002, 42, 897-899

[16] Perelló A.E., Moreno M.V., Occurrence of Ascochyta hordei var. europaea on wheat (Triticum aestivum) leaves in Argentina, Australas. Plant Path., 2003, 32, 565–566