

# First report of *Phomopsis diachenii* in Bulgaria

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**Abstract.** The occurrence of *Phomopsis diachenii* on caraway (*Carum carvi*) was established in 2001. This is the first report of *P. diachenii* in Bulgaria. The pathogen produced black pycnidia and two types of conidia (alpha- and beta-conidia) on infected plant tissues as well as on nutrient media. Another *Phomopsis* species was found on the same host plant in 2003. It produced mainly alpha-conidia. In addition, mature perithecia assigned to *Diaporthe angelicae* were observed on stem fragments taken from annual caraway (*C. carvi* var. *annuum*) in 2003. *Phomopsis* spp. were isolated from wild growing caraway and *Heracleum* plants collected in the region of Rila monastery, too. All fungi mentioned were studied on the host plant and in agar culture.

**Key words:** Bulgaria, caraway, *Carum carvi*, *Diaporthe angelicae*, *Phomopsis diachenii*, *Phomopsis* sp.

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## Introduction

*Phomopsis diachenii* Sacc. had been reported as the causal agent of a severe disease of caraway (*Carum carvi* L.) in the Czech Republic (Ondřej 1997) and in Germany (Gabler & Zielke 1998; Gabler & Ehrig 2000). The main symptoms were umbel browning and stem necrosis. The fungus produced both  $\alpha$ - and  $\beta$ -conidia. Besides *P. diachenii*, Ondřej (1997) mentioned on caraway another *Phomopsis* sp. that produced numerous pycnidia on the root neck and stem basis of the heavily diseased plants. These pycnidia had 1-2 ostioles, were 100-400  $\mu$ m in diameter, and contained only 6-8  $\times$  2-2.5  $\mu$ m  $\alpha$ -conidia. No teleomorph of *P. diachenii* is known.

In this article we report the results of our investigations on the presence of *Phomopsis* of caraway in Bulgaria, as well as attempts to induce the development of the teleomorph stage.

## Materials and Methods

Observations for disease incidence were made on one annual ('Sprinter') and three biennial ('Rekord', 'Konczewicki', and 'Arterner') cultivars of caraway cultivated at the Institute of Genetics (Sofia region, alt. 539 m) and at the Field Experimental Station (Bagrentsi village near Kyustendil, alt. 482 m). Most specimens were collected in June and July 2001-2003. Samples of wild growing caraway and of *Heracleum* sp. were also collected around Rila monastery (alt. 1147 m) in August 2003. The causal agents were isolated directly from fruit bodies or from plant parts carrying symptoms. A collection of isolates is preserved at the Institute of Genetics on potato-dextrose agar (PDA) (Difco Laboratories, Detroit, Michigan, USA).

In order to stimulate the production of the teleomorph, bundles of caraway stems bearing pycnidia were placed in tree and fences during the winter. These samples were periodically examined for development of teleomorphs. In addition, stem fragments with pycnidia were surface sterilized with 0.5% NaOCl for 5 min, rinsed with sterile distilled water and incubated in moist chambers.

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Ascospores and conidia were mounted in lactophenol and observed and measured by light microscopy (Kreisel & Schauer 1987).

## Results

The first symptoms of umbel browning were observed on the biennial varieties in the middle of June and on the annual one at the end of June. In the initial stage of attack, individual umbellets were damaged (Fig. 1). The most characteristic symptoms were umbel browning and the drooping of the diseased umbellets and umbels. The disease quickly spread downward, causing stem necrosis and eventually death of the plant (Fig. 2). Subepidermal or erumpent pycnidia were noticed on the attacked plant parts. Pycnidia were black, globose and 188–375  $\mu\text{m}$  in diameter. They appeared as black points on the umbel rays and on stems and extruded conidia were visible as a whitish slime (Fig. 3). Two types of conidia were observed:  $\alpha$ -conidia, fusiform, 2–3 guttulate, (5–) 7.5–10.0 (–11)  $\times$  (2.5–) 3.2–4.5 (–5)  $\mu\text{m}$  ( $n = 100$ ) and  $\beta$ -conidia, filiform, curved, (12.5–) 19.0–25.4 (–29)  $\times$  (1–) 1.1–1.8 (–2.5)  $\mu\text{m}$  ( $n = 100$ ) (Fig. 4). The fungus was identified as *P. diachenii* on the basis of the available descriptions (Saccardo 1915; Sutton 1980; Ondřej 1997; Gabler & Ehrig 2000). When the fungus was cultivated on PDA, the development of pycnidia began after 7–8 days. At first  $\alpha$ -conidia (6–) 7.2–10.9 (–14)  $\times$  (3–) 3.8–5.3 (–7)  $\mu\text{m}$  ( $n = 100$ ) were predominant but later a large number of  $\beta$ -conidia (14–) 19.5–27.7 (–35)  $\times$  (1–) 1.3–2.2 (–2.5)  $\mu\text{m}$  ( $n = 100$ ) could be observed. *Phomopsis diachenii* was consistently found on the plants examined during 2001–2003 at both stations. However, in 2003, a *Phomopsis* sp. differing from *P. diachenii* was detected at Bagrentsi. This was characterized by small pycnidia (153–175  $\mu\text{m}$ ) bearing mainly  $\alpha$ -conidia, (5–) 4.9–6.8 (–8.5)  $\times$  (2–) 2.3–3.3 (–5)  $\mu\text{m}$  ( $n = 100$ ). Only rarely  $\beta$ -conidia were found (13.5–) 14.6–19.4 (–21)  $\times$  (1–) 1.1–1.7 (–2)  $\mu\text{m}$  ( $n = 10$ ). The fungus formed whitish rose colonies on PDA (Figs 9–10) and produced pycnidia with abundant ovate to fusoid, (5–) 5.4–7.3 (–7.5)  $\times$  2.4–3  $\mu\text{m}$  ( $n = 100$ )  $\alpha$ -conidia, and scarce, filiform,  $17.0 \pm 0.2 \times 1.4 \pm 0.03 \mu\text{m}$  ( $n = 10$ ),  $\beta$ -conidia.

No perithecia were found on the over wintered diseased caraway stems, or on the samples from Sofia kept in moist chambers. Nevertheless, in one specimen from annual caraway originating from Bagrentsi and collected in 2003, perithecia were formed in moist chambers after 4–5 weeks of incubation at 22 °C. Many dark spots were found to develop at the surface of the host epidermis. Later perithecial beaks emerged (Fig. 5). These increased in length during the subsequent days, (175–) 194–271 (–300)  $\mu\text{m}$  and slimy masses of released ascospores appeared at their apex (Fig. 6). Well-developed, globose, often clustered, perithecial ascomata, 259–292  $\mu\text{m}$  in diameter, were found embedded in stem tissues adjacent to the pycnidia. Numerous asci were formed in the perithecia (Fig. 7). The ascospores were unicellular, colourless, ellipsoidal, guttulate, papillate, with rounded ends, (10–) 11.7–13.0 (–15)  $\times$

(4–) 4.9–5.4 (–6)  $\mu\text{m}$  ( $n = 100$ ) (Fig. 8). This fungus could be assigned to *Diaporthe angelicae* (Berk.) D.F. Farr & Castl. (Syn. *Diaporthopsis angelicae* (Berk.) Wehm.), according to Castlebury *et al.* (2003). Because of the association of these fungi on the same host plant, we are currently studying in more detail if an anamorph-teleomorph connection between *Diaporthe angelicae* and *Phomopsis* is involved.

Several *Phomopsis* isolates were also obtained from wild growing caraway and *Heracleum* sp. The characteristics of the colonies obtained on PDA were similar to that of the isolates from cultivated caraway, but darker. The isolates from *Heracleum* induced yellow discoloration of the nutrient media (Figs 9–10). The size of  $\alpha$ -conidia produced by isolates from wild caraway on PDA was (7.5–) 9.5–12.9 (–15)  $\times$  (2.5–) 2.8–3.8 (–4.5)  $\mu\text{m}$  ( $n = 100$ ) and by these from *Heracleum* sp. (8.5–) 9.8–13.0 (–15.5)  $\times$  (3–) 3.3–4.5 (–5)  $\mu\text{m}$  ( $n = 100$ ). The size of  $\beta$ -conidia was (20–) 23.0–28.0 (–32.5)  $\times$  (1–) 1.2–1.9 (–2.5)  $\mu\text{m}$  ( $n = 100$ ) and (17.5–) 21.0–26.8 (–31)  $\times$  (1–) 1.2–1.9 (–2.5)  $\mu\text{m}$  ( $n = 100$ ), respectively.

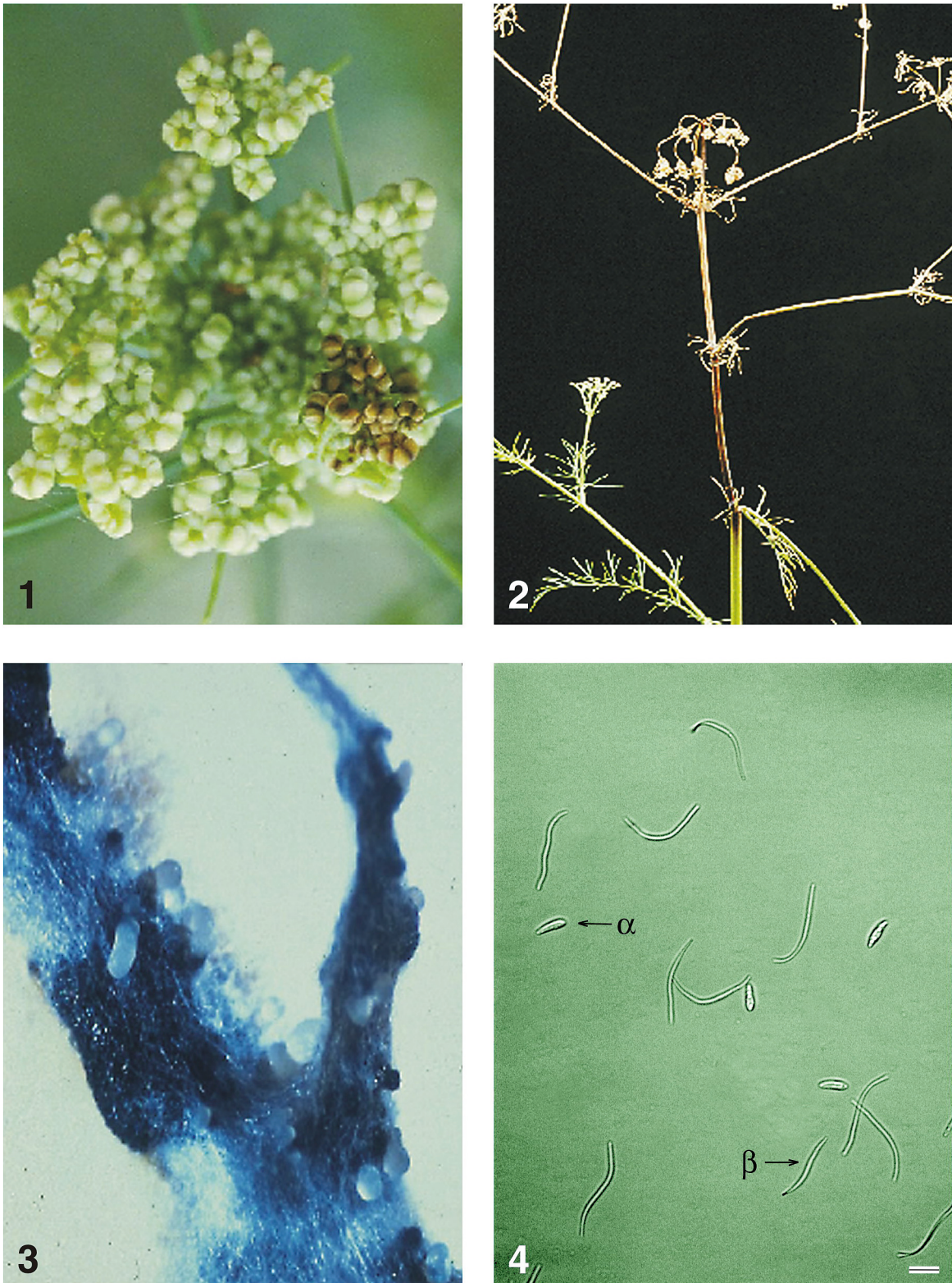
## Discussion

Our observations confirm the findings of Ondřej (1997) who reported the occurrence of two *Phomopsis* spp. on caraway. He made no attempts to cultivate the fungi. Marić *et al.* (1982) also recognized two different species of *Phomopsis* on diseased sunflower plants. The descriptions of *Phomopsis* species have mainly been based on host association, but now it is accepted that they are not necessarily host-specific (Rehner & Uecker 1994), and that more than one *Phomopsis* can occur on a single host species (Zhang *et al.* 1998; Mostert *et al.* 2001; Farr *et al.* 2002). Phillips (2003) found three morphologically different *Phomopsis* species on *Foeniculum vulgare*. The identification of *Phomopsis* by using morphological characteristics only is difficult. Furthermore, there is no modern taxonomic treatment of the genus. In addition, only a limited number of anamorph-teleomorph connections have been reported, and these are not based on experimental proof (Phillips 2003). Therefore, we agree with several authors (Mostert *et al.* 2001; Phillips 2003) that molecular techniques are the most promising tool for the identification of *Phomopsis* species.

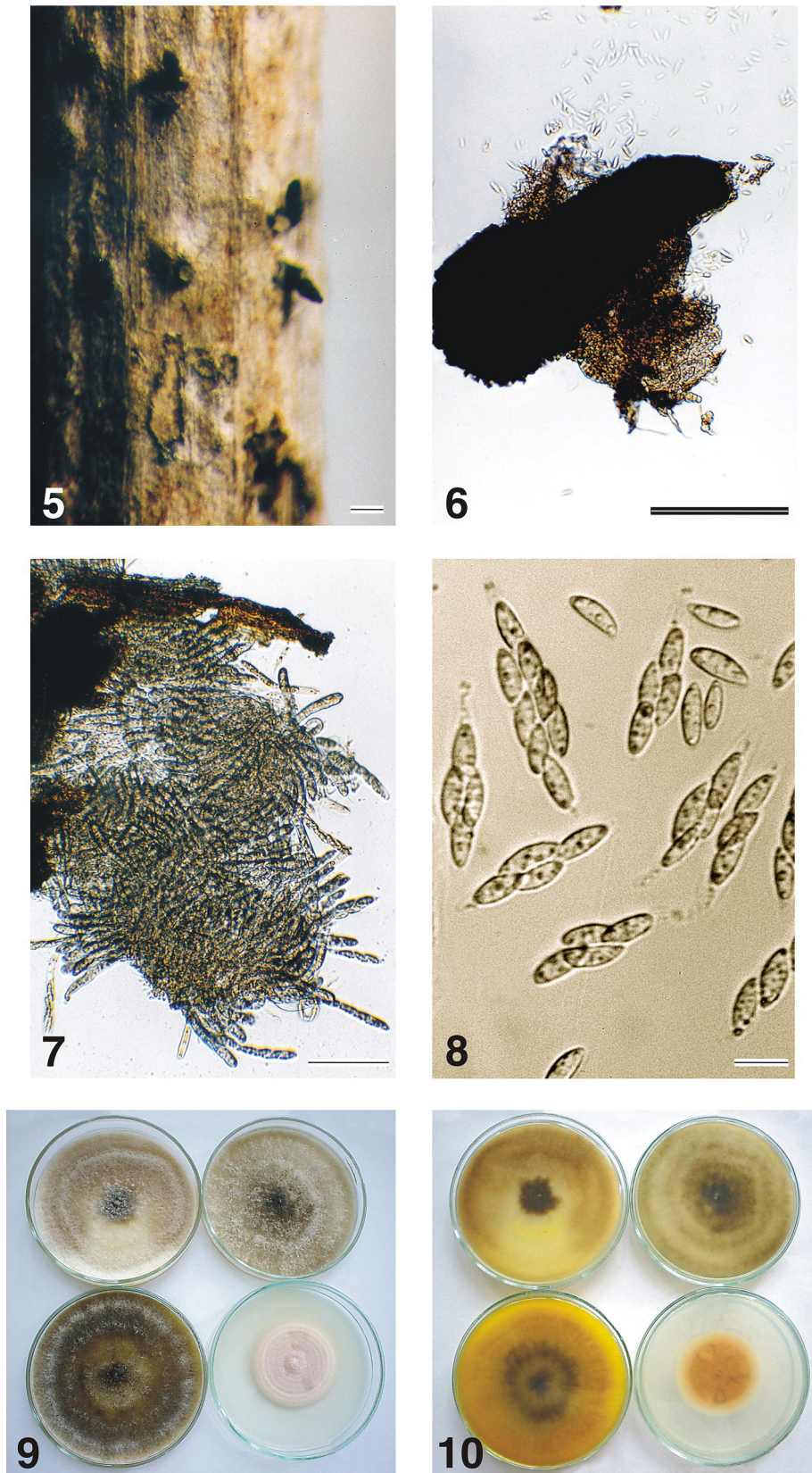
Wechtel (1990) observed both  $\alpha$ - and  $\beta$ -conidia in the large, stromatic pycnidia of *Phomopsis* spp. but only  $\alpha$ -conidia in smaller ones. However, such correlation between the size of pycnidia and the types of conidia is not consistent. The isolated by us *Phomopsis* sp. different from *P. diachenii* produced large number of small pycnidia bearing mainly  $\alpha$ -conidia.

The development of mature perithecia in moist chambers at 22 °C obtained by us suggests that this fungus does not necessitate a period of low temperature for the formation of teleomorph.

*Diaporthopsis* Fabre differs from *Diaporthe* Nitschke in having aseptate ascospores. According to Castlebury *et al.* (2003) septation of the ascospores is not a useful taxonomic



Figs 1-4. *Phomopsis diachenii*. 1. Early symptoms of umbel browning. 2. Diseased plant with umbel drooping and stem necrosis. 3. Pycnidia with extruding conidia. 4.  $\alpha$ - and  $\beta$ - conidia. Scale bar = 10  $\mu$ m



Figs 5-8. *Diaporthopsis angelicae*. 5. Stem fragment with perithecia. 6. Perithecium. 7. Asci. 8. Ascospores. 9-10. Cultures of *Phomopsis* spp. on PDA: 9. **Top left** – *P. diachenii* from cultivated caraway (Sofia region); **top right** – *Phomopsis* sp. from wild growing caraway (Rila monastery); **bottom left** – *Phomopsis* sp. from *Heracleum* sp. (Rila monastery); **bottom right** – *Phomopsis* sp. from cultivated caraway (Bagrentsi village). 10. The same isolates on the bottom. Scale bars: 5-6 = 250  $\mu$ m, 7 = 50  $\mu$ m, 8 = 10  $\mu$ m

character for distinguishing *Diaporthopsis* from *Diaporthe* and may not be useful in distinguishing genera in the Diaporthaceae. Based on morphological and molecular data, these authors transferred *Diaporthopsis angelicae* to *Diaporthe* and considered the genus *Diaporthopsis* as a synonym of *Diaporthe*.

Although *P. diachenii* was initially described from *Pastinaca sativa* (Saccardo 1915), it had proved later to be a caraway pathogen of great importance (Ondřej 1997; Gabler & Ehrig 2000). Our further research will also focus on pathogenicity tests by using isolates from wild caraway and *Heracleum* sp. to verify the hypothesis that they could serve as a source of inoculum (Ondřej 1997; Gabler & Ehrig 2000).

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