New or Unusual Disease Reports

First report of *Didymella fabae*, teleomorph of *Ascochyta fabae*, on faba bean crop debris in Tunisia

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Summary. Ascochyta blight of faba bean, caused by *Ascochyta fabae*, is one of the most destructive diseases of faba bean (*Vicia faba*) in Tunisia. Yield losses caused by the disease can reach 35% under conditions favourable for disease development. Despite its widespread occurrence, only the asexual part of Ascochyta life-cycle has been recovered in Tunisia. However, the sexual stage of the fungus is suspected to play an important role in the epidemiology of the disease, given the distribution of the primary infection symptoms. Pseudothecia of *Didymella fabae*, the teleomorph of *A. fabae*, were first observed in autumn-winter 2010–2011, on faba bean debris collected from the region of Beja (Tunisia) and placed in three different locations. Mature pseudothecia were first observed 2 months after the debris placement, with the typical characteristics of *Didymella fabae*. Isolation from ascospores resulted in typical cultures of *A. fabae* which produced typical symptoms of the disease when used to inoculate faba bean seedlings. This is the first report of *D. fabae* in Tunisia. The occurrence of the teleomorph has important implications for epidemiology of Ascochyta blight and therefore, on its management in faba bean crops.

Key words: Ascochyta blight, epidemiology, sexual stage, *Vicia faba.*

Introduction

Faba bean (*Vicia faba* L.) is a grain legume grown worldwide as a source of protein for both human food and animal feed. In addition to protein, cropping faba bean benefits the ecosystem with renewable inputs of nitrogen (N) available to the next crops in the rotation and soil from biological N_2 fixation, and a diversification of cropping systems (Jensen *et al.*, 2010). Even though the total area cropped to faba bean worldwide has declined by 56% in the past 10 years (Jensen *et al.*, 2010), faba bean acreage in Tunisia has increased from 42,800 ha in 2000 to 57,700 ha in 2008. However, crop yields are still variable (from 0.38 to 0.77 t ha^{-1}) because of many biotic (pests and diseases) and abiotic (mainly drought) stresses (Anonymous, 2008).

Ascochyta blight, caused by *Ascochyta fabae* Speg., and its teleomorph *Didymella fabae*, is one of the most common pathogens affecting faba bean in Tunisia and in several other faba bean producing countries (Kharrat *et al.,* 2006). This disease is considered one of the main factors contributing to the decrease in areas sown in faba bean crops in some countries (Hanounik, 1980). Ascochyta blight can cause considerable grain losses during both the wet cool and the hot dry seasons (Hanounik and Robertson, 1989). Yield losses due to attacks by this fungus can reach 90% when susceptible cultivars are sown and weather conditions are favourable for disease development (Hanounik, 1980). The disease is initiated in crops from few lesions on seedlings, then infections spreading to the upper leaves of plants, and the the stems and finally pods (Maurin and Tivoli, 1992). The

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disease can affect all the aerial parts of plants (stems, leaves, pods, and grain), and when epidemic onset is early in the crop growth stage, the commercial value of infected seeds is generally reduced. In addition, as the disease can directly develop on seeds, thess seeds can constitute a viable source of primary inoculum for the next season (Torres *et al.*, 2006).

Ascochyta blight is widely distributed owing to seed transmission of the pathogen. *Didymella fabae*, the teleomorph of *A. fabae*, was first discovered in 1989 in the United Kingdom (Jellis and Punithalingam, 1991), and has since been recorded in Australia (Kaiser, 1997), Canada (Kharbanda and Bernier, 1980), Poland (Filipowicz, 1983), Spain (Rubiales and Trapero-Casas, 2002), and Syria (Bayaa and Kabbabeh, 2000). Although not confirmed, the teleomorph may also be present in Algeria and Lebanon since the two compatible mating types have been found in debris collected from these countries (Kaiser, 1997). In Tunisia, the existence of the sexual stage (*D. fabae*) was suspected, given the distribution of disease symptoms in some faba bean fields. Many consequences arise from the existence of the teleomorph and its involvement in the disease spread, particularly, the choice of the stage in the fungus life-cycle to target and the means that should be used for disease management, whether through chemical, breeding and selection for disease resistance, or agronomic practices.

In Tunisia, *A. fabae* cycles as the asexual form. However, since it was shown for other *Ascochyta* species (e.g. *A. rabiei*), that the teleomorph is able to develop on debris (Rhaiem *et al.*, 2006), and given the distribution of primary symptoms of Ascochyta blight on faba bean, we strongly suspect the occurrence of *D. fabae* on the debris of faba bean crops. Based on these observations, the objective of the present study was to confirm the natural occurrence of *D. fabae* on faba bean debris in Tunisia.

Materials and methods

Stem debris of faba bean plants naturally infected with *A. fabae* were collected from the Oued Béja experimental station (36°44′05′′N, 9°13′35′′E, altitude 150 m) in June 2010. Stems were air dried and cut into pieces of 12 cm-length on which there were at least two necrotic lesions with pycnidia of *A. fabae*. Each of these stem sections was placed in a nylon mesh bag (15 × 20 cm). Sixty bags were prepared and 20 were placed during mid-October in three locations presenting different climatic conditions: Morneg (36°38′15′′N, 10°16′42′′E, altitude 47 m), Oued Béja and Oued Mliz (36°28′ 46′N, 8° 29′ 40′′E, altitude 178 m). Two months later, three stem pieces from each of the three regions were taken for sampling and washed under running tap water then dried on filter paper. Samples were then examined with a stereoscopic microscope (×45 magnification) to observe the fruiting bodies. These were removed, squashed, stained with cotton blue, and examined with a compound microscope (Leica ICC50 HD). The teleomorph of *A. fabae*, *D. fabae,* was identified by the morphology of the fruiting bodies and by pseudothecia, asci and ascospores sizes (Rubiales and Trapero-Casas, 2002; Rhaiem *et al.*, 2006).

Pseudothecia were removed from the stems, and positioned on the lids of Petri dishes containing water agar. After 24 h, germinating spores were transferred to malt extract agar medium and incubated at 20°C in a 12 h photoperiod for 2 weeks. Fruiting bodies were picked from the developing cultures and examined under a microscope to confirm identity. The cultures were used as conidial suspensions adjusted to a concentration of 10^5 spores mL^{-1} to inoculate ten seedlings of the susceptible faba bean cultivar "Badi". to confirm the Koch's postulates by evaluating the number of inoculated seedlings that developed the typical symptoms of Ascochyta blight.

Results

Some black fruiting bodies immersed in rows on faba bean straws (Figure 1A) were observed with the stereoscopic microscope. When observed with at ×100 magnification, some of these fruiting bodies were brown and some were blackish brown and subglobose, showing inconspicuous circular ostioles, as reported by Jellis and Punithalingam (1991) and Rubiales and Trapero-Casas (2002). Diameter of a sample of 30 fruiting bodies was assessed, and was shown to range from 200 to 240 μm (mean = 230 ± 2 μm: mode [the most often observed diameter] = 240 μm) (Figure 1B).

Crushing the observed fruiting bodies showed a pseudoparenchymatic brown layer in each (Figure 1B), and hyaline, cylindrical to subclavate and bitunicate asci that were each constricted near the base, forming a distinct foot (Figure 1C). Ascus length ranged from 55 to 70 μm (mean = 62.4 ± 1.01 μm:

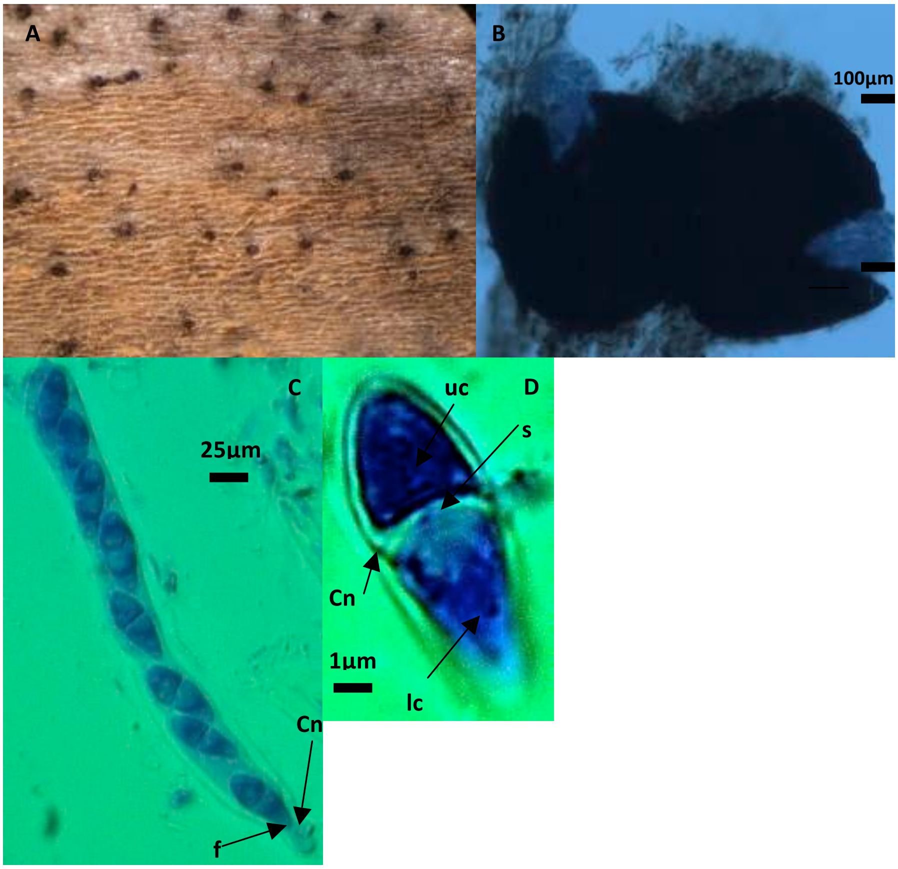

Figure 1. Sexual fruiting bodies of *Didymella fabae*. A, Pseudothecium inserted on stem tissue (×40). B, Pseudothecium with asci (×100). C, Ascus with ascospores (×1000). D, Ascospore (×1000). (f, foot; Cn, constriction; lc, lower cell; uc, upper cell; s, septum).

mode =f 60 µm). Ascus width was 10 to 12 µm (mean = 10.8 ± 0.08 µm; mode = 11 µm). In each ascus, eight hyaline and irregularly distichous ascospores were observed. These were each smooth, slightly biconic to ellipsoid, two-celled, constricted at the septum, and had an upper cell broader than the lower one. The ascospores ranged from 15 to 17.5 µm in length (mean = 15.5 ± 0.33 µm; mode = 15 µm) and width from 6.2 to 6.3 µm (mean = 6.32 ± 0.03 µm; mode = 6.25 µm) (Figure 1D).

Discharged ascospores on the malt extract agar resulted in typical colonies of *A. fabae* (Figure 2A) which developed pycnidial fructifications with the morphological characteristics of this fungus (Figures 2B and 2C). The use of these cultures in a pathogenicity test resulted in typical symptoms on the inoculated plants, confirming the identity of the fungus (Figure 2D).

Discussion

This study revealed for the first time, the existence of the teleomorph *D. fabae* in Tunisia. The morphological characteristics of pseudothecia (Figure 1A and1B), asci and ascospores (Figure 1C and 1D) observed on the debris sampled from the three locations (Morneg, Oued Béja and Oued Mliz) confirmed the fungus identity. Mean pseudothecium, ascus and ascospore dimensions measured in this study were 230 µm for pseudothecia, 62.4 µm length and 10.8 µm width for asci and 15.5 µm length and 6.32 µm width for ascospores. These dimensions are close to those reported by Bayaa and Kabbabeh (2000), Jellis & Punithalingam (1991) and Rubiales & Trapero-Casa (2002), where pseudothecia ranging from 180 to 240 µm in diameter, asci ranging from 55 to 70 µm in length and from 10 to 70 µm in width and ascospores

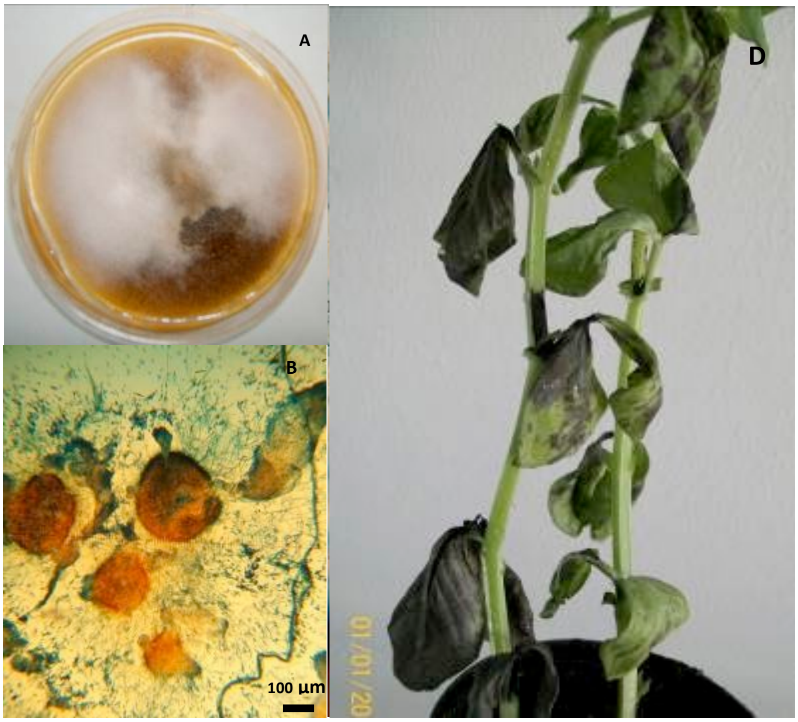

Figure 2. Asexual fruiting bodies of *Ascochyta fabae*. A, Pycnidia (×100). B, C, Pycnidium liberating pycnidospores (×400). D, symptoms induced by *A. fabae*.

ranging from 15 to 18 μm in length and from 5.5 to 6.5 μm in width were reported (Table 1).

A previous study by Kaiser (1997) on 50 isolates collected from 14 countries demonstrated that the fungus was heterothallic. Two compatible mating types designated MAT1 and MAT2 were identified and are required to develop fertile *D. fabae* pseudothecia. Observation of *D. fabae* on plant debris in Tunisia is a significant result, suggestings that these two mating types coexist in the location from which the debris were collected. Molecular studies should be carried out on populations of *A. fabae* collected from different faba bean cropping fields and regions to evaluate geographical distribution of the mating types in Tunisia. This would determine if they are closely related and able to produce the sexual stage, which would possibly allow more genetic variation in the fungus.

Identification of the sexual stage of the pathogen may have consequences for the management of Ascochyta blight in Tunisia. Historically, management strategies in this country were designed to mitigate or prevent asexual transmission of the disease from seeds, debris and volunteers. Disease control methods include using non-infected seed for crop establishment, and foliar fungicide treatments such as chlorotalonil and mancozeb (Stoddard *et al.*, 2010) applied to crops. Despite these efforts, control of the disease has not always been sufficient. The existence of the teleomorph of the pathogen means that disease management strategies must account for wide and rapid spread of the disease due to wind-dispersed spores (ascospores) that can travel up to 200 m (Jellis and Punithalingam, 1991). Additionally, the teleomorph may contribute to greater genetic diversity and new pathotypes in the populations of *A. fabae* in Tunisia and the greater region. This variability in the pathogen population could lead to fungicide insensitivity, or to rapid breakdown of the resistance in existing commercial cultivars. Consequently, management strategies aiming to development of multisite fungicides with multisite modes of action rather than monosite action, and cultivars with horizontal rather than vertical resistance, may be important for sustained faba bean production in the future.

Table 1. Observed dimensions of *Ascochyta fabae* reproductive structures compared to those reported in literature.

Reproductive structure		Observed			Reported	
		Range (µm)	Mean (µm)	Mode (µm)	Range (µm)	Reference
Pseudothecia		200–240	230	240	180–240 150–250	Jellis & Punithalingam (1991) Rubiales & Trapero Cassas (2002)
Asci	Length	55–70	62.4	60	50–70 55–80	Jellis & Punithalingam (1991) Rubiales & Trapero Cassas (2002)
	Width	10–12	10,8	11	10–14 12–15	Jellis & Punithalingam (1991) Rubiales & Trapero Cassas (2002)
Ascospores	Length	15–17.5	15.5	15	15–18 13–18	Jellis & Punithalingam (1991) Rubiales & Trapero Cassas (2002)
	Width	6.2–6.3	6.32	6.25	5.5–6.5 5–7	Jellis & Punithalingam (1991) Rubiales & Trapero Cassas (2002)

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