الكشف عن الطور الجنسي للفطرين *Alternaria* و *Stemphylium* على الكرمة في جنوب سورية

Detection of *Alternaria* and *Stemphylium* teleomorph on grapevine in the south of Syria

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الملخّص

وجد العديد من التركيبات الإثمارية على فروع كرمة تم جمعها من بساتين كرمة في محافظة السويداء جنوبي سورية. أظهر الفحص المجهري وجود شكلين من الثمار الزقية من الطراز *Perithecia*. وبالاعتماد على شكل الثمار الزقية وحجمها، وشكل الأبواغ الزقية، تبيّن أن الفطر يتبع للجنس *Pleospora*، إذ تم تعريف الفطر *Pleospora* sp. ذي الثمار الزقية من الشكل الأول والتي بلغت أبعادها 200 إلى 250 ميكرومتراً على أنه الطور الجنسي للفطر *Alternaria* sp.، بينما تم تعريف الفطر *Pleospora* sp. ذي الثمار الزقية من الشكل الآخر والتي بلغت أبعادها 320 إلى 400 ميكرومتراً على أنه الطور الجنسي للفطر Stemphylium sp. وتم تأكيد هذه النتائج بإجراء عملية عزل للفطرين *Alternaria* sp. و *Stemphylium* sp. من سطوح فروع الكرمة الميتة، وكذلك من الأبواغ الزقية. وتجدر الإشارة إلى أن هذه الفطور وجدت نامية بصورة رمية على أفرع كرمة ميتة بسبب الصقيع الذي ضرب المنطقة خلال شتاء 2015. وتعد هذه الدراسة التسجيل الأول للطور الجنسي للفطرين *Alternaria* و *Stemphylium* في سورية.

الكلمات المفتاحية : طور جنسي، *Pleospora*، *Stemphylium*، *Alternaria*.

Abstract

Many fruiting structures were found on the surface of dead branches collected from vineyards in Swaida govenorate (south of Syria). Microscopic observation showed the presence of two forms of ascomata (*perithecia*). According to their size, and the morphology of ascospores, ascomata of diameters of 200 - 250 µm were identified as *Pleospora* sp. the teleomorph of *Alternaria* sp. and the other type of ascomata (320 - 400 µm diam.) as *Pleospora* sp. the teleomorph of *Stemphylium*. These results were confirmed by isolation of *Alternaria* and *Stemphylium* anamorph from the surface of dead branches and from ascospores. These fungi have been grown saprophytically on dead branches affected by frost during the winter 2015 season. To the best of our knowledge, this is the first report of the sexual form of *Alternaria* and *Stemphylium* in Syria.

Keywords: *Alternaria*, *Stemphylium*, *Pleospora*, teleomorph.

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Introduction

Alternaria species are cosmopolitan. These ubiquitous fungi are phytopathogenic in a number of plant species, causing crop diseases which are classified under the group "Alternariose" (Thomma, 2003; Agrios, 2005). They can be found on different substrates such as senescent plants, vegetables, soil, food and various organic materials. The *Alternarias* are also known to be potent allergens, triggering seasonal reactions during the summer months. However, not all species are pathogenic or undesirable: some of them are used as biological agents to control invasive plants (Thomma, 2003).

The *Alternaria* species are imperfect filamentous fungi belonging to the class Deuteromycetes. Diagnostic characteristics of the genus included the production in chains of dark-colored multi-celled conidia with longitudinal and transverse septa (phaeodictyospores), and a beak of tapering apical cells (Honda *et al*., 1987; 1990, Neergaard, 1945).

Considering the diversity of conidium shapes and sizes among species in general, there have been efforts in developing sub-generic groupings of species based upon similar conidium characteristics. Elliot (1917) suggested that the genus could be organized into six groups based upon common conidium characteristics of length, width, and septation, with each group designated by a typical species. Neergaard (1945) proposed 3 sections for the genus based upon the formation of conidia in long chains (Longicatenatae), short chains (Brevicatenatae), or singly (Noncatenatae). Simmons (1992) expanded concepts from both Elliot and Neergaard in loosely organizing the genus into 14 species-groups based upon characteristics of conidia and catenulation. Additional species-groups discussed in other work include the arborescens, brassicicola, porri, and radicina groups (Roberts *et al*., 2000; Simmons, 1995; Pryor and Gilbertson, 1998, 2000, 2002).

Numerous atypical *Alternaria* and *Macrosporium* species were described that did not produce conidia in chains and/or have a conidium beak. Moreover, two additional genera were erected, *Stemphylium* and *Ulocladium*, which also were characterized by the production of phaeodictyospores, further complicating the taxonomic resolution of this group of fungi (Pryor and Gilbertson, 2002).

Although most *Alternaria* species do not have known sexual form, some of them have recognized teleomorphs within Pleosporaceae (Ascomycetes), although most are not commonly encountered. Most species of *Stemphylium*, the currently accepted sister group to *Alternaria*, have well established teleomorphs in the genus *Pleospora*.

Studies conducted by Simmons have suggested an exclusive teleomorph / anamorph relationship between *Pleospora* and *Stemphylium*. Subsequently, a more critical evaluation of the teleomorphic characters of well-known *Pleospora* spp. with anamorphs of *Alternaria*, namely *P. infectoria* and *P. scrophulariae* revealed that *Pleospora* spp. with *Stemphylium* anamorphs were morphologically distinct from *Pleospora* spp. with *Alternaria* anamorphs, particularly in the size of the ascomata and ascospores, and resulted in the designation of a new genus *Lewia* for Pleospora-like fungi with *Alternaria* teleomorphs (Simmons, 1986; 1995). Actually, *Clathrospora*, *Leptosphaeria*, *Lewia* and *Pleospora* are known as *Alternaria* teleomorphs and belong to the class Ascomycetes (Pryor and Gilbertson, 2002).

Material and Methods

Sampling: A field survey was conducted on some vineyards which showed dead branches on local cultivar (Baladi), in Swaida govenorate (south of Syria). during the spring period of 2015. Dead wood showing symptoms of black or brown blotches were collected randomly from two vineyards (figure 1).

Figure 1. Dead branches of grapevine showing black discoloration.

Fungus isolation from ascospores: dead branches were transported to the laboratory where they left to dry for examination of the presence or absence of the sexual forms. Fruiting bodies, asci and ascospores were examined by the microscope and morphologically described. The size average of approximately 20 ascomata, asci and ascospores were calculated. Preparation of ascospores was conducted from fruiting structures in sterilized water and was placed on PDA plates. After 24 h at 22 ± 2 ºC, individual germinated spores were transferred to new PDA plates.

Fungus isolation from dead branches: wood pieces of 0,5 mm were taken from the surface of the collected branches. Pieces were disinfected by immersion in sodium hypochlorite (NaOCl) (2%) for 3 min, after that they were rinsed and dried with sterilized filter paper. Then, they were placed on potato dextrose agar (PDA) plates and stored at 22 ± 2ºC. Morphological and microscopic characteristics of mycelia culture and fruiting bodies were described

Results and Discussion

On the surface of dead branches collected from two vineyards, many fruiting structures (ascomata) were found. Microscopic observation of these ascomata showed that they were perithecia with an reduced ostiole (fig.2). The results showed also the presence of two forms of fruiting structures: in the first one, ascomata are globose, dark brown, thin-walled, with no beak observed. Single mature ascomata measure 200 – 250 µm. Asci are subcylindrical, straight to slightly curved, contain 8 ascospores, and their size is 60–100 × 13–15 µm. Ascospores are fusoid, usually tapered at both ends, with four transversal septa and often with one series of longitudinal septa; end segments are often without septa, and their size is 15–22 × 6 – 9 µm. In the second one, ascomata are similar in shape to those described above, but they are larger (320- 400 µm diam.). Asci are cylindrical, elongate, with typically 8-ascospores, and measure 100–120 × 13–15 µm. Ascospores are cylindrical with five transversal septa and 1–2 series of longitudinal septa in each of the four original central segments; but end segments are often without septa, very rarely with one longitudinal, oblique or Y-shaped septum, and their size is 15–22 × 6.5–10.5 µm.

The isolates on PDA from both ascospores and dead branches were divided into two groups: in the first one, the isolates produced velvety gray to black colonies. Conidia are multiseptate, formed solitary, and originate from a conidiogenous cell at the tip of a hypha. These isolates were identified as *Stemphylium* according to the morphological characters described by Simmons (1967) for this genus. In the second group, the colonies were oblivious to grayish. Conidiophores are dark, septate and produce conidia in simple or branched chains. Conidia are multicellular divided by several transversal and longitudinal septa. Based on their appearance in culture, these isolates were typical of *Alternaria* genus. Morphological and microscopic characters were in according with those reported previously for the genus *Alternaria* (Nees, 1816; Kwaśna and Kosiak, 2003; Simmons, 2002; Pryor and Gilbertson, 2002).

In this present investigation, we report the presence of two forms of perithecia, and based on their morphological characters, they were identified as *Pleospora* sp. previously described as a teleomorph of some *Alternaria* and *Stemphylium* species (Inderbitzin *et al*., 2009). According to their size, and the morphology of ascospores, the small ascomata (200 - 250 µm diam.) were identified as a teleomorph of *Alternaria* sp. and the other type of ascomata (320 - 400 µm diam.) as a teleomorph of Stemphylium. In fact, *Stemphylium* is morphologically similar to the related and more widely known *Alternaria*. The conidia of both genera are multiseptate, pigmented and formed from hyphae in the mycelium. However, unlike *Alternaria* where conidia can remain connected and form chains, *Stemphylium conidia* are always solitary (Simmons, 1967; Inderbitzin *et al*., 2009). Our results are in according to those of other studies showing that the sexual state of *Stemphylium*, where known, is *Pleospora*. The type of *Pleospora*, *P. herbarum*, has a Stemphylium anamorph, *S. herbarum* Simmons (Simmons 1985). In fact, the observation of the sexual stage of *Alternaria*, where present, is very difficult because the formation of ascomata in pure culture needs a controlled environment, a low nutrient medium, and incubation at low temperature in the dark for a prolonged period (Kwasna and Kosiak, 2003).

Our identification of the two forms of *Pleospora* ascomata was confirmed by Dr. Barry Pryor from University of Arizona School of Plant Sciences in USA (personal communication).

Vine bushes have been affected by frost during the winter 2015 season when the temperature reached -16 ºC, which led to the death of most of the branches. It seems that these fungi grown saprophytically on the dead branches, where any symptoms of infection were observed later on the newly developing branches

nor leaves. In fact, Most *Alternaria* species are common saprophytes that derive energy as a result of cellulytic activity and are found in a variety of habitats as ubiquitous agents of decay. Some species are plant pathogens that cause a range of economically important diseases like stem cancer, leaf blight or leaf spot on a large variety of crops (Thomma, 2003).

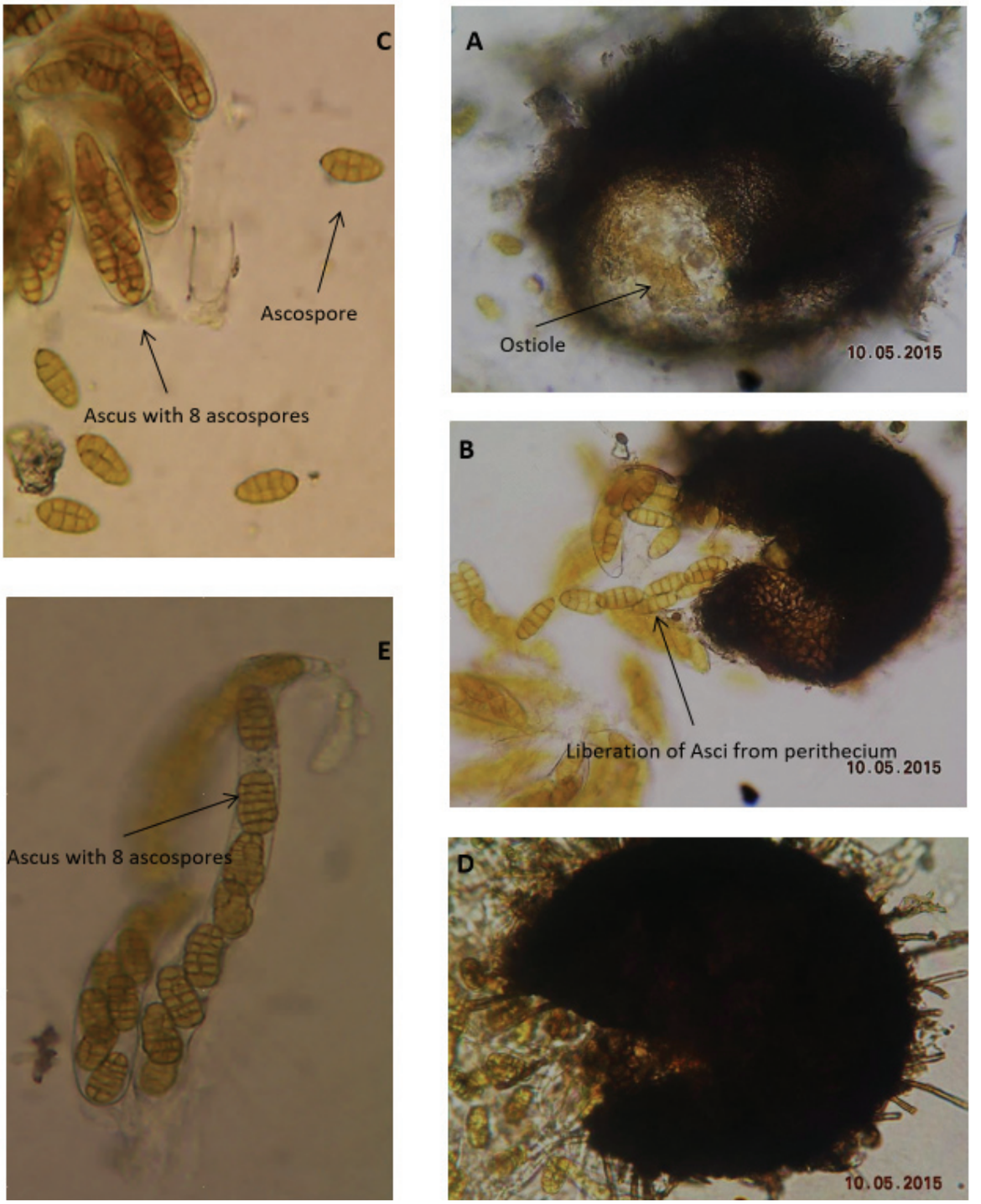

Figure 2. Fruiting structures of *Pleospora* sp. observed on dead branches of grapevine. (A) Ascotoma before it is ruptured in a slide mount. (B) Ascomata (200 - 250 µm) of *Pleospora* sp. teleomorph of *Alternaria* sp. (C) Asci and ascospores of *Alternaria* teleomorph. (D) Ascomata (320 – 400 µm) of *Pleospora* sp. teleomorph of *Stemphylium* sp. (D) Asci and ascospores of *Stemphylium* teleomorph.

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