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Management of Fusarium wilt of lettuce

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ABSTRACT

Fusarium wilt of lettuce, caused by *Fusarium oxysporum* f. sp. *lactucae*, is an important disease affecting lettuce production in many countries throughout the world. The pathogen can be seedborne, which provides a likely mechanism for long distance dispersal. Locally, *F. o. lactucae* can be moved between fields with contaminated soil on farming equipment. Minimizing opportunities for introduction of the pathogen with soil or seed is an important element of disease management. Once established, the pathogen will be difficult to eradicate, unless soil fumigation is an option. Propagules of *F. o. lactucae* have a half-life in soil of approximately six months, under fallow conditions. Persistence in soil may be enhanced if crops grown in rotation with lettuce support development of the pathogen. Cauliflower and broccoli appear to present a minor risk in this regard, whereas spinach is more extensively colonized and is therefore a less desirable crop to be grown in rotation with lettuce. Most commonly grown lettuce cultivars are susceptible to Fusarium wilt but some leaf and romaine types are highly resistant. Major gene resistance has been deployed in Japan, where three pathogenic races are known to occur. Symptom development is strongly influenced by ambient temperature, with higher temperatures resulting in more severe disease. For this reason, the risk of disease can be reduced by growing susceptible cultivars only during the cool part of the year.

1. Introduction

Fusarium oxysporum f. sp. *lactucae* (*F. o. lactucae*) was described by Matuo and Motohashi (1967) as the cause of root rot of lettuce (*Lactuca sativa*) in Japan, where it has since become a serious problem for lettuce producers. The disease was referred to as Fusarium root rot, and the pathogen was shown to be virulent on lettuce and not on 20 other vegetable crops that were tested (Matuo and Motohashi, 1967). The same forma specialis was discovered in the San Joaquin Valley of California (Fresno Co.) in 1990 and the disease was referred to as Fusarium wilt (Hubbard and Gerik, 1993) rather than root rot, although the latter name continues to be used (Yamauchi et al., 2001; Osigo et al., 2002; Fujinaga et al., 2005). Fusarium wilt subsequently became more widespread in California, where it is now found in all major lettuce growing regions. In 2001, the disease was discovered in Arizona (Matheron and Koike, 2003) and by 2003 had been identified in 27 fields (Matheron et al., 2005). Fusarium wilt of lettuce has also been reported to occur in Iran (Millani et al., 1999), Taiwan (Huang and Lo, 1998), Italy (Garibaldi et al., 2002), Portugal (Pasquali et al., 2007), Brazil (Ventura and Costa, 2008) and Argentina (Malbrán et al., 2014). Isolates of *F. o. lactucae* from Italy, Japan and the U.S. were shown to be somatically compatible (Pasquali et al., 2005), which suggests the observed global distribution of the pathogen reflects dissemination of a clonally propagated strain, rather than independent origins of the pathotype. Movement of seed contaminated by *F. o. lactucae* constitutes a likely mechanism by which the pathogen could be moved between continents (Garibaldi et al., 2004a).

2. Symptoms of Fusarium wilt

The nature and extent of symptom development is influenced by cultivar susceptibility, the density of inoculum in soil and ambient temperature, as described below. Stunting is common and may be severe. Older leaves become chlorotic and/or necrotic, and plants may die before the crop reaches maturity. Even young plants (having six to eight true leaves) can show foliar symptoms of Fusarium wilt. No external symptoms may be visible on roots, but internally the taproot typically shows a reddish brown

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discoloration. Rotting at the core of the root can be extensive. Such root symptoms may develop to a considerable extent without evidence of damage above ground. In fact, cultivars that are considered to be resistant may support extensive development of the pathogen within the root, as described more fully below.

3. Diagnosis and identification of the pathogen

3.1. Field diagnosis

Several pathogens other than *F. o. lactucae* can cause stunting and collapse of lettuce, with *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *S. minor*, and *Botrytis cinerea* being among the more common. The facility with which one can distinguish between these causal agents may depend on the cultivar, time of year, and the extent to which symptoms and signs have developed. What follows are some general characteristics of Verticillium wilt, caused by *V. dahliae*, lettuce drop, caused by *Sclerotinia* spp., and gray mold, caused by *B. cinerea*, that can serve to differentiate these diseases from Fusarium wilt (Table 1).

Plants affected by Verticillium wilt often reach harvest maturity, or nearly so, before foliar symptoms are visible, and progression of the disease is typically more rapid than what occurs in the case of Fusarium wilt. Microsclerotia forming along veins of senescent basal leaves would also be diagnostic of disease caused by *Verticillium dahliae* (Subbarao et al., 1997). Both Verticillium and Fusarium wilts cause internal discoloration of the taproot. A reddish to brown coloration is characteristic of disease caused by *Fusarium oxysporum*, whereas Verticillium wilt usually causes the internal taproot tissues to appear black. Ammonium buildup in soil can also result in a reddish brown vascular discoloration of the taproot and wilting of lettuce plants; this physiological disorder can be confused with Fusarium wilt (Koike et al., 2007). Lettuce drop is characterized by extensive soft rot of external crown tissues, and both *Sclerotinia* species usually produce black sclerotia and white mycelium on the surface of decayed crowns in contact with soil. Gray mold likewise is characterized by a very soft rot of the external crown tissues; fungal signs in this case consist of the fuzzy gray sporulation of *B. cinerea*. On lettuce, *Sclerotinia* species and *B. cinerea* do not cause any vascular discoloration. Of course, isolation of the pathogen may be required to confirm the identity of the causal organism.

Table 1
Comparison of symptoms associated with diseases caused by soilborne pathogens of lettuce.

Field symptoms	Disease[a]			
	Fusarium wilt	Verticillium wilt	Lettuce drop	Gray mold
Plants can be stunted	Yes	Yes	Yes	Yes
Plants can eventually collapse	Yes	Yes	Yes	Yes
Initial foliar symptoms on younger or older plants	Yes	No	Yes	Yes
Foliar symptoms initially occur only on mature plants	No	Yes	No	No
Vascular discoloration visible in taproot and crown	Yes	Yes	No	No
External crown and root tissues soft and rotted	No	No	Yes	Yes
Fungal mycelium and sclerotia may be present on crown	No	No	Yes	Yes

[a] Fusarium wilt is caused by *Fusarium oxysporum* f. sp. *lactucae*, Verticillium wilt is caused by *Verticillium dahliae*, lettuce drop can be caused either by *Sclerotinia sclerotiorum* or *S. minor*, and gray mold is caused by *Botrytis cinerea*.

3.2. Identification based on morphology

Although morphological criteria are generally sufficient to unambiguously identify *F. oxysporum*, not all strains of this species are pathogenic. Non-pathogenic strains are common in agricultural soils, where they persist as saprobes and colonizers of plant roots (Gordon and Martyn, 1997). Consequently, *F. oxysporum* isolates recovered from symptomatic plants may or may not be the cause of disease. This ambiguity pertains particularly to isolations from roots, with non-pathogenic strains being less likely to emerge from symptomatic crown and shoot tissue. Uncertainty can be dispelled by performing a pathogenicity test but owing to the time-consuming nature of this process, alternative means of pathogen identification have been sought.

A broad sampling of *F. o. lactucae* isolates from California and Arizona showed that the pathogen could be identified with a high level of confidence based on colony morphology on Komada's medium (Komada, 1975). The appearance associated with pathogenicity to lettuce was a pink pigmentation on the underside of the colony and white aerial mycelium organized into variously sized tufts. Three hundred and seventy three isolates were examined, and of 196 isolates that were pathogenic to lettuce, 195 (99%) had this colony morphology. All isolates with this appearance were pathogenic on lettuce (Scott et al., 2010a). The diagnostic value of colony morphology was established only for race 1 of *F. o. lactucae*, and may be contingent on morphological variation in the local population of non-pathogenic *F. oxysporum* strains, from which the pathogen must be distinguished.

3.3. Identification based on somatic compatibility

Puhalla (1985) showed that strains of *F. oxysporum* pathogenic to a particular host corresponded to one or two somatic compatibility groups. Subsequent work with a number of formae speciales, including *F. o. lactucae* (Osigo et al., 2002; Pasquali et al., 2005), have confirmed that isolates pathogenic to the same host tend to be associated with one or some small number of somatic compatibility groups. Once such a correlation has been established, compatibility with tester strains can serve as a substitute for a pathogenicity test. However, although this procedure may be less labor intensive than a pathogenicity test, there is still a substantial time delay before results are available.

3.4. Molecular detection

More rapid results may be obtained from a diagnostic test based on a specific DNA sequence that can be amplified using the polymerase chain reaction (PCR). This approach is appealing because it can yield results quickly and is especially valuable if it can be shown to have a high level of specificity. Such a test may not be needed for routine identification of *F. o. lactucae* emerging from symptomatic tissue, where recovery of non-pathogenic strains is unlikely, but can be of great value in testing seed lots, which may carry propagules of *F. o. lactucae* (Garibaldi et al., 2004a) as well as non-pathogenic strains of *F. oxysporum*. The need for a rapid and reliable test for *F. o. lactucae* in seed has motivated the design of PCR primers that produce an amplicon unique to this pathogen. Pasquali et al. (2007) used inter-retrotransposon amplified polymorphisms to develop primers that amplified a DNA fragment from *F. o. lactucae* race 1, and not from other isolates of *F. oxysporum* or other species of fungi that were tested. This assay was reported to have a detection threshold of approximately 50 colony forming units (CFUs) per gram of seed. Mbofung and Pryor (2010) developed a nested PCR assay that amplified a portion of the intergenic spacer of the rDNA. This test did not discriminate between *F. o. lactucae*