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
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 crows 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.

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 saprophages 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 the 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 specialiae, 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 portion of the intergenic spacer of the rDNA. This test did not discriminate between *F. o. lactucae* and *F. o. lactucae*

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**Table 1**

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

<table>
<thead>
<tr>
<th>Field symptoms</th>
<th>Disease&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fusarium wilt</th>
<th>Verticillium wilt</th>
<th>Lettuce drop</th>
<th>Gray mold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants can be stunted</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Plants can eventually collapse</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Initial foliar symptoms on younger or older plants</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Foliar symptoms initially occur only on mature plants</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Vascular discoloration visible in taproot and crown</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>External crown and root tissues soft and rotted</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Fungal mycelium and sclerotia may be present on crown</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 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*.
and certain other formae speciales, but those pathogens would not commonly be associated with lettuce. Tests with contaminated seed lots showed that infestation levels as low as 0.1% could be detected.

Whereas these and other tests represent progress toward a reliable and efficient means to detect *F. o. lactucae*, none are yet sufficiently sensitive to prevent dissemination of the pathogen with infested seed. Given that 75,000 seeds per hectare are commonly used to establish lettuce plantings, close to 100 contaminated seeds might be spread over each hectare through use of a seed lot in which *F. o. lactucae* was not detectable. It should also be noted that tests of assays reported to date have not included a broad range of non-pathogenic isolates, which are highly diverse and abundant in agricultural field soils (Gordon et al., 1992), and would not be unexpected as contaminants of seed.

4. Management of Fusarium wilt

4.1. Reducing the risk of introduction

A key to management of Fusarium wilt of lettuce is minimizing dissemination of the pathogen to areas where it is not already established. Long distance movement is most readily accomplished with contaminated seed, and hence the interest in improved methods for detection. Of course, it is also important to recognize the means by which seed becomes contaminated and to adopt practices that will minimize production of infested seed lots. Step one toward this objective would be to avoid growing seed crops in fields where the pathogen is present. Even if true seed infections do not occur, the proximity of pathogen propagules in soil and plant debris would pose a significant risk of external contamination of lettuce seed (Mbofung and Pryor, 2007). Additional insurance may be gained through the use of chemical seed treatments. Gilardi et al. (2005) reported carbendazim and prochloraz, among other fungicides, to be effective in treatment of seed artificially contaminated with *F. o. lactucae*. Several biological agents applied to seed were also shown to be beneficial. However, none of the treatments completely eliminated the pathogen from infested seed (Gilardi et al., 2005).

Local dissemination of *F. o. lactucae* is readily accomplished by movement of infested soil. The pathogen has been documented to survive in fallowed soil for at least 2.5 years following incorporation of a lettuce crop affected by Fusarium wilt, with a half-life estimated at approximately six months (Scott et al., 2012). The longevity of *F. o. lactucae* in soil may be extended by colonization of crops grown in rotation with lettuce (Scott et al., 2014). Furthermore, the pathogen can be resident in soil for an extended period of time before it reaches levels high enough to cause disease. Consequently, soil cannot be assumed to be pathogen-free based only on the absence of disease on a recent lettuce crop. For this reason, it is highly advisable to remove soil from farming equipment as completely as possible before it is moved between fields, even where there is no evidence of disease.

4.2. Intervention to reduce inoculum levels

Once *F. o. lactucae* is established in a field, eradication might be accomplished through fumigation of the soil, but the cost of this operation is generally prohibitive. To some degree, lettuce growers in California have benefited from prior year fumigations by following a strawberry crop, for which pre-plant fumigation has been a standard practice. However, owing to regulatory and economic constraints, the materials and application methods available to strawberry growers no longer consistently maintain soilborne pathogen populations below damaging levels. Consequently, a field previously cropped to strawberry will not necessarily present a lower risk of disease caused by a soilborne pathogen.

Other practices that modify the soil environment may help to lower the population of *F. o. lactucae* and/or inhibit its ability to infect and cause disease. Solarization has been reported to reduce inoculum density of numerous soilborne pathogens, including *F. o. lactucae*. Microplot studies in Arizona showed that the incidence of Fusarium wilt on lettuce sown in naturally-infested soil after solarization was reduced by up to 91% compared to non-solarized plots. Mean soil temperatures in solarized plots at a depth of 5 cm were 47 and 49°C, in two replications of this study (Matheron and Porchas, 2010). These results were obtained from solarization for a period of one month, and there was no significant benefit of extending the treatment interval to two months. Based on this study, solarization may be an option for lettuce growers in regions where temperatures are high enough to obtain the desired effect. Flooding soil to achieve anaerobic conditions has been shown to reduce populations of soilborne pathogens, but limited tests of this procedure did not demonstrate a beneficial effect on control of Fusarium wilt of lettuce (Matheron and Porchas, 2010).

4.3. Pathogen survival in soil

Crop rotation is a common practice in agriculture and, among other benefits, can allow for a reduction in the abundance of soilborne pathogens, as the viability of survival structures declines in the absence of a susceptible host. This strategy should be particularly effective against host-specific pathogens, such as formae speciales of *F. oxysporum*. The size of the *F. o. lactucae* population that remains after an interval when non-susceptible crops are grown will be a function of the rate of attrition of chlamydospores already present in the soil and the extent to which propagules are produced on rotation crops. These two components can be separated by monitoring inoculum density in fallow field soil, where population size will be determined principally by the mortality of propagules already present. As noted above, such studies indicate that viable propagules can be recovered 2.5 years after incorporation of a susceptible crop (Scott et al., 2012). The rate of decline is not linear, and most of the reduction occurs during the first year, with a more gradual decline thereafter (Fig. 1).

After 12 months, the inoculum density in naturally infested, fallow soil was reduced by 86%. An additional 22 months was required to achieve a comparable reduction (88%) from the level present at 12 months (Gordon, unpublished data). The relationship between the log number of viable propagules and the time after incorporation of a naturally infested crop is shown in Fig. 1. The size of the inoculum of *F. oxysporum f. sp. lactucae* in two replications of this study (Matheron et al., 2005) is shown in Fig. 1.
between soil inoculum density and disease severity is not well characterized, and will always be contingent on cultivar susceptibility and ambient temperatures (see below), but observations suggest that the population remaining after 34 months of fallow (17.5 ± 6.9 CFUs per gram) would pose a minimal risk of economic losses to Fusarium wilt. For example, the density of *F. o. lactucae* inoculum in soil adjacent to symptomatic lettuce plants in a commercial field in the San Joaquin Valley (CA) ranged from 50 to 300 CFUs per gram, as compared to a range of 5–27 CFUs per gram of soil just outside the affected area and adjacent to healthy plants (Gordon, unpublished).

4.4. Crop rotation

Of course fallowing soil for two or more years is rarely an option for growers, so in most cases, if lettuce is not grown, the field will be cropped to another marketable commodity. This being the case, the natural attrition of *F. o. lactucae* propagules may be offset to some extent by the production of survival structures on crops grown in rotation with lettuce. It is well known that pathogens causing Fusarium wilt may infect the roots of crops that do not show any symptoms (Katan, 1971; Elmer and Lacy, 1987; Gordon et al., 1989). *Fusarium oxysporum* f. sp. *lactucae* has been shown to colonize three crops that are commonly grown in rotation with lettuce: broccoli, cauliflower and spinach. Based on the frequency with which roots became infected, there was no significant difference between these three crops, but the extent of colonization was quite variable. Whereas growth of *F. o. lactucae* appeared to be limited to the root cortex on broccoli, the lettuce pathogen was recovered from within the vascular stele in 74 and 50% of cauliflower and spinach plants, respectively (Scott et al., 2014). This more invasive growth implies an expanded capacity for production of propagules, which would diminish the value of crop rotation.

An even greater contribution to the soil population of *F. o. lactucae* can result from growing lettuce cultivars that are resistant to Fusarium wilt, some of which sustain extensive colonization of the root vascular stele (above 2000 CFUs per gram of host tissue). In fact, inoculum production can be much higher on resistant cultivars, which grow to full size, than on susceptible cultivars, which are stunted and often die early in the season (Scott et al., 2014). Thus some cultivars, though not sustaining economic damage from Fusarium wilt, may allow inoculum levels to increase to the point that disease would have a significant impact on subsequent plantings of susceptible lettuce cultivars.

4.5. The effect of temperature on disease

The development of Fusarium wilt in lettuce is strongly influenced by ambient temperatures, with more severe disease occurring during warmer parts of the year (Matheron et al., 2005; Matuo and Motohashi, 1967). This effect is well illustrated by a comparison of disease severity in the susceptible cultivar Early Queen planted on different dates. In a June planting, mean disease severity rating was 2.9 at 18 days after planting, compared with a rating of 1.1 after the same interval when planting was in July (rating was on a 1–4 scale, with 1 corresponding to a healthy plant and 4 to a plant killed by Fusarium wilt). The differential progression of disease was associated with mean daily high/low soil temperatures for the June and July plantings of 30/20 °C and 25/19 °C, respectively (Scott et al., 2010a).

Observations in California’s coastal lettuce-growing districts also support the importance of temperature as a factor affecting development of Fusarium wilt (Scott et al., 2012). The disease has been observed both in the Pajaro Valley and the King City area. Whereas Fusarium wilt in the Pajaro Valley has remained insignificant, the incidence and severity of disease has increased in the King City area. In the Pajaro Valley, mean daily high temperatures during the main lettuce-growing season (May through October) remain below 23 °C; corresponding temperatures in the King City area are 25 °C or above, reaching a high of 29 °C for a 3 month period (July through September). Thus, higher temperatures may help to explain the greater incidence and severity of Fusarium wilt in the King City area. Where lettuce can be grown throughout the year, as in California, knowing how temperature influences disease severity can guide growers in selecting appropriate cultivars for each planting window, with the most susceptible cultivars being grown only during the cooler parts of the year.

4.6. Genetic resistance to Fusarium wilt

For many crops, management of Fusarium wilt has benefited from the use of genetically resistant cultivars, and differences in susceptibility to Fusarium wilt among lettuce cultivars have been documented (Fujinaga et al., 2001; Garibaldi et al., 2004b; Matheron et al., 2005; Scott et al., 2010b). In Japan, genetically resistant cultivars have been deployed, and this has revealed the existence of pathogenic races of *F. o. lactucae*. Fujinaga et al. (2003) described the use of differential cultivars that can distinguish between race 1, race 2 and race 3. With the exception of Taiwan, where race 3 was recently reported (Lin et al., 2014), outside of Japan, Fusarium wilt of lettuce is caused exclusively by race 1 of *F. o. lactucae*. According to Aruga et al. (2012), in Japan only single races of *F. o. lactucae* have been found in infested fields, and consequently, cultivars with resistance to only one race can still be useful. This pattern of occurrence is consistent with the fact that pathogenic races of *F. o. lactucae* are not closely related (Fujinaga et al., 2005), which suggests that each race arose independently and not as a mutant derivative of a pre-existing pathotype. This underscores the value of adopting measures that will reduce the risk of disseminating the pathogen from existing infestations.

Based on studies conducted in Italy (Garibaldi et al., 2004b) and the U.S. (Matheron et al., 2005; Scott et al., 2010b), it appears that although iceberg lettuce cultivars differ in susceptibility to Fusarium wilt, none are truly resistant. On the other hand, high levels of resistance are apparent in some leaf and romaine cultivars (Fujinaga et al., 2003; Garibaldi et al., 2004b). For example, both controlled environment and field studies have confirmed that the leaf cultivar, Lolla Rossa, and romaine cultivars Caesar and King Henry, remain healthy even under optimal conditions for disease development (Scott et al., 2010a). However, all cultivars tested were shown to sustain root infections at the same frequency, regardless of their susceptibility to Fusarium wilt (Scott et al., 2014). In fact, as noted above, pathogen growth in the taproot of resistant plants can be extensive. Differences in the extent of internal colonization may be indicative of differences in resistance that would not be apparent if only above-ground symptoms are assessed. For example, the cultivars Lolla Rossa and Caesar appear equally resistant, but colonization of the taproot vascular stele is significantly greater in Lolla Rossa than in Caesar (Scott et al., 2014). To the extent that such differences are heritable, cultivars supporting less development of the pathogen may be superior sources of resistance for introgression into the genetic background of susceptible iceberg types.

The genetic basis for resistance to Fusarium wilt is not completely understood, but recent work in Japan suggests that resistance to race 2 may be determined primarily by a single dominant locus, with lesser contributions from minor loci (Aruga et al., 2012). Studies of progeny of a cross between the iceberg cultivar Salinas, which has moderate resistance, and Valmaine, a highly resistant romaine cultivar, identified three quantitative trait loci associated with resistance to Fusarium wilt, each on a different
linkage group (Scott et al., 2012). At two of these loci, the alleles conferring resistance were derived from Valmaine, whereas at the third locus, the Salinas allele was responsible for resistance. Combining these positive alleles from both parents should provide a higher level of resistance than is conferred by either parent.

Overall, the genetic resources that have been identified augur well for more effective control of Fusarium wilt in the future. However, the potential for new pathogenic races to degrade the efficacy of genetic resistance argues for maintaining an integrated approach to disease management. This includes efforts to minimize dispersal of the pathogen with seed and soil, and where the disease is present, maintaining soil inoculum densities well below damaging levels.

Acknowledgments

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References


