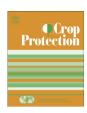


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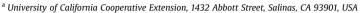
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## Management of Fusarium wilt of strawberry

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#### ABSTRACT

Fusarium wilt of strawberry was first described in the 1960s in Australia and Japan. Since then the pathogen, *Fusarium oxysporum* f. sp. *fragariae* (Fof), has been reported worldwide with the majority of cases being found in the 1990s and 2000s. Many of these recent reports are associated with changes in pre-plant soil fumigation practices. The disease is of significant economic importance because infected plants can collapse and die. Field diagnosis of Fusarium wilt is complicated by the fact that other soilborne diseases exhibit very similar symptoms. Methods for detection of Fof based on molecular criteria have been developed, but none have yet been shown to uniquely identify the strain of *F. oxysporum* causing Fusarium wilt of strawberry. Management of Fusarium wilt is best achieved through the use of resistant strawberry cultivars. Research indicates that sources of Fof resistance exist in strawberry germplasm, though cultivar reactions may differ depending on the Fof isolate. Pre-plant treatment of infested soil with fumigants remains a useful management tool. In addition, alternative treatments such as steam, solarization, anaerobic soil disinfestation, and the planting of brassicaceae crops are being assessed for their effectiveness in managing the disease. Standard integrated pest management practices of crop rotation with non-hosts, planting of pathogen-free transplants, and sanitation of equipment remain important measures that can reduce the risk of damage from Fusarium wilt.

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#### 1. Introduction to Fusarium wilt

Fusarium wilt of strawberry (Fragaria x ananassa) is an important disease of this crop worldwide. Caused by the pathogen Fusarium oxysporum f. sp. fragariae (Fof), the disease was first found in Australia in 1962 (Winks and Williams, 1965) and was then found in Japan in 1969 (Okamoto et al., 1970). Since that time, Fusarium wilt was confirmed on strawberry in Korea in 1982 (Kim et al., 1982), Mexico in the late 1980s-early 1990s (Castro-Franco and Davalos-Gonzalez, 1990; Cejas-Torres et al., 2008), China in 2005 (Huang et al., 2005; Zeng et al., 2006; Zhang et al., 2012; Zhao et al., 2009), the United States in 2006 (California: Koike et al., 2009) and 2011 (South Carolina: Williamson et al., 2012), Spain in 2007 (Arroyo et al., 2009), and Serbia in 2013 (Stankovic et al., 2014). In Mexico, researchers feel that Fusarium wilt is part of a disease syndrome in that the combined infections by Fof and a strawberry virus complex has contributed to the decline of strawberry in that region (Davalos-Gonzalez et al., 2014). For the more recent occurrences, Fusarium wilt outbreaks on strawberry have been associated with changes in pre-plant soil fumigation. In California, for example, virtually all of the initial outbreaks were associated with fields that no longer were flat-fumigated with methyl bromide + chloropicrin that was shank injected under plastic tarps (Koike et al., 2013). Thus, the pathogen may have been present for an extended period but not detected because fumigation maintained inoculum densities below damaging levels. A preliminary assessment of California isolates of Fof has identified three somatic compatibility groups and diverse sequences in the translation elongation factor, suggesting the population is not derived from a single recent introduction (Gordon, unpublished). Consistent with the narrow host ranges of almost all *formae specialis* of *F. oxysporum*, strawberry is the only known host to Fof (Kodama, 1974).

#### 2. Fusarium wilt symptoms

In fields with no or inadequate fumigation, strawberry plants can show initial symptoms of decline as early as 30 days after transplanting; these plants will stop growing and be stunted when compared to healthy plants. However, often the initial symptoms of Fusarium wilt in strawberry occur after the plants are well established and begin to flower or produce fruit, at which time the older

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leaves wilt, turn gray green in color, and begin to dry up. As disease progresses, virtually all of the foliage will collapse and dry up with the exception of the central, youngest leaves. Plants can eventually collapse and die. When internal tissues of plant crowns are examined, vascular and cortical tissues are dark brown to orange brown. Internal tissues of the main roots may also be discolored and dark brown in color. Environmental and other stresses can cause the disease to develop more rapidly and severely. Such factors may include the following: weather extremes, particularly high temperatures; water stress from insufficient irrigation or prolonged saturation in the root zone; poor soil conditions; pressure from pests such as mites.

#### 3. Diagnosis and confirmation of Fusarium wilt

#### 3.1. Diagnosis via culturing and isolation

For any crop, deploying effective controls and implementing integrated pest management (IPM) strategies depend on the accurate diagnosis of the disease. Diagnosis and confirmation of strawberry Fusarium wilt in the field is not possible, however. Strawberry is subject to at least four major soilborne pathogens that cause similar, if not identical, symptoms (Table 1). Poor growth, stunting, wilting and collapse of foliage, and plant death can be caused by Fof, Macrophomina phaseolina (causal agent of charcoal rot), Verticillium dahliae (causal agent of Verticillium wilt), and Phytophthora cactorum (causal agent of Phytophthora crown and root rot). Phytophthora crown and root rot differs from Fusarium wilt in that generally both younger and older foliage will collapse at the same time and the plant roots will be distinctly soft and rotted; this disease is also commonly associated with overly wet soil conditions. Verticillium wilt looks very similar to Fusarium wilt except the internal crown tissue of Verticillium-infected strawberry plants lacks the very dark orange to brown discoloration caused by Fof. However, charcoal rot of strawberry (Koike, 2008) causes symptoms that are identical to those of Fusarium wilt. Accurate field diagnosis of collapsing plants is therefore not possible to achieve without laboratory tests that mostly are based on the culturing and isolating of the causal agent.

#### 3.2. Diagnosis via molecular methods

Rapid and accurate identification of the particular *formae specialis* that causes Fusarium wilt of strawberry would also be of great

Table 1
Comparison of symptoms caused by four major soilborne pathogens of strawberry.

Field symptoms	Pathogens			
	F. oxysporum f. sp. fragariae	Macrophomina phaseolina	Verticillium dahliae	Phytophthora cactorum
Poor growth and stunting	yes	yes	yes	yes
Wilting, initially older leaves only	yes	yes	yes	no
Wilting, all leaves at once	no	no	no	yes
Plant collapse and death	yes	yes	yes	yes
Discolored internal crown tissue	yes	yes	no	yes
Soft, rotted roots	no	no	no	yes
Associated with stress factors	yes	yes	yes	no
Dependent on overly wet soils <sup>a</sup>	no	no	no	yes

<sup>&</sup>lt;sup>a</sup> The life cycle of *Phytophthora* depends on periods of saturated soil.

use. Such precision could be used to distinguish between Fof and non-pathogenic Fusarium oxysporum recovered from plant material and could also be employed to test field soil so as to gauge the risk of planting strawberry in that particular field. Toward this objective, Suga et al. (2013) developed PCR primers that amplified a diagnostic DNA sequence from Fof but not from other formae speciales of F. oxysporum or non-pathogenic strains of this species. Whereas there were no false negatives, six strains identified as Fof based on the PCR detection method were found to be nonpathogenic to strawberry (Suga et al., 2013). In addition, using the same primers, no amplicon was obtained from a subset of Fof isolates originating in the U.S. (Gordon, unpublished results). In China, Li et al. (2014) used both real-time PCR and a semi-selective medium to detect and quantify F. oxysporum following fumigant treatments. There was a strong correlation between results obtained with these two methods, both of which were predictive of disease incidence in a greenhouse trial. However, it appears that results of both procedures were based on total F. oxysporum and not solely the strain causing Fusarium wilt of strawberry. Thus, further work will be required to produce a robust method for detection of Fof based on PCR.

#### 4. Management of Fusarium wilt

The overall strategy for managing this disease depends on preventative measures. No effective therapeutic treatments for diseased plants are available. An IPM approach that incorporates planting resistant cultivars, pre-treating infested fields, rotating with non-hosts, and implementing various cultural practices can minimize damage from Fusarium wilt.

#### 4.1. Fusarium-resistant strawberry cultivars

The discovery, development, and planting of Fusarium-resistant strawberry cultivars is the most effective and preferred way to control Fusarium wilt. A number of breeding lines and cultivars have been tested in the various countries where the disease has occurred. Such research thereby demonstrated that sources of Fof resistance and diversity in strawberry germplasm exist. In Mexico a number of advanced selections and two cultivars (Cometa, Buenavista) released in 2003 exhibited resistance to Fof (Davalos-Gonzalez et al., 2006). Australian studies found cvs. Festival, Aromas, and Camino Real to be resistant, with Festival being the most resistant (Fang et al., 2012a). In California, cvs. Portola and San Andreas were found to be highly resistant when subjected to rootdip inoculations (Gordon, 2012, 2013). Breeders in Asia have been actively developing resistant cultivars for many years and have tested and released a number of them over time: Fukuba, Fogyoku, Yachiyo, Benituyu and Senga Gigana (1974); Hatsukuni (1982); Daehak 1, Line 10-2, Senga Sengana (1982); Asuka Wave (1994); Aisutoro and Hogyoku (2005); Komachi Berry (2007) (Kim et al., 1982; Kodama, 1974; Mori et al., 2005; Takahashi et al., 2007). Wild clones of Fragaria chiloensis, collected originally from California, also exhibited resistance to Fof and therefore could provide another source of genetic material for resistance breeding (Davalos-Gonzalez et al., 2006).

Interestingly, there are differing accounts on whether certain commercial cultivars are resistant to Fof. In inoculation tests conducted in Spain, cv. Ventana was proven to be very susceptible (Arroyo et al., 2009) while in California cv. Ventana was resistant in most, but not all, inoculation experiments and is considered to be resistant in commercial plantings (Gordon, 2012, 2013). Cv. Camino Real was very susceptible in a California study (Koike et al., 2009) but was the most resistant cultivar in an Australian investigation (Fang et al., 2012a). Such discrepancies could be accounted for if

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