The *Secreted in Xylem* Gene Profile of the Spinach Fusarium Wilt Pathogen

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Introduction (ICPP, Boston, MA, USA, 29 Jul. – 3 Aug. 2018)

The maritime Pacific Northwest (PNW) is the only region of the United States suitable for production of spinach seed, which requires a long day length for bolting and cool, dry summers to yield high quality seed **(Fig. 1).** The greatest limitation to spinach seed production in the PNW is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *spinaciae* (*Fos*) (2). There are no known races of *Fos* and resistance to this pathogen appears quantitative. Little is known about the genetics of *Fos* pathogenicity and host-specificity. Lineage-specific accessory chromosomes found in the tomato wilt pathogen, *F. oxysporum* f. sp. *lycopersici*, carry pathogenicity genes (4). These include 14 *Secreted in Xylem* (*SIX*) effector genes which encode small, cysteine-rich peptides secreted in the xylem of tomato plants during infection. Unique combinations of *SIX* genes found in some *F. oxysporum* ff. spp. may be responsible for host-specificity. **The objective of this study is to determine if *Fos* isolates have a unique *SIX* gene profile that might account for specificity to spinach.**

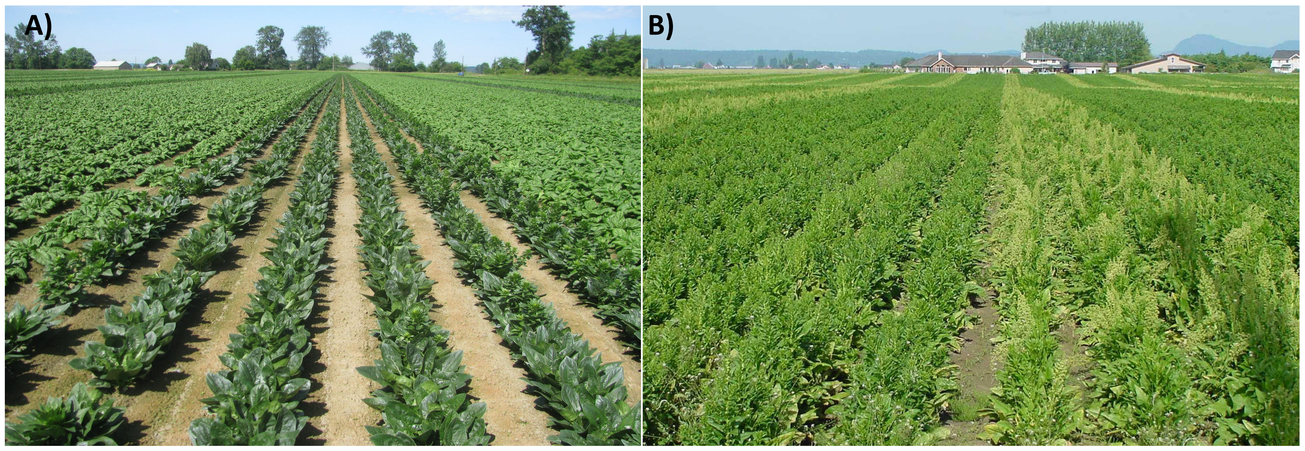

Fig. 1. A) Spinach seed crop prior to bolting (conversion from vegetative to reproductive growth). **B)** after bolting.

Materials and Methods

Phenotypic characterization: To date, **65** isolates of *Fusarium oxysporum*, including **42** isolates from spinach, were characterized for pathogenicity to spinach. Three inbred spinach lines ranging in degree of susceptibility to Fusarium wilt were each drench-inoculated with 15 mL of microconidial suspension (1 x 10^5 spores/mL) of each isolate in replicated trials. Wilt severity was rated weekly (**Fig. 2**) and spinach dry biomass measured.

Genotypic characterization: PCR assays for the 14 known *SIX* genes were completed for **65** isolates, which represent 6 ff. spp. as well as isolates for which the host range was not known (3,5,6). A portion of the translation elongation factor 1α (*TEF-1α*) gene also was sequenced for species confirmation. Phylogenies of the *TEF-1α*, *SIX8*, and *SIX14* regions were estimated (**Fig. 3**). The SIX14 amino acid sequences were compared among isolates of the various ff. spp. (**Fig. 4**).

Fig. 2. Spinach plants ranging in severity of Fusarium wilt from 0 (healthy) to 5 (dead).

Results

1. Of **42** isolates sampled from spinach tissue, soil, etc., 39 were pathogenic to spinach (i.e., verified as *Fos*) and 3 were classified as non-pathogenic to spinach (NPS).
2. Isolates of *F. oxysporum* ff. spp. *ciceris*, *cepae*, *lycopersici*, and *pisi* had *SIX* gene profiles that matched published profiles (1,3,6,7).
3. Among the **39** *Fos* isolates, 18 carried *SIX8* and 35 carried *SIX14*. Of the three NPS isolates, none carried *SIX8* (**Fig. 3B**) and all three carried *SIX14* (**Fig. 3C**).
4. Among the 35 *Fos* isolates carrying *SIX14*, 31 formed a monophyletic group in the *SIX14* phylogeny, but 4 carried divergent copies of *SIX14* that positioned these isolates in clades with other ff. spp. (**Fig. 3C**). All *Fos* isolates were monophyletic in the *TEF-1α* phylogeny, suggesting that pathogenicity to spinach has a single evolutionary origin (**Fig. 3A**).
5. One NPS isolate grouped with 31 of the 35 *Fos* isolates (**Fig. 3C**).
6. Translation of the full coding sequence of *SIX14* for 31 of the 35 *Fos* isolates demonstrated that residues 3 and 10 were unique from those of other ff. spp. (**Fig. 4**). These amino acid residues could not be determined for the four isolates with divergent *SIX14* sequences.

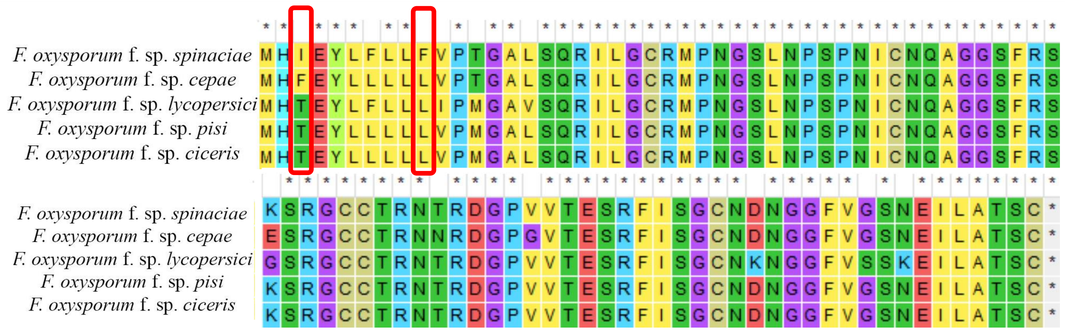

Fig. 4. Amino acid sequence alignment of the SIX14 protein translated *in silico* from *SIX14* gene sequence for isolates representing five *F. oxysporum* ff. spp.

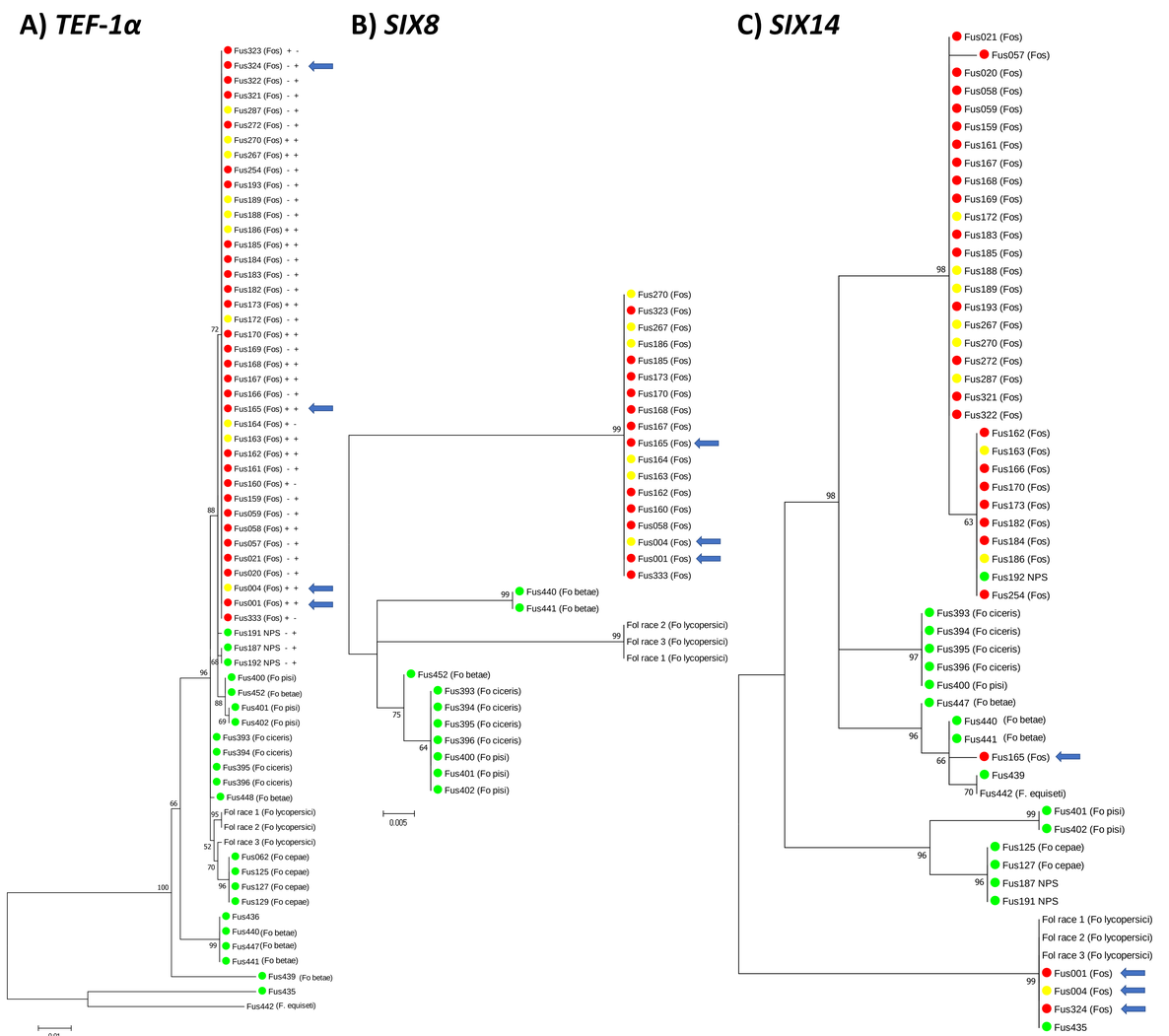

Fig. 3. Maximum likelihood phylogenies of *Fusarium oxysporum* isolates tested for pathogenicity on spinach and for the presence of *Secreted in Xylem* (SIX) genes. The number at each node represents the percentage of trees with the node among 1,000 bootstrap replicates. Each scale bar indicates the average number of substitutions per site. **A)** *Translation elongation factor* (*TEF*) *-1α*. '+' and '-' for the *F. oxysporum* f. sp. *spinaciae* and non-pathogenic spinach isolates indicate whether or not *SIX8* and *SIX14* were detected by PCR, respectively. **B)** *SIX8*. **C)** *SIX14* regions. ●, ●, and ● represent isolates that were pathogenic, moderately pathogenic, or non-pathogenic on spinach, respectively. Isolates without a circle have not yet been tested. NPS isolates = non-pathogenic to spinach. Blue arrows point to the *Fos* isolates with divergent *SIX14* sequences.

Discussion

1. Thirty-nine *Fos* isolates tested to date **did not have a distinct *SIX* gene profile** compared to published profiles of other ff. spp. However, the *SIX8* sequences of 18 *Fos* isolates with that gene were unique compared to those of the other ff. spp. In addition, the *SIX14* sequences and the predicted amino acid sequence of 31 of the 35 *Fos* isolates that tested positive for that gene were unique. The other 4 *Fos* isolates carrying *SIX14* had divergent sequences from the 31 *Fos* isolates.

2. **The results did not reveal the roles of *SIX8* and/or *SIX14* in host-specificity of *Fos*.** Only 18 of 42 *Fos* isolates were positive for *SIX8* and 35 were positive for *SIX14*, suggesting that pathogenicity to spinach does not require *SIX8* or *SIX14*. We found three NPS isolates carrying *SIX14*, one of which grouped with 31 of 35 *Fos* isolates. This suggests that *SIX14* alone may not be sufficient for pathogenicity. Furthermore, there was incongruence among the *TEF-1α*, *SIX8* and *SIX14* phylogenies. The *TEF-1α* phylogeny suggests that pathogenicity to spinach is monophyletic (**Fig. 3A**). The *SIX14* phylogeny indicates that gene is polyphyletic in *Fos* (**Fig. 3C**). Sequencing of additional housekeeping and *SIX* gene loci might resolve these conflicting evolutionary histories.

Future Directions

1. The genomes of *Fos* isolates as well as NPS isolates are being sequenced to identify potential genetic factors that distinguish isolates of *Fos* from NPS isolates and isolates of other ff. spp.

2. This information should lead to an understanding of how *Fos* causes Fusarium wilt of spinach, aid in development of molecular diagnostic tools for this pathogen and facilitate breeding for resistance to Fusarium wilt of spinach.

Select References

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