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Infection by *Fusarium proliferatum* in aerial garlic bulbils is strongly reduced compared to rates in seed cloves when both originate from infected bulbs

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ABSTRACT

Infection of bulbs of garlic (*Allium sativum*) by *Fusarium proliferatum* is increasingly documented worldwide and management of the pathogen is problematic. Garlic bulbs were harvested at the USDA-ARS Plant Introduction farm near Pullman, WA in fall 2015. From a sample of 18 bulbil-producing accessions whose bulbs were documented as infected by *F. proliferatum*, a mean of 64% of cloves from infected bulbs contained the pathogen. In umbels produced from infected bulbs, a mean of 11% were detected with the pathogen, with a mean infection rate of bulbils in infected umbels of 42%, resulting in a probability of bulbil infection of less than 5%. In bulbs harvested in fall 2016 from 15 accessions whose bulbs were previously documented as infected, a mean of 34% of cloves in infected bulbs contained the pathogen, but the pathogen was not detected in umbels or bulbils. Overall incidence of *F. proliferatum* in bulbs surveyed for infection in 2016 (99 accessions) at the same farm was assessed via a cumulative geometric distribution, and indicated occurrence in 87% of accessions, with probability of infection in a given bulb between 25 and 50%. In 2016, all but 0.01% of whole bulbs harvested for this survey of overall incidence were asymptomatic at harvest on the basis of firmness, but 77% of cloves were symptomatic (inclusive of all biological and abiotic causes) when peeled and plated to agar media 9–16 months after harvest. Bulbils take at least a year longer to mature into full size bulbs than do seed cloves, presenting a longer window for infection by several pathogens, but if planted to pathogen-free soil might represent a cost-effective means to strongly reduce infection of propagation material by *F. proliferatum*.

1. Introduction

Fusarium proliferatum, a fungal pathogen of garlic (*Allium sativum*), was first detected and confirmed as a pathogen in garlic in North America in 2001 (Dugan et al., 2003). The fungus was also reported from onion in Idaho (Mohan et al., 1997), garlic in Germany (Seefelder et al., 2002), and onion in Washington State (du Toit et al., 2003). It was documented as a pathogen of ornamental *Allium* in Korea (Shin and Kim, 2001). Subsequently, *F. proliferatum* has been documented in *Allium* species in a growing body of literature worldwide (e.g., Alberti et al., 2018; Alizadeh et al., 2010; Bayraktar and Dolar, 2011; Dissanayake et al., 2009; Fuentes et al., 2013; Haapalainen et al., 2016; Moharam et al., 2013; Palmero et al., 2010; Quesada-Ocampo et al., 2014; Ravi et al., 2014; Salvalaggio and Ridao, 2013; Sankar and Babu, 2012; Stankovic et al., 2007; Tonti et al., 2012). It is also a pathogen of other liliaceous hosts worldwide, e.g. asparagus (*Asparagus officinalis*) in Australia and elsewhere (Elmer et al., 1999) and *Gladiolus* hybrids in Oman (Mahmooli et al., 2013), in addition to other plants in multiple plant families (Farr and Rossman n.d.).

Chemical-based disease management has not been consistently cost-effective, largely because the pathogen often grows deeply within inner clove scales and even systemic fungicides cannot effectively penetrate (Dugan et al., 2007). Recent research on chemical-based management holds greater promise, but there are indications of fungicide resistance and of failure to control rots in storage (Patón et al., 2017). Garlic is typically propagated via seed cloves, but planting of aerial bulbils (borne in umbels at the apex of stalks known as scapes) is an alternative means of propagation for cultivars that produce them. We wished to know if the infection rate in bulbils would differ from the infection rate in cloves, when both bulbils and cloves originated from plants whose bulbs were known to be infected by *F. proliferatum*. If the infection rate in bulbils were substantially lower than that in cloves, it might indicate that planting of bulbils would represent a cost-effective means to manage *F. proliferatum* in garlic. Given the dramatic increase over the past decade in reports of *F. proliferatum* in *Allium* spp. referenced above, we also wished to survey incidence of infection at the USDA-ARS Plant Introduction farm near Pullman, WA, a unit of the USDA-ARS National Plant Germplasm System.

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Table 1
Accession numbers and GenBank numbers for representative isolates identified as *F. proliferatum* based on TEF1 partial gene sequences.

Accession, Isolate Name	GenBank Accession Number	Closest Blast Hit by total score and (%) Identity	Identity to K140108[b] (KT218533) and CBS 131574[c] (JX118983)	Identity to HYC1410080201[d] (MF448528)	Identity to NRRL 43617[e] (HM347124)	Identity to NRRL 22944[f] (AF160280)
W6 50 B4C1	MH383507	K140108 (99%)	99%	99%	99%	98%
W6 1885 B3C5	MH383508	K140108 (100%)	100%	99%	99%	98%
W6 8406 B2C3	MH383509	NRRL 32155[a] (FJ538242) (100%)	99%	99%	99%	99%
W6 8411 B2C1	MH383510	K140108 (100%)	100%	100%	99%	98%
W6 26172 B1C4	MH383511	CBS 131574 (100%)	100%	99%	99%	98%
W6 35677 B2C3	MH383512	K140108 (100%)	100%	99%	99%	98%
PI 540375 B1C1	MH383513	K140108 (100%)	100%	99%	99%	98%

[a] Isolated from *Cicer arietinum* in India (Gujar et al., 2009).
[b] Cause of tomato leaf spot in China (Gao et al., 2016).
[c] Isolated from *Fusarium* head blighted wheat in Iran (Davari et al., 2013).
[d] Cause of daylily (*Hemerocallis citrina*) flower rot in China (Li et al., 2018).
[e] Isolated from human blood (O'Donnell et al., 2010).
[f] Isolated from *Cymbidium* sp. in Germany (Nirenberg and O'Donnell, 1998).

2. Materials and methods

2.1. Identification of the pathogen

We wished to be certain that results and analyses focused on plants whose bulbs were confirmed as infected with *F. proliferatum* (Table 1). Data on infected cloves and bulbils (Tables 2 and 3) pertain to instances in which a minimum of one colony of *F. proliferatum* (provisionally identified on the basis of morphology as indicated below) originated from a clove plated to half strength V8 juice agar (Stevens, 1981) amended with antibiotics (streptomycin sulfate and tetracycline hydrochloride at 50 μg/L) (½ V8) following surface-disinfestation in 0.5% hypochlorite solution, thereby demonstrating infection in that bulb. For this data, as well as for data on the survey of infection in bulbs (Table 4), pathogens other than *F. proliferatum* were excluded. Two hundred and twenty-four putative isolates of the pathogen from the survey were initially selected on the basis of morphology (Fig. 2a) (Leslie and Summerell, 2006; Nirenberg and O'Donnell, 1998) and seven representative isolates from the survey were subsequently chosen for sequencing of partial translation elongation factor 1-alpha sequences (TEF1) as follows.

Isolates were grown on ½ V8 and conidia were gently washed from the agar surface and pelleted by centrifugation, washed twice with sterile water, and the pellet lyophilized. Fifteen mg of lyophilized conidial pellet was disrupted in the presence of three 3 mm glass beads in a Fast Prep™ 120 cell disruptor (speed 6 for 30 s). Genomic DNA was isolated immediately following tissue disruption using Qiagen DNeasy® Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. DNA was eluted from the column with 100 μl of sterile water. Amplification of the TEF1 partial gene sequence was accomplished using PCR primers EF-1 and EF-2 (O'Donnell et al., 1998). PCR was conducted in 50 μl reaction mixtures containing 2 μl of genomic DNA extract, 10 μl of GoTaq 5X reaction buffer, 1 μl of 10 mM dNTP mix, (50 pmol) of each primer, and 0.5 μl of GoTaq® polymerase

Table 2
Infection in bulbs and cloves versus bulbils: Results from 2015 harvest.

A	B	C	D	E	F	D x F
Accession number	Number of infected bulbs out of sample of 8	Number of infected cloves per number cloves sampled in infected bulbs and % infection[a]	Number of infected umbels from the infected bulbs, expressed as % of tested umbels	Number of infected bulbils in infected umbels	% Bulbil infection per infected umbel	Given the bulb was infected, chance of a bulbil being infected to nearest %
PI 497944	3	8/15 = 53%	0, 0%			0%
PI 515975	7	32/35 = 91%	3, 3/7 = 43%	7	47% (7/(3 × 5))	20%
PI 540334	3	7/13 = 54%	0, 0%			0%
PI 540335	7	19/29 = 66%	1, 1/7 = 14%	3	60% (3/(1 × 5))	8%
PI 540360	2	4/10 = 40%	1, 1/2 = 50%	1	20% (1/(1 × 5))	1%
PI 540365	7	24/35 = 69%	1, 1/7 = 14%	2	40% (2/(1 × 5))	6%
W6 8415	2	6/10 = 60%	0, 0%			0%
W6 12829	5	8/25 = 32%	0, 0%			0%
W6 12912	7	20/35 = 57%	2, 2/7 = 29%	5	50% (5/(2 × 5))	15%
PI 540343	8	29/40 = 73%	0, 0%			0%
PI 540361	7	27/35 = 77%	2, 2/7 = 29%	6	60% (6/(2 × 5))	17%
PI 540363	1	4/5 = 80%	0, 0%			0%
W6 1883	5	8/25 = 32%	1, 1/5 = 20%	1	20% (1/(1 × 5))	4%
W6 12832	6	17/30 = 57%	0, 0%			0%
W6 12837	8	25/40 = 63%	0, 0%			0%
W6 17281	7	28/35 = 80%	0,0%			0%
W6 35679	6	24/30 = 80%	0,0%			0%
W6 35689	7	29/35 = 83%	0,0%			0%
Mean	5.4 bulbs	**64%**	**11%**		**42%**	**5%**

Chance of infection in next planting propagule.
If planting cloves, same as column C.
If planting bulbils, chance of infection = D x F.
[a] Number of cloves sampled is not always a multiple of 5 because some bulbs had less than 5 cloves.