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Etiology and characterization of cucumber vine decline in Oman

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ABSTRACT

A long-term study was conducted between 2000 and 2009 to characterize the incidence, progress and causal agents of cucumber vine decline in Oman. A survey in 175 different greenhouses showed that disease incidence levels range from 0 to 50%, with the highest levels of mortality being in the hotter seasons. Detailed temporal disease increase data from 24 different greenhouses showed mortality progress consists of two phases. The first phase is characterized by attack of young seedlings (<3 weeks old), resulting in damping-off disease. The second phase was characterized by re-appearance of symptoms and plant death (vine decline) during the fruit setting period, 35–50 days after transplanting. Isolations from 148 declining adult cucumber plants yielded *Pythium aphanidermatum* (80%), *Pythium spinosum* (13%), *Fusarium equiseti* (12%), *Fusarium solani* (8%), *Rhizoctonia solani* (5%) and one isolate each of *Trichoderma hamatum* and *Bionectria* sp. *P. aphanidermatum*, *P. spinosum*, *R. solani* and *F. solani* were found to be pathogenic on cucumber, with *P. aphanidermatum* being the most aggressive. This appears to be the first report of association of *P. spinosum* with vine decline in greenhouse cucumbers and the first report of the high susceptibility of adult cucumber plants to vine decline during the initial period of fruit setting. In addition, this is the first report of association of *R. solani* and *F. solani* with declining adult cucumber plants in Oman and the first report of the occurrence of *T. hamatum* and *Bionectria* species in the country.

1. Introduction

Cucumber (*Cucumis sativus* L.), together with other cucurbits (mainly watermelon and muskmelon), are among the most important vegetable crops in Oman, with a combined total production of about 30,000 tons in 2008 (FAO, 2010). In Oman, cucumber is produced in greenhouses, with over 95% of the national greenhouses used for cucumber production. Production is mostly soil-based; however, about 2% of growers use hydroponic systems.

Despite the high reliance on cucumber cultivation, cucumber production is limited by a number of constraints. Damping-off and vine decline diseases and increasing irrigation water salinity levels are considered the most serious limiting factors (Al-Kiyumi, 2006; Al-Sa'di et al., 2007; Al-Sadi et al., 2010). Damping-off has been reported to occur in over 80% of the greenhouses in Oman and results in the mortality of 4–8% of seedlings (Al-Sa'di et al., 2007). It is a common problem in cucumber grown in soil-based systems in other parts of the world (Stanghellini and Phillips, 1975). Damping-off levels are higher under high salinity levels, resulting from the synergistic interaction between salinity stress and infection by the salinity tolerant *Pythium* species (Al-Sadi et al., 2010).

In addition to damping-off which occurs at the seedling stage, vine decline of cucumber plants which occurs at the adult stage also limits production of cucumbers in greenhouses in different parts of the world (Stanghellini and Phillips, 1975; Christensen and Thinggaard, 1999). Although some limited surveys have reported this disease to occur on some farms in Oman (Al-Hasani, 2004), there is a lack of knowledge concerning losses due to this disease and the effect of growing season on cucumber mortality. Despite reports from other parts of the world concerning timing and factors affecting vine decline in other cucurbits (e.g. melons; Pivonia et al., 1997), there is a lack of information on the timing of expression of vine decline in greenhouse cucumbers.

Different pathogens have been reported as associated with root rot and vine decline of cucumber including *Pythium aphanidermatum* (Edson) Fitzp., *Pythium ultimum* Trow., *Fusarium oxysporum* (Schlecht) f. sp. *cucumerinmum* and *Fusarium solani* (Mart.) f. sp. *cucurbitae* (Stanghellini and Phillips, 1975; Bourbos et al., 1997; Christensen and Thinggaard, 1999). In Oman, damping-off disease of cucumber has been reported to be associated with *P. aphanidermatum*, *Pythium spinosum* Swada and *Pythium splendens* Braun

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(Al-Sa'di et al., 2007). However, it is not clear whether the same pathogens are also associated with decline of adult plants.

The main purpose of this study was to investigate the temporal progress and etiology of adult cucumber plant decline. Specific objectives include: (i) to investigate percent mortality in greenhouse cucumbers due to damping-off and vine decline; (ii) to investigate the temporal progress of damping-off and adult cucumber decline over time during four different seasons under commercial growing practices; (iii) to identify pathogens associated with cucumber vine decline in Oman; and (iv) to test for pathogenicity of the isolated fungi and oomycetes. Findings from this study may provide a solid basis for the development of future management strategies to cucumber vine decline in Oman.

2. Materials and methods

2.1. Mortality in cucumber due to damping-off and vine decline

Two distinct surveys were conducted. The first aimed to document mortality due to damping-off and vine decline. Regular visits to greenhouses between 2000 and 2009 collected data on growing season, cucumber variety, plant number and the percent cucumber seedlings and plants showing mortality due to these diseases. The visits between the end of the 2000 to the end of the 2004 were at least 6 visits per growing season, while visits thereafter were 2–6 visits per growing season. A total of 175 different greenhouses were visited and the percent mortality in cucumber due to damping-off and adult vine decline was recorded.

In order to follow development of damping-off and vine decline for the whole period of the crop, 24 different greenhouses were randomly selected from different districts. Data was collected during 2001–2003 on the daily progress of damping-off and vine decline for 120 days following transplanting under current growers' practices. Data included information about year of planting, season, plant number per greenhouse, cucumber variety and general cultivation practice information (Table 1). Each day during the growing season the number of dead seedlings/plants was recorded.

2.2. Causal agents of cucumber vine decline

Since the causal agents of damping-off disease of cucumber in Oman have been characterized (Al-Sa'di et al., 2007), only samples of adult cucumber plants showing decline symptoms were collected. A total of 90, 3 and 39 plant samples were collected during surveys conducted in 2004, 2008 and 2009, respectively from 106 different greenhouses; one to three samples from each greenhouse.

Isolations from roots and shoot bases were carried out in 2.5% potato dextrose agar (PDA) after surface sterilization for 60s using 1% sodium hypochlorite. Emerging fungi were subcultured to new plates and purified using mycelial tips or single spore culture. This was followed by preservation in 2.5% PDA (for fungi) or 1.7% corn meal agar (CMA, for *Pythium* spp.) in slants at 20 °C for later use.

2.3. Identification

Preliminary identification of the isolated fungi and oomycetes was based on morphological characteristics (van der Plaats-Niterink, 1981; Barnett and Hunter, 1998; Leslie and Summerell, 2006). The identity of 40 *P. aphanidermatum*, 17 *P. spinosum*, 2 *F. solani*, 3 *Fusarium equiseti* isolates and one isolate each of *Trichoderma* and *Bionectria* was further confirmed using sequences of the internal transcribed spacers of the ribosomal DNA (ITS rDNA).

Identification based on sequences of the ITS rDNA was carried out following a modified protocol of Lee and Taylor (1990) as described by Al-Sa'di et al. (2007). Mycelium was harvested from 3 to 5-day old cultures grown on 2.5% PDA followed by freeze drying and addition of about 600 µl of lysis buffer (50 mM Tris–HCl, 50 mM EDTA, 3% SDS, 1% 2-mercaptoethanol) to about 50 mg of the ground

Table 1
Characteristics of greenhouses used in the study.

Year	District	Farm code	GH[a]	Cucumber variety	Season	Pre-transplanting treatment[b]	Post-transplanting treatment[b]
2001	Barka	B34-4	F	Delah	Fall	X	X
2001	Barka	B34-1	B	Delah	Fall	X	X
2002	Barka	B25-3	C	Salalah	Winter	0	0
2002	Barka	B06-2	D	Salalah	Spring	M	TM (2, 47)
2002	Barka	B25-2	E	Luna	Summer	0	0
2002	Barka	B34-3	G	Delah	Winter	X	X
2002	Barka	B06-1	J	Luna	Fall	0	PH (1), M (15, 18, 21), TM (35, 43, 48)
2002	Barka	B46-2	M	Salalah	Summer	M	M (2), PH (2), TM (18), H (54)
2002	Barka	B34-2	O	Delah	Winter	X	X
2002	Barka	B25-1	Q	Luna	Summer	0	PH (X)
2002	Barka	B15-1	R	Sondus	Fall	S	M (3)
2002	Barka	B50-1	U	Hana	Summer	PH	H (1), M (40)
2002	Shinas	S01-1	N	Hana	Fall	0	M (17)
2002	Khaboura	K01-1	S	Rawn	Winter	0	0
2002	Suwaiq	W03-1	V	Printo	Fall	0	0
2002	Shinas	S01-2	W	Hana	Winter	0	0
2003	Barka	B06-3	P	Printo	Spring	0	TM (1,5), PH (8)
2003	Barka	B15-2	T	Sindbad	Spring	M	M (24, 29, 36), MZ (31)
2003	Barka	B50-2	H	Salalah	Spring	0	PH (2, 18, 30)
2003	Barka	B46-3	I	Luna	Summer	M	M (11, 42)
2003	Suwaiq	W03-2	A	Salalah	Summer	S	0
2003	Suwaiq	W03-3	K	Muscat	Winter	0	0
2003	Musanaa	M03-1	L	Eskandaria	Spring	0	TM (20), M (28, 72)
2003	Khaboura	K01-2	X	Rawn	Spring	0	PH (5)

[a] Greenhouse code.
[b] The letters indicate the treatment (fungicide or solarization) used as follows: M = Metalaxyl, S = Solarization, PH = Propamocarb hydrochloride, TM = Thiophanate methyl, H = Hymexazol, MZ = Mancozeb, O = No treatment applied, X = No data available. Numbers in brackets indicate time (days after transplanting) after which application of fungicides was made.