Greenhouse Cucumber Cultivars Differ in Susceptibility to Fusarium Root and Stem Rot

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SUMMARY. Eighteen cucumber (*Cucumis sativus* L.) cultivars (long English type) were screened for their susceptibility to fusarium root and stem rot caused by *Fusarium oxysporum* Schlecht.: Fr. f.sp. *radicis-cucumerinum* D.J. Vakalounakis using seedlings at the third true-leaf stage. Roots were trimmed and dipped into a spore suspension (10^5 spores/mL) of the pathogen and the plants were re-potted. A disease severity index (DSI) was used to assess disease responses 4 or 8 weeks later based on plant mortality and the height of surviving plants compared to the noninoculated controls. 'Sienna', 'Amazing' and 'Dominica' were most susceptible to infection and the resulting DSI values were significantly ($P \leq 0.05$) higher compared to noninoculated control plants. The cultivars 'Korinda', 'Euphoria' and 'Aviance' displayed significantly lower DSI values which were not significantly different from noninoculated control plants. The remaining 12 cultivars displayed DSI values which were intermediate between the above two classes of responses. The results from this study indicate there is the potential to identify and develop cultivars and breeding lines of greenhouse cucumbers with enhanced resistance to fusarium root and stem rot.

Fusarium root and stem rot (FRSR), caused by the fungal pathogen *Fusarium oxysporum*

f.sp. *radicis-cucumerinum* (FORC), has been observed on cucumbers in commercial greenhouses in British Columbia, Canada since 1995 (Punja and Parker, 2000). The pathogen was first reported in Greece and the Netherlands (Vakalounakis, 1996). The disease has become a recurrent problem for growers throughout many regions of Canada and it has been recently reported from other parts of the world, including Israel, France, and the U.S. (Z.K. Punja, unpublished). The host range also includes muskmelon (*Cucumis melo* L. var. *cantalupensis* Naud.) and a few other cucurbits, such as pumpkin and squash (*Cucurbita pepo* L.) and watermelon (*Citrullus vulgaris* Eckl. and Zeyh.), when inoculated under experimental conditions (Punja and Parker, 2000). Presently, there are no fungicides registered for use against FRSR, and disease control methods include primarily sanitary measures to prevent pathogen spread and survival (Punja and Parker, 2000). While resistance to fusarium wilt of cucumber, caused by a related pathogen, *F. oxysporum* f.sp. *cucumerinum* J.H. Owen, is routinely incorporated into cucumber breeding efforts, cultivars with genetic resistance to FORC have not been identified. Pavlou et al. (2002) demonstrated resistance to this pathogen in rootstocks of *Cucurbita* L. species, including malabar gourd (*C. ficifolia* Bouche), winter squash [*C. moschata* (Duchesne) Duchesne ex Poir. and *C. maxima* Duchesne x *C. moschata*]. The identification of disease-resistant horticulturally acceptable cucumber cultivars or germplasm material would be of benefit to greenhouse growers and this has not yet been accomplished. The objective of this study was to screen a range of currently available cucumber cultivars to determine whether differences in susceptibility to FRSR could be identified.

Materials and methods

INOCULUM PREPARATION. Isolates of FORC were recovered from the crown and roots of diseased cucumber plants in several British Columbia greenhouses. Tissues were surface-sterilized in 10% Javex (containing 4.5% sodium hypochlorite) and plated onto *Fusarium oxysporum* selective agar medium (Komada, 1975). Established colonies were transferred to acidified potato dextrose agar (containing 1 mL·L⁻¹ of 90% lactic acid) (EM Science, Gibbstown, N.J.).

After 2 weeks of incubation at 23 to 25 °C (73.4 to 77.0 °F), colonies were flooded with 10 to 15 mL (0.3 to 0.5 fl oz) of sterile distilled water and conidia were suspended by rubbing the surface of colonies with a glass rod. The resulting suspension was filtered through a double layer of cheesecloth to separate conidia from mycelium. The concentration of conidia in the suspension was determined using a haemocytometer and diluted to the appropriate inoculum concentration for the experiments described below.

GROWTH ROOM TRIALS. A total of 18 cucumber cultivars (all long English type) were obtained from seed companies and propagators (Table 1). The experiments were initially conducted in a growth room [temperature range of 20 to 23 °C (68.0 to 73.4 °F)] with a 24-h photoperiod provided by 400 W high-pressure sodium lamps (light intensity of $100 \pm 15 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at canopy level). Seeds were planted into Sunshine Mix #1 (Sun Gro Horticulture Inc., Bellevue, Wash.) [one seed per 4-cm² (0.62-inch²) pot] and seedlings were grown for 2 weeks. At the third true-leaf stage, plants were gently uprooted, the roots were carefully washed in tap water, and trimmed using scissors [≈ 2.5 cm (1 inch) was removed]. The plants were dipped in a spore suspension (10^5 spores/mL) for 3 min and replanted into fresh potting medium and grown for an additional 8 weeks. Control plants of each cultivar were wounded as above and dipped in water. The number of dead plants and plant height were recorded at 4 and 8 weeks after inoculation from five replicate plants of each cultivar. A disease severity index (DSI) was used to quantify the cultivar responses to inoculation. The DSI was calculated from [(percent dead or diseased plants (with symptoms of wilting and crown lesions) at 4 weeks following inoculation)/14] + [(percent dead or diseased plants at 8 weeks following inoculation)/28] + [1 - (height of surviving plants/height of control plants)]. The trial was conducted once.

GREENHOUSE TRIALS. Cultivar evaluations were repeated in a research greenhouse located at the British Columbia Ministry of Agriculture, Fisheries and Food in Abbotsford, B.C., during August to November 2002 using the same potting medium and inoculation procedures as described above. Plants were randomly arranged on a bench. The number of diseased plants and plant height were recorded at 2 and 4 weeks

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following inoculation and a DSI was calculated as above. The range of ambient temperatures during the experiment was 23 to 28 °C (73.4 to 82.4 °F). Each cultivar had five replicate plants in each of two separate experimental trials that were conducted over 4 months.

DATA ANALYSIS. The DSI values from each of the three trials were averaged and the mean value and ranges were used to rank the relative susceptibility of each cultivar to FRSR. The three most susceptible or resistant cultivars (and noninoculated controls) were compared using one-way analysis of variance, and mean DSI values were separated using Tukey Kramer's honestly significant difference test at $P \leq 0.05$. The test of significance was done using JMP IN 4 (SAS Institute Inc., Cary, N.C.) software.

Results and discussion

Several cucumber cultivars consistently displayed no symptoms due to *FORC* under both growth room and greenhouse conditions in each of three trials (Fig. 1). The commercial cultivars 'Euphoria' (Rijk Zwaan, De Lier, The Netherlands) and 'Korinda' (Nunhems Zaden, Haalen, The Netherlands) had DSIs of -0.05 and 0.04, respectively,

averaged over three trials, and did not display root and stem rot symptoms in any of the trials (Table 1). The DSI values of these two cultivars were not significantly different ($P \leq 0.05$) from the noninoculated control plants (DSI = 0). 'Sienna' (Nunhems Zaden), 'Bodega' (Rijk Zwaan), and 'Mystica' (Rijk Zwaan), which are widely-grown in British Columbia, were all highly susceptible to root and stem rot, showing symptoms of plant collapse, wilting, or stunting in all three trials. The DSI values of these cultivars were significantly higher than DSI values of 'Korinda', 'Euphoria', and noninoculated control plants (Table 1). The remaining cultivars evaluated displayed degrees of susceptibility that ranged between the highly susceptible and resistant cultivars identified above (Table 1). Since cucumber plants were previously shown to be most susceptible to infection by *FORC* during the first 30 d after seeding (Punja and Parker, 2000), the trials reported here were conducted for 4 weeks (the growth room assay was conducted for an additional 4 weeks since the growth rate of plants was considerably slower compared to the greenhouse trials). However, these cultivars should be

evaluated to the mature plant stage under conditions of fruit set and to harvestable yield (16 weeks). These physiological stresses can exacerbate *Fusarium* development, especially under conditions of high temperature (Punja and Parker, 2000).

The prevalence of FRSR in British Columbia has likely been enhanced by the commercial use of desirable, yet highly susceptible, cultivars by growers. These cultivars display high fruit yield, good fruit quality, early production, and consistent production in a given area. In many cases, resistance to powdery mildew (*Sphaerotheca fuliginea*) is present in these cultivars. For example, none of the most susceptible cultivars identified in this study ('Sienna', 'Amazing', 'Dominica', 'Bodega') showed symptoms due to powdery mildew, whereas the two most resistant cultivars ('Korinda' and 'Euphoria') succumbed to powdery mildew infection from natural inoculum. Both diseases are prevalent in British Columbia greenhouses and therefore cucumber cultivars should carry resistance to both pathogens.

The method of inoculation used in this study provided consistent disease responses for most cultivars, indicating it

Table 1. Response of greenhouse cucumber cultivars long English type to root inoculation with *Fusarium oxysporum* f.sp. *radicis-cucumerinum*. Cultivars are ranked from most resistant to most susceptible.

Cultivar name ^z	Seed company ^y	Avg DSI ^x	Range of DSI values ^w	Cultivar resistance rank ^v
Euphoria	Rijk Zwaan	-0.05	-0.25-0.08	1 a
Korinda	Nunhems	0.04	-0.30-0.26	2 a
Aviance	Rijk Zwaan	0.28	-0.15-0.94	3 a
Tiffany	Daehnfeldt	0.71	-0.13-2.19	4
Europa	Rijk Zwaan	0.76	0.14-1.37	5
Logica	De Ruiter	1.17	0.66-1.73	6
Nicola	Nunhems	1.28	0.04-2.77	7
Millie	Bruinsma	1.73	0.47-3.15	8
Sabrina	Nunhems	1.77	1.17-2.31	9
Flamingo	Nunhems	2.03	1.36-2.49	10
Enigma	Rijk Zwaan	2.34	0.96-4.33	11
Naomi	Daehnfeldt	2.49	1.69-4.04	12
Ladner	Rijk Zwaan	2.83	0.19-5.79	13
Mystica	Rijk Zwaan	3.00	2.38-3.44	14
Bodega	Rijk Zwaan	3.38	2.36-4.57	15
Dominica	De Ruiter	3.56	1.73-6.00	16 b
Amazing	Daehnfeldt	3.99	1.75-6.00	17 b
Sienna	Nunhems	4.74	3.65-6.00	18 b

^zCultivars were evaluated in three trials, except for Europa two trials.

^yBruinsma = Bruinsma Seeds, Enkhuizen The Netherlands; Daehnfeldt = L. Daehnfeldt, Odense, Denmark; De Ruiter = De Ruiter Zaden, Bergschenhoek, The Netherlands; Nunhems = Nunhems Zaden, Haalen, The Netherlands; Rijk Zwaan = Rijk Zwaan, De Lier, The Netherlands.

^xDisease severity index DSI was calculated as: [(percent dead or diseased plants at 14 d following inoculation/14) + [(percent dead or diseased plants at 28 d following inoculation/28) + [1 - height of surviving plants/ height of control plants]]. Data represent the average of 3 trials, conducted in both growth room and greenhouse conditions.

^wThe range of DSI values from three trials is indicated in brackets.

^vCultivars are ranked from highly resistant ranking value of 1 to 4, DSI < 0.75, moderately susceptible 5 to 13 or susceptible ranking value of 14 to 18, DSI > 3.00. The highly resistant cultivars were not significantly different $P \leq 0.05$ from the noninoculated control DSI = 0. The highly susceptible cultivars were significantly different from the noninoculated control.

can be used in a more widespread screening of additional cultivars and breeding lines of greenhouse cucumber (long English type). Since the pathogenicity of isolates of *F. oxysporum* is known to be affected by prolonged growth on nutrient-rich media in the laboratory (Kim, 1997), re-isolations from diseased tissues were conducted frequently and fresh isolates used. However, several of the moderately susceptible cultivars (e.g., 'Ladner', 'Naomi', and 'Enigma') displayed varying degrees of relative susceptibility between trials (Table 1), suggesting there was some variation in response to inoculation. In other words, a few plants did not respond to the infection while others were diseased within the same trial. This resulted in a broader range of DSI values. The mechanism(s) by which seedlings of the cultivars that did not display symptoms in this study were able to withstand pathogen colonization of roots and prevent disease development is unknown and deserves further investigation. Unlike fusarium wilt of cucumber, FRSR is a relatively new and less well-recognized disease, and there are as yet no reports of this pathogen on field-grown cucumber or muskmelon. However, the disease has the potential to spread further and efforts to identify germplasm and incorporate disease resistance genes into greenhouse cucumber cultivars is recommended.

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Fig. 1. Fusarium root and stem rot development, caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum*, on greenhouse cucumber. (A) Lesion (arrow) on stem of 3-week-old seedling. (B) Lesion (arrow) and fungal sporulation at the base of 2-month-old plant. (C) Comparison of a moderately susceptible cultivar ('Flamingo', left) to a resistant cultivar ('Korinda', right) 28 d following inoculation. (D) Comparison of a susceptible cultivar ('Mystica', left), to a resistant cultivar ('Korinda', right) 28 d following inoculation.