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Short communication

Phytophthora nicotianae transmission and growth of potted kalanchoe in two recirculating subirrigation systems

Myung-Min Oh, Jung Eek Son *

Department of Plant Science and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea

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ABSTRACT

Recirculating subirrigation systems are frequently exposed to the risk of plant pathogens transmission, which may deteriorate the growth and quality of the plants. The transmission of *Phytophthora nicotianae* was examined using *Kalanchoe blossfeldiana* cv. New Alter in two recirculating subirrigation systems, a nutrient-flow wick culture (NFW) system and an ebb and flow (EBB) system. When the nutrient solution was infested, the pathogen was recovered from roots in both subirrigation systems. However, foliar blights and browning of roots appeared 4 and 7 weeks, respectively, after inoculation in the EBB system. Only a little discoloration appeared in the NFW system. The fresh and dry weights were lower in the EBB system than in the NFW system. When growing medium was inoculated, the pathogen was unable to be isolated from the plants in the NFW system. However, disease symptoms appeared in the EBB system 4 weeks after inoculation, and the pathogen was observed in the basal leaves and roots. Similar to the infested nutrient solution, the plant growth in the EBB system was inhibited. These results suggested that when the nutrient solution was infested, pathogen transmission could occur in plants in both systems, although differences existed with regard to disease symptoms and the time it took for symptoms to appear. However, we observed that when growing medium was inoculated the pathogen was not transmitted to adjacent plants in the NFW system using wick.

1. Introduction

Previous researches have well established that pathogens such as *Phytophthora* and *Pythium* threaten plants in recirculating irrigation systems (Bates and Stanghellin, 1984; Hoitink et al., 1992; Thinggaard and Andersen, 1995). In particular, *Phytophthora nicotianae* is a fungal pathogen known to be destructive to several commonly grown bedding plants, including kalanchoe (*Kalanchoe blossfeldiana*), because it produces zoospores that are motile in water (Han et al., 2001; Stanghellini and Rasmussen, 1994). In order to reduce possible damage from plant pathogens, a number of factors need to be controlled carefully. For instance, low irrigation frequency and high nutrient concentration inhibit the Phytophthora root rot of potted *Gerbera* (Thinggaard and Andersen, 1995). In addition, biosurfactants as a biological control of pathogens induce zoospore lysis (Stanghellini and Miller, 1997). Pathogens also spread faster in inoculated or infested nutrient solution than they do in soil and plants (Van der Gaag et al., 2001) due to increased motility.

The possible transmission of plant pathogens may hinder the adoption of the recirculating systems for practical use (Sanogo and Moorman, 1993). In an ebb and flow (EBB) system, which is representative of the recirculating system, the root disease caused by *Phytophthora* spp. is a common and serious problem (Stanghellini et al., 2000). Pathogen propagules may leach into irrigated water from infested pots and thus may act as a source of inoculum to other pots. However, in a capillary mat culture system, the irrigated mats typically reduce the transmission of propagules by acting as a filter (Van der Gaag et al., 2001), thereby reducing the pathogen infestation.

A nutrient-flow wick culture (NFW) system can supply nutrient solution in inclined troughs with specific irrigation time and frequency, allowing the water and nutrient to enter the pots via the capillary action of a wick (Son et al., 2006). It is possible that, like a mat filter, the NFW system can inhibit pathogen transmission. The objective of this study was to examine the role of subirrigation systems in the transmission of *P. nicotianae* and the subsequent plant growth of kanlanchoe.

* Corresponding author. Tel.: +82 2 880 4564; fax: +82 2 873 2056.
E-mail address: sjeenv@snu.ac.kr (J.E. Son).

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2. Materials and methods

2.1. Plant species and pathogen

Cuttings of *K. blossfeldiana* (cv. New Alter) grown in a venlo-type greenhouse in Seoul National University, Korea were planted in 6 cm diameter pots that contained a 7:3 mixture of peat moss and perlite (v/v). After rooting, young plants were subjected to short days (9/15 h, day/night). The nutrient solution of 11.7N–1.5P–5.5K, based on Sonneveld solution (Sonneveld, 1989), was maintained at 1.6 dS m^{-1} and pH 6.5 by adjusting it every 3 days with 0.1 M H_2SO_4 and 0.1 M NaOH. *P. nicotianae* isolated from the kalanchoe plants was used for producing the inoculum.

2.2. Subirrigation systems

The troughs in the NFW system were held at a 1% slope, and the nutrient solution was made available to the plants through a wick. A wick (cotton 90%, nylon 10%), 12 cm × 1.5 cm ($L \times W$) was inserted 3 cm into the pot, while maintaining a space of 3 cm between the bottom of the pot and the water surface. In the EBB system, water level was maintained at 1.5 cm when flooded. The nutrient solution was applied twice a day for 15 min each in the NFW system and once a day for 10 min in the EBB system. Irrigation time and water level in the EBB system were comparable to those used in previous reports (James and van Iersel, 2001; Morvant et al., 1998; Oh et al., 2007).

2.3. Effect of wick on pathogen transmission via nutrient solution (Experiment I)

Eighteen plants grown for 9 weeks after short-day exposure in the greenhouse were transferred to a growth chamber (DS-54GLPC-O, Dasol Co., Korea) with 25 °C, 9/15 h (day/night), and 240 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) on May 2, 2003. Three troughs, 20 cm × 8 cm × 3 cm ($L \times W \times H$), containing three pots each, were used as the NFW and EBB systems. Zoospores of *Phytophthora* were produced by growing the isolates in V8 juice agar (Mitchell and Kannwischer-Mitchell, 1992) for 5 days. After 10 days of plant transfer to the growth chamber, a zoospore suspension was poured into the nutrient solution reservoir, 21 cm × 12 cm × 7 cm ($L \times W \times H$), 20 min before irrigation. The inoculum level of zoospore suspension was 1×10^4 mL^{-1} (Raftoyannis and Dick, 2002).

Fresh and dry weights for shoots and roots were measured and disease symptoms were observed 7 weeks after inoculation. The disease symptoms were ranked using the following scale: 1, healthy; 2, some blight on leaf or stem; 3, blight and some necrosis or wilting; 4, dead. The causal fungus was isolated from roots and was cultured using water agar and a semi-selective medium for *Phytophthora* (Jee et al., 1997). The roots of each potted plant were cut into small pieces and were placed on the media and incubated for 2–3 days at 25 °C. The growing mycelial tips were isolated from the pieces and transferred onto corn meal agar (CMA; DIFCO, Detroit, USA, 17 g L^{-1}).

2.4. Effect of wick on pathogen transmission via infested root media (Experiment II)

The experiment was conducted in the greenhouse from May 16 to July 16, 2003. The cuttings, 2 weeks after rooting, were transferred to the NFW and EBB systems and subjected to the short days (9/15 h, day/night). All conditions with regard to root medium, irrigation, and nutrients were the same as those in the first experiment. Average daily air temperature and cumulative irradiation were 27.1 °C and 6.9 mol m^{-2} day^{-1} PPFD inside the greenhouse during the experiment, respectively. The NFW system contained three troughs, 60 cm × 8 cm × 3 cm ($L \times W \times H$), each with five pots placed at a spacing of 12 cm × 12 cm (between-row × within-row). Nutrient solution was recycled to the troughs from a single tank. In the EBB system, a 50-hole tray, 70 cm × 43 cm × 9 cm ($L \times W \times H$), containing 23 pots, was placed at a spacing of 6 cm × 12 cm (between-row × within-row). After 10 days of growth, two pots per treatment were inoculated using 10 mL zoospore suspension (1×10^4 mL^{-1}) in each system (Fig. 1). Disease symptoms were periodically observed and pathogens were isolated from the roots from the infested pots and pots nearest and farthest from the infested pots 7 weeks after inoculation. Dry and fresh weights of the plants were also measured.

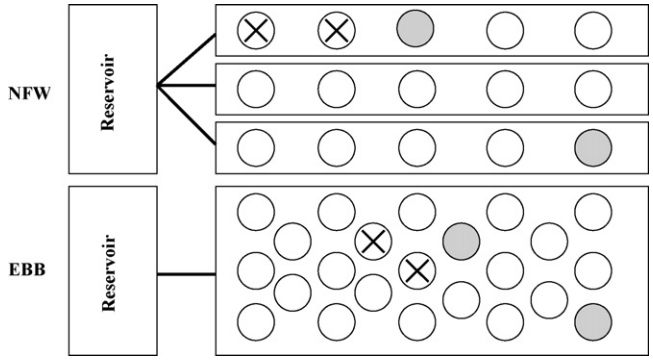

Fig. 1. Schematic diagrams of nutrient-flow wick culture (NFW) and ebb and flow (EBB) systems used for evaluating the effect of wick on the transmission of *Phytophthora nicotianae* via infested root medium. X and closed circles represent the location of infested pots and sampling at the end of the experiment, respectively.

2.5. Experimental design and data analysis

A completely randomized experimental design was used. Each treatment was replicated three times. Analysis of variance (ANOVA) was performed using statistical analysis system (SAS). Duncan's multiple range test (DMRT) was used to compare the means.

3. Results and discussion

3.1. Effect of wick on pathogen transmission via nutrient solution (Experiment I)

In the EBB system, kalanchoe plants started to show foliar blight symptoms after 4 weeks of inoculation, and six plants were observed to have some blight after 7 weeks of inoculation, while there were no visible disease symptoms in the NFW system (Table 1). After 7 weeks of inoculation, *P. nicotianae* was recovered from roots in both systems (Table 1). The root color was markedly

Table 1
Disease symptom rating of kalanchoe plants and isolation of *Phytophthora nicotianae* from infected roots in nutrient-flow wick culture (NFW) and ebb and flow (EBB) systems 7 weeks after inoculating the nutrient solution with the pathogen

Irrigation system	Disease symptom rating[a]	Isolation of *P. nicotianae*
NFW	1.00b[b]	Isolated
EBB	1.67a	Isolated

Values are the means of data from 18 pots.
[a] Disease symptom rating: 1, healthy; 2, some blight on leaf or stem; 3, blight and some necrosis or wilting; 4, dead.
[b] Mean separation within a column by Duncan's multiple range test at $P = 0.01$.

Fig. 2. Degrees of root browning of kalanchoe plants grown in nutrient-flow wick culture (NFW) and ebb and flow (EBB) systems 7 weeks after inoculating the nutrient solution with *Phytophthora nicotianae*.

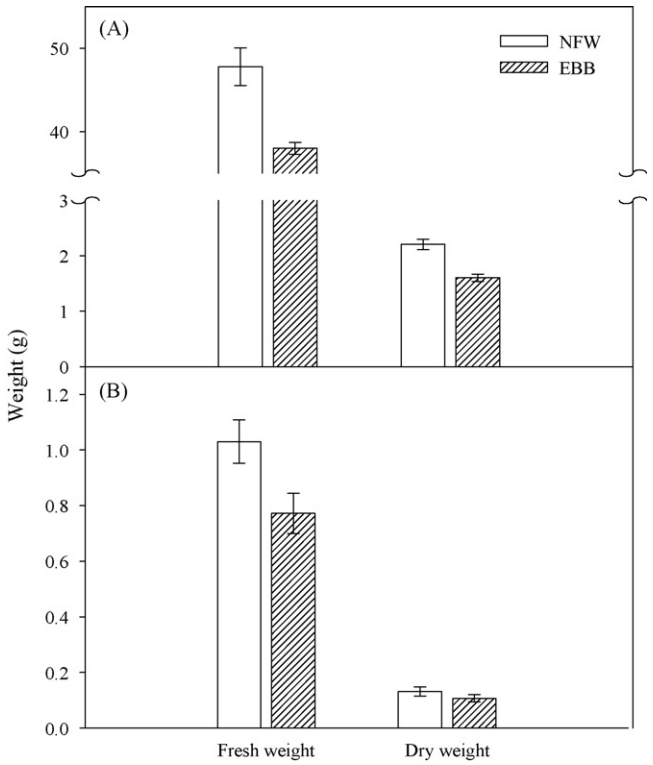

Fig. 3. Shoot (A) and root (B) growth of kalanchoe plants grown in nutrient-flow wick culture (NFW) and ebb and flow (EBB) systems 7 weeks after inoculating the nutrient solution with *Phytophthora nicotianae*. Vertical bars represent standard errors of means from three replications.

darker in the EBB system compared to that in the NFW system (Fig. 2). This indicates that *P. nicotianae* had attacked the roots in both irrigation systems, causing the weakening and browning of the root, which is a typical disease symptom of *P. nicotianae* as reported by Han et al. (2001). However, the appearance of disease symptoms and the root browning imply that wicks have some effects on the inhibition of *P. nicotianae* transmission, although it is important to note that the wicks could not completely protect the pot plants from the pathogen.

Raftoyannis and Dick (2002) reported that plant roots infested with a high inoculation level of *Phytophthora* and *Pythium* showed more of a dark discoloration and the disease severity was negatively related to plant age. In our preliminary study, the disease symptoms of leaves in the EBB system were not as severe in fully grown plants as in the younger plants (data not shown). Regardless of the subirrigation system, the disease symptoms were likely to be dependent on the plant age.

The fresh and dry weights were lower in the EBB system than in the NFW system (Fig. 3). In an evaluation of bedding plants for resistance to *Phytophthora*, Thomas and Marcia (2000) found the differences in growth rates between inoculated and non-inoculated plants although disease symptoms were not noted in these plants. In addition, it should be noted that the use of two different subirrigation systems is not a cause for this growth difference in our experiment because observations from our previous studies (Oh et al., 2007; Son et al., 2006) showed that EBB system typically produced better plant growth. Thus, the decreased biomass accumulation of plants in EBB system is perhaps due to the adverse effects by the pathogen.

3.2. Effect of wick on pathogen transmission via infested root medium (Experiment II)

After 4 weeks of inoculation, the plants located 6 cm away from the infested pots showed the disease symptom on a basal leaf in the EBB system. *P. nicotianae* was isolated from the basal leaf showing disease symptoms. Also, *P. nicotianae* was found in the primary roots, regardless of the location of inoculation at the end of the experiment. These results mean that *P. nicotianae* inoculated to one pot can be easily transferred to another pot via nutrient solution in the EBB system. Particularly, when the water level rises in the EBB bed during irrigation, the growing medium, which contains pathogens, may actually contaminate the nutrient stock solution. Although in previous studies, EBB system has been shown not to inhibit the transmission of pathogens like *Phytophthora* and *Pythium*, the actual infection level may vary depending on the combination of pathogen and plant species (Van der Gaag et al., 2001; Strong et al., 1997).

In the NFW system, except for infested pots no disease symptoms were detected in kalanchoe plants and the pathogen was not recovered from the roots, regardless of location at the end of the experiment. When mats were used in the subirrigation system, they inhibited the release of inoculum from pots and reduced the infective propagules moving into the pots from nutrient solution (Van der Gaag et al., 2001). The wicks used in this experiment played an important role in preventing the movement of zoospores from inoculated pots to the nutrient solution or the other pots: propagules might have a more difficult time moving downward along the wick to the nutrient solution rather than via the mats. Even if propagules could move throughout the wicks to some extent, they are less likely to survive on the wick as the wicks became completely dried after 60–90 min of irrigation in the NFW system.

Another cause of restricted pathogen transmission in the NFW system may be due to the soil conditions. Drier soil conditions might be helpful in reducing pathogen activity in the growing medium. In our study, the water content in the NFW system ranged from 20% to 30%, whereas growing medium in the EBB system had water content between 45% and 50%. Kuan and Erwin (1980) reported that saturated soil water conditions can induce severe root rot on alfalfa seedlings compared to unsaturated soil. Furthermore, with Gerbera root rot, low watering frequency made the infested-medium drier than did high watering frequency. Such a medium was unfavorable for the survival of the pathogen (Thinggaard and Andersen, 1995).

Table 2
Fresh and dry weights of kalanchoe plants grown in nutrient-flow wick culture (NFW) and ebb and flow (EBB) systems 7 weeks after inoculating the growing media of pots with *Phytophthora nicotianae*

Irrigation system	Treatment	Fresh weight (g)		Dry weight (g)	
		Shoot	Root	Shoot	Root
NFW	Inoculated	26.19b[a]	0.88b	1.51a	0.17a
	Not	28.88ab	1.01ab	1.84a	0.18a
EBB	Inoculated	13.93c	0.45c	0.94b	0.11b
	Not	34.11a	1.18a	1.85a	0.21a
Significance		***	***	***	**

Values are the means of data from 24 pots. Asterisks (**, ***) represents significant values at $P = 0.01$ and 0.001, respectively.
[a] Mean separation within columns by Duncan's multiple range test.

The plant growth significantly differed between the NFW and the EBB systems with pathogen inoculation. After 7 weeks of inoculation, the growth characteristics in the EBB system were much less than that in the NFW system (Table 2). Fresh weights of roots and shoots in the EBB system were about 50% of those in the NFW system. Furthermore, roots in the EBB system were short with fewer tertiary roots compared to those in the NFW system with pathogen inoculation. However, all kalanchoe plants grown in non-inoculated NFW and EBB systems showed normal growth. Thus, this result implies that the plant growth in the EBB system may be inhibited due to pathogen transmission through the nutrient solution.

In conclusion, the transmission patterns of *P. nicotianae* in two subirrigation systems were different which affected the plant infection and plant growth. Regardless whether the medium or the nutrient solution is inoculated, NFW system retarded the transmission of this pathogen compared to EBB system, resulting thereby in less infection and better plant growth.

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