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Epidemiology of Fusarium wilt in cyclamen in an ebb and flow system

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Abstract

Introduction of *Fusarium oxysporum* f. sp. *cyclaminis* either into the nutrient solution or into the potting soil, did not result in noticeable spread of disease in a cyclamen crop grown in pots on tables of an ebb and flow system. Immediately after its introduction, only a small amount of the inoculum was detected in the soil in the pots and in the nutrient solution flowing onto the tables.

Study of the epidemiology of the disease, after introduction of the pathogen into the system, revealed a mechanical process. Within 24 hours the amount of cfu found in the out-flowing nutrient solution and halfway down the container was sharply reduced. After 5-7 days few cfu could be detected. On the contrary on the bottom of the containers the fungus was observed in increasing amounts. In the system used the spores of the fungus settled down and were not transported during floods.

Introduction

During the last decade, a new irrigation system, known as the ebb and flow system, has been developed for potplant growing in the Netherlands. The nutrient solution in this system is pumped up from a storage basin into a watertight table on which the plants are placed (Fig. 1). The frequency of flooding depends on the water and nutrient requirements of the cultured crop and varies from 2-3 times a day to once in 2-3 days. The length of time of flooding is 4-5 minutes. Thereafter the nutrient solution flows back into the storage basin and the excess solution drains out of the pots.

Since the nutrient solution is re-used, dissemination of pathogen propagules introduced into the nutrient solution would be expected to occur, as was demonstrated for other systems in which a nutrient solution is recirculated (Rattink, 1983).

Previously it was shown for an ebb and flow system that inoculum of *F. oxysporum* f.sp. *cyclaminis* introduced into the nutrient solution in the storage basin caused a few diseased plants. Only after very large amounts of inoculum were added a few diseased cyclamen plants were found. Spread of the disease from diseased plants, placed among healthy plants on the table, did not occur (Rattink, 1989). Addition of propagules of

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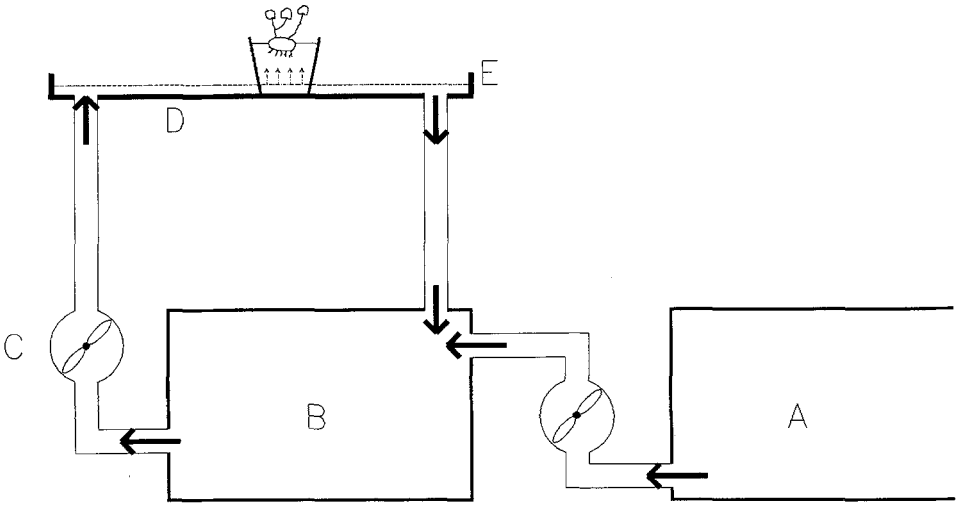

Fig. 1. Ebb and flow system. A) central storage basin; B) unit container; C) pump; D) water tight bench top; E) level during flooding.

Phytophthora either to the nutrient solution or to the soil in the pots, to *Gerbera*, *Peperomia* and *Saintpaulia* crops did not result either in disease (Rattink, unpublished data). In spite of this, viable propagules of *F. oxysporum* f.sp. *cyclaminis* were isolated from the nutrient solution. Long lasting viability and pathogenicity of *F. oxysporum* f.sp. *cyclaminis* after staying in nutrient solution at different temperatures has been proven before (Rattink, 1986).

To determine why Fusarium wilt did not develop in the cyclamen crop, in spite of the presence of the pathogen and its lasting viability and pathogenicity in the nutrient solution, a research programme on the epidemiology of the disease in the ebb and flow system itself was started in 1987.

Materials and methods

The research was performed on 4 separate ebb and flow units as illustrated in Fig. 1. The tables were flooded once each two days for a period of 5 minutes. Each day the small 70 l containers of the individual units were refilled from a large storage basin. Inoculum was prepared by growing *F. oxysporum* f.sp. *cyclaminis* on a shaker in a liquid Czapek-Dox medium during 10 days at 20 °C. Before inoculation the inoculum was adjusted to the required concentration of spores (microconidia) per ml.

Inoculation was done by adding a suspension of microconidia to the nutrient solution in the small containers or to the potting mix. The concentrations are given as number of spores per ml of nutrient solution. The potting mix was infested by adding 5 ml of a suspension of 10^6 microconidia per ml.

In each treatment 50 or 100 young cyclamen plants (pottable, transplanted twice) of the pastel-mix-group were used. They were potted in a sterilized (steamed) standard potting soil, one plant per pot.

The population of *F. oxysporum* f.sp. *cyclaminis* in the nutrient solution was deter-

mined by taking 5 ml samples at various locations in the system. These locations were at the inlet, the outlet, halfway down the container and at the bottom of the container. The population of *F. oxysporum* f.sp. *cyclaminis* in the potting soil was determined by making a suspension of the contents of a pot (1 : 5 or 1 : 10). After 2 minutes of sedimentation 5 ml samples were taken from the supernatant.

Dilution series of all samples were plated in quadruplicate on Komada-medium (Komada, 1975) and incubated at 23-25 °C for 6 days after which the number of cfu was determined.

Results

In none of the experiments diseased plants were found. In the first experiments after infestation of the nutrient solution dispersal of propagules to the tables was investigated. The number of cfu in the nutrient solution flooding the tables was determined 1, 2, 7 and 33 days after infestation with 0, 3.4×10^4, 3.4×10^5 or 7.9×10^6 microconidia per ml. The results are given in Table 1. Less than 25% of the original population was detected in the nutrient solution after the first flooding period, i.e. one day after inoculation of the nutrient solution. After 2 days less than 1.5 percent was recovered and after 33 days no propagules were detected.

Dispersal of the pathogen from the infected nutrient solution to the potting soil was investigated in the next experiment. At various times, viz. 0, 2, 4, 8, 10 and 16 days after introduction of the pathogen into the nutrient solution with 0, 7.8×10, 8.8×10^3 or 6.3×10^5 microconidia per ml, the number of cfu per gram of soil (dry wt) in the pots was determined (Table 2). Pathogen pupulations after the first flooding following infestation (i.e. at time 0), showed that relatively high numbers of *Fusarium* cfu were present in the potting mix at that time. However, from 2 days after inoculation and on, and regardless of the original level of the inoculum used, approximately the same low numbers of cfu were recovered from plants in all treatments. These data correspond with those found in an experiment where the potting mix was infested with various concentrations of microconidia (Table 3). Although large numbers of microconidia of *F. oxysporum* f.sp. *cyclaminis* were added to the potting mix only relatively low numbers were recovered from the nutrient solution applied to the plants or from the soil in the pots. Additional sampling revealed that propagules accumulated on the

Table 1. Population development of *F. oxysporum* f.sp. *cyclaminis* in the ebb and flow nutrient solution.

Concentration of inoculum (microcondidia ml^{-1}) added	Number of cfu ($\times 10^3$) recovered per ml of nutrient solution on the table at various days after infestation			
	1	2	7	33
0.0	0.0	0.0	0.0	0.0
3.4×10^4	3.7	0.5	1.5	0.0
3.4×10^5	81.0	3.4	0.3	0.0
7.9×10^6	120.0	96.0	4.0	0.0

Table 2. Population development of *F. oxysporum* f.sp. *cyclaminis* in the potting mix after infestation of the ebb and flow nutrient solution.

Concentration of inoculum (microconidia ml^{-1}) added	Number of cfu ($\times 10^3$) recovered per g of soil at various days after infestation					
	0	2	4	8	10	16
0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.5×10	9.9	1.7	6.3	0.01	0.9	1.2
8.8×10^3	22.2	1.0	0.6	0.0	0.1	0.6
6.3×10^5	1.1	2.9	3.9	0.0	0.8	4.8

Table 3. Population development of *F. oxysporum* f.sp. *cyclaminis* in the potting mix after infestation of the mix.

Concentration of inoculum (microconidia ml^{-1}) added	Number of cfu ($\times 10^3$) recovered per g of soil at various days after infestation		
	2	23	33
0.0	0.0	0.0	0.0
4.5×10^2	7.4	3.5	1.6
4.5×10^3	1.3	0.1	2.8
1.0×10^5	4.6	2.8	0.7

Table 4. Spatial distribution of propagules of *F. oxysporum* f.sp. *cyclaminis* in the container of an ebb and flow system after infestation of the nutrient solution.

Concentration of inoculum (microconidia ml^{-1}) added	Number of cfu ($\times 10^3$) at different days after inoculation							
	halfway down				bottom			
	0	2	7	14	0	2	7	14
47×10^3 (before 1st flooding)	4.1	3.3	0.05	0.0	—	16.9	23.3	33.3
47×10^3 (after 1st flooding)	—	3.5	0.1	0.05	—		15.8	13.8

bottom of the container, in a layer underneath the outlet, at levels up to 450 times the original inoculum density, especially if samples were taken before flooding.

The concentration of cfu in the nutrient solution halfway down and at the bottom of the containers after inoculation was checked several times. The samples were taken before flooding, which had a frequency of once every 2 days. The results are given in Table 4. Shortly after introduction many cfu of *F. oxysporum* f.sp. *cyclaminis* were found at the bottom of the containers, whereas very few were found in the nutrient solution halfway down the containers. Apparently the propagules sink down to the bot-

Table 5. Distribution of *F. oxysporum* f.sp. *cyclaminis* in a model of an ebb and flow container after sedimentation (S), mixing (M) or drainage at different periods after infestation at day X.

Day	Treatment	Number of cfu ($\times 10^3$) recovered		
		halfway down	bottom	drain
X	M	66	66	
	24^h S			
X + 1		16.5	87.5	
	M			
	24^h S			
X + 2		8.0	138.0	
	M			
	24^h S			
X + 3		4.0	23.5	18.5
	M	20.0	14.5	
	96^h S			
X + 7		0.0	22.0	

tom of the container in the periods between floodings. The results confirm the earlier findings.

In a specially constructed container with a funnel-shaped bottom the influence of the physical properties of the ebb and flow system on the distribution of *F. oxysporum* f.sp. *cyclaminis* was studied. After introduction the presence of the fungus halfway down and at the bottom of the container was determined. Samples were taken at different times after introduction before or after stirring the solution. At regular times the solution at the bottom of the container was drained to remove part of the deposited inoculum. The results are shown in Table 5. After stirring the infested solution, an even distribution of *Fusarium* was observed. However, after 24 h of sedimentation most of the *Fusarium* was settled at the bottom of the container. After a longer period of sedimentation, 7 days or more, all *Fusarium* had settled down. By drainage *Fusarium* could be removed from the containers.

Discussion

Although the nutrient solution in an ebb and flow system is recirculated, introduction of *F. oxysporum* f.sp. *cyclaminis* did not lead to a serious epidemic caused by Fusarium-wilt. This is contrary to earlier findings when *F. oxysporum* was introduced in other systems with a recirculating nutrient solution (Rattink, 1983). *F. oxysporum* f.sp. *cyclaminis* however, can survive and remain pathogenic for a year or more in a nutrient solution, as has been demonstrated before (Rattink, 1986). Therefor the mechanism underlying the lack of dispersal of the pathogen must be connected with the ebb and

flow system itself.

The data obtained in this work reveal that a mechanical process underlies the lack of disease transmission. When the nutrient solution in the container was not stirred, propagules settled down on the bottom of the container (Table 4 and Table 5). After stirring of the solution the propagules were dispersed in the nutrient solution. In the system used (Fig. 1) the outlet, from which the nutrient solution was pumped up to the tables, was located at some distance above the bottom of the container. When the solution was pumped up the layer below the outlet was not stirred, so no or few propagules of *Fusarium* were transported to the plants.

Although exact data on time of sedimentation of the propagules are lacking, the experiments show that most propagules had settled at the bottom of the containers within 24 h after stirring the solution in the containers. In cyclamen culture on an ebb and flow system, the frequency of flooding is every other day or at the most once every day. It provides ample opportunity for sedimentation.

The results from this and earlier research demonstrate that dissemination of fungi in systems in which the nutrient solution is re-used, depends largely on the technical characteristics of the systems itself. The physical mechanism, which takes place in an ebb and flow system and which is responsible for lack of dispersal of the pathogen through the system, may be used in other systems with recirculation of the nutrient solution. By collecting the back-flowing nutrient solution first in a sedimentation basin and storing it in this basin for 24 h or more before re-using and placing the outlet, from which the nutrient is pumped up again, at some distance above the bottom, it is possible te remove a high percentage of spores from the solution. It is even possible to catch away the sedimented spores by a drainage device.

Whether the found mechanical process also applies for spores of other fungi than *Fusarium* has to be investigated. Probably the process depends largely on the properties of the spores.

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Samenvatting

Epidemiologie van Fusarium verwelkingsziekte bij cyclamen in een eb-vloed-systeem

Bij cyclamenteelt op een eb-vloed-systeem werd, na introductie van *F. oxysporum* f.sp. *cyclaminis* via de voedingsoplossing of via de grond in de potten, vrijwel geen verspreiding van het pathogeen gevonden. In vrij korte tijd na de introductie werd in de uitstromende voedingsoplossing en in de potgrond slechts een zeer gering gedeelte van de oorspronkelijk geïntroduceerde hoeveelheid *Fusarium* teruggevonden.

Bij onderzoek omtrent de epidemiologie van de ziekte na introductie in het systeem waren op verschillende plaatsen na 24 uren slechts zeer geringe hoeveelheden en na 5-7

dagen vrijwel geen *Fusarium* aantoonbaar. Daarentegen werden op de bodem van de voorraadtanks in toenemende mate grote hoeveelheden *Fusarium* aangetroffen. In het gebruikte systeem bezonken de sporen en werden niet meegevoerd tijdens de vloed.

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