



# Reuse of untreated irrigation water as a vehicle of inoculum of pathogens in eucalyptus clonal nursery

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## ABSTRACT

The risk posed by reuse of untreated water for irrigation, collected from the effluents of mini-hedges, greenhouses, shade houses, growing and hardening areas of a eucalyptus cutting nursery, was evaluated in relation to the dissemination of *Botrytis cinerea* and *Cylindrocladium candelabrum*. The presence of inocula of these fungi was also evaluated on gravel used as soil cover. For pathogen detection, leaf discs of castor bean (*Ricinus communis*) were employed as biological bait. Periodical analyses of water samples, collected in the nursery effluents, and of gravel, showed that both pathogens are frequently disseminated in the water of the effluents and the gravel used as soil cover, although *C. candelabrum* is most common. The composition and salt concentration of three nutrient solutions, expressed in electric conductivity values (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mS.cm<sup>-1</sup>) did not affect conidial germination of these two fungi.

**Keywords:** hydropony, nursery diseases, floating, eucalyptus cuttings, nutrient solution.

## RESUMO

### Reaproveitamento de água de irrigação não tratada como fonte de inóculo de patógenos em viveiro clonal de eucalipto

Avaliou-se o risco do reaproveitamento de água não tratada, originária do efluente do minijardim clonal, da casa de vegetação, da casa de sombra, das áreas de crescimento e rustificação a céu aberto de um viveiro clonal de eucalipto, quanto ao potencial de disseminação de inóculo de *Botrytis cinerea* e *Cylindrocladium candelabrum*. Avaliou-se também a presença de inóculo desses fungos em brita usada como cobertura do piso das áreas do viveiro. Para a detecção dos patógenos, empregaram-se discos de folhas de mamoneira (*Ricinus communis*) como isca. Análises periódicas evidenciaram que ambos os patógenos são constantemente veiculados na água não tratada, proveniente das diferentes fases da propagação clonal e sobre a brita, sendo *C. candelabrum* mais frequentemente constatado. A composição e a concentração de sais de três soluções nutritivas, expressa em valores de condutividade elétrica (0, 0,5, 1,0, 1,5, 2,0, 2,5 e 3,0 mS.cm<sup>-1</sup>), não afetaram significativamente a germinação de conídios.

**Palavras-chave:** Hidroponia, inundação, doenças em viveiros, mudas de eucalipto, irrigação e solução nutritiva.

## INTRODUCTION

In Brazil, eucalyptus is currently multiplied mainly by the mini-cutting technique. The vegetative propagules, named mini-cuttings, are obtained from mini-stumps planted in mini-hedges normally established in trenches containing sand (sand beds) and drip ferti-irrigation or in floating systems. The mini-cuttings are planted in rooting substrate and kept in a greenhouse with intermittent mist irrigation for about 15 – 30 days, when they are transferred to a shade house (50% daylight intensity) for acclimation. Subsequently, the plants are grown and hardened in the open air, becoming ready for planting at about 90-120 days old (Alfenas *et al.*, 2004). Studies show that eucalyptus is one of the most efficient plant species in water uptake in the field (Novais *et al.*, 1996). However, during cutting production a relatively high amount of water is required and therefore efforts have to be made to optimize its rational use

and minimize the environmental impact in the nursery.

Minimization of water consumption can be effected by reusing irrigation water from the ferti-irrigation system in mini-hedges and other stages of cutting production. However, irrigation water (Newman, 2004) or water from closed hydroponic systems (Ehret *et al.*, 2001) is one of the most efficient vehicles for spreading plant pathogens. Contamination of nutrient solution in protected cultures can come from various sources, including from infested rainwater, from water tanks and growth substrate, as well as infected plant material (Stanghellini & Rasmussen, 1994; Menzies *et al.*, 1996).

Several root pathogens are easily spread in nutrient solution (Sanogo & Moorman, 1993; Stanghellini & Rasmussen, 1994; Menzies *et al.*, 1996; Toppe & Thinggaard, 1998; Ehret *et al.*, 2001). For example, a study with various inoculum densities of *Pythium aphanidermatum* has shown that the pathogen affected cucumber plant development in

hydroponic systems with recycled nutrient solution, since the fungus multiplied quickly in the nutrient solution, infecting all the plants as the water was re-used, independently of the inoculum concentration. As concentrations of the inoculum increased, so did the symptoms and mortality of the plants (Menzies *et al.*, 1996).

Production of eucalyptus cuttings or seedlings in nurseries is carried out under environmental conditions that favor multiplication and dissemination of pathogens (Alfenas *et al.* 2004). Among the nursery diseases, cutting rot and leaf blight caused by *Botrytis cinerea* Pers. and *Cylindrocladium* spp. are, currently, those that most frequently cause losses (Alfenas *et al.*, 2004). Considering the possibilities of re-using water in hydroponic systems or for mist irrigation, the present study aimed to evaluate the risk posed by recycled and untreated water as a vehicle for dissemination of these pathogens.

## MATERIAL AND METHODS

Collection of water and of soil cover material (gravel) was carried out in a forest nursery in Belo Oriente, MG (latitude 19°17'49" S; longitude 42°23'26" O; altitude 233m), between January and May of 2004.

### Calibration of the microbiological analysis of water

Before analyzing samples of water collected in the nursery, we adapted the biological bait method described for *Rhizoctonia* spp. (Sanfuentes *et al.*, 2002) and *Cylindrocladium* spp. (Gonçalves *et al.*, 2001) in soil and substrate samples. Calibration of the method used in the present study was made by preparing conidial suspensions at different concentrations of *B. cinerea* (1, 10, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> and 10<sup>6</sup> conidia/mL) and of *C. candelabrum* Viégas (1, 10, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup> and 10<sup>5</sup> conidia/mL), following evaluation by the bait method. Subsequently, 50mL samples of each inoculum suspension were applied in 25g of medium grade vermiculite, previously sterilized and placed in gerbox-type plastic boxes (13 x 13 x 4 cm). Thirty leaf discs (15 cm in diameter) of castor bean bait (*Ricinus communis*) previously disinfested in 50% ethanol solution/ 30 s and then in 5% NaClO/3 min, and finally washed twice consecutively in sterilized distilled water were inserted in the vermiculite, containing different suspensions of inoculum. After 48h incubation at 25°C in the dark, the baits were transferred to acidified PDA (pH = 5.0). After 48h and 96h of incubation in the same conditions, the percentage of colonized baits by each fungus was recorded. A completely randomized experimental design composed of 5 replicates for each inoculum concentration was employed.

### Microbiological analysis of irrigation water

Water samples were analyzed weekly in each of the sampled months, at 8 points in the nursery from the supply water tank and from the effluents of the ferti-irrigation systems. The samples corresponded to the irrigation systems from the clonal mini-hedges, rooting houses, shade houses,

three growth areas and one hardening area. Water samples of 1 L collected from each point were placed in a sterilized plastic container and transported under refrigeration for laboratory analysis. Each sample was divided into three 50 mL sub-samples, and analyzed by the bait method described elsewhere. The pH and electric conductivity (EC) of each sample were also determined.

### Microbiological analysis of soil cover material (gravel)

Samples of gravel were analyzed for presence of fungal inoculum using the same method as for water analysis. A sample of 1 kg of gravel was divided into 5 sub-samples; these were each placed in a beaker containing 150 mL of sterile water and then Vortex agitated for 3 min to allow inoculum release. The suspension obtained was divided into three aliquots of 50 mL and baited as previously described.

### Conidial germination in nutrient solution at different salt concentrations expressed by electric conductivity

The effect of salt concentration in the following three nutrient solutions, displaying different electric conductivity (EC), was tested. 1. *Growth area* (stock solution, quantities for 1000 L): iron sulfate 1 Kg; disodic EDTA 1.5 kg; boric acid 500 g; sodium molibidate 11 g; zinc sulfate 28 g; copper sulfate 50 g; manganese sulfate 222 g; ammonium sulfate 8.9 kg; phosphoric acid 3.3 L; potassium chloride 33.3 kg; magnesium sulfate 14.4 kg; and nitric calcium 27.8 L. 2. *Mini-hedge in floating system* (stock solution, quantities for 1000 L): iron sulfate 1.3 kg; disodic EDTA 2 kg; boric acid 311 g; sodium molibidate 11 g; zinc sulfate 28 g; copper sulfate 42 g; manganese sulfate 778 g; ammonium sulfate 27.8 kg; MAP 7.8 kg; potassium chloride 20 kg; magnesium sulfate 14 kg; and nitric calcium 83 l. 3. *Mini-hedge in sand beds* (stock solution, quantities for 1000 l): disodic EDTA 578 g; iron sulfate 389 g; boric acid 44 g; sodium molibidate 2 g; zinc sulfate 7 g; copper sulfate 7 g; nitric calcium 18.5 L; manganese sulfate 17 g; ammonium sulfate 7.4 kg; MAP 2.8 kg; potassium chloride 6.2 kg; magnesium sulfate 8 kg; and nitric acid 1.4 L. The stock solution was diluted to different concentrations with EC values of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mS.cm<sup>-1</sup>. An aliquot of 10 mL of the respective solution was added to colonies of each fungus and the conidial mass was scraped off with a soft hair brush. A 50 µL sample of each suspension was transferred to glass slides, containing wells. The slides were placed on glass stick supports inside a Gerbox, containing two sheets of moistened filter paper to favor spore germination. After incubating at 25°C for 20 h for *B. cinerea* and 6 h for *C. candelabrum*, a drop of lactofenol was added to stop spore germination. Thereafter, the number of germinated conidia was evaluated under a light microscope (200 x) by counting randomly 100 conidia per replicate. Conidia with germ tubes of the same size or longer than the conidium length were considered germinated. A completely randomized experimental design, with three replicates in a factorial scheme (three nutrient solutions x seven EC values) was used for each pathogen.

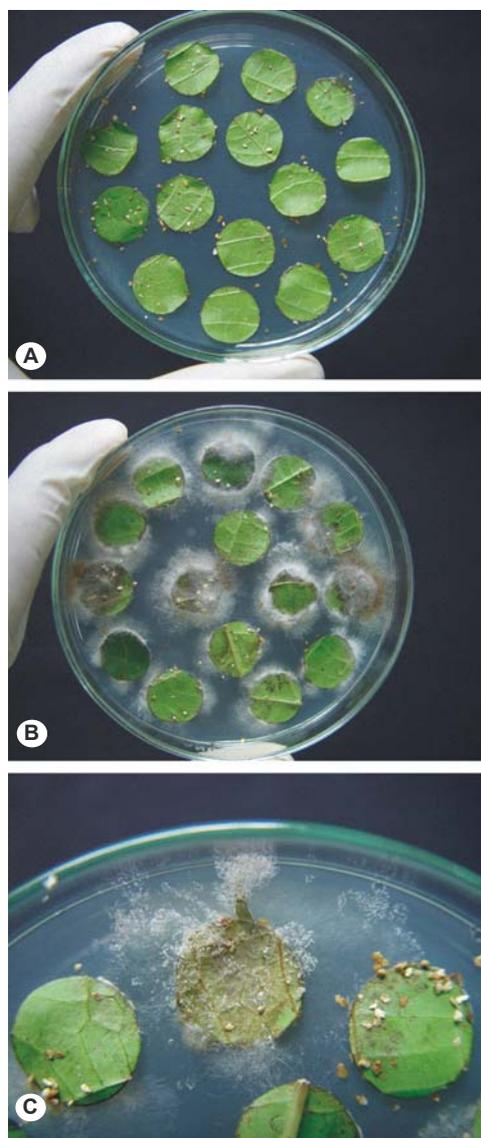
## RESULTS

### Calibration of microbiological analysis of water

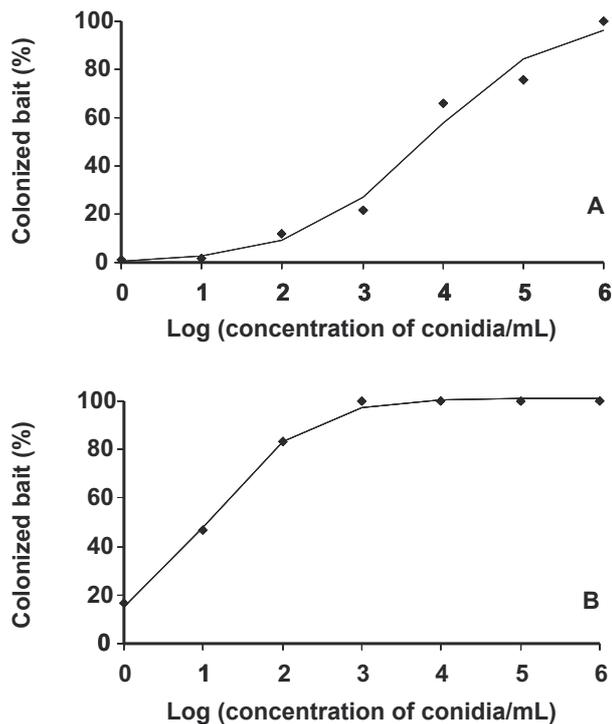
Colonies of *C. candelabrum* are easily differentiated from *B. cinerea* (Figure 1). Castor bean leaves were more efficient as bait for detection of *C. candelabrum* than for *B. cinerea*. A value of 100% of colonized baits by *C. candelabrum* and *B. cinerea* was observed at  $10^3$  and  $10^6$  spores/mL, respectively (Figure 2). Spore concentrations higher than these do not allow estimation of the inoculum concentration in water.

### Microbiological analysis of irrigation water

The periodical analysis of water showed that the two pathogens studied here are frequently carried in the various



**FIG. 1** – Biological bait (leaf discs) from castor bean; **A.** no colonization by pathogens (control); **B.** colonized by *Cylindrocladium candelabrum*; **C.** colonized by *Botrytis cinerea*.



**FIG. 2** – Percentage of castor bean leaf as bait (leaf discs) colonized by **A.** *Botrytis cinerea*,  $Y = 101,33/1+132,83 e^{-1,29 X}$  and **B.** *Cylindrocladium candelabrum*,  $Y = 101,14/1+5,74 e^{-1,64 X}$  in function of the inoculum concentration.

phases of clonal propagation of eucalyptus. In addition, *C. candelabrum* was found more often than *B. cinerea* (Table 1).

The presence of pathogenic inoculum varied according to the fungal species and time of sampling (Table 1). On average, the highest level of inoculum of both fungi was found in water from the floating mini-hedge system and from growth area 2. The greatest inoculum intensity of *C. candelabrum* and *B. cinerea* was found, respectively, in April and March, considering all irrigation systems evaluated. With the exception of the mini-hedge in sand bed and growth area 2, the inoculum level was higher in the effluent water than in the feedingwater (Table 1).

The pH values of the irrigation feedingwater were higher than those of the effluent in sand bed, floating, rooting house and shade house, but continued practically unchanged in other phases of clonal propagation. The highest reduction in pH was observed in the floating irrigation system, dropping on average from 7.08 to 3.82. The electric conductivity of the solutions as an indication of salt concentration was on average lower than  $1.53 \text{ mS}\cdot\text{cm}^{-1}$  (Table 2).

### Microbiological analysis of soil cover material (gravel)

The two pathogens were detected in soil cover material (gravel). Furthermore, greater intensity of the inoculum was found for *Cylindrocladium* in gravel from the rooting house, in one of the growth areas (number 3) and in

**TABLE 1** – Presence of *Cylindrocladium* (C) e *Botrytis* (B) inoculum in samples of water at different periods and from various cutting production phases, considering feedingwater and effluent water for irrigation over time

Place		Month												
		Jan		Feb		Mar		Apr		May		Average		
		C	B	C	B	C	B	C	B	C	B	C	B	C + B
Mini-clonal	Feedingwater	0.0	0.	0.0	2.2	1.1	13.3	0.0	3.9	2.2	0.0	0.7	3.9	4.6
hedge (sand bed)	Effluent	0.0	0.0	4.4	0.0	0.0	2.2	2.2	3.9	3.9	0.0	2.1	1.2	3.3
Mini-clonal	Feedingwater	0.0	0.0	18.9	0.0	1.7	5.0	19.4	2.8	0.0	0.0	8.0	1.6	2.4
hedge (floating)	Effluent	17.8	0.0	12.2	0.0	4.4	6.1	20.0	2.2	0.0	0.0	10.9	1.7	11.6
Rooting house	Feedingwater	0.0	0.0	0.0	0.0	1.1	0.0	1.1	1.7	1.1	0.0	0.7	0.3	1.0
	Effluent	0.0	16.4	1.1	0.0	0.6	2.2	1.1	3.3	2.2	0.0	1.0	4.4	5.4
Shade house	Feedingwater	0.0	0.0	1.1	0.0	0.0	6.1	0.0	3.9	1.1	0.0	0.4	2.0	2.4
	Effluent	0.0	0.0	0.0	1.1	1.1	7.2	18.8	10.0	0.0	0.0	4.0	3.7	7.7
Area of growth 1	Feedingwater	0.0	0.0	0.0	2.2	2.8	8.3	14.4	11.1	0.0	0.0	3.4	4.3	4.7
	Effluent	0.0	0.0	2.2	0.0	0.6	6.7	15.6	2.8	1.1	0.0	3.9	1.9	5.8
Area of growth 2	Feedingwater	0.0	0.0	2.2	1.1	1.7	7.2	43.9	1.1	0.0	0.0	9.6	1.9	11.5
	Effluent	0.0	0.0	1.1	0.0	0.0	0.0	2.2	4.4	0.0	0.0	0.7	0.9	1.6
Area of growth 3	Feedingwater	0.0	0.0	0.0	1.1	0.6	4.4	12.2	6.1	0.0	0.0	2.6	2.3	4.9
	Effluent	0.0	0.0	0.0	0.0	0.6	7.2	33.3	2.2	0.0	0.0	6.8	1.9	8.7
	Feedingwater	0.0	0.0	0.0	0.0	2.8	1.7	1.7	2.8	3.9	0.0	1.7	0.9	2.6
	Effluent	0.0	0.0	0.0	0.0	0.6	7.8	6.1	1.1	3.9	0.0	2.1	1.8	3.9
<b>Average</b>		1.1	1.0	2.7	0.5	1.2	5.3	12.0	4.0	1.2	0.0	3.6	2.2	5.1

the hardening off area (Table 3). Although lower inoculum intensity was found for *B. cinerea*, the fungus was more widespread in the nursery, occurring in gravel from all areas, with the exception of the clonal mini-hedges and the shade house. *C. candelabrum* was not detected in February, while *B. cinerea* was not found in January and March (Table 3).

#### Conidial germination in nutrient solution at different salt concentrations expressed by electric conductivity

Electric conductivity at the values tested did not significantly affect conidial germination, nor did the three different compositions of nutrient solution used in the ferti-irrigations of the various phases for eucalyptus cutting production. Germination was, on average, equal to 94.7% for *B. cinerea* and 60.0% for *C. candelabrum*.

### DISCUSSION

The reuse of the nutrient solution in ferti-irrigation systems aims to minimize water consumption, reduce fertilizer use and environmental impact caused by the liberation of nutrient solutions in the environment. However,

from the results obtained in the current work, it became evident that this strategy presents a high risk in terms of spreading eucalyptus pathogens such as *Cylindrocladium* spp. and *B. cinerea*.

The method adapted for microbiological analysis of water was very sensitive in detecting inocula, especially of *C. candelabrum*. Another study, Gonçalves *et al.* (2001) tested various baits to quantify the inocula of *Cylindrocladium* spp. and observed that leaf discs of castor bean also worked efficiently in the recovery of *C. candelabrum* from samples of naturally or artificially infested soil. As well as the high sensitivity of detection, this method presented other advantages, such as ease of use and low cost per sample analyzed. On the other hand, the method is time-consuming and does not permit the detection of bacterial pathogens, such as *Xanthomonas axonopodis* and *Ralstonia solanacearum*, causal agents of leaf blight and eucalyptus wilt, respectively. For *R. solanacearum*, for example, molecular methods such as use of PCR (Polymerase Chain Reaction) have made it possible to detect the pathogen in natural substrates, including water, seeds, samples of plant tissues and from soil (Poussier *et al.*, 2002).

**TABLE 2** – Values of pH and EC (mS.cm<sup>-1</sup>) in water samples at different periods and for various phases in clonal propagation, considering feedingwater and effluent for the ferti-irrigation system over time

Place		Jan		Feb		Mar		Apr		May		Average	
		pH	EC	pH	EC								
Mini-clonal hedge (sand bed)	Feedingwater	5.90	0.46	4.90	1.20	5.20	1.28	5.00	1.41	4.10	1.31	5.02	1.13
	Effluent	4.80	1.40	4.80	1.27	5.40	1.35	4.80	1.46	4.10	1.35	4.78	1.36
Mini-clonal hedge (floating)	Feedingwater	4.70	1.44	5.00	0.99	5.60	1.08	5.10	1.28	4.60	1.19	5.00	1.20
	Effluent	3.40	0.97	3.60	1.55	4.60	1.51	4.20	1.80	3.30	1.80	3.82	1.53
Rooting house	Feedingwater	7.10	0.14	6.90	0.13	6.90	0.13	7.00	0.15	7.50	0.19	7.08	0.15
	Effluent	5.50	2.29	4.90	2.50	5.70	2.43	5.40	0.23	5.20	0.21	5.34	1.53
Shade house	Feedingwater	7.00	0.14	6.90	0.14	6.70	0.50	6.90	0.31	5.50	0.48	6.60	0.31
	Effluent	5.60	0.79	5.90	0.81	5.50	0.90	5.50	1.28	5.10	1.16	5.52	0.99
Area of growth 1	Feedingwater	5.00	1.08	4.30	0.98	5.50	0.97	5.20	1.08	5.30	1.03	5.06	1.03
	Effluent	5.20	1.06	4.80	0.94	5.50	1.04	5.20	1.09	5.20	1.09	5.18	1.05
Area of growth 2	Feedingwater	5.10	0.81	4.90	0.82	5.60	0.97	5.10	1.06	5.50	0.89	5.24	0.91
	Effluent	6.10	0.38	4.70	0.70	5.70	4.71	5.10	1.00	5.00	0.90	5.32	1.54
Area of growth 3	Feedingwater	6.50	0.31	5.30	0.75	5.50	0.90	5.10	0.94	4.50	0.98	5.38	0.78
	Effluent	6.20	0.28	4.70	0.93	5.20	0.88	4.60	1.09	4.60	0.99	5.06	0.83
<b>Average</b>		5.58	0.83	5.11	0.98	5.61	1.33	5.30	1.01	4.96	0.97	5.31	1.02

**TABLE 3** – Detection of *Botrytis cinerea* and *Cylindrocladium candelabrum* inoculum in samples of gravel collected at different periods and in various phases of clonal propagation of eucalyptus

Place	<i>C. candelabrum</i>				<i>B. cinerea</i>			
	Jan	Feb	Mar	Average	Jan	Feb	Mar	Average
Mini-clonal hedge ((sand bed)	0	0	0	0	0	0	0	0
Mini-clonal hedge (floating)	0	0	0	0	0	0	0	0
Rooting house	0	0	1.10	0.37	0	1.10	0	0.37
Shade house	0	0	0	0	0	0	0	0
Area of growth 1	0	0	0	0	0	1.10	0	0.37
Area of growth 2	0	0	0	0	0	1.10	0	0.37
Area of growth 3	0	0	12.20	4.07	0	2.20	0	0.73
Hardening off shed	21.00	0	0	7.00	0	1.10	0	0.37
<b>Average</b>	2.33	0	1.48	1.27	0	0.73	0	0.24

Of the ferti-irrigation systems evaluated, *C. candelabrum* and *B. cinerea* occurred mainly in floating mini-clonal hedge and in one of the growth areas, respectively. The first of these pathogens may have been favored in the clonal mini-hedge by the constantly high level of humidity in the air, caused by the ongoing flow of ferti-irrigation, as well as by the infected leaves mixed with the nutrient

solution. For *B. cinerea*, it is believed that in the growth area, shaded and weakened plants may be infected, generating a large quantity of spores, which are easily spread by the ferti-irrigation water.

Periodical monitoring of the water showed, generally, a greater intensity in the pathogen inoculum in the effluent from the ferti-irrigation system, which indicates

an accumulation of inoculum when water is reused. *C. candelabrum* was more frequently detected over time in the various ferti-irrigation systems, probably because of its wider adaptation to the various environmental conditions, especially temperature (Alfenas *et al.*, 1979). In contrast, *B. cinerea* is more favored by temperatures from 20 to 24°C (Souza, 1991). In the present study, the average temperature was relatively high (data not shown), which partially explains the seasonal occurrence of *B. cinerea*, although other environmental factors may also exert an influence.

In relation to water quality, it is known that this should preferably be of drinking quality, treated by traditional methods (filtered and treated with chlorine-based products or, better, with ozone) or collected in artesian or semi-artesian wells. These precautions are to ensure not only the chemical but also the microbiological water quality. This is because water is one of the main vehicles of dissemination of plant pathogenic fungi and bacteria (Alfenas *et al.*, 2004).

In accordance with the production system of eucalyptus plants, as a strategy to reuse water in the open systems of sprinkler ferti-irrigation, it is necessary to collect water after making the soil impermeable, covering it again with gravel. The results obtained demonstrate that the two pathogens were recovered from this soil cover. It is thought, therefore, that the spores of these fungi can be washed off lesions by ferti-irrigation water and rain; then, when they reach the gravel, they colonize left-over substrate and plant material, mainly residue from dead and decomposing leaves, and are favored by the continual humidity and presence of nutrients. It is known, for example, that *B. cinerea* can survive as a saprophyte of leaves and organs on its hosts even when they are dead on the ground (Ferreira, 1989), in organic substrates, on the surface of containers and in tissues of eucalyptus plants as a component of the phylloplane (Souza & Ferreira, 1990; Souza, 1991; Sanfuentes & Ferreira, 1997). As well as sclerotia, studies have shown that conidia of *B. cinerea* can also be important for survival (Gindro & Pezet, 2001). The intense sporulation of *B. cinerea* on diseased eucalyptus plants is easily spread by wind and by irrigation water (Ferreira, 1989; Ferreira & Souza, 1999; Ferreira & Milani, 2002). This is similar for *Cylindrocladium* spp., as irrigation or rain water can move conidia from lesions to the soil and thus spread them to other plants, establishing secondary infections (Ferreira, 1989).

The two pathogens studied were not detected in the gravel that covered the soil of the clonal mini-hedges. In the two systems studied, water used in ferti-irrigations did not come into contact with the gravel, since the irrigation systems are closed. On the other hand, it is believed that inoculum multiplication can be enhanced in these systems by the presence of dead and decomposing plant material, which is likely to be spread over containers for plant production, on trays or within the nutrient solution.

Salt concentration of three nutrient solutions expressed by the electric conductivity (EC), at the values tested, did not affect the germination of *C. candelabrum*

or *B. cinerea* conidia. In contrast, Thinggaard & Andersen (1995) demonstrated that the frequency and concentration of soluble salts in the nutrient solution affect the propagation and aggressiveness of *Phytophthora cryptogea* in *Gerbera jamesonii*. An increase in the electric conductivity resulted in the inhibition of the pathogen, which was attributed to the high EC and/or high concentration of one or more of the specific ions. To confirm this result in another study, it was seen that the change in EC from 1.5 to 1.2 mS/cm, with no change in the concentration of copper ions, did not influence disease severity, while the addition of copper ions did inhibit the pathogen's activity (Toppe & Thinggaard, 1998). From these other studies it is possible to infer that the three nutrient solutions tested did not present ions in sufficiently high concentrations to inhibit the pathogens tested.

As highlighted by Alfenas *et al.* (2004) and by Vida *et al.* (2004), and in accordance with the results obtained in this work, water can constitute one of the sources of pathogen inoculum during production of plants in nurseries. It is, therefore, vital to carry out appropriate water treatment in ferti-irrigation systems where water is reused or discarded to avoid spread of pathogens in nature and reduce the environmental impact. There are different methods for water treatment in hydroponic systems, which can be divided thus: i) thermal treatment (pasteurization); ii) chemical treatment; iii) ultraviolet radiation and iv) filtration. In all these cases, there are variations in the efficiency, depending on a series of factors (Ehret *et al.*, 2001; Newman, 2004). The choice of any of these methods should be efficient in elimination of fungi and bacteria pathogenic to eucalyptus, although they have to be tested in further studies.

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Recebido 22 Maio 2007 - Aceito 17 Março 2008 - TPP 7057

Editor Associado: José Maurício Fernandes