European Journal of Plant Pathology **104:** 359–366, 1998.

Prevention of Phytophthora Root Rot in *Gerbera* by increasing copper ion concentration in the nutrient solution

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Accepted 4 February 1998

Key words: ebb-and-flow, electrical conductivity, greenhouse iron chelate, *Phytophthora cryptogea*, recirculation, zoospore

Abstract

Increased copper concentration in the nutrient solution was used as a means for reducing the severity of root rot caused by *Phytophthora cryptogea* in *Gerbera jamesonii* in three experiments. Plants were grown in pots on ebb-and-flow benches with separate systems for recirculating nutrient solutions. Eight nutrient solutions with two concentrations of copper ions (0.07 and 0.28 ppm), two electrical conductivity values (1.5 and 2.2 $mScm^{-1}$), and two iron sources (FeHEEDTA or $FeSO_4$) were combined in a factorial design. Plants were inoculated with zoospores of *P. cryptogea* via the recirculating nutrient solution. Disease incidence was significantly reduced in inoculated plants grown on nutrient solution with 0.28 ppm copper compared with 0.07 ppm copper, when $FeSO_4$was introduced as the iron source. No effects of increased copper concentration was observed when iron was added as FeHEEDTA. The change in electrical conductivity from 1.5 to 2.2 $mScm^{-1}$ without changing the Cu^{2+} concentration did not influence the disease severity in these experiments. The results suggest that increased copper ion concentration in the nutrient solution could be a component of disease management strategy for ebb-and-flow systems. Possible management of the cupric ion concentration in the nutrient solutions is discussed.

Introduction

Production of pot plants in systems with recirculation of the nutrient solutions is known to provide conditions conducive to dispersal of zoosporic pathogens like *Phytophthora* spp. and *Pythium* spp. (Hoitink et al., 1992; Sanogo and Moorman, 1993; Stanghellini and Rasmussen, 1994; Strong et al., 1997; Thinggaard and Andersen, 1995; Thinggaard and Middelboe, 1989). Root rot caused by *Phytophthora cryptogea* Pethybr. & Lafferty is one of the most serious diseases encountered in the production of *Gerbera jamesonii* H. Bolus ex J.D. Hook (Rattink, 1981; Sadowska-Ryback et al., 1996; Schickedanz, 1993; Thinggaard and Andersen, 1995). Recently, the availability of effective fungicides and surfactants has been reduced, due to e.g. regulatory activity. Therefore, alternative procedures for prevention of root diseases are needed in order to continue using recirculation systems.

In previous investigations, Thinggaard and Andersen (1995) have shown that both watering frequency and soluble salt concentration in the nutrient solution affect the spread and aggressiveness of *P. cryptogea* on *G. jamesonii*. A rise in the electrical conductivity (EC) from 1.5 $mScm^{-1}$ to 2.2 $mScm^{-1}$ resulted in pathogen inhibition which may be attributed to a general effect of high EC or to a elevated concentration of one or more specific ions.

Control of zoosporic pathogens has long been associated with various forms of copper (Kennedy and Erwin, 1961; Smith, 1979; Zentmyer and Marshall, 1959). For *Phytophthora* zoospores, Slade and Pegg (1993) recorded a LD_{50} for Cu^{2+} at 1.84 μm (0.12 ppm). Halsall (1977) was able to decrease the activity of *P. drechsleri* on safflower seedlings by watering

Table 1. Macro and micro nutrient concentration (ppm) and EC ($mScm^{-1}$) level in the eight nutrient solutions at the start of each experiment and in tap water. Nutrient solution 1 to 4 was used in experiment 1, nutrient solution 5 to 8 was used in experiment 2 and all eight nutrient solutions were used in experiment 3

Elements (ppm)	Nutrient solution 1	2	3	4	5	6	7	8	Tap water
EC ($mScm^{-1}$)	1.5	2.2	1.5	2.2	1.5	2.2	1.5	2.2	
Ca	153	153	153	153	153	153	153	153	153
K	163	268	163	268	163	268	163	268	2
Mg	23	38	23	38	23	38	23	38	13
N	141	232	141	232	141	232	141	232	...
P	17	28	17	28	17	28	17	28	...
S	57	74	57	74	57	74	57	74	41
B	0.26	0.44	0.26	0.44	0.26	0.44	0.26	0.44	...
Cl	43	43	43	43	43	43	43	43	43
Cu	0.07	0.07	0.28	0.28	0.07	0.07	0.28	0.28	...
Mn	0.4	0.7	0.4	0.7	0.4	0.7	0.4	0.7	...
Mo	0.05	0.09	0.05	0.09	0.05	0.09	0.05	0.09	...
Na	19	19	19	19	19	19	19	19	19
Zn	0.26	0.44	0.26	0.44	0.26	0.44	0.26	0.44	0.26
Fe									
- as $FeSO_4$	–	–	–	–	5.6	5.6	5.6	5.6	
- as FeHEEDTA*	1.3	2.2	1.3	2.2	–	–	–	–	

* Source 5.2%, Interfiller A/S, Køge, Denmark.

frequently with 1μM (0.06 ppm) Cu^{2+} over the critical period of infection. In a laboratory test he also found that infection of eucalypt cotyledons by *P. drechsleri* zoospores was inhibited by 1 μm Cu^{2+} (0.06 ppm), but the inhibition was reversed by addition of EDTA (10^{-4} M). Likewise Kennedy and Erwin (1961) showed that the inhibitory effect of Cu^{2+} on sporulation of *P. megasperma* was reversed by addition of the chelating agent KEDTA.

Based on previous experiments (Thinggaard and Andersen, 1995), the aim of the present study was to investigate the possibility of reducing attacks of *P. cryptogea* in an ebb-and-flow system by manipulation with copper ion concentration, EC and iron sources in the recirculating nutrient solution.

Materials and methods

Plant cultivation

Three experiments were carried out in the first half of 1996 in a 136 m^2 greenhouse section at Årslev, Denmark. *G. jamesonii*, cv. Hummingbird Orange Naeldebakke, Aps, Denmark, was grown from seeds in limed and fertilized peat (Pindstrup 3, Pindstrup Mosebrug A/S, Ryomgaard, Denmark) and potted in 11 cm plastic pots 5 weeks after sowing. Ten weeks after sowing 21 plants (39 plants/m^2) were placed on each of 27 ebb-and-flow benches (60 cm × 108 cm) with separate recirculation of the nutrient solution. Set points for air temperature and ventilation were 22 °C and 25 °C, respectively. To induce flowering, black screens were used for 12 h during night to obtain a short photoperiod. Whenever the PPFD (Photosynthetic Photon Flux Density) level inside the greenhouse dropped below 88 μmol m^2s^{-1}, 60 μmol m^2s^{-1} were supplied by 400 W high pressure sodium lamps (SON-T, Philips) for up to 10 h per day. The insecticide pirimicarb (Pirimor), the parasitoid *Aphidius colemani* and a biological insecticide, VectoBac 12AS (*Bacillus thuringiensis*), were used during cultivation to suppress aphids and sciarid flies. The entire ebb-and-flow system in the greenhouse was washed and surface-disinfected with a 3% solution of hydrogen peroxide and peracetic acid (Deosan Flora, Diversey, Denmark) prior to each experiment. No plants were removed during the period of experiment.

Nutrient solution

Eight different nutrient solutions were used (Table 1). The ratio of elements in nutrient solution no. 1 was selected to fit the Danish standard for fertilizing *Gerbera*. Two concentrations of copper (0.07 and 0.28 ppm) and two EC levels (1.5 and 2.2 $mScm^{-1}$) were combined in a factorial design. In the first experiment, an Fe-chelate (FeHEEDTA, Fe salt of hydroxyethylethylenediamine-triacetic acid) was used as iron source. In the second experiment, $FeSO_4$ was used as iron source. $FeSO_4$ was added to the solution at the same concentrations as used by Halsall (1977) for both high and low EC treatments. In the third experiment both iron sources were used, in combination with the two concentrations of copper and the two EC levels.

EC and pH were controlled in the nutrient solutions every third day by EC- and pH metres (Volmatic, Farum, Denmark). The pH was maintained between 5.5 and 5.9 by adding $CaCO_3$ or 1: 3 H_3PO_4: HNO_3. For determination of element concentrations of N, P, K, Ca, S, Mg, B, Cl, Zn, Cu, Fe, Mn, Mo and Na, in the growing medium and nutrient solutions, samples were collected at the beginning of each experiment.

Watering practice

When watering, the nutrient solutions were pumped onto the benches to a height of 2 cm, before they freely drained back into separate storage tanks (25 l). The total flooding period was 8 min. The frequency of irrigation ranged from three times a week when the plants were small, to twice a day for large, flowering plants. Treatments with the respective nutrient solutions started when the plants were 10 weeks old, one week before inoculation. Just prior to addition of inoculum (see below), the element concentrations in the nutrient solutions were adjusted as shown in Table 1. Additional nutrient solution was supplied to each tank whenever necessary (one to two times per fortnight).

Determination of copper in nutrient solutions and soil extracts

Total copper was determined by atom absorption spectrophotometry (Perkin Elmer 373), and the levels of Cu^{2+} by an ISE25 ion selective electrode (Radiometer Denmark A/S, Copenhagen, Denmark) in weekly samples of the nutrient solutions. Samples of soil extracts for determination of pH, EC and total copper were made once a week by pressing soil water out of the potting media one hour after watering.

Inoculum production and inoculation

An isolate of *P. cryptogea* obtained from roots of *G. jamesonii* in a Danish nursery (Thinggaard and Andersen, 1995) was used as inoculum. For zoospore production, five-day-old cultures of the pathogen grown on V8 juice agar with $CaCO_3$ (Ribeiro, 1978) in Petri dishes were overlayed with autoclaved pond water. The pond water was changed once a day for four days. Zoospore release was induced by chilling the cultures at 4 °C for 30 min.

In the first experiment, 10 Petri dishes with cultures of *P. cryptogea* were placed at each of the ebb-and-flow benches immediately after chilling the cultures. Inoculation was done by overflowing of Petri dishes at time of watering. The solutions were kept at the benches for two hours before they were drained off to the storage tanks. The same procedure was repeated the following day before the Petri dishes were removed from the benches. For determination of zoospore concentration in the nutrient solutions, ten samples of the solutions were collected and the number of zoospores determined by direct counting in a haemacytometer. The mean inoculum concentration after overflowing the Petri dishes the second day was 230 zoospores ml^{-1} nutrient solution.

In experiment 2 and 3, the inoculation was carried out by adding a suspension of zoospores to the storage tanks. Zoospore concentrations were determined by direct counting in a haemacytometer and adjusted to 90 and 75 zoospores ml^{-1} nutrient solution, respectively, in experiment 2 and 3. Two hours after adding zoospores to the nutrient solutions in the tanks inoculation was done by normal watering. Non-inoculated controls were nutrient solution no. 4 in experiment 1, and nutrient solution no. 8 in experiment 2 and 3 (see Table 1).

Detection of P. cryptogea *in the nutrient solutions*

Samples of the nutrient solution in each tank (250 ml) were examined weekly for the presence of *P. cryptogea* using the filtration method described by Thinggaard and Middelboe (1989). In addition to the filtration, four needles of *Cedrus deodara*, and four petiole pieces of *G. jamesonii* were each week placed in the nutrient solution to catch *Phytophthora* zoospores. The baits were removed after three days in the solution, surface

Table 2. Experiment 1–3: Mean percentage of dead *Gerbera jamesonii* plants at the end of the experiments (15 days after inoculation with *P. cryptogea* in experiment 1, 22 days after inoculation in experiment 2 and 3)(± = Standard error)

Treatments				Mean percentage of dead plants		
no	Fe	Cu	EC	Exp. 1	Exp. 2	Exp. 3
1	Fe-HEEDTA	0.07[a]	1.5[b]	95 ± 2.6	–	88 ± 4.3
2		0.07	2.2	97 ± 1.9	–	93 ± 3.6
3		0.28	1.5	87 ± 4.0	–	89 ± 4.3
4		0.28	2.2	89 ± 3.6	–	89 ± 4.3
5	$FeSO_4$	0.07	1.5	–	87 ± 4.2	80 ± 5.6
6		0.07	2.2	–	95 ± 2.7	64 ± 6.7
7		0.28	1.5	–	11 ± 4.0	21 ± 5.7
8		0.28	2.2	–	35 ± 6.1	20 ± 5.5

Sources of variance							
Exp.	1	2	3		1	2	3
Cu conc.	ns[c]	***	***	Cu × EC	ns	ns	ns
EC levels	ns	ns	ns	Cu × Fe	–	–	***
Iron Sources	–	–	***	EC × Fe	–	–	ns
Rep	ns	ns	ns	Cu × Ec × Fe	–	–	ns

[a] ppm, [b] $mScm^{-1}$, [c] ns = not significant, *** = $p \leq 0.0001$.

sterilized with 70% ethanol for 30 seconds, placed on *Phytophthora* selective medium (HMI) with hymexazol (Tsao and Guy, 1977) for five days at 22 °C in the dark, and examined for growth of *P. cryptogea*.

Recording disease incidence

Permanently wilted plants with brown decayed roots were considered dead, and they were recorded every second day. To confirm that the plants were infected with *P. cryptogea*, root sections were taken from one wilted plants from each of the 24 inoculated plots for isolation of the pathogen. Surface sterilized (70% ethanol for 30 seconds) root segments were plated on HMI, incubated for five days at 22 °C in darkness, and examined under the light-microscope for growth of the fungus.

At day 22 after inoculation (day 15 in experiment 1), the root system and foliage of all plants were evaluated with disease indices ranging from 1 to 4 (roots) and 1 to 3 (leaves). For root system, index 1 indicated white, healthy roots, index 2 = the start of root rot, with a few brown roots, index 3 = more extensive root rot and index 4 = total damage of the root system in the pot. For foliage, index 1 was given to healthy plants with no visible symptoms, index 2 when the foliage started to wilt, and index 3 when the foliage was completely wilted with symptoms of dark *Phytophthora* rot at leaf basis.

Statistical analysis

Each of the three experiments consisted of a full factorial design with three replicates and 21 plants per treatment. For each experiment the number of *Phytophthora* dead plants in each plot and the disease indices were analysed by factorial analyses of variances. Analyses and means were made by the GLM procedure (SAS Institute, 1989).

Results

Effect of copper in relation to iron source and EC level

No symptoms of Phytophthora Root Rot or copper toxicity were seen in the non-inoculated control plants in either experiment. Wilting of inoculated plants began within 5 to 10 days of inoculation. Disease developed much slower in plants grown with 0.28 ppm copper in combination with $FeSO_4$ in the nutrient solution. A significant reduction in the number of dead plants (Table 2), and a better root and foliage disease index score ($P \leq 0.001$) (Figure 1) was obtained at the con-

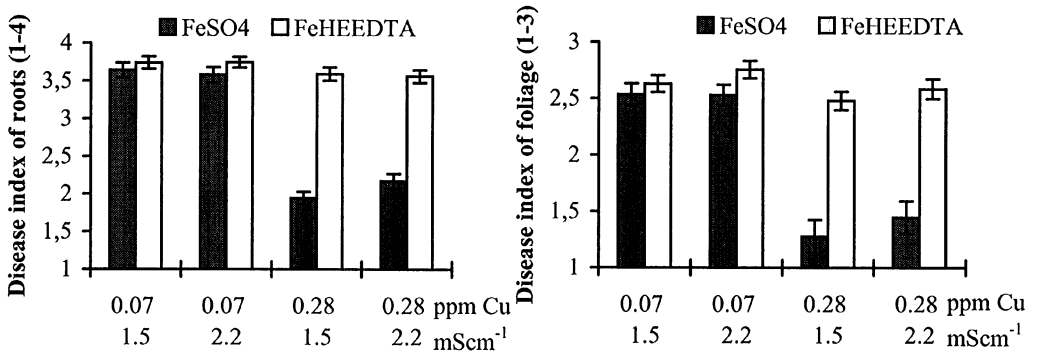

Figure 1. Mean index of disease symptoms caused by *Phytophthora* on root system and foliage of *Gerbera jamesonii* of experiment 1, 2 and 3 at two copper ion levels (0.07 and 0.28 ppm). Recorded at the end of the experiments (day 15 in experiment 1, day 22 in experiment 2 and 3). Index 1 represents no disease symptoms, and index 3 (4) the most developed disease symptoms. Vertical bars represent standard error.

clusion of the experiments with this copper and iron source. Copper failed to inhibit disease development when iron was added as FeHEEDTA. The rise in EC from 1.5 $mScm^{-1}$ to 2.2 $mScm^{-1}$ without changing the Cu^{2+} concentration did not reduce the infection of *P. cryptogea.*

Detection of P. cryptogea *in the nutrient solution*

In inoculated plots, *P. cryptogea* was isolated qualitatively by filtration and baiting from all storage tanks one hour after inoculation. With time the frequency of pathogen recovery declined, and at the conclusion *P. cryptogea* was found in 49 percent of the tanks. There was no difference in pathogen recovering between treatments. However, the third week after inoculation *P. cryptogea* was absent in all replicates of nutrient solutions containing 0.28 ppm copper, EC 1.5 $mScm^{-1}$ and $FeSO_4$ while it was isolated in at least one of the replicative storage tanks of the other nutrient solutions.

P. cryptogea was never isolated from the non-inoculated control tanks. Isolation of *P. cryptogea* happened in the present investigation more often by filtration than by baiting, although baiting with petioles of Gerbera leaves, or needles of *Cedrus deodara* in most cases also was successful (data not shown).

Levels of total copper and cupric ions

No significant differences between treatments were found for total copper in the potting media with dry weight 5.4 ($\pm$ 1.0) mg kg^{-1} at the start of the experiments and 6.7 ($\pm$ 1.2) mg kg^{-1} at the end of the experiments. Mean values of total copper and cupric ions in samples of the nutrient solutions and soil extracts are shown in Figure 2. The electrode used for measurements of cupric ions was not stable for standards of Cu^{2+} with concentrations lower than 10^{-6} M (0.06 ppm). However, values for Cu^{2+} (mV) were lower ($P \leq 0.01$) in nutrient solutions where FeHEEDTA was used, as compared to those containing $FeSO_4$. Where FeHEEDTA was used, no differences in Cu^{2+} level were noted between nutrient solutions with 0.07 ppm Cu (solution 1 and 2) and nutrient solutions with 0.28 ppm Cu (solution 3 and 4). At the conclusion of each experiment, no significant accumulation of total copper was recorded in the soil extracts or in the recirculating nutrient solutions.

EC, pH, and temperature

Ranges of EC in the nutrient solutions for high and low EC treatment was 1.4 – 1.6 $mScm^{-1}$ and 2.0 – 2.3 $mScm^{-1}$, respectively. Solution pH varied between 5.1 and 6.6 before being adjusted to pH 5.8 every third day.

In the weekly soil extract samples mean EC was 1.9 ($\pm$ 0.3) and 2.3 ($\pm$ 0.4) $mScm^{-1}$, respectively, for the two EC-treatments. Mean pH of these samples (average of all three experiments) was 5.5 ($\pm$ 0.5). Average air temperature in the greenhouse during the three experiments was 23.2 °C with maximum at 33 °C and minimum at 21 °C. Mean soil temperatures was 23.6 °C, with maximum of 40.7 °C and minimum at 20 °C (recorded only in the third experiment).

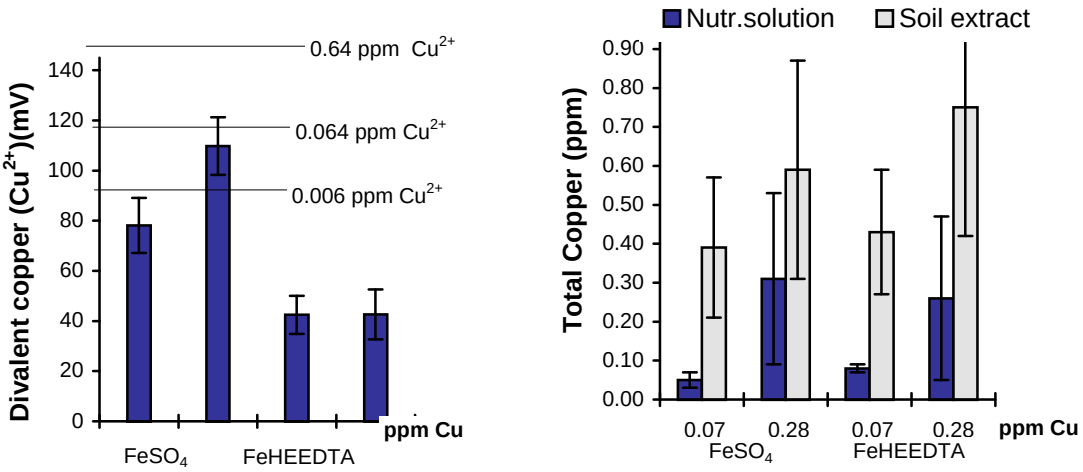

Figure 2. Mean levels of Cu^{+} (mV) in samples of the recirculated nutrient solutions and mean level of total copper (ppm) in samples of the nutrient solution and the soil extracts. Average values of weekly samples with conductivity 1.5 and 2.2 $mScm^{-1}$ in experiment 1, 2, 3. Known standard values in ppm of Cu^{+} are given as horizontal lines. Vertical bars represent standard deviation.

Discussion

Our results show that the concentration of cupric ions in the nutrient solution have a significant impact on the development of *P. cryptogea*-incited root rot of *G. jamesonii* grown in an ebb-and-flow system. When $FeSO_4$ was used as the iron source, an increase in copper concentration from 0.07 to 0.28 ppm in the nutrient solution reduced disease incidence between 40 and 70 percent. The efficacy of elevated copper concentration in the nutrient solution is most likely due to sensitivity of zoospores to copper. Even low concentrations of cupric ions (0.06-1 ppm) are known to be toxic to the zoospores and to reduce the sporangial production of *Phytophthora* spp. (Halsall, 1977; Kennedy and Erwin, 1961; Slade and Pegg, 1993; Smith, 1979; Zentmyer and Marshall, 1959). Another possible effect of the increased copper concentration is interruption of the process of infection of zoospores by disruption of the interactions between other cations e.g. mg^{2+}, K^{+}, Fe^{2+} in the infective process (Halsall, 1977).

The suppressive effect of copper against root rot in *G. jamesonii* was not evident if FeHEEDTA was used as the iron source. Reduced concentrations of cupric ions obtained with FeHEEDTA than with $FeSO_4$, is responsible for the decline in efficacy against *P. cryptogea*. This can be explained by exchange of Fe by Cu in the metal chelate, and thus removal of cupric ions from the solution (Guinn and Joham, 1963; Wallace et al., 1983). Metal chelates are used as iron source in most of the commercial fertilizers, and alternative sources must be identified for utilization of copper to suppress *Phytophthora* incited root rot diseases in ebb-and-flow systems. Since complex binding to humic substances may remove cupric ions from the nutrient solution (De Kreij and Basar, 1995), methods for practical management of cupric ion concentration in the growing systems need further development. This could be done by using more stable iron chelates which possibly could prevent the chelate-binding of cupric ions, or alternatively, to apply low concentrations of copper frequently to the tanks with nutrient solution. In that case, a method for measuring low concentration of cupric ions routinely in the nutrient solution would be required.

At the end of the experiments *P. cryptogea* was qualitatively identified in less than 50 percent of the storage tanks. Similar results were found in previous experiments (Thinggaard and Andersen, 1995), where *P. cryptogea* zoospores could not be detected in the nutrient solution beyond four weeks after inoculation. However, other researchers detected *P. parasitica* in a nutrient solution seven weeks after inoculated plants were placed on ebb-and-flow tables (Strong et al., 1997). This dissimilarity could be explained by differences in zoospore viability or inoculum concentration. In our experiments primary inoculum concentrations were relative high and the disease developed rapidly. For plants grown with 0.07 ppm copper in the nutrient solution, most of the roots quickly died and the absence of fresh roots declined the pathogen production of zoospores.

No significant effects of EC were observed in this study. This suggest the effect of EC seen in previous experiments (Thinggaard & Andersen, 1995) to be explained by increased level of specific ions in the solution, and not as an effect of EC. In addition to copper, other ions such as Ag^{+}, Al^{3+}, Mn^{2+}, Mo^{6+}, B^{+}, Cl^{-} and Ca^{2+} are known to inhibit the activity of root pathogens (Benson, 1995; Byrt et al., 1982; Halsall, 1977; Slade and Pegg, 1993; Smith, 1979; Vanachter et al., 1992; Von Broembsen and Deacon, 1997; Webster and Dixon, 1991). As far as known by the authors, the role of these ions in preventing fungal-incited root rot in ebb-and-flow systems with recirculating nutrient solutions has not been tested. However, in a flood irrigated system Von Broembsen and Deacon (1997) recently found the amendment of fertilizer solution with 10 or 20 mM $Ca(NO_3)_2$ to suppress the infection of container vinca seedlings inoculated by zoospores of *P. parasitica*.

For practical purposes the possibility of copper accumulation in the nutrient solution, and the risk of copper toxicity in plants must be considered. Cupric ions have the ability to displace other metal ions, in particular Fe^{2+}, and chlorosis, a commonly observed symptom of copper toxicity, superficially resembles Fe deficiency (Daniels et al., 1972). Although levels of copper used in this investigation did not cause toxicity symptoms in the plants, further research is necessary to investigate long term effects of copper in an ebb-and-flow system with recirculation of the nutrient solution.

Increased knowledge of integrated control of root pathogens (Chambers and Scott, 1995; Rattink, 1996; Stanghellini and Miller, 1997) should lead to investigations of consequences of increased copper levels on other pathogenic fungi and the rest of the microflora in the recirculating nutrient solution. Copper concentrations must be low, and treatments should be carried out only when there is a risk for introduction of *Phytophthora* in the growing system.

The possibility of manipulation of copper in the nutrient solution as a means of disease control in ebb-and-flow systems could reduce fungicide use, and is inexpensive when compared to disinfection of the nutrient solution by heat, ozone, UV or slow sand filtration (McPherson et al., 1995). Along with reduced disease severity, copper supplement could satisfy the plants requirements for accessible copper for optimal growth (Willumsen, 1986).

Acknowledgements

The assistance of the staff at Danish Institute of Agricultural Sciences, Årslev is greatly appreciated. This research was supported in part by a grant from the Norwegian Research Council and by the Danish research programme for quality, post harvest, performance, and vitality in ornamentals (OPKOT).

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