ELSEVIER

Crop Protection 21 (2002) 751–756

Crop Protection

www.elsevier.com/locate/cropro

Activity of calcium salts in controlling Phytophthora root rot of citrus

V. Campanella, A. Ippolito*, F. Nigro

Dipartimento di Protezione delle Piante dalle Mallatie, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy

Received 26 February 2001; accepted 29 January 2002

Abstract

The effect of 10 calcium salts in controlling Phytophthora root rot of citrus was evaluated both in vitro, on agar and liquid media, and in vivo, on sour orange citrus seedlings. In vitro, calcium salts were applied at 300, 600, and 1200 ppm. Calcium oxide and calcium carbonate significantly reduced the growth of *P. nicotianae*, in comparison with the control; minimum dry weight of the mycelium was observed with calcium oxide; moreover, this salt significantly reduced zoospore production and their viability. In vivo, calcium salts were applied at 1200 ppm on 4-month old sour orange seedlings. Calcium propionate and calcium lactate significantly reduced the inoculum density of the pathogen in the soil, whereas all the other calcium salts—except nitrate, pantothenate and gluconate—significantly reduced root infections in comparison with the control. Phytotoxicity symptoms on sour orange seedlings were induced only by calcium chloride. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Biological control; Calcium compounds; Zoospore production; Feeder root rot; *Phytophthora nicotianae*; Sour orange

1. Introduction

Phytophthora root rot is the most widespread and severe citrus disease of Apulia, Basilicata and Northern Calabria, in peninsular south Italy (Ippolito et al., 1990a, b), where, root rot is mainly due to *Phytophthora nicotianae* van Breda de Haan [syn. *P. nicotianae* van Breda de Haan var. *parasitica* (Dast) Watherh]. In these regions the citriculture is based on the use of resistant sour orange (*Citrus aurantium* L.) rootstock, which limits the damage caused by *Phytophthora* spp.; however, intrinsic factors, such as poor seed and seedling selection (Salerno and Cutuli, 1981), the incorrect application of cultural practices and environmental conditions conducive to the disease, as well as the grafted citrus varieties (Ippolito et al., 1997), may negatively affect the natural resistance of this rootstock. Besides the traditional cultural practices, the disease is managed by means of two systemic fungicides, specific against *Phytophthorae*: Fosetyl-Al and Metalaxyl (Ippolito et al., 1996, 2000). However, environmental pollution, hygienic-sanitary factors and the possible selection of resistant fungal strains have increased the interest for alternative control methods, more safe for both the environment and the people.

Calcium is an essential element, which influences growth and fruiting of plants. Calcium ions preserve the structural integrity and functionality of membranes and cell wall, increase host resistance to invasion by certain pathogenic micro-organisms and enhance tolerance to abiotic diseases (Yuen, 1993). Calcium accumulates in the cell wall, giving it stability and integrity and strongly affecting pectinolytic (Wisniewski et al., 1995; Biggs et al., 1997) as well as reproductive activities of pathogens (Conway et al., 1992). The activity of this element against Phytophthora root rot of citrus and avocado is well known (Chapman, 1965; Zentmyer and Lewis, 1975). Moreover, some inorganic calcium salts gave encouraging results in controlling various fungal diseases, both in vitro and in vivo trials (Brunelli, 1995; Nigro et al., 1997).

This paper reports the results of in vivo and in vitro trials on the activity of several calcium salts in reducing both Phytophthora root rot of citrus and the production of propagules by the pathogen.

2. Materials and methods

2.1. In vitro activity

The activity of Ca-sulphate, Ca-oxide, Ca-propionate, Ca-lactate, Ca-nitrate, Ca-acetate, Ca-pantothenate,

*Corresponding author. Tel.: +39-080-544-3053; fax: +39-080-544-2911.

E-mail address: antonio.ippolito@agr.uniba.it (A. Ippolito).

0261-2194/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0261-2194(02)00032-7

Ca-gluconate, Ca-chloride, and Ca-carbonate was evaluated on Corn Meal Agar (CMA 17 g/l) and on V8 broth. For each calcium compound, a stock solution at 2400 ppm was prepared. The solution was filter-sterilized (0.22 μm pore size Millipore membrane), and aliquots were added to a molten CMA (43–45°C) to achieve a final calcium concentration of 300, 600, and 1200 ppm; then, 12 ml of salt-amended CMA was poured into 100 mm Petri dishes. Unamended CMA was used as a control. A 6-mm diameter plug of *P. nicotianae*, taken from the edge of an actively growing colony (isolate Ph 117 from the collection of the Dipartimento di Protezione delle Piante e Microbiologia Applicata, University of Bari, Italy) was centrally seeded onto the plates; for each salt concentration, five replications were prepared. Dishes were incubated at 25°C, in the dark, and the growth was assessed after 3 and 6 days by measuring two colony diameters at right angles to each other.

In cleared V8 broth (20% V8 juice Campbell, 0.2% $CaCO_3$ clarified by centrifugation) (Ribeiro, 1978) calcium salts were added to obtain the same concentration as above. V8 broth added to sterile distilled water was used as a control. Ten milliliters of clarified V8 broth and 10 ml of each salt solution were poured into 100 ml Erlenmeyer flasks and inoculated with a plug (∅ 6 mm) of the Ph 117 isolate, taken from the margin of actively growing culture. For each salt and concentration, three replications were made. Flasks were incubated at 25±1°C in the dark and after 7 days, the mycelium was collected by filtration and dried at 40°C until constant weight. At the end of the trial, the pH of each V8 broth salt solution was measured.

The activity of Ca-oxide, Ca-propionate, Ca-lactate, Ca-acetate, and Ca-gluconate, on production of zoospores and on germination of *P. nicotianae* cysts was tested following the method reported by Von Broembsen and Deacon (1996). The effect of calcium salts on zoospore production of isolate Ph 117 was indirectly evaluated by counting the number of cysts in 1 ml of zoospore suspension, after encystment induction. The five calcium salts were applied at three different times: before (treatment 0 h) and during (treatment 24 and 48 h) the procedure to induce the production of sporangia, which corresponds to the incubation for 72 h under fluorescent tubes at 3000 lux (Von Broembsen and Deacon, 1996). After the growing phase in clarified V8 broth, the mycelium mat was immediately dipped in the different salt solutions at the final calcium concentration of 1200 ppm, and then exposed for 72 h (treatment 0 h) under fluorescent tubes, or left to grow for 24 or 48 h in the mineral salt solution [$Ca(NO_3)_2$ (3.08 g), $MgSO_4$ (1.49 g) and KNO_3 (0.51 g) in 1 l of distilled water and 1 ml of chelated iron solution composed of EDTA (0.65 g), KOH (0.37 g) and $FeSO_4$ (1.24 g) in 50 ml distilled water], normally used to induce the production of sporangia (Von Broembsen and Deacon, 1996). Distilled water, instead of calcium solution, was used as a control. Cysts number was assessed with a haemocytometer after induction of zoospore encystment, which was obtained by vortexing for 70 s in Eppendorf tube containing the zoospore suspension (Von Broembsen and Deacon, 1996). For each treatment, three replications were made.

The activity of Ca-oxide, Ca-propionate, Ca-lactate, Ca-acetate, and Ca-gluconate on cyst germination was also evaluated. Forty μl of cyst suspension of isolate Ph 117 and 40 μl of each calcium salt at the final concentration of 1200 ppm or water (control) were placed together on a sterile glass slide; slides were arranged on two glass stands in a Petri dish containing a thin layer of water. Dishes were seeded and incubated for 18 h at 21±1°C. On each slide 100 cysts were observed by using a light microscope and considered to have germinated if the germ tube length equalled or exceeded the cyst diameter. For each treatment, three replications were made.

2.2. In vivo activity

The efficacy of soil amendments with Ca-sulphate, Ca-oxide, Ca-propionate, Ca-lactate, Ca-nitrate, Ca-acetate, Ca-pantothenate, Ca-gluconate, Ca-chloride, and Ca-carbonate against Phytophthora root rot was evaluated on 4-month-old sour orange seedlings, growing in a greenhouse with an average temperature of 27°C. For each Ca-compound, 15 seedlings, transplanted into a plastic pot containing 1.5 l of a sterile mixture of soil and sand (70% and 30%, respectively), were used. Ten plants were artificially inoculated in the soil with isolate Ph 117, according to the Roiger and Jeffer's method (1991), whereas five plants were transplanted into uninoculated soil and used as a control. To promote root rot disease, the seedlings were water flooded for 3 days, by placing a plastic basin under each pot and keeping the basin full of water. Following the third day, the water was poured of and no watering occurred during the subsequent 7 days. For each calcium salt, 300 ml of an aqueous solution at the final concentration of 1200 ppm was added to the soil, 5 days after the end of water stress. Eight months after inoculation, the height of the canopy, the weight of the root system, the inoculum density expressed as the number of propagules of the pathogen per gram of dry soil (IDt) and per milligram of fresh root (IDr), and the percentage of infected root segments (RI) of each plant, were assessed. The inoculum density was determined by soil dilution plate method using the selective agar media BNPRAH (Masago et al., 1977), as reported by Ippolito et al. (1992). Ten grams of 2-mm-sieved soil from each sample was diluted in 90 ml of 0.1% agar and 1 ml was spread on each plate of the selective medium. Ten plates

from each soil replicate were incubated for 2 days in the dark at 19°C, then the soil suspension was washed off the agar surface under a slow stream of tap water. The number of colonies of *P. nicotianae* per plate was expressed as propagules/g dry soil. Soil moisture was determined by desiccating 20 g of soil for 24 h at 110°C. To determine the RI value, 50 root pieces about 10 mm long, randomly taken from the roots of each plant, were seeded in Petri dishes containing BNPRAH selective medium. Plates were then incubated as reported above. At the end of the trial, the pH of each calcium-amended soil was measured following the standard procedure (Lotti and Galoppini, 1980).

2.3. Experimental design and data analysis

In vitro and in vivo trials were arranged in a completely randomised design. Each experiment was repeated at least twice with similar results; however, the reported data are from representative experiments. Data were submitted to analysis of variance and means of the treatment compared by using Duncan's Multiple Range Test.

3. Results

Ca-carbonate, Ca-oxide, Ca-propionate, and Ca-nitrate at all the concentrations tested on agar medium, significantly ($P \leqslant 0.01$) reduced the radial growth of *P. nicotianae* in comparison with the control (Table 1). Ca-oxide was the most active reducing the growth by 46–57%. Ca-acetate and Ca-sulphate significantly ($P \leqslant 0.01$) reduced the growth of the pathogen only when applied at 300 and 600 ppm, respectively (Table 1). The results of the activity of calcium salts in liquid culture are reported in Table 2. Ca-oxide at all the concentrations tested reduced the dry weight of mycelium of *P. nicotianae* by 85–87%, in comparison with the control. The other calcium salts reduced the growth of the pathogen with different results among the three concentrations used.

The effect of calcium salts on zoospore production and cyst germination is reported in Figs. 1 and 2, respectively. Ca-oxide applied before (0 h) and during (24 and 48 h) the production of sporangia, significantly ($P \leqslant 0.01$) reduced the number of zoospores in comparison with the control and the other calcium salts (Fig. 1). In particular, this salt completely inhibited the zoospore production when applied in the first two stages (0 and 24 h); on the contrary, Ca-gluconate in the first stage (0 h), Ca-lactate and Ca-acetate, in the second stage (24 h), markedly promoted the zoospore production of *P. nicotianae*, as compared to the control. Similarly, Ca-oxide significantly ($P \leqslant 0.01$) reduced cyst germination in comparison with the control and the other calcium salts; conversely, Ca-lactate and Ca-gluconate stimulated cyst germination (Fig. 2).

Table 1
Activity of different Ca-salts applied at concentrations of 1200, 600, and 300 ppm on the growth of *P. nicotianae* on agar medium

Treatment	Colony diameter (mm) Salt concentration (ppm)		
	1200	600	300
Control	31.9 A	31.9 A	31.9 A
Carbonate	25.4 CD	23.4 D	20.5 CD
Oxide	12.8 D	14.7 E	17.7 E
Propionate	26.9 B	26.5 C	26.7 CD
Sulphate	29.7 AB	27.4 BC	28.8 AB
Nitrate	27.6 B	26.8 BC	27 BC
Lactate	32.1 AB	29.7 AB	29.4 A
Acetate	27 AB	29.4 ABC	30.3 CD
Pantothenate	30.6 AB	31.1 A	28.6 A
Chloride	30.9 A	31 A	30.8 A

In each column, values marked with the same letters are not statistically different at $P \leqslant 0.01$, according to the Duncans's Multiple Range Test.

Table 2
Activity of different Ca-salts applied at concentrations of 1200, 600, and 300 ppm on the growth of *P. nicotianae* in liquid medium

Treatment	Dry weight (mg) Salt concentration (ppm)		
	1200	600	300
Control	2.66 AB	2.75 A	2.67 A
Carbonate	2.25 CD	2.25 BC	2.11 ABC
Oxide	0.37 E	0.31 E	0.29 D
Propionate	1.94 D	2.22 BC	2.49 AB
Sulphate	2.36 AC	1.88 CD	2.2 ABC
Nitrate	2.33 BC	2.27 AB	2.39 AB
Lactate	2.56 AC	2.41 AB	1.67 C
Acetate	2.58 AC	2.37 AB	2.51 AB
Pantothenate	2.29 C	2.28 BC	2.02 BC
Chloride	2.7 A	2.52 AB	2.38 AB
Gluconate	2.32 BC	2.5 AB	2.38 AB

In each column, values marked with the same letters are not statistically different at $P \leqslant 0.01$, according to the Duncans's Multiple Range Test.

Considering the in vivo trials, sour orange seedlings treated with Ca-oxide and Ca-propionate showed higher values of canopy height and root weight than the inoculated control (Table 3). The other calcium salts did not affect the vegetative parameters of citrus seedlings, except for Ca-chloride, which induced the lowest values of root dry weight. Ca-propionate and Ca-lactate induced the lowest values of inoculum density (IDt and IDr), as compared to the inoculated control; on the contrary, Ca-gluconate, Ca-carbonate, and Ca-pantothenate induced the highest values of inoculum density. Almost all calcium salts significantly ($P \leqslant 0.01$) reduced RI in comparison with the control, except

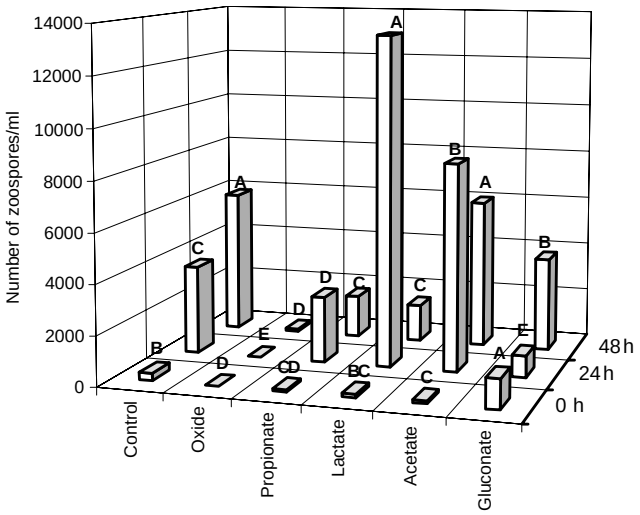

Fig. 1. Activity of different Ca-salt solutions (1200 ppm) on zoospore production. The salts were applied in three different stages of zoospore production of *P. nicotianae* (0, 24, 48 h). For each reading time, values marked with the same letter are not statistically different at $P \leqslant 0.01$, as determined by Duncan's Multiple Range Test.

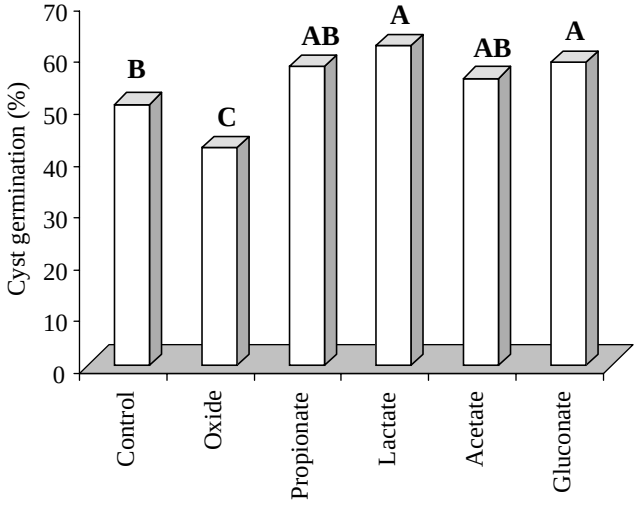

Fig. 2. Activity of different Ca-salt solutions (1200 ppm) on cyst germination of *P. nicotianae*. Values are the means of three assessments for each compound. Values marked with the same letter are not statistically different at $P \leqslant 0.01$, as determined by Duncan's Multiple Range Test.

Ca-nitrate, Ca-pantothenate, and Ca-gluconate. The lower RI values were observed in sour orange seedlings treated with Ca-sulphate, Ca-oxide, and Ca-acetate. No significant differences in soil pH were observed among treatments (Table 3).

4. Discussion

Some calcium salts effectively reduced the growth of *P. nicotianae*, both on agar and in the liquid medium. Ca-oxide, Ca-carbonate, and Ca-propionate were the most active, whereas the other Ca-salts moderately reduced the growth of the pathogen. The three concentrations used (300, 600, 1200 ppm) did not influence the activity, so an effect related to the osmotic potential should be excluded. Similar results were obtained in a trial in which the same calcium salts were assayed against *Botrytis cinerea* (McLaughlin et al., 1990). Ca-oxide and Ca-propionate also reduced zoospore production, while only the first salt reduced cyst germination. The efficacy of Ca-oxide in reducing fungal growth could be ascribed to the pH value in the media, which was 10.7 as this value is far from optimal for growth of *P. nicotianae*, namely a pH of 6–6.5 (Allen and Nandra, 1975; MacDonald and Duniway, 1978). The other research with calcium salts against *B. cinerea*, indicated that the effects were due not only to the pH but also to the ionic components, adversely affecting enzyme activities of the pathogen (Biggs et al., 1997, Nigro et al., 1998; Miceli et al., 1999). For Ca-propionate, in particular, the mechanism of action seems to be related to its lipophilic property, which is capable of interfering with the permeability of the microbial cell membrane (Biggs et al., 1997). However, in a research on effects of inorganic salts against sclerotia germination of *Sclerotium rolfsii*, only Ca-carbonate and Ca-bicarbonate had a fungicidal effect at pH 8.6, but not at 6.0, probably because of the prevalence of free HCO_3^- and CO_3^{2-} at the high pH value (Punja and Grogan, 1982).

The most effective Ca-salts in reducing the growth of the pathogen in liquid and solid media were also effective in reducing zoospore production. In particular, Ca-oxide almost completely inhibited zoospore production and interfered with the sporangia formation, which showed deep morphological alterations and were easily detachable; on the contrary, compounds such as Ca-gluconate, Ca-lactate, and Ca-acetate, increased zoospore production and cyst germination as compared to the control. The activity of these compounds in promoting production of propagules of the pathogen, not previously reported in literature, could be useful for in vitro production of zoospores of *P. nicotianae*. The different behaviours among Ca-compounds seem to further support the role of the anionic components as reported above, although in literature also there are evidences on the role of calcium ion: at a concentration from 2 to 10 mM, calcium ions promoted sporangia formation (Allen and Nandra, 1975), while higher concentration of this ion can reduce zoospore release from sporangia (Von Broembsen and Deacon, 1997) and their mobility (Byrt et al., 1982; Von Broembsen and Deacon, 1996). On the basis of these indications, almost complete inhibition of propagule production due to Ca-oxide could be due to the high Ca^{2+} concentration that for this Ca-compound was more than 20 mM.

Table 3
Activity of different Ca-salt solutions (1200 ppm) against *P. nicotianae* root rot of sour orange rootstock vegetating in soil mixture artificially inoculated with *P. nicotianae*

Treatment	Canopy		Root	Inoculum density		Root infection	Soil pH
	Height (cm)	Fresh weight (g)	Dry weight (mg)	(IDt)	(IDr)	(RI)	
Control (not inoculated)	8.6 ABC	1.4 AB	0.24 AB	—	—	—	7.41
Control (inoculated)	8.2 BC	1.2 ABC	0.18 ABC	3.8 CD	9.4 EF	7.4 A	7.41
Sulphate	7.8 C	1.2 ABC	0.21 ABC	4.9 BCD	14.2 CDE	0.8 C	7.39
Carbonate	8.1 BC	0.8 C	0.16 BC	6.2 B	22.9 B	2.4 BC	7.38
Oxide	9.6 A	1.4 AB	0.27 A	4.3 BCD	12.9 DEF	0.8 C	7.54
Propionate	9.5 A	1.7 A	0.24 AB	1.4 E	2.1 H	2.0 BC	7.47
Lactate	8.5 ABC	1.1 BC	0.22 ABC	1.1 E	2.6 GH	2.0 BC	7.46
Nitrate	9.1 ABC	1.1 BC	0.20 ABC	4.2 BCD	15.0 CDE	3.8 ABC	7.46
Acetate	8.4 ABC	1.1 BC	0.21 ABC	5.5 BC	16.0 CD	0.8 C	7.46
Pantothenate	9.2 AB	1.4 AB	0.20 ABC	6.0 B	18.8 BC	6.0 AB	7.43
Chloride	9.2 AB	0.8 C	0.14 C	4.4 BCD	24.3 B	3.0 BC	7.42
Gluconate	8.5 ABC	0.9 BC	0.18 ABC	13.0 A	53.8 A	3.8 ABC	7.41

Plant height, fresh weight of canopy, dry weight of roots system, inoculum density as the number of propagules of the pathogen/g of dry soil (IDt) and per milligram of fresh roots (IDr), percentage of infected root segments, and soils pH, are reported.

In each column, values marked by the same letters are not statistically different at $P \leqslant 0.01$, as determined by Duncan's Multiple Range Test.

About the in vivo trial (Table 3), Ca-compounds did not significantly influence the growth of sour orange plantlets, although seedlings transplanted in Phytophthora-infested soil treated with Ca-oxide and Ca-propionate showed a greater development. The lowest inoculum density of the pathogen was found in Ca-propionate-treated soil; while the degree of root infection was lower in almost all the treated plants, in comparison with the inoculated control. The treated soils showed no significant pH differences at the end of the trial, probably due to the high buffering capacity of the soil. The soil pH near the neutrality could partially explain the lack of activity of Ca-oxide in reducing propagule density of the pathogen as expected from the results of the in vitro trials. Ca-propionate confirmed its suppressive activity on production of propagules of the pathogen, probably because the mechanism of action was related to its lipophilic activity, rather than to the pH value. The results obtained with Ca-lactate that, as opposed to the in vitro trials, provided very low values of inoculum density, seems at present difficult to explain.

Almost all the Ca-treated plants showed a lower degree of root infection in spite of the high level of propagules in the soil. Generally, a high level of propagules of the pathogen in the soil corresponds to a high degree of root infection (Ippolito et al., 1991). Therefore, these results seem to point out an increased resistance by the sour orange feeder roots. A specific effect of strengthening of plant tissues to invasion by pathogens is widely documented for calcium ions. Calcium is a normal constituent of the cell wall and middle lamella and the relationship between calcium ions and pectins in the cell wall could partially explain the increased resistance to invasion by *P. nicotianae* (Conway et al., 1992). High levels of calcium are associated to the decrease in the host tissue of hydrosoluble pectins/pectate ratio, the latter being more stable and resistant to hydrolytic enzymes produced by decay-causing pathogens; moreover, calcium also seems to induce the synthesis of phytoalexins and/or phenolic substances (Kohle et al., 1985). In addition, high calcium level in the soil is one of the characteristics of *P. cinnamomi* suppressive soil (Broadbent and Baker, 1974) and more recently, an investigation carried out in citrus orchards located in Apulia and Basilicata, Italy, pointed out a negative correlation between calcium content in the soil and disease level (Ippolito et al., 1990a, b).

In conclusion, these trials further confirm that there is no correspondence between results obtained in vitro and those obtained in vivo, probably because of various factors acting in the soil environment. Most of the calcium salts utilized were effective in reducing the degree of root infection although the population density of the pathogen remained high. The use of higher concentration of the most active Ca-salts should reduce the inoculum density of the pathogen, but an increase of pH value in the soil above 8.5 should be avoided, considering the possible manifestation of toxicity and/or nutritional disorder in citrus plants (Chapman, 1968). Further researches are needed in order to confirm the behaviour of the most active calcium salts in field trials.

References

Allen, D.J., Nandra, S.S., 1975. Effects of pH and calcium concentration on the sporulation of *Phytophthora* isolates from agave. Plant Dis. Rep. 59, 555–558.

Biggs, A.R., El-Kholi, M.M., El-Neshawy, S., Nickerson, R., 1997. Effects of calcium salts on growth, polygalacturonase activity, and infection of peach fruit by *Monilinia fructicola*. Plant Dis. 81, 339–403.

Broadbent, P., Baker, K.F., 1974. Behaviour of *Phytophthora cinnamomi* in soil suppressive and conductive to root rot. Aust. J. Agric. Res. 25, 121–137.

Brunelli, A., 1995. I prodotti naturali nella lotta alle malattie fungine. La Difesa delle Piante. 18 (2), 57–69.

Byrt, P.N., Irving, H.R., Grant, B.R., 1982. The effect of cations on zoospores of the fungus *Phytophthora cinnamomi*. J. Gen. Microbiol. 128, 1189–1198.

Chapman, H.D., 1965. Chemical factors of the soil as they affect micro-organisms. In: Baker, K.F., Snyder W, .C. (Eds.), Ecology of Soil-Borne Plant Pathogens. University of California, Berkeley, CA, pp. 120–139.

Chapman, H.D., 1968. The mineral nutrition of citrus. In: Reuther, W., Batchelor, L.D., Webber, H.J. (Eds.), The Citrus Industry., Vol. 2. University of California, Berkeley, CA, pp. 86–122.

Conway, W.S., Sams, C.E., McGuire, R.G., Kelman, A., 1992. Calcium treatment of apples and potatoes to reduce postharvest decay. Plant Dis. 76 (4), 329–334.

Ippolito, A., De Cicco, V., Salerno, M., 1990a. Role of *Phytophthora* spp. in citrus decline in Apulia and Basilicata, Italy. Bull. OEPP/EPPO Bull. 20, 91–94.

Ippolito, A., De Cicco, V., Vanadia, S., Mauroantonio, V., Salerno, M., 1990b. Inoculum density and citrus root infection of *Phytophthora* spp. as related to same chemical and physical soil factors. Proceedings of the Eighth Congress of the Mediterranean Phytopathological Union, October 28–November 3, pp. 99–101.

Ippolito, A., De Cicco, V., Salerno, M., 1991. Aspetti eziologici ed epidemiologici del marciume radicale degli agrumi da *Phytophthora* spp. in Puglia e Basilicata. Phytopath. Mediterr. 30, 47–51.

Ippolito, A., De Cicco, V., Salerno, M., 1992. Seasonal variation in root infections and population levels of *Phytophthora* spp. in citrus orchards of Apulia and Basilicata, Italy. Riv. Pat. Veg., S.V 2, 57–65.

Ippolito, A., Nigro, F., Lima, G., 1996. Efficacia di Fosetyl-Al e di Metalaxyl contro il marciume radicale da *Phytophthora* spp. in piante di clementine innestate su arancio amaro. La Difesa delle Piante 19 (2–3), 81–88.

Ippolito, A., Nigro, F., Lima, G., 1997. Influence of the scion on the susceptibility of sour orange rootstock to Phytophthora gummosis and root rot. Phytopath. Mediterr. 36, 81–86.

Ippolito, A., Nigro, F., Ligorio, A., Schena, L., Campanella, V., Liguori, R., 2000. Effectiveness of Metalaxyl-M against Phytophthora root rot of citrus. Proceedings of the International Society of Citriculture, Orlando, FL, December 3–9.

Kohle, H., Jblick, W., Poten, F., Blaschek, W., Kauss, H., 1985. Chitosan-elicited callose synthesis in soy bean cells as a Ca^{2+} dependent process. Plant Physiol. 77, 544–551.

Lotti, G., Galoppini, C., 1980. Misure chimico-fisiche. In: Guida alle Analisi Chimico Agrarie. Edagricole, Bologna, pp. 94–104.

MacDonald, J.D., Duniway, J.M., 1978. Influence of soil texture and temperature on the motility of *Phytophthora cryptogea* and *P. megasperma* zoospores in soil. Phytopathology 69, 436–441.

Masago, H., Yoshikava, M., Fukada, M., Nakanishi, N., 1977. Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. Phytopathology 67, 425–428.

McLaughlin, R.J., Wisniewski, M.E., Wilson, C.L., Chalutz, E., 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. Phytopathology 80, 456–461.

Miceli, A., Ippolito, A., Linsalata, V., Nigro, F., 1999. Effect of preharvest calcium treatments on decay and biochemical changes of table grape during storage. Phytopathol. Mediterr. 38, 47–53.

Nigro, F., Ippolito, A., Ligorio, A., Romanazzi, G., 1997. In vitro and in vivo effectiveness of different salts toward Botrytis storage rot of table grapes. Preliminary results. Proceedings of the COST 914-COST 915 on Non Conventional Methods for the Control of Post-Harvest Disease and Microbiological Spoilage, October 9–11, Bologna, Italy, pp. 175–180.

Nigro, F., Ippolito, A., Romanazzi, G., Ligorio, A., 1998. Attività di sali contro il marciume botritico dell'uva da tavola in postraccolta. Proceedings of the II Workshop Nazionale "Postraccolta dei prodotti ortofrutticoli", Vallo di Diano (Sa), 1–2 October, p. 22 (Abstract).

Punja, Z.K., Grogan, R.G., 1982. Effects of inorganic salts, carbonate-bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. Phytopathology 72, 635–639.

Ribeiro, O.K., 1978. In: Cramer, J. (Ed.), A source book of the genus *Phytophthora*. Vaduz, Liechtenstein, pp. 417pp.

Roiger, D.J., Jeffer, S.N., 1991. Evaluation of *Trichoderma* spp. for biological control of *Phytophthora* crown and root rot of apple seedlings. Phytopathology 81, 910–917.

Salerno, M., Cutuli, G., 1981. The management of fungal and bacterial diseases of citrus in Italy. Proc. Int. Soc. Citriculture 1, 360–362.

Von Broembsen, S.L., Deacon, J.W., 1996. Effects of calcium on germination and further zoospore release from zoospore cysts of *Phytophthora parasitica*. Mycol. Res. 100 (12), 1498–1504.

Von Broembsen, S.L., Deacon, J.W., 1997. Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. Phytopathology 87, 522–528.

Wisniewski, M., Droby, S., Chalutz, E., Eilam, Y., 1995. Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum* in vitro and on the biocontrol activity of *Candida oleophila*. Plant Pathol. 44, 1016–1024.

Yuen, M.C., 1993. Postharvest handling of tropical fruits. In: Proceedings of the International Conference on Postharvest Handling of Tropical Fruit, July 19–21, Chaing Mai, Thailand.

Zentmyer, G.A., Lewis, L.L., 1975. Avocado root rot research program. Calif. Avocado. Soc. Yearb. 58, 89–92.