Use of Soil-Applied Calcium Chloride to Reduce Fire Blight and Brown Spot Susceptibility of Pear

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Abstract

The pear industry in Italy's north-eastern Po river region suffers from important diseases such as bacterial fire blight (*Erwinia amylovora*) and fungus brown spot (*Stemphylium vesicarium*) infections which are very difficult to control by chemical sprays. The aim of this study was to evaluate the effectiveness of soil-applied calcium chloride ($CaCl_2$) in reducing pear susceptibility to both diseases. In two distinct experiments, carried out on one-year-old, potted pear trees of cv. 'Abbé Fetel'/'Quince C', the following treatments were compared in a completely randomized design with 6 (fire blight) or 12 (brown spot) replicates: 1) control, well irrigated, 2) irrigation rate reduced by 50% of the control; and 3) full irrigation rate with water solution of $CaCl_2$ to obtain an electric conductivity of 8.3 mS cm^{-1}. Treatments were imposed when shoot length was around 10 cm and continued for 4-5 weeks. *E. amylovora* inoculum ($7x10^8$ cfu ml^{-1}) was applied on 3 apical leaves per shoot (3 shoots per tree), and the progressions of shoot blight symptoms were assessed after 8, and 21 days. *S. vesicarium* was applied by spraying an aqueous suspension of conidia ($2x10^4$ ml^{-1}), and disease incidence was determined as a percentage of infected leaves. Soil-applied $CaCl_2$ was effective in reducing pear susceptibility to both fire blight and brown spot as compared to control trees. A 50%-reduction of water supply decreased fire blight but not brown spot incidence. Soil-applied $CaCl_2$ and the reduction of water supply decreased shoot length, number of leaves and stem water potential as compared to control, although did not affect leaf osmotic potential. Pear susceptibility to fire blight and brown spot was positively related to stem water potential and negatively to leaf Ca concentration.

INTRODUCTION

In Italy, fire blight (*Erwinia amylovora*) and brown spot (*Stemphylium vesicarium*) are two of the most important diseases that cause heavy losses to the pear industry. Chemical control of both diseases is very difficult, and the increasing demand for sustainable orchard management drives research toward integrated control strategies, including different agronomic tools. Recently, a positive effect of salinity on pear resistance to fire blight was demonstrated (Toselli et al., in press), although it was not clear if this effect was related to leaf osmoregularity induced by an increased leaf concentration of sorbitol (Bieleski, 1982; Suleman and Steiner, 1994) or simply**,** by a lower tree water potential. Plants tolerate soil salinity induced by $CaCl_2$ better than that induced by NaCl (Bressan et al., 1998). The main aim of this study was to assess the effectiveness of soil salinity induced by $CaCl_2$ in lowering pear susceptibility to fire blight and brown spot.

MATERIAL AND METHODS

Fire Blight

Eighteen one-year-old pear trees of cv. 'Abbé Fétel'/ 'Quince C', potted in 20 liter pots filled with peat, sand and soil (1:1:1, v:v:v) and trained to 10 shoots, were treated

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outdoors for 4-5 weeks, starting when shoots were about 10 cm long, with one of the following soil water management: 1) control, well watered; 2) irrigation rate reduced by 50% (RIR-50%) of the control; 3) full irrigation rate with water solution of $CaCl_2$ to obtain a final electric conductivity of 8.3 mS cm^{-1}. To avoid any uncontrolled water supply by rain each pot was enclosed in a plastic bag. In a greenhouse chamber with controlled air temperature (25 °C) and humidity (80 %), trees were inoculated with young *Erwinia amylovora* cells, strain OMP-BO 1077.7/94 (7 x 10^8 cfu ml^{-1}) by cutting the apical portion of the 3 youngest leaves per shoot (3 shoots per tree) with scissors dipped in the bacterial suspension and symptom progression in shoots was determined as length of necrosis 8 and 21 days after experimental inoculation. Before inoculation, shoot length was recorded. Stem water potential (Ψw) was measured according to Naor et al. (1995) on one fully expanded healthy leaf per tree, wrapped with aluminum foil and enclosed in a plastic bag for 90 minutes. Leaves were then cut off from the shoot using a sharp blade, and the leaf lamina was immediately inserted into the pressure chamber, where the pressure was increased slowly until the first drop of water was forced out of the petiole (Scholander et al., 1965). Leaf osmotic potential (Ψs) was measured on the same leaf used for Ψw and stored at -20°C. After thawing, the leaf was squeezed with a garlic press to extract sap. The Ψs of 100 µl of diluted sap from each leaf was determined immediately without centrifugation by a micro osmometer 5B (Roebling, Analitical Control, Milan, Italy).

Brown Spot

Thirty-six one-year-old pear trees of cv. 'Abbé Fétel'/ 'Quince C', potted in 20 liter pots filled with soil and sand (1:1, v:v) and trained to 8-10 shoots were treated outdoors with the same treatments as in the fire blight experiment. After 5 weeks of treatments, trees were enclosed in a chamber of plastic film to increase relative humidity and inoculated at sunset by spraying a *Stemphylium vesicarium* suspension (2 x 10^4 conidia ml^{-1}) on the leaves. Plastic chamber was removed the following morning and brown spot incidence was determined as a percentage of infected leaves 23 days after inoculation. Prior to experimental inoculation, leaf samples (25 leaves per tree) were collected to determine leaf concentration of carbohydrates, calcium (Ca), chlorine (Cl), potassium (K) and nitrogen (N). Alcohol soluble sugars (fructose, glucose, sucrose) and sugar alcohols (sorbitol and inositol) were determined by gas chromatography. Leaf Ca and K concentrations were determined by flame atomic absorbtion spectrophotometry, Cl was evaluated, after extraction with water at room temperature, by ionic chromatography, and N was analyzed by Kjeldahl technique.

Analysis of variance was used, separately for the two experiments, to compare treatment effects and means were separated according to Least Statistical Difference (LSD) test ($P \leq 0.05$).

RESULTS

Soil-applied $CaCl_2$ was effective in lowering pear susceptibility to fire blight (Table 1) and brown spot (Table 2). A 50%-water supply reduction significantly lowered pear susceptibility to fire blight but not to brown spot (Tables 1 and 2). Unlike leaf osmotic potential (Ψs), stem water potential (Ψw) was decreased by soil-applied $CaCl_2$ and by a 50%-water supply reduction (Table 3). Soil-applied $CaCl_2$ and a 50%-water supply reduction limited vegetative growth (Tables 1 and 2), however, salt application was not phytotoxic. While increasing Ca and Cl leaf concentration, soil-applied $CaCl_2$ decreased leaf K concentration. Leaf N concentration was increased by both $CaCl_2$ application and a 50% water supply reduction (Table 4). Leaf sorbitol, inositol, glucose, fructose and sucrose were statistically unaffected by treatments (data not reported). The percentage of leaves showing brown spot lesions (data not reported), as well as the length of necrosis on blighted pear shoots were positively related with stem Ψw (Fig. 1). Leaf Ca concentration was negatively related to the percentage of leaves with brown spot lesions (Fig. 2).

DISCUSSION AND CONCLUSIONS

The effectiveness of $CaCl_2$ in lowering susceptibility of pear to fire blight might be related to soil salinity which reduced water availability and tree Ψw. A similar response was already observed (Toselli et al., in press) and it is in agreement with van der Zwet and Keil (1979) who reported, in commercial US pear orchards, a moderate water stress as an effective means of limiting fire blight symptom progression. This was also indirectly supported by the effect of the reduction of water supply that similarly lowered stem Ψw and susceptibility to the disease. On the other hand, $CaCl_2$ but not a 50%-reduction of water supply was effective in giving a resistance response to brown spot. This response might find an explanation on the high leaf Ca concentration induced by soil application of $CaCl_2$. It is well known the positive effect of Ca bound as a pectate in the middle lamella for the strengthening of the cell wall and plant tissue. The large proportion of calcium pectate in leaf cell wall of plants receiving high levels of Ca is also of importance for susceptibility of tissue to fungal and bacterial infections (Marschner, 1995). Beside that, Bressan et al. (1998) stressed that when plants are challenged with salinity stress, an increase in the concentration of Ca can often ameliorate the inhibitory effect on growth through a membrane stabilization and signaling roles. A number of cell responses appear to be mediated and/or stimulated directly by an elevated cytosolic concentration of Ca^{2+} ions: among these, a pathogen-induced oxydative burst, catalized by an activated NADPH oxydase after an essential Ca^{2+} influx, and reactive oxygen species (ROS; such as O_2^- and H_2O_2) occurring in cells during defense responses (Hutcheson, 1998; Sagi and Fluhr, 2001). Leaf osmotic potential (Ψs) was not associated with disease susceptibility; in particular, sorbitol leaf concentration did not significantly increase in treated trees. These findings confirm former studies on pear tolerance to fire blight under soil salinity (Toselli et al., in press) and, at the same time, disagree with Suleman and Steiner (1994) who explained the lower susceptibility of old apple leaves to fire blight as a result of the a higher concentration of sorbitol compared with young leaves. Agronomic strategies to control fire blight and brown spot should include an irrigation management that prevent any water excess and, possibly, an adequate Ca supply.

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Tables

Table 1. Effect of $CaCl_2$ and water supply on shoot length and necrosis progression 8 and 21 days after inoculation with *E. amylovora.*

Treatment	Shoot length (cm)	Necrosis length (cm)	
		8 days	21 days
Control	68.3a[1]	16.5a	29.5a
$CaCl_2$	59.3b	6.2b	15.1b
RIR 50%	59.9b	7.1b	11.6b
Significance	**	**	**

[1]Values followed by the same letter are not different (P≤ 0.05).

Table 2. Effect of $CaCl_2$ and water supply on total number and percentage of leaves infected by brown spot.

Treatment	Leaves	
	Total	Infected (%)
Control	223a[1]	21.3a
$CaCl_2$	191b	10.6b
RIR 50%	173b	18.4a
Significance	***	**

[1]Values followed by the same letter are not different (P≤ 0.05).

Table 3. Effect of $CaCl_2$ and water supply on stem water potential (*Ψw*) and leaf osmotic potential (*Ψs)* of tree infected by *E. amylovora.*

Treatment	Stem *Ψw* (-MPa)	Leaf *Ψs* (-mOsm L^{-1})
Control	0.87b[1]	628
$CaCl_2$	1.15a	654
RIR 50%	1.39a	613
Significance	*	ns

[1]Values followed by the same letter are not different (P≤ 0.05).

Table 4. Effect of soil-applied $CaCl_2$ and water supply on leaf Ca, K, N and Cl of tree infected by brown spot.

Treatment	Ca (%)	K (%)	N (%)	Cl (mg kg^{-1})
Control	1.31b[1]	2.17a	2.15b	624b
$CaCl_2$	1.59a	1.70b	2.45a	4451a
RIR 50%	1.45ab	1.96a	2.55a	897b
Significance	**	**	**	*

[1]Values followed by the same letter are not different (P≤ 0.05).

Figures

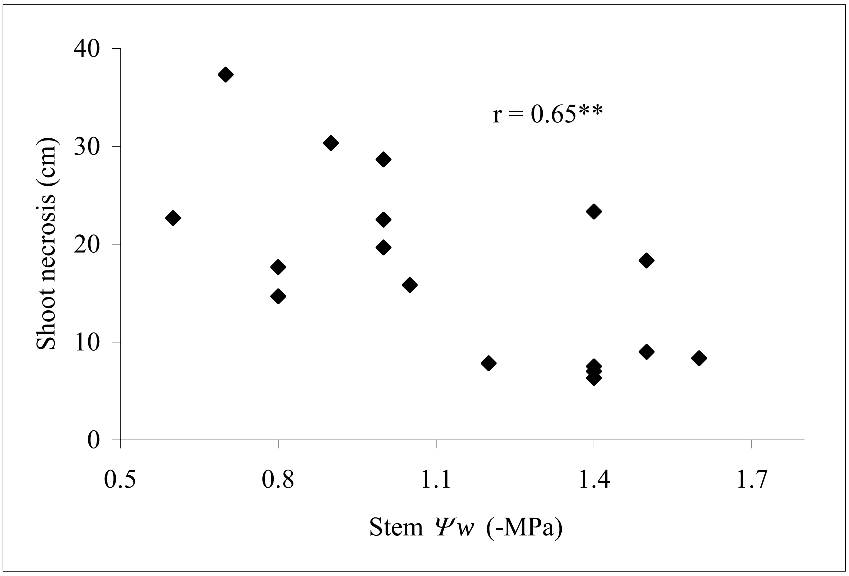

Fig. 1. Relationship between stem water potential (*Ψw*) and length of shoot necrosis, 21 days after inoculation with *E. amylovora*.

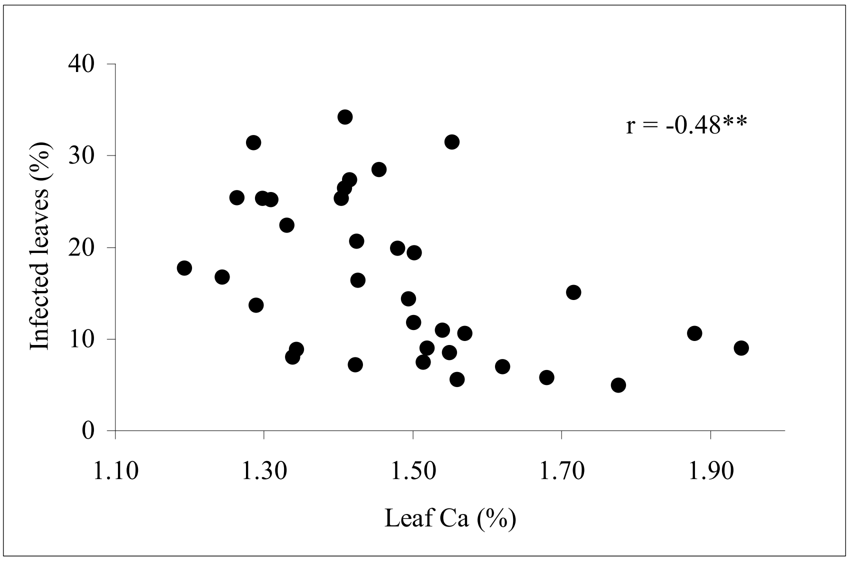

Fig. 2. Relationship between leaf Ca concentration and brown spot incidence on leaves.