Scientia Horticulturae 125 (2010) 775–778

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Effects of conventional and organic nitrogen fertilizers on soil microbial activity, mycorrhizal colonization, leaf antioxidant content, and *Fusarium* wilt in highbush blueberry (*Vaccinium corymbosum* L.)

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ARTICLE INFO

Article history:
Received 15 December 2009
Received in revised form 28 April 2010
Accepted 30 April 2010

Keywords:
Antioxidants
Blueberry
Conventional and organic farming systems
Mycorrhiza
Pot experiment
Soil biological activity

ABSTRACT

A study was conducted in the greenhouse to determine the effects of conventional and organic nitrogen (N) fertilizer on *Fusarium* wilt, caused by *Fusarium solani*, in northern highbush blueberry (*Vaccinium corymbosum* L. 'Legacy'). Root colonization by mycorrhizal fungi, soil microbial activity, and leaf antioxidants content was also measured the treatments. Plants grown with organic N fertilizer exhibited a lower incidence of *Fusarium* wilt than those grown with conventional N fertilizer (5% and 30% by the end of the experiment, respectively). Organic fertilizer also increased the soil biota activity, mycorrhizal colonization, and leaf antioxidant content relative to conventional N source. The results suggest that organic N fertilizer may improve the tolerance of blueberry to soil pathogens, such as *F. solani*.

1. Introduction

In the last 5 years, a significant number of Chilean conventional blueberry growers (near to 900 ha) have transitioned from conventional to organic management (Eguillor, 2008). Observations and farm registers suggest that once these farms are under organic management, some diseases are less frequent and the plants exhibit less pathogen damage (Montalba et al., 2008). Previous research in a number of crops suggests a consistent reduction of soil-borne diseases when managed organically instead rather than conventionally (van Bruggen, 1995). Several hypothesis have been proposed to explain the reduction of diseases in organically grown crops: (i) organic soil amendments coupled with no pesticides increase both microorganism diversity and biological activity in the soil (Zhu et al., 2003; van Bruggen and Semenov, 2000), which decreases the *inoculum* of diseases by competition and antibiosis (Bailey and Lazarovits, 2003; Raviv, 2008); (ii) organic agriculture increases defense mechanisms of plants, such as antioxidants production (Amor et al., 2008), and the increase of these compounds in turn reduces the incidence and intensity of damage caused by plant pathogens (Hain et al., 1993), and/or (iii) plants colonized by mycorrhizal fungi protect the plants from diseases (Azcón-Aguilar and Barea, 1997; Matsubara et al., 2004).

We hypothesize that the reduction of diseases related to organic management is specifically linked to the fertilization management and particularly to N fertilizer. In order to explore this effect, we conducted a pot experiment to compare conventional vs. organic N fertilization on *Fusarium* disease incidence in blueberry.

2. Materials and methods

2.1. Experimental design and plant growth conditions

Between September 2006 and May 2007, we conducted an experiment at the greenhouse facilities of the Universidad de La Frontera, located in southern Chile (latitude 38°45′S; longitude 72°37′W). We used highbush blueberry plants (*Vaccinium corymbosum*), 'Legacy', which we obtained by *in vitro* cultivation and grew in sterile soil substrate (1/3 organic soil and 2/3 pine leaves). In September 2006, we transplanted 40 healthy plants that were homogeneous in size, into individual 35 l pots filled with acidic Andisol (pH 5.5; Organic Matter%10.2; P-Olsen 20 $\mu g\ g^{-1}$). An aqueous suspension in sterile distilled water containing approximately 10^8 spores ml^{-1} of *Fusarium solani* was prepared from cultures

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doi:10.1016/j.scienta.2010.04.046

grown in potato dextrose agar (PDA, DIFCO) for 1 week at 27 °C and 2.5 ml of this suspension were inoculated per pot.

Each plant was grown with either conventional or organic N fertilizer. The fertilizer treatments were arranged in a randomized complete block design, with four replications and five plants per replication. The conventional N treatment was fertilized with ammonium sulfate added at a rate equivalent to 60 N ha^{-1} (i.e., 15 g/plant); the fertilizer was applied as a liquid in equal parts every 10 days through irrigation (i.e., 1.5 g/plant at each application). A mix of organic fertilizers (Compost 300 g/plant, N content 1.5%; blood meal 80 g/plant, N content 8%; legume flour 150 g/plant, N content 4%) were applied to the organic N treatment at a rate also equivalent to 60 kg N ha^{-1}; all the organic fertilizers were applied at once on the soil pots surface on the same day that the first dose of fertilizer was applied to the conventional N treatment.

Each plant also received the equivalent of 60 kg ha^{-1} P, applied as triple superphosphate, and 60 kg ha^{-1} K applied as sulfate potassium. In the conventional treatment the total amount of P and K was applied using these commercial fertilizers while in the organic treatment we (subtracted the amount of P and K provided by the compost (P, 0.8% and K, 0.5%) and legume flour (K, 0.9%).

2.2. Growth conditions and plant nutrients

The plants were placed in a greenhouse at planting with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 E m^{-2} s^{-1}, 400–700 nm, with a 16/8 h day/night cycle and a day/night temperature of 25/19 °C and an average relative humidity of 70%. Plant growth was determined in both treatments by counting the number of canes per plant and measuring the canes length and diameter every 30 days after planting. Plants were destructively harvested at the end of the experiment, separated into shoots and roots, dried at 60 °C for 72 h, and weighed. Additionally, the nutrient content of the leaves (N, P, K, Ca, and Mg) was determined by a leaf nutrients analysis taken in January 12, 2007.

2.3. Rizospheric biological activity

We sampled soil from each pot at 100 days after planting and determined FDA hydrolysis according to the method described by Schnurer and Rosswall (1982). Results were expressed as μg of fluorescein per gram of dry soil.

2.4. Antioxidant content

Just prior to the final destructive harvest, 25 mature leaves were sampled from the upper third part of the main stem of each plant and analyzed for total antioxidant content. Plants with symptoms of *Fusarium* wilt were excluded from this process. Antioxidants were determined by measuring free radical scavenging activity using the radical chromogen 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, St. Louis, MO, USA) photometric assay adapted from Grzegorczyk et al. (2007). Free radical scavenging capacity was calculated and expressed in percentage of antioxidant activity.

2.5. Root mycorrhizal colonization

At the end of the experiment we collected samples from the active roots of each plant to evaluate the level of mycorrhizal colonization. The root samples were taken from the entire root system at random, were cleared in KOH and stained with trypan blue in lactic acid (Phillips and Hayman, 1970), and the percentage of root length colonization was measured by the gridline intersect method under a binocular stereo microscope (Olympus SZ-PT) at 10× magnification (Giovanetti and Mosse, 1980).

2.6. Fusarium incidence determination

We determined the incidence of *Fusarium* disease in the plants every week during the experiment, by visually observing the plants for any wilt symptoms in them.

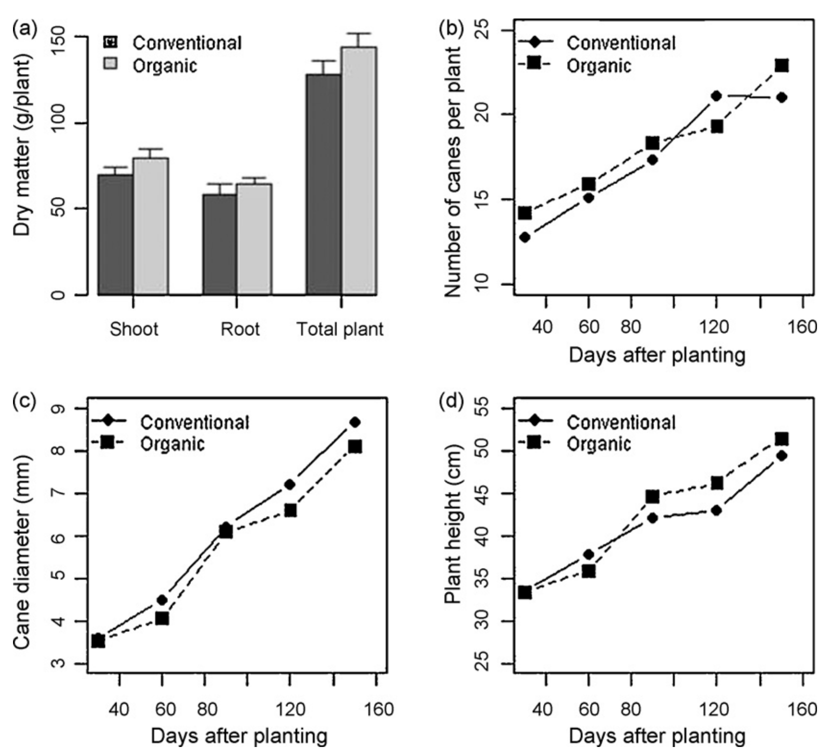

Fig. 1. Mean (a) dry matter of the shoot, roots, and total plant, (b) number of canes per plant, (c) cane diameter, and (d) average of height of 'Legacy' blueberry plants grown with either conventional or organic N fertilizer. All growth variables showed no significant difference between conventional and organic treatments. The data are the means of four replicates and error bars represent ± one standard error.

Table 1
Effect of conventional and organic N fertilizer on leaf macronutrient concentrations in 'Legacy' blueberry. Normal ranges are based on Hart et al. (2006). The data are the means of four replicates ± standard errors of means.

N fertilizer	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Conventional	1.84 ± 0.06	0.19 ± 0.03	0.49 ± 0.03	0.48 ± 0.04	0.11 ± 0.03
Organic	1.79 ± 0.09	0.19 ± 0.05	0.56 ± 0.04	0.55 ± 0.03	0.12 ± 0.04
Normal ranges	1.76–2.0	Above 0.10	0.41–0.70	0.41–0.8	0.13–0.25

Table 2
Rizopheric soil biological activity, root mycorrhizal colonization, and leaf antioxidant content of 'Legacy' blueberry plants grown with either conventional or organic N fertilizer. The data are the means of four replicates ± standard error.

Measured Variables	Conventional N fertilizer	Organic N fertilizer
Soil biological activity (fluorescence, $\mu g\, g^{-1}$ dry soil)	46.1 ± 2.3	76.9 ± 3.2
Root mycorrhizal colonization (%)	17.5 ± 3.8	36.4 ± 5.2
Leaf antioxidant activity (consumed DPPH, %)	47.3 ± 2.4	77.6 ± 3.1

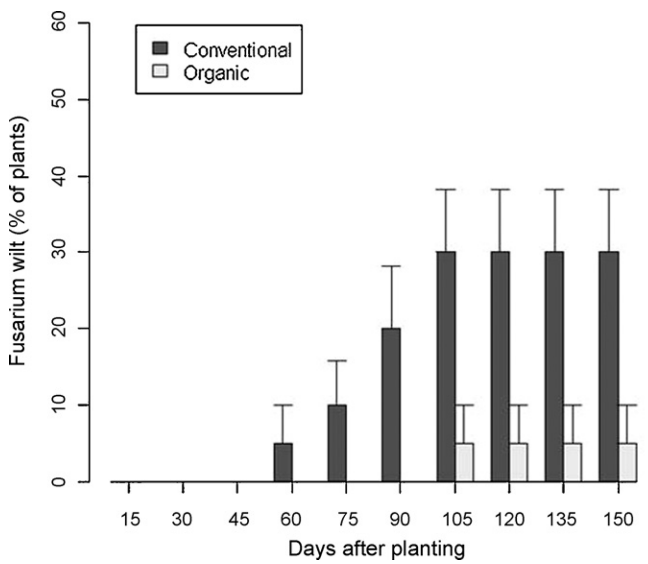

Fig. 2. Incidence of *Fusarium* wilt (caused by *F. solani*) in 'Legacy' blueberry grown with either conventional or organic N fertilizer. The data are the means of four replicates and error bars represent ± one standard error.

2.7. Statistical analysis

Data was analyzed by analysis of variance (ANOVA) using a randomized complete block design and repeated measures for each cane measurement and incidence of *Fusarium* wilt.

3. Results

Growth of healthy plants was similar between the conventional and organic N fertilizer treatments. All growth variables (dry matter, number of canes per plant, cane diameter, and plant height) were not significantly different between treatments (Fig. 1). Leaf nutrient concentrations were also similar between the treatments and were within normal range recommended for each nutrient according Hart et al. (2006) (Table 1).

Soil biological activity and leaf antioxidants were about 70% higher with organic N fertilizer than with conventional N fertilizer (Table 2). Mycorrhizal colonization was also higher in the organic treatment, averaging 36% with organic N fertilizer and only 17% with conventional fertilizer (Table 2).

Through periodical evaluation of the plants, we observed that since day 60 of initiating the experiment, some plants with conventional N fertilizer were affected with *Fusarium* disease, expressed symptomatically as a general wilt of the plants (Fig. 2). In plant with organic N fertilizer these symptoms were observed since day 105. At the end of the experiment 30% of the plants with conventional N fertilizer presented symptoms of *Fusarium* disease, while only 5% of the plants with organic N fertilizer developed the disease.

4. Discussion

In the present study, the use of ammonium sulfate fertilizer appeared to inhibit soil microbial activity and mycorrhizal colonization. High solubility of such fertilizers generates high soil N concentrations detrimental to many soil microorganisms and micorrhizal fungus (O'Donnell et al., 2001; Scagel, 2005). In contrast, organic amendments (e.g., compost) and fertilizers often promote activity and diversity of these microorganisms due to low-solubility and slow release of N and other nutrients released by microbial degradation of the organic compounds (Goulart et al., 1995; van Bruggen and Semenov, 2000). The higher levels of antioxidants found in plants produced under organic N fertilization is consistent with previous work by others (Rembialkowska, 2007; Amor et al., 2008; Wang et al., 2008); this effect is linked to higher levels of soluble N in the conventional sources which inhibits the synthesis of flavonoids and the establishment of anthocyanins (Mozafar, 1993; Dixon and Paiva, 1995).

In this study, the reduction of *Fusarium* wilt with organic fertilizer coincided with an increase in (1) soil biological activity, (2) mycorrhizal colonization, and (3) leaf antioxidants, each of which are linked directly to plant health. High soil biological activity indicates the presence of more microorganisms and possibly more complex trophic interactions. It also increases competition for resources and antibiosis effect reducing the ability of *Fusarium* to spread successfully throughout the rhizosphere (Raviv, 2008). Several diseases have been observed to be reduced by mycorrhizal colonization in plant roots (Azcón-Aguilar and Barea, 1997). It is plausible that in our study that higher root colonization by mycorrhizal fungi with organic fertilizer is partly responsible of the lower incidence of *F. solani* observed. The reduction of *Fusarium* with organic fertilizer is also probably linked to higher antioxidant activity, since this process acts as a defensive mechanism, preventing or reducing the penetration and proliferation of plant pathogen (Hain et al., 1993; Dixon and Paiva, 1995; Boutigny et al., 2008), such as *F. solani*.

5. Conclusions

Our results support the hypothesis that organic N fertilization reduces disease development in plants. In this particular case, organic N fertilizer reduced *Fusarium* in highbush blueberry compared to a conventional N source, and this reduction was correlated with an increase in soil biota, mycorrhizal colonization, and the antioxidant content in the plants grown with organic fertilizer.

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