

## Effect of chemical resistance inducers on the control of *Monosporascus* root rot and vine decline of melon

MARIA PIA ALEANDRI, ROBERTO REDA, VINCENZO TAGLIAVENTO, PAOLO MAGRO and GABRIELE CHILOSI

Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia,  
Via S. Camillo de Lellis, 01100 Viterbo, Italy

**Summary.** The effect of the resistance inducers methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH) and dipotassium hydrogenphosphate ( $K_2HPO_4$ ) was tested by seed soaking and by foliar application to determine whether these resistance inducers controlled root rot and vine decline of melon caused by *Monosporascus cannonballus*. Tests were carried out in pots and under field conditions in a two-year trial. Application of MeJA to melon seed significantly reduced symptoms of melon root rot and vine decline in soil artificially inoculated with *M. cannonballus*, and seeds treated with BTH and  $K_2HPO_4$  produced plants with a slightly greater resistance to the pathogen. Greenhouse experiments in soil naturally infected with *M. cannonballus* in 2006 showed that MeJA treatments by seed soaking followed by foliar applications decreased the severity of the disease. In 2007, both MeJA and BTH significantly reduced root rot and vine decline, but  $K_2HPO_4$  was ineffective. The resistance inducers differentially induced the synthesis of a number of pathogenesis related (PR) protein isoenzymes, markers of induced resistance in the root system. Using MeJA to induce resistance to root rot and vine decline of melon caused by *M. cannonballus* may provide a practical supplement to an environmentally-friendly disease management when it is combined with appropriate integrated agronomic practices.

**Key words:** chemically induced resistance, pathogenesis related proteins, *Cucumis melo*, *Monosporascus cannonballus*.

### Introduction

Root rot and vine decline of melon (*Cucumis melo* L.) is one of most destructive soil-borne diseases of melon in Italy (Chilosi *et al.*, 2008). Two fungal pathogens, *Monosporascus cannonballus* and *Acremonium cucurbitacearum* are the primary causal agents of this disease (Martyn and Miller, 1996; Cohen *et al.*, 2000; García Jiménez *et al.*, 2000). Other fungal species associated with the disease are *Plectosporium tabacinum* and *Rhizopycnis vagum* (Abad *et al.*, 2000; Armengol *et al.*, 2003). In central Italy, *M. cannonballus* is the main cause of the disease (Chilosi *et al.*, 2008). Aboveground symptoms are visible just prior to harvest: rapid

wilt of plants, premature fruit ripening and a low sugar content of the fruits. The typical symptoms of the disease however occur on the root system in the soil: root lesions, root rot and loss of the smaller feeder roots. Root rot and vine decline of melon by *M. cannonballus* is difficult to control. Fumigants are the most common control method. However, fumigants have a high environmental impact and are expensive. Melon rootstocks with inbred resistance are currently the most effective means to control a number of soil-borne diseases, such as *Fusarium* wilt of melon. Unfortunately, the melon genotypes resistant to those diseases are also particularly susceptible to root rot and vine decline. Growing melon on cucurbita rootstocks is becoming one of the most promising control strategies in Spain and Israel (Cohen *et al.*, 2007; Beltràn *et al.*, 2008), but in Italy this practice is not popular with growers since it represents an additional cost and requires a greater number of agronomical inputs. As a consequence of the rapid spread of melon root rot and

---

Corresponding author: G. Chilosi  
Fax: +39 0761 357473  
E-mail: chilosi@unitus.it

vine decline and difficulties with the disease prevention, alternative means of prevention and control are necessary.

Chemical resistance inducers are an additional means to control plant diseases, within an integrated crop protection system. Resistance inducers have been effective in a number of crops, including melon. Acibenzolar-S-methyl (BTH, ASM), a synthetic analogue of salicylic acid, protected cantaloupe against *Colletotrichum lagenarium* and cucumber mosaic virus (CMV) (Smith-Becker *et al.*, 2003). A BTH pre-flowering treatment combined with a fruit dip in guazatine at harvest substantially decreased infection by *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp. and *Trichothecium* sp. in stored melons (Huang *et al.*, 2000). The resistance inducer dipotassium hydrogenphosphate ( $K_2HPO_4$ ) produced high systemic resistance in cucumber by activating mechanisms that resembled the mechanisms of necrotising microorganisms triggering systemic acquired resistance (SAR) (Orober *et al.*, 2002).  $\beta$ -aminobutyric acid (BABA) induced resistance against downy mildew in melon (Ovadia *et al.*, 2000). Induced resistance by chemicals is also a promising approach to prevent diseases caused by soil-borne pathogens (Okubara and Paulitz, 2005). When screening for resistance inducers in melon, we found that soaking melon seeds in BTH and methyljasmonate (MeJA) strongly decreased the symptoms of the soil-borne fungi *Didymella bryoniae* and *Sclerotinia sclerotiorum* (Buzi *et al.*, 2004a). MeJA also protected melon against the wilt pathogen *Fusarium oxysporum* f. sp. *melonis* (Buzi *et al.*, 2004b) and so did BABA (Ovadia *et al.*, 2000). The greater resistance that melon seedlings grown from seeds treated with BTH and MeJA had to soil-borne pathogens has been associated with a rapid increase in the activity and differential induction of the pathogenesis related (PR) proteins chitinase and peroxidase (Buzi *et al.*, 2004a). PR proteins are inducible proteins implicated in active defence against disease and they could play a key role in restricting pathogen development and spread in the plant (van Loon *et al.*, 2006).

The aim of this study was to test whether the resistance inducers MeJA, BTH and  $K_2HPO_4$  controlled root rot and vine decline of melon in pot experiments as well as under field conditions in the greenhouse in soil naturally infected with *M. cannonballus*. Isoenzyme patterns of the PR proteins  $\beta$ 1,3 glucanase, chitinase and peroxidase in melon

roots, from treated seeds, were also analysed to see if they could serve as molecular markers of induced resistance.

## Materials and methods

### Plant and treatments

Melon seeds cultivar Bingo were soaked in solutions each containing one of the chemical resistance inducers until the solution was completely absorbed (0.75 ml solution  $g^{-1}$  seeds). The following doses were used: MeJA (Sigma Chemical Co., St. Louis, USA) 45.0  $\mu$ M in 0.1% (v:v) ethanol/distilled water; acibenzolar-S-methyl (BTH) (formulated as 50% a.i. in wettable granules, Syngenta Crop Protection, Milan, Italy) 50  $\mu$ g  $ml^{-1}$ ; dipotassium hydrogenphosphate ( $K_2HPO_4$ ) (Carlo Erba Reagents, Milan, Italy) 50mM. A solution of either 0.1% ethanol or distilled water was used as a control. Treated and control seeds were then separately sown in wet sterilised vermiculite and grown in the dark at 25°C.

### Fungus and inoculation procedures

*Monosporascus cannonballus* isolate MA1 was cultured on potato dextrose agar (PDA) (Oxoid, Unipath Ltd, Basingstoke, England) at 23±2°C. Resistance against *M. cannonballus* was induced on melon plants growth in artificially inoculated soil in pots as previously described with some modifications (Chilosi *et al.*, 2008). Inoculum was prepared utilising 50 grams of millet kernels imbibed with sterilised  $H_2O$  in 250 mL Erlenmeyer flasks and autoclaving twice for 30 min at 121°C. Each flask containing this substrate was then inoculated with one agar disc (6-mm diameter) cut from the edge of an actively growing fungal culture and incubated for 10 days at 23±2°C. The experiments were carried out in 2 L pots filled with sterilised potting mix (sand, peat, soil) (2:1:1 v:v) and 5 g inoculum. Plants were grown in the greenhouse under ambient conditions. Treated plants were grown in 10 pots each per treatment; plants grown from water-treated seeds served as control. The experiments were repeated twice, in March-May of 2005 and of 2006. Plants were harvested 60 days after sowing and graded for disease incidence, root growth and biomass. After that the vines were cut, and the roots were carefully extracted from the pots. The soil was washed off with tap water, then the roots were washed in distilled water. The roots were measured and

weighed. Roots from all treatments were surface sterilised for 1 min in 1.0% NaClO<sub>2</sub>, washed with ddH<sub>2</sub>O, and then plated on PDA with 200 µg mL<sup>-1</sup> streptomycin (Sigma Chemical Co.) for re-isolation of *M. cannonballus*. Symptoms on crown, primary and secondary roots were rated on the following scale: 0, healthy; 1, 25% root surface with lesions; 2, 50% root surface with lesions; 3, 75% root surface with lesions; 4, roots extensively lesioned; 5, roots badly decayed (Figure 1). The disease index was calculated using a modification of the McKinney's formula (McKinney, 1923).

The effect of the resistance inducers on root growth was evaluated both in terms of disease index and root biomass.

#### Field trial

The effect of the resistance inducers on the root rot and vine decline of melon was evaluated in a two-year field experiment in an unheated greenhouse. The greenhouse was located in the melon producing coastal area of North Latium (central Italy, Province of Viterbo) (42°23'09.31"N; 11°30'46.10"E) in soil naturally infected with *M. cannonballus* (Chilosi *et al.*, 2008). Melon seeds cultivar Bingo were soaked in solutions containing the chemical inducers, as above. Minimal inputs were made for bed preparation and maintenance in the greenhouse in order to minimise undesired movement of soil and redistribution of inoculum. Transplanting was done in June 2006 (summer crop) and February 2007 (spring crop). Melon was grown using standard local agronomic practices. Treatments were arranged in a randomised complete block design with three replications and ten plants per replication in each year. The leaves of some of the plants within the experimental design were also sprayed with the

resistance inducers three times at two-week intervals after transplanting. At the end of each trial, the plants were harvested, graded for disease, and the pathogen was isolated as above.

#### Induced resistance markers

The activity and isoenzyme patterns of selected PR proteins, β-1,3 glucanase, chitinase and peroxidase from roots raised from treated seeds were examined to see if they could serve as markers of induced resistance (Buzi *et al.*, 2004a). Inducer treated melon seeds were planted in sterilised vermiculite, watered daily and grown in the dark at 25°C at high RH for 6 days (Chilosi and Magro, 1998). The roots were then harvested, weighted, rapidly frozen in liquid nitrogen and immediately ground to a fine powder using a chilled mortar and pestle. Chilled extraction buffer (Tris-HCl 20 mM, pH 7.8) containing polyvinylpyrrolidone (1% w:v) (Sigma Chemical Co.) was added to the powder (1 mL g<sup>-1</sup> fresh wt). Extractions were carried out at 4°C for 120 min under continuous gentle stirring. The buffer extracts were then centrifuged twice at 12,000 g for 20 min at 4°C. Supernatant protein concentrations were determined by the method of Bradford (1976).

β-1,3-glucanase activity was measured spectrophotometrically according to Kim and Hwang (1994). Reaction mixtures contained 30 µl of the enzyme and, 80 µl of 0.1 M sodium acetate buffer (pH 5.2) containing laminarin 0.1% w:v (Sigma Chemical Co.). The amount of reducing sugars released by enzymatic cleavage of laminarin was determined according to Nelson (1944). An enzyme unit was defined as the amount of enzyme that catalysed the release of reducing sugar groups equivalent to 1 µmol of glucose for 1 min under the described assay conditions. Chitinase

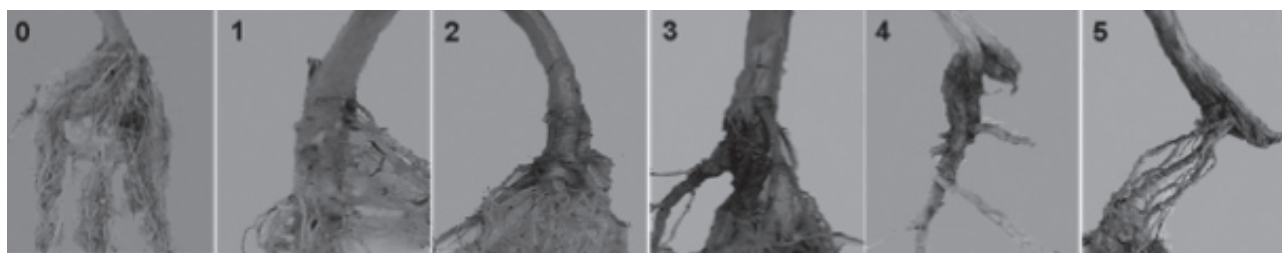


Figure 1. Representative symptoms on melon crown, primary and secondary roots upon artificial inoculation with *Monosporascus cannonballus* rated on a scale of severity: 0, healthy; 1, 25% root surface with lesions; 2, 50% root surface with lesions; 3, 75% root surface with lesions; 4, roots with very extensive lesions; 5, roots badly decayed.

activity was determined as previously reported (Fenice *et al.*, 1998) by measuring the amount of reducing sugars in a reaction mixture containing colloidal chitin in 0.05 M citrate-phosphate buffer; N-acetyl-D-glucosamine (Sigma Chemical Co.) was used for the standard curve. Under the assay conditions, one unit (U) of enzyme activity was defined as the amount of enzyme that released 1  $\mu\text{m}$  of N-D-acetylglucosamine  $\text{min}^{-1} \text{mg}^{-1}$  of protein. Peroxidase activity was assayed by measuring at 30°C the absorbance increase at 470 nm in a reaction containing 0.46 % (v:v) guaiacol (Sigma) and 13 mM  $\text{H}_2\text{O}_2$  in sodium phosphate buffer 0.1 M pH 5.4 (Magro, 1984). One unit of peroxidase activity was defined as the amount of enzyme required for the formation of 1 mM tetraguaiacol  $\text{min}^{-1} \text{mg}^{-1}$  total protein.

Isoenzymes was separated horizontally by isoelectric focusing (IEF) on a Multiphor II apparatus (Pharmacia Biotech, Uppsala, Sweden) using 0.4-mm-thick polyacrylamide gels containing 5% (v:v) ampholytes (Pharmacia Biotech) covering the pH range 3.5–10.0. The run was carried out at a constant power of 5W for approximately 1.5 h. The same amount of total protein was loaded for each extract.

To detect  $\beta$ -1,3-glucanase isoenzymes, after IEF the gels were washed with water and soaked for 10 min in 50 mM sodium acetate, pH 5.2, then incubated for 45 min at 40°C with 0.5% (w:v) laminarin in 50 mM sodium acetate buffer. After washing with water, the gels were soaked in 0.15% (w:v) 2,3,5-triphenyl tetrazolium chloride in 1 M sodium hydroxide at 100°C until red bands appeared on a clear and translucent background (Pan *et al.*, 1989). After staining, the gels were stored in 7.5% acetic acid. Chitinase isoenzymes were detected in overlaying gel containing glycol chitin (Trudel and Asselin, 1989). After IEF, the gels were equilibrated in 0.1 M sodium acetate buffer, pH 5.2, in a reciprocal shaker for 10 min, then covered with 7.5% polyacrylamide – chitin overlay gels containing 0.04 glycol chitin (Sigma) in 0.1 M sodium acetate buffer pH 5.2; the sandwich gels were incubated at 40°C for 2 h. After incubation, the overlay gels were separated from the sandwich gels and treated with 0.01% Calcofluor White M2R (Sigma) in 0.5 M Tris-HCl pH 8.8 at room temperature for 10 min. Chitinase isoenzymes were visualised as dark bands under UV lighting.

The activity stain for peroxidase was done by soaking the gel in sodium phosphate buffer 0.1 M,

pH 5.4 for 10 min and then immersing it in 40 mM guaiacol (Sigma) in the same buffer containing 5 mM  $\text{H}_2\text{O}_2$  at room temperature until the red bands appeared (Magro, 1984).

### Statistical analysis

Data were subjected to parametric and nonparametric analysis of variance using GraphPad Prism software (San Diego, CA, USA). The Tukey or Dunn tests ( $P=0.05$ ) were used to compare the means or medians.

## Results

### Disease development upon artificial inoculation

Symptoms caused by *M. cannonballus* were assessed on the roots and rootlets 60 d after sowing. On the untreated control plants the pathogen caused severe root rot of the lateral and taproots. Plants grown from seeds treated with BTH and  $\text{K}_2\text{HPO}_4$  became more resistant to the pathogen (Figure 2). Plants whose seeds had been treated with MeJA showed a significantly lower disease severity (i.e. greater resistance) than plants whose seeds had been treated with  $\text{K}_2\text{HPO}_4$  or than untreated control. Symptoms produced on control plants grown from ethanol-treated seeds are not shown since they were very similar to symptoms on plants grown from the water-treated seeds.

Root biomass, expressed as dry weight grams, was higher in the  $\text{K}_2\text{HPO}_4$ -treated plants than in the control, and significantly lower in MeJA and BTH-treated plants than in the control (Figure 3).

### Disease development in naturally infected soils

Differences in the disease severity of plants treated with the various chemical inducers were assessed in the summer of 2006 and the spring of 2007 in plants grown in naturally infected soil under greenhouse conditions. Plants took an average of 60 d from transplanting to maturity, in summer, and 80 d in spring.

In both summer and spring trials, the typical symptoms of the disease, rot of secondary and feeder roots, and reddish or corky lesions on the taproots, were recorded when the control plants were removed from the soil. *M. cannonballus* was constantly re-isolated from samples of infected roots.

Plants grown from seeds that were only soaked



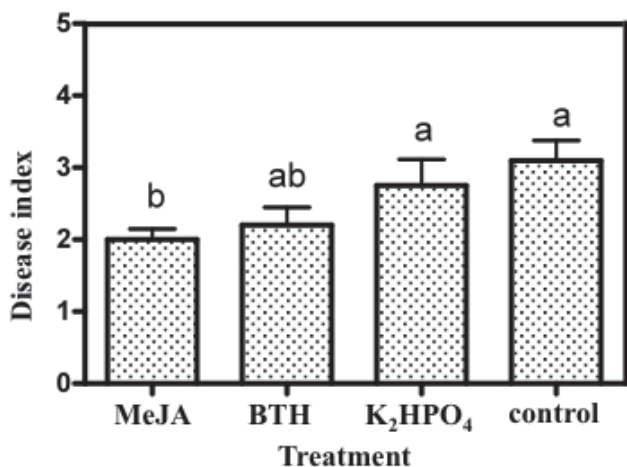


Figure 2. Effect of soaking melon seeds in methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH) and dipotassium hydrogenphosphate (K<sub>2</sub>HPO<sub>4</sub>) on the disease index in melon roots growth in soil artificially inoculated with *Monosporascus cannonballus*. Data are the means of two separate experiments ± SE. Data followed by the same letters are not significantly different at P≤0.05 (Dunn test).

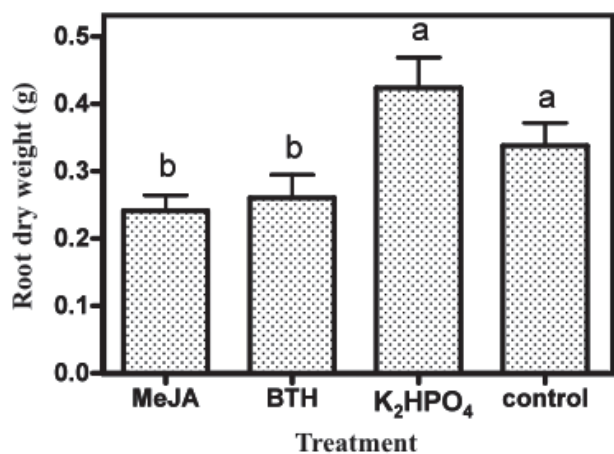


Figure 3. Effect of soaking melon seeds in methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH) and dipotassium hydrogenphosphate (K<sub>2</sub>HPO<sub>4</sub>) on the root biomass of melon plants grown in soil artificially inoculated with *Monosporascus cannonballus*. Data are the means of two separate experiments ± SE. Data followed by the same letters are not significantly different at P≤0.05 (Tuckey test).

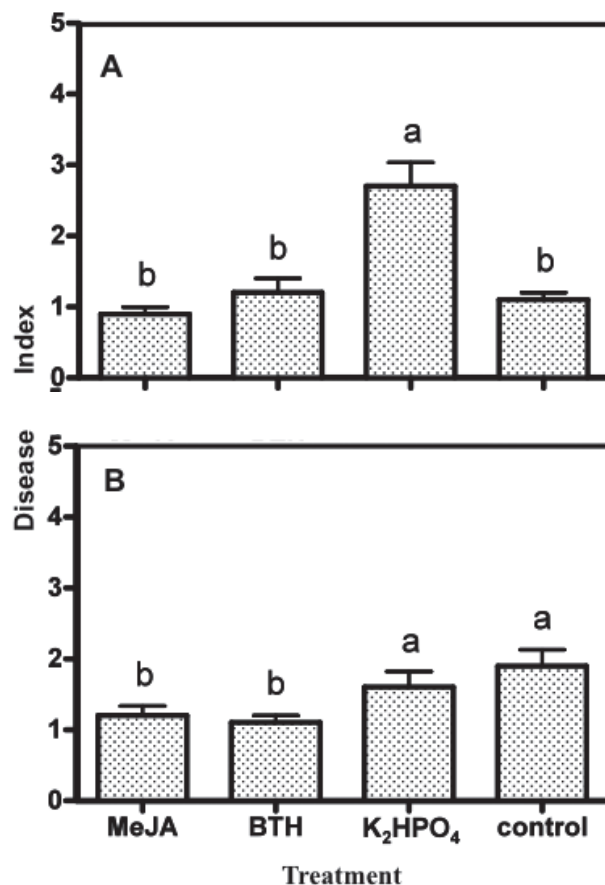


Figure 4. Effect of soaking melon seeds in methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH) and dipotassium hydrogenphosphate (K<sub>2</sub>HPO<sub>4</sub>) followed by foliar sprays, in reducing the disease index of melon plants grown in soil naturally infected with *Monosporascus cannonballus* in 2006 (A) and 2007 (B). Data are the means ± SD. Data followed by the same letters are not significantly different at P≤0.05 (Dunn test).

in chemical inducers did not differ significantly from control plants in their disease and yield parameters in either year (data not shown). However, plants whose seeds were soaked in the chemical inducer and that also received foliar sprays differed in the degree of disease incidence on the roots (Figure 4). In the summer trial (2006), treatments with BTH had no effect on the disease. After seed-treatment with K<sub>2</sub>HPO<sub>4</sub>, severe symptoms occurred on melon roots and the disease incidence was significantly higher than that of control. Seed-treatment with MeJA caused a slight decrease of disease incidence

as compared to the control. In 2007, control plants developed more severe symptoms than in 2006. Treatment with BTH and MeJA significantly decreased disease incidence, while  $K_2HPO_4$  was ineffective.

#### PR protein isoenzyme patterns.

On roots of plants grown from seeds soaked in MeJA, BTH and  $K_2HPO_4$ , the activity and isoenzyme patterns of  $\beta$ 1,3-glucanase, chitinase and peroxidase were analysed 4 d after germination to see if they could serve as markers of induced resistance.

$\beta$ 1,3-glucanase activity was higher in root extracts treated with the chemical inducers than in the control (Figure 5A).  $\beta$ 1,3-glucanase isoenzyme patterns from the roots of untreated plants were characterised by the faint expression of two acidic bands focalising at pI 5.8 and 6.0. The resistance inducers caused a more intense banding of these two isoenzymes (Figure 6). The inducers did not appreciably increase chitinase activity as compared to the control (Figure 5B). The chitinase isoenzyme pattern from the control roots showed three bands at pI 3.7, 6.9 and 8.3. Unlike what occurred with the chitinase quantitative assay, the intensity of chitinase profile from roots treated with MeJA and  $K_2HPO_4$  was higher than that of the control. Seed treatment with BTH induced two faint chitinase extra-bands, pI 8.1 and 8.9 (Figure 6). Total peroxidase activity was strongly enhanced by BTH compared with the other treatments and the control (Figure 5C). Activity staining of both treated and control plants after IEF revealed the expression of a considerable number of peroxidase isoenzymes, ranging from pI 3.8 to 9.3, with pI 3.8 being the most prominent band. All the inducers produced a faint band at pI 6.0. After BTH treatment, the pI <3.5 peroxidase isoenzyme was strongly expressed (Figure 6).

#### Discussion

Resistance inducers provide an additional option to manage plant diseases while maintaining sustainable production. Inducers are usually applied as foliar sprays, but they can also induce systemic resistance when applied to the seeds (Siegrist *et al.*, 1997; Jensen *et al.*, 1998; Morris *et al.*, 1998; Latunde-Dada and Lucas, 2001; Geetha and Shetty, 2002; Lopez and Lucas, 2002; Buzi *et al.*, 2004a; Faessel *et al.*, 2008). Treating seeds with resistance

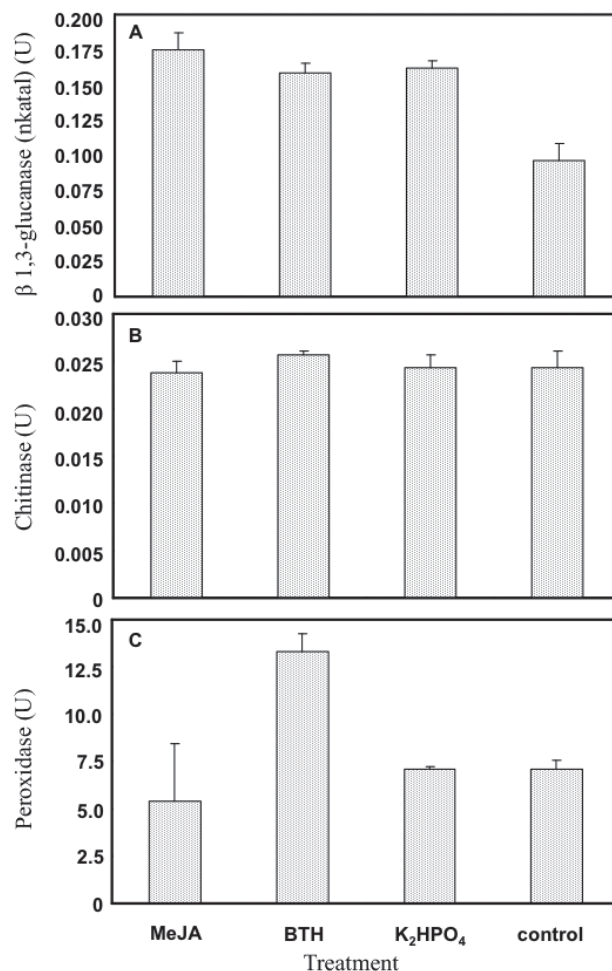


Figure 5.  $\beta$ 1,3-glucanase (A), chitinase (B) and peroxidase (C) activities in melon rootlets raised from seed treated with methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH), dipotassium hydrogenphosphate ( $K_2HPO_4$ ) and water (control) 48h after seed germination. Data represent the means of two separate experi-

inducers may represent a novel approach to control diseases in crop plants, particularly diseases caused by soil-borne pathogens. A comparative evaluation of MeJA, BTH and  $K_2HPO_4$  showed that these inducers varied in their effectiveness against root rot and vine decline caused by *M. cannonballus*. When melon plants were grown in soil artificially inoculated with *M. cannonballus*, soaking the seed of a susceptible cultivar in MeJA provided a long lasting protection against the pathogen. Soaking the seed in BTH and  $K_2HPO_4$  offered significantly

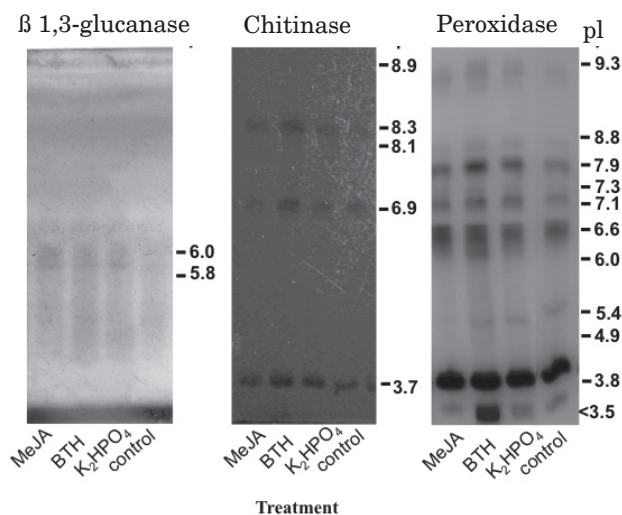


Fig. 6.  $\beta$ 1,3-glucanase, chitinase and peroxidase isoenzyme patterns in melon rootlets raised from seed treated with methyl jasmonate (MeJA), acybenzolar-S-methyl (BTH), dipotassium hydrogenphosphate ( $K_2HPO_4$ ) and water (control) 48h after seed germination. Equal amount of total protein (10  $\mu$ g) per lane was applied. Estimated pI values are indicated on the right.

less protection. Pivonia *et al.* (2002) reported that it is important for *M. cannonballus* to penetrate and colonise the melon roots at the beginning of the season if the disease is to be expressed close to fruit maturation. Thus, although disease symptoms also occurred later in the season in both artificially and naturally infected soils, the resistance induced by MeJA at the beginning of season had a significant influence on the severity of the disease later on. However, trials in naturally infected soil under intensive growing conditions indicated that seed treatments, even with MeJA, were not sufficient to prevent root rot and vine decline when the plants were grown in conditions particularly favourable to *M. cannonballus*. Field studies in soil naturally infected with the pathogen confirmed that soaking the seeds in MeJA, followed by three foliar applications with this inducer, significantly controlled the disease in both years of the study. BTH was ineffective in the 2006 trial, but in 2007, like MeJA, it significantly decreased symptoms. Conversely,  $K_2HPO_4$  promoted disease development in 2006, and was ineffective in 2007. The varying effectiveness of the resistance activators when used in the field

may have depended on the season at which the trial was conducted. In 2006 the trial was carried out in the summer (July–September) when young plants grew under constant warm temperatures, which increased during maturation, thus causing faster growth with limited symptoms in most plants. Conversely, in the 2007 trial in spring (February–May) low temperatures (<15°C) occurred during and after transplanting. This may have stressed the plants during early root development, delaying growth so that fruits matured under higher temperatures more conducive to *M. cannonballus*. Our findings are at variance with those of Pivonia *et al.* (2002). These researchers found that in Israel soil temperatures above 20°C during the early stages of plant growth are an important factor in the development and expression of melon root rot and vine decline caused by *M. cannonballus*. The onset and development of the disease may be thus affected by a multiplicity of factors, depending on the peculiar environmental conditions of the site of cultivation.

Resistance inducers caused differential changes in both the activity and the banding intensity of the defensive enzymes  $\beta$ 1,3-glucanase, chitinase and peroxidase from the total root protein extracts. Higher levels of PR proteins are associated with greater disease resistance, and so are useful biochemical markers of induced resistance (Oostendorp *et al.*, 2001; Buzi *et al.*, 2004a; Pozo *et al.*, 2005). We suggest, therefore, that soaking seeds in MeJA stimulates the systemic signalling of the defence responses, and this enhances the resistance of the plant to *M. cannonballus*. Treatments with MeJA as well as with BTH adversely affected melon growth and the root biomass. The negative influence of jasmonates on plant growth is well documented and is correlated to the inhibition of the synthesis of cell wall polysaccharides (Miyamoto *et al.*, 1997). The negative effect of BTH on plant growth has been explained as a consequence of the allocation cost arising from the metabolic competition between the processes involved in plant growth and the *de novo* synthesis of defence-related compounds (Heil *et al.*, 2000). Thus it is likely that BTH controls root rot and vine decline by activating the defence responses rather than by directly affecting root development and function. Phosphate salts induce SAR to several plant pathogens (Reuveni and Reuveni, 1998), and this phenomenon is associated with localised cell death (Orober *et al.*, 2002). In



the present study phosphate-treated plants had a greater root biomass than control plants and plants receiving other treatments, probably because phosphate improved the nutritional state of plants. However phosphate treatment did not prevent the disease. The signal pathway activated by  $K_2HPO_4$  most likely produced defence responses that are not effective against *M. cannonballus*. Similarly, jasmonic, salicylic and BTH - based resistance in Arabidopsis, melon and tomato is essential for these plants to be resistant to distinct pathogens with different lifestyles (Thomma *et al.*, 1998; Buzi *et al.*, 2004a; Thaler *et al.*, 2004).

Taken together, the resistance inducers MeJA, and to a lesser extent BTH, consistently enhanced resistance to *M. cannonballus*. Jasmonates are ubiquitous plant regulators of different aspects of plant biology including plant response to necrotrophic pathogens. That these compounds enhance resistance to disease was shown by the genetic analysis of mutants and transgenic plants whose biosynthesis or perception of pathogens is affected, and also by the fact that jasmonates often accumulate in response to pathogen attack, and that they affect plant resistance when they are applied exogenously (Poza *et al.*, 2005). Jasmonates have also a crucial role in the systemic resistance to soil-borne pathogens that is induced by various beneficial micro-organisms such as plant growth promoting rhizobacteria, fungal antagonists and arbuscular mycorrhizal fungi (Poza *et al.*, 2005; Shores *et al.*, 2005; Hause *et al.*, 2007). Moreover, exogenous application of jasmonates stimulates the mycorrhizal development (Regvar *et al.*, 1996; 1997). Further experiments are required to determine whether the jasmonates also activate beneficial micro-organisms to render melon more resistant to disease. The induction of resistance to root rot and vine decline by MeJA and BTH in melon may provide a practical supplement to environmentally-friendly disease management when they are combined with appropriate integrated agronomic practices.

## Acknowledgements

The research was supported by the Ministero dell'Università e della Ricerca Scientifica (MURST, Italy), COFIN 2004. Authors thank Dr. Diana Martignoni for technical support.

## Literature cited

- Abad P., A. Pérez, M.C. Marqués, M.J. Vicente, B.D. Bruton and J. García-Jiménez, 2000. Assessment of vegetative compatibility of *Acremonium cucurbitacearum* and *Plectosphaerella cucumerina* isolates from diseased melon plants. *EPPO Bulletin* 30, 199–204.
- Armengol J., A. Vicent, P. Martínez-Culebras, B.D. Bruton and J. García-Jiménez, 2003. Identification, occurrence and pathogenicity of *Rhizopycnis vagum* on muskmelon in Spain. *Plant Pathology* 52, 68–73.
- Beltrán R., A. Vicent, J. García-Jiménez and J. Armengol, 2008. Comparative epidemiology of *Monosporascus* root rot and vine decline in muskmelon, watermelon, and grafted watermelon crops. *Plant Disease* 92, 158–163.
- Bradford M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248–254.
- Buzi A., G. Chilosi, D. De Sillo and P. Magro, 2004a. Induction of resistance in melon to *Didymella bryoniae* and *Sclerotinia sclerotiorum* by seed treatments with acibenzolar-S-methyl and methyl jasmonate but not with salicylic acid. *Journal of Phytopathology* 152, 34–42.
- Buzi A., G. Chilosi, P. Magro, 2004b. Induction of resistance in melon seedlings against soil-borne fungal pathogens by gaseous treatments with methyl jasmonate and ethylene. *Journal of Phytopathology* 152, 491–497.
- Chilosi G., R. Reda, M.P. Aleandri, I. Camele, L. Altieri, C. Montuschi, L. Languisio, V. Rossi, G.E. Agosteo, C. Macri, A. Carlucci, F. Lops, M. Mucci, M.L. Raimondo and S. Frisullo, 2008. Fungi associated with root rot and collapse of melon in Italy. *EPPO Bulletin* 38, 147–154.
- Chilosi G. and P. Magro, 1998. Pectolytic enzymes produced *in vitro* by *Didymella bryoniae* and during colonisation of melon tissues. *Plant Pathology* 47, 700–705.
- Cohen R., J. Burger, C. Horev, A. Koren, M. Edelstein, 2007. Introducing grafted cucurbits to modern agriculture. The Israeli experience. *Plant Disease* 91, 916–923.
- Cohen R., S. Pivonia, M. Edelstein, A. Gamliel and J. Katan, 2000. Toward integrated management of *Monosporascus* wilt of melon in Israel. *Plant Disease* 84, 496–505.
- Faessel L., N. Nassr, T. Lebeau and B. Walter, 2008. Effects of the plant defence inducer, Acibenzolar-S-Methyl, on hypocotyl rot of soybean caused by *Rhizoctonia solani* AG-4. *Journal of Phytopathology* 156, 236–242.
- Fenice M., Di Giambattista R., Raetz E., Leuba J-L., Federici F., 1998. Repeated-batch and continuous production of chitinolytic enzymes by *Penicillium janthinellum* immobilised on chemically-modified macroporus cellulose. *Journal of Biotechnology* 62, 119–131.
- García Jiménez J., J. Armengol, R. Sales and B.D. Bruton, 2000. Fungal pathogens associated with melon collapse in Spain. *EPPO Bulletin* 30, 169–173.
- Geetha H.M. and H.S. Shetty, 2002. Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide - a comparative evaluation. *Crop Protection* 21, 601–610.



- Hause B., C. Mrosk, S. Isayenkov and D. Strack, 2007. Jasmonates in arbuscular mycorrhizal interactions. *Phytochemistry* 68,101–110.
- Heil M., A. Hilpert, W. Kaiser and K.E. Linsermair, 2000. Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs? *Journal of Ecology* 88, 645–654.
- Huang Y., B.J. Deverall, W.H. Tang, W. Wang and F.W. Wu, 2000. Foliar application of acibenzolar-S-methyl and protection of postharvest rock melons and hami melons from disease. *European Journal of Plant Pathology* 106, 651–656.
- Kim Y.J. and Hwang BK., 1994. Differential accumulation of  $\beta$ -1,3-glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of *Phytophthora capsici*. *Physiological and Molecular Plant Pathology* 45, 195–209.
- Jensen B.D., A.O. Latunde-Dada, D. Hudson and J.A. Lucas, 1998. Protection of *Brassica* seedlings against downy mildew and damping-off by seed treatment with CGA 245704, an activator of systemic acquired resistance. *Pesticide Science* 52, 63–69.
- Latunde-Dada A.O. and J.A. Lucas, 2001. The plant defence activator acibenzolar-S-methyl primes cowpea [*Vigna unguiculata* (L.) Walp.] seedlings for rapid induction of resistance. *Physiological and Molecular Plant Pathology* 58, 199–208.
- Lopez A.M.Q. and J.A. Lucas, 2002. Effects of plant defence activators on anthracnose disease of cashew. *European Journal of Plant Pathology* 108, 409–420.
- Magro P., 1984. Onion neck rot: isoperoxidase patterns in infected scales and the effect of *Botrytis alii* polygalacturonase on host peroxidase and phenolic compounds. *Rivista di Patologia Vegetale* 20, 124–132.
- Martyn R.D. and M.E. Miller, 1996. *Monosporascus* root rot and vine decline: An emerging disease of melons worldwide. *Plant Disease* 80, 716–725.
- McKinney H.H., 1923. Influence of soil temperature and moisture on the infection of wheat seedling by *Helminthosporium sativa*. *Journal of Agricultural Research* 26, 195–218.
- Miyamoto K., M. Oka and J. Ueda, 1997. Update on the possible mode of action of the jasmonate: focus on the metabolism of cell wall polysaccharides in relation to growth and development. *Physiologia Plantarum* 100, 631–638.
- Morris B.W., B. Vernooji, S. Titatarn, M. Starret, S. Thomas, C.C. Wiltse, R.A. Frederiksen, A. Bhandhufalck, S. Hulbert and S. Uknes, 1998. Induced resistance responses in maize. *Molecular Plant-Microbe Interactions* 11, 643–658.
- Nelson N., 1944. A photometric adaptation of the Somogyi method for determination of glucose. *Journal of Biological Chemistry* 153, 375–380.
- Okubara P.A and T.C. Paulitz, 2005. Root defense responses to fungal pathogens: A molecular perspective. *Plant and Soil* 274, 215–226.
- Oostendorp M., W. Kunz, B. Dietrich and T. Staub, 2001. Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* 107, 19–28.
- Orober M., J. Siegrist and H. Buchenauer, 2002. Mechanisms of phosphate-induced disease resistance in cucumber. *European Journal Plant Pathology* 108, 345–353.
- Ovadia A., R. Biton and Y. Cohen, 2000. Induced resistance to downy mildew and *Fusarium* wilt in cucurbits. *Acta Horticulture* 510, 55–59.
- Pan S.Q., X.S. Ye and J. Kúc, 1989. Direct detection of  $\beta$ -1,3-glucanase isozymes on polyacrylamide electrophoresis and isoelectrofocusing gels. *Analytical Biochemistry* 182, 136–140.
- Pivonia S., R. Cohen, J. Kigel and J. Katan, 2002. Effect of soil temperature on disease development in melon plants infected by *Monosporascus cannonballus*. *Plant Pathology* 51, 472–479.
- Pozo M.J., L.C. van Loon and C.M.J. Pieterse, 2005. Jasmonates - signals in plant-microbe interactions. *Journal of Plant Growth Regulation* 23, 211–222.
- Regvar M., N. Gogala and Zalar P., 1996. Effects of jasmonic acid on mycorrhizal *Allium sativum*. *New Phytologist* 134, 703–707.
- Regvar M., N. Gogala and N. Znidarsic, 1997. Jasmonic acid effects mycorrhization of spruce seedlings with *Laccaria laccata*. *Trees - Structure and Function* 11, 511–514.
- Reuveni R. and M. Reuveni, 1998. Foliar fertilizer therapy - a concept in integrated pest management. *Crop Protection* 17, 111–118.
- Shoresh M., I. Yedidia and I. Chet, 2005. Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology* 95, 76–84.
- Siegrist J., D. Glenewinkel, C. Kolle, M. Schmidtke, 1997. Chemically induced resistance in green bean against bacterial and fungal pathogens. *Journal of Plant Disease and Protection* 104, 599–610.
- Smith-Becker J., N.T. Keen and J.O. Becker, 2003. Acibenzolar-S-methyl induces resistance to *Colletotrichum lagenarium* and cucumber mosaic virus in cantaloupe. *Crop Protection* 22, 769–774.
- Thaler J.S., B. Owen and V. J. Higgins, 2004. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiology* 135, 530–538.
- Thomma B.P.H.J., K. Eggermont, I.A.M.A. Penninckx, B. Mauch-Mani, R. Vogelsang, B.P.A. Cammue and W.F. Broekaert, 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Science USA* 95, 15107–15111.
- Trudel J. and A. Asselin, 1989. Detection of chitinase activity after polyacrylamide gel electrophoresis. *Analytical Biochemistry* 178, 362–366.
- van Loon L.C., M. Rep and C.M.J. Pieterse, 2006. Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology* 44, 135–162.

Accepted for publication: November 28, 2009