Crop Protection 35 (2012) 58–63

Contents lists available at SciVerse ScienceDirect

Crop Protection

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

Management of *Macrophomina* wilt in melons using grafting or fungicide soil application: Pathological, horticultural and economical aspects

Ron Cohen [a,*], Nabil Omari [c], Asaf Porat [b], Menahem Edelstein [b]

[a] Department of Plant Pathology, Institute of Crop Protection, A.R.O., The Volcani Center, Newe Ya'ar, P.O. Box 1021, Ramat-Yishay 30095, Israel
[b] Department of Vegetable Research, Institute of Plant Science, A.R.O., The Volcani Center, Newe Ya'ar, P.O. Box 1021, Ramat-Yishay 30095, Israel
[c] Extension Service, Ministry of Agriculture, Gilboa 18120, Israel

ARTICLE INFO

Article history:
Received 18 May 2011
Received in revised form
22 November 2011
Accepted 11 December 2011

Keywords:
Azoxystrobin
Grafting
Fungicide
Macrophomina
Melon

ABSTRACT

Melon cultivation in the Yizre'el Valley of northern Israel is threatened by plant wilting that occurs toward harvest. *Macrophomina phaseolina* is the most common fungus isolated from the wilted plants. Disease management using grafted plants or soil application of fungicides to non-grafted melons during the growing season was studied. For the grafting experiments, two Ananas-type melons (*Cucumis melo* L.), cv. 6405 and Eyal, were grafted onto interspesific F_1 *Cucurbita* rootstock TZ-148 and transplanted at spacings of 60, 90, 120 and 180 cm within rows in *M. phaseolina* infested soil to test their ability to cope with the disease and to evaluate the profitability of grafted plant cultivation at different spacings. Grafted plants did not wilt, compared to 80 and 70% wilting of non-grafted melon plants in experiments conducted in 2006 and 2008, respectively. Cultivation of grafted melons in infested soil for the local market was shown to be profitable, even with a 50% reduction in transplant number for a given area (120 cm between transplants). In addition, selection of the right melon cultivar as scion was shown to be crucial to the success of the crop. In another set of experiments conducted in summer 2010 with non-grafted melon plants, application of the fungicides azoxystrobin alone or combined with chlorothalonil or medenoxam reduced disease incidence to 5% as compared to 45% in the untreated control. Disease management using both cultural and chemical approaches is discussed.

1. Introduction

Melon cultivation in the Yizre'el Valley of northern Israel is threatened by plant wilting that occurs toward harvest. The most common fungus isolated from the wilted plants is *Macrophomina phaseolina*, often accompanied by various *Fusarium* species, mostly *Fusarium solani*. *M. phaseolina* (Tassi) Goidanich is a pathogen of a wide number of cultivated and wild plant species in warm, temperate and tropical regions of the world (Manici et al., 1995). Diseases incited by *M. phaseolina* are often referred to as "charcoal rot" because of the parasitized host tissue's dark coloration. The pathogen survives in the soil as small black sclerotia which are the primary inoculum source (Bruton and Wann, 1996). The sclerotia form on infected tissues and are liberated into the soil during tissue disintegration. *M. phaseolina* causes crown or stem rot in cucurbits and it can be fairly serious in melons under hot and dry conditions (Bruton and Wann, 1996; Bruton et al., 1987). This pathogen was the leading cause of melon collapse and of the drastic reduction in melon cultivation in the northern Negev in Israel (Krikon et al., 1982; Reuveni et al., 1982). In the last few years, *M. phaseolina* has been the main fungus isolated from collapsed watermelon and melon roots in most growing areas in northern Israel (Cohen et al., 2002).

Wilting or plant collapse caused primarily by *M. phaseolina* toward fruit ripening is also referred to as "vine decline" syndrome, which has been reported worldwide, and which may involve one or more organisms acting in concert: any one of the organisms alone might not cause disease, but when acting as part of an army of organisms, disease can result (Martyn, 2009). In northern Israel melon wilting is more common in July and August plantings, but may also occur, albeit to a lesser extent, in early April–May plantings. Management of charcoal rot in cucurbits is problematic: soil fumigation has had little success, with its effectiveness being highly dependent on soil type (Bruton and Wann, 1996).

The use of grafted watermelons (*Citrulus lanatus* L.) and melons for reducing damages caused by soil-borne pathogens is expanding rapidly in Israel. Grafting was effective in reducing fusarium wilt and Monosporascus vine decline incidence (Cohen et al., 2007), but its efficacy was not tested against Macrophomina wilt in Israel. The price of a grafted transplant is approximately seven times higher than that of a non- grafted one, and thus growers have to carefully

* Corresponding author.
E-mail address: ronico@volcani.agri.gov.il (R. Cohen).

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doi:10.1016/j.cropro.2011.12.015

consider the economic benefits of using grafted plants, which depend on expected yield and income. Growing grafted Galia-type melon, for example, in the Arava Valley of southern Israel is profitable when grown in the winter for export to Europe. However, growing expensive grafted Ananas-type transplants in midsummer in the open field for the local market can be economically risky. One of the ways to increase profitability in grafted plant cultivation is to decrease expenses by reducing plant density in the field. With watermelon for example, growers commonly reduce the grafted transplant population by 50% compared to non-grafted transplants. This reduction is usually accompanied by a compensatory increase in plant productivity, and thus total yields are maintained and the crop is profitable despite the higher cost of the grafted transplants (Cohen et al., 2007). The same approach has been taken with grafted cucumber: decreasing plant density from 2 plants m^{-1} to 1.43 plants m^{-1} within a row did not decrease total fruit yield (Echevarria and Rodriguez Castro, 2002). In experiments conducted in Mexico (Ricardez-Salinas et al., 2010), it was shown that the number of grafted melon transplants per a given area can be reduced by 50% without lowering the yield. Nevertheless, maintaining the yield with lowering transplanting spacing is not obvious with melons and should be tested specifically for each rootstock and scion combination and growing location and seasons.

Fungicide application on existing vegetable crops for soilborne pathogens is used mainly for the management of seedling diseases such as *Pythium* and *Rhizoctonia* damping off. In these pathosystems, host-pathogen interactions occur during a short time window and in a relatively limited soil volume (Pivonia et al., 2010). Chemical treatment for managing pathogens that attack the deep root system of older plants, such as the vascular pathogens *Fusarium* and *Verticillium* (Erwin, 1981) or the root- and stem-rot pathogen *Macrophomina* are less common. Partial reduction in viability of *Macrophomina* sclerotia following soil treatment with benomyl was reported. Recovery of *M. phaseolina* from soybean stems buried in soils containing low vs. high organic matter were 48 and 62% respectively. Control efficacy was better when free sclerotia were used and less effective when sclerotia were attached to fresh stem pieces (Ilyas et al., 1976). Nevertheless this report refers to reduction in sclerotia viability in soil but not to disease control. An example for successful root rot disease reduction by fungicide was reported recently. Sudden wilt of melons caused by *Monosporascus cannonballus* was effectively suppressed under field conditions following fungicide treatments (Pivonia et al., 2010), the most effective fungicides being azoxystrobin and prochloraz. The experiments were conducted in sandy soil. However, fungicide efficacy against this pathogen has not been tested in heavy soils.

The purposes of this study were to examine cultural and chemical approaches for *M. phaseolina* management in melons in the heavy soils of the Yizre'el Valley in summer cropping. The specific goals for the cultural approach (grafted melons) were: 1) to test the grafted plants' ability to cope with *M. phaseolina* infection, or the phenomenon of "vine decline"; 2) to evaluate the performance of two different melon cultivars used as scions; 3) to evaluate the yield and profitability of grafted plant cultivation at different planting spacing. The objective of the chemical control approach was to select suitable fungicides for the management of vine decline and to evaluate their efficacy for non-grafted melons under field conditions.

2. Materials and methods

2.1. Fungus isolation

Fungi were isolated from diseased plant crowns and roots in the laboratory. Roots of wilted plants were washed under running tap water and dipped for 3 min in 3% sodium hypochlorite followed by rinsing in sterile water. Root and crown segments were incubated on potato dextrose agar (PDA) for 5 d at 27 °C. The fungi that grew out of the root segments were identified according to known anatomical structures using a light microscope.

2.2. Grafting: field experiments

Experiments were conducted in 2006, 2007 and 2008. The first experiment was transplanted on 13 Jul 2006, the second one on 5 July 2007 and the third one on 23 June 2008. The experiments were conducted in heavy soil (19% sand, 23% silt, 58% clay, 1.7% organic matter and pH 7.6) in the village of Sde Ya'akov in the Yizre'el Valley of northern Israel. Grafted and non-grafted transplants were planted in the center of 1.93-cm-wide raised beds. Melon transplants were planted in various spacing in order to evaluate the economical balance between plant number (transplants price per given area) and yield (the farmer income). Plant spacing within rows was 60, 90 and 120 cm in 2006 and 2007 and 60, 120 and 180 cm in 2008. Two Ananas-type melon cultivars commonly grown in midsummer in Israel, (cv. 6405 from HaZera Genetics, Brorim, Israel and Eyal from Zeraim, Gedera, Israel) were used as scions in 2006 and 2007 and only cv. 6405 was tested in 2008. Melons grafted onto the interspecific *(Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) commercial rootstock 'TZ-148' (Tesizer, France) and were compared to non-grafted plants. Grafting was performed in a commercial nursery (Hishtil, Ashkelon, Israel). Standard cultural practices, including drip fertigation, were applied. Wilt incidence was evaluated between fruit maturation and harvest. Both experiments were set up in a random block design with five 10-m long replicates.

2.3. Effect of fungicides on M. phaseolina – in vitro test

The activity of 16 different fungicides against *M. phaseolina* was determined *in vitro* (Table 1). Sterile aqueous solutions of fungicide were mixed with melted PDA medium to give concentrations of 0.1, 1 and 10 μg a.i. ml^{-1}. A 10-mm disk of agar with *M. phaseolina* mycelium from a 10-d-old culture was placed in the center of a petri dish containing the fungicide being tested. The dishes were incubated at 27 °C for 5 d, and then colony diameter was measured. Growth inhibition following exposure to the fungicides was calculated relative to the untreated control and presented as ED_{50} values. There were four replicates (petri dishes) for each fungicide and concentration.

2.4. Fungicide treatment – management of M. phaseolina in non-grafted plants – preliminary screening

Field trials with fungicides were conducted in naturally *M. phaseolina*-infested soil at the village of Sde Ya'acov. The experiments were conducted in the summer, the main growing season for Ananas-type melons destined to the local market.

In summer 2009, all fungicides evaluated *in vitro* (Table 1) were tested in the field. The goal of the observation was to select the best fungicides for detailed experiments. The screening consisted of 20 plants (10 plants in two replicates). Melon plants (cv. 6405) were transplanted on July 15. The fungicides were applied as soil drench three times during the growing season: on August 6, at male flowering, August 21, at fruit set and September 5, at fruit ripening. Since there are no recommendations for *M. phaseolina* control as soil treatment with these fungicides, estimated application rates were based on recommendations for other crop-disease systems. The fungicides were applied as soil drench to the crown zone. Each