Crop Protection 40 (2012) 83–90

Contents lists available at SciVerse ScienceDirect

Crop Protection

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

Evaluation of systemic acquired resistance inducers for control of downy mildew on basil

Zelalem Mersha [a], Shouan Zhang [a,*], Richard N. Raid [b]

[a] Tropical Research and Education Center, University of Florida, 18905 SW 280 St., Homestead, FL 33031, USA
[b] Everglades Research and Education Center, University of Florida, 3200 E Palm Beach Rd., Belle Glade, FL 33430, USA

ARTICLE INFO

Article history:
Received 10 January 2012
Received in revised form
17 April 2012
Accepted 22 April 2012

Keywords:
Ocimum basilicum
Acibenzolar-S-methyl
DL-3-aminobutyric acid
Isonicotinic acid
Salicylic acid
Sodium salicylate

ABSTRACT

Basil downy mildew, caused by *Peronospora belbahrii* Thines sp. nov., is a devastating foliar disease of fresh-cut basil first discovered in the U.S. in South Florida in 2007. Since then the pathogen has been found in over 20 U.S. states and has become a major threat to sweet basil production. In this study, acibenzolar-S-methyl (ASM, Actigard 50WG), DL-3-aminobutyric acid (BABA), isonicotinic acid (INA), salicylic acid (SA) and sodium salicylate (SS) were evaluated for their potential to control basil downy mildew in the greenhouse. Efficacy of these systemic acquired resistance (SAR) inducers varied in control of basil downy mildew depending on the rate, method and timing of application. Foliar sprays of ASM applied pre-, post- or pre- + post-inoculation at rates ranging from 25 to 400 mg l^{-1} significantly ($P = 0.05$) reduced disease severity compared to the non-treated control in all experiments. ASM sprayed at 50 mg l^{-1} three times on a weekly basis starting 3 and 7 days post- inoculation resulted in a 93.8 and 47.1% reduction in disease severity, respectively. Six weekly foliar sprays of BABA as pre- + post-inoculation at rates equal or higher than 125 mg l^{-1} significantly suppressed downy mildew compared to the non-treated control. Foliar treatments of ASM or BABA followed by one or two post-inoculation sprays of a mixture of potassium phosphite (Prophyt) and azoxystrobin (Quadris) significantly improved efficacy for disease control. Sporangia counted on ASM treated leaves were significantly lower than leaves sampled from the non-treated control. ASM and BABA at concentrations lower than 1.0 mM did not inhibit sporangial germination *in vitro*. The effect of INA, SA and SS on disease reduction was generally inconsistent and not significant compared to the non-treated control.

1. Introduction

Production of basil (*Ocimum basilicum* L.) in the United States has significantly increased in the past several years because of its high demand as a fresh or dried culinary herb, as a source of essential oil and oleoresin for food flavors, and as an ingredient for manufacture of perfumes, pharmaceuticals and aroma therapeutic products (Simon et al., 1990). Annual import of basil has also increased in the past years to meet this growing demand (Furth, 2001). US consumers, buyers and distributors, however, are actively seeking greater domestic production due to concerns of food safety (Lopez et al., 2001), increased costs of importation, transportation and storage.

A recently discovered disease, basil downy mildew, caused by the biotrophic oomycete *Peronospora belbahrii* Thines sp. nov. (Belbahri et al., 2005; Thines et al., 2009), is threatening the production of this important herb. Since the first report of the disease from southern Florida in the fall of 2007 (Roberts et al., 2009), many northern and southeastern US states have had major losses, in some instances up to 100%, due to this foliar disease (Wyenandt et al., 2010). The range of the pathogen has expanded to the central and Pacific regions of the US including the Hawaiian Islands (Zhang et al., 2009; McGrath et al., 2010; McGrath, 2011). Worldwide, the disease was first discovered in Uganda in 1933 (Hansford, 1933) and so far has been reported from fields and greenhouses in many countries (Garibaldi et al., 2004b, 2005; Khateri et al., 2007; McLeod et al., 2006; Ronco et al., 2008; Voglmayr and Piatek, 2008; Martínez de la Partea et al., 2010). Although there is no research on how the pathogen arrived in the US, infested basil seeds (Garibaldi et al., 2004a) and/or infected plant materials are believed to facilitate the spread.

Successful infection by *P. belbahrii* is favored by cool to warm temperatures accompanied by high humidity. Profuse sporulation is facilitated by warm and wet conditions. Sporangiophores of

* Corresponding author. Tel.: +1 305 246 7001; fax: +1 305 246 7003.
E-mail address: szhang0007@ufl.edu (S. Zhang).

0261-2194/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2012.04.031

P. belbahrii emerge from the stomata, branch dichotomously, and bear a single broadly-ellipsoidal to subglobose sporangium at the tip. An initial symptom of the disease is vein-bound chlorosis of the adaxial leaf surface followed by visually discernible gray to blackish sporulation on the abaxial leaf surface under humid conditions.

Because of the rapid spread and significant economic impact of this disease, tremendous efforts have been made to promote public awareness (Zhang et al., 2009; Raid et al., 2010b; McGrath, 2011), to evaluate the efficacy of fungicides and secure their registration (Raid, 2008a, 2008b, 2008c, 2009a, 2009b; Raid et al., 2010a; Babadoost, 2010; Thompson et al., 2010), and to explore varieties and breeding lines with high disease resistance (Wyenandt et al., 2010). However, research related to the etiology, epidemiology and management of basil downy mildew in the US is still very limited.

Currently, basil downy mildew is primarily controlled by fungicide application. Only a few biologicals such as Actinovate, the phosphoric acid fungicides such as Prophyt, and the strobilurin fungicide Quadris are registered for control of downy mildew on herbs, but not specifically on basil (McGrath, 2011). However, heavy reliance on fungicides is evoking concerns of the environment and the potential of downy mildew species to quickly develop resistance to Quinon inhibiters, copper and other fungicides. This necessitates soliciting environmentally friendly management strategies such as the use of systemic acquired resistance (SAR) inducers. Exogenous application of SAR inducing compounds such as acibenzolar-S-methyl (ASM), DL-3-aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), salicylic acid (SA) and sodium salicylate (SS) is one way to achieve induction of resistance against a wide range of microbial pathogens in plants (Kessmann et al., 1994; Sticher et al., 1997; Cohen, 2002; LaMondia, 2009; Walters et al., 1993, 2005).

In this study, greenhouse experiments were conducted to evaluate these SAR inducers for their potential to control downy mildew in greenhouse grown basil. Each inducer was evaluated at different rate, method and timing of application. Optimized application of the two best performing inducers, i.e. ASM and BABA, was explored through combining ASM or BABA at reduced rates with a mix of fungicides azoxystrobin and potassium phosphite. In addition, *in vitro* effect of the ASM and BABA on sporangial germination of *P. belbahrii* was determined.

2. Materials and methods

2.1. Experimental plants

Seeds of sweet basil (*O. basilicum* L.) variety 'Genovese' (Eden Brothers, Dohlonega, GA, USA) were planted in 10-cm diameter plastic pots containing the substrate "Fafard #2 Mix" (Fafard Inc., Agawam, MA) augmented with slow release fertilizer Scotts Osmocote Plus (Scotts Company LLC, Marysville, OH). The same seed batches were used for all experiments in this study. Plants were watered daily, supplemented with Miracle Gro (Miracle-Gro Lawn Products, Inc. Marysville, OH) as needed and grown for 26–36 days in a nursery greenhouse prior to pathogen inoculation.

2.2. Preparation of SAR inducers

Solutions of acibenzolar-S-methyl (ASM), DL-3-aminobutyric acid (BABA), isonicotinic acid (INA), salicylic acid (SA) and sodium salicylate (SS) were prepared with sterile deionized water according to treatment descriptions (Table 1). Actigard 50WG, a product of ASM from Syngenta Crop Protection (Greensboro, NC) was used in this study. BABA and INA were purchased from Sigma Aldrich (St. Louis, MO), and SA and SS were from Fisher Scientific (Waltham, MA). To assure complete solubility, each solution was stirred with a magnet bar for at least 1 h and also vigorously shaken before application. Deionized water was sprayed as the non-treated control and the chemical control was the mixture of potassium phosphite (Prophyt, Pamol Ltd., Memphis, TN) at 2.5 ml l^{-1} and azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC) at 0.7 ml l^{-1}.

2.3. Application of SAR inducers and experimental setup

Pre-inoculation treatments started about 12–20 days after planting in the nursery greenhouse. A total of three applications were made at weekly intervals. Post-inoculation treatments began at either 3 or 7 days after inoculation, and they were applied three times at weekly intervals. Pre- and post-inoculation treatments consisted of a total of six applications, i.e. three pre- and three post-inoculation treatments, all applied at weekly intervals. Treatments after pathogen inoculation were carried out in an air-conditioned greenhouse. Foliar applications were made until runoff using 1000-ml handheld sprayers. The average volume of foliar spray per pot ranged between 4.3 ml and 28.0 ml from the 1st to the 6th spray, respectively. Drenching was made by gently pouring 15 ml of a given concentration of ASM onto the center of the pots using a calibrated plastic test tube. All foliar sprays and drench treatments were preceded by 4 h and followed by an overnight without irrigation. Number of plants per pot and number of replications per treatment ranged from three to six considering one pot with three to six plants as a replication. All experiments were arranged in a completely randomized design (CRD).

Table 1
Summary of rate, timing and method of application of five systemic acquired resistance (SAR) inducers evaluated for control of basil downy mildew in greenhouses.

Treatment[a]	Factor tested	Experiment[b]				
		1	2 and 3	4 and 5	6 and 7	8 and 9[c]
ASM	Rate (mg l^{-1})	30	30, 50	50, 100, 200, 400	50, 100	25, 50
	Method	Foliar	Foliar	Foliar, Drench	Foliar, Drench	Foliar
	Timing[d]	Pre	Pre	Pre	Pre, Post[e], PP	Pre, Post[f], PP
BABA, INA, SA, SS	Rate (mg l^{-1})	50	50, 100	100, 400	250, 500	125, 250
	Method	Foliar	Foliar	Foliar	Foliar	Foliar
	Timing	Pre	Pre	Pre	Pre, PP	Pre, PP

[a] Treatments included: ASM = Actigard 50WG (acibenzolar-S-methyl); BABA = DL-3- amino butyric acid; INA = isonicotinic acid; SA = salicylic acid; SS = sodium salicylate. The standard chemical control was a mix of Prophyt (2.5 ml l^{-1}) and Quadris (0.7 ml l^{-1}). Plants for the non-treated control in all experiments were sprayed with deionized water.
[b] Experiments 3, 5, 7 and 9 were repetitions of experiments 2, 4, 6 and 8, respectively.
[c] Only ASM and BABA were tested in experiments 8 and 9. ASM and BABA were also evaluated in combination with either one post-inoculation fungicide spray at 1 week after inoculation or two post-inoculation fungicide sprays at 1 and 3 weeks after inoculation.
[d] Applied three times pre-inoculation (Pre), three times post-inoculation (Post) or six times pre- and post- inoculation (PP) each at weekly intervals.
[e] ASM application started 3 days after inoculation.
[f] ASM application started 7 days after inoculation.