Effect of Plant Age and Acibenzolar-S-methyl on Development of Downy Mildew of Basil

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Abstract. Sweet basil (*Ocimum basilicum*) is an important annual culinary herb grown in the United States. Recently, basil production was drastically affected by downy mildew caused by *Peronospora belbahrii*, a recently discovered foliar disease of basil in Homestead, FL. The disease has spread to more than 30 states in the United States causing significant losses to basil growers. As a result of the recent emergence of the disease, limited management tools are available for control of downy mildew, and it is critical for growers to apply management measures at appropriate times. This study was designed to evaluate 2- to 7-week-old basil plants for their susceptibility to downy mildew. Another objective of the study was to evaluate the effect of a pre-inoculation application of acibenzolar-S-methyl (ASM) for control of downy mildew. The results suggested that 2- to 3-week-old basil was more susceptible to downy mildew than 4- to 7-week-old plants. The area under the disease progress curve (AUDPC) was smaller for 5- to 7-week-old ASM-treated basil plants than for 2- to 4-week-old ASM-treated basil plants. This study indicated that 2- to 3-week-old basil plants need to be protected, and ASM should be applied before pathogen infection on 5- to 7-week-old plants to reduce downy mildew to a greater extent.

Sweet basil (*Ocimum basilicum* L.; Lamiales: Lamiaceae) is commercially the most important culinary herbal crop grown in the United States. Basil, a member of the mint family, is native to central Asia and north-west India and is grown for both fresh and dry consumption as well as used for manufacturing perfumes, food flavors, and aromatherapy products (Simon et al., 1990). The product quality of basil is ensured by appearance (color and absence of disease and insect damage), flavor, and moisture content for the fresh market and volatile oil content for the processing market.

Downy mildew of basil, caused by the oomycete *Peronospora belbahrii* Thines (Peronosporales: Peronosporaceae) (Thines et al., 2009), has become the most devastating disease of basil in the United States. Basil downy mildew was first discovered in Uganda in 1933 (Hansford, 1933). Until its first outbreak in the United States in 2007 (Roberts et al., 2009), basil downy mildew had been reported from Europe (Garibaldi et al., 2004, 2005), the Middle East (Khateri

et al., 2007), and Africa (McLeod et al., 2006). Since its first discovery in Florida, *P. belbahrii* has been spreading to other basil-growing regions in the United States and making commercially produced basil unsuitable for the fresh market. Presently, downy mildew of basil has occurred in more than 30 U.S. states including all eastern states, the Midwest region, California, and even the Hawaiian islands. In 2013, some growers who had produced basil in Florida for many years ceased this line of operation because of disastrous crop losses from downy mildew (Vern Meyer, personal communication).

P. belbahrii is believed to be seed-borne (Garibaldi, 2004) and spread by wind-borne sporangia and movement of seeds, infected plants, and cuttings. Initial symptoms of basil downy mildew on infected basil resemble a nutrient disorder such as nitrogen deficiency. Infected leaves of basil plants become chlorotic mainly between the veins, and like other downy mildew pathogens, *P. belbahrii* typically sporulates on the abaxial surface of infected leaves. In mass, sporulation is grayish to black and has a dirty appearance. In the case of severe infection, leaves may show necrotic lesions and finally defoliation of foliage. The most commonly grown sweet basil (*O. basilicum*) is substantially more susceptible to downy mildew than other cultivars such as red leaf, lemon, and blue spice basil (Wynandt et al., 2010). It appears that disease severity of basil downy mildew is strongly associated with the age of the plant, environmental conditions, and cultivar susceptibility. Cool, wet, and humid

weather, which are characteristics of the climate of Florida, favor infection by *P. belbahrii*, and blowing wind and water splashing help spread sporangia from infected to healthy plants. Moreover, there are very few fungicides and biologicals registered for the use on basil for control of downy mildew (Homa et al., 2014). The limited disease management tools, favorable weather conditions in south Florida, and lack of resistant cultivars make basil production very vulnerable to downy mildew. Downy mildew has the potential to destroy basil production as has already occurred in many regions of the United States where basil has been grown.

The use of resistant cultivars is the most economical means to manage crop diseases. Because downy mildew-resistant basil cultivars are not commercially available, it is important to determine if the susceptibility to downy mildew of available basil cultivars varies at the different stages of plant growth. Determining the susceptibility stage of the basil will help to understand the epidemiology of downy mildew of basil and to apply registered fungicides at an appropriate stage of the basil to prevent or slow down the advancement of this disease. However, little information is currently available on susceptibility of basil at different growth stages to downy mildew infection. The primary objective of this study was to evaluate 2- to 7-week-old basil plants for their susceptibility to downy mildew. In addition, we evaluated the effect of pre-inoculation application of ASM for control of downy mildew on 2- to 7-week-old basil.

Materials and Methods

Plant materials. Seeds of basil cv. Genovese (Eden Brothers, Dohlonoga, GA) were planted in 10-cm diameter plastic pots containing Fafard growing mix (Fafard Inc., Agawam, MA). Plants were supplemented with Miracle Gro 18-18-21 (Miracle-Gro Lawn Products, Inc. Marysville, OH) as required. The seeds were covered with a 5-mm deep layer of Fafard super fine germinating mix (Fafard Inc.). Basil was seeded each week for 6 consecutive weeks in a nursery greenhouse before ASM (Actigard™ 50 WG; Syngenta Crop Protection LLC, Greensboro, NC) treatment and pathogen inoculation. Plants were regularly watered four times a day each for 5 min using an automated overhead irrigation system.

ASM treatment and pathogen inoculation. One week after the last seeding, ASM at 50 mg·L⁻¹ was sprayed uniformly on basil in nine pots (as nine replications) for each seeding and water was sprayed on plants in other nine pots in the same seeding as the non-treated control. Each pot contained four basil plants. A week after ASM treatment, basil plants were inoculated with *P. belbahrii* by spraying the suspensions at 2 × 10³ sporangia/mL onto both ASM-treated and untreated basil plants until runoff. The inoculum was prepared by washing off freshly

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produced sporangia from underside of basil leaves into distilled water, filtering through four layers of cheesecloth, and determining the sporangial concentration using a hemocytometer under a microscope. Inoculated plants were maintained in a humidity chamber with greater than 85% humidity and $23 \pm 3^\circ\text{C}$ in an air-conditioned greenhouse for at least 24 h before they were moved to benches. At the time of inoculation, the age of the basil plants ranged from 2 to 7 weeks after seeding. The plants were watered three times a day each for 5 min by overhead irrigation using 360 NN-GR Nifty nozzles (Dramm Corporation, Manitowoc, WI). The temperature in the greenhouse was set to 23°C ; however, temperature varies ($\pm 5^\circ\text{C}$) each day depending on the ambient solar radiation. The experiment was conducted two times.

Data collection and statistical analysis. Disease severity was assessed as the percentage of symptomatic leaf area (sign and symptoms). Disease severity of basil downy mildew was rated 9, 13, 16, and 18 d after inoculation (DAI) in Expt. 1 and 10, 12, 15, and 17 DAI in Expt. 2. Disease severity was rated as percentage of leaves showing downy mildew symptoms and signs of the pathogen. The AUDPC was calculated based on the four ratings of disease severity for each seeding using the formula: $\sum_{i=1}^{n-1} \left[\frac{Y_i + (Y_{i+1})}{2} \right] [(T_{i+1}) - T_i]$, where Y_i = disease severity at i^{th} observation and T_i = days at i^{th} observation. To determine the effect of ASM on growth of the plant, the number of fully expanded leaves was counted from each of four plants per replication regardless of downy mildew infection in Expts. 1 and 2 on 13 and 17 DAI, respectively. Similarly, plant height was measured from four plants per replication in Expt. 2 on 17 DAI. Data were analyzed using GLM procedure in SAS 9.3 software (SAS Institute Inc., Cary, NC). Means of treatments were separated by Fisher's protected least significant difference test at $P = 0.05$.

Results

Effect of plant age and ASM on downy mildew of basil. In both experiments, the greatest disease severity of downy mildew was observed in 2-week-old basil regardless of the plants treated with ASM or not (Table 1). In Expt. 1, disease severity on non-treated 5- and 7-week-old plants was significantly ($P < 0.05$) lower than 2- and 3-week-old plants, and 6- and 7-week-old ASM-treated plants had significantly lower disease compared with 2- and 3-week-old plants. In Expt. 2, disease severities in non-treated 4-, 6-, and 7-week-old plants were significantly lower than in 2-week-old plants, and 4- to 7-week-old ASM-treated plants had significantly lower disease compared with the 2- to 3-week-old ASM-treated plants. The effect of interaction between plant age and ASM treatment on AUDPC values was significant in Expt. 1 but was not significant in Expt. 2 (Table 2). The effect of

Table 1. Effect of basil plant age and ASM treatment on downy mildew in the greenhouse.^z

Weeks after seeding	Disease severity (%)			
	Expt. 1 ^y		Expt. 2 ^x	
	ASM	Non-treated ^v	ASM	Non-treated ^v
2	59.3 a ^w	58.4 a	59.2 a	58.8 a
3	50.8 b	53.8 b*	56.0 ab	56.3 ab
4	49.6 bc	53.0 bc*	44.8 d	50.8 c*
5	49.7 bc	49.7 d	47.4 cd	55.6 ab*
6	47.4 c	51.6 bcd*	48.3 cd	52.7 bc*
7	47.3 c	51.0 cd*	46.4 d	52.7 bc*

^zASM at $50 \text{ mg}\cdot\text{L}^{-1}$ was sprayed uniformly on basil, and 1 week later, the plants were inoculated with *P. belbahrii* by spraying at 2×10^3 sporangia/mL onto both ASM-treated and untreated basil plants until runoff.

^yDisease severity was rated 18 d after inoculation (DAI).

^xDisease severity rated after 17 DAI.

^wValues are the means of nine replications; means followed by the same letter within a column are not significantly different ($P = 0.05$) among different weeks of seedings.

^vMeans followed by * are significantly different between the ASM-treated and non-treated basil of the same week of seeding.

ASM = acibenzolar-S-methyl.

Table 2. *P* values of factors affecting the AUDPC and number of the leaves on downy mildew infected basil.

Factors	Expt. 1		Expt. 2	
	<i>P</i> values		<i>P</i> value	
	AUDPC	Number of leaves	AUDPC	Number of leaves
Plant age	<0.001	<0.001	<0.001	<0.001
ASM	<0.001	0.001	<0.001	0.029
Replication	0.212	0.408	0.686	0.527
Plant age $\times$ ASM	<0.001	0.059	0.136	0.739

AUDPC = area under the disease progress curve; ASM = acibenzolar-S-methyl.

plant age was found to be significant on the development of downy mildew in both experiments. Similarly, ASM treatment on basil was significant in reducing downy mildew (Table 2). Two-week-old basil plants had significantly greater disease severity than 3-week-old basil in Expt. 1 but disease severity difference was not significant in Expt. 2 irrespective of ASM treatment. Similarly, 3-week-old basil had significantly greater disease severity than 4-week-old basil in Expt. 2 but disease severity difference was non-significant in Expt. 1 irrespective of ASM treatment. In Expt. 1, 6- and 7-week-old basil had significantly less disease severity than 2-week-old basil. However, there was no significant difference in disease severity between 6- and 7-week-old basil. In Expt. 2, 6- and 7-week-old basil had significantly less disease severity than 2-week-old basil, and there was no significant difference in disease severity between 6- and 7-week-old basil.

In both experiments, 2-week-old basil plants had the greatest AUDPC values regardless of whether they had been treated with ASM except 2-week-old basil treated with ASM in Expt. 2 (Fig. 1). The efficacy of ASM treatments increased with increase in plant age in Expt. 1, and the same pattern was seen in Expt. 2 except for 3- and 6-week-old basil plants (Table 3). Among plants without ASM treatment in Expt. 1, 4- to 7-week-old basil had significantly lower AUDPC values than 2- and 3-week-old plants; however, after treatment with ASM, the AUDPC values of

basil plants further decreased with increase in plant age (Fig. 1A). Except for 2-week-old plants, ASM treatment significantly reduced AUDPC values compared with the non-treated plants at the same age. In Expt. 2, a similar trend in the reduction of AUDPC values (except for 3-week-old basil) was observed among variously aged groups of plants, although the AUDPC values of ASM-treated 2-, 3-, 4-, and 6-week-old basil were not significantly different from the non-treated basil plants (Fig. 1B).

Number of basil leaves. In Expt. 1, the number of leaves on each plant was significantly ($P < 0.05$) greater in 5-, 6-, and 7-week-old ASM-treated basil plants than the non-treated basil of the same plant age group (Fig. 2A). In Expt. 2 (Fig. 2B), a similar albeit non-significant trend of more leaves on ASM-treated plants of 4-, 5-, 6-, and 7-week-old ASM-treated plants was observed compared with the control. In both experiments, the number of leaves from ASM-treated 3- and 4-week-old basil plants was not significantly different from the control. The effect of plant age and ASM on number of leaves was significant, whereas the effect of interaction between plant age and ASM on number of leaves was non-significant in both experiments (Table 2).

Height of the basil plants. Data of plant height were collected only in Expt. 2. No significant differences in height of the plants were found between ASM-treated and non-treated basil plants within each plant age group (data not shown).

Fig. 1. Area under the disease progress curve (AUDPC) of downy mildew of non-treated and ASM-treated 2- to 7- week-old basil in Expt. 1 (A) and Expt. 2 (B). Same letters outside the column indicate no significant difference ($P = 0.05$) between the treatments (ASM vs. non-treated) within the same age group of basil. Same upper case letters inside the column indicate no significant differences ($P = 0.05$) among plant age groups of non-treated basil, and same lower case letters inside the column indicate no significant difference among plant age groups of ASM-treated basil. ASM = acibenzolar-S-methyl.

Table 3. Percent reduction of the AUDPC values of downy mildew in ASM-treated basil compared with non-treated plants.^z

Weeks after seeding	Percent decrease of AUDPC values compared with non-treated controls	
	Expt. 1	Expt. 2
2	-1.0	5.9
3	8.0	-3.0
4	9.5	3.9
5	10.7	12.6
6	17.8	10.2
7	26.8	14.4

^zASM at 50 mg·L⁻¹ was sprayed uniformly on basil 1 week before plants were inoculated with *P. belbahrii* by spraying at 2×10^3 sporangia/mL onto both ASM-treated and untreated basil plants until runoff. AUDPC = area under the disease progress curve; ASM = acibenzolar-S-methyl.

Discussion

Data from this study indicate that 4- to 7-week-old basil plants after seeding developed significantly lower downy mildew disease compared with 2- and 3-week-old basil plants (Table 1; Fig. 1). One application of ASM before *P. belbahrii* inoculation significantly reduced disease severity and AUDPC values of downy mildew except for 2-week-old basil when plants are very susceptible to this disease. Leaf number per basil plant was increased by ASM treatment in 5-, 6-, and 7-week-old plants compared with the

Fig. 2. Average number of leaves per plant on 3- to 7-week-old basil plants with and without ASM treatment in Expt. 1 (A) and Expt. 2 (B). Same letters indicate no significant difference ($P=0.05$) between non-treated and ASM-treated basil within each age group. ASM = acibenzolar-S-methyl.

non-treated basil within the same plant age group. No effect on plant height was observed for ASM treatment. There are more than 35 articles on basil downy mildew published in Plant Disease Management Reports. Downy mildew on basil plants increases over time according to these published articles; however, none of the articles reported comparison of disease severity in younger basil plants compared with older plants for their susceptibility to downy mildew. We demonstrated for the first time that younger basil plants appear to be more susceptible than older plants to downy mildew under greenhouse conditions.

Development of downy mildew represented by AUDPC values of susceptible basil cultivar Genovese is shown in Figure 1. The measure of disease severity in this study was based on the percentage of the symptomatic leaf area (sign and symptoms) observed on basil plants, which clearly differentiated disease severity among 2- to 7-week-old basil. In each experiment, 2-week-old young plants developed more disease than 3- to 7-week-old plants (Table 1; Fig. 1). In both experiments, decrease in AUDPC values was observed as plant age at the time of inoculation increased from 2 to 4 weeks regardless of whether they had been treated with ASM treatment, whereas reduction in AUDPC values was not significant when plants were 5 to 7 weeks old when inoculated except for ASM-treated basil in Expt. 1. Therefore, two age groups at the time of inoculation can be readily distinguished based on the AUDPC values in both ASM and non-treated basil. The first group consists of 2- to 3-week-old plants, whereas the second group consists of 4- to 7-week-old plants. In both experiments, a clear pattern of reduction in AUDPC values by ASM treatment was observed in 4- to 7-week-old basil compared with the non-treated basil of the same age. However, no significant reduction in AUDPC was detected between ASM-treated and non-treated young (2 weeks old) plants. In Expt. 1, ASM treatment significantly increased percent decrease of the AUDPC values of 5- to 7-week-old basil compared with

non-treated basil plants (Table 3). Reduction in AUDPC values was the result of systemic acquired resistance (SAR) response incited by ASM in basil plants (Merisha et al., 2013). Similar reduction in disease severity was reported when ASM was applied at pre-, post-, and pre- + post-inoculation at rates ranging from 25 to 400 mg·L⁻¹ (Merisha et al., 2012). SAR induces the expression of pathogenesis-related (PR) genes and confers broad-spectrum resistance to pathogens including obligate oomycetes (Ryals et al., 1996). Reduction in AUDPC values by ASM treatment, compared with the non-treated plants, was also seen in the repeated experiment, but the decrease was not significant in 6-week-old basil (Fig. 1B). Overall, greater reduction in AUDPC values was observed in ASM-treated basil compared with non-treated basil in the second group, i.e., 4 to 7 weeks old when inoculated, indicating that ASM may induce greater resistance to diseases in older basil plants. Protection by effective fungicides from downy mildew when basil plants are young is warranted in commercial production, particularly in south Florida where *P. helbahrii* is present year-round. The results showed that ASM application significantly reduced AUDPC on 3- to 7-week-old basil in Expt. 1 but ASM treatment significantly reduced AUDPC only on 5- and 7-week-old basil in Expt. 2 (Fig. 1A–B). This study demonstrates that the effect of ASM treatment at 50 mg·L⁻¹ may not be practical in basil production to reduce disease severity and therefore treatment with ASM at 50 mg·L⁻¹ alone may not benefit commercial basil growers. This warrants further studies to determine which rate of ASM will provide adequate control of basil downy mildew at different stages of basil. Presently, basil growers may apply ASM followed by application of registered chemicals for effective control of downy mildew of basil (Merisha et al., 2012).

More leaves developed on ASM-treated basil than on non-treated plants, which were inoculated at 5 to 7 weeks and treated with ASM 1 week before inoculation. This is correlated with the significantly reduced

AUDPC values in ASM-treated plants compared with the non-treated basil. However, increases in the number of leaves were not significantly different from the non-treated basil. The increased number of leaves in both experiments suggested that if basil is protected from the pathogen attack, more basil leaves can be harvested.

The association between plant age and disease development has been investigated in many host–pathogen systems (Bateman and Lumsden, 1965; Griffey and Leach, 1965; Pretorius et al., 1988; Reuveni et al., 1986; Ward et al., 1981). Some crops become more susceptible to certain plant pathogens as they become older and other crops display greater susceptibility when they are younger plants. Zhang et al. (2001) investigated the effect of plant age of peanut on late leaf spot caused by *Cercosporidium personatum* and found that disease severity on 3-week-old peanut plants was significantly lower than on 4-, 5-, or 6-week-old plants. However, other studies indicated that older plants exhibit greater resistance to many diseases. Older wheat plants displayed increased resistance in the wheat–*Puccinia recondita* f. sp. *tritici* system (Pretorius et al., 1988). When older plants displayed reduced susceptibility to pathogens or increased resistance, this type of resistance is referred to as age-related resistance (Kus et al., 2002). Data from our study revealed that younger basil was more susceptible than older basil and therefore reduced downy mildew disease in older plants may be the result of age-related resistance. In the tobacco–*Peronospora tabacina* system, younger tobacco plants showed greater susceptibility than older plants (Reuveni et al., 1986). Susceptibility of lettuce to *Bremia lactucae* decreased with increased age (Dickinson and Crute, 1974). In another example, older pea leaves showed increased resistance to *Peronospora viciae* (Mence and Pegg, 1971). It appears that plants infected by obligate plant pathogens are more susceptible at early stages of plant growth. Response of plants to obligate biotrophic pathogens is different from that of necrotrophic pathogens (Glazebrook, 2005). According to the present study and

previous reports (Dickinson and Crute, 1974; Mence and Pegg, 1971; Reuveni et al., 1986), it is clear that protection is required at early growth stage of basil and other crops infected by obligate plant pathogens.

This study indicated that 2- to 3-week-old basil cultivar Genovese showed more susceptibility than 4- to 7-week-old basil in the greenhouse. More basil cultivars may need to be investigated to determine the level of susceptibility between young and old plant growth stages to downy mildew. To accurately identify resistant basil genotypes to downy mildew, plant breeders should consider using younger plants, i.e., less than 4 weeks old, after seeding at the time of inoculation because older basil plants appear to be more resistant to the disease. Results of this study also indicated that application of ASM should be made before pathogen infection on plants older than 5 weeks to maximize the efficacy of ASM for management of downy mildew on basil.

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