Basil Downy Mildew (*Peronospora belbahrii*): Discoveries and Challenges Relative to Its Control

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ABSTRACT

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Basil (*Ocimum* spp.) is one of the most economically important and widely grown herbs in the world. Basil downy mildew, caused by *Peronospora belbahrii*, has become an important disease in sweet basil (*O. basilicum*) production worldwide in the past decade. Global sweet basil production is at significant risk to basil downy mildew because of the lack of genetic resistance and the ability of the pathogen to be distributed on infested seed. Controlling the disease is challenging and consequently many crops have been lost. In the past few years, plant breeding efforts have been made to identify germplasm that can be used to introduce downy mildew resistance genes into commercial sweet basils while ensuring that resistant plants have the correct phenotype, aroma, and tastes needed for market acceptability. Fungicide efficacy studies have been conducted to evaluate current and newly developed conventional and organic fungicides for its management with limited success. This review explores the current efforts and progress being made in understanding basil downy mildew and its control.

Basil (*Ocimum* spp.) is a highly diverse genus that exhibits wide inter- and intraspecific variation in morphology, genetics, and composition of essential oil (Paton and Putievsky 1996; Vieira et al. 2003). Sweet basil (*O. basilicum* L.) is the most economically important basil species in the world, popularized by demand in the fragrance, flavor, fresh culinary and dried herb industries (Simon et al. 1990, 1999). While *O. basilicum* is the most popular basil grown in the United States, it is just one species of *Ocimum* which comprises more than 50 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America (Paton et al. 1999) from which species and phenotypes are available in the horticultural trade as ornamentals and as culinary herbs with a wide range of aromas and flavors (Simon et al. 1999; Vieira and Simon 2000, 2006). Basil plants accumulate a wide variety of terpenes and phenylpropanoids, secondary plant products that are constituents of the plant's aromatic volatile oils, also known as essential oils (Gang et al. 2001; Vieira and Simon 2000; Vieira et al. 2001). It is the presence and relative ratio of these major and minor volatile constituents such as linalool, methyl chavicol, citral, eugenol, methyl cinnamate, and others that impart particular aromas and flavors in basils (Charles and Simon 1990; Deschamps and Simon 2006; Simon et al. 1990; 1999). Sweet basils, for example, contain a high content of linalool followed by methyl chavicol, eugenol, and 1,8-cineole; anise or licorice basils are richer in methyl chavicol; lemon basils (*O. americanum* × *citriodorum*) are rich in citral such as in 'Sweet Dani' lemon basil (*O. basilicum* var. *americanum*); and cinnamon basils (*O. basilicum* var. *cinnamomum*) are rich in methyl cinnamate (Deschamps et al. 2006; Simon et al. 1990; Vieira and Simon 2006). Basils also vary significantly in their morphology relative to growth habit, size, shape, texture, and orientation of leaves; and color of leaf, stem, and flower, resulting in a wide variety of ornamental cultivars (Simon et al. 1999). Basils have largely been selected and only more recently bred for phenotypic characteristics and few if any have been purposefully bred for disease resistance. The one disease in which basil was purposefully and successfully bred for was Fusarium wilt caused by *Fusarium oxysporum* f. sp. *basilicum*. Several basil varieties entered into the commercial marketplace that were tolerant or resistant to Fusarium wilt including the popular 'Nufar', a purported hybrid, and others that were open pollinated such as 'Poppy Joe's'. As the problem became less of a threat and systems to test the seed for *Fusarium* infestation became commonplace, continued screening and development of Fusarium wilt resistant sweet basils subsided, and today those lines appear to have lost some of their resistance to current *Fusarium* isolates unless those isolates are genetically different and more virulent than those which were problematic earlier (J. E. Simon, *personal observation*).

TAXONOMICAL CHALLENGES

Phylogenetic classification within the basil genus is complicated by natural and artificial outcrossing (Nation et al. 1992; Putievsky et al. 1999) resulting in extensive variation in genetics, morphology, secondary metabolite chemistry, and ploidy (Simon et al. 1990). Taxonomic analysis by Paton et al. (1999) recognized 64 *Ocimum* spp. using various morphological descriptors to partition the genus into three subgenera: *Ocimum*, *Nautochilus*, and *Gymnocinum*. Sweet basil (*O. basilicum*) belongs to section *Ocimum* within subgenus *Ocimum*.

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More recently, additional traits have been incorporated into systematic evaluation of the genus including aromatic volatiles, flavonoids, and molecular markers (Vieira and Simon 2000; Vieira et al. 2001; Grayer et al. 2004; Carović-Stanko et al. 2011). Studies demonstrate that individual chemotypes can be represented by multiple plant morphologies, which has resulted in confusion and mislabeling of cultivars especially within the *O. basilicum* species (Paton et al. 1999). A number of genetic diversity studies have been conducted using plastids (Paton et al. 2004), random amplified polymorphic DNA (Vieira et al. 2003), and amplified fragment length polymorphism DNA markers (Labra et al. 2004) in conjunction with morphological and volatile composition data to better define phylogenetic relationships among and within species. However, a more extensive characterization of the relationship among basil accessions and/or varieties as well as greater clarification as to origin and species is greatly needed.

In addition to differences in genotype, *Ocimum* spp. exhibit variation in genome structure. Cytological and nuclear DNA content studies provide evidence for different basic chromosome numbers among basil species (Khosla and Sobti 1985; Paton and Putievsky 1996; Pushpangadan and Bradu 1995). Sweet basil is considered to be a tetraploid with $2n = 4\times = 48$ (Mukherjee et al. 2005), while *O. americanum* and *O. africanum* are most likely hexaploids ($2n = 6\times = 72$) (Carović-Stanko et al. 2010; Pushpangadan and Bradu 1995). Flow cytometric analysis of 23 basil genotypes representing eight species found a wide distribution of genome sizes ranging from 0.92 Gbp (*O. campechianum*) to 5.5 Gbp (*O. americanum*) with sweet basil (*O. basilicum*) being within the 2.97 to 3.39 Gbp range (Koroch et al. 2013). Wide differences in genome constitution among species result in barriers to sexual reproduction, which has limited interspecific crossing (Putievsky et al. 1999) and obstructed downy mildew resistance gene introgression (J. E. Simon, R. M. Pyne, and C. A. Wyenandt, *unpublished data*).

UNITED STATES AND WORLDWIDE PRODUCTION

Field and greenhouse production of fresh basil has increased significantly in recent years because of demand and its high dollar value (Dekalb et al. 2014; Homa et al. 2014). Basil production and value statistics are combined with all fresh market herbs, excluding parsley, in the 2012 U.S. Department of Agriculture (USDA) Census figures (http://agcensus.usda.gov). In 2010, approximately 4,400 ha of basil were grown in the United States. Based on information from buyers, distributors, seed companies, and growers, New Jersey, North Carolina, and Florida have approximately 710, 720, and 525 ha of fresh market herb production, respectively. Total fresh market herb production is split almost equally between the east and west coast (~2,185 ha versus ~2,225 ha in California, Washington, and Oregon) in the United States. In Hawaii, basil is grown on about 23 ha with an estimated annual farmgate value of $1.2 M USD (Hamasaki et al. 1992). The combined statistics make it difficult to determine the full economic significance of basil to farmers, though according to large buyers and distributors the U.S. basil production (>1,500 MT) generates a retail market of >$300 M USD. Basil is also grown for fresh-markets in most European and Mid-Eastern countries in greenhouses, high tunnels, or in the open field. In France, ~30 ha of sweet basil are grown annually for fresh and processed consumption (Garibaldi et al. 2005). In Germany, over 50 million pots with basil are produced annually in more than 25 ha of greenhouses (Farahani-Kofoet et al. 2012). Approximately 80 ha are grown annually in Italy (Garibaldi et al. 2004a). In Cyprus, approximately 4 ha are grown annually, either in greenhouses as a year-round crop or in open fields from April to November, with the majority of the production being exported to the European market (Kanetis et al. 2014). Basil commands a high price and is one of the most profitable of all fresh market herbs (Dekalb et al. 2014). Large U.S. growers and buyers report that basil serves as the leading market driver of all other culinary herbs such as arugula, oregano, rosemary, sage, thyme, and other specialty niche products thereby extending the economic impact to the same stakeholders. Additionally, there are numerous, small roadside conventional and organic vegetable production operations throughout the United States that also grow herbs with fresh basil being one of the most popular whose volumes are not included in the above estimates. Limitations and losses in basil production, particularly due to disease in the United States over the last few years, coupled with higher consumer demand have created a year-round opportunity (20% of the total basil production, or an estimated value of $60 million) for fresh sweet basil to be imported into the United States from other countries, mainly Colombia, Israel, Mexico, and Peru. However, importing basil into the United States is becoming increasingly difficult due to insect and disease problems found during customs inspections. With the discovery of the parasite *Cyclospora cayetanensis* by the USDA on sweet basil imported from South Africa that led to cases of human foodborne illness (http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6002a1.htm) such inspections are likely to continue and occur more often in the future. Importantly, food safety is now at the forefront of American and European agriculture with many large buyers and distributors now requiring basil growers to follow mandated food safety guidelines. Following these guidelines and guaranteeing safe product has become especially important for growers of all leafy greens, including basils. Coupled with increased importation costs, U.S. buyers and distributors are actively seeking greater domestic production from basil growers who follow food safety guidelines.

HISTORY OF BASIL DOWNY MILDEW

Basil downy mildew, caused by *Peronospora belbarhii* Thines, is the important disease of basil in the United States (Roberts et al. 2009). Basil downy mildew had been previously reported as a destructive disease in several countries and continents. The disease was first reported on basil in Uganda in 1932 as *Peronospora* spp. and again in 1937 as *P. lamii* causing defoliation and death of sweet basil (Hansford 1933, 1938). The disease was not reported again until 2001 in Switzerland (Belbahri et al. 2005). Following this confirmation, other countries throughout the world reported first occurrences: Italy in 2003, France and Belgium in 2004, South Africa in 2005, Iran in 2006, Cameroon in 2007, Argentina in 2008, Cuba and Taiwan in 2009, Hungary in 2010, Canada in 2011, Cyprus and the Czech Republic in 2012 (Belbahri et al. 2005; Garibaldi et al. 2004b, 2005; Martinez-de la Parte et al. 2010; McLeod et al. 2006; Nagy and Horvath 2011; Kanetis et al. 2014; Khateri et al. 2007; Petrzelova et al. 2014; Ronco et al. 2009; Safrankova and Holkova 2014; Saude et al. 2013). In the United States, the pathogen was first observed and reported in Florida in the fall of 2007 (Roberts et al. 2009). Shortly afterward, basil downy mildew was observed throughout the Northeast in the summer of 2008 (McGrath et al. 2010b; Wick and Brazee 2009) and since that time it has spread across the northern, southeastern, central, and pacific states including California and Hawaii (Fig. 1) (Blomquist et al. 2009; Mersha et al. 2012c, d). The pathogen that was first reported in the United States in 2007 was genetically identical to the pathogen reported in Switzerland in 2001 (Roberts et al. 2009). Like the United States, 100% of the sweet basil acreage in those regions of the world are at risk and already reported to be significantly affected by basil downy mildew since its introduction to Switzerland.

EPIDEMIOLOGY

Symptoms of basil downy mildew on an infected plant resemble and can be mistaken for a nutrient deficiency, such as nitrogen deficiency (Fig. 2). Like other downy mildew pathogens, basil downy mildew almost exclusively sporulates on the underside (abaxial surface) of infected leaves. Dark purplish-brown sporangia are produced on the underside of leaves during favorable weather

conditions for disease development (Fig. 3). Like other downy mildews, *P. belbahrii* is an obligate parasite and it is not known to produce oospores, thus it requires a living host in order to reproduce and survive (Fig. 4) (Farahani-Kofoet et al. 2012). Therefore, the pathogen can only overwinter in warm, temperate regions where basil will not freeze during cold winter months. In the continental United States, this only occurs in the southern most regions of the country (i.e., southern Florida, Texas, and California). In the rest of the United States, the obligate pathogen cannot survive outdoors during winter months between field-grown basil crops. The pathogen can survive on living plants in greenhouse production operations that produce basil year round. Under controlled conditions, basil downy mildew was particularly severe when plants were kept wet for 6 to 12 h after inoculation and leaf wetness was needed for at least 24 h after symptom appearance for sporulation with 20°C being optimal for infection and sporulation (Farahani-Kofoet et al. 2012; Garibaldi et al. 2007). At least 7.5 h of dark are needed to initiate sporulation (Cohen et al. 2013a). Exposure to light suppresses spore formation but still allows for sporangiophores to emerge from stomata (Cohen et al. 2013a). In the field or greenhouse, basil downy mildew can develop and spread rapidly throughout plantings during periods of high humidity, mild temperatures, poor air circulation, and extended durations of leaf wetness (Garibaldi et al. 2007; Spencer 1981).

The basil downy mildew pathogen can also be spread via contaminated seed (Farahani-Kofoet et al. 2012; Garibaldi et al. 2004a) where it most likely survives as sporangiophores and sporangia (R. Wick, *personal communication*). Sporangiophores and sporangia were recovered by washing a gram of seed (previously tested positive for basil downy mildew by polymerase chain reaction [PCR]) and centrifuging the wash water at high speed (R. Wick, *personal communication*). Farahani-Kofoet et al. (2012) detected *P. belbahrii* in 80 to 90% of randomly selected commercial seed stocks, and could detect systemic infections in stems and seed of basil plants artificially inoculated with *P. belbahrii*, but not showing symptoms. In a study examining 140 basil seed samples, 11.4% tested positive for *Peronospora belbahrii* by PCR. Samples received from seed companies had a high incidence of contamination; of 52 samples, 46% tested positive for the downy mildew pathogen. However, both PCR and quantitative (q)PCR assay results had been inconsistent and many of the tests were run multiple times (Wick et al., *unpublished data*). Little else is known about the etiology of basil downy mildew challenging our ability to make predictions about its development and spread. Thus, the rapid global spread of *P. belbahrii* has been attributed to the distribution of infested seed (Belbahri et al. 2005; Farahani-Kofoet et al. 2012). As noted by Farahani-Kofoet et al. (2012), this underlines the importance of developing and implementing seed testing, certification protocols, and standards for the basil seed industry.

ECONOMIC IMPACTS

Grown in the field, high tunnels, and in greenhouses in many regions of the United States and elsewhere in the world, basil is an important source of income for many vegetable and herb growers (Simon et al. 1990, 1999; Vieira et al. 2003). Since its introduction in 2007, losses to basil downy mildew in the United States alone are estimated to be in the tens of millions of dollars. Importantly, it is estimated the annual costs of trying to manage basil downy mildew with conventional or organic fungicides, sometimes with limited or no success, has also grown to tens of millions of dollars annually in the United States alone.

Conventional and organic growers in the United States have reduced their basil acreage and/or even temporarily suspended basil production due to crop losses from basil downy mildew. Crop losses can occur in the field during production and during postharvest handling. Environmental conditions which promote basil downy mildew development are similar to the conditions in which fresh sweet basil is packaged and shipped, and when the disease is undetected at the grading/sorting stage and packaged, often times by the time the basil reaches its destination, the product is unmarketable due to sporulation that develops during transportation and/or storage. While the above comments pertain exclusively to commercial growers, losses to home gardeners and horticulturalists growing basil for pleasure and their home culinary use have also been substantial.

CURRENT MONITORING PROGRAM

A spreadsheet-based online monitoring program developed by Margaret McGrath, Cornell University, was launched in 2009 (McGrath et al. 2010a). The priorities of this monitoring program were to determine where basil downy mildew was occurring and to use this information to address the question of whether the pathogen could move northward up the East coast similar to the cucurbit downy mildew pathogen (*Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovzev. In 2009, 49 reports of basil downy mildew were logged from 18 states and Canada (Fig. 1). These reports confirmed the first major basil downy mildew outbreak in the eastern United States and helped establish that conditions were favorable for the basil downy mildew pathogen to be disseminated over a wide geographic region in a single growing season in the United States (McGrath et al. 2010a, b). It was anticipated that extension pathologists would be the primary users of this service, so they were contacted about the monitoring program. Interestingly, since its inception home gardeners have logged the most reports as well as some large and small scale basil producers. Producers and gardeners while searching the internet for what was causing their basil to die found an online article (http://vegetablemdonline.ppath.cornell.edu/NewsArticles/BasilDowny.html) about the disease that included requests for occurrence information with links to each year's report page. Attempts were made to confirm all reports by requesting photographs from reporters not known to have expertise to accurately identify downy mildew. Symptom descriptions were also used as confirmation methods. After 6 years of reporting, not only is knowledge about basil downy mildew occurrence in the United States substantially better than it could have been without the help of the public, reports in the comments section of the dataset have provided valuable additional information including environmental conditions when the disease appeared, its impact, and management practices tried. Since the monitoring program began, basil downy mildew has been reported in a total of 42 states plus the District of Columbia. A total of 49, 63, 63, 75, 64, and 284 reports were logged in 2009, 2010, 2011, 2012, 2013, and 2014 from 20, 26, 22, 26, 20, and 37 states, respectively (Fig. 1). Reports of basil downy mildew have also come from Argentina, Australia, Mexico, Grand Cayman, Costa Rica, Puerto Rico, Jamaica, Quebec, Ontario, British Columbia, South Africa, and South Korea showing its global importance.

CONTROL STRATEGIES FOR BASIL DOWNY MILDEW

Fungicides for basil produced conventionally and organically. Since the introduction of downy mildew, commercial basil growers in the United States have relied on a slowly growing list of fungicides registered for its management (Homa et al. 2014; Wyenandt et al. 2010). Mefenoxam (Ridomil Gold SL; Syngenta Crop Protection) and azoxystrobin (Quadris; Syngenta Crop Protection) were labeled for use on conventionally produced basil in 2008, but not surprisingly, basil downy mildew was not specified on the label, and the fungicides could not be used in states like New York where the pest as well as the crop must be specified on the label. Their use was also prohibited in greenhouses. At the time, only two phosphorous acid fungicides, Pro-Phyt (Helena Chemical; Collierville, Tennessee) and K-Phite (Plant Food Company; Cranbury, New Jersey) were labeled for basil downy mildew control and not prohibited from use in greenhouses; additional products in this

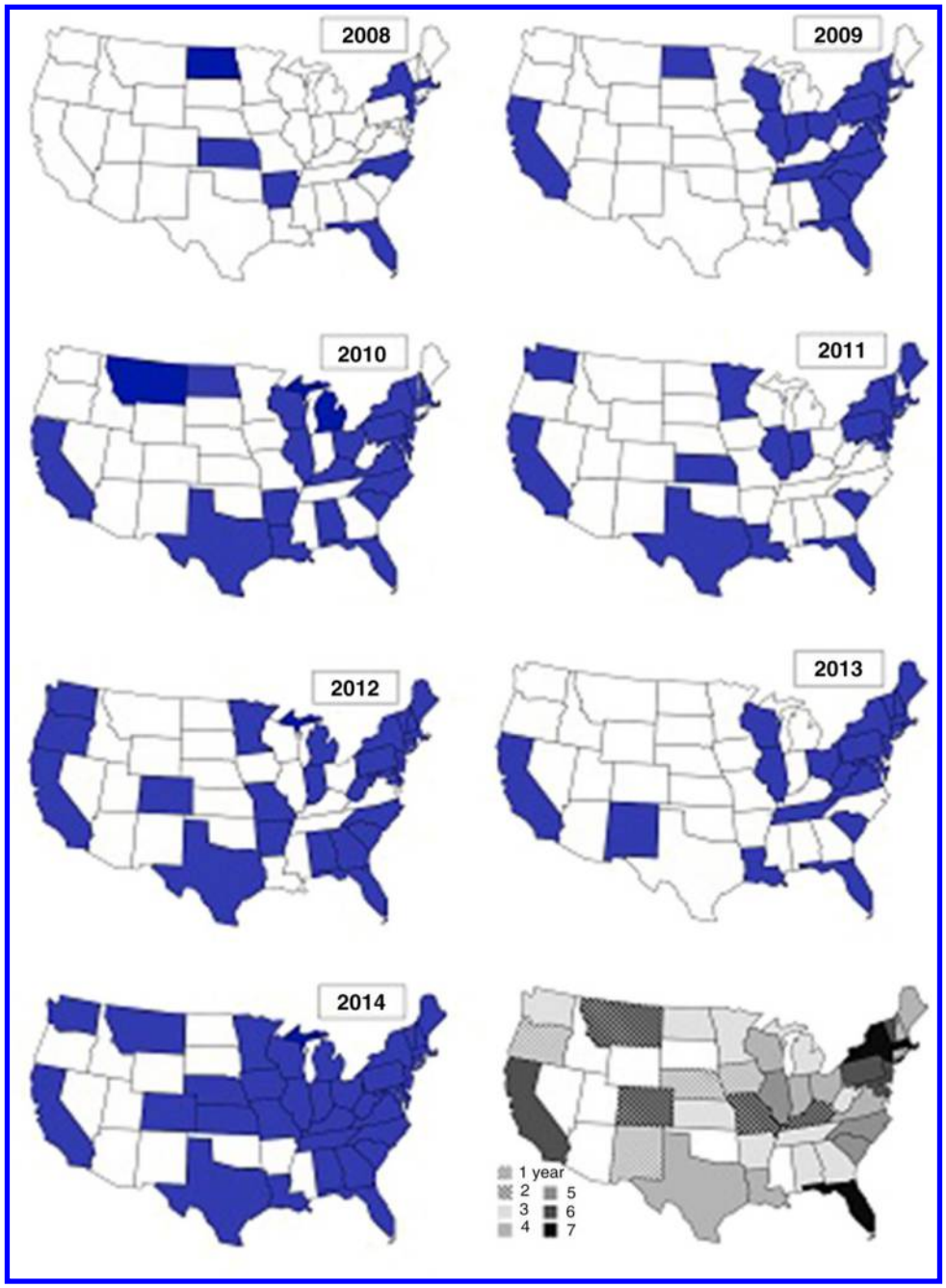

Fig. 1. Reports of basil downy mildew in the continental United States since 2008. Reports in states highlighted for 2009 to 2014 are based on individual reports logged in the Google Docs monitoring web pages. The bottom right map indicates the number of years that reports were received from each state.

group have subsequently been labeled for this disease. The only federally registered conventional fungicide products currently and specifically labeled for basil downy mildew are cyazofamid (Ranman; FMC Agricultural Products) and mandipropamid (Revus; Syngenta Crop Protection). Both were registered with assistance from the IR-4 Program based in Princeton, New Jersey. Cyazofamid is currently labeled for use in greenhouses. Some fungicide products have FIFRA Section 24 (c) Special Local Need Labels in the same states for use in greenhouses on plants intended for resale to consumers including azoxystrobin (Heritage; Syngenta Professional Products), mefenoxam (Subdue Maxx; Syngenta Professional Products), and mandipropamid (Micora; Syngenta Crop Protection) in Alabama and California. Conventional fungicide products in the IR-4 registration process include fenamidone (Reason 500 SC; Bayer CropScience), fluopicolide (Presidio; Valent U.S.A. Corporation Agricultural Products), oxathiapiprolin (experimental; Syngenta Crop Protection), and valifenalate (experimental; FMC Agricultural Products). Federally registered fungicide products that meet the standards of the National Organic Program (NOP) and are listed for use on basil by the Organic Materials Review Institute (OMRI) include *Streptomyces lydicus* (Actinovate AG; Monsanto BioAg), *Bacillus amyloliquefaciens* strain D747 (Double Nickel 55 and LC; Certis U.S.A.), extract of *Reynoutria sachalinensis* (Regalia; Marrone Bio Innovations), neem oil (Trilogy; Certis U.S.A.), potassium bicarbonate (Eco-Mate Armicarb O; Helena Chemical Company; Milstop, BioWorks Inc.), and hydrogen dioxide (Oxidate; BioSafe Systems LLC). Biopesticides that are not approved for organic production include mono- and di-potassium salts of phosphorous acid (K-Phite, Plant Food Company), potassium bicarbonate (Armicarb 100; Helena Chemical Company), potassium phosphite (Fosphite; JH Biotech, Inc.; Fungi-Phite, Verdesian Life Sciences, LLC; Prophyt, Helena Chemical Company; Rampart, Loveland Products, Inc.); Oxiphos, a combination of hydrogen peroxide, phosphorous acid, and mono- and di-potassium salts (Biosafe

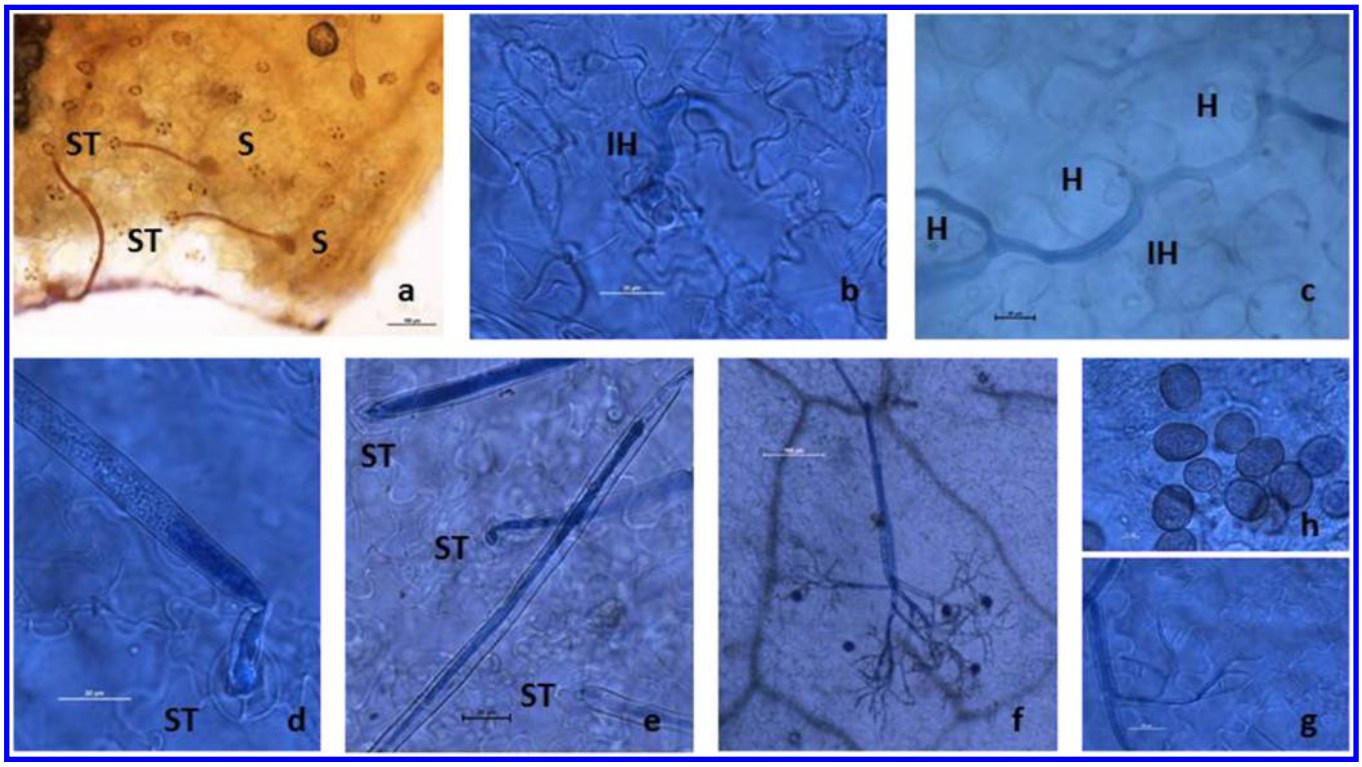

Fig. 2. *Peronospora belbahrii* morphology and stages of pathogenesis. **a,** Sporangia directly germinate and enter host through host stomata (Koroch et al. 2013). **b,** Hyphae penetrate through the lower epidermis. **c,** Hyphae advance intercellularly through mesophyll tissue. Haustoria invaginate mesophyll cells for nutrient acquisition. **d, e, and f,** Sporangiophores exit through stomata and produce secondary inoculum (sporangia) from the distal end of the hyphal tips. **h,** Sporangia. **g,** Dichotomous hyphal branching of sporangiophore. H, haustoria; IH, intercellular hyphae; S, sporangia; ST, stomata.

Fig. 3. Symptoms of *Peronospora belbahrii* infection on the adaxial side of a sweet basil leaf (*Ocimum basilicum*).

Fig. 4. *Peronospora belbahrii* sporulating on the abaxial side of an infected sweet basil leaf (*Ocimum basilicum*).

Systems, LLC); Phostrol, a combination of phosphorous acid, mono- and di-potassium salts, and ammonium salts (Nufarm Agricultural Products); and Rendition, a combination of hydrogen peroxide and peroxyacetic acid (Certis U.S.A.). Many of these conventional and organic products have been evaluated only in small efficacy studies in basil production areas of the United States with varying results (Allen and Patrie 2012; Allen and Saska 2013; Babadoost and DeYoung 2012, 2013; Gilardi et al. 2012; McGrath and Hunsberger 2011, 2012; McGrath and LaMarsh 2013, 2015; Mersha et al. 2011a, b; Mersha and Zhang 2012; Mersha et al. 2012a, b, c, d; Patel et al. 2013a; Raid 2008a, b, c, d, e, f, g, h, i; Raid, 2011a, b; Raid and Sui 2011a, b; Raid et al. 2011a, b; Raid et al. 2013). In both years of a study in New Jersey, mono- and di-potassium salts of phosphorous acid provided the most efficacious control of basil downy mildew compared with other conventional and organic fungicides (Homa et al. 2014). In fungicide efficacy trials in Florida and New York, mono- and di-potassium salts of phosphorous acid provided control that was not as high as other fungicides tested (McGrath and LaMarsh 2013; Raid 2008e, h, i). A direct comparison of results obtained in New Jersey with studies in other states is not possible because of differences in application rates (Raid 2008b, f, g; Raid et al. 2013), plant maturity (Patel et al. 2013b; Raid 2008a, c, e; Raid et al. 2011b;

Raid et al. 2013), number of applications (Raid 2008b, f, h), use of adjuvants (Allen and Patrie 2012; Allen and Saska 2013; Babadoost and DeYoung 2012; Babadoost and DeYoung 2013; McGrath and Hunsberger 2011; McGrath and Hunsberger 2012; McGrath and LaMarsh 2013; Raid 2008a, b, c, d, e, f, g, h, i; Raid and Sui, 2011a, b; Raid et al. 2011a, b), varying levels of disease pressure (Babadoost and DeYoung 2012; McGrath and Hunsberger 2011; McGrath and Hunsberger 2012), and the use of different rating scales (Allen and Patrie 2012; Allen and Saska 2013; Raid 2008a, b, c, d, e, f, g, h, i; Raid and Sui 2011a, b; Raid et al. 2011a, b). However, in many cases, those compounds providing better efficacy as well as those providing the least were similar across trials. In a recent fungicide evaluation with treatments initiated before symptoms were found, applying registered conventional fungicides on a weekly schedule provided 98% control while the program with organic fungicides applied twice weekly was ineffective (McGrath and LaMarsh 2015).

POTENTIAL FOR FUNGICIDE RESISTANCE

To effectively manage basil downy mildew while reducing the potential for fungicide resistance development, it is important for conventional basil growers to (i) initiate a regular fungicide maintenance program prior to the arrival of the pathogen, (ii) limit the number of applications of high-risk fungicides applied during the production season, and (iii) rotate fungicides with different modes-of-action as indicated by their Fungicide Resistance Action Committee (FRAC group) (Gilardi et al. 2012; Homa et al. 2014). Another standard fungicide resistance management practice of applying tank mixtures of high-risk fungicides with protectant fungicides is difficult to implement with basil because no conventional protectant fungicides (i.e., such as chlorothalonil and mancozeb) are labeled. Current fungicide recommendations for the management of downy mildew in field-grown basil across much of the United States include applying a phosphorous acid fungicide at a high rate on a weekly basis prior to the onset of disease (Homa et al. 2014). Once symptoms are observed in the region, phosphorous acid fungicides should be tank mixed and/or rotated on a weekly basis with other currently labeled fungicides with different modes-of-action (i.e., cyazofamid, mandipropamid, or azoxystrobin where permitted). Mefenoxam is recommended at planting if the pathogen is already present in the area. Resistance to mefenoxam, a fungicide widely-used for controlling downy mildew and other oomycete pathogens, has been reported in *P. belbahrii* in Israel (Cohen et al. 2013b). This is the first report of *P. belbahrii* developing resistance to a fungicide (Cohen et al. 2013b). With high-risk fungicides now registered (i.e., azoxystrobin and mandipropamid) and/or being developed for basil downy mildew control, it is expected that *P. belbahrii* will develop resistance to some of these fungicides over time. Organic products are considered to be at low risk for resistance development. However, all of the organic products evaluated have provided limited to no control, including when applied twice weekly on a preventive schedule to a moderately resistant variety (McGrath and LaMarsh 2015). Therefore, until highly resistant varieties are available, it appears the only options for organic producers are to grow basil when and where downy mildew is less likely to occur (e.g., in early summer in northern states) and in greenhouses where humidity can be managed and lights can be used at night to suppress spore production.

COMMERCIAL NEEDS AND CONTROL OPTIONS

Fungicide seed treatments or at-seeding fungicide applications have the potential to help manage downy mildew in basil (Garibaldi et al. 2004a). However, there are no fungicides labeled for use on seed in the United States. The only currently-available conventional option for an at-seeding application in the United States is mefenoxam applied to the soil in field-grown basil. Extract of *Reynoutria sachalinensis* (Regalia; Marrone Bio Innovations, Davis, CA) is the only organic fungicide currently labeled for ground application. Additional conventional and organic seed treatments and other potential methods for treatment, such as with steam or dry heat, need to be examined in order to reduce the wide-scale distribution of the pathogen in seed. Molecular techniques, such as the real-time PCR have been developed for detection of basil downy mildew from infected tissues (Belbahri et al. 2005) yet there appears to be challenges in obtaining consistency in test results within and between seed lots (R. Wick, *personal communication*). Improved seed production methods, along with the implementation of detection methods to identify infested seed lots, and the development of useful seed treatments and quarantine measures (Thines et al. 2009) will vastly improve basil seed quality and slow the global dissemination of *P. belbahrii*.

Control of basil downy mildew is most effective when full coverage of the underside of the leaves in the canopy is achieved. Additionally, some fungicides may not provide control once the pathogen has infected the plant and begun sporulating on the abaxial surface of infected leaves (Fig. 3). Targeted, oomycete fungicides such as cyazofamid and mefenoxam are able to translocate to the abaxial surface of the leaf. Additional systemic or translaminar fungicides with different modes-of-action (i.e., active ingredients) are needed to achieve more effective control, and to help alleviate the potential for resistance development in high risk fungicides. Current organic fungicides have not provided adequate control in fungicide evaluations, and thus result in a product that is not acceptable in the marketplace when conditions favor basil downy mildew development. Until effective organic control options are found, all basil grown organically in the United States and abroad is at risk for significant losses to basil downy mildew. The costs associated with and level of control, the timing of applications, residue levels, preharvest and re-entry intervals are all important factors that need to be considered when developing and evaluating conventional and organic fungicides for basil downy mildew control. Because there are no current sweet basil cultivars with a high level of resistance to basil downy mildew and none of the organic fungicides evaluated have shown to provide an adequate level of control, organic growers producing field-grown basil should consider planting sweet basils under protected conditions (i.e., high tunnels or greenhouses) as well as only produce basil in early spring and summer before inoculum reaches their region. Organic basil growers should aim for a single harvest or earlier harvest date(s) rather than multiple harvests that may extend later into the production season, especially in more northern regions. Production methods, such as transplanting instead of seeding, earlier planting dates, use of plastic mulch and drip irrigation instead of bare ground and overhead irrigation, and increased fertility that allow sweet basil to grow and be ready for harvest earlier in the season could also be useful for those in more northern temperate zones where disease pressure may be lower. Alternative options also include growing downy mildew tolerant basils such as lemon and spice types which show a higher tolerance to *P. belbahrii* (Farahani-Kofoet et al. 2012; Pyne et al. 2014; Wyenandt et al. 2010), if growers have the market for such unusual types. Additionally, all conventional and organic basil growers should adopt an integrated pest management strategy. Basil growers should know the symptoms of the disease and signs of pathogen to be able to accurately and rapidly identify the disease, follow regional and local reports for current outbreaks, and monitor their plantings on a daily basis for presence of the pathogen. There are a number of cultural practices that all basil growers should also consider to help reduce the chances for potential infection and dispersal of the pathogen. These include purchasing certified pathogen-free seed (if and when such becomes available), examining transplants for symptoms and signs of disease or pathogen and ensuring such plants are immediately rouged out, maintaining sanitation by eliminating weeds to prevent favorable microclimates (i.e., higher relatives humidity and reduced air movement), destroying contaminated crops immediately to reduce disease pressure, minimizing

leaf wetness and humidity in the plant canopy, increasing plant spacing, planting in an open field in full sunlight exposed to prevailing winds and using drip rather than overhead irrigation. Keeping humidity below 85% is considered key to managing basil downy mildew in protected culture. Recent work by Cohen et al. (2013a) demonstrated that light during the night, especially red light, inhibits sporulation in *P. belbahrii* and that its use may be of practical importance in protected culture such as in high tunnel or greenhouse basil production.

SCREENING AND BREEDING FOR RESISTANCE

Genetic resistance in commercial sweet basil (*O. basilicum*) is currently in high demand given the limited effectiveness of available chemical products and the manner in which fresh-market basil is consumed (Gilardi et al. 2012; Homa et al. 2014). The occurrence of basil downy mildew as an increasingly destructive plant disease in the United States and Europe has prompted the search and development of *Ocimum* spp. germplasm as sources of resistance and tolerance (Pyne et al. 2014; Wyenandt et al. 2010). Resistance was first identified in three commercial varieties of *O. americanum* exhibiting no sporulation, while lemon chemotype basil varieties within *O. basilicum* and *O.* × *citriodorum* demonstrated reduced levels of sporulation in response to downy mildew (Wyenandt et al. 2010). This disease response has since been confirmed at the cotyledon and true leaf stages in a greenhouse evaluation study (Pyne et al. 2014). Results of that study demonstrated that evaluation of basil cotyledons for resistance to downy mildew under controlled conditions is an effective in rapid selection tool for resistant genotypes (Pyne et al. 2014). Using a similar method, Farahani-Kofoet et al. (2012) examined the true leaf susceptibility of 236 basil genotypes under controlled conditions with comparable results to Pyne et al. (2014) and Wyenandt et al. (2010). These studies validate the use of high-throughput downy mildew screening systems focusing on early plant growth stages to identify resistant genotypes that can then be used in basil downy mildew breeding programs.

The most promising sources of resistance reported to date are from nonsweet basil phenotypes, outside the economically important *O. basilicum* species (Farahani-Kofoet et al. 2012; Wyenandt et al. 2010). In an effort to identify sources of resistance within *O. basilicum* commercial varieties as well as the entire collection of basils within the USDA-National Plant Germplasm System (NPGS) system was explored and screened for basil downy mildew resistance. The USDA-NPGS accessions PI 172996, 172997, and 172996 exhibited a tolerant disease response in which chlorosis or necrosis of leaf tissue occurred, but with very little or no sporulation (Pyne et al. 2014). Furthermore, all three accessions are phenotypically indistinguishable and likely utilize the same gene action in their defense response to *P. belbahrii* (Pyne et al., *unpublished data*). An additional PI, 652053, was completely resistant (McGrath et al. 2014) but exhibits F1 sterility when hybridized with sweet basil breeding lines suggesting the existence of significant genetic divergence between this accession and *O. basilicum* species. Sexual incompatibility and hybrid F1 sterility are common among basil species (Putievsky et al. 1999) and present major obstacles to introgression of resistance from *Ocimum* spp. to *O. basilicum* accessions. Furthermore, resistant *Ocimum* spp. may transfer morphological, flavor and aroma characteristics to sweet basil that are undesirable to the commercial market and can be difficult to breed out from basil downy mildew resistant phenotypes. Evaluation of *O. americanum* × *O. basilicum* and *O. kilimandsharicum* × *O. basilicum* interspecific hybrids for response to downy mildew demonstrated that full resistance was inherited from the resistant *Ocimum* spp. (McGrath et al. 2014), suggesting a dominant gene action. Restoration of fertility and substantial breeding will be required to introduce these sources of resistance into a commercial sweet basil with the correct phenotype and chemotype needed for the stability of the correct aroma and taste.

With downy mildew as a major threat to other crops such as *Cucumis* spp. and *Brassica* spp. for more than a decade, there has been extensive research into downy mildew resistance gene action in these economically important plant species (Olczak-Woltman et al. 2011; Vicente et al. 2012). Such studies are only now being implemented in basil, and thus, resistant varieties are not yet commercially available. However, current research strongly indicates that resistant varieties of sweet basil can be developed. Development of an MRI × SB22 full sibling family at Rutgers University provided the first insights into the inheritance and gene action of basil downy mildew resistance (Pyne et al. 2015). Six generations, including the MRI (P2) (resistant parent), SB22 (P1) (susceptible parent), F1, F2, BC1P1 and BC1P2 were evaluated for response to downy mildew over 2 years at New Jersey Agricultural Experimental Extension Stations located in southern (near Upper Deerfield) and northern (near Pittstown) New Jersey. Analysis of generation means and segregation ratios demonstrated that resistance in this family is controlled by two genes with digenic epistasis. χ^2 goodness of fit tests applied to segregating F2 and BC1P1 generations provided evidence for both a complementary ($P_{F2} = 0.11$; $P_{BC1P1} = 0.04$) and recessive epistasis ($P_{F2} = 0.03$; $P_{BC1P1} = 0.63$) two gene models. In both genetic systems recessive alleles at either locus have a resistance-reducing effect, while dominant alleles confer resistance (Pyne et al. 2015). Quantitative trait loci (QTL) are effective in providing a more precise explanation of gene action and recently revealed epistatic interactions between genes conferring resistance in lettuce (den Boer et al. 2014). This approach is being pursued with sweet basil at Rutgers in order to fully elucidate the gene action conferring resistance in the MRI × SB22 family. An evaluation of 240 di-, tri-, and tetranucleotide expressed sequence tag microsatellite markers yielded a 28% rate of polymorphism between the resistant (MRI) and susceptible (SB22) parents (J. E. Simon R. M. Pyne, and C. A. Wyenandt, *unpublished data*). This genetic polymorphism will allow for mapping of QTLs in the F2 population potentially linked to genes involved in downy mildew resistance.

UNDERSTANDING HOST RESISTANCE

Understanding the molecular basis of host resistance and pathogen virulence at the host-pathogen interface will help shed light on effective disease management for downy mildew disease. This is challenging since *P. belbarhii* is an obligate biotrophic pathogen requiring living basil plants to persist. Since the genomes of neither the host nor the pathogen have been sequenced, an alternative approach is to sequence the total RNA using the next-generation sequencing technologies (Li and Dewey 2011), which currently underway.

As a proof of principle, Ma et al. have generated a transcriptome from an infected basil plant (5 days postinoculation) and a control plant without infection using illumina Hi-seq technology (L. Guo, A. Madeiras, R. Wick, and L.-J. Ma, *unpublished data*). The transcriptome from the infected plant captures all actively transcribed genes from both the basil and the pathogen *P. belbarhii* at the host–pathogen interface, also referred to as metatranscriptomics. Since there is no reference genome to compare with, transcripts were assembled de novo using Trinity (Grabherr et al. 2011) after filtering low quality reads through Trimmomatic (Bolger et al. 2014). By comparing this pair of RNAseq experiments, the group has identified over 20,000 plant transcripts that are shared between the infected and the control basil plants (L. Guo, A. Madeiras, R. Wick, and L.-J. Ma, *unpublished data*). More importantly, this comparison revealed equal amounts of transcripts that are only expressed in the metatranscriptomic sample, representing transcripts of the pathogen and potential host transcripts induced upon pathogen challenge.

Among the 44 transcripts with the highest expression levels, 41 were oomycete homologs. These highly expressed oomycete homologs included ribosomal complex, as well as four core-histone proteins that were expressed at almost the same level. Most

interestingly, four secreted proteins: GH12 (endoglucanase), GH17 (glycoside hydrolase), an exo-1,3-beta-glucanase and a carbohydrate-binding protein homologous to *Phytophthora infestans* effector gene XP_002998388 were identified among the most abundantly expressed transcripts at the host-pathogen interface. Other potential virulence factors detected in the metatranscriptomics data were HSP and PLAC8 homologs; both were induced when the pathogen *Saprolegnia parasitica* infected its host (Jiang et al. 2013); and a cyclophilin, a known suppressor of immune systems (Gan et al. 2009) functioning in both plant and human pathogens (Chen et al. 2011; Viaud et al. 2002; Wang et al. 2001).

This preliminarily study reveals the untapped potential of metatranscriptomics, particularly for the study of the interaction of obligate pathogens with their hosts where available genomic information is lacking. Applying this technique to study interactions of resistant cultivars with pathogens could be used to identify resistant genes involved in host immune responses. RNAseq over a carefully designed time course that captures intimate host–pathogen interactions could be used to study virulence and the evolution of pathogenesis. Overall, application of such omic tools will facilitate identification of key molecular weapons of the pathogen and host defense machineries in the future.

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