RAPID STAINING METHOD TO DETECT AND IDENTIFY DOWNY MILDEW (PERONOSPORA BELBAHRII) IN BASIL.

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• Premise of the study: Demand for fresh-market sweet basil continues to increase, but in 2009 a new pathogen emerged, threatening commercial field/greenhouse production and leading to high crop losses. This study describes a simple and effective staining method for rapid microscopic detection of basil downy mildew (Peronospora belbahrii) from leaves of basil (Ocimum basilicum).

• Methods and Results: Fresh leaf sections infected with P. belbahrii were placed on a microscope slide, cleared with Visikol™, and stained with iodine solution followed by one drop of 70% sulfuric acid. Cell walls of the pathogen were stained with a distinct coloration, providing a high-contrast image between the pathogen and plant.

• Conclusions: This new staining method can be used successfully to identify downy mildew in basil, which then can significantly reduce its spread if identified early, coupled with mitigation strategies. This technique can facilitate the control of the disease, without expensive and specialized equipment.

Key words: basil; downy mildew; light microscopy; Peronospora belbahrii; staining; visualization.

Sweet basil (Ocimum basilicum L., Lamiaceae) is the most important annual culinary herb cultivated in the United States and a source of essential oils and oleoresins, which are used in the manufacturing of foods, flavors, perfumes, and aromatherapy products (Simon et al., 1990; Juliani et al., 2008). Popular commercial basils are not limited to traditional Italian sweet basil (O. basilicum) types, but include a number of different species and varieties. The demand for fresh-market basils has led to more intensive field and greenhouse production systems; however, high losses due to pathogens and pests have been reported (Garibaldi et al., 1997). Basil downy mildew (Peronospora belbahrii Thines) has been previously reported as a destructive disease in the United States (Roberts et al., 2009; Wick and Brazee, 2009) and in several foreign countries, and has recently become a serious concern worldwide (Hansford, 1933; Garibaldi et al., 2004, 2005; McLeod et al., 2006; Khateri et al., 2007; Ronco et al., 2009). A number of commercial varieties belonging to O. ×citrifolium Vis. and O. americanum L. have been identified as highly tolerant to downy mildew (Wyenandt et al., 2010).

Downy mildew (Oomycota) is caused by an obligate biotrophic pathogen and is responsible for diseases of many economically important plant species (Spencer, 1981; Savory et al., 2011). High disease severity can cause complete crop loss in both the greenhouse and field (Roberts et al., 2009; Wick and Brazee, 2009). Downy mildew has been reported to also infect several species of the Lamiaceae family including sage, coleus, and basil (Choi et al., 2009; Thines et al., 2009). Rapid sporulation and dissemination of the pathogen can be observed during periods of high humidity, mild temperatures, poor air circulation, and duration of leaf wetness (Garibaldi et al., 2005, 2007). The pathogen mainly affects the aerial plant organs with initial symptoms of infection identified as chlorosis confined to interveinal regions along the adaxial leaf surface. Thus, early symptoms of downy mildew infection resemble nitrogen deficiency, which can result in misdiagnosis and allow the pathogen to persist under the guise of an abiotic stress. Within a few days under favorable conditions, sporangiophores—visualized as a gray to black fuzzy growth on the abaxial leaf surface epidermis—emerge from stomata and produce asexual spores for secondary infection and disease epidemic in the absence of chemical control (Belbahri et al., 2005; Wyenandt et al., 2010).

The inconspicuous nature of early disease symptoms presents a serious obstacle for growers to prevent disease outbreaks.

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