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# Conditions influencing the development of sweet basil grey mould and cultural measures for disease management

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ABSTRACT

Sweet basil (*Ocimum basilicum*) is an annual herb crop that is harvested several times each season. *Botrytis cinerea* infects the fresh wounds that are created at harvest and grey mould also develops on harvested shoots. The aim was to characterize grey mould epidemics and to develop cultural means of controlling sweet basil grey mould in commercial plantings and postharvest. Two annual surveys at 82 sites revealed that grey mould epidemics are polycyclic in nature. The incidence of grey mould was found to be unaffected by the planting date or crop age, but related to weather conditions (i.e., rain events outside the greenhouse) and the limiting factor for grey mould development was the need for a high level of humidity in the greenhouse. Higher planting density, restricted aeration and the use of narrower walk-in tunnels or greenhouse structures with lower ceilings all contribute to epidemics. Latent infection was found in the leaves of harvested shoots as determined by *B. cinerea*-specific molecular probes. The effectiveness of plant spacing, soil mulch, thermal screens, greenhouse aeration, floating covers and combinations of some of these practices was tested under semi-commercial conditions for four years. A planting density that was half that of the common practice suppressed grey mould incidence in the field experiments with no significant yield losses. This effect can be attributed to the reduced amount of receptive host tissue and better aeration within the canopy at the initial stages of growth. The shoots harvested from the lower-density plots were less susceptible to *B. cinerea* infection. Floating covers and thermal screens were ineffective in reducing the incidence of grey mould in the field experiments and increased the susceptibility of harvested shoots of sweet basil to rot development. Polyethylene soil cover reduced grey mould in sweet basil planted in soil whereas the same mulch failed to control the disease on plants growing in a detached medium. The combination of increased plant spacing and the use of a polyethylene soil cover synergistically improved the yield of sweet basil shoots. Aeration of the tunnels decreased grey mould intensity and this positive effect was enhanced when the aeration treatment was applied in combination with lower planting density. In conclusion, cultural measures are capable of grey mould suppression in sweet basil and result in reduced susceptibility of the host tissues.


## 1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an annual herb crop from the Labiatae family. It is a leading crop among the herbs in Israel grown in polyethylene-covered structures and shoots are harvested several times a season. In Israel, sweet basil production is mainly

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found along the ridge above the Syrian-African Rift, south of the Sea of Galilee and around and north of the Dead Sea. Winter crops are planted from October to December in detached growth media or directly in soil. The first harvest is carried out when plants are 30–50 cm in height. At that point, 15- to 20-cm-long pieces of shoot are harvested. Plants continue growing by branching and the shoots are harvested repeatedly. The fresh wounds that are created by the harvesting are susceptible to infection by *Botrytis cinerea*, which leads to the development of grey mould. Stem infection can develop until the entire plant dies and a large amount of conidia are discharged to the air (Sharabani et al., 1999). *B. cinerea* not only infects sweet basil plants in the greenhouse, but also develops on the crop postharvest (Aharoni et al., 2010). Increasing energy costs over the last 10 years have discouraged growers from heating their greenhouses and, consequently, the prevalence of grey mould and postharvest rots have increased significantly.

*B. cinerea* infects more than 200 plant species and produces large numbers of conidia on conidiophores (Elad et al., 2004). In basil, stem wounds are infected mainly during the winter, from mid-December until March. *B. cinerea* conidia germinate in free water (Williamson et al., 1995; Yunis et al., 1994), which is readily available on the sweet basil stem wounds. However, the optimal relative humidity for stem wound infection is between 75 and 85% (Edden et al., 1996; O'Neill et al., 1997; Sharabani et al., 1999). The optimal temperature for conidia germination is 15–25 °C, but the pathogen can infect at temperatures as low as 2 °C, as well as at temperatures above 25 °C (Jarvis, 1980; Marois et al., 1988; Salinas et al., 1989). Conditions in sweet basil greenhouses during Israeli winter are suitable for stem wound infection and these infections occur within half a day of the creation of harvesting wounds (Sharabani et al., 1999). When the relative humidity drops, conidia are released by a hygroscopic mechanism and these conidia are then carried by air currents (Dik and Wubben 2004; Jarvis, 1980). Leaves of sweet basil harvested in the greenhouse can carry *B. cinerea* inoculum, allowing the development of postharvest rot. Unlike other fresh produce, sweet basil cannot be kept at temperatures cooler than 12 °C, since the low temperature physiologically damages the tissues (Aharoni et al., 2010). Leaves and shoot tips injured by low temperature turn black and are quite susceptible to grey mould (Aharoni et al., 2010).

Currently, greenhouse aeration is used to control grey mould in sweet basil (Garibaldi et al., 1997). In Israel, fungicide treatments such asfludioxonil + cyprodinil, pyrimethanil, fenhexamide, an extract of *Melaleuca alternifolia* and polyoxin AL may be considered, but most synthetic fungicides cannot be used because of restrictions regarding residues on the produce (Ministry of Agriculture Extension Service, *personal communication*).

It is assumed that the conditions favorable for infection may be manipulated, in order to prevent infection (Shtienberg and Elad, 1997). Alternative nonchemical means of control may be effective in grey mould management (Dik and Wubben, 2004). The hypothesis of the present research was that in the absence of active heating, the conditions in sweet basil greenhouses are favorable for *B. cinerea* infection and that it is possible to agrotechnically manipulate those conditions to make them less favorable for the development of *B. cinerea* grey mould. The aim of the present research was to characterize grey mould epidemics and to evaluate cultural means of controlling sweet basil grey mould in the greenhouse, as well as *B. cinerea*-incited postharvest rot. Specifically, we report here the results of a broad survey and the effectiveness of a lower planting density, soil polyethylene mulch, thermal screens, greenhouse aeration, floating covers and combinations of some of these techniques for the control of grey mould in sweet basil.

## 2. Materials and methods

### 2.1. Surveys in sweet basil plots

Surveys were conducted during the growing seasons of 2008–2009 and 2009–2010 in sweet basil plots spanning from EmekHama'ayanot in the north down to KikarSedom, south of the Dead Sea. The surveys included fields of variable size, different covered production structures and different disease levels, cropping practices, locations, altitudes, planting dates and production histories (the number of previous sweet basil crops). The first phase of this project included 12 walk-in tunnels and 12 greenhouses at various locations in the surveyed regions. Four scouts recorded grey mould incidence data once every 7 days at fixed evaluation sites in each of the 24 structures. Each evaluation site consisted of 50 plants. In the walk-in tunnels, 6–13 evaluation sites were visited along the two side beds and the central bed, for a total of 18–39 evaluation sites pertunnel, depending on the length of the tunnel. In the greenhouses, 6–10 evaluation sites were visited along the central bed in each second greenhouse bay. There were 36–60 evaluation sites per greenhouse, depending on the total number of bays in the particular greenhouse.

The data from the first survey (first half of 2008–2009) were used to determine the minimal number of evaluation sites needed to accurately represent the level of disease in a particular structure. We calculated the average disease incidence across the 24 structures and the standard error (SE) of that average. In addition, we randomly chose samples of 40, 35, 30, 25, 20, 15, 10, 8, 7 and 6 individual evaluation sites per structure .The average disease incidence (and SE) of each of these different-sized samples were compared with the average and SE of the original full data set in each field from which the data originated. A second survey was conducted in 25 walk-in tunnels and 33 greenhouses. Based on the results of the first survey, 9–12 evaluation sites were visited in each structure. In the second survey, 25 walk-in tunnels and 33 greenhouses were visited in two consecutive seasons (2008–2009 and 2009–2010). In each structure, there were three sampling sites along each sampled bed and 3–4 beds were surveyed in each structure. Data regarding cultural practices and the location of each of the surveyed structures were recorded as described in Section 3.

### 2.2. Dynamics of grey mould epidemic

In order to characterize the nature of the spread-of-disease foci in the field plots, we plotted the correlation between the natural log (ln) of average disease incidence and the ln of the standard deviations of disease incidence in the greenhouses. A slope $<1.0$ represents the organized spread of disease in the greenhouse and a slope $>1.0$ represents non-random spread of disease in the greenhouse (Madden et al., 2007). The graphic mode of surface contour (Microsoft Excel) was used to visually describe the spatial spread of disease severity over greenhouse plots. Gray scale color gradient was used where lighter gray represents lower disease incidence and darker color represents higher incidence.

Logit transformation of disease data was performed in order to characterize epidemics as monocyclic or polycyclic, based on their development over time after the planting date or after a specific fixed date (1 December). Regression analysis was performed and the $R^2$ values were calculated using logit transformations for monocyclic $[\ln(100/(100-y))]$ and polycyclic $[\ln(y/(100-y))]$ epidemics.