Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Passive heat treatment of sweet basil crops suppresses *Peronospora belbahrii* downy mildew

Y Elad[1], C. Omer[1,2], Z. Nisan[1,2], D. Harari[3], H. Goren[4], U. Adler[5], D. Silverman[6] & S. Biton[6]

1 Department of Plant Pathology and Weed Research, Agricultural Research Organization, Bet Dagan, Israel
2 Section Agroecology and Plant Health, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
3 Central and Northern Arava R&D, Arava, Israel
4 Eden Research Station, Emek Hama'aynot R&D, Emek Hama'aynot, Israel
5 Agricultural Growers Council, Yehud, Israel
6 Extension Service, Israel Ministry of Agriculture and Rural Affairs, Bet Dagan, Israel

Keywords
Downy mildew; greenhouse crop; heat treatment; passive heat; plant disease; systemic induced resistance.

Correspondence
Yigal Elad, Department of Plant Pathology and Weed Research, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel. Email: elady@volcani.agri.gov.il.

Received: 9 April 2015; revised version accepted: 26 October 2015.

doi:10.1111/aab.12269

Abstract

Sweet basil (*Ocimum basilicum*) is an annual herb crop grown in polyethylene-covered structures in Israel. It is Israel's leading herb crop, grown in warm regions of the country. Downy mildew (caused by *Peronospora belbahrii*) is a severe disease in Israel and in many other crop-growing regions worldwide. Experiments were carried out to identify potential climate-management techniques for suppression of this disease on basil in non-heated greenhouses. Disease severity was evaluated under commercial-like conditions in three experiments, with 8–10 walk-in tunnels at each location. Pathogen inoculum was introduced into all walk-in tunnels. Regression analysis was performed between the disease values and air temperature, relative humidity (RH) and soil temperature. Downy mildew severity was negatively related to high (>25°C) air temperature, RH in the range of 65–85% and high (>21°C) soil temperature. The increase in air temperature did not result in a significant increase in leaf temperature; canopy surface median temperatures only reached 30°C. Symptomless plants from relatively warmer tunnels (peak temperatures of 45–48°C) that were transferred to conditions that promote downy mildew (22 ± 2°C, RH > 95%) became severely diseased, showing sporulation of *P. belbahrii*, suggesting that infection occurred but at the high temperatures symptom expression/tissue colonisation was suppressed. Pot experiments in which aerial and subterranean plant organs were differentially heated revealed that treating the roots with a high temperature (26–31°C), similar to the soil temperatures in the warmer greenhouses, while maintaining the upper plant parts at ambient temperature (20°C), suppresses canopy downy mildew. The effect lasted for 1–2 weeks after the plants were removed from the heated soil treatments and maintained under optimal conditions for pathogen development. Furthermore, oospores were found in the symptomatic leaves. Oospores are minimally affected by high temperature, and therefore the high temperature presumably did not affect pathogen survival. In conclusion, the effect of high greenhouse temperature on basil downy mildew may not result from a direct negative effect of high temperature on the pathogen but from an indirect high-temperature effect on the host, rendering it less susceptible to pathogen development.

Introduction

Sweet basil (*Ocimum basilicum* L.) is an annual herb crop grown in polyethylene-covered structures in Israel. Sweet basil production is mainly found along the ridge above the Syrian–African Rift, south of the Sea of Galilee and around the Dead Sea. It is the leading herb crop in Israel with an annual production of 11 000 t. During the cooler season in sweet basil-cropping regions, temperatures may reach minima of 5–10°C at night and maxima of 10–25°C during the day. Increasing energy costs over the last 10 years have discouraged growers from heating their greenhouses. Relative humidity (RH) in the greenhouses can reach over 95%, and as a consequence, diseases such as grey mould [caused by *Botrytis cinerea* Pers.:Fr.; (Elad *et al.*, 2014a)], white mould [*Sclerotinia sclerotiorum* (Lib.) de Bary; (Elad *et al.*, 2015)], and recently downy mildew commonly develop in sweet basil greenhouses, where the latter can become severe (Cohen *et al.*, 2013).

Downy mildew of basil (*Peronospora* sp.) was originally reported in Uganda (Hansford, 1932). Recently, sudden and severe epidemics of downy mildew of sweet basil have been reported in the Riviera region of Italy and France (Garibaldi *et al.*, 2004; Garibaldi *et al.*, 2005), Switzerland (Belbahri *et al.*, 2005), Malta (Porta-Puglia & Mifsud, 2006), Iran (Khateri *et al.*, 2007), USA (Roberts *et al.*, 2009), Argentina (Ronco *et al.*, 2009) and a few other locations where the crop is grown. The causal agent was identified as an oomycete pathogen and named *Peronospora belbahrii* Thines (Belbahri *et al.*, 2005; Thines *et al.*, 2009). This pathogen mainly infects the leaf blades of sweet basil but has been found on other green organs of this crop, such as petioles and stems (Belbahri *et al.*, 2005). Infected leaves are distorted, asymmetric chlorosis develops on the leaf blades, and dark spores (on sporangiophores) form on the underside of the leaves (Garibaldi *et al.*, 2007; Roberts *et al.*, 2009). The leaves may become necrotic and abscise. Although leaves may bear symptoms of chlorosis or sporulation, these symptoms do not necessarily co-exist on the same leaf (Y. Elad, personal observation). Garibaldi *et al.* (2007) found the disease to be particularly severe when plants were kept wet for at least 6–12 h immediately after inoculation; the pathogen was most active at 20°C, whereas at 12 and 27°C, the disease was suppressed. Garibaldi *et al.* (2007) reported that 24 h at high humidity is needed for sporulation, and Cohen *et al.* (2013) found that sporulation occurs when infected plants are incubated for at least 7.5 h in a dark, moisture-saturated atmosphere at 10–27°C, and that light suppresses it.

Control methods for basil downy mildew (BDM) may involve fungicides, seed treatment, and/or breeding for resistance. Although the latter might serve to manage downy mildew in sweet basil – resistance is present in wild *Ocimum* species – a long-term cross-breeding programme is required to establish new resistant cultivars (Römer *et al.*, 2010; Wyenandt *et al.*, 2010). Seeds have been found to be vehicles for pathogen transmission. It was therefore recommended that a seed-certification scheme be established and that the pathogen be controlled on the seeds (Djalali Farahani-Kofoet *et al.*, 2012). Fungicides were tested for their efficacy in controlling BDM; mefenoxam with copper hydroxide, azoxystrobin and mandipropamid were found to effectively suppress BDM but their economic value was not demonstrated (Gilardi *et al.*, 2013). Furthermore, experiments with the resistance inducers acibenzolar-S-methyl and beta-aminobutyric acid significantly reduced the disease (Mersha *et al.*, 2013). Following the appearance of downy mildew of sweet basil in Israel in 2011 (Cohen *et al.*, 2013), chemical control agents (fungicides) were gradually registered: dimethomorph with mancozeb, chlorthalonil, boscalid with pyraclostrobin, azoxystrobin, potassium phosphite and mefenoxam with mancozeb. This last fungicide was potentially the most effective among the tested fungicides. Unfortunately, pathogen resistance to mefenoxam was found (Cohen *et al.*, 2013) and by 2014, it was already associated with control failure. Moreover, synthetic fungicides can only be used within limited time intervals during the growing season because of restrictions regarding residue on the produce (Ministry of Agriculture Extension Service). In the absence of control measures, total devastation of the crop can occur in commercial greenhouses with BDM.

Heating crops grown under a polyethylene cover is common practice during the cooler seasons. Temperatures in the greenhouse are usually raised with heaters or passively using the sun's irradiation during the day. Active heating can also be used to manage foliar plant diseases (Morgan, 1985; Hausbeck *et al.*, 1996; Dik & Wubben, 2004) by essentially drying the canopy and reducing RH (Elad & Shtienberg, 1995). Reducing RH can reduce the level of humidity-promoted diseases such as grey mould, white mould, downy mildew and bacterial infections (Elad & Shtienberg, 1995; Elad, 1999; Elad *et al.*, 2014a, 2015). Similarly, aerating greenhouses with the aim of reducing the humidity load is effective at reducing the pressure of humidity-promoted foliar diseases.

Experiments have been conducted to identify potential climate-management techniques for the suppression of humidity-promoted and powdery mildew diseases in greenhouse-grown sweet pepper, tomato and sweet basil. Elevated daytime temperatures were obtained by closing the side walls of the greenhouses, resulting in significant suppression of pepper powdery mildew (*Leveillula taurica;* anamorph: *Oidiopsis sicula*) and tomato powdery

mildew (*Oidium neolycopersici*). On the other hand, severe epidemics developed in aerated greenhouses maintained according to common practices (Elad *et al.*, 2014b). Temperatures in polyethylene-covered greenhouses with sweet basil that were kept closed for 6 h each day reached 42°C during the winter period; the incidence of grey mould in these greenhouses was significantly reduced and basil yields increased (Elad *et al.*, 2014b). Passive heating of greenhouse walk-in tunnels is possible during the winter and results in increased yields (Shapiro *et al.*, 2014). The range of temperature elevation reaches ca 7–20°C above outside air temperature during the daytime. These initial observations and results led us to test the effect of elevated daytime temperatures, temperature durations, root zone temperatures, RH and RH durations on the severity of downy mildew in sweet basil and which parameter is most important to BDM suppression.

Materials and methods

Walk-in tunnels in experimental stations

Walk-in tunnel experiments that simulated commercial production conditions were carried out at experimental stations in the winter and spring of 2012/2013 and 2013/2014. Sweet basil cv. Peri (Dudai *et al.*, 2002) plants were used in all experiments. This cultivar is susceptible to *P. belbahrii*. Plug containing 3–5 plants were prepared in a commercial nursery (Hishtil, Ashkelon, Israel) and transplanted 3–4 weeks after seeding. Experiments were carried out over two consecutive seasons; the planting dates were 14 November 2012 (exp. 1 below) and 1 November 2013 (exp. 2 below). Exp. 3 (below) was carried out in the 2014 season, planted on 9 February 2014.

Eden Experimental Station, Emek Hama'ayanot Research and Development Center (site 1, exps. 1 and 2)

Experiments 1 and 2 were conducted at site 1, the Eden Experimental Station, Emek Hama'ayonot Research and Development Center (32°30′N, 35°30′E; elevation 120 m below sea level). The regional climate is Mediterranean, semiarid with winter rain and a dry, hot summer (daily averages and maxima, 29–35 and 35–46°C, respectively). At this site, there were 9 and 10 walk-in tunnels in exps 1 and 2, respectively. Each walk-in tunnel measured 30 m × 6 m (180 m^2). The structures were covered with 150-μm thick Sunsaver Clear IR AV polyethylene (Ginegar Plastic Products, Kibutz Ginegar, Israel). The front and rear openings were covered by 50-mesh nets and polyethylene. The tunnels were oriented east to west. The polyethylene was rolled up daily to allow aeration from 1000 to 1400 h. The plants were grown in soil (calcareous silty clay: 30% sand, 32.5% silt, 37.5% clay; Xeric Haplocalcid according to USDA Soil Taxonomy) developed on tufa. The soil in this area is ca 30 cm deep.

Plants were irrigated daily according to local extension service recommendations, and fertigated proportionally with 5–3–8 N–P–K fertiliser (Fertilizers & Chemicals Ltd., Haifa, Israel) at a rate of 2 L fertiliser per 1000 L water. The corresponding nutrient concentrations were 8.6 mM, 1.0 mM and 4.0 mM (120 ppm, 30 ppm and 160 ppm) for N, P and K, respectively. Fertigation was performed with a 17-mm drip-irrigation pipe with a 1-L h^{-1} dripper embedded in the pipe every 20 cm. There were three 30-m long beds in each tunnel, and six evaluation plots of 1 m × 15 m were allocated per tunnel, two plots in each bed. Plugs were planted at a density of 32 plug m^{-2}. For disease evaluation, each plot consisted of 416 planted plugs (13-m long bed) excluding buffer areas of one m along each of the plot edges. Disease severity was evaluated three times in each experiment, from 28 March 2013 until 24 April 2013 and from 16 December 2013 until 4 May 2014, respectively. Disease was not detected 24 and 26 days, respectively, prior to these evaluation dates.

Different climate conditions were obtained inside the walk-in tunnels by using passive heating methods: standing black polyethylene water tubes placed along the north side of the structures, polyethylene soil mulch on all tunnel surfaces, horizontal water tubes along the beds and entire double layers of polyethylene tunnel-covering material. Each tunnel had different temperature and RH conditions (Elad *et al.*, 2014b; Shapiro *et al.*, 2014) as shown in the results section (Figs 1–3, Supporting Information).

Zohar Experimental Station, Northern Arava Research and Development Center (site 2, exp. 3)

Experiment 3 was conducted at site 2, the Zohar Experimental Station, Kikar Sedom (30°57′N, 35°23′E; elevation 400 m below sea level). The regional climate is arid with scarce winter rain and a dry, hot summer. The work at the Zohar Experimental Station was carried out in eight walk-in tunnels, each 40 m × 5 m (200 m^2). The structures were covered with 100-μm thick Sunsaver Clear IR AV polyethylene. The front and back openings of each tunnel were covered with 50-mesh netting. Temperature in the greenhouses varied according to the number of aeration openings made in the polyethylene cover, as shown in the results section; three to six round aeration openings (50 cm diam.) were cut along the tunnels and covered with 50-mesh netting. Sandy soil beds (1 m wide) were planted at a density of 30 plugs with three to five plants each per m^2.

Plants were irrigated with local brackish water (4 dS m^{-1}) according to local extension service recommendations, and fertigated with 1.0 L 8–2–4 N–P–K fertiliser per 1000 L water. Corresponding nutrient concentrations were 6.9 mM, 0.33 mM and 1.0 mM (96 ppm, 10 ppm and 40 ppm) for N, P and K, respectively. Fertigation was performed using a 17-mm drip-irrigation pipe with a 1.2-L h^{-1} dripper embedded in the pipe every 20 cm. Each plot consisted of two 9-m long beds, and there were four plots along the walk-in tunnel. Each plot consisted of 420 planted plugs excluding 1 m of buffer plants along each side of the bed. Disease evaluations were carried out six times from 20 March 2014 until 11 June 2014. Disease was not detected 18 days before 20 March 2014.

Pot experiments under controlled conditions

Sweet basil cv. Peri was obtained from a commercial nursery (Hishtil) and transplanted into 1-L pots containing a coconut fibre:tuff (unsorted to 8 mm) (7:3 v/v) potting mixture. Plants were fertigated proportionally with drippers two to three times per day with 5–3–8 fertiliser ($N–P_2O_5–K_2O$, Fertilizers & Chemicals Ltd, Haifa, Israel); irrigation water was planned for total N, P and K concentrations of 120 mg L^{-1}, 30 mg L^{-1} and 150 mg L^{-1}, respectively, and EC of 2.2 dS m^{-1}, allowing for 25–50% drainage. Plants were maintained for 35–42 days at 20–30°C in a pest- and disease-free greenhouse and then transferred to a greenhouse compartment where BDM was allowed to develop following inoculation as described below.

The potted plants were treated with each of two sets of treatments to allow whole-plant temperature variations, or root temperature variations (a and b, respectively) as follows: (a) illuminated growth rooms (2 m × 3 m × 2.8 m) with full temperature control were used for whole potted plant treatments with air temperatures set to 20 ± 1°C, 26 ± 1°C and 31 ± 1°C during the day (0700–1700 h), and to 11 ± 1°C at night (1700–0700 h); (b) a greenhouse compartment with supplemental light (daytime) and air temperature maximum of 22–24°C during the day and minimum of 16°C at night was used. Each pot was placed in an external plastic envelope covering all of its sides except the soil surface. Each envelope was equipped with a spiral polyethylene pipe (2 mm diam.) in which warm water flowed. The water was warmed in thermostat-controlled 0.4-m^3 water tanks and pumped to allow pot temperature maxima of 28°C (initial experiments) and 22°C, 26°C and 31 ± 0.5°C (later experiments). In each of the experimental sets, treatments lasted 2 weeks and inoculation with *P. belbahrii* was carried out at either the beginning or end of the plants' exposure to the temperature treatments. The plants that were inoculated after the temperature treatments were incubated under optimal conditions for BDM as described below. Each set of experiments was repeated three to four times, with six potted plant replicates per experiment.

Inoculation with *P. belbahrii* and disease evaluation

Spores of *P. belbahrii* were harvested in water by washing sporulating leaves of sweet basil plants that were kept in an experimental greenhouse at the Volcani Center, Agricultural Research Organization, Israel. The suspension was then filtered through cheesecloth. The concentration of spores was determined using a haemocytometer and a light microscope, and adjusted to 1×10^3 cell mL^{-1}. Potted sweet basil plants were inoculated by spraying with a spore suspension (5 mL $plant^{-1}$), incubated at high RH (>95%) in the dark in a growth chamber at 22 ± 1°C for 12 h, incubated in a greenhouse chamber at 22 ± 2°C for 1 week, incubated at high RH (>95%) in the dark in a growth chamber at 22 ± 1°C for 12 h, and then incubated in a greenhouse chamber at 22 ± 2°C for symptom development.

Potted sweet basil plants subjected to this artificial inoculation served as inoculum sources to ensure even inoculum loads across the walk-in tunnels in experiments 1–3. Plants were placed at the border of each adjacent plot, six to eight infected plants per tunnel. Disease-severity evaluation in the walk-in tunnel field plots included all plants except those along the 1 m edges of each plot. The severity of BDM was determined every 2–3 weeks in all plants of each plot in each experiment on a graded scale of 0–100 where 0 = all plants visually healthy, 10 = 10% of the leaf area in the plot is covered by typical downy mildew symptoms of either chlorosis, dry necrotic lesions or sporulation of *P. belbahrii* on their underside, and 100 = all leaves on all plants in the plot showing typical downy mildew symptoms.

Infection of potted plants in controlled-condition experiments was carried out by a suspension of 1×10^3 spores mL^{-1} of *P. belbahrii* (5 mL $plant^{-1}$) as described above. Disease was evaluated on 10 leaves of each plant with symptoms of chlorosis, dry necrotic lesions or sporulation on the underside of the leaf. Severity of BDM was documented for each evaluated leaf on a 0–100 scale, where 0 = leaf shows no signs or symptoms, and 100 = entire leaf surface displays signs and/or symptoms. Incidence of infected leaves was also recorded.

Microclimate data recording and analysis

Temperature and RH were recorded using portable electronic data loggers (Hobo, Onset Computer Corp., Bourne, MA). Air temperature and RH were recorded

at canopy height (30–40 cm, exps 1–3) and soil temperature was recorded at 12–15 cm depth (exps 1 and 2) in the middle of each walk-in tunnel. Measurements were recorded in all walk-in tunnels every 20 min and averaged for every hour of the day starting 3 weeks after planting and ending when experiments were terminated.

Temperature and RH data were mathematically characterised according to the calculated averages for specified time intervals each day, the number of hours per day at a certain air temperature (i.e. 0–5°C, 5–10°C, 10–15°C, 15–20°C, 20–25°C, 25–30°C, 30–35°C, 35–40°C and 45–50°C), soil temperature (i.e. 3–6°C, 6–9°C, 9–12°C, 12–15°C, 15–18°C, 18–21°C, 21–24°C, 24–27°C, 27–30°C and 30–33 °C), or RH (i.e. 10–15%, 15–20%, 20–25%, 25–30%, 30–35%, 35–40%, 40–45%, 45–50%, 50–55%, 55–60%, 60–65%, 65–70%, 70–75%, 75–80%, 80–85%, 85–90%, 90–95% and 95–99%) ranges, the sum of temperature degrees (Celsius) × hours per day or RH percentages × hours in certain ranges (details in Tables 1–3, Supporting Information). The relationship between disease severity data and microclimate variables was quantified by using regression analysis. The regression analysis between the microclimate variables averaged for 7 days and/or 14 days before disease evaluation and disease severity was calculated for the walk-in tunnels in each experiment (exp. 1, $n = 9$; exp. 2, $n = 10$; exp. 3, $n = 8$). Regression analyses were linear, logarithmic and polynomial. The tables present the formulas describing these types of regression analysis, correlation coefficient (r) values and α significance levels and the figures present the best fit. Canopy surface and ground surface temperatures were evaluated using a FLIR Ebx-Series Thermal Imaging Camera. Linear, logarithmic and polynomial regression analyses were calculated for air versus ground temperatures, air versus canopy surface temperatures, and ground versus canopy surface temperatures. Table 1 presents the formulas describing these types of regression analysis, correlation coefficient (r) values and α significance levels.

Pot soil, air and leaf temperatures were measured in the controlled-condition experiments with Type T (copper – constantan) thermocouples. Air temperature was measured at canopy height (25–30 cm) and leaf temperature was measured on the underside of the leaf. The temperature data were recorded hourly by a CR23X Micrologger data logger (Campbell Scientific, Logan, UT).

Microscopic observations

Sweet basil leaves showing symptoms of BDM were sampled randomly from ca 100 infected plants at the end of the experiments, stained with Cotton blue (0.065% Aniline blue in lactic acid:glycerol:double-distilled water [1:1:1, v/v], Sigma, St. Louis, MO) and observed under a light microscope (Zeiss Axiolab, Carl Zeiss Microscopy GmbH, Goettingen, Germany).

Experimental design and statistics

Walk-in tunnels were allocated randomly in the experimental sites. Regression analysis between disease severity and microclimate variables was calculated as described above. Experimental treatments in pot experiments were allocated randomly and replicated as specified above. The BDM was described as percent disease severity. Data in percentage of disease severity were arcsine-transformed before further analysis. Area under the disease progress curve (AUDPC) values was calculated by integrating the area between each couple of evaluation dates. AUDPC values were presented as percent × days. Standard errors (SEs) of the mean were calculated and presented alongside the number of degrees of freedom (DF = n-1 for controlled-conditions experiments and DF = n−2 for regression analysis calculated for the walk-in tunnel experiments' data). Disease severity (as AUDPC) data in controlled conditions pots experiments were analysed using analysis of variance (ANOVA) and Fisher's protected LSD test.

Results

Downy mildew development and microclimate conditions in walk-in tunnels

The sweet basil was harvested every 2–4 weeks by removing young shoots. The disease evaluation was carried out before harvest, every one to three harvests. Severity of BDM reached at the end of cropping 78% at 133 days after planting (DAP), 57.5% at 185 DAP and 82.5% at 114 DAP in exps. 1, 2 and 3, respectively. In some walk-in tunnels, disease severity in all experiments was low, from 0 to up to ca 10% (Fig. 1), despite massive *P. belbahrii* inoculum at the experimental locations, ensured by placing sporulating sweet basil plants with downy mildew symptoms in all of the tunnels. This pointed to the fact that microclimate may have a suppressing effect on BDM development.

In addition, plants that originated from tunnels where no BDM symptoms developed were subsequently transferred to a disease-free growth chamber with optimal conditions for disease development. Disease severity reached 75 ± 4.9% after 10 days with no application of external inoculum, again pointing to conditions that suppress the appearance of disease symptoms in the walk-in tunnels with high temperatures.

Examples of air temperature and RH in the various walk-in tunnels in the three experiments, and of soil

Table 1 Measured temperatures of sweet basil canopy surface in Zohar experiment (exp. 3)

					Regression analyses[b] between temperature sets (formula, r value and α level)			
Time range (h)[a]	Tunnel (number)	Air temp. (°C)	Ground temp. (°C; range, and median)	Canopy surface temp. (°C; range, and median)	Type	Air temp–Ground temp	Air temp–Canopy surface temp	Ground temp–Canopy surface temp
0703–0718	1	16.9	15.0–16.0, 15.4	10.2–14.1, 12.5	Linear	n.s.[c]	n.s.	n.s.
	2	15.8	13.6–16.0, 15.2	9.3–12.0, 10.5				
	3	19.6	15.2–18.0, 16.5	12.2–16.0, 13.8	Log.	n.s.	n.s.	n.s.
	4	17.0	15.0–18.0, 16.6	12.0–16.2, 14.5				
	5	19.6	17.2–20.0, 18.6	13.2–15.0, 13.8	Polynomial	n.s.	$y = -0.51x^2 + 18x - 155$	n.s.
	6	17.5	15.5–18.0, 16.5	12.0–15.0, 14.0			$r = 0.714$, $\alpha < 0.05$ (negative corr.)	
	7	18.3	14.2–15.0, 14.5	11.5–15.5, 13.4				
	8	16.6	15.2–17.0, 16.2	14.0–15.0, 14.6				
0912–0930	1	31.3	20.0–25.2, 23.5	26.2–28.0, 27.2	Linear	$y = 1.75x - 30$		
	2	30.5	19.2–24.5, 22.0	23.0–26.4, 24.0		$r = 0.940$, $\alpha < 0.001$	n.s.	n.s.
	3	28.9	19.2–21.5, 20.5	26.2–28.0, 27.1	Log.	$y = 57\ln(x) - 173$		
	4	29.5	21.0–23.2, 22.0	24.2–28.2, 25.8		$r = 0.938$, $\alpha < 0.001$	n.s.	n.s.
	5	34.7	32.0–33.4, 33.0	28.5–30.2, 29.4	Polynomial	$y = 0.04x^2 - 0.76x + 10$		
	6	34.2	28.2–32.0, 30.5	21.4–25.2, 23.6		$r = 0.941$, $\alpha < 0.001$	n.s.	n.s.
	7	37.6	34.2–38.0, 36.0	27.0–29.2, 28.5				
	8	35.1	25.1–27.2, 27.0	26.0–28.5, 27.1				
1117–1132	1	45.4	36.3–39.4, 38.2	27.2–29.0, 28.3	Linear	$y = 1.2x - 15$	$y = 0.79x - 5.3$	
	2	40.3	34.7–35.9, 35.0	23.2–25.1, 24.5		$r = 0.748$, $\alpha < 0.05$	$r = 0.784$, $\alpha < 0.05$	n.s.
	3	45.0	43.0–46.1, 45.1	30.8–33.5, **31.2**	Log.	$y = 54\ln(x) - 167$	$y = 36\ln(x) - 106$	
	4	38.5	36.2–38.0, 37.5	24.2–26.2, 25.5		$r = 0.743$, $\alpha < 0.05$	$r = 0.793$, $\alpha < 0.02$	n.s.
	5	50.2	44.8–48.2, 46.3	30.0–34.8, **31.5**	Polynomial	$y = 0.04x^2 - 1.9x + 55$	$y = -0.07x^2 + 7.3x - 151$	$y = 0.06x^2 - 4.0x + 95$
	6	41.4	26.0–31.0, 28.0	26.1–31.8, 29.5		$r = 0.752$, $\alpha < 0.05$	$r = 0.826$, $\alpha < 0.02$	$r = 0.813$, $\alpha < 0.02$
	7	49.5	40.5–49.0, 43.5	33.1–38.0, **34.2**				
	8	42.6	28.0–34.8, 32.3	25.0–29.2, 26.8				
1329–1341	1	43.1	42.5–47.3, 45.5	29.0–31.0, 29.8	Linear			
	2	42.7	28.5–32.0, 30.5	26.0–29.5, 27.3		n.s.	n.s.	n.s.
	3	46.5	42.0–47.0, 45.0	25.0–31.0, 28.5	Log.			
	4	42.9	40.0–44.5, 42.1	26.0–30.0, 27.8		n.s.	n.s.	n.s.
	5	48.4	38.0–42.0, 39.5	29.0–32.0, 29.7	Polynomial	$y = -0.58x^2 + 54x - 1230$		
	6	42.9	42.0–45.0, 43.3	27.2–30.0, 28.6		$r = 0.714$, $\alpha < 0.05$	n.s.	n.s.
	7	46.4	46.0–48.5, 47.0	27.2–30.0, 28.5				
	8	49.2	44.0–47.5, 46.5	28.2–31.2, 29.5				

[a]Measured on 7 April 2014 using an infrared temperature detector.

[b]Correlation coefficient (linear [upper], logarithmic [middle] and polynomial [lower]) between temperature sets (measured temps. [air] or median of measured values [ground and canopy temp.]) was calculated for each sampling period ($n = 8$, df = 6).

[c]n.s. = not significant, correlation formula and *r* values are not presented.

temperature in exps 1 and 2, are given in the supporting information Figs 1–3. In the example taken from exp. 1, air temperature ranged from 10 to 48°C, soil temperature from 16–32°C and RH from 34 to 96% (Fig. 1, Supporting Information), and respective data from exp. 2 were 7.5–48.8°C, 13–33°C and 26–94% (Fig. 2, Supporting Information). In exp. 3, air temperature ranged between 13 and 44°C and RH between 24 and 98% (Fig. 3, Supporting Information). The microclimate conditions varied between the walk-in tunnels in each location, ranging from higher to lower levels than those given in supporting information Figs 1–3. For instance, air temperature recorded in exp. 3 was 56°C but most temperature peaks were at 45–48°C. The disease and microclimate data were used to quantify relationships, as described below.

Quantified relationship between microclimate variables recorded under walk-in tunnel conditions and downy mildew severity

Regression analyses between disease severity at various time points of epidemic development and the microclimate variables recorded 1 and 2 weeks before disease evaluation in the walk-in tunnels for the three walk-in tunnel experiments at the two experimental sites are

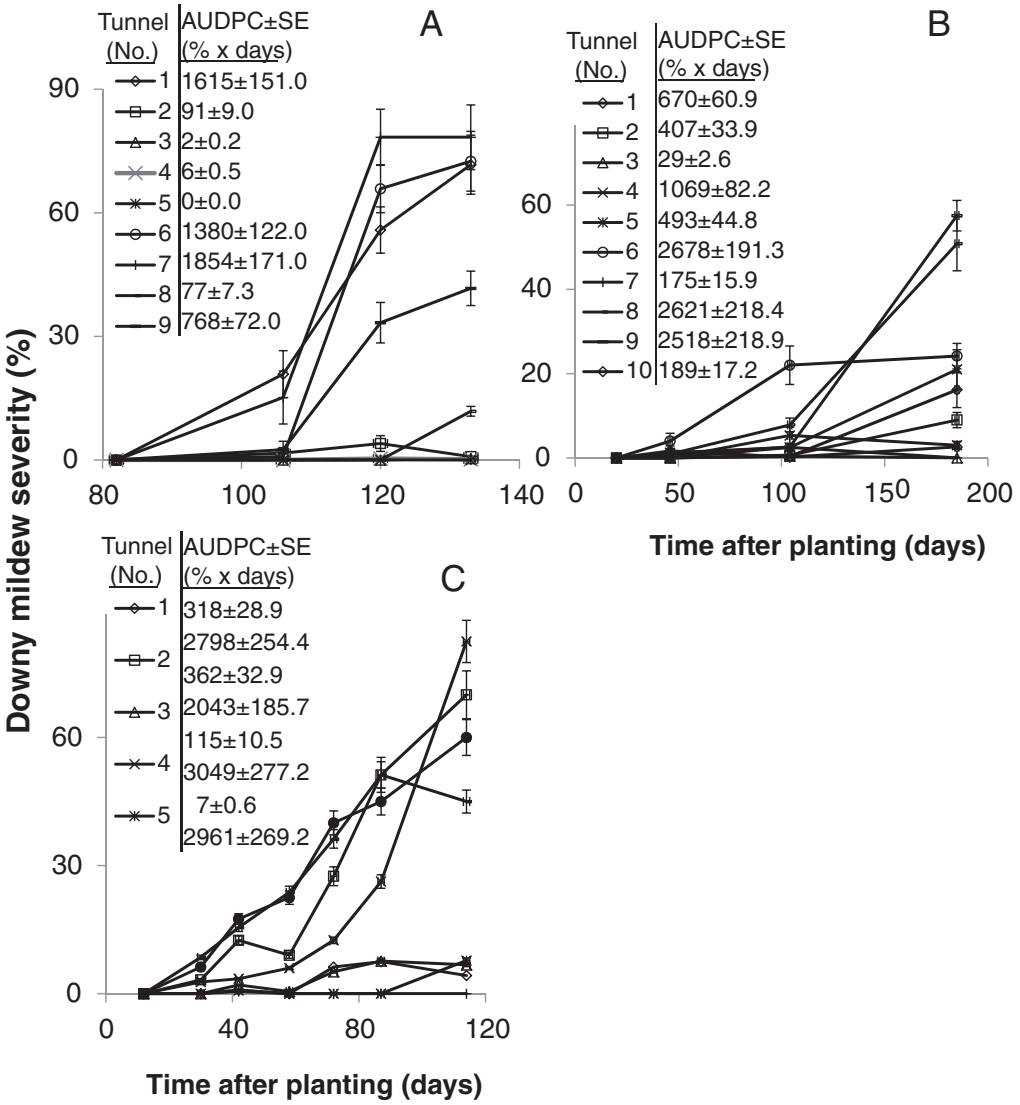

Figure 1 Severity of downy mildew (*Peronospora belbahrii*) on sweet basil plants grown in polyethylene-covered walk-in tunnels at site 1, exp. 1 (A, spring 2013), site 1, exp. 2 (B, spring 2014) and site 2 (C, spring 2014). Severity is described at each sampling date (% ± SE [bars], 5 degrees of freedom) and as area under the disease progress curve [AUDPC] throughout the entire period of severity evaluation.

summarised in the supporting information Tables 1–3. Selected data are presented in Figs 2–4.

In exp. 1 (site 1, 2012/2013 growing season), average daily (24 h) air temperatures were negatively related to disease severity. Average duration per day (number of hours) at air temperatures of 16–25°C was positively related to BDM severity, whereas duration per day of temperatures above 20°C, 25°C and 30°C was negatively related to BDM (Table 1A, Supporting Information; Fig. 2A and 2B).

Average RH levels over an entire day, during the daytime and at night, and number of hours per day at RH above 85% were positively related to BDM severity in exp. 1. Number of hours at RH below 65 and 75% and at RH between 65 and 85% were negatively related to BDM severity (Table 1B, Supporting Information; Fig. 2C and 2D).

The averages of soil temperature over an entire day, during the daytime and at night, as well as the number of hours at soil temperature above 24°C, 27°C and 30°C, were negatively related to BDM in exp. 1 (Table 1C, Supporting Information; Fig. 2E and 2F).

In exp. 2 (site 1, 2013/2014 growing season), average air temperatures over an entire day, during the daytime and at night, and daytime peak hours and

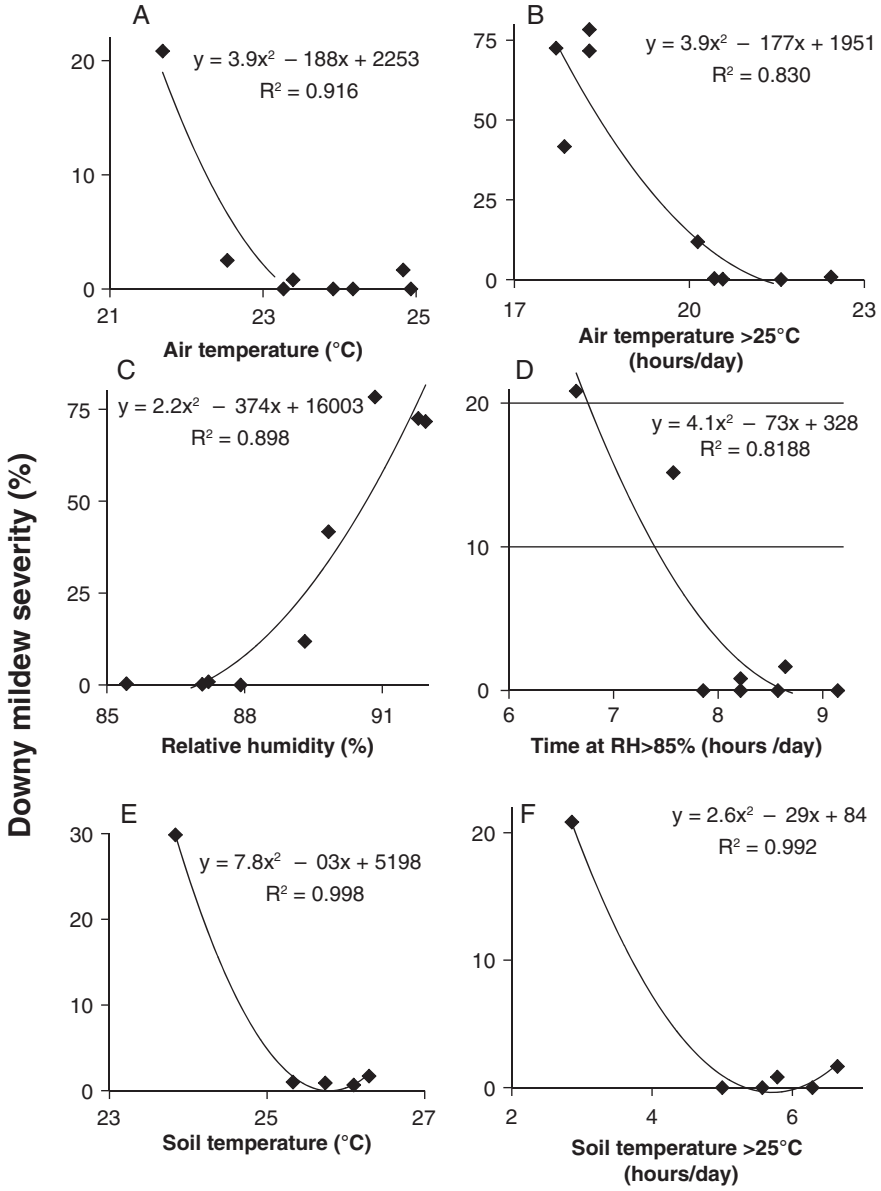

Figure 2 Regression analysis between basil downy mildew (BDM) disease severity and measured or calculated microclimate variables at site 1, exp. 1 (spring 2013). Each graph shows the lines of the calculated linear, logarithmic and polynomial regression analyses, the formulas that describe these types of regression analysis, and correlation coefficient (*r*) values. Regression analyses are presented for average air temperature from 0700–1700 h versus BDM severity on 28 March 2013 (A), and number of hours per day with air temperature >25°C versus BDM severity on 24 April 2013 (B); average relative humidity (RH) from 0700–1700 h versus BDM severity on 24 April 2013 (C), and number of hours per day with RH <75% versus BDM severity on 28 March 2013 (D); average soil temperature from 0700–1700 h versus BDM severity on 12 May 2013 (E), and number of hours per day with soil temperature >27°C versus BDM severity on 28 March 2013 (F). Only significant correlations are presented (7 degrees of freedom).

lower-temperature hours 1 and 2 weeks prior to the disease-evaluation dates were negatively related to disease severity on both sampling dates. The values of temperature × time variables at temperatures above 15°C, 20°C, 25°C and 30°C and in the ranges of 20–30°C, 25–30°C and 25–35°C were negatively related to BDM severity. Similarly, the number of hours with air temperatures above 25°C, 30°C and 35°C and in the range of 25–30°C were negatively related, whereas duration at air temperatures below 25 and 30°C were positively related to BDM severity at the presented sampling times and for the 1- and 2-week periods prior to disease evaluation (Table 2A, Supporting Information; Fig. 3A).

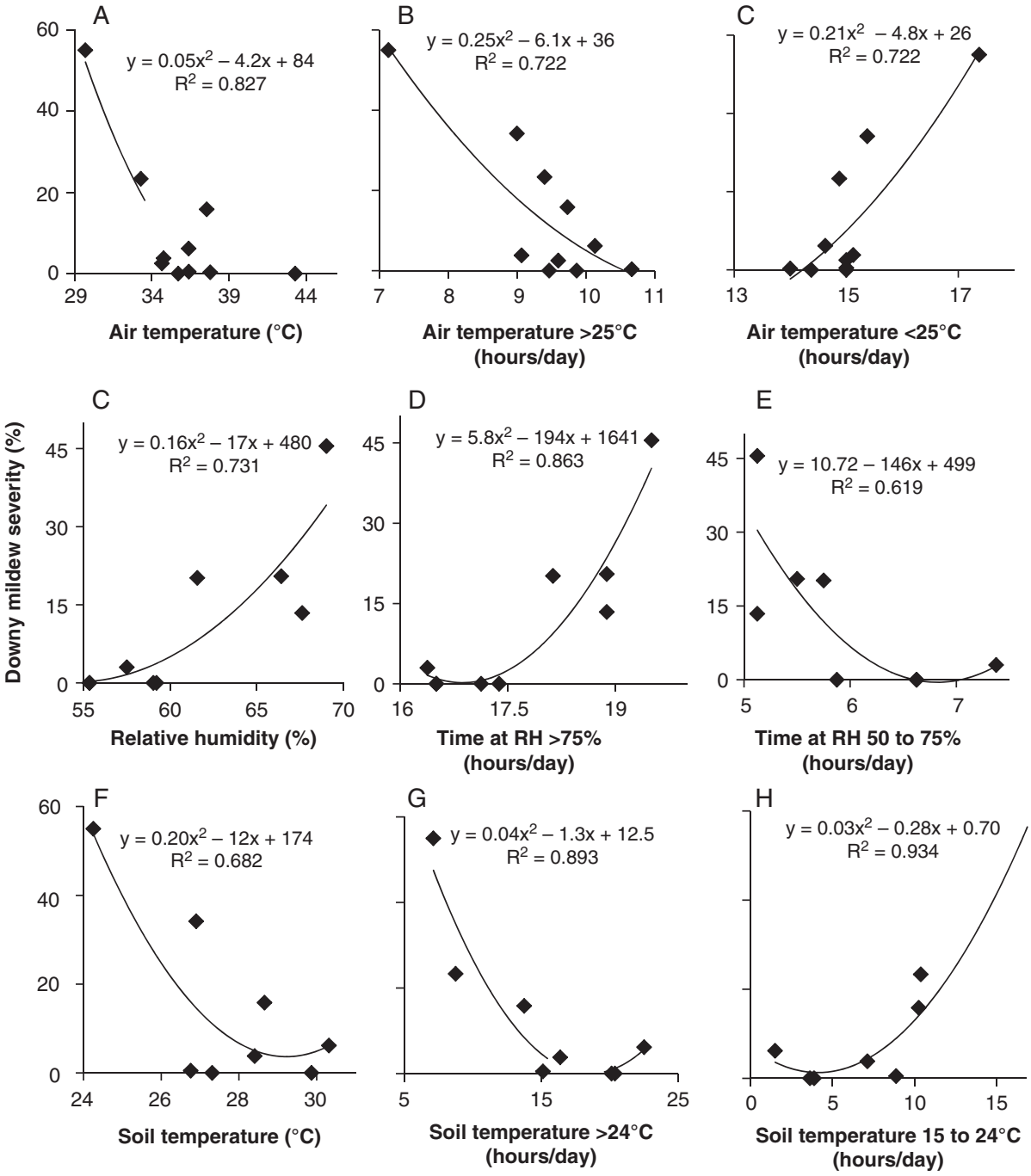

Figure 3 Regression analysis between basil downy mildew (BDM) disease severity and measured or calculated microclimate variables at site 1, exp. 2 (spring 2014). Each graph shows the lines of the calculated linear, logarithmic and polynomial regression analyses, the formulas that describe these types of regression analysis, and correlation coefficient (*r*) values. Regression analyses are presented for average air temperature from 0700–1700 h versus BDM severity 153 days after planting (DAP) (A), number of hours per day with air temperature >25°C versus BDM severity 153 DAP (B), and number of hours per day with air temperature <25°C versus BDM severity 153 DAP (C); average relative humidity (RH) from 0700–1700 h versus BDM severity 104 DAP (D), number of hours per day with RH >75% versus BDM severity 104 DAP (E), and number of hours per day with RH of 50–75% versus BDM severity 104 DAP (F); average soil temperature from 0700–1700 h versus BDM severity on 153 DAP (G), number of hours per day with soil temperature >24°C versus BDM severity 153 DAP (H), and number of hours per day with soil temperature of 15–24°C versus BDM severity 153 DAP (I). Only significant correlations are presented (8 degrees of freedom).

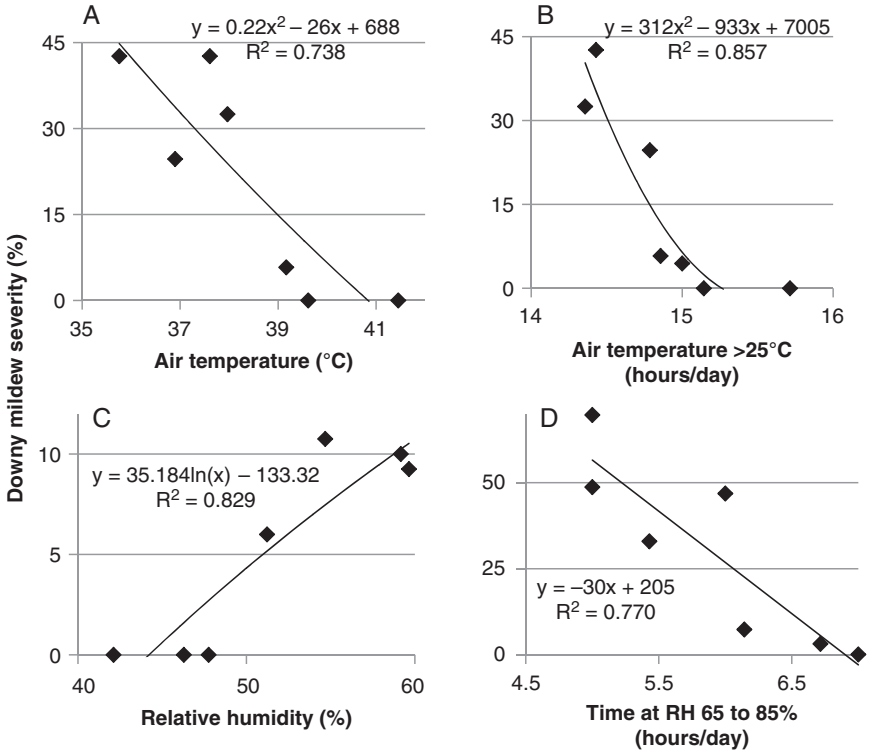

Figure 4 Regression analysis between basil downy mildew (BDM) disease severity and measured or calculated microclimate variables at site 2 (spring 2014). Each graph shows the lines of the calculated linear, logarithmic and polynomial regression analyses, the formulas that describe these types of regression analysis, and correlation coefficient (*r*) values. Regression analyses are presented for average air temperature from 1200 to 1500 h versus BDM severity 87 DAP (A), and number of hours per day with air temperature >25°C versus BDM severity 87 DAP (B); average relative humidity from 0700–1700 h versus BDM severity 87 DAP (C), and number of hours per day with RH of 65–85% versus BDM severity 114 DAP (D). Only significant correlations are presented (6 degrees of freedom).

In exp. 2, average RH levels over an entire day, during the daytime and at night were positively related to BDM severity. The values of RH above 75 and 85% × time variables and the duration per day at RH above 65%, 75% and 85% were also positively related to BDM severity. In contrast, the duration at RH between 50 and 75% and between 65 and 85% was negatively related to BDM severity (Table 2B, Supporting Information; Fig. 3B).

In exp. 2, average soil temperatures over an entire day, during the daytime and at night, and daytime peak hours and lower-temperature hours were negatively related to disease severity on the two sampling dates and for microclimate values that prevailed 1 and 2 weeks prior to the disease-evaluation dates. The values of soil temperature × time variables, and the number of hours with soil temperatures above 21°C, 24°C, 27°C and 30°C were negatively related to BDM. In contrast, a positive relation between soil temperature and disease severity was observed for time intervals at 15–21 and 15–24°C in the two microclimate periods related to the second evaluation date. A positive correlation between soil temperature and disease severity was also observed for temperatures below 27°C, 24°C and 21°C (Table 2C, Supporting Information; Fig. 3C).

In exp. 3 (site 2, 2013/2014 growing season), average air temperatures over an entire day, during the daytime and at night, and daytime peak hours and lower-temperature hours were negatively related to disease severity on the three sampling dates. The number of hours and values of temperature × time variables at temperatures above 25°C, 30°C and 35°C were negatively related to BDM severity. Temperatures below 25 or 20°C and temperatures between 20 and 25°C were positively related to disease severity (Table 3A, Supporting Information; Fig. 4A).

In exp. 3, average RH levels during the daytime and at night were positively related to BDM severity, whereas the values of RH above 50%, 65% and 75% × time variables were negatively related to BDM severity. This negative correlation originated from the values of 65–85% RH, whereas the accumulation of RH hours above 85% was positively related to BDM (Table 3B, Supporting Information; Fig. 4B).

In all three locations, RH × temperature was calculated for all of the data taken each hour in the walk-in tunnels. Regression analyses between the RH × temperature averages over an entire day, during the daytime and at night, and from 0400–0700 to 1400–1700 h were calculated as described above for the respective air temperature and RH values alone. Correlation coefficients of RH × temperature versus disease severity were similar or lower than the respective RH and temperature correlation coefficients, and therefore the RH × temperature values are not presented.

Climate–canopy temperature relationships

Canopy surface temperature was measured four times during the day. In the first round of measurements, air and ground surface temperatures in the eight walk-in tunnels were moderately low (up to 19.6°C air temperature), and the respective canopy surface was comparably cool – up to 14.5°C (Table 1). In the second round of measurements, the air temperature rose to over 30°C in six out of the eight tunnels, whereas none of the canopy surface median temperatures reached 30°C. The third round of measurements revealed air and ground surface temperatures of up to 50.2°C and somewhat higher median canopy surface temperatures of 30°C (31.2–34.2°C) in three tunnels.

Despite air temperatures that were mostly above 40°C in the fourth round of temperature measurements, the canopy surface median temperatures were below 30°C (Table 1). Air and ground temperature data sets were positively related in the three latter measurement rounds. Air and canopy temperature data were only positively related in the third round of measurements, and ground and canopy temperature sets were only positively related by polynomial formula in the third round of measurements (Table 1). Thus, there was no strong relation between air and canopy temperatures in most of the measurement rounds, confirming the minor influence of air temperature on the temperature of the canopy.

Effect of air and root temperature on BDM under controlled conditions

Experiments were conducted with potted plants under controlled conditions to clarify the role of air and root zone temperature in BDM development. Infected potted sweet basil plants were kept under controlled conditions of daytime temperatures at 20 ± 1 to 31 ± 1°C for 2 weeks. BDM did not develop at 31°C. The disease was more severe at 26°C than at 20°C, but the incidence of BDM was similar at these temperatures (Fig. 5A and 5B).

For plants grown at the same temperatures but subjected to *P. belbahrii* inoculation only after incubation in the growth chambers, low disease severity was observed on plants previously kept at 26–31°C. The epidemic in the plants grown at 31°C was the least severe, as indicated by the AUDPC results. At the end of the disease-promoting incubation period, the incidence of BDM was lower on plants previously kept at 31°C. The AUDPC results indicated lower incidence on plants that had been incubated at 26–31°C (Fig. 5C and 5D).

The temperature data from the walk-in tunnel experiments indicated that root zone temperature might play a role in the susceptibility of sweet basil plants to *P. belbahrii* infection. The root zone temperatures in the growth chamber experiment were 4–7°C lower than the air temperature (Fig. 6A). Since field data indicated the potential importance of soil temperature, experiments with potted sweet basil plants were especially designed to maintain daytime root zone temperatures of 22 ± 1 to 31 ± 1°C (Fig. 6B), while the daytime leaf temperature was kept at 22 ± 1°C (Fig. 6C).

The effect of duration of root zone heating on BDM of potted sweet basil plants was tested. In these initial experiments, plant infection during 8 and 24 h day^{-1} exposure to root zone heating at 28°C resulted in 18.0 ± 2.04 and 4.5 ± 1.27% disease severity, respectively, whereas no root zone heating resulted in 38.2 ± 1.23% severity.

A root temperature of 31°C resulted in lower BDM severity and incidence, whereas a root temperature of 26°C resulted in the highest BDM severity and incidence (Fig. 7A and 7B). Infection with *P. belbahrii* after root zone heating at 26 and 31°C resulted in lower disease severity (Fig. 7C), but the disease incidence was similar in the three root zone temperature treatments, resulting in 100% infection of the evaluated leaves 20 days after infection (not shown).

Oospore production by *P. belbahrii*

Symptomatic leaves collected from the walk-in tunnels commonly bore the mycelium, typical sporangiophores and spores of the oomycete on their surface. Surprisingly, thick-walled oospores were also observed on the leaves (Fig. 8). The oospores were attached to the leaf surface in older parts of the infection area and were also found in water washes of the leaf surface (Fig. 8).

Discussion

Downy mildews have been reported to develop at moderate temperatures (Spencer, 1981). For instance, rose downy mildew (*Peronospora sparsa*) develops at temperatures up to 25°C (Aegerter *et al.*, 2003). *Peronospora*

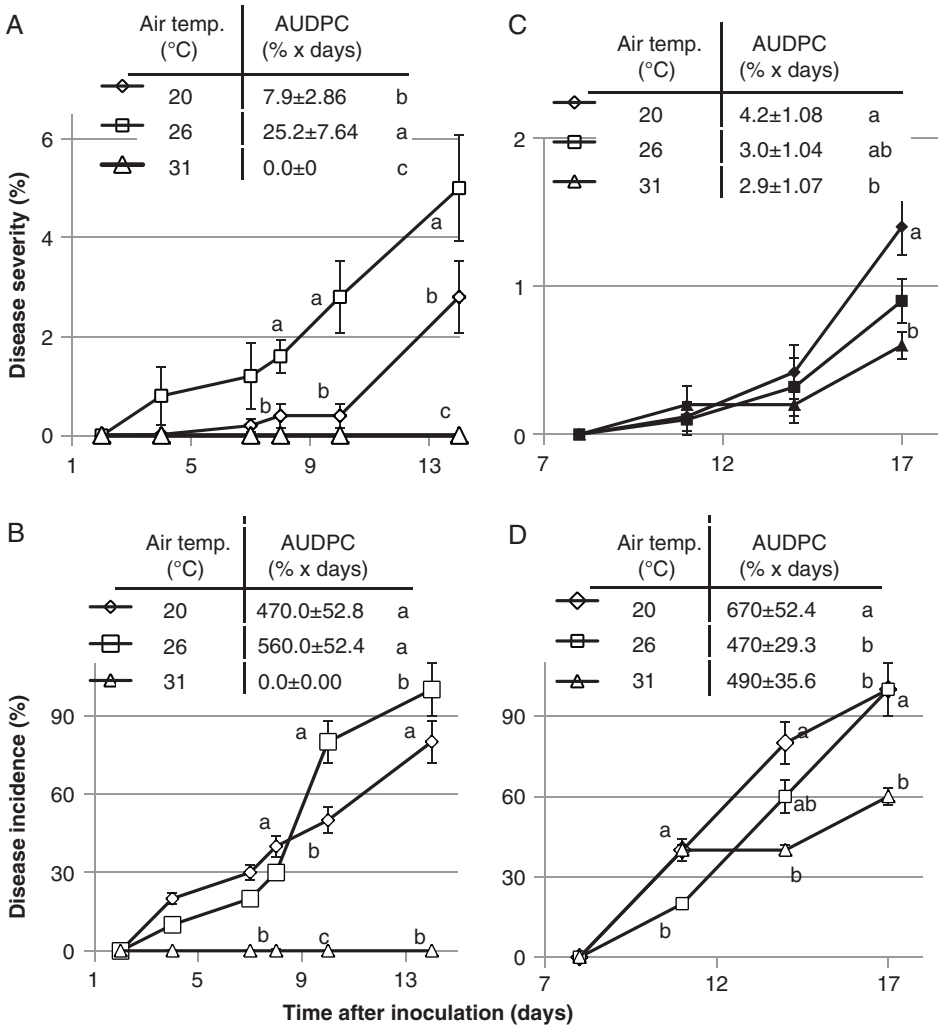

Figure 5 Severity (A and C) and incidence (B and D) of basil downy mildew (BDM) on potted plants incubated under conditions of whole-plant temperature variations with daily peak air temperatures set to 20 ± 1°C, 26 ± 1°C, and 31 ± 1°C during the day (0700–1700 h) and 11 ± 1°C at night. Plants were infected before incubation at the various air temperatures and disease developed during the incubation (A and B), or plants were infected after incubation at the various air temperatures and incubated under optimal conditions for BDM development (C and D). Disease variables are described at each sampling date (% ± SE [bars] 4 degrees of freedom). Data points at each date and AUDPC comparison labelled by a common letter are not significantly different according to Fisher's protected LSD test.

belbahrii develops at temperatures up to 27°C (Garibaldi *et al.*, 2007) or 29°C (Y. Cohen, personal communication). It takes *Peronospora destructor*, the causal agent of onion downy mildew, 3 h to penetrate host tissue via the stomata, and the incubation time is 10–17 days from infection to symptom expression at temperatures between 3.5 and 25°C. A relatively high temperature of 25–30°C lengthens the incubation period to 18 or 20 days (Viranyi, 1981). In 1970, Rotem and Cohen reported that lesion appearance and sporulation of *Peronospora tabacina* on tobacco leaves is delayed and ultimately suppressed with increasing duration of exposure to temperatures between 30 and 40°C – intercalated in a 20°C incubation period. This effect was observed when previous colonisation had occurred at a temperature that favoured the pathogen (Rotem & Cohen, 1970). Thus, more extreme temperatures also play a role in the development of downy mildews. In the present work, air temperature in the walk-in tunnels reached 44–49°C and soil temperature reached 32–33°C. Disease severity was high in walk-in tunnels with moderate temperatures, i.e. temperatures that were more than 5°C lower than the maximal temperatures; on the other hand, disease severity was low in tunnels with the high-temperature regimes, despite the presence of pathogen inoculum in those tunnels. Furthermore, symptomless plants from the warm tunnels

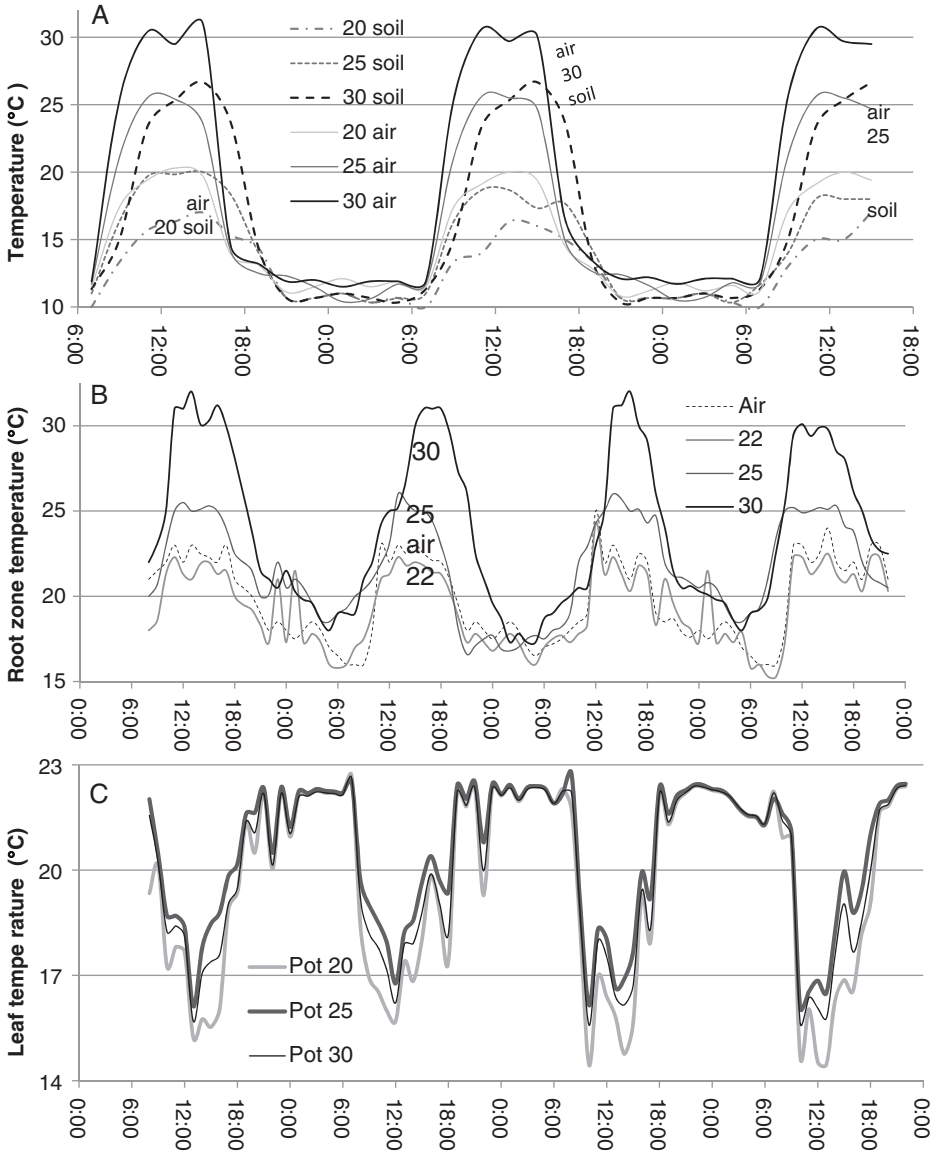

Figure 6 Air and root zone temperatures in experiments with potted sweet basil plants. Air and root temperature measurements in the air-temperature variation experiment (A); air and root temperature measurements in the root temperature variation experiment (B), and leaf temperature measurements in the root-temperature variation experiment (C).

that were transferred to conditions that promote BDM became severely diseased, showing sporulation of *P. belbahrii* on the leaves. Thus, there are microclimate conditions that suppress the appearance of disease symptoms in the walk-in tunnels with high temperatures.

Taken together, the three experiments that were carried out under commercial conditions revealed that BDM is negatively related to the occurrence of air temperature >25°C, RH in the range of ca 65–85%, and relatively higher soil temperature. Surprisingly, under the conditions in the walk-in tunnels, the increase in air temperature did not result in a similar increase in leaf temperature. Combined with the fact that the plants hosted live *P. belbahrii* thalli, it can be concluded that the effect of high greenhouse temperature on BDM may not result from a direct negative effect of high temperature on the pathogen that populates the leaves, but from an indirect high-temperature effect on the host, rendering it less susceptible to pathogen development. Temperature might have affected the survival of *P. belbahrii* mycelium and spores in the greenhouse environment in places where the temperature reached high levels. However, we

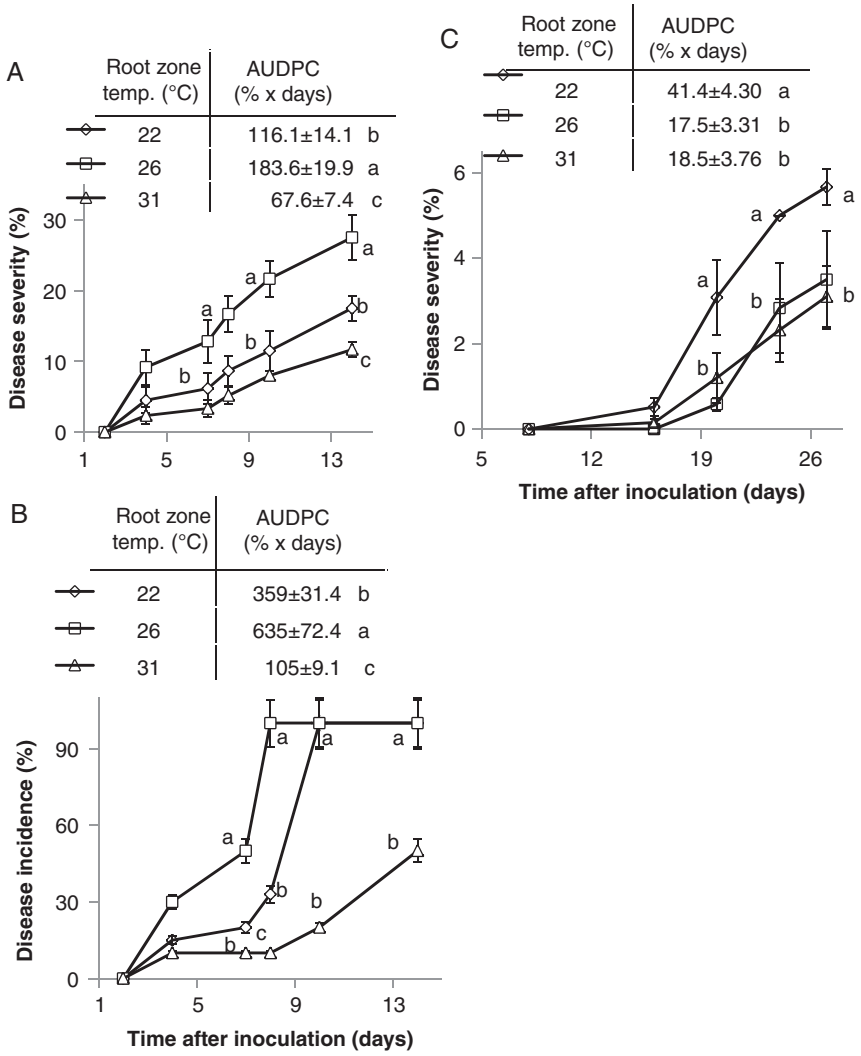

Figure 7 Severity (A and C) and incidence (B) of basil downy mildew (BDM) on potted plants incubated with root temperatures set to maxima of 22°C, 26°C and 31°C and air temperature set to a maximum 22.5°C during the day (0700–1700 h). Plants were infected before incubation at the various root zone temperatures and disease developed during the incubation (A and B), or plants were infected after incubation at the various root zone temperatures and incubated under optimal conditions for BDM development (C). Disease variables are described at each sampling date (% ± SE [bars], 4 degrees of freedom). Data points at each date and AUDPC comparison labeled by a common letter are not significantly different according to Fisher's protected LSD test.

found oospores on the symptomatic sweet basil leaves; survival of these pathogen structures is generally associated with a minimal effect of high temperature (Van der Gaag *et al.*, 1993; Van der Gaag & Frinking, 1997). The occurrence of *P. belbahrii* oospores has been reported previously (Cohen *et al.*, 2013). Thus, high air temperature might also not have been instrumental in limiting *P. belbahrii* survival in the walk-in tunnels.

The pot experiments, in which a separation was maintained between the heated plant part and the infected plant organs, revealed the mode of heat activity. Exposing the roots to high temperature that was similar to the soil temperatures prevailing in the warmer greenhouses resulted in suppression of canopy BDM, even though the canopy itself was not exposed to the high temperatures. Furthermore, the effect lasted for 1–2 weeks after the plants were removed from the heated soil treatments and maintained under optimal conditions for BDM development. We suggest that the sweet basil plants reacted to the heat-treatment signal by becoming resistant to infection or disease development, or that they developed in a way that rendered them less susceptible to the disease, as suggested further on. This is despite the fact that inoculum of downy mildew pathogens spreads better when temperatures are high and RH is comparatively low (Granke & Hausbeck, 2011). The latter authors monitored airborne

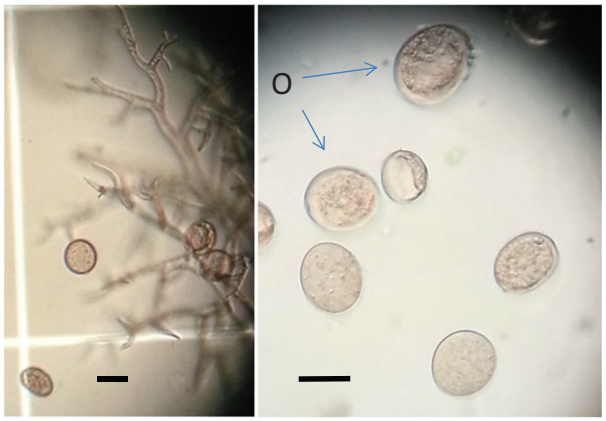

Figure 8 Micrographs of *Peronospora belbahrii*-infected sweet basil leaf surfaces water washes. Note the oospores' (O) thick cell walls and sporangia. Bar = 30 μm.

concentrations of *Pseudoperonospora cubensis* sporangia in a commercial cucurbit field and calculated their correlation with temperature, RH, leaf wetness and rainfall. It was concluded that sporangia are likely to be airborne above the crop canopy during periods of high temperature and low RH with leaf wetness (Granke & Hausbeck, 2011).

Heat treatment has been shown to be beneficial in sweet basil. Aharoni *et al.* (2010) demonstrated that postharvest hot-air treatment consisting of 38°C for 8 h applied to afternoon- or evening-harvested basil markedly reduces its susceptibility to chilling injury and decay caused by *B. cinerea* and *Erwinia carotovora*, and enables storage of some basil cultivars at 9°C, a temperature that normally results in substantial injury. Furthermore, heat treatment of basil in the greenhouse 2–3 days before harvest eliminated black spots (due to various pathogens, mainly *Alternaria* spp.) almost completely. The treatment involved closing the greenhouse and allowing the temperature to rise above 42°C for several hours (Kenigsbuch *et al.*, 2010).

Li *et al.* (2012) characterised a cucumber cDNA, designated CsHsp45.9, which encodes a putative heat-shock protein. CsHsp45.9 was significantly induced in cucumber leaves inoculated with *P. cubensis* downy mildew in an incompatible interaction. The authors concluded that CsHsp45.9 harbours broad-spectrum responses to both biotic and abiotic stresses and may play a role in downy mildew resistance in cucumber (Li *et al.*, 2012).

Chang *et al.* (2005) showed that sweet basil plants grown at 25°C for 2 weeks are taller and have higher dry matter content and larger leaves than plants grown at other temperatures. Moreover, the total volatile oil contents in fresh leaves of plants grown at 25 or 30°C for 2 weeks were three times the levels found in leaves of plants grown at 15°C. Temperature also affected the composition of the volatile oils. Warm conditions (25°C) resulted in the accumulation of eugenol and cis-ocimene (Chang *et al.*, 2005). Eugenol has antimicrobial properties (Dhara & Tripathi, 2013) and has been shown to induce resistance in tomato plants (Wang & Fan, 2014). The volatile oil of *Ocimum sanctum* includes eugenol as a major constituent (55.3%), and this oil exhibited complete inhibition of *Rhizoctonia solani* and *Fusarium oxysporum* mycelial growth (Ray *et al.*, 2012). Eugenol also showed antifungal activity against 12 species of microorganisms, including *Rhizopus*, in *in vitro* and *in vivo* tomato fruit systems (Tejeswini *et al.*, 2014). The antimicrobial spectrum of eugenol compounds of clove leaves included the oomycete *Phytophthora capsici* (Tombe *et al.*, 1995).

Conclusions

Heat treatment induces disease resistance in a systemic manner in root zone heated sweet basil plants grown in pots. This treatment may act similarly in the sweet basil–BDM pathosystem under conditions of commercial cropping in walk-in tunnels where the heated root zone volume induces the suppression of symptoms of BDM in the canopy while the leaves themselves do not increase in temperature. Although under commercial conditions both high air temperature and high soil temperature were negatively correlated with BDM severity, the controlled conditions experiments and the fact that the pathogen inoculum are not significantly affected by the heat treatment lead to the conclusion that the increase in soil temperature is instrumental in inducing decreased susceptibility of the leaves to disease development. Passive heating of the crop, which is essentially effective via an increase in root zone temperature, is inexpensive. It was tested in the spring in Israeli regions of basil cropping and will require some adaptations before it can be tested in other regions. Furthermore, it may be hypothesised that the heat treatment induced eugenol production and release by sweet basil, and that vapours of this compound have a direct effect on the causal agent and/or cause indirect induction of resistance to the pathogen.

Acknowledgments

The authors acknowledge the help of Ran Shulhani, Menahem Borenshtein from the Volcani Center; Shimon Pivonia, Ami Maduel, Avihai Vain and Ton Grunvald from R&D Northern and Central Arava and Tzion Dako from Eden Research Station, Emek Hama'aynot R&D. This research was funded by the Chief Scientist of the Israeli Ministry of Agriculture and Herbs Growers Board project no. 132-1702.

References

Aegerter B.J., Nuñez J.J., Davis R.M. (2003) Environmental factors affecting rose downy mildew and development of a forecasting model for a nursery production system. *Plant Disease*, **87**, 732–738.

Aharoni N., Kenigsbuch D., Chalupowicz D., Faura-Mlinski M., Aharon Z., Maurer D., Ovadia A., Lers A. (2010) Reducing chilling injury and decay in stored sweet basil. *Israel Journal of Plant Sciences*, **58**, 167–181.

Belbahri L., Calmin G., Pawlowski J., Lefort F. (2005) Phylogenetic analysis and real time PCR detection of a presumably undescribed *Peronospora* species on sweet basil and sage. *Mycological Research*, **109**, 1276–1287.

Chang X.-M., Alderson P.G., Wright C.J. (2005) Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum* L.) *The Journal of Horticultural Science and Biotechnology*, **80**, 593–598.

Cohen Y., Vaknin M., Ben-Naim Y., Rubin A.E., Galperin M., Silverman D., Bitton S., Adler U. (2013) First report of the occurrence and resistance to mefenoxam of *Peronospora belbahrii*, causal agent of downy mildew of basil (*Ocimum basilicum*) in Israel. *Plant Disease*, **97**, 692.

Dhara L., Tripathi A. (2013) Antimicrobial activity of eugenol and cinnamaldehyde against extended spectrum beta lactamase producing Enterobacteriaceae by *in vitro* and molecular docking analysis. *European Journal of Integrative Medicine*, **5**, 527–536.

Dik A.J., Wubben J.P. (2004) Epidemiology of *Botrytis cinerea* diseases in greenhouses. In *Botrytis: Biology, Pathology and Control*, pp. 319–333. Eds Y. Elad, B. Williamson, P. Tudzynski and N. Delen. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Djalali Farahani-Kofoet R., Römer P., Grosch R. (2012) Systemic spread of downy mildew in basil plants and detection of the pathogen in seed and plant samples. *Mycological Progress*, **11**, 961–966.

Dudai N., Chaimovitsh D., Reuveni R., Ravid U., Larkov O., Putievsky E. (2002) Breeding of sweet basil (*Ocimum basilicum*) resistant to *Fusarium oxysporum* f. sp. *basilicum*. *Journal of Herbs, Spices & Medicinal Plants*, **9**, 45–51.

Elad Y. (1999) Plant diseases in greenhouses. In *The Greenhouse Ecosystem*, pp. 191–210. Eds G. Stanhill and H.Z. Enoch. Amsterdam, the Netherlands: Elsevier.

Elad Y., Shtienberg D. (1995) *Botrytis cinerea* in greenhouse vegetables; chemical, cultural, physiological and biological controls and their integration. *Integrated Pest Management Reviews*, **1**, 15–29.

Elad Y., Fogel M., Rav David D., Messika Y., Jacob D., Silverman D., Shapiro D., Adler U., Esquira I., Yitzhak S., Deko T., Harary D., Maduel A., Pressman E. (2014a) Daytime solar heat treatment for the suppression of foliar plant pathogens in polyethylene-covered greenhouses. *Acta Horticulturae*, **1015**, 89–94.

Elad Y., Israeli L., Fogel M., Rav David D., Kenigsbuch D., Chalupowicz D., Maurer D., Lichter A., Silverman D., Biton S., Yitzhak S., Harari D., Maduel A., Pivonia S., Adler U. (2014b) Conditions influencing the development of sweet basil grey mould and cultural measures for disease management. *Crop Protection*, **64**, 67–77.

Elad Y., Fogel M., Silverman D., Biton S., Yitzhak S., Harari D., Adler U. (2015) White mould of sweet basil: conditions influencing its development in greenhouses and cultural measures for disease management. *Plant Pathology*, **64**, 951–960.

Garibaldi A., Minuto A., Minuto G., Gullino M.L. (2004) First report of downy mildew on basil (*Ocimum basilicum*) in Italy. *Plant Disease*, **88**, 312.

Garibaldi A., Minuto A., Gullino M.L. (2005) First report of downy mildew caused by *Peronospora* sp. on basil (*Ocimum basilicum*) in France. *Plant Disease*, **9**, 683.

Garibaldi A., Bertetti D., Gullino M.L. (2007) Effect of leaf wetness duration and temperature on infection of downy mildew (*Peronospora* sp.) of basil. *Journal of Plant Diseases and Protection*, **114**, 6–8.

Gilardi G., Demarchi S., Garibaldi A., Gullino M.L. (2013) Management of downy mildew of sweet basil (*Ocimum basilicum*) caused by *Peronospora belbahrii* by means of resistance inducers, fungicides, biocontrol agents and natural products. *Phytoparasitica*, **41**, 59–72.

Granke L.L., Hausbeck M.K. (2011) Dynamics of *Pseudoperonospora cubensis* sporangia in commercial cucurbit fields in Michigan. *Plant Disease*, **95**, 1392–1400.

Hansford C.G. (1932) Annual report of the mycologist. Annual Report of the Department of Agriculture. *Annual Review of Applied Mycology*, **21**, 421.

Hausbeck M.K., Pennypacker S.P., Stevenson R.E. (1996) The use of forced heated air to manage botrytis stem blight of geranium stock plants in a commercial greenhouse. *Plant Disease*, **80**, 940–943.

Li J., Zhang H., Hu J., Liu J., Liu K. (2012) A heat shock protein gene, CsHsp45.9, involved in the response to diverse stresses in cucumber. *Biochemical Genetics*, **50**, 565–578.

Kenigsbuch D., Chalupowicz D., Aharon Z., Maurer D., Ovadia A., Aharoni N. (2010) Preharvest solar heat treatment for summer basil (*Ocimum basilicum*) affects decay during shipment and shelf life. *Acta Horticulturae*, **880**, 161–166.

Khateri H., Calmin G., Moarrefzadeh N., Belbahri L., Lefort F. (2007) First report of downy mildew caused by *Peronospora* sp. on basil in Northern Iran. *Journal of Plant Pathology*, **89**(Suppl.3), S70.

Mersha Z., Zhang S., Fu Y.Q., Mo X.D., Raid R.N., Hau B. (2013) Efficacy of acibenzolar-S-methyl and β-aminobutyric acid for control of downy mildew in greenhouse grown basil and peroxidase activity in response to treatment with these compounds. *Journal of Phytopathology*, **161**, 154–164.

Morgan W.M. (1985) Influence of energy-saving night temperature regimes in *Botrytis cinerea* in an early-season glasshouse tomato crop. *Crop Protection*, **4**, 99–110.

Porta-Puglia A., Mifsud D. (2006) First record of downy mildew caused by *Peronospora* sp on basil in Malta. *Journal of Plant Pathology*, **88**, 124.

Ray D.P., Srivastava S., Dutta D., Roy C., Tarafdar J.C. (2012) Antifungal activity of the essential oil of *Ocimum sanctum* L. against plant pathogens, *Rhizoctonia solani* and *Fusarium oxysporium*. *Pesticide Research Journal*, **24**, 17–20.

Roberts P.D., Raid R.N., Harmon P.F., Jordan S.A., Palmateer A.J. (2009) First report of downy mildew caused by a *Peronospora* sp on basil in Florida and the United States. *Plant Disease*, **93**, 199.

Römer P., Grosch R., Kofoet A., Djalali Farahani-Kofoet R. (2010) Selection of basil (*Ocimum basilicum*) breeding material resistant against downy mildew (*Peronospora* sp.) and tolerant to low temperature. *Acta Horticulturae*, **860**, 147–152.

Ronco L., Rollán C., Choi Y.J., Shin H.D. (2009) Downy mildew of sweet basil (*Ocimum basilicum*) caused by *Peronospora* sp. in Argentina. *Plant Pathology*, **58**, 395.

Rotem J., Cohen Y. (1970) The effect of temperature on the pathogen and on the development of blue mold disease in tobacco inoculated with *Peronospora tabacina*. *Phytopathology*, **60**, 54–57.

Shapiro D., Deko T., Itshak I., Silverman D., Sacks M., Adler U., Esquira I., Stigliz Y. (2014) An innovative way for heating greenhouses using solar energy during the winter for summer crop production. *Acta Horticulturae*, **1015**, 37–44.

Spencer D.M. (Ed.) (1981) *The Downy Mildews*. London, UK: Academic Press. 636 pp.

Tejeswini M.G., Sowmya H.V., Swarnalatha S.P., Negi P.S. (2014) Antifungal activity of essential oils and their combinations in *in vitro* and *in vivo* conditions. *Archives of Phytopathology and Plant Protection*, **47**, 564–570.

Thines M., Telle S., Ploch S., Runge F. (2009) Identity of the downy mildew pathogens of basil, coleus, sage with implications for quarantine measures. *Fungal Biology*, **113**, 532–540.

Tombe M., Kobayashi K., Oniki M., Ogoshi A. (1995) Toxicity of clove eugenol against several pathogenic fungi. *Indonesian Journal of Crop Science*, **10**, 11–18.

Van der Gaag D.J., Frinking H.D. (1997) Survival characteristics of oospore populations of *Peronospora viciae* f. sp. *pisi* in soil. *Plant Pathology*, **46**, 978–988.

Van der Gaag D.J., Frinking H.D., Geerds C.F. (1993) Production of oospores by *Peronospora viciae* f. sp. *fabae*. *Netherlands Journal of Plant Pathology*, **99**(Supplement 3), 83–91.

Viranyi F. (1981) Downy mildew of onion. In *The Downy Mildews*, pp. 461–472. Ed D.M. Spencer. London, UK: Academic Press.

Wang C., Fan Y. (2014) Eugenol enhances the resistance of tomato against tomato yellow leaf curl virus. *Journal of the Science of Food and Agriculture*, **94**, 677–682.

Wyenandt C.A., Simon J.E., McGrath M.T., Ward D.L. (2010) Susceptibility of basil cultivars and breeding lines to downy mildew (*Peronospora belbahrii*). *HortScience*, **45**, 1416–1419.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Microclimate data recorded outside (out) and inside 9 walk-in tunnels (1–9) over 3 days on 25 through 27 March 2013 at site 1, exp. 1 (spring 2013). Air temperature (A), relative humidity (B) and soil temperature (C) were recorded

Fig. S2. Microclimate data recorded outside (out) and inside 10 walk-in tunnels (1–10) over 3 days on 15 through 17 March 2014 at site 1, exp. 2 (spring 2014). Air temperature (A), relative humidity (B) and soil temperature (C) were recorded

Fig. S3. Microclimate data recorded outside (out) and inside 8 walk-in tunnels (1–8) over 3 days on 20 through 22 March 2014 at site 2 (spring 2014). Air temperature (A) and relative humidity (B) were recorded

Table S1. Effect of microclimate variables on downy mildew severity in Eden walk-in tunnel ($n = 9$, 7 degrees of freedom) during 2012/2013. Regression analysis was calculated between disease severity values on sampling dates and calculated climate data collected during the preceding 7 days

Table S2. Effect of microclimate variables on downy mildew severity in Eden walk-in tunnels ($n = 10$, degrees of freedom) at two sampling times in 2014. Regression analysis was calculated between disease severity and microclimate variables calculated for 1–7 and 1–14 days preceding disease evaluation

Table S3. Effect of microclimate variables on downy mildew severity in Zohar walk-in tunnel ($n = 8$, 6 degrees of freedom) in 2014. Regression analysis was calculated between disease severity values at each of three sampling dates and calculated climate data collected during the preceding 7 days