European Journal of Plant Pathology **104:** 753–763, 1998.

Significance of leaf infection by *Botrytis cinerea* in stem rotting of tomatoes grown in non-heated greenhouses

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Accepted 8 July 1998

Key words: cultural control, Integrated Pest Management, *Lycopersicon esculentum*, sanitation

Abstract

The most serious symptom of *Botrytis cinerea* in tomatoes grown in greenhouses is stem rotting. Lesions on the stem may result from direct infection or from progression of the rot along infected leaves, until infection approaches the stem. In a set of experiments conducted in commercial greenhouses, an experimental greenhouse and growth chambers, the significance of the two types of stem infections was studied. In non-heated greenhouses most of the stem lesions originated from progression of the pathogen along infected petioles. The rate at which *B. cinerea* had progressed on infected petioles was 0.3–0.5 cm/day, an average of ca. 6 weeks was needed for a leaf infection to approach the stem. Application of *Trichoderma harzianum* T39 extended this time by 1–2 weeks and application of chemical fungicides by 3 weeks. Influence of the environment on the progression of *B. cinerea* along infected petioles was then determined. Within range of 5–30 °C, the higher the temperature, the more rapid was the rate of disease progression. The fungus progressed more rapidly on tomato petioles incubated at high vapour pressure deficit (VPD) rather than at low VPD. The source-sink relationship of the plant governed the rate of *B. cinerea* progression along the petioles as well: it was more rapid when the source was restricted (by shading) and slower when the sink was restricted (by removal of flowers and small fruits). The possibility that sanitation of infected leaflets would reduce the incidence of stem rotting was examined in two experiments. In plots not treated with a fungicide, the sanitation treatment substantially decreased the incidence of stem lesions and this treatment was as effective as weekly application of chemical fungicides.

Introduction

Botrytis cinerea Pers.: Fr., the causal agent of grey mould, attacks flowers, fruits, leaves and stems of tomato plants grown in greenhouses. The relative importance of the various symptoms is governed by the environmental conditions and by the cultural practices: in non-heated or partially heated greenhouses, the pathogen infects primarily leaves, but lesions on the stems are also apparent. In heated greenhouses, the occurrence of leaf and fruit infections is limited but infections on stems are common. Stem rotting caused by *B. cinerea* has become an increasing problem in long season (up to 8-11 months) tomato crops (Elad and Shtienberg, 1995) and it is often the reason why a crop is finished earlier than planned (Chastagner et al., 1977; Elad et al., 1992; Jarvis, 1989, 1992; O'Neill, 1994; Yunis et al., 1990). Stem rotting may be devastating: for example, in an experiment conducted recently in a commercial greenhouse in Israel under a natural epidemic of *B. cinerea*, 72% of untreated plants died prematurely from stem rotting (Shtienberg and Elad, 1997).

Stem rotting may develop from two distinct sources: (i) direct infection of the stems, usually on wounds that are created during pruning of lower leaves or side branches; and (ii) subsequent progression of the pathogen from an infected leaflet along the petiole, until it approaches the stem (Verhoeff, 1968). Excessive heating and ventilation limits canopy wetness and reduces the intensity of grey mould on leaves, flowers and fruits (Morgan, 1985; Winspear et al., 1970), but is generally less effective in preventing stem infections. Consequently, growers apply fungi-

cides directly to wounds as a protective measure or for curative purposes after detection of infection. Application of biocontrol agents such as *Trichoderma harzianum* T39 may provide adequate protection to the wounds as well (O'Neill et al., 1996).

Some information is available in the literature on various aspects related to direct infection of stems by *B. cinerea*. Wilson (1962) found a clear difference in susceptibility to stem infection by *B. cinerea* between plants of different physiological ages. The change from resistance to susceptibility was related to the plant age and also to the location of the node in the plant: lower nodes were more resistant than upper nodes. Verhoeff (1967) tried to exploit this tendency as a means of preventing the fungus from reaching the stems. He recommended deleafing by cutting the petioles of old leaves as a method of avoiding stem infection. Stem age had no effect on spore germination or on the germ-tube growth, indicating that there is no specific effect on pathogen behavior prior to penetration. The resistance mechanism appeared to be a temporary inhibition of subsequent mycelium growth (Wilson, 1962). Stem infection may be quiescent for up to 12 weeks before becoming aggressive (Jarvis, 1989; Wilson, 1963). Stem susceptibility to infection decreases with time after the occurrence of wounding: 24 h after wounding, the tissue heals over and becomes relatively resistant to infection (O'Neill et al., 1997; Verhoeff, 1967).

The effect of temperature on infection of tomato stems by *B. cinerea* was similar to the reported effects on infection of other plant organs and other hosts, i.e., the optimum temperature for infection is between 10 and 20 °C, but infection occurred even at 5 °C and 26 °C (O'Neill et al., 1997). Low vapour pressure deficit (VPD), free moisture on plant surface and cool weather are considered the most important environmental factors that promote infection by *B. cinerea* on leaves. However, several lines of evidence indicate that ambient relative humidity has only a slight effect on infection of stem wounds. O'Neill et al. (1997) demonstrated that tomato stem wounds could be infected even at a VPD as high as 1.30 kPa and that infection developed at a similar rate under low and high VPD conditions. Fluctuation between low and high VPD regimes had an insignificant effect on rot development. Similarly, Wilson (1963) reported that infection of fresh scars was independent of ambient relative humidity and Eden et al. (1996) found a trend of increasing infection with higher RH, but it was insignificant at the 5% level. Yunis et al. (1990) reported the same findings for *B. cinerea* infections on cucumber plants.

In non-heated tomato greenhouses, symptoms of grey mould are apparent on all plant organs. The influence of the environment, the host and various cultural practices on infection of leaves by *B. cinerea* is well documented (e.g. Jarvis, 1980; Elad and Shtienberg, 1995) and will not be reviewed here. Protecting the foliage with fungicides is, in general, an effective approach to suppress stem rotting. However, the desire to reduce the number of chemical sprays and the risks for development of fungal populations resistant to common fungicides, motivate the search for alternative management methods. If a large proportion of stem lesions in non-heated tomato greenhouses had originated from infected leaves, then blocking the progression of the fungus along the petioles would prevent, or at least lower, the incidence of stem infections. In order to develop such an approach, there is a need to quantify the significance of leaf infection as an origin of stem lesions. Furthermore, knowledge of the factors affecting the progress of *B. cinerea* along tomato petioles is essential (Verhoeff, 1967; Wilson, 1963). Little is known about these subjects. In this study we examine the influence of various factors affecting the progression of *B. cinerea* along infected petioles to identify implications for disease management. The specific objectives were to: (i) find out whether the origin of stem rotting in non-heated greenhouses is through direct infection of stem wounds or via progression of *B. cinerea* along infected petioles; (ii) study the influence of the environment on the progression of grey mould from infected leaves to the stems; and (iii) evaluate the efficacy of removing infected leaflets as a measure to preclude the occurrence of stem lesions.

Materials and methods

Experiments were conducted in commercial greenhouses, an experimental greenhouse and growth chambers. Objective (i) was studied in the experimental greenhouse. Analysis of disease progress curves recorded in commercial greenhouses enabled us to define the rate at which *B. cinerea* progressed along the petiole until it approached the stems and the influence of chemical fungicides and a biocontrol agent on that rate. For objective (ii), the effects of temperature, VPD and source-sink relationship on the rate of progression of *B. cinerea* along petioles were studied in an experi-

mental greenhouse and in growth chambers. Finally, the possibility of managing stem rotting by removing infected leaflets was evaluated in the experimental greenhouse and in a commercial greenhouse.

Experiments in commercial greenhouses

Six experiments were conducted in commercial greenhouses located in the central and southern coastal plain of Israel. Experiments 1 and 2 were conducted in 1992, exp. 3 in 1993, exps. 4 and 5 in 1995, and exp. 6 in 1996. Tomato seedlings (*Lycopersicon esculentum* Mill., cvs. F175, F144 and 198) were planted in August-September of each year. All cultivars were susceptible to *B. cinerea*. Polyethylene-covered greenhouses were not heated or ventilated automatically, but had side vents that were opened for 5-8 h daily when weather permitted. Plants were spaced 0.5 m apart within rows and 1.25 m between rows and grown in a high-wire cropping system. The crop was maintained according to the recommendations of the extension service in the region. No fungicides were applied against *B. cinerea* other than those mentioned for specific experiments below.

Experimental design

The experiments were designed to determine the efficacy of various fungicides and biocontrol agents (Elad et al., 1995) and to develop guidelines for integration of biological and chemical measures for suppression of grey mould (Shtienberg and Elad, 1997). All the experiments were arranged in randomized blocks with four or five replicates per treatment. Each plot consisted of one row, 8-12 m in length (15–25 plants). Each experiment included 4–8 treatments, viz., fungicides or spraying schedules against *B. cinerea.* In this report disease progress recorded in three of the treatments will be considered; details about the other treatments have been given elsewhere (Elad et al., 1995; Shtienberg and Elad, 1997). The three treatments were: (i) untreated control; (ii) the standard commercial practices for management of *B. cinerea*: alternative application on a weekly basis of fungicides from different chemical groups and with different modes of action; and (iii) weekly application of *T. harzianum* T39. The fungicides used were as follows: iprodione (0.5 g a.i./L, Rovral 50 WP, Rhone Poulenc, Lyons, France); vinclozolin (0.5 g a.i./L, Ronilan 50 WP, BASF AG, Ludwigshafen, Germany); chlorothalonil (1.75 g a.i./L, Bravo 50 SC, ISK BioScience, Plainsville, OH, USA); fenbuconazole (0.1 g a.i./L, Indar 5 EC, Rohm & Haas Co., Philadelphia, PA, USA); pyrimethanil (0.75 g a.i./L, Mythos 30 SL, AgrEvo, Berlin, Germany). In addition, the following fungicide mixtures were applied: carbendazim + diethofencarb (0.25 + 0.25 g a.i./L, Resec 25+25 WP, Sumitomo Chemicals Co., Osaka, Japan); prochloraz + folpet (0.45 + 1.8 g a.i./L, Mirage F 15 + 60 WP, Makhteshim Chemical Works Ltd., Be'er Sheva, Israel); and tebuconazole + dichlofluanid (0.25 + 0.62 g a.i./L, Silvacure 10+25 WP, Bayer AG, Leverkusen, Germany). The rate of the biocontrol agent *T. harzianum* T39 was 0.5 g a.i./L (10^9 CFU/g), Trichodex 25 SP (Makhteshim, Israel). The number of fungicide and *T. harzianum* T39 sprays in the various experiments varied between 8 and 13; the specific products and times of application are listed elsewhere (Elad et al., 1995; Shtienberg and Elad, 1997).

Natural epidemics of *B. cinerea* developed in the commercial greenhouses during the winter seasons. Spraying was initiated in all experiments when the first grey mould symptoms were observed: in early December in exp. 4 and in early to mid-January in the rest of the experiments. The chemical and biological fungicides were applied with a backpack sprayer adjusted to 275 kPa of pressure or by a gun sprayer. Care was taken to avoid drift to adjacent plots. Spray volume was calibrated to 1000-1500 L of water/ha.

Disease assessment and data analysis

Disease incidence was assessed every 7–10 days on 8–10 plants in the center of each experimental plot. The number of infected leaves and the number of stem lesions (leaf and stem incidence, respectively) were counted on each plant. Unless otherwise stated, diseased leaves were not removed. The data were used to construct disease progress curves for leaf infection and for stem lesions. These curves enabled us to determine the time that passed from infection of a leaflet until the fungus had approached the stem (by progression along the petiole). This period is equivalent to the time span between the progress curve of infected leaves and the progress curve of stem lesions. It will be referred to hereafter as Δt. For estimation of Δt, a time-series analysis was performed, as follows. The incidence of stem lesions at time t (S_t) was plotted against the incidence of infected leaves at n weeks before (time t-n;L_{t-n}). Then, a regression analysis was performed where S_t was the dependent variable and L_{t-n} was the independent variable. The analysis for each pair of S_t and L_{t-n} data included all disease records from

experiments 1–4 and 6 that still had the relevant time interval (*t-n*). Similar analyses were conducted for all possible comparisons of S_t and L_{t-n} (i.e., *t-n* = 4, 5, 6, 7, 8 and 9 weeks). Since disease was assessed every 7–10 days, and because the smaller time interval in the analysis was one week, there were some cases where interpolation was carried out. For each analysis the slope of the regression equation was compared with a value of 1 by means of the *t*-test. A slope of 1 indicates that the number of stem lesions at time *t* was equal to the number of infected leaves *t-n* weeks previously. It was assumed that this was a good estimation of Δt. To identify the influence of chemical and biological sprays on the progress of *B. cinerea* along the petioles, a separate time series analysis was performed for these treatments, and Δt was defined for them as well.

In exp. 5 a strict sanitation program was employed. Sample plants in the center of each experimental plot (10 plants/plot) were inspected carefully every week. When an infected leaflet was observed, it was manually removed by cutting off the infected tissue together with few (2–4) cm of a healthy section of the leaf. Since most of the diseased leaflets were observed soon after they appeared, at least some part of the leaves remained, in most cases. However, when the infection site was near the stem, the entire leaf was removed. The number of infected leaves and of stem lesions per plant was recorded. For data analysis, the cumulative number of infected leaves was considered.

Experiments in experimental greenhouse

Tomato plants (cv. 144) were planted in a peat/-compost/tuff mixture placed in 1.2 $\times$ 0.70 $\times$ 0.25 m boxes in a polyethylene-covered experimental greenhouse. Air was forced into the greenhouse by means of fans. Ten boxes were placed adjacent to each other, forming a bed 12 m long. All together there were four beds, spaced 1.2 m apart; each bed comprised two rows, ca. 0.50 m apart, intra-row spacing being 0.40 m. Planting was done in early September of each year. Plants were maintained according to the common cultural practices for high wire tomato production. Unless mentioned otherwise, plants were not inoculated artificially and fungicides were not applied.

The origin of stem rotting

The origin of stem lesions (direct infection of stem wounds or progression of *B. cinerea* along infected petiole) was determined in an experiment conducted in 1994. Starting in early January, the plants were carefully examined weekly until late April. Grey mould lesions on the leaves and on the stems were marked by means of numbered plastic bands that were placed around the affected organ. In the case of stem lesions, the origin of infection was recorded. There were four replicate beds (4 m long), with ten plants being monitored in each replicate. For the leaf infections, measurements were taken to determine the rate at which *B. cinerea* progressed along the petiole. Once a week, the gap between the boundary of infection along the petiole and the stem was measured. There were ten leaves per replicate. When infection approached 2 cm or less from the stem, this leaf was not included in further measurements. The rate of disease progression along the petiole (cm/day) was calculated by dividing the difference between two measurements of each leave by the time span. The rates on individual leaves in a certain week were used to calculate the average rate (mean $\pm$ SE) for that week.

Effects of source – sink relationship on progression of B. cinerea *along the petioles*

The influence of source-sink relationship on the rate at which *B. cinerea* progressed along infected petioles was studied in the experimental greenhouse in 1995. The four beds were divided into four subplots, each containing two adjacent 6–m–long beds (four rows of tomato plants). In one of the subplots the crop was maintained according to the usual commercial cultural practices. To regulate the size of the sink, all flowers and small fruits were removed from all plants in two subplots, starting soon after the initiation of blooming. Flower and fruit removal continued weekly, until the end of the experiment; therefore these plants did not bear any fruit. To regulate the source, a net, transmitting only 50% of the incoming sun irradiation, was stretched above the plants in two subplots. The net was placed in early January, ca. 0.5 m above the upper wire to which the plants were attached, to allow air exchange. The net did not alter temperature, nor VPD, as compared with non-covered plots. The experiment was arranged in a factorial arrangement and all four possible treatments were included, viz., (i) with fruits – no shading (‘normal’ crop); (ii) with fruits – with shading (regulation of the source); (iii) no fruits – no shading (regulation of the sink); and (iv) no fruits – with shading (regulation of both the source and the sink). This particular layout of the experiment was chosen because of space limitations. It was not optimal, because it did not enable estimation of the error term associated with differences among the subplots

(there was no true replication of the treatments). Consequently, only the main effects of the treatments and a measure of the variance (SE) were computed. No attempt was made to compare the means of the various treatments statistically.

In each of the four subplots, leaves were artificially inoculated with *B. cinerea* in mid-January. Leaves to be inoculated were designated in the inner-rows of the two beds of each treatment at a height of 0.8–1.4 m above ground (nodes 4–7 above ground); they were marked by means of numbered plastic labels. There were 15-20 leaves (replicates) for each of the four subplots (treatments). A naturally infected cucumber fruit (ca. 5 cm long) was folded in the terminal leaflet of sampled leaves and bound with metal wire. Within a week, ca. 95% of the inoculated leaflets showed typical grey mould symptoms and the disease then started to progress along the petiole. Only successful infections were followed. The gap between the boundary of infection along the petiole and the stem was measured weekly, six times in total. The rate at which *B. cinerea* progressed was calculated for each of the petioles, using regression analysis. The dependent variable was the gap between the boundary of infection along the petiole and the stem, and the independent variable was time. The slope of the regression equation is a measure of lesion progression.

By early April (ca. 10 weeks after inoculation) there were numerous lesions on the stems in all treatments and the rate of lesion expansion was defined as well. Stem lesions were examined on the middle section of the plants (nodes 9–13; 0.8–1.4 m above ground), 15–25 lesions per treatment. The size of each lesion (length and width) was measured on the stem surface three times, at weekly intervals. It was assumed that the shape of the lesion is close to rectangular and its area (mm^2) was so calculated. The rate of lesion expansion was determined by dividing the difference between two consecutive area measurements by the time span. For data analysis, the rates calculated for each lesion (two data) were averaged and then the mean rate (mm^2/day $\pm$ SE) was calculated for each of the treatments.

The effects of leaflet removal

The possibility of reducing the number of stem lesions by removing infected leaflets was examined in an experiment in 1996. The experiment was laid out in a randomized block design, with four replicates, each 4 m long. Each plot consisted of 18 plants in two rows. There were three treatments: (i) untreated control; (ii) weekly removal of all infected leaf tissue; and (iii) weekly application of chemical fungicides. A natural epidemic developed and the treatments were initiated soon after the onset of the disease, in early January. In treatment (ii) all infected leaflets were removed as described above. In treatment (iii) the fungicides iprodione, pyrimethanil and prochloraz+folpet were applied in alternation, at the rates and with the equipment described above. The number of infected leaves and the number of lesions on the stems were recorded on ten plants in the middle of each experimental plot.

Experiments in growth chambers

Effects of temperature and VPD on the rate at which *B. cinerea* progressed on leaf petioles were studied in growth chamber experiments. Tomato plants (cv. 144) were grown in a peat/perlite/sand mixture (1:1:1) in 17-cm pots in a growth chamber maintained at 15 °C by night and 25 °C by day. Plants were watered every 1-2 days and fertilized weekly with a fertilizer solution containing NPK, 5:3:8%. When the plants were ca. 60 cm in height, fully expanded leaves were removed from the main stem and placed in plastic boxes (280 $\times$ 160 $\times$ 120 mm) on a plastic net placed on wet paper toweling. A small cut was made with a scalpel on the terminal leaflet and a 6-mm agar disk containing mycelium of 6-day-old *B. cinerea* culture was placed, face down, on that cut. The isolate of the pathogen used was originally isolated from a naturally infected cucumber flower and maintained and grown on potato dextrose agar (PDA, Difco) at 20 °C. This isolate sporulates abundantly in the dark and is capable of infecting various plant hosts, including tomato. All boxes were enclosed in polyethylene bags and placed in a growth chamber at 20 °C for 8 days. At the end of that time, the fungus had invaded the terminal leaflet and had started to progress along the petiole. Then, humidity and temperature treatments were initiated. There were two humidity treatments, viz., high relative humidity and low relative humidity. The Relative humidity measurements (measured by Testo 175-2 data logger, Testo, Germany) at the specified temperatures, were used to calculate VPDs. For the high humidity treatment, the base of the incubation boxes was flooded to a depth of 5 mm with distilled water and the polyethylene bags were closed. For the low humidity treatment, no water was added and boxes remained uncovered (O'Neill et al., 1996). Boxes were then distributed to different incubators ad-

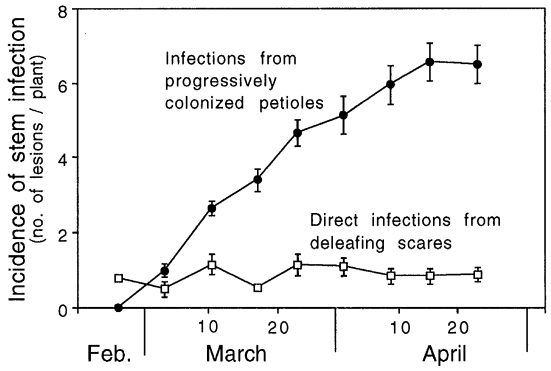

Figure 1. Changes in the number of grey mould lesions on tomato stems in an experimental greenhouse in 1994. Lesions on the stems originated from direct infection of the stems or via progression of the pathogen along the petiole. Plants were not protected with fungicides. Bars indicate the SE.

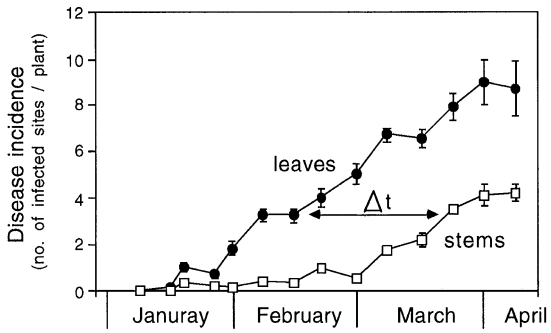

Figure 2. Grey mould progress curves on the leaves and stems of tomato plants grown in a commercial greenhouse in 1994. Infected leaf parts were not removed during the season. Δt = the time (indicated by the arrow) that passed from infection of a leaf until the rot had approached (by progression along the petiole) the stem. Plants were not protected with fungicides. Bars indicate the SE.

justed to nominal temperatures of 5, 10, 15, 20, 25 and 30 °C. Average VPDs for each of the respective temperatures and the low and high relative humidity treatments were: 0.26 and 0.09; 0.37 and 0.12; 0.51 and 0.17; 0.70 and 0.23; 0.95 and 0.31; 1.27 and 0.42 kPa. The progression of *B. cinerea* along the petioles was measured daily for 10 days. In cases where the rotted part had approached 2 cm from the end of the petiole, measurements for that leaf were terminated. The rate of progression of *B. cinerea* was calculated by means of regression analysis as described above. There were five replicates (leaves) per treatment and the experiment was repeated three times. Since conclusions based on different trials were similar, findings of one experiment are presented in the results.

Table 1. Statistics for the regression equation describing the relationship between the incidence of *Botrytis cinerea* incited lesions on tomato stems at time *t* (the dependent variable) and the incidence of infected leaves at time *t-n* (the independent variable). Analyses were performed for various *t-n* comparisons. Data were recorded in plots not treated with fungicides, and in plots treated weekly with *Trichoderma harzianum* T39, or with chemical fungicides*

Treatment	*t - n* (weeks)	b	r^2	*P*
Untreated	4	0.709 (0.051)	0.857	0.0001
	5	0.867 (0.068)	0.856	0.0002
	6	1.052 (0.112)	0.808	0.007
	7	1.188 (0.152)	0.780	0.01
Trichodex	5	0.721 (0.074)	0.777	0.0001
	6	0.806 (0.085)	0.909	0.0001
	7	0.970 (0.086)	0.918	0.0002
	8	1.048 (0.143)	0.870	0.0001
Fungicide	6	0.566 (0.052)	0.894	0.0001
	7	0.640 (0.059)	0.961	0.0001
	8	0.832 (0.122)	0.824	0.003
	9	1.030 (0.173)	0.979	0.04

* A slope of 1 indicates that the number of stem lesions at time *t* was equal to the number of infected leaves at time *t-n*. The analysis included all disease records from exps. 1–4 and 6 that had a time interval (*t-n*). Plots for two of the regression analyses are presented in Figure 3. Numbers in parentheses indicate the ± SE. *P* values denote the significance of the regression equation.

Results

The origin of stem lesions

Lesions on tomato stems may originate from direct infection of stem wounds or via progression of mycelium along infected petioles. Direct infection of the stems occurred early in the season and the number of lesions incited by that type of infection did not change considerably thereafter. On the other hand, the number of stem lesions which originated from leaf infections increased gradually throughout the season. By the end of the epidemic, the number of stem lesions that originated from direct infection (0.8 lesions/plant) was markedly lower than the number that originated from infected leaves (6.8 lesions/plant) (Figure 1). Stem infections originated from truss peduncles were relatively rare.

In commercial greenhouses disease symptoms were first observed when the rainy season started (early December – mid January) and followed a similar pattern. As an example, disease progress curves in exp. 4 are presented in Figure 2. Symptoms were observed almost at the same time on leaves and on stems. However, as time passed the pattern of dis-

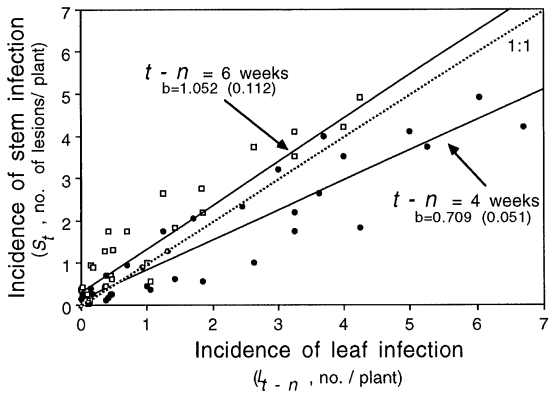

Figure 3. The relationship between grey mould incidence on the stems (S_t) at time *t* and disease incidence on leaves (L_{t-n}) at time *t-n* at two intervals of *t-n*: 4 weeks (circles) and 6 weeks (squares). The analysis included all disease records from experiments 1–4 and 6 that still had the relevant time interval (*t-n*). Solid lines indicate the regression equations describing the coincidence between the two variables. b = slope of the regression equation (± SE) Line 1:1 is a theoretical line with an intercept of 0 and a slope of 1, which represents a perfect coincidence between the variables. In such a case, the number of stem lesions at time *t* is equal to the number of infected leaves at time *t-n*. Plants were not protected with fungicides. Data of the regression statistics for these analyses are presented in Table 1.

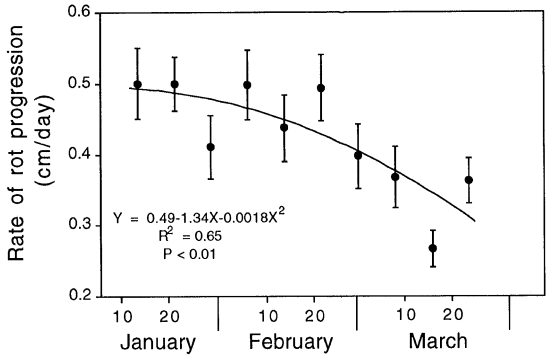

Figure 4. The rate at which *Botrytis cinerea* progressed along petioles of tomato in the experimental greenhouse in 1995. Plants were not protected with fungicides. For regression analysis, January 1 was considered as 'day 0'. Bars indicate the SE.

ease progress curves on these organs differed. On leaves, disease incidence increased gradually until the end of the rainy season (late March – mid April), by which time the increase in disease incidence on leaves had ceased. On stems, the number of lesions did not change much over a period of ca. 6 weeks. Only at the beginning of March did the incidence of stem lesions start to increase. From that time on, the stem lesions progress curve followed a pattern similar to that observed for the leaves, except that it was delayed in time, Δt (Figure 2).

Since most of the stem lesions had originated from infected leaves, it was possible to identify Δt, an estimation of the time in which an infection had progressed along the petioles towards the stem. An example of the analyses conducted for the untreated control treatment is presented in Figure 3. When the time gap between S_t and L_{t-n} was 4 weeks (*t-n* =4), the slope of the regression equation (0.709 ± 0.051) was significantly lower than 1, indicating that the number of stem lesions was less than the number of infected leaves recorded 4 weeks earlier. However, when the time gap was 6 weeks (*t-n* =6), the slope (1.052 ± 0.112) did not differ significantly from 1, indicating that the number of stem lesions at a certain date was close to the number of infected leaves recorded 6 weeks earlier. This was our definition for Δt. Statistics of the regression analyses conducted for various *t-n* comparisons are presented in Table 1. The same analyses were conducted for data recorded in plots treated weekly with *T. harzianum* T39 and in plots treated weekly with chemical fungicides. For the former treatment Δt was 7–8 weeks and for the latter treatment, 9 weeks (Table 1). These results indicate that both the biocontrol agent and chemical fungicides lessened the rate at which infection progressed along the tomato petioles.

Factors affecting the progression of B. cinerea *in petioles*

The rate at which *B. cinerea* had progressed along the petioles was determined in the experimental greenhouse in 1994. The rate changed slightly throughout the growing season. It was ca. 0.5 cm/day at the beginning of the epidemic (in January) but gradually slowed down thereafter, and by late March it was ca. 0.3 cm/day (Figure 4). Measurements did not continue after that time because most infections already reached the stems.

Experiments conducted under controlled conditions enabled us to identify the influence of temperature and VPD on the rate at which *B. cinerea* progressed along the petiole. At the range of temperature tested (5–30 °C), this rate increased linearly with increasing temperatures. At the lower temperatures (5–10 °C) the humidity regime did not alter the rate of progression of *B. cinerea* along the petioles; however, at temperatures above 20 °C, low humidity promoted a more rapid progression of *B. cinerea* along the petioles than under high humidity. At 30 °C the rate of progression of *B. cinerea* along petioles incubated under dry

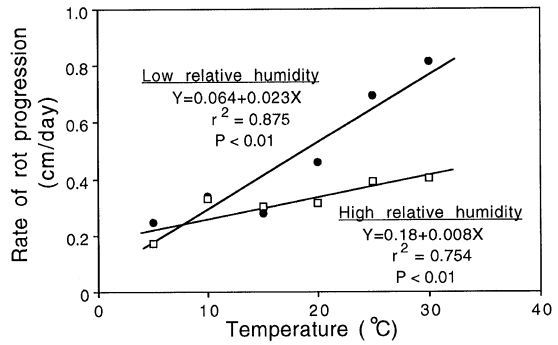

Figure 5. Effects of temperature and relative humidity on the rate at which *Botrytis cinerea* progressed along petioles of tomato leaves.

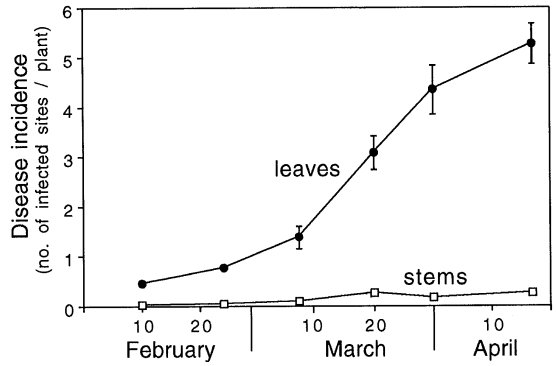

Figure 6. Botrytis cinerea progress curves on leaves and stems of tomato plants in a commercial greenhouse in 1995. Infected leaf parts were removed weekly. Plants were not protected with fungicides. Bars indicate the SE.

conditions was ca. 0.8 cm/day, whereas under humid conditions it was only 0.4 cm/day (Figure 5).

The rate at which *B. cinerea* progressed along the petioles and the expansion of lesions on the stems were affected by the source-sink relationship of the plants. Under normal growth conditions (plants bearing fruits and grown under normal light), the rate of progression of *B. cinerea* along the petioles was 0.38 cm/day. Reducing the sink capacity by removing all flowers and small fruits, decreased that rate by 24%; however, reducing the magnitude of the source (by interception of 50% of the incoming solar radiation) increased the rate by 45%. When both the source and the sink were reduced, the rate of progression of *B. cinerea* along the petioles was not affected as compared with the rate recorded under normal growth conditions (Table 2). Similar effects were observed for these treatments when changes in lesion expansion rate on stems were considered (Table 2).

Table 2. Effects of source-sink manipulation on the rate at which rot caused by *Botrytis cinerea* progressed along tomato petioles and on the rate of stem lesion expansion

Treatment*		Progression along	Stem lesion expansion
Fruits	Shading	the petiole (cm/day)	rate (mm^2/day)
+	−	0.377 (0.031)**	0.137 (0.033)
+	+	0.546 (0.052)	0.172 (0.050)
−	−	0.288 (0.027)	0.102 (0.026)
−	+	0.371 (0.040)	0.147 (0.038)

* To reduce the capacity of the sink, flowers and small fruits were removed once a week from all plants, starting soon after the initiation of blooming (+, flowers and small fruits were not removed and fruits developed normally; −, flowers and small fruits were removed). To reduce production of assimilates at the source, a net transmitting 50% of the incoming solar radiation was stretched above the plants in two subplots (+, with shading; −, without shading).
** Numbers in parentheses indicate the ± SE.

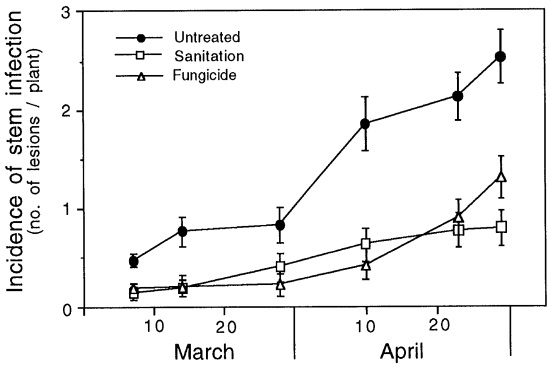

Figure 7. Effects of sanitation (removal of infected leaflets) and fungicides on the incidence of *Botrytis cinerea* on stems of tomatoes grown in the experimental greenhouse in 1996. Sanitation and fungicides were applied weekly. Bars indicate the SE.

Removing infected leaflets as a measure to preclude stem lesions

In one of the experiments conducted in a commercial greenhouse (exp. 5) all infected leaflets were removed weekly. The cumulative number of infected leaves per plant increased gradually with time, and by the end of the winter season there were 5.3 infected leaves/plant (Figure 6). However, the number of stem lesions did not increase significantly and by the end of the season there were only 0.3 stem lesions per plant (Figure 6). Moreover, it did not follow the pattern observed for stem lesions as in cases where the infected leaflets were left on the plants (Figure 2).

In another trial conducted in the experimental greenhouse in 1996 the efficacy of a sanitation treatment was compared with that of the traditional fungi-

cide spraying. The sanitation treatment was as effective as a weekly application of chemical fungicide: the number of stem lesions/plant did not differ significantly between the two treatments, and they both differed significantly from the untreated control (Figure 7). Yield in the sanitation treatment was not affected and it did not differ significantly from that in the fungicide treatment (data not shown).

Discussion

The most devastating symptom of grey mould on tomatoes grown in high wire cropping systems is stem rotting. Although not every stem lesion necessarily results in plant death, eliminating or at least minimizing the incidence of stem lesions is the current goal of grey mould management in tomato greenhouses. Several lines of evidence now indicate that the origin of stem lesions in heated greenhouses differs from the origin in non-heated greenhouses. In heated greenhouses, the pathogen infects the stem directly on wounds that are created during pruning of lower leaves and side-branches even at relatively high temperatures and VPD (Eden et al., 1996; O'Neill et al., 1997; Wilson, 1963). Similar results were observed for stem infection of cucumber (Dik and Koning, 1996) and sweet basil (Shaharabani et al., 1996). However, in non-heated greenhouses only a small proportion of the stem lesions was found to have had originated from direct infection of the stem. Most of the stem lesions originated from progression of the pathogen along infected petioles. Direct stem infections occurred only at early stages of the epidemic and did not increase in number thereafter. On the other hand, stem infections that originated from infected leaves occurred later and the incidence increased gradually with time (Figures 1 and 2). The rate at which *B. cinerea* progressed along infected petioles under commercial conditions was relatively rapid: 0.3–0.5 cm/day (Figure 4, Table 2). Assuming that a typical tomato leaf is 30–40 cm long, and that infection would occur (on the average) in the middle of the leaf (ca. 15–20 cm away from the stem), it would take 35–50 days before an infection approached the stem. Indeed, estimation of Δt, a measure for that time period, revealed that in untreated crops it was approximately 6 weeks (Table 1). Application of *T. harzianum* T39 extended this period by 1–2 weeks and application of chemical fungicides by 3 weeks (Table 1).

Several environmental factors governed the rate at which *B. cinerea* progressed along the petioles. In the temperature range of 5 to 30 °C, the higher the temperature, the more rapid was the rate of pathogen progression. This finding contradicted somewhat the reported response to temperature of other stages in the life cycle of *B. cinerea*. Temperatures of 10 to 20 °C are optimal for spore germination, infection and sporulation; at higher temperatures these processes decrease gradually (Elad and Shtienberg, 1995; Jarvis, 1980, 1989; O'Neill et al., 1997). Our findings corroborated previous reports that the rate of lesion expansion increases with temperature even above 20 °C (Berg van den and Lentz, 1968; Hyre, 1972).

High relative humidity and free moisture on the plant surface are considered the most important environmental factors that promote *B. cinerea* epidemics. We were surprised to find that the pathogen progressed more rapidly along tomato petioles incubated at low rather than at high relative humidity (Figure 5). However, these results are corroborated by other studies. Dik and Koning (1996) reported that significantly more cucumber plants had died from stem lesions induced by *B. cinerea* under high than under low VPD conditions. Similarly, P. Nicot (personal communication) found that the rate of *B. cinerea* progression along infected tomato branches was, in some cases, more rapid at 50% than at 90% relative humidity. The reason why the pathogen is more aggressive at high VPD in xerophytic plant organs (such as petioles and stems) is not known. It is possible that at high VPD, when the pathogen does not sporulate, all resources contribute to growth within the water-soaked host tissue. On the other hand, under low VPD conditions, mycelium growth is restricted due to allocation of assimilates to the sporulating process. Nevertheless, the implication is that this process is less sensitive to the environment as compared with other stages in the life cycle of the fungus, such as spore germination, infection and sporulation.

The source-sink relationship of the host plant influenced the rate of *B. cinerea* progression along the petioles (Table 2). The rate was more rapid when the source was restricted (by shading) and slower when the sink was restricted (by removal of all fruits). Although this experiment was not repeated, the conclusions drawn are supported by evidence from other experiments in our work and from previous studies. Shading crops from the sun has long been recognized as predisposing to grey mould diseases. However, this was attributed mainly to the effect of the sun on early

drying of the host tissue and, to a lesser extent, to effects on the plant's metabolism (Jarvis, 1980). Our data suggest that the latter mechanism is more important, at least in regard to progression of the fungus along the petiole.

The fact that *B. cinerea* progressed less rapidly when assimilates were excessive, provides an explanation for the observation that the rate of its progression was more rapid in January than in March (Figure 4). In January (winter), the intensity of solar radiation in the coastal plain of Israel (ca. 1000 μE/m^2/sec) is a limiting factor for photosynthesis and the production of assimilates. This is the lowest radiation intensity of any month in the year. By March (early spring), solar radiation intensity increases to ca. 1500 μE/m^2/sec. Our results are supported from another direction as well. It has been repeatedly reported that a high nitrogen level in the soil reduces the incidence of infection by *B. cinerea*. This was attributed to the more rapid vegetative development of tomato plants under high nitrogen regimes (Gullino et al., 1991; Verhoeff, 1965). Increased vegetative growth enables excessive production of assimilates. Moreover, Verhoeff (1968) found that the growth of the fungal mycelium through petioles was limited at higher soil nitrogen levels. This suggests that in non-heated greenhouses, where manipulation of the environment is not feasible, it might be possible to restrict the incidence of stem rotting by adjusting the nitrogen fertilizer.

Since stem rotting is the most devastating symptom of grey mould in tomatoes, and because most of the stem lesions originated from leaf infections in nonheated greenhouses, we examined the possibility of reducing stem rot damage by removing all infected leaf material. Implementing this treatment enabled us to reduce the incidence of stem lesions substantially even in plots not treated with fungicides (Figure 6). This treatment was as effective as weekly application of fungicides (Figure 7). These results are very encouraging, because they imply that it might be possible to manage the most devastating symptom of a very important disease even without spraying. It could be argued that the suggested practice is time consuming and would involve intensive manual labor. However, since pruning of lower leaves and side-branches is common practice in tomato production, and because these activities are conducted periodically, it might be possible to train the workers also to remove infected material. This would reduce stem rotting and be cost-effective.

Acknowledgments

The research was supported in part by the Chief Scientist of the Israel Ministry of Agriculture and by grant no. 93/32 from DIARP, the Dutch-Israeli Agricultural Research Program.

The authors acknowledge the help of A.J. Dik, J. Köhl, N.J. Fokkema, the Netherlands; Y. Kock, Zafit Education greenhouse, Israel; and the involvement of farm advisors and farmers throughout Israel.

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