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Disease control/Moyens de lutte

Integrated control of garlic leaf blight caused by *Stemphylium solani* in China

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Abstract: Leaf blight caused by *Stemphylium solani* is a major fungal disease of garlic (*Allium sativum*) in central China where it has caused severe crop losses during the winter growing season from the end of autumn to the middle of spring. Epidemiology, cultivar resistance, and chemical controls were investigated during the 2006 to 2008 growing seasons in Dangyang County to improve disease control methods. Disease severity monitoring revealed that the activity of *S. solani* was variable between growing seasons, and this may have been due to weather conditions. Disease severity was positively correlated with increasing temperatures, but no consistent relationship was found between total rainfall and disease. Additionally, the study demonstrated that conidia and mycelium of *S. solani* could survive in garlic debris for long periods and serve as the primary inoculum source for the subsequent season. Relatively few of the commonly grown cultivars had high levels of resistance to leaf blight. Garlic cultivars 'Qingganruanye', 'Ruanruanye' and 'Zixuan-2' were among the most resistant, but except for 'Zixuan-2', did not produce sufficient harvestable bolts as would be desirable for the local market. All fungicide treatments applied to cloves used as planting material seemed to promote seedling emergence, but significant effects (P = 0.05) were observed only with fludioxonil (0.05 g kg^{-1}) and thiram (1.25 g kg^{-1}). Fungicide applications in the field were effective in controlling leaf blight, and flusilazole (50 g ha^{-1}), flusilazole plus famoxadone (50 g plus 104 g ha^{-1}) or mancozeb (350 g ha^{-1}) had the highest efficacy in reducing leaf blight severity.

Keywords: chemical control, cultivar susceptibility, disease progression, inoculum survival, *Allium, Stemphylium solani*

Résumé: La brûlure de la feuille causée par *Stemphylium solani* est une des principales maladies fongiques de l'ail (*Allium sativum*) du centre de la Chine où elle a causé de lourdes pertes durant la période de croissance hivernale, c'est-à-dire de la fin de l'automne au milieu du printemps. De 2006 à 2008 dans le comté de Dangyang, durant les saisons de croissance, l'épidémiologie, la résistance des cultivars et la lutte chimique ont été étudiées afin d'améliorer les méthodes de lutte contre la maladie. La surveillance de la gravité de la maladie a révélé que l'activité de *S. solani* variait d'une saison de croissance à l'autre et que cela était peut-être dû aux conditions du temps. La gravité de la maladie était positivement corrélée à l'augmentation des températures, mais aucune relation constante n'a pu être établie entre les précipitations et la maladie. De plus, l'étude a démontré que les conidies et le mycélium de *S. solani* pouvaient survivre dans les débris d'ail durant de longues périodes et servir de source d'inoculum primaire pour la saison suivante. Un petit nombre relatif de cultivars utilisés couramment possédait de hauts degrés de résistance à la brûlure de la feuille. Les cultivars d'ail 'Qingganruanye', 'Ruanruanye' et 'Zixuan-2' étaient parmi les plus résistants, mais ils n'ont pas produit assez de feuilles récoltables de qualité acceptable pour le marché, sauf 'Zixuan-2'. Tous les traitements fongiques appliqués aux gousses utilisées pour les semis ont semblé promouvoir leur émergence, mais des résultats significatifs (P = 0.05) ont été observés seulement avec le fludioxonil (0.05 g·kg^{-1}) et le thirame (1.25 g·kg^{-1}). Au champ, on a pu maîtriser la brûlure de la feuille par l'application de fongicides. Le flusilazole (50 g ha^{-1}), le flusilazole plus le famoxadone (50 g + 104 g·ha^{-1}) et le mancozebe (350 g·ha^{-1}) ont été les plus efficaces pour réduire la gravité de la maladie.

Mots clés: *Allium*, lutte chimique, progression de la maladie, sensibilité du cultivar, *Stemphylium solani*, survie de l'inoculum

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Introduction

Garlic (*Allium sativum* L.) is one of the most widely grown vegetable crops in the world. China is by far the largest producer of garlic, with approximately 10.5 billion kilograms annually, accounting for over 77% of world output (Zohary & Hopf, 2000). Leaf blight caused by *Stemphylium solani* G.F. Weber is one of the most important diseases of garlic in Hubei province, China (Zheng *et al*., 2008). In early 1975, leaf blight of *Allium* was found for the first time in India (Rao & Pavgi, 1975), and has now been observed in many countries worldwide, including the USA (Miller *et al*., 1978), South Africa (Aveling & Naude, 1992), Spain (Basallote-Ureba *et al*., 1993), Brazil (Mehta, 1998), Australia (Suheri & Price, 2000), Egypt (Hassan *et al*., 2007) and China (Zheng *et al*., 2008).

From autumn 2004 to spring 2008, leaf blight was found on garlic leaves in Dangyang County, Hubei, China, with the crop area affected estimated to be over 7000 ha. Garlic yield was reduced by 30% on average, with up to 70% yield losses in some fields during the winter growing season. Early symptoms were observed as white spots (1–3 mm), which enlarged to produce sunken purple lesions, extending until the leaves withered (Zheng *et al*., 2008). Healthy leaves contacted diseased tissues of neighbouring plants, allowing for abundant mycelial growth with profuse conidial production on the newly infected tissues. These conidia were presumed to be rapidly disseminated since many white spots were observed on garlic leaves within a few days, and repeated cycles led to epidemic levels of disease.

In general, garlic is planted throughout many regions of China with moderate rainfall and under cool temperate conditions. Crop growth stage and weather, in particular temperature and humidity favouring long duration of leaf wetness, are considered to be associated with foliar blight development caused by *Stemphylium* spp. (Falloon *et al*., 1987; Suheri & Price, 2000; Prados-Ligero *et al*., 2003). Previous observations suggest that the combined effects of optimal temperature, high relative humidity and prolonged periods of leaf wetness (≥8 h) caused by rainfall or dew are environmental conditions conducive for Stemphylium leaf blight, and these factors have been well investigated in studies of *S. versicarium* (Wallr.) E.G. Simmons which causes leaf spot in garlic, onion and asparagus (Mwakutuya, 2006). Sinha & Singh (1993) had confirmed the importance of temperature (18 °C) and relative humidity (RH 85–90%) for successful development of Stemphylium leaf blight in lentil. Species of *Stemphylium* occur over a wide range of environmental conditions on many host plants (Falloon *et al*., 1987; Sinha & Singh, 1993; Suheri & Price 2000, 2001). However, there are limited experimental data on factors affecting the epidemiology of *S. solani* on garlic in China.

An association between the amount of debris from the previous growing season left on the soil surface and the incidence of Stemphylium leaf blight of asparagus has been observed (Falloon et al., 1987). The burial of the infected plant debris may reduce the disease in the coming season (Johnson, 1990). Long crop rotations with unrelated crops have also been recommended for fields that are infected by *Stemphylium* spp. (Maude, 1966). However, *S. solani* from garlic has a wide host range (Zheng *et al*., 2009) and crop rotation might not be effective.

Traditionally, control of leaf blight on *Allium* crops has primarily depended on applications of fungicides. Only applications of tebuconazole and procymidone have been reported to provide effective control of Stemphylium leaf spots in garlic (Basallote-Ureba *et al.*, 1998). However, the fungus *S. versicarium* had been reported to be resistant to procymidone, which was the most frequently used fungicide in Italy in previous years (Alberoni *et al*., 2005). In the past 10 years, control of leaf blight in local farms in Dangyang County has been accomplished with two to three sprays (commonly using the fungicides chlorothalonil or thiophanate-methyl) during the growing season. However, applications have frequently been observed to provide poor efficacy. In lab studies, we found that triazole fungicides showed strong suppression of mycelial growth of *S. solani* (data not shown). For fungicidal seed treatments, Aveling *et al.* (1993) used anilazine, benomyl and procymidone among others on onion seed, but disease suppression was not considered adequate.

An evaluation of garlic cultivars for resistance to Stemphylium leaf blight conducted in Australia (Suheri & Price, 2000) showed that five of 26 cultivars were more resistant than the others. However, an issue with cultivar introduction is that some resistant cultivars do not bolt, that is, they do not produce flower stalks after the seedling stage, and hence are not suitable for the local market where seedling foliage and immature flower stalks are consumed.

The objectives of this paper were to (a) study the disease progression of garlic leaf blight on cultivated garlic crops in central China; (b) assess the relationship between disease severity in the field and weather data; (c) assess survival of *S. solani* in plant debris under different field conditions; (d) test the susceptibility of garlic cultivars to *S. solani*; and (e) test the effectiveness of selected fungicides as seed treatments or foliar sprays in controlling Stemphylium leaf blight of garlic under field conditions.

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Materials and methods

Experimental site

Except for the inoculum survival study, the experiments were conducted during the 2006/2007 and 2007/2008 growing seasons in Dangyang County. The plots were located on the western part of Jianghan Plain in Dangyang County, 31° North and 112° East, 90 m above sea level. The test plots were placed in fields where crops susceptible to *S. solani* had been grown for over 10 years (including several cycles of oilseed rape [*Brassica napus* L.] and wheat [*Triticum aestivum* L.]). The soil type was a clay-sand, lightly compacted, with 1.5% organic matter, available nitrogen 75.9 mg kg^{-1}, available phosphorus 99.2 mg kg^{-1} and pH 7.9. Each year before sowing, a fertilizer containing nitrogen, phosphorus and potassium was applied at a rate of 150 kg ha^{-1} for each nutrient. The garlic cloves were sown on raised beds in August of each year within three days of regular commercial planting on neighbouring farms. Plots were located in 6 m long beds with 2.5 m width, and distance between beds of 25 cm. Each plot consisted of 10 rows of plants, 10 cm apart, with plants spaced 15 cm apart within rows. Approximately 1500 cloves were sown per plot, and the plants were maintained and cultivated following practices of commercial garlic growers in this region.

Disease progression

The survey sites were established during two seasons (2006/2007 and 2007/2008) in Dangyang County using susceptible garlic 'Changbanpo'. Five 0.1 ha garlic fields at least 0.5 km apart were selected for the disease progression trial. In 2006, garlic cloves were sown on 22 August, and in 2007 on 20 August. In both years, seedlings had emerged after 20 days. After first disease symptoms were observed in each year, DSI was assessed at 7 to 30 day intervals as follows. A total of 1000 plants per field were chosen by systematically selecting 40 plants in five staggered sections along each of the five plots at a distance of at least 0.5 m from the plot edge. Every plant leaf was examined for disease and DSI calculated for each plot. Air temperature, relative humidity and precipitation data for 2006/2007 and 2007/2008 were obtained each growing season from an automated weather station at Dangyang County near the experimental site (~3 km away).

Disease assessment

Disease evaluation was completed by visual observation by walking between the rows multiple times each season. Three hundred plants for each plot (60 plants at five locations in two diagonals of each plot) were assessed for each trial except for evaluation of disease progression which is described above. Symptoms on every plant were evaluated on a scale of 0–9 modified from Mehta (1998): 0 = healthy leaves; 1 = minute white spots, less than 5% leaf tissue diseased; 3 = small white to reddish purple lesions, 5–25% diseased; 5 = dark purple necrotic lesions with chlorosis, 26–50% diseased; 7 = necrotic lesions >10 cm long, 50–75% diseased; 9 = lesions coalescing, 76–100% diseased. A disease severity index (DSI) ranging from 0 (no disease on any leaves) to 100 (all leaves in disease category 9), was calculated for each trial using the formula of Falloon *et al*. (1987): DSI = $\sum$ (disease category $\times$ no. leaves in that category) $\times$ 100/(total leaves $\times$ 9).

Conidial viability of S. solani *in garlic debris*

An experiment was conducted to test the survival of *S. solani* under different conditions. Plant debris of garlic 'Changbanpo' was collected on 13 April 2006 from field plots of the disease progression study where leaf blight was observed during the previous growing seasons (Dangyang County). The pieces were cut into 10 cm long segments, and 30 segments were packed together in 0.2 mm thick vapour-permeable polyethylene film (Jiayang TPU-2290, Wenzhou, China), for use as an experimental unit. The 120 packages were divided into five groups and incubated under different conditions: glasshouse (25 °C, 30% RH); cool storage (4 °C, 90% RH); and in the field on the soil surface, buried in soil at 10 cm, or buried at 20 cm at Huazhong Agricultural University, Wuhan, China. Samples were collected four times between 20 March 2006 until 25 September 2006 to assess the survival of conidia and mycelium in plant debris.

Conidia were collected from samples of each environmental condition, by unwrapping and washing each sample with 100 mL autoclaved distilled water. Conidial suspensions were filtered through four layers of cheesecloth, and conidial concentrations were adjusted with water to a concentration of 1×10^6 mL^{-1}, with the addition of sucrose (1 µg mL^{-1}) to enhance germination. To assess spore viability, spore suspensions from each tube were placed on concave microscope slides and incubated in a moist chamber at 25 °C. After 24 h, up to 300 conidia per sample were examined by microscopy (160×), and the percentage that had germinated was recorded. However, these numbers may be conservative because there were likely some viable spores that had failed to germinate. A spore was considered to have germinated if the germ tube was over half the width of the spore. Conidia of *S. solani* were differentiated from other fungi by characteristic conidial morphology.

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Recovery of S. solani *from plant debris*

Entire packages of debris were recovered and leaves aseptically cut into 1 × 1 cm sections from five samples per treatment and washed with 3 L sterile-distilled water (SDW), and then surface-sterilized by soaking leaf pieces in 0.1% mercuric chloride solution for 1 min followed by three rinses in SDW. After air drying under aseptic conditions, the pieces were placed onto modified PSA medium (1 L potato sucrose medium containing 0.05 g penicillin, 0.05 g streptomycin and 2.5 mL lactic acid), and incubated at 25 °C on a 12 h light–dark cycle. Fungal growth was examined daily for up to 10 days. The percentage recovery of colonies initially resembling *S. solani* was recorded, and some subcultures were made to confirm the presence of *S. solani* based on microscopic characteristics.

Susceptibility of garlic cultivars

Sixteen garlic cultivars grown in wide acreages across China were selected, representing two sets recommended for cultivation in southern China (‘Qingganruanye’, ‘Ruanruanye’, ‘Zhengyuezao’, ‘Hongqixing’, ‘Chengsuanzao-2’, ‘Chengsuanzao-3’, ‘Dusuan’, ‘Wenerzao’ and ‘Ershuizao’ from Sichuan province; ‘Taicang’ from Jiangsu province; and ‘Zixuan-1’, ‘Zixuan-2’ and ‘Changbanpo’ from Hubei province) and northern China (‘Tainuo-1’, ‘Shandongjinan’ and ‘Lusuan-1’ from Shangdong province). Two trials consisting of 11 and 13 commercial cultivars were evaluated in Dangyang County in 2006/2007 and 2007/2008, respectively. A randomized complete block design was used, with three blocks.

For all garlic cultivars, cloves were planted on 23 August 2006 and on 21 August 2007. No fungal inoculum was applied and no fungicide applications were made during the course of the experiments, but occasional insecticide sprays were used. Leaf blight development on the plots was evaluated five times for DSI at 15 to 30 day intervals from 29 October to 2 March in both growing seasons using the assessment system described above. Samples of seedling foliage (late December), bolts (late March) and bulbs (late April) were collected from plots in both years, and fresh weights were recorded.

Chemical control of garlic leaf blight – clove treatments

To determine the fungicidal efficacy of different clove soaks against *S. solani*, experiments were conducted in 2006/2007 and 2007/2008. Five treatments (hymexazol, thiram, fludioxonil, carbendazim and carbendazim plus KH_2PO_3) were tested for efficacy. Cloves of ‘Changbanpo’ collected from a diseased field were used in these experiments. Each clove sample of 1 kg was soaked in 2 L solutions of 0.1% carbendazim or 0.1% carbendazim plus 0.2% KH_2PO_3 for 6 h. Other 1 kg clove samples were sprayed with 100 mL water solution containing 0.05 g fludioxonil, 1.25 g thiram or 1.9 g hymexazol. The seeds treated with water for 6 h were considered as a control. Cloves were planted as described above on 24 August 2006 and on 22 August 2007, and per cent emergence was determined after 40 days. Disease was assessed four times at 15 to 30 day intervals from 29 October to 20 December in both growing seasons. This period included the seedling stage during the most critical period for leaf blight development.

Chemical control of garlic leaf blight – foliar fungicide applications

In 2006/2007, a 24-plot field trial with ‘Changbanpo’ was established to investigate two concentrations of three fungicides applications for control of garlic leaf blight. Water and thiophanate-methyl at 233 g ha^{-1} was used as negative and positive controls, respectively (thiophanate-methyl was used frequently by local growers in previous years and served as the standard fungicide control). Other treatments were difenoconazole at 25 and 50 g ha^{-1}, flusilazole at 25 and 50 g ha^{-1}, and mancozeb at 175 and 350 g ha^{-1}. Each plot consisted of 10 rows of plants, 10 cm apart, with plants spaced 15 cm apart within rows, in a randomized complete block design with three replications. After first observation of symptoms on garlic leaves in the field plots, fungicide treatments were made three times at 10 day intervals and applied in 500 L of water per ha with a high-pressure sprayer (1000 kPa, Solid cone swirl nozzle). The use of three spray applications after first symptoms are observed is a routine practice by growers in the region to control garlic leaf blight. In 2007/2008, the same treatments as used in the previous year were evaluated in fields 50 m away. In addition, azoxystrobin at 63 and 126 g ha^{-1}, difenoconazole plus propiconazole at 37.5/37.5 and 75/75 g ha^{-1} and flusilazole plus famoxadone at 26/26 and 52/52 g ha^{-1} were evaluated in fields 100 m away. Fungicides were applied three times at 10 day intervals ending 28 November 2006 or 14 December 2007 only during the early seedling stage because the lower mean temperatures in December (<5 °C) were considered to be unsuitable for further infection. Disease assessment, as described in the section above on susceptibility, was done four times at 7 to 15 day intervals from 29 October to 20 December in both growing seasons.

Statistical analyses

Data were analyzed with DPS statistical analysis software (version 3.01, China Agric. Press, Beijing). The area under the disease progress curve (AUDPC) was calculated for each garlic cultivar and chemical treatment from DSI values using the formula described by Shaner & Finney (1977): AUDPC = $\sum [(1/2(x_i + x_{i+1})] (t_{i+1} - t_i)$, where x_i = DSI (%) ^{i}th assessment; t_i = the time in days after appearance of the disease at ^{i}th days; and n = the total number of observations. In order to facilitate a better visual comparison among treatments over seasons of testing, AUDPC was normalized by dividing by the number of days for each season (Fry, 1978). The normalized AUDPC was referred to as the relative area under disease progress curve (RAUDPC).

Germination and recovery data were subjected to analysis of variance. The data met the assumptions of ANOVA without transformation. Cultivar disease and yield ratings were subjected to correlation analysis, and the Pearson Correlation Coefficient was calculated for cultivars which were rated in 2006/2007 and 2007/2008. All RAUDPC values, yield data and percentage data concerning germination of conidia, recovery of pathogen, clove emergence were subjected to analysis of variance (ANOVA), and when significant treatment differences were found ($P \leq 0.05$), means were compared by Tukey's test ($P = 0.05$).

Results

Disease progression

Disease was rated 24 times from 1 October 2006 until 30 April 2007, and 16 times for the same period in 2007/2008 (Figs. 1 and 2). The level of disease was compared with rainfall, relative humidity and temperature data for 2006/2007 or 2007/2008 (Fig. 1). The disease severity in the field plots generally increased towards the end of the growing season as plants matured (247 to 251 days after sowing). This allowed for a significant correlation between temperature and disease severity when temperatures were

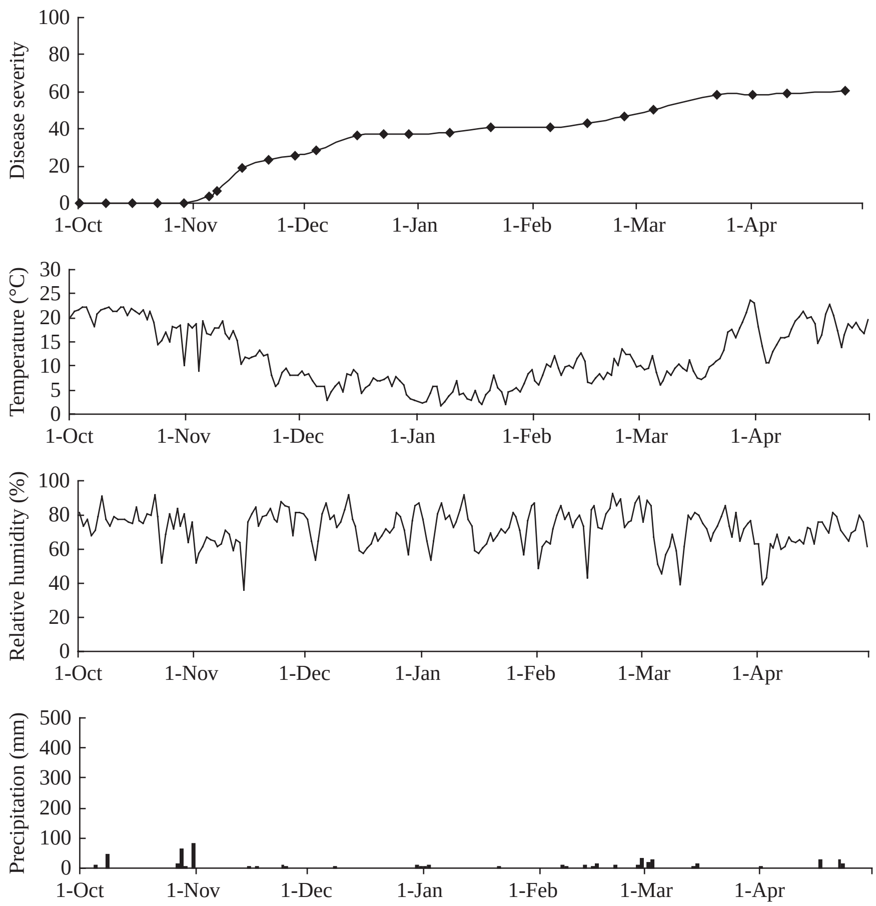

Fig. 1. Disease severity of garlic leaves infected by *Stemphylium solani*, mean daily air temperature, relative humidity and precipitation at Dangyang County, Hubei, China in the 2006/2007 growing season.

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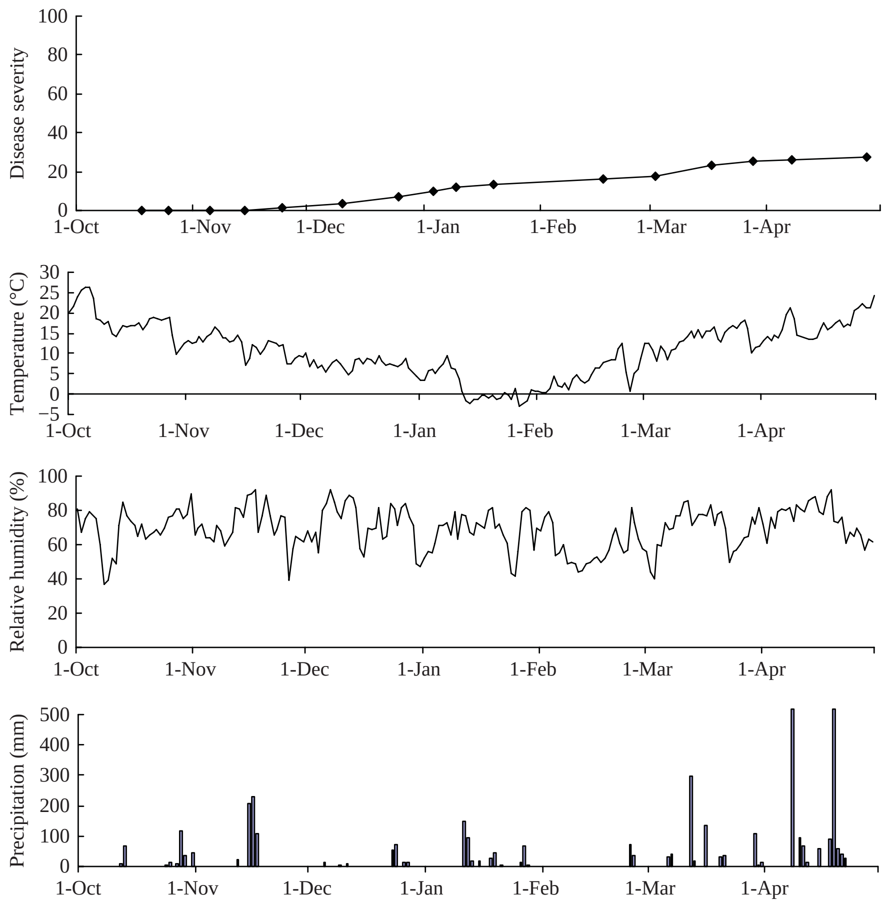

Fig. 2. Disease severity of garlic leaves infected by *Stemphylium solani*, mean daily air temperature, relative humidity and precipitation at Dangyang County, Hubei, China in the 2007/2008 growing season.

increasing in 2006 (20 January to 26 April, r = 0.79, P < 0.05) and 2007 (21 January to 27 April, r = 0.94, P < 0.05). However, there were no significant correlations between disease severity and rainfall or relative humidity in either year. There was greater disease severity during the 2006/2007 growing season than in 2007/2008.

In 2006/2007, the initial symptoms of small white spots were observed on 5 November, and a high disease severity (DSI = 60.6 on a scale of 0 to 100) was also recorded at final harvest. The months of November, December and March had the highest rates of disease increase, with 37%, 14% and 13%, respectively. In 2007/2008, the occurrence of leaf blight was delayed until 24 November, and leaf blight increased very slowly with DSI reaching only a maximum of 27.5 by final harvest. Examination of weather data from Dangyang automatic station revealed no major deviations in weather patterns that would explain the differential occurrence of garlic leaf blight. In 2006/2007 and 2007/2008, mean daily temperature and relative humidity showed similar trends; however, average temperatures were lower by approximately 1 °C in 2006/2007 than in 2007/2008 during most days of the garlic-growing season. Mean daily temperatures decreased from sowing day until late January in both growing seasons. In particular, the days between late October and early November (considered critical for leaf blight development across central China) was cooler in 2007 than 2006. Nevertheless, total rainfall during this period was 167 and 249 mm, respectively, and the corresponding numbers of rainy days were five and eight.

Survival of S. solani *in plant debris*

Diseased garlic leaves with visible sporulation of *S. solani* were first collected from standing plants in garlic fields on 13 April 2006 and the percentage of viable conidia on these leaves was ~95% during that time. Samples of diseased tissue were packaged in vapour-permeable polyethylene film, and incubated under five conditions. The percentage of viable conidia of *S. solani* in the different treatment conditions declined significantly throughout samplings in May, July, August and September (Table 1).

Table 1. Viability of conidia of *Stemphylium solani* in garlic debris incubated starting 13 April 2006 under five different environmental conditions at Huazhong Agricultural University, Wuhan, Hubei, China.

Environmental condition	Percentage germination of conidia of *S. solani* (%)[a]			
	20 May	5 July	15 August	25 September
Glasshouse	73.4 a	48.5 b	12.6 def	10.8 def
Cool storage	26.2 c	6.9 fg	6.4 fg	5.7 fg
Soil surface	19.0 cd	13.1 def	0.2 g	0.0 g
10 cm soil depth	16.9 de	10.7 def	0.0 g	0.0 g
20 cm soil depth	13.4 def	8.7 efg	0.0 g	0.0 g

Note: [a]Analysis of variance was performed using a completely randomized design. Means followed by a letter in common are not significantly different at P = 0.05 using Tukey's test.

Table 2. Recovery of *Stemphylium solani* from garlic debris incubated starting 13 April 2006 under five different environmental conditions at Huazhong Agricultural University, Wuhan, Hubei, China.

Environmental condition	Percentage recovery of *S. solani* (%)[a]			
	20 May	5 July	15 August	25 September
Glasshouse	80.0 a	68.6 ab	62.9 abc	40.0 bcd
Cool storage	68.6 ab	20.0 defg	8.6 efg	2.9 g
Soil surface	42.9 bcd	34.4 cdef	31.4 defg	25.7 defg
10 cm soil depth	37.1 cde	28.6 defg	17.1 defg	17.1 defg
20 cm soil depth	31.4 defg	28.6 defg	14.3 defg	5.7 fg

Note: [a]Analysis of variance was performed using a completely randomized design. Means followed by a letter in common are not significantly different at P = 0.05 using Tukey's test.

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By 25 September (165 days after treatment), viable conidia of *S. solani* were detected only in debris kept in the glasshouse (10.8%) or under cool storage (5.7%, Table 1). Viable conidia of *S. solani* were found for up to 124 days on plant debris at the soil surface, or for up to 83 days at soil depths of 10 or 20 cm. The conidial suspensions with non-germinating conidia were plated onto PSA to further check for viability and delayed germination, but no colonies of *S. solani* were observed even after incubation at 25 °C up to 14 days. For all treatments, *S. solani* was consistently recovered from diseased plant debris until the end of September (165 days after treatment) with recovery rates of 2.9% in cool storage to 40.0% in the glasshouse (Table 2). The percentage recovery of *S. solani* was highest from glasshouse storage for each collection period, while viability in samples incubated at 4°C and 90% RH in cool storage decreased rapidly from the start.

Susceptibility of garlic cultivars

In 2006/2007 and 2007/2008, a continuous increase in leaf blight severity on garlic leaves was observed on all of the cultivars throughout both growing seasons. In general, leaf blight susceptibility rankings of different cultivars were consistent between two growing seasons. Values for RAUDPC are shown in Table 3. In 2006/2007, severe attacks of leaf blight developed, but no symptoms were seen before early November. There were significant differences in blight severity between garlic cultivars in the field. Cultivars 'Hongqixing' and 'Wenerzao' were the most susceptible with RAUDPC of 67.3 and 64.4. These more susceptible cultivars took an average of 70 days after planting to show initial symptoms. Cultivars 'Qingganruanye' and 'Ruanruanye' were the least susceptible with an average RAUDPC at 10.0 and 10.4, respectively, under field conditions. These took longer to show white spots, an average of 74 days. In 2007/2008, 'Zixuan-2,' 'Qingganruanye' and 'Ruanruanye' were again among the most resistant cultivars whereas 'Tainuo-1' and 'Shandongjinan' were the most susceptible. The ranking of the cultivars with moderate to high susceptibility varied slightly during the seasons with a Pearson Correlation Coefficient (r) of 0.964 ($P < 0.05$) between RAUDPC of the eight cultivars ranked in both seasons. The most resistant cultivars had significantly less disease in comparison with the most susceptible cultivars in both seasons.

The cultivars responded to leaf blight infections with significantly different yield (Table 3). In 2006/2007, 'Zhengyuezao' gave the highest (by a significant amount) seedling leaf yield at 41.1 t ha^{-1}, while 'Ruanruanye' had the lowest yield at 25.5 t ha^{-1}. Bolts were harvested at different dates for different cultivars. Cultivars 'Qingganruanye' and 'Ruanruanye' did not bolt by harvest time (or even later) while 'Zhengyuezao' had the highest weight of harvested bolts (13.3 t ha^{-1}). The highest marketable bulb yield was recorded from 'Qingganruanye' and 'Ruanruanye' (37.8 and 36.9 t ha^{-1}), while other cultivars gave relatively lower yields. In 2007/2008, 'Zixuan-2' had the highest seedling leaf yield with 50.0 t ha^{-1}, and 'Zhengyuezao', 'Zixuan-1' and 'Qingganruanye' resulted in a consistently higher harvested leaf yield compared with other cultivars. There were significant differences in leaf, bolt and bulb yield and RAUDPC among the cultivars (P = 0.05) within and between the seasons, with Pearson Correlation Coefficients (r) of 0.67 ($P > 0.05$), 0.90 ($P < 0.05$), –0.51 ($P > 0.05$), and 0.96 ($P < 0.05$), respectively, between the ratings of the eight cultivars tested in both seasons.

Table 3. Evaluation of relative susceptibility to Stemphylium leaf blight by garlic cultivars in the 2006/2007 and 2007/2008 growing seasons, at Dangyang County, Hubei, China.

Cultivar[a]	RAUDPC[b]		Seedling leaf yield (t ha^{-1})		Bolt yield (t ha^{-1})		Bulb yield (t ha^{-1})	
	06/07	07/08	06/07	07/08	06/07	07/08	06/07	07/08
Qingganruanye	10.0 d[c]	3.4 ef	31.4 bc	37.5 ab	0.0 c	0.0 d	37.8 a	17.7 ef
Ruanruanye	10.4 d	3.9 ef	25.5 c	28.0 bc	0.0 c	0.0 d	36.9 a	18.4 ef
Changbanpo	33.4 c	9.9 d	34.3 abc	33.9 bc	12.2 ab	10.8 b	25.8 b	25.7 abcd
Zhengyuezao	36.1 c	7.6 def	41.1 a	39.3 ab	13.3 a	10.4 b	23.9 b	16.8 f
Chengsuanzao-2	37.6 c	no	30.8 bc	no	10.2 ab	no	18.8 b	no
Chengsuanzao-3	37.7 c	9.0 de	31.8 abc	34.0 bc	10.2 ab	10.4 b	20.5 b	21.8 cdef
Zixuan-1	42.8 bc	12.7 cd	36.1 ab	37.7 ab	10.1 ab	12.8 a	22.8 b	28.7 ab
Dusuan	49.5 b	no	27.0 bc	no	8.1 b	no	22.3 b	no
Ershuizao	51.5 b	11.9 cd	29.0 bc	34.4 bc	9.5 ab	11.4 b	25.4 b	24.8 abcd
Wenerzao	64.4 a	16.2 c	32.7 abc	27.8 bc	12.7 ab	8.4 c	21.2 b	22.0 cdef
Hongqixing	67.3 a	no	28.4 bc	no	8.6 ab	no	20.0 b	no
Zixuan-2	no[d]	2.7 f	no	50.0 a	no	10.9 b	no	20.0 def
Taicang	no	15.7 c	no	35.5 b	no	0.0 d	no	29.7 a
Lusuan-1	no	26.6 b	no	28.6 bc	no	7.6 c	no	22.0 cdef
Shandongjinan	no	32.8 a	no	22.9 c	no	0.0 d	no	26.0 abc
Tainuo-1	no	33.0 a	no	22.2 c	no	0.0 d	no	23.3 bcde

Notes: [a]Garlic cultivars were planted on 23 August 2006 or 21 August 2007. Fresh weight yields of seedling foliage (28 December), bolts (24 March) and bulbs (26 March) were collected in both growing seasons.
[b]RAUDPC = relative area under the disease progress curve, which is AUDPC per day calculated from the disease severity index evaluated five times at 15 to 30 day intervals from 29 October to 2 March in both growing seasons.
[c]Means followed by a letter in common are not significantly different at P = 0.05 using Tukey's test.
[d]no = not observed.

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Chemical control of garlic leaf blight

The effect of different clove treatments on garlic leaf blight was assessed four times in the early seedling stage. The garlic emergence rates among the treatments were increased consistently except by hymexazol. In 2006/2007 and 2007/2008, the lowest RAUDPC values were found for plots treated with fludioxonil or thiram while the untreated control, hymexazol and carbendazim had the highest RAUDPC values (Table 4).

The efficacy of fungicides tested against leaf blight varied significantly (Table 5) as measured by individual assessments on garlic leaf as well as from calculations of RAUDPC from four disease assessments. In 2006/2007, flusilazole at 50 g ha^{-1} and mancozeb at 350 and 175 g ha^{-1} gave the best control of leaf blight with RAUDPC at 11.5, 13.0 and 14.6, respectively. In 2007/2008, disease progression on plots was also significantly different (P = 0.05) among fungicide treatments. Relative area under disease progress curve values were significantly lower for plots treated with flusilazole (50 and 25 g ha^{-1}), mancozeb (350 and 175 g ha^{-1}) and flusilazole plus famoxadone (104 g ha^{-1}) compared to the untreated control and thiophanate-methyl (233 g ha^{-1}) which provided less disease control.

Discussion

Leaf blight caused by *S. solani* is the major constraint for garlic cultivation in central China where garlic actively grows from the end of autumn to the middle of spring. The data on disease severity showed variation between years with high disease pressure in 2006/2007 and low disease pressure in 2007/2008. In 2006/2007, disease severity levels of up to DSI = 60.6 (on a scale of 0 to 100) were recorded, whereas levels were less than 27.5 on crops grown during 2007/2008. This might have been due to differences in weather between the two seasons at the test plots. The present work was conducted through autumn, winter and spring conditions in central China. The relatively lower temperatures in 2007/2008 apparently limited the onset of infection by *S. solani*. Despite the absence of rainfall, the high relative humidity occurring in the Jianghan Plain as a result of high levels of residual moisture, along with foggy days that could favour long hours of wetness on leaf surfaces, provided conditions that allowed establishment and multiplication of *S. solani*. Within several weeks before bulb harvest in 2006/2007, a rapid increase in disease severity also occurred with little rainfall but with the presence of fog. No consistent relationship was found between the

Table 4. Clove emergence and mean RAUDPC of Stemphylium leaf blight on leaves of garlic cultivar 'Changbanpo' after various clove treatments in the 2006/2007 and 2007/2008 growing seasons at Dangyang County, Hubei, China.

		Emergence (%)		RAUDPC[a]	
Treatment	Concentration	06/07	07/08	06/07	07/08
Fludioxonil	0.05 g kg^{-1}	94.2 bc[b]	90.7 a	23.0 b	5.7 c
Thiram	1.25 g kg^{-1}	95.1 b	82.3 ab	23.7 b	6.3 bc
Carbendazim	0.1%	92.1 c	84.0 ab	25.3 ab	7.6 a
Carbendazim/ KH_2PO_3	0.1%/0.2%	98.2 a	92.0 a	26.3 ab	7.1 ab
Hymexazol	1.9 g kg^{-1}	92.9 bc	75.0 b	27.2 ab	7.2 ab
Untreated control	–	83.7 d	78.3 ab	29.3 a	8.0 a

Notes: [a]RAUDPC = relative area under the disease progress curve, which is AUDPC per day calculated from the disease severity index evaluated four times at 15 to 30 day intervals from 29 October to 20 December in both growing seasons.
[b]Means followed by a letter in common are not significantly different at P = 0.05 using Tukey's test.

Table 5. Mean RAUDPC of Stemphylium blight severity on leaves of garlic cultivar 'Changbanpo' after foliar fungicide applications in 2006/2007 and 2007/2008 growing seasons.

		RAUDPC[b]	
Fungicide[a]	Rate (g ha^{-1})	06/07	07/08
Flusilazole	50	11.5 e[c]	4.4 e
	25	15.2 cd	6.4 cde
Mancozeb	350	13.0 de	4.7 de
	175	14.6 de	6.5 cde
Flusilazole/famoxadone	52/52	no	5.2 de
	26/26	no	8.0 bc
Azoxystrobin	126	no	6.8 cd
	63	no	8.1 bc
Difenoconazole/propiconazole	75/75	no	7.4 c
	37.5/37.5	no	8.5 bc
Difenoconazole	50	15.3 cd	8.1 bc
	25	18.2 bc	8.2 bc
Thiophanate-methyl	233	19.3 b	9.5 ab
Untreated control	–	25.1 a	11.1 a

Notes: [a]Fungicides were applied three times at 10-day intervals ending 28 November 2006 or 14 December 2007.
[b]RAUDPC = relative area under the disease progress curve, which is AUDPC per day calculated from the disease severity index evaluated four times at 7 to 15 day intervals from 29 October to 20 December in both growing seasons.
[c]Means followed by a letter in common are not significantly different at P = 0.05 using Tukey's test.
[d]no = not observed.

amount of rainfall and leaf blight severity, implying that the duration of wetness was likely more important than the amount of rain.

The teleomorph of *Stemphylium* species is found in *Pleospora*, and the sexual structures generally form under prolonged cold conditions (Simmons, 1969). However, there is no record of the occurrence of the teleomorph of *S. solani*, either from tomato or cotton (Mehta, 2001). The *Pleospora* stage of *S. solani* was not observed in garlic fields of Dangyang County throughout the 2006 to 2008 growing seasons. Ascospores are probably not important as a primary inoculum source in this region of central China, but further research on genetic diversity of isolates in neighbouring fields would be needed to confirm this.

We speculate that garlic leaves in the field are the most important source of oversummering conidia and mycelia of *S. solani*, which survived in plant debris for long periods. Our results showed that cold and wet conditions, and burial in soil were not optimal for survival of *S. solani*. In order to minimize the risk from leaf blight, farmers should incorporate the debris into the soil or decrease the risk of Stemphylium leaf blight with rice paddy–upland rotation. Delay of the sowing date for fall grown garlic could also be used to reduce disease as the pathogen population decreases with cooler temperatures. Field surveys on other hosts near garlic fields in Dangyang County were conducted and *S. solani* was also isolated from white leaf spots on leek, Chinese cabbage, oilseed rape and maize (Zheng *et al*., 2009). However, the role of secondary hosts in the epidemiology of garlic leaf blight remains unknown.

Resistance or tolerance is considered the most economical and environmentally friendly way to control this disease. The local cultivar 'Changbanpo' currently used in Dangyang County is susceptible to leaf blight. In fact, farmers had tried to use several other cultivars to curtail the disease, but did not succeed because the other cultivars were also susceptible. This is the first report of screening of garlic cultivars in China for resistance to garlic leaf blight. None of the cultivars used in this study were completely resistant to garlic leaf blight. Cultivars 'Qingganruanye', 'Ruanruanye' and 'Zixuan-2' were more resistant and could be used to reduce the disease risk. Some variation in the ranking of the cultivars was seen between the two seasons, but the results were similar for the most resistant cultivars and the most susceptible cultivars. Several cultivars did not adapt to the low temperatures in the spring of 2008. This affected the bulb yield in the 2007/2008 growing season. So the cultivars which had high bulb yields in 2006/2007, showed relatively lower yields in 2007/2008, resulting in a negative relationship ($r = -0.51$, $P > 0.05$) among cultivars between the two seasons. Development of effective resistant cultivars that may be

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grown in Hubei province is under way, but may take several years before commercial use. However, local farmers sometimes chose cultivars with less resistance because of favourable agronomic characteristics, including better bolt yield, since some more resistant cultivars such as 'Qingganruanye' and 'Ruanruanye' do not have the desired bolting characteristics.

Approximately 15% of the garlic seed lots collected from Dangyang County in 2006/2007 were infected with *S. solani* assessed using an *in vivo* method described by Aveling *et al*. (1993) (data not shown). It is likely that seed was one of the main sources of inoculum for these plants. Garlic cloves treated with fungicides delayed the onset of visible leaf blight infections until approximately 71 days after sowing (3 Nov 2006) compared with 66 days (29 Oct 2006) for non-treated clove. Similarly in 2007, initial disease symptoms on clove-treated plants were observed 90 days after sowing (20 Nov) compared to 84 days (14 Nov) for the untreated control. In both growing seasons, fludioxonil and thiram were found to be more effective than other clove treatments. Thiram as a seed treatment has also been reported to eliminate *Stemphylium* sp. from carrot seeds (Maude, 1966). However, clove treatments are not effective over an entire growing season since garlic leaf blight is a foliar disease whose inoculum is spread through air or rainfall.

Among the screened fungicides, flusilazole (± famoxadone) and mancozeb were found to be more effective than others. Flusilazole is a triazole compound with protective and eradicative activity (Scheinpflug & Kuck, 1987), and becomes well distributed within the plant tissue (Smith *et al*., 1992). It provided effective and persistent control of leaf blight in the present study. Mancozeb is classified as a contact fungicide with preventative activity (USEPA, 1987), and the second highest level of protection against leaf blight was obtained in this work. Mancozeb efficacy was found to be very similar to that reported by Yang *et al*. (2000). The RAUDPC values of fungicides were quite different between two growing seasons.

The findings from this study can be used to develop management alternatives for *S. solani* in garlic fields in central China. Cultivar susceptibility should be considered along with fungicides for an integrated protection against leaf blight. When relatively resistant cultivars are planted, the start of the epidemic is delayed, and presumably the number of fungicide sprays can be reduced throughout the whole season. It may be possible to apply these concepts also to other pathogens where host–pathogen interactions are similar to those described here for *S. solani* in garlic fields.

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In conclusion, the use of garlic 'Qingganruanye', 'Ruanruanye' or 'Zixuan-2' which naturally show high resistance to garlic leaf blight can be combined with fludioxonil or thiram for seed treatment to decrease disease levels in this region. As well, once symptoms are observed, field application of the fungicides flusilazole (± famoxadone) or mancozeb are recommended to further combat disease.

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