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Black Leaf Mold Development on *Lycopersicon* spp. and Associated Yield Losses

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ABSTRACT

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Black leaf mold of tomato, caused by *Pseudocercospora fuligena*, has become a serious threat to tomato production in Taiwan in recent years. In this study, black leaf mold development and its effect on yield were studied in 12 Lycopersicon entries representing a range of resistance levels. Disease developed more rapidly on cultivars and lines of *Lycopersicon esculentum* than *L. hirsutum, L. pimpinellifolium,* and *L. glandulosum* accessions which were included in this study. The *L. hirsutum* entries received significantly lower area under disease progress curve (AUDPC) values and produced significantly fewer conidia per cm^2 of leaf area than the other entries. There was a significant positive correlation between the conidia produced per cm^2 of leaf area and AUDPC values. Black leaf mold caused a significant reduction in the yield of all *L. esculentum* entries, but not the *L. hirsutum, L. pimpinellifolium* and *L. glandulosum* accessions. There was also a significant positive correlation between the percentage of yield loss and both measurement parameters for black leaf mold, i.e. AUDPC values and the number of conidia produced per unit of leaf area.

Key words: *Pseudocercospora fuligena*, black leaf mold, tomato, Lycopersicon, yield loss.

INTRODUCTION

Black leaf mold (BLM) of tomato is caused by *Pseudocercospora fuligena* (Roldan) Deighton (Syn. *Cercospora fuligena* Roldan)(6,11). The disease and pathogen were first described in the Philippines in 1938 (11). Although BLM has been reported in Africa (10) and the U.S.A. (2), most reports originate from Asia (3, 7,9,14). Recent reports have shown that BLM causes extensive damage to tomato in Taiwan (3,4). The pathogen infects leaves causing irregular shaped lesions of up to 10-cm diameter with some associated chlorosis. The lesions are initially gray, but turn dark black with age as the fungus sporulates profusely, and the leaves dry prematurely. Yield losses of 32% with a reduction in fruit number and weight have been reported from experimental plots (4). Several reports have noted that most tomato cultivars are highly susceptible to BLM (1,2,4,14). Cultivars tolerant or resistant to BLM have been identified and used in Florida, U.S.A. to minimize losses from disease (2,8).

The objectives of our study were to assess and monitor BLM under field conditions and to determine the relationship between disease parameters and yield components.

MATERIALS AND METHODS

The studies were conducted at the Asian Vegetable Research and Development Center (AVRDC). On 27 September 1993, 30-day-old seedlings of 12 cultivated and wild tomato entries (see below) were transplanted to the field in double rows, with 50 cm between rows and 50 cm between plants on 1.0 × 6 m raised beds with a 0.5 m furrow width. The 12 entries included *L. glandulosum* (PI126434), *L. hirsutum* (PI134417, 134418, 308182 and 365904), *L. pimpinellifolium* (LA1236 SAL317), and *L. esculentum* [LA403, L5235, Known You 301 (KY301), Tainan selection No.2 (TN-2), Taichung ASVEG No. 4, and Hualien ASVEG No. 5]. These entries were selected because they represent a wide range of reactions to

black leaf mold (5). The experiment was arranged in a split-plot design with four replications. The experimental design consisted of a main-plot with 12 tomato entries and two subplots which were fungicide-protected and fungicide-nonprotected. Fungicide-protected plots were not inoculated and were sprayed with 50% benomyl W. P. (1 gm of 50% W. P. per liter) combined with 80% mancozeb W. P. (2.5 gm of 80% W. P. per liter) every 2 weeks. The nonprotected plots were inoculated with *P. fuligena* (isolate Pf-2) 3, 4, 5 and 6 weeks after transplanting using a conidial suspension (10^4 spores/ml) harvested from 10-day-old plate cultures with tomato leaf juice and oatmeal agar (TOA)(3).

The percentage leaf area affected by BLM was visually estimated 13 times at weekly intervals starting 8 November and ending 31 January 1994. Area under the disease progress curves (AUDPC) were calculated (12) based on days after transplanting and percentage leaf area affected. Ten leaves from the middle of the canopy were randomly collected from each experimental plot on 29 November 1993. Five discs, 1.5-cm-diam were cut from each sample leaf with a No. 8 cork borer, suspended in 40 ml of sterile water, shaken for 30 sec, and the concentration of conidia was determined with a hemacytometer. Data were transformed to conidia per cm^2 of leaf area. Fruits were harvested from the middle 5 m of each 6 m plot 14 times from 29 November 1993 to 28 February 1994. Total fruit weight and the number of fruit per plot were recorded from each harvest. Average fruit weight was calculated by dividing the total fruit weight by the number of fruit.

Data were analyzed by ANOVA and means were separated by LSD ($P<0.01$). Regressions of yield components to AUDPC and conidia/cm^2 of leaf area across 12 tomato entries were calculated. Two entries, PI308182 and PI365904 flowered, but no fruits were set, therefore they were not included in the regression calculation.

RESULTS

Only trace amounts of BLM occurred on plants in the non-inoculated, fungicide-protected plots. No other foliar diseases occurred at damaging levels during the study. BLM symptoms began to appear on plants about 10 days after the first inoculation in the inoculated, fungicide-nonprotected plots, and the symptoms increased in severity over time in each successive disease severity assessment (Fig. 1). Tomato entries varied greatly in their BLM reactions, with disease severity ratings (percent leaf area affected) ranging from 5 to 98 among entries at 125 days after transplanting. Based on disease severity ratings, entries could be grouped into three distinct categories within species. Five of the *L. esculentum* lines formed the highest disease severity rating group, *L. hirsutum* entries the lowest disease severity rating group, and single entries of *L. esculentum, L. pimpinellifolium,* and *L. glandulosum* forming an intermediate disease severity group (Fig. 1 & 2).

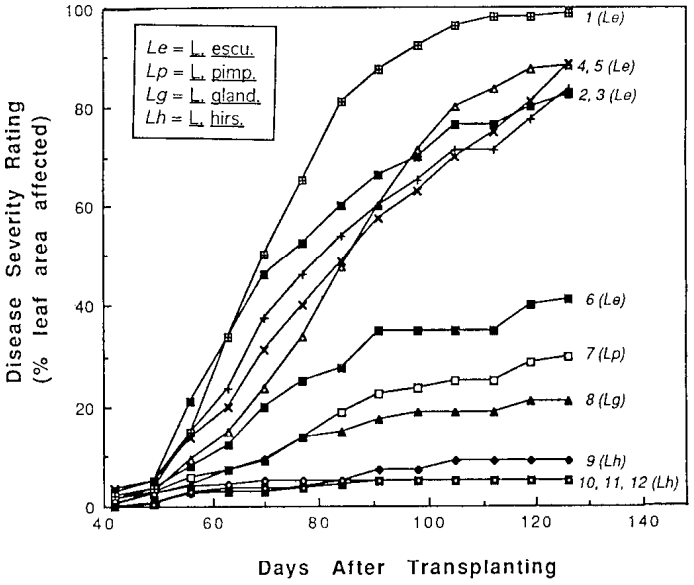

Fig. 1. Development of black leaf mold on 12 tomato entries grown in the field and inoculated with *Pseudocercospora fuligena*, but did not receive any fungicidal treatments. Entries: 1) TN-2, 2) KY301, 3) Taichung ASVEG No. 4, 4) L5235, 5) Hualien ASVEG No. 5, 6) LA403, 7) LA1236 SAL317, 8) PI126434, 9) PI134418, 10) PI365904, 11) PI308182, and 12) PI134417.

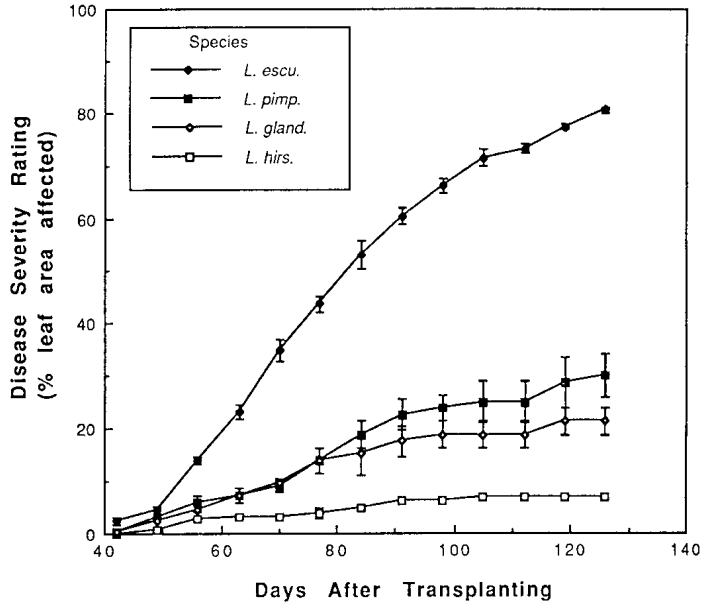

Fig. 2. Development of black leaf mold on field-grown plants of *Lycopersicon esculentum* (six accessions), *L. pimpinellifolium* (one accession), *L. glandosum* (one accession), and *L. hirsutum* (four accessions), that were inoculated with *Pseudocercospora fuligena*, but received no fungicidal application.

Similar results were obtained when the data were transformed and expressed as AUDPC, with *L. esculentum* receiving significantly higher ($P<0.01$) AUDPC values than all other entries and the *L. hirsutum* entries significantly lower ($P<0.01$) AUDPC values (Table 1 and Fig. 2). The *L. pimpinellifolium* and *L. glandulosum* entries received intermediate AUDPC values that were significantly higher ($P<0.01$) than those for *L. hirsutum* entries and significantly lower ($P<0.01$) than those for *L. esculentum* entries. AUPDC values among the four *L. hirsutum* entries did not differ significantly, but AUDPC values of some *L. esculentum* entries were significantly different (Table 1). TN-2 received the highest AUDPC value, followed by KY301, then Taichung ASVEG No. 4, L5235, and Hualien ASVEG No. 5 with similar values, and finally LA403 with the lowest AUDPC value.

Conidial production was significantly greater ($P<0.01$) on *L. esculentum* entries compared to entries of other species, and there were no significant differences among the *L. esculentum* entries (Table 1). Conidial production was significantly lower ($P<0.01$) on *L. hirsutum* entries than on *L. esculentum* entries, whereas sporulation was intermediate to these species on the *L. pimpinellifolium* and *L. glandulosum* entries. There was a significant positive correlation ($P<0.01$, $r^2=0.87$) between the number of conidia produced per cm^2 of leaf surface area and the AUDPC values for the same lines.

Plants of PI308182 and PI365904 (*L. hirsutum*) flowered, but failed to set fruit in our field plots. Yield reductions of entries that produced fruit ranged from 5.2 to 63.1% in BLM-affected plots (Table 2). Significant yield reductions that ranged from 21.6 to 63.1 % occurred in all *L. esculentum* entries in the BLM-inoculated plots receiving no fungicidal sprays. Yields were not significantly reduced in the *L. hirsutum*, the *L. pimpinellifolium*, nor the *L. glandulosum* entries. Yield losses among the 10 entries that set fruit were positively correlated with parameters used to measure BLM development. For example, there was a highly significant correlation ($P<0.01$, $r^2=0.93$) between AUDPC values and percent yield reduction (Table 1 & 2). There was also a highly significant correlation ($P<0.01$, $r^2=0.84$) between the number of conidia produced per cm^2 of leaf area on each of the 10 entries and the percent yield reduction measured (Table 1 & 2).

Average fruit weights were reduced by 3.6 to 33.4% among the 10 entries that produced fruit in the BLM-affected plots compared to BLM-free plots. However the reductions in fruit size were significant in only three *L. esculentum* entries: TN-2, Hualien ASVEG No. 5, and LA403.

DISCUSSION

Both AUDPC values and number of conidia produced per cm^2 of leaf area were highly correlated with percent of yield reduction. Furthermore, there was a highly significant correlation between the number of conidia produced on the foliage and the AUDPC values for the tomato entries evaluated. These data suggest that either parameter could be used to evaluate tomato lines for their BLM reactions, although

TABLE 1. Black leaf mold development and sporulation on plants[1] of 12 field grown Lycopersicon entries inoculated with *Pseudocercospora fuligena*

Entries[2]	Species	AUDPC[3]	Conidia/cm^2 leaf area (Log_{10})
TN-2	*L. esculentum*	5401	6.4
KY301	*L. esculentum*	4414	6.4
Taichung ASVEG No. 4	*L. esculentum*	3987	6.4
L5235	*L. esculentum*	3919	6.0
Hualien ASVEG No. 5	*L. esculentum*	3868	6.4
LA403	*L. esculentum*	2059	6.0
LA1236 SAL317	*L. pimpinellifolium*	1384	5.5
PI126434	*L. glandulosum*	1109	5.1
PI134418	*L. hirsutum*	450	5.0
PI365904	*L. hirsutum*	377	4.3
PI308182	*L. hirsutum*	322	4.7
PI134417	*L. hirsutum*	317	4.8
LSD ($P<0.01$)		256	0.5
Correlation coefficient		$r^2=0.87$	

[1] Data based on four replications of each tomato line in field plots receiving no fungicidal protection.
[2] Entries with prefixes PI, LA and L are accessions from the USDA, Univ. California-Davis, and Genetic Resources & Seed Unit at AVRDC, respectively; others are cultivars.
[3] Area under disease progress curve.

TABLE 2. Effect of black leaf mold on yield and average fruit weight of Lycopersicon entries based on harvests from fungicide-protected plots and nonprotected plots

Line	Yield (t/ha)[1]			Weight (g/fruit)[1]		
	Fungicide-protected[2]	Non-protected[3]	Loss (%)	Fungicide-protected[2]	Non-protected[3]	Loss (%)
TN-2	36.5	13.5	63.1	49.9	33.2	33.4
L5235	31.0	16.0	48.3	44.8	41.6	7.1
KY301	18.1	9.8	46.0	48.6	44.2	9.1
Taichung ASVEG No. 4	66.6	36.3	45.5	51.5	46.6	9.5
Hualien ASVEG No. 5	54.8	32.1	41.4	64.0	53.6	16.3
LA403	17.5	13.7	21.6	31.0	25.4	18.0
PI126434	4.9	4.6	5.9	1.7	1.6	5.8
PI134417	2.4	2.3	5.4	2.2	2.1	3.6
PI134418	2.6	2.5	5.4	2.2	2.1	5.5
LA 1236 SAL317	12.4	11.7	5.2	2.1	1.9	7.3
LSD (P<0.05)	8.6[4]		17.1[5]	4.0[4]		10.3[5]

[1] Mean of four replications.
[2] Benomyl (1 g of 50 W. P. per liter) combined with mancozeb (2.5 g of 80% W. P. per liter) applied at 2-wk intervals; plants were not artifically inoculated.
[3] No fungicidal protection was applied; plants were inoculated 3, 4, 5, and 6 wk after transplanting.
[4] Differences between columns within the same line.
[5] Differences within column.

AUDPC values are much more precise. AUDPC values were much more discriminating in this study, for distinguishing levels of resistance that could not be recognized by comparison of conidial numbers produced on each entry.

Results from this study show that a high potential exists for serious economic damage to tomato crop in Taiwan from BLM. Yield losses of 63.1, 46.0, 45.5, and 41.4% were observed in commonly grown tomato cultivars TN-2, KY301, Taichung ASVEG No. 4, and Hualien ASVEG No. 5, respectively, when grown in inoculated field plots receiving no fungicidal protection and compared with yields from non-inoculated plots receiving fungicidal-protection. A yield loss of 32% in TN-2 was recently reported in a similar study (4). Yield losses appear to be due to foliar damage caused by the disease, because the fruit are not infected by the pathogen. Although all the Taiwan cultivars tested were shown to be susceptible based on their high AUDPC values, TN-2 was significanly ($P<0.01$) more susceptible than KY301 which, in turn was, significantly ($P<0.01$) more susceptible than Taichung ASVEG No. 4 and Hualien ASVEG No. 5. In spite of the differential responses of these cultivars to BLM, none of them possesses a resistance level sufficient to be useful for BLM management under Taiwan conditions.

Included in this field study, in addition to commercial cultivars, were accessions of *Lycopersicon* spp. found previously in laboratory studies to possess various levels of BLM-resistance (5). These accessions were included in the present study to ascertain their reactions to BLM under field conditions in an attempt to determine whether their resistance levels were sufficient to be useful. Based on the AUDPC values, most of the accessions tested expressed some levels of resistance, but the four *L. hirsutum* accessions were significantly more resistant than all other entries in the study. Furthermore, yield reductions among these accessions were statistically insignificant (about 5%) even under the severe BLM pressure in this test. These results suggest that if levels of resistance found in these accessions were introgressed into tomato cultivars, it might provide them with a high level of protection. The *L. hirsutum* accessions which evolved in more humid conditions than most of the other *Lycopersicon* spp. may also possess useful resistance to other foliar diseases of tomato (13). BLM-resistant cultivars would offer growers an alternative to their current BLM management practice which is total reliance on protective fungicide applications. In fact, concurrent with this study some progress has been made in a tomato breeding study at AVRDC which has shown BLM-resistance in a *L. hirsutum* accession, PI134417, and a *L. esculentum* line, PI254655, to be a heritable trait governed by two recessive genes (T. C. Wang et al, *unpublished*).

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摘　　要

王添成[1]、白若威[2]、謝文瑞[1]. 1994. 番茄黑黴病病勢之進展與產量損失之關係. 植病會刊 3:181-185. (1. 台中市　國立中興大學植物病理學研究所, 2. 台南縣　亞洲蔬菜研究發展中心)

最近幾年來，由 *Pseudocercospora fuligena* 引起之番茄黑黴病已經對台灣地區番茄生產造成嚴重之威脅。本研究乃是利用十二種對黑黴病具不同程度抗性之番茄品系探討黑黴病病勢進展對產量之影響。結果發現屬於 *Lycopersicon esculentum* 之品種及品系之病勢進展比 *L. hirsutum*、*L. pimpinellifolium* 及 *L. glandulosum* 之品系較爲快速，而且 *L. hirsutum* 之品系得到病勢進展曲線下面積值及每平方公分葉面積所產生之分生胞子量均顯著低於其他品系。因此，每平方公分葉面積所產生之分生胞子量與病勢進展曲線下面積值呈現正相關。黑黴病之危害可使所有 *L. esculentum* 供試品系呈現顯著地減產，但未對 *L. hirsutum*、*L. pimpinellifolium* 及 *L. glandulosum* 之品系造成顯著減產。本研究結果顯示番茄產量損失百分率與黑黴病之病勢進展曲線下面積值及每單位葉面積產生分生胞子量呈正相關之關係。

關鍵詞：黑黴病、產量損失、番茄、*Pseudocercospora fuligena*。