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SOME EPIDEMIOLOGICAL ASPECTS OF STRAWBERRY ANGULAR LEAF SPOT IN EGYPT.

~~SOME EPIDEMIOLOGICAL ASPECTS OF STRAWBRRY ANGULAR LEAFE SOPT IN EGYPT.~~

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ABSTRACT

In this study, various epidemiological aspects of strawberry angular leaf spot caused by *Xanthomonas fragariae* including the disease distribution and intensity in strawberry fields, incidence of *X. fragariae* on cooled stored transplants, aggressiveness of numerous Egyptian strains of *X. fragariae*, and cultivars reaction were investigated under Egyptian conditions. Among four governorates surveyed in 2007 and 2009 growing seasons, the disease occurred in Beheira and Qalyubia and did not occurre in Sharqia and Ismailia. In Qalyubia governorate, the number of fields that exhibited the angular leaf spot disease in both seasons was generally fewer than those in Beheira governorate. Disease intensity was higher in Beheira than Qalyubia during both seasons and was higher in 2009 than 2007 in both governorates. Among all surveyed localities, Markaz Bader had the highest disease intensity whereas Al-Deer locality had the lowest. *X. fragariae* was detected in plants stored in cold storage facilities at Beheira and Qalyubia governorates and the pathogen was more frequently isolatedion incidence was higher in Beheira than Qalyubia. Little variation was observed among the 15 local strains of *X. fragariae* with regard to their virulence and none of these strains was exceptionally virulent. There was no resistant cultivars among the 14 strawberry cultivars tested. All cultivars ranged from moderate to highly susceptible. The highest susceptible cultivars were Camarosa and Sweet Charlie.

INTRODUCTION

Angular leaf spot of strawberry *(Fragaria xananassa* Duchesne), caused by *Xanthomonas fragariae* Kenn. & King (Kennedy and King 1962a), is a troublesome disease that occurs in many parts of the world (Maas *et al.* 1995). Disease symptoms first appear as small (1-2 mm) water-soaked lesions bounded by leaf veins, resulting in an angular-shaped lesions. These lesions appear translucent with transmitted light and eventually ooze bacteria under moist conditions. With time, they may coalesce, become necrotic, and develop a purple border (Maas 1998). The pathogen is able to move systemically in the plant from the leaves to the crown, roots, and newly formed runners (Milholland *et al*. 1993; Stefani and Mazzucchi 1989), sometimes causing collapse of the crown and plant (Hildebrand *et al*. 1967). In case of severe outbreak, also the petals show disease spots, which make the fruit unmarketable (Mass 1998).

Due to the unintentional international movement of infected plants, the European Plant Protection Organization (EPPO) has listed *X. fragariae* as a quarantined pest and has prescribed phytosanitary procedures (Roberts *et al.* 1996). Also, FAO/IPGRI has classified angular leaf spot as a potential risk in international distribution of strawberry germplasm (Maas *et al.* 1995).

In Egypt, strawberry producers have had to deal with many fungal pathogens, but they have never concerned about bacterial diseases in the past. However, they have recently faced an increasing occurrence of bacterial angular leaf spot, which has become increasingly destructive to strawberry production. In Egypt, the disease was first observed in 2003-2004 by different strawberry production companies, but the disease was first documented in Egypt in 2006 (Tolba 2006).

The epidemiology of *X. fragariae* must be understood before effective control strategies can be devised. The delineation of disease distribution, severity and pathogen population are imperative to such studies. Studies to date on angular leaf spot of strawberry have been merely on the reporting of the presence of infection, the identification of the bacterium, and the proof of its pathogenicity. Very little research on the epidemiology of angular leaf spot of strawberry has been reported (Roberts *et al.* 1996).

The objectives of these studies were to investigate some epidemiological aspects of angular leaf spot of strawberry including the distribution and intensity of the disease in strawberry fields, incidence of *X. fragariae* on transplants stored in cold storage facilities, and quantify the aggressiveness of various Egyptian strains of *X. fragariae* and the reaction of several commercial strawberry cultivars to this pathogen.

MATERIALS AND METHODS

Distribution of angular leaf spot in strawberry growing fields.

Strawberry-growing fields in different localities in Beheira, Qalyubia, Sharqia and Ismailia governorates were surveyed during January in 2007 and 2009 growing seasons. Plants were visually assessed for presence of angular leaf spot symptoms and some symptomatic leaves were collected from diseased plants and attempts to isolate the pathogen from putative lesions were done. Lesioned tissue was removed and macerated in 200 µl of sterile distilled water. A loopful of the suspension was streaked onto Wilbrink-N medium (Koike, 1965) and the culture plates were incubated at 27°C for 5 days and checked for development of typical colonies of *X. fragariae*. In every sampling, several colonies were selected to confirm its identification following procedures described by Schaad *et al.* (2001). The pathogenicity of the isolated strains was tested on Sweet Charlie strawberry plants.

Intensity of angular leaf spot in strawberry growing fields.

Diseased strawberry-growing fields in five localities in Beheira and Qalyubia Governorates were surveyed from January to March in 2007 and 2009 growing seasons to determine the intensity of angular leaf spot disease depending on visual symptoms. Incidence (percentage) of plants showing typical symptoms of bacterial angular leaf spot was assessed in five randomly

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selected groups of 20 consecutive plants by a total of 100 plants per field (Fourie, 2002). When the area of the field was more than one feddan, an area of this field equal to one feddan (60 x 70 m^2 approximately) was chosen. Disease severity was evaluated for each plant on a, 0-100 scale as follows: 0 =No signs of disease; 25 =Minute water soaked lesions fewer than 50 / leaflet without apparent exudates; 50 =Small water soaked lesions, 50 - 100/leaflet, beginning to coalesce, moderate amounts of exudates present; 75 =Coalesced large water soaked lesions, 100 or more/leaflet, beginning to turn brown with slight necrosis of the tissue between them, abundant exudates present; 100 =Same as the previous class but the necrosis is extensive. This rating scale is a modification of the disease severity rating scale, which was used by Patel and Walker (1965) in the case of bacterial halo blight of bean. The rating scale was adapted to become convenient with symptom characters of angular leaf spot of strawberry. Incidence and severity values were used to calculate a disease intensity index *(DI)* using the model: $DI = (I \times S)/ M$. Where I = incidence of diseased plants (%), S = mean severity of foliar symptoms and M = maximum severity value. (Bejarano-Alcazar *et al.* 1996).

Incidence of *Xanthomonas fragariae* in strawberry storage facilities.

In summer of 2009, frigo strawberry transplants were sampled while in cold storage at facilities in some localities at Beheira and Qalyubia governorates. The groups of plants (comprised of different cultivars) were sampled. Three boxes were selected at random from within a group and 20 plants were removed from each box. The crown of each single plant was excised and blended with 25 ml of sterile phosphate buffer in a lab blender for 3 min. The resulted suspension was centrifuged for 5 minutes at 12000 rpm, then the pellet was resuspended in 2 ml of sterile phosphate buffer and spread on plates of Wilbrink's medium plus 150µg/ml cycloheximide. The plates were incubated at 27°C for 5 days. Incidence of *X. fragariae* typical colonies was recorded for each individual plant.

Aggressiveness of *Xanthomonas fragariae* strains.

Susceptible strawberry cultivars, Camarosa and sweet Charlie, were inoculated with 15 strains of *X. fragariae* (obtained from various Egyptian localities during the surveys) to determine the virulence of these strains. Following the method of Hildebrand *et al*. (2005), a handheld automotive paint sprayer was mounted on a retort stand. The trigger of the sprayer was kept in an open position, and airflow through the sprayer was (620 kPa). Bacterial inoculum was delivered to the sprayer through a rubber hose from a flask. Inoculum was directed downward through a 9-cm^2 opening in a thin metal template that held a strawberry leaflet flat against the base of the retort stand. The template was mounted on a hinge that enabled easy and rapid insertion of leaves to be inoculated. The inoculator was adjusted to 20 cm above a strawberry leaf and an inoculation time of 10 s. Prior to inoculation, the plants were transferred from the greenhouse to the humid chamber overnight and exposed to 4 h of light to encourage stomatal openings (Hildebrand *et al*. 2005). After inoculation, the plants were returned to the humid chamber and evaluated for lesion density after 21days. Lesion density was assessed on a scale of 0-7 (Hildebrand *et al*. 2005), where 0 = approximately 3, 1 = 6, 2 = 12, 3 = 24, 4 = 48, 5 = 96, 6 = 192, and 7 > 384

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lesions within the inoculated area (9 cm^2) of the template. A key of Hildebrand et al.(2005) for lesion density (Fig. 1) was used to aid in the evaluations; increasing numbers of small dots, approximating the size angular leaf spot lesions and corresponding to the lesion density scale,

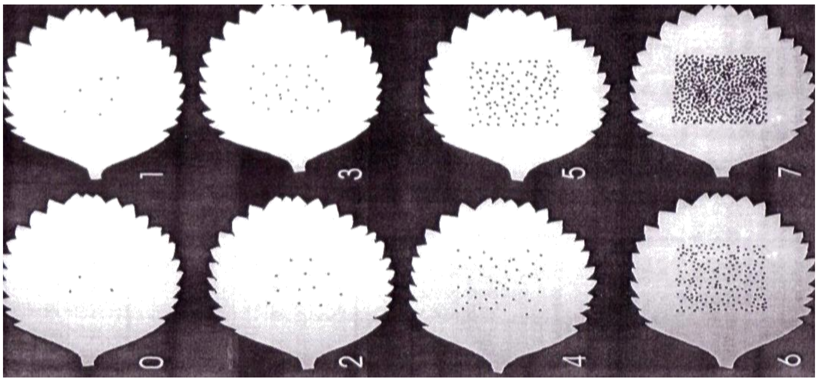

Fig. 1. Key of lesion density on strawberry leaflets inoculated with *X. fragariae.* Densities are 3, 6, 12, 24, 48, 96, 192, and 384 lsions/9 cm^2, respectively for the rating scale 0-7.

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Cultivar reaction.

A pots trial was conducted to evaluate the susceptibility of 14 strawberry cultivars to *X. fragaria* under greenhouse conditions. The tested cultivars were Albion, Camarosa, Camino Real, Chandler, Candonga, CalGiant, Festival, Palomar, Red Delight, Sweet Charlie, Suzana, Tamar, Ventana and Yael. Strawberry plants were obtained from several local nurseries in Egypt and propagated from runner tips in a quarantined green house facility away from other strawberry plants. For inoculum preparation, two strains of *X. fragariae* designated Xf 7 and Xf 10 were seeded onto Wilbrink-N medium and incubated at 27°C for 3 days. Bacteria were washed from culture plates with sterile distilled water and the suspensions were diluted to a final optical density of 0.100 at OD595 corresponding to ≈ 10^8 CFU/mL (Maas *et al*. 2000). Bacterial suspension was drawn into a 3-mL syringe. The needle was removed and the syringe aperture placed firmly against the abaxial surface of a leaflet, and the syringe plunger carefully pressed until a water-soaked area become visible. Care was taken not to injure the leaflet with pressure from the syringe body. In all tests, each leaflet was inoculated at four sites away from the midrib. Inoculated plants were placed in individual clean plastic bags for 3 days in a lighted laboratory at room temperature (23±3°C). Plants then were moved to greenhouse where they were misted two times per day. The mean of daytime temperatures during the study was 22°C. Plants were watered and fertilized as needed. After 21 days, inoculated sites were evaluated and rated using the criteria of Maas *et al*. (2000) as follows: 0 = no reaction, transient water-soaking from the inoculation no longer evident; 1 = transient water-soaking evident in the inoculation site; 2 = slight chlorosis or necrosis in the center of the inoculation

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site; 3 = water-soaking expanding beyond inoculation site and often bacterial exudates evident; 4 = necrosis spreading beyond the inoculation site and/or secondary infections evident; 5 = total necrosis of the inoculation area and leaflet changing color from chlorotic to reddish-brown. At the conclusion of the test, inoculation sites were cut from leaflets, surface sterilized, fragmented, placed in sterile water for 10 min and agitated on a vortex mixer for 10-20 seconds. The extract was streaked into Wilbrink's medium. The culture plates were incubated at 27°C and checked for development of typical colonies of *X. fragaria* (Roberts *et al* 1996). The experiment was replicated three times. The data were analyzed with the ANOVA directive in Genstat 5, release 3 (Genstat 5 Committee 1993). The probability level for significance testing was set at $P < 0.05$. Results were reported on the rating scale ($\log_2$).

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RESULTS

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Distribution of angular leaf spot in strawberry-growing fields.

results of surveys, which were conducted in January 2007 and 2009 for determining the distribution of angular leaf spot in strawberry-growing fields at four Egyptian governorates (Table 1) showed that, the disease occurred in Beheira and Qalyubia and did not occur in Sharqia and Ismailia during the two seasons. In Beheira governorate, the disease occurred in the two surveyed localities (Noubaria and Markaz Bader). There were 11 (20.7%) of 53 fields contained plants with symptoms of angular leaf spot and *X. fragariae* was isolated from 10 (90.9) of these infested fields in 2007.In 2009, a total of 18 (30.5%) form 59 fields contained plants with symptoms of angular leaf spot and *X. fragariae* was isolated from 17 (94.4%) of diseased fields. In Qalyubia governorate, among six localities that were surveyed, the disease did not occur in three localities (Met-Kinana, Tukh and Kafr Shibin) in 2007. In 2009, only one locality (Tukh) remained free from the disease. The percentage of fields, which were contained symptomatic plants in the two seasons was generally lower when compared with Beheira governorate.

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Table (1). Distribution of angular leaf spot of strawberry in four Egyptian governorates in 2007 and 2009 growing seasons.

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Governorate	Locality	2007 No. of fields surveyed	2007 Diseased fields No.	2007 Diseased fields %	2007 Isolation incidence No.	2007 Isolation incidence %	2009 No. of fields surveyed	2009 Diseased fields No.	2009 Diseased fields %	2009 Isolation incidence No.	2009 Isolation incidence %
Beheira	Noubaria	8	5	62.5	4	80	11	9	81.8	8	88.9
	Markaz Bader	45	6	13.3	6	100	48	9	18.7	9	100
	Total	53	11	20.7	10	90.9	59	18	30.5	17	94.4
Qalyubia	Al-Deer	25	6	24.0	6	100	28	8	28.5	8	100
	Met-Kinana	29	0	0.0	0.0	0.0	20	6	30.0	5	83.3
	Qaha	7	3	42.8	3	100	10	5	50.0	5	100
	Tukh	24	0	0.0	0	0.0	15	0	0.0	0	0.0
	Khanka	18	4	22.2	4	100	21	3	14.2	2	66.7
	Kafr Shibin	17	0	0.0	0	0.0	16	3	18.7	2	66.7
	Total	120	13	10.8	13	100	110	25	22.7	22	88
Sharqia	Al-Salhia	15	0	0.0	0.0	0.0	17	0	0.0	0.0	0.0
	Bilbeis	12	0	0.0	0.0	0.0	15	0	0.0	0.0	0.0
	Total	27	0	0.0	0.0	0.0	32	0	0.0	0.0	0.0
Ismailia	Serapium	20	0	0.0	0.0	0.0	24	0	0.0	0.0	0.0
	Al-Kassasin	9	0	0.0	0.0	0.0	24	0	0.0	0.0	0.0
	Total	29	0	0.0	0.0	0.0	48	0	0.0	0.0	0.0

LSD between localities = 15.6, LSD between Governorates = 8.7, LSD between seasons = 9.4

Among 120 surveyed fields, there were 13 fields (10.8%) contained symptomatic plants and X. *fragariae* was isolated from 13 (100%) of these diseased fields in 2007.

~~In Beheira governorate, the disease occurred in the two surveyed localities (Noubaria and Markaz Bader). There were 11 (20.7%) of 53 fields contained plants with symptoms of angular leaf spot and *X. fragariae* was isolated from 10 (90.9) of these infested fields in 2007.In 2009, a total of 18 (30.5%) form 59 fields contained plants with symptoms of angular leaf spot and *X. fragariae* was isolated from 17 (94.4%) of diseased fields. In Qalyubia governorate, among six localities that were surveyed, the disease did not occur in three localities (Met-Kinana, Tukh and Kafr Shibin) in 2007. In 2009, only one locality (Tukh) remained free from the disease. The percentage of fields, which were contained symptomatic plants in the two seasons was generally lower when compared with Beheira governorate. Among 120 surveyed fields, there were 13 fields (10.8%) contained symptomatic plants and X. *fragariae* was isolated from 13 (100%) of these diseased fields in 2007.~~ In 2009, the percentage of infected plants relatively increased to reach 22.7% but *X. fragariae* isolation precentage decreased (88%).

Intensity of angular leaf spot in strawberry-growing fields.

Results of surveys conducted in 2007 and 2009 to determine the intensity of angular leaf spot in two Egyptian governorates are illustrated in Table (2). In general, intensity of the disease significantly differed between the two governorates but insignificantly differed between the two seasons. The mean of disease intensity was higher in Beheira than Qalyubia during the two seasons (16.3 versus 6.1). In addition, the mean of disease intensity was insignificantly higher in 2009 than 2007 in both governorates. Among all surveyed localities, Markaz Bader locality (Beheira governorate) had the highest disease intensity mean (19.3) whereas Al-Deer locality (Qalyubia governorate) had the lowest disease intensity mean (5.8) during the two seasons.

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Table (2): Intensity* of angular leaf spot of strawberry in two Egyptian governorates in 2007 and 2009

Governorate	Locality	2007				2009				Mean *DI* in 2007& 2009 (%)
		No. of fields surveyed	*I* (%)	*S*	*DI* (%)	No. of fields surveyed	*I* (%)	*S*	*DI* (%)	
Beheira	Nubariyah	5	21.6	54.3	11.7	8	27.5	55.5	15.2	13.4
	Markaz Bader	25	34.2	57.4	19.6	24	32.8	58.3	19.1	19.3
Mean			27.9	55.8	15.6		30.1	56.9	17.1	16.3
Qalyubia	Al-Deer	10	15.4	36.7	5.6	12	14..9	41.2	6.1	5.8
	Qaha	7	22.1	34.8	7.8	10	24.4	37.3	9.1	8.4
	Khanka	8	18.2	41.2	7.4	11	22.7	30.4	6..9	7.1
Mean			18.5	36.2	6.9		25.2	35.1	7.3	6.1

LSD between localities = 2.4, LSD between Governorates = 3.5,
LSD between seasons = 3.8

***Disease intensity index *(DI)* was calculated using the model: *DI = (I x S)/ M,* where *I* = incidence of diseased plants (%), *S* = mean severity of foliar symptoms, and *M* = maximum severity value. (Bejarano-Alcazar *et al.* 1996). Disease severity for each plant was evaluated according to Patel and Walker (1962) on a, 0-100 scale as follows: 0 =No signs of disease; 25 = Minute water soaked lesions fewer than 50/leaflet without apparent exudates; 50 = Small water soaked lesions, 50-100/leaflet, beginning to coalesce, moderate amounts of exudates present; 75 = Coalesced large water soaked lesions, 100 or more/leaflet, beginning to turn brown with slight necrosis of the tissue between them, abundant exudates present; and 100 = same as the previous class but the necrosis is extensive.**

Incidence of *Xanthomonas fragariae* in strawberry storage facilities.

In general, *X. fragariae* was detected in plants stored in the cold storage facilities at both sampled governorates (Table 3). In Beheira governorate, three of ten groups sampled contained infected plants. In one of these groups, infected plants were found in all of the boxes sampled. In the other two samples, only one box contained plants infected by *X. fragariae*. In Qalyubia governorate, only one of twelve groups contained infected plants but all boxes of this group contained infected plants. Pathogenicity of *X. fragariae* strains on strawberry plants was confirmed.

Table (3): Incidence of *Xanthomonas fragariae* on strawberry plants stored in cold facilities in Beheira and Qalyubia governorates during the summer of 2009.

Governorate	Beheira										Qalyubia											
Group	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	11	12
Infected boxes / 3	0	0	0	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Infected plants / 60	0	0	0	6	15	0	0	4	0	0	0	0	0	0	0	0	0	0	9	0	0	0

Infected plants (%)	0	0	0	10	25	0	0	6.6	0	0	0	0	0	0	0	0	0	0	15	0	0	0

Aggressiveness of *Xanthomonas fragariae* strains.

Fifteen strains of *X. fragariae* were screened for their aggressiveness on the two susceptible strawberry cultivars, Camarosa and Sweet Charlie (Table 4). There was no cultivar x isolate interaction. All strains showed high level of aggressiveness but Xf 10 strain was insignificantly more aggressive among the tested strains whereas Xf 3 and Xf 14 were the lowest.

Table (4). Aggressiveness of 15 strains of *Xanthomonas fragariae* on strawberry cultivars, Camarosa and Sweet Charlie.

Strain		Xf 1	Xf 2	Xf 3	Xf 4	Xf 5	Xf 6	Xf 7	Xf 8	Xf 9	Xf 10	Xf 11	Xf 12	Xf 13	Xf 14	Xf 15
Origin		B	B	B	B	B	B	B	B	B	B	Q	Q	Q	Q	Q
Density rating	Camarosa	5.7	5.4	4.6	4.9	5.5	5.6	5.3	4.9	5.8	6.4	5.6	5.1	4.8	4.8	5.3
	Sweet Charlie	5.5	5.4	4.8	4.8	5.5	5.7	5.5	5.1	5.8	6.2	5.8	5.4	4.8	4.8	5.6
Mean		5.6	5.4	4.7	4.8	5.5	5.6	5.4	5.0	5.8	6.3	5.7	5.2	4.8	4.8	5.4

LSD between means = 1.4, **B** = Beheira **governorate,** **Q** = Qalyubia **governorate.**

Table (4): Aggressiveness of 15 strains of *Xanthomonas fragariae* on strawberry cultivars, Camarosa and Sweet Charlie.

Strain		Xf 1	Xf 2	Xf 3	Xf 4	Xf 5	Xf 6	Xf 7	Xf 8	Xf 9	Xf 10	Xf 11	Xf 12	Xf 13	Xf 14	Xf 15
Origin		B	B	B	B	B	B	B	B	B	B	Q	Q	Q	Q	Q
Density rating	Camarosa	5.7	5.4	4.6	4.9	5.5	5.6	5.3	4.9	5.8	6.4	5.6	5.1	4.8	4.8	5.3
	Sweet Charlie	5.5	5.4	4.8	4.8	5.5	5.7	5.5	5.1	5.8	6.2	5.8	5.4	4.8	4.8	5.6
Mean		5.6	5.4	4.7	4.8	5.5	5.6	5.4	5.0	5.8	6.3	5.7	5.2	4.8	4.8	5.4

LSD between means = 1.4, **B** = Beheira **governorate,** **Q** = Qalyubia **governorate.**

Cultivar reaction:

Among 14 strawberry cultivars, no resistant cultivars were found (Table 5). All cultivars ranged from moderate to highly susceptible. The highest susceptible cultivars were Camarosa and Sweet Charlie, which had mean reaction of 4.9 and 4.8, respectively. Cultivars that had lowest mean reaction were Suzana, Festival, Red Delight, Cal Giant, Albion, Candonga and Camino Real, respectively.

Table (5~~).~~): Reaction of 14 strawberry cultivars against two strains of *X. fragariae*, Xf 7 and Xf 10.

Cultivar		Albion	Camarosa	Camino Real	Candonga	Cal Giant	Chandler	Festival	Palomar	Red Delight	Suzana	Sweet Charlie	Tamar	Ventana	Yael
Mean reaction	Xf 7	3.7	4.9	3.8	4.2	4.1	4.6	3.9	4.3	3.9	4.6	4.8	4.4	4.2	4.7
	Xf 10	3.9	4.8	4.1	4.2	3.8	4.2	4.2	4.2	3.6	4.2	5.2	4.8	4.4	4.6
Total mean		3.8	4.8	3.9	4.2	3.9	4.4	4.1	4.2	3.7	4.4	5.0	4.6	4.3	4.6

LSD = 0.66

~~Cultivar~~		~~Albion~~	~~Camarosa~~	~~Camino Real~~	~~Candonga~~	~~Cal Giant~~	~~Chandler~~	~~Festival~~	~~Palomar~~	~~Red Delight~~	~~Suzana~~	~~Sweet Charlie~~	~~Tamar~~	~~Ventana~~	~~Yael~~
~~Mean reaction~~	~~Xf 7~~	~~3.7~~	~~4.9~~	~~3.8~~	~~4.2~~	~~4.1~~	~~4.6~~	~~3.9~~	~~4.3~~	~~3.9~~	~~4.6~~	~~4.8~~	~~4.4~~	~~4.2~~	~~4.7~~
	~~Xf 10~~	~~3.9~~	~~4.8~~	~~4.1~~	~~4.2~~	~~3.8~~	~~4.2~~	~~4.2~~	~~4.2~~	~~3.6~~	~~4.2~~	~~5.2~~	~~4.8~~	~~4.4~~	~~4.6~~
~~Total mean~~		~~3.8~~	~~4.8~~	~~3.9~~	~~4.2~~	~~3.9~~	~~4.4~~	~~4.1~~	~~4.2~~	~~3.7~~	~~4.4~~	~~5.0~~	~~4.6~~	~~4.3~~	~~4.6~~

Inoculation sites were evaluated and rated using 1-4 scale where 0= no reaction, transient water-soaking from inoculation site no longer evident; 1 = transient water-soaking evident in the inoculation site; 2 = slight chlorosis or necrosis in the center of the inoculation site; 3 = water-soaking expanding beyond inoculation site and often bacterial exudates evident; and 4= necrosis spreading beyond the inoculation site and/or secondary infections evident.

~~**LSD = 0.66**~~

~~Inoculation sites were evaluated and rated using 1-4 scale where 0= no reaction, transient water-soaking from inoculation site no longer evident; 1 = transient water-soaking evident in the inoculation site; 2 = slight chlorosis or necrosis in the center of the inoculation site; 3 = water-soaking expanding beyond inoculation site and often bacterial exudates evident; and 4= necrosis spreading beyond the inoculation site and/or secondary infections evident.~~

~~DISSECTION~~DISCUSSION

An understanding of the epidemiology of angular leaf spot is necessary to determine the effectiveness and practicability of any control method. The surveys of farmers' fields and the cold storage facility revealed that, the disease occurred in Beheira and Qalyubia and did not occur in Sharqia and Ismailia during both seasons. This probably accounts for introducing infected transplants into the field from imported diseased plants to one or more production companies, and then the disease established itself and spread gradually to other places. *X. fragariae* can be disseminated over short and long distances by runner plants in the market (Calzolari and Mazzucchi 1989). At importation, the bacteria are usually dormant on the daughter plants, showing no visual symptoms. This makes it difficult to stop the importation of the disease into a new region. Roberts *et al.* (1996)

reported that, strawberry plants arriving from the nurseries for transplanting in the fall were frequently infected with angular leaf spot. Maas *et al.* (1995) also reported that, infected unrooted runner tips were imported into North Carolina from Canada for rooting as plug plants. In this way, we belief that the progressed occurrence of angular leaf spot in Egyptian strawberry fields is associated with importation of nursery plants from regions where the environmental conditions are more conducive for this disease.

The slight difference in disease development between the two seasons did not appear to be from differences in rainfall, since mean rainfall was approximately the same in both years (data not shown). The appearance of new disease in either year could not be correlated to earlier, specific rain events. Mean temperature also was similar between years; however, the number of days with temperature below 15°C in November and December were much greater in 2007 than in 2009. The primary source of inoculum in a new field is contaminated transplants and secondary inoculum comes from bacteria that exude from lesions under high moisture conditions (Maas 1998). *X. fragariae* can survive on dry infested leaves and tissue buried in the soil for up to 1-year (Roberts *et al.* 1997). Open field cultivation in combination with a highly susceptible host, as the current cultivars Camarossa and Sweet Charle, leading to a further spread of the disease and higher losses. The rapid spread of the disease is influenced by the increasing rate of interchange of plant material. The pathogen can be spread easily by harvesting operations when wet and cool conditions favor the production of bacterial exudates. If the pathogen invades the vascular system of the plant, the disease will be difficult to control. Overhead irrigation, fog, high humidity, and rain are positive factors in disease development and spread (Epstein 1966; Hildebrand *et al.* 1967; Kennedy and King 1962b). Accordingly, the trend towards drip irrigation in commercial production fields should have a negative effect on the spread and survival of the bacterium. Epstein (1966) reported a 75-80% loss in a seven hectares fruiting field in the cultivar Sparkle in Wisconsin. Roberts *et al.* (1996) concluded that disease severity of 25 and 10% in each of two years of that study resulted in 10% yield loss each year among Sweet Charlie plants due to this disease. Due to unintentional international movement of infected plants, the European Plant Protection Organization (EPPO) has listed *X. fragariae* as a quarantined pest and has prescribed phytosanitary procedures (Roberts *et al.* 1996). The FAO/IPGRI also listed angular leaf spot as a potential risk in international distribution of strawberry germplasm (Maas *et al.* 1995).

Some variation in aggressiveness was observed among 15 strains of *X. fragariae* and none were exceptionally more or less aggressive (Table 4). Pooler *et al.* (1996) were able to differentiate strains from diverse geographic sources into four genotypic ERIC groups, but the aggressiveness of some of these same strains in Hildebrand *et al.* (2005) study did not correspond to the ERIC designations. The similarity in aggressiveness supports their conclusion that while genotypic differences can be discerned, strains of *X. fragariae* are closely related and are likely spread geographically through the movement of plant material. The relationship *of X. fragariae* to other members of the genus *Xanthomonas* has been examined (Hildebrand *et al*. 1990; Vauterin et al., 1995), but the genetic variability of strains within the species has not been reported (Maas *et al*. 1995). The information from studies to compare strains of *X. fragariae* at the genetic level could be useful to identify the origins and spread of specific strains or genetic types. This information could be applied in international tracking of pathogen populations for quarantine programs.

No resistance to *X. fragariae* was noted in the 14 strawberry cultivars. Some cultivars like Albion, Camino Real, Festival and Red Delight showed less susceptibility than the others (Table 5). Several studies have shown that resistance to angular leaf spot varies among commercial strawberry cultivars (Hazel and Civerol. 1980; Howard 1971; Kennedy and King 1962b; Maas *et al.* 1995, 2000; Hildebrand *et al.* 2005 and Desmet *et al.* 2009), but none was completely resistant. However, few studies have quantified disease severity among the cultivars. Hazel and Civerolo (1980) reported a continuum of increasing resistance among different cultivars. Historically, many cultivars have been evaluated using various inoculation techniques, but significant levels of resistance useful for breeding purposes have not been identified. Interestingly, Tristar cultivar showed the highest level of resistance in the study of Hildebrand *et al.* (2005), but Maas *et al.* (1995) reported a serious outbreak in this cultivar after floods had occurred. Under those severe conditions, it is possible that tissues became water congested, favoring disease development. An aspect that has yet to be explored is the differential proneness of cultivars to water congestion thereby possibly affecting their susceptibility to infection. Likewise, Maas *et al.* (2000, 2002) and Lewers *et al.* (2003) reported on and released two clones that, under their conditions of inoculation, appeared to be symptom-free. However, when these clones had tested by Hildebrand *et al.* (2005) using the syringe infiltration method or spray method, typical symptoms developed, albeit at a reduced intensity, but nevertheless indicating that these clones are not completely resistant. It is not clear why symptoms developed under these conditions. In addition, the identification of genetic types is a prerequisite to identify sources of resistance in strawberry to the pathogen. While strawberry cultivars exhibited levels of susceptibility or tolerance to *X. fragariae,* only *F. moschata* Duch. appeared to be immune (Hazel, 1981; Hazel and Civerolo, 1980; Kennedy and King, 1962b). A screening program to identify genes for resistance must incorporate representatives of the genetic variants in the screening process otherwise the resistance may be overcome quickly by genetic variants.

Conclusion

We need clear identification of the *X. fragariae* problem and development of effective measures to stop new pathogen introductions via latently infected planting material and to suppress existing contamination of field soils. Growers should avoid harvesting and moving equipment through infected fields when the plants are wet. Minimizing the use of overhead sprinklers during plant establishment and for freeze protection will also reduce the spread of the disease.

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بعض السمات الوبائية لمرض التبقع الزاوى البكتيرى على الفراولة فى مصر

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يعد محصول الفراولة من المحاصيل ذات الأهمية الاقتصادية في مصر؛ و نظراً للتأثير الضار لمرض التبقع الزاوي البكتيري على هذا المحصول ؛ فقد أجريت هذه الدراسة للتعرف على العديد من مظاهر وبائية هذا المرض والتي شملت مدى وجوده وانتشاره وشدته في أهم مناطق زراعة الفراولة في مصر، وكذلك تواجد المسبب المرضى على شتلات الفراولة المبردة فى الثلاجات المعدة لذلك. كما شملت الدراسة مدى التباين بين العزلات من ناحية قدرتها المرضية و مدى الإختلاف بين أصناف المحصول من ناحية قابليتها للإصابة بهذا المرض. وكانت نقاط الدراسة ونتائجها كالتالي:-

أولا: إنتشار المرض:

تم عمل حصر لانتشار المرض فى مناطق زراعة الفراولة فى محافظات البحيرة و القليوبية و الشرقية و الإسماعلية خلال موسمى ٢٠٠٧ و ٢٠٠٩م وكانت النتيجة كالتالي :

- ظهر المرض فى محافظتي البحيرة والقليوبية ولم يلاحظ تواجده فى محافظتى الإسماعيلية والشرقية، وكانت نسبة الحقول المصابة بالمرض فى محافظة البحيرة أعلى منها فى محافظة القليوبية وذلك خلال موسمى الدراسة.
- كانت هناك زيادة معنوية فى نسبة الحقول المصابة فى موسم ٢٠٠٩ عن موسم ٢٠٠٧ وذلك فى كلتا المحافظتين حيث بلغ متوسط نسبة الحقول المصابة فى البحيرة ٣٠.٥ % خلال ٢٠٠٩ مقابل ٢٠.٧ % خلال ٢٠٠٧ ، فى حين بلغ فى القليوبية ٢٢.٧ % خلال ٢٠٠٩ مقابل ١٠.٨ % خلال ٢٠٠٧.

ثانيا: شدة المرض:

- متوسط شدة المرض كان أعلى فى محافظة البحيرة (١٦.٣ %) عنه فى محافظة القليوبية (٦.١ %) خلال الموسمين، ولم يكن هناك فرقا معنويا فى شدة المرض بين الموسمين ٢٠٠٧ و ٢٠٠٩
- من بين المناطق المختبرة كانت منطقة مركز بدر (بحيرة) هى الأعلى (١٩.٣ %) من حيث شدة المرض بينما كانت منطقة الدير (قليوبية) هى الأقل(٥.٨ %) على الإطلاق.

ثالثا: تواجد المسبب المرضى على شتلات الفراولة المبردة :

بفحص شتلات الفراولة أثناء فترة التخزين فى الثلاجات فى محافظتى البحيرة و القليوبية فى صيف ٢٠٠٩ إتضح الآتى :

- تم عزل المسبب المرضى من بعض هذه الشتلات فى كلتا المحافظتين
- فى محافظة البحيرة كانت هناك ثلاثة مجموعات من أصل عشرة تحتوى على نباتات مصابة بينما فى محافظة القليوبية كانت هناك مجموعة واحدة فقط من اصل إثنتى عشرة تحتوى على نباتات مصابة.

رابعا: القدرة المرضية للعزلات :

بإجراء إختبار القدرة المرضية لخمسة عشر عزلة من العزلات المحلية للمسبب المرضى على صنفين من أصناف الفراولة القابلة للإصابة إتضح الأتى:

جميع العزلات أعطت معدل مرتفع من القدرة المرضية ، فى حين لم تكن هناك فروقا معنوية بين عزلة بعينيها وبقية العزلات الأخرى

- جميع العزلات أعطت تقريبا نفس معدل الإصابة على كلا الصنفين حيث لم تكن هناك فروقا واضحة لأى من العزلات على صنف دون الاخر

خامسا: تفاعل الأصناف:

فى إختبار لقابلية أربعة عشر صنفا من أصناف الفراولة التى تزرع فى مصر للإصابة بعزلتين من المسبب المرضى إتضح الآتى:

- جميع الأصناف أعطت معدل إصابة تراوح ما بين المتوسط إلى العالى من حيث القابلية الإصابة و

لم يكن هناك أى صنف مقاوم

- كانت أعلى الأصناف قابلية للإصابة تحت ظروف التجربة هما الصنفين "كماروزا" و "سويت شارل" .

قام بتحكيم البحث

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