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Studies on Stemphylium Leaf Spot of Broad Bean

I. Pathological Studies

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Stemphylium leaf spot disease of Vicia faba is a recently recorded disease in Egypt. A survey of this disease through broad bean growing cultivated areas, revealed its wide distribution all over the country. Symptoms of the disease are characterised by the appearance of brownish black necrotic irrigular lesions, mostly covering the leaflet surface within 7-10 days. Several isolates of Stemphylium sp. were either isolated from spotted broad bean leaves or from other hosts. All S. botryosum isolates proved to be pathogenic and produced typical symptoms on broad bean plants. One isolate, identified as S. vescarium Wallroth was the least pathogenic on this host. The disease developed, on inoculated plants at temperatures ranging from 12-27°C with an optimum of 20-27°C. Disease severity progressively increased with the increase of spore density in the inoculum. The fungus was able to attack some other hosts such as soybean, clover, lupines, tomato, flax and maize. Peanuts, beans, lentils, chick-pea and cotton were not attacked by this fungus.

Stemphylium leaf spot of Vicia faba caused by Stemphylium botryosum Wallroth, was reported in Arab Republic of Egypt(1).

Nelson[2] found that Stemphylium leaf spot of alfalfa had reduced both yield and quality of seeds. Edwardson et al.[3] reported that lines of blue lupine susceptible to S. botryosum produced less seed and green weight than resistant ones and attributed the former to defoliation during maturity.

Stemphylium botryosum had a wide host range. It had bean reported to produce leaf spot on many other crops including legumes such as Medicago arabica, Luccerne and Melilotus alba[4], alfalfa[5]. The fungus was also found to cause foliage blight and leaf spot to tomato plants[6,7]

As Stemphylium leaf spot was firstly recorded on broad bean, it was found necessary to make a general survey on its occurrence and distribution and to study symptoms, effects and the pathological aspects of the causal organism.

Material and Methods

Isolations of Stemphylium sp. were carried out from infected broad bean plants "varieties Rebaya 40 and Acquadulce". Two other isolates were obtained from the phyllosphere of maize and flax seeds and were included in the pathogenicity tests. Stock cultures were maintained on

P.D.A. slants and kept in the refrigerator at 6–8°C for further studies, however, fresh cultures were periodically prepared, every 20–30 days. Spore suspension of about 90,000 spores/ml was used in inoculation in all the experiments, unless otherwise stated. A casein sticker was added to the inoculum at the rate of 0.2 % (w/v) to give uniform infection over the entire plant. The inoculum was applied to potted plants under greenhouse conditions with the aid of hand atomizer, then the plants were irrigated and covered with polyethylene bags to maintain high relative humidity necessary for infection. Neither temperature nor light was controlled, but the range of air temperature fluctuations was recorded during each experiment. The bags were removed after 12-24 hr incubation according to the prevailing air temperature, in which the time exposure to high relative humidity was increased with the decrease in air temperature. Pathogenicity tests were carried out in the laboratory using healthy detached leaves. The plants were gently sprayed with water to remove the adhering soil particles, and mature leaves were carefully detached from the middle of the plant using flamed scissors, then inoculated with standard spore suspension. Inoculated leaves were incubated on filter paper in a moist chamber to maintain high relative humidity under room temperature. Seeds of the

susceptible local variety "Rebaya 40", was used in all experiments. All inoculations were carried out at least 45-60 days after planting. Disease readings were determined, 10-15 days after inoculation according to the prevailing temperature, when clear symptoms appeared on the inoculated plants. Disease readings were determined for each leaf according to disease severity rating which was made to include the size and frequency of the lesions/leaf. The following numerical rates were suggested to facilitate visual determination and to give a satisfactory comparison:

"0" = No symptoms.

"1" = Scattered small spots.

"2" = Spots coalescing and covering about ¼ - ½ leaf area.

"3" = More than ½ of the leaf area was infected.

Readings were converted to disease index according to the equation suggested by Townsend and Heuberger[8] as follows:

$$\text{Disease index \%} = \frac{\text{Sum } (n \times r)}{3N} \times 100$$

where; n is the number of leaves in which numerical rate "r" and "N" is the total number of inoculated leaves multiplied by the maximum numerical rate "3".

Experimental Results

Symptoms and Effects

A. On leaves

First symptoms of the disease appear on the mature leaves of naturally infected broad bean plants as circular or irregular brownish-black and slightly sunken spots, 2-5 mm in diameter, and the healthy tissues gradually lose their normal green colour. Further development of the disease resulted in irregular, lightly concentric, brownish-black necrotic lesions mostly covering the majority of the leaflet surface within one week. The lesions mostly appear at the margins of the leaflets and extend thereafter to cover all the leaflet surface. In final stages of lesions development, the leaves dry out and usually defoliate, but sometimes they remain attached to the plant for a short period before defoliation. No signs of disease symptoms were recorded on young leaves less than two weeks old.

Symptoms also appear on the stipules as dark lesions, 1-2 mm in diameter, then the whole stipula rapidly turns brownish-black in colour and almost dry out but remains attached to the leaf base.

Infected petioles show restricted, elongated necrotic streaks.

B. On flowers and pods

The whole parts of the infected flower suddenly turn black within 24-48 hr and dry out. The flowers may remain attached for a while, but may drop off when the plants are gently shaken.

Infection of buds may occur at any stage of their development, however, fully mature pods are not affected. All flower parts attached to the newly formed diseased buds usually can be infected. Symptoms often begin from the styler region and rapidly progress to the other parts causing complete necrosis to the pericarp. When the diseased pods are opened, the premature seeds are found to be somewhat shrinked and their colour turn olive-brown to brownish-black. Infected newly developed pods usually defoliate, but older pods mostly remain attached to the plant and the necrotic area may remain restricted if the pod rapidly reaches maturity after infection.

Occurrence and distribution

The disease was firstly reported in 1964 on a new broad bean variety named "Acquadulce", which was introduced from England and planted in about 300 fed. in El-Tahrir Province (Abdou and Fahim[1]). During the course of this

study (1969-1973), inspection of broad bean cultivated areas revealed that the disease was present in most broad bean growing areas throughout the country including, Kalubia, Menufia, Behiera, Gharbia, Giza, Beni-Suef, Minia and El-Tahrir areas. Examination of diseased fields showed that the first symptoms of the disease appeared during the second half of January and early February. It was also noticed that disease severity on leaves increased by plant maturity. Early defoliation was observed in severely infected fields.

Isolation and identification of the causal organisms

Three isolates identified as Stemphyliu botryosum (isolates 1, 2 and 3) were obtained from naturally infected broad bean plants "variety Rebaya 40" collected from several localities and from seeds obtained from infected plants "variety acquadulce". Another two isolates of the same genus were obtained from the phyllosphere of maize and flax and were identified as S. botryosum (isolate 4) and S. vesicarium (isolate 5), respectively. The five isolates were maintained in pure cultures and were all used in pathogenicity tests.

Pathogenicity tests

Potted healthy broad bean plants "variety Rebaya 40"

(about 60 days old) were inoculated as previously mentioned, using the five isolates of Stemphylium. Readings for disease indices were taken ten days after inoculation and defoliation percentage was recorded after 30 days.

Results presented in Table 1 show that all Stemphylium isolates proved to be highly pathogenic as they produced high incidence of leaf spot and defoliation. Stemphylium vesicarium was the least pathogenic and produced small restricted necrotic spots at a low frequency.

Table 1. Pathogenicity of different isolates of Stemphylium

Isolate No.	Identified as:	Disease index*	Defoliation (%)**
1	(S. botryasum)	22.0	15.0
2	" "	18.3	13.4
3	" "	17.3	14.3
4	" "	17.4	13.7
5	(S. vesicarium)	3.3	10.4
Control		0.0	0.0
L.S.D. at 0.05		8.5	3.08

* Readings were taken 10 days after inoculation.
** Readings were taken 30 days after inoculation.

Young leaves (less than 15 days old) seem to be resistant to infection with the pathogen, and they did not show any signs of disease symptoms.

The same results were obtained when detached leaves with different ages were inoculated with all the isolates of Stemphylium.

Isolate 1 which was considered the most virulent isolate was used in the subsequent experiments unless otherwise mentioned.

Pods of different stages of development were harvested, inoculated with a spore suspension and kept in moist chambers at room temperature. On the immature pods, first disease symptoms appeared after 10 days from inoculation as necrotic streaks, usually began from the styler region and extending downwards. Pods in the middle stage of development (prematured) were usually necrosed and rotted more rapidly; whereas, fully mature pods remained non-infected as those observed in nature.

Effect of temperature on disease development

a) In vivo (Greenhouse studies)

In this experiment, healthy potted broad bean plants, were inoculated under natural conditions outside the green-

house, with a standard spore suspension at different intervals during the growing season (from December to April). Fluctuations of temperature during the experimental period were recorded. Readings for disease indices and defoliation were taken as mentioned before.

Data presented in Table 2 show that there was an apparent positive correlation between disease incidence and temperature. Maximum infection occurred on plants inoculated during March and April. This was clearly found to coincide with the increase in air temperature during this perio where sometimes a maximum of 20° and 26°C was reached. Low temperature during December and January (11.9° and 13.5°C respectively) seems to be a limiting factor in leaf spot incidence and defoliation.

Table 2. Effect of temperature on disease development under natural conditions (season 1970-1971)

Date of inoculation	Temperature (°C)	Disease index (%)	Defoliation (%)
19/12	11.9 (9.0 - 15.0)*	9.4	5.8
9/1	13.5 (11.0 - 16.0)	13.8	8.5
17/2	15.6 (15.0 - 17.0)	13.4	11.0
11/3	17.2 (13.0 - 20.0)	19.4	15.0
22/4	18.9 (15.0 - 26.0)	24.7	20.0

* Figures between brackets are the minimum and maximum readings during the experimental period.

b) In vitro (Laboratory studies)

Detached mature leaves were inoculated with a standard spore suspension and incubated in moist chambers at temperatures ranging from 10-30°C. Disease development was recorded after 3 and 6 days (Table 3).

Table 3. Effect of temperature on disease development in the laboratory

Temperature (°C)	Disease index* (%)	
	3 days	6 days
10	0.0	0.0
18	11.1	44.4
21	16.6	55.5
24	36.6	76.6
27	50.0	83.3
L.S.D. at 0.05	31.2	

* Readings are the average of 20 leaves replicated 4 times.

Results showed that the optimum temperature for disease development was between 24 and 27°C, whereas a maximum of 50.0 and 83.3 disease indices was reached after 3 and 6 days, respectively. The incidence of the disease was considerably low after 3 days inoculation at temperature below 24°C especially at 18°C; however, a moderate

amount of infection was reached after 6 days.

Effect of different inoculum levels on disease incidence

Potted plants as well as detached mature leaves were inoculated with conidial suspensions containing 9,000; 50,000; 90,000; 180,000 and 360,000 conidia/ml and incubated at room temperature in moist chambers. Disease assessment of potted plants was carried out after 15 days from inoculation. Defoliation percentage was determined after 30 days from inoculation.

From the results shown in Table 4, it is clear that disease severity increased progressively as the inoculum concentration increased; however, the increase was not proportionally parallel to the degree of inoculum concentration.

Considering defoliation as a measure of relative reaction, it was also clear that defoliation increased with the increase of inoculum concentration.

The greenhouse results were emphasized by a laboratory test which showed the same trend, when assessment of the disease reaction was taken after 5 days.

Table 4. Effect of different inoculum levels on disease incidence

Conidia/ml	In greenhouse		On detached leaves
	Disease index (%)	Defoliation (%)	Disease index (%)
9×10^3	6.2	3.0	10.0
5×10^4	10.3	6.5	14.0
9×10^4	19.5	11.5	26.6
1.8×10^5	22.4	15.5	33.0
3.6×16^5	28.0	20.3	46.6
L.S.D. at 0.05	3.3	3.0	7.9

Host range studies

Further studies were made to determine the effect of the fungus on different plant species. All tested plants (about 60 days old) were inoculated as usual.

Bean, lentils, chick-pea, pepper and cotton did not show any signs of symptoms. Leaves of soybean and berseem were moderately infected as symptoms of infection appeared in the form of irregular brownish-black lesions (about 2 mm in diameter) with the edge being darker than the centre. Chlorosis then defoliation of infected leaves resulted several days after symptoms appearance.

Infection of lupines was accompanied by general chlorosis of the whole infected leaves. Symptoms on leaflets appeared as small grayish to black lesions with darker edges.

Moderate infection occurred on tomato leaves and appeared as small lesions, about 2 mm in diameter, dark olive to black in colour.

Flax plants were severely infected, where the disease attacked leaves causing irregular dark olive lesions, rapidly increased to cover most of leaf surface. Leaves dried out and mostly defoliated.

Typical leaf spot symptoms appeared on maize plants. Marginal region of leaves was pale yellow, whereas edges were brown coloured indicating some degree of necrosis.

The fungus sporulated giving typical Stemphylium spores on all the previous plant species which were infected.

Discussion

Leaf spot of broad bean caused by Stemphylium botryosum was reported for the first time on this crop in Arab Republic of Egypt in 1969. At this time, studies(1) indicated that this disease was restricted to El-Tahrir Province area on

both the introduced "Acquadulce" and local "Giza 2" varieties. The other broad bean growing areas such as El-Fayoum and Beni-Suef Provinces were free from this disease at that time.

Further studies during 1969-1973 seasons, showed that S. botryosum was present on this crop throughout the country including Kalubia, Menufia, Behiera, Gharbia, Giza, Beni-Suef, Minia and El-Tahrir areas. These findings indicated the rapid widespread of this pathogen during the previous period.

The isolation and pathogenicity studies also showed that this pathogen attacks other hosts in this country like Berseem, Soybean, Lupine, Tomato, Flax and Maize. It was recorded that this fungus also can infect alfalfa, red clover, white clover, Medicago arabica, lucerne, Melilotus alba, lettuce, Cucurbita sp., and tomato (2,4,5,7,9-12). This indicated that it is not specific to broad bean but can find suitable hosts to survive on throughout the year. Furthermore, in the present studies it was found that there are no apparent differences in virulence between the different isolates of S. botryosum when tested on the local broad bean variety "Rebaya 40", indicating the similarity between these isolates probably due to similar genetic pathogenic

capability. These studies also showed the possible infection of broad bean by different species of Stemphylium such as S. vesicarium isolated from flax seeds which proved to be less pathogenic than all the isolates of S. botryosum on broad bean producing typical leaf spots. This indicates that S. botryosum is the major pathogen of the disease.

Studies on effect of temperature on disease development showed that there is an apparent positive correlation between temperature and disease incidence. The disease progressed at temperatures ranging from about 12 to 27°C; however, the disease was more severe with the increase in temperature within the previous range. The optimum temperature for disease development was about 20-27°C. Nelson[2] in his study on leaf spot of alfalfa caused by S. botryosum, found that the optimum temperature for initial infection ranged from 18-24°C and that further disease development was promoted by moderate temperature. Other species of Stemphylium such as S. solani was found to cause heavy infection at higher temperatures; i.e. 26°C[13]. Disease severity progressively increased as the concentration of spores in the inoculum increased. However, this increase in disease severity was not proportionally parallel to the degrees of spore concentration.

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