PREDOMINATING MICROORGANISMS IN THE PHYLLOSPHERE OF BROAD BEAN VARIETIES IN RELATION TO INFECTION WITH STEMPHYLIUM BOTRYOSUM WALLROTH

By

F. M. BARAKAT,* and N. A. EISA **

Abstract

Qualitative and quantitative composition of the phyllosphere of 3 local and introduced broad bean varieties was studied in relation to pathogenesis of **Stemphylium botryosum** the cause of Stemphylium leaf spot. The three varieties differed in their disease reaction to the pathogen. Higher counts of epiphytic microflora were enhanced by the introduced variety «N.A. 29», than the local Romy and Giza 2 varieties. An apparent positive relation was found between absolute numbers of actinomycetes and disease resistance. However, a negative correlation was shown between the relative density of fungal population in the phyllosphere and resistance. Population pattern of microorganisms in the phyllosphere was also studied. Some bacterial groups (i.e. **Bacillus** spp., micrococci and non pigmented rods) were common to all varieties, but certain other groups were associated with a particular variety. **Alternaria** spp. and **Penicillium** spp. were also common fungi in the phyllosphere of all varieties, but other genera of fungi were associated with one variety. Considerable numbers of antagonists to the Stemphylium leaf spot pathogen were detected in the phyllosphere of all varieties. Antibiotic values of different groups of microorganisms varied with the variety. Maximum antibiotic value was recorded by the epiphytic bacteria of N.A. 29 variety. Two isolated fungi arising from the phyllosphere of the resistant Giza 2 variety were active antagonists to the pathogen. From this study, it could be concluded that the phyllosphere microflora play an important role in the pathogenesis of leaf parasites.

Introduction

In a previous work, Eisa (1974) has found that different local and introduced broad bean varieties differ considerably in their reaction to infection with **Stemphylium botryosum**

* Dept. of Agric. Bot. and Plant Pathol., Fac. of Agric., Cairo Univ.

** Dept. of Agric. Bot., High Inst. Agric. Moshtohor, Kalubia.

Wallr. In searching for disease resistance, many factors are involved, from which the role of leaf surface microorganisms (phyllosphere) in the pathogenesis of leaf parasites was investigated. Interaction between phyllosphere microflora and foliar pathogens and its effect on the degree of resistance of host plants was studied by some investigators (Last and Deighton, 1965 and McBride, 1971).

This work was thus carried out to investigate the qualitative and quantitative composition of leaf surface microflora of 3 local and introduced broad bean varieties, as well as their antagonistic propensities towards the Stemphylium leaf spot pathogen.

Materials and Methods

Three local and introduced varieties of broad bean (**Vicia faba** L.) namely; Romy, Giza 2 (local) and N.A. 29 (introduced), produced from the Legume Section, Agric. Research Centre, were employed in this study. Plants were grown in greenhouse in a limited area to ensure uniform conditions regarding the climate and air spora.

Susceptibility of different varieties was carried out in the laboratory. Detached mature leaves were inoculated with a standard spore suspension containing 90,000 conidia/ml, prepared from 10 days' old PDA cultures to give a satisfactory clear symptom expression (Eisa, 1974). A casein sticker was added to the inoculum at the rate of 0.2% (w/v) to give uniform infection over the leaves. Disease readings were determined according to disease severity rating to include the size and frequency of the lesions/leaf. Readings were thus converted to disease index according to the equation suggested by Townsend and Heuberger (1943).

Microflora were isolated using a leaf washing technique (Sinha, 1971). Young, mature and old leaflets were sampled from each grown variety when plants were about 10 weeks old. Three leaflets of each stage of maturity were selected from each of five plants. The washings of all leaflets were mixed thoroughly to make one sample, which was then diluted serially for plate count according to the technique adopted by Kiraly **et al** (1970). Five plates were inoculated from each of the following media ;

1. Nutrient glucose (1%) agar, for total microbial count.
2. Knight and Proom (1950) medium, for microflora of simple nutritional requirements.
3. Jensen's ager medium (Allen, 1961), for actinomycetes count.

4. Waksman's medium with rose bengal, for fungal count.

All plates were incubated at 28°C and colonies were counted after 48 hours for bacteria and 4 days for fungi and actinomycetes. Counts were calculated per cm^2 of leaf area.

Bacterial or fungal colonies appearing on plates containing 40 — 100 colonies were subcultured either on nutrient agar slants for bacteria and actinomycetes or PDA slants for fungi. From 20 — 30 colonies were subcultured from each plate at random at the highest dilution.

Identification of bacterial groups was carried out according to the morphological and cultural characters of each organism and compared with that present in Bergey's manual (1957). Genera of fungi were identified according to the description shown by Gilman (1957) and Barnett (1958).

Antagonistic effect of isolated microflora was tested on Czapeck's agar plates adjusted to pH 6.8. A disc of a well grown culture of **Stemphylium botryosum** was placed on the plate, and antagonistic microorganisms were tested using the following techniques :

a) For bacteria and actinomycetes, the antagonists were streaked at two places at the periphery of the dish, 3 cm apart from the fungal disc.

b) For fungi, the antagonist and the pathogen were placed on media opposite to each other at the periphery of dishes. placed media opposite to each other at the periphery of dishes.

Zones of inhibition were measured in mm., 8 days after incubation at 28°C. «Antibiotic index» and «antibiotic value» were calculated for each group of microorganisms according to Cooper and Chilton (1950).

Results

Disease reaction :

From the results of relative reaction of the three varieties tested in this experiment (Table 1), it was shown that they greatly differ in their reaction to infection with **S. botryosum** based on disease index. The local variety «Romy» was the highly susceptible, whereas Giza 2 was the more resistant. The introduced variety «N.A. 29» showed moderate resistance.

Table 1 : Reaction of three broad bean varieties to infection with **S. botryosum.**

Variety	Disease index %	Relative reaction
Romy	44.1	Susceptible
N, A. 29	16.6	Moderately resistant
Giza 2	7.7	Resistant

Microbial counts in the phyllosphere :

The results (Table 2), show that the phyllosphere of the introduced variety «N.A. 29» is inhabited by the highest numbers of microorganisms followed by Giza 2 and Romy varieties. The same trend was simultaneously found in simple nutritional microorganisms although their relative density (% of total count) was somewhat lower in the resistant variety Giza 2.

Actinomycetes population was apparently higher in the phyllosphere of Giza 2 representing a relatively high percentage of the total microbial count. On the other hand, the susceptible Romy variety enhanced the lower counts of this population, whereas N.A. 29 was inhabited by considerable numbers. Thus, a clear positive correlation could be manifested between tho absolute density of actinomycetes in the phyllosphere and resistance of broad bean varieties to infection with **S. botryosum.**

On the other hand, an apparent negative relation between the relative density of fungi (as % of total count) and disease resistance. The higher the percentage is, the less the variety resistance. This may show that the leaf surface of the susceptible variety «Romy» provides a more suitable habitat for fungal population than the more resistant ones i.e. N.A. 29 and Giza 2. The resistant variety Giza 2, though has a similar fungal count as the susceptible Romy variety, yet its relative density was much lower in Giza 2 variety. This suggests a Suppressive action on fungal development in the resistant variety.

Table 2 : Microbial counts in the phyllosphere of three broad bean varieties.

Variety	Counts (in thousands / cm^2) of ;							
	Total microflora		Simple nutritional microflora		Actinomycetes		Fungi	
	Absol no.	% *	Absol. no.	%	Absol. no.	%	Absol. no.	%
Romy	2.0	100	0.05	2.50	0.07	3.50	0.02	1.00
N.A. 29	18.0	100	0.53	2.94	0.50	2.72	0.03	0.17
Giza 2	7.6	100	0.11	1.44	0.69	9.15	0.02	0.03

* % Percentage to total count.

Population pattern of microorgonisms in the phyllosphere :

A) Bacteria and actinomycetes :

As shown in Table 3, the population pattern of bacteria in the three varieties includes common groups of bacteria namely ; spore-forming rods (**Bacillus** spp.), micrococci and non-pigmented rods. N.A. 29 variety has the higher frequency of spore-forming rods, while Giza 2 is the highest in micrococci. The occurrence of fluorescent pseudomonads on Romy and N.A. 29 leaves indicates their existence in a high frequency on these varieties. Lactobacilli and **Sarcina** spp, were only detected on Romy leaves.

Concerning the simple nutritional microflora, spore-forming bacilli and yeasts were dominant on all varieties. Micrococci were exceptionally undetected on dilution Knight and Proom plates of N.A. 29 variety, indicating their low frequency on this variety. Highest frequency of simple nutritional bacilli was recorded on N.A. 29 compared with Romy and Giza 2 varieties.

All colonies appearing on Jensen's agar, of all varieties, belonged to **Streptomyces** spp. No other genera of actinomycetes were detected.

B) Fungi :

It is apparent (Table 4) that **Alternaria** spp. and **Penicillium** spp. are dominant in the phyllosphere of broad bean varieties. Resistant Giza 2 variety stimulates the growth and development of more genera of fungi including **Cladosporium** spp., **Stemphylium** spp. and **Aspergillus** spp. **Helminthosporium** spp. was common on N.A. 29 variety, while **Cladosporium** and **Stachybotrys** predominate on Romy leaves.

Table 3 : Frequency of bacteria in the phyllosphere of broad bean varieties.

Variety	Bacterial group: On nutrient agar	% frequency	On Knight and Proom	% frequency
Romy	Spore-forming rods	28.56	Bacillus	28.5
	Non-pigmented rods	7.14	Micrococci	42.8
	Lactobacilli	14.32	Yeasts	28.7
	Fluorescent pseudo-monads	7.14		
	Micrococci	28.56		
	Sarcina spp.	7.14		
	No growth on transfer	7.14		
N.A. 29	Spore-forming rods	40.87	Bacillus	85.7
	Non-pigmented rods	8.33	Yeasts	14.3
	Fluorescent preudo-monads	16.15		
	Micrococci	29.10		
	No growth on transfer	5.55		
Giza 2	Spose-forming rods	9.08	Bacillus	33.4
	Non-pigmented rods	27.24	Micrococci	33.3
	Yellow pigmented rods	18.16	Yeasts	33.3
	Micrococci	45.52		

Table 4 : Predominating fungi in the phyllosphere of three broad bean varieties.

Genera	% frequency on the phyllosphere of variety ; Romy	N.A. 29	Giza 2
Alternaria spp.	40.0	50.0	27.2
Penicillium spp.	20.0	16.6	9.1
Cladosporium spp.	20.0	0.0	36.4
Aspergillus spp.	0.0	0.0	9.1
Helminthosporium spp.	0.0	33.4	0.0
Stemphylium spp.	0.0	0.0	18.2
Stachybotrys spp.	20.0	0.0	0.0

Interaction between phyllosphere microflora and S. botryosum:

As indicated in Table 5. it is clear that the phyllosphere of different varieties is inhabited by considerable numbers of antagonists although most microflora were symbiotic or synergistic to the Stemphylium leaf spot pathogen (**S. botryosum**). Epiphytic bacteria in the phyllosphere of N.A. 29 variety showed the maximum antibiotic value indicating a considerable antagonistic action towards the pathogen. All isolates from Romy variety were either of no or weak antibiotic action. Although most isolates arising from Giza 2 phyllosphere were non antagonistic, yet two isolated fungi belonging to the genera **Alternaria** and **Aspergillus** were quite active in antagonising **S. botryosum.** It could be concluded therefore, that these antagonistic microorganisms would partially play an important role in the pathogenesis of **Stemphylium botryosum** to broad bean varieties.

Table 5 : Interaction between some isolated microorganisms from the phyllosphere of broad bean varieties on **S. botryosum.**

Variety	Cultures isolated from ;	No. of isolates according to ; Diam. of inhibition zone (in mm.)			Anti-biotic index	Anti-biotic value
		0.0	1-3	4-6		
Romy	N.A.	7	6	0	0.9	1.80
	K & P	1	4	0	1.6	0.08
	Fungi	3	3	0	1.0	0.02
N.A. 29	N.A.	15	13	6	1.6	28.80
	K & P	0	7	0	2.0	1.06
	Fungi	5	2	1	1.1	0.03
Giza 2	N.A.	14	1	0	0.13	0.99
	K & P	2	1	0	0.67	0.07
	Fungi	7	1	2	1.20	0.02

N.A. = Cultures isolated from nutrient agar medium.
K & P = Cultures isolated from Knight and Proom medium.

Discussion

The tested local and introduced broad bean varieties were found to differ in their reaction to infection with **Stemphylium botryosum** Wallroth based on disease indices on mature detached leaves. The local variety «Romy» was the highly susceptible, whereas Giza 2 was the more resistant. Introduced

Population pattern of microorgonisms in the phyllosphere :

A) Bacteria and actinomycetes :

As shown in Table 3, the population pattern of bacteria in the three varieties includes common groups of bacteria namely ; spore-forming rods (**Bacillus** spp.), micrococci and non-pigmented rods. N.A. 29 variety has the higher frequency of spore-forming rods, while Giza 2 is the highest in micrococci. The occurrence of fluorescent pseudomonads on Romy and N.A. 29 leaves indicates their existence in a high frequency on these varieties. Lactobacilli and **Sarcina** spp, were only detected on Romy leaves.

Concerning the simple nutritional microflora, spore-forming bacilli and yeasts were dominant on all varieties. Micrococci were exceptionally undetected on dilution Knight and Proom plates of N.A. 29 variety, indicating their low frequency on this variety. Highest frequency of simple nutritional bacilli was recorded on N.A. 29 compared with Romy and Giza 2 varieties.

All colonies appearing on Jensen's agar, of all varieties, belonged to **Streptomyces** spp. No other genera of actinomycetes were detected.

B) Fungi :

It is apparent (Table 4) that **Alternaria** spp. and **Penicillium** spp. are dominant in the phyllosphere of broad bean varieties. Resistant Giza 2 variety stimulates the growth and development of more genera of fungi including **Cladosporium** spp., **Stemphylium** spp. and **Aspergillus** spp. **Helminthosporium** spp. was common on N.A. 29 variety, while **Cladosporium** and **Stachybotrys** predominate on Romy leaves.

ties due to the dilution plating technique employed in this study. Thus, microorganisms appearing on dilution plates are mostly those present in a high frequency.

It is also of interest to find that the resistant variety Giza 2 is inhabited with **Stempyhium** spp. spores probably **S. botryosum.** Thus, it could be suggested that the spores of the pathogen might survive as epiphytes on plant leaves without producting infection. Similarly, Leben (1963) found that **Xanthomonas vesicatoria** invaded the surface of tomato seedlings from infected seeds and multiplied there without inducing symptoms.

Crosse (1971) proposed that there are two possible ways in which saprophytes might interfere with the development of disease in the field ; by inhibiting infection, or by modifying the course of the disease after infection. In this study, the leaf surface of different varieties was shown to be inhabited by considerable numbers of antagonists to Stemphylium leaf spot fungus. Maximum antibiotic value was obtained by bacteria of N.A. 29 variety indicating a promising antagonistic action towards the pathogen. Most isolates from the phyllosphere of Giza 2 variety were almost non antagonistic to **S. botryosum** in culture plates, yet 2 genera of fungi undergo a clear antagonistic action. Last and Deighton (1965) suggested that foliar diseases might be reduced as a result of the antagonism between microorganisms or through their competition for nutrients. McBride (1971) in his study on microorganism interactions in the phyllosphere of larch, stated that the presence and activities of the microflora might be of critical importance to the degree of host resistance and the natural control of pathogen populations. Therefore, it could be concluded that the phyllosphere microflora are important factors in the pathogenesis of leaf parasitic fungi and their composition could be purposefully modified in the sake of biological control of such pathogens, a point which needs further investigation.

References

Allen, O.N. 1961. Experiments on soil Microbiology. Burgess Publ. Co., Minnesota, U.S.A.

Barnett, H.L. 1958. Illustrated Genera of Imperfect Fungi. Burgess Publ. Co., Minnesota, U.S.A.

Breed, R.S., E.G.D. Murray, and N.R. Smith (Eds.) 1957. Bergey's Manual of Determinative Bacteriology. Baillier, Tindall and Co., London.

Cooper, W.E. and S.J.P. Chilton. 1950. Studies on antibiotic soil microorganisms. I. Actinomycetes antibiotic to **Pythium arrehenomanes** in sugar cane soils of Luisiana. Phytopathology, 40 : 544-552.

Crosse, J.E. 1971. Interactions between saprophytic and pathogenic bacteria in plant disease. In Preece, T.F. and C.H. Dickinson (Eds.) 1971.

Eisa, N.A. 1974. Studies on Stemphylium leaf spot of broad bean (**Vicia faba L.**). Ph.D. Thesis, Fac. of Agric., Univ. of Cairo.

Gilman. J.C. 1957. A manual of Soil Fungi. Oxford Ibh Publ. Co., New Delhi, 450 pp.

Kiraly, Z., Z. Klement, F. Solmosy and J. Voros. 1970 Methods in Plant Pathology. Akademiai Kiado, Budapest, 507

Knight, B.C.J. and H. Proom. 1950. A comparative study on nutrition and physiology of mesophilic species of the genus **Bacillus.** J. Gen. Microbiol. 4 : 508-538.

Last, F.T. and F.C. Deighton. 1965. The non parasitic microflora on the surfaces of living leaves. Trans. Brit. Mycol. Soc. **48** : 83-99.

Leben, C. 1963. Multiplication of **Xanthomonas vesicatoria** on tomato seedlings. Phytopathology, **53** : 778-784

McBride, R.P. 1971. Microorganism interactions in phyllosphere of larch. In Preece, T.F. and C.H. Dickinson (Eds.) 1971.

Naim, M.S., S.A.Z. Mahmoud, and A.M. Hussein. 1957. Qualitative and quantitative studies on the rhizospheric microflora of some Egyptian cotton varieties. Ain Shams Sci. Bull., 2 : 65-83.

Preece, T.F. and C.H. Dickinson (Eds.) 1971. Ecology of Leaf Surface Microorganisms. Acad. Press, London, 640 pp.

Sinha, S. 1971. The microflora on leaves of **Capsicum anuum** (L.) Watt E.D., **Solanum melongena L., Solanum tuberosum** L. and **Lycopersicum esculentum** Mill. In Preece T.F. and C.H. Dickinson (Eds.) 1971.

Townsend G.R. and J.W. Heuberger. 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis. Reptr., 24 : 340-343.

دراسات على الميكروبات السائدة فى مجال الأوراق لاصناف الفول البلدى وعلاقتها بالاصابة بالفطر ستمفيليوم بوتريوزم

دكتور فاروق محمد بركات دكتورة نوال عبد المنعم عيسى

درس المحتوى الميكروبى للمجال الورقى لثلاثة أصناف من الفول البلدى وعلاقتها بالقابلية للاصابة بالفطر ستمفيليوم بوتريوزم المسبب لمرض تبقع الأوراق الأستمفيلى .

بينت النتائج أن الثلاث أصناف تختلف فى قابليتها للاصابة حيث كان الصنفجيزة ٢ هو أكثرها مقاومة أما الصنف رومى فكان الأكثر قابلية للاصابة فى حين الصنف ن . ا . ٢٩ المستورد كان متوسط القابلية للاصابة .

كما بينت النتائج أن الصنف ن . ا . ٢٩ يشجع نمو أعداد كبيرة من الميكروبات على سطح الأوراق أكثر من الأصناف المحلية جيزة ٢ ورومى . وجدت علاقة أيجابية بين العدد الكلى للأكتينومايستس فى مجال الأوراق وقابلية الصنف للاصابة بالفطر . بينما كانت هناك علاقة عكسية بين النسبة المئوية للفطريات فى المجال الورقى والقابلية للاصابة .

درست مجاميع الميكروبات فى المجال الورقى ووجد كثير من المجاميع البكتيرية سائدة فى جميع الأصناف مثل جنس باسلس ومجموعة ميكروكوكى ومجموعات من الميكروبات العصوية الغير ملونة . ولكن وجد مجموعات أخرى محددة كانت تصاحب صنف معين .

وجد أن الفطريات التابعة لجنسى الترناريا وبنسليوم كانت منتشرة فى المجال الورقى لكل الأصناف مع ظهور بعض أجناس معينة مرتبطة بأحد الأصناف دون الآخر . وجد أن بعض الميكروبات ذات التأثير المضاد للفطر ستمفيليوم بوتريوزم فى المجال الورقى لكل الأصناف وكانت درجة التضاد تختلف من صنف لآخر . وجدت أعلى درجة تضاد للبكتيريا على الصنف ن . ا . ٢٩ .

وجدت عزلتين من الفطريات فى المجال الورقى للصنف جيزة ٢ كانتا ذات نشاط عالى فى التضاد للفطر ستمفيليوم بوتريوزم . ومن ذلك يمكن أستنتاج أن ميكروبات المجال الورقى تلعب دورا كبيرا فى قابلية الأوراق للاصابة بالميكروبات المتطفلة .