Neth. J. Pl. Path. 78(1972) 89–98

The effect of pollen on the fungal leaf microflora of Beta vulgaris L. and on infection of leaves by Phoma betae

R. C. WARREN

Phytopathologisch Laboratorium 'Willie Commelin Scholten', Baarn

Accepted 14 December 1971

Abstract

Studies were made on the leaf-inhabiting fungus flora of two plots of flowering sugarbeet. Flowers were removed from plants of one plot as they appeared. Changes in the numbers of micro-organisms on leaves were recorded by leaf washing and leaf homogenization techniques, and were found to follow closely the changes in numbers of pollen grains seen on cleared leaf discs. The main colonizers, grouped into pink yeasts, white yeasts, *Cladosporium* spp. and *Aureobasidium pullulans*, were all influenced by the natural presence of pollen and reached high numbers on leaves in the plot bearing flowers. Rainfall was not found to influence numbers of pollen grains or micro-organisms greatly, but high temperature led to isolation of smaller numbers of pink and white yeasts. Recovery of populations was swift after hot weather, and may be linked with the high contamination of field-collected sugarbeet pollen by components of the microflora. The spectrum of fungi growing from washed leaf discs differed qualitatively from assessments made by washing and homogenization.

Rye pollen stimulates *Phoma betae* to aggressive infection of sugarbeet leaves on which, due to the prevention of a natural pollen deposit, the development of the leaf microflora is meagre. Sugarbeet plants in both plots were inoculated with conidia of *P. betae* with or without added rye pollen. The presence of a dense microflora, associated with a high natural pollen deposit, reduced the incidence of aggressive infection normally stimulated by the addition of rye pollen to inoculum.

Introduction

Studies by Last (1955), Kerling (1958), Dickinson (1967) and others have shown the presence of an active and changing fungal flora in the phyllosphere of living leaves. Fokkema (1968, 1971) has shown that pollen is important in influencing the numbers of these fungi on leaves of rye, an anemophilous plant, and strongly increases numbers of the four groups of saprophytic fungi distinguished as pink yeasts, white yeasts, *Aureobasidium pullulans* and *Cladosporium* spp.. Fokkema also found that pollen could enhance lesion production on rye leaves caused by *Helminthosporium sativum*, although this enhancement was lost if inoculations were made some time after pollen was shed naturally onto leaves, and when the saprophytic fungal leaf microflora had reached a high density.

This paper reports observations on numbers of pollen grains and of micro-organisms on leaves in two plots of sugarbeet, one of which was allowed to flower normally (plot A), while flower buds on plants in plot B were removed on appearance. Defined areas of leaf, bearing the natural microflora and pollen deposit, were inoculated with conidia of *Phoma betae* Frank. Water suspensions of conidia generally cause small

non-expanding necrotic flecks on healthy leaves of sugarbeet, but the addition of pollen to inoculum results in the production of expanding aggressive lesions such as those produced when pollen is added to *Botrytis cinerea* on strawberry and broad bean plants (Chou and Preece, 1968). Such fast growing lesions, which soon bear spores, are called aggressive.

Methods

Roots of sugarbeet (*Beta vulgaris* L. 'Kleinwanzleben E.') were planted at the end of April 1971 at Baarn, at intervals of 50 cm, in rows 50 cm apart. Two such plots, called A and B, each of 100 m^2, were planted at a distance of about 200 m apart. Leaves emerged during the second week of May and were sampled from May 18 until Sept. 3 inclusive, on the dates shown in Fig. 1. The first sample from leaves at 25 cm above soil level, was made by punching out discs of 7.0 mm diameter. Later samples were taken from leaves of equivalent height to those already marked by punching.

At each sampling date, two collections were made from each plot, each containing 50 discs from separate leaves. These 4 samples were each placed in a 100 ml round-bottomed flask containing 50 ml of 0.01 % Tween 80 solution and shaken for 3 hours at 5 °C. The foam was allowed to settle before appropriate dilutions were made to obtain about 20–50 colonies per plate from 0.1 ml aliquots on dry cherry agar in sterile plastic petri dishes. The remaining solution was decanted from the leaf discs which were rinsed twice with sterile distilled water before blotting dry on sterile filter paper. From each sample of 50 discs, 25 were placed onto sterile water agar, 5 to each plate. The remaining 25 were placed with 5 ml distilled water in a 2.5 cm diameter tube and homogenized for 30 seconds. The homogenizer blades were then washed in a further 5 ml water which was mixed with the homogenate before further dilutions were made and aliquots spread onto cherry agar. Plates were incubated at 25 °C in the dark until identification of the colonies was possible. Results were expressed as the number of colonies per cm^2 of leaf surface. Fungi not recognized at once were sub-cultured onto oatmeal agar slants and examined when fruiting structures appeared.

Furthermore at each sampling date, 25 discs of 12 mm diameter were taken from separate plants in each plot and bleached over 'Stardust' (Daft and Leben, 1966) before staining with aniline blue to aid identification and counting of sugarbeet pollen grains.

Cultures of *Phoma betae* Frank strain 523.66 were obtained from the Centraalbureau voor Schimmelcultures, Baarn, and maintained on potato carrot agar. Conidia were produced after 4 days incubation at 25 °C. When inoculations of field grown plants were made, plastic inoculation chambers lined with moist filter paper were placed over the plants which had their flowering shoots all uniformly removed to fit into the chambers. Separate droplets of 0.01 ml, each containing about 3000 conidia of *P. betae* were placed, 5 to each leaf, on 10 leaves of each of three plants in both plot A and plot B. To each set of 150 inoculations in each plot was added either an 0.01 ml doplet of water or a similar droplet containing pollen at 30 mg per ml. Each inoculation was covered with a moist filter paper disc of 1.1 cm^2 area, which remained moist for 72 hours. The results were assessed at 11 days after inoculation.

Rye pollen was used in inoculations as sugarbeet pollen collected in the field at Wageningen was seriously contaminated with organisms belonging to the four groups

mentioned above. However, the effect of pollen in stimulating infection appears to be independent of the origin of the pollen (Chou and Preece, 1968; Fokkema, 1971).

Measurements of mean daily temperature were taken from the records published by the Royal Dutch Meteorological Institute (KNMI) at De Bilt, 15 km from Baarn. Rainfall was measured daily on the laboratory roof using an automatic recording rain gauge.

Fig. 1. The effect of flower removal on numbers of pollen grains and micro-organisms, plotted logarithmically, on leaves of sugarbeet in 1971. Flowers were removed from plants in plot B until July 22; plants in plot A flowered normally. Mean daily temperature and rainfall are also recorded.

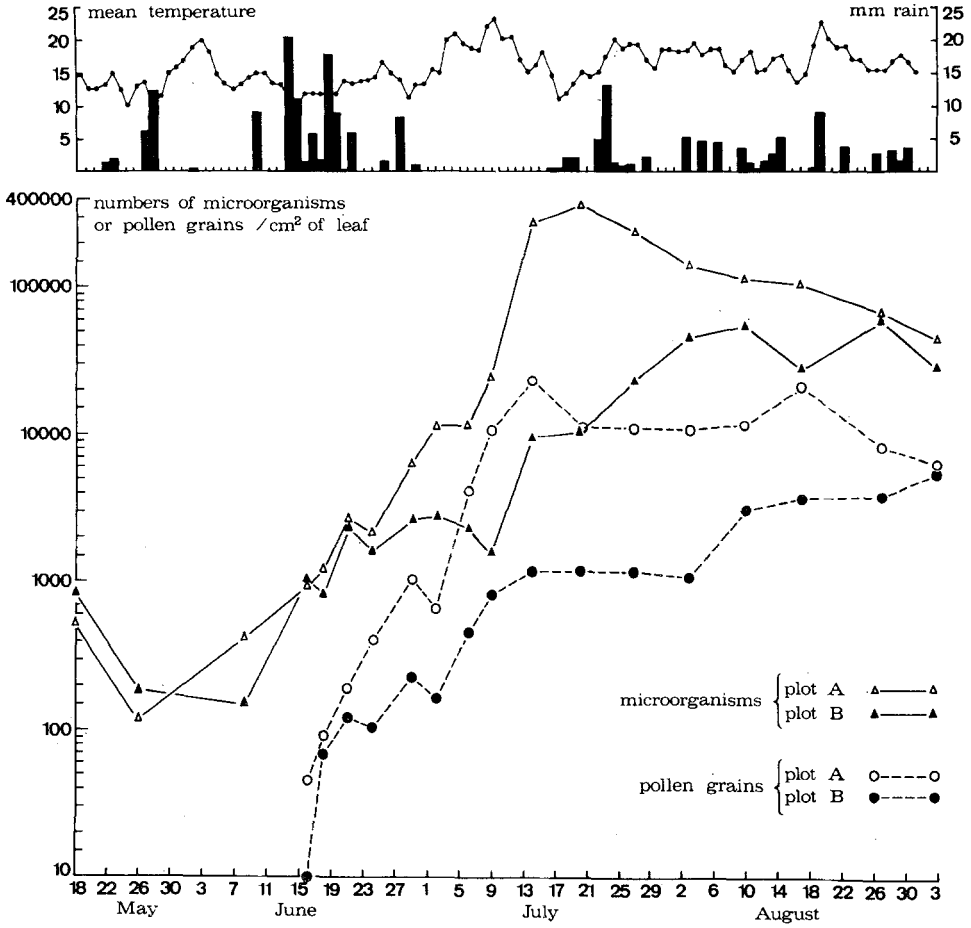

Fig. 1. Het effect van het verwijderen van bloemen op de aantallen stuifmeelkorrels en micro-organismen, logaritmisch uitgezet, op bladeren van suikerbiet in 1971. Van de planten in proefveld B werden tot 22 juli alle bloemen verwijderd; planten in proefveld A kwamen normaal in bloei. De gemiddelde dagtemperatuur en regenval zijn ook aangegeven.

Results

Assessment of pollen deposit

Flowering began during the second week of June, and although some background contaminant pollen of other plants was present before June 16, beet pollen was first observed on this date. The removal of flowers from plants in plot B did not completely exclude sugarbeet pollen from the leaves, but numbers of pollen grains rose more quickly on leaves in plot A than on leaves in plot B (Fig. 1). Numbers fell on the sample of July 2 in both plots, but thereafter continued to rise, reaching a maximum on leaves of plot A at the sample of July 14. After July 22, flowers were no longer removed from plants in plot B, and the pollen deposit increased thereafter. Increases were slower on leaves in plot B, but equivalent values to those recorded in plot A were finally reached on September 3. Rainfall between samples did not seriously reduce numbers of pollen grains seen on leaf discs and further increases in numbers occurred in spite of rain between August 3 and 17. Pollen was frequently seen in preparations as clumps of grains still attached to anthers, and in such cases only superficially visible grains were counted. The calculated estimates shown in Fig. 1, each the mean of 100 high power (0.2 mm^2) microscope fields, are therefore conservative values.

Assessment of micro-organisms

Numbers of micro-organisms per cm^2 of leaf of the four main groups of colonizers (pink yeasts, white yeasts, *Cladosporium* spp. and *Aureobasidium pullulans*) mentioned by Fokkema (1971) were recorded in Fig. 1. Each point in Fig. 1 represents the bulked total of numbers obtained from leaf washings and leaf homogenate. Until the appearance of pollen in the sample of June 16, numbers of micro-organisms isolated were low and began to increase on leaves in plot A at about one week after pollen was first recorded. Extensive rainfall was recorded before the samples of June 18 and 21 without large decreases in the numbers of micro-organisms. The samples taken on July 2, 6 and 9 were made during very hot weather with maximum temperatures of around 30 °C in the shade. Decreases in the numbers of pink and white yeasts were found in these samples. This is recorded as a net decrease in the total micro-organism count in plot B but in plot A, this decrease is masked in the samples of July 6 and July 9 by substantial increases in the numbers of *A. pullulans* colonies recovered (Table 1).

Within a week after the increase in pollen number on leaves in plot A, large increases in numbers of micro-organisms were recorded, the largest numbers being found in the pink and white yeast populations, although high numbers of *Cladosporium* spp. and *A. pullulans* were also found. Numbers of micro-organisms on leaves in plot B rose more slowly, but finally were similar in extent to figures obtained for plot A. (Table 1 also shows the numbers obtained for the occurrence of other fungi through the growing season).

Table 2 shows the importance of information obtained from homogenizing washed leaf tissue. When the large increases in total colony numbers were recorded on July 14 an increase was also found in leaf homogenates from plot A. The percentage of the total colony count at this date was low. Higher numbers and a high percentage value were obtained at the next sample on July 27. Thereafter both values remained high. In plot B, although the percentage of total colonies recovered rose at the same time as in plot A, the actual numbers remained relatively low until the end of sampling.

Table 1. Numbers of fungi per cm² recorded on sugarbeet leaf discs from plots A and B. The figures are the bulked totals from leaf washings and leaf homogenates.

Organism	Plot	Sampling date											
		18/5	16/6	21/6	2/7	6/7	9/7	14/7	20/7	27/7	3/8	10/8	17/8	27/8
Pink yeasts	A	20	340	1210	6000	3250	2500	152500	173700	98600	72700	32900	34000	25300
	B	–	400	1070	1350	760	100	6300	5800	15600	28200	15300	17400	21100
White yeasts	A	430	140	460	3500	330	1700	78500	122800	68100	41200	57000	32100	25100
	B	700	80	370	530	150	180	1400	2400	2300	4600	25500	4200	24400
Cladosporium spp.	A	–	250	480	560	600	400	18600	36700	61700	19100	15600	32000	15500
	B	60	280	760	500	500	200	900	1200	3100	5900	5700	2900	8100
A. pullulans	A	100	–	590	1500	7700	19700	30700	25500	11100	16200	12600	8000	2900
	B	110	–	280	550	900	1100	1200	1200	2500	6700	8900	4000	9700
Epicoccum purpurascens	A	–	–	–	–	–	–	50	500	–	–	60	60	390
	B	–	–	–	–	–	–	4	–	–	–	–	–	290
Altenaria spp.	A	–	–	20	–	6	–	30	300	330	–	880	30	320
	B	–	–	20	20	7	2	30	–	180	–	160	–	310
Mucor hiemalis	A	–	–	–	–	–	–	–	–	300	–	3250	310	160
	B	–	–	–	–	–	–	–	–	–	–	–	160	320
Acremonium spp.	A	–	–	–	–	–	–	–	–	–	–	–	670	80
	B	–	–	20	–	–	–	–	–	–	–	–	500	320
Apiospora montagnei	A	–	–	20	–	–	–	–	–	–	–	60	160	–
	B	–	–	–	–	–	–	–	–	–	–	–	30	–
Botrytis cinerea	A	–	–	–	80	130	–	–	–	–	–	–	–	–
	B	–	–	–	80	–	–	20	–	–	–	–	–	–
Tilletiopsis spp.	A	–	–	–	–	–	–	–	–	–	–	–	–	220
	B	–	–	–	–	–	–	–	–	–	–	–	–	130
Sterile forms	A	–	–	–	–	–	–	–	–	180	–	–	–	130
	B	–	–	30	–	–	–	4	–	60	250	–	–	130

Tabel 1. Aantal schimmels per cm² suikerbietbladschijf uit proefveld A en B. De getallen werden verkregen door steeds het totaal uit bladspoelwater verkregen aantal bij het totaal uit bladhomogenisaat verkregen aantal op te tellen.

Table 2. Percentage of total numbers per cm^2, and actual number of colonies which were obtained by leaf homogenization.

Sampling date	16/6	21/6	2/7	14/7	27/7	17/8	27/8
Plot A	120	370	1930	10200	59700	34300	23200
	12%	14%	17%	4%	25%	32%	33%
Plot B	190	390	220	900	7400	9000	22600
	18%	16%	8%	9%	31%	32%	36%

Tabel 2. Aantal per cm^2 en percentages van het totaal aantal kolonies dat uit bladhomogenisaat werd verkregen.

Table 3 reports the figures obtained from observing the fungal colonies growing from washed leaf discs incubated on water agar. As 50 discs were used per sample, the number of discs bearing one particular fungus has been doubled to express the incidence as a percentage. Substantial differences were not obtained between discs from the two plots, which showed similar figures for the incidence of the yeasts, *A. pullulans*, *Cladosporium* spp. and *Alternaria* spp. The appearance of *Fusarium tabacinum* (Gams and Gerlagh, 1968) in both plots was notable at the end of sampling as this fungus was rarely isolated from leaf washings or homogenate. Many other fungi were identified, but their incidence was so low that it was considered unnecessary to list them.

Leaves from various weed species were also sampled in each plot on July 9 in the manner described. Pollen present was not assessed, and the numbers of micro-organisms recovered were 46300/cm^2 in plot A and 2860/cm^2 in plot B. The percentage of these totals found by homogenization were 3% and 4% respectively for plot A and B.

Leaves were also sampled from beet plants bearing symptoms of beet yellows virus. The figures obtained for numbers of micro-organisms isolated from such leaves on two occasions did not differ from those made on normal leaves at the same time. On both weed leaves and leaves bearing symptoms of beet yellows virus, the organisms recovered belonged to the four main groups already mentioned, and occurred in similar ratios to their occurrence on normal leaves.

Inoculations with Phoma betae

Inoculations were made on July 14 as described and the numbers of expanding lesions were counted on July 25. The results are shown in Table 4. Similar results were obtained by inoculation of leaves detached from plants in the two plots and maintained in humid chambers in the glasshouse with their petioles dipping into tap water. It is clear that the addition of rye pollen to the inoculum on leaves of plot A did not significantly increase the number of aggressive lesions that developed. On leaves in plot B, however, aggressive lesions developed on the majority of inoculated spots which were supplemented with pollen.

Discussion

The development of a fungal microflora on leaves of sugarbeet (Fig. 1) shows close similarities to the results with rye (Fokkema, 1971), both in the kinds of micro-orga-

Table 3. Percentage incidence of fungi seen on washed leaf discs incubated on water agar.

Organism	Plot	Sampling date									
		16/6	21/6	2/7	14/7	20/7	27/7	3/8	10/8	17/8	27/8
Yeasts	A	22	50	64	74	72	76	90	92	90	94
	B	24	28	74	68	84	80	86	96	96	100
A. pullulans	A	4	46	12	2	6	–	–	2	4	–
	B	20	34	12	6	14	–	4	4	4	–
Cladosporium spp.	A	68	94	66	84	88	94	90	98	92	96
	B	86	92	86	86	94	86	92	100	100	100
Alternaria spp.	A	20	12	48	88	98	96	100	98	100	100
	B	8	18	38	74	82	96	96	100	100	100
Epicoccum purpurascens	A	–	10	10	8	–	14	22	2	16	6
	B	2	2	10	16	4	12	2	6	22	–
Fusarium tabacinum	A	–	–	–	–	–	–	14	12	40	22
	B	–	–	–	–	–	–	2	16	24	52
Phomopsis sp.	A	10	22	–	–	–	–	–	–	–	–
	B	6	–	–	–	–	–	–	–	–	–
Botrytis cinerea	A	2	2	–	–	–	–	–	–	–	–
	B	–	–	–	–	4	–	–	–	–	–
Apiospora montagnei	A	2	–	–	2	–	6	–	–	2	–
	B	–	–	–	–	–	2	–	–	–	–
Stemphylium botryosum	A	4	2	2	16	6	8	–	6	–	8
	B	–	–	6	14	6	2	2	4	2	–
Fusarium spp.	A	–	–	–	–	–	2	–	2	–	6
	B	–	–	2	–	2	–	–	4	–	–
Acremonium spp.	A	–	–	–	–	–	4	–	2	–	–
	B	–	–	–	–	–	–	–	–	44	–
Mucor hiemalis	A	–	–	–	–	–	2	–	–	–	2
	B	–	–	–	–	–	–	2	–	–	–
Verticillium spp.	A	–	–	–	–	2	–	10	–	34	12
	B	–	–	–	–	–	–	18	–	14	18
Sterile forms	A	12	–	2	2	–	–	–	–	–	–
	B	6	2	10	–	2	–	8	–	–	–

Tabel 3. Procentueel voorkomen van schimmels, microscopisch waargenomen op gewassen bladschijven, die op water-agar werden geïncubeerd.

nism reported most frequently, and in the way these fungi were influenced by the presence of pollen on the leaves. The results do not compare closely with those of Kerling (1958) who worked with fodder beet which was not flowering. Numbers of micro-organisms recorded in her work did not exceed 5000 per cm^2 at any sample, resembling records made before flowering on sugarbeet. Other fungi did not appear to be influenced by the presence of pollen as judged by the numbers of species isolated or by the numbers of propagules found. However, the figures listed are not too reliable for any fungus apart from those comprising the main four groups as the dilutions needed to count the large numbers of those occurring most frequently were very high. In general, changes in the numbers of micro-organisms isolated by leaf washings and homogenization lagged behind changes in the numbers of pollen grains by about one

Table 4. The number of aggressive lesions developing from 75 inoculations on sugarbeet leaves with differing natural pollen deposits, at 11 days after inoculation with conidia of *P. betae* with or without added rye pollen.

	Number of micro-organisms per cm^2 leaf	Number* of aggressive lesions after inoculation with	
		conidia	conidia + pollen
Plot A (flowering)	280,500	2 (3%)	4 (5%)
Plot B (flowers removed)	9,700	4 (5%)	66 (88%)

*Percentage figures in parenthesis

Tabel 4. Het optreden van agressieve lesies op suikerbietbladeren met verschillende concentraties langs natuurlijke weg afgezet stuifmeel, 11 dagen na inoculatie met conidiën van P. betae waaraan al of geen rogge stuifmeel was toegevoegd.

week (Fig. 1). This is more noticeable in the figures obtained from leaves of plot A. Rainfall was not found to affect numbers of micro-organisms or pollen grains greatly, unlike the results of Fokkema (1971) who worked with rye. This difference in results may relate to the different pattern of pollen release in the two plants. In rye, pollen is released within a few days, while pollen release in sugarbeet can last for 5 weeks, (Scott, 1970). A deposit of rye pollen could be more easily affected by rain, either by leaching when on the leaves or by washing out of the air after anthesis.

The effect of high temperatures on the populations of pink and white yeasts is similar to that recorded on rye leaves and tested by Fokkema (1971). It is noticeable that *A. pullulans* was less affected by high temperature than were the pink yeasts, white yeasts and *Cladosporium* spp.. This may point to a closer relation of this fungus to the leaf than for fungi of the other groups. Recently, Pugh and Buckley (1971) have shown that *A. pullulans* probably lives inside leaves of *Acer* (sycamore). The remarkable increase in numbers of micro-organisms following the hot spell at the beginning of July, during which many of the yeasts may have been killed, suggests that a heavy source for reinoculation was available nearby. Since field-collected sugarbeet pollen was heavily contaminated with the four main groups of fungi, it is likely that this rough-surfaced pollen is the source of the large increase in numbers of micro-organisms found in the sample of July 14.

The importance of the figures obtained from leaf homogenization is seen, particularly at and after the sample made on July 14 (Table 2). The great increase in numbers of micro-organisms isolated by washing preceeds the increase in the percentage of the total found in homogenates. This suggests that, at an early stage, micro-organisms are loosely seated, while they are later more firmly attached or may even penetrate the cuticle. The high percentage of the total isolated by homogenization after July 27 stresses the importance of this method of assessment in conjunction with leaf washings.

The technique of plating-washed leaf discs (Table 3) gave a different impression of the leaf microflora to the other methods mentioned. No major difference was found between leaf samples from the two plots. *A. pullulans* was rarely seen on discs and was probably overgrown by other fungi. The incidence of yeasts is probably underesti-

mated by this method. The observations on *Alternaria* spp. deviate most from other methods of assessment, and may reflect the preference of this genus for colonizing detached leaves to living leaves.

Inoculations with a pathogenic fungus were made when great differences in numbers of micro-organisms and pollen were apparent between leaves in plot A and plot B. It is probable that the dense microflora present on leaves in plot A interfered with the development of *Phoma betae* on such leaves even when extra pollen was added to the inoculum. The presence of a relatively sparse microflora, composed of fungi from the same four groups, in plot B may explain why aggressive lesions could develop from 88% of inoculations made with added rye pollen. The influence of the natural microflora, or components of it, on infection has been studied by van den Heuvel (1969), McBride (1969) and Fokkema (1971) all of whom conclude that such components of the microflora may have an inhibitory effect on the development of infection by certain pathogens. The value of a natural microflora as a barrier to infection and spread by fungal pathogens deserves further study, particularly in relation to the use of fungicides.

The complex relationship between the phyllosphere microflora, pollen and infection by *P. betae* has been followed up using glasshouse-grown plants, and the results will be reported elsewhere.

Samenvatting

De invloed van stuifmeel op de schimmelflora van suikerbietbladeren en op de infectie door Phoma betae

De bladmicroflora van bloeiende suikerbietplanten werd bestudeerd in twee proefvelden. Van de planten van één van beide proefvelden werden de bloemen vóór de bloei verwijderd. Veranderingen in aantallen micro-organismen op de bladeren, geregistreerd door uitplaten van bladspoelwater en bladhomogenisaat (Fig. 1, Tabel 1), vertoonden een nauwe correlatie met veranderingen in aantallen stuifmeelkorrels waargenomen op opgehelderde bladschijven. De voornaamste blad-koloniserende micro-organismen, gegroepeerd in rode gisten, witte gisten, *Cladosporium* spp. en *Aureobasidium pullulans*, werden in hoge mate beïnvloed door de aanwezigheid van stuifmeel. Hun aantallen waren zeer hoog op bladeren van bloeiende planten. Regen bleek weinig invloed te hebben op aantallen stuifmeelkorrels en micro-organismen op het blad. Hoge luchttemperatuur gaf echter duidelijk minder rode en witte gisten. Na een periode van warm weer herstelden de populaties zich snel, hetgeen waarschijnlijk verband houdt met de grote verontreiniging van in het veld verzameld bietenstuifmeel met elementen van de bladmicroflora. De microscopisch waargenomen aanwezigheid van schimmels op gewassen bladschijfjes verschilt kwalitatief van die bepaald met bladspoel- en bladhomogenisatie-technieken (Tabel 3).

Roggestuifmeel stimuleert *Phoma betae* tot agressieve infectie van bladeren van bieteplanten waarop de ontwikkeling van de bladmicroflora, door het voorkómen van de natuurlijke stuifmeelafzetting, beperkt is gebleven. Door suikerbietplanten in beide proefveldjes te inoculeren met al of niet met stuifmeel vermengde conidiën van *P. betae*, werd aangetoond dat deze stimulatie door aanwezigheid van een door stuifmeelafzetting gestimuleerde microflora wordt gereduceerd (Tabel 4).

Acknowledgments

The author is grateful for a grant from the Royal Society under the European Science Exchange Programme. Thanks are also due to the staff (and foundation) of the Phytopathologisch Laboratorium 'Willie Commelin Scholten' for providing facilities for this work, and especial thanks for encouragement and help from Dr N. J. Fokkema and Professor K. Verhoeff.

Thanks are also expressed to Dr K. W. Gams and Mr H. A. van der Aa at the Centraalbureau voor Schimmelcultures, Baarn, for identification of many cultures.

References

Chou, M. Chu & Preece, T. F., 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Ann. appl. Biol. 62:11–22.

Daft, G. C. & Leben, C., 1966. A method for bleaching leaves for microscope investigation of microflora on the leaf surface. Pl. Dis. Reptr 50:493.

Dickinson, C. H., 1967. Fungal colonization of *Pisum* leaves. Can. J. Bot. 45:915–927.

Fokkema, N. J., 1968. The influence of pollen on the development of *Cladosporium herbarum* in the phyllosphere of rye. Neth. J. Pl. Path. 74:159–165.

Fokkema, N. J., 1971. The effect of pollen in the phyllosphere of rye on colonization by saprophytic fungi and on infection by *Helminthosporium sativum* and other leaf pathogens. Diss. Amsterdam. Meded. Phytopath. Lab. 'Willie Commelin Scholten' 87, 60 pp. Neth. J. Pl. Path. 77(1971) Suppl. 1.

Gams, W. & Gerlagh, M., 1968. Beiträge zur Systematik und Biologie von *Plectosphaerella cucumeris* und der zugehörigen Konidienform. Persoonia 5:177–188.

Heuvel, J. van den, 1969. Effect of *Aureobasidium pullulans* on numbers of lesions on dwarf bean leaves caused by *Alternaria zinniae*. Neth. J. Pl. Path. 75:300–307.

Kerling, L. C. P., 1958. De microflora op het blad van *Beta vulgaris* L. Tijdschr. PlZiekt. 64:402–410.

Last, F. T., 1955. Seasonal incidence of *Sporobolomyces* on cereal leaves. Trans. Br. mycol. Soc. 38: 221–239.

McBride, R. P., 1969. A microbiological control of *Melampsora medusae*. Can. J. Bot. 47:711–715.

Pugh, G. J. F. & Buckley, N. G., 1971. *Aureobasidium pullulans*: an endophyte in sycamore and other trees. Trans. Br. mycol. Soc. 57:227–231.

Scott, R. K., 1970. The effect of weather on the concentration of pollen within sugarbeet crops. Ann. appl. Biol. 66:119–127.

Present address

Institute of Tree Biology, c/o Dept. of Forestry & Natural Resources, Kings Buildings, Edinburgh EH9 3JU, Scotland