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# Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vulgaris*)

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**Abstract**

Bacteria, yeasts and filamentous fungi colonizing immature, mature and senescing primary leaves of field grown *Beta vulgaris* (sugar beet) were analysed over a complete growing season. Greatest microbial numbers were detected on senescing primary leaves and these numbers increased over most of the season. The number of colonizers detected on mature leaves was found to be stable over most of the study.

Filamentous fungi and yeasts were identified to the genus level and the communities found to have greatest diversity during the summer months. There was no consistent pattern of diversity according to leaf type. Two genera of filamentous fungi, *Cladosporium* and *Alternaria* and two yeast genera, *Cryptococcus* and *Sporobolomyces* were the most numerous fungal populations isolated. Only 8 filamentous fungi and 3 yeast genera were commonly isolated on PDA (potato dextrose agar).

Bacterial strains (1236) were isolated on Tryptic Soy Broth (TSB) agar and identified to species, or in some cases sub-species level, by analysis of their fatty acid methyl ester (FAME) profiles. Isolated bacteria were grouped into 78 named and 37 unnamed species clusters. Greatest number of bacterial species were isolated from young plants and leaves, sampled during the autumn months. Bacterial community diversity was lowest in mid-summer and winter months. *Pseudomonas* was the most commonly isolated genus and *Erwinia herbicola* the most common species. *P. aureofaciens* was the only species isolated from soil that was also isolated from the phyllosphere of *B. vulgaris* throughout the season.

## Introduction

The leaf is a habitat exposed to acute environmental fluctuations. Frequent, and at times catastrophic disturbances, such as extremes of temperature, moisture and solar radiation are common features of the phyllosphere (Cullen and Andrews, 1984). The selective nature of the phyllosphere has a marked influence on microbial numbers. Microbial counts on an array of plant species are known to fluctuate markedly over the growing season (Lindow et al., 1978; Mew and Kennedy, 1982). This can lead to significant variation in microbial counts both

spatially and temporally, between and within plants (Hirano and Upper, 1986). Environmental fluctuations in the phyllosphere may also influence the composition of colonizing microbial communities. Often only a few species dominate at any one time and in some instances characteristic populations have been isolated at specific times of the season (Dickinson et al., 1975; Ercolani, 1991). Ercolani (1978) studied phyllosphere bacteria of field grown *Olea europaea* over three growing seasons and observed distinctive populations colonizing leaves at different times of the year. For instance, *Pseudomonas fluorescens* was detected only in the winter months and *Serratia marcescens* was only isolated from samples taken in July. Thus, despite the fluctuating environmental conditions in the phyllosphere, in some plant species distinctive patterns of microbial colonization have been reported over successive years.

Microbial studies of natural habitats such as the phyllosphere have been limited by difficulties involved in identifying large numbers of microorganisms. Numerical taxonomic analysis has been successfully used to study fluctuations in bacterial communities from a range of plant species (Austin et al., 1978; Ercolani, 1978; Goodfellow et al., 1976). This approach is, however labour intensive and reliant on organisms being reactive to diagnostic tests. Chemotaxonomic techniques such as analysis of whole cell protein patterns (Lambert et al., 1990) or fatty acid profiles, which group organisms by chemical composition, are not limited by biochemical inactivity of isolates (Collins et al., 1982; Minnikin and Goodfellow, 1980). Gas liquid chromatography of bacterial fatty acid methyl esters (FAME) has been extensively used in clinical and phytopathological microbiology as either a primary or adjunctive means of identifying bacteria (De Boer and Sasser, 1986; Dees et al., 1981; Stead, 1988). Thompson et al. (1993) recently demonstrated that analysis of bacterial FAME profiles, using a semi-automated procedure (Microbial Identification System; Miller, 1984) was a rapid and accurate method of identifying bacteria from *Beta vulgaris* (sugar beet). Furthermore, they found that its sensitivity enabled differentiation of isolates of the same species on the basis of when and where they were isolated.

The work described in this paper forms the basis of several studies aimed at investigating the microbial ecology of *B. vulgaris* in one field site. In order to design statistically significant studies aimed at determining the sensitivity of microbial communities to perturbation, a base line understanding of variation and fluctuations within the community is required. The aims of this study were: (1) to follow the seasonal quantitative and qualitative fluctuations in the filamentous fungi, yeast and bacterial communities on *B. vulgaris* over a complete growing season; (2) to determine the variability in the numbers of organisms isolated from replicated leaf samples; (3) to select a suitable candidate for genetic modification and subsequent field release.

## Materials and methods

### *Field site*

Sugar beet (*B. vulgaris* var. Amethyst) was sown in a 15-m by 15-m plot of previously fallow land in mid April 1990, at the University Field Station, Wytham, Oxford. The seed was commercially pelleted [EB3 pellets, Germains (UK) Ltd, Kings Lynn, UK] incorporating aqueous Thiram solution (Agrichem Flowable Thiram), Tachigaren (hymexazol) and Mesurol (methiocarb). The soil was classified as a heavy clay (sand 24.88%, silt 21.60% and clay 53.52%), pH 7.7, with an organic content of 8.57%. Seeds were sown approximately 15 cm apart to a depth of 3 cm. The soil was irrigated daily until germination (day 14) and the crop was sprayed with herbicide (Goltex, Bayer) on day 50 and insecticide (Gammacol, ICI) on day 100.

### *Soil and crop sampling*

On the day of sowing (April 1990) 30 g of soil from three randomly selected parts of the plot, were placed into self-sealing bags. On days 15 and 52 three plants were randomly selected, all the leaves removed and placed into separate bags. After day 52, plants were sufficiently differentiated to be divided into three separate

leaf types; (i) primary leaves, that were in various states of senescence, (ii) mature, fully expanded leaves and, (iii) immature, growing leaves. Between three to ten leaves (depending on size) of each leaf type, primary senescing, mature and immature, were taken from three plants and each group of leaves treated separately (3 leaf types from 3 plants, i.e. 9 leaf bulks per sampling occasion). Samples were taken 15 (May), 52 (June), 79 (July), 114 (August), 148 (September), 183 (November), 220 (December) and 272 (January) days from sowing. All samples were analysed on the day of collection. Leaf and soil samples were dried to 80°C and 105°C respectively to determine dry weight.

### *Microbial sampling and identification*

Soils samples (5 g) taken on the day of sowing were placed into 500 mL of quarter strength Ringer solution (Oxoid) and mixed with a magnetic stirrer bar, for 30 minutes at maximum speed. The phyllosphere microbial community, including the epiphytic (phylloplane) and endophyte colonizers were sampled by homogenising whole leaf samples. Weighed leaf material (10 g wet material, 1 g wet weight of *B. vulgaris* leaf material was determined to be equivalent to a surface area of approximately 115 $cm^2$ of leaf) was placed into a 1 litre Waring blender with 200 mL quarter strength Ringer solution and homogenised at maximum speed for 1 minute. Samples of the resulting suspensions (soil or leaf) were serially diluted and plated on Potato Dextrose Agar (PDA, Oxoid) containing 320 mg $L^{-1}$ aureomycin (Cyanamid) for isolation of filamentous fungi and yeasts and 3% Tryptic Soya Broth (TSB) Agar (Difco) with 50 mg $L^{-1}$ cycloheximide (Sigma) for isolation of bacteria. Fungal isolation plates were incubated at 15°C for 10 days and bacterial isolation plates for 4 days at the same temperature. After incubation the number of colony forming units isolated on triplicate dilution plates with between 20 and 200 colonies were determined. These plates were marked into sections containing 20 to 30 colonies. One section was randomly selected per plate and every colony it contained was picked off and purified. In total, 50 bacterial colonies were selected from TSB agar plated with primary senescing, mature and immature leaves (150 per sampling occasion). The number of filamentous fungi and yeasts detectable in soil samples was not determined. One hundred and fifty filamentous fungi/yeasts were selected from isolation plates in the same manner as described for the bacterial community. All colonies were transferred to the original isolation media for purification. Filamentous fungi and yeast isolates were treated as part of the same community since these originated from the same isolation plates. These were identified to genus level by microscopic analysis of colonies.

Bacterial isolates were identified using the Microbial Identification Systems (MIS-Microbial ID, Inc., Newark, Delaware, USA). Harvested cells were saponified and the fatty acid component extracted after methylation. Fatty acid methyl esters (FAME) were analysed using Gas-Liquid Chromatography (Hewlett Packard 5890A GC with a 25 m × 0.2 mm methyl phenyl silicone fused capillary). Extraction and chromatographic procedures were as described in the MIS instruction manual (Miller, 1984). FAME peaks were named by the MIS Library Generation Software 'Aerobe Library' (Revised version 3.3). FAME profiles were compared with library entries using a pattern recognition algorithm that named and provided a similarity indices for the species and subspecies most similar to the unknown isolate.

### *Reproducibility of fatty acid analysis*

The reproducibility of the chromatographic technique was regularly checked, every 11 samples, with the calibration standard mixtures (MIS, Newark, Delaware). Reproducibility of FAME profiles were determined by repeated analysis of one *Pseudomonas aureofaciens*, originally isolated from *B. vulgaris*. This strain was included as a standard in every group of 30 to 60 isolates analysed.

### *Meteorological data*

Rainfall and temperature were monitored throughout the growing season, 500 m from the site. These data are expressed as average weekly

rainfall and average weekly temperature taken at 4 am and 4 pm.

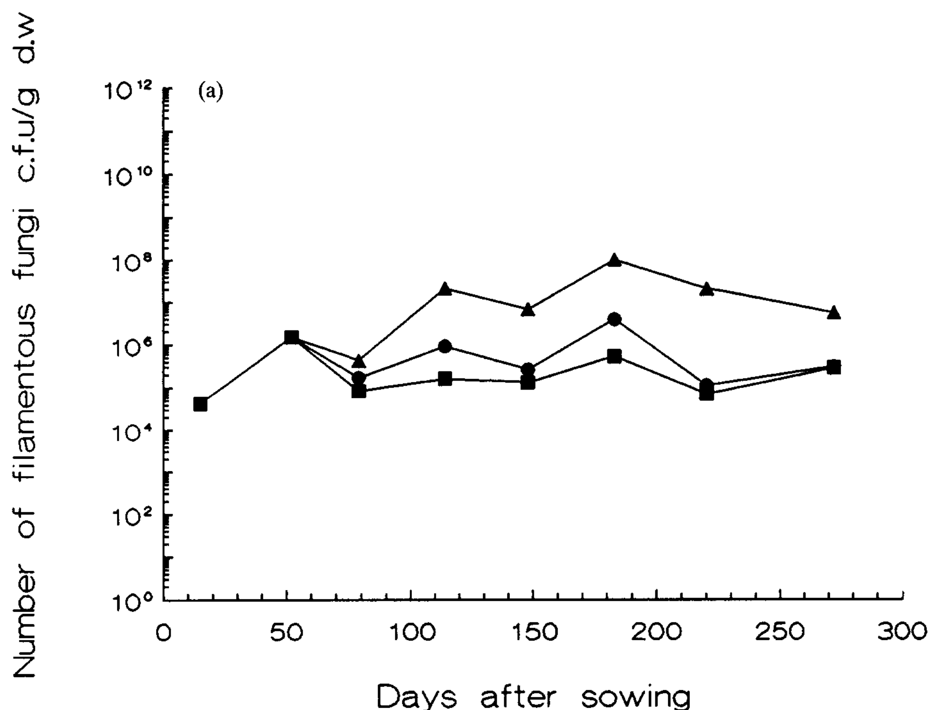
## Results

### *Quantitative changes in phyllosphere microbial communities*

The number of colony forming units (c.f.u.) of filamentous fungi, yeasts and bacteria isolated per gram dry weight of leaf are shown in Figures 1a to c.. Statistical analysis of these data were undertaken in two stages. A simple analysis of variance (ANOVA) was performed on $\log_{10}$ transformed data of colony forming units for each community and each sampling occasion. The range of standard errors within each community was limited: standard error of the difference (s.e.d) between two means ranged from 0.090 to 0.138, 0.090 to 0.217, and 0.058 to 0.293, respectively, for the filamentous fungi, yeast and bacterial communities.

With such limited heterogeneity of variance it was deemed acceptable to determine a combined ANOVA across sampling occasions for each community. This analysis revealed a difference in microbial numbers on all three leaf types over the season ($p < 0.001$). It also revealed a significant interaction between leaf type and microbial numbers ($p < 0.001$). From day 114, to the end of the study significantly greater numbers of colony forming units of filamentous fungi and yeasts were detected on senescing compared to mature and immature leaves ($p < 0.001$). Both communities showed a gradual increase in numbers on senescing primary leaves from days 79 to 183 and thereafter declined. Numbers of filamentous fungi and yeasts on mature and immature leaves showed little fluctuation over the season.

The pattern of colonization observed in the bacterial community on all three leaf types studied was similar to that of the communities of filamentous fungi and yeasts, although the magnitude of these fluctuations was greater for bacteria. From day 114 to the end of the study, significantly greater numbers of bacteria were



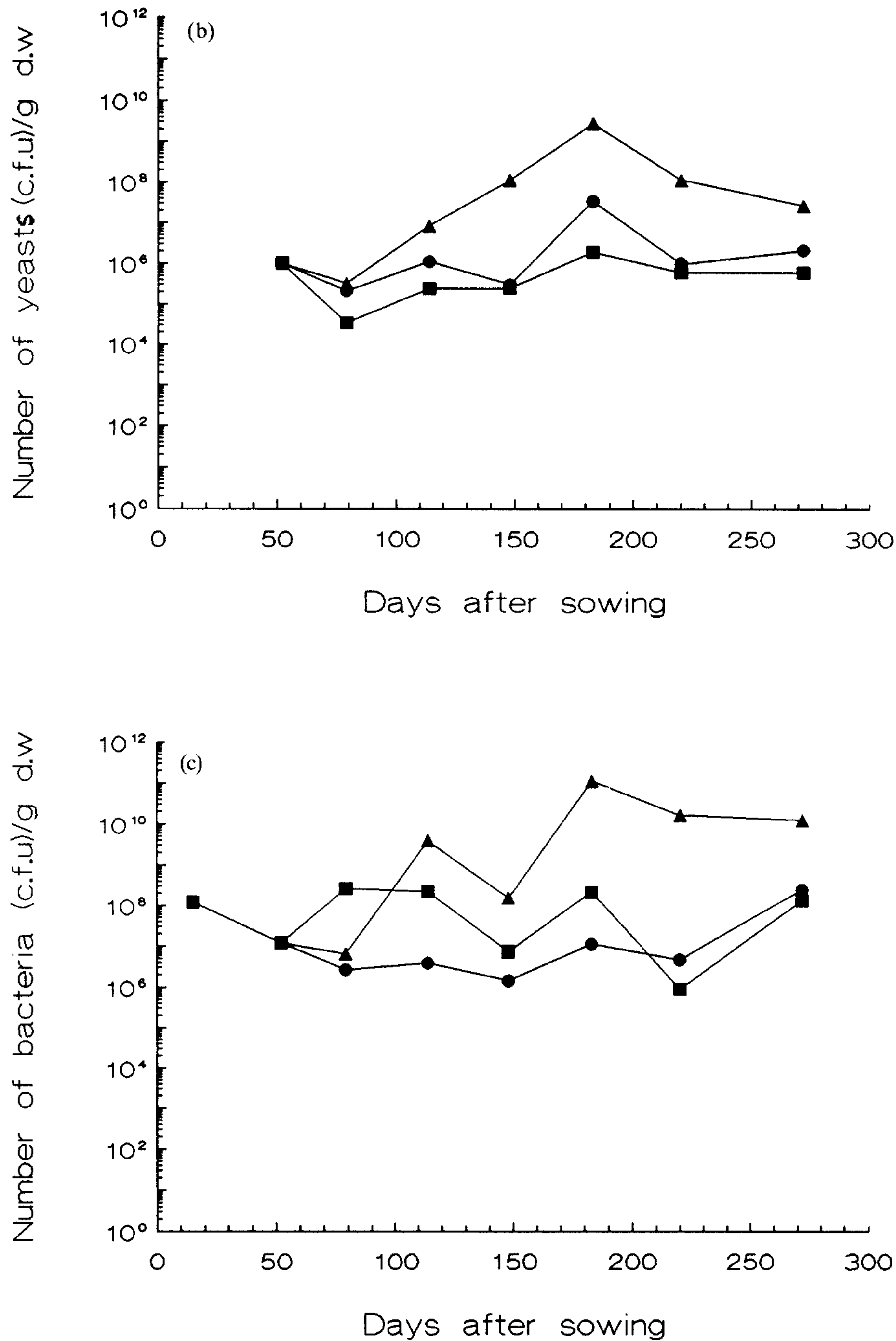



*Fig. 1.* Number of colony forming units of filamentous fungi (**a**), yeasts (**b**) and bacteria (c) isolated from the phyllosphere of senescent primary (▲), mature (●) and immature (■) *B. vulgaris* leaves.

detected on senescing primary leaves than on mature or immature leaves ($p < 0.001$). With the exception of the number of bacteria counted on senescing primary leaves on day 148, the community increased in numbers from day 79 to 183 and then declined. Bacterial numbers on mature

leaves were more stable than those observed on senescing primary leaves. Between days 52 and 220 bacterial numbers showed little significant fluctuation. Over most of the season the numbers of bacteria colonizing immature leaves also showed less fluctuation than on senescing primary leaves. The exceptions being on days 148 and 220 which had lower numbers of detectable bacteria than observed over most of the season.

*Qualitative changes in the phyllosphere microbial communities*

*Filamentous fungi and yeasts*

The identity and distribution of filamentous fungi and yeast populations isolated (620 filamentous fungi and 581 yeasts) from the three leaf types studied over one growing season are shown in Table 1. Over seventy-six percent of the isolates were identified to be members of just two filamentous fungi genera, *Cladosporium* and *Alternaria*, and two yeast genera *Cryptococcus* and *Sporobolomyces*. Only two fungal genera, *Fusarium* and *Mucor*, were isolated from 15 day old plants.

Figure 2 shows the number of filamentous fungi and yeast genera isolated from each leaf type on each sampling occasion. Taking all leaf types together, the greatest number of genera were isolated mid-season (days 114 and 148, 12 genera). There was no consistent difference between numbers of genera isolated and leaf type.

*Bacteria*

FAME profiles of 1236 bacterial isolates were analysed by the MIS, the results of this are shown in Table 2. Within each batch analysed, at least one FAME extract of the standard *P. aureofaciens* was included. This strain was identified correctly on every occasion, at the similarity index level (Gower, 1966) of 0.812 or more. FAME profiles were found to be highly reproducible. The coefficient of variation (standard deviation/mean × 100) for fatty acids representing more than 3.17% of the standard strains total content was <13.1% (Mukwaya and Welch, 1989).

Members of 78 named species from 34 named genera were isolated. Nineteen percent of samples (235) did not match any entries in the MIS Aerobic Bacteria library and consequently were unnamed. These isolates were clustered according to their FAME profiles and grouped into 12 separate, unnamed genera and 37 species clusters, containing between 1 and 97 isolates (<10% of any sampled community). Most unnamed isolates bore some distinct relationship to recognised genera, indicating that they were representatives of species not present on the MIS Aerobic Bacteria library, which was derived from analyses of bacteria from culture collections. Unnamed isolates were clustered and prefixed by 'No Match'. Four clusters did not group with any genus, so were considered to be members of genera unrepresented in the library. Members of one group, 'No Match 37', were identified by biochemical and morphological examination to be coryneform bacteria, represented the third largest population isolated from all phyllosphere samples.

*Pseudomonas* species (13 named, 7 unnamed) represented 30% (371) of the bacteria population isolated (Table 2) of which *P. aureofaciens* (119, 9.6%), *P. syringae* (77, 6.2%) and No Match SBW 4 (34, 2.7%) were the commonest. *P. aureofaciens* represented the largest single pseudomonad population isolated. *Erwinia herbicola* was the single most commonly named species isolated, representing 11.2% (139) of the bacteria isolated. Gram positive bacteria were also isolated in large quantities, representing over one third (37.7%) of isolates. *Arthrobacter oxydans* (60, 4.8%), *Micrococcus roseus* (36, 2.9%) and *Microbacterium lacticum* (35, 2.8%) were the most commonly isolated Gram positive species isolated, however No Match SBW 37 (94, 7.6%) represented the single largest Gram positive population.

The number of bacterial species isolated from each leaf type on each sampling is shown in Figure 3. There was no consistent difference in the number of species isolated from each leaf type throughout the season. When the total number of species isolated from all leaf types at each sampling was determined, species diversity was lowest mid-season (day 79, 15 species) and highest early (day 15, 29 species) and late in the season (day 220, 38 species).

Only one species, *P. aureofaciens* isolated

*Table 1.* Percentage composition of filamentous fungi and yeast populations isolated (50 colony forming units per sampling occasion from each leaf type) from the phyllosphere of *B. vulgaris*

| Fungi and yeast genera | Day 15 | Day 52 | Day 79 | | | Day 114 | | | Day 148 | | | Day 183 | | | Day 220 | | | Day 272 | | | % Total population |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | |
| **Fungi** | | | | | | | | | | | | | | | | | | | | | |
| *Cladosporium* | – | 36 | 51 | 48 | 76 | 16 | 24 | 24 | 16 | 46 | 51 | 20 | 30 | 58 | 7.0 | 42 | 56 | 28 | 18 | 24 | 33.3 |
| *Alternaria* | – | 7.1 | 38 | 18 | 2.9 | 32 | 11 | 3.4 | 11 | 21 | – | 10 | 6.0 | – | – | 2.0 | 2.0 | 6.0 | – | – | 9.2 |
| *Acremonium* | – | – | – | – | – | 21 | – | – | 5.5 | – | 5.1 | 20 | – | – | 25 | 8.0 | 12 | 12 | 14 | 14 | 6.8 |
| *Mucor* | 75 | – | – | 2.0 | 8.8 | – | 4.5 | – | – | 5.0 | 1.3 | – | – | – | – | – | – | – | – | – | 4.8 |
| *Aureobasidium* | – | 3.5 | 5 | 2.0 | 8.8 | 5.2 | 7.5 | – | – | – | 2.5 | – | 2.0 | – | – | – | – | – | – | 2.0 | 1.9 |
| *Fusarium* | 25 | 1.0 | – | 2.0 | 2.9 | 5.0 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 1.8 |
| *Penicillium* | – | – | – | 2.0 | – | – | 13 | 10 | 2.0 | 1.7 | – | – | – | 2.0 | – | – | 2.0 | – | – | – | 1.6 |
| *Asperigillus* | – | – | 2.6 | 2.0 | – | – | 11 | 7 | – | 1.7 | 7.6 | – | – | – | – | – | – | – | – | – | 1.6 |
| **Yeast** | | | | | | | | | | | | | | | | | | | | | |
| *Cryptococcus* | – | 3.5 | 2.6 | 15 | – | 11 | 18 | 45 | 36 | 12 | – | 43 | 44 | 23 | 64 | 22 | 16 | 40 | 56 | 50 | 25.0 |
| *Sporobolomyces* | – | 47 | – | 2.0 | – | – | 6.0 | 10 | 24 | 8.6 | 26 | 2.0 | 15 | 10 | 2.0 | 10 | – | 4.0 | 4.0 | – | 8.5 |
| *Rhodotorula* | – | – | – | – | – | – | – | – | 5.5 | – | – | 2.0 | 2.0 | 2.0 | 2.0 | 4.0 | 8.0 | 2.0 | 2.0 | 8.0 | 1.9 |
| **Others** (less than 1% of the total)[a] | – | 1.9 | 0.8 | 7.0 | 0.6 | 9.8 | 5.0 | 0.6 | – | 4.0 | 6.5 | 3.0 | 1.0 | 5.0 | – | 12 | 4.0 | 8.0 | 6.0 | 2.0 | 3.6 |

*Key*: 1°-senescent primary leaf, 3°-mature leaf, 5°-immature leaf.
[a] Includes *Rhizopus*, *Botrytis*, *Stemphylium*, *Phoma*, *Epicoccum*, *Trichosporium* and unknowns.

*Table 2.* Percentage composition of bacterial populations isolated (50 colony forming units per sampling occasion from each leaf type) from the phyllosphere of *B. vulgaris*

| Species | Soil | Day 15 | Day 52 | Day 79 | | | Day 114 | | | Day 148 | | | Day 183 | | | Day 220 | | | Day 272 | | | % Total population |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | 1° | 3° | 5° | |
| **Pseudomonads** | | | | | | | | | | | | | | | | | | | | | | |
| *P. aureofaciens* | 0.6 | 1.5 | 12.7 | 9.4 | 37.0 | 43.9 | 2.4 | 42.0 | 24.5 | – | – | 17.6 | – | – | 14.5 | 2.1 | – | – | 15.0 | 2.4 | 2.1 | 9.6 |
| *P. chlororaphis* | – | – | – | – | – | – | – | 4.0 | 6.1 | – | – | – | – | – | – | – | – | – | – | – | 1.9 | 0.5 |
| *P. cichorii* | – | – | 1.6 | – | – | 7.3 | – | – | – | – | – | – | – | 2.1 | – | – | 4.2 | – | – | – | – | 0.6 |
| *P. fluorescens* | 1.9 | – | 0.8 | – | 1.9 | 2.4 | – | – | 4.1 | – | – | – | – | – | 1.8 | – | – | – | 2.5 | | 8.3 | 1.1 |
| *P. marginalis* | – | – | 0.8 | 17.0 | 1.9 | 7.3 | – | – | – | – | – | – | – | 4.2 | – | – | – | – | 20.0 | 2.4 | 6.3 | 2.2 |
| *P. mendocina* | – | – | – | – | – | – | – | – | – | – | – | – | 4.5 | – | – | 6.4 | – | – | 2.5 | – | – | 0.5 |
| *P. pseudoalcaligenes* | – | – | – | – | – | – | – | – | – | – | – | – | 2.3 | – | – | 17.0 | – | – | 2.5 | – | 2.1 | 0.9 |
| *P. putida* | – | 0.7 | 0.8 | – | 13.0 | 14.6 | – | – | – | – | – | 2.0 | – | – | – | – | – | – | 5.0 | 7.1 | 4.2 | 1.8 |
| *P. syringae* | 11.2 | 2.2 | 0.8 | 9.4 | – | 12.2 | – | – | – | – | – | 3.9 | | – | – | – | 4.2 | 2.3 | 17.5 | 42.9 | 35.4 | 6.2 |
| *P. vesicularis* | – | – | – | – | – | – | – | – | – | – | – | – | 13.6 | – | – | 14.9 | – | – | – | – | – | 1.0 |
| *P. viridiflava* | – | – | 1.6 | – | – | – | – | – | – | – | – | – | – | – | – | – | 6.3 | – | 2.5 | 2.4 | – | 0.6 |
| No Match SBW-1 | – | – | – | – | – | – | – | – | – | 2.3 | 6.4 | – | – | – | – | – | – | – | – | – | – | 0.3 |
| No Match SBW-2 | – | – | – | – | – | – | – | – | – | – | 4.3 | – | – | – | – | – | 8.3 | 6.8 | – | – | – | 0.7 |
| No Match SBW-3 | – | – | – | – | – | – | – | – | – | – | 6.4 | – | 2.3 | – | – | – | – | – | – | – | – | 0.3 |
| No Match SBW-4 | – | – | – | – | – | – | 2.4 | – | – | – | – | – | 72.7 | – | – | 2.1 | – | – | – | – | – | 2.7 |
| Others[a] | 0.6 | 0.7 | 0.8 | 1.9 | – | – | – | – | – | – | – | 2.0 | – | – | – | 2.1 | 2.1 | 2.3 | 2.5 | 4.8 | 4.2 | 1.0 |
| **Enterobacteriacae** | | | | | | | | | | | | | | | | | | | | | | |
| *Aeromonas caviae* | 20.5 | – | – | – | – | – | – | – | – | – | – | – | – | – | 1.8 | – | – | – | – | – | – | 2.7 |
| *Cytophaga johnsonae* | 1.9 | – | 1.6 | – | – | – | – | – | – | – | – | – | – | – | – | 6.4 | 2.1 | – | 5.0 | – | 10.4 | 1.3 |
| *Erwinia herbicola* | – | – | 31.7 | 58.5 | 29.6 | – | 19.0 | – | – | – | – | 15.7 | – | 10.4 | 45.5 | – | 4.2 | 13.6 | – | – | 2.1 | 11.2 |
| *E. rhapontici* | – | – | – | – | – | – | – | 2.0 | – | – | – | – | – | 2.1 | 5.5 | – | – | – | – | – | – | 0.4 |
| *Flavobacterium indologenes* | – | – | – | – | – | – | – | – | 12.2 | – | – | – | – | – | – | – | – | – | – | – | – | 0.5 |
| *Hafnia alvei* | – | – | – | – | – | – | – | 4.0 | – | – | – | 5.9 | – | – | – | – | – | – | – | – | – | 0.4 |
| *Klebsiella planticola* | – | – | – | – | – | – | – | – | 14.3 | – | – | – | – | – | – | – | – | – | – | – | – | 0.6 |
| *K. terrigena* | 5.6 | 1.5 | – | – | 1.9 | 2.4 | 11.9 | – | – | – | – | – | – | 2.1 | 29.1 | 2.1 | 2.1 | 9.1 | – | – | – | 3.2 |
| *Serrratia liquefaciens* | – | – | – | – | – | – | 2.4 | 46.0 | 6.1 | – | – | – | – | – | – | – | – | – | – | – | – | 2.1 |
| *S. plymuthica* | – | – | – | – | 5.6 | 2.6 | – | – | – | – | – | – | – | – | – | – | – | – | – | 2.4 | – | 0.4 |
| *Yersinia enterocolitica* | – | – | – | – | 3.7 | 7.3 | – | – | 4.1 | – | – | 2.0 | – | – | – | – | – | – | – | – | – | 0.6 |
| Others[b] | 11.2 | 3.6 | 3.2 | – | – | – | – | – | 2.0 | – | – | – | – | – | – | 8.5 | – | – | 2.5 | 2.4 | 4.2 | 3.1 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Other gram negatives** | | | | | | | | | | | | | | | | | | | | | |
| *Agrobacterium rubi* | – | – | – | – | – | – | – | – | – | – | – | 7.8 | – | – | – | 2.1 | – | – | – | – | – | 0.4 |
| *Xanthomonas maltophilia* | – | – | 7.1 | – | – | – | – | – | 14.4 | – | – | 3.9 | – | – | 1.8 | – | – | 8.0 | – | – | 2.1 | 1.9 |
| No Match SBW-12 | – | – | – | – | – | – | – | – | – | – | – | – | 2.3 | – | – | 8.5 | – | – | – | – | – | 0.4 |
| No Match SBW-13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 2.1 | – | 7.5 | – | 2.1 | 0.4 |
| No Match SBW-6 | – | 0.7 | – | – | – | – | – | – | 8.2 | – | – | 2.0 | – | – | – | – | – | – | 2.5 | – | – | 0.6 |
| Others[c] | 0.6 | 10.3 | 2.3 | – | 1.9 | – | – | – | 2.0 | – | 2.1 | 3.8 | – | – | – | 6.4 | – | – | – | 7.0 | 2.1 | 2.2 |
| **Gram positives** | | | | | | | | | | | | | | | | | | | | | | |
| *Arthrobacter globiformis* | – | 5.1 | 3.2 | 3.8 | – | – | – | 2.0 | – | 4.7 | – | – | – | – | – | – | 2.1 | – | – | – | – | 1.3 |
| *A. mysorens* | – | – | – | – | – | – | 14.3 | – | – | – | – | – | – | – | – | – | – | 6.8 | – | – | – | 0.7 |
| *A. oxydans* | 8.1 | 27.7 | 7.9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 4.8 |
| *A. viscosus* | 5.6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.7 |
| *Aurobacterium saperdae* | 1.2 | – | 4.8 | – | – | – | – | – | – | – | – | 29.4 | – | – | – | – | – | – | – | – | – | 1.8 |
| *Bacillus sp.*[d] | 9.9 | 2.9 | 1.6 | – | – | – | 4.8 | – | – | – | – | – | – | – | – | – | – | – | – | 2.4 | – | 2.0 |
| *Curtobacterium flaccumfaciens* | – | – | 0.8 | – | – | – | – | – | – | 4.7 | – | – | – | 4.2 | – | – | 8.3 | – | – | – | – | 0.7 |
| *Kurthia zopfi* | 0.6 | – | – | – | – | – | 19.0 | – | – | – | – | – | – | – | – | 2.1 | – | 2.3 | – | – | – | 0.9 |
| *Microbacterium lacticum* | – | – | – | – | – | – | – | – | – | 2.3 | 2.1 | – | – | 56.3 | – | – | 6.3 | 4.5 | – | – | 2.1 | 2.8 |
| *Micrococcus kristinae* | 1.2 | – | – | – | – | – | – | – | – | 2.3 | – | 2.0 | – | – | – | 2.1 | – | 9.1 | 2.5 | – | – | 0.8 |
| *M. lylae* | 4.3 | 8.8 | 3.2 | – | – | – | – | – | – | – | – | – | – | – | – | 2.1 | – | 6.8 | – | – | – | 2.1 |
| *M. roseus* | 3.1 | 15.3 | 7.1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 4.5 | – | – | – | 2.9 |
| No Match SBW-26 | – | – | – | – | – | – | – | – | – | 2.3 | 6.3 | – | – | 6.3 | – | – | 6.3 | 2.3 | – | – | – | 0.9 |
| No Match SBW-29 | – | – | – | – | – | – | 11.9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.4 |
| No Match SBW-37 | – | – | 0.8 | – | – | – | 2.4 | – | – | 74.4 | 68.1 | – | – | 4.2 | – | – | 31.3 | 13.6 | 5.0 | 14.3 | – | 7.6 |
| Others[e] | 11.9 | 19.0 | 4.8 | – | 3.5 | – | 9.5 | – | 2.0 | 7.0 | 4.2 | 2.0 | 2.3 | 8.1 | – | 15.1 | 10.1 | 8.0 | 5.0 | 9.5 | 10.4 | 7.3 |

*Key*: 1°-senescent primary leaf, 3°-mature leaf, 5°-immature leaf.

[a] Includes *Comamonas acidovorans*, *C. testosteroni*, *Ochrobacterium anthropi*, *Pseudomonas corrugate*, *P. stutzeri*, No match SBW 2a, 4a, 5, 8, 9 and 38.

[b] Includes *Aeromonas salmonicida*, *A. sobria*, *Enterobacter amnigenus*, *E. intermedius*, *Erwinia chrysanthemi*, *Flavobacterium balustinum*, *F. esteraromaticum*, *Flavimonas oryzihabitans*, *Hydrogenophaga pseudoflava*, *Morganella morganii*, *Sphingobacterium multivorum*, No match SBW 10, 11, and 11a.

[c] *Agrobacterium radiobacter*, *A. tumefaciens*, *Acinetobacter calcoaceticus*, *A. genospecies* 9, *A. Iwoffi*, *Janthinobacterium lividum*, No match SBW 12a, 14, 15 and 18.

[d] Includes *Bacillus aminovorans*, *B. chitinosporus*, *B. globisporus*, *B. gordonae*, *B. megaterium*, *B. pabuli*, *B. polymyxa*, and *B. pumilus*.

[e] *Arthrobacter atrocyaneus*, *A. aureus*, *A. crystallopoietes*, *A. protophormiae*, *A. ureafaciens*, *Aureobacterium barkeri*, *A. liquefacients*, *Cellulomonas cartae*, *Clavibacter michiganese*, *Corynebacterium bovis*, *Micrococcus luteus*, *Oerskovia turbata*, *Rhodococcus rhodochrous*, *Staphylococcus haemolyticus*, *S. hominis*, No match SBW 6, 7, 17, 19 to 25, 26a to 28, 30 to 36, 37a, 39 and 40.

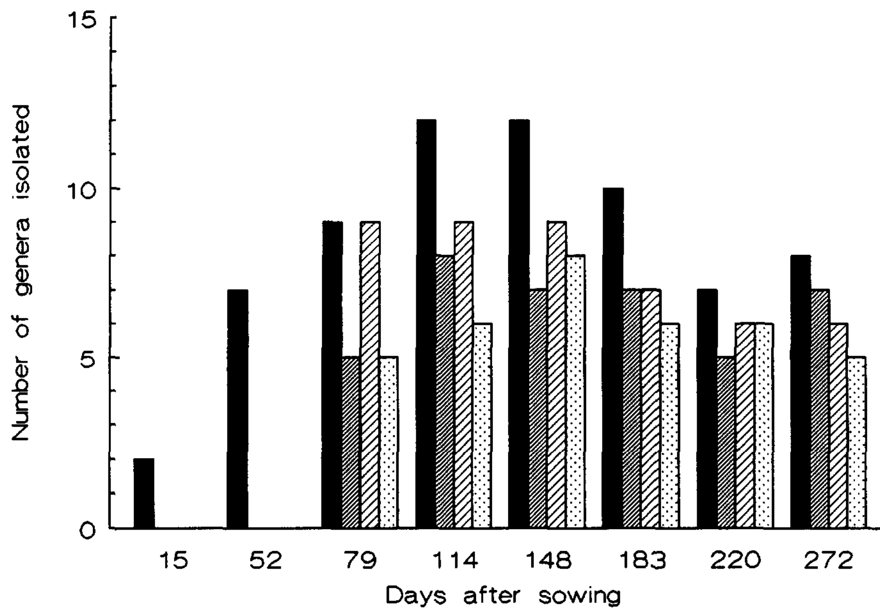



*Fig. 2.* Temporal fluctuations in numbers of filamentous fungi and yeast genera isolated from all three *B. vulgaris* leaf types sampled (■) and senescent primary (▨), mature (▨) and immature (▭) leaves.

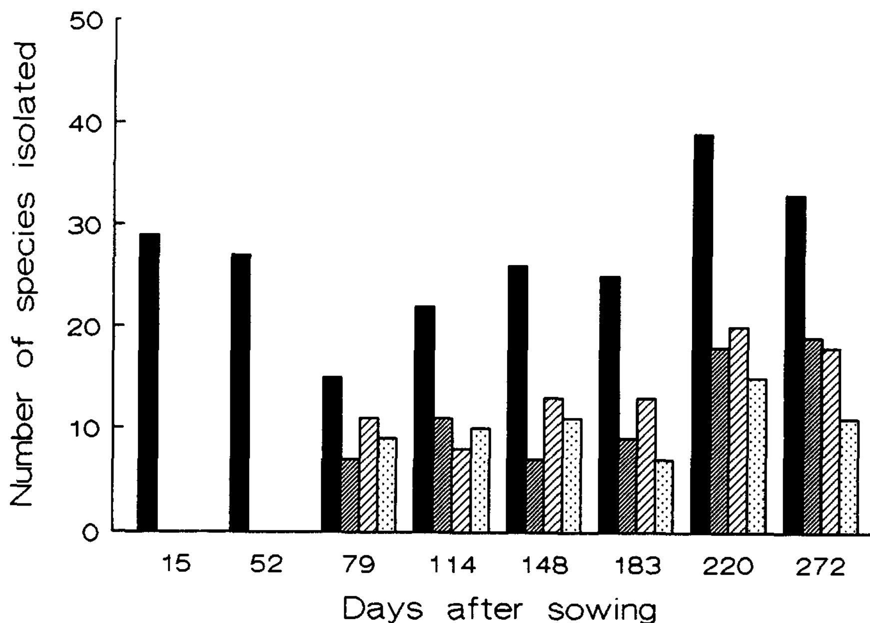



*Fig. 3.* Temporal fluctuations in the number bacterial species isolated from all three *B. vulgaris* leaf types sampled (■) and senescent (▨), mature (▨) and immature leaves (▭).

from soil on the day of sowing was also isolated from leaf samples on each sampling occasion. *E. herbicola* was not detected in soil or 15 day old plants, however once established, it accounted for up to 58.5% (senescent primary leaves on day 79) of the isolated bacteria community sample. *Arthrobacter*, *Bacillus* and *Micrococcus* occurred commonly in soil and on young plants,

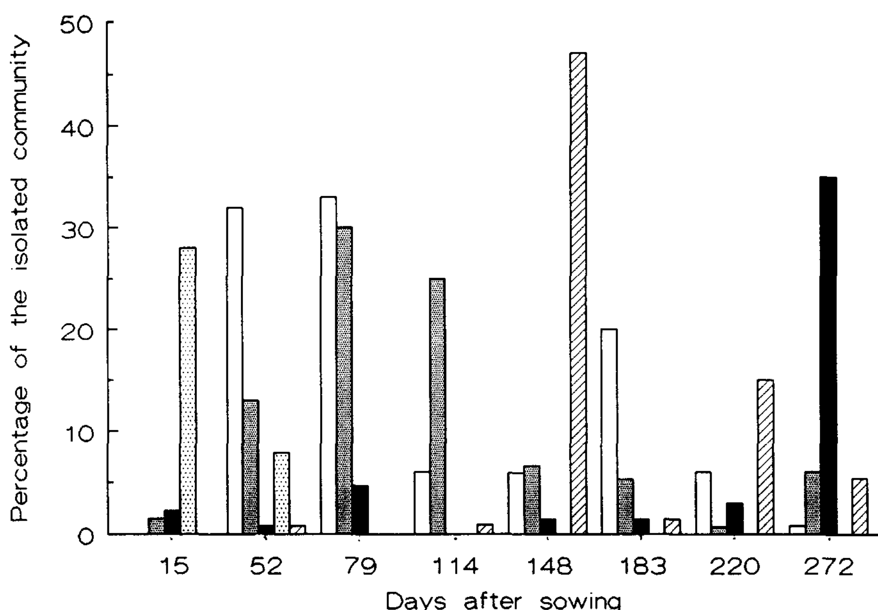



*Fig. 4.* Temporal fluctuations in the five most abundant bacterial species isolated from *B. vulgaris*, *Erwinia herbicola* (▭), *Pseudomonas aureofaciens* (▩), *P. syringae* (■), *Arthrobacter oxydans* (▭) and No Match 37 (▨).

but as the crop matured these were less frequently isolated.

*E. herbicola* (11.2%), *P. aureofaciens* (9.6%), *P. syringae* (6.2%), *Arthrobacter oxydans* (4.8%) and one unnamed species, No Match SBW 37 (7.6%), together represented 39.4% of the bacteria strains isolated. The temporal fluctuation of these five most abundant populations on all leave types sampled is shown in Figure 4. The remaining isolates were grouped into 12 unnamed genera and 37 unnamed species many of which were isolated on no more than two occasions, or were present more often, but in low numbers (<10% of any population).

## Discussion

The size and nature of the phyllosphere microbial community is a reflection of the host plant leaf characteristics together with environmental fluctuations (Dickinson, 1986). Leaf age and physiological condition have been identified as important factors influencing phyllosphere colonization (Dickinson, 1967, McBride and Hayes, 1977). In this study greatest microbial numbers were detected on senescing primary leaves. This may have been a consequence of ageing tissue; the leakage of nutrients from dying leaves can benefit microbial growth (Last and Deighton, 1965). However many other factors such as leaf position and growth may also have influenced the distribution of microorganisms (Andrews and Kenerley, 1980; Wildman and Parkinson, 1979).

Fluctuations in numbers of filamentous fungi, yeasts and bacteria were similar on all three leaf types, although differing in magnitude. With the exception of the number of yeasts colonizing senescing primary leaves on day 148, the numbers determined for all three microbial communities studied declined between days 114 and 148, and 183 and 220 on all three leaf types. This suggests that environmental factors were of particular importance and that leaf state was of less consequence in determining quantitative fluctuations in the field. Rainfall and temperature were measured throughout the study (Fig. 5) but fluctuations in these parameters did not correlate with detected changes in microbial numbers. However, other environmental factors such as solar radiation may have had an important influence on the microbial community.

Results presented here confirm previous ob-

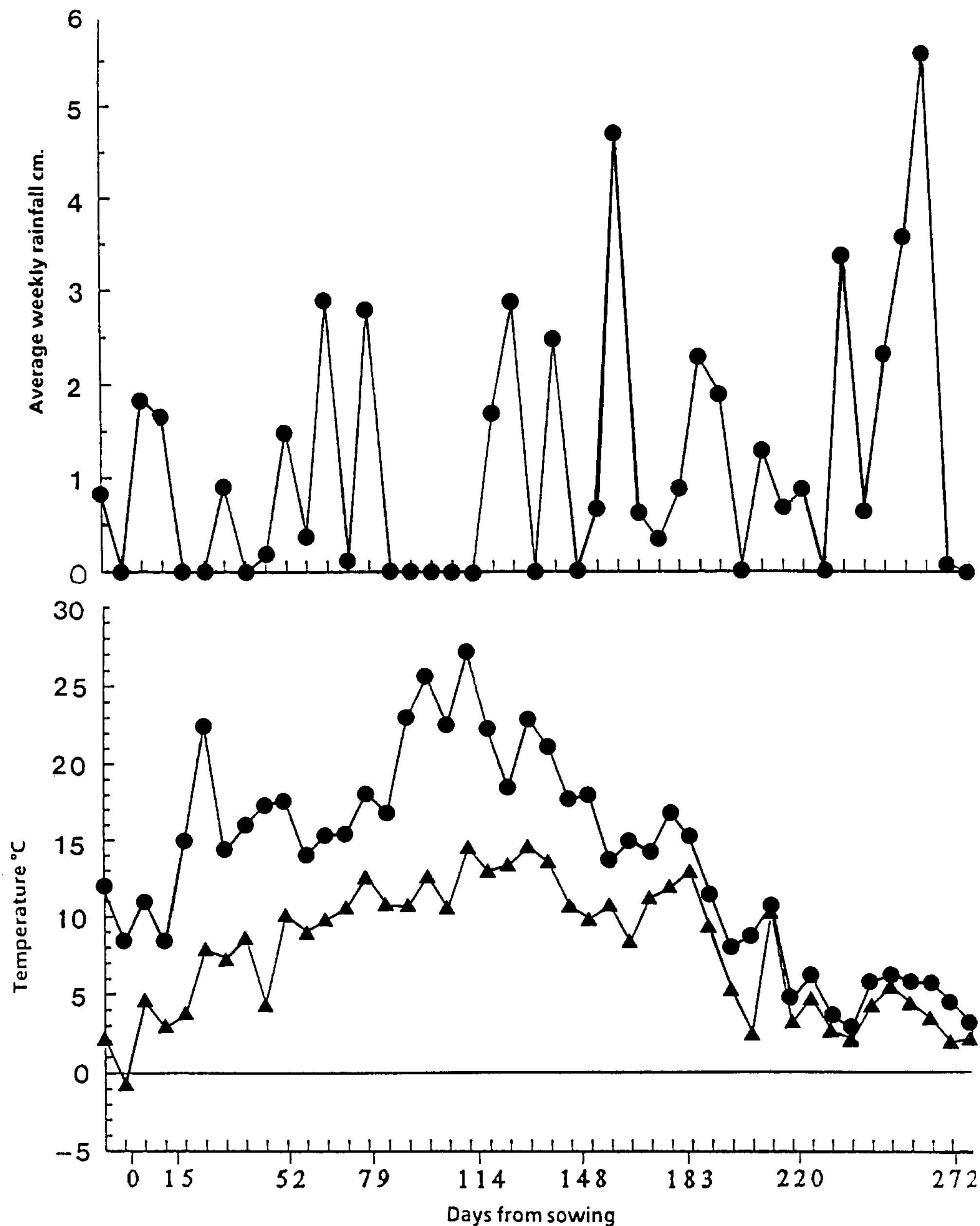



*Fig. 5.* (**a**) Average weekly rainfall and temperature (**b**) at Wytham Field Station from April 1990 to January 1991. Temperatures were taken at 4 am (▲) and 4 pm (●) respectively.

servations that the phyllosphere is a highly selective habitat for microorganisms (Lamb and Brown, 1970). On average, only 13 bacterial species were detected on each leaf type throughout the growing season, compared to 40 in soil, on the day of planting. The bacterial community of any given leaf type, sampled on any sampling occasion was invariably numerically dominated by one or other of only five species (Fig. 4). The low diversity within the phyllosphere bacterial community observed in this study is in good agreement with previous reports (Austin et al., 1978; Dickinson et al., 1975; Dickinson, 1976; Ercolani, 1991; Goodfellow et al., 1976). In this

study, bacterial community diversity (species number) was lowest mid-season (day 79 – Fig. 3) which corresponded to the warmest, driest period of the study and greatest (day 220) when rainfall was high and temperature low (Fig. 5). The decline in numbers of bacterial species isolated between days 220 and 272 may have been due to freezing night temperatures. The limited diversity observed in the bacterial community (34 named genera) was even more striking for the filamentous fungi and yeast communities. At most, only 12 genera were isolated from the whole plant on any single sample occasion. Limited diversity within the phyllosphere fungal community has previously been observed (Dickinson, 1976). However, as with most phyllosphere studies, broad range media were used in this investigation, thus specialized microbes that may have represented a significant component of the phyllosphere community may not have been isolated. All isolation media are selective to some extent and together with incubation conditions, extraction procedures and inhibitory interactions on the isolation plate, can greatly influence the nature of populations isolated (Curl and Truelove, 1986).

Two genera of filamentous fungi, *Cladosporium* and *Alternaria*, and two genera of yeast, *Cryptococcus* and *Sporobolomyces* represented three quarters of the cultures isolated on PDA. These four genera are known to be amongst the most common colonizers of deciduous annual plants (Dickinson, 1976; Hudson, 1971). The predominance of *Cladosporium* and *Alternaria* on *B. vulgaris* has been previously observed (Kerling, 1958) and have been detected in the phyllosphere of many other plant species (Dickinson and O'Donnell, 1977; Stout, 1960). The persistence of these four genera throughout the growing season corroborates previous reports of their excellent adaptation to the phyllosphere (Durrell, 1968; Pugh and Buckley, 1971; Simpson et al., 1971). In contrast to the bacterial community, the number of fungal genera sampled reached a peak mid-season, during the warmest driest period when bacterial diversity was at its lowest.

Microbial community studies have always been limited by the availability of rapid identification methods. Analysis of bacterial FAME profiles using the semi-automated Microbial Identification System (MIS) was found to be an accurate and comparatively rapid (60 samples in 24 hours) means of identifying large numbers of bacteria, in some cases below the species level. The system has several advantages for such studies, not least its ability to store numerous FAME profiles. These comprehensive profiles can be analysed subjectively by computer, thus reducing error and increasing the ability of the process to differentiate strains of the same species (Mukwaya and Welch, 1989). From such a data base the characteristics of fresh isolates taken over the year and subsequent seasons can be rapidly compared. In this way specific strains of species can be monitored over consecutive seasons.

Over 41% of the identified bacteria strains in this study were members of two genera *Pseudomonas* (371) and *Erwinia* (139). Pseudomonads are considered to be ubiquitous in the phyllosphere (Blakeman and Brodie, 1976; Dickinson, 1982). Twenty *Pseudomonas* species were isolated, the most common of which was *P. aureofaciens* (119–9.6%). This was the only species in this study that was isolated from soil and detected in leaf samples throughout the study period. Because of its persistence in the phyllosphere a *P. aureofaciens* isolate was selected for genetic marking and further study. *P. syringae*, the second largest pseudomonad population isolated, represented 11.2% of bacteria isolated from soil on the day of sowing. It was detected early in the season on young plants but was rarely isolated during summer months. Wimalajeewa (1987) also observed reduced *P. syringae* numbers during warmer months and attributed this to susceptibility to low humidity, high temperatures and ultra violet radiation. Some *P. syringae* are known to catalyse ice formation at temperatures close to 0°C and although this was not examined in the present study, *P. syringae* was isolated most commonly (42.9% of the mature leaf bacteria community) on day 272, after exposure to severe frost. *Erwinia herbicola* represented the largest named bacteria population isolated in this study. The abundance of *Erwinia* in the phyllosphere has previously been observed (Starr, 1981; Van Outrye et al., 1989). Last and Warren (1965) found that strains of *E. herbicola* were abundant on the leaves of various Gramineae and Leguminosae.

In order to determine the potential of soil as a

source of phyllosphere organisms soil samples were taken on the day of planting. On germination young plants grow upwards through the soil and in the process emerging plants are likely to be colonized by soil residents. *Arthrobacter*, *Bacillus* and *Micrococcus* are common soil residents (Goodfellow, 1969; Lowe and Gray, 1972; Stout, 1960), were isolated from soil in this study (representing 32% of the soil bacterial population) and represented over 59% of the bacterial community colonizing young *B. vulgaris* plants. Successional changes from a typical soil to phyllosphere community were also observed in the fungal community. Fifteen day old plants were colonized by *Mucor* and *Fusarium*, both common soil inhabitants (Waid, 1960) rarely isolated from mature plants. These findings indicate that soil represents a source of phyllosphere organisms, at least in young plants.

The size and manner of sample selection is one of the most important factors to consider in studies of natural habitats such as the leaf (Donegan et al., 1991). Microbial numbers detected on individual leaves can vary significantly (Crosse, 1959; Hirano and Upper, 1986). Because of this, in most phyllosphere studies bulked leaf samples have been analysed (Lindemann et al., 1982; Lindow et al., 1978). Statistical analysis (ANOVA) of microbial numbers in this study indicated that bulking sugar beet leaves allowed relatively rapid, yet representative quantification. However, no attempt was made to determine the variability in species/genera composition between replicate leaf samples. This is currently being attempted in a second year study of *B. vulgaris* plants grown on the same site. The fluctuation in species composition and colonizer numbers over two seasons will also be compared. The occurrence of specific bacterial species strains isolated in this first year study will be monitored.

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## References

Andrews J H and Kenerley C M 1980 Microbial populations associated with buds and young leaves of apples. Can. J. Microbiol. 58, 847–855.

Austin B, Goodfellow M and Dickinson C H 1978 Numerical taxonomy of phylloplane bacteria isolated from *Lolium perenne*. J. Gen. Microbiol 104, 139–155.

Blakeman J P and Brodie I D S 1976 Inhibition of pathogens by epiphytic bacteria on aerial plant surfaces. *In* Microbiology of Aerial Plant Surfaces. Eds. C H Dickinson and T F Preece. pp. 529–557. Academic Press, London.

Collins M D, Goodfellow M and Minnikin D E 1982 A survey of the structures of mycolic acids in *Corynebacterium* and related taxa. J. Gen. Microbiol. 129, 129–149.

Crosse J E 1959 Bacterial canker of stone-fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Appl. Biol. 47, 306–317.

Cullen D and Andrews J H 1984 Epiphytic microbes as biological control agents. *In* Plant-Microbe Interactions: Molecular and Genetic Perspective. Vol. 1. Eds. T Kosuge and E W Nester. pp 381–399. Macmillan, New York.

Curl E A and Truelove B 1986 The Rhizosphere. Eds. D R F Brommer, B R Sabey, G W Thomas, Y Vaadia, V D van Vleck. Springer–Verlag, Heidelberg. 288 p.

De Boer S H and Sasser M 1986 Differentiation of *Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica* on the basis of cellular fatty acid composition. Can. J. Microbiol. 32, 796–800.

Dees S B, Powell J, Moss C V, Hollis D G and Weaver R E 1981 Cellular fatty acid composition of organisms frequently associated with human infections resulting from dog bites: *Pasteurella mitocida* and groups EF-4, Ilj, M-5 and DF-2. J. Clin. Microbiol. 14, 612–616.

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

Dickinson C H, Austin B and Goodfellow M 1975 Quantitative and qualitative studies of phylloplane bacteria from *Lolium perenne*. J. Gen. Microbiol. 91, 157–166.

Dickinson C H 1976 Fungi on the aerial surfaces of higher plants. *In* Microbiology of Aerial Plant Surfaces. Eds. C H Dickinson and T F Preece, pp 293–324. Academic Press, London.

Dickinson C H and O'Donnell A 1977 Behaviour of phyllosphere fungi on *Phaseolus* leaves. Trans. Brit. Mycol. Soc. 68, 193–199.

Dickinson C H 1982 The phylloplane and other aerial plant surfaces. *In* Experimental Microbial Ecology. Eds. R G Burns and J H Slater. pp 412–430. Blackwell Scientific Press, Oxford.

Dickinson C H 1986 Adaptation of microorganisms to climatic conditions affecting aerial plant surfaces. *In* Microbiology of the Phyllosphere. Eds. N J Fokkema and J van den Heuvel. pp 77–100. Cambridge University Press, Cambridge.

Donegan K, Matyac C, Seidler R and Porteous A 1991 Evaluation of methods for sampling, recovery and enumeration of bacteria applied to the phylloplane. Appl. Environ. Microbiol. 57, 51–56.

Durrell L W 1968 Studies of *Aureobasidium pullans* (De-Bary) Arnaud. Mycopath. et Mycologia Applicata 35, 113–120.

Ercolani G L 1978 *Pseudomonas savastanoi* and other bacteria colonizing the surface of olive leaves in the field. J. Gen. Microbiol. 109, 245–257.

Ercolani G L 1991 Distribution of epiphytic bacteria on olive leaves and the influence of leaf age and sampling time. Microbiol. Ecol. 21, 35–48.

Goodfellow M 1969 Properties and composition of the bacterial flora of a pine forest soil. J. Gen. Microbiol. 109, 245–257.

Goodfellow M, Austin B and Dickinson C H 1976 Numerical taxonomy of some yellow-pigmented bacteria isolated from plants. J. Gen. Microbiol. 97, 219–233.

Gower J C 1966 Some distance properties of latent root and vector methods used in multivariate analysis. Biometri. 53, 325–338.

Hirano S S and Upper C D 1986 Temporal, spatial and genetic variability of leaf associated bacterial populations. *In* Microbiology of the Phyllosphere. Eds. N J Fokkema and J van den Heuvel. pp 235–251. Cambridge University Press, Cambridge.

Hudson H J 1971 The development of the saprophytic fungal flora as leaves senesce and fall. *In* Ecology and Leaf Surface Microorganisms. Eds. T F Preece and C H Dickinson. pp 447–455. Academic Press, London.

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

Lamb R J and Brown J F 1970 Non-parasitic microflora on leaf surfaces of *Paspalum dilatatum*, *Salix babylonica* and *Eucalyptus stellulata*. Trans. Brit. Mycol. Soc. 55, 383–390.

Lambert B, Meire P, Joos H, Lens P and Swings J C 1990 Fast-growing, aerobic, heterotrophic bacteria from the rhizosphere of young sugar beet plants. Appl. Environ. Microbiol. 56, 3375–3381.

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

Last F T and Warren F C 1965 Non-parasitic microflora colonizing green leaves; their form and functions. *Endeavour* 31, 143–150.

Lindemann J H A, Constantinidou H A, Barchet W R and Upper C D 1982 Plants as sources of airborne bacteria, including ice nucleation-active bacteria. Appl. Environ. Microbiol. 44, 1059–1063.

Lindow S E, Arny D C and Upper C D 1978 *Erwinia herbicola*: A bacterial ice nucleus active in increasing frost injury to corn. Phytopathology 68, 523–527.

Lowe W E and Gray T R G 1972 Ecological studies on coccoidal bacteria in alpine forest soil. I. Classification. Soil Biol. Biochem. 4, 459–467.

McBride R P and Hayes A 1977 Phylloplane of European larch. Trans. Brit. Mycol. Soc. 58, 329–331.

Mew T W and Kennedy B W 1982 Seasonal variation in populations of pathogenic pseudomonads on soybean leaves. Phytopathology 72, 103–105.

Miller L 1984 Gas liquid chromatography of cellular fatty acids as a bacterial identification aid. Hewlett Packard Application Note 228, 37.

Minnikin D E and Goodfellow M 1980 Lipid composition in the classification and identification of acid fast bacteria. *In* Microbiological Classification and Identification. Eds. M Goodfellow and R G Board, pp 189–256. Academic Press, London.

Mukwaya G M and Welch D F 1989 Subgroupings of *Pseudomonas cepacia* by cellular fatty aid composition. J. Clin. Microbiol. 27, 2640–2646.

Pugh G J F and Buckley N G 1971 *Aureobasidium pullans*: An endophyte in sycamore and other trees. Trans. Brit. Mycol. Soc. 57, 227–231.

Simpson K L, Chichester C O and Phaff H J 1971 Carotenoid pigments of yeast. *In* The Yeasts II. Eds. A H Rose and J S Harrison. pp 493–515. Academic Press, London.

Starr M P 1981 The genus *Erwinia*. *In* The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Eds. M P Starr, H G Truper, A Balows and H G Schege. pp 1260–1271. Springer-Verlag, Berlin.

Stead D E 1988 Identification of bacteria by computer-assisted fatty acid profiling. Acta Horticult. 225, 39–46.

Stout J D 1960 Biological studies of some tussock grassland soils. XV. Bacteria of two cultivated soils. N. Z. J. Agric. Res. 3, 214.

Thompson I P, Bailey M J, Ellis R J and Purdy K J 1993 Subgrouping of bacterial populations by cellular fatty acid composition FEMS Microbiology Ecology 102, 75–84.

Van Outrye M F, Gossele F, Kersters K and Swings J 1989 The composition of the rhizosphere of chicory (*Cichorium intybus* L. var. *foliosum* Heigi). Can. J. Microbiol. 34, 1203–1208.

Waid J S 1960 The growth of fungi in soil. *In* Ecology of Soil. Eds D Parkinson and J S Waid. pp 55–75. Liverpool University Press, Liverpool.

Wildman H G and Parkinson D 1979 Microfungal succession on living leaves of living leaves of *Populus tremuloides*. Can. J. Microbiol 57, 2800–2811.

Wimalajeewa D L S 1987 Seasonal variations in susceptibility of apricot to *Pseudomonas syringae* pv. *syringae* (bacterial canker) and sites of infection in apricot and cherry. Aus. J. Exp. Agri 127, 475–479.

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