THE EFFECT OF LEAF AGE AND POSITION ON THE DYNAMICS OF MICROBIAL POPULATIONS ON AERIAL PLANT SURFACES

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INTRODUCTION

Epiphytic microbial communities of terrestrial plants are non uniformly distributed in space and in time on leaf surfaces. The size and composition of microbial populations vary under the influence of biotic and abiotic factors related to the micro-organisms themselves (traits conferring epiphytic fitness, nutritional resource utilisation, abilities to compete for space, resistance or production of toxic compounds), to the host (its genotype, the age and the position of the leaves), and to the environmental conditions (micro- and macro-climate, activity of vectors and pathogens, application of pesticides and other chemicals). Moreover, the microbial population dynamics results from four processes -immigration, emigration, multiplication and death of cells as discussed in a previous chapter (Lindow, this volume) - which are under the influence of the previously mentioned factors. Establishing the relative importance of the different factors and processes that affect microbial population dynamics is important not only for answering unresolved fundamental ecological questions but also to develop practical solutions for the control of plant pathogenic micro-organisms. The development of novel strategies aimed at controlling plant pathogenic epiphytic micro-organisms rely on gaining a deeper insight into the functioning of populations of phytopathogenic and beneficial members of the community. Therefore some basic questions have to be answered. For example, what factors affect the composition of microbial communities? Do these factors influence immigration/emigration or growth/death? To limit proliferation of plant pathogenic micro-organisms is it more effective to try to limit their immigration than their growth or is the relative importance of these factors and processes similar for a given population?

When considering approaches for the study of epiphytic microbial community structure, one may be tempted to adopt either a factor-oriented analysis or a process-oriented analysis. The diversity of the involved factors and the difficulty in discerning their influence on each process
usually dictates that the impact of each factor has to be studied separately. The choice of which factor to investigate normally depends on the model system available and the aims of the investigation. In our laboratory, we are interested in the mechanisms implicated in the colonisation of broad-leaved endive leaves during field-cultivation and storage by epiphytic bacteria implicated in post-harvest decay. The role of bacteria in the decay of these salads is presented in detail in a previous chapter (Morris and Nguyen-the, this volume). Broad-leaved endive forms loose heads similar to butterhead type lettuces; The outer leaves are spread out and the inner leaves are closed tightly together. The outer leaves are relatively well-exposed to airborne microbial contamination while inner leaves are covered by adjacent leaves and hence relatively more protected from aerial microbial contamination. As a consequence of this morphology and in an attempt to evaluate sorting methods based on predicted microbial load of minimally processed salads (pre-cut, washed and packaged), we were particularly interested in a factor-oriented approach. The factor of interest for our studies is the age and the position of the leaves.

This chapter focuses on the effect of leaf age and position on microbial population sizes and composition in the phyllosphere. A brief review of the literature concerning the effect of this factor on microbial populations (and particularly on bacterial populations) will be presented. This will be followed by a discussion of the hypothetical mechanisms by which leaf age has its effect and the implications for the experimental approach taken in studies of epiphytic microbial population structure. Specific examples will be provided from studies of the influence of leaf age and position on population dynamics of total mesophilic, fluorescent and pectolytic epiphytic bacteria for broad-leaved endive (Jacques and Morris, 1995a; Jacques et al., 1995). This work allows us to show how leaf age and position may interact with bacterial immigration and growth influencing the quality of leaves within the canopy as a habitat for bacteria.

MICROBIAL COMMUNITY STRUCTURE

Characterisation of microbial community structure requires the design of a sampling strategy which considers collection of samples in time and space, the choice of a sampling unit and methods for liberating micro-organisms from the leaf surface and recovering them for further analyses. These different issues were recently reviewed by Jacques and Morris (1995b) and earlier by Hirano and Upper (1991) and Kinkel (1991). Here we will focus only on the choice of the sampling unit which is fundamental to increase precision and soundness of the data on microbial community structure.

The choice of a sampling unit has received increasing attention since the analysis of the statistical implications of the use of individual samples vs. bulked samples for estimating population sizes of epiphytic bacteria (Hirano et al., 1982). These authors have shown that epiphytic bacterial populations are often lognormally distributed on individual leaves of various crops leading generally to over-estimations of the true means of bacterial population sizes if bulked samples are used (depending on the variance among population sizes and the number of leaves in the bulk). Moreover, it has also been shown that the use of bulked samples leads to under-estimations of variances if microbial populations follow skewed distributions such as the lognormal distribution (Hirano et al., 1982; Kinkel, 1992).

Individual leaves are often used as the sampling unit for various biological reasons. Incidence, severity and progress of foliar diseases of plants in the field or processed vegetable products in storage are often assessed on individual leaves (Rouse et al., 1985) or leaf pieces (Jacques and Morris, 1995a). This is the case, because inoculum thresholds for symptom expression are more meaningful for single plant organs than for a whole plant or several plants. Furthermore, interactions among micro-organisms are more likely to take place in restricted sites than across groups of leaves or entire plants. Hence, an understanding of the