AN ABSTRACT OF THE THESIS OF

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Title: PHYLLOPLANE MICROORGANISMS ON THE LEAF SURFACES OF BEAN (Phaseolus vulgaris L.), HOP (Humulus lupulus L.), MINT (Mentha piperita L.) AND TOMATO (Lycopersicon esculentum Mill.)

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Phyllopane microorganisms on the leaf surfaces of bean (Phaseolus vulgaris L.), hop (Humulus lupulus L. 'Fuggle-H'), mint (Mentha piperita L. 'Mitcham') and tomato (Lycopersicon esculentum Mill.) has been observed by cultural methods. A leaf impression method was used to examine both actively growing and inactive propagules present on the surfaces of the leaves. A surface sterilization method was also used to detect the presence of any fungi occurring inside the living tissues. All leaf samples were collected from different locations in the Willamette Valley, i.e., Benton, Lane, Linn, Marion, Polk, Washington and Yamhill Counties. A total of 38 species of microorganisms were found on the surface of healthy leaves of all four host plants. They are divided into five categories which are as follows.

First, several microorganisms are very commonly found on the leaf surfaces of all plants and at almost every location; with frequencies of occurrence from 0.01-97 percent. There are 16 species in this first category and examples are Alternaria tenuis Nees ex Per., Aureobasidium pullulans (de Bary) Arn., Cladosporium cladosporioides (Fres.) de Vries, C. herbarum Pers. ex Fr., Epicoccum purpurascens Ehrenb. ex Schlecht., Sporobolomyces roseus Kluy. & van Niel, and so forth.

Second, microorganisms which occurred mainly on bean, mint and tomato leaf surfaces, with the range of frequencies between 0.01-29 percent. Thirteen species were isolated, e.g. Acremoniella atra (Corda) Sacc., Aspergillus niger Tiegh., Fusarium oxysporum Schlecht., and Phoma sp. etc.

Third, microorganisms which grow mainly on tomato leaves with some occurrence on mint or bean leaves.

Fourth, microorganisms which grow on tomato and mint leaves only. The frequency of occurrence was between 0.01-29 percent.

Fifth, other epiphyllous microorganisms besides fungi, include species of actinomycetes and bacteria that were not identified. These microorganisms, especially the bacteria, abounded on all of the crop leaves and at every location.

Numbers of the internal fungi were found in lesser numbers than those on the leaf surfaces. Seven species were isolated from

inside of the healthy leaf tissues of the same crops. They were Alternaria tenuis, Cladosporium cladosporioides, Epicoccum purpurascens, Phoma sp., Stemphylium botryosum Wallr., Ulocladium atrum Preuss, and Verticilium sp. A. tenuis was the most commonly present at the cut edges of bean leaf squares with average 57 percent. The rest of them were varied, and ranged from 1-17 percent.

Phylloplane Microorganisms on the Leaf Surfaces of Bean (*Phaseolus vulgaris* L.), Hop (*Humulus lupulus* L.), Mint (*Mentha piperita* L.) and Tomato (*Lycopersicon esculentum* Mill.)

by

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PHYLLOPLANE MICROORGANISMS ON THE LEAF SURFACES OF BEAN (*PHASEOLUS VULGARIS* L.), HOP (*HUMULUS LUPULUS* L.), MINT (*MENTHA PIPERITA* L.) AND TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

INTRODUCTION

The occurrence of microorganisms on the surface of healthy plants has been known for a long time. For example, a common fungus, *Dematium pululans* de Bary (*Aureobasidium pululans*),[1] was reported in 1886. The earliest descriptions of the sooty molds are of the brown-walled cells found on succulent fruits by Pasteur. During the last 20 years, there has been increasing interest in the leaf surface as a habitat of fungi (Pugh and Buckley, 1971a) because the leaf surface consists of several distinct microhabitats which are inhabited by a varied assemblage of saprophytic and parasitic organisms (Dickinson, 1971). These organisms have been found on healthy leaf surfaces of both dicotyledonous and monocotyledonous plants as well as on senescent or dead leaves (Webster, 1957; Hudson and Webster, 1958; Hudson, 1962; Dickinson, 1965; Hogg and Hudson, 1966).

Last (1955) and Ruinen (1956) adopted the term phyllosphere to characterize the habitat of saprophytic mycoflora growing on leaves. This habitat is now recognized as the phylloplane. The saprophytes

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[1]See Table 2 for authorities.

may be actively growing on the leaf surface or they may be present only as inactive propagules. In terms of the microflora in temperate zones, the principal groups of organisms present are bacteria, yeasts and filamentous fungi (Last, 1955). Leben (1965) distinguishes two groups of epiphytes: residents and casuals. Residents multiply on the surface of healthy plants, or on debris on these surfaces, without noticeably affecting the host. Casuals are epiphytes that are on the plant by accident, that cannot grow directly on plant surfaces, but may grow saprophytically on foreign debris.

Ecology

Populations of microorganisms do not remain static but constantly vary and respond to changes of environment (Last and Deighton, 1965). Meteorological factors such as atmospheric temperature, humidity and rain may influence the microorganisms on leaf surfaces. Both the total number and relative abundance of epiphytic microorganisms are affected by the weather conditions, some persist throughout the growing period whereas others are exclusively associated with a particular period of climatic conditions (Sinha, 1971).

The most profuse development of all types of epiphytes appears to be favored by moderate to warm temperature and virtually continuous rain or high humidity. Humidity at night is often high and dews

are heavy, even in a semiarid climate. Moreover, dew may be deposited several hours before the relative humidity reaches 90 percent. Air near leaves may carry more water than the general air mass; thus with air at relative humidity of 50 percent, the relative humidity near the lower surface of the leaf may be 20 percent higher. These considerations, added to the fact that plant parts near the soil remain moist longer than other parts, indicate that microbial epiphytes may be exposed to fairly lengthy periods during which the air relative humidity is greater than 70 percent (Leben, 1965).

Periods of alternating wet and dry conditions, together with extended dry periods also influence, sometimes decisively, the activity or even the viability of epiphyllous microorganisms. Thus, in order to succeed the fungi of the phyllosphere must, immediately after germination, be able to resist dryness caused by a decrease of ambient humidity (Diem, 1971).

Diem (1971) also studied the reaction of the Dematiaceae (Alternaria tenuis,[2] Stemphylium botryosum,[3] Helminthosporium sativum Pamm. King & Bakke, Cladosporium herbarum[4] and C. cladosporioides)[5] and the hyaline group of fungi (Colletotrichum

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[2]See Table 2 for authorities.

[3]Ibid.

[4]Ibid.

[5]Ibid.

graminicola (Ces.) Wils, Aspergillus sp. and Penicillium sp.) to low humidity. This humidity effect perhaps enables us to understand the ability of diverse fungi to live on the leaf surface. A large number of fungi can grow on leaves in the rainy season because the cuticle is constantly wet. But in the dry period would interrupt the activity of this mycoflora and eliminate some of its constituents. In the phyllosphere, hyaline fungi, because of their fragility, are less able to survive than the Dematiaceae.

Last and Deighton (1965) reported that populations of epiphytic microorganisms are often decreased by rain because the individuals are dispersed in rain droplets like pathogenic fungi. Members of genus Sporobolomyces forcibly discharge their spores. They have dispersal mechanisms similar to the drop excretion or gas bubble method found in Basidiomycetes which are favored by high relative humidity. In humid conditions, either early in the morning of a dry day or in rainy weather, 10^5-10^6 spores/m^3 air of Sporobolomyces and Tilletiopsis were found. Peak concentrations of these genera occurred in July/August and August/September respectively in Britain.

The maturity of the leaf may also influence the species composition of epiphyllous microbial populations. The numbers of fungi increase with increasing leaf age and yellowing leaves (Last and Deighton, 1965; Sinha, 1971), for example, Cladosporium and

Alternaria colonies are more common on senescent leaves of rye than on young ones. On the other hand, Aureobasidium pullulans has been isolated in large numbers from very young tissues. Numbers of Sporobolomyces colonies per unit area increase progressively as leaves age and there were more on old leaves in summer than in winter. Tilletiopsis spp. are exceptional among the colonizers of living leaves in the temperate zone in being predominantly mycelial fungi. Their numbers, like those of Sporobolomyces, increase as leaves age but, in contrast, large populations are found during a more restricted and later period of the year. When Sporobolomyces and Tilletiopsis occur together on cereal leaves, colonies of Sporobolomyces tend to be fewer than usual and concentrated along leaf margins (Last and Deighton, 1965).

The surface of a leaf consists of several microhabitats and some microorganisms are characteristic of specific habitats. Last and Deighton (1965) reported that numbers of Micropeltaceae and Chetothyriaceae occur in tropical Africa on a wide range of hosts, but are most common on those with waxy leaves. They are usually more numerous on the upper surface of leaves than on the lower. Other fungi, such as Brooksia tropicalis Hansf. and the hyphomycetes Grallomyces portoricensis Stev., Pithomyces cupaniae (Syd.) M. B. Ellis and Acarocybella jasminicola (Hansf.) M. B. Ellis, also occur on a wide range of hosts but are restricted mainly to the lower leaf

surface (Last and Deighton, 1965). Sporobolomyces, pink yeast colonies, tend to be arranged in rows parallel to the veins (Last, 1955; Pugh and Buckley, 1971a). The most definite rows of colonies usually occur along the leaf margins, with the number of colonies per square centimeter significantly more on the distal region than on the proximal one. The colonies are superficial; they occur mainly on the furrows between the veins and on the tissue between the peripheral veins and the leaf margins. They are not aggregated about the stomata (Last, 1955). And recently, by use of fluorescent staining technique, it has apparently shown that fungi plugged the stomatal pores of Douglas fir needles. Their fungal strands spread out from the stomatal pores and likely parallel to the lines of the stomates (Bernstein, Howard and Carroll, 1973).

Phylloplane fungi may subsist on materials leached from plant surfaces and plant leachates can affect the growth of fungal conidia as well as the growth of fungi (Ruinen, 1956; Last and Deighton, 1965). They may derive benefit from nutrients diffused not only from the leaf but also from algae, pollen grain, from the honey dew which is an abundant source of food when leaves are infested with aphids (Pugh and Buckley, 1971a) and from the organic volatiles or polluted air in the atmosphere (Rasmussen and Hutton, 1972).

Epiphytic Microflora on Leaf Surface

Aureobasidium pullulans, Cladosporium herbarum, Sporobolomyces roseus[6] are commonly found on the surface of green leaves of a wide range of species of plants which have been studied by many workers: i. e., Crosse (1959), Last and Deighton (1965), Hollomon (1967) Pugh and Buckley (1971a), and Stott (1971). Derx (1930) (cited in Last, 1955) found Sporobolomyces and Tilletiopsis colonies from a range of monocot and dicot plants (Last, 1955). Hogg and Hudson (1966) found S. roseus, Bullera alba (Hanna) Derx, Tilletiopsis minor[7] and T. perplexans on leaves of Fagus sylvatica L. Yeasts also form an important component of the phyllosphere microflora of many plant species (Last, 1955; di Menna, 1959; Ruinen, 1963; Last and Deighton, 1965; Dickinson, 1967; Hislop and Cox, 1969). Ruinen (1963) studied the tropical foliage in Java (Indonesia). She found 22 species of genera Hansenula, Cryptococcus, Candida, Sporobolomyces, Rhodotorula and Pullularia occurred on leaves of trees and shrubs. Stott (1971) also stated that the most prominent species were Alternaria chartarum Preuss (≡ Ulocladium chartarum (Pr.) Simmons) Aureobasidium pullulans, Botrytis cinerea,[8]

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[6] See Table 2 for authorities.

[7] Ibid.

[8] Ibid.

Cladosporium cladosporioides, Epicoccum purpurascens[9] and Phoma sp., and they were frequently isolated from green leaves of Halimione portulacoides (L.) Aell, Pisum sativum L., Solanum tuberosum L., Hordeum vulgaris L., Secale cereale L. and Fragaris spp. Sinha (1971) isolated 44 species of microorganisms from tomato leaves by washing technique. He found Mucor hiemalis Wehmer, Spicaria, Fusarium moniliforme,[10] Aspergillus niger,[11] A. flavus Link, Cladosporium cladosporioides, Alternaria tenuissima (Kunze ex Pers.), A. solani Sorauer and Streptomyces occur throughout the growing season.

Role of Leaf Surface Microorganisms

Some phylloplane organisms, presumably bacteria not higher fungi fix nitrogen from the atmosphere in vitro. Ruinen (1965) found that Beijerinckia, Azotobacter spp., which are abundant on tropical foliages in Java, are able to fix nitrogen. Jones (1970) was able to isolate N-fixing bacterium from the leaf surface of Douglas fir. Last and Deighton (1965) also reported that some epiphyllous microorganisms for example Beijerinckia, can fix atmospheric nitrogen, while others secrete root stimulants and antifungal metabolites. However,

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[9] See Table 2 for authorities.

[10] Ibid.

[11] Ibid.

they did not know whether these will benefit the host in vivo. Last and Warren (1972) found that some saprophyte phylloplane microorganisms not only can fix nitrogen, but they also degrade plant waxes, produce plant growth regulators, compete with plant parasites, and stimulate plants to produce phytoalexins.

The interactions between parasites and saprophytes on leaf surfaces are very interesting. Saprophytic microorganisms probably play an important role in controlling the incidence of plant diseases by utilizing energy sources present on the leaf surface. The saprophytes may produce substances which either stimulate or inhibit the growth of pathogens. Aureobasidium pullulans produces a substance that strongly inhibits the growth of Sclerotinia fructicola (Wint.) Rehm, S. laxa Aderh & Ruhl., Botrytis cinerea, Verticillium albo-atrum Reinke & Berth, Colletotrichum lini (Westerdijk) Tochinai, Physalospora obtusa (Sehw.) Cooke and Penicillium spp. in dual cultures on potato dextrose agar (PDA) and this antibiotic production increases with time (Baigent and Agawa, 1960). Meria laricis Peace & Holm and Cladosporium herbarum which occur on larch leaves were shown to be inhibited by bacteria and yeasts (Sporobolomyces roseus) isolated from the phyllosphere of larch (McBride, 1972). There are some other evidences for the production of inhibitory substances. For example, Alternaria tenuissima on bean leaves (Phaseolus vulgaris L.) can produce substances which inhibit

spore germination in A. zinniae Pape and which probably inhibit lesion formation (Heuvel T. van den, 1971).

In addition to their interaction with pathogens, some saprophytes can be secondary pathogens, i. e., Alternaria chartarum, a saprophyte on green leaves of sugarbeet, can behave as a secondary pathogen when it follows Beet Mild Yellow Virus (Russell, 1965).

Internal Fungi and Their Roles

The presence of latent infection of fungi inside healthy tissues has frequently been neglected in the search for phylloplane microorganisms since these internal fungi are symptomless. Some pathogenic fungi probably live inside the healthy leaf tissues and can cause disease when the host becomes weak or lacks nutrients. Rayner (1948) studied healthy green coffee leaves; he found that Colletotrichum coffeanum Noack, Phoma spp. and Phomopsis spp. were frequently isolated and they are present as latent infections on almost all healthy coffee tissues. They fructify on tissues which have died from carbohydrates or nitrogen-deficiency, sun scorch and so on. Hollomon (1967) isolated Alternaria tenuis, Botrytis cinerea and Fusarium spp. from the surface-sterilized potato leaflets, and found out that these organisms again present as latent infection on the potato. Botrytis cinerea, particularly, is present within the leaf rather than on the leaf surface. Moreover, many plants have been

reported to have symptomless, latent fungal infections; for instance, Nothofagus truncata (New Zealand hard beech) leaves (Ruscoe, 1971), Acer pseudoplatanus L. (Pugh and Buckley, 1971b) and many more evergreen conifers (Kendrick and Burges, 1962; Bernstein, Howard and Carroll, 1973; Millar, 1974); Carroll (personal communication). Aureobasidium pullulans is a common microorganism that can be isolated from sterilized leaf discs of sycamore (Acer pseudoplatanus) (Pugh and Buckley, 1971b).

Recently, Bernstein (1974) studied latent infection of Douglas fir needles ages 1, 3, 5 and 8 years. She found that two species of fungi are the main colonizers of interior part of the needle, but she did not indicate the species of those fungi. Her conclusion is that the older needles tend to have more internal fungi than the round ones.

MATERIALS AND METHODS

Healthy leaves of bean (Phaseolus vulgaris L.), hop (Humulus lupulus L. 'Fuggle-H'), mint (Mentha piperita L. 'Mitcham') and tomato (Lycopersicon esculentum Mill.) were collected from different locations in the Willamette Valley; i. e. , Benton, Linn, Lane, Marion, Polk, Washington and Yamhill Counties during the month of July, 1974 (Table 1). Five samples of each crop from different locations were removed arbitrarily, placed in clean polyethylene bags and stored in an ice chest, then transferred to a cold room (4°C) where they were kept at the OSU Botany and Plant Pathology Department. Time between collection and isolation was about 1-5 days.

Leaf Impression Method

Pieces of leaf 3 mm square were cut arbitrarily from the middle, edges and apex of leaves with a sterilized razor blade. Fifty pieces were cut per sample. These leaf squares were pressed into the surface of Corn Meal Agar (CMA) in petri dishes (9 cm ϕ) at a rate of 10 squares per plate. Five were placed with the abaxial surface exposed and the other five with the adaxial surface exposed. The cultures were incubated at 25°C in the incubator for 24 hours; after that all leaf squares were removed and the plates incubated for another 2 to 3 days. Microorganisms from imprinted leaves

Table 1. Locality and collection date of bean, hop, mint and tomato leaf samples.

Locality	Collection date			
	bean	hop	mint	tomato
Benton County				
Corvallis	7-10-74	-	-	7-10-74
Irish Bend	-	-	7-12-74	-
Lane County				
Eugene	7-11-74	-	-	7-11-74
Junction City	-	-	7-12-74	-
Linn County				
Albany	7-10-74	-	-	7-10-74
Botany Farm	-	7-10-74	-	-
Jefferson	-	-	7-12-74	-
Marion County				
Salem	7-10-74	-	-	7-10-74
Talbot	-	-	7-12-74	-
Polk County				
Buena Vista	-	-	7-12-74	-
Rickreall	7-10-74	-	-	7-10-74
Washington County				
Beaverton	7-10-74	-	-	7-10-74
Yamhill County				
Newberg	7-10-74	-	-	7-10-74

were identified under compound microscope and the percentage frequency of occurrence was counted.

Surface Sterilization Method

Leaf squares (3x3 mm) were also cut arbitrarily (50 squares per sample) and were immersed in undiluted bleach (Clorox, NaClO 5. 25 percent) for 30 seconds to 1 minute depending upon the thickness of the leaf squares. They were then rinsed in sterile distilled water and placed on Water Agar (WA, 1. 5 percent), 10 squares per plate. The cultures were incubated for 10 days under the same conditions as the leaf impressions. All plates were examined under the compound microscope and internal fungi were identified and counted to determine the percentage frequency of occurrence.

RESULTS

Leaf Impression Method

In my observations of leaf squares taken from four crops in seven different locations (250 leaf squares from each crop in each location), I found several species of microorganisms inhabiting the leaf surfaces. Certain species were found on all four crops at each of the seven locations. Other species appeared on only one, two or three host crops.

Bean (Phaseolus vulgaris)

A total of 33 species of microorganisms were isolated from bean leaf surfaces. Of these, 11 species were the most common at all locations. Cladosporium cladosporioides, C. herbarum, Epicoccum purpurascens. Sporobolomyces roseus and bacteria occurred at a high frequency (above 50 percent). Alternaria tenuis, Aureobasidium pullulans and Cryptococcus sp. occurred at a frequency of 30-49 percent. The rest were found at a very low frequency (below 29 percent). Certain fungi, besides the 11 species mentioned above, were observed on leaves from every location except Marion and Lane counties. For example, Penicillium spp., Phoma sp., Rhodotorula sp. and so on. Fusarium solani[12] was found at five

[12]See Table 2 for authorities.

locations but was absent in Polk and Washington Counties (Appendix I).

Hop (Humulus lupulus)

The hop leaf samples were taken only from Linn County since hops are grown in only a few areas. The cultivar I studied was 'Fuggle-H', five samples of which were collected from a USDA experimental farm. From these five samples 15 species of microorganisms were identified on the leaf surfaces. Among fungi, Cladosporium herbarum and Sporobolomyces roseus were found at the highest frequency of occurrence (97 percent); Aureobasidium pullulans occurred on 64 percent, and the rest of them were below 50 percent.

Mint (Mentha piperita)

The leaf samples of mint were collected from Benton, Lane, Linn, Marion and Polk Counties because they are the major growing areas in the Willamette Valley.

Thirty-three species of phylloplane microorganisms were identified; 11 species were predominant on leaf samples collected from all locations. Alternaria tenuis, Cladosporium cladosporioides, C. herbarum, Sporobolomyces roseus and bacteria were observed at the highest frequency (above 50 percent). Aureobasidium pullulans, Cryptococcus sp. and actinomycetes occurred at 30-50 percent. The

rest were found at low percentages (below 30 percent) Cephalosporium sp., Epicoccum purpurascens, Tilletiopsis minor and T. washingtonensis[13] were found in almost all the counties. Many fungi appeared only on specific crops and/or at specific locations (Appendix III).

Tomato (Lycopersicon esculentum)

A total of 36 species of microorganisms were associated with tomato leaf surfaces; 10 species were observed on all crop leaves. Aureobasidium pullulans, Cladosporium cladosporioides, C. herbarum, Epicoccum purpurascens, Sporobolomyces roseus and bacteria had an occurrence of more than 50 percent. Alternaria tenuis, Cryptococcus sp. occurred at 30-49 percent. Actinomycetes and Ulocladium atrum[14] appeared below 20 percent. The other 26 species were scattered among different varieties and locations (Appendix IV).

In this study, phylloplane microorganisms can be divided into five categories (Table 2).

First, microorganisms which were very common on the leaf surfaces of every kind of host crop and at almost every location, with the range of percentage frequency of occurrence from 0.01 to above 50 percent. But most of them occurred between 10-30 percent (Fig. 1).

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[13] See Table 2 for authorities.

[14] Ibid.

Table 2. Phylloplane microorganisms isolated from leaves of bean, hop, mint and tomato collected from different locations in the Willamette Valley.

Microorganism	Bean	Hop	Mint	Tomato
Alternaria tenuis Nees ex Pers.	xx	x	xxx	xx
Aureobasidium pullulans (de Bary) Arn.	xx	xxx	xx	xxx
Botrytis cinerea Pers. ex Fr.	x	x	x	x
Cladosporium cladosporioides (Fres.) de Vries	xxx	xx	xxx	xxx
C. herbarum Pers. ex Fr.	xxx	xxx	xxx	xxx
Cryptococcus sp.	xx	x	xx	xx
Epicoccum purpurascens Ehrenb. ex Schlecht.	xxx	xx	xx	xxx
Mucor sp.	x	x	x	x
Penicillium spp.	x	x	x	x
Rhodotorula sp.	x	x	x	x
Sporobolomyces roseus Kluy. & van Niel	xxx	xxx	xxx	xxx
Tilletiopsis minor Nyland	x	x	x	x
T. washingtonensis Nyland	x	x	x	x
Ulocladium atrum Preuss	x	x	x	x
Acremoniella atra (Corda) Sacc.	x	-	x	x
Aspergillus niger Tiegh.	x	-	x	x
Cephalosporium sp.	x	-	x	x
Curvularia inequalis (Shear) Boedijn	x	-	x	x
C. clavata Jain	x	-	-	x
Fusarium oxysporum Schlecht.	x	-	x	x
F. semitectum Berk. & Rav.	x	-	x	x
F. solani (Mart.) Sacc.	x	-	x	x
Nigrospora sphaerica (Sacc.)	x	-	x	x
Phoma sp.	x	-	x	x
Polyscytalum sp.	x	-	x	x
Rhizopus stolonifera (Ehrenb. ex Fr.) Vuill.	x	-	x	x
Stemphylium botryosum Wallr.	x	-	x	x
Chaetomium cochliodes Palliser.	-	-	x	x
Helminthosporium biforme Mason & Hughes	x	-	-	x
Verticillium sp.	-	-	x	x
Bipolaris sp. cf. kusanoi (Nisikado) shoem.	-	-	x	-
Colletotrichum sp.	-	-	-	x
Fusarium equiseti (Corda) Sacc.	-	-	-	x
F. moniliforme Sheldon	-	-	-	x
Actinomycetes	x	x	xx	x
Bacteria	xxx	xxx	xxx	xxx

xxx = percentage frequency of occurrence between 50-100; xx = 30-49 percent; x = 0.01-29 percent; - = none.

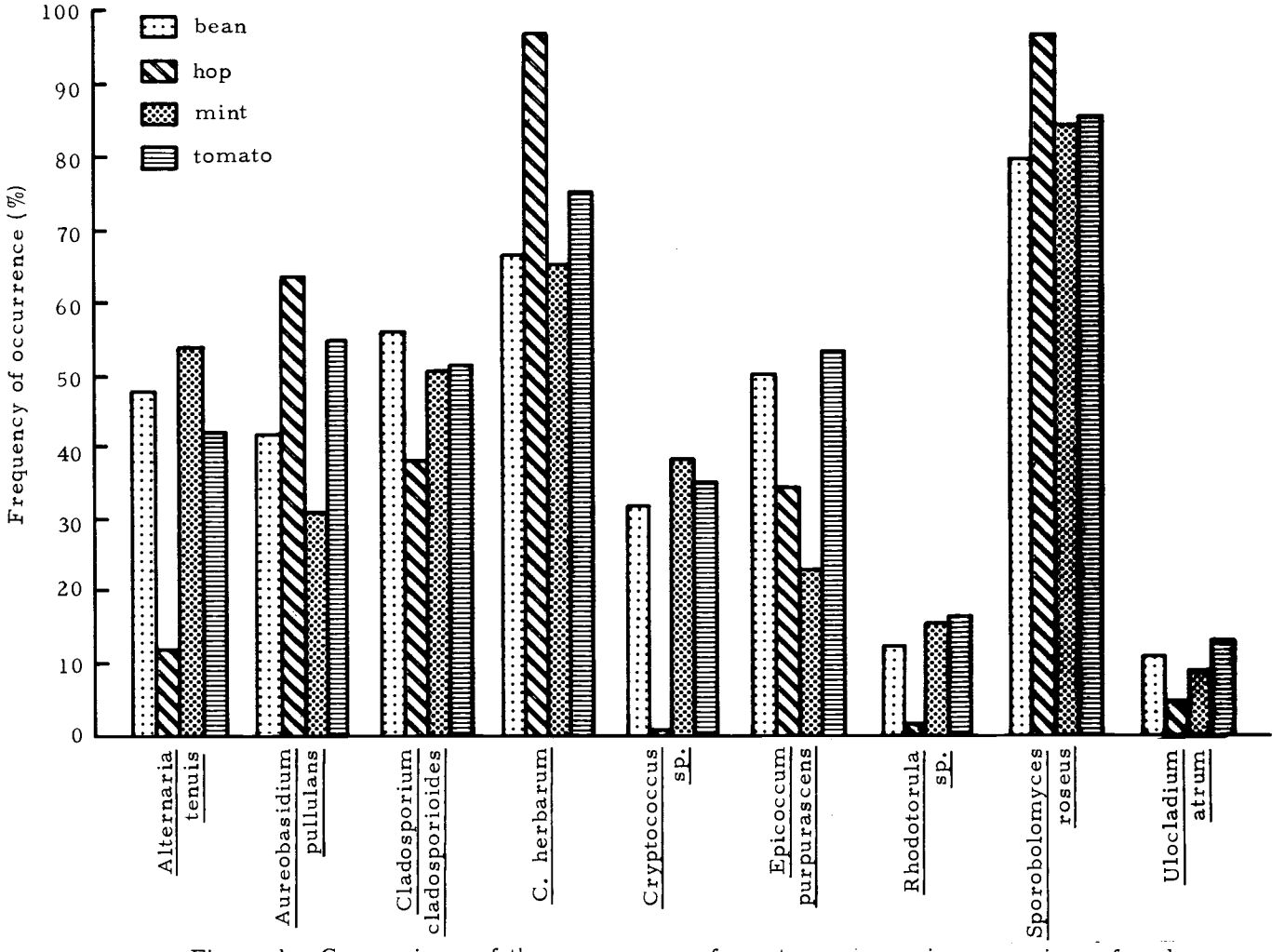

Figure 1. Comparison of the occurrence of most common microorganisms found on leaf surfaces of all four host species.

Second, microorganisms which occurred mainly on bean, mint and tomato leaf surfaces, with the range of frequency of occurrence between 0.01-29 percent.

Third, microorganisms which grow mainly on tomato leaves and some on mint or bean leaves.

Fourth, microorganisms which grew on tomato and mint leaves only. And the percentage was between 0.01-29 percent.

Fifth, other microorganisms besides fungi; i.e., species of actinomycetes and bacteria that were unidentified. These microorganisms, especially bacteria, abound on all kinds of crop leaves and at every location.

Surface Sterilization Method

Seven species of microorganisms were isolated from inside the living tissues of all four crop leaves collected from all seven locations. They were: Alternaria tenuis, Cladosporium herbarum, Epicoccum purpurascens, Phoma sp., Stemphylium botryosum, Ulocladium atrum and Verticillium sp. The colonies came out around the cut edges; sometimes they appeared at one corner. Fig. 2 showed the characteristic of some fungi gowing out from the leaf margins. If the leaf fragments are incubated longer than 10 days, the colonies will spread over the entire leaf squares.

Fig. 2-5. Internal fungi growing out from the cut edges of bean leaf squares.

Fig. 2. Conidial chains of Alternaria tenuis (X110).

Fig. 3. Chains of Cladosporium herbarum (X110).

Fig. 4. Mass of Epicoccum purpurascens with muriform conidia (X110).

Fig. 5. Pycnidia of Phoma sp. (X55).


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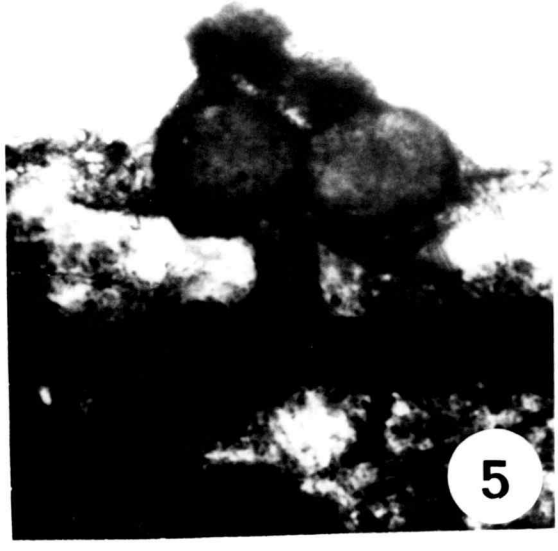
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Bean (Phaseolus vulgaris)

Alternaria tenuis, Cladosporium herbarum, Epicoccum purpurascens, Stemphylium botryosum and Ulocladium atrum were isolated from all locations at percentages between 2-57 percent. The rest of them were found at one and three locations (Appendix V).

Hop (Humulus lupulus)

Every species cited above, with the exception of Verticillium sp., were isolated from hop leaves with frequencies between 0.4-17 percent (Appendix VI).

Mint (Mentha piperita)

All seven species were found inside the healthy mint leaves collected from Benton, Linn, Lane, Marion and Polk counties. Their frequencies were between 0.16-12 percent (Appendix VII).

Tomato (Lycopersicon esculentum)

Among seven fungi, Alternaria tenuis, Cladosporium herbarum and Ulocladium atrum were the most commonly isolated, with 3-20 percent frequency of occurrence (Appendix VIII).

Table 3 lists the internal fungi found inside the leaves of these four crops from several locations. Alternaria tenuis showed the

Table 3. Incidence of internal fungi inside healthy leaves of bean, hop, mint and tomato collected from different locations in the Willamette Valley (in percent).

Fungi	Bean	Hop	Mint	Tomato
Alternaria tenuis	57. 77	8. 80	12. 00	20. 11
Cladosporium herbarum	13. 20	17. 60	0. 82	10. 51
Epicoccum purpurascens	2. 40	2. 40	0. 32	0. 51
Phoma sp.	1. 20	0. 40	0. 88	0. 17
Stemphylium botryosum	9. 34	2. 80	0. 16	2. 05
Ulocladium atrum	7. 77	4. 40	0. 40	3. 57
Verticillium sp.	0. 17	--	0. 80	0. 17

highest percentage inside bean leaves. Verticillium sp. was the only one that has not been observed from inside healthy leaves of hop.

DISCUSSION

Four kinds of cultivated crops, i. e., bean, hop, mint and tomato, were selected for observation of phylloplane microorganisms. These microorganisms have not been reported before on hop and mint leaf surfaces. 'Fuggle-H' variety and 'Mitcham' peppermint were chosen because they are the most important varieties grown in Oregon, particularly in the Willamette Valley area. Although the phylloplane microflora of bean and tomato have been studied before (Sinha, 1971; Last and Warren, 1972, different methods were used.

The examination of the surfaces of healthy leaves of four test crops has revealed the presence of several species of phylloplane microorganisms. Most of them have been found previously on the phylloplane of other plants (Last, 1955; Dickinson, 1965; Last and Deighton, 1965; Hogg and Hudson, 1966; Hollomon, 1967; Hislop and Cox, 1969; Lamb and Brown, 1970; Pugh and Buckley, 1971a; Sinha, 1971; Godfrey, 1974). The principal groups of these microorganisms are pigmented and non-pigmented fungi, yeast-like fungi (including members of the Cryptococcaceae and Sporobolomycetaceae), actinomycetes and bacteria. Alternaria tenuis, Aureobasidium pullulans, Cladosporium cladosporioides, C. herbarum, Cryptococcus sp., Epicoccum purpurascens, Sporobolomyces roseus, actinomycetes

and bacteria were usually present and numerous on the leaves of four of the test plants. Botrytis cinerea, Mucor sp., Penicillium spp. and Tilletiopsis spp. occurred on all hosts but were not as abundant as those mentioned above. Some fungi were associated exclusively with three and two of the hosts. From the second block downward, except the last one, in Table 2 are listed the name of species as well as their hosts. Helminthosporium biforme Mason & Hughes and Bipolaris sp. cf. kusanoi (Nisikado) Shoem. so far have not been reported on these four crops. They are rare in this study also.

Since most phylloplane microorganisms have been found elsewhere and are widespread on all hosts and locations in the Willamette Valley, it can be concluded that they have very wide geographic range and they are adapted to live on the leaf surfaces of many different hosts. These phylloplane microorganisms are apparently commensals which cause no symptoms nor evident pathological condition on the host plants. Some of them, found in this research, such as Phoma sp. and Verticillium sp., are probably pathogens or facultative pathogens. Both have been reported to be capable of causing diseases on these host plants, especially mint (Horner, 1971; Skotland, 1971; Melouk and Horner, 1972). That they did not show any sign of symptoms at the time of collection may be due to lack of virulence or they may have been present only as dormant spores. The percentage frequency of these species is less than 4 percent.

Common soil-inhabiting fungi such as Fusarium spp., Chaetomium cochliodes Palliser etc. (Table 2) were isolated from bean, mint and tomato only but none was found from hop leaves. Perhaps this difference is due to the proximity of bean, mint, and tomato to the soil, resulting in contamination by splashing which would not reach the higher hop leaves.

The method of collection and storage of leaf specimens for microbial examination may influence the results of the examination. It has been recently reported by Millar and Richards (1974) that a polyethylene bag is not a suitable container for storing the leaf samples during transit and storage, even in the cold room. The reason is that a polyethylene bag develops an extremely high humidity level and functions as a good moist chamber, allowing some microorganisms like Hendersonia acicola (Tebeuf) (New Zealand hard beech) and yeast to multiply prior to isolation (Millar and Richards, 1974). This study was done before the Millar and Richards report was published. They recommended surface-sterilization of leaf samples at the time of collection, then storage in paper bags during transit. This method cannot be used to study propagules on the leaf surface, since the surfactant will reduce the amount of population.

A leaf impression method (one type of cultural method) was used in this study. There are some advantages and disadvantages over the other methods. Some advantages are: 1) the leaf impression method

is relatively simple, requires no elaborate equipment and costs less to utilize; 2) it reveals living materials, i. e., microorganisms that actively growing and sporulating on the leaf surfaces (resident), detachable propagules which may be actively growing or propagules deposited on leaf but which have not germinated (transit) and fungi growing vegetatively on the leaf surfaces; 3) it allows isolation, culture and subsequent identification. Some disadvantages are: 1) it will not reveal the presence of fungi which do not grow at all in culture (obligate parasites, e. g., powdery mildew), or which grow poorly on the media used for isolation; 2) it does not show detailed spatial arrangement (although, since small pieces are used, one could distinguish petiolar end from tip; margin from center, etc.); 3) it does not distinguish between resident and transient fungi nor between resident fungi and transient (but viable) spores; 4) it is not able to detect all microorganisms even if they can grow in culture; and 5) it is slow, comparison with fluorescent microscopy.

A combination of several methods would probably give the best results, for instance, using a cultural method combined with scanning electron microscopy. This would show what species occur, how they are arranged, and in what part of the leaf they are established and so on.

Internal fungi were found inside living tissues of all four host crop leaves by surface sterilization and subsequent cultural isolation.

Those species which grew from the cut edges of surface-sterilized leaf squares were similar to those that occurred on leaf surfaces. It could be speculated that mycelia of these fungi, which normally grow on healthy leaves, penetrate the living tissues and persists as a latent infection. The fungi may develop further when the leaves senesce and aid in the decomposition of the leaf after leaf-fall. This has already been reported for other fungi on other hosts in other areas (Hogg and Hudson, 1966; Ruscoe, 1971 etc.). Some fungi, like Phoma sp. or Verticillium sp., have also been reported to cause diseases. Whether they are the same species of Phoma and Verticillium and, if so, under what conditions they become pathogenic, needs further study.

It is interesting to note that Aureobasidium pullulans has not been isolated from interior part of any of the crops in this study even though it commonly occurred on the surface of unsterilized leaf squares. This observation is in contrast to the experience of other workers (Kendrick and Burges, 1962; Pugh and Buckley, 1971b; Ruscoe, 1971; Beech and Davenport, 1971; Millar, 1974), who isolated Aureobasidium pullulans from leaf interiors.

BIBLIOGRAPHY

Baigent, N. L., and J. M. Ogawa. 1960. Activity of the antibiotic produced by Pullularia pullulans. (Abstr.) Phytopath. 50:82.

Beech, F. W., and R. R. Davenport. 1971. A survey of methods for the quantitative examination of the yeast flora of apple and grape leaves, p. 139-157. In T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Bernstein, M. E., H. M. Howard, and G. C. Carroll. 1973. Fluorescence microscopy of Douglas fir foliage epiflora. Can. J. Microbiol. 19:1129-1130.

Bernstein, M. E. 1974. Microbial populations of Douglas fir needles. Doctoral dissertation. University of Oregon, Eugene. 122 pp.

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

Dickinson, C. H. 1965. The microflora associated with Halimione portulacoides. III. Fungi on green and moribund leaves. Trans. Brit. Mycol. Soc. 48:603-609.

Dickinson, C. H. 1967. Fungal colonization of pisum leaves. Can. J. of Bot. 45:915-927.

Dickinson, C. H. 1971. Cultural studies of leaf saprophytes, p. 129-137. In T. F. Preece and C. H. Dickinson (eds.). Ecology of leaf surface microorganisms. Academic Press, London.

Diem, H. G. 1971. Effect of low humidity on the survival of germinated spores commonly found in the phyllosphere, p. 211-219. In T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Godfrey, B. E. S. 1974. Phylloplane microflora of bracken, Pteridum aquilinium. Trans. Brit. Mycol. Soc. 62:305-311.

Heuvel, J. van den. 1971. Antagonism between pathogenic and saprophytic Alternaria species on bean leaves, p. 537-544. In

T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Hislop, E. C., and T. W. Cox. 1969. Effects of Captan on the non-parasitic microflora of apple leaves. Trans. Brit. Mycol. Soc. 52:223-235.

Hogg, B. M., and H. J. Hudson. 1966. Micro-fungi on leaves of Fagus sylvatica. I. The micro-fungal succession. Trans. Brit. Mycol. Soc. 49 185-192.

Hollomon, D. W. 1967. Observation on the phylloplane flora of potatoes. Europe Potato Journal 10:53-61.

Horner, C. E. 1971. Rhizome and stem rot of peppermint caused by Phoma strasseri. Plant Dis. Reptr. 9:814-816.

Hudson, H. J. 1962. Succession of micro-fungi on aging leaves of Saccharum officinarum. Trans. Brit. Mycol. Soc. 45:395-423.

Hudson, H. J., and J. Webster. 1958. Succession of fungi on decaying stems of Agropyron repens. Trans. Brit. Mycol. Soc. 41:165-177.

Jones, K. 1970. Nitrogen fixation in the phyllosphere of the Douglas fir, Pseudotsuga douglasii. Ann. Bot. N. S. 34:239-244.

Kendrick, B. and A. Burges. 1962. Biological aspects of decay of Pinus sylvestris leaf litter. Nova Hedwigia 4:313-342.

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

Last, F. T. 1955. Seasonal incidence of Sporobolomyces on cereal leaves. Trans. Brit. Mycol. Soc. 38:221-239.

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.

Last, F. T., and R. C. Warren. 1972. Non-parasitic microbes colonizing green leaves; their form and functions. Endeavour 31:143-150.

Leben, C. 1965. Epiphytic microorganisms in relation to plant disease. Ann. Rev. Phytopath. 3:209-230.

McBride, R. P. 1972. Larch leaf waxes utilized by Sporobolomyces roseus in situ. Trans. Brit. Mycol. Soc. 58:329-331.

Melouk, H. A. and C. E. Horner. 1972. Growth in culture and pathogenicity of Phoma strasseri to peppermint. Phytopath 62:576-578.

diMenna, M. E. 1959. Yeasts from the leaves of pasture plants. New Zealand J. Agr. Res. 2:394-405.

Millar, C. S. 1974. Decomposition of coniferous leaf litter, p. 105-128. In T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Millar, C. S. and G. M. Richards. 1974. A cautionary note on the collection of plant specimens for mycological examination. Trans. Brit. Mycol. Soc. 63:607-610.

Pugh, G. J. F., and N. G. Buckley. 1971a. The leaf surface as a substrate for colonization by fungi, p. 431-445. In T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Pugh, G. J. F., and N. G. Buckley. 1971b. Aureobasidium pullulans: an endophyte in sycamore and other trees. Trans. Brit. Mycol. Soc. 57:227-231.

Rasmussen, R. A., and R. S. Hutton. 1972. Utilization of atmospheric organic volatiles as an energy source by microorganisms in the tropics. Chemosphere 1:47-50.

Rayner, R. W. 1948. Latent infection in Coffea arabica L. Nature, London 161:245-246.

Ruinen, J. 1956. Occurrence of Beijerinckia species in the phyllosphere. Nature, London 177:220-221.

Ruinen, J. 1963. The phyllosphere. II. Yeasts from the phyllosphere of tropical foliage. Antonie van Leeuwenhoek 29:425-438.

Ruinen, J. 1965. The phyllosphere. III. Nitrogen fixation in the phyllosphere. Plant and Soil 22 375-394.

Ruscoe, Q. W. 1971. Mycoflora of living and dead leaves of Nothofagus truncata. Trans. Brit. Mycol. Soc. 56:463-474.

Russell, G. E. 1965. The control of Alternaria species on leaves of sugar beet infected with yellowing viruses. I. Some effects of four fungicides on two beet varieties. Ann. Appl. Biol. 56: 111-118.

Sinha, S. 1971. The microflora on leaves of Capsicum annuum (L.) Watt E. D., Solanum melongena L., Solanum tuberosum L. and Lycopersicum esculentum Mill., p. 175-189. In T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Skotland, C. B. 1971. Pathogenic and nonpathogenic Verticillium species from south central Washington. Phytopath. 61:435-436.

Stott, M. A. 1971. Studies on the physiology of some leaf saprophytes, p. 203-210. In T. F. Preece and C. H. Dickinson (eds.), Ecology of leaf surface microorganisms. Academic Press, London.

Webster, J. 1957. Succession of fungi on decaying cocksfoot culms. J. Ecol. 45:1-30.

APPENDICES

Appendix I. Percentage frequency of occurrence isolated from leaf surfaces of bean (Phaseolus vulgaris L.) collected from seven locations.

Microorganism	Location							Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk	Washington	Yamhill		
Acremoniella atra	0.4		1.6		3.2			5.2	0.74
Alternaria tenuis	53.6	10.8	56.0	63.6	84.0	25.6	40.8	334.4	47.77
Aspergillus niger						1.2	0.8	2.0	0.28
Aureobasidium pullulans	27.2	55.6	15.2	64.4	25.6	82.0	24.8	294.8	42.11
Botrytis cinerea	0.4	0.4			2.8		0.4	4.0	0.57
Cephalosporium sp.	0.8		0.8		3.2			4.8	0.68
Chaetomium cochliodes	1.2							1.2	0.24
Cladosporium cladosporioides	74.0	27.2	36.8	81.6	57.6	50.4	65.2	392.8	56.11
C. herbarum	72.0	32.8	37.2	85.6	93.2	58.0	90.4	469.2	67.03
Cryptococcus sp.	60.4	1.6	81.2	7.2	11.2	57.2	6.8	225.6	32.23
Curvularia clavata					0.4			0.4	0.06
C. inequalis						0.4		0.4	0.06
C. lunata					0.8			0.8	0.11
Epicoccum purpurascens	69.2	7.6	23.6	65.6	81.2	41.7	71.2	360.1	51.44
Fusarium oxysporum	0.8	24.4	0.4	4.4	1.2	3.6	1.2	36.0	5.14
F. semitectum		0.8		0.8	3.2	0.4		5.2	0.74
F. solani	0.8	0.8	0.4	0.8			0.4	3.2	0.46
Helminthosporium biforme						0.4		0.4	0.06
Mucor sp.		0.4		0.4		0.8		1.6	0.23
Nigrospora sphaerica	0.4		0.6	1.2				2.2	0.31
Penicillium spp.	0.4	1.2	0.6		0.4	4.8	1.2	8.6	1.23
Phoma sp.	2.8		0.4	2.4	1.2	1.6	7.2	15.6	2.23
Polyscytalum sp.	0.8				1.2			2.0	0.29
Rhizopus stolonifera	0.4	6.8	0.4					7.6	1.09
Rhodotorula sp.	39.2		16.8	4.4	16.8	3.2	7.6	88.0	12.57
Sporobolomyces roseus	66.0	79.2	72.4	90.8	81.6	86.0	81.0	557.2	79.60

Appendix I. (Continued)

Microorganism	Location							Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk	Washington	Yamhill		
Stemphylium botryosum	0.4		0.8		4.4		7.2	12.8	1.83
Tilletiopsis minor	2.0							2.0	0.29
T. washingtonensis					0.4		0.4	0.8	0.11
Ulocladium atrum	17.2	17.6	3.4	14.8	7.6	12.0	7.2	79.8	11.40
Actinomycetes	14.4	25.2	4.4	4.0	8.0	3.6	0.8	60.4	8.63
Bacteria	54.0	61.6	54.4	54.4	57.6	32.4	44.8	359.2	51.31

Appendix II. Percentage frequency of occurrence of microorganisms isolated from leaf surfaces of hop (Humulus lupulus L.) collected from Linn County.

Microorganism		Frequency of occurrence (%)
Alternaria tenuis	28	11.2
Aureobasidium pullulans	162	64.8
Botrytis cinerea	3	1.2
Cladosporium cladosporioides	96	38.4
C. herbarum	244	97.6
Cryptococcus sp.	2	0.8
Epicoccum purpurascens	87	34.8
Myrothecium verrucaria	1	0.4
Mucor sp.	1	0.4
Penicillium spp.	4	1.6
Rhodotorula sp.	3	1.2
Sporobolomyces roseus	244	97.6
Ulocladium atrum	11	4.4
Actinomycetes	9	3.6
Bacteria	227	90.8

Appendix III. Percentage frequency of occurrence of microorganisms isolated from leaf surfaces of mint (Mentha piperita L. 'Mitcham') collected from five locations.

Microorganism	Location					Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk		
Acremoniella atra	2.4	0.4				2.8	0.56
Alternaria tenuis	66.0	69.6	20.8	53.6	60.00	270.0	54.00
Aspergillus niger					0.4	0.4	0.4
Aureobasidium pullulans	15.6	32.4	63.6	23.0	21.2	155.8	31.16
Bipolaris sp. cf. kusanoi			1.6			1.6	0.32
Botrytis cinerea			0.4			0.4	0.08
Cephalosporium sp.	0.4	0.8		0.8	0.4	2.4	0.48
Chaetomium cochliodes		0.4				0.4	0.08
Cladosporium cladosporioides	54.0	51.2	43.2	48.0	58.0	254.4	50.88
C. herbarum	58.4	69.6	62.0	65.2	71.2	326.5	65.28
Cryptococcus sp.	32.8	41.6	27.6	41.6	47.6	191.2	38.24
Curvularia inequalis			4.0			4.0	0.80
C. lunata			0.4			0.4	0.08
Epicoccum purpurascens	44.0	39.6	18.4	12.6		114.6	22.92
Fusarium oxysporum	4.4		0.4	0.4	8.0	13.2	2.64
F. semitectum	1.2					1.2	0.24
F. solani	0.8					0.8	0.32
Mucor sp.	0.8		1.2	0.4		2.4	0.48
Nigrospora sphaerica	0.8				0.4	1.2	0.24
Penicillium spp.	0.8	2.0	0.8	0.8	1.6	6.0	1.20
Phoma sp.	3.6	2.0	12.0	1.2	1.2	20.0	4.0
Polyscytalum sp.	2.0				0.4	2.4	0.48
Rhizopus stolonifera	0.4					0.4	0.08
Rhodotorula sp.	22.4	17.2	3.6	0.8	24.4	78.4	15.68
Sporobolomyces roseus	78.8	82.0	92.8	84.0	84.0	421.6	84.32

Appendix III. (Continued)

Microorganism	Location					Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk		
Stemphylium botryosum		0.8		1.2		2.0	0.40
Tilletiopsis minor	0.4	3.2		10.4	8.0	22.0	4.40
T. washingtonensis	2.0	2.0		8.8	1.6	14.4	2.88
Trichothecium roseum					0.4	0.4	0.08
Ulocladium atrum	14.8	13.6	7.2	2.4	3.2	41.0	8.20
Verticillium sp	0.4			0.4		0.8	0.16
Actinomycetes	66.4	32.0	1.6	48.8	28.8	177.6	35.52
Bacteria	38.4	56.8	11.6	32.0	61.6	200.4	40.08

Appendix IV. Percentage frequency of occurrence of microorganisms isolated from leaf surfaces of tomato (Lycopersicon esculentum Mill.) collected from seven locations.

Microorganism	Location							Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk	Washington	Yamhill		
Acremoniella atra	2.4	6.0			1.6		2.0	12.0	1.71
Altemaria tenuis	65.6	82.4	31.2	18.8	56.0	19.6	22.4	296.0	42.29
Aspergillus niger			1.2	0.4		0.4		2.0	0.29
Aureobasidium pullulans	35.2	33.6	33.6	90.8	49.2	85.6	58.0	396.0	55.14
Botrytis cinerea	3.2	0.4					0.4	4.0	0.57
Cephalosporium sp.	2.8							2.8	0.40
Cladosporium cladosporioides	74.4	1.6	45.2	55.2	67.2	58.0	61.2	362.8	51.83
C. herbarum	88.0	97.6	62.0	63.2	87.2	64.4	72.0	534.4	76.34
Chaetomium cochliodes	1.2							1.2	0.17
Colletotrichum sp.				0.4				0.4	0.06
Cryptococcus sp.	52.8	92.4	1.6	19.6	14.0	40.8	26.4	247.6	35.37
Curvularia clavata	0.4	1.2			0.4			2.0	0.29
C. inequalis			0.8					0.8	0.11
Epicoccum purpurascens	85.6	64.8	30.4	58.0	70.4	33.2	34.0	376.4	53.77
Fusarium equiseti			0.4					0.4	0.06
F. moniliforme				2.8				2.8	0.40
F. oxysporum		0.8	4.8	6.4	0.4			12.4	1.77
F. semitectum	0.4				0.4	1.6		2.4	0.34
F. solani	6.4		1.2	0.8		2.4	0.4	11.2	1.60
Helminthosporium biforme			0.4			0.8	0.4	1.6	0.23
Mucor sp.	0.8				0.8	1.2		2.8	0.40
Nigrospora sphaerica		1.6						1.6	0.23
Penicillium spp.	1.6		2.8	2.0		1.2	2.0	7.6	1.09
Phoma sp.		0.4	0.8	4.0		1.6		6.8	0.97
Polyscytalum sp.	5.2	2.8			0.4			8.4	1.20
Rhizopus stolonifera			0.8			1.6		2.4	0.34

Appendix IV. (Continued)

Microorganism	Location							Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk	Washington	Yamhill		
Rhodotorula sp.	52.4	29.6	12.8	14.8	7.2	1.2		118.0	16.86
Sporobolomyces roseus	69.2	88.0	78.8	84.4	97.6	90.4	92.8	601.2	85.86
Stemphylium botryosum	0.4	0.4			4.4			5.2	0.74
Tilletiopsis minor	0.4				0.4			0.8	0.11
T. washingtonensis	0.8				1.6			2.4	0.34
Ulocladium atrum	18.0	9.6	18.8	13.6	6.4	14.6	4.8	90.8	12.97
Verticillium sp.			0.4					0.4	0.06
Actinomycetes	12.8	6.0	28.8	2.4	2.0	3.2	1.2	56.4	8.06
Bacteria	56.0	69.2	80.4	50.8	40.8	50.8	52.0	400.0	57.14

Appendix V. Percentage frequency of occurrence of microorganisms isolated from surface-sterilized leaf squares of bean (Phaseolus vulgaris L.) collected from seven locations.

Microorganism	Location							Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk	Washington	Yamhill		
Alternaria tenuis	68.0	51.2	72.8	47.2	76.8	47.6	40.8	404.4	57.77
Cladosporium herbarum	2.4	4.8	11.2	19.6	5.6	19.2	29.6	92.4	13.20
Epicoccum purpurascens	4.0	0.4	0.4	5.6	2.8	0.4	3.2	16.8	2.40
Phoma sp.			6.8		0.8	0.8		8.4	1.20
Stemphylium botryosum	2.8	8.2	7.6	13.2	6.0	19.2	8.4	65.4	9.34
Ulocladium atrum	7.2	2.6	3.6	8.8	2.8	7.2	1.2	33.4	4.77
Verticillium sp.	0.8							0.8	0.11

Appendix VI. Percentage frequency of occurrence of microorganisms isolated from surface-sterilized leaf squares of hop (Humulus lupulus L.) collected from Linn County.

Microorganism		Frequency of occurrence (%)
Alternaria tenuis	22	8. 80
Cladosporium herbarum	44	17. 60
Epicoccum purpurascens	6	2. 40
Phoma sp.	1	0. 40
Stemphylium botryosum	7	2. 80
Ulocladium atrum	11	4. 40

Appendix VII. Percentage frequency of occurrence of microorganisms isolated from surface-sterilized leaf squares of mint (Mentha piperita L. 'Mitcham') collected from five locations.

Microorganism	Location					Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk		
Alternaria tenuis	6.0	23.6	5.6	12.8	12.0	60.0	12.00
Cladosporium herbarum			0.4	1.3	2.4	4.1	0.82
Epicoccum purpurascens			1.2	0.4		1.6	0.32
Phoma sp.			2.8		1.6	4.4	0.88
Stemphylium botryosum				0.8		0.8	0.16
Ulocladium atrum	0.4	1.2	0.4			2.0	0.40
Verticillium sp.	1.2	2.4			0.4	4.0	0.80

Appendix VIII. Percentage frequency of occurrence of microorganisms isolated from surface-sterilized leaf squares of tomato (Lycopersicon esculentum Mill.) collected from seven locations.

Microorganism	Location							Total	Frequency of occurrence (%)
	Benton	Lane	Linn	Marion	Polk	Washington	Yamhill		
Alternaria tenuis	22.8	38.0	26.0	20.4	12.0	15.6	6.0	140.8	20.11
Cladosporium herbarum	7.2	24.8	6.0	20.4	7.6	5.2	2.4	13.6	10.51
Epicoccum purpurascens		2.0			0.8	0.4	0.8	4.0	0.57
Phoma sp.			0.8		0.4			1.2	0.17
Stemphylium botryosum	1.6			6.0	1.6	4.0	1.2	14.4	2.05
Ulocladium atrum	1.2	8.0	10.8	0.4	0.4	4.0	1.2	26.0	3.57
Verticillium sp.	1.2							1.2	0.17