Technical Report No. 42

THE ROLES OF FUNGI IN HAWAIIAN ISLAND ECOSYSTEMS
I. FUNGAL COMMUNITIES ASSOCIATED WITH LEAF SURFACES OF THREE ENDEMIC VASCULAR PLANTS IN KILAUEA FOREST RESERVE AND HAWAII VOLCANOES NATIONAL PARK, HAWAII

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ABSTRACT

Three vascular plants endemic in native forests of the Hawaiian Islands were assessed for their phylloplane communities of fungi. The total Metrosideros collina var. polymorpha community (residents and transients) was three times greater than that of Acacia koa. The Cheirodendron trigynum var. trigynum community was not considered significant because of fewer samples. Community overlap of the total populations was 14% for Metrosideros and Acacia; 10.6% for all three leaves. Resident populations, if determined as fungi recovered only by maceration, were represented by 40 species for Metrosideros and 30 for Acacia. The difference between total and resident populations or species abundance can be equated to leaf anatomy, particularly the surface as a trapping and supporting layer. Of the resident fungi only a few could be considered endemic species. This does not preclude the possibility that others may exist. Distribution of populations by elevation was sporadic and reflected no clear evidence for altitudinal control.

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INTRODUCTION

If the community approach to the study of ecology provides a means of assessing (1) species distinctiveness with respect to habitat utilization; (2) the nature of ecological separation and/or overlap within the community; and (3) the influence of cardinal environmental parameters on habitat selection, species abundance, distribution, and diversity (Conant 1972), then leaf surfaces as a habitat for fungi (the phylloplane) should provide an elegant system for such analysis. For phylloplane fungi the leaf surface offers a distinctive, and limiting, substratum depending on the particular leaf species (Dickinson 1971). Given communities can be compared for similarities and differences among their constituent members. The role of environmental factors may be primary or secondary in influence (Pugh and Buckley 1971).

Studies of the phylloplane fungi associated with selected vascular plants were in progress from September 1970 to August 1972. The phylloplane communities selected were those of *Metrosideros collina* (Forst.) Gray var. *polymorpha* (Gaud.) Rock, known as ohia lehua; *Acacia koa* Gray var. *hawaiiensis* Rock, or koa; and *Cheirodendron trigynum* (Gaud.) Heller var. *trigynum*, called olapa. The three are endemic in the native forests of the Hawaiian Islands. Besides offering a means of assaying the relationship between fungal communities and specific leaf substrata, the selection of endemic leaves may also provide an indication of whether or not the relationship between fungi and leaf is close enough to warrant the recognition of endemism among the fungi.

METHODS

Study areas

The major sampling sites for these studies are located on the island of Hawaii: in Kilauea Forest Reserve, at an elevation of 5,400 ft; and on the east-flank of

Mauna Loa in Hawaii Volcanoes National Park (FIG. 1). These sites are on Transect Profiles 1, 2, and 3 established for the Island Ecosystems IRP, U. S. International Biological Program (Mueller-Dombois 1972:22 ff.). The Park locations are on an altitudinal gradient extending from 2,250 ft to 8,250 ft. TABLE 1 summarizes the characteristics of the sites on the transects. Samples were taken in their approximate locations. The actual altitude of each sample position was determined by a Thommen Everest pocket altimeter, Model 3-D6.

Metrosideros occurs over the entire gradient. *Acacia koa* does not occur above 6,700 ft (Mueller-Dombois and Krajina 1968). FIG. 2 illustrates their biomass distribution as developed on Mauna Loa and Kilauea (Mueller-Dombois 1973). *Cheirodendron trigynum* var. *trigynum* is best developed at 4,200 ft in the Bird Park. The distribution of vegetation is shown in FIG. 3, which also indicates the climate associated with each site. For comparative purposes, leaves of *Metrosideros* and *Acacia* were collected in upper Manoa Valley on Oahu at an elevation of approximately 600 ft.

Climatically the two areas offer considerable contrast. Kilauea Forest is considered as having a rain forest climate and the sample sites on Mauna Loa as occurring in a summer-dry climate (Doty and Mueller-Dombois 1966). The annual rainfall for Kilauea Forest is near 2,000 mm. Temperatures are equable, with a mean annual temperature of 14^{o}C. Both rainfall and temperature vary along the Mauna Loa transect. At 8,000 ft, annual mean rainfall is $<$ 1,000 mm and the mean annual temperature near 10^{o}C (see FIG. 3).

Leaf samples

Samples along the transect were collected at approximately 1,000-ft intervals between 2,250 and 8,250 ft elevation. Healthy leaves were placed aseptically in sterile plastic bags. After returning to the laboratory the collections were held in refrigerated storage at 5^{o}C until processing, which was usually within 24 hrs.

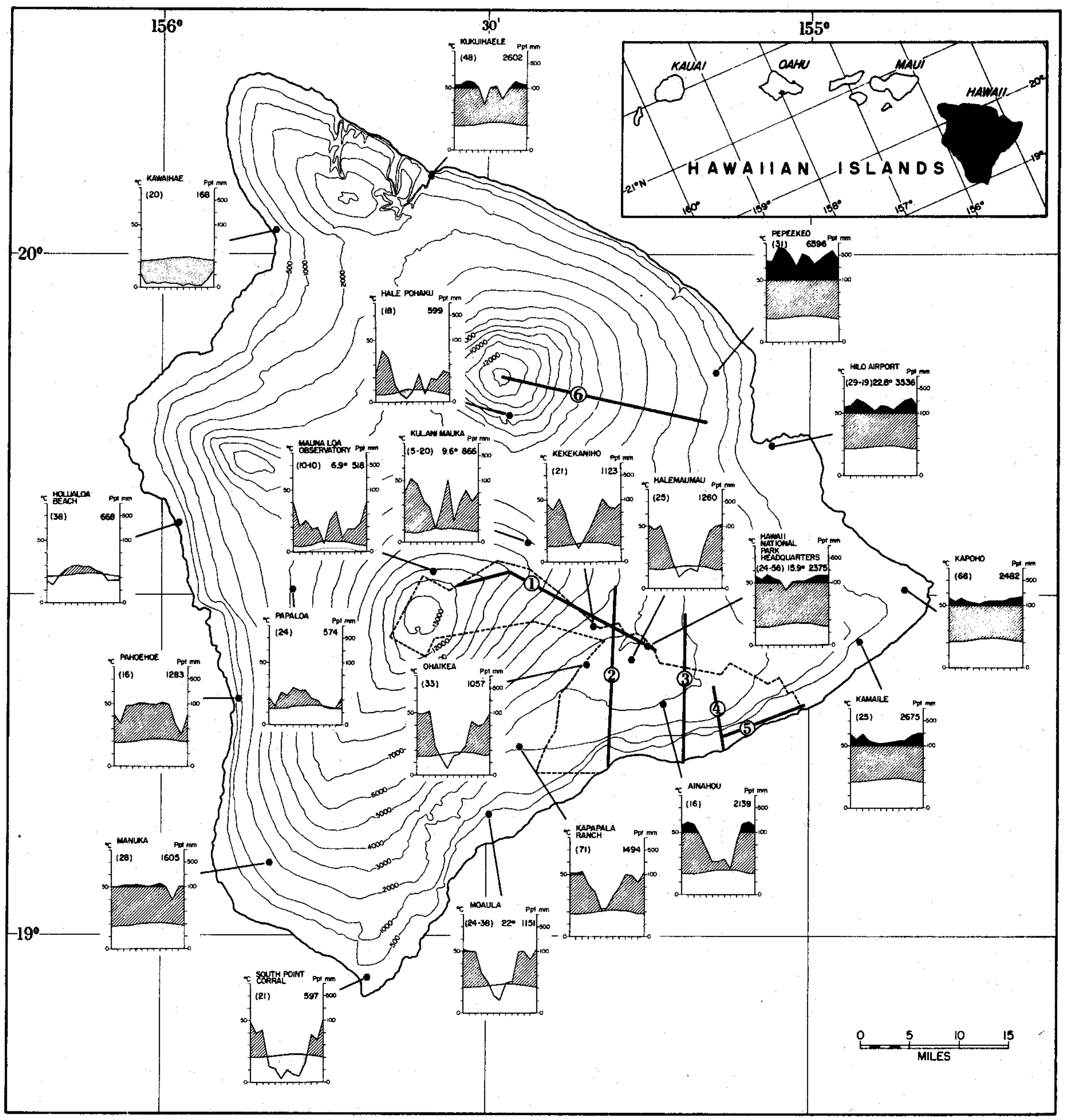


FIG. 1. Location of IBP transects 1-6. The Kilauea rain forest site is at the north end of T-2. Dashed lines indicate the limits of Hawaii Volcanoes National Park. Mean monthly rainfall (mm), temperature curves (0°C) and mean annual rainfall at 21 weather stations are also indicated. (From: Mueller-Dombois 1972:22).

TABLE 1. Segments of transect-profiles: TP 1, Mauna Loa, east-flank; TP 2, Kilauea Forest-Hilina Pali; and TP 3, Olaa Forest-Apua Point used as sampling sites.

TP	Segment	Description	Elevation in ft of sampling sites
1	5	Metrosideros tree line ecosystem	8,250
1	6	Open subalpine Metrosideros-Sophora scrub-forest	7,250
1	7	Mountain parkland ecosystem (Acacia koa tree colonies, Styphelia-Dodonaea tall-scrub communities, both in a matrix of subalpine grassland)	5,250
1	8	Acacia koa savanna ecosystem	4,250
3	5	Open Metrosideros-Andropogon forest with native shrubs on shallow ash over pahoehoe lava (seasonal Metrosideros montane forest)	3,250
2	4	Perennial grassland (Andropogon glomeratus and A. virginicus)	2,250
2	11	Acacia koa-Metrosideros-Cibotium (tree fern) forest; a 200-acre IBP study site in Kilauea Forest Reserve owned by Bishop Estate. This area is protected by a fence against invasion of cattle from the neighboring ranchland	5,400

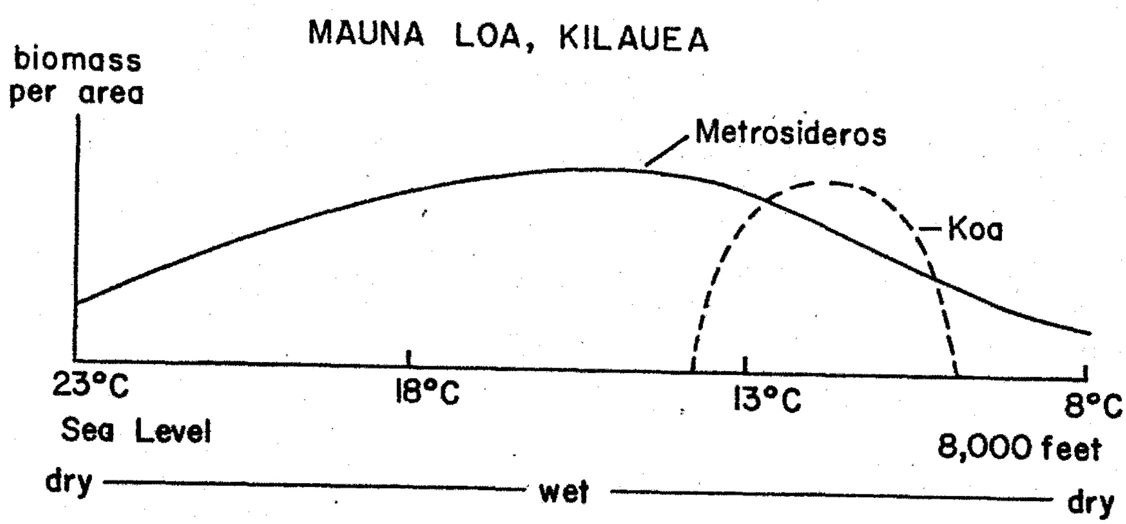


FIG. 2. Distributional characteristics of two major dominant trees. (From: Mueller-Dombois, 1973)

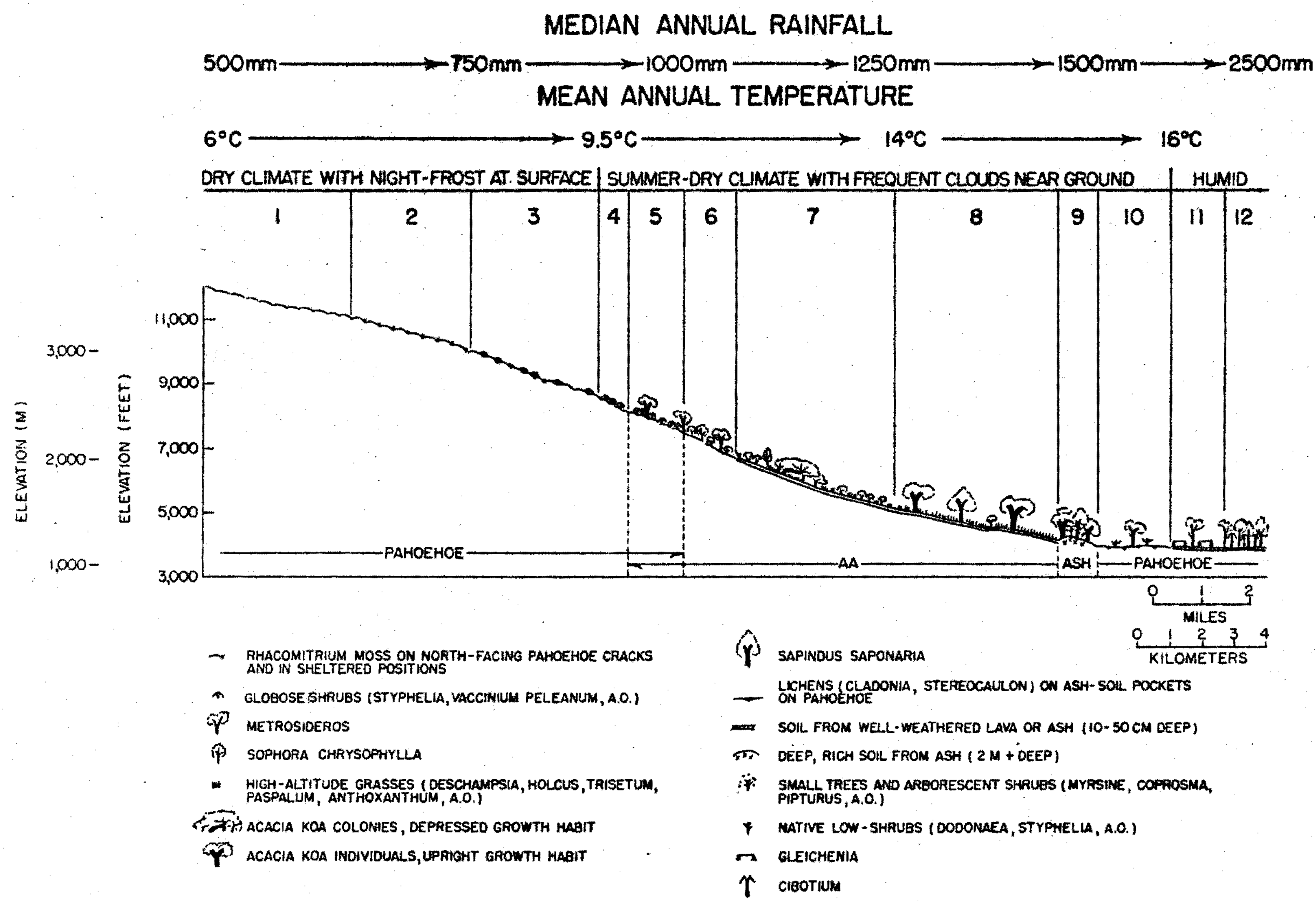


Fig. 3. Transect-profile 1, Mauna Loa - Thurston Lava Tube. Ecosystem types on the east-flank of Mauna Loa from 12,000 feet down to 3900 feet on prehistoric and dominant substrates, 22 miles, starting from Ohaku O Hanalei over Puu Ulaula, Kipuka Puaulu (segment 9) to Kilauea Iki surroundings. (From: Mueller-Dombois, 1972).

Sampling sites and collection data are enumerated in TABLE 2.

Three approaches were employed routinely for the isolation of the fungal taxa associated with the leaf surfaces (Lee and Baker 1971, Baker and Dunn 1972). A fourth method was used initially only for Cheirodendron samples as the other methods proved preferable.

1. Direct observation (moist-chamber method). Washed fresh leaves were incubated in moist chambers for 1 week, then examined microscopically for fungi on both dorsal and ventral surfaces.
2. Leaf washings. Twenty fresh leaves were washed and shaken in several changes of 200 ml sterile distilled water plus 0.1% Tween-80, a wetting agent. The first washing was plated as 10^{-2} and 10^{-3} after further dilution with the same diluent.
3. Leaf maceration. Well washed leaves were cut and then macerated in a sterile Waring blender. The resulting suspension was diluted with sterile distilled water before plating as 10^{-1} and 10^{-2}.
4. Swabbing leaf surfaces. Leaf surfaces were swabbed with a sterile calcium alginate swab (Calgiswab, COLAB), the swab placed in a test tube with 10 ml of sterile distilled water to which an equal amount of sterile 10% Calgon (Calgon Corp.) was added. The suspensions were agitated by hand until the Calgiswabs dissolved, then plated as 1:20, 1:40, and 1:200 dilutions (Marsh 1966).

Three media, sodium caseinate (BBL 11626), Cooke's rose bengal (Difco 0703) and Roth's medium (1964) were used for plating. All media were supplemented either with 50 ug streptomycin per ml or 100 ug penicillin plus streptomycin (50 ug each) per ml to inhibit bacteria, then further supplemented with 0.1% Tergitol NPX (nonionic) detergent (J. T. Baker Chemical Co.) to slow down rapidly growing fungi. Plates were prepared in replicates of 5 and incubated at approximately 22°C on a diurnal light

TABLE 2. Sampling data.

TP2-11 5.297*	TP2-4 2.250	TP3-5 3.500	TP1-8 4.500	TP1-7 5.250	TP1-7 6.600	TP1-6 7.500	TP1-5 8.250	Air All elevations
Acacia koa:								
4-15-72	10- -71	+	+	+	+	--**	--**	+
	1- -72	+	+	+	+	--	--	+
	4-15-72	+	+	+	+	--	--	--
Cheirodendron trigynum var. _trigynum_:								
	9-24-70		4-15-72					--
	4-15-72							--
Metrosideros collina var. _polymorpha_:								
9-24-70	10- -71	+	6- 1-71	--	+	+	+	+
2-14-71	1- -72	+	10- -71	+	+	+	+	--
4-15-72	4-15-72	+	1- -72	+	+	+	+	+
			4-15-72	+	--	--	+	--

* Elevation in ft as 10^3

** No _Acacia koa_ above 6600 ft

cycle (12 hrs light, 12 hrs dark) either by natural light or by fluorescent illumination.

Identifications were made from the original plates as well as from subcultures on other media. All Penicillia and Aspergilli were grown on Czapek's solution agar and malt agar according to standard procedures (Raper and Thom 1949, Raper and Fennell 1965). At the time of sampling, plates of the isolation agars were exposed for air spora at the sample sites (TABLE 2).

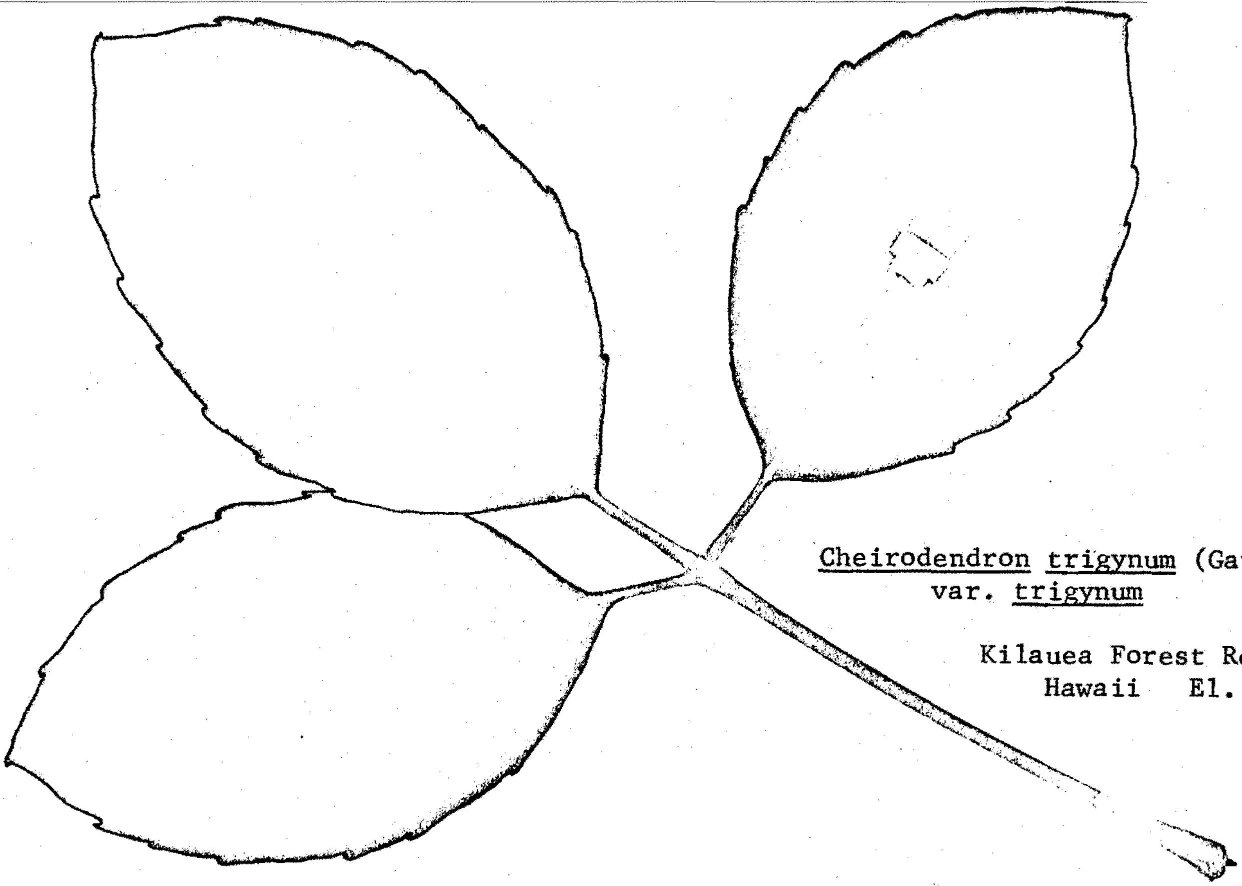
Leaf structure

Free-hand sections of fresh leaves collected on Hawaii were cut for microchemical analysis of the three substrata. Areas selected for sectioning were recorded by making direct xerox impressions of the leaves (FIG. 4). The impressions also illustrate the gross form of the three leaf substrata. Sections were tested for pectinaceous substances with ruthenium red (Jensen 1962); cutin, suberin, and fats with Sudan IV (Jensen, ibid.); and lignin with phloroglucinol (Jensen, ibid.). Sections were also cut with a Hooker Plant Microtome (Lab-Line Instruments, Inc., No. 1225) and were photographed unstained to show morphological characteristics.

Differences in leaf surfaces were studied by EM scan microscopy. Fresh leaves of *Acacia koa* and *Metrosideros collina* var. *polymorpha* collected in Manoa Valley, Oahu, were cut into 2-cm squares and freeze dried by immersion in freon-412 in a liquid nitrogen bath followed by vacuum sublimation using a Virtis 10-010 Automatic Freeze-Dryer. The freeze dried material was gold coated by vacuum evaporation using a Denton DV-502 Vacuum Evaporator. Material was viewed and photographed with a Cambridge Stereoscan S-4 operated at 10 kv using Polaroid 55 p/N film. All EM studies were done by William A. Sakai.

Analysis of data

The fungus taxa recovered by all isolation methods were compiled in a single

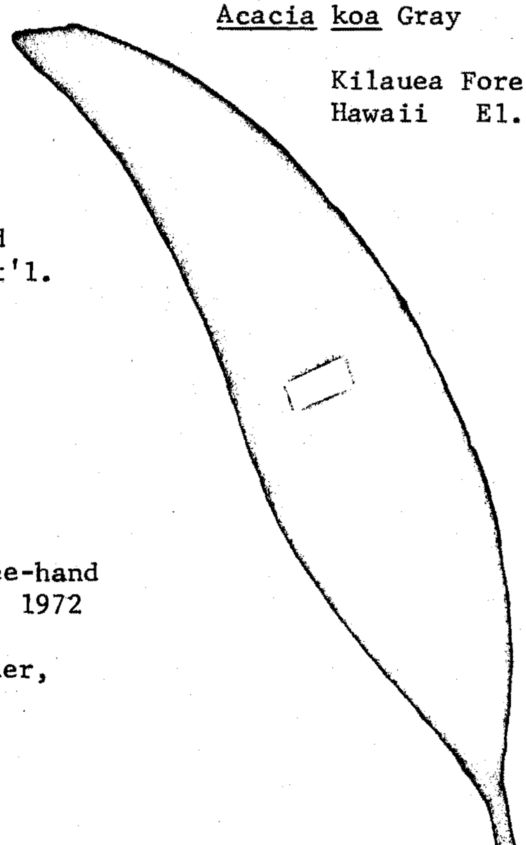


Cheirodendron trigynum (Gaud.)Heller
var. trigynum

Kilauea Forest Reserve,
Hawaii El. 5,297 ft

Acacia koa Gray

Kilauea Forest Reserve
Hawaii El. 5,297 ft



Metrosideros collina (Forst.)Gray
var. polymorpha
(Gaud.) Rock

Mauna Loa strip road
Hawaii Volcanoes Nat'l.
Park El. 5,250 ft

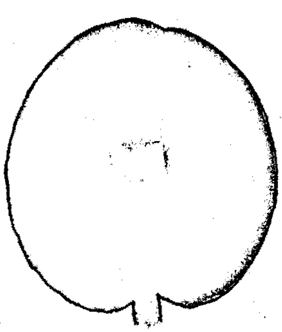


FIG. 4. Xerox impressions of leaves used for free-hand sections and microchemical tests May 1, 1972

Leaves coll. by P. H. Dunn and G. E. Baker,
April 15, 1972

list using a classification system based on Martin (1961) (TABLE 3). Distribution by taxonomic groups was recorded for each leaf individually and in all combinations of the three substrata. The number of species on each of the three leaves per sample site at all elevations was determined with and without surface washings (macerations) (TABLE 4). This data is presented graphically in FIG. 5.

The numbers of fungi by elevation and leaf surface were subjected to Sorensen's method (1948) for establishing groups of equal amplitude based on similarity of species. Comparisons were made for similarity quotients (SQ) of fungi at all elevations for *Metrosideros* and *Acacia*; and for air isolates recovered at sites on TP 1 (TABLES 5, 6, 7). Isolates from leaves were tested both with and without washings. Similarity quotients were determined between pairs of leaf substrata at all elevations (TABLE 8) for isolates obtained by direct observation and maceration. The number of species per leaf surface by elevation and the frequencies of their occurrence are recorded in TABLES 9 and 10. The resident population of each community was determined from those recovered only through maceration after washing.

Data were subjected to computer analysis (after Bray and Curtis 1957) for distributional similarities for fungi isolated from *Metrosideros*, *Acacia*, and air (FIGS. 6, 7, 8) both with and without washings.

RESULTS

The leaf substrata

Differences in leaf anatomy among the three leaf substrata are striking. Externally, the mature form of *Acacia koa* phyllodes has very smooth surfaces on both sides. *Cheirodendron* has palmately compound leaves; the three leaflets are smooth on both sides. *Metrosideros* leaves are distinguished by a continuous layer of abaxial hairs, well developed and intertwined (FIGS. 9 a,e).

TABLE 3

Fungi isolated from leaf surfaces of three vascular plants endemic to Hawaii, and from air at the same sites

	Kinds of leaves*:			Air:	Total Number Isolations**
Phycomycetes	One	Two	Three		
Gilbertella sp.				+	
Mortierella isabellina Oudemans & Koning	M				1
Mortierella sp.	M				1
Mucor sp.	C				1
Ascomycotina					
Chaetomium globosum Kunze ex Fr.	M				1
Coniochaeta sp.		M, C			2
Leptosphaeria sp.	M				4
Meliola koae Stevens	A				1
Mycosphaerella metrosideroi Stevens & Young		A, M			2
Nectria sp.		A, C			3
Pleospora herbarum (Pers. ex Fr.) Rabenhorst	A			+	2
Trichopeltis reptans Speg. op. cit.	C				1
Capnodiaceae Morphological form I, Stevens	M				9
Basidiomycotina					
Schizophyllum sp.	M				1
Trametes sp.	M				1

* M = *Metrosideros*; A = *Acacia*; C = *Cheirodendron*

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
Sp. indet. (clamp connections present)	M				1
Kordyana sp. ?	A				1
Fungi Imperfecti					
Sphaeropsidales					
Asteronella sp.	M				1
Botryodiplodia sp.	M				1
Coniothyrium sp.	M				3
Chaetophoma sp.	M				3
Dinemasporium sp.	M				1
Diplodina sp.	M				1
Phyllosticta sp.		A, M			3
Pycnidial type 1			A, C, M		47
Pycnidial type 2		A, M		+	7
Pycnidial type 3		A, M			23
Pycnidial type 4	A				6
Pycnidial type 5			A, C, M	+	33
Pycnidial type 6			A, C, M		1
Pycnidial type 7	A				1
Pycnidial type 8	C				1
Pycnidial type 9			A, C, M		3
Sphaceloma sp.	M				1
Sphaeropsis sp.		C, M			3
Sporonaema sp.	M				1

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
<u>Stagonospora</u> sp.	M				1
Melanconiales					
<u>Gloeosporium</u> sp.	M				3
<u>Marssonina</u> sp.	M				1
<u>Melanconium</u> sp.	M				3
<u>Monochaetia</u> sp.		A, M		+	4
<u>Perisporium</u> <u>vulgare</u> Corda			A, C, M	+	1
<u>Pestalotia</u> (<u>cocculi</u> type)			A, C, M	+	54
<u>Pestalotia</u> (<u>palmarum</u> type)		C, M			5
<u>Pestalotia</u> <u>stevensonii</u> Peck		A, M			3
<u>Pestalotia</u> sp. 1		C, M			3
<u>Pestalotia</u> sp. 2	M				6
Moniliales					
Moniliaceae					
<u>Acremonium</u> <u>curvulum</u> W. Gams	M				1
<u>Acremonium</u> <u>strictum</u> W. Gams		A, M		+	16
<u>Acremonium</u> sp.			A, C, M		5
<u>Acrostaphylus</u> sp.	C				1
<u>Alysidium</u> sp.				+	
<u>Aspergillus</u> <u>niger</u> Van Tieghem	C				1
<u>A</u>. <u>panamensis</u> Raper & Thom	M			+	1
<u>A</u>. <u>terreus</u> Thom				+	
<u>A</u>. <u>ustus</u> (Bain.) Thom & Church	M				1

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
Beauveria sp.	M				1
Botrytis sp.	M				2
Calcarisporium sp.	M				1
Coremiella sp.	M				1
Cylindrocladium sp.	A				2
Fusidium sp.	M			+	1
Geotrichum sp.	C				1
Gliocladium deliquescens Sopp.	M				1
G. roseum Link		A, M			7
Gliocladium sp.	A				1
Hansfordia grewiae (Hansford) Hughes	A				1
Hansfordia sp. nov.?	A			+	1
Malbranchea sp.	M			+	1
Monilia sp.	M			+	1
Monocillium sp.		M, C			2
Nodulisporium gregarium (Berk. & Curtis) Meyer		M, C			13
Oidiodendron sp.		M, C			3
Paecilomyces sp.			A, C, M		14
Penicillium canescens Sopp			A, C, M	+	12
P. casicolum Bain.	M				1
P. citreo-viride Biourge	M				1
P. citrinum Sopp	M				4
Penicillium sp. (coremial)	M			+	6

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
P. cyaneum (Bain. & Sart.) Biourge	M				2
P. diversum Raper & Thom	M				1
P. frequentans Westling			A, C, M		22
P. funiculosum Thom	M				1
P. herquei Bain. & Sart.	M				2
P. janthinellum Biourge		A, M		+	10
P. lilacinum Thom				+	
P. miczynskii Zaleski	M			+	1
P. nigricans (Bain.) Thom				+	
P. piscarium Westling	M				1
P. raistrickii Smith	A				1
P. simplicissimum (Oud.) Thom	C			+	1
P. spinulosum Thom	M				1
P. steckii Zaleski	M				1
Penicillium sp.		C, M			3
Trichoderma viride Pers.			A, C, M	+	15
Tritirachium sp. nov.?			A, C, M	+	3
Verticillium lateritium Berk.				+	
Verticillium sp.	M				4
Rhodotorula sp.		A, M			5
Yeasts		A, M		+	4
Dematiaceae					
Alternaria alternata (Fr.) Keissler		A, M			12

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
Arthrinium sporophleum Kunze	M				5
Aureobasidium pullulans (de Bary) Arnaud			A, C, M	+	65
Cercospora sp.		C, M		+	2
Chalara sp. 1	M				1
Chalara sp. 2		A, M			7
Chalara sp. 3			A, C, M		5
Cladosporium cladosporioides (Fresen.) de Vries			A, C, M	+	66
C. elatum (Harz.) Nannfeldt				+	
C. oxysporum Berk. & Curt.				+	
Cladosporium sp..		C, M			6
Cladosporium state of Venturia carpophila Fisher	C				4
Curvularia lunata (Walker) Boedijn				+	
Dendryphiella salina (Southerl.) Pugh & Nicot				+	1
Drechslera australiensis (Bugnicourt) Subram. & Jain. ex M. B. Ellis	A			+	2
D. biseptata (Sacc. & Roum.) Richardson & Fraser ?	M			+	2
Drechslera state of Pyrenophora avenae Ito & Kuribayashi	A				1
Drechslera state of Cochiobolus sativus (Ito & Kuribayashi) Drechsler ex Dastur				+	
Drechslera sp.		A, M		+	2
Gilmaniella sp.	M				1

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
Gliomastix murorum (Corda) Hughes var. *felina* (Marchal) Hughes	M				3
Humicola fuscoatra Traaen	M				2
Monodictys glauca (Cooke & Harkn.) Hughes	M				1
Nigrospora sphaerica (Sacc.) Mason	A				1
Nigrospora state of *Khuskia oryzae* Hudson		A, M			2
Nigrospora sp.	M				1
Periconia byssoides Pers. ex Merat		A, C			2
P. echinochloae (Batista) M. B. Ellis				+	
Periconia sp.	M			+	1
Periconiella angusiana M. B. Ellis ?	A			+	1
Phialophora sp.	M				1
Piricauda sp.	M				2
Pithomyces atro-olivaceus (Cooke & Harkn.) M. B. Ellis	A			+	1
P. chartarum (Berk. & Curt.) M. B. Ellis	M				2
P. cynodontis M. B. Ellis				+	
Pyricularia sp. nov.?	M				1
Rhinocladiella state of *Dictyotrichiella mansonii* Schol-Schwarz	M				2
R. cellaris (Pers. ex S. F. Gray) M. B. Ellis		C, M			3

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
Rhinocladiella sp.			A, C, M		9
Schizotrichella sp.	A				1
Scolecobasidium constrictum Abbott				+	
S. humicola Barron & Busch		A, M			4
S. terreum Abbott	C				1
S. variabile Barron & Busch	A				1
Scopulariopsis sp.		A, C			2
Spegazzinia tessarthra (Berk. & Curt.) Sacc.				+	
Sporodesmium sp.	M				1
Stachybotrys atra Corda	M				1
Stemphylium state of Pleospora herbarum (Pers. ex Fr.) Rabenh.	A			+	2
Stemphylium sp.				+	
Sympodiella sp.	M				2
Thielaviopsis sp.	M				1
Thermomyces lanuginosus Tsiklyinsky.	M				1
Torula sp.	M				1
Zygosporium gibbum (Sacc., Rouss., & Bomm.) Hughes		A, M			8
Tuberculariaceae					
Cylindrocarpon sp.		A, M			3
Epicoccum purpurascens Ehrenb. ex Schlecht		A, M			23

TABLE 3

	Kinds of leaves:			Air:	Total:
	One	Two	Three		
Fusarium episphaerica (Tode) Snyder & Hansen	M				1
F. lateritium Nees		A, M			5
F. merismoides Corda	A			+	1
F. nivale (Fr.) Cesati	M				1
F. roseum Link emend. Snyder & Hansen		C, M			4
F. solani (Mart.) Sacc.				+	
Fusarium sp. 1	M				2
Fusarium sp. 2	M				2
Metarrhizium sp.		A, M			2
Volutella sp.	A				1
Mycelia Sterilia					
Papulospora sp.	A				1
Sclerotium rolfsii Sacc.				+	
Non-sporulating mycelium *					
Moniliaceae			A, C, M	+	
Dematiaceae			A, C, M	+	

* Not included in tally for number of species

** Without air isolates

TABLE 4

Occurrence of fungi on leaf surfaces of three endemic vascular plants* in Hawaii and the air at the sample sites

	Total number	Only on M	Only on A	Only on C	On M + A	On M + C	On A + C	On M+A+C	Air only	Adjusted total - air
Phycomycetes	4	2		1					1	3
Ascomycotina	9	3	2	1	1	1	1			9
Basidiomycotina	4	3	1							4
Fungi Imperfecti	151	61	18	7	20	11	2	16	16	134
Sphaeropsidales	20	9	2	1	3	1		4		20
Melanconiales	10	4			2	2		2		10
Moniliales	121	48	16	6	15	7	2	10	16	105
Moniliaceae	53	24	5	4	5	4		6	5	48
Dematiaceae	54	20	8	2	6	3	2	4	9	45
Stilbaceae	0									
Tuberculariaceae	12	4	2		4	1			1	11
Mycelia Sterilia	2		1						1	1
Non-sporulating mycelium **								+		
Total number of species	168	69	21	9	21	12	3	16	18	150
Percentage with air isolates		41.0	12.5	5.3	12.5	7.1	N.S. ***	9.5	10.1	
Percentage without air isolates		45.7	14.0	5.9	14.0	8.0	N.S.	10.6	11.3	

* M = Metrosideros; A = Acacia; C = Cheirodendron

** Both moniliaceous and dematiaceous non-sporulating types

*** Not significant

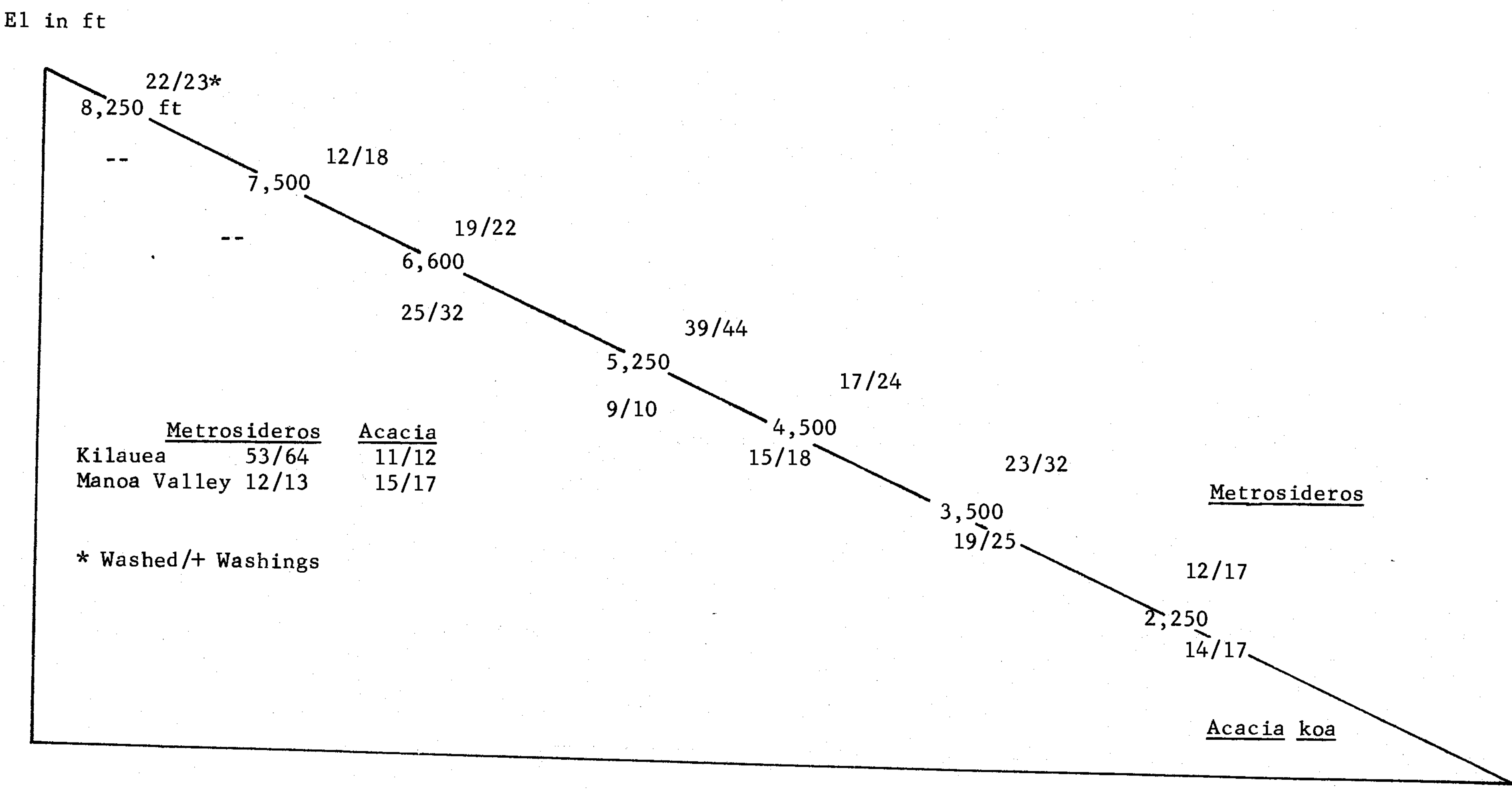


FIG. 5. Number of fungus species recovered from leaves of two dominant forest trees at seven stations along the Mauna Loa altitudinal gradient, Kilauea Forest, Hawaii and Manoa Valley, Oahu.

TABLE 5. Number of fungus taxa recovered from leaves of three endemic vascular plants with and without surface washings.

Elevation in ft x 10^3	Metrosideros + W*	Metrosideros - W	% difference	Acacia koa + W	Acacia koa - W	% difference	Cheirodendron + W	Cheirodendron - W	% difference
2.25	17	12	29	17	14	17.6			
3.50	32	23	28	25	19	24			
4.50	24	17	20.8	18	15	16.6	18	15	16.5
5.25*	13	12	7.6	17	15	11.7			
5.25	44	39	11.3	10	9	10			
6.60	22	19	13.6	32	25	21.6			
7.50	18	12	33.3	--	--	--			
8.25	23	22	4.3	--	--	--			
0.6 **	64	53	17.1	12	11	8.03			

* Kilauea Forest

** Manoa Valley

TABLE 6. Frequency of occurrence of fungus species by segments on TP 1.

Number of: Segments	Species on *Metrosideros*	Occurrence at other sites	Occurrence on *Acacia koa* Number of segments	Other sites
7	5			
	Capnodiaceae I	KF, M*	0	0
	Penicillium frequentans	KF	2	0
	Aureobasidium pullulans	KF, M	4	KF, M
	Cladosporium cladosporioides	KF, M	5	KF, M
	Epicoccum purpurascens	KF	5	KF
6	1			
	Pestalotia cocculi	KF, M	4	M
5	3			
	Pycnidial #1	KF, M	4	M
	Pycnidial #5	M	5	KF, M
	Zygosporium gibbum	0	1	0
4	3			
	Acremonium strictum	KF	4	KF, M
	Nodulisporium gregarium	KF	0	0
	Penicillium canescens	M	2	M
3	2			
	Paecilomyces sp.	0	3	0
	Penicillium sp. (coremial form)	0	0	0

* Kilauea Forest; Manoa Valley

TABLE 7. Similarity of fungi from Metrosideros between elevations along TP 1, TP 2, TP 3, Kilauea Forest and Manoa Valley.

Including washings:	Elevations (ft)								
Elevations	2250	3500	4500	5250	6600	7500	8250	Manoa	Kilauea
2250	100	57.1	68.3	29.5	61.5	51.4	55.0	46.7	22.2
3500	57.1	100	64.3	28.9	48.1	48.0	50.9	40.0	29.2
4500	68.3	64.3	100	26.5	43.5	47.6	59.6	48.6	29.5
5250	29.5	28.9	26.5	100	24.2	22.6	29.9	21.1	48.1
6600	61.5	48.1	43.5	24.2	100	60.0	53.3	51.4	23.3
7500	51.4	48.0	47.6	22.6	60.0	100	53.7	38.7	24.4
8250	55.0	50.9	59.6	29.9	53.3	53.7	100	33.3	25.3
Manoa	46.7	40.0	48.6	21.1	51.4	38.7	33.3	100	18.2
Kilauea Forest	22.2	29.2	29.5	48.1	23.3	24.4	25.3	18.2	100
Without washings (macerations):									
2250	100	45.7	69.0	23.5	51.6	41.7	47.1	58.3	15.4
3500	45.7	100	65.0	32.3	47.6	40.0	53.3	45.7	28.9
4500	69.0	65.0	100	25.0	44.4	48.3	51.3	55.2	28.6
5250	23.5	32.3	25.0	100	24.1	15.7	32.8	23.5	41.3
6600	51.6	47.6	44.4	24.1	100	58.1	53.7	51.6	22.2
7500	41.7	40.0	48.3	15.7	58.1	100	52.9	41.7	21.5
8250	47.1	53.3	51.3	32.8	53.7	52.9	100	35.3	26.7
Manoa	58.3	45.7	55.2	23.5	51.6	41.7	35.3	100	21.5
Kilauea Forest	15.4	28.9	28.6	41.3	22.2	21.5	26.7	21.5	100

TABLE 8. Similarity of fungi from Acacia koa between elevations TP 1, TP 2, TP 3, Kilauea Forest and Manoa Valley.

Including washings:	Elevations (ft)						
Elevations	2250	3500	4500	5500	6600	Manoa	Kilauea
2250	100	42.9	51.4	37.0	49.0	47.1	41.4
3500	42.9	100	51.2	22.9	42.1	57.1	32.4
4500	51.4	51.2	100	35.7	48.0	57.1	53.3
5250	37.0	22.9	35.7	100	28.6	29.6	27.3
6600	49.0	42.1	48.0	28.6	100	36.7	27.3
Manoa	47.1	57.1	57.1	29.6	36.7	100	34.5
Kilauea Forest	41.4	32.4	53.3	27.3	27.3	34.5	100
Without washings (macerations):							
2250	100	36.4	48.3	43.5	51.3	48.3	32.0
3500	36.4	100	47.1	14.3	40.9	52.9	26.7
4500	48.3	47.1	100	33.3	45.0	60.0	38.5
5250	43.5	14.3	33.3	100	23.5	25.0	20.0
6600	51.3	40.9	45.0	23.5	100	45.0	22.2
Manoa	48.3	52.9	60.0	25.0	45.0	100	30.8
Kilauea Forest	32.0	26.7	38.5	20.0	22.2	30.8	100

TABLE 9. Similarity of fungi between elevations isolated from the air on TP 1.

Including washings	Elevations (ft)					
Elevations	2250	3500	4500	6600	7500	8250
2250	100	40.0	41.2	35.3	22.9	27.6
3500	40.0	100	36.8	47.4	25.6	24.2
4500	41.2	36.8	100	43.8	36.4	22.2
6600	35.3	47.4	43.8	100	36.4	29.6
7500	22.9	25.6	36.4	36.4	100	28.6
8250	27.6	24.2	22.2	29.6	28.6	100

TABLE 10. Similarity quotients by elevation between fungi occurring on two leaf substrata recovered by direct observation and leaf maceration.

Leaf Substrata	Elevations in ft, x 10^2								
	22.5	35.0	45.0	52.5	66.0	75.0	82.5	KF*	Manoa
Metrosideros & *Acacia*	53.8	57.1	62.5	12.5	45.5	--	--	15.6	74.1
Metrosideros & *Cheirodendron*	--	--	43.8	--	--	--	--	43.6	--
Acacia & *Cheirodendron*	--	--	53.3	--	--	--	--	22.2	--

* Kilauea Forest

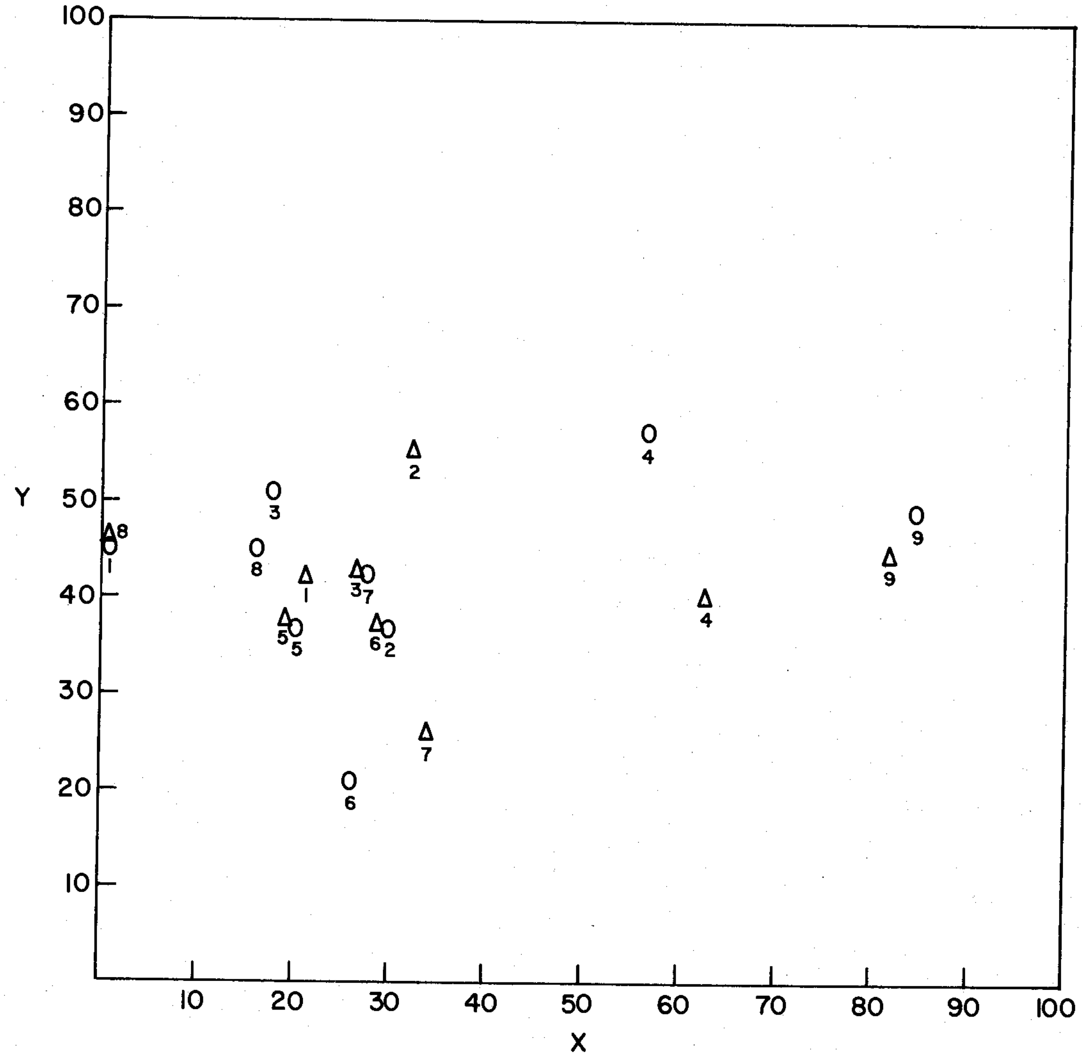


FIG. 6. Ordination of the fungal communities associated with <u>Metrosideros</u> <u>collina</u> var. <u>polymorpha</u>.

Site	With Washings (Δ) X	With Washings (Δ) Y	Without Washings (0) X	Without Washings (0) Y	Location
1	21.3	42.1	00.0	45.0	2,250 ft, Mauna Loa
2	32.2	55.6	29.9	36.6	3,500 ft, Mauna Loa
3	26.7	43.1	17.9	50.9	4,500 ft, Mauna Loa
4	62.6	40.1	56.5	57.2	5,250 ft, Mauna Loa
5	19.3	37.8	20.4	36.5	6,600 ft, Mauna Loa
6	28.9	37.5	26.0	21.0	7,500 ft, Mauna Loa
7	34.0	26.2	27.1	42.5	8,250 ft, Mauna Loa
8	00.0	46.1	16.2	45.0	Manoa
9	81.8	44.4	84.6	48.9	Kilauea Forest

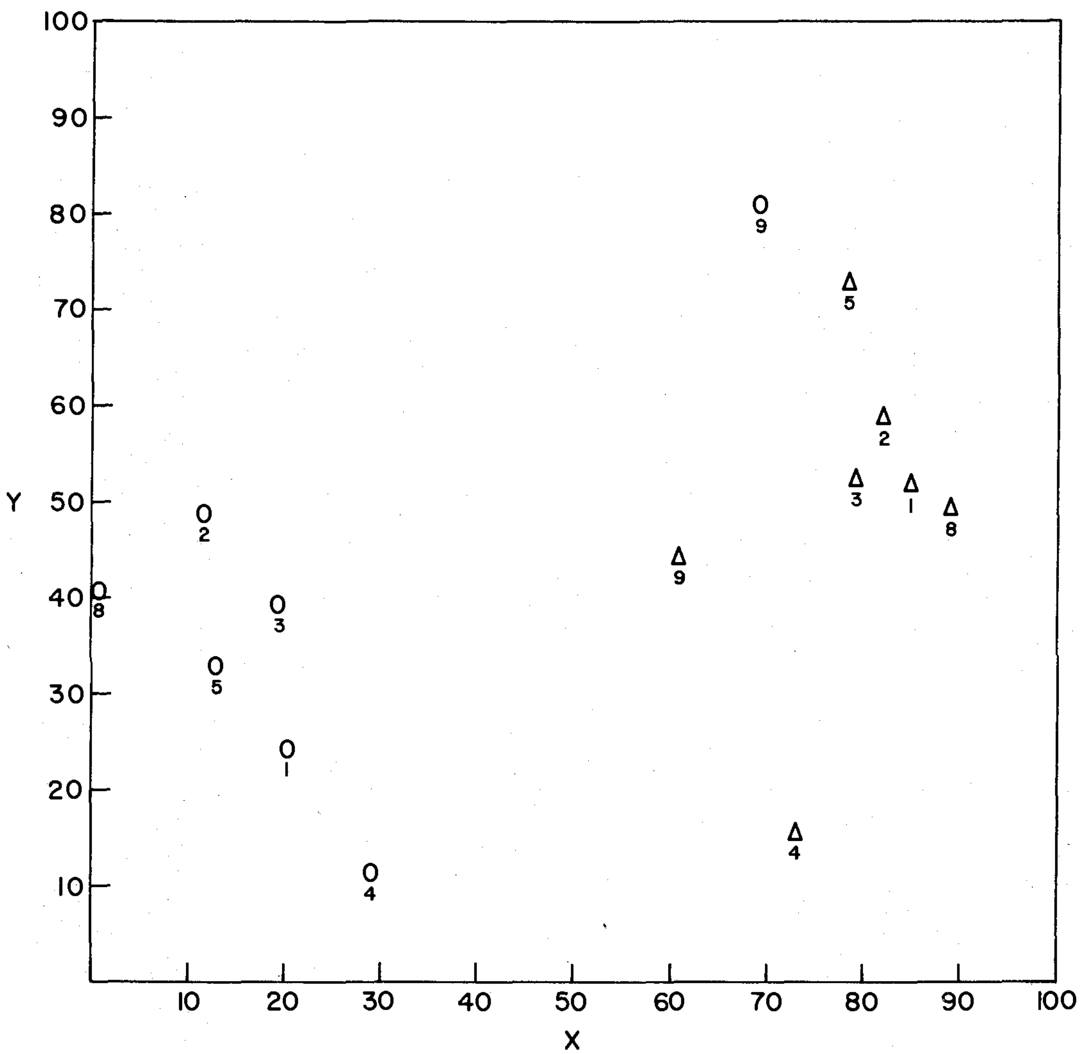


FIG. 7. Ordination of the fungal communities associated with *Acacia koa*.

Site	With Washings (Δ) X	With Washings (Δ) Y	Without Washings (0) X	Without Washings (0) Y	Location
1	84.9	52.1	20.5	24.3	2,250 ft, Mauna Loa
2	82.0	59.1	11.8	48.8	3,500 ft, Mauna Loa
3	79.1	52.5	18.8	39.4	4,500 ft, Mauna Loa
4	72.8	15.7	29.0	11.6	5,250 ft, Mauna Loa
5	78.2	73.1	12.8	33.2	6,600 ft, Mauna Loa
6					No sample
7					No sample
8	88.9	49.8	00.0	40.6	600 ft, Manoa
9	60.6	44.4	69.2	80.8	5,400 ft, Kilauea Forest

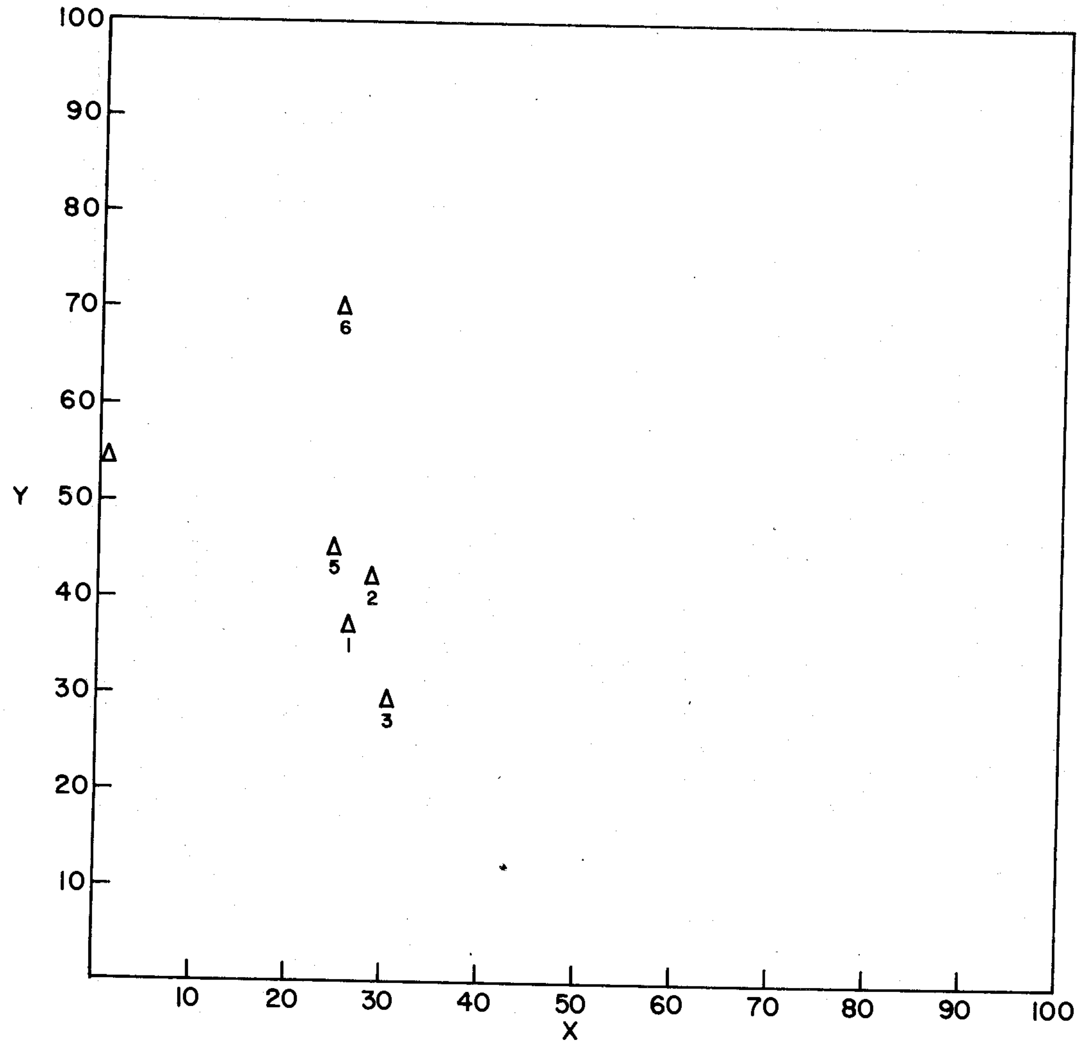


FIG. 8. Ordination of air fungal communities.

Site	X	Y	Location
1	26.2	37.5	2,250 ft, Mauna Loa
2	28.7	42.3	3,500 ft, Mauna Loa
3	30.2	29.8	4,500 ft, Mauna Loa
4			No sample
5	24.8	45.6	6,600 ft, Mauna Loa
6	25.5	70.2	7,500 ft, Mauna Loa
7	00.0	54.7	8,250 ft, Mauna Loa
8			No sample
9			No sample

FIG. 9. (a) Free-hand section of fresh *Metrosideros* leaf from approximately 5,200 ft, Mauna Loa, Hawaii Volcanoes National Park. Note well developed abaxial hairs.

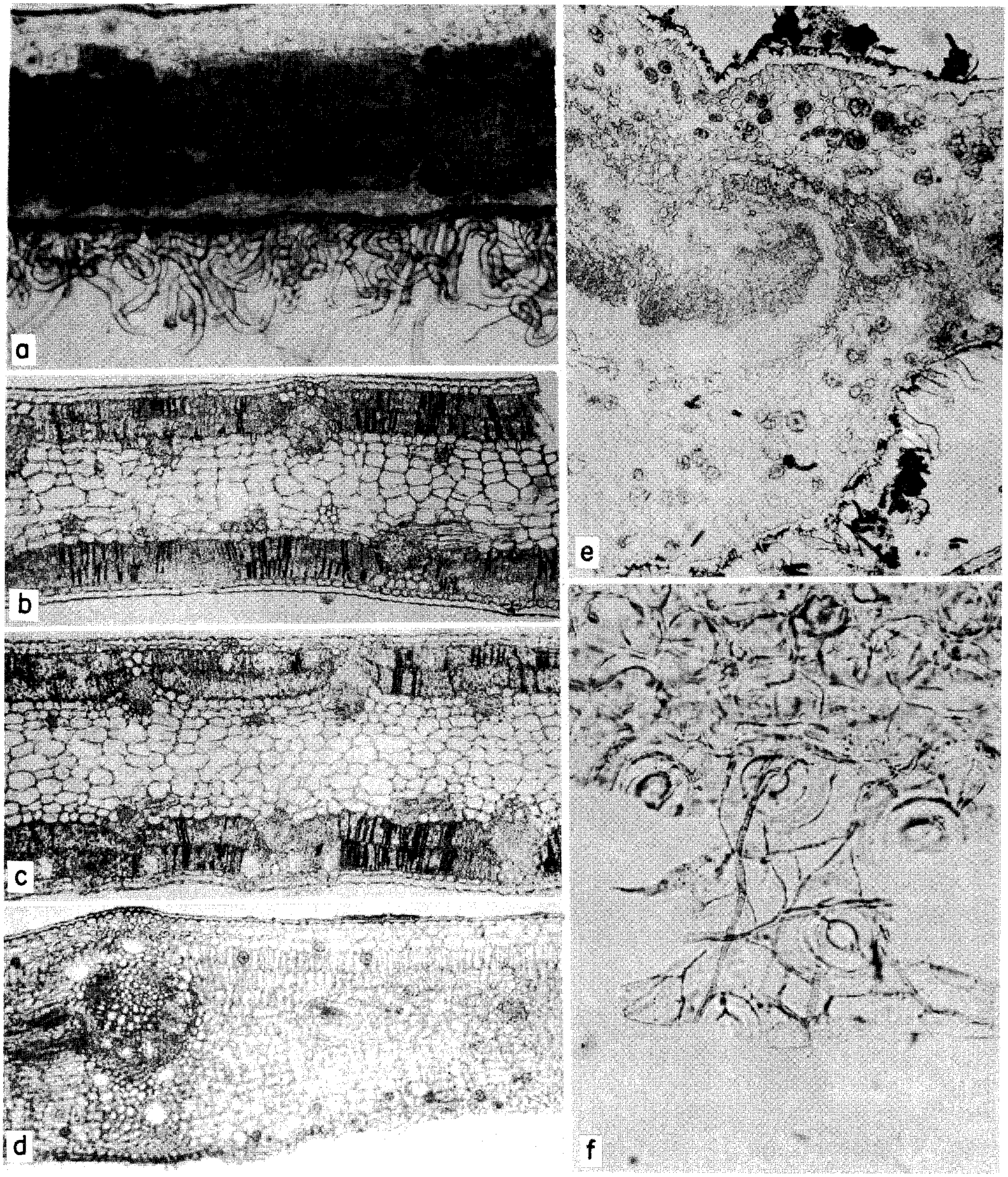
(b) Free-hand section of *Acacia koa* phyllode from 6,600 ft Mauna Loa transect.

(c) Free-hand section of *Acacia koa* phyllode from 600 ft Manoa Valley, Oahu; unstained.

(d) Unstained free-hand section of *Cheirodendron* leaf from 4,300 ft, Hawaii Volcanoes National Park.

(e) Unstained free-hand section of *Metrosideros* leaf through the mid-rib region. Note sooty molds on and among the abaxial hairs and bacteria on adaxial surface.

(f) Epidermis of *Cheirodendron* showing septate hyphae; abaxial surface.


a
b
c
d
e
f

Sections of fresh leaves examined with and without microchemical tests were employed to show the detailed structure of each leaf (FIG. 9, a-e). The mesophyll of Metrosideros and the palisade layers of Acacia had intensive reactions to the pectin test. Cheirodendron had the least diversified internal structure (FIG. 9 d). The hairs of Metrosideros gave a lignin reaction as well as a pectin reaction. Abortive hairs may be found on the adaxial surface which often showed phylloplane inhabitants, notably bacteria and fungi (FIG. 9 e). Sunken stomata are characteristic of both surfaces of Metrosideros and Acacia. Sections of Acacia koa from 6,600 ft, Mauna Loa TP 1 and from 600 ft Manoa Valley, Oahu, showed no significant anatomical differences (FIG. 9 b, c). Both Cheirodendron and Metrosideros have striking crystalline inclusions (FIG. 9 d, e). An epidermal strip of Cheirodendron showed fungal hyphae in association with stomata (FIG. 9 f).

By EM scan microscopy, differences in surfaces and distribution of fungal elements were delineated for all three leaf substrata. The smooth surface of Acacia koa is shown in FIG. 10 g, h. The hairy abaxial surface of Metrosideros is illustrated in FIG. 10 a, b, with sooty molds visible on the leaf surface. FIG. 10 c, d, e, f illustrate fungus hyphae in and around the hairs. The Cheirodendron abaxial surface displayed a remarkable network of hyphae which often encircled, crossed, or entered the stomata (FIG. 11 c, d, e, f). There was also an impressive hyphal net on the adaxial side with evidence of spore production (FIG. 11 a, b).

Fungal communities

The fungi identified represented 168 taxa. This total is the accrual of isolates from the three leaf substrata at all sample sites plus those from the air spora plates exposed at each site. Their distribution by leaf type and in the air is designated in TABLE 4. Exclusive of air spora the total number of taxa is 150. Air isolates totaled 52 species of which 18 were recovered only from air. Frequency

FIG. 10. (a) EM scan of *Metrosideros* abaxial surface showing sooty molds and hairs.

(b) Detail of *a*.

(c), (d) Detail of sooty molds and hairs.

(e), (f) Abaxial hairs with fungus filaments entwined and encircling them.

(g) EM scan of *Acacia koa* showing stomata.

(h) Detail of *g*.

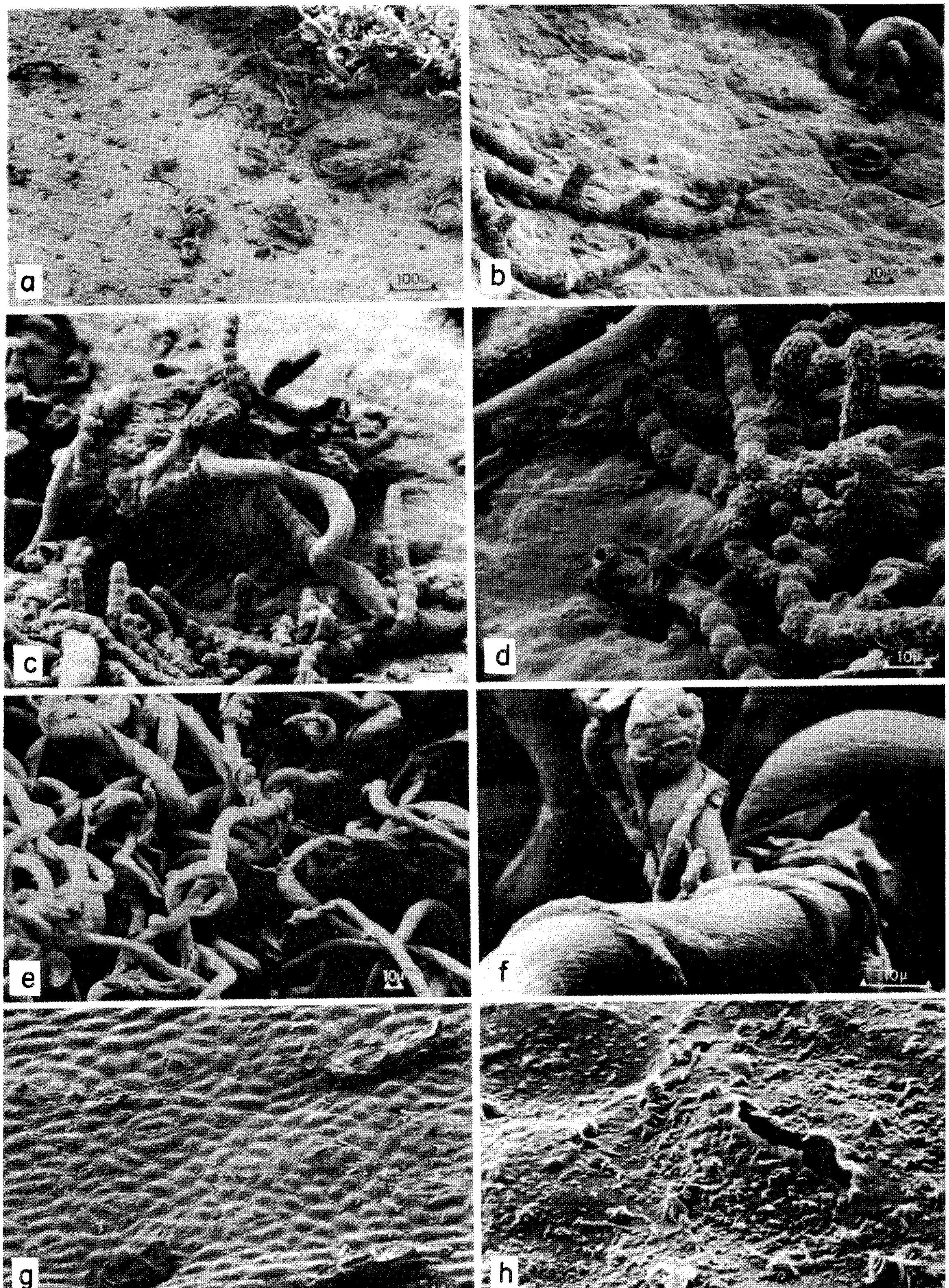

a
100μ
b
10μ
c
d
10μ
e
10μ
f
10μ
g
h

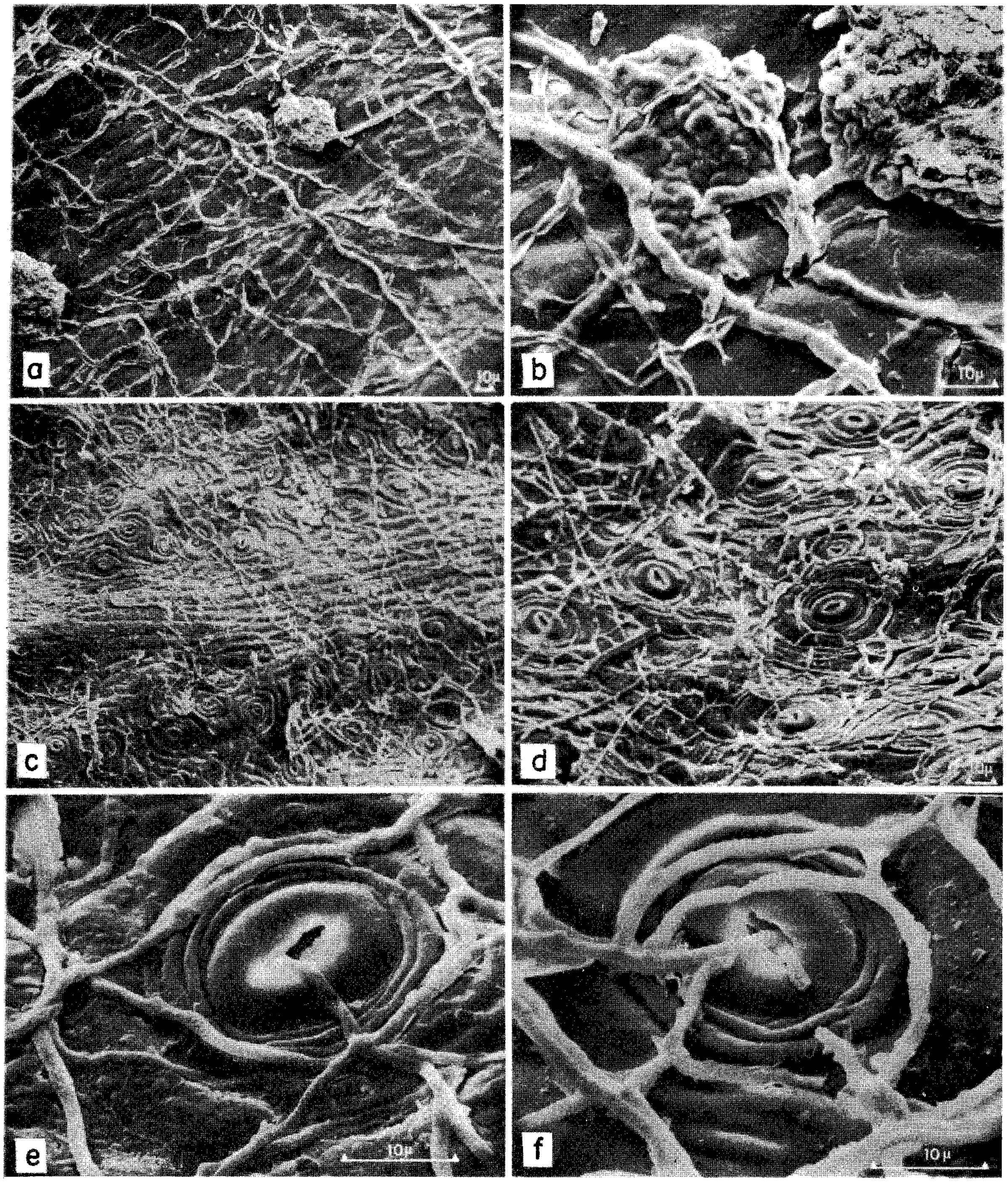
FIG. 11. (a) EM scan of Cheirodendron leaf, adaxial surface showing extensive network of fungus hyphae and spore clusters.

(b) Detail of a.

(c) EM scan of Cheirodendron abaxial surface. Note well developed net of hyphae.

(d) Detail of c.

(e), (f) EM scan of Cheirodendron stomata with entering hyphae.


a
10µ
b
10µ
c
d
e
10µ
f
10µ

of isolation is indicated for each taxon exclusive of air isolates (TABLE 3). When frequency of isolation is considered for the taxa 43% are represented by single isolations. As the number of repeated isolations increased the number of species involved decreased. In excess of seven repeated isolations only single taxa are represented for the most part. Two species occurred in frequencies of 9 and 12. Four species were recorded approximately 50 times or more (TABLE 3).

Considering the 168 taxa as representatives of taxonomic groups the dominant one is the Fungi Imperfecti (TABLE 4) accounting for 89.9% of the isolates. For the 150 taxa on leaves only, the percentage is 90. Curiously no Phycomycetes, Ascomycetes or Basidiomycetes occurred on all the leaf types but three of the nine Ascomycetes isolated were recovered from all paired leaf combinations (TABLE 3). Only one Phycomycete was recorded from the air.

Occurrence according to leaf substrate (TABLE 4) showed 69 taxa on Metrosideros only; 21 limited to Acacia; and 9 to Cheirodendron. In combinations of any two leaf substrata, 20 species occurred on Metrosideros and Acacia; 12 on Metrosideros and Cheirodendron; and three on Acacia and Cheirodendron. On all three leaves there were 16 species of which 10 were recorded also from air plates.

Distribution by numbers of taxa on the three leaf substrata was tabulated by elevation (TABLE 5) and graphically represented for two of them (FIG. 5). Counts were obtained from leaves by tabulating isolates using populations derived with and without washings. By either derivation the numbers are parallel for counts by elevation, but without washings the counts are from 4.3 to 33% lower. The highest counts for washed Metrosideros were, in order of magnitude, from Kilauea Forest (5,400 ft), 5,250 and 3,500 ft on the Mauna Loa transect (TP 1). The peak count for Acacia occurred at 6,600 ft with that for 3,500 ft next. The Kilauea Forest count is second lowest; it is very close to that at 5,250 ft on the transect. Data for Cheirodendron are incomplete.

On a community basis that of Metrosideros was the largest with 45% of the isolates exclusively on that substrate; Acacia had 14%; and Cheirodendron about 6% (TABLE 4). The last community is small but admittedly its size is skewed through fewer samplings so it cannot be regarded as a reliable representation for that leaf surface.

The Metrosideros community is richer in kinds of fungi than the Acacia community (TABLE 4), but the populations of each group present are in fair agreement on a percentage basis. The Fungi Imperfecti are represented respectively by 61 and 18 taxa for Metrosideros and Acacia which equate to 88% and 85% of their total populations. Other groups have percentages in similar correspondence. Significant differences, if any, therefore are at the taxon level.

Only one fungus, Cladosporium cladosporioides, was recovered from both Metrosideros and Acacia at all elevations (seven for Metrosideros and five for Acacia) on Transect Profile 1, Kilauea Forest and Manoa Valley. Epicoccum nigrum occurred at all these sites on both leaves on the island of Hawaii but was not found on either leaf substrate in Manoa Valley. Aureobasidium pullulans, well-known not only as a cosmopolitan fungus adapted to a wide range of conditions but as a consistent member of many phylloplane communities, was found at all elevations on TP 1 on Metrosideros, at four out of five elevations for Acacia, as well as in Kilauea Forest and Manoa Valley. Several pycnidial forms occurred on both leaves at several elevations of the Mauna Loa Transect, Kilauea Forest, and Manoa Valley. The fungus recognized as Capnodiaceae Morphological Form I, Stevens, was consistently present on Metrosideros along TP 1, in Kilauea Forest and Manoa Valley. It was never found in relationship with Acacia or Cheirodendron. Another taxon, Penicillium frequentans, occurred on Metrosideros at all TP 1 elevations and in Kilauea Forest, but on Acacia it was limited to two elevations.

Community overlap can be expressed in percentages (TABLE 4). It ranges from

7.1 to 12.5% for combinations of two substrata and is approximately 10% for all three leaf surfaces. Overlap can also be measured by Sorensen's (1948) similarity quotient (TABLES 7, 8, 9). Measurement of fungus SQ's by elevation for single substrata and air isolates is best evaluated by a summary of values obtained which are 50 or above for all combinations of elevation using data with and without washings.

Number of SQ combinations 50 at:	Metrosideros		Acacia		Air isolates
	Plus washings	Minus washings	Plus washings	Minus washings	
2,250 ft	5	3	1	1	0
3,500 ft	3	2	2	1	0
4,500	3	4	4	1	0
5,250	0	0	0	0	-
6,600	4	4	0	1	0
7,500	3	2	-	-	0
8,250	5	4	-	-	0
Kilauea Forest (5,400)	0	0	1	0	-
Manoa Valley	1	3	1	2	-

The greatest dissimilarities obtain from combinations involving the sites at an elevation of 5,250 ft and 5,400 ft for both Metrosideros and Acacia. This is clearly seen in the ordination of this data (FIG. 6 and 7). None of the air isolates had values above 50 in any combination of elevations. Similarity among Metrosideros fungi is usually reduced when the washings are deleted and only the taxa recovered by maceration are counted. There were only three exceptions to this reduction by washing; two for Metrosideros and one for Acacia. SQ values in excess of 50 are obviously fewer for Acacia.

Similarity quotients between fungi recovered from two leaf substrata were derived from direct observation, and with and without washings, meaning transient and resident populations. The dissimilar values occurred for both Metrosideros and

Acacia at the 5,250-ft and 6,600-ft level on TP 1 and also in Kilauea Forest at 5,400 ft. Values for the combination of Metrosideros and Cheirodendron were both less than SQ 50 and practically identical for two levels tested. Acacia plus Cheirodendron, again limited to two sites, had an SQ 53 at 4,500 ft at Kilauea Forest the SQ was reduced to 22. The dissimilarity of the SQ values for air samples would be expected of such a population.

Ordination shows that the Metrosideros fungal community (FIG. 6) associated with samples taken from the Mauna Loa transect are very similar except for the 5,200 ft level on Mauna Loa where there was a much larger group of isolates. This is also true of the Kilauea Forest sample. There are no major differences in the ordination when comparing samples with washings and without washings.

Ordination of the fungal community from Acacia koa (FIG. 7) shows a different situation from Metrosideros. The fungal communities before and after washing are not so similar, probably reflecting the greater efficiency of washing removing the surface flora of the smooth Acacia koa leaf as compared with the pubescent Metrosideros leaf. The patternsof distribution both with and without are similar showing the great similarity of both transient and permanent communities. As in Metrosideros, the Kilauea Forest and 5,250 ft samples are distinctive. In this case the community size is smaller than average, which would tend to allow differences to show more easily.

Ordination of air plates (FIG. 8) shows the lower elevation of Mauna Loa to be very similar but the two elevations above cloud line (7,500 and 8,250 ft) are much different as would be expected due to the extreme changes in climatic conditions.

DISCUSSION

The leaf as a substratum for fungi

Surfaces of living leaves of different plants support varied, distinctive, and saprobic communities of microorganisms. This ecological niche originally was designated the phyllosphere (Last 1955, Ruinen 1956), but the term phylloplane is now considered more definitive as the surrounding medium, the air, is microbiologically inactive (Last and Warren 1973).

The establishment of fungal communities in the phylloplane probably follows bacterial colonization. Yeasts precede filamentous fungi in the phylloplane succession. The source of the fungal community members is from air spora and other fungal propagules. Controlling factors associated with air as a source of inoculum include air currents, rain drops and rain splash. Deposition on the leaf surface is favored also by the anatomy of the leaf and its capacity to act as a spore trap. Following successful deposition the fungi may be inactive, or transient, or actively growing or resident, with or without sporulation. The non-sporulaters are considered potentially active, becoming active after leaf senescence and fall. The transient population is recovered with the inclusion of leaf washings. The resident population is represented by those recovered through maceration, and possibly should include those observed directly especially if well established and sporulating.

Actual establishment on the leaf substratum is dependent upon the microclimate of the leaf controlled by temperature, relative humidity, radiation and wind speed (Burrage 1971). Support of the community depends on the availability of leachates and the nature of the cuticle including leaf waxes. Variation resulting from age of the leaf and character of other microbial communities present on the leaf is important in the maintenance of the community (Pugh and Buckley 1971).

The three leaf substrata considered in this phylloplane study displayed differences in their potentials for spore trapping. Metrosideros with well developed abaxial hairs presents the best trapping surface. However, Metrosideros leaves are exceedingly variable in degree of pubescence (Corn and Hiesey 1973), especially at mid-elevations from 1,000 to 4,000 ft. There was observable difference in

Metrosideros pubescence among the samples taken. In Kilauea Forest the leaves are thinner, less hairy compared to leaves in Manoa Valley and along TP 1 at its lower levels. When differences in counts with and without washings are placed on a percentage basis, the Kilauea Forest leaves show little difference but leaves from Manoa Valley had one of the largest differences, indicating that the greater hairy surface trapped more transients.

Cheirodendron and Acacia present smooth surfaces less likely to snare wind borne particles and possibly more subject to removal by rain wash. If this is correct the quantitative returns for populations including washings should be larger for Metrosideros than for the other two leaves. This was clearly reflected by the total counts at all elevations. It also indicates that the transient population is greater on Metrosideros. If Metrosideros has a higher transient population because of greater trapping potential than in combination with Acacia and Cheirodendron the similarity quotients should be lower when the communities compared include the washings, as they were. Ordination (FIG. 6 and 7) also demonstrated the importance of hairs as spore traps. There was no major change in communities before and after washing in Metrosideros (FIG. 6) but there was a major shift in Acacia (FIG. 7) due to better washing of this smooth surface.

Establishment on leaves is influenced also by cuticle development (Last and Warren 1973). The cuticles of Metrosideros and Acacia are both well developed. This could favor establishment of resident fungi. The highly developed network of hyphae on Cheirodendron with indications of sporulation seen by EM scan microscopy shows successful colonizing of the living leaf. The fungus shown (FIG. 11 b) is tentatively identified as Gliocladium sp.

Granted that the three leaf substrata present different trapping surfaces one can look at the community returns in toto for each substrate as represented by their populations, by the diversity of their members, by overlap of members among them, for those which are resident members, and finally for close relationship between resident fungi and substrate which could ultimately reflect endemism.

The number of fungi comprising the total (transient and resident) Metrosideros community are three times greater than those of the comparable Acacia community. The total community is an important aspect of the phylloplane but only the resident population will be significant in close relationship between fungus and leaf. The numbers from Cheirodendron are unreliable for comparison because the sampling was limited. Samples for Metrosideros and Acacia were taken over a period to two and a half years. Therefore, they are considered representative. However, it should be noted that Acacia grows at fewer elevations which reduced the number of sample sites and consequently the number of samples.

Diversity of taxa by substratum can be measured by those limited to each substratum based on the resident population of each. When such an accounting is made Metrosideros displays the most diversity usually. Analyzed by taxonomic groups the ratios for Metrosideros:Acacia:Cheirodendron read: Phycomycetes 1:0:0; Ascomycetes 1:3:1; Basidiomycetes 3:1:0; Sphaeropsidales 9:2:1; Melanconiales 4:0:0; Moniliaceae 17:3:1; Dematiaceae 16:7:2; and Tuberculariaceae 4:1:0.

If the populations of the Metrosideros and Acacia communities are limited to resident members by eliminating those derived from leaf washings, Metrosideros species are still in excess of Acacia species. From the former substrate 76 of 116 species recovered by all methods can be considered resident by virtue of maceration isolation only. Comparable returns for Acacia show 30 species out of 60 can be considered residents. These figures represent 65% and 50% of their respective communities. In turn the resident members can be designated the active community members. The genera Alternaria, Aureobasidium, Botrytis and Cladosporium are well known as primary colonizers of leaves; Epicoccum more recently has been accepted as an active colonizer (Pugh and Buckley 1971). These taxa were recorded from both

Metrosideros and Acacia except for Botrytis which was limited to Metrosideros. All were recovered from macerations except Alternaria which was observed directly on the leaves. The majority, therefore, of accepted active phylloplane colonizers are present in the Pacific world on native trees of Hawaii.

Overlap of particular taxa on different leaves is to be expected when inoculum is wind borne. There was significant overlap of species (14%) between Metrosideros and Acacia. Overlap or similarity established by Sorensen's SQ method can be considered significant for figures exceeding SQ 50. Those SQ values exceeding 50 were greater for total communities than for resident groups, as would be expected. Both percentage of overlap and SQ determinations reflect this difference between transient and resident members.

Overlap by elevation showed that no clear cut pattern was established. The air isolates had no SQ values over 50 which is reasonable as the wind is the chief source of inoculum. Indirectly, this dissimilarity enhances the similarity of those established on the leaves.

To evaluate the members of a given community for close relationship to a given substratum one can consider frequency of isolation (multiple and single) for those resident members which are limited to one substratum. There was great variation in frequency of isolation for individual taxa. The extreme for multiple isolations was 65 for Aureobasidium. Since it occurred at all elevations on all leaves at all times, it can scarcely be ranked as an indicator of substrate association. A better example might be Penicillium sp., a distinctive coremial type recorded six times on Metrosideros only and from four elevations. Yet it was recovered more frequently from washings than macerations as well as from air so it is more likely a transient member. Other notable multiple isolations were nine pycnidial forms with as many as 47 recoveries yet only two of these were limited to a single leaf substrate.

Forty-seven percent of the taxa were single isolations. These could represent only chance associations or include those with truly close affinity for the substratum, leading to endemism if endemism and substrate enjoy a 1:1 relationship. Those taxa most likely though to qualify are the ones with multiple records from a single substrate isolated by maceration or possibly by direct observation. For Metrosideros those offering the greatest promise are:

Basidiomycete with 4 isolations, 4 elevations, direct observation

Leptosphaeria sp., 4 isolations, 3 elevations, maceration and direct observation

Capnodiaceae Morphological Form I, 9 isolations at all elevations, Kilauea Forest and Manoa Valley; direct observation only.

Other records are confused by isolation from washings which makes them suspect. Mycosphaerella metrosideri Stevens and Young called endemic by Stevens (1925) who recorded it only from Metrosideros, was recovered from Acacia but by washing. So possibly it can be considered still to have relationship only to Metrosideros. Obviously it is well established on that substratum. Meliola koae with one report on Acacia (Kilauea Forest) was based on direct observation. Its position as an endemic is therefore inviolate.

Although the pin-pointing of species with endemic affinity for any one of these substrata is poor, the correlation of leaf surface and phylloplane colonization is clear. The EM scan pictures clearly illustrate the presence of sooty molds on Metrosideros as well as the affinity of Metrosideros hairs for fungal trapping and growth. The well developed Metrosideros cuticle is revealed by EM scan as a site for fungal and bacterial growth. The Cheirodendron surface as seen by EM scan reflects not only the successful establishment of fungal hyphae but their potential for reproducing on the leaf surface. Stomatal penetration suggests direct entry for internal establishment and subsequent secondary activity, perhaps in senescent leaves and ultimate contribution to the litter zone.

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