ISOLATION, IDENTIFICATION AND ROLES OF FUNGI FROM CREOSOTE TREATED PINE POLES[1] /

*J. CARRANZA**

Resumen

El presente trabajo se realizó en la ciudad de Syracuse, Nueva York. La muestra consistió de 46 postes de pino tratados con creosota y de diferentes años de servicio (10-50 años). De dichos postes se aislaron 71 hongos pertenecientes a 12 géneros y 8 especies, incluyendo 11 hongos que no pudieron ser identificados.

Se llevaron a cabo pruebas de podredumbre y de tolerancia al preservante para determinar la función que pueden desempeñar dichos hongos en su invasión a la madera. Dicho estudio se completó con observación de la madera atacada al microscopio de luz y de barrida, para relacionar los cambios anatómicos de la madera con la actividad de los hongos.

Se concluye que tres grupos de hongos interactúan en estos postes. El primer grupo incluye a los llamados "invasores primarios", los cuales son muy tolerantes a la creosota y pueden actuar destoxificando el sustrato; el segundo grupo incluye a los "oportunistas", los cuales aprovechan el sustrato modificado y pueden ejercer reacciones antagonistas contra los otros hongos, sin producir podredumbre; el tercer grupo lo constituyen los hongos que producen "podredumbre", los cuales son algunas veces tolerantes a los preservantes y causan podredumbre. En este último grupo se incluyen a los himenomicetes y a los hongos causantes de podredumbre suave (ascomicetes).

Introduction

Wood is a renewable natural resource of major importance for fuel, construction, and as a chemical raw material. Despite the advantages wood has over many other construction materials, it has several disadvantages which limit its usefulness. A major one is that it is biodegradable under some conditions of use.

Although wood degradation by microorganisms has been studied extensively, additional research is needed to understand more fully the microorganisms involved, how they invade the wood, and the interactions among them that lead to complete breakdown of wood. Added information on these processes may lead to more economical and effective ways to control decay.

This research deals mainly with the identification, anatomical effects on wood structure, relative tolerance to a preservative, and elucidation of the roles and interactions among fungi associated with decay development in creosoted pine poles from utility lines.

Literature review

A thorough review of the current literature on decay in wood products has been assembled by Scheffer (42). Important contributions and methodologies directly pertaining to the research topic are briefly reviewed here.

Primarily it is fungi (Basidiomycetes and Ascomycetes) that invade, digest, and thereby reduce the advantageous properties of wood (14, 23, 38, 41).

1 Received for publication in March 30, 1983.
This research was part of a thesis completed for an MS degree at the State University of New York, College of Environmental Science and Forestry, at Syracuse, N. Y., under the direction of Dr. R. A. Zabel. Thanks are due to Dr. Chunk Wang for assistance in identifying many of the fungi reported.

* Tucson, Arizona, 3334 E. Presidio Rd. 85716, USA

If wood is properly handled and used, decay can be greatly minimized. Where the wood product is in constant contact with soil, or in other uses where the moisture content of the wood frequently rises above the fiber saturation point, preservatives are needed to minimize decay losses and to insure an economical service life. Although the use of wood preservatives has reduced decay significantly, losses still occur. Some wood-inhabiting organisms not necessarily responsible for major decay (ascomycetes and fungi imperfecti) are capable of modifying these poisonous substances to less toxic forms, thus rendering it less effective in protecting wood from decay by basidiomycetes wood destroyers (8, 30).

Some of these fungi are now known also to be soft rotters (10, 15, 33, 36).

Tolerance to certain preservatives occurs also among many of the wood-decay fungi (12, 13, 14, 26, 55); therefore, it is important in the selection of preservatives to consider the major decay fungi of a product and their tolerances to toxicants.

In some laboratory experiments the tolerance of fungi to preservatives increases with the time of incubation, and the same may occur in nature where, after a period of time, the substrate is detoxified (5).

The organisms associated with decay development in living trees, in fence posts and untreated stakes have been studied and a predominance of typical rot fungi are found in the interior and ascomycetes, fungi imperfecti, and bacteria are commonly found in the outer zone (2).

Such studies have also been carried out for treated wood and recently in chip piles (24, 30).

Data on the organisms involved assist in the selection of test fungi from knowledge of the most susceptible species in the system, and also develop a deeper insight into the decay process.

Eslyn (19) in a study of the decay of utility poles treated with creosote, found no evidence that the preservative influenced the species of fungi invading the central or untreated interior of the poles. He believed that decay fungi gain entry primarily through seasoning checks or, perhaps to a lesser extent, through zones of inadequately treated wood.

Studies on the interactions and related roles of the organisms which degrade wood have many complications, such as time of sampling, sampling techniques, isolation difficulties, and identification of the many associated organisms (24, 32, 44).

Dwyer and Levy (16) developed an objective analysis approach by investigating methods to process in a statistical way the data obtained from organisms colonizing wood. Previous studies of organisms invading at various times wood can be described as subjective analyses of objectively obtained data; these studies included those by Corbett and Levy (11), Merrill and French (36), Käärik (28, 29), and Banerjee and Levy (2). Butcher (6) was the first to attempt to carry out an objective analysis based on the methods commonly applied to higher plant communities, using frequency of isolation as a measure of abundance.

Selective media for separating the different groups of wood-inhabiting fungi have been used by many investigators (9, 13, 24).

A complete review of selective media for the isolation of basidiomycetes from wood is given by Hale and Savory (25).

Many researchers have suggested the possibility of succession and related interactions among organisms associated in the process of discoloration and decay of treated and untreated wood (22, 31, 35, 37, 46, 47, 48, 49, 51).

Some recent investigations have been concerned with the development and use of natural preservatives to minimize problems in the environment or to nontarget organisms. Antibiotic toxicants have been suggested as possible preservatives (40, 53).

Biological control, the employment of biological antagonists, has been proposed as a feasible alternative to the use of toxic chemicals for prevention of wood decay (27, 45).

This research deals mainly with the identification, anatomical effects on wood structure, relative tolerance to a preservative, and elucidation of the roles and interactions among fungi associated with decay development in creosote pine poles from utility lines.

Materials and methods

The general design of this study was to select randomly from creosoted pine poles in utility lines, four groups of poles representing service ages of 20, 30, 40, and 50 years. A total of 46 southern yellow pine poles were made available for the study by the New York Bell Telephone Co. in Syracuse, N. Y.

Sampling technique

Core samples were collected with an increment borer during the period January-August 1978. Ap-

proximately 10 poles in each age group were sampled at the ground line (pH of the soil varied from 4.5–5).

Media

Since some fungi grow better on certain media, ten media were tested initially to determine which would yield the largest number of organisms from the cores (Table 3), at pH values varying from 5.7 to 6.2 as follows:

2% malt agar: malt extract 25 g; agar 15 g; water 1000 ml.

3% malt agar: malt extract 30 g; agar 15 g; water 1000 ml.

5% malt agar: malt extract 50 g; agar 15 g; water 1000 ml.

Nutrient agar: nutrient broth 8 g; agar 15 g; water 1000 ml

Duncan's modified media: NH_4NO_3 6 g; K_2HPO_4 5 g; KH_2PO_4 5 g; $MgSO_4 \cdot 7H_2O$ 4 g; agar 15 g; water 1000 ml

Benomyl agar: malt extract 12.5 g; benomyl 0.05 g streptomycin sulphate 30 μg/ml; penicillin G 40 units per ml; agar 15 g; water 100 ml.

Copper sulphate: malt extract 25 g; agar 15 g; copper sulphate hydrated 10 g; water 1000 ml.

2% malt agar–0.5 malic acid: malt extract 20 g; agar 15 g; 0.5 g malic acid; water 1000 ml

2% malt agar-0.06 g o-phenyl phenol: malt extract 20 g; agar 15 g; 0.06 o-phenyl phenol; water 1000 ml.

Sodium caseinate: sodium caseinate 2 g; glucose 1 g; K_2PO_4 0.2 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; $FeSO_4$ 0.01 g; agar 16 g; water 1000 ml

Several chips were placed per plate per core position. The cultures were incubated at 28°C.

Isolation and identification of organisms

Identification and frequencies of the major microorganisms were determined by isolations from outer, middle, and inner core positions. Identification of the fungi was made mostly by cultural and microscopic means using general keys (1, 3, 4, 17, 18).

Tests

The roles and interactions of the major isolates, grouped by radial position in the core and in service age classes, were determined by decay and preservative tolerance tests, and anatomical study

Preservative tolerance test

For the preservative tolerance test, crude creosote previously sterilized (United States Steel Corporation; Clairton Coal, Coke, and Chemical Works, Clairton, Pa.), was added aseptically to sterilized 2.5% malt extract agar in the following quantities:

Concentration selected	Creosote added
%	g
0.05	0.25
0.10	0.50
0.50	2.50
1.00	5.00
5.00	25.00
10.00	50.00

The plates were inoculated with 14 major representative fungi, and incubated for two weeks at 28°C. Radial measurements of growth were made daily during the period of incubation. Plates without preservatives were also included as controls. Dosage response curves were plotted on semi-log graph paper and 50% inhibition values determined by extrapolation.

Wood decayers test

The fungi with clamps connections and putative decayers (the ones that appeared to be decayers but lacked clamps) were selected for the decay test. A well-known decayer [*Poria placenta* (Fr.) Cke.], commonly isolated from poles, was included in the test for comparison.

The procedure used was the agar type decay chamber with malt extract agar and wood blocks (3 x 2 x 1.5 cm). The inoculated French square bottles were incubated for one week at 28°C.

After one week when the mycelium had spread over the surface, sterilized glass V-support rods were placed aseptically at the center of the mycelium, and small sapwood blocks of southern yellow pine (3 x 2 x 1.5 cm) (previously oven-dried, water-satured and surface steam-sterilized) were introduced with the cross section surface down. The oven-dried weight for each block was recorded for determination of weight loss at the end of the test.

Chambers with blocks and no inoculum were assembled as controls. The decay chambers were incubated at 28°C in the dark for four months.

After two months of exposure, blocks for decay-rate determinations and sectioning were removed from some chambers. Sections from fixed (FAA)

blocks were cut (20-30 microns thick) from radial face of the block with a sliding microtome and stained using the picroaniline blue procedure (7). The blocks for weight loss determinations were oven-dried 24 hours at 105°C and reweighed.

After four months of exposure, the rest of the blocks were removed from the chambers. The same procedures were followed as in the two-month exposure.

Soft-rotters test

A modification of the Nilsson (38) method was used for the soft-rot test on the major microfungi isolated.

Two southern yellow pine sapwood blocks (3 x 2 x 1.5 cm) treated as previously described and with filter paper squares at their bases (to stimulate fungal growth), were buried in 10 g of vermiculite contained in eight-ounce French square bottles fitted with cotton filter caps. The blocks were oven-dried and weighed before introducing them into the bottles.

Thirty milliliters of the following nutrient solution were added aseptically to the bottles after introducing the blocks:

NH_4NO_3	6.0 g
K_2HPO_4	4.0 g
KH_2PO_4	5.0 g
$MgSO_4 \cdot 7H_2O$	4.0 g
Glucose	2.5 g
Water	1000 ml

After the addition of the nutrient solution, the bottles were sterilized 30 minutes at 121°C.

Inoculations were made with suspensions of isolate mycelium incubated in 5 ml of malt extract solution for 48 hours at 28°C. Bottles without inocula were prepared for checks. All the chambers were incubated in the dark at 32°C for four months.

Microscopic studies

After two and four months of exposure to different fungi, representative blocks from the decay and soft-rot tests were removed for anatomical studies. After four months, oven-dry weights for the test blocks were determined as described previously.

Permanent slides were prepared and studied under the light microscope for evidence of decay, such as bore holes, cell wall erosion, cell wall thinning of channeling on the inner lumen wall, and the dissociation or destruction of parenchyma cells. Special attention was directed to detect the two types of attack caused by soft rotters, i.e., type 1) longitudinal cavity formation and type 2) cell wall erosion (34).

Small sections were cut from the blocks with a sharp razor blade for scanning electron microscope observations. They were fixed in 2% glutaraldehyde or 2% OSO_4, rinsed in sodium cacodylated buffer or distilled water and dehydrated in a graded ethanol series, and then dried by the method of critical-point drying with CO_2, and sputter-coated with gold palladium, and observed under the scanning electron microscope Model ETEC Autoscan for evidence of decay.

Results

Isolation and identification of fungi

Seventy-one fungi assignable to 12 genera and including also 11 unknows were isolated from the 46 poles. The genera and species are listed in Table 1.

Table 1. Isolation frequency of the fungi obtained from 33 poles with service ages ranging from 10 to 50 years.

Genus and/or Species	Isolation frequency	Pole service age (10-50 years)
Frequent (Isolated 7-20 times)		
Cladosporium resinae	21	10-40
Exophiala jeanselmei	8	10 and 30
Hyalodendron sp	7	10, 30, and 40
Acremonium spp	7	20, 30, and 50
Infrequent (Isolated 2-6 times)		
Phialophora spp	5	10-30
Unknown decayers	4	30 and 40
Phialophora lagerbergii (Melin & Nannf.) Conant	2	20 and 30
Phialophora heteromorpha (Nannf.) Wang	2	30
Sporothrix sp.	2	30
Scytalidium album Pesante	2	10 and 30
Rare (Isolated only once)		
Scytalidium lignicola Pesante	1	10
Paecilomyces varioti Bainer	1	50
Penicillium sp.	1	20
Talaromyces sp	1	40
Rhinocladiella atrovirens Nannf.	1	30
Ramichloridium sp	1	30
Unknowns	7	10, 20 and 30

Table 2. Identity, location, and frequency of the fungi obtained from cores of 33 creosoted pine poles.

Outer position	Frecuency
Putative decayers	2
Phialophora sp	1
Sporothrix sp	1
Phialophora heteromorpha	1
Cladosporium resinae	12
Hyalodendron sp	4
Exophiala jeanselmei	4
Talaromyces sp	1
Acremonium spp	3
Phialophora spp	2
Paecilomyces varioti	1
Penicillium sp	1
Middle position	
Wood decayers	
Unknown decayer	1
Soft rotters	
Phialophora sp	1
Non-decayers	
Sporothrix sp	1
Phialophora heteromorpha	1
Scytalidium album	1
Phialophora spp	4
Cladosporium resinae	14
Hyalodendron sp	3
Exophiala *jeanselmei*	2
Phialophora lagerbergii	2
Acremonium spp	5
Paecilomyces varioti	1
Scytalidium lignicola	1
Unknown	1
Center position	1
Putative decayers	1
Acremonium sp	1
Unknowns	2

Cont.

Outer position	Frecuency
Sporothrix sp	1
Scytalidium album	1
Phialophora spp	4
Cladosporium resinae	12
Hyalodendron sp	2
Exophiala jeanselmei	5
Phialophora lagerbergii	1
Acremonium spp	4
Talaromyces sp	1
Rhinocladiella atrovirens	1
Scytalidium lignicola	1
Ramichloridium sp	1
Unknown	4

according to the frequency of isolation. The most common fungus was *Cladosporium resinae*, followed by *Exophiala jeanselmei*, *Phialophora* spp., *Hyalodendron* sp., and *Acremonium* spp. Four isolates of unknown (clamps) or putative decayers were obtained.

The majority of the fungi were isolated from the center (42) and middle portions (38) of the three core positions and the lowest number from the outer core position (33) (Table 2). The poles in 10 to 30 years of service range had the greater number of fungi (60); few fungi were isolated from 50-year-service poles (10) (Table 1).

Media

The majority of the fungi were isolated on the malt extract agar (112) and sodium caseinate media (47). Some of the fungi isolated grew in all media except the copper sulphate medium where no growth was observed. Benomyl medium reduced the growth of the microfungi and often growth was restricted to the surface of the wood chip. Some fungi grew on 2% malt agar ortho-phenyl phenol and 2% malt agar malic acid media. No specificity of media for a particular fungus was observed, eg., *C. resinae* grew on all media except benomyl, where the growth was reduced (Table 3).

Creosote tolerance test

The test fungi showed appreciable variation in resistance to creosote at the one week exposure (Table 4). *Poria placenta*, a very sensitive fungus and *Lentinus lepideus* Fries, a very tolerant fungus, used as controls, grew on 0.05% (1.0 cm/week) and 0.1% (0.5 cm/week) (Controls 3 cm/week). Some growth was observed in the case of *Lentinus lepideus*

Table 3. Types and frequency of microorganisms isolated from creosoted pine poles by using various selective media.

Media	Ascomycetes[a]			Basidio-mycetes[a]	Bacteria[a]		
	F	I	R	R	F	I	R
					Rod Coccus P	Rod Coccus P	P
					+ – + –	+ – + –	
Malt agar 2%	0	16	2	–	0 0 0 0 0	2 7 0 2 3	–
Malt agar 3%	45	0	0	–	4 1 0 1 1 8	0 0 0 0 0	–
Malt agar 5%	49	0	0	–	0 0 0 0	4 5 0 1 1 4	–
Nutrient agar	0	0	3	–	0 0 0 0	3 4 0 1 4	–
Malt agar 2% –0.5 malic acid	0	22	0	–	– – – – –	– – – – –	8
Malt agar 2% –0.06 g 0-phenyl phenol	0	23	0	–	– – – – –	– – – – 14	–
Sodium caseinate	46	0	0	1	8 6 3 5 1 4 –	– – – – –	–
Benomyl agar	–	24	–	3	– – – – – –	6 4 2 2 7	–
Copper sulphate	0	0	0	–	0 0 0 0 0 –	0 0 0 0 0	–

a/ F = frequent (30-50); I = infrequent (10-29); R = rare (1-9); P = non-identified

Table 4. Tolerances to creosote concentrations after one week of exposure.

Fungi	Concentration (%)		
	Growth[a] Reduction 50% (LD-50)	Threshold Toxicity	Minimum for Growth
Hyalodendron sp	4.50	15.0	10.0
Cladosporium resinae	0.90	15.0	10.0
Acremonium sp	0.05	1.0	0.5
325-3E	0.04	0.5	0.1
Exophiala jeanselmei	0.04	1.0	0.5
Lentinus lepideus	0.04	0.5	0.1
334-1B	0.03	5.0	1.0
119-1B	0.03	0.5	0.1
Poria placenta	0.03	0.5	0.1
328-3E	0.03	0.5	0.1
334-2E	0.03	0.5	0.1
326-3B	0.02	0.5	0.1
394-3M	0.02	0.1	0.05
Phialophora sp		0.05	

a Values extrapolated from the dosage response curves

on 0.5% after the seventh day (0.2 cm); in the case of *Poria placenta* only a trace of growth was observed on 0.5% (0.1 cm).

The unknown decayer and the putative decayers were resistant to the preservative and grew on 0.05% (0.5 cm/week) and 1.0% (0.2 cm) after the fifth day.

Decoloring of the media was observed with some fungi at the high concentrations of creosote previous to any fungal growth on the media.

Phialophora sp., a vigorous soft rotter, was very susceptible and did not grow on any of the creosote concentrations. The other soft rotters grew on 0.05% (1.0 cm/week) (Controls 2.0 cm/week). *Acremonium* sp., a weak soft rotter, grew on 0.1% (0.6 cm/week), on 0.5% some growth was observed on the eight day (0.1 cm) but did not continue (Controls 2.0 cm/week). Another soft rotter (325-3E) also grew on 0.5% (0.5 cm/week) but the growth did not increase after the sixth day (Controls 3 cm/week).

Exophiala jeanselmei and 328-3E, non-decay fungi, grew on 0.05% (0.4 cm/week) and 0.1%

(0.2 cm/week) (Controls 1 cm/week). *E. jeanselmei* also grew on 0.5% (0.1 cm/week) but no increase in growth was observed after the sixth day. Some growth started on 1% on the tenth day of exposure (0.1 cm). Two non-decay fungi, *Cladosporium resinae* and *Hyalodendron* sp., were highly tolerant to creosote. They grew on all media and abundant sporulation was observed after the fourth day (Controls 2.0 cm/week).

Decay studies

Substantial weight loss of the wood test blocks was obtained with three of the four putative decayers after a four-month exposure. The weight losses ranged from 20.74 to 27.99%. One of the suspect decay fungi showed less ability to decay wood, and the weight losses ranged from 3.51% to 17.95%. Four microfungi which caused more than 5% weight loss are grouped tentatively as soft rotters. A high weight loss (13.91-15.31%) was obtained with one of the soft rotters (*Phialophora* sp.). The weight loss for the other fungi ranged from 3.30% to 11.15%.

The remaining fungi tested were judged to be non-decayers when weight losses were less than 5% (Table 5).

Some of the wood decayers and soft rotters showed great variability as indicated by the high values

Table 5. Weight losses[a] of southern yellow pine blocks exposed to fungal isolates for four months.

Fungus	Weight loss percent	Standard deviation	
Wood decayers[b]	Mean	Range	
119–1B	27.99	23.70–31.15	2.767
334–2E	25.43	12.00–34.70	8.156
326–3B	20.74	14.25–26.45	3.903
334–1B	12.74	3.51–17.95	7.081
Poria placenta	59.28	27.70–65.75	13.970
Soft rotters			
Phialophora sp. (334–2A)	15.31	10.00–21.25	4.313
Phialophora sp. (334–1E)	14.55	7.55–21.00	4.847
Phialophora sp. (334–2D)	13.91	10.80–20.15	3.412
394–3M	9.78	9.00–11.15	0.701
325–3E	6.65	4.85–9.05	1.261
Acremonium sp. (335–3D)	5.15	3.30–7.70	1.756
Non–decayers			
Acremonium sp. (325–3D)	3.90	2.65–5.20	0.792
328–3E	3.84	3.40–4.70	0.418
Phialophora lagerbergii	3.48	1.90–4.55	0.992
Phialophora sp. (338–2A)	2.95	1.65–4.15	0.894
Exophiala jeanselmei	2.91	1.35–4.00	0.918
Phialophora sp. (335–2A)	2.71	1.60–3.95	0.830
Cladosporium resinae	2.70	1.60–3.45	0.543
Hyalodendron sp.	2.60	1.70–4.15	0.808
Rhinocladiella atrovirens	2.46	1.75–3.65	0.704
Acremonium sp. (371)	2.11	1.75–3.05	0.419
Phialophora heteromorpha	1.91	1.20–3.35	0.698
Sporothrix sp.	1.68	0.05–3.20	0.980

a Values for individual block weight losses were corrected by reference blocks subjected to all aspects of the test other than exposure to a fungus. These adjustments include a 2% correction in weight loss for all blocks due to loss of water solubles during water soaking to adjust moisture contents.

b Decayers: fungi with clamps, formed bore holes as large or larger than the hyphae and / or causing weight loss in a block exceeding 12%. Soft rotters: fungi which produce longitudinal bore holes and with weight losses exceeding 5%. Non-decayers: less than 5% weight loss.

obtained in the standard deviation (Table 5). These were associated with appressed mycelial mats and the difficulty in achieving uniform inoculation of the blocks

Microscopic studies of wood decayers

In some cases initially hyphae were concentrated in ray parenchyma which were later obliterated (334-1B; 119-1B; 334-2E; 326-3B), and ray tracheids, also in springwood tracheids (119-1B); or heavily and uniformly distributed in the wood (334-2E; 326-3B).

Hyphae were frequently or infrequently branched, with clamps (medalin type 334-2E) or without clamps (119-1B; 326-3B; 334-1B) and with a general diameter of 1-4 μ.

Small bore holes (119-1B) or some larger than the diameter of the hyphae were commonly observed (326-3B; 334-2E). Pit passages were very common (119-1B; 334-2E) (Figure 1). Longitudinally oriented erosion channels (334-2E; 334-1B) followed the fibril angle (Figure 2) and cell wall thinning were very common (334-2E). Pit apertures were eroded (326-3B).

Microscopic studies of soft rotters

Hyphae were abundant in wood (*Phialophora* sp 334-1E, 2A, 2D); concentrated in ray parenchyma

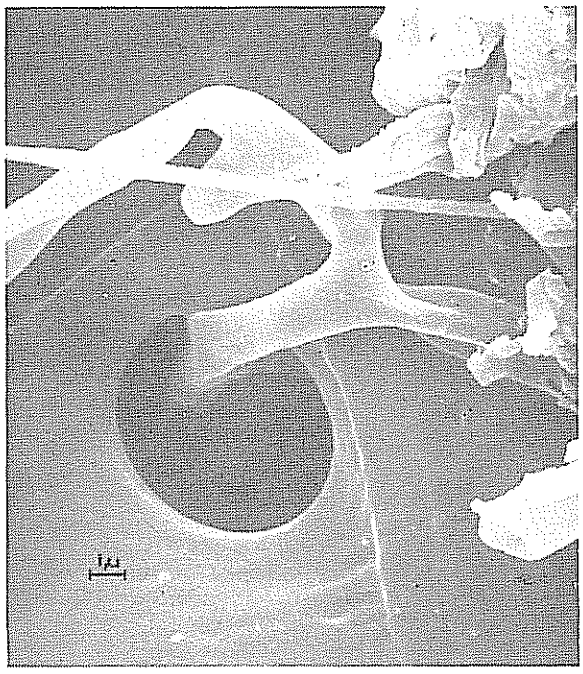

Fig 1 A radial longisection of decay fungi (119-1B) growing in southern yello pine sapwood showing a bordered pit penetration. SEM Mag 4400 X

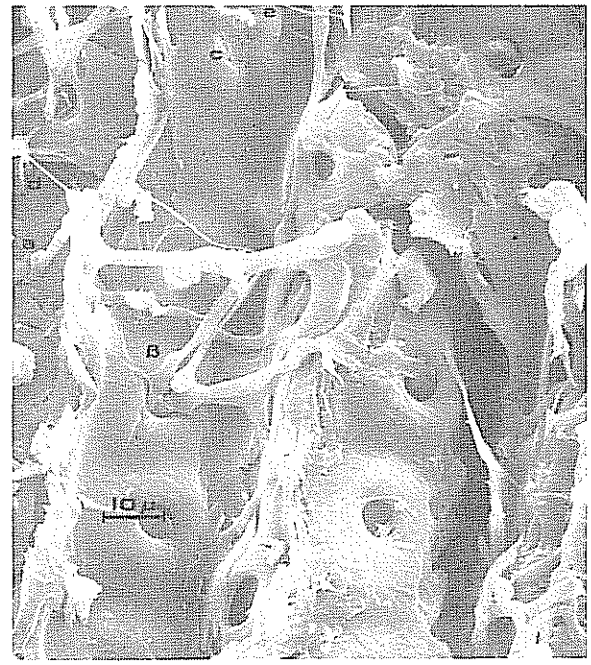

Fig 2 A radial longisection of decay fungus (334-2E) growing southern yellow pine sapwood, after a 4 month exposure in an agar wood-block decay test. Special features are designated by letter as follows: a) clamp connection, b) bordered pit penetration, c) differential cell wall erosion, d) desicatted slime tendrils, and e) possible microphyphae. SEM Mag. 925 X

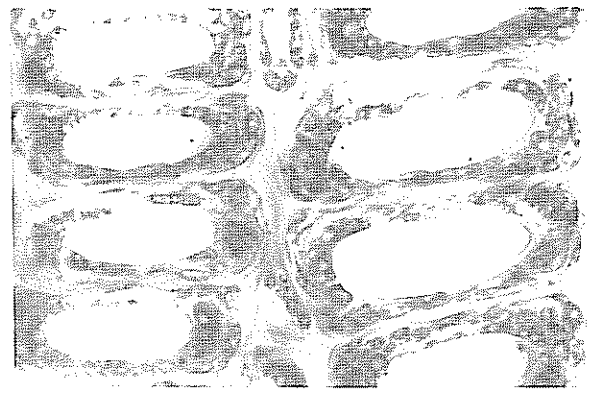

Fig 3 A transverse section of a soft-rot fungus (*Phialophora* sp.) growing in southern yellow pine sapwood after a 4 month-exposure in a decay chamber (Nilsson 1973) showing several states in the severity of secondary wall invasion by the fungus. LM Mag. 72 X.

and springwood tracheids (394-3M; 325-3E; 355-3D); or concentrated in the secondary wall of summerwood cells (*Acremonium* sp. 335-3D). The secondary wall was removed in several springwood cells (334-1E); in others, the secondary wall had numerous small cavities (334-1E; 325-3E; 394-3M).

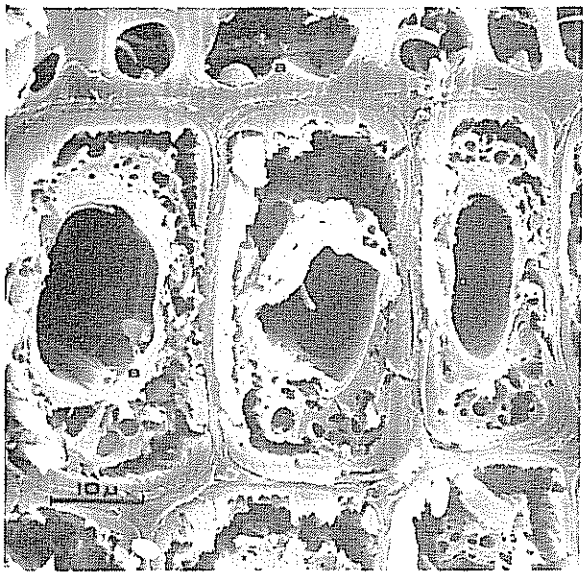

Fig 4 An enlarged cross section of southern yellow pine sapwood invaded by a soft-rot fungus (*Phialophora* sp.) showing a hypha transversely penetrating the cell wall (a) and hyphal filaments lining the inner lumen wall (b) SEM Mag 1950 X

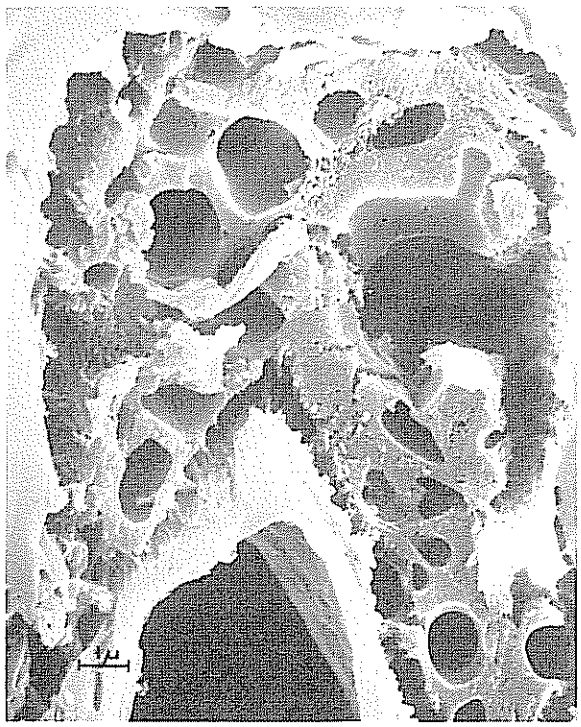

Fig 5 An enlarged section of a tracheid showing substantial cell wall erosion associated with the longitudinal bore holes SEM Mag 6250 X

The summerwood cells were heavily attacked and in some cells the secondary wall had been removed or appeared swollen (334-1E). Small cavities were observed in the tangential sections parallel to the microfibrills (Figures 3-5). Cell wall erosion (*Acremonium* sp. 335-3D) and pit passages were common (394-3M; 325-3E).

Hyphae were frequently or infrequently branched, without clamps, and with a general diameter of 1-4-3 μ

Pictures were taken with light microscope and scanning electron microscope of some of the wood decayers and one of the soft rot fungi (Figures 1-5).

Discussion

The 71 isolates totaling 8 species and 12 genera suggest a rather restricted range of fungi inhabiting creosote-treated wood. Although many researchers have reported difficulty in isolating wood decayers from creosoted wood, these techniques using selective media and daily subculturing obtained four decayers, representing approximately 6% of all isolations. Daily subculturing was judged to be as important as the selective media in obtaining pure cultures of the wood decay fungi from creosoted pine.

The isolation of fungi from only 33 poles, of the 46 sampled, demonstrated that the conventional creosote preservative treatment frequently protects poles effectively up to 40-50 years. There was no clear relationship between increasing pole age and the numbers of fungi isolated. This suggests a wide variation in the treatment effectiveness of poles and the probable concentration of the best treatments (by replacements) in older age classes. The small number of isolates obtained may reflect in part also the difficulty encountered in finding suitable media for the isolation of the fungi present. Also some slow-growing fungi may be missed by the growth-retardation effect of fast growing fungi which quickly cover the plates and dominate the media.

The kinds and numbers of fungi isolated were closely related to the external conditions of the poles as mechanical injuries or deep checks.

Based on these studies the fungi found in creosote-treated poles may represent a specialized fungi that can be divided into three groups.

The first are those "toxicant tolerant primary invaders" which include the fungi able to tolerate high levels of creosote in the wood and that primarily utilize wood components of low molecular weight

for their energy requirements. *Cladosporium resinae* is in this group. It has been reported as a fungus very tolerant to creosote by several investigators (8). *Hyalodendron* sp., *Acremonium* sp., and *Exophiala jeanselmei* were demonstrated also in this study to be tolerant to creosote. They did not produce visible cell wall erosion or appreciable weight losses. This suggests a possible role in the wood as detoxifiers or wood modifiers, facilitating the more creosote-susceptible fungi to invade the wood later. Most of them were found in the middle position of the cores.

A second group are those fungi that can be called "non-tolerant opportunists" because they follow and appear to invade the wood when the preservative level has been diminished by the detoxifiers. As a group, they were sensitive to creosote. They did not cause any decay as indicated by the very low weight losses obtained with these fungi (Table 5). They were commonly isolated from the middle and center position of the cores.

A third group the "wood decayers" include both hymenomycetes and soft-rot-type decayers. This group showed very special characteristics. Some of the decay fungi were highly tolerant to creosote and were fast growing fungi, which retard the growth of the non-decayers; others were more sensitive to creosote. One of the more aggresive soft rotters (*Phialophora* sp.) was totally inhibited by low concentrations of creosote. This fungus might be called a non-tolerant opportunist because its attack is delayed until the substrate is detoxified.

Some of the decayers produced substantial weight losses, copious bore holes, cell wall erosion, and cell wall thinning, and in the case of the soft rotter *Phialophora* sp., the secondary wall was completely destroyed.

The interaction roles of the soft rotters with the hymenomycetous decayers is unknown. In this study, the aggresive soft rotter (*Phialophora* sp.) was retarded by the fast growing decayers, but no visible distortion of the hyphae was observed and the fungus sporulated abundantly.

Association of bacteria with the fungi was observed frequently but studies correlating their processes in the decay of wood were not carried out.

A succession of fungi in the invasion and decay of wood has been suggested by many investigators (31, 37, 46, 47, 48, 49, 50, 51).

From this limited study, successional sequences can be postulated as the fungi from the first and second group were commonly isolated from the same position and from poles with the same service life. Also, the wood decayers which were very tolerant to creosote can appear early in the pole and not until the substrate is modified to start the invasion. A clear interaction between the three groups can be postulated on the basis that the "opportunists" were commonly found first on positions with low preservative concentrations and the detoxifiers on the highly concentrated; wood decayers were isolated from the outer portions of poles where preservatives were absent or in low concentrations.

In studies of wood preservative effectiveness and in the determination of the treatments to be used, it may be important to consider the possible toxicant modifying roles of the tolerant fungi.

The possible damaging roles of soft rot fungi in poles may be important and needs additional study.

Summary

Forty-six creosoted pine poles representing four ages of service (10-50 years) were sampled in the Syracuse area for wood-inhabiting fungi.

Seventy-one fungi assignable to 12 genera and 8 species including 11 unknowns were isolated from the poles.

Creosote tolerance and decay tests were carried out to determine the possible roles and interactions of the fungus. Anatomical studies with light and scanning electron microscopes were related to changes in wood by the decay fungi.

Three groups of fungi appeared to interact in creosoted pine poles. The first group includes the so-called "tolerant primary invaders" which are highly tolerant to creosote and may serve as detoxifiers. the second group include the "non-tolerant opportunists" which take advantage of the modification of the wood and may exert antagonistic reactions to other fungi, but do not cause decay. The third group were the "conventional decayers" which sometimes are tolerant to preservatives, and cause extensive decay. This group included both hymenomycetes and soft-rot fungi.

Literature cited

1. ARX, J. A. von. The genera of fungi sporulating in pure culture. Germany J. Cramer 1970 288 p.

2. BANERJEE, A. K. and LEVY, J. F. Fungal succession in wooden fence posts. Mat. und Org. 6(1):1-25. 1971.

3. BARNETT, H. L. and HUNTER, B. B. Illustrated genera of Imperfect Fungi. Third Edition. Minnesota. Burgess Publishing Co. 1972. 241 p.

4. BARRON, G. L. The genera of Hyphomycetes from soil. Baltimore. Williams and Wilkins Co. 1968. 364 p.

5. BRAVERY, A. F. Determining the tolerance of soft-rot fungi to wood preservatives: A comparison of test methods. Mat. und Org. 3:213-227. 1968.

6. BUTCHER, J. A. Techniques for the analysis of fungal floras in wood. Mat. und Org. 6:209-232. 1970.

7. CARTWRIGHT, K. ST. G. A satisfactory method of staining fungal mycelium in wood sections. Annals of Botany London. 43:412-413. 1929.

8. CHRISTENSEN, C. M. *et al. Hormodendrum resinae* (L.) an inhabitant of wood impregnated with creosote and coal tar. American Journal of Botany 29:552-558. 1942.

9. CLUBBE, M. and LEVY, J. F. Isolation and Identification of fungal flora in treated wood. Devised technique. The International Res. Group on Wood Preserv. Princes Risborough Lab. England. 1977. Doc. No IRG/WP/159. 3 p.

10. CORBETT, N. H. Micro-morphology studies on the degradation of lignified cell walls by Ascomycetes and Fungi Imperfecti. Journal Institute of Wood Science 14:18-29. 1965.

11. CORBETT, N. H. and LEVY J. F. Ecological studies on fungi associated with wooden fence posts. I. Brit. Wood Press. Ass. News Sheet No. 27. 1963.

12. COWLING, E. B. The relative preservative tolerance of 18 wood destroying fungi. Forest Products Journal 7(10):355-359. 1957.

13. DUNCAN, C. G. and DEVERALL, F. Degradation of wood preservatives by fungi. For. Prod. Lab. For. Service USDA. Applied and Environmental Microbiology 12(1):57-62. 1964.

14. DUNCAN, C. G. and LOMBARD, F. Fungi associated with principal decays in wood products in the United States. US. For. Service Research Paper WO-4 DA. 1965. pp. 1-13.

15. DUNCAN, C. G. and ESLYN, W. Wood-decaying Ascomycetes and Fungi Imperfecti. Mycologia 58:642-645. 1966.

16. DWYER, G. and LEVY, J. F. The colonization of wood by microorganisms: An objective approach. Beihefte zu Mat. und Org. 3:13-20. 1976.

17. ELLIS, M. B. Dematiaceous Hyphomycetes. England. Commonwealth Mycological Institute. 1971. 608 p.

18. ELLIS, M. B. More Dematiaceous Hyphomycetes. England. Commonweath Mycological Institute. 1976. 507 p.

19. ESLYN, W. E. Utility pole decay. Part II. Basidiomycetes associated with decay in poles. Wood Science Technical 4:97-103. 1970.

20. GAMS, W. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes) Urgarn: Gustav Fischer Verlag. 1971. 262 p.

21. GAMS, W. and HOLUBOVA-JECHOVA, V. *Chloridium* and some other dematiaceous hyphomycetes growing on decaying wood. Studies in Mycology 13:97. 1976.

22. GREAVES, H. Microbial ecology of untreated and copper-chrome-arsenic treated stakes exposed in a tropical soil. I. The initial invaders. Canadian Journal of Microbiology 18:1923-1931. 1972.

23. GREAVES, H. Influence of a mixed microbial population on basidiomycetes decay. Mat. und Org. 7(1):11-25. 1972b.

24. GREAVES, H. and SAVORY, J. G. Studies of the microfungi attacking preservative-treated timber, with particular reference to methods for their isolation. Journal Institute of Wood Science 15:45-50. 1965.

25. HALE, M. D. C. and SAVORY, J. G. Selective agar media for the isolation of basidiomycetes from wood. A review. International Biodetn. Bulletin 12(4):112-115. 1976.

26. HENNINGSSON, B. and NILSSON, T. Some aspects on microflora and the decomposition of preservative-treated wood in ground contact. Beihefte zu Mat. und Org. 3:307-318. 1976.

27. HULME, M. A. and SHIELDS, J. K. Biological control of decay fungi in wood by competition for non-structural carbohydrates Nature 227:300-301. 1970.

28. KAARIK, A. Colonization of pine and spruce poles by soil fungi after six months. Mat. und. Org. 2:97-108. 1967.

29. KAARIK, A. Colonization of pine and spruce poles by soil fungi after 12 and 18 months. Mat. und Org. 3:185-198. 1968.

30. KERNER-GANG, W. Effect of microorganisms on creosote. Beihefte zu Mat. und Org. 3:319-330. 1976.

31. LAFLAMME, G. and LORTIE, M. Microorganisms in discoloured and decayed wood of *Populus tremuloides*. Canadian Journal and Forestry Research 3(2):155-160. 1973.

32. LEIGHTLEY, L. E. and EATON, R. A. A method for studying the growth of soft-rot fungi in wood veneers. Int. Biodetn. Bulletin 12(2):44-48. 1976.

33. LEVY, J. F. The soft-rot fungi. Their mode of action and significance in the degradation of wood. **In** R.D. Preston Advances Botany Research 2:323-357. 1965.

34. LEVY, J. F. and STEVENS, M. G. The initiation of attack by soft-rot fungi in wood. Journal Institute of Wood Science 16:49-55. 1966.

35. LEVY, M. P. The effects of microorganisms on chromated-copper-arsenate preservatives in wood. Beihefte zu Mat. und Org. 3:297-306. 1976.

36. MERRILL, W. and FRENCH, D. W. Colonization of wood by soil fungi. Phytopathologist 56:301-303. 1966.

37. MORTON, L. H. G. and EGGINS, H. O. W. Studies of interactions between wood-inhabiting microfungi. Mat. und Org. 11(3): 197-214. 1976.

38. NILSSON, T. Studies on wood degradation and cellulolytic activity of microfungi. Studia Forestalia Suecica Nr. 104. Sweden. 1973. 40 p.

39. NOBLES, M. K. Studies in Forest Pathology VI. Identification of cultures of wood-rotting fungi. Canadian Journal Research 26:281-431. 1948.

40. RICHARD, J. L. Biological control of decay in standing creosote-treated poles. Journal Institute Wood Science 7(4):6-9. 1976.

41. SAVORY, J. G. Breakddown of timber by Ascomycetes and Fungi Imperfecti. Annals of Applied Biology 41:336-347. 1954.

42. SCHEFFER, T. C. Microbiological degradation and the causal organisms. In Wood Deterioration and its Prevention by Preservative Treatments. Vol. 1. Degradation and Protection of Wood. Syracuse: Syracuse University Press. 1973. pp. 31-106.

43. SCHOL-SCHWARZ, M. B. Revision of the genus *Phiolophora* (Moniliales) Persoonia 6(1):59-94. 1970.

44. SHARP, R. F. and EGGINS, H. O. W. A perfusion technique for culturing fungi in wood. Institute Wood Science Journal 4(4): 22, 24-31. 1969.

45. SHIELDS, J. K. and ATWELL, E. A. Effect of a mold *Trichoderma viride*, on decay of birch by four storage-rot fungi. Forestry Products Journal 13:262-265. 1953.

46. SHIGO, A. L. Succession of organisms in discoloration and decay of wood. International Review Forestry Research 2:237-285. 1967.

47. SHIGO, A. L. Interaction of organisms in the process of decay in forest trees. Bulletin Fonds. Rech. For. University Laval 13:43. 1970.

48. SHIGO, A. L. Succession of microorganisms and patterns of discoloration and decay after wouding in red oak and white oak. Phytopathology 62(2):256-259. 1972.

49. SHIGO, A. L. Biology of decay and wood quality. Biological transformation of wood by microorganisms. Proceedings Sessions Wood Prod. Path. Second International Congress Plant Pathology, Sept. 10-12. Minn. USA. 1973.

50. SHIGO, A. L. Compartmentalization of discolored and decayed wood in trees. Mat. und Org. Beihefte 3:221-226. 1976.

51. SHIGO, A. L. and HILLIS, W. E. Heartwood, discolored wood and microorganisms in living trees. Annual Review of Phytopathology 11:197-222. 1973.

52. SIGLER, L. and CARMICHAEL, J. W. Taxonomy of Malbranchea and some other Hyphomycetes with arthroconidia. Mycotaxon 4(2):349-488. 1976.

53. STILLWELL, M. A. *et al.* Production, isolation and antifungal activity of scytalidin, a metabolite of *Scytalidium* species. Canadian Journal of Microbiology 19:597-602. 1973.

54. VAN BEYMA THOE KINGMA, F. H. Beschreibung der im Centraal-bureau voor Schimelcultures vorhandenen arten der gattungen *Phialophora* Thaxter und *Margarinomyces* Laxa, Nebst schussel zu Ihrer Bestimmung. Antonie von Leenwenhoek. Journal Microbiology of Serology 9:51-76. 1943.

55. ZABEL, R. A. Variations in preservative tolerance of wood-destroying fungi. Journal Forestry of Production Research Society 4:166-169. 1954.

Reseña de libros

FRENEY, J. R y SIMPSON, J. R. ed. Gaseous los of nitrogen from plant-soil systems. M. Nijhoff y Dr. W. Junk Publication. La Hoja. 1983. 317 p.

Este volumen altamente especializado es el No. 9 en la serie de este editorial en el campo del desarrollo en ciencias de suelo y de plantas. Lo escribieron 21 especialistas escogidos de Europa, USA, Nueva Zelandia y Australia, de donde proceden los dos editores. Ellos han tratado de resumir los estudios voluminosos sobre las emisiones gaseosas de diferentes sistemas suelo-planta y las maneras de como influirlos.

El material se presenta en 12 capítulos con bibliografía amplia que llega incluso al año previo a la publicación de la obra.

El primer capítulo discute la volatilización del amoniaco que es importante a partir de abonos y de excrementos de animales. El proceso lo influyen una serie de factores biológicos, químicos y físicos. Aunque varios de estos factores son bien estudiados, falta información sobre las pérdidas de muchos sistemas naturales o artificiales.

La bibliografía se presentó con 188 referencias sobre este tópico.

El Dr. Fillery del IRRI resume la información sobre denitrificación biológica particularmente importante en suelos inundados.

Más de 200 referencias resumen la información hasta 1982. Se discuten aquí los microorganismos involucrados en el proceso, la bioquímica del mismo y la biología de la denitrificación en el suelo.

La denitrificación química es el tópico del tercer capítulo. El material es presentado más que todo con base en experimentos de laboratorio. Se discute el efecto de diferentes condiciones en el suelo y de los inhibidores de nitrificación. La bibliografía de 92 trabajos ilustra el progreso en este campo.

El cuarto capítulo se dedica a las difíciles mediciones de la denitrificación. Se discuten aquí técnicas con trazadores, cromatografía de gases, análisis infrarojo y la técnica de inhibición de formación de acetileno. 189 citas que llegan hasta 1982 y son dominantemente de los últimos años, documentan el progreso en esta área.

En el quinto capítulo, uno de los más cortos, se presentan los métodos micrometológicos para medir las pérdidas de N en el campo. La bibliografía de 44 trabajos resume lo conocido en este campo. El capítulo es presentado con un enfoque matemático que permite una generalización de los conceptos presentados y asume conocimientos de matemática avanzada.

La pérdida de nitrógeno gaseoso de plantas es el tópico del sexto capítulo, otro de los relativamente cortos. La magnitud de este proceso se discute, algunos autores indican hasta 38 mg para un período de 10 semanas de una cosecha con gran superficie foliar. La bibliografía de 97 trabajos permite a los interesados profundizarse en este tópico poco explorado.

El séptimo capítulo, el más corto, presenta la información sobre la pérdida de nitrógeno de excrementos de animales y residuos municipales aplicados a tierras agrícolas. A pesar de la importancia de estas prácticas, solamente 29 referencias amplían la información, ya que existen pocos trabajos que en forma precisa informan sobre el proceso y los factores que lo determinan.

La pérdida del amoniaco de abonos aplicados a pastos tropicales es el tópico del octavo capítulo. La información usa ampliamente datos de Australia tratando de explicar las pérdidas de 20 a 80% de N que se aplicó. El crecimiento de pastos y las lluvias parecen ser algunos de los factores principales que influyen aquí. La información se completa con 55 referencias.

En el noveno capítulo se presenta los adelantos sobre el intercambio gaseoso del nitrógeno para potreros en uso. El cuadro de este ecosistema complejo influye muchos aspectos poco entendidos, particularmente lo que se refiere a los de contaminación ambiental.

Por el momento queda mucho para determinar en lo referente a la importancia y significancia agronómica de este proceso, sobre el cual se citan 99 referencias.

La suerte del nitrógeno que se aplica al arroz inundado es el tópico del décimo capítulo, uno de los más largos del volumen y las 136 referencias correspondientes al capítulo son de las más completas de la obra. A pesar de los amplios trabajos, parece que mientras no existan experimentos de campo donde se midan directamente la denitrificación y la volatilización de NH_3, no se podrán determinar cuáles son las pérdidas importantes de N de abonos aplicados a suelos inundados.

El onceavo capítulo discute la suerte de los compuestos de nitrógeno en la atmósfera. Se presenta un balance de estos compuestos aunque para partes del sistema la información es muy escasa. La bibliografía del capítulo llega a 100 referencias.

En el doceavo capítulo se discute los enfoques tecnológicos y agronómicos para reducir al mínimo las pérdidas de N gaseoso de tierras agrícolas. Esta parte del volumen es hasta cierto grado un resumen donde se refleja la aplicación de los procesos antes expuestos, indicando en la bibliografía las 108 referencias más pertinentes con base al excelente conocimiento de la bibliografía que usualmente caracterizan las publicaciones del Dr. Hauck de la TVA, autor del capítulo.

El volumen tiene un breve índice de materia a su final y contribuye en forma significativa a un mejor conocimiento de las pérdidas de N del ecosistema suelo-planta. El libro es útil para las bibliotecas agrícolas de centros de conservación de recursos y dedicados a la ecología.

ELEMER BORNEMISZA
FACULTAD DE AGRONOMIA
UNIVERSIDAD DE COSTA RICA