SUCCESSION OF ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH ENGELMANN SPRUCE AND SUBALPINE FIR IN WYOMING

by

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(ABSTRACT)

Fungi associated with Engelmann spruce and subalpine fir were studied in a high altitude area of western Wyoming. Thirty permanent plots were established and their mean stand age determined. Three age classes were delineated: young (78 years), mature (127 years), and old-age (216 years). Stand and soil parameters including density, basal area, and soil pH, P, K, Ca, organic matter, and Mg concentrations were used to define similarities in stand characteristics. Sporocarps of higher basidiomycetes and ascomycetes were collected and identified, and ectomycorrhizal root tips sampled from each plot. Pure cultures of the basidiomycetes were routinely attempted. Mycorrhizal syntheses were subsequently attempted with both tree species using successful pure cultures. Thirty-nine species of higher fungi were collected during the study. Distribution of sporocarps throughout the age classes revealed a distinct fungal flora in each age class. Greatest density of species appeared in the mature stands. Non-mycorrhizal fungi were more abundant in the mature stand while mycorrhizal species were more abundant in the old-age stand. Mycorrhizal root counts increased from young to old-age stands. Low numbers of sporocarps and mycorrhizal rootlets were collected from the young stand. It seems evident from this study that a more diverse assemblage of higher fungi is present in mature and old-age forests and a progressive increase of fungal species from young to old-age stands supports the hypothesis that fungal succession is occurring in the study area.

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INTRODUCTION

Increased oil, gas and mineral exploration in the west has opened previously undisturbed land to development. Deserts, grasslands, rivers and mountains have all felt the impact of large-scale exploitation, yet little is known concerning their responsiveness to the shock of development. Current environmental policies allow exploitation even before a minimum of information has been amassed.

The overthrust belt of western Wyoming, eastern Idaho, and portions of central Montana is among those areas forcasted for concentrated development. Containing immense deposits of oil, coal and natural gas, the overthrust belt lies beneath some of the most prized recreation and wilderness land in the world. Consequently a diversity of important rare or unusual habitats, including montane and alpine meadows, riparian boarderlines, and old-growth climax forests are in danger of being lost. Some endangered areas may be regained through proper management practices; old-growth forests with their associated animal, plant and fungal species however, may never be replaced.

The importance of mycorrhizal fungi to forest trees of all ages is widely accepted, however much is still to be learned in almost every aspect of study. The ecology and biology of mycorrhizae and their function in forest ecosystems and community dynamics are among the areas where research is needed.

The present study outlines the importance of mycorrhizal fungi to young, mature and old-growth Engelmann spruce-subalpine fir forests in western Wyoming. Fungal succession, indicated by a shift in species and number of ectomycorrhizal fungi between different age classes of trees may reflect an association between the fungal community and the age and successional status of the stand. Adequate knowledge of fungal succession may lead to significant revision of nursery and forest management practices as well as the current concept of vascular plant succession.

LITERATURE REVIEW

Abies lasiocarpa (Hook.) Nutt.(Subalpine fir), and *Picea engelmannii* Parry (Engelmann spruce), although extremely difficult to work with in the laboratory are thought to be obligately mycorrhizal (Meyer 1973). Erratic germination rates, length of growing time, and low short-root production have caused investigators to avoid using fir and spruce for mycorrhizal research. Consequently, little is known about spruce and fir mycorrhizae, and their fungal symbionts compared to other conifer species.

Dominik (1956) in classifying ectotrophic mycorrhizae based on morphological and anatomical characteristics, described a generalized spruce mycorrhiza to be monopodially branched, smooth, or pubescent with a closely woven mantle containing lance-shaped setae. Fir mycorrhizae were similar, but contained smaller, hymeniform setae, and a conspicuous difference in mantle pigmentation as viewed in cross-section. Both spruce and fir mycorrhizae generally lacked rhizomorphs. Many additional types of spruce and fir mycorrhizae have since been described. Wojciechowska (1969) found 16 mycorrhizal types on *Picea excelsa* and designated each type as a distincive "genus" by assigning symbolic letter-names. Similarly, Dominik (1961) differentiated 37 genera on *P. excelsa*. Nine different types of mycorrhizae were observed on naturally occurring White fir(*Abies concolor*) seedlings (Alvarez and Cobb 1977). Consequent resynthesis in the laboratory indicated that the synthesis-medium could influence morphology of mycorrhizae formed, although no positive identification of the mycobionts could be provided (Alvarez, Rowney and Cobb 1979).

Identification of ectomycorrhizal fungi has been based primarily on the observation of sporophores in the vicinity of mycorrhizal rootlets (Trappe 1967, Zak 1969, Laiho 1970). Confirmation that the fungi are indeed mycorrhizal must be achieved by synthesis under aseptic conditions (Hacskaylo 1953; Bryan and Zak 1961; Trappe 1967). A listing of ectotrophic mycorrhizae and their fungus associates has been prepared by Trappe (1962). Putative and confirmed mycorrhizal fungi reported from the literature are catalogued by fungus and tree species. *Cenococcum geophillum* Fr. and *Russula delica* Fr. are listed as putative associates of both *A. lasiocarpa* and *P. engelmannii*. *Amanita pantherina*(DC. ex Fr.) Secr., *Lactarius deliciosus* (L. ex Fr.) S. F. Gray, *Russula emetica*(Schaeff. ex Fr.) S. F. Gray and *Suillus ruber* Sing. and Sipe are also listed as associated with Engelmann spruce. Confirmation of mycorrhizal synthesis with

Engelmann spruce or subalpine fir is lacking, but a variety of known ectomycorrhizal fungi have been reported with these and other spruce and fir species.

The extent to which fungal sporocarps reflect the mycorrhizal flora of a given species is debatable (Trappe 1967). The temptation to equate fruiting body occurrence with mycorrhizal occurrence, however, is great. Trenching experiments showed that mycorrhizal fungi associated with spruce are unable to form fruiting bodies without symbiosis with their host (Romell 1938). Likewise, Masui (1926) found that sporocarps of *Cantharellus floccosus* Schw. are found in areas where young roots of *Abies* are distributed. Thus sporocarp production indirectly indicates the presence of mycorrhizae. Watling (1977) however, emphasized that fruiting of mycorrhizal fungi is erratic and that sporocarp production is the end-product of a process which may take a particular species several seasons to complete. Parker-Rhodes (1956) also noted that some species are found fruiting as frequently as every ten years and that distributional data based on sporocarp occurrence can be entirely misleading. Therefore presence of fruiting bodies are a good indication of mycorrhizal presence, but the fungi fruiting in any one season may be representative of only a small portion of the mycorrhizal flora (Lamb 1979).

None-the-less, Hueck (1953) points out that, in the field, one is restricted to the study of carpophores and that valuable information may be gained from these data.

Several interesting mycosociological studies have been reported, especially concerning changes in seasonal sporocarp production within a particlar successional stage or sere leading to a climax forest type. Graham (1927) noted that flowering plants have a distinct seasonality which is paralleled by fungi in the Chicago region. *Morchella* and *Peziza* species for example, were typically early season genera. Greater diversity of fungi appeared during summer, dominated by numerous species of *Russula* and *Lactarius*, while *Tricholoma*, *Hebeloma* and *Cortinarius* dominated the autumn. *Flamulina velutipes* (Fr.) Karst. was restricted to fruiting in the colder months. Similarly, Wilkins and his co-workers (1937, 1938, 1939, 1940, 1946) showed that in grasslands, pinewoods and beechwoods, greatest density and diversity of sporocarps occurred in late fall. Few fungi fruited in the spring or winter, and only a low number of species overlapped one or more seasons. Friedrich (1936,1937) indicates that seasonality may change if climatic conditions are unusual, but some fungal species will maintain their seasonality though their fruiting period may be shortened or shifted somewhat. Friedrich then identified a midsummer *Russula* "aspect", a late summer *Lactarius* "aspect" and a late autumn *Mycena* "aspect". Likewise, thirty-three common species were compared by Lange (1948) who found that five temporal aspects could be distinguished for fungi including: winter, vernal, pre-aestival, aestival and autumnal. Grainger (1942) and Grainger (1946) also supported seasonality in fungal fruiting and indicated that soil nitrogen was the factor controlling sporocarp production. Nitrates and ammonia were present in low amounts in the summer but peaked in autumn, corresponding with high fungal densities. Thus, seasonality of fruiting and succession within a seral stage or fungal succession over a particular sere may be closely related.

The constancy of association and fungal fruiting is the primary means by which fungal succession may be understood. Murrill (1949) found that in Florida particular fungi are characteristic of certain forest types. The poorly drained flatwoods occupied by *Pinus elliottii* Engelm. is the habitat of an entirely different flora of fungi than is found in the well-drained "high-pine woods" areas where *Pinus palustris* Mill. and *Pinus taeda* L. dominate. Slipp and Snell (1944) found a high correlation between the occurrence of bolete species and different stages of forest succession in the climax *Thuja-Tsuga* zone of northern Idaho. Likewise, the bog-birch-spruce sere of Denmark was shown by Lange (1948) to have characteristic fungal floras represented at each seral stage. Few species were found in conjunction with more than one seral stage. Thoen (1974) described the fungi associated with birch, birch-spruce, and pure spruce forest types in Europe. Birch, the pioneering forest type and spruce, the climax forest type, each showed a distinctive fungal flora. The mixed forest type, however, displayed a mixture of the two discrete floras. Fungal succession following burning of conifer forests in Austria was studied by Moser (1949). Within one to four months, a "Pezizaceae stage" developed, characterized by the presence and fruiting of a number of pyrophilous Discomycetes. Each subsequent stage Moser characterized as being dominated by fungi which were: anthracobious-living only on burned material, anthracophilous-prefering burned areas, anthroxenous-tolerating burned areas and the last stage, anthracophobous-not tolerating burned areas. Successional patterns of fungi in newly developed river-bar communities in Alaska were studied by Baxter (1937, 1947). *Picea glauca* was the climax dominant, preceded on young terraces by *Salix*, then *Populus* stages. Baxter found that the rate of forest succession was increased by various rusts, cankers and wood rots which eliminated *Salix* more quickly than if the plants remained healthy. Baxter claimed that where a series of hosts occur together, a series of fungi occur with them. Miller (1982) concludes that coevolution of ectomycorrhizal fungi and their hosts is commonplace and serves to elucidate patterns of specificity at the family or generic level.

Schramm (1966) observed an obvious successional sequence following plant colonization of mining spoils in Pennsylvania. Sporophores of several ectomycorrhizal fungi including: *Astraeus hygrometricus* (Pers.) Morg., *Inocybe* sp., and *Pisolithus tinctorius* (Pers.) Coker and Couch were commonly found on fully insolated refuse near young pine trees. *Thelephora terrestris* Ehrhart ex Fr.was found associated with a slight build-up of litter. A few sporocarps of *Pisolithus tinctorius* were also found, however most species associated with the young trees no longer fruited in the older plots. Likewise, only *Amanita rubescens* (Pers. ex Fr.) S. F. Gray, *Scleroderma aurantium* Pers. and several species of *Boletus* were found near the oldest trees on refuse with considerable litter-cover. Miller (1981) also inferred that *P. tinctorius* was an early successional species. Masui (1926) found that *Cantharellus floccosus* does not fruit around young fir trees, but is restricted to fir trees thirty years or older. Statistical analysis by Parker-Rhodes (1956) of the distribution of basidiomycetes in a mixed hardwood forest also showed that the fruiting of macrofungal species could be correlated with age of the forest cover.

Obligate mycorrhizal fungi and many species of forest trees require mutual dependence to initiate and support healthy growth (Trappe 1967). The utilization of mycorrhizae in modern forestry practices has not always been successful due to ignorance or disbelief in fungal ecology and succession. Kessell (1927) for example reported a complete failure to establish pines on a previously treeless site. Shading, irrigation, or fertilization could not reverse the symptoms of nutrient starvation. In a similar study, a treeless site in Puerto Rico failed to support a pine nursery until the soil was inoculated with humus containing mycelium of a suitable mycorrhizal fungus (Hacskaylo and Vozzo 1967). Moser (1956, 1958, 1963, 1967) and Gobl (1965) in attempting to restore climax subalpine forest after clear-cutting in the European alps found that active mycorrhizal mycelium no longer occurred in the soil. *Suillus plorans* (Roll.) Sing., the most important mycorrhizal fungus with *Pinus cembra* L., was then inoculated in nurseries before outplanting. Utilization of mycorrhizal fungi to establish and promote tree growth is now common-place. Difficulties in using mycorrhizal fungi are abundant however, and are usually severe.

Benecke and Gobl (1974) showed that the mycorrhizae of *Pinus mugo* seedlings did not develop further when outplanted from a nursery to a natural soil. Reexamination of outplanted seedlings revealed that inoculated fungi from the nursery soil were totally superseded by other species. Mikola (1965) and Laiho (1967) also found that one year after outplanting, nursery-inoculated fungi could not be recognized on the roots of seedlings. Commonly inoculated mycorrhizal fungi were suggested by Lamb (1979) to be dominant in nurseries, but upon transfer to the field are replaced by a diverse unidentified mycoflora. Lamb further stated that the replacement in time of an entire mycorrhizal flora has great implications for artificial inoculation programs. Thomas and Jackson (1979) cautioned however, that mycorrhizae synthesized in the laboratory may not be stable nor morphologically similar to those occurring in nature. A change in appearance therefore, after outplanting might be expected.

Outplanting experiments offer good circumstantial evidence that mycorrhizal fungi change with time under certain conditions. However more definitive reports are available. Melin (1923) suggested that a single mycorrhiza may contain more than one fungal symbiont but that this rarely occurred. Zak and Marx (1964) illustrated two rootlets of *Pinus elliottii* colonized by multiple mycobionts, one rootlet with two mycorrhizal fungi, the other with three. Double and triple associations on individual short-roots were also noted by Marks (1965) on *Pinus radiata*. Mikola and Laiho (1962) in observing senescence and aging of mycorrhizae found that multiple colonization of single rootlets was common. Tips of old mycorrhizae began growing in the spring and at that point the fungal association often changed. Mikola and Laiho also noted that the boundaries of annual growth are often visible as constrictions in the mycorrhizal rootlet. A study devoted entirely to succession of mycorrhizal fungi on roots of *Pinus radiata* D. Don indicated that over four per cent of mycorrhizal rootlets clearly showed dual associations (Marks and Foster 1967). Intimate mixtures of fungal types on and in the mantle were common and supercession of one fungus by another occurred when root growth was resumed after a dormant period. Marks and Foster further suggested that replacement of mycorrhizal types occurred only after the continuity of the Hartig net in the cortex was broken by a zone of dead, lignified cells.

The dynamics of mycorrhizal succession may be inferred from Dominik (1961) who found that the mycorrhizal associates of spruce vary considerabley in the course of a growing season. Mycorrhizae of many individual trees, when studied over a limited period also were shown to approximate mycorrhizae of a few trees studied for a longer period. Dominik, therefore agrees with a growing number of mycorrhizal specialists who feel that succession of mycorrhizae does commonly occur in plant communities.

SITE DESCRIPTION

Field data were collected from a site 7.5km from Jackson Hole, Wyoming, .93km south of highway 22 at the top of Teton Pass (approximately 41N-117W, Teton County quadrangle). The site lies on a flat bench at the junction of the Teton and Snake River Ranges at an elevation of 2926m. Maximum relief at the site is 27m.

The Teton and Snake River Ranges developed at or near the close of the Paleocene in the Laramide Revolution (Love 1956). Blackwelder (1918) proposed the name Darby formation for Devonian strata exposed along Darby creek on the western slopes of the Teton Range. In Wyoming the Darby rests on Ordovician carbonates. Andrichuk (1956) states that:

> " The Darby is composed of varying amounts and proportions of carbonates (Dolomites and Limestones), shales, siltstones and arenaceous beds. In general shaly, silty, and sandy strata are prominently developed. The upper part consists of brownish-gray, dense to fine crystalline, thin-bedded limestonen and shaly limestone with interbeds of gray, brown and green shale and silty shale. The overlying gray, crinoidal limestones are of the Madison group".

The biota of the area is very diverse, with a number of unusual montane and alpine endemics. A number of conifer species are found in the area. *Pinus albicaulis* Engelm. (whitebark pine), found at lower elevations, and *Pseudotsuga* *menziesii* (Mirb.) Franco (douglas fir), found on western slopes of Teton Pass often occur admixed with *Picea engelmannii* and *Abies lasiocarpa*, although not at the study site.

Three classes of spruce-fir forest occurred at the study site. Young stands, distinguished by high density of small-sized trees, abundant layering, and profusion of unpruned limbs occupies the smallest proportion of forested area. Litter is scarce in the young stands and mortality is high. Mature stands occupy the largest proportion of forested area and are characterized by larger trees, the absence of layering and self-pruned lower limbs. Organic matter accumulation is generally high and mortality low. Old-age stands at the site are similar to the mature stands but contain fewer and much larger trees, greater litter accumulation and slightly greater mortality. Recruitment generally is low in both mature and old-age stands.

METHODS AND MATERIALS

FIELD METHODS

Thirty permanent one hundred square meter quadrats were established at the study site; ten quadtats representing each of three stand age-classes: young, mature, and old-age. Quadrat corners were marked with metal stakes and cotton string demarcated the boundaries.

The three largest trees in each quadrat were cored with an increment borer to give the maximum mean age of trees in each quadrat. Cores were placed in plastic soda straws for transportation and later glued to wood blocks and stained with lactophenol-cotton blue for tree ring analysis under a binocular dissecting scope. Diameter at breast height measurements were also taken from each tree cored, and basal area calculated following Husch, Miller and Beers (1972). Density of trees was calculated by counting the number of each species in each quadrat and extrapolating to hectare units.

Sporocarps of higher Basidiomycetes and Ascomycetes were collected from 14 June to 8 September 1980 and from the 26th to the 30th of July 1981. Quadrats were visited approximately every week, in the 1980 season, with no longer than two weeks between visits. Sporocarps were recorded by quadrat, photographed, described, identified if possible, and dried as voucher specimens or for further identification.

Tissue and spore cast isolates were attempted from each fruiting body in order to confirm possible mycorrhizal associations in laboratory synthesis. Nutrient-agar and modified Hagem's agar (Modess 1941)(see table 1) slant tubes were used for isolation and maintainance of cultures. Cultures were referigerated or iced for transportation back to Virginia Tech.

Enumeration omycorrhizae were made using the techniques of Harvey, Larsen, and Jurgensen (1976) and Marks, Ditchburne, and Foster (1968). Three cores were taken from each quadrat, using a standard 1334cm soil corer, and stored in Whirl-pak bags on ice for transportation. Core samples were then wet-sieved to remove soil. Individual mycorrhizal tips were counted and categorized according to gross morphological characteristics.

Soil samples were collected in conjunction with soil cores. Loose litter and organic debris were scraped away to reveal mineral soil. Adequate samples were placed in Whirl-pak bags until analyzed by the Virginia Tech Soil Testing Laboratory.

Engelmann spruce and subalpine fir seeds were collected on 14 September 1980 for use in mycorrhizal syntheses. Current-year spruce cones were collected from tops of trees in the mature stand, while fir cones were collected from trees in old-age stands with the aid of a 22-caliber rifle. Cones were then air dried for one week and seeds mechanically removed.

LABORATORY METHODS

Inoculum for mycorrhizal synthesis was prepared by growing tissue isolates in a peat-vermiculite mixture modified from Molina (1979)(see table 1). Glass petri plates were used to contain the inoculum and were sealed with masking tape. Inoculum was used after one monthnd isolates were maintained by monthly transfers into the peat-vermiculite medium and onto Hagem's agar. All techniques described were performed aseptically.

The spruce and fir seeds, stripped of wings and chaff, were stratified for 30 days on water-agar at 5° C. Stratified seeds were then soaked overnight in sterile distilled water with Tween-20 added as a surfactant and surface sterilized in 30% hydrogen peroxide for one hour. N-agar or water-agar plates served as moist chambers and as contamination screens. Contaminated seeds were carefully discarded. Seedlings were used for synthesis trials when the radicle was approximately 2.5-3cm long and the seed coat had detached itself from the primary leaves.

Glass culture tubes 38mm by 250mm were used in syntheses. The peat-vermiculite medium was added to the tubes until two-thirds full. Inoculum was added as a band in the upper quarter of the tube. Seedlings, placed radicle downward, were buried until the radicle was fully covered. Syntheses were conducted at 18° C in Sherer CEL-25 growth chambers with the fluorescent-incandescent light adjusted to 1200 ft. candles. Slanting the tubes to approximately 80-85 degrees compensated for light blocked by the cap.

Seedlings were harvested after six months. Root systems were carefully excavated, and the peat-vermiculite dissected away to expose the rootlets. Representative rootlets were described, photographed, fixed in FAA, and later dehydrated using an alcohol dehydration series. Paraffin imbedded rootlets were sectioned and stained using a safranin-fast green staining schedule. Presence of a well developed mantle accompanied by a Hartig net was regarded as the most rigorous demonstration of mycorrhizal formation.

Analysis of soil and silvical characteristics was aided by Ordiflex (Gauch 1977). Polar ordination with the "PD" similarity index was used. The program was allowed to select the endpoints for both the X and Y axes.

TABLE 1

Growth media for culture and synthesis of ectomycorrhizal fungi.

Hagem's Agar

Bacto agar	15.0 gm
Malt extract	5.0 gm
Glucose	5.0 gm
KH_2PO_4	0.5 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
NH_4Cl	0.5 gm
$FeCl_3$ (1% soln.)	0.5 ml
Biotin	5.0 g
Thiamine HCL	1.0 g
H_2O	to 1000 ml

Peat-Vermiculite-Mixture

Fine Peat (with large particles sifted out)	8 ml
Coarse Vermiculite (with fine particles sifted out)	90 ml
Liquid Hagem's (without agar)	60 ml

RESULTS

STAND DATA

Stand characteristics of the thirty quadrats chosen to represent young, mature, and old-age Engelmann spruce-subalpine fir stands were distributed along a gradient from young through old-age stands. Mean maximum age of trees in each quadrat ranged from 53 years in the youngest quadrat to 264 years in the oldest quadrat (table 2). The average age of trees in young, mature, and old-age categories was 78, 121, and 216 years respectively. Density of trees in each quadrat decreased with increasing stand age. Subalpine fir predominated in the younger stand and Engelmann spruce predominated in the old-age stand, while spruce and fir were equally common in the mature stand. Basal area values showed a reverse trend, with larger values in the old-age stand and progressively smaller values in the mature and young stands. Relative basal area values were higher for subalpine fir than for Engelmann spruce in the young stand, but Engelmann spruce predominated in both mature and old-age stands (table 2, figure 1). One-way analysis of variance of silvical characteristics suggests that age and basal area values varied more among stands than within quadrats, while density values overlap a great deal among quadrats in the young, mature, and old-age stands (table 3). Linear regression analysis of diameter breast-height measurements and tree age suggests that tree diameter is correlated with age only in older trees (figure 2).

Soil analysis for each plot is shown in table 4. Mean soil values showed that organic matter and P concentrations increased with stand age, while Ca, Mg, and pH decreased with stand age (figure 3). The highest mean K concentration occurred in the mature stands followed by the old-age and young stands. Duncan's Multiple Range Test (table 5) indicated no statistical difference in P levels in the three age classes. Mean pH, organic matter, and K and Mg levels were significantly different in the young stand versus the mature and old-age stands at the .05 alpha-level. All stands were significantly different in terms of Ca concentration. One-way analysis of variance for pH, organic matter, Ca, and Mg indicates a greater variance among stands than within quadrats. Variances of K and P concentration however are greater within quadrats than among stands (table 6). The higher Ca and Mg concentrations appear to peak at 1200 and 120 ppm respectively (table 4), but the similarity indicates only the highest value measureable by the Virginia Tech Soil Testing Laboratory.

Two dimensional linear ordination of stand and soil characteristics shows that quadrats containing young trees are most dissimilar to quadrats containing old trees (figure 4). Transitional quadrats however,such as 3 and 5 in the young stand, are similar to quadrats with mature trees. Likewise, transitional quadrats containing mature trees such as 12 and 17 are similar to quadrats containing old trees. Overall, a gradient exists throughout the young, mature, and old-age stands.

TABLE 2

Silvical characteristics of young, mature, and old-age Engelmann spruce-subalpine fir stands.

Stand[1]	Plot	Total Density (trees/ha)	RD[2] Spruce (%)	RD Fir (%)	Total Basal Area (m /ha)	RBA[3] Spruce (%)	RBA Fir (%)	Mean[4] Age (years)
1	1	1700	29.4	70.5	0.68	30.5	69.5	114
1	2	1500	33.3	66.7	0.39	27.9	72.1	68
1	3	1300	46.1	53.8	0.79	50.7	49.3	97
1	4	1100	45.5	54.5	0.41	44.8	55.2	83
1	5	1200	33.3	58.5	0.53	36.7	63.2	91
1	6	1500	33.3	66.7	0.75	38.5	61.5	66
1	7	1000	50.0	50.0	0.75	49.0	51.0	66
1	8	1400	35.7	64.3	0.99	34.2	65.8	53
1	9	800	62.5	37.5	0.57	77.2	22.8	59
1	10	1100	27.2	72.7	0.54	31.4	68.6	60
2	11	800	62.5	37.5	7.50	68.6	31.4	106
2	12	700	71.4	28.6	7.30	74.2	25.8	153
2	13	700	42.8	57.1	4.50	40.1	59.9	130
2	14	800	37.5	62.5	3.90	48.2	51.7	136
2	15	900	33.3	66.7	5.10	49.3	50.7	132
2	16	1300	38.5	61.5	2.10	32.4	67.6	106
2	17	1000	50.5	50.5	6.50	50.7	49.3	159
2	18	1000	60.0	40.0	7.70	61.2	38.8	138
2	19	1200	50.0	50.0	0.98	54.7	45.3	106
2	20	1300	38.5	61.5	0.70	61.2	39.0	102
3	21	500	60.0	40.0	16.50	71.4	28.6	210
3	22	500	80.0	20.0	15.10	86.6	13.4	208
3	23	400	75.0	25.0	16.20	77.4	22.6	205
3	24	500	80.0	20.0	21.20	82.7	17.3	236
3	25	700	57.1	42.9	21.30	52.7	47.3	264
3	26	600	66.6	33.3	10.50	77.0	23.0	156
3	27	700	57.1	42.9	18.10	57.9	42.1	227
3	28	800	50.0	50.0	12.80	60.0	40.0	186
3	29	800	50.0	50.0	16.70	51.7	48.3	228
3	30	800	50.0	50.0	11.10	61.0	39.0	234

[1] 1=young stand. 2=mature stand, 3=old-age stand

[2] 2=Relative Density

[3] Relative Basal Area

[4] Mean of the three largest trees in each quadrat

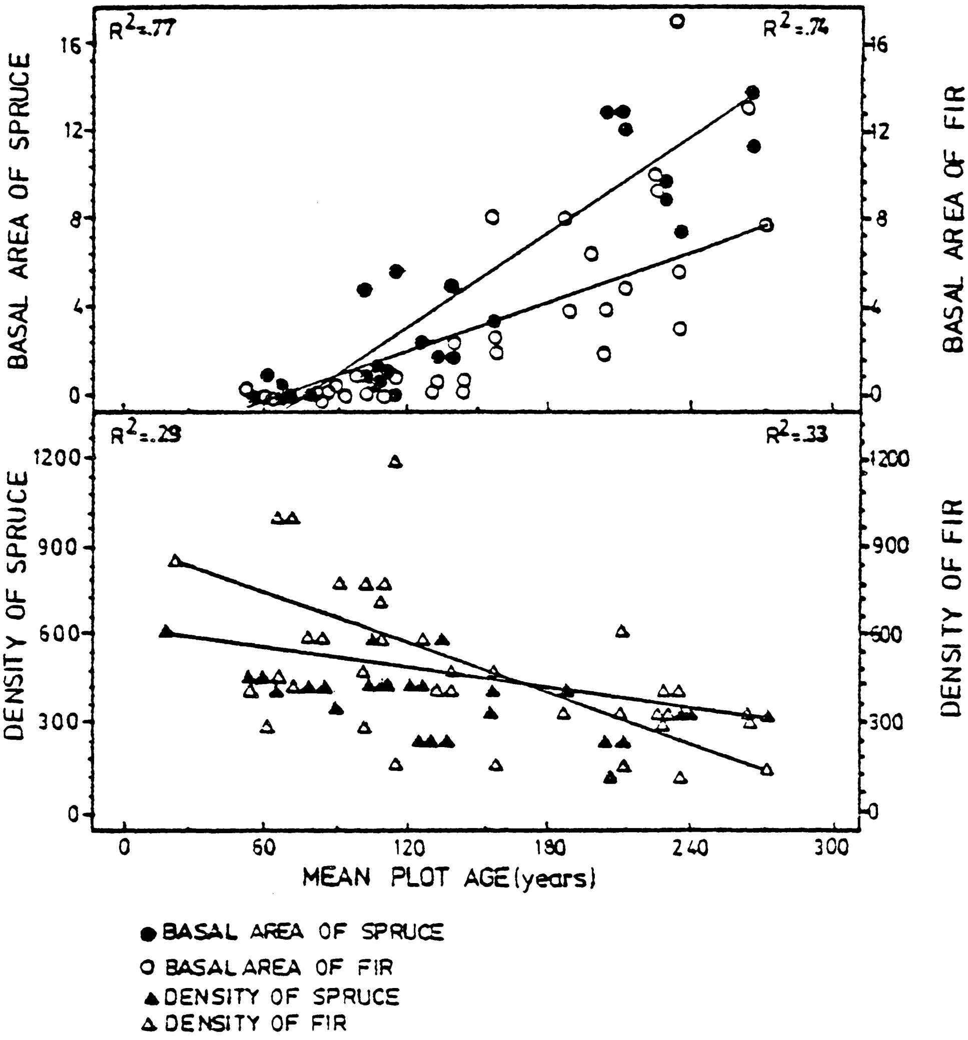


Figure 1: *Linear regression of density, basal area and mean plot age for Engelmann spruce-subalpine fir stands.*

TABLE 3

Anova table for silvical data

ANOVA FOR TREE AGE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Tree age	2	97565.	48783.	88.67
Error	27	14854.	550.	
Total	29	112419.		

ANOVA FOR DENSITY OF FIR

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Den. Fir	2	1064000.	532000.	11.56
Error	27	1241997.	46037.	
Total	29	2306997.		

ANOVA FOR DENSITY OF SPRUCE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Den. Spr.	2	74667.	37333.	6.30
Error	27	160000.	5926.	
Total	29	234666.		

ANOVA FOR TOTAL TREE DENSITY

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Density	2	1698666.	849333.	16.03
Error	27	1430997.	53000.	
Total	29	3129663.		

ANOVA FOR BASAL AREA OF FIR

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
BA of Fir	2	125.66	62.83	22.40
Error	27	75.73	2.8	
Total	29	201.39		

ANOVA FOR BASAL AREA OF SPRUCE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Ba of Spr.	2	596.56	298.28	62.77
Error	27	128.31	4.75	
Total	29	724.87		

ANOVA FOR TOTAL BASAL AREA

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
BA	2	1231.46	615.73	93.06
Error	27	178.64	6.62	
Total	29	1410.10		

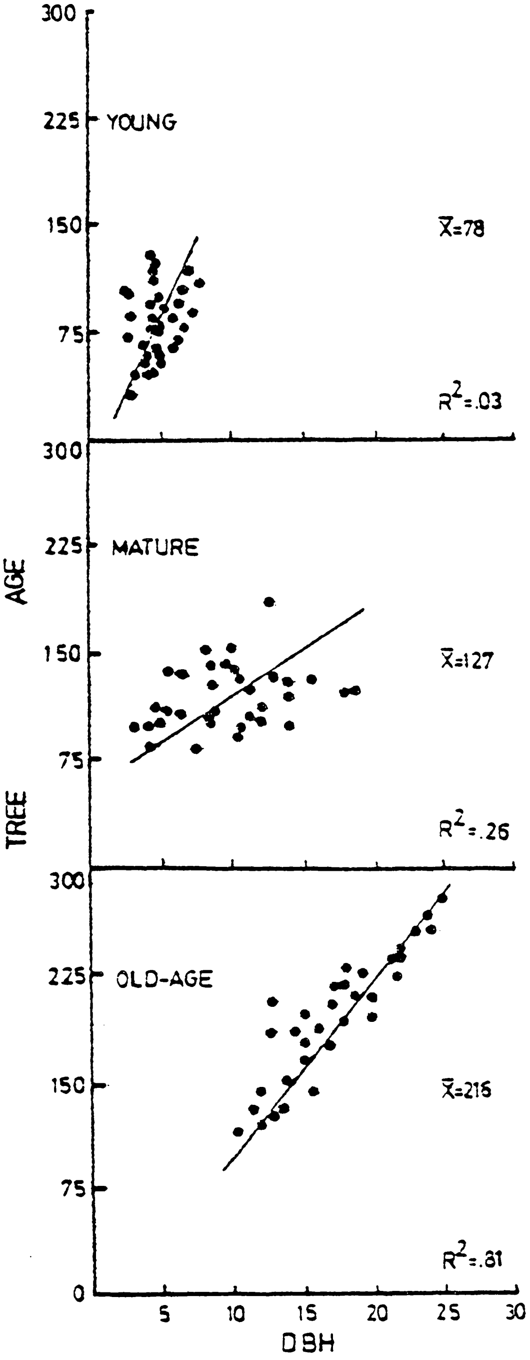


Figure 2: *Linear regression of diameter breast height and tree age for young, mature and old-age spruce-fir stands.*

TABLE 4

Soil analysis of young, mature, and old-age Engelmann spruce-subalpine fir stands in Wyoming.

Stand[3]	Plot	pH	P[1]	K	Ca	Mg	Organic[2] Matter
1	1	7.1	16	78	1200	105	3.0
1	2	7.5	15	86	1200	120	3.3
1	3	7.5	13	75	1200	120	3.1
1	4	7.7	12	83	1200	120	4.6
1	5	7.3	16	70	1200	120	3.1
1	6	7.6	10	78	1200	120	5.0
1	7	7.0	11	69	1200	120	2.9
1	8	7.2	12	93	1200	120	6.0
1	9	7.0	11	74	1200	120	7.5
1	10	7.1	17	85	1200	120	8.6
2	11	5.2	33	143	1007	87	10.3
2	12	5.0	47	145	924	108	12.4
2	13	5.1	33	132	876	105	11.8
2	14	5.1	31	147	948	95	15.0
2	15	4.7	33	155	1067	105	11.0
2	16	6.6	31	140	953	95	13.6
2	17	6.2	35	143	975	97	12.1
2	18	5.5	27	135	1176	120	11.7
2	19	5.1	23	147	925	114	12.4
2	20	5.0	33	155	948	105	10.3
3	21	5.3	36	155	800	99	12.5
3	22	5.3	34	155	800	108	12.5
3	23	5.2	16	74	776	87	14.6
3	24	5.5	13	93	800	85	15.1
3	25	5.3	55	156	813	94	15.6
3	26	4.9	54	155	824	93	14.1
3	27	4.7	56	145	788	95	14.9
3	28	5.3	54	750	790	87	13.6
3	29	4.8	54	155	750	89	13.0
3	30	4.7	11	78	800	87	12.9

[1] Values are in parts per million

[2] Values are in per cent

[3] 1=young stand, 2=mature stand, 3=old-age stand

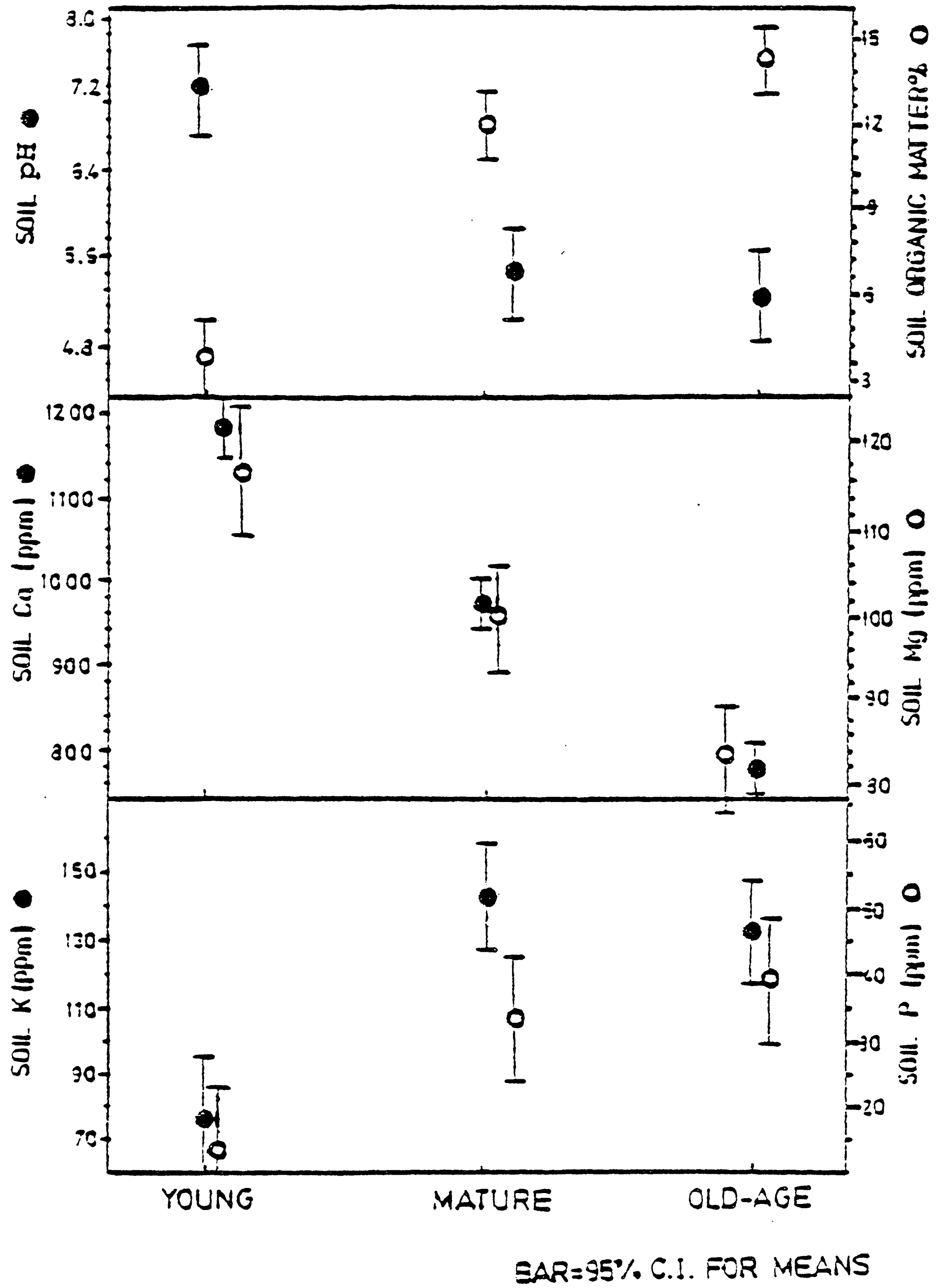


Figure 3: *Mean concentration of six soil characterists in young, mature, and old-age spruce-fir stands.*

TABLE 5

Duncan's Multiple Range Test for silvical and soil data.

	DUNCAN'S MULTIPLE RANGE TEST		
	Grouping	Mean	Age
Non-Mycorrhizal Sporocarps	A	13.0	127
	A	10.3	216
	A	0.5	78
Mycorrhizal Root Tips	A	14.7	216
	A	11.3	127
	A	2.9	78
Mycorrhizal Sporocarps	A	1.4	216
	A	1.2	127
	A	0.0	78
Spruce Density	A	500.0	78
	A	460.0	127
	A	380.0	216
Fir Density	A	710.0	78
	A	510.0	127
	A	250.0	216
Spruce Basal Area	A	10.7	216
	A	2.7	127
	A	0.3	78
Fir Basal Area	A	5.3	216
	A	2.0	127
	A	0.4	78
Soil pH	A	7.3	78
	B	5.3	127
	B	5.1	216
Soil Organic Matter	A	13.9	216
	A	12.0	127
	B	4.7	78
Soil P	A	38.6	216
	A	32.6	127
	A	13.3	78

Table 5 continued

	Grouping	*Mean*	*Age*
Soil K	A	144.0	127
	BA	132.0	216
	B	78.0	78
Soil Ca	A	1200.0	78
	B	980.0	127
	C	794.0	216
Soil Mg	A	119.0	78
	BA	03.0	127
	B	92.0	216

TABLE 6

Anova table for soil data.

ANOVA FOR SOIL PH

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Soil pH	2	30.134	15.067	86.39
Error	27	4.709	0.174	
Total	29	34.843		

ANOVA FOR SOIL PHOSPHORUS

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Soil P	2	3433.	1717.	12.75
Error	27	3637.	135.	
Total	29	7070.		

ANOVA FOR SOIL POTASSIUM

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Soil K	2	23843.	11922.	26.80
Error	27	12009.	445.	
Total	29	35852.		

ANOVA FOR SOIL CALCIUM

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Soil Ca	2	825735.	412868.	158.77
Error	27	70210.	2600.	
Total	29	895945.		

ANOVA FOR SOIL MAGNESIUM

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Soil Mg	2	3442.9	1721.4	30.58
Error	27	1519.8	56.3	
Total	29	4962.7		

ANOVA FOR SOIL ORGANIC MATTER

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Soil OM	2	473.62	236.81	94.39
Error	27	67.74	2.51	
Total	29	541.36		

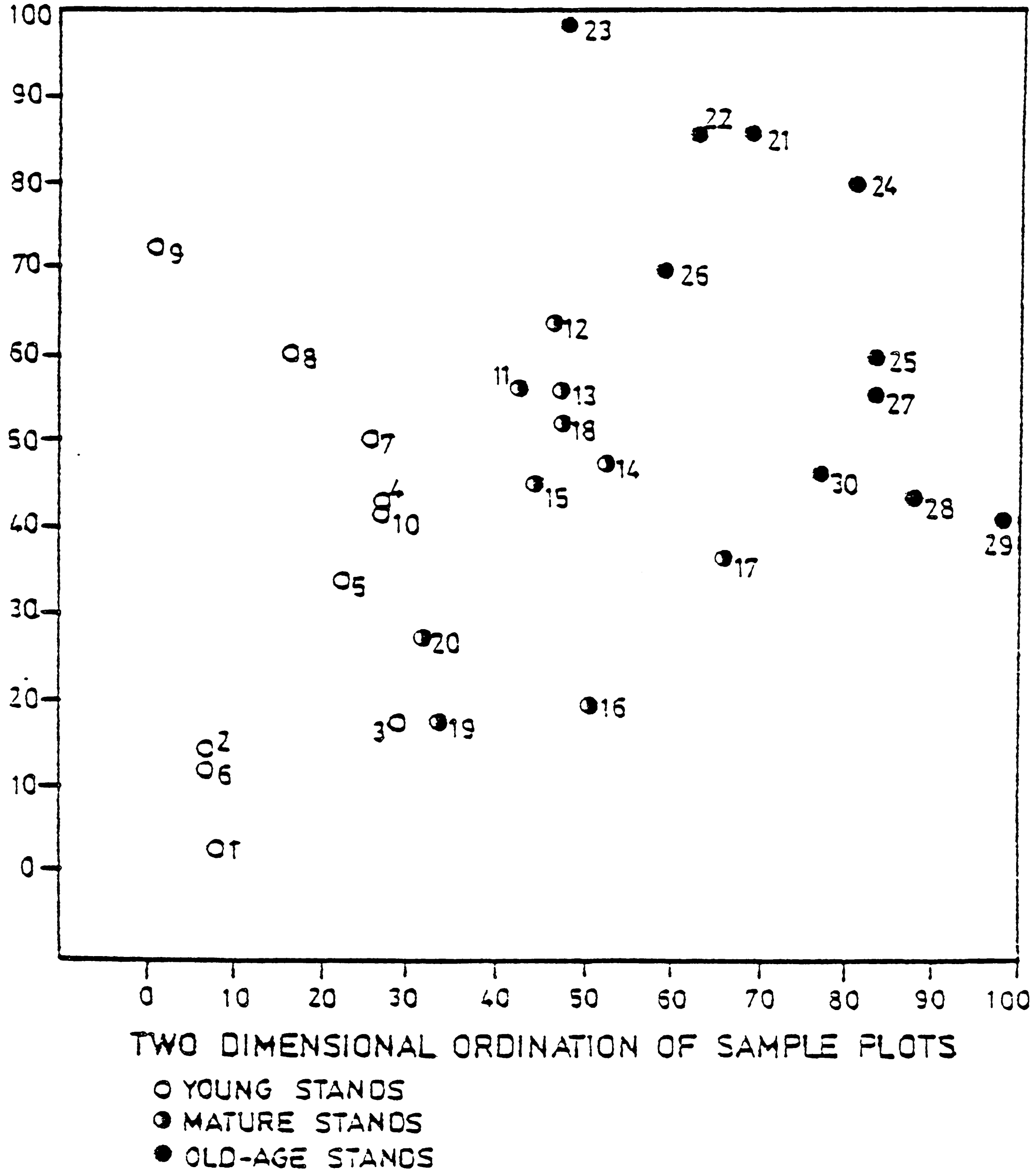


Figure 4: *Two dimensional ordination of stand and soil characteristics using youngest and oldest quadrats as endpoints.*

FUNGAL SPOROCARP AND MYCORRHIZAL DATA

Sporocarps collected and identified were primarily Basidiomycetes, represented by thirteen families, twenty-eight genera, and thirty-eight species. A single species, *Scutellinia scutelata* was the only ascomycete found (table 7).

The mature stand showed the greatest density of sporocarps, followed by the old-age and young stands. Diversity was approximately equal in the mature and old-age stands but much lower in the young stand. The mature and old-age stands shared only four species in common. No overlap occurred among species in young and mature or young and old-age stands (table 8).

Fewer sporocarps were produced by mycorrhizal species in the mature stand than in the old-age stand. No sporocarps were produced by mycorrhizal species in the young stand. Pathogenic sporocarps conversely, were more numerous in the young stand and progressively decreased in number with increasing stand age. The highest number of sporocarps were produced by wood saprophytes in the mature stand, followed by the old-age and young stands. Sporocarps produced by litter saprophytes were highest in the old-age stand, decreasing slightly in the young stand (figure 5, table 9).

Dry weight of mycorrhizal sporocarps was highest in the old-age stand, followed by the mature stand. Dry weight of pathogenic sporocarps in the young stand far exceeded dry weight in the other two age-classes due to a large "conk" produced by *Fomitopsis pinicola*. This species however, is a perennial fruiting body showing only a small biomass increase each year. Wood saprophytes showed the highest dry weight accumulation in the mature stands, while in the old-age stand litter saprophytes accumulated more sporocarp dry weight (figure 6). Overall, the mature stand showed the largest number of sporocarps and the largest dry weight accumulation, followed by the old-age and young stands (figure 7).

The number of species collected throughout the season increased from spring to fall. Similarly, the number of new species collected in each month of the season remained high (figure 8).

Ectomycorrhizal root tips obtained from soil cores were separated into five categories: smooth white, feathery white, smooth brown, cottony yellow, and smooth black (table 10). Positive identification of the mycobionts involved was not possible although the cottony yellow mycorrhizae were similar to *Corticium bicolor* and the smooth black mycorrhizae were similar to *Cenococcum geophilum* (Miller, Miller, and

Palmer 1982). The smooth white and feathery white mycorrhizal types appeared to be common in all stand age-classes. The smooth white mycorrhizae predominated. The smooth brown, cottony yellow, and smooth black mycorrhizae appeared only in the mature and old-age stands (table 10). One-way analysis of variance for each of the five mycorrhizal types however, indicates that there is a greater amount of variance within quadrats than among stands, and that none ot the mycorrhizal types are characteristic of a particular age-class (table 11).

A comparison of non-mycorrhizal sporocarps, mycorrhizal sporocarps, and active ectomycorrhizal tips (table 12) indicates that mycorrhizal and non-mycorrhizal fungi frequently occur together. Figure 9 shows that the highest number of ectomycorrhizal root tips and mycorrhizal sporocarps were produced in the mature and old-age stands, while few were produced in the young stand. Similarly, fewer non-mycorrhizal sporocarps were produced in the young stand than in the mature and old-age stands. One-way analysis of variance for ectomycorrhizal tips, mycorrhizal sporocarps, and non-mycorrhizal sporocarps shows that a large amount of variation exists among quadrats (table 13).

TABLE 7

Major groups of fungi and species collected in young, mature, and old-age spruce-fir stands.

- Ascomycetes
  - Pyronemataceae
    - *Scutellinia scutelata*
- Basidiomycetes
  - Tricholomataceae
    - *Mycena lilacifolia*
    - *Mycena overholtzii*
    - *Mycena stannea*
    - *Lentinellus montanus*
    - *Lepista tarda*
    - *Lyophyllum montanum*
    - *Tricholoma terreum*
    - *Tricholoma* sp.
    - *Xeromphalina campanella*
    - *Xeromphalina cauticinalis*
  - Russulaceae
    - *Russula chamaeleontina*
    - *Russula decolorans*
    - *Russula queletii*
    - *Russula silvicola*
  - Cortinariaceae
    - *Cortinarius* sp.
    - *Galerina autumnalis*
    - *Galerina megalocystis*
  - Agaricaceae
    - *Agaricus abruptibulbis*
    - *Agaricus silvaticus*
  - Hygrophoraceae
    - *Hygrophorus pudorinus* var. pudorinus
    - *Hygrophorus pudorinus* var. subsinereus
  - Strophariaceae
    - *Kuehneromyces vernalis*
    - *Pholiota flammans*
    - *Pholiota lubrica*
  - Amanitaceae
    - *Amanita vaginata*
  - Boletaceae
    - *Suillus sibericus*
  - Lycoperdaceae
    - *Lycoperdon pyriforme*
  - Polyporaceae
    - *Cryptoporus volvatus*
    - *Fomitopsis pinicola*
    - *Phellinus nigrolimitatus*
    - *Haplopilus albo-luteus*
  - Thelephoraceae
    - *Amphinema byssoides*
    - *Columnocystis abietina*
    - *Haematostereum sanguinelentum*
    - *Scytinostroma galactinium*
  - Auriculariaceae
    - *Auricularia auricula*
  - Dacromycetaceae
    - *Guepiniopsis alpinus*

TABLE 8

Number and location of fungai sporocarps collected in young, mature, and old-age spruce-fir stands.

Species	Relative Stand Age		
	Young	Mature	Old-age
Agaricus abruptibulbis	0	0	16
Agaricus silvaticus	1	0	0
Amanita vaginata	0	2	0
Amphinema byssoides	0	0	1
Auricularia auricula	0	15	0
Clitocybe albirhiza	0	7	7
Columnocystis abietina	0	14	0
Cortinarius sp.	0	1	0
Cryptoporus volvatus	0	1	0
Cystoderma fallax	0	0	6
Fomitopsis pinicola	1	0	0
Galerina autumnalis	0	0	3
Galerina megalocystis	0	2	0
Guepiniopsis alpinus	0	25	17
Haematostereum sanguinelentum	1	0	0
Haplopilus albo-luteus	1	0	0
Hygrophorus pudorinus var. pud.	0	0	2
Hygrophorus pudorinus var. sub.	0	3	0
Kavinia alboviridis	0	1	0
Lentinellus montanus	1	0	0
Lepista tarda	0	44	0
Lycoperdon pyriforme	0	8	0
Lyophyllum montanus	0	0	9
Mycena lilacifolia	0	0	1
Mycena overholtzii	0	0	26
Mycena stannea	0	0	6
Phellinus nigrolimitatus	0	5	0
Pholiota flammans	0	0	5
Pholiota lubrica	0	0	1
Russula chamaeleontina	0	4	0
Russula decolorans	0	1	1
Russula queletii	0	1	4
Russula silvicola	0	0	2
Scutellinia scutellata	0	1	0
Scytinostroma galactinum	0	0	1
Suillus sibericus	0	1	0
Tricholoma terreum	0	0	3
Tricholoma sp.	0	0	1
Xeromphalina campanella	0	8	0
Xeromphalina cauticinalis	0	0	5
	5	144	117

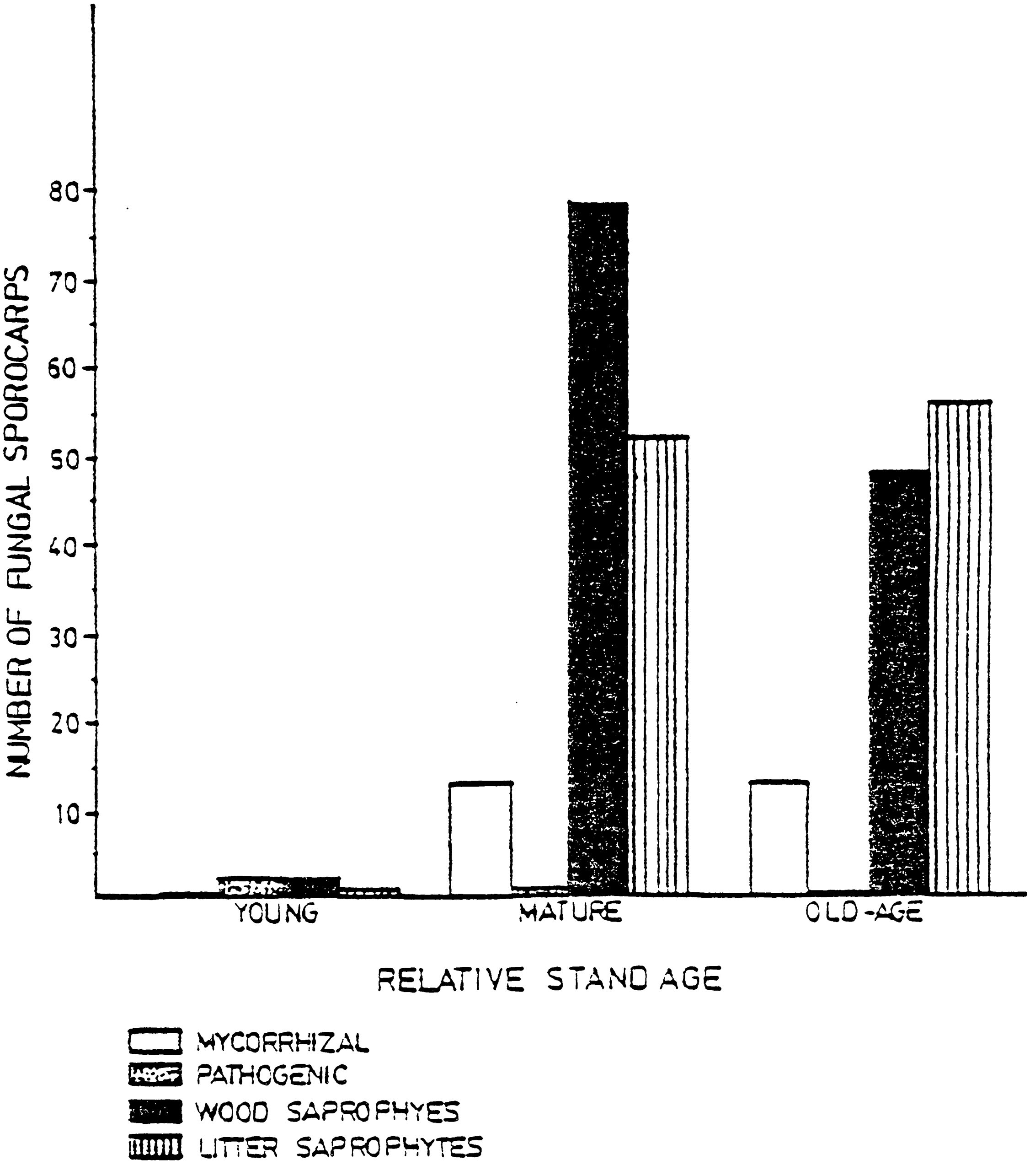


Figure 5: *Number of sporocarps categorized by niche collected from young, mature and old-age spruce-fir stands.*

TABLE 9

Number and dry weight of fungal sporocarps collected in young, mature, and old-age spruce-fir stands.

	Relative Stand Age					
Fungal Niche	Young		Mature		Old-Age	
	Number	Dry wt. (gm)	Number	Dry wt. (gm)	Number	Dry wt. (gm)
Mycorrhizal	0	0.0	13	16.2	13	24.1
Pathogenic	2	58.9	1	0.8	0	0.0
Wood Saprophytes	2	25.9	78	220.1	48	6.2
Litter Saprophytes	1	4.9	52	16.4	56	60.4
Total	5	89.7	144	253.5	117	90.7

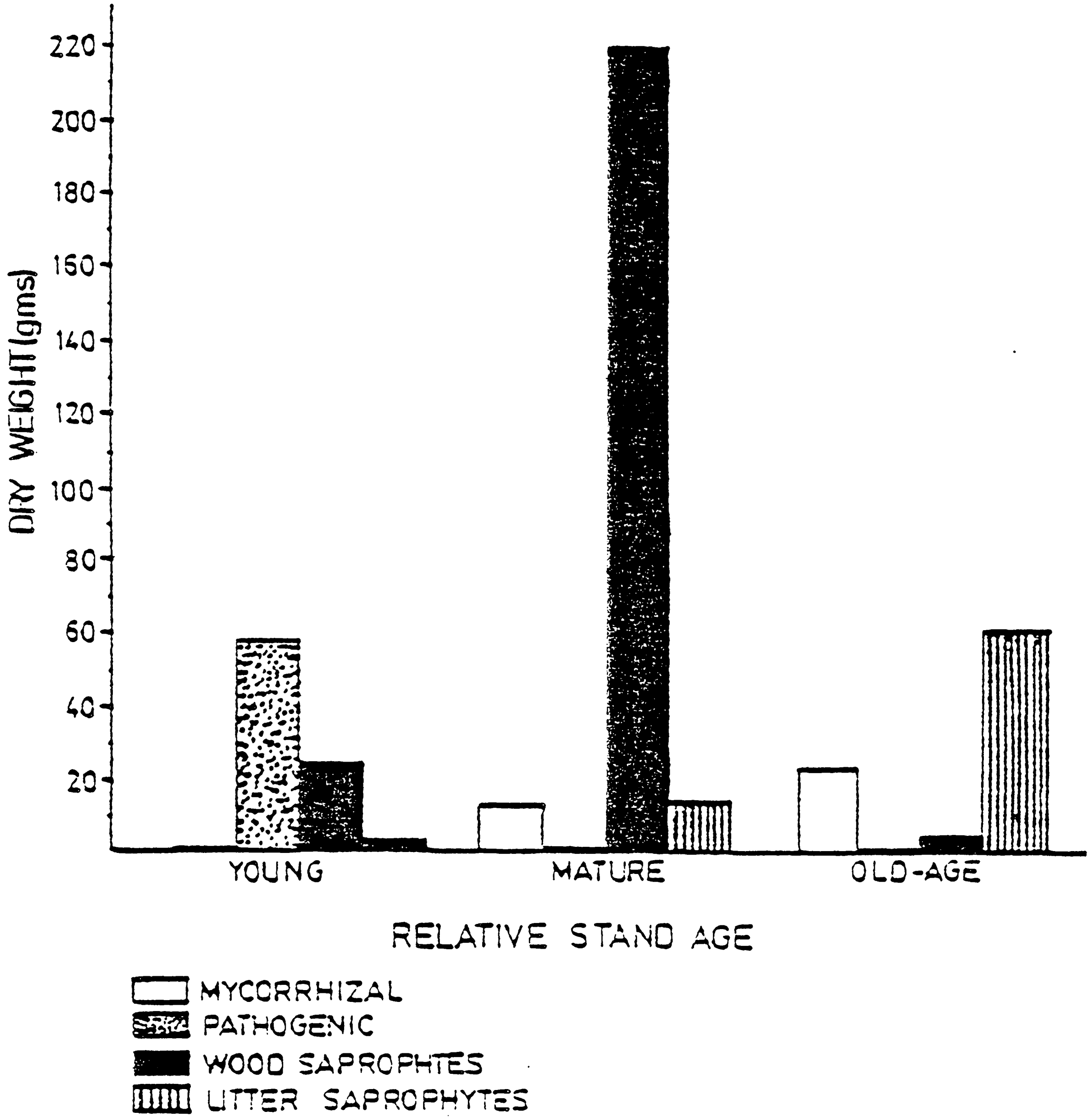


Figure 6: *Dry weight of sporocarps categorized by niche collected from spruce-fir stands.*

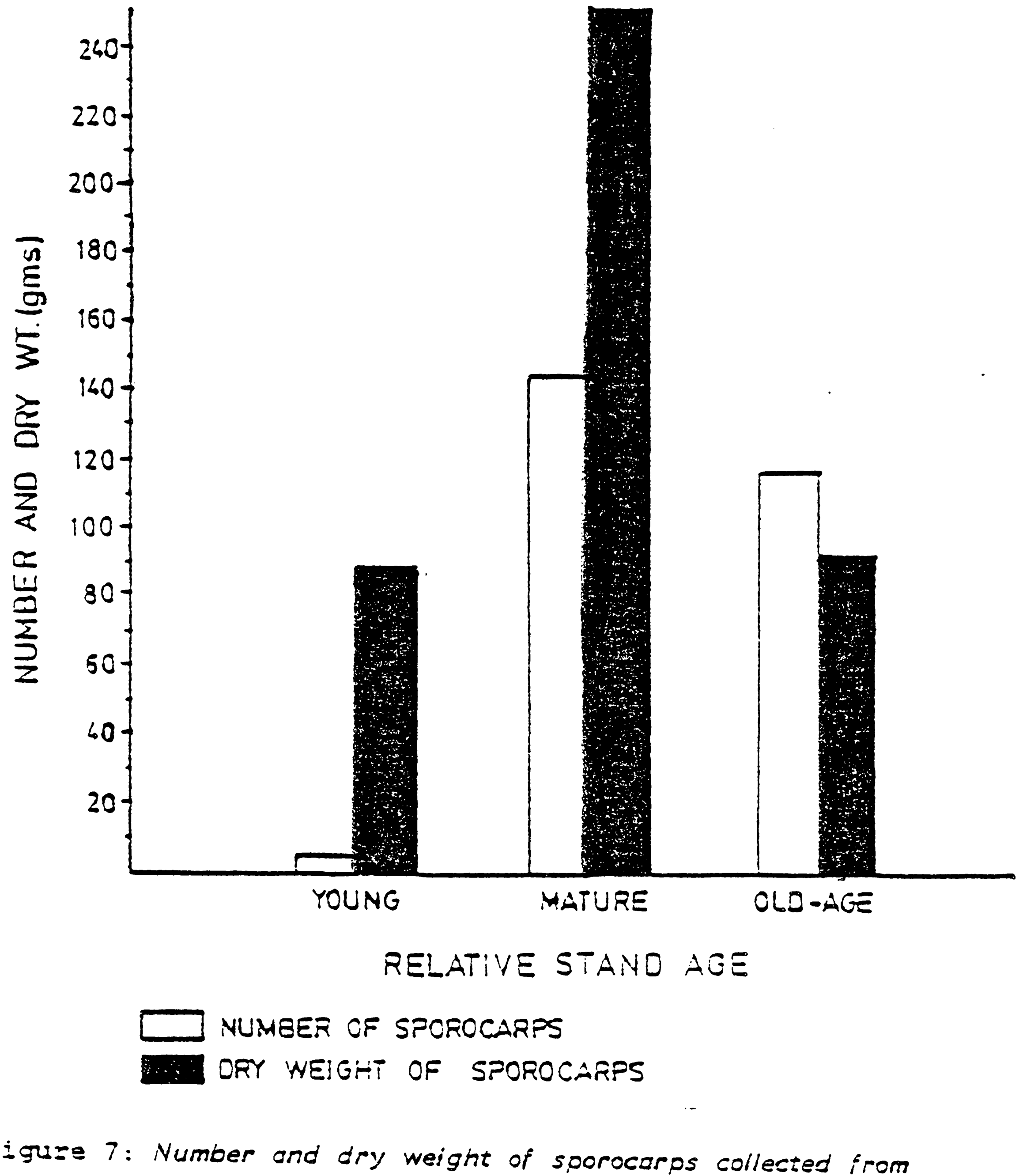


Figure 7: *Number and dry weight of sporocarps collected from young, mature and old-age spruce-fir stands.*

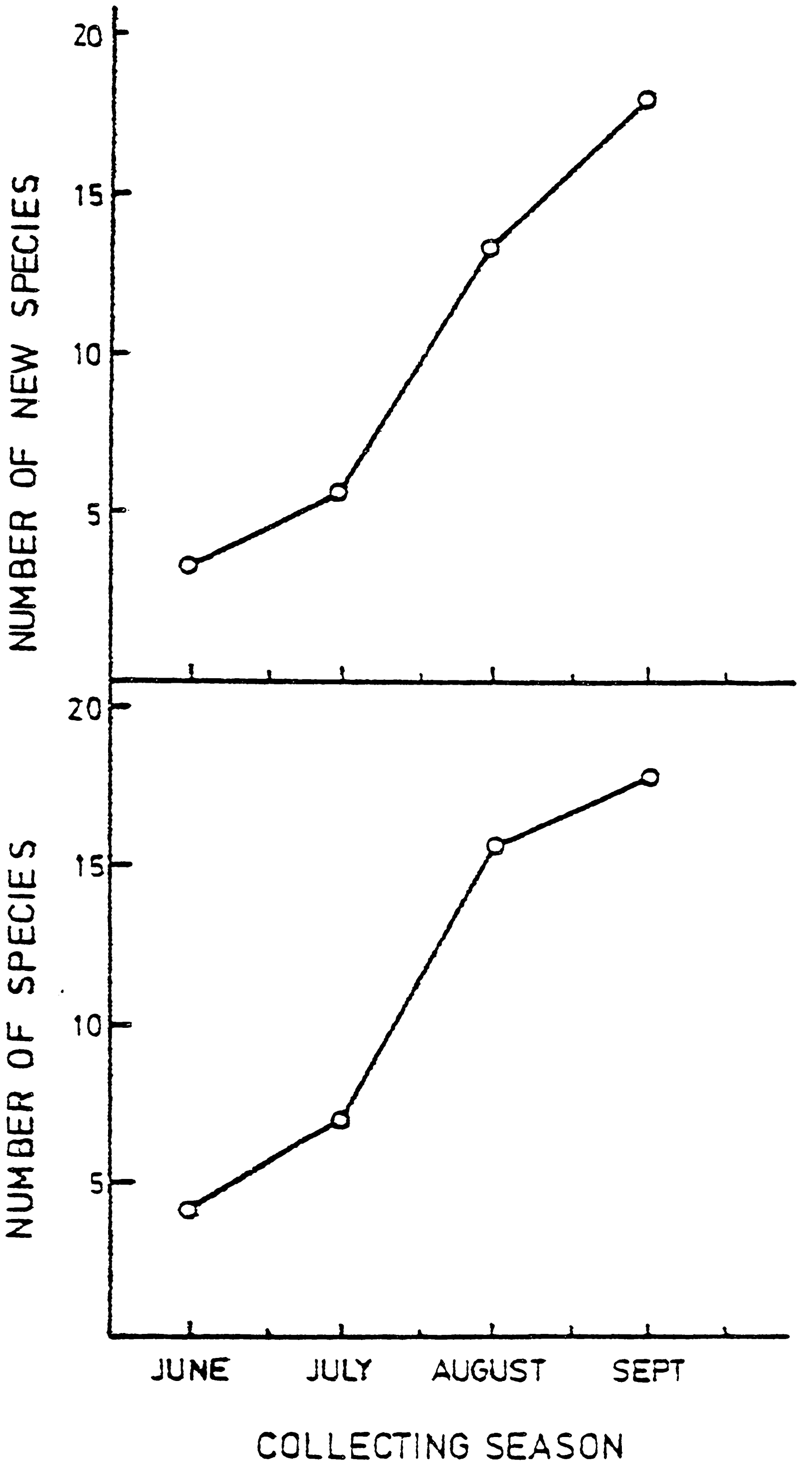


Figure 8: *Number of species and number of new species collected from spruce-fir stands.*

TABLE 10

Enumeration of ectomycorrhizal tips collected from young, mature, and old-age spruce-fir stands.

		Mycorrhizal Types				
Stand[1]	Plot	Smooth White	Feathery White	Smooth Brown	Cottony Yellow	Smooth Black
1	1	3	0	0	0	0
1	2	1	1	0	0	0
1	3	3	3	0	0	0
1	4	4	0	0	0	0
1	5	0	2	0	0	0
1	6	1	0	0	0	0
1	7	2	5	0	0	0
1	8	4	0	0	0	0
1	9	0	0	0	0	0
1	10	0	0	0	0	0
2	11	2	0	0	0	0
2	12	7	2	2	0	0
2	13	1	3	2	0	3
2	14	0	0	0	0	2
2	15	3	3	5	3	2
2	16	7	2	0	6	7
2	17	3	4	3	5	0
2	18	11	4	3	5	0
2	19	1	3	0	0	0
2	20	2	1	0	3	5
3	21	5	6	4	5	2
3	22	3	4	2	2	3
3	23	1	7	5	7	4
3	24	4	2	0	2	0
3	25	0	4	0	0	0
3	26	5	3	0	0	0
3	27	0	1	0	3	8
3	28	8	2	3	2	0
3	29	7	3	0	0	0
3	30	8	9	5	4	2
	Total	96	74	34	47	38

[1] 1=young stand, 2=mature stand, 3=old-age stand

TABLE 11

Anova table for mycorrhizal types.

ANOVA FOR SMOOTH WHITE MYCORRHIZAE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
SWM	2	30.20	15.10	1.87
Error	27	218.60	8.10	
Total	29	248.80		

ANOVA FOR FEATHERY WHITE MYCORRHIZAE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F VAlue*
FWM	2	46.07	23.03	6.01
Error	27	103.4	3.83	
Total	29	149.47		

ANOVA FOR SMOOTH BROWN MYCORRHIZAE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
SBM	2	20.07	10.03	3.79
Error	27	71.40	2.64	
Total	29	91.47		

ANOVA FOR COTTONY YELLOW MYCORRHIZAE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
CYM	2	37.27	18.63	4.83
Error	27	104.10	3.86	
Total	29	141.37		

ANOVA FOR SMOOTH BLACK MYCORRHIZAE

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
SBM	2	24.07	12.03	2.81
Error	27	115.80	4.29	
Total	29	139.87		

TABLE 12

Number of sporocarps and ectomycorrhizal root tips in young, mature and old-age spruce-fir stands.

Stand	Plot	Number of Mycorrhizal Tips	Number of Sporocarps	
			Mycorrhizal	Non-mycorrhizal
1	1	3	0	0
1	2	2	0	0
1	3	6	0	1
1	4	4	0	0
1	5	2	0	2
1	6	1	0	1
1	7	7	0	1
1	8	4	0	0
1	9	0	0	0
1	10	0	0	0
2	11	2	0	25
2	12	11	0	14
2	13	7	0	3
2	14	2	3	6
2	15	16	2	4
2	16	22	1	15
2	17	15	0	17
2	18	23	0	24
2	19	4	3	2
2	20	11	3	20
3	21	22	1	16
3	22	14	0	19
3	23	24	4	20
3	24	8	0	2
3	25	4	3	8
3	26	8	1	35
3	27	12	0	2
3	28	15	0	1
3	29	10	3	0
3	30	30	2	0
	Total	289	26	240

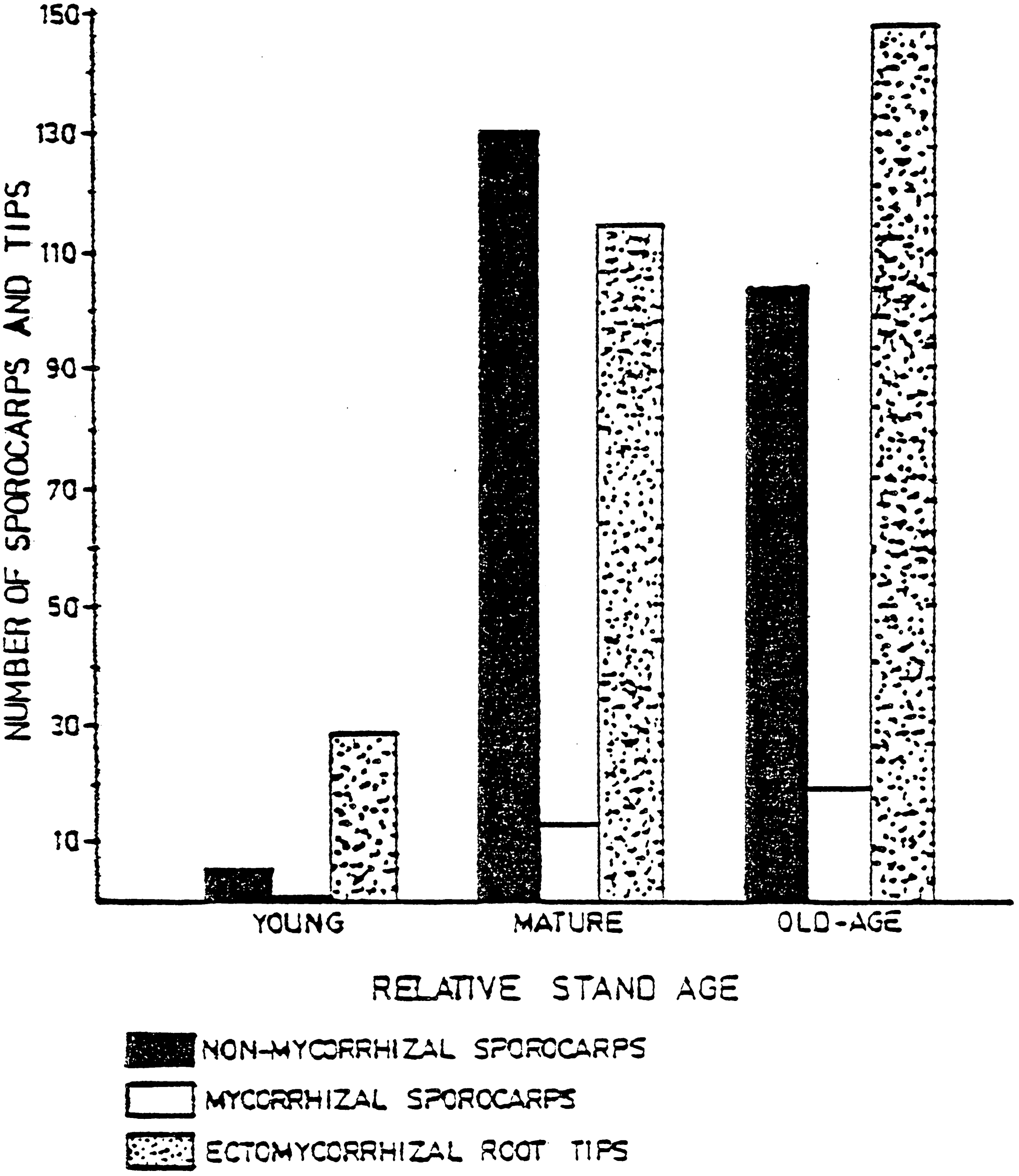


Figure 9: *Number of sporocarps and root tips collected in young, mature, and old-age spruce-fir stands in.*

TABLE 13

Anova for fungal collections.

ANOVA FOR MYCORRHIZAL TIPS

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
Tips	2	737.9	368.9	8.36
Error	27	1191.1	44.1	
Total	29	1929.0		

ANOVA FOR MYCORRHIZAL SPOROCARPS

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
MS	2	11.47	5.73	4.07
Error	27	38.00	1.41	
Total	29	49.47		

ANOVA FOR NON-MYCORRHIZAL SPOROCARPS

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*
NMS	2	865.3	432.6	6.01
Error	27	1944.6	72.0	
Total	29	2809.9		

STAND CHARACTERISTICS AND FUNGAL DATA

Scatter diagrams of ectomycorrhizal root tips, mycorrhizal and non-mycorrhizal sporocarps, along with plot age, density and basal area are presented in figures 10-14. Distribution of mycorrhizal and non-mycorrhizal sporocarps showed little relationship with mean plot age (figure 10). A larger number of root tips however, were found in the older aged stands. The relationship between spruce density, sporocarps and ectomycorrhizal root tips is masked (figure 11), because only four different density levels were measured for spruce. These densities are 300, 400, 500, and 600 trees/ha. The relationship between fir density and fungal entities is similar with fungi and ectomycorrhizal root tips occurring throughout all density levels (figure 12). Distribution of sporocarps and ectomycorrhizal root tips showed no solid relationship with basal area of either spruce or fir (figures 13 and 14).

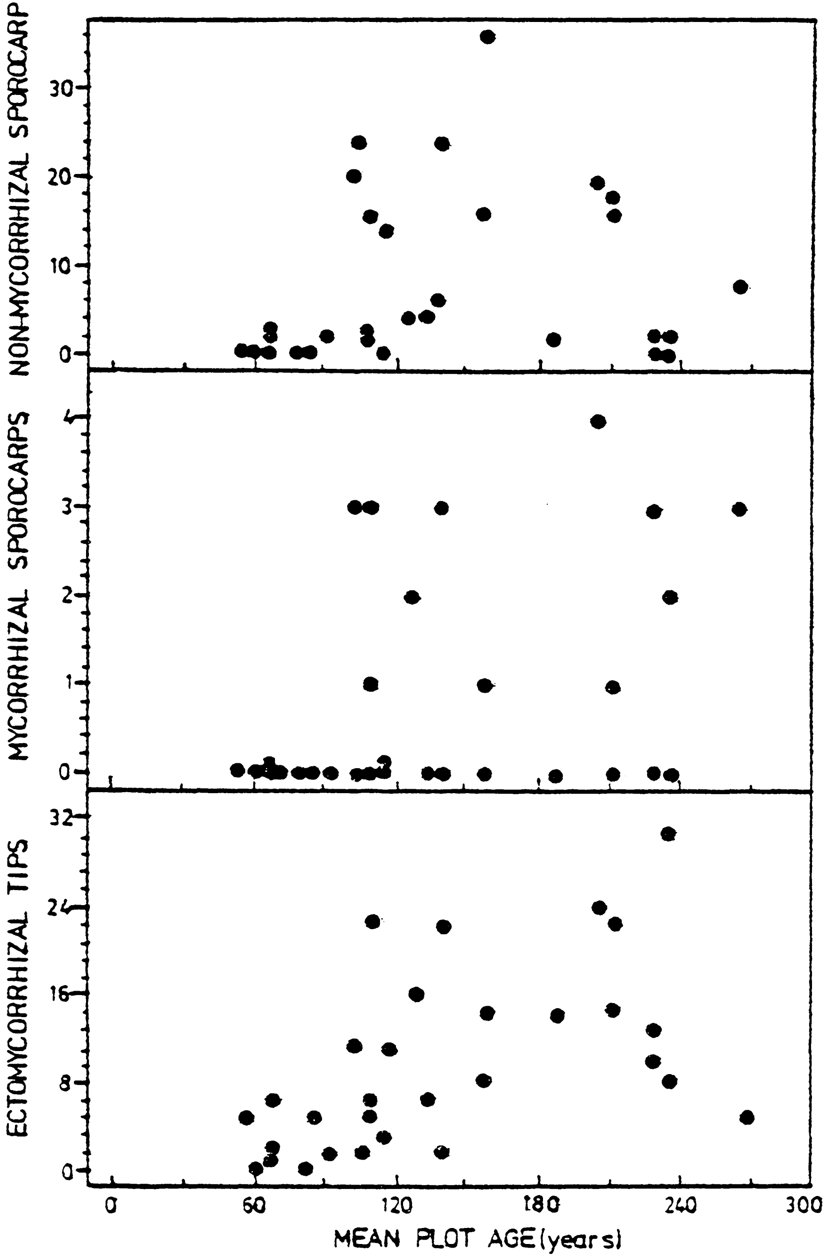


Figure 10: *Scatter diagram of mycorrhizal and non-mycorrhizal sporocarps, mycorrhizal root tips and age in spruce-fir forest.*

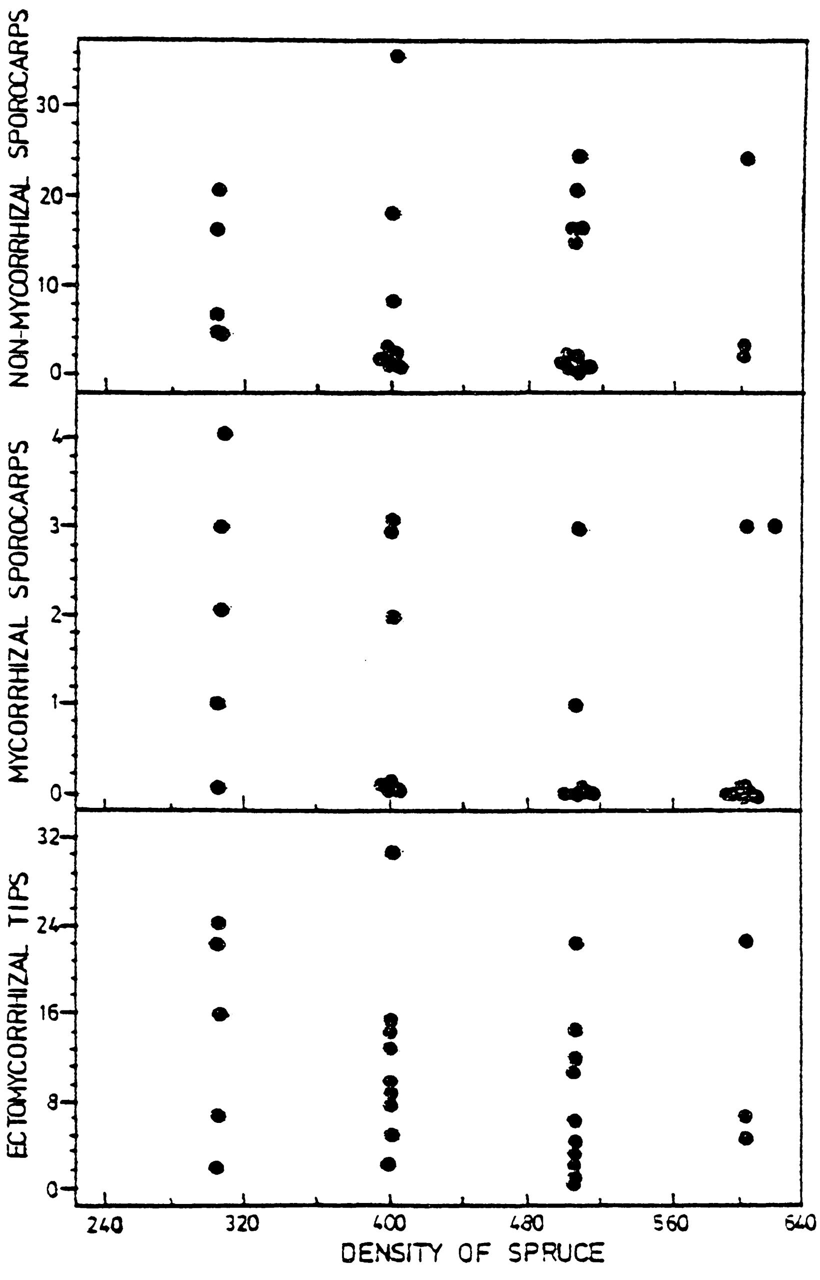


Figure 11: *The relationship between mycorrhizal root tips, mycorrhizal and non-mycorrhizal spororocarps and spruce density.*

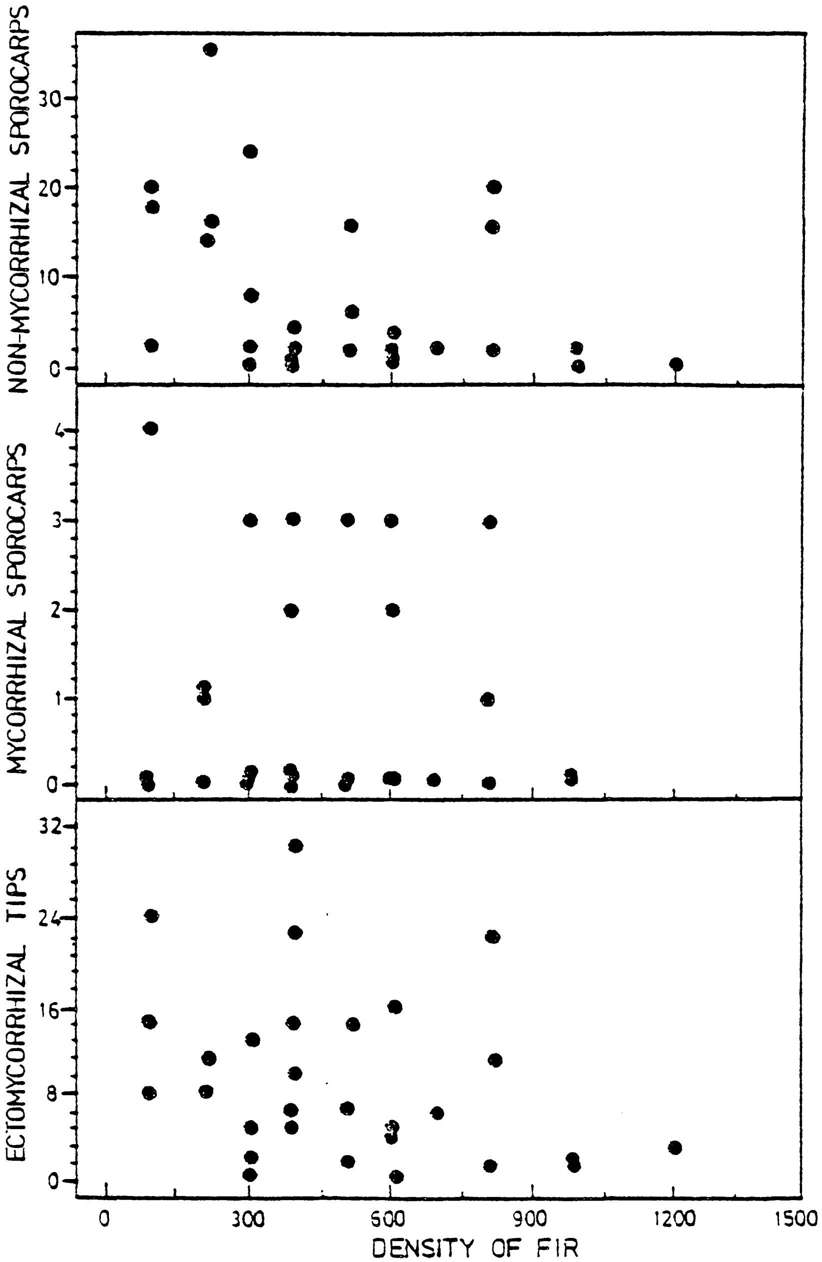


Figure 12: *The relationship between mycorrhizal root tips, mycorrhizal and non-mycorrhizal sporocarps and fir density.*

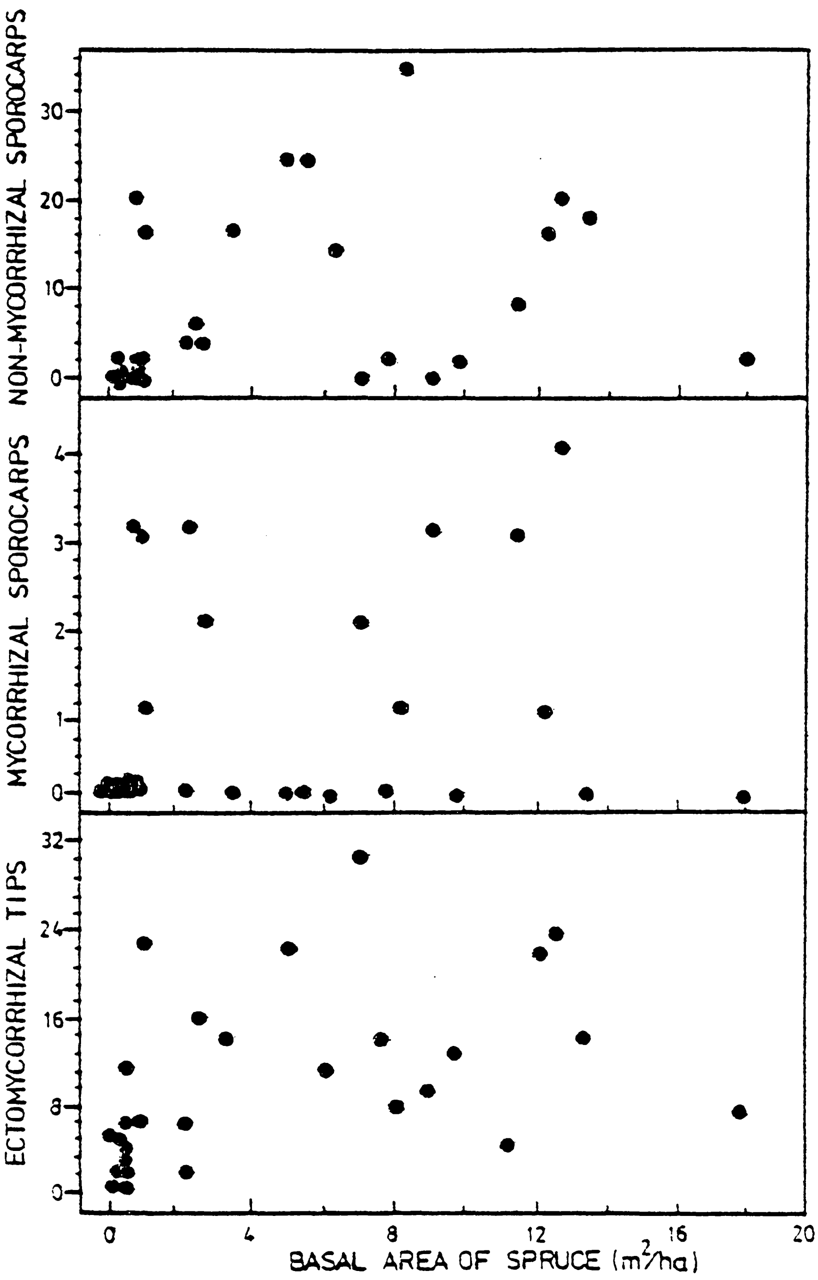


Figure 13: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and basal area of spruce.*

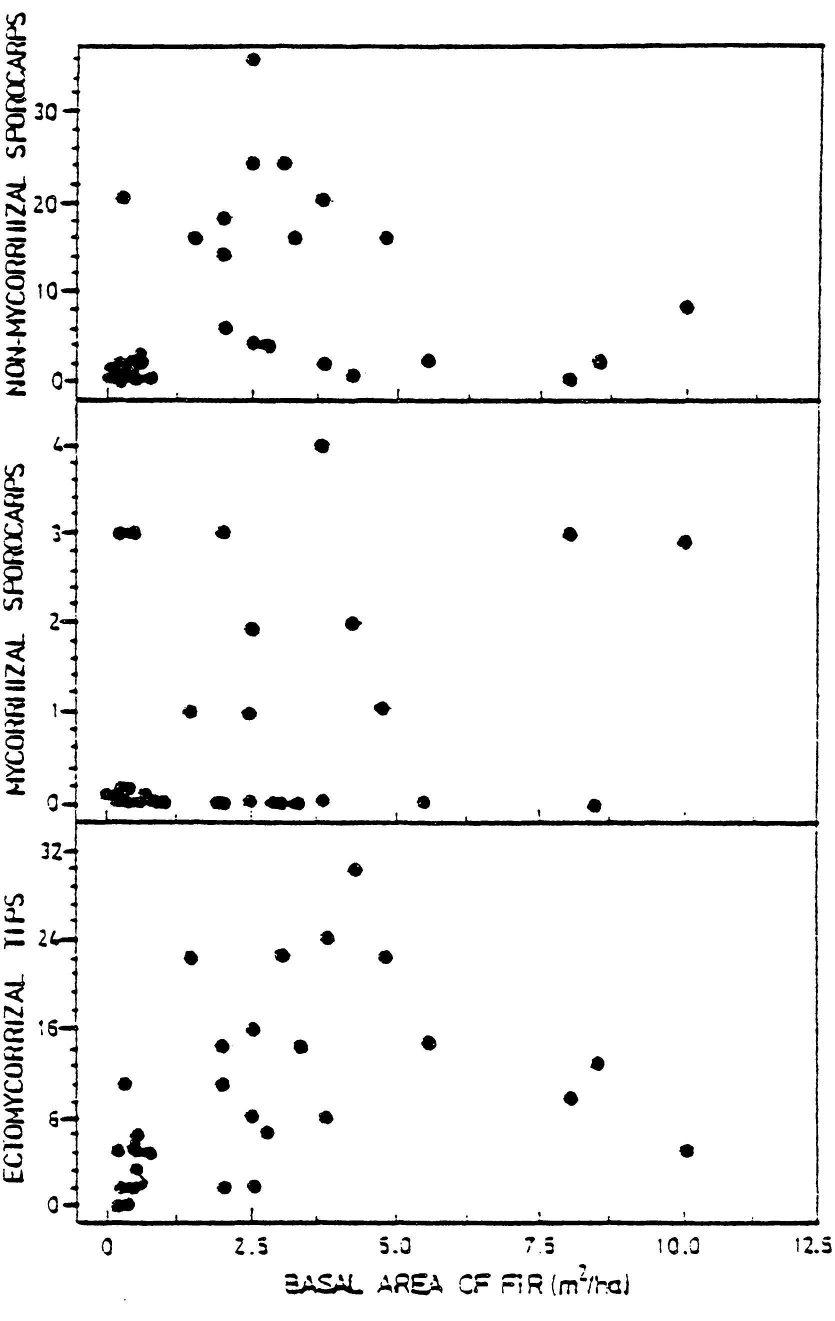


Figure 14: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and basal area of fir.*

SOIL CHARACTERISTICS AND SOIL DATA

Scatter diagrams of ectomycorrhizal root tips, mycorrhizal and non-mycorrhizal sporocarps, and soil pH, organic matter, P, K, Mg, and Ca are presented in figures 15-20. Numbers of ectomycorrhizal tips and both mycorrhizal and non-mycorrhizal sporocarps were most numerous at pH 5.0, corresponding to mature and old-age stands. Non-mycorrhizal and mycorrhizal sporocarps showed a second, smaller grouping at neutral pH, while a second clustering of ectomycorrhizal tips occurred under slightly alkaline conditions (figure 15). Figure 16 showed that non-mycorrhizal and mycorrhizal sporocarps as well as ectomycorrhizal tips occurred most frequently at higher organic matter concentrations. Organic matter concentrations between 12.0 and 15.0 per cent corresponded to concentrations found in the old-age stand.

The relationship between soil P and sporocarp and mycorrhizal distribution appearred to be approximately equal at all P levels (figure 17). Non-mycorrhizal sporocarps, mycorrhizal sporocarps and ectomycorrhizal root tips showed clustering at two K concentrations, 80ppm and 150ppm (figure 18). The grouping at the highest K concentration was more pronounced, but may be an artifact because K concentrations exceeded measurable levels.

Soil Mg and Ca concentrations did not affect distribution of non-mycorrhizal or mycorrhizal sporocarps or ectomycorrhizal root tips. High values for Mg and Ca again exceeded measurable concentrations (figures 19 and 20).

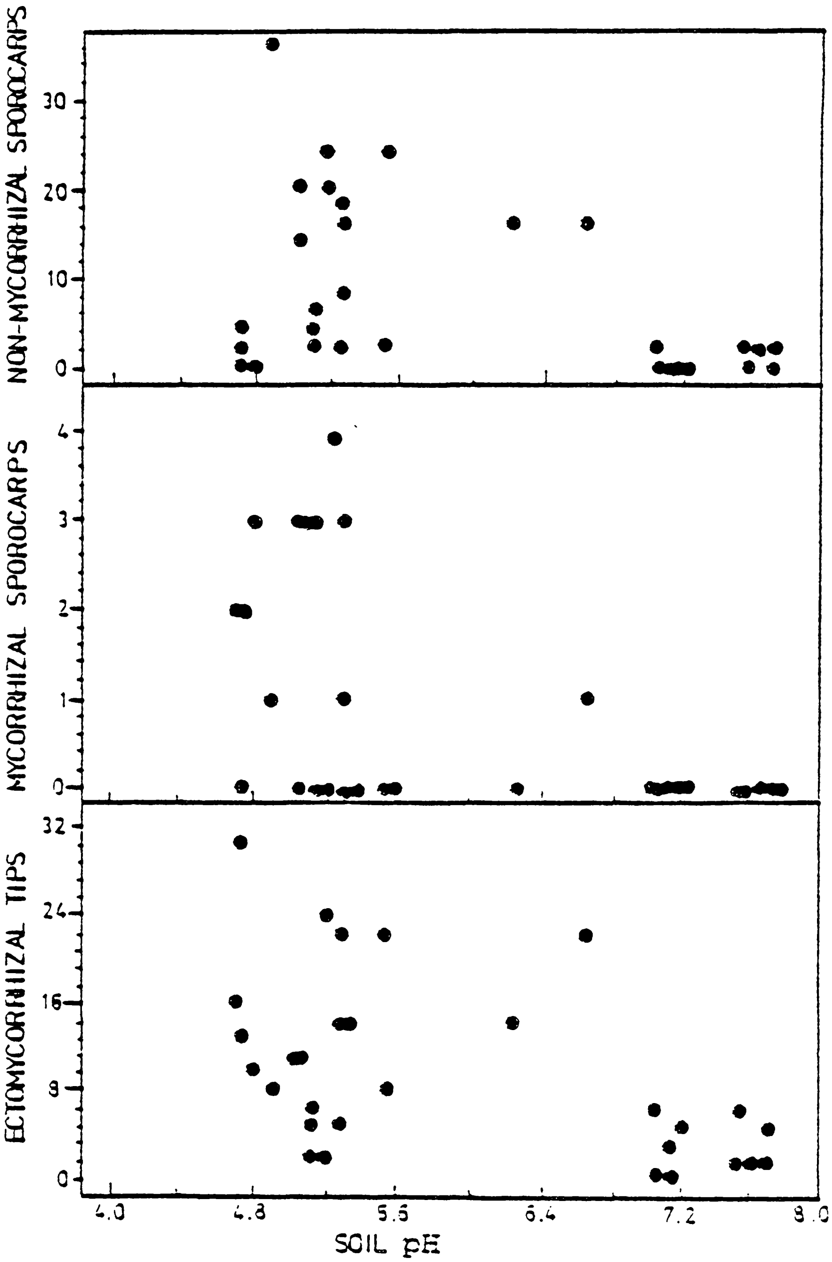


Figure 15: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and soil pH.*

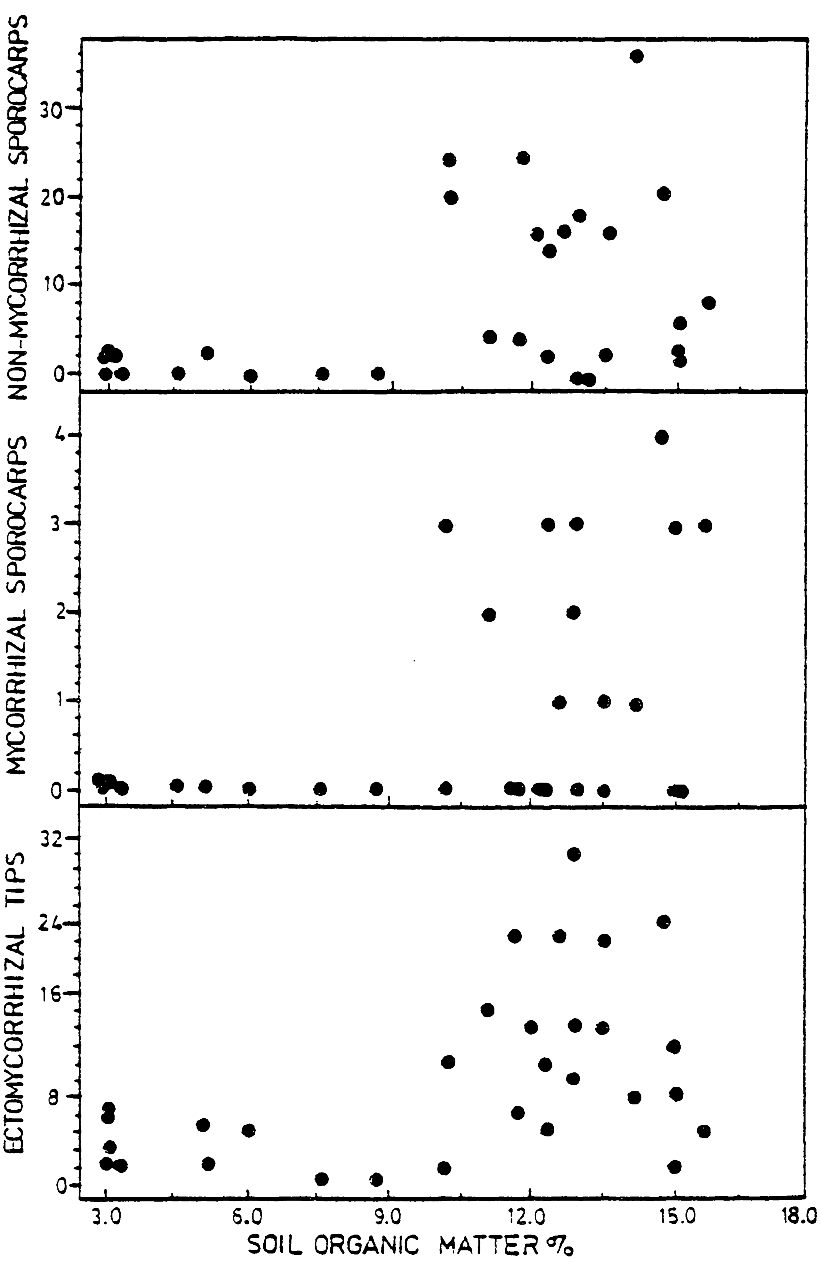


Figure 16: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and soil organic matter.*

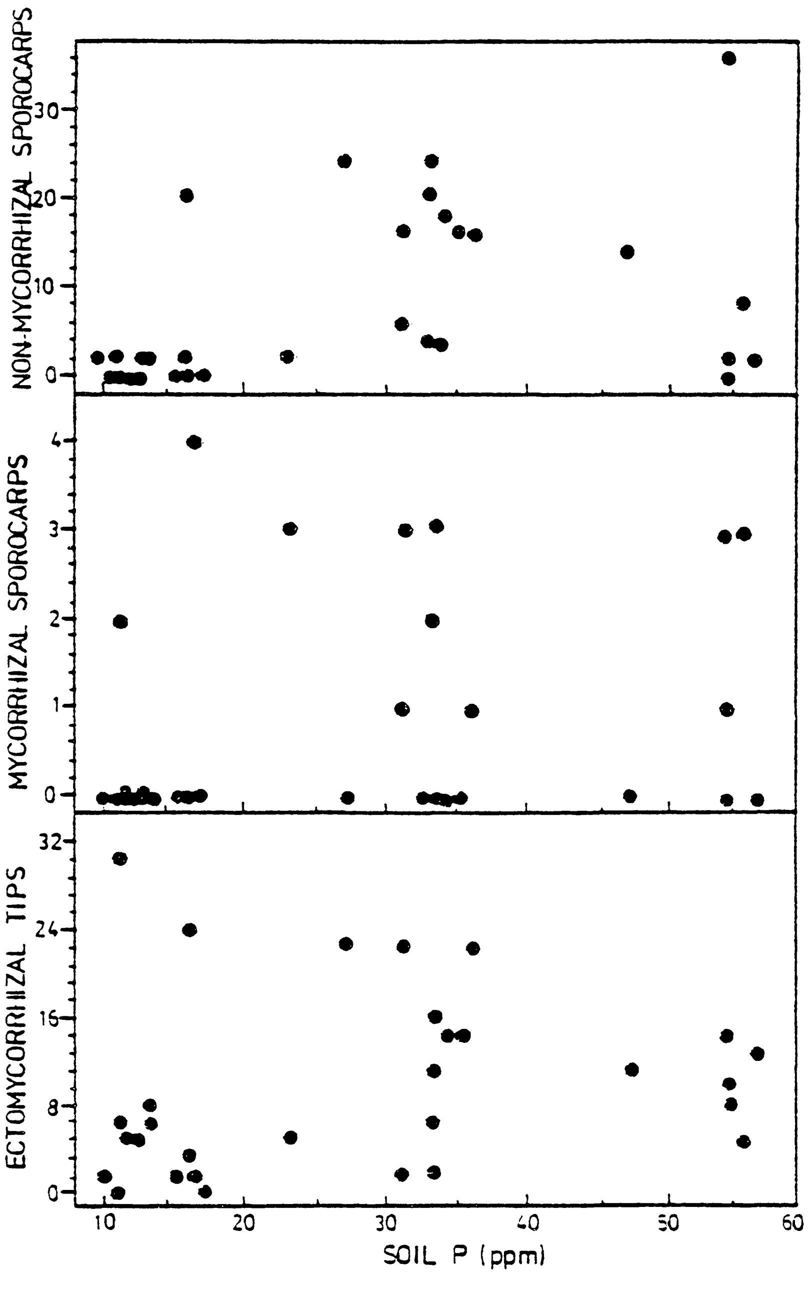


Figure 17: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and soil P.*

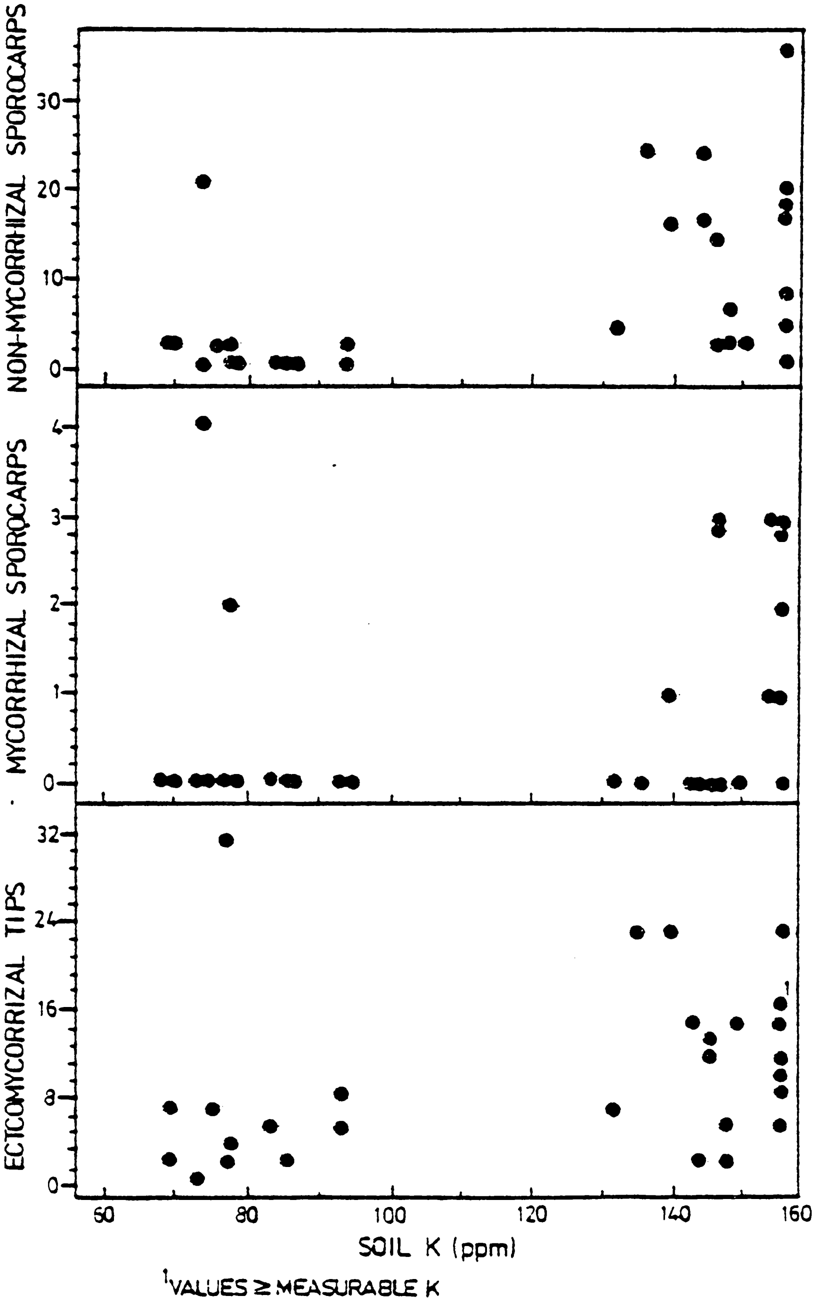


Figure 18: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and soil K.*

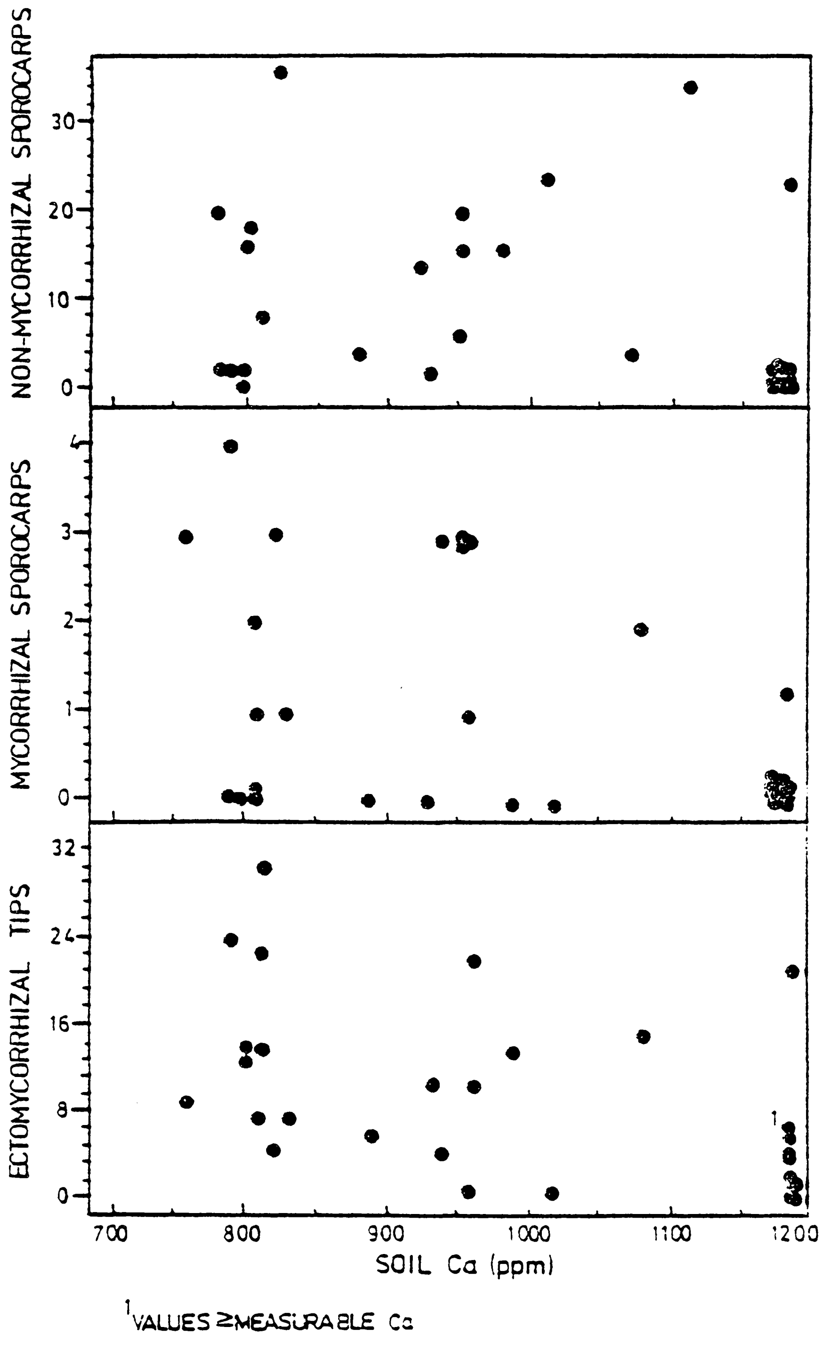
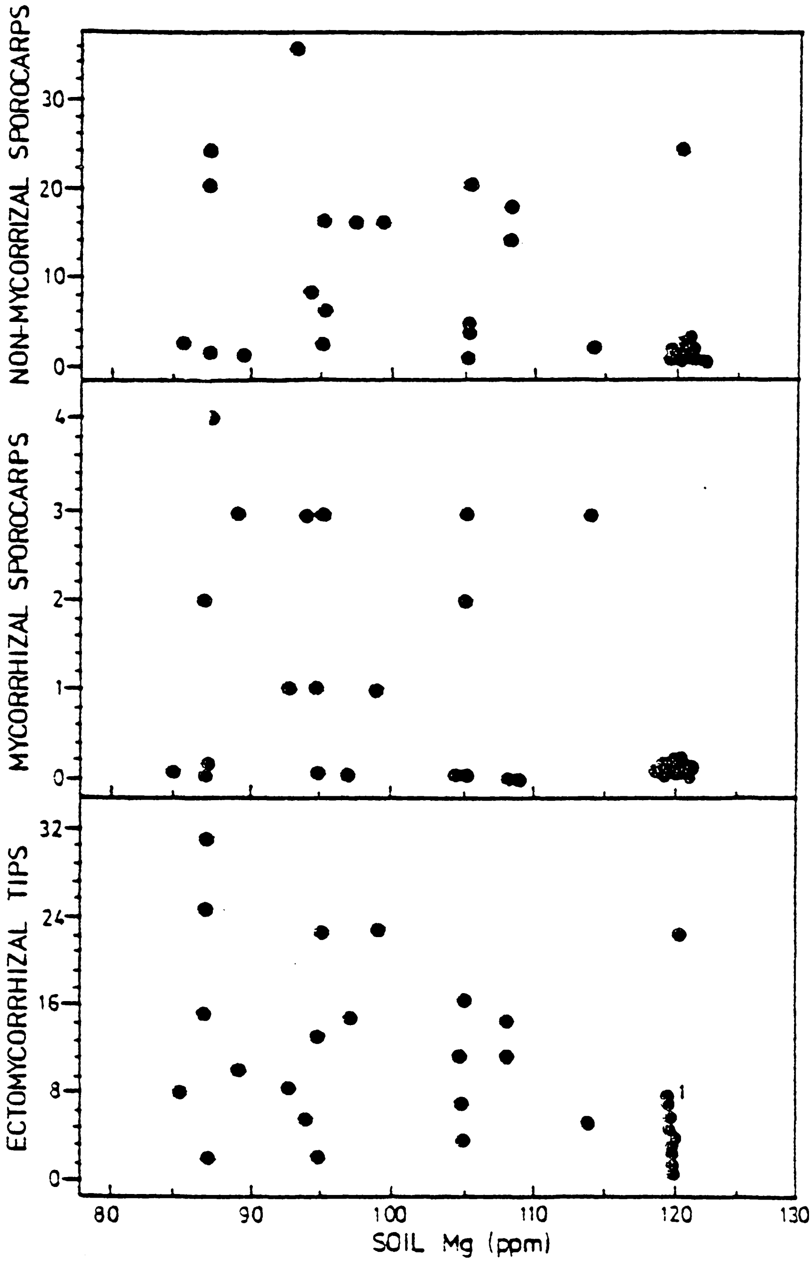


Figure 19: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and soil Ca.*



[1]VALUES ≥MEASURABLE Mg

Figure 20: *The relationship between mycorrhizal and non-mycorrhizal sporocarps, root tips and soil Mg.*

MYCORRHIZAL SYNTHESIS

Fungal species successfully isolated from spore-drop or tissue culture are listed in table 14. Isolation attempts from 38 species resulted in successful isolation from 8 species. Subsequent laboratory synthesis of the 8 fungi with both Engelmann spruce and subalpine fir confirmed that only *Suillus sibericus* is mycorrhizal with subalpine fir (table 15). No fungi were successfully synthesized with Engelmann spruce. The remaining synthesis attempts showed various effects on the tree. *Agaricus abruptibulbus* caused little effect on the tree; the root system was well developed, and the tree grew well. *Pholiota lubrica* however, parasitized the roots. *P. lubrica* caused epidermal and cortical cell collapse, and heavy wound response by cortical and stelar cells. Fungal hyphae generally penetrated throughout the root and often enveloped the entire seedling.

TABLE 14

Fungal species successfully cultured from Engelmann spruce-subalpine fir stands.

Non-mycorrhizal species	Mycorrhizal species
Agaricus abruptibulbis	*Suillus sibericus*[1]
Agaricus silvaticus	
Clitocybe albirhiza	
Lepista tarda	
Lyophyllum montanum	
Pholiota lubrica	
Tricholoma terreum	

[1] Synthesized with subalpine fir.

TABLE 15

Results of attempted mycorrhizal synthesis using eight fungi with Engelmann spruce and subalpine fir.

Fungi	Engelmann spruce	Subalpine fir
Agaricus abruptibulbis	nonmycorrhizal, epidermal cells sloughed, cortical and stelar cells well developed	nonmycorrhizal, epidermal cells sloughed, cortical and stelar cells well developed
Agaricus silvaticus	nonmycorrhizal, epidermal cells encrusted, collapsed, cortical cells heavily staining in safranin	nonmycorrhizal, parasitized, epidermal cells collapsed, cortical cells heavily staining in safranin, mycelium filled
Clitocybe albirhiza	nonmycorrhizal, cortical cells deeply staining in safranin, encrusted	nonmycorrhizal, parasitized, cortical cells heavily safranin stained, mycelium filled
Lepista tarda	parasitized, epidermal cells collapsed, cortical cells collapsed, mycelium filled	nonmycorrhizal, epidermal cells collapsed, cortical cells collapsed, heavily safranin stained
Lyophyllum montanum	nonmycorrhizal, root parasitized, epidermal cells encrusted, collapsed, heavily safranin stained	nonmycorrhizal, parasitized, epidermal cells collapsed, cortical cells heavily staining in safranin, mycelium filled

Table 15 continued

Fungi	Engelmann spruce	Subalpine fir
Pholiota lubrica	parasitized, epidermal and cortical cells mycelium filled	parasitized, epidermal and cortical cells staining, encrusted, mycelium filled
Tricholoma terreum	parasitized, epidermal cells sloughed, cortical and stelar cells mycelium filled	parasitized, epidermal cells sloughed, cortical and stelar cells mycelium filled
Suillus sibericus	nonmycorrhizal, cortical cells tannin filled, deeply staining in safranin	mycorrhizal, mantle loosely woven, 120-130μm thick, hyphae 4-6μm thick, clamped, Hartig net well developed, 4-5 deep

DISCUSSION AND CONCLUSIONS

The idea of succession is not new. The concept of fungal succession likewise, is not new, but not intensively investigated. The results of this study suggest that succession of ectomycorrhizal fungi and non-mycorrhizal fungi does occur in Engelmann spruce-subalpine fir forests in Wyoming. Consideration in this study was given to age and successional status of the stand as causative agents in ectomycorrhizal succession. The need for attention to other considerations including tree physiology, seasonal fluctuation and fungal function was reaffirmed as a result of this study.

Succession or shift in number and species of fungi within a sere is difficult to prove. A list of fungi in each stand as shown in table 7 strongly suggests that a distinct fungal flora exists at each developmental stage. Few species were found common to any two age-classes, and none were common to all three. *Russula decolorans* and *Russula queletii* are putative mycorrhizal species, which occurred in quadrats transitional between mature and old-age stands. A greater number of mycorrhizal species was found fruiting in the mature stand than in the old-age stand. This is consistent with the results of a study by Malajczuk, Molina and Trappe (1982), who infer that natural succession of fungi occurs as stands mature. They further indicate that this succession trends from broad-host-ranging fungi toward dominance by fewer host-specific fungi.

Mycorrhizae have been neglected in nutrient cycling studies of forest ecosystems (Fogel 1980). Fogel further claimed that mycorrhizae account for over 50% of the annual throughput of biomass and 43% of the annual nitrogen released in a Douglas fir ecosystem. These numbers, tremendously high by classical standards, could initiate further study of ectomycorrhizal function in ecosystems. Vitousek and Reiners (1975) concluded that essential nutrients are retained more effectively in mature ecosystems than in old-aged ecosystems. Mycorrhizal fungi could play an important nutrient obtaining role in the less effective old-age forests. Sporocarp production and dry weight of sporocarps, assumed in this study to be evidence of fungal activity, shows that the older stands consistently show greater density, diversity, and activity than the young stand. Mycorrhizal activity is greater in the old-age stand than in the mature stand (figures 5, 6, and 9). However, non-mycorrhizal activity is greatest in the mature stand, which indicates that decomposers have assumed importance at this stage of succession. Fungal activity is a new concept somewhat analogous to the importance value index of classical ecology. Activity in this sense refers to the amount of energy a particular fungus is capable of expending to reproduce in an area. Problems with this concept is that large perennial polypores produce enormous, single fruiting structures, while other fungi produce numerous small, dainty sporocarps from a single mycelium. Thus with some fungi the concept is difficult to apply when comparing grossly different fungi. The dominance of fungi with different niches in mature as opposed to old-age stands is a strong indication of change over time. The prevalence of non-mycorrhizal fungi over mycorrhizal fungi in the young stand suggests that the stand history contains some catastrophic disturbance. Logging, fire, and other disturbances have been shown to reduce mycorrihzal populations (Moser 1956, 1958, 1963, and 1967) and Gobl (1965).

Detailed examination of seres or components within a sere in classical ecology have been used to elucidate succession. "Succession" however may be an artifact evolving from comparison of too dissimilar components. Silvical data (table 2) and soil data (table 3) for the spruce-fir stands show fairly distinct ranges in each stand age-class. Statistically, pH, organic matter, K and Mg levels are different for the young versus the mature and old-age stands. Biologically however, even small differences in the mature and old-age forest may be quite important. A different fungal flora would be expected to be found in areas differing greatly in overall characteristics. Two dimensional ordination of stand and soil data for each quadrat however shows a gradient in overall quadrat similarity from young to mature to old-age stands (figure 4). Quadrats and stands used in this study therefore adequately simulated a young stand developing through maturity to an old-age stand. Stand and soil characteristics which differ throughout the sequence and affect the distribution of fungi (figures 10-20) change only in relation to ecosystem maturation (Smith 1974). The alternative would be to treat fungal succession as an artifact. In this case the results would be expressed as three distinct clusters of plots representing total dissimilarity among the stands.

The distribution of fungi and mycorrhizae as a function of age, density or basal area relates directly to forest maturation level. Figures 10-14 show that sporocarps and ectomycorrhizal tips tend to occur in mature or old-age stands or in stands with similar characteristics. Age and basal area were more closely related to fungal distribution than to density. The relationship with age cannot be distinguished from changes in stand characteristics, nor should it be. Change in stand characteristics is a function of ecosystem age or maturity. Basal area increases with age and is also a function of age in many cases. In cases where age differences are important it plays a major role in the increase in ectomycorrhizae and mycorrhizal sporocarp distribution. Sukachev and Dylis (1964) described root development as a function of basal area. Root biomass was shown to be approximately 25% of the above ground biomass in Russian spruce forests. Taskey (1982) however, found that root systems of fir in Washington comprised about 64% of total tree biomass. Saprophytes, non-mycorrhizal rhizosphere dwellers, and other non-mycorrhizal fungi also appear to be affected by increased basal area and root biomass in older stands.

The distribution of fungi as a function of soil characteristics is also a function of age and stand maturity. Distribution, however, as a function of soil pH and K often occurs at two distinct levels indicating a different set of requirements or a different function by fungi at each level. Palmer (1980) indicated that most common mycorrhizal fungi grow best at pH 5.0. Vinopal (1978) however, found optimum growth for many ectomycorrhizal species in the nutrient poor north Florida soils at highly alkaline pH, around 9.0. This may also serve to explain distribution of the different mycorrhizal types found in this study (table 9). Mycorrhizal fungi not tolerating high soil pH would occur most frequently in mature or old-age stands. Fungi growing best at high pH would be found in young stands. The argument is similar with K concentration with different fungi acting at different K levels. Soil P concentration as shown in Figure 17 shows little relationship with fungal distribution. Ford (1982) however, suggested that P uptake, regardless of concentration in the soil, is mediated by mycorrhizal fungi and not the tree. Therefore no definate relationship would be expected at any level.

Fungal fruiting and occurrence of ectomycorrhizal root tips are clearly influenced by age and successional status of the spruce-fir stands. These key variables however, are extremely complex and encompass several factors which could more directly influence fungal community structure. Tree physiology, which is indirectly related to stand age, could influence distribution of mycorrhizal and non-mycorrhizal fungi, as well as the entire soil microflora. Robbins (1957) concluded that several physiological changes occur as individual trees age including: decreased absorption and conduction of water, increased photosynthetic capacity, decreased resistance to decay, increased protein degeneration, and a decrease in the ratio of energy produced in photosynthesis to energy used in respiration. Smith (1970) found that the concentration and types of amino acids, sugars, organic acids, and hormones in root exudates are dramatically different in seedling and mature sugar maple trees. Ectomycorrhizal fungi therefore, which require specific physiological compensation from their host as suggested by Hacskaylo (1957, 1973), Harley (1959), and Bjorkman (1970), would theoretically undergo succession as the physiology of their host changed. Likewise, tree physiology could directly influence stand characteristics. Gail and Cone (1929) observed a significant increase in pH of ponderosa pine leaves with increasing tree age. Needles dropped every second or third year would increase soil pH and therefore affect nutrient availability.

The seasonality of sporocarp production (figure 8) coincides with previous reports (Lange 1948; Lamb 1979). The peak season for mycorrhizal and non-mycorrhizal fruiting is fall, with a smaller peak in the spring. The high number of new species being added late in the fall therefore suggests that more species could have been found following conclusion of the study. Interpretation of results is limited by the fact that this study was conducted during two extremely dry collecting seasons. Ideally, Watling (1977) suggests that at least ten moist, bountiful collecting seasons are required to obtain a representative sample of the mycoflora. Wilkins et al (1938) suggest that only one "good fruiting season" is required for an adequate sample. The flora of high-altitude spruce-fir forests in Wyoming, as depicted in this study, probably represents only a small cross section of the mycorrhizal and non-mycorrhizal fungi which occur there. Further, the fungi which fruited during this study may be characteristic of dry weather conditions. None-the -less, reduced xeric fruitings yeild small cross sections of the usually larger fungal flora.

Laboratory synthesis of mycorrhizae in pure culture confirmed one mycorrhizal relationship. This was the ectomycorrhizae formed by *Suillus sibericus* and *Abies lasiocarpa* (table 9). Failure of *Suillus sibericus* to synthesize with *Picea engelmannii* supports the conclusion of Chilvers (1973), that host-specific fungi often show specificity at the host and fungus genus level. The effects that the remaining fungi had on the trees appeared largely pathogenic (table 14). Molina (1981) showed that epidermal and cortical cells in immediate contact with incompatible or pathogenic mycelium accumulate polyphenolic compounds. Kosuge (1969) found that phenolic compounds are associated with host reactions to pathogenic invasion. The heavy safranin staining areas in the epidermal and cortical cells may be attributed to accumulation of phenolic compounds. It is unlikely therefore that fungi other than *Suillus sibericus* used in synthesis experiments are mycorrhizal even under field conditions. The extreme difficulty in culturing obligate mycorrhizal fungi (Trappe 1967) such as species of *Russula, Amanita, Cortinarius,* and *Tricholoma* spp. accounts for the low isolation success.

Theoretical and practical applications of ectomycorrhizal succession are numerous. The concept of fungal community and its placement with vascular plant communities has been explored by Hueck (1953). The difference in fungal strategy as opposed to vascular plant strategy warrants unification of fungal communities. The relationship between ectomycorrhizal fungi and host must be examined more closely. A vascular plant seral stage may be nothing more than a reflection of its associated fungal community. Shift in species and number of ectomycorrhizal fungi on a particular host with age seems quite probable and could indicate that in order to maintain optimum tree growth and health, inoculation of certain fungi at precise intervals must occur as the tree ages. Inoculation of nursery seedlings with specific mycorrhizal fungi before outplanting could also be an effective tool in fast early growth and survival. Economically it would be more practical to inoculate with a fungus which would not be immediately superceded upon outplanting. Study of the fungal community will clarify which fungi are present at various successional stages in tree development. The knowledge will be used to pinpoint the conditions and factors which favor their growth in the plant community and to gain an understanding of the advantages they provide the host. These results could then be expressed in terms of good silvacultural practice to benefit the goals of foresters to improve forest site yields.

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