THE ECOLOGY AND POPULATION BIOLOGY OF
TWO LITTER DECOMPOSING BASIDIOMYCETES

by

John F. Murphy

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Biology

APPROVED:

G. K. Miller, Chairman

E. T. Wilson

D. A. West

S. E. Scheckler

May, 1992

Blacksburg, Virginia
THE ECOLOGY AND POPULATION BIOLOGY OF
TWO LITTER DECOMPOSING BASIDIOMYCETES

by

John F. Murphy

Committee Chairman: Orson. K. Miller
Biology

(ABSTRACT)

Four 286m² plots on alternate sides of the spur ridges on
Brush Mt. were established and their plant communities
characterized. Agaric and Bolete basidiomycetes were
sampled for two years. Fifty species were recorded on the
plots. Phenologically, decomposer species were highly
dependent upon rainfall events, whereas mycorrhizal species
were more seasonal. Two species emerged as dominant litter
decomposers. Marasmiellus praeacutus (Ellis) Halling is
dominant on southwest facing slopes and occurs on a wide
variety of coniferous and hardwood detritus. Collybia
subnuda (Ellis ex. Peck) Gilliam is dominant on northeast
facing slopes, and occurs on hardwood leaves and small woody
detritus. The population structure of both of these species
was investigated using tests of somatic incompatibility.
Genets of both species are able to persist for more than one
year. The observed minimum population density is 0.071 –
0.121 genets/m² for M. praeacutus and 0.039 – 0.093
genets/m² for C. subnuda. Mating tests indicate that M.
praeacutus is heterothallic and tetrapolar, and that C.
subnuda is heterothallic and bipolar. Preliminary crosses
between monokaryotic tester sets indicate a surprisingly low number of mating alleles in both species. Decomposition studies suggest that while the restricted distribution of *C. subnuda* to the northeast slopes may be affected by substrate specificity, the restriction of *M. praecutus* to the southwest slopes is due to other factors.
ACKNOWLEDGEMENTS

The financial support offered by the Department of Biology, and the logistical support given by the Horton Center and the Virginia Museum of Natural History were of great assistance to this work.

The guidance offered by my committee members, Drs. Miller, Nilsen, Scheckler and West is greatly appreciated. The independence allowed by these professors was balanced by intelligent advice and stimulating discussion when it was needed. I thank them for their commitment to education and I admire their skill. I especially thank Dr. Miller for the many hours of patient listening and for the gentle and excellent advice and encouragement he offers. The sensible advice and warm hospitality offered by Mrs. Hope Miller was also invaluable.

Tim Flynn is responsible for making me aware of the unique opportunity provided by Brush Mountain. Mr. Clowers Woodyard kindly permitted the use of his land in the first year of this study. The personnel at the Blacksburg ranger station of the Jefferson National Forest were very helpful.

My colleagues in the Mycology Lab provided an unflagging source of entertainment and support. To Dr. Rajendra Bhatt, Cathy Cripps, Kathy Jacobsen, Laurel Kuehnel, and Dr. Roland Treu, I extend my most sincere gratitude.
The enduring friendships I share with Jim Stearns and Laurie Webber proved sound at critical times despite the time and distance which separate us.

The love and support of my family were crucial to the success of this project. To Laura, Bruce, Mary, Phil, Joe III, Concetta, Kate, Stefano, Anna, George, Joe Jr., and Janice, you have helped me more than you know. Thank you from the bottom of my heart.
# TABLE OF CONTENTS

Abstract......................................................ii
Acknowledgements..............................................iii
Table of contents.............................................vi
List of tables.................................................vii
List of figures................................................viii

Chapter one - Introduction.................................1

Chapter two - Biotic surveys.................................7
I. Introduction...............................................7
II. Plant survey.............................................12
Methods.....................................................12
Results....................................................17
Discussion...............................................25
III. Fungal survey...........................................27
Methods....................................................27
Results....................................................30
Discussion...............................................47

Chapter three - Genetic studies.........................54
I. Introduction...............................................54
II. Methods..................................................61
  1. Somatic incompatibility tests..61
  2. Mating tests........................................63
  3. Di - mon tests......................................64
III. Results................................................66
  1. Somatic incompatibility tests..66
  2. Mating tests.......................................84
     a) Mating system determination..................84
     b) Mating allele distributions...................84
  3. Di - mon tests.....................................86
IV. Discussion...........................................87

Chapter four - Substrate specificity tests..........102
I. Introduction............................................102
II. Methods...............................................103
III. Results................................................105
IV. Discussion...........................................111

Chapter five - Conclusions................................113

Bibliography.................................................117
Appendices ..................................................126
Vita..........................................................139
LIST OF TABLES

Table 1. Tree species and relative importance values on plots 1-4 ........................................ 19
Table 2. Shrub species and relative importance values on plots 1-4 ........................................ 20
Table 3. Mean and standard deviations of quadrat stem counts for *Vaccinium* sp. and *Gaylussacia baccata* ................................................................. 21
Table 4. Plant species separating plots 1 and 3 (northeast facing slopes) from plots 2 and 4 (southwest facing slopes) ......................................................... 23
Table 5. Plant species separating plot 1 from plot 3 ......................................................... 24
Table 6. Decomposer species occurring on plots 1-4 ......................................................... 32
Table 7. Mycorrhizal species occurring on plots 1-4 ......................................................... 33
Table 8. Number of species of fungi on each plot ......................................................... 34
Table 9. Occurrences of *M. praecutus* and *C. subnuda* by plot number and year .................. 40
Table 10. Mycorrhizal species occurring on both northeast or both southwest facing slopes ........ 46
Table 11. Summary of incompatibility reaction types .................................................... 70
Table 12. Observed minimum population density of *M. praecutus* and *C. subnuda* ............... 75
Table 13. Dikaryotization rates of *M. praecutus* monokaryotic isolates JFM 635.01 and JFM 635.04 by dikaryon JFM 593 ............................................................. 90
Table 14. Results of *C. subnuda* di-mon selfcross .................................................... 91
Table 15. *Marasmiellus* praecutus di-mon cross results ................................................ 92
Table 16. Fruiting substrate summary, *M. praecutus* and *C. subnuda* .................................. 106
Table 17. Comparison of conspecific isolates in mean percent decrease in dry mass .................. 109
LIST OF FIGURES

Figure 1 Marasmiellus praecutus (Ellis) Halling, JFM 108. .............................................5
Figure 2 Collybia subnuda (Ellis ex Peck) Gilliam, JFM 213. ..........................................6
Figure 3 Brush Mountain, Montgomery Co., Virginia .................................................8
Figure 4 Brush Mountain spur ridge. .................................................................9
Figure 5 Plot locations. .................................................................................14
Figure 6 Plot design. ....................................................................................15
Figure 7 Phenogram, tree and shrub data. ......................................................22
Figure 8 Phenology of fungi in 1990. ..............................................................37
Figure 9 Phenology of fungi in 1991. ..............................................................38
Figure 10 Species/area curves. ..................................................................39
Figure 11 Collybia subnuda occurrences on plot 1. .........................41
Figure 12 Collybia subnuda occurrences on plot 3. .................................42
Figure 13 Marasmiellus praecutus occurrences on plot 2 ......................43
Figure 14 Marasmiellus praecutus occurrences on plot 4 ......................44
Figure 15 Phenograms, fungal data. ...........................................................45
Figure 16 Variation in somatic incompatibility results .............................69
Figure 17 Genet distribution of Collybia subnuda on plot 1. ..................71
Figure 18 Genet distribution of Collybia subnuda on plot 3 ..................72
Figure 19 Genet distribution of Marasmiellus praecutus on plot 2 ..........73
Figure 20 Genet distribution of Marasmiellus praecutus on plot 4 ..........74

viii
Figure 41  Mycorrhizal species occurrences on plot 1...135
Figure 42  Mycorrhizal species occurrences on plot 2...136
Figure 43  Mycorrhizal species occurrences on plot 3...137
Figure 44  Mycorrhizal species occurrences on plot 4...138
CHAPTER ONE - INTRODUCTION

A primary problem in our understanding of population biology in the Fungi is the lack of understanding of allele frequencies in situ. Without this knowledge, mycologists are unable to address the problems of microevolution and speciation. This situation inspired J. H. Burnett, in his presidential address to the British Mycological Society, to say:

Mycology and mycologists, on the whole, have contributed very little to the mainstream of ideas concerning the modes of origin of species. . . . Virtually nothing is known about natural selection nor, indeed, about selection of any kind in fungi . . . . Moreover, the true measure of variation present in any fungal population, let alone a species, has hardly begun to be assessed (Burnett, 1983).

Even in the economically important wood-rotting basidiomycetes, detailed population studies have been neglected (Rayner and Bodd, 1986). Endler (1989) remarks that few studies have linked ecology and population genetics. A similar claim could be made for fungal species. The present study addresses these problems.

The primary intent of this study was to collect the baseline information necessary to investigate the population biology and microevolution of one or more woodland Agaric decomposers in an area in which selection pressure may significantly influence allele frequencies. The purpose of
such a study is to add to our knowledge of microevolutionary processes in these fungi and to compare and contrast the population structure of woodland litter decomposers to the more thoroughly studied wood-decomposing basidiomycetes.

In order to understand the processes which lead to genetic differentiation and speciation, detailed knowledge must be obtained about specific populations. In particular, it is important to understand the interplay between population size, gene flow, natural selection, mutation rates, and the degree of outbreeding. Characterizing these things is problematic, particularly in the fungi. The problem centers around determining the presence of an individual which can only be detected sporadically by its fruiting bodies. In addition, the limits between individuals, so easily perceived between most animals and plants, can be determined only with great difficulty, if, indeed, at all. In addition, considerable information about the ecology of candidate species must be collected, particularly if one is interested in the effects of natural selection and gene flow. This is labor intensive, but the gap between ecology and population biology recognized by Endler (1989) is a serious one which must be addressed. Lastly, a complete understanding of population dynamics requires observations repeated over time (Antonovics, 1984); studies of brief duration may miss significant temporal
variation. However, the relatively recent acceptance of the fungal thallus as a genetically discrete individual and the development of a simple test for individuality (Childs, 1963; Adams and Roth, 1967; Barrett and Uscuplic, 1971; Rayner, 1991; see chapter 3) make detailed studies of fungal population genetics both timely and promising.

This investigation into microevolutionary processes began with the following hypothesis: a species within the Agaricales, distributed along a steep cline in some environmental parameter, will experience selective pressures which will result in a corresponding cline in some allele frequencies. This same hypothesis was supported by the research of (Aston and Bradshaw, 1966) in Agrocybe stolonifera; in the higher fungi, however, almost nothing is known about the effect of natural selection (Burnett, 1983).

The scope of this study, therefore, was as wide as possible at its beginning and progressively narrowed its focus on the ecology and population biology of particular species of basidiomycetes. It was necessary first to identify a site likely to present a steep cline in environmental parameters. Once a site was chosen, permanent plots were established. A survey followed which characterized plant and fungal community structure and identified promising taxa for intensive sampling. Lastly, in vitro tests of these taxa were included to determine
various aspects of their ecology and population biology.

Among the dominant decomposers observed in the fungal survey, two species presented themselves as promising candidates for closer investigation; these were *Marasmiellus praeacutus* (Ellis) Halling (fig. 1), and *Collybia subnuda* (Ellis ex Pk.) Gilliam (fig. 2). Despite their obvious ecological importance as dominant components of the ecosystem studied in this project, no studies have involved these taxa beyond morphological taxonomic descriptions (Halling, 1987; Halling, 1983; Gilliam, 1976).

Of particular interest was the elucidation of the population structure of these two fungi; for example, do they exist as genetically distinct individuals and, if so, what is their distribution, and how large an area does each genotype occupy? What particular mating systems do they possess, and how are the individuals in the population related to each other? Finally, what is the ecological role of each fungus, and how does this role affect its distribution?

Chapter two gives the results of the plant and fungal surveys. Chapter three covers the identification of genetic individuals and mating systems of *M. praeacutus* and *C. subnuda*, the two dominant litter decomposers identified during the fungal survey. Chapter four covers an investigation of the nutritional needs of these two taxa.
Figure 1. *Marasmiellus praeacutus* (Ellis) Halling, JFM 108.
Figure 2. *Collybia subnuda* (Ellis ex Peck) Gilliam, JFM 213.
CHAPTER TWO - BIOTIC SURVEYS

I. INTRODUCTION

The choice of a study site was determined by several factors. Convenient location was important since the sites needed to be sampled frequently over several seasons and opportunistically as weather dictated. Of particular importance was the identification of areas of steep environmental gradient. Brush Mountain (fig. 3) provides both of these factors.

Brush Mt. is in the Ridge and Valley Physiographic Province of the Appalachian Mts. The dominant geologic features of this region are gently rolling valleys underlain by Cambrian and Ordovician limestones and shales and high narrow ridges of Silurian sandstones (Hoffman, 1969). The mountain lies within the oak-chestnut (now mostly oak-hickory) forest classification of Braun (1950), and most of it lies within the Jefferson National Forest. Brush Mt. is approximately 40 kilometers long, its main crest is oriented from southwest to northeast, and it can be accessed approximately 3 km north from Blacksburg, VA.

The northwest side of Brush Mt. is repeatedly dissected by small watersheds which create numerous "spur ridges," each with a northeast and a southwest facing slope (fig. 4). These slopes are typified by the Berks and Weikert soil
Figure 3. Brush Mountain, Montgomery Co., Virginia, as seen from the northwest.
Figure 3. Brush Mountain spur ridges. The southwest-facing, *Pinus* dominated slopes are sunlit. The northeast-facing *Quercus* dominated slopes are in the shade.
series, which is a shallow, well drained, loamy-skeletal, mixed, mesic lithic to typic dystrochrept (Creggar et al., 1985). The slopes are steep and the soils have low fertility, making them unsuitable for agriculture or timber production (Creggar et al., 1985; Williams, 1989).

Most of Brush Mt. was probably logged during the late 1800's and early 1900's, although no detailed records exist (Williams, 1989). There is no evidence of recent logging on the study sites. Fires have historically been important in maintaining the Pinus pungens Lamb. stands which occur on the mountain, but, although a few carbonized snags bear witness to past fires, there is no evidence of recent fire activity at the study sites.

The northeast slopes of the spur ridges support a mixed hardwood forest dominated by Quercus prinus L.; the southwest slopes are a mixed hardwood-coniferous forest dominated by Pinus pungens Lamb. The transition from one forest type to the other is quite sudden and is caused by differences in abiotic factors including irradiance, soil temperature, and possibly soil moisture availability (Lipscomb, 1986). The strong contrast between the forest types, and the repeating spur ridges, make Brush Mt. a "natural laboratory" for environmental studies (Schiffman, 1990). For the purposes of the present study, the strong environmental gradients which separate the forest types, the
proximity to Blacksburg, and the data base from previous botanical studies (Lipscomb, 1986; Schiffman, 1990; and Williams, 1989) make the spur ridges on Brush Mt. an ideal study site.

It became clear, soon after embarking on this project, that natural selection in the wild could not be detected with the resources available. I therefore gathered the baseline data necessary for a more complete study in the future. The following questions were addressed: what are the important biotic components on the study site? How similar or different are the plant and fungal flora on opposite sides of the spur ridges? What are the dominant components of the fungal community, and do any taxa occur on both sides? What are their particular ecological roles and constraints? Despite the scientific interest in Brush Mt. as a "natural laboratory," there have been no previous studies of the fungi in these communities. Since the fungal community structure ultimately depends upon the plant community, both trees and shrubs were surveyed. The fungi were surveyed over a two year period, and all agaric and bolete sporocarp occurrences were mapped.

The specific hypotheses addressed in this chapter are as follows:

1. The plots chosen on the successive NE facing slopes of Brush Mt. contain similar plant populations as will the
plots on the successive SW facing slopes; however, the plant populations will be significantly different between NE and SW facing slopes.

2. Different Agaric species will occur on the spur ridges, reflecting the species richness of both the ectomycorrhizal and decomposer components of the ecosystem. However, the taxa on the successive NE facing slopes will be similar to each other but differ from those of the successive SW facing slopes, and thus reflect the responses of the fungi to the biotic and abiotic differences on either side of the spur ridges.

II. Plant Survey

Methods

The study sites were chosen near those used by Lipscomb (1986). Precise locations are shown on fig. 5. Latitude longitude are as follows:

<table>
<thead>
<tr>
<th>Plot</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30° 16' 19&quot; N</td>
<td>80° 27' 18&quot; W</td>
</tr>
<tr>
<td>2</td>
<td>30° 16' 16&quot; N</td>
<td>80° 27' 21&quot; W</td>
</tr>
<tr>
<td>3</td>
<td>30° 16' 7&quot; N</td>
<td>80° 27' 44&quot; W</td>
</tr>
<tr>
<td>4</td>
<td>30° 16' 6&quot; N</td>
<td>80° 27' 49&quot; W</td>
</tr>
</tbody>
</table>

Plots were located in the approximate center of the northeast and southwest facing slopes of two spur ridges.
The exact location was subjectively chosen to represent the vegetation type dominating each slope. Plot characterization and interplot comparisons of the vegetation is described in detail below.

Bills (1985) found that a plot size of 150 m$^2$ was the minimal area in a mixed hardwood forest necessary to sample most of its ectomycorrhizal fungal species. In this study, rectangular plots were designed to encompass 256 m$^2$, plus room for 0.5 m access strips (fig. 6). Each plot was divided into eight 2 m columns (up the slope) and eight 2 m rows (oriented on the contour) creating 64 subplots, each 2 X 2 m. Each subplot was marked with a 22 mm (1/2") diameter PVC stake marked with a code identifying the plot, row and column numbers. Three access strips, each 0.5 m wide and parallel to the columns, were included. During sampling, effort was made to stay on these strips and outside the plot perimeter in order to minimize any disturbance of the litter layer. Direct sampling was restricted to the subplots adjacent to the access strips as well as to the strips themselves. Observation was therefore limited to a zone 2.0 - 2.5 m on either side of the line described by the plot stakes.

Shrubs were surveyed in the fall of 1991. For the purposes of this study, shrubs were defined as perennial plants with a DBH < 3 cm. Stem counts were obtained for
Figure 5. Plot location. Plots 1 and 2 are indicated in the enlargement on the top right. Plots 3 and 4 are indicated in the enlargement in the top left. Taken from the Newport, Virginia USGS topographic map N3715-W8022.5/7.5, 1965.
Figure 6. Plot design. Numbers on X and Y axes indicate column and row numbers, respectively. Each quadrat is 2 m x 2 m. The access strips (crosshatched) are 0.5 m wide.
each species for each subplot. Trees with a DBH ≥ 3 cm were surveyed in the spring of 1990. The precise location of each tree was determined from the subplot code and the subplot X and Y coordinates measured with a meter stick to the nearest 0.1 m from the subplot stake; these data were entered into a DBASE IV database for later analysis. Importance values for trees were calculated by the following equation:

\[ \text{Importance Value} = \text{Relative frequency} + \text{Rel. basal area} + \text{Rel. density} \]

Importance values for shrubs were calculated as a sum of relative frequency and relative density.

Phenetic relationships between plots were determined using the NT-SYS package of multivariate statistical programs developed by Rohlf (1988). Plots were considered operational taxonomic units (OTU's), and the calculated importance values for trees and shrubs were the character states used.

To determine approximate time elapsed since the last major ecological disturbance on the plots, the largest tree on each plot was cored. Cores were obtained from breast height, wrapped in aluminum foil and stored at -15°C. Core age was determined by counting rings under a dissecting scope.
Results

Maps of the tree locations on each plot are included in the appendix. Importance values for trees are given in table 1. On plots 1 and 3, the northeast facing plots (fig. 5), the dominant tree is *Q. prinus* (importance value = 1.84 and 1.21, respectively). The next most important tree is the *Acer rubrum* (importance value = 0.92 and 0.54, respectively). *Pinus pungens* is dominant on plots 2 and 4 (fig. 5), the southwest facing plots (importance values 1.63 and 2.10, respectively). *Quercus prinus* and *Nyssa sylvatica* are close seconds, the former having importance values of 0.59 and 0.45, the latter having importance values of 0.55 and 0.51. The dominant trees on all plots are ectomycorrhizal. In addition, some of the minor trees present on the plots are also ectomycorrhizal; these include *Carpinus caroliniana*, *Castanea dentata*, *Quercus velutina*, and *Carva glabra* (Trappe, 1962). *Acer rubrum* is endomycorrhizal (O. K. Miller, personal communication).

The number of rings counted from trees cored on plots 1 (*Quercus prinus*) and 2 (*Pinus pungens*) fixes the largest tree ages at 79 and 80 years, respectively. The number of rings counted from trees cored on plots 3 (*Q. prinus*) and 4 (*P. pungens*) fixes the largest tree ages at 145 and 123.

Shrub importance values are given in table 2. *Vaccinium sp.* has a high importance value on plots 2, 3, and 4.
Gaylussacia baccatta has high importance values on plots 2 and 4 but is absent on plots 1 and 3. Mean and standard deviation of quadrat stemcounts are given in table 3. Together, these two species create a dense shrub layer over plots 2 and 4 which is much less dense on plots 1 and 3. Vaccinium sp., Gaylussacia baccata and Menziesia pilosa are all ericaceous shrubs and can be expected to have ericoid ectendomycorrhizae. Many of the other shrubs on the plots are saplings of tree species, some (for instance, Castanea dentata) may contribute to the ectomycorrhizal flora.

The similarity matrix used in the phenetic analysis was derived from the species and importance values given on tables 1 and 2. The phenogram resulting from the cluster analysis of this data is included in fig. 7. The plots cluster together as expected. Plots 1 and 3, located on northeast facing slopes, have similar floral composition dominated by Quercus prinus, whereas plots 2 and 4 are dominated by Pinus pungens, although Q. prinus is also present on these plots. Plots 2 and 4 are 97.2% similar, plots 1 and 3 are 75.5% similar, and plots 1 and 3 are only 18.0% similar to plots 2 and 4. The first three eigenvectors account for 100% of the variation in the data. The first eigenvector accounts for 62.45% of this variation. It separates all the plots from each other, but it mostly separates plots 1 and 3 from plots 2 and 4. The second
Table 1. Tree species and relative importance values on plots 1-4.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Plot 1</th>
<th>Plot 2</th>
<th>Plot 3</th>
<th>Plot 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Quercus prinus</em> L.</td>
<td>1.84</td>
<td>0</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Pinus pungens</em> Lamb.</td>
<td>0</td>
<td>0</td>
<td>1.63</td>
<td>2.10</td>
</tr>
<tr>
<td><em>Acer rubrum</em> L.</td>
<td>0.92</td>
<td>0.54</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Nyssa sylvatica</em> Marsh.</td>
<td>0</td>
<td>0.60</td>
<td>0.55</td>
<td>0.51</td>
</tr>
<tr>
<td><em>Cornus alternifolia</em> L. f.</td>
<td>0.15</td>
<td>0.36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Carpinus caroliniana</em> Walt</td>
<td>0.08</td>
<td>0.07</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td><em>Castanea dentata</em> (Marsh.) Borkh.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Quercus velutina</em> Lam.</td>
<td>0</td>
<td>0</td>
<td>0.17</td>
<td>0.22</td>
</tr>
<tr>
<td><em>Carya glabra</em> (Mill.) Sweet</td>
<td>0</td>
<td>0.23</td>
<td>0</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table 2. Shrub species and relative importance values on plots 1-4

<table>
<thead>
<tr>
<th>Shrub species</th>
<th>Plot 1</th>
<th>Plot 2</th>
<th>Plot 3</th>
<th>Plot 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vaccinium sp.</em> L.</td>
<td>0.41</td>
<td>0.86</td>
<td>0.91</td>
<td>0.76</td>
</tr>
<tr>
<td><em>Kalma latifolia</em> L.</td>
<td>0.01</td>
<td>0.07</td>
<td>0.21</td>
<td>0.28</td>
</tr>
<tr>
<td><em>Gaylussaccia baccata</em> (Wang.) K. Koch</td>
<td>0</td>
<td>0</td>
<td>0.40</td>
<td>0.53</td>
</tr>
<tr>
<td><em>Menziesia pilosa</em> (Michx.) Juss</td>
<td>0.12</td>
<td>0.12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Quercus prinus</em> L.</td>
<td>0.23</td>
<td>0.44</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td><em>Sassafras albidum</em> (Nutt.) Nees</td>
<td>0.61</td>
<td>0</td>
<td>0.22</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Nyssa sylvatica</em> Marsh.</td>
<td>0</td>
<td>0.15</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Pinus pungens</em> Lamb.</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Quercus cf. velutina</em> Lam.</td>
<td>0</td>
<td>0</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Rhododendron sp.</em></td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Acer rubrum</em> L.</td>
<td>0.16</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Acer pensylvanicum</em> L.</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Carpinus caroliniana</em> Walt.</td>
<td>0.02</td>
<td>0.11</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Carya glabra</em> (Mill.) Sweet</td>
<td>0.07</td>
<td>0.03</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Castanea dentata</em> (Marsh.) Borkh.</td>
<td>0.04</td>
<td>0.09</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Cornus alternifolia</em> L. f.</td>
<td>0.19</td>
<td>0.08</td>
<td>0</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Table 3. Mean and standard deviations of quadrat stem counts for *Vaccinium* sp. and *Gaylussaccia baccata*.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Vaccinium sp.</th>
<th>Gaylussaccia baccata</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4 (+/- 2.9)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20.0 (+/- 12.3)</td>
<td>11.1 (+/- 10.4)</td>
</tr>
<tr>
<td>3</td>
<td>8.1 (+/- 5.0)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>17.0 (+/- 10.2)</td>
<td>11.0 (+/- 16.8)</td>
</tr>
</tbody>
</table>
Figure 7. Phenogram, tree and shrub data. Constructed from the similarity matrix generated by the UPGMA option of NT-SYS. Character states used in this analysis are tree and shrub importance values. Horizontal scale indicates percent similarity.
Table 4: Plant species separating plots 1 and 3 (northeast facing slopes) from plots 2 and 4 (southwest facing slopes).

<table>
<thead>
<tr>
<th>Species</th>
<th>Higher importance value on plots 1 and 3</th>
<th>Higher importance value on plots 2 and 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus prinus (tree)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Acer rubrum (tree)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Cornus alternifolia (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Menziesia pilosa (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pinus pungens (tree)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Quercus velutina (tree)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pinus pungens (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Quercus sp. (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Gaylussacia baccata (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Kalmia latifolia (shrub)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Species</td>
<td>Higher importance value on plot 1</td>
<td>Higher importance value on plot 3</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Sassafras albidum (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Rhododendron sp. (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Carya glabra (tree)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Nyssa sylvatica (tree)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Nyssa sylvatica (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Carpinus caroliniana (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Castanea dentata (shrub)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Vaccinium sp. (shrub)</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
eigenvector accounts for 31.63% of the variation and also contributes to the separation of plots 1 and 3 from plots 2 and 4. The second eigenvector also separates plot 1 from plot 3. The species which account for these separations are listed in tables 4 and 5.

Discussion

The plots cluster together as expected. The vegetation of the two northeast facing plots is very similar as is the vegetation of the two southwest facing plots. The stands on plots 1 and 2 are at least 33 years younger than those on plots 3 and 4. The similarity of the floral composition of the plots, despite their difference in age, indicates that the plant communities on all plots are in an advanced stage of secondary succession. However, differences in age may account for part of the floristic variation observed between plots on the same side of the spur ridges, particularly between plots 1 and 3. Ectomycorrhizal trees exhibit an ectomycorrhizal fungal succession as they mature (Miller, 1983), so the observed difference in stand age could affect the species composition of the mycoflora.

The dominance of Quercus prinus on the northeast facing plots and Pinus pungens on the southwest facing plots is expected to have a strong affect on the structural component of the fungal communities. Both Pinus and Quercus are
ectomycorrhizal genera. The field mycorrhizal associations of *Pinus pungens* have been studied by Palmer, et al. (in press). To our knowledge, no similar study has been made of the ectomycorrhizal associates of *Quercus prinus*. Since many different host trees have quite different mycorrhizal associates, it is possible that some fungal symbionts will be present on only one side of the spur ridges.

The decomposer element of the fungal community on Brush Mt. is also expected to be affected by the observed differences in the plant community. The composition of the litter layer largely reflects the composition of the plant community which creates it, although wind, sheet floods, and gravity can distort these relationships. Pine detritus is almost completely absent from the litter layer on the successive northeast slopes but comprises a large portion of the layer on the successive southwest slopes; oak leaves predominate in the litter of the northeast slopes and are also present in smaller amounts on the southwest slopes. These differences in the litter composition between the plots are hypothesized to effect the distribution of substrate specific decomposer taxa.

The plots were situated on or near the midpoint of the spur ridge slopes of Brush Mt. The objective was to select sites representative of the forest types on these successive slopes. Species composition changes quite suddenly over the
shoulder of each spur ridge and less suddenly, but significantly, from the crest of Brush Mt. to the valley below (pers. obs.). Quantifying biotic and abiotic gradients on the spur ridges and correlating these to changes in fungal species diversity would be an interesting and informative addition to this work. However, it would be a large undertaking beyond the scope of this work.

III. FUNGAL SURVEY

Methods

The four plots were visited approximately every two weeks during spring and early summer and approximately every week during the main fruiting season. Additional unscheduled visits were made after rainfall events, particularly in the second year. The field season was brought to a close by autumn leaf fall and reduced fruiting.

Collecting was restricted to members of the Agaricales and Boletales. Collections of species not previously noted from the study site were assigned a collection number and deposited in the VPI Mycological Herbarium. Sporocarps of species previously identified from the plots were listed as "occurrences" of that species; distributional data on these occurrences were collected, but voucher specimens were not.

In order to compare plots phytosociologically and to determine species dominance, a species importance index was required.
However, quantifying species importance is problematic in the fungi. Since factors such as density and basal area cannot yet be applied to fungi, species importance values such as those used in the vegetation analysis could not be calculated. Instead, a frequency index was used which scored each fungal species by the percent of subplots on which it occurred. This index, termed "percent quadrat occupancy," was used to estimate species dominance. A phenetic analysis of the plots was performed using fungal species as characters, and percent quadrat occupancy was the character state used in this analysis.

Data collection in the field included genus, species, plot, row and column number, X and Y coordinates, date, fruiting substrate, and collection number. These data were entered into a computer database (DBASE IV). A copy of the database is stored on disc and is available for inspection at the Mycology lab, Department of Biology, VPI. Large sporocarps were wrapped in wax paper; smaller sporocarps were placed in a divided plastic box with a small piece of wet moss to prevent desiccation. Collections and data were brought to the laboratory as soon as possible for fresh descriptions and successful tissue isolation. Whenever possible, tissue isolates were obtained from sporocarps. Tissue from the sporocarp interior of mycorrhizal fungi was aseptically removed and grown on modified Melin-Norkrans
medium (Marx, 1969). The small size of the sporocarps of C. subnuda and M. praeacutus necessitated surface sterilization. A 1 cm stipe segment of C. subnuda or the entire stipe of M. praeacutus was immersed in 30% H₂O₂ for 30 sec., rinsed in sterile distilled water for approximately 10 sec., and plated onto 1.5% malt extract agar. Isolates were checked for the presence of clamp connections and monitored for contaminants. Petri plates of these cultures were kept at 5°C until needed. Agar slants in pure culture were inoculated and deposited in the Virginia Tech Culture Collection.

Small voucher collections of specimens for tissue isolates were obtained from one cluster of sporocarps. This approach was used in order to make every effort to include only sporocarps which originated from the same vegetative thallus. Very often, however, sporocarps were scattered and did not occur in clusters. Enough material remains after making a tissue isolate from a sporocarp of C. subnuda to justify keeping it as a voucher specimen. M. praeacutus is minute and presents a problem since virtually the entire sporocarp is used in the isolation process. In 1990, single sporocarps of M. praeacutus were noted as occurrences, but no tissue isolation of these was attempted. In 1991, however, a higher resolution of the population structure on the plots was desired, and therefore tissue isolation of
singly-occurring sporocarps were obtained, but these lack vouchers.

Results

The four plots were sampled 14 times each year. In 1990, the mean number of days between sampling periods was 9.0 (st. dev. = 5.6); in 1991 it was 7.9 (st. dev. = 6.4). Fifty species of higher fungi were identified including eighteen decomposers and 32 species ectomycorrhizal species. These species and their calculated percent quadrat occupancy is given in tables 6 and 7. Sporocarp fruiting patterns were complex, and ranged from scattered, infrequent occurrences to "fairy ring" associations. The location of sporocarp occurrences of mycorrhizal and minor decomposer species is illustrated in the appendix.

There are some apparent differences in the species richness of the fungi on the plots. For instance, 28 species were observed on plot 1, while only 18 species were observed on plot 3 (table 8).

Nearly all macrofungi were in the Boletales or Agaricales; others were infrequently observed, with the exception of the ascomycete Galiella rufa (Schw.) Nannf. & Korf, which was observed on plots 1 and 3 in the spring of 1990 when the plots were being set up. Although occurrences of G. rufa were not included in the survey, it appeared to
be an important decomposer of small woody detritus. It, too, was observed infrequently in 1991.

Decomposer and mycorrhizal species were found to have different phenology (figs. 8 and 9). Decomposers tended to produce sporocarps after rain events while mycorrhizal fungi are more seasonal in their occurrence. Fruiting was more abundant in 1990 than in 1991, but the pattern of fruiting in both years was similar. On the basis of these observations in 1990, the plots were sampled more often during wet periods and during the peak mycorrhizal fruiting season in 1991. Species/area curves for northeast and southwest slopes are presented in figure 11. In both cases, the number of new mycorrhizal species observed with increased sampling area is high at first and then levels off at about 200 m. However, the number of new decomposer species observed with increased sampling area is relatively low and linear.

Three species stand out as dominant members of the decomposer fungi in this study. Marasmiellus praeacutus and Collybia subnuda fruited abundantly and frequently both years (table 9). The general pattern of sporocarp occurrence illustrates the overall dominance of both taxa (figs. 11-14). Collybia subnuda occurs mostly on the northeast slopes, although it was found 14 times on the southwest slopes. In contrast, M. praeacutus was more
Table 6. Decomposer species occurring on plots 1-4. Numbers indicate calculated percent quadrat occupancy.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plot 1</th>
<th>Plot 3</th>
<th>Plot 2</th>
<th>Plot 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armillaria mellea (Vahl in Fr. Dan. ex Fr.) Karst Hallimasch</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Collybia subnuda (Ellis ex Peck) Gilliam</td>
<td>26.6</td>
<td>35.9</td>
<td>6.3</td>
<td>9.4</td>
</tr>
<tr>
<td>Lentinellus sp.</td>
<td>0</td>
<td>4.7</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Marasmius androsaceus (L. ex Fr.) Fr.</td>
<td>0</td>
<td>0</td>
<td>14.1</td>
<td>12.5</td>
</tr>
<tr>
<td>Marasmius scorodonius (Fr.) Fr.</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marasmiellus opacus (Berk &amp; Curt.) Sing.</td>
<td>25</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Marasmiellus praeactus (Ellis) Halling</td>
<td>6.3</td>
<td>3.1</td>
<td>73.4</td>
<td>62.5</td>
</tr>
<tr>
<td>Mycena haematopus (Fr.) Quel.</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Mycena pura (Pers. ex Fr.) Kummer</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mycena subcaerulea (Pk.) Sacc.</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mycena sp.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mycena vulgaris (Pers. ex Fr.) Quel.</td>
<td>0</td>
<td>0</td>
<td>9.4</td>
<td>0</td>
</tr>
<tr>
<td>Panellus stipticus (Fr.) Karst.</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Tricholomopsis platyphylla (Fr.) Sing.</td>
<td>9.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hygrocybe cf. laeta (Pers. ex Fr.) Karst.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Hygrocybe subminutula Murr.</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Crepidotus nephrodes (Berk. &amp; Curt.) Sacc.</td>
<td>0</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Simocybe centunculus (Fr.) Sing.</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pluteus cervinus (Schaeff. ex Fr.) Kummer</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 7. Mycorrhizal species occurring on plots 1-4. Numbers indicate calculated percent quadrat occupancy.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plot 1</th>
<th>Plot 2</th>
<th>Plot 3</th>
<th>Plot 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amanita brunnescens Atk.</td>
<td>1.6</td>
<td>7.8</td>
<td>42.2</td>
<td>21.9</td>
</tr>
<tr>
<td>A. citrina (Schff.) S. F. Gray</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>A. daucipes (Mont.) Lloyd</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>A. flavoconia (Atk.)</td>
<td>3.1</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. rubescens (Pers. ex Fr.) Gray</td>
<td>3.1</td>
<td>6.3</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>A. virosa (Fr.) Quél.</td>
<td>10.9</td>
<td>4.7</td>
<td>0</td>
<td>9.4</td>
</tr>
<tr>
<td>Boletellus betula (Frost) Gilbert</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>21.8</td>
</tr>
<tr>
<td>Boletus affinis Peck</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Boletus pallidus Frost</td>
<td>23.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phylloporus rhodoxanthus (Schw.) Bres.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Stroblomyces floccopus (Vahl in Fl. Dan. ex Fr.) P. Karst</td>
<td>1.6</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Suillus granulatus (L. ex Fr.) O. Kuntze</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Suillus hirtellus (Peck) Kuntze</td>
<td>0</td>
<td>0</td>
<td>3.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Tylopilus badiceps (Peck) Smith &amp; Thiers</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tylopilus indecisus (Peck) Murrill</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cantharellus cornucopioides Fr.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.1</td>
</tr>
<tr>
<td>Cantharellus minor Peck</td>
<td>0</td>
<td>3.1</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Lactarius chrysorheus Fr.</td>
<td>4.7</td>
<td>4.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lactarius corrugis Peck</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Lactarius piperatus (L. ex Fr.) S.F. Gray</td>
<td>6.3</td>
<td>3.1</td>
<td>0</td>
<td>3.1</td>
</tr>
<tr>
<td>Lactarius subisabellinus Murrill</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lactarius vellereus (Fr.) Fr.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15.6</td>
</tr>
<tr>
<td>Lactarius volemus (Fr.)</td>
<td>1.6</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Russula cf. emetica Fr.</td>
<td>4.7</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Russula laurocerasi Melzer</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Russula variata (Bann. apud Peck)</td>
<td>15.6</td>
<td>9.4</td>
<td>7.8</td>
<td>6.3</td>
</tr>
<tr>
<td>Tricholoma sejunctum (Sow. ex Fr.) Quél.</td>
<td>0</td>
<td>6.3</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Laccaria laccata (Scop. ex Fr.) Bk&amp; Br.</td>
<td>3.1</td>
<td>3.1</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Cortinarius sp.</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Phaeocollybia christinae (Fr.) Heim</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rozites caperata (Pers. ex Fr.) Karst.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Entoloma sp.</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. Number of species of fungi on each plot.

<table>
<thead>
<tr>
<th></th>
<th>Decomposers</th>
<th>Mycorrhizal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot 1</td>
<td>10</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Plot 2</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Plot 3</td>
<td>9</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Plot 4</td>
<td>6</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>
rigidly restricted to the southwest slopes, although it was also found twice on the northeast slopes. Extensive observations revealed that *M. praeacutus* is common in coniferous litter throughout the southwest slopes, but its frequency decreases sharply over the shoulder of the spur ridges. Its distribution is apparently correlated with the sudden change from coniferous to deciduous litter. In general, *C. subnuda* has a more scattered distribution, and the decrease in its frequency over the shoulder of the spur ridges is not as steep as is that of *M. praeacutus*. A third decomposer, tentatively identified as *Mycena* sp., was common on all plots. Detailed analysis of this species was postponed pending its identification or formal description.

The phenograms resulting from the phenetic analyses are included in fig 15. Phenetic analysis by decomposer species gave results similar to the phenetic analysis of vegetation. The northeast facing plots 1 and 3 group together (percent similarity = 64.2), the southwest facing plots 2 and 4 group together (percent similarity = 98.8), but plot 1 and 3 are distant from plots 2 and 4 (percent similarity = 10). The analysis is strongly influenced by single occurrences of species unique to one plot (6 species). These occurrences separate plot 1 and plot 3 from each other and from plots 2 and 4. The analysis is also strongly influenced by the high percent quadrat occupancy of *Marasmiellus praeacutus* and
Collybia subnuda. These two species separate plots 1 and 3 from plots 2 and 4.

The phenetic analysis of mycorrhizal species by percent quadrat occupancy resulted in a phenogram with an r value of 0.779, indicating only a fair representation of the data. The plots did not group as expected. Although the southwest facing plots 2 and 4 group together on the phenogram, they are only 57% similar. Plots 1 and 3 are only 33.8% similar, and plot 3 is grouped closer to plots 2 and 4 than it is to plot 1.

The low similarity in the mycorrhizal species analysis can be attributed to species which occurred only on one plot or had exceptionally large percent occupied quadrat values on a single plot. For instance, six species were unique to plot 1 and six species were unique to plot 4. Boletellus betula had an exceptionally high percent quadrat occupancy value on plot 4, and Amanita brunnescens had an exceptionally high value on plot 2. The effect of these results is to separate the plots in the phenetic analysis. Nevertheless, certain species were characteristic of the successive northeast or southwest facing slopes. These are given in table 10.
Figure 8. Phenology of fungi observed in 1990. A) Mycorrhizal species. B) Decomposer species. Superimposed rain data was obtained from the Department of Geology weather station at Virginia Polytechnic Institute and State University.
Figure 9. Phenology of fungi observed in 1991. A) Mycorrhizal species. B) Decomposer species. Superimposed rain data was obtained from the Department of Geology weather station at Virginia Polytechnic Institute and State University.
Figure 10. Species/area curves. A) Southwest slopes. B) Northeast slopes. Data points were calculated by sequential summation of the area of contiguous quadrats for the X axis and simultaneously summing the newly observed species for the Y axis.
Table 9. Occurrences of M. praecutus and C. subnuda by plot number and year.

<table>
<thead>
<tr>
<th>Year</th>
<th>M. praecutus</th>
<th>C. subnuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot 1</td>
<td>1990</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1991</td>
<td>0</td>
</tr>
<tr>
<td>Plot 2</td>
<td>1990</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>1991</td>
<td>78</td>
</tr>
<tr>
<td>Plot 3</td>
<td>1990</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1991</td>
<td>0</td>
</tr>
<tr>
<td>Plot 4</td>
<td>1990</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>1991</td>
<td>51</td>
</tr>
</tbody>
</table>
Figure 11. *Collybia subnuda* occurrences on plot 1. (*) = no tissue isolate available.  = Tissue isolate available. Arrow points north.
Figure 12. *Collybia subnuda* occurrences on plot 3. (*) = no tissue isolate available. **= Tissue isolate available. Arrow points north.
Figure 13. *Marasmiellus praecutus* occurrences on plot 2. (*) = no tissue isolate available. = Tissue isolate available. Arrow points north.
Figure 14. *Marasmiellus praecutus* occurrences on plot 4. (*) = no tissue isolate available. ● = Tissue isolate available. Arrow points north.
Figure 15. Phenograms, fungal data. Constructed from the similarity matrix generated by the UPGMA option of NT-SYS. Character states are percent quadrat occupancy of A) decomposer species and B) mycorrhizal species. Horizontal axis indicates percent similarity.
Table 10: Mycorrhizal species occurring on both northeast or both southwest facing slopes.

<table>
<thead>
<tr>
<th>Species only on northeast slopes</th>
<th>Species only on southwest slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amanita flavoconia</td>
<td>Suillus granulatus</td>
</tr>
<tr>
<td>Strobilomyces floccopus</td>
<td>Suillus hirtellus</td>
</tr>
<tr>
<td>Lactarius chrysorheus</td>
<td></td>
</tr>
<tr>
<td>Lactarius volemus</td>
<td></td>
</tr>
<tr>
<td>Russula cf. emetica</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Adequate mycosociological studies require at least three years of observation, and preferably more (E. Horak, pers. comm). Bills, et al. (1986) report that even three years of sampling may be insufficient to obtain the overall pattern of fruiting phenology. The two seasons included in this study have almost certainly underrepresented some taxa. However, the pattern of fruiting of decomposer fungi following rain events has allowed identification of the dominant decomposers. A similar conclusion concerning the mycorrhizal taxa must await additional sampling. Until such time, the conclusions concerning mycosociology on the sites must be considered tentative.

For instance, the differences in species richness between mycorrhizal fungi and decomposers on the plots may reflect reality or may be an artifact caused by incomplete sampling. Fungal species may be present in the vegetative state but, due to environmental or genetic reasons, did not form sporocarps during the sampling period. Bills, et al. (1986) have shown that low rainfall during late summer and early autumn affected sporocarp production and the observed phenology. In this study, low rainfall occurred both years during the late summer and fall.

The difference noted between mycorrhizal and decomposer species diversity on the plots may not be a sampling
artifact, however. The litter layer is a more stressful habitat than the soil layer (Hedger, 1984), and stressful environments are more likely to support fewer species than less stressful habitats. While mycorrhizal fungi may also be found in the litter layer, their connection with a plant host may mitigate environmental extremes, making the mycorrhizal habit less stressful then that of the litter decomposer.

The occurrence of a species of Mycena on all plots and almost all quadrats is very interesting. It appears to occur deeper in the litter layer than either M. praecutus or C. subnuda, and may occupy a later seral position in the process of litter decomposition. Its surprising ubiquity contrasts with that of all other decomposer species noted, and it is certainly worthy of additional study.

The occurrence of Galiella rufa on the successive northeast facing slopes on woody debris suggests that this species may compete directly with C. subnuda. Competitive interactions between C. subnuda, G. rufa, and possibly other species may account for the lower percent quadrat occupancy of C. subnuda relative to M. praecutus.

The phenological differences observed between mycorrhizal and decomposer species on the plots have important biological as well as practical implications. The phenological pattern exhibited by the decomposers in this
study has been observed in other small decomposers (Hedger, 1984). The ability of a decomposer to fruit repeatedly throughout late spring and early summer allows it to increase the number of propagules it produces per year, which may be especially significant for species which occupy small volumes of substratum and which produce small sporocarps, such as *M. praecutus*. Practically speaking, the regular fruiting of decomposer species after rainfall events allowed extensive sampling of their populations throughout the growing season.

The species/area curves (fig. 10) emphasize the difference in species richness between mycorrhizal and decomposer fungi. The curves indicate that most of the mycorrhizal species occurring on these slopes have been recorded. In contrast, the linear shape of the decomposer species/area curve is similar to the species/area curves for soil fungi reported by Christensen (1981. See also Christensen, 1989). In her study, repeated isolation of soil fungi within a plant community resulted in increasing numbers of species with no tendency for the species/area curve to level off. These observations indicate that additional area would be required to thoroughly sample the decomposer community on Brush Mt. However, since the purpose of the fungal survey was to identify the dominant decomposers occurring on the spur ridges of Brush Mt., an
increase in the area of the plots was not necessary.

Despite the infrequent occurrence of many of the decomposers on the study sites, two agarics stand out as dominant species. *Collybia subnuda* fruits on hardwood leaf litter, twigs and small sticks. Close observation around sporocarp occurrences indicates that the vegetative thallus also occupies these substrata and does not produce rhizomorphs like many other wood and leaf decomposers (see, for example, Holmer and Stenlid, 1991; Rayner and Boddy, 1986). *Marasmiellus praeacutus* produces sporocarps on a wide range of coniferous as well as deciduous detritus, although it is most commonly found on pine needles. Close observation around sporocarp occurrences indicates that the vegetative thallus is active on the same substratum on which it fruits and, like *C. subnuda*, does not produce rhizomorphs.

The dominance of these fungi on closely adjacent but opposite slopes raises the question of what limits their distribution on the spur ridges. Clearly, each species is capable of colonizing both northeast and southwest facing slopes since they are both found on all plots, yet both are predominantly limited to one side of a spur ridge. Only careful field observation and *in vitro* hypothesis testing will answer this question, but several possibilities exist. Substrate specificity may be an important limiting factor,
since *C. subnuda* appears restricted to hardwood detritus even when it occurs on southwest facing slopes. However, this does not account for the distribution of *M. praeacutus*, which usually occurs on coniferous detritus but occasionally colonizes hardwood litter on northeast as well as southwest slopes. Autecological factors such as temperature, moisture, and pH optima may restrict both species distributions. Syneccological factors which may be important include competitive as well as other interspecific interactions. A good example of an unexpected synergistic effect on a fungus' competitive ability has been demonstrated by Newell (1984a, 1984b), who showed that the relative competitive ability of two litter decomposers (*Mycena galopus* (Pers. ex Fries) Kummer and *Marasmius androsaceus*) was reversed by selective grazing of an arthropod (the collemboalan *Onychiurus latus*). It remains to be seen if similar interactions affect the distribution of *M. praeacutus* or *C. subnuda*.

The phenetic analysis of the plots using percent subplot occupancy of decomposer species (fig. 15A) results in a cluster similar to the vegetation phenetic analysis (fig. 7). This result supports the hypothesis that similar plant communities will harbor similar fungal communities. However, it should be noted that several species of decomposers occurred on only one plot, indicating that a
Phylogenetic analysis may mask the complexity that actually exists on a fine scale within an ecosystem. The phylogenetic analysis by percent subplot occupancy of decomposers was strongly affected by the values for C. subnuda and M. praecutus, once again indicating the dominance of these species on their respective plots.

Phylogenetic analysis of mycorrhizal species did not result in the same clustering of plots as did phylogenetic analyses of the vegetation and the decomposer fungi. Unexpected differences exist among all plots. These differences are caused by the high diversity of mycorrhizal species, the large number of species which occur on a single plot, and the lack of any clearly dominant species on the same facing slopes. Possible explanations for these observations include an age effect, because the forest community on plots 1 and 2 is approximately 40 years younger than the community on plots 3 and 4. Another possibility is that the mycorrhizal species are more unevenly distributed than plant species, and the observed distribution of mycorrhizal fungi is the result of a random sample of a poorly mixed group of mycorrhizal species. A final possibility is that the two year sampling period was too limited to completely sample the mycorrhizal population and the observed distribution underrepresents many species. Very likely these alternatives could be explored with additional seasons of
sampling on the study sites. In order to control for a possible age effect, new study sites with similar-aged forest communities could be examined.

In conclusion, the biotic surveys indicate that the plots on the same slopes of the study site spur ridges are similar to each other and different from the plots on opposite slopes of the spur ridges. The observed similarities and differences are based on tree, shrub, and decomposer importance values. After two years of sampling, decomposer populations appear to consist of a few dominant species and many infrequent species, while mycorrhizal populations appear to consist of many common species as well as many infrequent species. More mycorrhizal species were observed than decomposer species. Three decomposer species are clearly dominant: Mycena sp., which occurred on almost all quadrats of all plots, but was not intensively sampled, Collybia subnuda, which is dominant on northeast facing slopes, and Marasmiellus praeacutus, which is dominant on southwest facing slopes. Both M. praeacutus and C. subnuda are occasionally found on slopes where they are not dominant. These dominant species were chosen for the fungal population surveys.
CHAPTER THREE ~ GENETIC STUDIES

I. INTRODUCTION

Determining the taxon to which an amorphous white mycelium belongs is often difficult. Even when sporocarps are present, defining the limits of the genetic individual has been nearly impossible. This combination of problems has greatly limited advances in our understanding of fungal population biology. The earliest markers used to investigate fungal population biology were mating alleles. Fungi can be either homothallic (self-fertile) or heterothallic, which means that two haploid mycelia of different types must fuse for the completion of the life cycle (Raper, 1966). Heterothallism in the Homobasidiomycetes was first discovered by Kniep in 1915, and independently by Bensaude in 1917 (Raper, 1966). Kniep was the first to elucidate the details of the mating system of Schizophyllum commune Fr., a system which later came to be known as tetrapolar incompatibility. In tetrapolar systems, successful fusion between two haploid mycelia will only occur if they possess different alleles at each of two loci. Bipolar systems are governed by only one locus; successful fusion is governed by differences in alleles at this locus. The tetrapolar mating system is unique to the homobasidiomycetes. Raper (1966) reports that 65% of the homobasidiomycete species studied
are tetrapolar and most of the remaining 35% are bipolar. He further states that the two systems are indiscriminantly distributed among the homobasidiomycetes, but Gordon and Petersen (1991) have found correlations between mating systems and sections of the genus *Menasmius*.

The discovery of the mating systems of the basidiomycetes allowed mating alleles to be used as markers to investigate fungal evolution and population biology. On an interspecific level, tests of interfertility are now commonly used to define biological species (see, for example, Ullrich and Anderson, 1978; Vilgalys, 1982). On an intraspecific level, the distribution of mating alleles has been used to investigate gene flow. For instance, the nonrandom distribution of mating alleles in the gasteromycetes *Cyathus striatus* Pers. and *Crucibulum vulgare* Tulasne is probably due to the short-range spore dispersal inherent in the splash-cup mechanism used by these bird's-nest fungi. In most other homobasidiomycetes investigated, the number of alleles at each locus is on the order of 100, and these appear to be distributed randomly throughout the population (for a review of this subject, see Raper, 1966). However, nonrandom allele distribution in non-gasteroid homobasidiomycetes has been observed in many wood-rotting fungi within the level of a single substratum (Eggertson, 1953; Lindsey and Gilbertson, 1977; Kay and Vilgalys, in
press). It should be noted, however, that studies of mating allele distributions have been strongly biased towards wood-rotting fungi; almost nothing is known about the mating allele distribution of basidiomycetes which exhibit different ecological strategies, such as litter decomposers and ectomycorrhizal fungi. Quantifying mating allele distributions in order to determine the degree of outbreeding within a population and to identify possible constraints to gene flow is, therefore, important.

Historically, there has been controversy over whether or not basidiomycetes exist as genetically discrete individuals. Buller (1931) observed different mating-type alleles carried by sporocarps of Coprinus cinereus (Schaef. ex Fr.) Fr. on the same dung ball. Doubting that genetically distinct mycelia could occupy the same small resource unit and still be able to fruit, he hypothesized that the mycelium consisted of a collective of conspecific but genetically unlike nuclei. This became known as the "unit mycelium" hypothesis and was the accepted doctrine in mycology (Raper, 1966 and Burnett, 1976) until quite recently. Burnett and Partington (1957) observed different mating alleles present in adjacent sporocarps of two basidiomycetes, Piptoporus betulinus (Bull. ex Fr.) Karst and Coriolus versicolor (L. ex Fr.) Quél. They interpreted these observations as support for the unit mycelium
hypothesis. The unit mycelium hypothesis greatly complicates studies of evolution and population biology in the fungi, since evolutionary theory itself relies heavily on the existence of genetically distinct individuals. However, other studies have cast great doubt on the unit mycelium hypothesis.

Childs (1937; 1963) noted that a "line of demarcation" forms when conspecific mycelia from different sources confront each other on agar plates. He reported this for Phaeolus schweinitzii (Fr.) Patt. and Poria weirii Murr, and used the technique to distinguish between what he called "clones," but he did not investigate the basis for the reaction. Adams and Roth (1967) reported "demarcation lines" in paired cultures of Fomes cajanderi Karst., and, by crossing parents, offspring, and sib-composed dikaryons in different combinations, concluded that formation of the line is a "sound basis for distinguishing genetically distinct mycelia." Barrett and Usćuplic (1971) paired a large number of related dikaryotic cultures and also observed the formation of "interaction zones;" however, their results were variable and they hesitated to conclude that absence of interaction between two cultures indicated that they were clones. Coriolus versicolor and Piptoporus betulinus were reinvestigated by Todd and Rayner (1978) and Adams, Todd, and Rayner (1981). Three dimensional analysis of the
heavily colonized substrate (dead trees, in these cases) revealed a mosaic of occupied territories, each piece of the mosaic defined by a dark line similar to the interaction zones noted in culture. Analysis of crosses among cultures isolated from the substrate and their associated sporocarps resulted in patterns of interaction which could not be explained by the unit mycelium hypothesis. They concluded that fungal mycelia exist as a genetically and physiologically discrete entities, which they termed "genets" (Brasier and Rayner, 1987). In subsequent studies by Rayner and colleagues, the pattern of individuality of mycelia has been observed in many species (for a review, see Rayner, 1991). *Coprinus cinereus* (Schaeff. ex Fr.) Fr. has been reinvestigated by May (1988) and has also been found to exist as genetically discrete mycelia on the basis of somatic incompatibility reactions. The genet hypothesis has been supported by simultaneous analysis of somatic incompatibility, mating allele distribution and isozyme analysis in *Heterobasidion annosum* (Fr.) Bref. (Stenlid, 1985).

The acceptance of the individuality of the fungal mycelium has breathed new life into fungal population biology, for it provides a practical method for determining the extent of individual mycelia in situ. Tests of somatic incompatibility have been used now in many wood-rotting
fungi (e.g., Rayner, 1991; Chamuris and Falk, 1987; Lindsey and Gilbertson, 1977; Adaskaveg and Gilbertson, 1987). So far, however, few studies have used tests of somatic incompatibility in fungi with other ecological roles. Only three studies have tested somatic incompatibility in ectomycorrhizal fungi (Fries, 1987; Sen, 1990; Dahlberg and Stenlid, 1990). Only two studies so far have used somatic incompatibility tests to determine the vegetative extent of a litter decomposer (Frankland, 1984; Holmer and Stenlid, 1991).

Since different ecological strategies may result in profoundly different evolutionary strategies, there is clearly a need for population studies of non-wood decomposing basidiomycetes.

The objective of the present study is to investigate the population biology of the dominant decomposers on the study sites. An integrated approach was chosen which includes classic mating compatibility tests and the more recent somatic incompatibility tests in order to determine the distribution of genets as well as the mating alleles they possess.

The objectives of this portion of the study are as follows:

1. To evaluate the use of in vitro tests of somatic incompatibility in the determination of genetically distinct
mycelia of *M. praecutus* and *C. subnuda*.

2. To determine if the genets of *M. praecutus* and *C. subnuda* are perennial or annual.

3. To determine the size of the genets.

4. To determine the mating systems of *M. praecutus* and *C. subnuda*.

5. To investigate the distribution of mating alleles in order to explore the relatedness of different genets and to determine the level of gene flow.

The following hypotheses determined the particular somatic incompatibility tests used.

1. Compatible reactions are more likely to occur between tissue isolates from adjacent sporocarps than from more distant sporocarps.

A "nearest neighbor" approach was used to determine which specific somatic incompatibility crosses to make. When tissue isolates were obtained in a clustered group, crosses were made among all members of the cluster.

2. If tissue isolate "A" is compatible with tissue isolate "B," and "B" is compatible with "C," then "A" will also be compatible with "C."

When an incompatibility reaction occurred among more than two tissue isolates, crosses were made in all possible combinations of these isolates.
3. Two tissue isolates determined to be compatible will also test compatible after one year's storage. Somatic incompatibility tests which resulted in compatible reactions in 1990 were repeated in 1991.

4. Tissue isolates obtained from precisely the same location in alternate years will be compatible. A few tissue isolates were obtained from sporocarps which occurred in nearly the exact same position in consecutive years. These were crossed with each other.

5. A tissue isolate obtained from the area between two compatible tissue isolates will be compatible with each of them. Isolates obtained in 1991 from sporocarps occurring between isolates testing compatible in 1990 were crossed in all possible combinations.

II. METHODS
1. Somatic incompatibility tests

Variability in somatic incompatibility reactions was observed in trial runs on different media types and concentrations. Potato dextrose agar (Difco) prepared at recommended strength optimized the strength of the somatic incompatibility reaction on C. subnuda and M. praeacutus;
this was the standard medium used in all somatic tests in this study. Other media gave a weaker reaction; these included malt extract agar prepared at 15%, 10% and 5%, and Hagem's medium (Hagem, 1910, modified as follows: 4g malt extract, 1g yeast extract, 5g d-glucose, 0.5g NH₄Cl, 0.5g KH₂PO₄, 0.5g MgSO₄·7HO, 0.5ml FeCl₃ (1% aqueous stock solution), 100μL biotin (50μg/mL aqueous stock solution), 100μL thiamine, (1 mg/mL aqueous stock solution), 15g agar, 1000mL H₂O).

Both false positive and false negative results were a concern. The junction line between mycelia inoculated from the same source (i.e., genetically identical inocula which should be somatically compatible) occasionally forms a slightly more dense zone of mycelium than in the surrounding mycelia. After some experience reading the plates, most compatible and incompatible reactions are easily distinguished. However, incompatibility reactions were variable (see results) and often not manifest for several weeks. Controls were therefore included in each incompatibility test to ensure accurate results.

Each test of somatic incompatibility consisted of a 60 X 15 mm plastic petri dish with two 5 mm plugs from one tissue source paired with two similar plugs from another source, each plug approximately 1 cm from all others (fig. 16). The plates were wrapped with parafilm (Amer. Nat. Can),
incubated in the dark at 20°C, and examined once a week for six weeks for the formation of somatic incompatibility reactions.

2. Mating tests

Single spore isolates were obtained by affixing a wedge of pileus (in C. subnuda) or an entire pileus (in M. praeacutus) to the under surface of a petri plate lid with a dab of petroleum jelly. Plates were steeply tilted such that discharged spores fell across the agar surface. After approximately 5 to 10 minutes, the wedge was removed. A variety of agar media were tried, including malt extract agar, potato dextrose agar, modified Melin-Norkrans (MMN; Marx, 1969), and modified Haagem's, but most often MMN was used. Individual colonies were transferred to fresh plates and allowed to grow. Colonies which had clamp connections were eliminated while those which lacked clamp connections were preserved for mating studies. In an extensive review of cultural characters of basidiomycetes by Lamoure (1989), all species in Collybia and Marasmius were reported to have dikaryotic secondary mycelia. Therefore, in this study, unclamped hyphae were assumed to be monokaryotic and clamped hyphae were assumed to be dikaryotic.

In order to determine the type of mating system of M. praeacutus and C. subnuda, single spore isolates from the
same sporocarp were crossed with each other in all possible combinations. Small plugs (approximately 5mm) were cut from a stock culture and placed within 5mm of a plug from another culture on malt extract agar and allowed to grow for two weeks, when they were observed microscopically for the formation of clamp connections. In some cases, subcultures were made from the contact zone and subsequently examined. For those groups of single spore isolates which contained all mating types, "tester sets" were chosen. These were composed of one isolate of each mating type and were used in other mating crosses.

In order to investigate the number and distribution of mating alleles on the plots, intraspecific crosses were made between sets of single spore isolates using the technique described above. Mating allele designations were arbitrarily assigned to one set from each species, and allele designations for other sets were determined relative to it.

3. Di-mon tests

Tester sets from JFM 498 and JFM 217 were chosen for the monokaryotic sources. These were grown on 1.5% malt extract agar in 60 mm X 15 mm petri dishes until the mycelium covered approximately 75% of the agar surface. When possible, monokaryotic sets were crossed with their
parent dikaryon. In addition, a nearest neighbor approach was used to determine which dikaryons to include in tests (fig. 29). The dikaryon was inoculated onto the vacant portion of the agar surface, one dikaryon inoculating each member of a monokaryotic set. The petri plates were wrapped in parafilm, incubated at 25°C in the dark and the monokaryon was periodically examined microscopically for the presence of clamp connections. When clamps were not observed, subcultures were made and also observed for clamp formation.

Trial di-mon crosses gave inconsistent results, and so the following experiment was designed to determine the rate of dikaryotization in a di-mon cross of \textit{C. praeacutus}. Two members of the tester set from JFM 635 were tested against dikaron JFM 593. Growth rate of the dikaryon was determined by inoculating 1 cm diameter plugs of JFM 593 onto 1.5% malt agar, incubating in the dark at 21°C, and measuring colony growth at 3 day intervals. Di-mon crosses were made as described for other di-mon crosses, but larger (100 mm X 15 mm) petri dishes were used. Six, nine, and twenty three days after the dikaryotic and monokaryotic mycelia made contact, small isolates were taken 1.5, 3.0, 4.5 and 6.0 cm from the monokaryotic side of the junction of the two mycelia, subcultured, and examined for the presence of clamp connections.
III. RESULTS

1. Somatic incompatibility tests

_**Marasmius praecutus**_ was recorded 205 times and _**Collybia subnuda**_ 105 times on the study plots. Of these occurrences, 84 (40.9%) tissue isolates were obtained from _**M. praecutus**_, and 38 (36.2%) tissue isolates were obtained from _**C. subnuda**_. More than 232 somatic crosses were made with this material. Both _**M. praecutus**_ and _**C. subnuda**_ gave strong somatic incompatibility reactions when dikaryons were paired on PDA. Somatic incompatibility reactions manifest themselves in three to six weeks. After six weeks, no further changes in the reaction type was observed.

The following four types of reactions were observed (fig. 16):

1. The mycelia intermingled freely without the formation of pigments or an interaction zone. These reactions were scored as compatible.

2. Pigments formed in the agar surrounding the contact zone of the two isolates, but no dense zone of differentiated hyphae occurred at the contact zone (_i_1).

3. A dense zone of slightly upraised hyphae occurred at the interaction zone without the formation of pigments in the adjacent agar (_i_2). Microscopic examination of this type of interaction zone revealed abnormally swollen and branched hyphae which were often highly vacuolated.
(4) A dense zone of slightly upraised hyphae and the formation of pigments in the agar occurred together (i₃).

The last three (I₁, I₂, and i₃) reactions were scored as incompatible. The variability in reaction type is nutritionally sensitive, since pigment formation was not as intense on MEA as it was on PDA. Since all the tests made in 1991 were on PDA, reaction variability was recorded and is summarized in Table 11. For *M. praecutus*, there was no obvious relationship between reaction type and distance between isolate source. For *C. subnuda*, any two isolates which originated more than 5 m from each other in the field gave an i₃ type reaction. I₁ reactions were always from isolates less than 4 m apart, and i₂ reactions were always from isolates less than 5 m apart.

Crosses scored as compatible in 1990 were repeated in 1991 in order to test the *in vitro* stability of the reaction. Four crosses were available (three *M. praecutus* pairs and one *C. subnuda* pair), and all tested compatible again in 1991.

Three pairs of isolates of *M. praecutus* and two pairs of *C. subnuda* were obtained from nearly the same location in alternate years. These were crossed in order to determine if genets of these species of fungi are annual or perennial. One of the *M. praecutus* pairs and one of the *C. subnuda* pairs tested compatible. The rest tested incompatible.
The results of all somatic crosses are summarized in figs. 17 - 20. There are many isolates of both species which are somatically incompatible with all other isolates tested. Somatically compatible isolates occurred in both species and on all plots. The most distant isolates of *M. praecutus* which tested compatible were 4.6 m apart. The most distant compatible *C. subnuda* isolates were 2.3 m apart. Isolates taken from sporocarps occurring between compatible sporocarps always tested compatible with them. The somatic incompatibility test was found to be fully transitive; that is, if isolate "A" was compatible with isolate "B," and "B" was compatible with "C," then "A" was always compatible with "C."

Tissue isolates taken from closely adjacent sporocarps often tested incompatible, indicating a high population density. Assuming that somatically incompatible isolates are genetically unique and randomly distributed, and that an isolate incompatible with an adjacent isolate is not compatible with a more distant isolate, a lower limit to the average population density can be calculated by dividing the plot area by the number of observed somatically incompatible groups. These calculations are summarized in table 12.
Figure 16. Variation in somatic incompatibility reactions. C) Compatible reaction. I$_2$) Dense interaction zone only. I$_3$) Both pigments and interaction zone. I$_1$) Pigments in agar only.
Table 11. Summary of incompatibility reaction types. Numbers indicate the number of times a particular reaction type was observed.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>M. praeacutus</th>
<th>C. subnuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>i₁</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>i₂</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>i₃</td>
<td>17</td>
<td>36</td>
</tr>
</tbody>
</table>

i₁ = pigments in agar only.
i₂ = dense interaction zone only
i₃ = both pigments and interaction zone.
Figure 17. Genet distribution of *Collybia subnuda* on plot 1. (* *) = tissue isolate incompatible with all other isolates tested. Each group of letters stands for a group of isolates which are somatically compatible with each other. Arrow points north.
Figure 18. Genet distribution of *Coryphia subnuda* on plot 3. (*) = tissue isolate incompatible with all other isolates tested. Each group of letters stands for a group of isolates which are somatically compatible with each other. Arrow points north.
Figure 19. Genet distribution of *Marasmiellus praecactus* on plot 2. (*') = tissue isolate incompatible with all other isolates tested. Each group of letters stands for a group of isolates which are somatically compatible with each other. Arrow points north.
Figure 20. Genet distribution of *Marasmiellus praecutus* on plot 4
(*) = tissue isolate incompatible with all other isolates tested. Each group of letters stands for a group of isolates which are somatically compatible with each other. Arrow points north.
Table 12. Observed minimum population density of *M. praecutus* and *C. subnuda*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plot</th>
<th>genets/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. praecutus</em></td>
<td>2</td>
<td>0.121</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.071</td>
</tr>
<tr>
<td><em>C. subnuda</em></td>
<td>1</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.093</td>
</tr>
</tbody>
</table>
Figure 21. Collybia subnuda self-cross results. Single spore isolates from JFM 621. (+) = clamp connections formed. (-) = clamp connections not formed.
Figure 22. *Collybia subnuda* self-cross results. Single spore isolates from JFM 637. (+) = clamp connections formed. (-) = clamp connections not formed
Figure 23. *Marasmiellus praeacutus* self-cross results. A) Single spore isolates from JFM 498. B) Single spore isolates from JFM 635. (+) = clamp connections formed. (−) = clamp connection not formed.
Figure 24. *Marasmiellus praecutus* self-cross results. A) Single spore isolates from JFM 489. B) Single spore isolates from JFM 217. (+) = clamp connections formed. (-) = clamp connection not formed.
Figure 25. Results of *Collybia subnuda* inter-set cross. (+) = clamp connections formed. (-) = clamp connections not formed.
Figure 26. Results of *Marasmiellus praecalcox* inter-set cross between JFM 498 and JFM 635. Dikaryons of these two isolates were somatically compatible. (+) = clamp connections formed. (-) = clamp connections not formed.
### Figure 27. Results of *Marasmiellus praecutus* inter-set cross. (A) JFM 498 X JFM 217. (B) JFM 498 X JFM 489. (+) = clamp connections formed. (-) = clamp connections not formed.
Figure 28. Results of *Marasmiellus praeacutus* inter-set cross. (A) JFM 635 X JFM 489. (B) JFM 217 X JFM 489. (+) = clamp connections formed. (-) = clamp connections not formed.
2. Mating tests

a) Mating system determination

In both species, but especially *C. subnuda*, spores often failed to germinate or, upon germination, ceased growing. Occasionally, colonies of single-spore isolates of *C. subnuda* cannot be successfully transferred. Despite these difficulties, four groups of single spore isolates of *M. praecutus* and two of *C. subnuda* were obtained in sufficient quantity to determine mating system.

No difference in macroscopic appearance between homo- and heterokaryotic mycelia was noted. No microscopic differences were noted aside from the formation of clamp connections, which are common on the septa of heterokaryotic mycelia. The results of self-crosses among single-spore isolates are given in figs. 21 - 24. These results indicate that *M. praecutus* is heterothallic and tetrapolar, and *C. subnuda* is heterothallic and bipolar.

b) Mating allele distributions

The results of intraspecific crosses between tester sets are given in figs. 25 - 28. Although in some basidiomycetes, the presence of common A and B alleles can be determined by the formation of pseudoclamps or colony morphology (Raper, 1966), no such phenomena were observed in either *M. praecutus* or *C. subnuda*. The A and B loci were, therefore, assigned arbitrarily. The mating alleles of the
tester set from JFM 489 were designated $A_1A_2$, $B_1B_2$. Mating allele genotypes of the other tester sets were assigned relative to JFM 489 based on the results of inter-set crosses. The location and mating allele genotypes of the sporocarps from which the single-spore isolates were taken is as follows:

**Marasmiellus praeacutus**

JFM 489 - Poverty Hollow, approximately 10 km from the study site. Mating allele genotype = $A_1A_2/B_1B_2$

JFM 635 and JFM 498 - Plot 2. Tissue isolates from these sporocarps tested somatically compatible with each other. The sporocarps were less than 2 m apart. Mating allele genotype = $A_1A_4/B_3B_5$

JFM 217 - Plot 2, approximately 16 m from JFM 636 and JFM 498. Mating allele genotype = $A_1A_3/B_3B_4$

**Collybia subnuda**

JFM 621 - Plot 1. Mating allele genotype = $A_1A_2$

JFM 637 - Plot 2. Mating allele genotype = $A_1A_2$

**Marasmiellus praeacutus** isolates JFM 635 and JFM 498 share all four mating alleles, as expected since they are somatically compatible. Allele $A_1$ is common to all isolates. Allele $B_3$ is common to JFM 217, JFM 498 and JFM 635; all are isolates from the same plot. The two sets of
monokaryotic isolates from \textit{C. subnuda} have identical mating alleles.

3. Di-mon tests

The results of the dikaryotization rate experiment indicated that dikaryotization in \textit{M. praeacutus} proceeds at approximately 0.2 cm / day (table 13). The growth rate of the dikaryon was 0.27 - 0.3 cm per day. Unexpected or inconsistent results from di-mon crosses were usually resolved upon subculturing. Approximately 25\% of the pairings needed subculturing before clamps were observed.

Since \textit{C. subnuda} has a bipolar mating system, 100\% of the di-mon crosses are expected to result in dikaryotization of the monokaryon (fig. 30). Two monokaryons of each mating type from each set were crossed with their parental dikaryon (table 14). Three of the crosses resulted in dikaryotization of the monokaryon. JFM 621.05 was not dikaryotized. It possesses the same mating allele genotype as JFM 621.07, which was dikaryotized.

Since \textit{M. praeacutus} has a tetrapolar mating system, di-mon crosses will result in 50\% dikaryotization if all mating alleles are shared between the dikaryotic source of monokaryotic sets and the dikaryon (fig. 30). Crosses between monokaryotic sets and their parent dikaryon resulted in the expected 50\% dikaryotization. JFM 498.07 and JFM
498.01 were dikaryotized by the parent dikaryon, indicating that these monokaryons possess the same nuclear mating allele combinations as their parent and that JFM 498.02 and JFM 498.06 possess recombinant mating allele combinations. Crosses between JFM 217 and its monokaryotic tester set indicate that JFM 217.01 and JFM 217.03 possess the parental nuclear mating allele combinations.

75% of the monokaryons will be dikaryotized if three mating alleles are shared, and 100% will be dikaryotized if two or fewer mating alleles are shared. All non-parental dikaryons crossed with tester sets from both JFM 498 and JFM 217 were dikaryotized (table 15).

IV. DISCUSSION

Both M. praeacutus and C. subnuda exhibit strong somatic incompatibility reactions when paired on nutrient agar. The abnormally swollen and branched hyphae of the interaction zone indicates a post-fusion physiological rejection mechanism (Rayner, 1991). The observed incompatibility responses suggest that thalli of these species exist in nature as genetically distinct individuals. The genetic basis for somatic incompatibility in the fungi is poorly understood (Rayner, 1991; Wilson, 1992). Hypothetically, very closely related individuals might be somatically compatible and tests of somatic incompatibility
Figure 29. Sources of di- and mono-karyons used in di-mon tests. Diagram is of plot 2. (A) = Location of the sporocarp which gave the JFM 217 tester set. (1) = dikaryon sources for di-mon crosses with JFM 217. (B) = Location of the sporocarp which gave the JFM 498 tester set. (2) = dikaryon from JFM 635, which was somatically compatible with JFM 498. (3) = other dikaryon sources for di-mon crosses with JFM 498.
Figure 30. Expected results from di-mon self-crosses. A) Bipolar mating system. B) Tetrapolar mating system.
Table 13:  Dikaryotization rates of *M. praecutus* monokaryotic isolates JFM 635.01 and JFM 635.04 by dikaryon JFM 593.

<table>
<thead>
<tr>
<th># Days</th>
<th>Distance (cm)</th>
<th>Monokaryon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JFM 635.01</td>
<td>JFM 635.04</td>
</tr>
<tr>
<td>1.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>3.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+ = clamps formed; – = clamps not formed.
Table 14. Results of *C. subnuda* di-mon selfcross. (+) = clamp connections formed. (-) = clamp connections not formed. Numbers indicate JFM collection number.

<table>
<thead>
<tr>
<th>Dikaryons</th>
<th>Monokaryons</th>
</tr>
</thead>
<tbody>
<tr>
<td>621</td>
<td>621.04¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>637</td>
<td>637.08¹</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

¹ Mating allele genotype = A₁
² Mating allele genotype = A₂

<table>
<thead>
<tr>
<th>Dikaryon</th>
<th>Monokaryotic tester set</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(.07) (.01)</td>
<td>(.02)</td>
<td>(.06)</td>
</tr>
<tr>
<td>498 (self-cross)</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>635</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>569</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>654</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>219</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>496</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>636</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>574</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>633</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>308</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>623</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

1 JFM 635 is somatically compatible with JFM 498.

<table>
<thead>
<tr>
<th>Dikaryon</th>
<th>Monokaryotic tester set</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(.10) (.03)</td>
<td>(.05)</td>
<td>(.06)</td>
</tr>
<tr>
<td>217 (self-cross)</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>624</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>606</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>554</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>558</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>247</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>243</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>353</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>218</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>307</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
would not distinguish between them. However, Todd and Rayner (1978), working with the wood decomposer *Coriolus versicolor*, demonstrated somatic incompatibility between a parent dikaryon and dikaryons synthesized from its monokaryotic products, regardless of mating allele genotype. Holmer and Stenlid (1991), working with the litter decomposer *Marasmius androsaceus*, observed strong somatic incompatibility reactions between synthesized dikaryons paired against their parent progenitors. In addition, evidence from mitochondrial inheritance (May, 1988), and isozyme studies (Stenlid, 1985) indicates that any nuclear genetic variability between isolates results in somatic incompatibility. Additional studies using molecular techniques would be very helpful in determining the minimum genetic distance necessary to elicit an incompatibility reaction.

The correlation between somatic compatibility and mating allele types observed in this study supports the working hypothesis used here that somatically compatible isolates are of the same genetic individual, and somatically incompatible isolates are from different genetic individuals.

The variability in the somatic incompatibility reaction has been observed in most other comparable studies. In some, the variation has been used as an indicator of
relatedness. According to Adams and Roth (1967); Todd and Rayner, (1978) and Rayner and Boddy (1986), more closely related genets produce less pigment formation. Stenlid (1985), working with Heterobasidion annosum (Fr.) Bref., observed pigment formation only between paired dikaryons from different intersterility groups. However, May (1988) did not find a correlation between relatedness and pigment formation in Coprinus cinereus (Schaeff. ex Fr.) Fr. and somatic incompatibility tests. It seems, therefore, that the amount of pigment formed by somatic incompatibility reactions may be species specific. Furthermore, pigment formation in this study was observed to be sensitive to media formulation, since paired dikaryons of both C. subnuda and M. praeacutus showed more pigment formation on potato-dextrose agar than on malt-extract agar. Although the distance separating tissue isolate sources in C. subnuda may be related to the type of incompatibility reaction observed, generalizations are premature until more detailed genetic analysis is performed.

Somatically compatible isolates originating from nearly the same location in consecutive years indicate that genets of both species can be perennial. Since all of the investigations so far indicate that somatic compatibility indicates genetic identity, I conclude that genets of Collybia subnuda and Marasmiellus praeacutus are potentially
perennial.

Full-scale somatic incompatibility testing including isolates obtained in both years was, therefore, justified. Hedger (1984), working with marasmioid tropical litter decomposers, assumed that individuals of these species were "ephemeral." While the substrate supporting these species may be ephemeral, one should not assume that the fungus that decomposes it is also ephemeral, since regular replacement of substrate may allow an individual fungus to last for many years. Such a system appears to be operating with M. praeacutus and C. subnuda. However, incompatibility between tissue isolates taken from nearly the same location in consecutive years can be interpreted several ways. One possibility is that two different but closely adjacent, stationary thalli were sampled. Alternatively, the thalli may be migratory, and one replaced another after the first died or moved away. More thorough sampling of smaller units would help clarify the situation in the two fungi studied. In this study, sporocarp occurrences were measured to the nearest 10 cm. Finer resolution would help define the limit of the individual as well as provide a more complete understanding of age structure.

Until more is understood about the genetic basis of the somatic incompatibility response, care should be taken to ensure that mutations affecting compatibility have not
occurred during storage. In this study, isolates which were somatically compatible in 1990 were also compatible in 1991. These results indicate that the mechanisms which determine compatibility are stable after one year's storage in vitro.

Full scale somatic incompatibility tests indicate that both species are composed of a large number of genetically distinct individuals. In well sampled locations, thallus size may be roughly approximated by observing the limits of compatibility, as defined by incompatible crosses. However, many areas are not well sampled and the absolute limits of individuality cannot be determined. A single tissue isolate incompatible with all neighbors may occupy the entire area between its neighbors or any proportion of it. Only thorough, repeated sampling can resolve thallus size and dynamics.

Nevertheless, some interesting generalizations can be made. For instance, the vegetative size of both species studied is roughly equivalent to those reported for Mycena galopus and Marasmius androsaceus. Frankland (1984) reports the maximum distance that separated compatible isolates of M. galopus to be 2.5 m. Holmer and Stenlid (1991) report the maximum distance that separated compatible isolates of M. androsaceus to be 3.5 m apart. In contrast, cord forming lignicolous species are considerably larger. For instance, Thompson and Rayner (1982) found compatible isolates of
Tricholomopsis platyphylla (Fr.) Sing. as much as 150 m apart. Childs (1963) reported compatible isolates of Poria weirii Murr. taken from 100 feet apart. Anderson et al (1979) reported clones of Armillaria mellea Fr. to extend at least 450 m. A single genet of Armillaria bulbosa (Barla) Romagnesi has been reported to encompass 15 hectares in a northern Michigan hardwood forest (Smith, et al., 1992). Mycorrhizal basidiomycetes may also extend for considerably longer distances than litter decomposers, although the data are extremely limited. Dahlberg and Stenlid (1990) reported compatible isolates of Suillus bovinus (L ex Fr.) O. Kuntze up to 30 m apart.

For several reasons, many of the individuals on the plots were probably not sampled. Some genets may not have produced sporocarps, and some that did may not have been sampled. On the other hand, genet fragmentation can result in the formation of ramets (discontinuous clones). The occurrence of ramets was not investigated in this study, but has been reported in another litter decomposer, Marasmius androsaceus (Holmer and Stenlid, 1991). If the number of undetected ramets is less than the number of unsampled genets, then the number of genets observed represents a conservative lower limit to the actual number of genets on the plot. From this number, an estimate of the lower limit of population density was calculated by dividing the number
of observed genets by plot area. The upper limit to
population density cannot be estimated with the data
available, nor can the area occupied by individual genets.

The limited data concerning the mating systems of
Marasmiellus species indicate that this genus may be
consistently tetrapolar (Gordon and Petersen, 1991), and the
observation of tetrapolarity in Marasmiellus praeacutus is
consistent with this hypothesis. Collybia is also reported
to be strictly tetrapolar (Gordon and Petersen, 1991;
Lamoure, 1989; Halling, pers. comm.). The observation in
this study that C. subnuda has a bipolar mating system is,
therefore, surprising and should be carefully evaluated.

Gordon and Petersen (1991) report that in some species
of Marasmius, spores may adhere to one another during and
after discharge, resulting in multispore isolations which
yield clamped mycelia or mycelia of incompatible mating
genotypes. Desjardin (pers. comm.) reports that "a number
of these marasmioid fungi are amphithallic," resulting in
dikaryotic spores which contain random combinations of
mating alleles. Some of these spores would contain nuclei
of incompatible mating types. In both of these cases,
mating studies would yield inconclusive results.

Inconclusive results, however, were not observed in
this study. All unclamped isolates behaved in a manner
fully consistent with a bipolar mating system. The
possibility that the mating system of \textit{C. subnuda} is actually tetrapolar, and that only two of the four mating types were isolated from JFM 621 is \((0.5)^\text{13}\), or \(1.22 \times 10^{-4}\). The probability drops much lower when the isolates from JFM 637 are taken into account. Nevertheless, the sample size is small, and additional selfcrosses of monokaryons from different sources are necessary before a determination of the mating system of \textit{C. subnuda} can be achieved with absolute confidence.

Dikaryotization rates in \textit{M. praeacutus} are approximately equal to the growth rate of dikaryotic hyphae. Inconsistent results from di-mon crosses are probably attributable to premature observation or a low frequency of clamp formation in the mycelium sampled. Subculturing resolved unexpected or inconsistent results, with one exception. In this case, the unsuccessful dikaryotization of JFM 621.05, the mycelium may have been prematurely sampled.

Di-mon test results indicate that, except for JFM 498 and JFM 635, none of the pairs tested shared three or more mating alleles. This suggests that outcrossing is occurring in \textit{M. praeacutus}.

The identical mating allele genotypes of JFM 498 and JFM 635 are not surprising since the dikaryons isolated from these sources are somatically compatible. However, the
discover of shared mating alleles between somatically incompatible isolates is surprising, especially when compared with the number and distribution of mating alleles observed in wood decomposing basidiomycetes. JFM 217, located approximately 16 m from the compatible isolates JFM 498 and JFM 635, shares an A₁ and a B₂ allele. The A₁ allele was also found in JFM 489, which occurred approximately 10 km away. The two monokaryons isolated from Collybia subnuda have identical mating alleles, which is especially remarkable since the sporocarps from which these monokaryons were isolated occurred on separate plots, at least 100 m apart. In a review of mating systems in the higher fungi, Whitehouse (1949) concluded that "... apart from special instances such as the species of Nidulariales, it is probable that the allelomorphs at the loci for heterothallism are distributed at random through the population of a species, except where geographical or other barriers restrict their spread." In addition, he determined that the number of alleles at a mating locus in Hymenomycetes and Gasteromycetes is on the order of 100, and that these are randomly distributed within populations. Subsequent studies have supported these observations (Eggertson, 1953; Raper, 1966; Lindsey and Gilbertson, 1977; Kay and Vilgalys, in press). In each of these studies, nonrandom distribution of mating alleles was observed only
at the level of the individual substrate; i.e., log or stump. According to Fries, species of Nidulariales, or bird's nest fungi, have nonrandom distribution of mating alleles presumably because of their unusual short-range dispersal of spores in peridioles (in Whitehouse, 1949).

The sample size of monokaryotic tester sets obtained in this study is small, but the results indicate that, in the populations studied of *Maraasmiellus praeacutus* and *Collybia subnuda*, there may be significantly fewer mating alleles than in wood decomposing basidiomycetes, and that these mating alleles may be nonrandomly distributed. Di- mon testing indicates that outcrossing is occurring in *M. praeacutus*. However, the relatively small sporocarps produced by litter decomposing agarics and their low position in the air column may significantly restrict spore dispersal. This could result in a high degree of selfing and the low mating allele diversity observed.

Additional sampling both within and between populations of these fungi is needed to determine the true number and distribution of mating alleles in these populations and the relative importance of selfing and outcrossing. The possible contrast between the population biology and ecology of wood versus litter decomposing basidiomycetes makes such a study particularly intriguing.
CHAPTER FOUR - SUBSTRATE SPECIFICITY TESTS

I. INTRODUCTION

From the results of the fungal survey, the distributions of *Marasmiellus praeacutus* and *Collybia subnuda* are seen to barely overlap. *Marasmiellus praeacutus* is a dominant decomposer on the southwest facing slopes of the spur ridges and is nearly absent from the northeast facing slopes. *Collybia subnuda* occurs on all slopes, but is infrequent on the northeast facing slopes and is dominant on the southwest facing slopes.

The ultimate goal of this project was to examine the distribution of a taxon or taxa of agarics that grow along a cline of selective pressures. The objective is to seek correlations between gene frequencies and this gradient. This would be accomplished by determining their frequencies, distributions, *in vitro* growth characteristics, and, ultimately, allele frequencies. The dominant decomposers identified above are good candidates for such a study. A crucial component of such a study is the identification of nutritional requirements and potentials of the fungi under study (i.e., their substrate specificity) and the relationship between these requirements and the observed distribution of the fungi. A three-pronged approach was taken to achieve this goal. Firstly, field observations of sporocarp occurrences on particular substrata were collected
and tabulated. Secondly, both species were tested for the presence of the laccase enzyme system, a metabolic component required for the breakdown of lignin. Thirdly, decomposition rates of oak leaves and pine needles were determined for both fungi under controlled conditions.

The hypothesis being tested is that the observed distribution of these fungi is due to their substrate specificity. In particular, M. praeacutus more rapidly decomposes pine needles, and C. subnuda more rapidly decomposes oak leaves.

II. METHODS

The substrata upon which sporocarps occurred was recorded in the field. In addition, the location of the mycelium giving rise to the sporocarps was examined to determine if the fruiting substrata also served as nutritional substrata.

Syringaldezine was prepared for the laccase enzyme test according to the method of Harkin, et al. (1974). Gum guaiac solution was prepared by dissolving 0.5 g gum guaiac (Sigma) in 30 mL of 90% EtOH. The reagents were applied directly to mycelium growing on 1.5% malt extract agar in 100 X 15 mm plastic petri plates, and color changes were recorded over the next fifteen minutes.

Needles of Pinus pungens and leaves of Quercus prinus
for the decomposition chamber tests were collected from near
the plots on 1/28/91. They were transported in large
plastic bags to VPI where they were removed from the bag,
dried at 60°C for at least two days, and stored in zip-lock
plastic bags at -10°C until needed.

The variable factors used in the decomposition
experimental design were species of fungus (C. subnuda and
M. praecutus), strain (two different isolates of each
species included as a block to control for intraspecific
variation), substrate (oak leaves and pine needles) and time
(two, three, and four weeks). Four replicates of each
treatment combination were used.

Five g of oak leaves or 10 g of pine needles were
placed in 250 mL glass flasks. These were closed with
aluminum foil and autoclaved for 30 min. When cool, enough
sterile water was added to cover the substrate; excess
standing water was aseptically removed one day later. Each
flask was then inoculated with three 1 cm agar plugs taken
from the edge of a vigorous mycelium. The covered flasks
were incubated at 20°C in the dark for 2, 3 or 4 weeks.
Four flasks of each substrate were prepared like the rest
but otherwise not inoculated; these served as controls.
However, they were not run concurrently with the treatments.

At the end of the specified time period a small amount
of the substrate was removed and plated onto agar to test
for contaminants. The small amount of substrate removed was not accounted for in the subsequent calculations, but was considered too trivial to affect the results. The flasks were then uncovered and placed in a dryer at 60°C for not less than two days. Dry mass of the contents was determined by weighing the flasks before and after the contents were removed and calculating the difference. Percent decrease in mass was then calculated using the following equation:

\[
100 \times \frac{\text{(Original mass of substrate} - \text{final mass of substrate})}{\text{Original mass of substrate}}
\]

In order to preserve normality, percents were arc-sine transformed according to Zar (1974).

III. RESULTS

Table 16 summarizes the substrata upon which sporocarps occurred. In most cases, the vegetative thallus appeared to be decomposing the fruiting substrate.

Both species reacted with syringaldehyde and exhibited a red color change. Treatment with gum guaiac resulted in a blue color change. These results indicate that both species have an active laccase enzyme system and are capable of decomposing lignin (Harkin, et al., 1974).

The results of the decomposition tests are illustrated in figures 31 and 32. The presence of contaminating fungi

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves(^1)</td>
<td>77.9</td>
</tr>
<tr>
<td>Sticks(^2)</td>
<td>22.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus</em> needles</td>
<td>64.0</td>
</tr>
<tr>
<td>Leaves(^1)</td>
<td>16.7</td>
</tr>
<tr>
<td>Sticks(^2)</td>
<td>24.7</td>
</tr>
<tr>
<td>Bark pieces(^3)</td>
<td>13.5</td>
</tr>
<tr>
<td><em>Pinus</em> male strobili</td>
<td>5.1</td>
</tr>
<tr>
<td><em>Pinus</em> female strobili</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Quercus</em> acorn</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Quercus</em> (?) insect gall</td>
<td>0.6</td>
</tr>
</tbody>
</table>

1. Mixed leaves, predominantly *Quercus*.
3. Mixed bark, predominantly *Pinus*.
Figure 31. Percent decrease in mass of *Quercus* leaf substrate.
Figure 32. Percent decrease in mass of Pinus leaf substrate.
Table 17: Comparison of conspecific isolates in mean percent decrease in dry mass.

<table>
<thead>
<tr>
<th>Weeks incubated</th>
<th>Collybia subnuda</th>
<th>Marasmiellus praeacutus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oak</td>
<td>Pine</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>0.19</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Numbers indicate p values calculated from t-tests on arcsine transformed data (see text). The two isolates of Collybia subnuda tested were JFM 256 and JFM 323. The two isolates of Marasmiellus praeacutus tested were JFM 190 and JFM 218.
observed on re-isolation plates, indicated that the
treatment combination C. subnuda isolate JFM 323 incubated
for three weeks on pine needles, was not a pure culture.
Mean decrease in dry mass of this treatment combination,
however, was not significantly different ($p = 0.63$) from the
same treatment combination using C. subnuda isolate JFM 256.
A comparison between the two different strains of a
particular species, at a given treatment combination,
revealed two significant ($p \leq 0.05$) and three near-
significant ($p \leq 0.10$) differences between the strains
(table 17).

After two weeks incubation, all the oak substrate
treatment combinations had a significantly greater decrease
in dry mass than the controls. However, the pine substrate
treatment combinations had a significantly greater decrease
in dry mass than the controls in only one instance; in the
remainder of the pine treatment combinations, the dry mass
decrease of the controls was equal to or significantly
greater than the dry mass decrease of the treatments.

Analysis of variance showed no significant difference
between the two species' ability to decompose either
substrate ($p = 0.136$ on oak leaves, $p = 0.79$ on pine
needles) during three weeks incubation, but at 2 and 4 weeks
incubation, M. praeacutus had a significantly greater
decrease in dry mass of both substrata ($p \leq 0.05$).
IV. DISCUSSION

The significantly greater decrease in dry mass of the controls relative to the treatments indicates that running the controls asynchronously with treatments may have seriously affected meaningful interpretation of the results. The unexpectedly large decrease in dry mass of the controls may have resulted from one or a combination of several different factors, all relating to the drying regime. The high drying temperature (60°C) may have volatilized certain compounds in the pine needles, which would account for the high change in dry mass of the pine controls relative to the oak controls. Inconsistent drying times could have resulted in a variable amount of moisture removed from the substrata. Inconsistent treatment of samples after removal from the dryer could have resulted in a variable amount of atmospheric moisture reabsorbed by the substrata. These observations and considerations make it clear that quantitative predictions are not possible based on these data. Certain qualitative conclusions can be made, however, contingent on further testing.

Both species possess the laccase enzyme system, which is necessary for the decomposition of lignin. Lignin is an important constituent of leaves and needles as well as wood. Lignin composition varies from 16 - 42% of the dry weight of angiosperm leaf litter (Jensen, 1974) and is about 20% of
the dry weight of coniferous needles (Millar, 1974). The laccase enzyme system must certainly confer a competitive advantage to both of these species in vivo.

In vitro, M. praeacutus decomposes both pine and oak leaf litter faster than C. subnuda. This conclusion is supported by direct observation of mycelial growth within the decomposition flasks as well as by statistical analysis of the results. Although C. subnuda is occasionally found on the conifer-dominated southwest slopes, it appears to be utilizing woody detritus and hardwood leaves, and not pine needles, where it is probably outcompeted by M. praeacutus. Growth of M. praeacutus on oak leaves in vitro is vigorous and rapid. Its near absence on northeast slopes is, therefore, not due to an inability to decompose oak leaf litter. Other factors must, therefore, prevent its growth on northeast slopes. These may include a combination of biotic factors, such as direct competition, and abiotic factors, such as temperature, moisture or Ph optima. Elucidating the role of these different factors on the distribution of M. praeacutus and C. subnuda would be an important contribution to understanding their biology.
CHAPTER FIVE - CONCLUSIONS

The primary motivation for this study was to investigate the occurrence of natural selection in the higher fungi. However, without baseline data, such an investigation is impossible. The present study partially fills this vacuum by providing the following: the construction of permanent plots at either end of a steep gradient in biotic and abiotic factors, and the characterization of the plant and fungal communities on these plots. In the course of the survey, two dominant litter decomposing agarics were identified, *Marasmiellus praecutus* and *Collybia subnuda*. Because of their dominance, occurrence on opposite sides of the gradient, and their favorable growth characteristics, these two species presented themselves as excellent candidates for population studies.

The two year survey of the fungi occurring on Brush Mountain resulted in the identification of 50 species of agarics and boletes. Cluster analysis of the plots by mycorrhizal species did not group the plots similar to cluster analysis by plant species. Additional field seasons may change this result. Alternatively, the observed distribution of mycoflora may represent differences in plant communities that are not reflected by vegetation importance
values, such as forest stand age. Cluster analysis by decomposer species grouped the plots similarly to cluster analysis by plant species. This analysis is heavily influenced by the dominance of *Marasmiellus praeacutus* on the southwest facing slopes, and *Collybia subnuda* on the northeast facing slopes.

Investigation of the population biology of these two fungi provide important baseline data for possible future inquiries into the occurrence of natural selection. Somatic incompatibility in both species was demonstrated. Genets are able to survive for more than one year *in vivo*. They are numerous and small; the largest observed distance between two isolates of one genet was 4.5 m, similar to the two other litter decomposers reported (Frankland, 1974; Holmer and Stenlid, 1991). This small size contrasts strongly to the large size of Agaric wood decomposing genets.

Additional investigations into the population biology of *C. subnuda* and *M. praeacutus* included mating system determination and mating allele distributions. *Marasmiellus praeacutus* has a heterothallic, tetrapolar mating system. Only two tester sets of *Collybia subnuda* were obtained, but these indicate that *C. subnuda* has a heterothallic, bipolar mating system. This is the first report of bipolarity in the genus *Collybia*, a conclusion which will be tested.
further when additional tester sets are available.

Although the material available for mating allele distribution was also limited, the results support the working hypothesis that somatically compatible isolates indicate genetic identity. In addition, a surprisingly low number of mating alleles appear to exist in the populations sampled of both fungi. These results also contrast with the population biology of wood decomposing agarics, which have a high number of mating alleles.

Investigations into the substrate requirements of *M. praeacutus* and *C. subnuda* reveal that both species possess the laccase enzyme system, which is necessary for the decomposition of lignin. *Marasmiellus praeacutus* produces sporocarps on leaves, pine needles, and a wide range of small woody detritus, but is mostly limited to the southwest, *Pinus* dominated slopes. *Collybia subnuda* produces sporocarps on leaves and small branches, occurs mostly on the northeast, *Quercus* dominated slopes, but is less restricted in its distribution than *M. praeacutus*. Preliminary *in vitro* decomposition tests indicate that *M. praeacutus* more rapidly decomposes both pine needles and oak leaves than *C. subnuda*. Additional studies are needed, but these results indicate that while the distribution of *C. subnuda* may be due to its specificity for leaf detritus, the observed distribution of *M. praeacutus* is not due to
substrate specificity alone.
BIBLIOGRAPHY


Barret, D. K., and M. Uscuplic. 1971. The field distribution of interacting strains of *Polyporus*


Burnett, J. H., and M. Partington. Spatial distribution of


Vilgalys, R. 1991. Speciation and species concepts in the


Figure 33. Location of the dominant vegetational cover on plot 1. (A) = Quercus prinus. (C) = Acer rubrum. (E) = Cornus alternifolia. (F) = Carpinus caroliniana. Arrow points north.
Figure 34. Location of the dominant vegetational cover on plot 2. 
(A) = *Quercus prinus*. (B) = *Pinus pungens*. (D) = *Nyssa sylvatica*. (F) = *Carpinus caroliniana*. (H) = *Quercus velutina*. Arrow points north.
Figure 35. Location of the dominant vegetational cover on plot 3. (A) = *Quercus prinus*. (C) = *Acer rubrum*. (D) = *Nyssa sylvatica*. (E) = *Cornus alternifolia*. (F) = *Carpinus caroliniana*. (I) = *Carya glabra*. Arrow points north.
Figure 36. Location of the dominant vegetational cover on plot 4. (A) = *Quercus prinus*. (B) = *Pinus pungens*. (C) = *Acer rubrum*. (D) = *Nyssa sylvatica*. (G) = *Castanea dentata*. (H) = *Quercus velutina*. (I) = *Carya glabra*. Arrow points north.
Figure 37. Location of minor decomposers on plot 1. Does not include C. subnuda. or Mycena sp. (O) = Marasmiellus opacus. (P) = Tricholomopsis platyphylla. (C) = Simocybe centunculus. (V) = Pluteus cervinus. (S) = Panellus stipticus. (B) = Mycena subcaerulea. (M) = Marasmiellus praeacutus. (A) = Armillaria mellea. Arrow points north.
Figure 38. Location of minor decomposers on plot 2. Does not include Marasmiellus praeacutus or Mycena sp. (V) = Mycena vulgaris. (L) = Lentinus sp. (C) = Crepidopus omphalodes. (O) = Marasmiellus opacus. (A) = Marasmius androsaceus. (H) = Mycena haematopus. (S) = Panellus stipticus. Arrow points north.
Figure 39. Location of minor decomposers on plot 3. Does not include C. subnuda or Mycena sp. (C) = Crepidotus omphalodes. (H) = Hygrocybe subminutulus. (L) = Lentinus sp. (M) = Mycena pura. (S) = Panellus stipticus. Arrow points north.
Figure 40. Location of minor decomposers on plot 4. Does not include *Marasmiellus praecutus* or *Mycena sp.* (A) = *Armillaria mellea*. (C) = *Collybia subnuda* (H) = *Hygrocybe cf. laeta*. (M) = *Marasmius androsaceus*. Arrow points north.
Figure 42. Mycorrhizal species occurrences on plot 2. (A) = Amanita brunnescens. (B) = A. citrina. (C) = A. rubescens. (H) = Cantharellus minor. (U) = Russula variata. (Q) = Cortinarius sp. (V) = Suillus granulatus. (W) = S. hirtellus. (X) = Tricholoma sejunctum. Arrow points north.
Figure 43. Mycorrhizal species occurrences on plot 3. (A) = Amanita brunnescens. (D) = A. flavoconia. (C) = A. rubescens. (E) = A. virosa. (H) = Cantharellus minor. (I) = Laccaria laccata. (J) = Lactarius chrysorheus. (K) = L. piperatus. (M) = L. volvens. (O) = Russula cf. emetica. (Q) = R. variata. (R) = Strobilomyces confusus. (X) = Tricholoma sejunctum. (Y) = Boletus affinis. (Z) = Entoloma sp. Arrow points north.
VITA

John (Jack) Murphy was born near Woodstock, New York, on October 17, 1960. His mother, Anna Sochor Chapman, encouraged outdoor activities, travel, and biological investigations and instilled in him a love of nature and strong environmental ethic. After graduating from high school in 1978, Jack attended Deep Springs College, a small (24 student) combination ranch/college located on the edge of the Great Basin in eastern California. The combination of intense, interdisciplinary academics, small classes, hard labor, and wilderness experience at Deep Springs set an educational standard to which Jack will always aspire. After Deep Springs, Jack attended Humboldt State University in Arcata, California, where he earned a Bachelor of Arts in Biology. It was at Humboldt that Jack learned about mushrooms from Dr. David Largent and from the northwest coastal Sitka Spruce, Douglas Fir, Coast Redwood forest association. For two years after graduating, Jack worked as a Teacher/Naturalist at Caritas Creek Environmental Education Center in Sebastopol, California, an experience which affirmed his commitment to education, and which ultimately led to his enrollment at Virginia Tech. Some of Jack's favorite non-mycological pastimes include playing ultimate frisbee, rock climbing, and backpacking.