THE BIOLOGY OF *TYPHULA ERYTHROPUS* FR.

by

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We accept this thesis as conforming to the
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ABSTRACT

The life cycle of *Typhula erythropus* was studied in the field and in culture. An attempt was made to correlate observations from both and to explain the behavior of this organism in nature.

The effect of temperature, pH, various carbon:nitrogen ratios, and nutrient concentration on mycelial growth, sclerotium formation, and basidiocarp production was examined. Vegetative growth, including sclerotium formation, was favored by temperatures above 10°C, high strength media, and a pH of 4-5. Basidiocarp production was maximal at lower temperatures, on low strength media, at pH 6, and at a low C/N ratio.

The conditions for sclerotium germination were determined, and sclerotium viability was investigated. Sclerotia produced at 4°C did not germinate until exposed to 15-20°C. These heat activated sclerotia and sclerotia grown to maturity at 15°C germinated rapidly when incubated at 4°C on water agar. A low germination rate resulted when sclerotia were incubated at 15°C.

The growth zone of *T. erythropus* basidiocarps is sub-apical. Expansion of the *Typhula* fructification occurs in the same manner as that in the common mushroom, *Agaricus bisporus*.

A complex relationship between the particular stage of development of the organism and cultural conditions was evident. The interaction of environmental and nutritional factors was especially obvious during sclerotium formation in culture.

*T. erythropus* appears to be facultatively homothallic. In the absence of a compatible mating strain, all nine monokaryotic isolates became dikaryotic. However, when crossed in all
combinations, the monosporic isolates exhibited typical tetrapolar heterothallism. This ambivalence in mating is of rare occurrence in the fungi.

Species identification in the genus *Typhula* is based on such characters as basidiospore measurements, basidiocarp dimensions, coloration, substrate, number of basidiocarps per sclerotium, and sclerotial anatomy. The stability of these features under various conditions has never been tested. Four isolates of *T. erythrops* were examined in culture, and the constancy of certain taxonomically important characteristics was noted. Basidiospore measurements, sclerotial micro-morphology, and the coloration of basidiocarps arising from sclerotia were stable features in all isolates. Other characteristics were variable.
# TABLE OF CONTENTS

| Introduction | 1 |
| Literature Review | 2 |
| Materials and Methods | 7 |
| (1) Isolates Employed | 7 |
| (2) Culture Techniques and Growth Conditions | 7 |
| (3) Assessment of Growth and Reproduction | 9 |
| (4) Methods of Inoculation | 9 |
| (5) Monokaryons and Mating | 10 |
| (6) Staining Techniques | 10 |
| Results and Observations | 11 |
| I. Life Cycle in Nature | 11 |
| II. Basidiospore Germination | 12 |
| III. Growth of Monokaryotic Mycelium | 12 |
| IV. Matings of Monokaryons | 16 |
| A. Intraspecific Pairings | 16 |
| B. Interspecific Pairings | 17 |
| V. Growth of Dikaryotic Mycelium | 19 |
| A. General Characteristics | 19 |
| B. Temperature Effects | 21 |
| C. pH Effects | 21 |
| D. Nitrogen Utilization | 22 |
| E. Vitamin Requirements | 24 |
| VI. Sclerotium Formation | 24 |
| A. Morphology of Mature Sclerotia | 24 |
| B. Development | 25 |
| C. Time of Formation | 27 |
TABLE OF CONTENTS (Cont'd.)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Temperature Effects</td>
<td>28</td>
</tr>
<tr>
<td>E. pH Effects</td>
<td>32</td>
</tr>
<tr>
<td>F. Carbon: Nitrogen Ratio Effects</td>
<td>34</td>
</tr>
<tr>
<td>G. Nutrient Concentration Effects</td>
<td>34</td>
</tr>
<tr>
<td>H. Nitrogen Utilization</td>
<td>37</td>
</tr>
<tr>
<td>I. Wheat Germ Effects (Medium C)</td>
<td>39</td>
</tr>
<tr>
<td>VII. Sclerotium Germination</td>
<td>44</td>
</tr>
<tr>
<td>A. General Observations</td>
<td>44</td>
</tr>
<tr>
<td>B. Temperature Effects</td>
<td>45</td>
</tr>
<tr>
<td>(1) Temperature During Germination</td>
<td>45</td>
</tr>
<tr>
<td>(2) Temperature During Production</td>
<td>46</td>
</tr>
<tr>
<td>(3) Freezing of Sclerotia</td>
<td>48</td>
</tr>
<tr>
<td>C. Other Physical Factors</td>
<td>48</td>
</tr>
<tr>
<td>(1) Drying of Sclerotia</td>
<td>48</td>
</tr>
<tr>
<td>(2) Washing of Sclerotia</td>
<td>48</td>
</tr>
<tr>
<td>(3) Sclerotium Diameter</td>
<td>48</td>
</tr>
<tr>
<td>(4) Illumination of Sclerotia</td>
<td>49</td>
</tr>
<tr>
<td>D. Medium Composition Effects</td>
<td>49</td>
</tr>
<tr>
<td>E. In Situ Germination</td>
<td>50</td>
</tr>
<tr>
<td>VIII. Basidiocarp Formation and Growth</td>
<td>50</td>
</tr>
<tr>
<td>A. General Observations</td>
<td>50</td>
</tr>
<tr>
<td>B. Morphology of the Mature Basidiocarp</td>
<td>51</td>
</tr>
<tr>
<td>C. Basidiocarp Development</td>
<td>52</td>
</tr>
<tr>
<td>D. Expansion of the Basidiocarp</td>
<td>53</td>
</tr>
<tr>
<td>E. Temperature Effects</td>
<td>56</td>
</tr>
<tr>
<td>F. pH Effects</td>
<td>59</td>
</tr>
</tbody>
</table>
### TABLE OF CONTENTS (Cont'd.)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. Photo Effects</td>
<td>59</td>
</tr>
<tr>
<td>H. Gravity Effects</td>
<td>61</td>
</tr>
<tr>
<td>I. Carbon:Nitrogen Ratio Effects</td>
<td>61</td>
</tr>
<tr>
<td>J. Nutrient Concentration Effects</td>
<td>62</td>
</tr>
<tr>
<td>K. Nitrogen Utilization</td>
<td>64</td>
</tr>
<tr>
<td>L. Vitamin Requirements</td>
<td>64</td>
</tr>
<tr>
<td>Discussion</td>
<td>66</td>
</tr>
<tr>
<td>I. General Considerations</td>
<td>66</td>
</tr>
<tr>
<td>II. Mating System and Monokaryons</td>
<td>66</td>
</tr>
<tr>
<td>III. Mycelial Growth, Dikaryotic</td>
<td>69</td>
</tr>
<tr>
<td>IV. Sclerotium Development</td>
<td>70</td>
</tr>
<tr>
<td>V. Sclerotium Germination</td>
<td>74</td>
</tr>
<tr>
<td>VI. Basidiocarp Growth and Development</td>
<td>77</td>
</tr>
<tr>
<td>VII. Interaction of Nutritional/Environmental Factors</td>
<td>82</td>
</tr>
<tr>
<td>VIII. Taxonomic Aspects and Implications</td>
<td>85</td>
</tr>
<tr>
<td>Bibliography</td>
<td>88</td>
</tr>
<tr>
<td>Appendix A: Collection Data</td>
<td></td>
</tr>
<tr>
<td>(1) <em>Typhula erythropus</em></td>
<td>93</td>
</tr>
<tr>
<td>(2) <em>Typhula sclerotioides</em></td>
<td>93</td>
</tr>
<tr>
<td>Appendix B: Culture Media</td>
<td></td>
</tr>
<tr>
<td>(1) Medium A.</td>
<td>94</td>
</tr>
<tr>
<td>(2) Medium B.</td>
<td>95</td>
</tr>
<tr>
<td>(3) Medium C.</td>
<td>95</td>
</tr>
<tr>
<td>(4) Malt-Yeast-Peptone (MYP)</td>
<td>95</td>
</tr>
<tr>
<td>(5) Water Agar</td>
<td>95</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table I: The Effect of Incubation Temperature on Sclerotium Germination................. 47

Table II: Duration of the Stages of Basidiocarp Development........................................ 58
FIGURES AND ILLUSTRATIONS

Plate 1: Life Cycle of *T. erythropus* in Nature .............. 13
Plate 2: Effect of Temperature on Basidiospore Germination 14
Plate 3: Pairing of Monokaryotic Isolates ...................... 18
Plate 4: Growth of the Colony on Medium A ...................... 20
Plate 5: Environmental Effects on Mycelial Growth .............. 23
Plate 6: Relationship of Sclerotium Development and
Mycelial Growth ........................................... 29
Plate 7: Effect of Temperature on Sclerotium Development .... 31
Plate 8: Effect of pH on Sclerotium Development ................ 33
Plate 9: Effect of C/N on Sclerotium Formation .................. 35
Plate 10: Sclerotium Development ................................ 36
Plate 11: Effect of Medium Strength on Sclerotium
Formation ..................................................... 38
Plate 12: Nitogen Sources for Sclerotium Formation ............ 40
Plate 13: Relationship of Sclerotium Development and
Mycelial Growth on a Wheat Germ Medium ..................... 42
Plate 14: Effect of Wheat Germ on Sclerotium Formation .... 43
Plate 15: Effects of Incubation Temperature on the
Rate of Sclerotium Germination ................................ 47
Plate 16: Growth of the Basidiocarp, 1 ......................... 55
Plate 17: Growth of the Basidiocarp, 2 ......................... 57
Plate 18: Effect of Temperature on Basidiocarp
Development .................................................. 58
Plate 19: Effect of pH on Basidiocarp Production ............. 60
Plate 20: Effect of C/N on Basidiocarp Production ............ 63
Plate 21: Basidiocarp Development ................................ 65
Plate 22: Interrelationship of Nutritional and Environmental Factors and Fungal Development ........... 83
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INTRODUCTION

The genus *Typhula* was established by Fries (1821) to include clavarioid species with a cylindrical fertile clavule and a distinct sterile stalk. Fries placed this genus in the order Clavati of the Hymenomycetes. Karsten (1882) emended the genus to retain only those species with basidiocarps arising from sclerotia. Remsberg (1940) and Corner (1950) accepted Karsten's definition and placed all sclerotium-forming clavarioid fungi in *Typhula*. In 1970, Corner proposed the family Clavariadelphaceae to include *Typhula*, *Chaetotyphula*, *Pistillaria*, *Pistillina*, *Myxomycidium*, *Araecoryne*, *Ceratellopsis*, and *Clavarialdelphus*.

The distinction between *Typhula* and *Pistillaria*, a sclerotium-free *Typhula* (Corner, 1950), was questioned by Koske and Perrin (1971) who found sclerotia in the life cycle of *Pistillaria setipes* Grev. Corner (1970) reported a sclerotium present in the "*Typhula*-state" of *P. petasitidis* Imai. In addition, a sclerotium was found in *Pterula scleroticola* Berthier (Berthier, 1967), a species placed in a different family of the clavarioid fungi.

Species identification in *Typhula* is very difficult. Such characteristics as spore size, basidiocarp dimensions, coloration, substrate, and sclerotium anatomy are used as major criteria in distinguishing species (Corner, 1950, 1970). There is evidence that sclerotium anatomy, at least, may be a useless character for this purpose (Røed, 1969 and see literature review). The stability of these morphological features under various environmental conditions has not been tested.

The aim of the present investigation was to determine the
effects of environmental and nutritional factors on the developmental morphology of different phases in the life cycle of *T. erythropus* Fr. It was further hoped that a detailed study of a well defined species might provide insight as to which morphological features are stable and taxonomically useful in this genus.

**LITERATURE REVIEW**

Much of the literature dealing with *Typhula* concerns the symptoms and control of diseases caused by pathogenic species, but some cultural studies have been reported. In 1887, deBary described sclerotium formation in *T. variabilis* Riess and *T. phacorrhiza* Fr. and basidiocarp development in the former. Part of my work concerned the examination of these processes in another species.

Although *T. erythropus* is the most common *Typhula* in Europe (Corner, 1950), it has been studied little in culture. Lehfeldt (1923) germinated spores of this species on malt extract agar. He followed the movement of nuclei during dikaryotization, demonstrated heterothallism, and remarked on its psychrophily. Tasugi (1929, 1935) examined *T. incarnata* Lasch ex Fr. (as *T. graminum* Karst.) and found that UV light was necessary for the development of fertile sporophores. The temperature range for growth was 5–23°C, with an optimum between 8–15°C. Maximum growth occurred at pH 7.

MacDonald (1934) reported no special light requirement for sporophore development in *T. sclerotioides* (Pers.) Fr. which he misidentified as *T. gyrans* Fr. (Corner, 1950). Basidiocarps arose from colonies on potato dextrose agar (PDA) and from sclerotia incubated on moist soil. The fungus grew at tempera-
tutes from 0-25 C. The optimum temperatures for mycelial growth, sclerotium formation, and basidiocarp production were 15-17, 17-20, and 13-15 C, respectively. Details of sclerotium development and basidiocarp anatomy were also presented. MacDonald concluded that sporophore growth was apical and that basidiocarps were positively phototropic and negatively geotropically.

Nuclear migration in *T. trifolii* Rostr., a pathogen of clover, was studied by Noble (1937). She also described the morphological characteristics of the haploid and dikaryotic mycelia, clamp formation, and the development of basidiocarps. Sclerotia germinated on moist soil after an undetermined dormancy period. Heterothallism was demonstrated, but only five monosporic isolates were made, and she could not determine if the species was bipolar or tetrapolar.

A major contribution to our knowledge of *Typhula* was made by Remsberg (1940) when she cultured and studied 14 North American species. She determined the optimum temperature for mycelial growth of each species, noted general cultural characteristics, and verified Tasugi's observations on the UV requirement. She found that light of a wavelength of 2650-3250 Å was necessary for the production of fertile fructifications. All 14 species grew at 0 C, and the optimum temperatures for growth varied from 6-12 C. Stressing sclerotium morphology, she devised a key to the species in her study. Complete descriptions were given for the 14 species, and an historical account of the genus was presented.

The age of the host plant was shown to determine resistance to *Typhula* snow blight by Tomiyama (1952). He also noted the
mutual antagonism between mycelia of *T. incarnata* (as *T. itoana* Imai) and *T. ishikariensis* Imai. In 1955, he cultured both species and reported that spore germination of *T. incarnata* occurred at 0°C and that maximum growth in this species was made at 7-15°C.

Ekstrand (1955) determined the optimum temperature for mycelial growth of *T. borealis* Ekstrand (10°C) and *T. hyperv borea* Ekstrand (5°C). Both species are pathogens of winter cereals; W. C. MacDonald (1961) considers them synonyms of *T. idahoensis* Remsb.

In 1960, Potatosova published three papers on *Typhula* and typhulosis in the USSR. In the first study (1960a) she surface sterilized sclerotia of *T. incarnata* (as *T. itoana*), *T. variabilis*, *T. idahoensis*, and *T. trifolii* and buried them in moist sand. The pots of sand were covered with glass and placed outdoors. Fertile basidiocarps arose from sclerotia of *T. variabilis* and *T. trifolii* when the outside temperature dropped to 8-15°C. Sporophores of *T. incarnata* were produced at 1.4-13.5°C and of *T. idahoensis* at 1.4-4.6°C. Basidiocarps in the latter two species remained sterile. Maximum germination resulted from sclerotia buried at a depth of 5 mm; below 10 mm no germination occurred. Her second paper (1960b) reported the range and optimum temperature for growth of *T. incarnata* (0-18°C; opt. 10°C) and *T. idahoensis* (0-16°C; opt. 10°C). She found that the development of sclerotia was stimulated by diffuse light but inhibited by direct light. A description of the 14 species of *Typhula* found in the USSR was the subject of her third paper (1960c). In it she discussed methods of overwintering, periods of sclerotium formation, specialization, and variability of some
morphological features.

The effect of long wave UV radiation on sclerotium germination and basidiocarp formation was investigated briefly by Leach (1962). Sclerotia of several undetermined Typhulas were irradiated with UV at 12 hours/day for 3-10 days. Sporophores arose from sclerotia incubated on moist sand.

Jackson (1963) reported a snow scald of turf caused by \textit{T. incarnata}. He verified that basidiocarps were autumnal. Basidiospores of this species germinated readily on PDA, and sclerotia were produced after 7-10 days in culture. Lehmann (1965) examined some aspects of the pathogenic nature, growth, and respiration of \textit{T. incarnata}.

The first investigation of the ultrastructure of \textit{Typhula} sclerotia was performed by Scurti and Converso (1965). They described types of cells in the medulla: storage cells with polysaccharides and fats and metabolically active cells. Hyphal cells in the sclerotal rind (the outer layer of the cortex) of this undetermined species were devoid of contents.

Lockhart (1967) examined another undetermined \textit{Typhula} species parasitic on strawberry plants. He cultured it on PDA at -1, 10, and 19 C. At 10 C vigorous mycelial growth ensued, filling a 90 mm petri plate in 11 days. Increasing the concentration of CO$_2$ above 0.05% caused a decrease in the growth rate. In a CO$_2$-free atmosphere, mycelial growth was similarly retarded.

The germination of sclerotia of \textit{T. idahoensis} on sterile soil was studied by Protosenko (1967) and Huber and McKay (1968). Protosenko found that germination occurred in October. He also reported that the fungus grew faster on PDA at 6-8 C than at
18-20 C. Huber and McKay observed that low temperatures (1-2 C) or burial in soil had little effect on sclerotium viability. At higher temperatures (24 C), however, marked reductions in germination ability were noted.

Of particular importance to the taxonomy of the genus was Røed's (1969) interspecific crossing of *T. graminum* and *T. incarnata*. Corner (1970) placed these two species in different subgenera on the basis of their distinctly dissimilar sclerotial morphology. Røed found that they were completely interfertile and relegated the former to synonymy with *T. incarnata*.

Corner (1970) examined specimens of *T. ishikariensis*, a parasitic species on winter cereals in Japan and Scandanavia. He suggested that it might be the same as *T. erythropus*. However, *T. erythropus* has not been reported as a parasite. Also, W. C. MacDonald (1961) considered *T. ishikariensis* to be a synonym of *T. idahoensis*, a well known parasitic species. Unfortunately, neither investigator attempted interspecific matings of the species, and the problem remains unsolved.

The most recent cultural investigation of *Typhula* was that of Dejardin and Ward (1971). They grew *T. incarnata*, *T. trifolii*, and *T. idahoensis* in agar cultures and determined the optimum temperature and pH for growth of each species. Maximum growth occurred at pH 7 on a malt extract-yeast extract-glucose medium at 5-10 C. Poor growth was observed at -5 and 20 C. Sclerotia were produced more abundantly above 10 C. Oxygen uptake by *T. idahoensis* was optimal at 20 C, nearly 15° higher than the temperature for maximum growth. A variety of sugars were utilized as respiratory substrates.
MATERIALS AND METHODS

(1) Isolates Employed

Four isolates of T. erythropus were examined in culture and the detailed investigation was carried out with one of them (isolate T-4). Cultures were obtained from basidiospores released by mature basidiocarps. Collection data are given in Appendix A.

The culture of T. sclerotioides, used for attempted matings with T. erythropus, was obtained from basidiospores shed from mature basidiocarps. Collection data for this species are also given in Appendix A.

(2) Culture Techniques and Growth Conditions

Since a defined medium has not been used for any studies of Typhula except for the respiration work of Dejardin and Ward (1971), comparisons based on earlier cultural data are of questionable value. For this reason, and to ensure reproducible results, T. erythropus was grown on a defined medium.

The standard medium used was a modification of the synthetic basal medium of Lilly and Barnett (1951). Its composition is given in Appendix B. This basal medium was designated Medium A. Medium B was prepared by reducing the asparagine concentration in Medium A from 2.0 g/l to 0.2 g/l. Medium C, used for sclerotium production, contained flakes of wheat germ instead of asparagine in Medium A. Details of its preparation are given in Appendix B. For basidiocarp development studies, Medium A was prepared with 1.0 g/l of vitamin free casein hydrolysate in place of asparagine. Water agar (Appendix B) was routinely used for sclerotium germination investigations.

Stock cultures were maintained on Medium A at 15 C.
For nitrogen utilization studies Medium A was prepared with various compounds in place of asparagine to give the same concentration of N. The asparagine used was a Difco product, and other amino acids were supplied by Nutritional Biochemicals. All other chemicals were of reagent grade.

Vitamin requirement studies were carried out in dichromate cleaned glassware, and conclusions were based on data from three serial transfers.

The ingredients of all media were autoclaved together at 15 lbs. for 15 minutes. Twenty mls (±2) were dispensed into 90 mm glass or sterile plastic petri plates. When the phosphate was autoclaved separately and added to the remainder of the cooled medium, no difference was observed in the growth response of the fungus.

The pH of all media was adjusted to 5.0-5.4 with KOH before autoclaving. In pH-effect studies, the pH of the media was adjusted with 1N KOH or 1N H₂SO₄ and checked with a Radiometer M28 pH meter before autoclaving. Addition of bromcresol green or bromcresol purple to the medium was useful in detecting a pH change following sterilization.

Cultures were grown at 4 C (±1), 10 C (±1), 15 C (±2), and 20 C (±1). Those at 10 and 15 C received 12 hours/day illumination at a distance of 6-12" from Westinghouse cool-white 20W fluorescent lamps. At 20 C, 12 hours/day illumination was provided by overhead fluorescent lamps in a walk-in incubator. Cultures at 4 C were grown in a refrigerator and exposed to diffuse overhead fluorescent light only when the door was open.

A minimum of three plates was used for each experiment, and major experiments were repeated at least twice. For sclerotium
germination studies, 15-50 sclerotia were used, and experiments were repeated at least once.

Investigations of the light requirement for fruiting were performed by wrapping petri plates in aluminum foil and allowing the fungus to develop in darkness. Since light-grown sclerotia and mycelia were able to give rise to atypical basidiocarps in darkness, it was necessary to make serial transfers from dark-grown cultures to eliminate possible carryover of fruiting-inducing substances. The dark transfers were accomplished in a darkened room by the faint light of an alcohol lamp placed four feet away.

Assessment of Growth and Reproduction

Observations on sclerotium formation and germination and basidiocarp production were performed only on structures derived from dikaryotic colonies.

Relative growth rates were determined by measuring the increase in colony diameter. For temperature and pH studies, this method was adequate, but total growth on different nitrogen sources could not be related to linear extension. Rapid and very sparse growth was associated with a nutrient-poor medium. Sclerotium production and fruiting activity were rated by the average number of mature structures formed per petri plate. Dry weights were obtained by filtration and drying of the mycelial mat on pre-weighed filter papers at 50 C for 24 hours.

Methods of Inoculation

It was found that the age, source, and manner of application of the inoculum was very important in the growth response of cultures of T. erythropus. Small agar blocks 3 mm² cut from colonies grown on Medium A at 15 C for 1-2 months were used as
the standard inoculum source. The mycelium growing from these blocks responded to cultural conditions in the same manner as an inoculum of basidiospores. Inocula from colonies grown on Medium A for less than one month or from other media did not produce colonies that behaved predictably. The growth response was usually different from that of a basidiospore suspension inoculum. Growth rate, basidiocarp development, and sclerotium formation were erratic when the standard inoculum was not used.

In an effort to shorten the time required to obtain sporo-phores in culture, fresh plates of Medium A were inoculated with a water suspension of Medium A-grown colonies macerated in a Waring blender. Basidiocarp development was retarded, and most basidiocarps did not mature when this inoculation method was used.

(5) Monokaryons and Matings

Monosporic cultures were obtained by the dilution and plating out of a water suspension of basidiospores. A microscopic examination insured that basidiospores did not stick together when released. The recovered monosporic cultures were all derived from two fructifications from isolate T-4. Monokaryons were microscopically examined for clamp connections and observed for basidiocarps. Both were present in dikaryotic cultures and absent from young monokaryotic cultures.

Monokaryons were crossed in all combinations on malt extract-yeast extract-peptone agar (MYP, Appendix B) at 10 C. Small blocks of mycelium (with agar) to be crossed were set side-by-side on fresh media, and observations were made on the mycelium growing from the zone of contact.

(6) Staining Techniques

For routine observation and photography, hyphae were
stained with KOH-phloxine (Martin, 1952). Nuclei in developing and mature basidiospores were stained with a mixture of aceto-orcein and aceto-carmine (1:1). Furtado's (1970) toluidine blue method was used to stain nuclei in monokaryotic and dikaryotic hyphae.

RESULTS AND OBSERVATIONS
I. Life Cycle in Nature

Observations were made on the life history of *T. erythropus* in nature and in culture. The findings from the field studies are presented in this section.

Sclerotia produce basidiocarps from late September until mid-December in the Vancouver area. Most of my collections of sporophores were made in the moist leaf litter on petioles of *Acer macrophyllum* Pursh. These petioles were detached from leaf blades and had been on the ground approximately one year. The fungus was also found fruiting on old fruits of *Acer*, petioles of *Rubus parviflorus* Nutt., petioles of *Alnus rubra* Bong., and stems of *Urtica lyallii* Wats.

Mature basidiocarps shed basidiospores that germinate and give rise to limited haploid mycelia on suitable substrates (Corner, 1950). The fleshy petioles of *Acer* leaves, dropped a few months prior to basidiospore discharge, are the usual substrate in Vancouver. Following dikaryotization of the mycelia, sclerotia are produced in the cortex of the petioles on either side of the ring of perivascular fibers. Immature sclerotia are present by mid-December, and, by mid-January, mature sclerotia are abundant. Often petiole and blade are still attached when sclerotia first develop.

Mature sclerotia remain dormant throughout the spring and
summer. Sporophores appear in the autumn with the onset of cool, moist conditions. In my collections up to 60% of the basidiocarps and sclerotia on a petiole were located in the basal 1 cm of the petiole. This area of the petiole is the most fleshy.

A diagramatic representation of the life cycle of *T. erythropus* is shown in figure 1 to illustrate the stages of development.

II. Basidiospore Germination

Mature basidiospores of *T. erythropus* are haploid when shed and each contains one nucleus. In my studies, the spores germinated by the formation of one to four germ tubes. Basidiospores shed onto a sterile glass slide at 4 C were placed in a drop of water on Medium A and incubated at 4, 10, 15, and 20 C. The appearance of a germ tube was taken as an indication of germination. Germination occurred most rapidly at 15 C, with 83% of the spores germinating within 60 hours (fig. 2). At 10 C, a lag period slowed the initial rate of germination, but total germination after 60 hours was comparable to that at 15 C. Germination at 4 C was retarded by a 24 hour lag period before 63% germination was achieved at the termination of the experiment. At 20 C, less than 30% of the spores germinated.

III. Growth of Monokaryotic Mycelium

The mycelium of a young colony started from a single basidiospore was monokaryotic and lacked clamp connections. All nine single-spore isolates grew much more slowly in culture than did dikaryotic mycelia. Cultures were grown on Medium A and MYP, a natural, but undefined, medium. After 33 days growth on Medium A at 4, 10, 15, and 20 C, the average colony diameter
Figure 1: Life cycle of *T. erythrops* in nature.
Figure 1
Figure 2: Effect of temperature on basidiospore germination on Medium A. Each point on the graph represents 100 basidiospores counted.
Figure 2

HOURS

% GERMINATION

15°
10°
4°
20°

0 10 20 30 40 50 60

Figure 2
was 18, 22, 7, and 4 mm, respectively. Mycelial growth on MYP was slightly improved, the above colony diameters being reached in 21 days at the same four temperatures.

At 10 C or above, the colonies were dark brown; at 4 C, the cultures were a greyish-white, comparable to dikaryotic cultures. The mycelium of all isolates was submerged, and the colony surfaces were shiny. Haploid cultures grown at 4 C were indistinguishable microscopically from dikaryotic mycelium except that the former lacked clamp connections. Haploid cultures grown at 10 and 15 C showed frequent bulges in the hyphae that were absent from dikaryotic mycelia.

Three to four week old monosporic cultures grown at 4 and 10 C produced 0.2-0.6 mm sporophores in the center of the colony. These sporophores resembled the cone-shaped stage I basidiocarps (see section VIII) of dikaryotic cultures, but bore basidia and basidiospores. The basidia were four-spored, and the spore dimensions were similar to those of spores formed on dikaryotic fructifications. The presence of clamp connections on the hyphae of the basidiocarp and at the colony margin was especially interesting. These clamp connections indicated that homodikaryotization had occurred.

Colonies became dikaryotic at the time of basidiocarp formation, prior to basidiospore production. In contrast, cultures started from a multispore inoculum were dikaryotic in approximately three days. The rate of mycelial extension on the agar surface did not increase following homodikaryotization. Homokaryotic cultures also differed from heterodikaryotic cultures in not forming sclerotia at any temperature.
IV. Matings of Monokaryons

A. Intraspecific Pairings

Several difficulties were encountered in attempting to obtain monosporic cultures and cross them. Since maximum extension of dikaryotic hyphae and maximum basidiospore germination occurred at 15 C, dilution plates of basidiospores were incubated at 15 C. Usually only dikaryotic cultures were recovered from these plates. When no monosporic cultures were visible after 10 days, the plates were discarded. As was later discovered, the growth of haploid mycelia was negligible at this temperature.

By incubating dilution plates at 10 C, I was able to isolate nine monosporic cultures. These were crossed in all combinations, in duplicate, on MYP at 10 C. It had previously been ascertained that the morphological characteristics of the dikaryotic mycelium on MYP were the same as those on Medium A. MYP was used because the monosporic isolates grew faster on it than on Medium A.

Clamp formation and basidiocarp formation could not be taken as indications of successful mating since these structures were present in older monosporic cultures. However, basidiocarps produced by heterokaryotic mycelia were distinguishable from those of homodikaryotic mycelia by their larger size. Both types of basidiocarps were atypically cone-shaped at 10 C. The homokaryotic sporophore was very narrow and lacked the plumpness and length (to 1.5 mm) of heterodikaryotic basidiocarps. In addition, basidiospores shed from heterodikaryotic sporophores soon produced a clamped mycelium where they landed on the agar near the parent colony. Basidiospores from homo-
dikaryotic fructifications did not produce a clamped mycelium for several weeks.

Because of inoculum-induced variation (see below) sclerotium production on Medium A at 4 or 10°C was not indicative of heterodikaryotization. Transfer of a piece of the mycelium growing out from the contact zone to plates of Medium A for standardization was not feasible for two reasons. In some cases no mycelium grew into the agar from two mated strains. Secondly, during the initial inoculum standardization testing of dikaryons, I observed that erratic results were obtained when the inoculum source was less than one month old, grown at a temperature other than 15°C, or grown on a medium other than Medium A. Inocula from such sources gave rise to colonies that failed to produce sclerotia under conditions usually favorable for sclerotium production. Production of basidiocarps from the agar surface was also influenced by the inoculum source.

On the basis of the production of larger, heterodikaryotic basidiocarps, the compatibility reactions of their basidiospores, and the increased growth rate following heterodikaryotization, I grouped the nine isolates into four mating types. Isolates #3, 9, and 10 were compatible only with isolates #4, 5, and 6. Isolate #8 was compatible only with isolates #1 and 2. These mating reactions fit the tetrapolar pattern. The pairing reactions are shown in figure 3.

B. Interspecific Pairings

Monosporic isolates #1, 3, 4, 8, 9, and 10, representing all four mating strains of *T. erythropus*, were crossed with two monosporic cultures of *T. sclerotiorum*. Except for their coloration, fructifications of *T. sclerotiorum* are very similar
Figure 3: Results of pairings of nine monosporic isolates of *T. erythrops*. Heterodikaryon formed = +; heterodikaryon not formed = - . In keeping with standard mycological practice, the mating types are arbitrarily designated AB, Ab, aB, and ab.
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*Figure 3*
to those of *T. erythropus*. No dikaryons were formed in any of the pairings. A definite barrage effect (Burnett, 1968) was apparent in all crosses.

V. Growth of the Dikaryotic Mycelium

A. General Characteristics

The mycelium of all four isolates of *T. erythropus* grew slowly, taking over 30 days to cover a 90 mm petri plate under optimal conditions on all media tested. Most hyphal growth occurred on or below the agar surface; aerial mycelium was limited in young colonies and was restricted to a layer of short, thick-walled hyphal cells in the center of older cultures (figs. 14, 17). The cells that make up this crust were often encrusted with crystals and were similar in appearance to the sheathing hyphae at the base of a basidiocarp.

Clamp connections were common but were not present at all septa. Branches frequently arose from the clamps. Hyphal cells varied in size from 2-10μ x 40-150μ.

After one month on Medium A at 15 C, the colony was a transparent greyish-white becoming light reddish-brown in the center where the crust was forming. The surface was uneven, interrupted by numerous lumps of hyphae and basidiocarps arising directly from the mycelium.

The rate of mycelial extension in a petri plate followed the sigmoid curve (fig. 4). After an initial lag period of approximately seven days, the log phase commenced, with the colony diameter increasing at the rate of 2-3 mm per day. Before the plate became filled the growth rate declined, possibly in response to an autoinhibitor produced during growth. Other isolates grew at rates equivalent to that recorded for
FACING PLATE 4

Figure 4: Growth of a colony of *T. erythrops* on Medium A at 15 C.
isolate T-4 on all media tested.

The reddish-brown crust that formed in the center of the colony and expanded radially was influenced in its development by temperature, pH, and carbon and nitrogen sources. Factors favoring rapid mycelial growth also favored maximum crust formation. When T. erythropus was grown on Medium A containing glucose at 1.0, 3.0, 5.0, 10.0, and 15.0 g/l, maximum growth and most extensive crust development occurred at the highest concentration. Least growth and no crust were found at 1.0 g/l glucose. The amount of asparagine in the medium similarly influenced crust development. Increasing the concentration of asparagine in Medium A from 0.1 to 3.0 g/l resulted in thicker mycelial growth and increased crust formation (fig. 14).

B. **Temperature Effects**

The optimum temperature for mycelial growth, as measured by both dry weight and linear extension, was 15 C. Good growth also occurred at 10 C and fair growth at 4 C. A very low rate of growth was observed at 0 and 20 C. Figure 5 shows the total linear growth of 21 day old colonies incubated at four different temperatures and the dry weight of 11 day old colonies grown at three temperatures. At 20 C, there was a tendency for the slow growing colony to pile up, producing a hard amorphous lump covered with a bloom of white mycelium. A slight crust was produced at 20 C, abundantly at 10 and 15 C, and not at all at 4 C on Medium A.

C. **pH Effects**

The optimum pH for vegetative growth was determined by measuring linear extension of mycelium at 15 C on Medium A. The most vigorous spread of mycelium occurred near pH 5 (fig. 6).
but cultures grew well over a pH range of 4.0-6.0. Above pH 6.0 the growth rate declined. Linear extension could not be determined below pH 4 because the medium did not solidify at lower pH values. The final pH of all culture media was determined after 36 days growth with the indicators bromcresol green, bromcresol purple, phenol red, and pH paper. The final pH of media with initial pH values of 4.0, 5.0, 6.0, 7.0, 7.7, and 8.5 was 6.5, 6.8, 7.6, 7.8, 7.8, and 7.8, respectively.

At 10 C, the optimum pH for linear extension of hyphae shifted to 4.0 (fig. 6). Cultures were grown for 25 days. Media with initial pH values of 4.0, 5.0, 6.0, and 7.0 had a final pH of 7.2, 7.2, 7.5, and 6.8, respectively.

D. Nitrogen Utilization

*T. erythrophus* was able to utilize nitrate, ammonium, amides, and amino acids as nitrogen sources for mycelial growth. A visual estimation of growth was made on the basis of colony diameter and density of the hyphal mat. On medium A, DL-asparagine and casein hydrolysate supported the most vigorous growth. DL-alanine, ammonium sulfate, and ammonium chloride gave good growth, but a rapid pH drop (to 3.8) on media with ammonium salts quickly curtailed growth. Nitrates as calcium and potassium salts induced rapid linear extension, but growth was sparse. L-(-)-phenylalanine, L-proline, and L-tyrosine supported poor growth, and DL-methionine inhibited growth.

The optimum concentration of KNO₃ for hyphal growth was investigated. Cultures were grown at 15 C with KNO₃ added at 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 10.0 g/l. At all concentrations greater than 0.0 g/l the rate of linear extension was nearly equal, and no optimum could be determined.
FACING PLATE 5

Environmental Effects on Mycelial Growth

Figure 5: Effect of temperature on mycelial growth.
Colony diameters were measured after 21 days, dry weights determined after 11 days. All cultures were grown on Medium A.

Figure 6: Effect of pH on mycelial growth at 10 and 15 C. Cultures at 10 C were grown for 25 days, those at 15 C for 36 days. All cultures were grown on Medium A.
Figure 5

Figure 6
E. Vitamin Requirements

A stock solution of the vitamins thiamine, biotin, pyridoxine, and inositol was routinely included in the preparation of Medium A. When these vitamins were omitted, vegetative growth was very poor. Two serial transfers with 1 mm² inoculum plugs were executed to exclude the possibility of carryover from the original inoculum source. When thiamine (100 ug/l) was added to the Medium A lacking the vitamin stock solution, vigorous growth was restored. From these data it appeared that thiamine was required for the growth of *T. erythropus*.

VI. Sclerotium Formation

A. Morphology of Mature Sclerotia

Corner (1950) described in detail the micro-morphology of *T. erythropus* sclerotia. At that time he believed, as did Remsberg (1940), that certain species could be distinguished solely on the basis of their sclerotial morphology. The stability of this character was never established. In my studies, all four isolates of *T. erythropus* retained their characteristic sclerotial features under a variety of nutritional conditions and incubation temperatures. Mature sclerotia from culture were dorsiventrally flattened bodies 0.7-2.9 mm in diameter. The final size of these subspherical sclerotia was dependent upon the medium and the incubation temperature.

The reddish-brown to nearly black coloring was limited to the cuticle. The unistratose cortex and prosenchymatous medulla were white. A cross section of a mature sclerotium is shown in figure 15.

The dark pigment of the cuticle was slightly soluble in
water and insoluble in ethanol, ethyl ether, petroleum ether, chloroform, acetone, 12N HCl, 1N KOH, and a saturated aqueous solution of FeCl₃.

The pH of mature sclerotia was near 7. This was determined by reaction with bromcresol green, bromcresol purple, and phenol red. Sclerotia from culture retained only 36% of their fresh weight after drying at room temperature for one week. On drying, the sclerotia darkened and became slightly wrinkled and hard.

B. Development

Four distinct stages, illustrated in figure 11, were evident during the formation of sclerotia and their growth to maturity. For the purpose of this study, a mature sclerotium was defined as one able to produce a basidiocarp. The knot of hyphae destined to become a sclerotium first became visible in stage Ia. This Ia stage developed on or beneath the agar surface. The stage Ib commenced when the sclerotial primordium was a well-defined 0.8-1.2 mm diameter white sphere. At the top of the Ib sclerotium a reddish-brown spot appeared that slowly and evenly spread over the sclerotium. Stage II began with the first appearance of this spot and continued until the entire sclerotium was darkened. One to several drops of clear liquid frequently were exuded from sclerotia during stages Ib and II. Their occurrence was especially common on sclerotia produced at 4°C. When sclerotia were completely darkened, stage III and maturity were reached.

Stage III sclerotia averaged 1.2 mm in diameter when produced at 15°C. If left on the parent colony, these sclerotia continued to increase in size up to 1.4 mm.
The four stages of development that were observed in cultured sclerotia also appeared to be present during sclerotium formation in the field. Developing sclerotia on petioles incubated in moist chambers at 4 C were observed in stages Ib and III. It was not determined if the darkening process of stage II occurred in as orderly a manner as seen in culture. Sclerotium formation in all four isolates grown in culture followed the stages of development cited for isolate T-4.

The details of development of sclerotia from one or two parent hyphae were described by deBary (1887), MacDonald (1934), and Remsberg (1940) for several species of Typhula. Sclerotium formation in T. erythropus was similar to that in other species, but the medullary hyphae did not become thick-walled in T. erythropus. Sclerotial initiation was of the "strand type" described by Townsend and Willets (1954). Increased branching, growth, and septation from a few intercalary hyphal cells produced the nucleus of the sclerotial primordium (fig. 12). These developments were completed during stage Ia. Growth of the young sclerotium by cell division and expansion built up the diffuse knot of hyphae to a white sphere (stage Ib). The outer layer of cells, constituting the cortex, was not much differentiated from the medullary cells until late in stage Ib. The prosenchymatous nature of the expanding, intertwining cells of the medulla was constant throughout development (fig. 16).

During stage II, cortical cells developed a characteristic jigsaw piece shape. It was not determined whether this distortion was a result of stretching of the cells by internal expansion of the sclerotium, as deBary suggested, or by the differential growth of the cells to accommodate the increased
sclerotium size. Regardless, the resulting jigsaw-like pattern that was formed on the sclerotium surface was a constant feature of all mature sclerotia (fig. 13). This surface pattern is not restricted solely to *T. erythropus*. *T. viburni* Remsb. and *T. phacorrhiza* have similar patterns on their sclerotia. However, the internal anatomy of these species is much different from that of *T. erythropus*.

While the cortical cells became deformed, a dark reddish-brown cuticle was deposited on their outer walls. In cross section this cuticle, with its short perpendicular projections down the radial walls of the cortex, resembled the cuticle of higher plants and its relationship to the epidermis. The dark cuticle was separable from the hyphal walls. Often drops of liquid exuded between the cortex and the cuticle during stage II lifted the cuticle from the sclerotium surface. After the drop evaporated, the cuticle returned to the sclerotium surface. This acellular cuticle sometimes appeared as a membrane-like structure around the drops. When examined microscopically, it carried the jigsaw pattern of the sclerotium surface. Patches of the cuticle were readily scraped from the surface of mature sclerotia.

The morphology of mature sclerotia was very constant under all cultural conditions tested. All isolates and all field collections displayed the same sclerotial morphology.

C. **Time of Formation**

The relationship of sclerotium development to mycelial growth in culture is shown in figure 7. Since sclerotium production was best studied on Medium B at 15°C, this medium was used to correlate the events. Stage Ia sclerotia were first
visible on 12 day old colonies in the log phase of mycelial growth. Sclerotia had advanced to stage Ib four days later, at the end of the log phase. The sclerotia began to darken six days later as the growth rate of the colony declined. Mature sclerotia were present after an additional four days. The average time from initiation to maturity was 14 days. This was the shortest average time recorded. The interval was longer on other defined media and at different incubation temperatures. The above description applies only to the 6-10 sclerotia that mature first on a colony. The interval between each stage was relatively constant, but the initiation time for all sclerotia that formed on a plate was variable. Sclerotia first were formed close to the point of inoculation in the oldest hyphae of the colony. As the colony grew, young sclerotia appeared behind the margin. Mature sclerotia appeared first in the center of the colony, forming a gradient of development to the immature sclerotia near the margin. New sclerotia were initiated and matured after the log phase of growth had passed.

D. Temperature Effects

The effect of temperature on sclerotium formation was related to the carbon:nitrogen ratio and the pH of the medium. At 15 C, few sclerotia were formed on Medium A. These sclerotia were not well-defined and were difficult to separate from the colony. Cultures grown at 4 and 10 C on this medium produced up to 80 distinct sclerotia per plate. Medium B supported the production of 15-30 well-defined sclerotia per plate at 15 C; at 4 and 10 C, the average number of sclerotia per plate was 5-15. It was discovered later that up to 200 sclerotia per plate could be obtained on Medium B at 15 C if the inoculum was
Figure 7: Sclerotium development and its relationship to mycelial growth. Cultures were grown on Medium B at 15 C.
taken from a culture grown on Medium A prepared with \( \text{KNO}_3 \) in place of asparagine.

A two to three week old culture, grown at 15 C on Medium A, produced numerous typical sclerotia when incubated at 0, 4, or 10 C. Return of the culture with stage III sclerotia to 15 C often was accompanied by the incorporation of the sclerotia into the spreading crust.

Medium C supported excellent sclerotium production at 15 C, with up to 90 sclerotia formed per plate. At lower temperatures fewer sclerotia were produced.

In addition to affecting the number of sclerotia formed per plate, the incubation temperature also influenced the time of sclerotium initiation and development. Mature sclerotia were formed earliest on Medium B, of the defined media employed. The time between stages and the time to initiation at 4, 10, and 15 C are presented in figure 8. Data from the 4 and 10 C experiments are from observations of sclerotium formation on Medium A. The 15 C data are taken from cultures on Medium B. Sclerotium production on Medium A was greater than that on Medium B at these temperatures. When cultures were grown on Medium A, the time required to produce sclerotia at 4 C was similar to that observed in nature.

Stage II sclerotia appeared first in cultures grown at 10 C. However, the darkening process was retarded at this temperature, and stage III sclerotia were formed earliest at 15 C. The time of initiation and the duration of maturation were considerably greater at 4 C than at 10 or 15 C. I later discovered that mature sclerotia could be produced in 35 days instead of 67 days at 4 C when the asparagine in Medium A was
FACING PLATE 7

Figure 8: The effect of temperature on the rate of sclerotium development. Cultures grown at 4 and 10 °C on Medium A; cultures at 15 °C on Medium B.
Figure 8
replaced by 1.0 g/l of casein hydrolysate.

At 10 and 15 C, sclerotia were well separated from each other. At 4 C, they tended to coalesce, frequently forming compound sclerotia.

The incubation temperature also affected the diameter of mature sclerotia. The average diameter of 30 sclerotia produced at 15 C was 1.2 mm. At 4 C, sclerotia from Medium A were more than twice as large, averaging 2.6 mm. The diameter of sclerotia produced at 10 C was intermediate, averaging 1.5 mm.

Isolates T-182 and T-29 produced numerous sclerotia on Medium A at 4 and 10 C. Isolate T-18 formed less than 15 sclerotia per plate on this medium at 4 C.

E. pH effects

The effect of pH on sclerotium formation was examined at 10 and 15 C. The results of these experiments are presented in figure 9. Cultures were grown at 15 C on Medium A adjusted to pH values of 4.0, 5.0, 5.5, 6.0, 7.0, 7.7, and 8.5. After 26 days, the average number of sclerotia per plate was determined. The final pH of the respective media was 6.5, 6.5, 7.6, 7.6, 7.8, 7.8, and 7.8. Sclerotia were produced at pH 4.0 and 5.0, but these were not well-defined from the colony surface. No sclerotia were produced at other pH levels. The experiment was terminated after 26 days because the spreading crust threatened to grow over the sclerotia.

Cultures at 10 C were grown on Medium A adjusted to pH values of 4.0, 5.0, 6.0, and 7.0. After 32 days, the average number of sclerotia per plate was determined, and the final pH was measured. The final pH for these media was 7.2, 7.2, 7.5, and 6.8, respectively. Maximal sclerotium production occurred
Figure 9: The effect of initial pH on sclerotium formation. Cultures were grown at 10 and 15°C on Medium A.
MATURE SCLEROTIA PER PLATE

Figure 9

Graph showing Mature Sclerotia per Plate at pH levels 4, 5, 6, and 7 with temperature conditions of -10° and -15°.
at pH 4.0, with 32 sclerotia per plate; at pH 5.0, 6.0, and 7.0 the average number of sclerotia per plate was 16, 7, and 6.

F. Carbon:Nitrogen (C/N) Ratio Effects

The optimum C/N ratio for sclerotium production was influenced markedly by the temperature of incubation. For the 15°C investigation, media with C/N ratios from 3.8:1 to 190:1 (g C/g N) were prepared by adjusting the amount of asparagine in Medium A. The pH of all media was adjusted to 5.0, and cultures were grown for 60 days. The final pH of media with C/N ratios of 47:1 to 190:1 was 5.2. Media with ratios of 19:1 and 9.5:1 had a final pH of 7.0 and 8.4, respectively. Maximum sclerotium production occurred at a C/N ratio of 95:1, with an average of 16 sclerotia formed per plate (fig. 10). Media with ratios of 140:1, 63:1, 47:1, and 38:1 supported approximately one half the production achieved on the 95:1 medium. No sclerotia were formed at 15°C when the C/N ratio was lower than 19:1.

In contrast to the 15°C results, excellent sclerotium production occurred at 4 and 10°C on a medium with a C/N ratio of 9.5:1 (Medium A). Cultures were grown on media with C/N ratios of 9.5:1 and 95:1. At 4 and 10°C, the average number of sclerotia per plate was 80 at 9.5:1 and 5-15 at 95:1 (Medium B).

G. Nutrient Concentration Effects

The concentration of glucose and asparagine in Medium A was adjusted to make media of 1/2 to 5X regular strength. A C/N ratio of 95:1 was maintained in all media, and the pH was set at 4.0, optimal for sclerotium formation. Cultures were grown at 15°C for 31 days; the results are shown in figure 18. The average number of sclerotia per plate was proportional to the strength of the medium. No stage III sclerotia were produced on
FACING PLATE 9

Figure 10: The effect of various C/N ratios on sclerotium formation at 15 C. The media were prepared by adjusting the concentration of asparagine while the glucose concentration was maintained at 10 g/l.
Figure 10

SCLEROTIA PER PLATE

C/N

Figure 10
Sclerotium Development in T. erythropus

Figure 11: Stages of sclerotium development. The darkness of the Ia and Ib sclerotia is a result of the accumulation of bromcresol green. X2.

Figure 12: Young stage Ia sclerotium. X300.

Figure 13: Surface of the mature sclerotium. X400.

Figure 14: The effect of several C/N ratios on sclerotium production at 15°C. The numbers on the plates indicate the g/l of asparagine in Medium A. The values 0.2, 0.5, 1.0, 2.0, and 5.0 are equivalent to C/N ratios of 95:1, 38:1, 19:1, 9.5:1, and 3.8:1 respectively. Sclerotia at 1.0 g/l are not well defined from the colony. Note crust development.

Figure 15: X.S. of a mature sclerotium. The dark edge is the outer surface of the sclerotium. Note the homogeneous nature of the cells of the medulla. The cortex, of one cell thickness, is not evident at this magnification. X70.

Figure 16: Cells of the medulla of the sclerotium. Note their prosenchymatous nature. X1000.

Figure 17: Crusting hyphae from the surface of the colony. Note thickened, dark walls. X400.
the 1/2 strength medium (5.0 g/l glucose, 0.1 g/l asparagine). On the plates of full, 1.5X, 2X, and 5X strength media, the average number of sclerotia produced was 2, 4, 21, and 24, respectively.

The radial spread of the colony paralleled the increase in sclerotium production. Maximum mycelial growth occurred on the 5X strength medium. The correlation of colony diameter at 31 days to nutrient concentration is also illustrated in figure 18.

H. Nitrogen Utilization

Sclerotia of *T. erythropus* were produced on media made with a variety of nitrogen sources. However, it was difficult to determine which compounds were utilizable for this process since sclerotia could be formed on simple water agar inoculated with basidiospores.

Nitrogen sources were added to medium A in place of asparagine. The influence of temperature on the optimum C/N ratio for sclerotium production has already been noted. For this reason, media for use at 10 C were prepared with a C/N ratio of 9.5:1 and media for use at 15 C with a 95:1 ratio. The pH of all media was adjusted to 5.0, and cultures were grown for 35 days. The results are shown in figure 19. At 15 C, asparagine supported the maximum production of sclerotia. Consistently less than 10 sclerotia per plate were formed on media made with DL-alanine, DL-methionine, casein hydrolysate, *KNO₃*, and *Ca(NO₃)₂*. The appearance of sclerotia on these poorly utilized substrates was also much delayed. Mature sclerotia were not present on methionine media until the cultures were over 110 days old. No sclerotia were produced on L-tyrosine or *NH₄Cl*. 

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Figure 18: The effect of medium strength on sclerotium formation and radial growth of a colony. Standard strength Medium A contains 10 g/l glucose and 2.0 g/l asparagine. Cultures were grown at 15 °C for 31 days.
Figure 18
The pH drop associated with ammonium utilization possibly was the reason for the failure of this source to support sclerotium production.

At 10 °C, optimum sclerotium production was made on asparagine with 80 sclerotia formed per plate. Casein hydrolysate was the next most productive, with 45 per plate. Alanine and \( \text{KNO}_3 \) media supported less than 10 sclerotia per plate. L-proline and L-(-)-phenylalanine inhibited sclerotium formation.

I. Wheat Germ Effects (Medium C)

Sclerotium production on Medium C was exceptionally vigorous in 15 °C grown cultures. Mature sclerotia were formed rapidly and in large numbers. The relationship of mycelial growth to sclerotium development is shown in figure 20. The log phase of mycelial growth on Medium C commenced approximately four days after inoculation when cultures were grown at 15 °C. Stage Ia sclerotia appeared in 9 day old colonies. The other stages followed at nearly the same intervals as those recorded on Medium B at 15 °C. The relationship of sclerotium formation to the growth rate of the colony also was the same as that on Medium B. Mature sclerotia were present in 22 day old cultures. This was four days less than the time required on Medium B. The lag period of mycelial growth also was four days less than on Medium B. At 4 and 10 °C, sclerotium production on Medium C was inferior in numbers of sclerotia and maturation rate to that observed on Medium A at these temperatures. All isolates produced numerous sclerotia when cultured on Medium C at 15 °C.

To determine the optimum concentration of wheat germ in Medium C for sclerotium production, petri plates containing 1.0 to 25.0 g/l of wheat germ were prepared. Cultures were
Figure 19: Nitrogen sources for sclerotium formation. The rating system refers to the number of stage III sclerotia per plate.
<table>
<thead>
<tr>
<th></th>
<th>15°</th>
<th></th>
<th>10°</th>
</tr>
</thead>
<tbody>
<tr>
<td>ala</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>asn</td>
<td>++</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>met</td>
<td>+</td>
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<tr>
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<td>-</td>
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<tr>
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</tr>
<tr>
<td>CaN</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>KN</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amCl</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sclerotia per plate

+++ = >40
++  = 10-39
+   = 1-9
-   = 0

Figure 19
incubated at 15 C. After 27 days, an average of 85 mature sclerotia were present in cultures grown with 3.5-4.5 g/1 of wheat germ. In comparison, only 6-10 mature sclerotia were produced in the same period of time on Medium A. As figure 21 shows, wheat germ concentrations from 2.5-9.0 g/1 supported the formation of large numbers of mature sclerotia, and these sclerotia were well-defined and easily lifted from the colony. At concentrations below 2.5 g/1 the sclerotia remained a light reddish-brown and, though viable, were difficult to separate from the cartilaginous colony. On media with wheat germ concentrations greater than 7.5 g/1, the dark mycelial crust soon developed and overgrew many mature sclerotia, rendering them inseparable from the colony.

The active fraction of the wheat germ was soluble in cold water but not soluble in ethanol, ethyl ether, or chloroform. A quantity of wheat germ flakes was extracted with 100 ml of water agitated by a magnetic stirrer for 20 minutes at 20 C. The water solution was filtered and then used to make up 100 ml of Medium A lacking asparagine. Sclerotium production was compared with that on media prepared with the extract of 2.0, 4.0, and 6.0 g/1 of wheat germ. Cultures were grown at 15 C for 47 days. The results are shown in figure 22. Maximum sclerotium production occurred when the extract of 6.0 g/1 was used.

The water extract of wheat germ did not support the production of as many sclerotia as did wheat germ flakes. This water soluble factor(s) was found to be dialyzable and heat stable. No further attempt was made to characterize the active fraction of wheat germ.
Figure 20: The relationship of sclerotium development to mycelial growth on Medium C at 15°C.
Figure 21: The effect of wheat germ concentration on the production of sclerotia. Cultures were grown at 15 C for 27 days.

Figure 22: Sclerotium production on media prepared with three concentrations of a water extract of wheat germ. Cultures were grown for 47 days at 15 C.
Figure 21

Figure 22
VII. **Sclerotium Germination**

A. **General Observations**

In nature, and in the laboratory, the sclerotia of *T. erythropus* germinate to produce the diminutive clavate basidiocarps characteristic of the species. Sclerotia also germinate in culture by producing dikaryotic hyphae when set on fresh media. Germination of the first type was the subject of this part of the investigation.

Mature sclerotia from culture germinated well in petri plates of water agar. On Medium A mycelial growth from sclerotia placed on the agar surface was much more vigorous than that on water agar, and no basidiocarps arose from sclerotia incubated on the nutrient medium.

The temperature during production of sclerotia and the temperature during germination were the most important factors affecting sclerotium germination. The viability of sclerotia was also influenced by the medium upon which they were produced.

The young basidiocarp originates in the medulla of the sclerotium. The hyphal details of germination in *T. erythropus* are comparable to those reported for *T. sclerotioides* by MacDonald (1934). The sporophore primordium in the medulla pushes outward, eventually causing the outer layer of cells (the cortex in the *T. erythropus* sclerotium) to rupture and allowing the young basidiocarp to grow out through a jagged hole. One to eight sporophores were produced from germinating *T. erythropus* sclerotia.

Corner (1950) suggested that the location of the basidiocarp may be determined during sclerotium formation. Observations on germinating sclerotia of *T. erythropus* confirmed this
opinion. The rounded upper side of a sclerotium when attached to the parent colony for convenience was designated the dorsal surface, and the flattened lower side was the ventral surface. Sclerotia removed from culture and inverted on water agar first produced basidiocarps from the original dorsal side of the sclerotium. These sometimes grew downward into the agar. When sclerotia were not inverted before germination, sporophores arose from the dorsal surface. This apparent predetermination effect was observed in sclerotia produced at 4, 10, and 15°C.

No dormancy period prior to germination was required for sclerotia from cultures grown at 10 and 15°C. However, sclerotia grown to maturity at 4°C rarely germinated if not given a 15-20°C treatment for 10-14 days before germination (see next sub-section).

B. Temperature Effects

(1) Temperature During Germination

Viable sclerotia from cultures grown on Medium C at 15°C were placed on water agar and incubated at 4, 10, 15, and 20°C. Some sclerotia were also given a five day treatment at 4°C prior to incubation at 15°C on water agar. As shown in Table I, 95-100% of the sclerotia incubated at 4 and 10°C germinated. At 15°C, less than 20% produced basidiocarps, but after the 4°C treatment the germination rate was 70%. At 0 and 20°C, no germination occurred.

The rate of germination (time to appearance of young basidiocarps) was also affected by the temperature at germination. Sclerotia incubated at 4°C germinated quickly, with maximum germination reached in 14-16 days (fig. 23). Up to 35 days were required for maximum germination at 15°C following
the 4 C pretreatment. This 35 day figure includes the five days of pretreatment.

Although sclerotia germinated at 15 C, basidiocarps developing from sclerotia at this temperature often remained sterile. At 10 and 4 C, sporophores matured quickly and frequently grew to more than 20 mm in length. Temperature effects on basidiocarp development are treated in another section of this paper.

Both percentage and rate of germination were also influenced by the medium on which the sclerotia were grown. This is discussed in another part of this section.

Sclerotia collected on petioles in the field in January and May did not germinate when incubated in clear plastic moist chambers at 4, 10, 15, or 15 C after one week at 4 C. Sclerotia from August collections germinated readily at 4 C and 15 C following 4 C pretreatment. No germination occurred at 15 C without pretreatment.

(2) Temperature During Production

Sclerotia grown at 10 and 15 C on suitable media were viable after reaching stage III. Up to 100% germination was obtained from these sclerotia when incubated at 4 C. However, sclerotia produced on Medium A at 4 C germinated only after a pretreatment at 15-20 C for 10-14 days. Following treatment, germination occurred at 4 C on water agar. The 15-20 C treatment was effective on sclerotia still attached to the parent colony or separated from it on water agar plates. The treatment was ineffective when sclerotia were desiccated. A germination rate of 85% was recorded from treated sclerotia grown at 4 C.

The addition of casein hydrolysate or wheat germ did not
Figure 23: The effect of incubation temperatures on the rate of sclerotium germination. Sclerotia were produced on Medium C at 15°C.
Table I: The effect of Incubation Temperature on sclerotium germination.

<table>
<thead>
<tr>
<th>GERM. TEMP</th>
<th>PRE-TREAT</th>
<th>% GERM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>15°C</td>
<td>-</td>
<td>0-20</td>
</tr>
<tr>
<td>15°C</td>
<td>4°C-7days</td>
<td>70</td>
</tr>
<tr>
<td>10°C</td>
<td>-</td>
<td>95-100</td>
</tr>
<tr>
<td>4°C</td>
<td>-</td>
<td>95-100</td>
</tr>
</tbody>
</table>

Figure 23
alleviate the necessity of the heat treatment.

Since it was possible that a light stimulus was involved in the production of viable sclerotia, the 20 C treatment was performed in darkness. Treated and untreated sclerotia in the 4 C incubator were thus exposed to the same amount of light.

Sclerotia grown to stage II at 4 C and matured at 15 C on the parent colony were identical in their viability to sclerotia produced at 15 C.

(3) Freezing of Sclerotia

Viable sclerotia were frozen at -5 C for two weeks on water agar plates. They were then placed on fresh plates and incubated at 15 C. A germination rate of 40% was observed, 30% less than the 4 C pretreated control.

C. Other Physical Factors

(1) Drying of Sclerotia

Viable sclerotia were air dried at 20 C for 25 days and then placed on water agar at 4 C for germination. After 30 days, 55% of the sclerotia had produced basidiocarps. A 95% germination rate occurred in the control.

(2) Washing of Sclerotia

Sclerotia grown on Medium C at 15 C were removed from the colony and washed in flasks of sterile water at 20 C. To simulate the leaching effect of rain, the flasks were set on a reciprocating shaker for 48 hours. The washed sclerotia were pretreated at 4 C for 5 days and incubated on water agar at 15 C. After 36 days, 71% germination had occurred, the same as the unwashed control.

(3) Sclerotium Diameter

It is known that the larger sclerotia of *Claviceps purpurea*
(Fr.) Tul. are more viable than smaller ones (Cooke and Mitchell, 1966). Sclerotia from several cultures of *T. erythropus* were measured and a record kept of their germination to determine if a similar correlation existed. Of the sclerotia with diameters greater than 0.7 mm, no correlation between size and viability was evident. Sclerotia measuring less than 0.7 mm were seldom observed to germinate. In nature, and in culture, the average sclerotium diameter was greater than 1.0 mm.

(4) **Illumination of Sclerotia**

Sclerotia grown to maturity in the light were able to germinate when placed on water agar and incubated in darkness. Although the sporophores that arose in darkness usually were palmately branched and sterile, some typical fertile basidiocarps were produced under these conditions.

**D. Medium Composition Effects**

Sclerotia were formed in culture on a variety of media at several different temperatures, but sclerotia from different sources were not equally viable. Sclerotia grown on Medium B at 10 C germinated poorly (less than 5%) in contrast to a high rate (95%) for 15 C grown sclerotia from Media A, B, and C. Sclerotia from Medium C made with the water extract of wheat germ were equal in viability to those produced on Medium C. Varying the C/N ratio from 19:1 to 190:1 in Medium A (refer to section VI) did not alter the germination rate of sclerotia produced at 15 C.

The sclerotia that formed on water agar were small (0.5-0.8 mm) and had a low germination rate. Whether their low viability was an effect of size or of nutrients was not determined.
E. **In Situ Germination**

Stage III sclerotia that had developed on a colony on Medium A or B did not usually germinate in place. If lifted from the colony and relocated on the same plate, no germination resulted. Placement of a square of dialysis tubing between the sclerotium and the parent colony did not encourage germination. Exceptions to this failure to germinate in situ were noted in several month old colonies grown on Media A and C. Fertile basidiocarps arose from sclerotia in cultures on Medium C when incubated a 4 C after maturation at 15 C. Seven month old cultures on Medium A grown at 4 C produced basidiocarps from sclerotia; the basidiocarps lacked heads and were sterile.

Sclerotia whose development was arrested between stage II and III often gave rise to fertile sporophores in culture. This occurred most commonly on media with a low C/N ratio in cultures incubated at 10 and 15 C.

VIII. **Basidiocarp Formation and Growth**

A. **General Observations**

In nature, basidiocarps of *T. erythropus* are known to arise only from sclerotia (Corner, 1950). No sclerotium-free specimens were found in any of the collections from British Columbia. However, in culture, basidiocarps were produced from sclerotia, directly from the colony surface, and from the edges of the inoculum plug. Often a medium that did not support fruiting from the agar surface induced some sporophore development from the inoculum block. When plugs were inverted prior to inoculation, the appearance of basidiocarps was delayed, and the first fructification arose from the lower side, nearest the agar. This orientation effect appeared to be a result of
the much denser mycelial mat formed on the upper surface of a culture.

Small sporophores arose from inoculum plugs on water agar plates and sometimes developed in the agar away from the inoculation point. On more favorable media basidiocarps arose directly from the agar as well as from the inoculum plug. Occasionally sporophores were produced from sclerotium-like lumps in the colony. These ill-defined lumps were reported to be abortive sclerotia in cultures of *T. sclerotioides* (MacDonald, 1934).

Since it was possible to obtain basidiocarps without sclerotia, the effects of nutritional and environmental factors on basidiocarp formation could be investigated free from the interference of this physiologically active structure.

B. Morphology of the Mature Basidiocarp

Corner (1950) has described the structural details of mature *T. erythropus* basidiocarps. Except for the possibility of *T. ishikariensis*, the many recombinations and synonyms associated with other *Typhulas* are not found in *T. erythropus*. The characteristic coloring of the head and stalk, the general spore dimensions, and the structure of the sclerotium make this an easily identifiable species. Lehfeldt (1923) found this species convenient to use because of the certainty of its identification. I chose it for the same reason.

Basidiocarps were typically unbranched, but in culture branching was occasionally present. These branched fructifications were always found on plates in conditions unfavorable for development (i.e. on water agar or in darkness). No branched basidiocarps were seen in field collections.
C. **Basidiocarp Development**

Sporophores followed the same pattern of development regardless of their source of initiation (i.e. from sclerotia, the agar surface, or inoculum plugs). For convenience, their development has been divided into four stages, I, II, III, and IV. At stage I the basidiocarp primordium first becomes visible as a pure white cone, 0.1 x 0.2 mm. This body elongates with little increase in diameter (0.2 mm), assuming a gradually tapered candle-like shape up to 1.2 mm long. Stage II commences when the base of the young basidiocarp begins to darken (fig. 31). This darkening is caused by the sheathing cells of the stalk becoming thick-walled and pigmented, much like the crust that forms on the surface of older colonies. The tapered stage II sporophore elongates for 36-48 hours when the upper 0.4 mm suddenly swells to delimit a well-defined clavate head (fig. 35). The appearance of the head indicates the onset of stage III. No hymenium is present at this time. Sporophore elongation and head development continue. When the head is approximately 1 mm long, it becomes fertile, and stage IV commences (figs. 30, 32, 34). Sporulation continues for 10-15 days under favorable conditions of temperature and humidity. During this interval the stage IV basidiocarp continues to elongate (see later for details).

Sporophores past maturity took on a watery appearance and collapsed. The head of a fallen sporocarp gave rise to a dikaryotic mycelium or was converted to a sclerotium on the agar surface.

The zone of encrusting hyphae that first appeared in stage II spread up the stalk a few mm behind the apex of the growing
basidiocarp. Mature sporophores are typified by this reddish-brown, horny stalk (Corner, 1950). Every basidiocarp that arose from a sclerotium possessed this characteristic. However, not all basidiocarps that were produced from the mycelium had the reddish-brown stalk. The temperature of incubation and the medium composition affected pigmentation. These effects are detailed in a later sub-section.

The length of basidiocarps during different stages of development was relatively constant. Stage I carpophores measured from 0.1 to 1.2 mm, stage II from 1.2 to 3.2 mm, stage III from 3.2 to 6.3 mm, and stage IV from 6.3 to 25 mm. The head of a 25 mm basidiocarp averaged 4 mm in length.

The duration of each stage and, thus, the time from initiation to maturity were variable, influenced by temperature and medium composition. These effects are presented in detail in another sub-section.

Sporocarps arising from the mycelial mat first appeared 13-19 days after inoculation of Medium A and incubation at 15°C. These first-formed basidiocarps were situated close to the inoculum plug. As the colonies grew, basidiocarps were produced further from the inoculation point (fig. 33). A 60 mm diameter colony included up to 60 fructifications within a 30 mm diameter inner circle.

All isolates produced sporophores from cultures in an identical manner to that described for isolate T-4.

D. Expansion of the Basidiocarp

The growth of the stage I primordium into a mature basidiocarp was brought about almost entirely by the inflation of
pre-existing cells. After early stage I, few new cells formed at the apex of the sporophore. This was demonstrated by the application of marks to sporophores and by microscopic examination. Basidiocarps in various stages of development from sclerotia and inoculum plugs were marked with spots of black ink or spores of *Equisetum* along their length. The distance between the spots was measured daily with an ocular micrometer, and the zone of expansion was determined. Unmarked basidiocarps developed at the same rate as those that were marked. The growth of a sporophore from 1 mm to 8.3 mm is shown in figure 24. Only the upper part of a basidiocarp expanded, leaving behind a non-expanding trunk. The tip (0.2-0.4 mm) of a stage I or II sporophore elongated little, contributing only to the head. When the head had been delimited as a 0.4 mm long body, its growth was independent of the elongation of the stalk.

The growth zone of a stage II basidiocarp was confined to a 1-2 mm region approximately 0.4 mm behind the apex. In stage III and IV sporocarps, the growth zone was located just below the head. Relative to the base, the growth zone traveled up the stalk. The percentage of a basidiocarp elongating was inversely proportional to the total length of the fructification. Figure 25 illustrates this relationship. Approximately 70% of the length of a young 2 mm sporophore was elongating. The hyphal cells at the base (the remaining 30%) had ceased inflation. Less than 40% of the length of a 7 mm basidiocarp was in the process of elongation.

The rate of extension of the sporophore increased as the structure developed. At 10 C, a stage IV basidiocarp elongated at an average rate of 3.2 mm/24 hours; a stage I grew at just
FACING PLATE 16

Figure 24: The growth of a basidiocarp from 1 mm to 8.3 mm. Note that the lower part ceases elongation before the upper part. The growth zone is located just below the head.
Figure 24

LENGTH
mm

STAGE

Figure 24
1.1 mm/24 hours. The elongation rates of the four stages are illustrated in figure 25.

E. Temperature Effects

Basidiocarps arose from inoculum plugs placed on Medium A after 2-3 days incubation at 4, 10, and 15 C. Since very vigorous sporophore production occurred when the plugs were placed on Medium A with 1.0 g/l of casein hydrolysate in place of asparagine, this medium was used to study the effects of temperature. Basidiocarps were formed rapidly at 4 and 10 C; stage IV sporophores were observed on the inoculation plug 6-8 days after inoculation. At 15 C, development was retarded, requiring up to 10 days for basidiocarps to reach stage IV. The effect of temperature on the duration of each stage is shown in Table II and figure 27. These data represent average values. Not all stage I primordia completed their development. The delay in reaching stage IV at 15 C was seen in the transition from stage II to III. Head formation frequently was delayed or totally inhibited at 15 C.

The production of basidiocarps from the surface of the colony was limited to a much narrower temperature range than that for their development from inoculation plugs. On Medium A and Medium A with casein hydrolysate, sporophores were rarely produced at 0, 4, or 10 C. Basidiocarps that did develop at these temperatures usually were very short and were composed of of a sub-globose head on a tiny stalk. These 1-2 mm high fertile sporophores lacked pigmentation. At 15 C, however, typical basidiocarps developed freely from the mycelial mat of cultures grown on both media. Sixty or more sporophores commonly were produced in a single petri plate. These mature fructifications
FACING PLATE 17

Growth of the Basidiocarp - 2

Figure 25: The relationship of the stage of development to the rate of extension of basidiocarps produced at 10 C.

Figure 26: The correlation of basidiocarp length to the length of the growth zone in elongating basidiocarps.
Figure 27: The effect of temperature on the rate of basidiocarp development. The rate for sporophores produced at 4 °C was very similar to that at 10 °C.
Table II: Duration of the stages of Basidio-carp development

<table>
<thead>
<tr>
<th>TEMP</th>
<th>0-I</th>
<th>I-II</th>
<th>II-III</th>
<th>III-IV</th>
<th>0-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°</td>
<td>2-3</td>
<td>1.5</td>
<td>3</td>
<td>2</td>
<td>8.5-9.5</td>
</tr>
<tr>
<td>10°</td>
<td>2-3</td>
<td>1.5-2</td>
<td>1.5-2</td>
<td>1-1.5</td>
<td>6-8</td>
</tr>
<tr>
<td>4°</td>
<td>2-3</td>
<td>2-3</td>
<td>1.5</td>
<td>1-2</td>
<td>6.5-8.5</td>
</tr>
</tbody>
</table>

Figure 27
were indistinguishable from those arising from sclerotia.

Basidiocarps developing from the colony surface at 18 C lacked a hymenium. At 20 C, no sporophores were produced.

The effect of temperature on basidiocarps produced from sclerotia was similar to that observed on sporophores arising from inoculation plugs. At 4 and 10 C, large, typical carpophores were produced from viable sclerotia. However, basidiocarps arising from sclerotia at 15 C frequently grew to just 5-12 mm in length and remained at stage II. When these stage II sporophores were incubated at 4 or 10 C they resumed development and reached stage IV. No differences were noted between basidiocarps produced from sclerotia from culture or from the field.

F. pH Effects

The effect of pH on basidiocarp formation was determined by counting the number of mature sporophores produced in cultures on Medium A at 15 C. The final pH was also determined at this time. Cultures were grown for 31 days at pH 4.0, 5.0, 5.5, 6.0, 7.0, 7.7, and 8.5. The final pH of these media was 6.5, 6.5, 7.6, 7.6, 7.8, 7.8, and 7.8, respectively. Maximum fruiting occurred where the initial pH was 5.5-6.0, with 15-18 basidiocarps formed per plate (fig. 28). At an initial pH of 7.0, fruiting was less vigorous and was negligible on media with and initial pH of 4.0, 5.0, 7.7, or 8.5.

G. Photo Effects

Basidiocarps of T. erythropus are positively phototropic in all stages of development. Apices of growing sporophores grow toward the light source. This growth response took place in the zone of elongation, not at the apex. When the position
Figure 28: The effect of initial pH on basidiocarp production on Medium A at 15 C. Cultures were grown for 31 days.
of the light source was shifted, the growth of the sporophore toward the new source was evident in less than nine hours (fig. 35). Blue and white light were the most effective in eliciting the response. Light passing through red cellophane did not evoke a growth adjustment.

Exposure to light appeared to be necessary for the development of mature basidiocarps. Dark-grown cultures started from light-grown mycelia frequently produced branched stage II basidiocarps or did not produce any sporophores. Sclerotia grown to maturity in the light sometimes gave rise to fertile basidiocarps when germinated in darkness, but most basidiocarps did not develop beyond stage II. Stage I fructifications were formed on the colony surface in dark-grown cultures started from dark-grown mycelia. No fertile sporophores were produced in these cultures. A control of dark-grown mycelium inoculated onto a plate and grown in the light produced fertile sporophores.

H. Gravity Effects

Observations of basidiocarps produced in darkness in inverted petri plates showed no signs of a positive or negative geotropic response. Sporocarps arose at various angles away from the agar surface. Since light has been implicated in the geotropic response of some basidiomycetes (Taber, 1966), light-grown cultures were incubated at several angles. The orientation of the basidiocarps was noted. No cultured sporophores of this species were geotropic.

I. Carbon:Nitrogen (C/N) Ratio Effects

The asparagine concentration in Medium A was adjusted to give C/N ratios (g C/g N) of 3.8:1 to 190:1. The pH of all media was 5.5, and cultures were incubated at 15 C. After 26
days the number of stage IV basidiocarps was counted, and the final pH of the media was determined. The results are illustrated in figure 29. Basidiocarp production was highest at a C/N ratio of 19:1, with an average of 40 stage IV basidiocarps per plate. Fruiting vigor was one half this value at C/N ratios of 9.5:1 and 38:1. At 62:1 only 15 sporophores were present, and at greater than 95:1 no fruiting occurred. The final pH of media with C/N of 19:1 to 190:1 was 5.5. Media with ratios of 9.5:1, 4.7:1, and 3.8:1 had final pH values of 5.8, 7.0, and 8.0, respectively.

There was a good correlation between the degree of fruiting and basidiocarp length. The medium that yielded the maximum number of basidiocarps also produced the longest ones as well. Basidiocarps produced at a C/N ratio of 19:1 were long and slender, whereas those at 3.8:1 were short and stout.

Other isolates grown on C/N ratios of 95:1 and 9.5:1 responded in the same manner as isolate T-4.

J. Nutrient Concentration Effects

The concentration of glucose and asparagine in Medium A was adjusted to make media of 1/10 to 2X regular strength. A C/N ratio of 9.5:1 was maintained in all media. Cultures were grown at 15 C for 26 days. Maximum basidiocarp production from the colony surface occurred on the 1/10 strength medium (1.0 g/l glucose, 0.2 g/l asparagine). On this medium 60 mature sporophores were formed per plate. Fruiting decreased as the nutrient concentration increased. The average number of mature basidiocarps on 1/2, full, 1.5X, and 2X strength media was 40, 25, 20, and 2 per plate, respectively. Sporophores were formed earliest on media with low nutrient concentrations.
Figure 29: The effect of various C/N ratios on basidio-carp production at 15 C. The concentration of asparagine was adjusted while the glucose concentration was maintained at 10.0 g/l.
In contrast to the decrease in basidiocarp production as the strength of the medium increased, mycelial growth was most vigorous on the high strength media.

K. Nitrogen Utilization

The difficulties already mentioned with regard to nitrogen nutrition of sclerotium development apply to this section as well. Of the compounds tested, DL-asparagine and casein hydrolysate (1.0 g/l) supported maximum basidiocarp production. Few sporophores were formed on media made with DL-alanine, KNO₃, or Ca(NO₃)₂. Although nitrates were poorly utilized, they did support some basidiocarp production after three serial transfers. No basidiocarps were produced from cultures on media made with DL-methionine, L-(-)-phenylalanine, L-proline, or L-tyrosine. Ammonium chloride and ammonium sulfate also supported no fruiting. The rapid drop in pH, prohibitive to growth, probably was responsible for the lack of basidiocarps. Cultures grown on 2% malt-extract agar (Difco), used in preliminary work with T. erythropus, did not produce carpophores. Addition of 2.0 g/l of ammonium sulfate or ammonium tartrate induced the formation of robust sporophores on the medium. It is expected that ammonium salts could be utilized for basidiocarp formation if the pH of the medium could be maintained at a satisfactory level.

L. Vitamin Requirements

No basidiocarps were produced from cultures grown on Medium A prepared without the vitamin stock solution. Addition of 100 ug/l of thiamine to the medium in place of the vitamin stock solution restored normal sporophore production. Two serial transfers verified that thiamine was the required vitamin.
Figure 30: Stage IV basidiocarps on a petiole of *Acer macrophyllum*. X1.5

Figure 31: Stage II basidiocarps developing from the inoculum plug on the casein hydrolysate medium. Culture was grown at 15°C. Note the strong phototropic response. X7.

Figure 32: Stage IV basidiocarps developing from the inoculum plug. Note heavy spore drop below the heads of the fructifications. Culture was grown at 10°C on Medium A with casein hydrolysate. X7.

Figure 33: Basidiocarps on Medium A with casein hydrolysate. Note development from agar surface. A few stage III basidiocarps are present, but most are stage II. Apparent branching is an optical illusion caused by overlaying sporophores. X0.8.

Figure 34: Germinating sclerotia on water agar. Most sporophores are at early stage IV. Germination was at 4°C. Approx. normal size.

Figure 35: Stage III basidiocarps. Plate had been rotated twice (90° each time) from the original position. The second rotation occurred 9 hours before the photograph was made. Culture was grown on Medium A at 15°C.
I. General Considerations

The cultural studies performed with *T. erythrops* provided data that may elucidate the life cycle of this fungus in nature. Except for the 15-20°C activation of sclerotia, *T. erythrops* is able to complete its entire life cycle at 4-10°C. These temperatures are comparable to those prevalent during the growing season of the organism. In nature, sclerotia are formed in the winter but remain dormant throughout the spring and summer. They germinate in the fall when the habitat is cool and moist. It appears likely that sclerotium activation occurs in the late spring when sclerotia are turgid. Desiccated sclerotia from culture could not be activated. Mature sclerotia collected on petioles in May 1970, did not germinate when incubated at 4, 10, or 15°C or after a pretreatment of 4°C for 5-10 days followed by 15°C incubation. Sclerotia collected in August, 1970, produced basidiocarps readily when incubated at 4°C but not at 15°C.

Not all Typhulas are autumnal. Some species sporulate in spring and autumn (Corner, 1950). Corner ascribed this single fruiting season to a failure of the sclerotia to mature by spring, when they should otherwise germinate. This explanation does not agree with my field observations. Mature sclerotia of *T. erythrops* were present on petioles by February. *T. sclerotioides*, another autumnal species, also was observed on *Acer* petioles. Sclerotia of this species were also mature before the end of winter.

II. Mating System and Monokaryons

*T. erythrops* appears to be facultatively homothallic.
In the absence of a compatible mating strain, single-spore haploid cultures are able to become dikaryotized. However, before dikaryotization, single-spore mycelia are potentially heterothallic. In its heterothallic reactions, *T. erythropus* is tetrapolar. Four mating strains were identified from the nine monosporic isolates derived from isolate T-4. The incompatibility system in only one other *Typhula* is known; *T. incarnata* is also tetrapolar (Røed, 1969).

Lehfeldt (1923), a student of Kniep, crossed single-spore mycelia of *T. erythropus* and observed fusion and clamp formation in successful matings. He could not determine if this species were bipolar or tetrapolar. Kniep (1928) reported the formation of haploid and "diploid" fructifications by cultures of the same species. It is not known if these haploid fructifications were actually homodikaryotic.

In *Sistotrema brinkmanni* three "subspecies" are recognized, one homothallic, one with bipolar heterothallism, and one with tetrapolar heterothallism (Lemke, 1969). The same situation may exist in *T. erythropus*, but further study is needed.

Noble (1937) was able to obtain only five monosporic isolates of *T. trifolii*. She demonstrated heterothallism but could not determine if the species were bipolar or tetrapolar.

Lemke (1969) noted that dikaryons could be homokaryotic or heterokaryotic and used the terms "homodikaryotic" and "heterodikaryotic" to denote these situations. His terminology was used in the present study.

Homodikaryotic cultures of *T. erythropus* grew more slowly than heterodikaryons. This slow growth rate and the delay of self-dikaryotization for 3-4 weeks after inoculation would seem
to be favoring outbreeding.

Dikaryotization of the monokaryotic colonies was first observed in the young basidiocarps that arose in the center of the colony. Later, clamp connections were formed on the hyphae at the margin of the colony. The homodikaryotization process might be similar to that described in *Taphrina* (Kramer, 1960). In this genus the dikaryotic condition arises as a result of the mitotic division of the single haploid nucleus during the germination of a blastospore or an ascospore. *Typhula* mycelium, like the *Taphrina* blastospore, can produce haploid cells for an indeterminate period. Under suitable conditions a homodikaryon is initiated, and the life cycle can be completed.

The slower growth rate of monokaryons is common in the higher basidiomycetes (Fincham and Day, 1963; Raper, 1966). It should be emphasized that all nine single-spore isolates were slow growing, and all developed basidiocarps and clamps within one week of each other. This would suggest that this facultative homothallism is a well established genetic character in *T. erythropus* and that homodikaryotization was not a result of a contamination with a compatible mating strain.

*T. trifolii* produced haploid basidiocarps in culture (Noble, 1937). These sporophores had the appearance of miniature dikaryotic sporophores. Four basidiospores were produced per basidium. The basidiospores were approximately one half the size of basidiospores from the dikaryon. Only three of Noble's five haploid isolates formed basidiocarps in culture. These same three also formed haploid sclerotia in culture.
**T. erythropolis** basidiospores from basidiocarps formed on single-spore cultures were equal in size to normally produced basidiospores. Hanna (1928) reported that spores from haploid and dikaryotic fructifications of *Coprinus lagopus* were of equal size.

III. Mycelial Growth, Dikaryotic

The general morphological features of mycelial growth of **T. erythropolis** are similar to those reported for **T. sclerotioides** by MacDonald (1934). The growth rate of 2-3 mm/day increase in colony diameter in cultures of **T. erythropolis** was less than the rates recorded for other species. Dejardin and Ward (1971) noted rates of 3.5 mm/day for **T. incarnata**, 3.8 mm/day for **T. idahoensis**, and 4.4 mm/day for **T. trifolii**. An undetermined *Typhula* species showed a rate of 8 mm/day (Lockhart, 1967).

**T. erythropolis** was able to grow at temperatures from 0 to 20 C. Basidiospore germination occurred over the same range. The psychrophilic nature of many *Typhula* species is well documented (MacDonald, 1934; Tasugi, 1935; Remsberg, 1940; Terui, 1941; Ekstrand, 1955; Tomiyama, 1955; Potatosova, 1960a; Jackson, 1963; Lockhart, 1967; Corner, 1970; Dejardin and Ward, 1971). Doubtless, this ability to grow at very low temperatures when other fungi are inhibited is important to their mode of existence.

The optimum pH for mycelial growth of *T. erythropus* was 4-6. Dejardin and Ward (1971) reported maximum linear extension in *T. trifolii*, *T. incarnata*, and *T. idahoensis* at pH 5-7. Their work verified Tasugi's (1935) report on the optimum pH for *T. incarnata* (as *T. graminum*).

My isolates of *T. erythropus* were thiamine deficient; a deficiency of this type is common in the higher basidiomycetes (Cochrane, 1958), but has not been investigated in other species of *Typhula*. The scant growth that did occur in the apparent absence of thiamine probably can be attributed to impurities in the other ingredients of the medium. Agar, asparagine, and glucose frequently are contaminated with biologically significant amounts of the vitamin (Cochrane, 1958).

Asparagine and casein hydrolysate as nitrogen sources supported maximum mycelial growth. They were also superior to other sources for sclerotium and basidiocarp production. Nitrates did not support the formation of a dense mycelial mat. The rapid drop in pH to inhibitory levels during ammonium utilization by *T. erythropus* has been noted in many other fungi (Apparao, 1956; Cochrane, 1958; Ward, 1964; Curren, 1968). The initial growth that was made on media with ammonium salts was dense and comparable to that with asparagine. It appeared that ammonium would have supported the production of a dense mycelial mat had the pH not changed.

IV. Sclerotium Development

The development of sclerotia of *T. erythropus* was similar to that described for other *Typhulas* (deBary, 1887; MacDonald, 1934; Remsberg, 1940). Sclerotia of *T. sclerotiorides* took
9-10 days to reach maturity after initiation (MacDonald, 1934) in comparison to the 14-16 days required by T. erythrops.
The same four stages of development were noted during the formation of T. sclerotioidees sclerotia as were present in their formation by T. erythrops. The exudation of liquid from maturing sclerotia was first reported by deBary in 1887. He and later investigators (MacDonald, 1934; Remsberg, 1940) believed that the liquid was mainly water that was expelled during the compaction of the sclerotium. When the liquid from T. erythrops sclerotia was evaporated on a glass slide, a white crystalline deposit remained. Remsberg observed the same phenomenon in other Typhula species.

The effects of temperature on sclerotium production were difficult to assess. A definite interaction between temperature of incubation and the C/N ratio of the medium was established. Sclerotium production was maximal at 15 C on a medium with a C/N of 95:1; at 4 and 10 C, the optimum C/N was 9.5:1. Dejardin and Ward (1971) mentioned that the greatest number of sclerotia were formed on a malt extract-yeast extract-glucose medium (note high C/N) at temperatures above 10 C. If the same temperature-C/N relationship exists for the species in their study as for T. erythrops, their observation is of little value without more experimental data. The same may be said for MacDonald's (1934) observation that maximal sclerotium production by T. sclerotioidees was at 13-15 C on PDA.

The incubation temperature also influenced the time to sclerotium initiation and to maturity. These processes were most rapid at 10 and 15 C, sclerotia appearing 8-12 days after
inoculation of suitable media. Remsberg (1940) reported sclerotium formation occurring 5-14 days after inoculation in the 14 species she examined. MacDonald (1934) recorded a time of 21 days for sclerotia of T. sclerotiodes, and Dejardin and Ward (1971) 4 days for T. trifolii and T. idahoensis and 6 days for T. incarnata. The latter species was reported by Jackson (1963) to require 7-10 days on PDA.

The coalescence and fusion of sclerotia at lower temperatures in T. erythropus was described previously by Remsberg for other species. This effect was evident only when cultures were grown on a rich medium or one with a low C/N ratio. Corner (1950) proposed that the absence of compound sclerotia from field collections was probably a result of the lack of rich substrates colonized by these fungi. The C/N ratio of forest litter is 40:1 to 50:1 (Brock, 1966).

Townsend (1957) examined sclerotium formation in several species of fungi imperfecti, some of which had perfect states in the hymenomycetes. She recognized three different stages of sclerotium formation: initiation, growth to full size, and maturation. Each stage differed in its nutritional requirements. These stages correspond to stages Ia, Ib, and II in the present investigation. Stage III of the T. erythropus sclerotium is equivalent to the "mature sclerotium" in Townsend's work.

Nutritional differences were noted among the stages of sclerotium formation in T. erythropus. On Medium A prepared with galactose, sclerotia did not develop beyond stage Ia. Stage Ib sclerotia often failed to become pigmented when produced on a medium with nitrate as the sole nitrogen source.
Except for the abortive sclerotia that developed on Medium A at 15 C, stage II sclerotia, once formed, continued on to stage III.

Townsend (1957) and others (Hawker, 1950; Cochrane, 1958) have reported that factors favoring vegetative growth also favor sclerotium production. This correlation was observed in cultures of *T. erythropus* grown on media of various strengths. The richest medium supported maximal sclerotium production and the highest rate of linear extension of hyphae. Furthermore, the optimum temperature for mycelial growth and sclerotium formation was 15 C. This would indicate that sclerotium formation can be classified as vegetative growth in contrast to reproductive growth. Cochrane (1958) suggested that, "...sclerotium development, as a prelude to sexual reproduction, may be expected to be influenced by the same factors." However, he presented no evidence in support of this. Such factors as higher temperatures, high strength media, and high C/N ratios at 15 C, which favored sclerotium production in *T. erythropus* frequently were inhibitory to basidiocarp formation.

Wheeler and Waller (1965) found that the initiation of *Sclerotium rolfsii* Sacc. sclerotia in culture was delayed until the lateral extension of the mycelium had been checked. Sclerotia did not appear until the plate was filled with mycelium. This effect was not observed in cultures of *T. erythropus*. Sclerotium initiation, growth, and maturation were often completed before the plate was covered.

The stimulatory effect of wheat germ on sclerotium formation was evident in two ways. First, sclerotia were formed
earlier on Medium C than on any other media. It has been shown previously that factors favoring mycelial growth also favor sclerotium production. The short lag period for the mycelial growth rate was reflected in the more rapid appearance on mature sclerotia on Medium C. The second effect was to increase the number of sclerotia produced per plate. These beneficial effects of wheat germ could result from the inclusion of new ingredients into the medium (i.e., vitamins, sugars, amino acids), the alteration of the proportion of the other ingredients, or both. The factor or factors in wheat germ were water soluble, heat stable, and dialyzable.

V. Sclerotium Germination

All previously reported attempts to germinate sclerotia of Typhulas were done by placing them on moist sterile sand or soil. The use of water agar for this purpose in the present investigation was superior to previous methods for three reasons: 1) it facilitated observations, 2) results were easily reproducible, 3) nutritional factors were minimized.

No dormancy period or special treatment was necessary for sclerotium germination in any species previously tested. Mature sclerotia of *T. trifolii* germinated readily on the parent colony but required an undetermined resting period when sown on moist soil (Noble, 1937). It was not stated in earlier publications if sclerotia produced at temperatures similar to those in the field were as viable as those produced at the optimum temperature for mycelial growth. I found that sclerotia of *T. erythropus* produced at 4 C or from field collections in May did not germinate under conditions favorable for the germination of sclerotia grown at 10 and 15 C. The 4 C grown
sclerotia required treatment at 15-20 C for germination to occur.

A good correlation between sclerotium size and viability has been shown in *Claviceps purpurea* (Cooke and Mitchell, 1966). Large sclerotia of this species were significantly more viable than smaller sclerotia. This relationship was not noted in germinating sclerotia of *T. erythrospus*, except for very small sclerotia (0.7 mm) that never germinated.

Corner (1950) interpreted the *Typhula* sclerotium as an adaptation to low temperature, but my results indicate that its function is to carry the fungus through the warm, dry summer. During this time no fleshy, newly fallen petioles are available to support mycelial growth. At temperatures above 20 C, mycelial growth was essentially nil. Species of this genus, including *T. erythrospus*, will grow at very low temperatures. In regions with very severe winters the sclerotium may have the dual function of overwintering and oversummering.

At least two phases of sclerotium germination were influenced by temperature. First, sclerotia produced at 4 C normally required a treatment of 15-20 C for germination to occur. Ten to fourteen days at the elevated temperature were sufficient to ensure germination. The occasional germination of these sclerotia without heat activation may have been caused by their frequent exposure to room temperature during examination. Only 6-10 month old sclerotia were observed to germinate without heat activation, and this was sporadic. A minimum time of heat treatment was not determined.

It was noted that sclerotia produced at 4, 10, and 15 C
had the areas of basidiocarp origin established within them during sclerotium formation. Corner (1950) suggested that such a prelocation occurred. The effectiveness of the 15-20°C treatment on sclerotia grown at 4°C seems to be in bringing the basidiocarp primordium to a level of differentiation comparable to that in 15°C grown sclerotia. Cross sections of sclerotia produced at 10 and 15°C frequently showed areas of compacted hyphae and alignment in the medulla indicative of a sporophore primordium. No such area were noted in sclerotia produced at 4°C. Thus, while the location of the basidiocarp origin may be physiologically determined during sclerotium formation at 4°C, morphological differentiation apparently does not occur until the sclerotium is exposed to temperatures of 10°C or higher.

Sclerotia had to be turgid for the heat activation to be effective. Sclerotia of *Claviceps purpurea* require a cold treatment for germination, and this could be met only when the sclerotia were water soaked (Mitchell and Cooke, 1968).

The second temperature effect concerned the germination of sclerotia produced at 15°C and of activated sclerotia grown at 4°C. The cell or cells that differentiated in the basidiocarp primordium prior to germination seldom developed further when left at 15°C. Rather, most rapid germination resulted when sclerotia were incubated at 4°C. At the reduced temperature, basidiocarp formation was stimulated. This would explain the effectiveness of the 5-7 day treatment at 4°C on the increased germination rate of 15°C grown sclerotia. When these sclerotia were returned to 15°C to germinate, the germination process had already been triggered. Continued sporophore devel-
Potatosova (1960a) reported a temperature effect on sclerotium germination similar to the one discussed above. Sclerotia of *T. variabilis*, *T. trifolii*, *T. incarnata*, and *T. idahoensis* were placed outdoors in pots of sterile sand and covered with glass. Fructifications did not appear until the outside temperatures had dropped several degrees. Sporophores of *T. variabilis* and *T. trifolii* were produced at 8-15.5 C, of *T. incarnata* at 1.4-13.5 C, and of *T. idahoensis* at 1.4-4.6 C.

*T. erythropus* sclerotia produced at 10 C germinated on water agar at 10 C, although more rapid germination occurred at 4 C.

VI. Basidiocarp Growth and Development

Remsberg (1940) and Tasugi (1929, 1935) encountered considerable difficulty in obtaining fertile sporophores from germinating sclerotia of 15 *Typhula* species. They found that UV radiation (2650-3250 Å), which was not transmitted through Pyrex glassware, was necessary for the development of fertile heads. In the absence of this quality UV light, long, sterile stalks were produced. However, not all *Typhula* species require this wavelength of UV to sporulate. *T. sclerotioides* (MacDonald, 1934), *T. trifolii* (Noble, 1937; Potatosova, 1960a), and *T. variabilis* (Potatosova, 1960a) produced fertile sporophores when grown under glass. *T. erythropus* belongs to this group of species able to sporulate under glass.

The basidiocarps of *T. erythropus* are strongly phototropic, as are those of *T. sclerotioides* (MacDonald, 1934). Considering the microhabitat, the advantage to the fungus in possessing this positive phototropic response is obvious. In
the shaded leaf litter a positively phototropic sporophore would grow toward a light source, avoiding contact with the moist debris piled around the sclerotium. A negative geotropic response would reduce the effectiveness of spore discharge by driving the basidiocarp into the litter above the sclerotium. The sporophore of *T. erythrophus* is ensured of maximum efficiency in spore release by growing toward open areas in the litter where basidiospores are most likely to be dispersed by the wind. MacDonald (1934) commented that the basidiocarps of *T. sclerotioiides* are negatively geotropic, but he presented no evidence.

Blue and white light were the most effective in eliciting the phototropic response. The growth adjustment of a basidiocarp toward a new light source occurred in the zone of elongation, below the head. It was not determined where the light stimulus was received.

It was not conclusively demonstrated that light was required for basidiocarp initiation. Light was necessary for the formation of the head and hymenium on basidiocarps arising from the mycelium. However, sclerotia grown in conditions of light were sometimes able to produce fertile basidiocarps when germinated in darkness. Thus it seems that the light stimulus for head development might be stored in the sclerotium until an emergent basidiocarp had reached stage II. It was previously noted that sclerotium germination and basidiocarp development were independent events.

The development of the *T. erythrophus* basidiocarp was arbitrarily divided into four stages of primordium, headless sporophore, head formation, and sporulation. A similar
approach using four stages has been followed in studies of carpophore development in Agaricus bisporus (Lange) Imbach (Bonner et al., 1956; Gruen, 1963). Komagata and Okunishi (1969) recognized seven stages in development of carpophores of Coprinus kimuriae.

In 1887, deBary recognized that the growth zone in sporophores of T. variabilis was limited to the apex. Moreover, he believed that cell division continued in the growing tip until the basidiocarp had attained its full length. He also noted that no further augmentation occurred at the base of the sporophore. MacDonald (1934) commented that growth was apical in the sporophore of T. sclerotioides.

Corner (1950) stated, in reference to Typhula sporophores, that, "... apical growth may be arrested very early and inflation may be so prolonged that the fruit-bodies appear to emerge as though from a button-stage, as in typical agarics, but development is never really indirect with a period of apical growth followed by a separate period of expansion." This would indicate that the period of cell inflation overlaps part of the period of cell division. Further, inflation continues after cell division has ceased. What Corner said is true for T. erythropus, but he misjudged the nature and the duration of the overlap of division and inflation.

The stage I primordium is built up by cell formation and slight inflation. When the basidiocarp elongates rapidly during stages II-IV, it is as a result of inflation. However, new cells are formed at a low rate in the head region. These new cells produce basidia on the slowly growing head. Basidio-
carp elongation and basidium formation are concurrent events that cease at approximately the same time.

There appears to be no significant difference between the development of the T. erythropus sporophore and that of an agaric sporophore. Bonner et al (1956) marked carpophores of Agaricus bisporus with dots of carmine and noted the region of basidiocarp expansion and the movement of this zone up the stipe of a developing basidiocarp. They found, as had Buller (1924) and Borriss (1934), that maximum elongation occurs in the upper part of the stipe, below the cap. This region is responsible for the great increase in the height of a carpophore. After the 2 cm button stage, no new cells are formed in the stipe, and cell inflation accounts for basidiocarp expansion. The expansion of the agaric pileus is independent of stipe elongation.

In stage I T. erythropus basidiocarps, the region 0.2-0.5 mm below the tip is the growth zone. As this zone moves upward, it increases to a 1-2 mm long region traveling away from the previously expanded lower part of the stalk. Head formation and expansion are independent of stalk growth.

In both agaric and Typhula fructifications, the length of the growth zone (zone of expansion) is inversely proportional to the height of the basidiocarp. A smaller percentage of the length of a tall, older sporophore is expanding than that of a short, younger sporophore. No new cell formation occurs at the base of either type of basidiocarp.

The time from initiation to maturity of basidiocarps is not known for most species of Typhula. Corner (1950) recorded a time of 15-20 days for T. variabilis, and MacDonald (1934)
reported 1-4 days for growth to full size of *T. sclerotioides*. This time interval in *T. erythropus* is dependent upon incubation temperature. At 4 and 10 °C, a sporulating basidiocarp is present 5-6 days after the appearance of stage I. Growth to full size requires another 3-5 days. At 15 °C, sporulation is frequently inhibited; sporophores do not exceed stage II in their development.

Basidiocarp production was not so subject to the interaction between incubation temperature and C/N ratios that influenced sclerotium formation. At temperatures below 15 °C, sporophores seldom were produced from the colony surface. Similar responses to incubation temperature were observed in all sporophores, regardless of their origin (i.e., sclerotia, colony surface, or inoculum plug).

The optimum pH for basidiocarp formation from the colony surface was 6.0. This contrasts with the lower pH optima for mycelial growth and sclerotium formation. The internal pH of mature sclerotia is near 7.0.

The optimum C/N ratio for basidiocarp production was 19:1. Madelin reported maximum sporocarp formation from cultures of *Coprinus lagopus* grown on a medium with a C/N ratio of 15:1 when glucose and alanine were the main constituents (Madelin, 1956). The relevance of a low C/N ratio for fruiting from the colony in *T. erythropus* to fruiting from sclerotia on maple petioles in the field is not entirely clear. Brock (1966) gives the C/N ratio of "microorganisms" as approximately 10:1. Since the *Typhula* sclerotium is rich in stored glycogen and fats (Scurti and Converso, 1965), the C/N of a sclerotium is
probably higher than 10:1. This would agree with data from culture. Sclerotia contained sufficient reserve materials to support the production of up to eight basidiocarps without an exogenous nutrient supply.

Maximum sporophore formation from the medium occurred on a 1/10 strength medium. This response is in accord with the usual distinctions noted between vegetative and reproductive growth (Cochrane, 1958). Reproduction in T. erythrops is favored by a weak medium, and vegetative growth, including sclerotium formation, is favored by a rich medium.

VII. Interaction of Nutritional/Environmental Factors

As results from experiments with T. erythrops and other fungi have shown, a single ingredient of the medium does not operate independently of the other ingredients, their proportions, environmental conditions, or the phase of growth of the organism. The effects of temperature, pH, the molecular configuration and concentration of carbon and nitrogen sources, the C/N ratio, vitamins, salts, and fungal response are complexly interrelated. In figure 36 I have attempted to depict these relationships graphically. Manipulation of one factor changes the optimum level of the other 6 factors. The particular fungal structure cited in the chart may be the mycelium, the sclerotium, or the basidiocarp in any stage of its development.

Temperature appears to be the most important single factor. Although a definite correlation between temperature and the optimum C/N ratio for sclerotium production was noted, this relationship is probably of little importance in the field. Cultures on high or low C/N ratio media produced only sclerotia
The Interrelationship of Nutritional and Environmental Factors and Fungal Development

Figure 36: A change in any of the 7 factors will influence the uptake of certain substances, the optimum concentration of certain substances, or the morphological response of the fungus. Each factor in the hexagon affects and is affected by the others, and all may operate as limiting factors in the development of a particular fungal structure.
particular fungal structure

Figure 36
when incubated at temperatures prevalent during sclerotium formation in nature.

The uptake of carbon and nitrogen compounds by certain fungi has been found to be affected by temperature (Burnett, 1968). It is possible that the effective C/N ratio available to the hyphae of *T. erythropolis* when grown on Medium A at 15 C is different than that in cultures incubated at 4 or 10 C. The failure of typical sclerotia to form on Medium A at 15 C could have been the result of several factors. The pH was seen to be very important in sclerotium formation at 15 C. Cultures grown on media with a pH of 4 or 5 did form some sclerotia. The uptake of organic and inorganic nitrogen compounds by certain fungi is dependent upon pH and the concentration of the compounds (Fries, 1956; Jones, 1963; Nicholas, 1966). Amino acid synthesis in some fungi has been reported to be a limiting factor at higher temperatures (Deverall, 1966).

Certain compounds are not as readily utilized as others. Thus, a C/N ratio of 40:1-50:1 cited for litter (Brock, 1966) cannot tell us very much of the suitability of a substrate for a particular fungus. Much of the C or N may be in forms unavailable to the organism.

Inorganic salts and vitamins have been reported to be limiting factors under certain conditions of growth (Cochrane, 1958; Casselton, 1966). The interaction of environmental and nutritional factors must be examined in a two-way relationship with the particular stage in the life cycle of an organism. Hawker (1957) and Cochrane (1958) reported that the nutritional requirements and optimum physical conditions often differ in
in different stages of development of the same species.

VIII. Taxonomic Aspects and Implications

The small size and simplicity of the *Typhula* fructification makes morphological characteristics of taxonomic value at a premium. Corner (1950, 1970) uses head size and shape, badidiospore dimensions, stalk length and width, coloration, substrate, number of sporophores per sclerotium, and sclerotium structure as major taxonomic criteria in distinguishing species. The validity of sclerotial characters for taxonomic perposes was negated by Røed (1969) when he successfully crossed *T. incarnata* with *T. graminum*. Corner (1950, 1970) had placed these two species in different sub-genera on sclerotial differences.

The failure of successful mating between the monokaryotic isolates of *T. erythropus* and *T. sclerotioides* helped to define the limits of both species. Corner (1950) stressed that, "But for the red-brown color of the stem and sclerotium it *T. erythropus* would hardly be distinguishable from *T. sclerotioides*." Although the fructifications are similar, the sclerotial structure of the two species is quite different. The sclerotial hyphae of *T. sclerotioides* are thick-walled and those of *T. erythropus* are thin-walled. Also, the surface pattern and thickness of the cortex differ.

Cultural data from the present study showed that the length of the head and stalk was not constant but increased with age. While a sporulating basidiocarp grew from 6.3 to 25 mm, the head length increased from 1.2 to 4.0 mm. Coloration and stalk proportions were relatively constant in basidiocarps produced from sclerotia. However, when sclerotium-free sporophores were formed on a medium with a low C/N ratio, the
stalks were thick and short and were a plain brown color.

The number of basidiocarps per sclerotium was also variable. Although Corner (1950) gives the number for *T. erythrops* sclerotia as one or rarely two to three, up to 8 sporophores were produced from cultured sclerotia. Sclerotia that gave rise to this number of basidiocarps were no larger than those from field collections.

Basidiospore size from cultured sporophores of *T. erythropus* varied from 6-8 $\mu$m x 2-3.6 $\mu$m. Ekstrand (1955) has placed considerable importance on the length/width ratio of basidiospores of two species he described, and, in fact, the species were distinguished by this single character. W. C. MacDonald (1961) did not agree and relegated both to synonymy with *T. idahoensis*, a species described by Remsberg. Species in this genus can be divided into a large-spored group and a small-spored group; *T. erythropus* is in the latter. Although J. A. MacDonald (1934) thought that, "spore size may turn out to be the only stable character in the group," the variation in certain species is so great that this prediction is unlikely. A single basidiocarp of *T. phacorrhiza* produced spores measuring from 11-20$\mu$m x 4.7-7.5$\mu$m (Corner, 1950). The length/width ratio varied from 1.6 to 2.9.

The nature of the substrate in identifying saprophytic species might be more the result of limited collection data than an indication of substrate specialization (Corner, 1950). Thus, nearly all currently used taxonomic criteria in this genus are quite variable. Until crosses are made between many species, we cannot know the limits of morphological var-
iation within a single species.

In addition to the problem of species determination, the distinction between *Typhula* and *Pistillaria* is questionable. Species of both genera produce basidiocarps from the colony surface without sclerotia (Koske and Perrin, 1971). Sclerotia have been found in the life cycle of *P. petasitidis* (Corner, 1970) and *P. setipes* (Koske and Perrin, 1971).
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APPENDIX A
Collection Data

(1) *Typhula erythropus* Fries
   (a) isolate T-4; UBC culture collection # 5019
       Location: U.B.C. campus, Vancouver, B.C.
       Date: 30 September, 1969
       Habitat: on petioles of *Acer macrophyllum*
   (b) isolates T-181 and T-182; UBC culture # 5020, 5021
       Location: U.B.C. campus, Vancouver, B.C.
       Date: 15 October, 1970
       Habitat: on petioles of *Acer macrophyllum*
   (c) isolate T-29; UBC culture # 5022
       Location: U.B.C. campus, Vancouver, B.C.
       Date: 12 November, 1970
       Habitat: on petioles of *Acer macrophyllum*

(2) *Typhula sclerotioides* (Pers.) Fries
   (a) isolate T-19; UBC culture # 5023
       Location: U.B.C. campus, Vancouver, B.C.
       Date: 13 October, 1970
       Habitat: on petioles of *Acer macrophyllum*

Dried specimens of all collections have been deposited in the U.B.C. mycological herbarium.
APPENDIX B
Culture Media

(1) **Medium A**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>glucose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>asparagine</td>
<td>2.0 g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1.0 g</td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Vitamin stock soln.</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Micro-elements stock soln.</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Agar (K&amp;S brand, high gel strength)</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.0 l</td>
</tr>
</tbody>
</table>

**Vitamin stock solution**

- thiamine: 50 mg
- pyridoxine: 0.5 mg
- inositol: 25 mg
- biotin: 25 μg

Dissolve in 500 ml of 20% ethanol, store in refrigerator.

**Microessential elements stock solution**

- Fe(NO$_3$)$_3$·9H$_2$O: 181 mg
- ZnSO$_4$·7H$_2$O: 110 mg
- MnSO$_4$·4H$_2$O: 51 mg

Dissolve in 150 ml distilled water. Add 1N H$_2$SO$_4$ until solution becomes colorless. Then add 110 mg CuSO$_4$·5H$_2$O and 100 ml dist. water. Store in refrigerator.
(2) **Medium B**

Same as Medium A except asparagine concentration is 0.2 g/l instead of 2.0 g/l.

(3) **Medium C**

Make up Medium A lacking asparagine. Pour 20 ml of hot sterile medium into 90 mm petri plates containing 0.12-0.17 g of autoclaved wheat germ flakes (Rockhill brand, Wild Rose Mills, Vancouver, B. C.).

(4) **Malt extract-yeast extract-peptone agar (MYP)**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>malt extract (Difco)</td>
<td>7.5 g</td>
</tr>
<tr>
<td>yeast extract (Difco)</td>
<td>0.5 g</td>
</tr>
<tr>
<td>peptone</td>
<td>1.0 g</td>
</tr>
<tr>
<td>agar (K&amp;S brand)</td>
<td>12.0 g</td>
</tr>
<tr>
<td>distilled water</td>
<td>1.0 l</td>
</tr>
</tbody>
</table>

(5) **Water Agar**

1.2% K&S brand high gel strength agar in distilled water.