DISEASES OF WESTERN SWORD-FERN, 
POLYSTICHUM MUNITUM (KAULF.) PRESL.

by

JAMES LOUIS SANDENO

A THESIS
submitted to
OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of
MASTER OF SCIENCE

June 1962
APPROVED:

Redacted for Privacy
Professor of Plant Pathology
In Charge of Major
Redacted for Privacy
Head of Department of Plant Pathology and Botany
Redacted for Privacy
Chairman of School Graduate Committee
Redacted for Privacy
Dean of Graduate School

Date thesis is presented February 20, 1962

Typed by Barbara Snook Cameron
ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Roy A. Young, advisor and major professor, for his guidance and encouragement during the preparation of this study.

Thanks also to Dr. H. J. Jensen, for his help with the section dealing with nematodes, and to Dr. E. K. Vaughan and Dr. R. O. Belkengren for their valuable criticism of the manuscript.

The author also wishes to express his gratitude to Mr. Frank Skewis and Mr. Orvile Noice of Callison's Incorporated for some of the information and plant material used for this study.

Finally, financial assistance provided by Callison's Incorporated is gratefully acknowledged.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>2</td>
</tr>
<tr>
<td>The host</td>
<td>4</td>
</tr>
<tr>
<td>Fern diseases</td>
<td>8</td>
</tr>
<tr>
<td>Taphrina faulliana Mix</td>
<td>9</td>
</tr>
<tr>
<td>Milesia polystichii Wineland and Milesia Vogesiaca (Syd.) Faull.</td>
<td>10</td>
</tr>
<tr>
<td>Aphelenchoides fragariae (Ritzema Bos 1891) Christie</td>
<td>12</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>15</td>
</tr>
<tr>
<td>Source of ferns</td>
<td>15</td>
</tr>
<tr>
<td>Isolation of microorganisms</td>
<td>15</td>
</tr>
<tr>
<td>Media</td>
<td>17</td>
</tr>
<tr>
<td>Identification of microorganisms</td>
<td>18</td>
</tr>
<tr>
<td>Bacterial inoculation of fern pinnae</td>
<td>19</td>
</tr>
<tr>
<td>Isolation, mounting and staining of nematodes</td>
<td>21</td>
</tr>
<tr>
<td>Identification of nematodes</td>
<td>23</td>
</tr>
<tr>
<td>Photomicrographs and photographs</td>
<td>23</td>
</tr>
<tr>
<td>Measurement of organisms</td>
<td>24</td>
</tr>
<tr>
<td>LEAF BLISTER CAUSED BY TAPHRINA FAULLIANA MIX</td>
<td>25</td>
</tr>
<tr>
<td>DRY ROT</td>
<td>30</td>
</tr>
<tr>
<td>FUSARIIUM</td>
<td>34</td>
</tr>
<tr>
<td>IDENTIFICATION OF BACTERIA ISOLATED FROM FERN</td>
<td>38</td>
</tr>
<tr>
<td>LEAF NEMATODE - APHELENCHOIDES FRAGARIAE (RITZEMA BOS 1891) CHRISTIE</td>
<td>42</td>
</tr>
<tr>
<td>FERN RUSTS - MILESIA POLYSTICHI WINELAND AND MILESIA VOGESIACA (SYD.) FAULL</td>
<td>50</td>
</tr>
<tr>
<td>BUCKSKIN</td>
<td>54</td>
</tr>
<tr>
<td>OTHER MICROORGANISMS FOUND ON WESTERN SWORD-FERN</td>
<td>56</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>63</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>67</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>69</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Forest habitat of Western Sword-fern. June, 1961.</td>
</tr>
<tr>
<td>2</td>
<td>Appearance of leaf blister caused by <em>Taphrina faulliana</em> in June 1960.</td>
</tr>
<tr>
<td>3</td>
<td>Asci of <em>Taphrina faulliana</em>. 350X</td>
</tr>
<tr>
<td>4</td>
<td>Blastospores of <em>Taphrina faulliana</em>. 5,000X</td>
</tr>
<tr>
<td>5</td>
<td>Early stage of dry rot. June 1960. Blister areas progressively becoming brown or black.</td>
</tr>
<tr>
<td>6</td>
<td>Spread of dry rot from blistered areas. June 1961.</td>
</tr>
<tr>
<td>7</td>
<td>Advanced stage of dry rot. June 29, 1961.</td>
</tr>
<tr>
<td>8</td>
<td>Fusarium growth on surface of pinnae from moisture chamber.</td>
</tr>
<tr>
<td>9</td>
<td>Typical spores of <em>Fusarium</em> from fern.</td>
</tr>
<tr>
<td>10</td>
<td>Spread of dry rot from areas inoculated with <em>Fusarium</em> plugs.</td>
</tr>
<tr>
<td>11</td>
<td>Typical water-soaked areas caused by <em>Aphelenchoides fragariae</em> on Western Sword-fern.</td>
</tr>
<tr>
<td>12</td>
<td>Stained tissue showing the presence of nematodes in pinnae.</td>
</tr>
<tr>
<td>13</td>
<td>Nematode from fern.</td>
</tr>
<tr>
<td>14</td>
<td>Nematode head showing spear.</td>
</tr>
<tr>
<td>15</td>
<td>Nematode tail showing mucor and thorn-shaped spicule.</td>
</tr>
<tr>
<td>16</td>
<td>Uredia of <em>Milesia polystichii</em>. July 1961. 6X</td>
</tr>
<tr>
<td>17</td>
<td>Section through a uredum from above. 240X.</td>
</tr>
<tr>
<td>19</td>
<td><em>Milesia vogesiaca</em> urediospores. Nov. 1961.</td>
</tr>
</tbody>
</table>
LIST OF FIGURES continued

20. Mosaic-like pattern on upper surfaces of fern pinnae. ........................................ Page 55

21. Two Alternaria sp. isolated from fern.
   August 1961. ................................................ 58

22. Spores from upper half of above plate.
   1,500X .......................................................... 58

23. Spores from lower half of above plate.
   1,500X .......................................................... 58

24. Microthyrium sp. ascocarps on surface of fern pinnae. 5X. ........................................ 60

25. Shield-shaped ascocarps of Microthyrium sp. ...................................................... 60

26. Ascocarp of Chaetomium sp. from pinnae surface. ............................................... 62
INTRODUCTION

The use of fern, along with salal, huckleberry, holly, pine, hemlock, scotch broom and laurel, as background or accent material in floral decorations, has led to the development of a substantial "Greenery" industry in the Pacific Northwest. The fern most commonly used for floral decorations in the United States is Western Sword-fern, Polystichum munitum (Kaulf.) Presl. This fern is native to western Oregon and Washington where its fronds are picked and kept in cold storage for eventual sale to florists throughout the country. Because this fern is used for decorative purposes, the packers of Western Sword-fern are bothered by diseases which affect the appearance of their product.

The objectives of this study were: 1) to describe the types of disfigurations and discolorations that occur on Western Sword-fern in its natural habitat and in storage, 2) to determine the cause of the diseases found and 3) to evaluate the importance of the different diseases of this fern. Organisms such as Pythium, Rhizoctonia, and Botrytis, which may injure the prothallium, and certain insects, which may cause disfiguration of the fronds in their natural habitat (17), were not included in this study.
More than 9,000 species and varieties of ferns are grouped in the division Pteridophyta and belong to a series of non-flowering plants collectively known as Cryptogams. Unlike most other plants, ferns are found in the wild state all over the world. They are found at various elevations from the equator to the arctic. Most flourish in the shaded habitats of forest floors and in deep ravines where moderate temperatures and moist conditions prevail; some are even aquatic (21, p. 457 and 26, p. 554). The climbing ferns, Schizaeaceae, in the United States reach a height of 3 to 4 feet, but in tropical countries some varieties may climb to 50 feet (52, pp. 132-133). The succulent Ceratopteridaceae and free floating Salviniaceae are the aquatic ferns. Tree ferns, Cyatheaceae and Dicksoniaceae, can be found growing to a height of over 80 feet. The Polypodiaceae, of which Western Sword-fern is a member, have an erect underground stem or rhizome with the fronds clustered in dense crowns (26, p. 554).

The ferns are usually considered as of little economic importance to man. In the past they have been thought to bestow magical powers on the finder (52, pp. 106-107). In the tropics their fronds and stems have been used for building purposes. The epidermal hairs of other ferns have been used as stuffing in pillows and upholstery. Some
drugs, which supposedly had the power to remove parasitic worms from animal bodies, have been made from ferns (21, p. 457).

Ferns, however, do have some economic importance. In Great Britain it has been suggested that the Bracken fern, which is considered a weed, may be treated as a crop to be used in paper making and as a substitute for straw (58, p. 56). In the United States it has been shown that this same fern is a persistent carrier of Rhizoctonia which causes a disease of strawberries grown in the same areas as the fern (62, p. 12). There are several other diseases on ferns that can attack other economic crops such as coffee, tobacco, sugar-cane, and tomatoes (56, pp. 145-147). Graf lists some 240 species and varieties of fern that can be used as house plants (26, pp. 556-592) and one can find such plants for sale in florist shops, dime stores, and in some markets. Although ferns have now lost much of their popularity as house plants, due to the exacting care they require (33, pp. 281-282), the grace and beauty of some fern fronds has led to their use as background or accent material in floral decorations. In tropical areas ferns are picked and sent to florists in nearby cities (34, pp. 55-57). In the United States, Florida and the Pacific Northwest have been a good source of fern for the cut-leaf fern market. Retail prices range from a few cents per leaf
for the "Easter fancy fern", *Dryopteris intermedia*, to 30-40 cents for a 4 foot leaf of "woodwardia", *Woodwardia chamissoi* (9).

Formerly, one of the most commonly used ferns in floral decorations was the Christmas-fern or Eastern dagger-fern, *Polystichum acrostichoides*, which came from Florida (7, p. 1217). This fern has now been entirely replaced by a fern that grows in the Pacific Northwest, *Polystichum munitum*, which is known in the New York trade as Western Dagger-fern (9), but has also been called Giant Holly-fern, Pacific Christmas-fern, and Western Sword-fern (59, p. 887). Western Sword-fern, however, is the accepted common name for *Polystichum munitum* (31, p. 225).

The host

*Polystichum munitum* (Kaulf.) Presl. - Western Sword-fern.

Synonym: *Aspidium munitum* Kaulf.

*Polystichum* is a Greek name meaning many rows. The name probably refers to the numerous regular rows of sori of the type species *Polystichum lonchitis* (1, p. 9). The common name, Sword-fern refers to the pinnae, and the frond as a whole, which resembles a short broad sword (46, p. 3). Western obviously refers to the fact that it is found growing in the western part of the United States.

The fronds are several, 30-140 cm. long, rigidly
ascending in a distinctive crown; stipes and rachis are densely paleaceous; stipes stout, 5-6 cm. long, the scales large, ascending, bright glossy brown, ovate to oblong-acuminate; blades linear-laceolate, pinnate; pinnae numerous, spreading, lance-linear to linear-attenuate, 25-100 cm. long, 5-25 cm. broad, often chaffy beneath, sharply serrate or biserrate; sori abundant, borne on the underside of the pinnae in one to several rows on either side of the midrib extending almost to the margin; leaf tissue coriaceous, evergreen; indusia papillose-dentate to long-ciliate (1, p. 10; 42, p. 49; and 46, p. 3).

This fern can be found on damp, wooded slopes in the Humid Transition Zone: Alaska to southern California, northwestern Montana, and northern Idaho (1, p. 10 and 42, p. 49). It is extremely variable, but grows best throughout the Pacific Northwest under dense fir, hemlock, and spruce forests of the coast range (46, p. 3 and Fig. 1.). Although this area may be covered with a blanket of snow in the winter the fern does not seem to suffer. This may be due to the relatively short, mild winters and the Antarctic origin of the genus *Polystichum* (13, p. 109).

Although the commercially available Western Sword-fern is supplied, about equally, from both Oregon and Washington, most of the following information, as obtained from Callison's Incorporated, pertains to practices in Oregon.
Fig. 1. Forest habitat of Western Sword-fern. June, 1961.
These do not, however, differ much from those in Washington.

The fern is picked on private or federal lands in wooded areas of the coastal range and on the western slopes of the Cascade Mountains. Except for keeping access roads in repair, owners of private land usually do not charge pickers for picking privileges. To pick fern on federal land, the pickers have to pay $\frac{1}{2}$ to 1 cent per bundle of 50 fronds picked. The pickers can remove as much as 25% of the fronds from each plant without damaging the plant or next year's crop (44, p. 3).

Individuals doing the picking are usually small farmers or loggers and their families. The packers of fern do not hire the pickers, but merely buy the fern from the individual pickers for 14 to 20 cents per bundle of 50 fronds. Acceptable fronds must be about 30 inches long and free of disease, disfiguration, and surface dirt. The price varies with the season; summer and fall fern brings the lowest price and winter fern brings the highest. Spring fern, depending upon supply and demand, sells at prices somewhere between the two extremes.

Individual pickers sometimes may bring their fern to a town for sale directly to local florists in the area, but most pickers sell to dealers who buy the picked fern, crate it in its original bundles of 50 fronds, and keep it in cold storage for eventual distribution to wholesale houses.
The crated fern is sold in either half cases or full cases containing 25 to 50 bundles of fronds respectively. The cases themselves are made of wood and wire, similar in construction to orange crates, and are lined with wax paper or polyethylene sheets.

Although Western Sword-fern can be found on thousands of acres of Northwest timberland, scarcely 1% of this land is covered by the pickers. Even though only a small amount of the potentially acceptable fern is picked, the output from Oregon and Washington supports a multimillion dollar business (46, p. 3). Not much information on losses is available, however, Loring estimates that the two major suppliers of Western Sword-fern in Oregon sustained losses of about $14,000.00 in 1959 alone (35, p. 15), but this figure is probably low.

**Fern diseases**

Fern has not been considered an economic crop, thus one will find little reference to diseases of fern in the literature. There are several articles about specific diseases on specific ferns, namely those used as house plants, but only a few works on fern disease in general are available (3; 17; 54; and 56, pp. 141-158).

Several host indexes list numerous microorganisms on different ferns (49 and 59, pp. 882-890), however, Seymour has the largest list with approximately 200 species of
fungi that can be found on nearly 100 fern species
(48, pp. 23-27).

**Taphrina faulliana Mix**

Mix lists some 24 species of Taphrina as occurring on
fern (41, p. 3), but only 2, *Taphrina polystichi* and *Taphri­
na faulliana*, occur on ferns of the genus *Polystichum* (59, pp. 887-888). *Taphrina polystichi* occurs on *Polystichum
acrostichoides*, which grows in the eastern part of the
United States, and *Taphrina faulliana* is listed as occurring
on *Polystichum munitum* (59, pp. 887-888).

The original collection of *Taphrina* on Western Sword­
fern was made by J. H. Faull at Rhododendron, Oregon, on
September 6, 1931. This material was sent to Mix who pub­
lished a description of the fungus and named it *Taphrina
faulliana*, in honor of Faull, in 1938 (40, p. 573).

The original description by Mix stated that the organ­
ism caused small roundish or elliptic, brown spots on the
pinnae with no thickening of the pinnae tissue (40, p. 573).
The fern was not fresh when it was examined and thus several
errors were made in the original description. These errors
were, however, corrected by Mix in 1949 (41, p. 28).

The corrected description states that the organism
causes small, up to 5 mm. in diameter, round to oval,
lemon-yellow, slightly thickened spots on the pinnae. These
spots become brown on aging or on drying. Because of this
thickening of the pinnae tissue, the disease is commonly referred to as leaf blister (44, p. 353; 59, p. 888; and 60, p. 552).

The mycelium is subcuticular; asci hypophyllous, sometimes also epiphyllous, closely packed, long-clavate, 43-76 by 6-9 μ. Asci provided with a stalk cell 13-33 by 4-7 μ. Ascospores were reported as not being seen, but that all of the asci examined contained numerous blastospores which were described as long-elliptic to bacilliform with dimensions of 4.6-6.5 by 1.5-2 μ. (41, p. 28). Kramer states that these so-called blastospores are the result of continuous nuclear division to produce a large number of ascospore nuclei (32, pp. 317 and 319).

Milesia polystichii Wineland and Milesia vogesiaca (Syd.) Faull.

Both of these rusts are reported on Western Swordfern in Oregon (6, pp. 7 and 9; 30, p. 214; and 59, p. 888). Both appear similar; uredia abundant, hypophyllous, grouped on greenish or brownish areas, bullate, dehiscent by a central pore; urediospores obovoid or ellipsoidal; wall colorless. Teliospores in epidermal cells, vertically septate, one to many-celled; wall colorless, about 1 μ thick, smooth. The urediospores of Milesia vogesiaca average 18 by 36 μ and the wall is 1 μ thick. The urediospores of Milesia polystichii average 17 by 28 μ and the wall is 1.5-2.5 μ thick.
The main difference, aside from size and shape, is that the urediospore walls of *Milesia vogesiaca* are smooth, while those of *Milesia polystichii* are strongly echinulate (6, pp. 7-9 and 30, p. 214).

Shaw also lists these 2 fungi as occurring on Western Sword-fern in Oregon. He refers, however, to the genus as *Milesina* (49, p. 82); Gregor also refers to this rust as *Milesina* (56, p. 145). Rogers, in discussing Article 57 of the International Rules, states that *Milesia*, not *Milesina*, is the valid generic name for this rust (45, p. 251).

There also seems to be some doubt as to the validity of the species name *polystichii*. Jackson, who first described *Milesia polystichii*, made a collection of Western Sword-fern at Grants Pass, Oregon, in September of 1916 and labeled this collection as being infected with *Hyalopsora laeviuscula*, which is listed as a synonym of *Milesia laeviuscula* by Arthur (6, p. 7 and 30, p. 214). A Miss Grace O. Wineland found that some of this fern material was infected with a rust with echinulate urediospore walls, while the urediospore walls of *Milesia laeviuscula* were described as being smooth. Thus Jackson described the fungus and gave it the name *Milesia polystichii* Wineland; Jacks. (30, p. 241). Shaw lists the species of this organism as *winelandii* (49, p. 82). The name, *Milesia polystichii* Wineland, which is listed in the Index of Plant Diseases in
the United States (59, p. 888) is the one that will be used in this paper.

The alternate host for these 2 species of Milesia, and 3 others that occur in Pacific Coast region, is unknown. It has been suggested that, like the eastern species of Milesia, Abies may be found as the alternate host (29, p. 291 and 54, pp. 97-99). Because of this relationship to fir, the Melampsoraceae, of which this fungus is a member, have been referred to as the Fir-fern rusts (29, p. 289 and 60, p. 381).

Generally rusts have been considered as unimportant in the growing of ornamental ferns (44, p. 354).

Aphelenchoides fragariae (Ritzema Bos 1891) Christie

In much of the literature the Aphelenchoides sp. found on fern is listed as Aphelenchoides olesistus (22, p. 144; 23, p. 218; 44, p. 355; 59, p. 888; and 60, pp. 252-253). Many investigators, however, consider Aphelenchoides olesistus to be a synonym of Aphelenchoides fragariae (2, p. 109; 16, p. 156; 19, p. 3; 50, p. 90; and 51, 622).

Franklin states that originally Ritzema Bos seems to have differentiated these two nematodes in their action on the host; Aphelenchoides fragariae is usually ectoparasitic and Aphelenchoides olesistus is endoparasitic. Franklin also states that the original drawing and written description of Aphelenchoides olesistus, or Aphelenchus olesistus...
as it was then known, do not agree (19, p. 3).

In 1932 Steiner reported the successful transfer of _Aphelenchoides_ from fern to strawberry with the production of typical dwarf symptoms (50, p. 90). Crossman, in 1936 (16), agreed with Steiner and Bührer (51, p. 622) that some of the _Aphelenchoides_ species could not be differentiated on morphological characteristics and thus should be considered as host varieties or strains of _Aphelenchoides fragariae_. Franklin also stated that there are no important morphological differences between _Aphelenchoides olesistus_ and _Aphelenchoides fragariae_ as originally described by Ritzema Bos (19, p. 3).

Allen set up a new classification of the nematodes related to _Aphelenchoides fragariae_ in 1952. His suggested classification listed _Aphelenchoides olesistus_ as a synonym of _Aphelenchoides fragariae_ (2). In 1960 Allen also stated that whether _Aphelenchoides fragariae_ acts as an endoparasite or an ectoparasite depends upon the host (28, p. 614). Westcott, in 1960, lists the nematode on fern as _Aphelenchoides olesistus_ only because of the familiarity of that name (60, pp. 252-253). Throughout this paper Allen's classification will be followed and the nematode on Western Sword-fern will be referred to as _Aphelenchoides fragariae_.

This nematode occurs on ferns which belong to many
different genera (16, pp. 157-164 and 23, pp. 213-214). Under conditions of high relative humidity, moisture is deposited as a film on the leaf surfaces. Nematodes migrate through this film of water until they enter the leaves through stomata or wounds (22, p. 152; 23, p. 306; and 39, p. 558).

Once the nematode enters the leaf it lives within the mesophyll spaces where it may secrete a toxin that causes destruction and discoloration of the surrounding cells (22, p. 152). This discoloration causes characteristic patchy or blotched areas that first appear to be water-soaked (4, p. 892), but later turn reddish, brown, or black (38; 44, p. 355; and 60, pp. 252-253).

Movement of the nematode within the leaf tissue is restricted to that portion of parenchyma circumscribed by the veins (29, p. 559); thus the discolored areas are limited to bands of interveinal tissue (4, p. 892; 23, p. 210; 38; 44, p. 355; and 60, pp. 252-253).
MATERIALS AND METHODS

Source of ferns

Observations for the presence of disease organisms on Western Sword-fern were made on material personally collected in the fern's natural forest habitat and on material supplied by a packer of greenery.

From July 1960 to September 1961 fern fronds were collected, at about 2 week intervals, from a forest area approximately 8 miles east of Triangle Lake, Oregon and just off of state highway 36. Other material was collected less regularly, until November 1961, from Mary's Peak. Single or very irregular collections were made from other locations so that the area of the Oregon coastal mountain range from Salem, on the north, to Eugene, on the south, was covered at the time of this study.

Picked fern fronds were supplied by Callison's Incorporated from their packing plant in Eugene, Oregon. Most of this material was also picked in the coastal mountain range of Western Oregon.

Isolation of microorganisms

Two methods were used to isolate microorganisms from fern tissue; (1) Incubation of frond sections in small moisture chambers and (2) direct plating of sectioned fern tissue on agar media in Petri plates.
Small, clear plastic boxes, 6½ X 5½ X 1½ inches, with a layer of moistened paper toweling at the bottom were used as moisture chambers. Since the fern fronds are quite large, only sections of such fronds could be put into each chamber. The paper toweling was moistened with sterile distilled water and the closed boxes were kept at room temperature for periods of 1 to several weeks. Chambers that contained tissue to be incubated for more than 1 week required the addition of more sterile distilled water once a week.

When using Petri plates, about 20 ml. of media was poured into each clean sterile plate and allowed to harden. The plates were then kept in a cold room, about 2°C., until used.

All tissue to be plated was cut to a size that could easily be handled and then washed in running tap water. The tissue was then immersed for 1 minute in a 5% Clorox solution, removed from the Clorox solution, cut with flamed scissors into portions about 1/8 of an inch thick, and placed on the surface of an agar medium. The sections were then pushed down into the agar with a flamed forceps. Four sections were thus plated in each Petri plate and the plates were incubated at room temperature (26-28°C.) for 4 to 6 days.
Media

Two types of media were used in attempting to isolate organisms present in discolored fern pinnae.

1. Streptomycin-Potato-Dextrose Agar (SPDA)

Streptomycin nitrate was added to standard PDA to reduce bacterial growth. The medium was prepared as follows;

- Potatoes 200.0 gm.
- Dextrose 20.0 gm.
- Phytomycin 0.5 ml.
  (20% streptomycin nitrate)
- Agar 20.0 gm.
- Distilled water 1,000.0 ml.

Two hundred grams of peeled potatoes were cut into thin slices, added to about 500 ml. of water, and autoclaved for 20 minutes. The agar was also added to about 500 ml. of water and autoclaved, in a separate container, along with the potatoes. After autoclaving the potato broth was separated from the potato slices, by pouring through cheesecloth, and added to the melted agar. The dextrose and Phytomycin were then added to the melted agar and potato broth and the volume of the medium was brought up to 1 liter. The medium was put into flasks, autoclaved for 20 minutes, and then poured into sterile Petri plates.
2. Bacterial medium

This medium is used by the Plant Clinic at Oregon State University and to my knowledge is thus far unpublished. The medium is said to have been developed by M. P. Starr. It is made up as follows:

- Proteose peptone Difco #3: 20.0 gm.
- Glycerol: 15.0 gm.
- MgSO₄: 1.5 gm.
- K₂HPO₄: 1.5 gm.
- Agar: 15.0 gm.
- Distilled water: 1,000.0 ml.

All the constituents were dissolved in distilled water, autoclaved for 20 minutes, and then poured into sterile Petri plates. The medium has a pH of about 7.0. This medium was also used in liquid form by not adding agar. After the constituents were dissolved in distilled water, 50 ml. of the medium were placed in 125 ml. Erlenmeyer flasks that were then plugged with non-absorbent plugging cotton and autoclaved for 20 minutes.

**Identification of microorganisms**

The Genera of the Fungi, by Clements and Shear (12), and The Fungi Imperfecti, by Barnett (8), were used for general identification of fungi. Specific articles, or
texts, will be mentioned as each organism is discussed.

After obtaining pure cultures of bacteria by dilution plates, Bergery's Manual of Determinative Bacteriology (10) was the main source for identification of the bacteria isolated from fern. Procedures and media for such identification were obtained from the Laboratory Manual for General Bacteriology by Peltier et al. (43). Some of the media were obtained, already prepared, from the Department of Microbiology at Oregon State University.

**Bacterial inoculation of fern pinnae**

Four different methods were used to inoculate healthy-looking fern pinnae with the fluorescent and non-fluorescent types of bacteria isolated from discolored pinnae.

1. One ml. of a 24 hour culture, that had been incubated at room temperature, was diluted 1:100 with distilled water and 1 ml. of the diluted bacterial suspension was placed on the surface of a solidified agar medium. By rotating the plate, the entire surface of the agar was covered with the bacterial suspension. Agar plugs were made with a flamed #3 cork borer after 36 hours of incubation at room temperature. Forty sections of fern fronds, which had been washed with tap water and with distilled water, were put into the plastic moisture chambers. Four agar plugs were placed,
with the bacterial growth side down, on the pinnae surfaces of 30 frond sections. Two of the 4 plugs were placed on the lower surface and 2 on the upper surface of the pinnae. Agar plugs, with no bacterial growth, were placed on the remaining 10 frond sections to be used as controls.

2. The procedure was the same as above, except that after the plugs were placed on the pinnae surfaces a flamed dissecting needle was pushed through the agar plug and on into the pinnae tissue.

3. A diluted bacterial suspension was atomized over 30 frond sections. Distilled water was atomized over the surface of the remaining 10 frond sections for control purposes.

4. Forty frond sections were washed and carborundum was sprinkled on the lower surface of 4 pinnae per section and on the upper surface of 4 other pinnae of the same section. A clean piece of cotton was dipped into the bacterial suspension, prepared as described above, and rubbed over the carborundum-sprinkled surfaces of 30 frond sections. The 10 remaining sections were rubbed with clean cotton dipped in distilled water to be used as control.

The pinnae were treated as described with both the fluorescent and non-fluorescent bacteria, and a 1:1 mixture
of the two. Following treatment, the frond sections were placed in plastic moisture chambers, 5 sections per chamber. The moisture chambers were allowed to remain at room temperature for a period of 2 weeks and then checked for the appearance of discolored tissue.

**Isolation, mounting, and staining of nematodes**

Nematodes were removed from the fern tissue in 3 ways:

1. Sections of fern pinnae were placed in a Syracuse watch glass partially filled with water. The fern tissue was then pulled apart with dissecting needles to allow the nematodes to migrate from the tissue into the surrounding water. Observation with a dissecting microscope permitted removal of the nematodes from the water and torn tissue with a sharpened bamboo splinter.

2. Sections of fern fronds, about 5 inches long, were placed in water in a plastic moisture chamber. After 18 hours the water was poured into Syracuse watch glasses. Nematodes could then be removed from the water as previously described.

3. The Baermann funnel technique was also used to remove nematodes from the pinnae tissue. About 10 grams of fern pinnae were placed, along with 200 ml. of water, in a Waring Blender. The blender was turned on for 30 seconds, the water and
ground tissue removed and placed on a piece of cheesecloth in the top of a funnel. A wire screen supported the cheesecloth at the top of the funnel; rubber tubing and a pinch clamp at the bottom of the funnel kept the liquid from draining out of the funnel. After 2 days the water, containing the nematodes, was drained from the funnel for observation.

Temporary mounting techniques were used to observe and identify the nematodes found. With a bamboo splinter, several nematodes were removed from a Syracuse watch glass and placed in a drop of water on a clean microscope slide. The nematodes were then inactivated by gently heating the slide over an alcohol flame. Three glass rods, about the same diameter as the nematodes, were arranged to form a triangle to support a glass cover slip on the slide. A round glass cover slip was then placed over the drop of water containing nematodes and glass rods, and excess water was removed from around the edge of the cover slip with strips of blotting paper. After removal of excess water, the cover slip was sealed to the slide with melted wax from a birthday candle. The slide was then ready for microscopic observation.

The method used for staining nematodes within the fern tissue was similar to that described by Goodey (24, p. 138)
and Franklin (18, p. 92). Pinnae tissue was washed with tap water and then placed in boiling lactophenol and 0.5% acid fuschin for about 2 minutes. After the lactophenol-acid fuschin solution had cooled, the fern tissue was removed, washed in running tap water, and then transferred to liquid phenol to remove the stain from the tissue. Observation of the tissue under a dissecting microscope showed the presence of the red-stained nematodes within the tissue.

Identification of nematodes

The nematodes were identified with the aid of a key prepared by Goodey (25), and by the de Man formula (25, p. xxvi) which states:

\[
L = \text{total body length in millimeters.}
\]

\[
a = \frac{\text{total body length}}{\text{greatest width}}
\]

\[
b = \frac{\text{total body length}}{\text{length of esophagus}}
\]

\[
c = \frac{\text{total body length}}{\text{length of tail}}
\]

\[
v = \frac{\text{distance from head to vulva}}{\text{total body length}}
\]

\[
T = \frac{\text{length of testes}}{\text{total body length}}
\]

spear = length of spear in microns.

spicule = length of spicule in microns.

Photomicrographs and photographs

Objects to be photographed were first positioned on a
microscope equipped with 10X, 43X and 97X oil immersion objectives and a binocular head. The binocular head was then replaced with a monocular head to which was attached a 35 mm. Praktica FX-2 camera. The camera lens had been removed and replaced by an extension tube to make the distance of film plane to eyepiece about 3 inches. A 10X eyepiece was retained in the monocular head.

Kodak High Contrast Copy film, developed in Agfa Rodinal diluted 1:100 for 18 minutes, was used for all photomicrographs.

Stage micrometer negatives, photographed through all 3 objectives and enlarged to the same size as the photomicrographs, were used to determine magnification.

All photographs were taken with a 2 1/4 X 3 1/2 Crown Graphic camera fitted with a 120 roll film back. The film used was Kodak Plus X, and it was developed in Kodak Microdol.

**Measurements of organisms**

The size of mycelium and spores was determined by the use of an ocular micrometer placed in a 10X eyepiece.

Body length and placement of the various organs of the nematodes was obtained with the aid of a Camera Lucida and a map mileage indicator. These relative measurements were then used with the forementioned de Man formula.
LEAF BLISTER CAUSED BY TAPHRINA FAULLIANA MIX

By June, most of the new growth fern fronds have uncurled, the weather has become warmer, and the cold winter rains have started to subside. At this time, and throughout June, small round to oval lemon-yellow spots can be found on the new growth fern fronds in the Coastal mountains of western Oregon. The spots range in size from a few millimeters to several centimeters and were most numerous on the lower parts of the fronds although some spots could be found on pinnae at the tips of the fronds.

Tissues in the smallest spots were slightly thickened, while the tissues in the larger spots, which most commonly extend to the margin of the pinnae, were thickened and curled. The thickening and curling make the pinnae appear blistered (Fig. 2).

Microscopic observation of sections of the yellowed tissues revealed numerous closely packed asci arising from both the upper and lower surfaces of the pinnae (Fig. 3). The symptoms, along with the presence of the long asci, provided with a stalk cell, corresponded to the description of Taphrina faulliana by Mix (41, p. 28). The dimensions of the asci averaged about 55 by 6 µ and those of the stalk cell 20 by 5.5 µ. These dimensions fall within the range given by Mix. Material was collected at 1 week intervals.
Fig. 2. Appearance of leaf blister caused by *Taphrina faulliana* in June 1960. 2.5X
Fig. 3. Asci of Taphrina faulliana. 350X
for the entire month of June 1961 and at no time were asco-
spores present. However, numerous rod-shaped objects,
measuring 5 by 2 μ, could be seen within the asci (Fig. 4).
These would correspond to what Mix calls blastospores.

Some of the blistered pinnae were taped to the inside
surface of a Petri plate top and the top was then placed
over a Petri plate bottom containing SPDA. Every few hours
the top was rotated 60 degrees. The growth produced on the
agar was like that of Torulopsis; single cells, reproduced
only by budding, and neither mycelium nor spores were
formed (20, p. 38 and 41, pp. 6-7).

A suspension of these cells was sprayed on the surface
of growing Western Sword-fern, but there was no effect on
the plant. This was done in July, and conditions may not
have been proper or optimum for infection. Also this stage
of the organism may not be able to cause infection.
Fig. 4. Blastosporas of *Taphrina faulliana*. 5,000X
DRY ROT

The condition known as dry rot results in the destruction of forest fern fronds throughout the summer. Dry rot is also responsible for a substantial loss of packed fern throughout the entire year.

By the end of June the lemon-yellow blistered areas had started to turn brown and eventually became almost black (Fig. 5). The pinna tissue around many of these discolored blistered areas also began to discolor (Fig. 6), and eventually this discoloration spread to the rachis. After the discoloration had reached the rachis the portion of the frond distal to this point collapsed (Fig. 7).

Fronds that exhibit any discoloration are not accepted by packers of Western Sword-fern, but some fronds that have small blister areas may be overlooked. Thus some of these infected fronds are packed along with healthy-looking fronds. In storage, the dark discoloration may spread from the infected fronds to the healthy-looking fronds and thus an entire crate of 2,500 fronds may be rendered unfit for sale. This discoloration of fronds in the forest and in packing crates is referred to as dry rot by the packers of Western Sword-fern.

Both a fungus and a bacterium were consistently isolated from dry rot tissue that had been placed in a moisture
Fig. 5. Early stage of dry rot. June 1960. Blister areas progressively becoming brown or black.

Fig. 6. Spread of dry rot from blistered areas. June 1961.
Fig. 7. Advanced stage of dry rot. June 29, 1961.
chamber or plated on the bacterial medium and SPDA. The fungus was a *Fusarium* sp. and the bacterium was a fluorescent *Pseudomonas* sp.
A Fusarium sp. was found in the forest growing over the surface of fronds that exhibit advanced discoloration. If fronds with blister areas, or those already exhibiting tissue discoloration, were placed in a moisture chamber, this fungus was found growing profusely over the pinnae surfaces in about a week (Fig. 8). This fungus had the typical macrospores of Fusarium (Fig. 9).

Only 1 reference to a Fusarium attacking fern was found in the literature (56, p. 147), but the original article was not obtainable in this country.

The Fusarium was grown on SPDA and after a period of 1 week, plugs, made with a cork borer and containing both agar and fungus mycelium, were placed on the surface of fern pinnae. The section of fronds that contained these pinnae and fungus plugs were then placed in the plastic moisture chambers. At the end of 1 week the tissue in the area of the plugs had become discolored and the discoloration was spreading (Fig. 10). One cannot positively attribute the discoloration to Fusarium alone since a bacterium was consistently found in association with Fusarium in inoculated tissue and was even present in apparently healthy uninoculated tissue.

Discolored tissue from the forest, packing crates, and moisture chambers was plated on both SPDA and the
Fig. 8. Fusarium growth on surface of pinnae from moisture chamber.

Fig. 9. Typical spores of Fusarium from fern.
Fig. 10. Spread of dry rot from areas inoculated with Fusarium plugs.
bacterial medium. Several fungi, along with the *Fusarium*, were isolated from such areas on SPDA. Bacterial growth was also present even though Phytomycin had been incorporated into the medium. On the bacterial medium the bacterial growth was so great that, in most cases, the fungi present in the fern tissue did not grow.
IDENTIFICATION OF BACTERIA ISOLATED FROM FERN

Three bacteria were apparent in dilution plates from incubated fern fronds.

1. A Gram positive, spherical bacterium was found only a few times. Since most plant pathogenic bacteria are Gram negative rods (11, p. 182 and 57, p. 96), this bacterium was considered to be either a contaminant or a saprophyte (20, p. 44).

2. A Gram negative, yellow, rod-shaped bacterium was found several times but not consistently.

3. A Gram negative, rod-shaped bacterium which produced a greenish pigment that dissolved into the medium and fluoresced under a General Electric 40 watt "black light blue" fluorescent lamp was consistently isolated from dry rot areas.

According to Bergey's Manual, the yellow, non-fluorescent bacterium could be a *Xanthomonas* and the greenish-yellow fluorescent bacterium is a *Pseudomonas* (10, pp. 88-89). The greenish-yellow fluorescent bacterium had the following characteristics: rods about 1 X 3 μ, motile, Gram negative; gelatin liquified; beef-extract-peptone colonies were grayish white and produced a slight fluorescence in the medium; the colonies were smooth with entire edges; nutrient broth turned turbid in 24 hours and no pellicle was formed; litmus milk became alkaline and no
curd was produced; nitrites and ammonia not produced in nitrate broth; hydrogen sulfide not produced; acid but no gas from glucose and sucrose. Lactose fermentation uncertain; facultative aerobe; best growth at 25-30°C, and the least amount of growth at 5°C. Based on the above characteristics the fluorescent bacterium isolated from fern could be either *Pseudomonas asplenii* (5 and 10, pp. 123-124) or *Pseudomonas syringae* (10, p. 128).

*Pseudomonas syringae* has a very wide range of unrelated hosts (10, p. 128), and *Pseudomonas asplenii* is found on Bird’s-nest fern, *Asplenium nidus* (5). Ark stated that the bacterial disease on Bird’s-nest fern starts as small water-soaked, translucent spots which are usually found on the upper surfaces of the leaves (5, p. 758).

Symptoms of dry rot on Western Sword-fern did not include water-soaked areas as described by Ark, thus the identification of this bacterium is still uncertain.

As described in the section on materials and methods, the fern fronds were inoculated with the bacteria isolated from dry rot areas in several ways: 1) plugs, containing bacterial growth, were placed on both the upper and lower surfaces of healthy-looking pinnae; 2) a dissecting needle was run through the plugs and on into the pinnae tissue; 3) a bacterial suspension was atomized over frond sections; and 4) the pinnae were inoculated by rubbing the pinnae surfaces with carborundum and a bacterial suspension. All
the frond sections were kept in plastic moisture chambers for 2 weeks at room temperature after treatment. Both the upper and lower surfaces of the pinnae were inoculated with both the fluorescent and non-fluorescent bacterium as well as a 1:1 mixture of the two. At no time did any of the dry rot symptoms occur. The bacterium alone seemed to have absolutely no effect upon the tissues of the pinnae.

Even though the pinnae exhibited no reaction to the forementioned treatments, sections of these treated pinnae that were plated on the bacterial medium showed growth of the fluorescent bacterium. The control pinnae also showed growth of the fluorescent bacterium when they were plated. This indicated that the fluorescent bacterium may normally be present in the fern pinnae even though the pinnae showed no symptoms of dry rot.

From September, 1960, to September, 1961, 855 pinnae from healthy-looking fronds of different ages and different locations, were sectioned and plated on bacterial media. Of these 855 pinnae, 726 showed bacterial growth. In most cases, only the fluorescent type of bacteria was present. Growth of the bacteria from new growth pinnae showed up in 2 to 3 days, but growth of bacteria from older pinnae took almost 1 week to appear. Roots of 25 plants were plated in September, 1960, and 21 of these also showed bacterial growth.
As mentioned earlier, tissue discoloration started in about June and spread, from the areas of the pinnae that have been infected with *Taphrina* (Figs. 5 and 6), until whole parts of the frond have become discolored (Fig. 7). Tissue discoloration was also associated with the *Fusarium* found in the packing crates and plugs of *Fusarium* placed in contact with fern pinnae (Fig. 10). All such discolored tissue showed a great amount of fluorescent bacterial growth in as little as 24 hours after plating on bacterial agar media. Thus dry rot of fern seems to be due to a succession of 2 fungi and a systemic bacterium.
The wedge-shaped blotches, characteristic of nematode infestation, were first found on fern pinnae in the forest in March 1961. At this time the forest was still very damp, due to the intermittent rains, and the blotched areas showed up as water-soaked wedges on the underside of the old growth pinnae (Fig. 11). When these fronds were picked and allowed to dry, the water-soaked appearance soon disappeared and the tissue became a dull brown.

In May the forest was still fairly damp, and the new fronds were starting to appear as fiddlenecks. The nematode water-soaked areas were still visible on the old growth pinnae. By June most of the new fronds had uncurled and the forest was drying out. At this time water-soaked areas could no longer be seen, but dull to dark brown areas were in evidence on the old growth pinnae that still showed some green. At no time were water-soaked, or brownish, wedge-shaped areas seen on the new growth pinnae until the beginning of the fall rains in September. At that time they were once again present on the underside of the pinnae.

These water-soaked areas were stained with a hot lactophenol solution, containing 0.5% acid fuschin. Observation of the stained tissues with a dissecting microscope showed that numerous nematodes were present within
Fig. 11. Typical water-soaked areas caused by *Aphelencho-ides fragariae* on Western Sword-fern.
these water-soaked tissues (Fig. 12). Similar staining of dull or dark brown areas did not reveal the presence of many, if any, nematodes.

By pulling these water-soaked tissues apart, nematodes could be isolated from the tissue (Fig. 13). The presence of a spear (Fig. 14), mucor, and a thorn-shaped spicule (Fig. 15) made it possible to readily identify this nematode as belonging to the genus *Aphelenchoïdes*.

According to the de Man formula (25, p. xxvi), the nematodes found on Western Sword-fern would be listed as:

**Male;** $L = 0.506 - 0.606 \text{ mm.}$
- $a = 45 - 50$
- $b = 9 - 10$
- $c = 16 - 17.5$

**Female;** $L = 0.615 \text{ mm.}$
- $a = 51 - 58.7$
- $b = 9.33$
- $c = 15 - 18.7$
- $V = 69$

By using the above information with Allen's classification, the nematode from Western Sword-fern was identified as *Aphelenchoïdes fragariae* (Ritzema Bos 1891) Christie (2, p. 109).

The presence of this nematode in the forest was most marked during the fall and winter months when vegetative
Fig. 12. Stained tissue showing the presence of nematodes in pinnae.
Fig. 13. 
Nematode from fern.

Fig. 14. 
Nematode head showing spear.

Fig. 15. 
Nematode tail showing mucor and thorn-shaped spicule.
growth was at its lowest. During the spring and summer months, when fern growth was active, the presence of the nematode was masked. This relationship between fern growth and visible presence of the nematodes follows the same sequence as occurred with *Aphelenchoides* nematodes and ferns grown in ferneries (23, p. 210).

Most references in the literature did not mention the nematode infested areas of the tissue as having a water-soaked appearance. This fern tissue, however, seemed to be very sensitive to any kind of injury and these injured areas became easily water-soaked. Ark described a nematode disease of Bird's-nest fern, caused by *Aphelenchoides fragariae*, in which the first symptoms of nematode infestation consisted of a slight water-soaking. The water-soaking soon disappeared and the tissue turned a dull brownish-black (4). The disease symptoms of Western Sword-fern follow those of Bird's-nest fern very closely.

Ark also described a bacterial leaf blight of Bird's-nest fern caused by *Pseudomonas aspleni*. The disease starts as small, water-soaked, translucent spots which were usually found on the upper surfaces of the leaves (5). Such symptoms were not found on Western Sword-fern, but bacteria was readily isolated from the water-soaked nematode areas.
Aggerly described a discoloration on the lower surface of fern pinnae and states that *Aphelenchoides* nematodes were found in these discolored areas (3, p. 134). He also stated, however, that this discoloration was caused by bacteria, and that the nematode simply performs a mechanical function by carrying the bacteria into the pinnae. Thus the bacteria, not the nematode, was the parasite which caused the damage observed (3, p. 161).

Nematodes are also thought to provide the means of entry of a bacterial pathogen in the case of bacterial wilt of carnations (53), and in the case of Granville wilt of tobacco (36). Horsfall states that nematodes may break down the resistance of a host to attack by fungi and bacteria (28, p. 614), Sasser states that nematodes may contribute some biochemical alteration of the host cells that makes them a more congenial substrate for the attacking pathogen (47).

In the case of strawberries, the association of both the nematode *Aphelenchoides ritzema-bosi* and the bacterium *Corynebacterium fascians* was required to produce cauliflower disease of strawberries (15, p. 484). *Aphelenchoides* species isolated from strawberry plants were often heavily contaminated with the bacterium and at times the bacterium formed a complete sheath around the nematode (14, p. 138). The nematodes isolated from Western
Sword-fern were also heavily contaminated with the fluorescent *Pseudomonas* bacterium. The exact relationship, if any, between nematode and bacteria on Western Sword-fern is not known.
Fern pinnae containing rust uredia were first found on overwintered fronds collected near Triangle Lake, Oregon, on July 24, 1961. The uredia were hypophyllous, grouped in brownish areas on the underside of the pinnae, and measured about 0.2 mm. across (Fig. 16). Sectioned tissue showed the presence of urediospores within the uredia (Fig. 17) and many-celled teliospores were found in the epidermal cells of the pinnae. The urediospores averaged 16 x 26 μ; the urediospore walls were about 1.5 μ thick and were echinulate. The above fits the description of Milesia polystichi as given by Jackson (30, p. 214) and Arthur (6, p. 9).

Collections of fern pinnae from Mary's Peak, Oregon, on November 6, 1961 also showed the presence of rust uredia. This time both echinulate (Fig. 18) and smooth walled (Fig. 19) urediospores were present. The uredia of both rusts were about the same size; 0.2 to 0.3 mm. in diameter. At this later observation, the central pore opening in the uredium was quite evident whereas this was not previously true. The echinulate urediospores measured 18-20 by 24-28 μ, while the smooth walled urediospores measured 15-20 by 31-42 μ. The dimensions of the smooth walled urediospores fit those of Milesia vogesiaca as
Fig. 16. Uredia of *Milesia polystichii*.
July 1961. 6X.

Fig. 17. Section through a uredum from above. 240X.
Fig. 18. *Milesia polystichi* urediospores.
Nov. 1961. 750X.

Fig. 19. *Milesia vogesiaca* urediospores.
Nov. 1961. 750X.
given by Arthur (6, p. 7). The cell walls of the smooth walled urediospores were about 1 μ thick, or slightly thinner than the echinulate urediospore walls. The urediospores of *Milesia polystichii* were ellipsoid, but the urediospores of *Milesia vogesiaca* were oblong (Figs. 18 and 19). The uredia of *Milesia polystichii* were grouped together in brownish areas (Fig. 17), while those of *Milesia vogesiaca* seemed to be more scattered with little or no browning of the tissue.

As far as the packers of Western Sword-fern are concerned, this fungus causes no trouble; diseased fronds picked early in the year are not too discolored, and diseased fronds found later in the year are so discolored that they are not picked.
The only other discoloration that occurred on Western Sword-fern was a yellow mosaic-like pattern that appeared, during late summer and throughout the winter months, on the upper surfaces of the pinnae (Fig. 20). Under storage conditions the pattern becomes more pronounced, but does not spread to healthy looking fronds. The packers of Western Sword-fern refer to this condition as buckskin.

Gregor (56, p. 154) refers to 2 papers by I. Hino, which unfortunately were unavailable, in which 10 species of fern were said to have "mosaic and mosaic-like diseases" which may have been caused by a virus. Since no experimental work was presented, Gregor felt that Hino may have been referring to many types of non-infectious variegation known to occur on fern (56, p. 154).

One hundred sections of fern pinnae exhibiting such mosaic-like patterns were plated. Aside from the fluorescent bacterium, no organism was found to be present in these tissues. More work involving the inoculation of virus indicator plants with juice from fern tissue will have to be done in order to say if this yellow pattern is caused by a virus.
Fig. 20. Mosaic-like pattern on upper surfaces of fern pinnae.
OTHER MICROORGANISMS FOUND ON WESTERN SWORD-FERN

In August of 1961 it was noticed that several of the old discolored fronds in the forest had numerous, small (0.5 mm. in diameter), brown spots scattered over the upper surface of the pinnae. These were identified as pycnidia of a *Phyllosticta* sp. Each pycnidium contained a copious number of small, 6.4 - 8.8 by 2.4 - 3.2 μ, spores. The literature lists several ferns that are attacked by *Phyllosticta*, but the only species names given are *Phyllosticta pteridis* and *Phyllosticta adianticola*. Halsted, who named *Phyllosticta pteridis*, gave a description of the symptoms of *Phyllosticta* tip blight as it occurred on *Pteris cretica* var. *magnifica*, but no description of the organism itself could be found (27, pp. 419-424). Young described and named *Phyllosticta adianticola*, which infects *Adinatum tenerum* in Porto Rico. The conidia of this fungus were described as hyaline, ovate, slightly pointed at one end, 4.8 - 7.2 by 2.4 μ (61). The conidia found on Western Sword-fern were slightly larger and the spores were rounded at both ends. This organism has caused the packers of Western Sword-fern little or no trouble because it only appears on very discolored fronds and these are not picked.

Plating of some old discolored fronds on agar showed 2 species of *Alternaria* to be present.
1. Colony growth of the fungus on SPDA had a gray, cottony, concentrically zonate appearance (Fig. 21, upper half of plate). The spores were ellipsoid to elongate; 9 - 11 by 12 - 34 μ; septate mostly transverse but also longitudinal; hyaline beak at the apex; olive to dark brown in color (Fig. 22).

2. Growth on SPDA resulted in the production of numerous spores that gave the colony a black, suppressed appearance; no zones appeared to be formed (Fig. 21, lower half of plate). The spores were mostly ellipsoid, 11 - 19 by 18 - 30 μ; septate both transversely and longitudinally; no pronounced beak; mostly very dark brown in color (Fig. 23).

Major had reported an Alternaria disease on Polypodium vulgare. He named the fungus Alternaria polypodii and the appearance of the spores are like that of Fig. 22 except that the spore size of Alternaria polypodii was given as 10.5 - 19.25 by 24.5 - 70.0 μ (37). Major had some trouble inoculating greenhouse fern with the fungus, and Weiss listed this fungus as a possible secondary organism (59, p. 887). In the case of Western Sword-fern this organism may also be secondary. It was found only on very badly discolored, old growth, fronds in the forest and thus caused the packers of this fern no loss due to
Fig. 21. Two Alternaria sp. isolated from fern. August 1961.

Fig. 22. Spores from upper half of above plate. 1,500X.

Fig. 23. Spores from lower half of above plate. 1,500X.
discoloration of fern in storage.

Two species of *Aspergillus* were isolated on SPDA and were also found growing on the surfaces of discolored pinnae in moisture chambers. One of these species had a green colony color; conidiophores were rough; conidia more or less rough; heads hemispherical. This may put it in the *Aspergillus flavus-oryzae* group as described by Thom and Raper (55, pp. 259-272). The other isolate of *Aspergillus* had a brown colony color and the conidiophores were smooth. This species fits into the *Aspergillus wentii* group as described by Thom and Raper (55, pp. 241-249). Both these types are common in soils, may be found on decomposing plant material, and thus are probably secondary organisms on Western Sword-fern. As in the case of the *Alternaria*, the *Aspergillus* fungus was only found on fronds that were too badly discolored to pick.

Two other fungi were found on the surfaces of Western Sword-fern pinnae; a *Microthyrium* sp. and a *Chaetomium* sp.

The typical shield-shaped ascocarps of *Microthyrium* were found scattered, or grouped together, over the surface of otherwise healthy-looking pinnae (Figs. 24 and 25). In July, asci and typical two-celled, hyaline, ascospores were found. The pinnae pictured (Fig. 24) were not typical; most pinnae had very few scattered ascocarps which were so small that they were not readily seen. The organism caused
Fig. 24. Microthyrium sp. ascocarps on surface of fern pinnae. 5X.

Fig. 25. Shield-shaped ascocarps of Microthyrium sp.
no discoloration. The ascocarps were easily dislodged during normal handling by the pickers and the packers of Western Sword-fern, thus they do not lower the value of such fern used for decorative purposes.

The Ascocarps of the Chaetomium sp. (Fig. 26) were also very small and scattered. They too caused no discoloration and were very easily dislodged through normal handling processes of picking and packing. The ascocarps did not grow to any noticeable size until infected fern pinnae were placed in a moisture chamber which was kept at room temperature. After 1 week the fungus had spread to the moistened paper toweling in the moisture chamber and the ascocarps then contained numerous asci and ascospores.
Fig. 26. Ascocarp of Chaetomium sp. from pinnae surface.
DISCUSSION

The picking and eventual selling of fern fronds for use by florists throughout the United States is a big industry. Western Sword-fern, *Polystichum munitum* (Kauf.) Presl., from Oregon and Washington is the most commonly used fern for background and accent material in floral decorations. As with any decorative plant material, anything that causes the fern to become unsightly renders it unfit for sale. The types of disfigurations and discolorations that occur on Western Sword-fern are commonly known as leaf blister, dry rot, water-soaking, undefined brownish areas, and buckskin.

Leaf blister, caused by the fungus *Taphrina faulliana* Mix, consisted of round to oval lemon-yellow spots which appeared on the pinnae of fern in the forest in June. The spots varied in size, were more numerous on the lower portions of the fronds, and did not spread among stored fern. The tissues within the infected areas were thickened, hence the name leaf blister.

The dark discoloration known as dry rot spread among fern in the forest until entire fronds became discolored and had a dried appearance. A similar discoloration also spread throughout pinnae in packing crates. This discoloration occurred throughout the year, but was most active during the summer months. Under forest conditions, dry rot
appeared to be initiated in spots caused by *Taphrina faulliana*. The spread of dry rot, in the forest and in storage, was associated with the spread of a *Fusarium* sp. Both forest and storage dry rot areas also contained a fluorescent bacterium of the genus *Pseudomonas*. Spread of dry rot in the packing crates was more closely related to the presence of the *Fusarium* fungus than to that of *Taphrina* and the *Taphrina* did not need to be present to cause serious dry rot losses of stored fern. Much of the fern growing in Oregon seemed to be systemically infected with the *Pseudomonas* bacterium. This bacterium alone did not cause dry rot symptoms to develop, nor did it seem to reduce the vigor of infected plants, thus the disease known as dry rot may be caused by a complex consisting of *Taphrina faulliana*, *Fusarium* sp. and a *Pseudomonas* sp., or by the latter 2 organisms alone.

During the fall and winter months, fern pinnae in the forest, and in packing crates, exhibited wedge-shaped water-soaked areas on their under surfaces. Upon aging, the upper surfaces of these pinnae turned a dull brown. The water-soaking was due to the activity of the nematode *Aphelelenchoids fragariae* (Ritzema Bos 1891) Christie.

The rusts *Milesia polystichii* Wineland and *Milesia vogesiaca* (Syd.) Faull. produced small, undefined brownish areas on lower pinnae surfaces of fern in the forest during
the late summer months. Upon aging, the upper surfaces of these infected pinnae also turned a dull brown.

During late summer and throughout the winter a yellow mosaic-like pattern occurred on the upper pinnae surfaces of fern in the forest. This mosaic pattern became more pronounced, but did not spread, on pinnae under storage conditions. The packers of Western Sword-fern refer to the condition as buckskin. Its cause is unknown.

Leaf blister, water-soaking, rust damage, and buckskin did not spread among stored fern and thus accounted for little economic loss of the packers product. Dry rot, on the other hand, spread readily and rapidly among stored fern fronds. Since dry rot seemed to be initiated by the fungus *Taphrina faulliana* in the forest, and to some extent in the packing crate, it was logical to believe that control of this fungus would reduce storage losses due to dry rot. Western Sword-fern is not grown in fields, but is picked in its natural forest habitat, thus programs aimed at controlling *Taphrina* on the plant are impractical. Another way to minimize losses due to dry rot would be to stop its spread among healthy-looking fronds in storage. This would require the application of some fungicide or antibiotic to control the *Fusarium* or the bacterium. Since fern is used as decorative material, the applied chemicals should not leave a residue.
Phyllosticta sp., Alternaria sp., and Aspergillus sp. were found on old discolored fronds in the forest, but they did not seem to damage stored fern. Microthyrium sp. and Chaetomium sp. were also found on Western Sword-fern, but caused no discoloration and thus did not affect the decorative appearance of this fern.
1. The most destructive disease of the florist's cut fern, *Polystichum munitum* (Kaulf.) Presl., is dry rot. The disease occurs in the fern's natural habitat, and also accounts for extensive discoloration of cut and stored fronds.

2. In the forest the discoloration known as dry rot seems to be due to a succession of several microorganisms: *Taphrina faulliana* Mix, a *Fusarium* sp., and a systemic fluorescent *Pseudomonas* bacterium. Under storage conditions extensive dry rot damage can occur due to the action of the latter two organisms alone.

3. Absence of dry rot symptoms at the time of packing, rapid spread within the packing crate, and lack of a suitable control make this disease important to the packers of Western Sword-fern.

4. The water-soaked areas resulting from the action of *Aphelenchoides fragariae* (Ritzema Bos 1891) Christie and the brownish areas caused by *Milesia polystichii* Wine-land and *Milesia vogesiaca* (Syd.) Faull. result in only minor damage to Western Sword-fern.

5. The yellow mosaic-like pattern of buckskin does not seem to reduce the decorative value of Western Sword-fern. The cause of buckskin is unknown.

6. *Phyllosticta* sp., *Alternaria* sp., and *Aspergillus* sp. occur on old fern fronds in the forest, but these
fronds are not picked and thus cause no damage to fern in storage.

7. The fungi Microthyrium and Chaetomium also occur on Western Sword-fern, but are not pathogenic.


