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Selected aspects of the biology of *Hyalopsora polypodii* (Pers) Magn on *Cystopteris fragilis* (L) Bernh

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HYALOPSORA POLYPODII (PERS.) MAGN.
ON CYSTOPTERIS FRAGILIS (L.) BERNH.

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SELECTED ASPECTS OF THE BIOLOGY OF
HYALOPSORA POLYPODII (PERS.) MAGN.
ON CYSTOPTERIS FRAGILIS (L.) BERNH.

by

Michael Randy McGinnis

A Dissertation Submitted to the
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Approved:

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1969

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	2
MATERIALS AND METHODS	5
Rust Collections	5
Urediospore Viability Under Various Storage Conditions	5
Urediospore Germination on a Nutrient Agar Medium and on Host Tissue	6
Infection	7
Mycelium	8
Teliospores	8
RESULTS	10
Field Distribution of <u>Hyalopsora polypodii</u> in Iowa	10
Urediospore Viability Under Various Storage Conditions	10
Urediospore Germination on a Nutrient Agar Medium and on Host Tissue	15
Infection	15
Mycelium	16
Teliospores	17
DISCUSSION	18
SUMMARY	21
LITERATURE CITED	23
ACKNOWLEDGEMENT	25

INTRODUCTION

Hyalopsora polypodii (Pers.) Magn., a fern rust, is commonly associated with many fern genera throughout the world. In the wooded areas of Iowa, it often develops on fronds of Cystopteris fragilis (L.) Bernh. Golden-yellow, pulverulent urediospores are abundantly produced within subepidermal uredia from May to October.

In some regions, vertically septate teliospores develop within the epidermal cells of C. fragilis during the growing season (6). Teliospores appear to be extremely rare and have not been found in Iowa. Spermogonia and aecia are unknown (23).

This study has been undertaken to investigate selected aspects of the biology of H. polypodii on C. fragilis in Iowa. These include methods of overwintering, establishment of the host-parasite relationship, and general elucidation of the life cycle of H. polypodii.

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REVIEW OF LITERATURE

In 1801, Persoon as cited in Hiratsuka (10) described a rust on fronds of Polypodium fragilis L. as Uredo linearis var. polypodii Pers. Ninety-eight years later, teliospores were discovered within host epidermal cells (7) and U. linearis var. polypodii Pers. was transferred to the genus Pucciniastrum. Magnus (17) transferred the rust to Melampsorella and, in 1901, erected a new genus, Hyalopsora, to accommodate M. polypodii (Pers.) Magn. and M. aspidiotus (Peck) Magn., thus naming the rust Hyalopsora polypodii (Pers.) Magn. (17). H. polypodii was believed to differ from other members of Melampsorella by lacking a peridium in the uredium and having germ pores in the urediospore wall (18). Hiratsuka (11) gave Dietel as the authority, and considered the rust as H. polypodii (Dietel) Magn. This was an error, since Persoon, and not Dietel was the original authority.

Arthur (2) included H. polypodii as a member of the Pucciniastreae in the Melampsoraceae because it formed vertically septate sessile teliospores within the host tissue. Hyalopsora is differentiated in the field from the closely allied fern rust genera, Uredinopsis and Milesia by its golden-yellow, pulverulent urediospores. Hyalopsora, Uredinopsis, Milesia, Pucciniastrum, Melampsorella, Melampsoridium, Pucciostele, Cronartium, and Miyagia possess the unique character of having a uredium with a peridium (6). Melampsorella and Hyalopsora were originally differentiated primarily on the bases of Hyalopsora lacking a peridium and having germ pores in the urediospore wall. Based upon teliospore morphology, the two genera are distinct. Melampsorella forms one-celled,

intraepidermal teliospores, while Hyalopsora forms two- to five-celled, vertically septate, intraepidermal teliospores (2, 6).

Hyalopsora polypodii parasitizes 23 species in 8 fern genera, of which Woodsia obtusa Torr., Athyrium filix-femina Roth., and Cystopteris fragilis occur in Iowa. The rust is not known from W. obtusa nor A. filix-femina in Iowa. The rust has been reported from Story County (9), Fayette County (22) and Winneshiek County (9), Iowa. H. polypodii has been recorded on C. fragilis from Great Britain, Europe, Japan, Canada, and from Massachusetts to Alaska, and southward to Mississippi and northern California (2, 11).

Thick-walled and thin-walled urediospores may occur within the same uredium, and as the season progresses, thick-walled urediospores increase proportionately (18) In 1907, Arthur (1) concluded that the thick-walled spores present in sori were not urediospores, but aeciospores. His misinterpretation was not corrected until Bartholomew (3) demonstrated the dikaryotic nuclear condition for all mycelium associated with the sorus. Thus, both thin-walled and thick-walled spores were true urediospores.

Dietel (8) first demonstrated that urediospores could overwinter on the fern host in Germany. In 1918, Weir and Hubert (21), working in eastern Washington, stated that urediospores propagate the rust and that teliospores were not encountered. They erroneously concluded that H. polypodii must overwinter by teliospores because it is similar to H. aspidiotus in habitat and morphology. Kern, et al. (15) studying rusted ferns collected in Pennsylvania, postulated a third overwintering method, perennial mycelium within the fern host.

Two- to four-celled, vertically septate, colorless teliospores 14 to 18 microns in diameter develop within host epidermal cells (2). Cummins (6) reported that teliospores developed in overwintered fronds, while Wilson (22) stated they developed within living fronds from June to September. Teliospores have been reported only from California (4) and Washington (13) in the United States. They seem to be extremely rare and are unknown from most regions, including Iowa.

MATERIALS AND METHODS

Rust Collections

Thirty-eight collections of Hyalopsora polypodii urediospores were obtained during July and August of 1967, and 6 during May, July, September, and October of 1968. The term urediospore will be used in this dissertation to refer to both thick- and thin-walled urediospores. Rusted fronds were procured from two moist, shaded, west-facing sites along the Des Moines River near Madrid, from three sites at the Ledges State Park near Boone, and from three locations within Pammel Woods at Ames.

Urediospore Viability Under Various Storage Conditions

Urediospores collected from the field in 1967 were stored in the laboratory for three weeks on dried pinnae at 20 to 25°C. They were then transferred with a dissecting needle to plates of 2% water agar and incubated at 5, 10, 15, 20, 25, 30, or 35°C for 24 hours in the dark. Germination percentages were determined by counting at least 200 spores per plate in numerous random fields.

Urediospores on detached pinnae collected from the field were exposed for three days to 20% relative humidity over a saturated aqueous potassium acetate solution in a desiccator (5). The pinnae were then transferred to size zero gelatin capsules for storage at -15, -10, 0, 5, 10, 15, 20, 25, or 20 to 25°C. Germination percentages were ascertained prior to the initial treatment by counting at least 200 urediospores per plate in

numerous random fields on plates of 2% water agar after 24 hours in the dark at 20°C. Following storage for 220, 229, or 288 days, urediospores in uredia on detached pinnae were transferred to glass vials and either placed in a 40°C waterbath for 5 minutes or exposed for 24 hours at 20°C at 100% relative humidity in a moist chamber in the dark. After final treatment, urediospores were transferred with a dissecting needle from the uredia in detached pinnae to plates of 2% water agar and kept at 20°C in the dark. Germination percentages were determined after 24 hours by counting at least 200 urediospores per plate in numerous random fields.

Urediospore Germination on a Nutrient

Agar Medium and on Host Tissue

Studies pertaining to development following germination were conducted on a nutrient agar medium and on the fern host. Urediospores collected from the field were transferred with a dissecting needle from the uredium to plates of a nutrient agar medium containing: 0.2M magnesium nitrate, 2.5 ml; 0.5M calcium nitrate, 1.5 ml; 0.5M ammonium nitrate, 0.5 ml; 0.5% ferric tartate, 0.5 ml; 0.2M potassium (monobasic) phosphate, 1.5 ml; 0.2M potassium (dibasic) phosphate, 1.5 ml; 1 to 10% glucose; agar, 20 grams, and de-ionized water to make one liter (14, 19). The autoclaved medium had a pH reaction near 6.0. Germ tube development was observed after 48 hours at 20°C in the dark.

Urediospores collected from recently infected ferns maintained in the laboratory in December, 1968, were transferred with a dissecting needle to the stomate containing dorsal or lower surfaces of detached

young pinnae. The detached pinnae were maintained on a 5% aqueous sucrose solution for 2 days at 20°C in the dark (10). The pinnae were transferred to 80% ethanol and then placed in a 5% sodium hydroxide solution for several days to dissolve the cellular contents. The pinnae were given 3 washings for 15 minutes each in de-ionized water prior to being placed in a 0.3% chloral hydrate solution for several hours. The chloral hydrate solution cleared the tissue (16), after which the pinnae were examined microscopically for germinated spores and mycelium.

Infection

Infected and non-infected living ferns to be used for inoculation studies were transferred from the field to clay pots in July, 1967. Six ferns with rust and seven healthy plants were moved to the laboratory, where they were maintained near a north window until February, 1969. Polyoxyethylene sorbitan monolaurate¹, an inert oil, and Trend² were sprayed as wetting agents with an atomizer as aqueous solutions of 0.25% on mature living fronds. The wetting agents caused necrosis and were discontinued. They were replaced with an aqueous mist applied with an atomizer. Urediospores collected from ferns in the field were placed on the moistened dorsal surfaces of pinnae with a dissecting needle. The living fronds were maintained in the dark at 20°C for 2 days in a moist chamber, then returned to the laboratory and kept in the moist chamber an

¹Tween 20.

²Purex Corp. Ltd., Lakewood, California.

additional 8 days. Rusted fronds collected from the field during October, 1968, were placed directly on young croziers emerging from the soil in 4 pots maintained in the laboratory. Non-rusted fronds obtained from ferns in the laboratory were placed on croziers in a fifth pot. All plants were kept in a moist chamber for one week, and then examined periodically until February, 1969.

Mycelium

Five pots containing plants with rusted fronds were placed out of doors at Ames during September, 1967, to overwinter under natural conditions until May, 1968. They were then moved to the laboratory for study.

Fern meristems, rhizomes, scales, and petioles collected in the field during July and August of 1967, were fixed in Craff III, processed through an ethanol dehydration series, and embedded in tissuemat (20). Microtome sections were cut at 10, 13 and 15 microns and stained with aqueous safranin and fast green. Cleared fronds utilized in the teliospore study were examined for the presence of perennial mycelium.

Teliospores

Fronds with chlorotic and necrotic areas collected in July, 1967, were placed in 80% ethanol to remove the chlorophyll. The fronds were transferred to a 5% sodium hydroxide solution for several days to dissolve the cellular contents, put through 3 changes of de-ionized water for 15 minutes each, and then placed in a 0.3% chloral hydrate solution for several hours. The chloral hydrate solution cleared the tissue (16).

After processing the fronds through an ethanol dehydration series of 3 changes for 15 minutes each in 50, 80, and 95% ethanol, the tissue was stained with aqueous safranin and/or fast green in 95% ethanol, then mounted with Piccolyte³ and examined for teliospores.

³General Biological, Inc., Chicago, Illinois.

RESULTS

Field Distribution of Hyalopsora polypodii in Iowa

Cystopteris fragilis is often found on moist, shaded slopes.

Hyalopsora polypodii was found restricted in distribution to local areas within eight of the nine fern populations studied. No rust was observed in one fern population. The diseased plants were heavily infected with H. polypodii, while adjacent ferns were rust free.

The recorded distribution for H. polypodii in Iowa was extended in the central part of the state. H. polypodii has been recorded from Story County, Boone County, Webster County, Fayette County, and Winneshiek County. Collections from Story County and Webster County are deposited in the Iowa State University Herbarium.

Urediospore Viability Under Various Storage Conditions

Urediospores collected from plants in the field germinated on 2% water agar plates within 24 hours when maintained at 5, 10, 15, 20, 25, 30, or 35°C. The spores remained golden-yellow with no distortion of the spore wall. Urediospores kept in the laboratory on dried pinnae for three weeks at 20°C had 13.9% germination, while those placed on plates immediately germinated 12.3%. No differences in germination were noted between randomly dispersed spores or groups of spores.

Germinating urediospores on plates of 2% water agar at 15 to 25°C formed 1 to 3 hyaline germ tubes within 24 hours (Fig. 1). Maximum germination, 13.9%, occurred at 20°C (Table 1). The greatest development

Fig. 1. Germinating urediospore of H. polypodii on 2% water agar after 48 hours at 15°C, 430 X

a: urediospore; g: germ tube

Fig. 2. Germinating urediospore of H. polypodii on a nutrient agar medium at 15°C, 430 X

a: urediospore; g: branched germ tube

Fig. 3. Cross section of uredium of H. polypodii in fern pinna, 380 X

n,a: dikaryotic urediospore;
m: mycelium; b: sporogenous cells;
e: fern epidermis; p: fern
parenchyma cell; d: rust peridium

Fig. 4. H. polypodii hypha in cross section of fern pinna, 830 X

p: fern parenchyma cell;
s: septum; n: dikaryotic nuclei

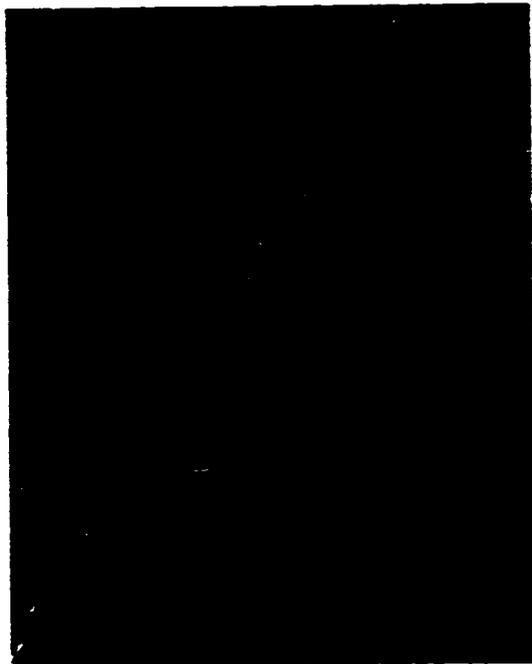


Table 1. Germination percentages of urediospores given different treatments and stored at different constant temperatures when germinated on 2% water agar at 20°C for 24 hours

Treatment	Temperature (centigrade)									
	-15	-10	-5	0	5	10	15	20	25	20-25
None	--	--	--	--	1.6	4.0	4.1	13.9	8.1	--
Initial: 3 days at 20% relative humidity Final: 24 hours at 100% relative humidity at 20°C ^a	0.5	3.0	1.5	1.0	1.5	0	0.5	0.5	4.0	0.5
Initial: none Final: 5 minutes in a 40°C waterbath ^b	4.0	3.0	0	3.0	4.0	3.0	1.0	1.0	3.0	0
Initial: 3 days at 20% relative humidity Final: 5 minutes in a 40°C waterbath ^c	3.0	5.0	3.5	4.5	8.0	6.5	1.5	1.0	6.0	1.0
Initial: 3 days at 20% relative humidity Final: none ^c	1.0	0	0	1.0	4.0	3.0	3.0	3.0	4.0	0

^aStorage duration 220 days.

^bStorage duration 229 days.

^cStorage duration 288 days.

of germ tubes on 2% water agar occurred at 15 to 25°C, extending to more than 150 microns in 48 hours. No germination, or germination by a single germ tube from each urediospore, occurred at 5° and 35°C. The single germ tubes were similar to those produced on plates of nutrient agar medium at 20°C (Fig. 2). Branched germ tubes developed at all temperatures and consisted of two hyphae which developed parallel and adjacent to each other (Fig. 2).

Urediospores stored on pinnae for 220, 229, or 288 days, showed a decrease in germination with increased storage time and extreme temperatures when compared to the average check germination of 12.3% (Table 1). The highest germination percentage was obtained with urediospores initially exposed in uredia on detached pinnae to an atmosphere of 20% relative humidity for 3 days, and then to 5 minutes at 40°C in a water-bath after storage. Germinating spores developed a single, branched, hyaline terminal germ tube 20 to 35 microns long. The germ tubes were like those produced on the nutrient agar medium at 20°C (Fig. 2). Masses of living protozoans were frequently encountered surrounding urediospores on 2% water agar after 24 hours at 20°C. The protozoans appeared not to affect urediospore germination.

Many urediospores stored near 0°C and below became distorted and hyaline. A few spores contained a golden-yellow central sphere surrounded by a hyaline region. Distorted spores, hyaline spores, and spores containing a central golden-yellow sphere were not viable.

Urediospore Germination on a Nutrient Agar Medium
and on Host Tissue

Urediospores placed on the nutrient agar medium for 24 hours at 15° or 20°C, developed single, terminal, short, hyaline, branched germ tubes (Fig. 2). The germ tubes reached a maximum length of 20 to 35 microns within 24 hours. Further development did not occur.

Urediospores placed on the dorsal surface of young detached crozier pinnae germinated and produced a single, hyaline, terminal, branched germ tube. Penetration had not occurred after 48 hours at 20°C in the dark. It had been estimated that 48 hours would be sufficient time for H. polypodii to complete penetration and establishment within the fern host. Lack of urediospores made further experimentation with penetration impossible.

Infection

Urediospores placed on moistened mature attached fronds germinated and developed single, hyaline, branched, terminal germ tubes 20 to 35 microns long within 24 hours. The germ tubes did not penetrate the fern leaf. Urediospores in uredia on detached fronds placed directly on emerging croziers apparently germinated, successfully penetrated and established a mycelium. Randomly scattered uredia developed on the rachis and pinnae surfaces within 14 days. Uredia were always associated with the veins in the pinnae.

Mycelium

Rain splashing upon the unprotected pots containing living overwintered ferns, dislodged and removed the upper soil layer and associated rusted fronds. No rust development occurred after the ferns resumed vegetative growth in the laboratory.

Histological studies of material collected in the field showed that urediospores apparently germinated, successfully penetrated and established mycelium in the fern leaf. Each individual uredium developed from a localized mycelium, which radiated about 150 to 200 microns in all directions in the fern leaf (Fig. 3). Interwoven mycelium was found associated with numerous adjacent uredia.

The intercellular hyphal elements were composed of large, coarse, regularly septate cells. Numerous short side branches and haustoria, and two large distinctly staining dikaryotic nuclei (Fig. 4) per cell were readily visible in most sections. The haustoria were bulb-like structures about 20 microns in diameter. The dikaryotic nuclear condition was observed in urediospores, stalk cells, sporogenous cells, and the cells of the peridium.

A limited amount of intercellular and intracellular mycelium was found in some sections of roots, rhizomes, and scales. This mycelium in these structures was fine, hyaline, sparsely septate, lacked haustoria and dikaryotic nuclei, and was definitely not rust mycelium. Cleared overwintered fronds possessed large amounts of superficial, intercellular, and intracellular mycelium associated with immature fungal fruiting bodies. This mycelium was composed of thick-walled, dark, rounded cells, and was

not rust mycelium. No mycelium other than that associated with a uredium was encountered in living fronds. Thus, no evidence was found indicating extensive development of rust mycelium that might function as perennial mycelium.

Teliospores

Twenty overwintered fronds and 35 living fronds obtained from the field during the growing season were cleared and examined for teliospores. Rust mycelium and uredia were found, but no teliospores were observed.

DISCUSSION

Hyalopsora polypodii is considered a "primitive" rust by many workers (1, 2, 10, 18, 23) because it occurs on ferns, develops teliospores within the host tissue, and possesses a uredium with a peridium. Primitive or not, H. polypodii is extremely specialized and highly successful.

H. polypodii is restricted to local areas in the fern population. These local areas of infection may represent specific microclimates or consist of genetically distinct fern clones. Further study is needed in this area.

Germination experiments have demonstrated that a resting period is not required prior to thick-walled urediospore germination. Germinating urediospores did not produce structures such as vesicles, appressoria, or fusion bodies on a nutrient agar medium. Present data tend to support the hypothesis that they do not develop in the host, since vesicles, appressoria, or fusion bodies were not observed in thin sections, nor in cleared tissues. Penetration of detached crozier pinnae may have failed because specific environmental conditions such as dew, relative humidity, or temperature were not met in the experiment.

No extensive development of mycelium that might function as perennial mycelium was observed within the fern host. Therefore, the conclusion of Kern, et al. (15) that the rust may survive by being perennial on C. fragilis, was not confirmed. Rust mycelium associated with uredia in pinnae may persist during winter, and form a crop of urediospores in the spring.

In the United States, there are two questionable (4, 13) and one confirmed report of the occurrence of teliospores⁴. Adequate collections have not been made to justify any conclusion concerning the presence or absence of teliospores in most regions, and certainly not in Iowa. In the present study, three general areas in two counties of Iowa have been collected intensively in the search for teliospores.

Absence of teliospores supports the hypothesis that teliospores are rarely, if ever, formed in Iowa, and that they are not significant in the propagation of the fungus.

Teliospores may have been found, but not reported in the literature for various reasons. It is also possible that the rust does not have the genetic ability to produce teliospores in some regions. Since the only confirmed report is based upon a collection made at 10,600 foot elevation at Lake Marie, Medicine Bow Mountains, Wyoming, environmental conditions typically associated with high altitudes may be required for teliospore formation. These conditions could include such factors as low oxygen content, low carbon dioxide content, temperature variation, light intensity and quality, and high radiation as compared to lower elevations. Regardless of the factor or factors controlling their development, teliospores are rare.

In conclusion, the following life cycle for Hyalopsora polypodii on Cystopteris fragilis is postulated. During May, urediospores from uredia on overwintered fronds germinate and penetrate the young emerging croziers through the dorsal pinna surface. A uredium develops from a

⁴Cummins, George, Lafayette, Indiana. Hyalopsora polypodii teliospores found in Wyoming. Private communication. 1969.

single localized mycelium in about 14 days. Thin-walled urediospores form initially within uredia and as the season progresses, thick-walled urediospores increase proportionately. New infections occur throughout the summer, whenever young croziers come into contact with urediospores. Mature fronds apparently are resistant to infection. Teliospores may develop within the epidermal cells in some regions, but none are known from Iowa. During August and September, rusted fronds die and remain attached to the rhizome. Pinnae curl back upon themselves exposing the uredia on the dorsal or lower surfaces. During the following spring, urediospores are exposed to elevated temperatures as the ferns resume growth. Newly emerging croziers come into contact with germinating overwintered urediospores on dead fronds, and are invaded. Thus, the rust seems to be propagated solely by means of urediospores in Iowa.

SUMMARY

Viable, golden-yellow urediospores of Hyalopsora polypodii can germinate without a rest period. One to three hyaline germ tubes may emerge from any of several germ pores. Maximum germination, 13.9%, and the greatest germ tube elongation, 150 microns, occurred at 15 to 25°C within 48 hours on 2% water agar. No germination, or germination by a single, branched germ tube from each urediospore, occurred at 5° and 35°C on 2% water agar. Branched germ tubes were initiated at all temperatures and consisted of two hyphae which developed parallel and adjacent to each other. Germination was similar on a nutrient agar medium, and the hyphae did not continue to grow.

Urediospores stored for 220, 229, or 288 days showed a decrease in germination with storage time. Urediospore germination showed a decrease following storage at 20 to 25°C and 0 to -15°C. The highest germination occurred with urediospores initially exposed to an atmosphere of 20% relative humidity for three days before storage, and held for 5 minutes at 40°C in a waterbath following storage. Urediospores after storage germinated by single, branched, terminal, hyaline germ tubes that were 20 to 35 microns long after 24 hours in the dark on 2% water agar at 20°C.

Mature fronds are apparently resistant to infection, while young croziers are susceptible. Rust mycelium was localized beneath the uredium, but some hyphae radiated 150 to 200 microns in all directions in the living fern leaf.

A limited amount of intercellular and intracellular mycelium of other fungi was found in some sections of roots, rhizomes, and scales. Cleared

overwintered fronds contained large amounts of superficial, intercellular, and intracellular mycelium that was not rust mycelium. This mycelium was associated with immature fungal fruiting bodies. In cleared living and overwintered fronds, localized rust mycelium was associated with uredia. No evidence was found indicating extensive development of mycelium that might function as perennial mycelium in the fern meristems, rhizomes, scales, or petioles.

Rust mycelium and uredia were found, but no teliospores were observed in overwintered or living fronds. These observations support the hypothesis that teliospores are rarely, if ever, formed in Iowa, and that they are not significant in the survival of the fungus. Thus, H. polypodii propagates itself by urediospores in Iowa.

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