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Observations on Chytridiaceous Parasites of Phanerogams

XV. Host range and species concept studies in Physoderma

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In a previous paper (SPARROW, GRIFFIN and JOHNS[1] 1961) we described the morphological and developmental features of a species of the chytrid genus *Physoderma* found on common quack grass in Northern Michigan. At that time we did not attempt to place our fungus in any published taxon but merely compared it with other congeneric forms described from the same host and uniformly assigned by their discoverers to *P. graminis* (Büsgen) de Wild. We noted that our fungus was distinct from these primarily in not producing host stunting or suppressing flowering and in possessing smaller resting spores. We expressed hope at this time that "extensive cross-inoculation work" would enable us to place our fungus with some certainty taxonomically. In view of the chaotic conditions prevailing generally at the moment in differentiating species of *Physoderma*, a situation which is seen perhaps at its worst in supposed new taxa described from grasses, the need for such studies is imperative. Indeed, the general paucity of information on host-range studies in the *Physodermataceae* is appalling, and concrete data derived from experimental evidence defending the supposed host specificity of many species ("*P. menthae* Schroeter", "*P. aponogetonis* Sparrow", "*P. marsiliae* Brewster", "*P. digitariae* Pavgi and Thirumalacher", etc.) is almost non-existent.

Therefore, we set up a series of cross-inoculation studies to determine whether other members of the Tribe *Hordeae*, and other grasses on which various species of *Physoderma* had been described, were susceptible to our parasite. Furthermore, it seemed of interest to discover if certain non-gramineous swamp plants from the same area on which we had also found *Physoderma* could be infected by the *Agropyron* parasite. The list of prospective host plants is by no means comprehensive for the purpose needed but represents what materials were available to us at the time. Needless to say, it is being extended as our own collections of seeds and plants continues.

[1] Deceased, 1963.

Two other objectives suggested themselves. Since spore size and host symptoms had oftentimes been the only other characters used in describing new species, it seemed of value to determine how static these were when our fungus was introduced into different hosts.

Methods and Materials

All resting spores of *Physoderma* used in these experiments were collected from the same site in Northern Michigan and have been kept frozen since their collection in 1960 and 1962[1]. Conditions provided for germination were the same at all times, namely, resting spores which had been scraped from fragments of infected material were suspended in charcoal-treated, glass-distilled water and placed in shallow watch glasses in petri dish damp chambers. These dishes were then placed in constant light from a cool, white fluorescent lamp delivering 460 foot candles at nine inches distance, and at a temperature of approximately 23.5° C. Under these conditions zoospores in favorable trials were released within forty-eight hours.

Inoculations were carried out by two methods. Both used the aforementioned zoospores from resting spores, swimming in water, as inoculum. In one, seedlings at second leaf stage were immersed in the inoculum in watch glasses. These were maintained in damp chambers which were left in the aforementioned light room. They were examined at irregular intervals up to 10—15 days. A great advantage here was that the developmental stages were readily seen if formed, since the whole seedling could be subjected to microscopic examination at relatively high powers. Another method used plants growing in flats or pots. These were, in the case of the grasses, seedlings of the same age as those employed by the first method. Bits of cotton previously washed in sterile distilled water were soaked in inoculum and applied to the prospective host at appropriate places, usually at points of most recent growth. Swabbing with a mild detergent of these parts was tried but since this did not seem to influence infection it was discontinued. Drawings, slides and herbarium specimens were kept of most of the successfully inoculated plants.

The F-test for analysis of variance as outlined by SNEDECOR (1956) was used in our study of the significance of resting spore size.

Results

We do not wish to imply that the results obtained under these inoculation procedures may necessarily be readily duplicated in the field. We do emphasize, however, that our fungus is a true obligate parasite and does not fare on moribund tissue. Furthermore, SPARROW and JOHNS (1959) have shown that infection, the epibiotic and endobiotic stages, and even resting spores, do occur regularly in nature on various hosts in the early growing season under submerged, completely aquatic conditions.

We have elucidated the results of our cross-inoculation work by indicating in Table 1 the degree of development undergone by the parasite, i.e., which phase or phases of the life cycle were developed on the host presented.

Table 1 shows that in our fungus occurrence of resting spores on a particular host is not diagnostically significant, since these may be

[1] Fungi of both collections were identical in morphology and host range.

Table 1

Results of cross-inoculations using Physoderma on Agropyron repens as inoculum

Family/Tribe	Host	Epibiotic stage	Endobiotic stage	Resting spores	T/P[1]
Gramineae					
Festuceae	*Briza maxima*	+	—	—	1/4
	B. media	+	p.t.c.[2]	—	3/19
	Festuca megalura	+	+	—	1/5
	Glyceria borealis	+	—	—	10/37
	Poa annua	+	—	—	3/6
	P. palustris	+	+	—	1/4
Hordeae	*Aegilops speltoides*	+	+	—	2/7
	A. ventricosum	+	+	+	1/4
	Agropyron elongatum	+	+	+	1/4
	A. intermedium	+	+	+	1/7
	A. pectiniforme	+	+	+	1/4
	A. repens	+	+	+	4/12
	A. scribneri	+	+	+	3/7
	A. sibiricum	+	+	+	2/3
	A. smithii	+	+	+	1/4
	A. spicatum	+	+	+	1/1
	A. subsecundum	+	+	+	2/7
	A. trichophorum	+	+	+	4/16
	Elymus canadensis	+	+	+	5/22
	E. caput-medusae	+	p.t.c.	—	2/8
	E. riparius	+	+	+	1/4
	Hordeum bulbosum	+	+	+	3/8
	H. jubatum	+	+	+	2/8
	H. vulgare	+	+	+	5/18
	Hystrix patula	+	+	—	6/22
	Lolium perenne	+	+	—	7/26
	Secale cereale	+	+	+	4/9
	Sitanion histrix	+	—	—	1/4
	Triticum aestivum				
	Dual	+	+	+	4/6
	Genessee	+	+	+	4/6
	Monon	+	+	+	4/6
	T. monococcum	+	—	—	3/16
	T. polonicum	+	+	+	1/4
	T. spelta	+	+	+	1/6
Aveneae	*Avena byzantina*	—	—	—	1/3
	A. sativa	—	—	—	1/3
	A. sterilis	+	—	—	1/1
Agrostideae	*Agrostis alba*	+	—	—	5/20
	A. pulchellus	+	+	—	1/4
	Alopecurus pratensis	+	—	—	3/17
	A. soongoricus	+	—	—	3/6
	Calamogrostis pseudophragmites	+	+	—	1/4
	Phleum pratense	+	—	—	1/3
Chlorideae	*Cynodon dactylon*	—	—	—	1/5

Table 1 (Continued)

Family/Tribe	Host	Epibiotic stage	Endobiotic stage	Resting spores	T/P
Phalarideae	*Phalaris arundinacea*	+	+	—	11/45
Paniceae	*Echinochloa crusgalli*	—	—	—	4/13
	Paspalum sp.	+	p.t.c.	—	5/15
Andropogoneae	*Andropogon* sp.	+	+	—	1/3
Maydeae	*Zea mays*	—	—	—	1/6
Alismataceae	*Alisma plantago-aquatica*	—	—	—	2/6
Juncaceae	*Juncus pelocarpus*	—	—	—	2/4
Ranunculaceae	*Ranunculus acer*	—	—	—	6/6
	R. pennsylvanicus	—	—	—	4/8
Rosaceae	*Potentilla anserina*	+	+	+	3/3
	P. palustris	—	—	—	2/3
Umbellifereae	*Sium suave*	—	—	—	12/24
Labiateae	*Lycopus americanus*	—	—	—	9/22
Scrophulariaceae	*Mimulus ringens*	—	—	—	2/5
Campanulaceae	*Lobelia cardinalis*	—	—	—	4/8

[1] T/P = total number of trials/total number of test plants.
[2] p.t.c. = primary turbinate cells only present.

Table 2. *Measurements of spores from different hosts used in the analysis of variance, and of spores from field-collected Agropyron repens*

Host	Length		Width	
	mean μ	S.D.[1]	mean μ	S.D.
Triticum aestivum (Monon)	19.3	3.2	16.8	2.4
Hordeum bulbosum	19.4	1.9	16.8	1.7
Agropyron trichophorum	20.1	2.6	17.0	1.6
Elymus canadensis	20.1	2.7	17.2	2.1
Agropyron repens	20.4	2.0	17.6	1.9
Triticum spelta	21.4	2.6	17.8	2.1
Agropyron sribneri	21.0	1.9	18.3	1.7
Potentilla anserina	23.0	1.9	19.8	2.4
Agropyron repens (field-collected)	22.1	2.5	17.8	2.2

[1] S.D. = Standard deviation.

formed on any of twenty species of grasses, in six genera of the *Hordeae*, and in *Potentilla anserina (Rosaceae)*, which is not even closely related to the host of the inoculum. The epibiotic stage, moreover, is present in representatives of six of the eight other grass tribes used. No stages of any kind were formed on the non-gramineous hosts save on *P. anserina*.

Although the effect of resting spore formation on seedlings and more mature plants was in many cases indistinct, the following general types

Table 3. *F-test for analysis of variance of the measurements of the two axes of resting spores of Physoderma from Agropyron repens formed in eight different hosts*

Source	d. f.	S. S.	s^2	F
		Long axis		
Between groups	7	987.81	141.1	25.0[1]
Within groups	792	4470.75	5.6	
Total	799	5458.56		
		Short axis		
Between groups	7	741.16	105.9	25.8[1]
Within groups	792	3218.20	4.1	
Total	799	3959.36		

[1] The probability therefore is much less than 5% that all spores could come from the same collection (SNEDECOR 1956, Table 10.5.3).

of host reaction caused by our fungus can be cited: indiscrete red pigmented areas, as on the blade of *Elymus canadensis*, light brown indiscrete spots as on *Agropyron sibericum*, 1 mm spots varying to coalescent streaks 30—40 mm long, as on *Secale cereale* and *Triticum aestivum*, and tiny leaden flecks up to 1 mm long as on *Hordeum vulgare*.

As earlier indicated, resting spore measurements attainable as a by-product of these cross-inoculations add another dimension to this study. Here we have a means of determining whether resting spore size in the *Physoderma* on *Agropyron* is a reliable taxonomic character.

From each of seven grass seedlings (*Triticum aestivum* var. Monon, *T. spelta*, *Elymus canadensis*, *Hordeum bulbosum*, *Agropyron repens*, *A. scribneri*, and *A. trichophorum*) and from one dicotyledonous plant (*Potentilla anserina*) used experimentally as hosts for the *Physoderma* originally collected on *Agropyron repens*, 100 mature resting spores were measured. Table 2 gives the means and standard deviations of these measurements in microns. For purposes of ready comparison, measurements of field-collected spores from mature *Agropyron repens* are also included.

To determine the statistical significance of our measurements of samples of spores from each of the several cross-inoculations made with the *Agropyron* fungus, an analysis of variance was carried out on the data obtained (Table 3).

The analysis of variance of resting spores in laboratory-inoculated seedlings shows that the chances of all these spores coming from the same sample is much less than 0.5 per cent. In other words, the resting spore size varies significantly among these hosts. It might further be noted here (Table 2) that a non-statistical comparison of spore sizes in laboratory-infected seedlings of *Agropyron repens* with those from field-collected, mature *Agropyron* plants also shows evident size differences.

Discussion

Historically, many species of *Physoderma* have been based (1) on host specificity, in most cases, as earlier indicated, merely assumed rather than tested, (2) on differences in resting spore size, and (3) on host reaction. Within the limits imposed by the materials used in our work, certain generalities can be made concerning these points. Our fungus on *Agropyron repens* infects all other nine species of that genus tried. Within the *Hordeae*, of the nine genera (including *Agropyron*) represented, all supported at least the epibiotic stage. Since the resting spore stage is the over-wintering one, the fungus could survive in that form on species of *Agropyron*, *Aegilops*, *Elymus*, *Hordeum*, *Secale*, and *Triticum* but on no other members of the *Hordeae* or of other tribes of grasses used here.

The epibiotic stage, somewhat surprisingly, seems not to be so particular as to its hosts. It can develop to maturity and sporulate on all members of the *Hordeae* and, in other tribes: in the *Aveneae*, on one species of *Avena*; all species, representing three genera used belonging to the *Agrostideae*; on *Phalaris* of the *Phalarideae*; on *Paspalum* of the *Paniceae*; and *Andropogon* of the *Andropogoneae*. We have shown earlier (Sparrow, Griffin and Johns 1961) that epibiotic zoospores can form the epibiotic stage as well as resting spore zoospores. Hence, our fungus could maintain itself epibiotically for considerable periods and through many sporangial generations on a variety of hosts if conditions favoring infection continued to prevail.

A note of economic interest is struck with the well-authenticated capacity of our quack-grass parasite to live on barley, rye and wheat and to produce both epibiotic and resting spore stages on these hosts. No great damage is brought about and we are not implying that such might be accomplished under field conditions. It is, however, a fact that they are attacked successfully by a parasite of an extremely common roadside weed. It should also be pointed out that any weeds harboring the epibiotic stage alone can, presumably, also act as a reservoir of infection. It will be recalled (Sparrow, et al. 1961) that epibiotic zoospores can form the endobiotic as well as epibiotic stages.

As indicated, the non-gramineous plants used as possible hosts were all ones growing in the study area and known to harbor *Physoderma*. No infection occurred on them with the exception of the single and unquestioned instance of *Potentilla anserina*, an European pond-margin weed. This rosaceous host seems particularly susceptible, since a *Physoderma* on the umbellifer *Sium suave* can also form both stages on it. This raises the question of the susceptibility of *Agropyron repens* itself to *Physoderma* spp. from other hosts, a matter which must await study. It may be mentioned at this time, however, that a *Physoderma* on maize ("*P. maydis* Miyabe"), one on *Sium suave* ("*P. palustris* Sparrow"), one

on *Potentilla anserina* ("*P. vagans* Schroeter"), and one on *Phalaris arundinacea* ("*P. gerhardti* Schroeter") will all infect it.

As may be seen, at present no clear pattern emerges as to the limits of host range of our *Physoderma* on *Agropyron*. Most certainly, the greatest "degree of infection" as expressed in the formation of resting spores, is produced on members of the *Hordeae* but also on *Potentilla anserina*. Representatives of other grass tribes, however, are infected, in the sense that there is established in them by the epibiotic stage a positive host-parasite relationship eventuating in sporulation. Clearly, in delineating species of *Physoderma* on a basis of host plant, experimental evidence must accompany the claim for such specificity.

A second aspect of this study is the quantitative one. Reference has already been made to the separation of species on differences in size of resting spores. In some instances a case might be made for this. For example, resting spores of *Physoderma maculare* Wallr. have means of approximately 28×34 μ (SPARROW 1964) whereas in those of *P. calami* Krieger, they are 13×17 μ (SPARROW 1964). Where the differences are not great, however, (for example, less than 3 μ) justification for species separation on the basis of resting spore size alone is hardly warranted in the light of our findings. From our data it appears that the particular strain or species of *Physoderma* with which we worked, under the conditions imposed by our procedures, does not have a resting spore size which is strictly limited by inheritance. Rather, the size varies as much as 3 μ depending upon the host and even upon the degree of maturity of the host in which the spore is formed. This may well depend, in turn, upon host cell size, number of maturing resting spores in a cell, and hence, availability of nutrients. It should be emphasized that the magnitudes of differences in measurements recorded by us in this one fungus attain and even exceed those intended to distinguish between certain published species of *Physoderma*.

Lastly, host reactions may now be considered. Since they are not, strictly speaking, a part of the fungus, but only a function of its presence on the host and age of infection at time of collection, they are in essence a matter of secondary consideration. Our fungus can produce differing effects on various hosts as indicated earlier. We do not minimize the fact that some *Physoderma* spp. do produce very marked and very different host reactions. On grasses, however, except for stunting and associated effects, the variation seems small indeed, and the descriptions of these reactions are often too subjective to be significant.

Applying our findings to actual described graminicolous species[1] we present the following recent descriptions. Both of these are by the

[1] In utilizing these two species as examples, the senior author wishes to add that he, too, has indulged in similar malpractices, and that had his "species" been concerned with gramineous hosts, would have used them.

same authors and concern two supposedly different species of *Physoderma*. The hosts belong to different genera of the tribe *Paniceae*.

Species A

"Inciting dark olive-green to brownish streaks on leaf sheaths and stems, 1 to 1.5 mm long, coalescent with each other, slightly raised. Rhizomycelium tenuous, intracellular. Resting sporangia intracellular, 2 to 3 in each cell, globose to ovate, pale yellowish-brown, flattened on one side, smooth, measuring $17.2—33 \times 14.3—24.3$ μ with a mean of 22.6×18.7 μ. Germination not observed."

Species B

"Infection spots minute, brown, 1 to 1.5 mm in diameter, slightly raised, often coalescent. Rhizomycelium intracellular, tenuous. Resting sporangia intracellular, ovate globose to reniform, occasionally irregular, yellowish-brown, smooth, thin-walled, with a lateral depression, measuring $15.7—26 \times 11.5—24.3$ μ with a mean of 20.8×17 μ."

The noted differences in host reactions described above can all be duplicated by the *Agropyron* fungus on various grasses. Thus, on *Triticum*, streaks are formed whereas on *Hordeum*, spots are produced. The lack of information on the epibiotic stage, whether or not it even exists, and the paucity of data on the endobiotic thallus gives a very fragmentary picture of these organisms. Differences in sizes of resting spores of the two are not very meaningful in distinguishing them since the ranges of lengths and widths overlap and the means of these measurements differ by only 1.8 μ and 1.7 μ, respectively. Our fungus, it will be recalled, formed resting spores with mean measurements on different hosts varying as much as 3.7 μ in length by 3.0 μ in width. Certainly, size differences alone do not distinguish species A and B. Lastly, differences in host genera, within the same tribe, as these are, to be significant at the species level, would have to be well-authenticated, since we found our fungus would form resting spores on five other generic representatives of the tribe to which *Agropyron*, the original host, belongs. We can not, of course, disprove that species A and B are host specific, but our findings would make us want substantial proof of this fact. Other characters cited by the authors could apply to any of a wide range of species.

It is evident that we cannot make sweeping conclusions as to the descriptive data necessary for proper diagnoses of all species of *Physoderma* on the basis of this study of one fungus. We can emphasize, however, that a meaningful species description can only be derived from a developmental study of the living fungus, including both epi- (if formed) and endobiotic stages. Furthermore, an effort should also be made to discover something of the host range of the parasite. Spore color and

size, host plant and host reactions should most certainly be indicated but these should not constitute the major part of the information used in delineating the species as was done a century ago.

Summary

1. A *Physoderma* on *Agropyron repens*, common quack grass, infects in the laboratory all other congeneric hosts used.

2. Within the Tribe *Hordeae*, to which *Agropyron* belongs, representatives of nine genera support growth of some phase of the fungus. The roseaceous host *Potentilla anserina* is also infected. Other plants associated in the field with infected *Agropyron* and known to harbor *Physoderma* are not susceptible to it.

3. Statistical study of resting spore size of the same *Physoderma* on different gramineous hosts indicates there are significant size differences on these as well as on seedlings when compared with mature plants. These size differences are greater than those utilized by some investigators in distinguishing supposed new species.

4. Host reaction differences seem more a function of the host and not of the fungus in this study.

5. Two specific published examples of supposedly different species are analyzed in the light of findings from the *Agropyron* parasite and are shown to be inadequate in the criteria used to distinguish them.

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Literature

SNEDECOR, G. W.: Statistical methods. XIII + 534 pp. 5th Ed. Iowa State College Press, Ames. (1956).

SPARROW, F. K.: Observations on chytridiaceous parasites of Phanerogams. XIII. *Physoderma maculare* Wallroth. Arch. Mikrobiol. **48**, 136—149 (1964).

— Observations on chytridiaceous parasites of Phanerogams. XIV. *Physoderma calami* Krieger. Amer. J. Bot. (in press) (1964).

—, and R. M. JOHNS: Observations on chytridiaceous parasites of Phanerogams. IX. Epibiotic sporangial stages of *Physoderma* collected in the field. Arch. Mikrobiol. **34**, 93—102 (1959).

— JOYCE E. GRIFFIN, and R. M. JOHNS: Observations on chytridiaceous parasites of Phanerogams. XI. A *Physoderma* on *Agropyron repens*. Amer. J. Bot. **48**, 850—858 (1961).

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