STUDIES ON THE MORPHOLOGY, PHYSIOLOGY AND PARASITISM OF THE GENUS *PIRICULARIA* IN MADRAS*

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The genus *Piricularia* belonging to the group *Hyphomycetes* is well known as the causal organism of the ‘blast’ disease of rice (*Oryza sativa* Linn.), ‘ragi’ (*Eleusine coracana* Gaertn.), and ‘tenai’ or the Italian millet (*Setaria italica* Beauv.) bringing about a heavy loss of food grains—as heavy as 76% or more (McRae, 1922). Other important hosts are the banana (‘pitting disease’—Hoette, 1936; Van Hook, 1926), *Triticum vulgare* Vill. (Anstead, 1924), *Theobroma cacao* Linn. (Saccardo, 1875) and *Zingiber officinale* Rosc. (Nisikado, 1927). Several grasses also serve as hosts.

The genus has a wide distribution, being present in all tropical regions, especially the rice-growing tracts. Work on *Piricularia* has been carried out in all these regions, chiefly Italy, Japan and India and rice being the most important of the hosts, *Piricularia* on rice (called *Piricularia oryzae* Cav.) has received most attention. This form which causes the ‘brusone’ of rice (as the disease is called in Italy) was first recorded in Italy by Briosi e Cavara (1892) and much work has since been done on this fungus. In Japan work on ‘blast’ of rice (called ‘Ine Imochibyo’) was started by Hori (1898) and was continued by Kawakami (1901, 1902) and Miyake (1909, 1910). Much useful work on the host parasite relationship, leaf anatomy in relation to resistance, entry of the pathogen into the host, viability, breeding for resistance and methods of control was being done in research centres like Hokkaido and Formosa by numerous workers. Nisikado (1917, 1927) has made an attempt to assign a few Japanese isolates of *Piricularia* from different hosts to their systematic position within the genus with the help of their morphology, physiology and parasitism. In India the Madras Department of Agriculture has been tackling the ‘blast’ disease of cereals since 1918, when it was first recorded (McRae, 1920) as a serious outbreak in the Tanjore delta; much work has been done on the economic side of the problem, viz., devising methods of control, breeding for resistance, varietal trials for selecting resistant varieties of cereals and cultural practices to eliminate the disease (McRae, 1920–1923; Sundaraman, 1922–1936; and Thomas, 1930, 1931 and 1936–1941).

* Formed part of a thesis accepted for the M.Sc. degree of the University of Madras.

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Morphology, Physiology & Parasitism of the Genus Piricularia

The present communication includes a study of the morphology, physiology and parasitism of four isolates of *Piricularia* isolated from important cereals like *Oryza sativa* Linn., *Setaria italica* Beauv., and *Eleusine coracana* Gaertn., and the grass *Digitaria marginata* Link.

**MORPHOLOGICAL STUDIES**

**Materials and Methods.**—*Piricularia* was isolated from infected leaf tissues of rice (*Oryza sativa* Linn.—strain Adt. 10), 'ragi' (*Eleusine coracana* Gaertn.—strain E.C. 593 of the Millets Specialist, Coimbatore), ‘tenai’ or the Italian millet (*Setaria italica* Beauv.—local Coimbatore variety) and *Digitaria marginata* Link by single spore isolation method. Stock cultures were maintained on oat-meal-agar slants and on sterilised leaf bits of the respective hosts in Roux tubes. All work, which necessitated the study of fresh mycelia or spores direct from the hosts, was done during November to January, when the crop at Coimbatore showed maximum infection in the fields.

**Symptoms of infection.**—In rice, ‘ragi’, ‘tenai’ and *Digitaria* the fungus causes the characteristic leaf spots. In rice and ‘ragi’, in addition to the leaf spots in their younger stages, there is a darkening of nodes and necks of earheads after the flowering of the crops. The blackening of the necks and nodes is, however, absent in the case of ‘tenai’ and *Digitaria*. That the attack by *Piricularia* is restricted to the foliage in *Digitaria* sp. has been observed by Hansford (1943) in Uganda.

**Nature of leaf-spots.**—Spindle shaped dark-brown leaf spots are met with on leaves of rice, ‘ragi’ and *Digitaria* while spots on the leaves of ‘tenai’ are more or less circular. The spots invariably appear on either side of the mid-rib. On leaves of rice and ‘ragi’ the spots are found to be 1 to 3 cm. long. Weather conditions favouring development, neighbouring spots coalesce and very long spindles are formed and the leaf tissue rots in the middle and gets torn. The spots are greyish in the centre and brownish in the periphery. In ‘tenai’ the leaf spots are smaller and scattered. The spots are about 2 to 5 mm. in diameter, light brown in the centre and dark brown in the periphery.

**Mycelia.**—There is no appreciable difference between the mycelia of the different isolates. They are all thin, hyaline and straight when young. In older cultures they are thicker, hyaline and straight when young. In older cultures they are thicker and attain a slightly brownish tinge and are variously contorted, developing swellings. In all the isolates the breadth of the mycelium varies from 1.4 to 5.8 µ.

**Conidiospores.**—The spores of all the isolates are hyaline and top-shaped. They are mostly three-celled (rarely two- or four-celled). The
spores may be straight or slightly bent. Some of them are very long and narrow while some are fairly broad. In any case the spores are longer than broad. They are borne in a scorioid manner on conidiophores which are hyaline, their septa being very prominent. The fungus puts forth the conidiophores through the stomata of the affected parts of the plant and conidia are exposed to the air. In leaves the conidia are met with in the spotted region both on the upper side and on the lower side of the leaf-blade but more abundantly on the upper side. The spores vary in length from about 19 to 37 μ and in breadth from 7 to 15 μ and show no appreciable difference between the isolates.

The following table gives the average measurement of 200 spores taken of the four isolates:

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Measurements expressed in microns)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pircularia from</th>
<th>Spores from the living host</th>
<th>From sterile leaf bits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Breadth</td>
</tr>
<tr>
<td>Rice</td>
<td>Mode</td>
<td>26.56</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>29.22</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>20-75 to 37-35</td>
</tr>
<tr>
<td>' Tenai'</td>
<td>Mode</td>
<td>24.90</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>26.00</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>19.92 to 31.54</td>
</tr>
<tr>
<td>' Ragi'</td>
<td>Mode</td>
<td>24.50</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>26.00</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>19.60 to 30.00</td>
</tr>
<tr>
<td>Digitaria</td>
<td>Mode</td>
<td>24.00</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.80</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>19.20 to 30.30</td>
</tr>
</tbody>
</table>

In all the four strains the end cells of the spores germinate readily when kept in a drop of water in a Van Tieghem cell.

Chlamydospores.—These are almost alike in all the isolates, round in shape, thick-walled, varying from 4 to 10 μ in diameter. These are brownish green in colour. Both terminal and intercalary chlamydospores are met with.

It is thus evident that morphological characters of the isolates do not afford much basis for their classification as they do not show significant differences. Nisikado (1917), though he has obtained similar results, has separated the strains on Setaria italica Beauv. into a new species—Pircularia
Setaria Nisikado, basing his classification on the size of the individual cells of the spores, the basal appendage and the size of the germinating hyphae. But the variations obtained by him within the groups, viz., Oryza form and Setaria form, are too great and, therefore, any generalisation to include a particular spore in one group or the other may appear empirical and not rigidly scientific. Taking for instance the measurements of the conidia they vary from 14 to 40 μ × 6 to 13 μ in the Oryza form and 14 to 35 μ × 5 to 12 μ in the Setaria form. In the case of the individual cells of the conidium, taking the middle cell, it varies from 4.8 to 12.0 μ in the Oryza form and 4.8 to 8.3 μ in the Setaria form. It will now be difficult to say whether spores measuring say between 14 and 35 μ in length or 5 and 12 μ in breadth or having middle cells measuring between 4.8 to 8.3 μ belong to the Setaria or the Oryza form. The importance of taking morphological evidence in collaboration with physiological needs, therefore, no emphasis and this will be discussed at a later stage.

Physiological Studies

Materials and Methods.—The cultures of the four isolates used for the morphological studies were used for the physiological studies as well. Throughout the investigation standard mycological technique was followed and all the experiments were conducted at a constant temperature of 30° C. unless otherwise specified. For all the experiments a synthetic medium (Ramakrishnan, 1941) of the composition, glucose 10 gm., peptone 6 gm., K₂HPO₄ 1.75 gm., MgSO₄ 0.75 gm., water 1 litre, agar 2% (for all solid media) was used unless otherwise stated. This is referred to as the Standard medium. In the case of growth studies care was taken to transfer equal quantities of the inoculum to the media in dishes or flasks. For obtaining the dry weight of mat the isolates were grown for a definite period in 50 ml. of the medium in 100 ml. flasks, the mat filtered through a gauze crucible, washed and dried in a hot water oven, to constant weight. All pH determinations were made with the help of a Quinhydrone Electrometric pH indicator and colour determinations with Ridgeway’s (1912) colour nomenclature.

Growth of the strains on media.—Linear growth of the colonies of the four isolates on standard medium agar, Richard’s agar, Brown’s agar, oat-meal agar, French been agar and the decoction agars made of the leaf material of rice, ‘ragi’ and ‘tenai’, was determined. The amount of mat produced by the isolates in the Standard medium, Richard’s medium Brown’s medium and decoctions of leaf material of rice, ‘ragi’ and ‘tenai’ was also determined. The results are represented diagrammatically below:
Graph I.—Showing the average daily growth in diameter of the colonies of the four isolates in millimetres on different agar media.

O.M.A. for oat meal agar; F.B.A. for French bean agar; R.A. for Richard's agar; B.A. for Brown's agar; P.D.A. for rice leaf decoction agar; R.D.A. for 'ragi' leaf decoction agar; T.D.A. for 'tenai' leaf decoction agar; St.M.A. for standard medium agar.

O, E, S and D represent isolates from O. sativa, E. coracana, S. Italica and D. marginata.

Graph II.—Showing the comparative amounts of mat produced by the isolates in different media.


O, E, S and D represent isolates from O. sativa, E. coracana, S. Italica and D. marginata.)
The results of the above experiments agree with those of Nisikado (1927) in that the Madras isolates also produce good growth on the decoctions of their host material as did the Japanese isolates. The Madras isolates produced yellowish olive colouration in synthetic media containing sugars. While linear growth is best on 'ragi'-leaf decoction agar, the isolates produce maximum amount by weight of mat in 'tenai'-leaf decoction agar, showing that measurement of linear growth in terms of increase in diameter of the colonies does not always give a correct idea of the amount of mat produced.

**Optimum temperature for growth.**—Linear growth of the colonies of the isolates and the weight of mat produced by them at different temperatures are given in the following graphs.

![Graph III](image_url)

**Graph III.**—Showing the average growth in diameter of the colonies in millimetres at different temperatures.

--- isolate from *O. sativa.*
--- isolate from *E. coracana.*
--- isolate from *S. italica.*
--- isolate from *D. marginata.*

Thomas (1940) obtained best growth of a strain of *Piricularia,* isolated from 'ragi' at 29.5°C. Abe (1930) and Yoshii (1936) showed that 28°C was the best for isolates from *O. sativa.* Nisikado (1927) has reported best growth of isolates from *Setaria* and ginger between 23 and 28°C, and the maximum temperature at which growth of the strains from rice was possible at 36 to 37°C.

**Effect of pH of the medium on the growth of the isolates.**—The following graphs show the rate of linear spread of the isolates and the weight of mat produced by them in the Standard medium at different pH levels.
GRAPH IV.—Showing the comparative amounts of mat produced by the isolates in liquid medium at different temperatures.

- - - - isolate from *O. sativa*.
- - - - isolate from *E. coracana*.
- - - - isolate from *S. italicca*.
- - - - isolate from *D. marginata*.

GRAPH V.—Showing the average rate of daily growth of colonies of the isolates at different pH levels.

- - - - isolate from *O. sativa*.
- - - - isolate from *E. coracana*.
- - - - isolate from *S. italicca*.
- - - - isolate from *D. marginata*. 
Graph VI.—Showing the comparative amounts of mat produced by the isolates at different pH levels.

- isolate from O. sativa.
- isolate from E. coracana.
- isolate from S. italicca.
- isolate from D. marginata.

Nisikado (1927), with isolates from O. sativa, obtained best growth between pH values 5 and 10. A highly pathogenic strain grew best at pH 4.4. Thomas (1940) reports optimum growth of a strain from 'ragi' between pH 5 and 6. The present investigations show that round about pH 7 is the optimum for both dry weight and for radial spread irrespective of the isolates used. Though the variation in the weight of mat produced by the isolates is prominent the peak production of the mycelium both on dry weight basis and on radial spread was optimum for all the isolates at pH 7. During growth the isolates tend to bring the pH of the medium to round about 5 and 6.

Influence of different sources of carbon in the medium on growth.—One of the following, viz., glucose, maltose, lactose, sucrose, soluble starch and
cellulose was tried as the source of carbon in the medium at the 2% level. The results are shown below:

**Graph VII.**—Showing the average growth in diameter of the colonies of the isolates in standard medium to which different carbohydrates were added.


According to Tochinai and Nakano (1940) certain strains of *P. oryzae* appear to be capable of utilising higher alcohols like glycerine and mannite as the carbon source. Among the carbohydrates tried here they prefer maltose, soluble starch and glucose, in the order of their mention. Yoshii (1936) reports pectin as a good source of carbon for growth of *P. oryzae*. 
Influence of different sources of nitrogen on growth.—The capacity of the isolates to utilise nitrogen from different sources was investigated using the following nitrogenous compounds in pure form: potassium nitrite, potassium nitrate, ammonium sulphate, urea, asparagin and peptone. Five grams of potassium nitrate was added to a litre of the standard medium.

**Graph IX.**—Showing the average daily increase in diameter of the colonies of the isolates in media with different nitrogen sources.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.

**Graph X.**—Showing the comparative amounts of mat produced by the isolates in media with different nitrogen sources.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*. 
The other nitrogenous compounds were used in quantities calculated to contain an equivalent amount of nitrogen, i.e., potassium nitrate 5 gm., potassium nitrate 4·25 gm., ammonium sulphate 3·1 gm., urea 1·5 gm., asparagin 3·3 gm., and peptone 5·9 gm. The above diagrams show the results.

Yoshii (1936) observed that P. oryzae did not reduce nitrate and was found to be injured by nitrite. Tochinai and Nakano (1940) observed that the best source of nitrogen for P. oryzae was peptone followed by sodium nitrate, asparagin, glutonic acid and acetamide. In the present investigation all the isolates do not favour potassium nitrite as the source of nitrogen in an agar substratum and growth is totally inhibited. Peptone gives the best growth both on solid and in liquid media showing that they prefer organic nitrogen to inorganic.

*Effect of different Carbon/Nitrogen ratios on the growth of the isolates.*—Different proportions of carbon and nitrogen sources were used keeping the total weight of glucose and peptone at 16 gm. per litre of the Standard medium. The experiment was conducted in liquid medium and the weights of mat produced by the isolates at different C/N ratios is given in the accompanying graph.

![Graph XI](image-url)  

*Graph XI.*—Showing the comparative amounts of mat produced by the isolates in liquid medium at different C/N levels.

- --- isolate from *O. sativa.*
- --- --- isolate from *E. coracana.*
- --- --- --- isolate from *S. italicca.*
- Δ---Δ---Δ---Δ isolate from *D. marginata.*
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It is found that the weight of mat produced increases as the C/N ratio increases and best growth is obtained at C/N = 5/1. Ramakrishnan (1941) also obtained similar results with isolates of Colletotrichum falcatum.

Enzymes produced by the isolates during metabolism.—The production by the isolates of the enzymes diastase, inulase, lipase, erepsin, amidase and trypsin was tested quantitatively by the method adopted by Uppal and Kulkarni (1937) in their work on Fusarium, based on the method of Crabill and Reed (1915). The production of maltase, sucrase, lactase and urease was tested in vivo by the method followed by Garren (1938) in his studies on Polyporus abietinus.

**Table II**

Table showing the production of the different enzymes by the isolates

<table>
<thead>
<tr>
<th>Name of enzyme</th>
<th>Medium used</th>
<th>pH of medium</th>
<th>Piricularia isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Oryza sativa</em></td>
</tr>
<tr>
<td>Diastase</td>
<td>Soluble starch</td>
<td>6.62</td>
<td>+</td>
</tr>
<tr>
<td>Inulase</td>
<td>Inulin</td>
<td>6.78</td>
<td>+</td>
</tr>
<tr>
<td>Lipase</td>
<td>Litmus cream agar</td>
<td>7.29</td>
<td>+</td>
</tr>
<tr>
<td>Erepsin</td>
<td>Casein agar</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Amidase</td>
<td>Asparagin Rosalic acid agar</td>
<td>6.44</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Egg albumen agar</td>
<td>6.35</td>
<td>+</td>
</tr>
<tr>
<td>Sucrase</td>
<td>Sucrose</td>
<td>6.82</td>
<td>+</td>
</tr>
<tr>
<td>Maltase</td>
<td>Maltose</td>
<td>5.93</td>
<td>+</td>
</tr>
<tr>
<td>Lactase</td>
<td>Lactose</td>
<td>6.26</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>Urea</td>
<td>8.80</td>
<td>-</td>
</tr>
</tbody>
</table>

[+ shows production. – shows non-production.]

It is seen from these results that all the four isolates produce diastase, inulase, lipase, amidase, trypsin, sucrase, maltase and lactase and do not produce urease. The isolates from *E. coracana* and *S. italica* produce erepsin, while those from rice and *D. marginata* do not. Yoshii (1936) has detected oxidase and dehydrase in cultures of *P. oryzae*.

**CROSS INOCULATION STUDIES**

Cultures of the isolates were maintained on sterilised leaf bits of the respective hosts. This facilitated a ready supply of spores whenever required as the fungi produced abundant conidia on these media and remained viable for long periods.

Seedlings were raised from healthy seeds in small pots. Garden soil was used for raising *S. italica* and *E. coracana* while soil from rice fields was used for raising rice and *Digitaria* seedlings. In each experiment 25 pots of
the seedlings were raised for each host inside glass cages which had been previously disinfected with a spray of 2 in 1,000 mercuric chloride solution, five pots in each cage. When the seedlings had grown to a height of about a foot, the seedlings were thinned out so that each pot contained about four healthy seedlings. The chambers were all kept in the shade in the pot-culture house. The experiments were conducted during the months November to January as infection by *Piricularia* was maximum in the fields during these months at Coimbatore.

Spore suspensions of the isolates were prepared in sterile atomisers. The inside of the cages was given a spray of clean water with a sprayer to raise the humidity, as high humidity favours infection by *Piricularia*. The spore suspensions of the isolates were sprayed on the plants in the four chambers each chamber receiving the spores of one of the isolates. The cages were kept closed. The plants in the fifth chamber formed the un-inoculated controls.

Observations were made daily for the characteristic symptoms of infection by *Piricularia*. The symptoms, namely the leaf spots, appeared within 4 to 7 days after inoculation. The experiments were repeated thrice over two seasons. The following hosts of *Piricularia* were taken up:

1. *Oryza sativa* Linn. .. rice strain G.E.B. 24 of the Paddy Specialist, Coimbatore.
2. .. .. *Eleusine coracana* Gaertn. · ‘ragi’ strain E.C. 593 of the Millets Specialist, Coimbatore.
4. *Digitaria marginata* Link. .. seeds obtained from the Millets Specialist, Coimbatore.

Variety G.E.B. 24 of rice is a fairly resistant strain evolved by the Government Economic Botanist, Coimbatore. Adt. 10 is a very susceptible variety grown in the deltaic tracts of the Tanjore District of Madras.

The results are given below:

<table>
<thead>
<tr>
<th><em>Piricularia</em> isolated from</th>
<th>Hosts tried</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rice G.E.B. 24</td>
</tr>
<tr>
<td><em>O. sativa</em></td>
<td>..</td>
</tr>
<tr>
<td><em>E. coracana</em></td>
<td>..</td>
</tr>
<tr>
<td><em>S. italica</em></td>
<td>..</td>
</tr>
<tr>
<td><em>D. marginata</em></td>
<td>..</td>
</tr>
<tr>
<td>Control</td>
<td>..</td>
</tr>
</tbody>
</table>

+ indicates infection.  - indicates non-infection.
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It may be mentioned here that the isolate from *E. coracana* when infecting *S. italica* forms circular spots on leaves while the isolate from *S. italica* forms spindle-shaped leaf spots on *E. coracana* when infecting it. This shows that the nature of the leaf spot is due to certain qualities of the leaves of the host and not of the pathogen.

It may be concluded from the results of the experiments that the isolate from rice is distinct in that it infects only rice. The isolates from 'ragi' and 'tenai' each infects the host of the other in addition to its own. The isolate from *Digitaria*, in addition to its own host, infects 'ragi' and rice. These observations agree with those of Thomas (1940), who reports that the strain from 'ragi' and 'tenai' both infected 'ragi' and 'tenai' but not rice. Nisikado (1927) was not also able to get infection on grasses and cereals other than rice with *Piricularia* isolated from rice.

**Discussion and Conclusion**

There has been endless controversy and difference of opinion whether characters relating to morphology alone should form the basis for classification of the fungi into groups or whether their physiology, pathogenicity and host relationship should also be taken into consideration. Where there is marked morphological difference between the given members of the genus, it is an easy matter. But, when morphological differences are not very marked or are absent, the question must be asked whether classification should be based on such hair-splitting differences as variation of one or two microns in spore and mycelial measurements.

Turner (1940) compared *Ophiobolus* sp. from oats with isolates of *O. graminis* Sacc. from wheat, and found it difficult to distinguish one group from the other with the help of physiological characters. On the other hand marked differences were noticed in the length of the ascospores of the isolates from wheat and the ones from oats. All the isolates, however, produced similar symptoms in susceptible hosts. In spite of the differences in the morphological characters and in the host relationship, Turner (1940) considered the isolates from oats only as a new variety of *O. graminis* Sacc., *viz.*, *O. graminis* Sacc. var. *Avena* Turner, because of their similarity in cultural behaviour and symptoms produced in the susceptible hosts.

Leonian (1932), working on the pathogenicity and viability of *Fusarium moniliforme* Sheldon, deplores the common notion that dissociants "played havoc with taxonomy while on the contrary they are of inestimable aid in bringing order out of chaos. We have been in the habit of describing the species according to its morphological characters; we have usually failed to make intensive cultural studies, and have endeavoured to formulate fundamental
truths by superficial observations. To many of us form and size of spores and reproductive bodies constitute the sum total of the mycological concept, and the vital life processes of the fungi have little taxonomic value in our scheme of classification.”

Leonian (1925) in an earlier work employed certain physiological features as manifest on solid agars and the host relationship to overcome the uncertainty of a purely morphological classification of some Phytophthora, for the specific distinction within the genus were very limited, as its members exhibited remarkable uniformity of morphological characters. He believes that the average of all the morphological, physiological and pathological features should form the specific sphere.

Padwick (1939), while criticising Wollenweber and Reinking (1935) for their “indiscriminate use” of morphological and physiological characters in their scheme of classification of the Fusaria, advocates the adherence to their system for, he says, “Inadequate though it may be, there is nothing better.” He would base specific rank upon characters easily recognisable “under standard conditions available in all moderately equipped mycological laboratories”. According to him, classification based on the use of host parasite relationship must be given a rank lower than the species, for it demands the use of a pure line of host and definite conditions such as temperature and moisture as has been shown by workers at Wisconsin, and soil conditions as shown by Mundkur (1936) working with the cotton wilt in India.

In the light of the above and the results obtained during these studies, it might be considered how the four isolates studied could be grouped. Of the four isolates, the one on Oryza sativa Linn. has been given a specific name P. oryzae Br. e Cav. ever since 1892. Nisikado (1917) working on isolates of Piricularia from O. sativa, Setaria spp., Zingiber spp. and some grasses reports that these forms “showed many differences among themselves morphologically and physiologically” and has, on this basis given the form of Piricularia on Setaria spp. the status of a species namely, P. Setariae Nisikado. A reference to the data put forth by him by way of morphological differences would show that emphasis could not be laid on measurements of the kind envisaged. The variations of individual measurements within the groups, namely, Oryza form and Setaria form, are too great and, therefore, generalisation to include any particular spore in one group or the other, may appear empirical and not rigidly scientific. It does not, therefore, appear proper that the separation of the Setaria form should have been based on such data. The isolates from Eleusine
coracana Gaertn. and Digitaria marginata Link. have not been assigned their systematic status so far.

From the results of the present observations it may be said that a study of the morphological characters does not help in distinguishing between the four isolates, there being no characteristic difference in the mycelia, conidio-phores, conidia and chlamydospores. The only differences observed are in the nature of infection—(only foliar infection in the case of Digitaria and Setaria; and foliar, nodal and earhead infection in O. sativa and E. coracana, and the nature of the leaf spot, more or less roundish in S. italicca and spindle-shaped in others). Even the nature of the leaf spots depends only on the host, as the Setaria isolate, when infecting E. coracana forms spindle-shaped spots and the Eleusine isolates when infecting S. italicca form roundish spots. From the results of the physiological studies, the Digitaria isolate may be said to be distinct from the other three.

(1) It forms slate grey growth on oat-agar, while the others give pale gull grey growth. Unlike the other isolates, it does not grow well on Brown's agar.

(2) The Digitaria isolate does not very much favour cellulose as the carbon source, while the other isolates grow well in agar medium with cellulose as the carbon source.

(3) The Digitaria isolate does not grow in agar media with ammonium sulphate as the nitrogen source, while the other isolates, though they do not very much favour ammonium sulphate, grow fairly well with ammonium sulphate in the agar medium.

(4) 30° C. is the optimum temperature for growth, in liquid cultures of the isolates except the one from Digitaria which shows optimum growth at 15° C.

(5) Isolates from E. coracana and S. italicca produce the enzyme erepsin, whereas the isolates from O. sativa and D. marginata do not.

It may thus be seen that the Digitaria isolate has certain physiological characters of its own.

It is very difficult to distinguish between the isolates from O. sativa, S. italicca and E. coracana from their physiology, as they do not differ very much in their behaviour in culture media. The only differences are:

(1) The isolate from O. sativa differs from the other two by its inability to produce erepsin.

(2) The isolate from E. coracana shows better growth on agar medium with ammonium sulphate as the nitrogen source than with urea, whereas the others prefer urea to ammonium sulphate.
(3) In liquid medium maltose is the best source of carbon for the isolate from *E. coracana* while the other isolates grow best with lactose.

Cross inoculation experiments show that the *Piricularia* isolate from *O. sativa* is distinct from the other three isolates from *E. coracana*, *S. italica* and *D. marginata* in that it does not infect any host other than *O. sativa*. Nisikado (1927) also did not get infection with isolates from *O. sativa* on any host other than *O. sativa*.

The isolate from *S. italica* infects its host and *E. coracana* but not *D. marginata* or *O. sativa*. Similarly, the isolate from *E. coracana* infects in addition to its host *S. italica* and not *O. sativa* and *D. marginata*.

Nisikado (1917, 1927) did not study isolates from *E. coracana* and *D. marginata* nor did he use these hosts in his studies. He has doubted the ability of the isolates from *Setaria* spp. to infect *O. sativa*.

The isolate from *D. marginata* is able to infect *O. sativa* and *E. coracana* in addition to its host. It should be noted here that isolates from *O. sativa* and *E. coracana* do not infect *D. marginata*.

Summing up, the various *Piricularia* isolates can be classified into three groups:

(a) Isolates that attack only their natural hosts, *e.g.*, isolate from *O. sativa*.

(b) Isolates that attack their own hosts and one other, *e.g.*, isolates from *E. coracana* and *S. italica*.

(c) Isolates that attack their own hosts and two more, *e.g.*, isolate from *D. marginata*.

If morphology could alone be depended upon as the basis of classification it will be impossible to place the isolates systematically. If Leonian's (1925) suggestion of taking the sum-total of all the characters, morphological, physiological and pathological, is to be followed, it may be necessary to study more forms before arriving at some conclusion. The system of Wollenweber and Reinking (1935) of studying physiological characters in addition to morphological is somewhat similar to Leonian's (1925) suggestion and, indeed, has been emphasised by Padwick (1939) who has recommended the use of characters easily recognisable under standard conditions in all moderately equipped mycological laboratories and this presumably includes cultural studies as well. Padwick (1939) has also admitted of a classification based on the host relationship, though as inferior in rank to the species, if a pure line of the host and definite conditions of temperature and moisture
were assured. In the present studies, the hosts used were the susceptible varieties and the experiments were conducted under conditions most favourable for infection (under ideal weather conditions in Coimbatore during the months November to January, when the crops showed maximum infection in the field) thus incorporating the suggestions of all the workers in the experimental technique.

Nevertheless, it has been found difficult, if not impossible to draw bold lines of demarcation between subtle differences that exist among the different isolates of *Piricularia* studied here. Although very prominent and specific differences between the physiological behaviour and degrees of pathogenicity have been noticed among the isolates, it has been found impossible to detect significant morphological differences. This study, without making extravagant claims, has established the importance of attacking problems of classification of fungal parasites from three angles, namely, physiology, pathology and morphology, and the final analysis of the results to be undertaken by studying the three aspects interdependently and not independently. Further work with larger number of isolates from more hosts that the fungus *Piricularia* is known to attack, possibly grasses under the monocots and other hosts like *Theobroma cacao* Linn. among the dicots would enable significant comparisons easier and may result in establishing different species or varieties of the same species. Until such time, this work enables us to conclude that the various strains of *Piricularia* studied here can only be considered from a broad evolutionary point of view and they have to be provisionally placed as physiological races within the genus and species *Piricularia oryza*.

**Summary**

The morphology of four isolates of *Piricularia* from *Oryza sativa* Linn., *Eleusine coracana* Gaertn., *Setaria italica* Beauv., and *Digitaria marginata* Link., has been studied. No appreciable difference in the morphological characters of the isolates was noticed.

The physiology of the isolates was studied. A study of their growth on different media, the optimum temperature and pH range for their growth was made. The effect of different sources of carbon and nitrogen and their different ratios in the medium on the growth of the isolates was investigated. The enzymes produced by the isolates were qualitatively tested for.

Cross-inoculation studies were conducted under optimum conditions for infection of the hosts.

The studies reported in this paper have established the importance of attacking problems of classification of fungal parasites from three angles,
namely, morphology, physiology and pathology and these aspects considered interdependently and not independently. With the results obtained an attempt has been made to classify the isolates within the genus.

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EXPLANATION OF PLATE

1. Photograph of two varieties of rice — one resistant and the other susceptible — seen side by side in an experimental plot at Coimbatore.
   A. Susceptible variety completely destroyed by *Piricularia* attack.
   B. Resistant variety unaffected.

2. Photomicrograph of mycelia from young cultures of *Piricularia* (mycelia straight and thin) × 360.

3. Photomicrograph of mycelia from older culture (mycelia variously contorted) × 360.


5. Rice (*Oryza sativa* Linn.) affected by *Piricularia* (spindle-shaped leaf spots and darkened neck, node and grains) — diagrammatic.

6. 'Ragi' (*Eleusine coracana* Gaertn.) affected by *Piricularia*. Note spindle shaped leaf spots with neighbouring spots coalescing. Also showing affected ear-heads — diagrammatic.

7. 'Tenai' (*Setaria italica* Beauv.) affected by *Piricularia* (Circular leaf spots, ear-heads not affected) — diagrammatic.

*Note.*—For photograph 1 the author is indebted to the Govt. Mycologist, Coimbatore and to Dr. T. S. Sadasivan.

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