GROWTH FACTOR REQUIREMENTS OF *PIRICULARIA* SPP. AND *SCLEROTIUM ORYZÆ**

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EVER since Schopfer (1934) demonstrated the necessity of an external supply of thiamine to *Phycomyces blakesleeanus*, a great many fungi have been studied for their growth factor requirements and the literature in this regard has been reviewed by Robbins and Kavanagh (1942), Schopfer (1943), Fries (1948), Hawker (1950) and Lilly and Barnett (1951). However, work of this nature has received little attention in India except for studies on a few fungi, *viz.*, *Chætomium brasiliensis* (Basu, 1952), *Corticium microsclerotia* (Mathew, 1953) and *Pellicularia koleroga* (Mathew, 1952, 1954). A preliminary screening of several crop-infecting fungi for their vitamin deficiencies in this laboratory indicated quite a few of them to be heterotrophic and these have been reported (Sadasivan and Subramanian, 1954). The present paper deals with the more elaborate studies conducted on different species and isolates of *Piricularia* and *Sclerotium oryzæ* for their vitamin deficiencies. Of the few species of *Piricularia*, only *Piricularia oryzæ* has been studied for its growth factor needs by other workers. The vitamin deficiencies of other species and isolates of *Piricularia* from different hosts and of *Sclerotium oryzæ* have not been reported.

The beneficial effect of the addition of the vitamin-like 'oryzanin' to the nutrient medium was observed to accelerate the formation and germination of conidia of *Piricularia oryzæ* (Ito and Terui, 1931). Working under more exacting conditions and with synthetic vitamins Leaver *et al.* (1947) indicated the heterotrophy of this fungus to thiamine and biotin. More recent work in Japan with this organism showed it to be mainly biotin deficient, thiamine being complementary (Otani, 1952, Tanaka and Katsuki, 1953). However, earlier studies made by the author with a strain of this fungus isolated in India revealed mainly thiamine deficiency, biotin being complementary (Appa Rao *et al.*, 1955).

Apart from the vitamin studies of this fungus little work has been done on its nutritional physiology. Little or no information is available regard-

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ing the physiological behaviour of the different species and isolates of *Piricularia* and *Sclerotium oryzæ* especially from the aspect of their growth factor requirements. Even though knowledge is still lacking on the exact significance of growth factors in host-parasite relationship, their importance in fungal physiology cannot be overlooked and to quote Foster (1949), "Much of the earlier work on the ability of an organism to utilize various forms of nitrogen nowadays must be re-examined in the light of accumulated knowledge of vitamins and growth factors. The inability of an organism to develop on a form of nitrogen in a synthetic medium does not necessarily mean that the particular nitrogen compound was not assimilable; it may rather mean that certain vitamins also essential for growth were lacking and that their need was imperceived in 'pre-vitamin' times."

Material and Methods

The methods of investigation in this field are exacting. Hence, the factors that tend to vitiate the results were kept at the minimum by strictly adhering to the codes of procedure indicated by Robbins and Kavanagh (1942). Slight improvements and modifications were made as and when necessary.

Source and maintenance of cultures.—Most of the cultures were kindly supplied by the Government Mycologist, Agricultural Research Institute, Coimbatore. Others were either isolated by the author or obtained from Centraalbureau Voor Schimmelcultures, Baarn, Holland. The details of the source and their maintenance are given below:

The above cultures were subcultured once a fortnight.

Glassware, water, chemicals and vitamins.—Pyrex Erlenmeyer flasks of 250 ml. capacity and test-tubes of the same brand were employed. Cleaning of glassware was done after Mathew (1953). Metal distilled water redistilled in a Pyrex still was used in earlier experiments and later, water distilled in an all-glass Pyrex electric still was found satisfactory. Chemicals of utmost purity, vitamins and their components referred to by Mathew (1953) were used.

Basal medium.—The basal medium consisted of: $NaNO_3$: 2·5 g., KH_2PO_4: 1·0 g., $MgSO_4.7H_2O$: 0·5 g., Sucrose: 5·0 g. and glass distilled water to make 1,000 ml. supplemented with 0·1 ml. of a trace element solution of Robbins (1939 *b*), containing B, Cu, Fe, Mn, Mo and Zn. An aliquot of 25 ml. of this, pipetted out in each flask or 5 ml. in each tube was maintained throughout the investigation. In studies, on the influence of different nitrogen and carbon compounds, substitution to the above medium was always made on an equivalent of nitrogen or carbon. The pH was adjusted

Name of the fungus	Host	Maintenance	Source
Piricularia oryzæ Cav. (Strain 1)	*Oryza sativa*	2% P.D. agar	Govt. Mycologist, Coimbatore
P. oryzæ (Strain 2)	,,	Sterilized host bits	Isolated by author
P. setariæ Nisikado (Strain 1)	*Setaria italica*	2% P.D. agar	Govt. Mycologist, Coimbatore
P. setariæ (*Strain* 2)	,,	Sterilized host bits	Isolated by author
P. zingiberi Nisikado	*Zingiber* sp.	2% P.D. agar	C. B. S., Holland.
Piricularia sp. (Strain 1)	*Eleusine coracana*	,,	Govt. Mycologist, Coimbatore
Piricularia sp. (Strain 2)	,,	Sterilized host bits	Isolated by author
Piricularia sp.	*Triticum vulgare*	2% P.D. agar	Govt. Mycologist, Coimbatore
Piricularia sp.	*Brachiaria mutica*	,,	,,
Piricularia sp.	*Leersia hexandra*	,,	,,
Sclerotium oryzæ	*Oryza sativa*	,,	,,

P.D.: Potato-dextrose.

to 6·0 before autoclaving with 6 N.NaOH or 6 N.HCl with the aid of a Hellige comparator. Agar when used was purified with 5% aqueous pyridine (Robbins, 1939 *a*). Vitamins were added before autoclaving. Both solid and liquid media were sterilized at 15 lb. pressure for 15 minutes.

Aerial mycelium without any agar adhering to it formed the inoculum for all *Piricularia* spp. and a single sclerotium was inoculated in the case of *Sclerotium oryzæ*. In later experiments the method of Wikén *et al.* (1951) was found to be more satisfactory. This consists in growing the fungus for six days in the liquid medium described above with sub-optimal doses of thiamine and biotin and macerating the fungal mat with glass beads in sterile glass distilled water in a Pyrex boiling tube. A loopful of this mycelial suspension was added to each flask or tube as the case may be. This procedure had the definite advantage of tending to greatly reduce the variation between replicates as well as minimizing the carry over of growth factors along with the inoculum. All cultures were incubated at room temperature (29 ± 3° C.).

Growth determination and data.—Growth in test-tubes, either on solid or liquid, media were visually rated. Mats from liquid cultures were harvested by filtering through Gooch crucibles, washed thoroughly with distilled water, dried over filter-papers at 55–60° C. for 48 hours, cooled in a desiccator and weighed. The mean value of three replicates have been presented along with statistical deductions following the methods of Paterson (1939) and Morony (1951).

EXPERIMENTAL

Preliminary screening of *Piricularia oryzæ*, *P. setariæ*, *P. zingiberi* and isolates of *Piricularia* from *Eleusine coracana*, *Triticum vulgare*, *Brachiaria mutica*, *Leersia hexandra* and *Sclerotium oryzæ* on an asperagine-sucrose medium solidified with 2% pyridine washed agar showed them to be incapable of growth on the vitamin-free synthetic medium, thereby indicating vitamin deficiency. The following experiments represent a closer examination of the nature and type of heterotrophy of these organisms.

1. *Vitamin deficiency of species and isolates of* Piricularia

Vitamin deficiencies of the six *Piricularia* referred *supra* were evaluated using the liquid medium (*vide* 'Materials and Methods') by having a vitamin mixture (+ All) of the following composition: (1) thiamine 5·0 μg., (2) biotin 2·5 μg., (3) pyridoxine 5·0 μg., (4) nicotinic acid 5·0 μg., (5) inositol 1·0 μg., (6) *p*-amino benzoic acid 5·0 μg., (7) riboflavin 2·5 μg. and (8) pantothenic acid 5·0 μg. Flasks without added vitamins served as controls. The effect of excluding any one vitamin at a time from the mixture (+ All) was studied. The trial was conducted in triplicates for each treatment.

A pin-head size of aerial mycelium removed from a ten-day old potato-dextrose slant formed the inoculum. The cultures were incubated for 15 days, the mats harvested and weighed (*vide* 'Methods').

The data are presented in Table I.

Results.—In the controls, without added vitamins, slight growth occurred with all the seven isolates which amounted to as little as 2% in the case of the isolate from *Leersia hexandra* to as much as 19% with that from *Brachiaria mutica*. Thiamine, when omitted from the vitamin mixture (+ All) decreased

TABLE I

Effect of omitting a single vitamin from a mixture (+All) on the growth of Piricularia *spp.*

Treatments	Mat weight (mg.)						
				Isolates from			
	A *P. oryzæ*	B *P. setariæ*	C *P. zingiberi*	D *E. coracana*	E *T. vulgare*	F *B. mutica*	G *L. hexandra*
1 Control ..	2·5	5·7	1·1	5·9	3·1	4·2	0·4
2 — Thiamine ..	5·1	7·3	0·8	3·9	4·8	11·2	1·1
3 — Biotin ..	24·1	35·1	26·6	17·1	15·9	20·7	16·3
4 — Pyridoxine ..	33·0	50·1	37·1	29·4	19·7	30·0	35·3
5 — Nicotinic Acid	33·7	50·5	34·6	30·4	20·8	28·5	31·3
6 — Inositol ..	29·6	45·7	34·7	29·5	20·7	29·5	31·3
7 — Pantothenic Acid	31·2	47·1	34·4	28·8	19·1	31·0	33·3
8 — *p*-A.B. Acid ..	35·4	51·4	35·5	32·4	19·0	31·7	35·1
9 — Riboflavin ..	32·7	43·0	37·3	29·7	17·9	29·4	26·6
10 + All ..	33·2	43·3	34·9	31·7	20·0	29·1	25·9
Significance by F. test ..	Yes	Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level ..	4·1	12·8	4·3	3·3	3·0	2·6	4·1

Conclusions

A: 8 5 10 4 9 7 6 3 2 1
B: 8 5 4 7 6 10 9 3 2 1
C: 9 4 8 10 6 5 7 3 1 2
D: 8 10 5 9 6 4 7 3 1 2
E: 5 6 10 4 7 8 9 3 2 1
F: 8 7 4 6 9 10 5 3 2 1
G: 4 8 7 5 6 9 10 3 2 1

growth considerably. This reduction in growth was equal, slightly more, or less than that obtained in the controls. Exclusion of biotin, however, brought down growth only to a smaller extent. Withholding of other vitamins had no retarding effect on growth of the organisms. Even though withdrawal of certain vitamins other than thiamine and biotin tended to increase growth with certain isolates, the effect was significantly marked only in the case of the isolate from *Leersia hexandra* where pyridoxine, nicotinic acid, inositol, pantothenic acid and *p*-amino benzoic acid proved to be definitely toxic to growth.

2. *Effect of thiamine and biotin added singly on the growth of* Piricularia *spp.*

Experiment 1 has shown that excluding thiamine from the (+ All) mixture, markedly reduced growth of all *Piricularia* spp. while withholding of biotin decreased growth only to a slight extent.

The effect of thiamine and biotin added singly to the medium on growth of these fungi was next studied. Thiamine at 1·0 µg. and biotin at 0·1 µg. levels were added to 5 ml. of the standard medium in Pyrex test-tubes. The tubes were inoculated with a loopful of mycelial suspension (*vide* 'Methods') and the growth of the different fungi was visually rated on the 15th day and the data are presented in Table II.

TABLE II

Growth of different isolates of Piricularia *spp. with thiamine and biotin*

Name of the fungus	Treatments			
	Control	Thiamine 1 µg.	Biotin 0·1 µg.	Thiamine 1 µg. + Biotin 0·01 µg.
P. oryzæ ..	–	+	–	++++
P. setariæ ..	–	+	–	++++
P. zingiberi ..	–	+	–	++++
Piricularia sp. on *Eleusine coracana*	–	+	–	++++
Piricularia sp. on *Triticum vulgare*	–	+	–	++++
Piricularia sp. on *Brachiaria mutica*	–	+	–	++++

– Negligible growth.
+ Slight growth.
++++ Full growth.

Results.—All the different species and isolates of *Piricularia* made negligible growth in the controls or with biotin alone. Very slight growth was observed with thiamine. Full growth of the fungi occurred only when both thiamine and biotin were present in the medium.

3. *Vitamin deficiency of* Sclerotium oryzæ

The vitamin deficiency of this fungus was investigated on slants of purified agar and the results are presented in Table III and Plate XXI.

TABLE III

Vitamin deficiency of Sclerotium oryzæ

Treatments	Growth ratings		Sclerotial production	
	6th day	12th day	6th day	12th day
Control ..	–	–	–	–
– Thiamine ..	–	–	–	–
– Biotin ..	++	++++	++	++++
–Pyridoxine ..	++	++++	++	++++
– Nicotinic acid ..	++	++++	++	++++
– Inositol ..	++	++++	++	++++
– Pantothenic acid ..	++	++++	++	++++
– *p*-Amino benzoic acid ..	++	++++	++	++++
– Riboflavin ..	++	++++	++	++++
+ All ..	++	++++	++	++++

– Negligible.
++ Fair.
++++ Full.

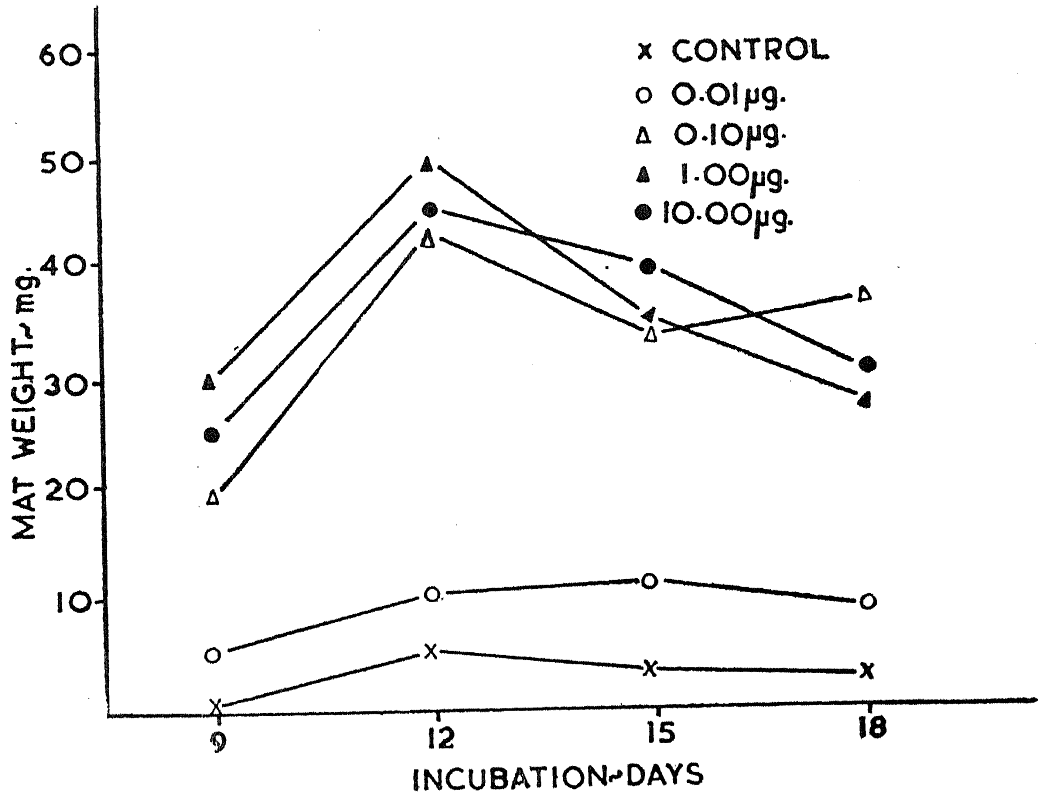
Results.—Growth was negligible in the control and in the thiamine omitted series (Plate XXI). Withdrawal of other vitamins did not affect growth or sclerotial reduction. Sclerotia were observed to form from the sixth day after inoculation. The different vitamins did not apparently influence the amount of sclerotial production.

4. *Levels of thiamine and biotin as a function of time for* Piricularia *spp.*

A priori all the species and isolates of *Piricularia* and *Sclerotium oryzæ* appear to be heterotrophic to thiamine and biotin and thiamine respectively. However, the nature of such a heterotrophy needs to be considered by a study of the effect of different levels of the concerned vitamins as a function of time. Besides, the experiment had in it the object of fixing an optimum period of incubation for the fungi in question and to be followed in further investigation.

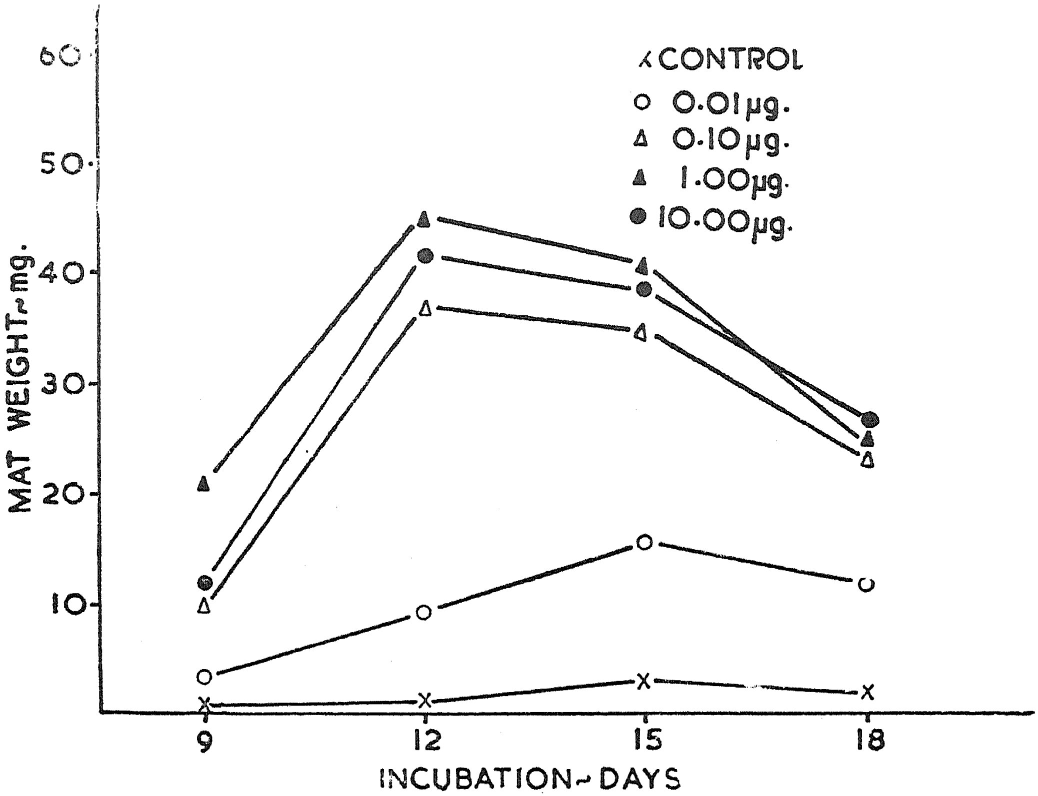
Methods already mentioned in previous experiments were followed. Thiamine and biotin at 0·01 μg., 0·10 μg., 1·00 μg. and 10·00 μg. were added to each flask. The controls had no added vitamins. Triplicate cultures in each treatment were harvested at periodic intervals of three days from the 9th to 18th day after inoculation.

The data are presented in Text-Figs. 1–6.



TEXT-FIG. 1. Growth of *Piricularia oryzæ* with various levels of thiamine and biotin at different incubation periods.

Results.—Slight or negligible growth occurred in the controls throughout the different incubation periods. With 0·01 μg. of the vitamins, growth was accelerated but significant growth was obtained only with 0·1, 1·0,



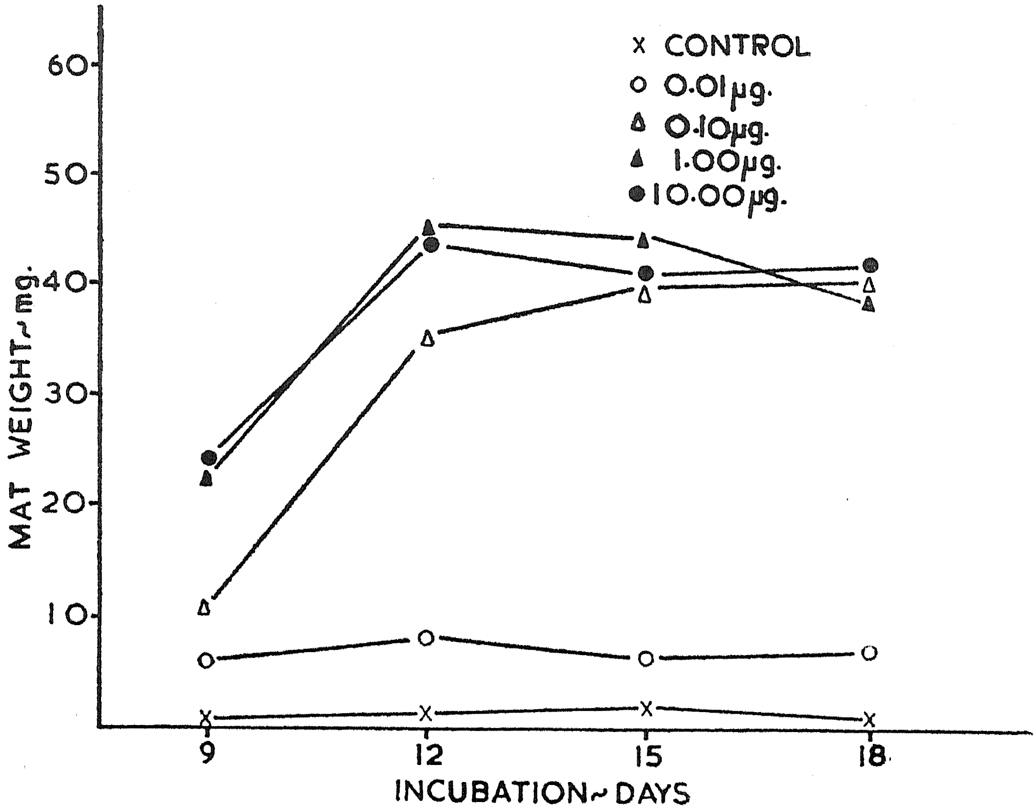
TEXT-FIG. 2. Growth of *Piricularia setariæ* with various levels of thiamine and biotin at different incubation periods.

10·0 μg. of the vitamins. Maximum growth of all isolates took place with 1·0 μg. level of both the vitamins on the 12th day, growth beginning to decline thereafter indicating autolysis. Active growth of the fungi was evident only between the 9th and 12th day and levels of vitamins higher than 0·1 μg. were required to promote significant growth. Incubating the controls upto 18 days did not materially benefit the organisms to any advantage.

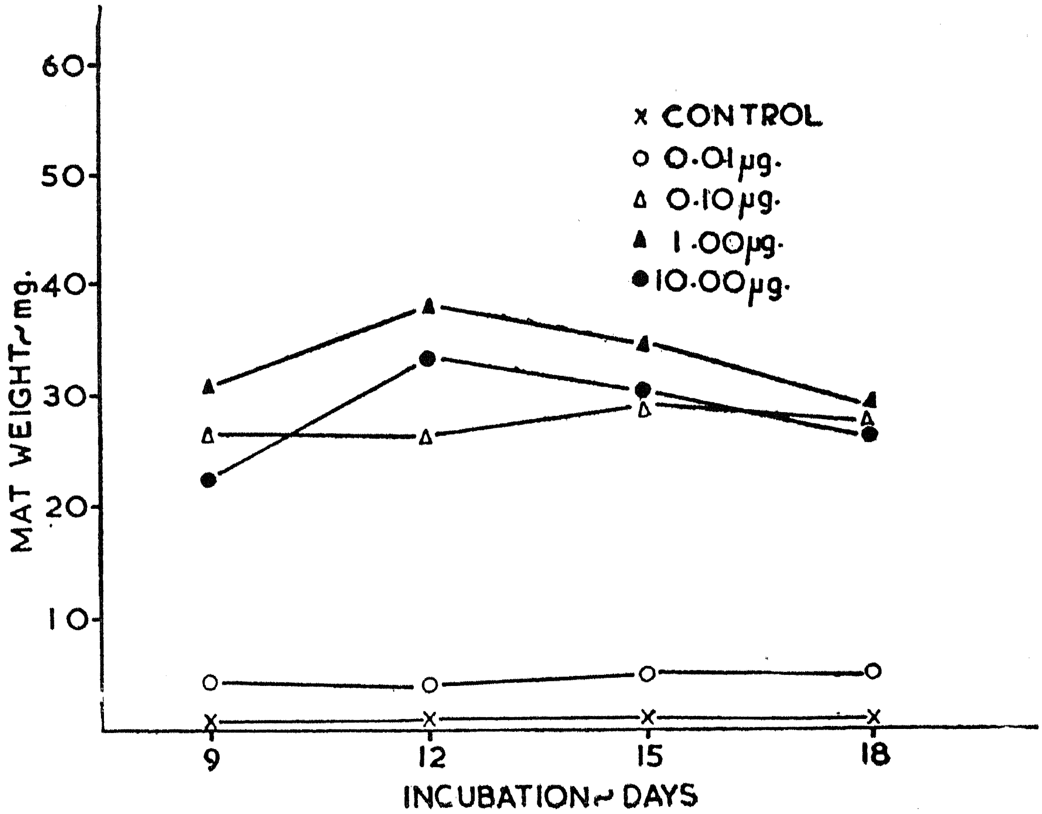
5. *Levels of thiamine as a function of time for* Sclerotium oryzæ

Experiment 3 has shown this organism to be heterotrophic only to thiamine. The object and experimental procedure followed was the same as the preceding one.

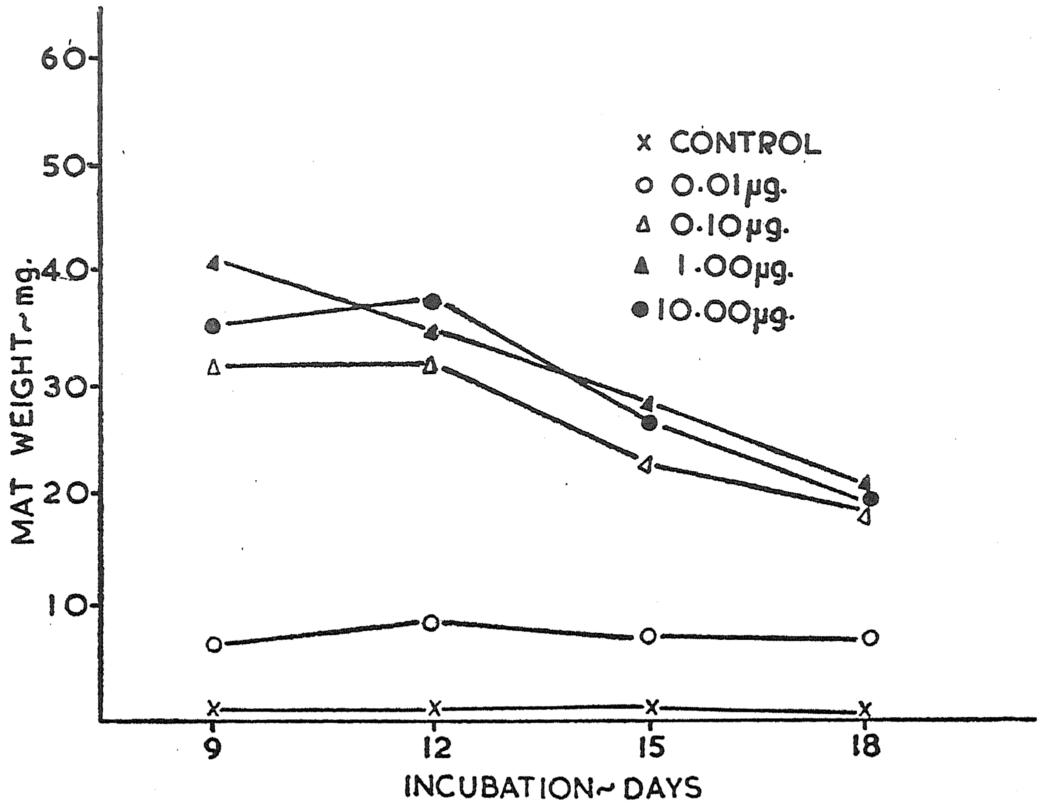
Single sclerotium was used for inoculation. Mats were harvested at intervals of 6 days and the incubation period ranged from 6 to 24 days. The amounts of thiamine added per flask were: none, 0·001 μg., 0·01 μg., 0·10 μg. and 10·0 μg.



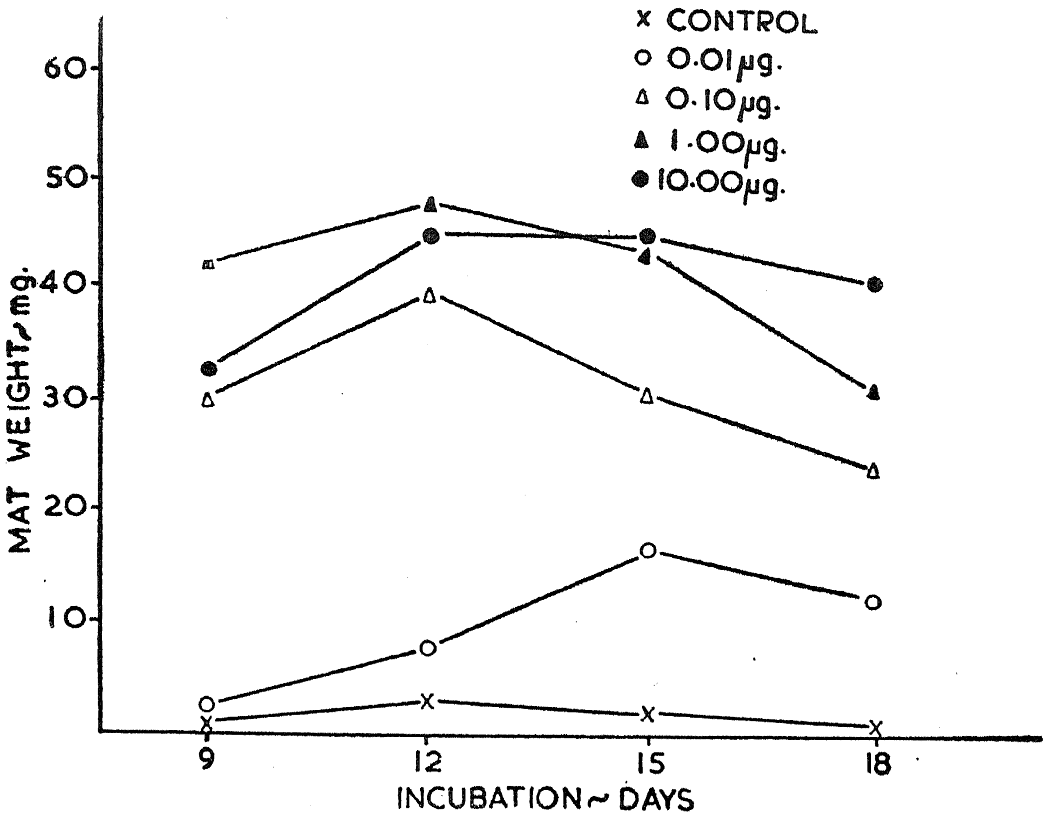
TEXT-FIG. 3. Growth of *Piricularia zingiberi* with various levels of thiamine and biotin at different incubation periods.



TEXT-FIG. 4. Growth of *Piricularia* sp. from *Eleusine coracana* with various levels of thiamine and biotin at different incubation periods.



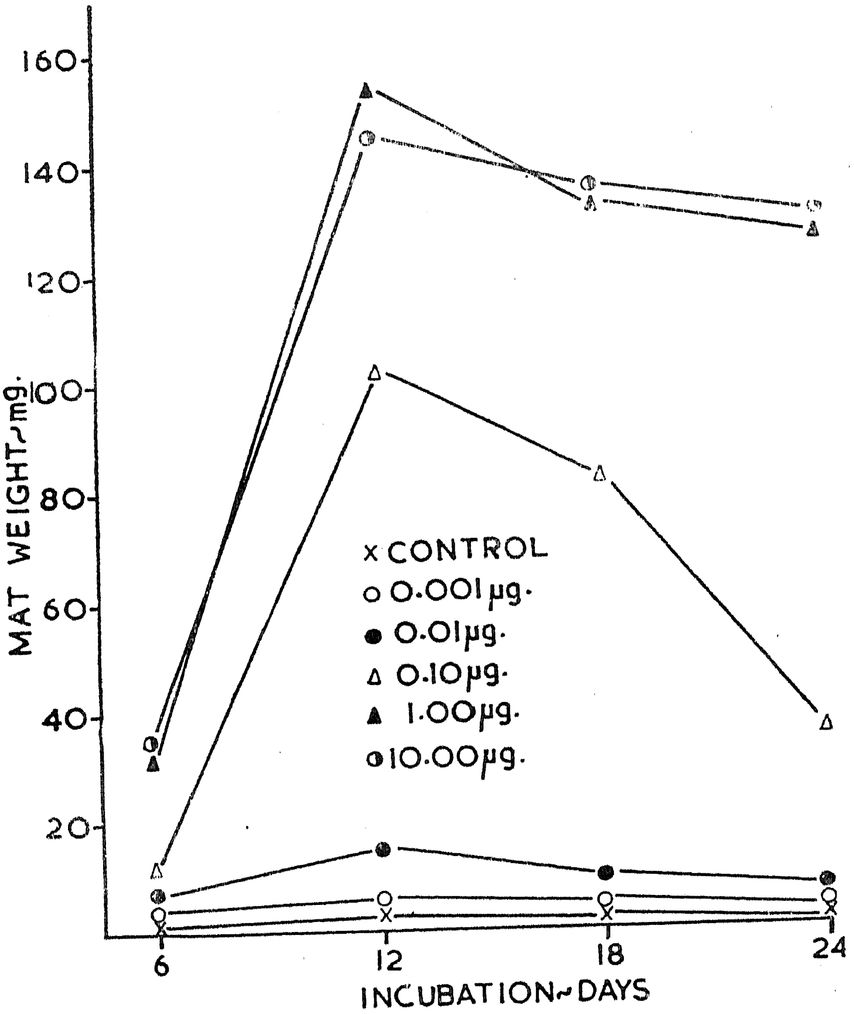
Text-Fig. 5. Growth of *Piricularia* sp. from *Triticum vulgare* with various levels of thiamine and biotin at different incubation periods.



Text-Fig. 6. Growth of *Piricularia* sp. from *Brachiaria mutica* with various levels of thiamine and biotin at different incubation periods.

The data are presented in Text-Fig. 7.

Results.—Growth was negligible in the controls at all incubation periods. Addition of 0·001 μg. and 0·01 μg. of thiamine did not bring about any change. Growth of the fungus with 0·1 μg. of the vitamin was negligible at the end of 6 days while this level promoted 66% growth by 12 days. Significant growth occurred even by 6 days with 1·0 μg. and 10·0 μg. of the vitamin. Maximum growth was reached by 12 days with 1·0 μg. and 10·0 μg. of the vitamin. Autolysis had set in after 12 days with all levels of the vitamin.



TEXT-FIG. 7. Growth of *Sclerotium oryzæ* with various levels of thiamine at different incubation periods.

6. *Optimum level of thiamine and biotin for* Piricularia *spp.*

Experiment 4 indicated that the optimum period of incubation for all the three species of *Piricularia, viz., P. oryzæ, P. setariæ, P. zingiberi* and the isolate from *Brachiaria mutica* was about 12 days and that thi amine and biotin in excess of 0·01 μg. per flask were needed to promote significant growth of all the six fungi. However, the optimum period was only 9 days (as evident from the statistical treatment of the data) for the isolates from *Eleusine coracana* and *Triticum vulgare*.

In order to determine the optimum levels of thiamine and biotin for these fungi, various levels of thiamine, ranging from 0·01 μg. to 5·0 μg. together with biotin at 0·001 μg., 0·01 μg. and 0·1 μg. levels were added per flask. The flasks were inoculated as before and incubated for 12 or 9 days, as the case may be.

The data are presented in Table IV.

Results.—The results in general indicate 1·0 μg. of thiamine and 0·01 μg. of biotin to be the optimum, irrespective of the species or isolate.

7. *Optimum level of thiamine for* Sclerotium oryzæ

The experimental procedure was the same as that of the previous experiment. Thiamine levels ranging from 0·01 μg. to 5·0 μg. in the order of 0· 01 μg., 0·10 μg., 1·00 μg., 2·00 μg. and 5·00 μg. were tried as this fungus has been shown to be heterotrophic only to thiamine (*vide* Expt. 3).

The data are presented in Text-Fig. 8.

Results.—The optimum amount of thiamine was found to be 1·0 μg. per flask.

8. *Vitamin deficiencies of pathogenic and non-pathogenic isolates of* Piricularia oryzæ, P. setariæ *and* Piricularia *sp. from* Eleusine coracana

The isolates of *Piricularia oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* mentioned in the foregoing experiments were maintained on 2% potato-dextrose agar. Repeated inoculation trials showed these isolates to have lost their virulence and not to infect their respective hosts, *viz.*, *Oryza sativa*, *Setaria italica* and *Eleusine coracana*. It was, therefore, thought worthwhile to study if qualitative differences would exist in the vitamin requirements of fresh and old isolates. The experiment had also another end in view, *i.e.*, if cultures maintained on sterilized host bits would differ in their vitamin needs from those on media like potato-dextrose agar.

A number of single spore isolates of *P. oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* were made and maintained on sterilized host bits as well as on potato-dextrose agar. They were tested for their virulence by spraying spore suspensions and found to infect their respective

TABLE IV

Growth of Piricularia *spp. with different levels of thiamine and biotin*

	Levels of vitamins in μg./flask		Mat weight (mg.)					
						Isolates from		
	Thiamine	Biotin	A *P. oryzæ*	B *P. setariæ*	C *P. zingiberi*	D *E. coracana*	E *T. vulgare*	F *B. mutica*
1	0·01	0·001	4·7	9·1	6·3	6·8	7·5	5·9
2	0·10	0·001	19·7	25·7	33·3	35·4	23·9	14·7
3	1·00	0·001	39·1	25·1	31·2	37·5	26·6	16·5
4	2·00	0·001	35·2	25·2	33·3	36·5	26·9	19·1
5	5·00	0·001	43·9	26·6	29·9	40·2	23·1	20·7
6	0·01	0·01	6·7	10·6	5·7	6·7	6·1	6·8
7	0·10	0·01	36·3	24·2	30·0	36·8	27·2	24·1
8	1·00	0·01	44·3	37·7	45·3	42·1	33·7	34·1
9	2·00	0·01	43·3	35·3	44·1	36·5	29·9	34·5
10	5·00	0·01	40·2	35·6	41·5	39·3	27·5	30·9
11	0·01	0·10	6·3	10·3	6·0	5·8	7·8	9·6
12	0·10	0·10	24·9	32·1	34·9	37·1	26·7	24·1
13	1·00	0·10	44·3	31·8	43·9	42·8	28·8	33·9
14	2·00	0·10	37·5	36·5	41·1	42·3	27·2	32·9
15	5·00	0·10	38·3	36·9	45·1	41·2	26·6	35·1
Significance by F. test			Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level ..			3·5	5·8	5·8	1·6	3·5	7·4

Conclusions

A: 8 13 5 9 10 3 15 14 7 4 12 2 6 11 1

B: 8 15 14 10 9 12 13 5 2 4 3 7 6 11 1

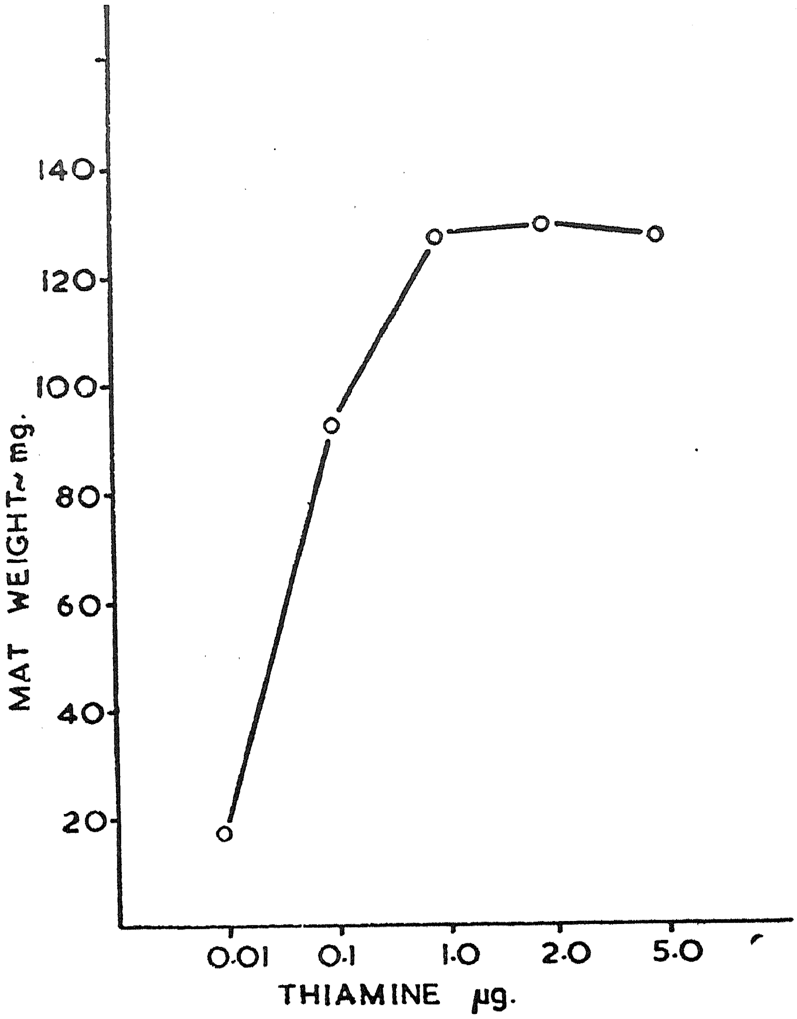
C: 8 15 9 13 10 14 12 2 4 3 7 5 1 11 6

D: 13 14 8 15 5 10 3 12 7 4 9 2 1 6 11

E: 8 9 13 12 7 14 4 12 3 15 2 5 11 1 6

F: 15 9 8 13 14 10 8 12 5 4 3 2 11 6 1

hosts. These isolates have been termed as *P. oryzæ* (Strain 2), *P. setariæ* (strain 2) and *Piricularia* sp. from *E. coracana* (Strain 2).



TEXT-FIG. 8. Growth of *Sclerotium oryzæ* with different levels of thiamine.

Comparative studies were made on the vitamin deficiencies of these pathogenic strains together with the non-pathogenic strains used in the earlier part of this investigation, hereinafter to be called as *P. oryzæ* (Strain 1), *P. setariæ* (Strain 1), and *Piricularia* sp. from *E. coracana* (Strain 1). These isolates were studied for their vitamin deficiencies in liquid cultures in test-tubes. Only four of the vitamins for which more often fungi are known to be deficient, *viz.*, thiamine, biotin, pyridoxine and inositol were used in this study. The treatments comprised of: no vitamins, thiamine 1·0 µg., biotin 0·1 µg., thiamine 1·0 µg. + biotin 0·1 µg., and thiamine 1·0 µg. + biotin 0·1 µg. + pyridoxine 1·0 µg. + inositol 25 µg. per tube.

Growth ratings were made visually after 12 days incubation and the results are presented in Table V.

TABLE V

Growth of non-pathogenic and pathogenic isolates of Piricularia oryzæ, P. setariæ *and* Piricularia *sp. on* Eleusine coracana *with different vitamins*

Name of fungus	Nature of the isolate	Vitamins				
		None	T	B	T+B	T+B+Py+I
P. oryzæ (Strain 1)	Non-pathogenic	–	+	–	++++	++++
P. oryzæ (Strain 2)	Pathogenic	–	+	–	++++	++++
P. setariæ (Strain 1)	Non-pathogenic	–	+	–	++++	++++
P. setariæ (Strain 2)	Pathogenic	–	+	–	++++	++++
Piricularia sp. on *E. coracana* (Strain 1)	Non-pathogenic	–	+	–	++++	++++
Piricularia sp. on *E. coracana* (Strain 2	Pathogenic	–	+	–	++++	++++

– Negligible growth.
+ Slight growth.
++++ Full growth.
T: Thiamine.
B: Biotin.
Py: Pyridoxine.
I: Inositol.

Results.—The results indicated that all the isolates were heterotrophic only to thiamine and biotin regardless of their time of isolation and maintenance on either potato-dextrose agar or sterilized host bits.

9. *Influence of the forms of nitrogen on the vitamin deficiency of non-pathogenic and pathogenic isolates of* Piricularia

It has been known that the vitamin requirements of a fungus may change with a change in the nitrogen source. The object of this experiment was to compare the vitamin needs of non-pathogenic and pathogenic isolates of *P. oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* with different nitrogen sources.

The experimental procedure, the nature and amounts of vitamins added were the same as in the preceding experiment. Ten different nitrogen compounds were used representing the inorganic nitrate and ammonium nitrogen and organic nitrogen.

The data are presented in Table VI.

Results.—Both the pathogenic and non-pathogenic isolates of *Piricularia* behaved alike not only in their vitamin needs but also in their ability to utilize the different forms of nitrogen. They were heterotrophic to thiamine and biotin regardless of the nitrogen source. In general, these organisms were found to utilize organic nitrogen and inorganic nitrate nitrogen. Inorganic ammonium nitrogen was less satisfactory and nitrite inhibited growth completely. Presence of pyridoxine and inositol along with thiamine and biotin did not enable any of the fungi to better utilize the inorganic ammonium nitrogen.

10. *Influence of the source of carbon on the vitamin deficiencies of non-pathogenic and pathogenic isolates of* Piricularia

Following the procedure of Expt. 8, the influence of different carbon sources on the vitamin deficiencies of pathogenic and non-pathogenic isolates of *Piricularia* were studied by replacing sucrose of the standard medium with 9 different sugars on an equivalent basis.

The data are presented in Table VII.

Results.—All the three isolates, irrespective of their virulence, were heterotrophic only to thiamine and biotin with all the sources of carbon. In general, addition of pyridoxine or inositol was not beneficial to any of the fungi. All the six isolates practically made no growth with mannitol, dulcitol and glycerol. The pathogenic strains were found to utilize the sugars better than the non-pathogenic strains, though their ability to utilize the different carbon sources did not differ. Glucose, fructose, sucrose and maltose appeared to be better sources of carbon than the other sugars for all the three fungi. However, *P. setariæ* and *Piricularia* sp. from *E. coracana* could use with equal facility lactose and xylose respectively.

11. *Influence of pH of the medium on the vitamin needs of non-pathogenic and pathogenic isolates of* Piricularia

Studying the influence of pH on the vitamin deficiencies of non-pathogenic and pathogenic isolates of *Piricularia* on the same lines as Expts. 9

TABLE VI

Growth factor requirements of non-pathogenic (NP) and pathogenic (P) isolates of Piricularia oryzæ *(PO),* P. setariæ *(PS) and* Piricularia *sp. from* Eleusine coracana *(EC) with different nitrogen sources*

Treatments	Control						Thiamine						Biotin						Thiamine+Biotin						+ All					
Nitrogen Source	PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
Sodium nitrate	0	0	0	0	0	–	1	2	2	1	2	2	1	1	0	1	0	–	4	4	4	4	3	4	4	4	4	4	4	4
Potassium nitrate	0	0	0	0	0	–	1	2	2	1	2	2	1	1	0	0	1	–	4	4	4	4	4	4	4	4	4	4	4	4
Potassium nitrite	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonium nitrate	0	0	–	0	–	1	1	1*	2	2	2	2	1	1	–	0	2	–	2	2	2	3	4	3	2	2	3	3	4	3
Ammonium chloride	–	–	–	–	–	–	1	1	2	1	2	2	–	–	–	–	–	–	2	2	2	2	3	2	2	2	2	2	3	2
Ammonium sulphate	1	1	1	0	1	1	1*	2	2	1	1	2	1	1	–	–	–	1	2	2*	2	2	3	2	2	2*	2	2	3	2
Ammonium oxalate	–	–	–	0	0	1*	1	1	2	1*	2	2	–	–	–	0	0	2	3	3	3	4	4	4	3	3	3	4	4	4
Ammonium tartrate	–	–	–	0	–	1	1	1	2	2	2	2	1	–	–	–	1	–	3	3	4	4	4	3	3	3	4	4	4	3
Urea	0	–	0	0	0	–	1	1*	1	2	2	2	–	–	–	0	–	–	[illegible]	3	3	3	3	3	3	3	3	3	3	3
Asparagine	0	–	–	–	–	–	1	1*	1	2	2	2	–	1	–	–	1	1	4	4	3	4	4	4	4	4	3	4	4	4

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0:	Nil.	2:	Little.
–:	Negligible.	2*:	Fair.
1:	Very slight.	3:	Good.
1*:	Slight.	4:	Excellent.

TABLE VII

Growth factor requirements of non-pathogenic (NP) and pathogenic (P) isolates of Piricularia oryzæ *(PO),* P. setariæ *(PS) and* Piricularia *sp. on* Eleusine coracana *(EC) with different carbon sources*

Treatments ..	Control						Thiamine						Biotin						Thiamine+Biotin						+All					
	PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC	
Carbon source	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
Xylose ..	1	–	–	0	–	–	1	–	1	0	2	1	1	1	–	0	–	–	2	3	2	4	4	4	2	3	2	4	4	4
Glucose ..	1	–	–	0	–	0	2	–	2	–	2	1	1	–	–	0	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Galactose ..	1	–	–	–	–	0	2	–	1	–	2	1	1	1	–	–	–	–	2*	3	2*	3	2*	3	2*	3	2*	3	2*	3
Fructose ..	–	–	–	0	–	0	2	–	1	–	2	1	–	–	–	0	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Sucrose ..	–	0	–	0	–	0	2	–	1	1	2	1	1	–	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Lactose ..	1	–	–	0	–	–	1	–	1	–	2	1	1	–	–	1	–	–	2	2*	4	4	2*	3	2	2*	4	4	2*	3
Maltose ..	–	–	–	–	–	–	2	–	1	–	2	1	–	–	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4
Mannitol ..	0	0	–	–	–	0	0	0	–	–	1	–	0	0	–	–	1	0	0	0	–	–	1	–	0	0	–	–	1	–
Dulcitol ..	0	0	–	–	–	0	0	0	–	–	–	–	0	0	–	–	–	0	0	0	–	–	–	–	0	0	–	–	–	–
Glycerol ..	0	0	–	–	–	0	0	0	–	–	–	–	0	0	–	–	–	0	0	0	–	1	–	1	0	0	–	1	1	–

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil.
–: Negligible.
1: Very slight.
2: Little.
2*: Fair.
3: Good.
4: Excellent.

and 10, a range of pH from 4·0 to 8·0 with unit intervals was chosen. pH was adjusted to the desired level with 6 N HCl or NaOH.

The data are presented in Table VIII.

Results.—Complete growth of the fungi occurred at all pH levels only when both thiamine and biotin were added to the medium. The presence of pyridoxine and inositol along with thiamine and biotin had no added benefit to the fungi at any of the pH levels tried. No marked differences were noted in growth of the fungi at the different pH levels. The results in general showed that pH of the medium did not alter the thiamine and biotin heterotrophy of the *Piricularia* spp., irrespective of their virulence.

12. *Nitrogen sources and vitamin deficiency of* Sclerotium oryzæ

The influence of different nitrogen sources on the vitamin deficiency of this fungus was studied on similar lines as Expt. 9.

The data are presented in Table IX.

Results.—Negligible or slight growth occurred in the controls with all the nitrogen sources. Thiamine alone promoted as much growth as a combination of the vitamins. Biotin, added singly, was without effect. This organism could not utilize ammonium tartrate. Nitrite was inhibitory. All other sources of nitrogen representing the inorganic nitrate and ammonia and organic nitrogen were utilized by this fungus. However, not all nitrogen sources were equally favourable for sclerotial production. While sodium nitrate, potassium nitrate, ammonium oxalate, urea and asparagine were very favourable for sclerotial production, ammonium nitrate, ammonium chloride and ammonium sulphate were not favourable. In general, the source of nitrogen did not alter the heterotrophy of this organism to thiamine.

13. *Carbon sources and vitamin deficiency of* Sclerotium oryzæ

Ten different sugars were tried in this study for their influence on the vitamin deficiency of this fungus. The procedure of Expt. 10 was followed.

The data are presented in Table X.

Results.—In the controls, where no vitamins were added, only negligible or slight growth occurred with all the carbon sources. Biotin alone did not promote growth. Thiamine when added singly or in conjunction with other vitamins promoted good growth of the fungus. No differences were noted between the different vitamin treatments, indicating thereby that the type of carbon source did not affect the thiamine heterotrophy of this organism.

TABLE VIII

Effect of pH on the vitamin deficiency of non-pathogenic (NP) and pathogenic (P) isolates of Piricularia oryzæ *(PO),* P. setariæ *(PS) and* Piricularia *sp. on* Eleusine coracana *(EC)*

Treatments	Control						Thiamine						Biotin						Thiamine+Biotin						+All					
	PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC		PO		PS		EC	
pH	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
4·0	0	0	0	0	0	0	1	2	1	2	2	2	–	–	–	0	–	0	4	4	4	4	4	4	4	4	4	4	4	4
5·0	0	0	–	–	0	0	2	2	2	2	2	2	–	–	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4
6·0	–	0	–	–	0	0	4	2	2	2	2	2	–	–	–	–	–	–	4	4	4	4	4	4	5	4	4	4	4	4
7·0	0	0	–	–	0	0	2	2	2	2	2	2	–	1	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4
8·0	0	0	–	0	0	0	2	2	2	2	2	2	1	–	–	–	–	–	4	4	4	4	4	4	4	4	4	4	4	4

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil. 2: Slight.

–: Negligible. 4: Good.

1: Very slight 5: Excellent.

TABLE IX

Growth factor requirements of Sclerotium oryzæ *with different nitrogen sources*

Nitrogen source	Control	Thiamine	Biotin	Thiamine+ Biotin	+All	Sclerotial production in (+All)
Sodium nitrate ..	+	++++	+	++++	++++	Present
Potassium nitrate	+	++++	+	++++	++++	Present
Potassium nitrite	0	0	0	0	0	No growth
Ammonium nitrate	+	++++	+	++++	++++	Absent
Ammonium chloride	+	++++	+	++++	++++	Absent
Ammonium sulphate	+	++++	++	++++	++++	Absent
Ammonium oxalate	+	++++	—	++++	++++	Present
Ammonium tartrate	+	+	+	+	+	Absent
Urea ..	0	++++	—	++++	++++	Present
Asparagine ..	+	++++	+	++++	++++	Present

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

0: Nil. ++: Little.

—: Negligible. ++++: Good.

+: Slight.

Among the carbon sources tried, glucose, fructose and sucrose were better than xylose, galactose, lactose, maltose, mannitol and glycerol. No growth occurred with dulcitol. For sclerotial production, glucose, fructose, sucrose, lactose and maltose were more favourable than xylose, galactose, mannitol and glycerol.

14. *Growth of* Piricularia *spp. with different nitrogen sources in the presence of thiamine and biotin*

Sodium nitrate of the standard medium was replaced equivalently with 9 different nitrogen sources and the comparative ability of the different *Piricularia* spp. to utilize the various sources in the presence of 1·0 μg. of thiamine and 0·01 μg. of biotin are reported here. Cultures were grown in 250 ml. Erlenmeyer flasks (*vide* 'Methods').

TABLE X

Growth factor requirements of Sclerotium oryzæ *with different carbon sources*

Carbon source	Con-trol	Thia-mine	Bio-tin	Thiamine+ Biotin	+ All	Sclerotial production in (+All)
Xylose ..	+	+++	+	+++	+++	Absent
Glucose ..	+	++++	+	++++	++++	Present
Galactose ..	+	+++	+	+++	+++	Absent
Fructose ..	+	++++	+	++++	++++	Present
Sucrose ..	+	++++	+	++++	++++	Present
Lactose ..	+	+++	+	+++	+++	Present
Maltose ..	+	+++	+	+++	+++	Present
Mannitol ..	+	++	+	++	++	Absent
Dulcitol ..	–	–	–	–	–	Absent
Glycerol ..	+	+++	+	+++	+++	Absent

+ All: Thiamine + Biotin + Pyridoxine + Inositol.

Growth Ratings

–: Negligible. +++: Fair.
+: Slight. ++++: Good.
++: Moderate.

The data are presented in Table XI.

Results.—All *Piricularia* spp. could utilize organic amide or amino nitrogen and inorganic nitrate nitrogen. They could not, however, utilize inorganic ammonia nitrogen, as in ammonium nitrate, ammonium chloride and ammonium sulphate. Potassium nitrite was without exception toxic. In general, asparagine, ammonium tartrate, potassium nitrate, sodium nitrate and ammonium oxalate were better sources of nitrogen than the rest.

15. *Growth of* Piricularia *spp. with different carbon sources in the presence of thiamine and biotin*

Comparative studies on the ability of different *Piricularia* spp. to utilize various carbon sources in the presence of 1·0 μg. thiamine and 0·01 μg. biotin are reported here.

TABLE XI

Growth of Piricularia *spp. with different nitrogen sources in the presence of thiamine and biotin*

Nitrogen source	Mat weight (mg.)					
				Isolates from		
	P. oryzæ A	*P. setariæ* B	*P. zingiberi* C	*E. coracana* D	*T. vulgare* E	*B. mutica* F
1 Sodium nitrate	25·4	30·2	46·5	42·7	23·5	46·7
2 Potassium nitrate	34·1	33·1	41·7	38·4	24·1	48·8
3 Potassium nitrite	0·0	0·0	0·0	0·0	0·0	0·0
4 Ammonium nitrate	7·0	11·3	3·3	7·2	12·7	10.8
5 Ammonium chloride	4·1	7·0	2·9	3·6	7·1	7·4
6 Ammonium sulphate	3·3	6·8	3·1	4·7	6·5	8·4
7 Ammonium oxalate	27·5	30·3	4·2	35·7	27·5	53·3
8 Ammonium tartrate	30·7	43·2	24·0	31·7	34·5	52·9
9 Urea	31·1	34·9	24·9	37·2	27·7	41·5
10 Asparagine	42·5	47·5	48·7	85·0	46·7	46·7
Significance by F. test	Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level	2·1	6·0	4·9	4·2	4·4	4·6

Conclusions

A: 10 2 9 8 7 1 4 5 6 3

B: 10 8 9 2 7 1 4 5 6 3

C: 10 1 2 9 8 7 4 6 5 3

D: 1 2 9 7 10 8 4 6 5 3

E: 10 8 9 7 2 1 4 5 6 3

F: 7 8 2 1 10 9 4 6 5 3

Sucrose of the standard medium was replaced with 10 different carbon sources and the data are presented in Table XII.

TABLE XII

Growth of Piricularia *spp. with different carbon sources in the presence of thiamine and biotin*

Carbon source	Mat weight (mg.)					
				Isolates from		
	P. oryzæ A	*P. satariæ* B	*P. zingiberi* C	*E. coracana* D	*T. vulgare* E	*B. mutica* F
1 Xylose	25·1	35·8	15·7	7·9	17·9	35·7
2 Glucose	28·9	21·1	43·0	41·2	19·5	37·3
3 Galactose	2·5	14·2	4·6	2·5	17·8	4·8
4 Mannose	25·1	19·3	35·4	32·2	18·2	35·2
5 Fructose	28·8	21·7	30·9	38·3	17·3	34·1
6 Sucrose	32·3	23·2	40·1	34·3	16·9	41·4
7 Lactose	23·3	31·4	25·7	6·2	20·5	27·9
8 Maltose	20·9	16·8	9·1	4·3	17·0	41·5
9 Mannitol	4·9	3·1	1·1	1·1	7·8	38·3
10 Dulcitol	1·1	1·8	5·3	0·3	3·1	16·2
11 Glycerol	2·0	0·0	0·0	0·2	0·0	6·8
Significance by F. test	Yes	Yes	Yes	Yes	Yes	Yes
C.D. at 1% level ..	5·1	7·0	8·1	9·2	2·5	4·4

Conclusions

A: 6 2 5 1 4 7 8 9 3 11 10

B: 1 7 6 5 2 4 8 3 9 10 11

C: 2 6 4 5 7 1 8 10 3 9 11

D: 2 5 6 4 1 7 8 3 9 10 11

E: 7 2 4 1 3 5 8 6 9 10 11

F: 8 6 9 2 1 4 5 7 10 11 3

Results.—The utilization of the different carbon sources varied with the type of isolate. They were: *Piricularia oryzæ*: Sucrose, glucose and fructose were the best sources of carbon. Higher alcohols and galactose were poor sources.

P. setariæ: Xylose and lactose were the best sources. This isolate could, however, utilize galactose to a certain extent. Higher alcohols were poorly utilized.

P. zingiberi: Glucose, sucrose and mannose were the best. Maltose, galactose and the higher alcohols proved to be poor sources of carbon.

Piricularia sp. from *Eleusine coracana*: Glucose, frcutose, sucrose and mannose were the best sources. All others were definitely inferior.

Piricularia sp. from *Triticum vulgare*: Lactose was the best source of carbon compared to xylose, galactose, fructose and maltose. Glucose and mannose were as good as lactose. Higher alcohols were poor sources.

Piricularia sp. from *Brachiaria mutica*: Maltose and sucrose were the best compared to xylose, mannose and fructose. Among the higher alcohols, mannitol and to a certain extent dulcitol were used by this isolate. Glycerol and galactose were poorly utilized.

Discussion

While a number of filamentous fungi have been reported for their vitamin deficiencies (Robbins and Kavanagh, 1942), admittedly, quite a few of them have been investigated intensively with regard to several important factors that are known to influence the vitamin heterotrophy of a fungus. The present work, wherein attention has been particularly paid to such limiting factors as the carry over of growth factors with the inoculum, the influence of the source of nitrogen and carbon, pH of the medium and behaviour of different strains of the same fungus, is discussed in the light of some general concepts derived from the accumulated knowledge on vitamins and growth factors for fungi.

Robbins and Kavanagh (1942) recognized two kinds of heterotrophy, *viz.*, complete and partial. The former is termed by Lilly and Barnett (1951) as total. Both complete and partial deficiencies may be single, for one vitamin or multiple, for more than one vitamin. The widely worked out fungus *Phycomyces blakesleeanus* is a classical example of complete deficiency for a single vitamin, thiamine (Schopfer, 1943). In the case of *Lenzites trabea* (Lilly and Barnett, 1948) the deficiency is partial since the rate of growth of this organism doubled in the presence of thiamine, while in its absence, the fungus still attained maximum growth, though slowly. *Sordaria fimicola* for biotin (Lilly and Barnett, 1947) and *Phytophthora* spp. for thiamine (Robbins, 1938) may be cited as other examples of fungi deficient for a single vitamin. *Ceratostomella pini* (Robbins and Ma, 1942 *b*), *Ophiobolus graminis* (White, 1941), *Melanospora destruens* (Hawker, 1939 *a*) and *Lambertella pruni* (Lilly and Barnett, 1951) are some of the fungi reported to be deficient for thiamine and biotin, illustrating multiple deficiencies. Such deficiencies may again be total or partial. *Ophiobolus graminis* has been found to be highly or completely deficient for both thiamine and biotin (White, 1943). The individual vitamins themselves had no effect on this fungus. On the contrary, thiamine or biotin alone could promote some growth of *Lambertella pruni* indicating a partial deficiency of this organism to the respective vitamins (Lilly and Barnett, 1951). *Balstocladia pringsheimii* (Cantino, 1948) and *Ceratostomella ips* no. 255 (Robbins and Ma, 1942 *a*) are fungi showing deficiencies for more than two vitamins.

On the basis of the above classification, the *prima facie* evidence offered by the results of Expts. 1 and 2 indicates that all the species and isolates of *Piricularia* investigated show a deficiency for both thiamine and biotin (Tables I and II). *Sclerotium oryzæ*, on the other hand, has but a single deficiency for thiamine (Table III and Plate XXI).

Examination of Table I indicates that, while omission of thiamine from the vitamin mixture reduced growth of the different isolates by 62–84%, withdrawal of biotin brought down growth only by 10 to 29% except in the case of *Piricularia* sp. on *Eleusine coracana* where this reduction amounted to 46%. This naturally leads to the *a priori* assumption that the isolates of *Piricularia* are highly deficient or near totally so for thiamine and partially for biotin. However, none of these isolates were able to grow with thiamine or biotin, added singly to the medium (Table II). Complete growth resulted only in the presence of a combination of both vitamins showing thereby that the fungi in question are heterotrophic to thiamine as well as biotin.

The discrepancy in the results between the two experiments may be explained as being due to the different methods of inoculation used. In Expt. 1 a pin-head size of aerial mycelium without any adhering agar was used as inoculum after Robbins and Ma (1945). But, a loopful of mycelial suspension (*vide* 'Methods') formed the inoculum in Expt. 2. The very fact that growth amounting to 3 to 19% occurred in the controls in Expt. 1 is suggestive of the carry over of some quanta of the vitamins with the inoculum. It is not, therefore, surprising that biotin, carried over with the inoculum, perhaps in sub-optimal doses, has, nevertheless, contributed to appreciable growth of the fungi in the biotin-omitted treatments, in view of its activity in extremely minute quantities.

Further proof of the totality of the deficiencies of the species and isolates of *Piricularia* to thiamine as well as biotin and of *Sclerotium oryzæ* to thiamine is offered by a consideration of the response of these fungi to different levels of the vitamins over a sufficiently long period of incubation facilitating maximum growth (Text-Figs. 1–7). Based on the results of such a response the following generalizations can be made:

1. Growth of all the seven fungi is negligible in the absence of the vitamins in the medium. 2. Positive effect of the vitamins is felt only when they are present in amounts excess of 0·01 μg. per flask. 3. Significant growth is promoted by 0·1, 1·0, 10·0 μg. levels of vitamins and with these levels maximum growth is reached on the 12th day in the case of *P. oryzæ*, *P. setariæ*, *P. zingiberi*, *Piricularia* sp. from *Brachiaria mutica* and *Sclerotium oryzæ* and on the 9th day by *Piricularia* spp. from *Eleusine coracana* and *Triticum vulgare*. The fact that none of these fungi is able to make any appreciable growth even after prolonged incubation is indicative of the totality of their deficiencies. However, this total deficiency is multiple (for thiamine and biotin) for *Piricularia* spp. and single (for thiamine only) for *S. oryzæ*.

The optimum levels of the vitamins required by these fungi are found to be 1·0 μg. of thiamine and 0·01 μg. of biotin for all the species and isolates of *Piricularia* and 1·0 μg. of thiamine for *S. oryzæ* (Table IV and Text-Fig. 8). Even though the six isolates of *Piricularia* consisted of three distinct species and three isolates from different hosts, they did not exhibit any qualitative or quantitative differences in their vitamin requirements under the cultural conditions of the investigation.

The vitamin deficiencies of *Piricularia oryzæ*, *P. setariæ*, *Piricularia* sp. on *Eleusine coracana* and *Sclerotium oryzæ* are further considered with reference to the influence of the cultural environment. It must be emphasized that much attention has not been paid in this regard with many of the fungi reported to be vitamin-deficient. However, on the available evidence, Robbins and Kavanagh (1942) recognized two classes of vitamin deficiencies, *viz.*, absolute and conditioned. The total deficiency of *Phycomyces blakesleeanus* (Hawker, 1950; Lilly and Barnett, 1951) and *Ceratostomella fimbriata* (Lilly and Barnett, 1951) to thiamine has not been known to be altered by any environmental conditions. The deficiency of such fungi is termed absolute whereas in other cases the vitamin requirement of a fungus is largely dependent on the cultural environment. The influence of the source of nitrogen is reported with a mutant of *Neurospora sitophila* (Stokes *et al.*, 1943) where the fungus is found to require pyridoxine with nitrate, amino or amide sources of nitrogen. But, the need for an external supply of the vitamin is obviated in the presence of ammonium salts. The different *Piricularia* spp. and *Sclerotium oryzæ*, on the contrary, need only thiamine and biotin and thiamine respectively with inorganic nitrate, organic ammonium and amide sources of nitrogen. Pyridoxine or inositol is neither essential nor stimulatory with any of the nitrogen sources tried (Tables VI and IX). The red yeast *Rhodotorula sanniei* may be quoted as an example of the influence of the source of carbon on the vitamin requirement of a fungus. This organism requires thiamine when glucose formed the carbon source. But, when glycerol replaced glucose, thiamine is not needed (Fromageot and Tschang, 1938). Investigations in this direction on the *Piricularia* spp. and *Sclerotium oryzæ* have shown that replacing sucrose of the standard medium with nine different sugars does not alter the heterotrophy of these fungi, *i.e.*, thiamine and biotin for *Piricularia* spp. and thiamine to *S. oryzæ* (Tables VII and X). Further, the results indicate that the *Piricularia* spp. used in these experiments do not grow with glycerol. Even a combination of thiamine, biotin, pyridoxine and inositol is without effect with this source of carbon. On the other hand, *S. oryzæ* which is able to grow with glycerol still needs thiamine (Table X). Yet another such instance is the pH of the culture medium influencing the vitamin deficiency of a fungus and is illustrated by *Sordaria fimicola* (Lilly and Barnett, 1947). While partial thiamine deficiency of this fungus is noted at the pH range of 3·4 to 3·8, no such deficiency is apparent at pH 4·0 and above. This, however, is not the case with *Piricularia* spp. A range of pH from 4·0 to 8·0 does not alter the thiamine and biotin heterotrophy of these fungi (Table VIII).

Thus, in view of the consistent heterotrophy of these fungi to thiamine and biotin (*Piricularia* spp.) and thiamine (*S. oryzæ*), regardless of the source of nitrogen, carbon and pH of the culture medium, they may be considered to have an aboslute and total deficiency for the respective vitamins.

In the above discussion, the vitamin deficiencies of *Piricularia* spp. have been comparatively dealt with. Speaking of *P. oryzæ*, in particular, the results confirm its total multiple deficiency to thiamine and biotin. This result, while in conformity with that obtained by Leaver *et al.* (1947), differed in the following respects with the later work in Japan. Otani (1952) found that biotin is essential for this organism with thiamine as a complemental factor. Tanaka and Katsuki (1953) confirmed the findings of Otani (*loc. cit.*) and stated that thiamine was only stimulatory. Such divergent results in the vitamin deficiencies of one and the same species of a fungus are not uncommon. While certain strains of *Boletus granulatus* had an absolute demand for thiamine, others were only strongly stimulated by this vitamin (Melin and Nyman, 1941). Similar observations were made on *Marasmius perforans* (Lindeberg, 1944) and *Ophiostoma ulmi* (Fries, 1943).

The study of the vitamin requirements of *Piricularia* spp. was further extended to a consideration of the vitamin needs of non-pathogenic and pathogenic isolates. But the pathogenic strains of *P. oryzæ*, *P. setariæ* and *Piricularia* sp. from *Eleusine coracana* scarcely differed from the non-pathogenic strains in their heterotrophy to thiamine and biotin, quite independent of the source of nitrogen, carbon or pH of the medium (Tables VI–VIII). That vitamin deficiencies have no corrleation with loss in pathogenicity has been similarly reported in the case of *Venturia inaequalis* (Leben and Keitt, 1948).

Mathew (1954) observed that the fungus *Pellicularia koleroga* responded to different vitamins equally well when the isolate was fresh but ultimately became heterotrophic only to thiamine on repeated sub-culturings on artificial media. Such a phenomenon was not noticed with any of the *Piricularia* spp. tried. The fresh isolates maintained on 2% potato-dextrose agar for well over three years did not show any difference in their heterotrophy, with time. Further, that the substrate of the stock culture has no bearing on the vitamin heterotrophy has been shown (Expt. 8 and Table V).

Even though all *Piricularia* spp. used in this work are uniform in their heterotrophy to thiamine and biotin, their physiological abilities to use various nitrogen and carbon compounds varied somewhat with the type of the isolate (Tables XI and XII). But, some broad generalizations can be made which are considered below incidentally with the physiology of *S. oryzæ*. While organic ammonium and amide nitrogen as well as inorganic nitrate nitrogen were assimilable by all the *Piricularia* spp. inorganic ammonium sources of nitrogen proved non-assimilable under the cultural conditions of the investigation. This was found not to be due to a conditioned deficiency of pyridoxine or inositol (Table VI).

Sclerotium oryzæ, on the other hand, utilized all the three forms of nitrogen though sclerotial production was sparse with inorganic ammonium sources of nitrogen (Table IX). These results do not agree with those obtained by Tomizowa (1953) with *P. oryzæ* where this fungus has been reported to grow profusely with ammonium sulphate. But this inability to use inorganic ammonium nitrogen has been likewise noted with *Metarrhizium glutinosum* (Brian *et al.*, 1947).

Foster's (1949) stand that the inability of an organism to utilize various forms of nitrogen must be re-examined in the light of its growth factor requirements is very well brought out by this work on different *Piricularia* spp. Working on *P. oryzæ*, *P. setariæ* and *Piricularia* sp. on *Eleusine coracana*, Ramakrishnan (1948) obtained very poor growth of these fungi on KNO_3 but comparatively better growth with asparagine, urea and peptone. However, in this work, mention is not made regarding the purity of chemicals or addition of vitamins to the medium. Since the present work has shown the ability of all *Piricularia* spp. to use both potassium nitrate and urea equally well, the poor growth of these fungi with potassium nitrate in the previous case is evidently due to the lack of an external supply of thiamine and biotin. The better growth observed with asparagine, urea and peptone can be explained on the basis of the presence of growth factors in these chemicals. That commercial asparagine carries some biotin as contaminant has been reported (Robbins and Schmidt, 1939).

All the *Piricularia* spp. and *Sclerotium oryzæ* were found to grow with a variety of carbon sources, though their relative capacities to utilize the different sugars somewhat varied (Tables XII and X). Higher alcohols, in general, proved to be poor sources of carbon. Similar observations were made on *P. oryzæ* by Otani (1953) and Tomizowa (1953).

As to the possibility of using the fungi investigated for bioassays, these may not be preferred despite their consistent heterotrophy in view of the availability of several mutants of *Neurospora* (Beadle and Tatum, 1945) which by virtue of their rapid growth rate facilitate quick assays.

Finally, the present study may be examined on its possible significance in the taxonomy of *Piricularia* and host-parasite relationships.

Apart from the four species of *Piricularia* recognized by Padwick (1950), the taxonomic position of other isolates of *Piricularia* infecting various hosts is still uncertain. The present work has revealed the uniformity of the different species and isolates of *Piricularia* in their heterotrophy to thiamine and biotin. This naturally tends to reduce the importance of this study in the taxonomy of *Piricularia*. Nevertheless, further studies on the response of these fungi to different substitution products of thiamine and biotin might aid in fixing up the taxonomic position of *Piricularia* spp. infecting *Eleusine coracana*, *Triticum vulgare*, *Brachiaria mutica* and a number of other graminaceous plants.

The probable significance of the growth factor requirements of fungi in relation to the presence of growth substances in host cells has been discussed by Gäumann (1950) and Allen (1954). Work in this laboratory (Subramanyan and Suryanarayanan, unpublished) has indicated that desthiobiotin not only does not replace normal biotin for *P. oryzæ* but also inhibits growth. Speculatory as yet, it is not unlikely that such unsuitable analogues are present in the resistant types especially on the leaf surface as products of exosmosis and their vital role at the "infection-court" in the pre-penetration stages can hardly be ruled out.

Summary

Detailed studies made on *Piricularia oryzæ*, *P. setariæ*, *P. zingiberi*, *Piricularia* spp. on *Eleusine coracana*, *Triticum vulgare* and *Brachiaria mutica* and *Sclerotium oryzæ* showed them to have a total heterotrophy to vitamins which seemed to be absolute. All the species and isolates of *Piricularia* had a multiple deficiency to thiamine and biotin while *S. oryzæ* had a single deficiency to thiamine.

The optimal amounts of vitamins for these fungi were found to be about $1 \cdot 0\,\mu g$. of thiamine and $0 \cdot 01\,\mu g$. biotin per flask in the case of *Piricularia* spp. and $1 \cdot 0\,\mu g$. thiamine for *S. oryzæ* under the cultural conditions of investigation.

The heterotrophy of these fungi to the respective vitamins was not altered by the type of nitrogen or carbon source and at various pH ranges, thereby indicating their vitamin deficiencies to be absolute and not conditioned by the cultural environment. No correlation was found to exist between the vitamin requirements of these fungi and their virulence.

The physiology of these fungi, considered from the aspect of their growth factor requirements, revealed the inability of *Piricularia* spp. to utilize inorganic ammonium sources of nitrogen. *S. oryzæ*, on the other hand, utilized inorganic nitrate, ammonium nitrogen and organic nitrogen. Nonetheless, inorganic ammonium sources of nitrogen were not favourable for sclerotial production by this fungus. The unsuitability of this form of nitrogen to the growth of *Piricularia* spp. and sclerotial formation by *S. oryzæ* was found to be not due to a conditioned deficiency of pyridoxine or inositol.

The fungi investigated were found to use a variety of carbon sources. In general, sugars were better sources of carbon than higher alcohols. The importance of the study of growth factors in fungal physiology is discussed.

The utility of this study in the taxonomy of *Piricularia*, the usefulness of the organisms for bioassays and the probable significance of this

investigation in understanding disease resistance to *Piricularia* are discussed in a general way.

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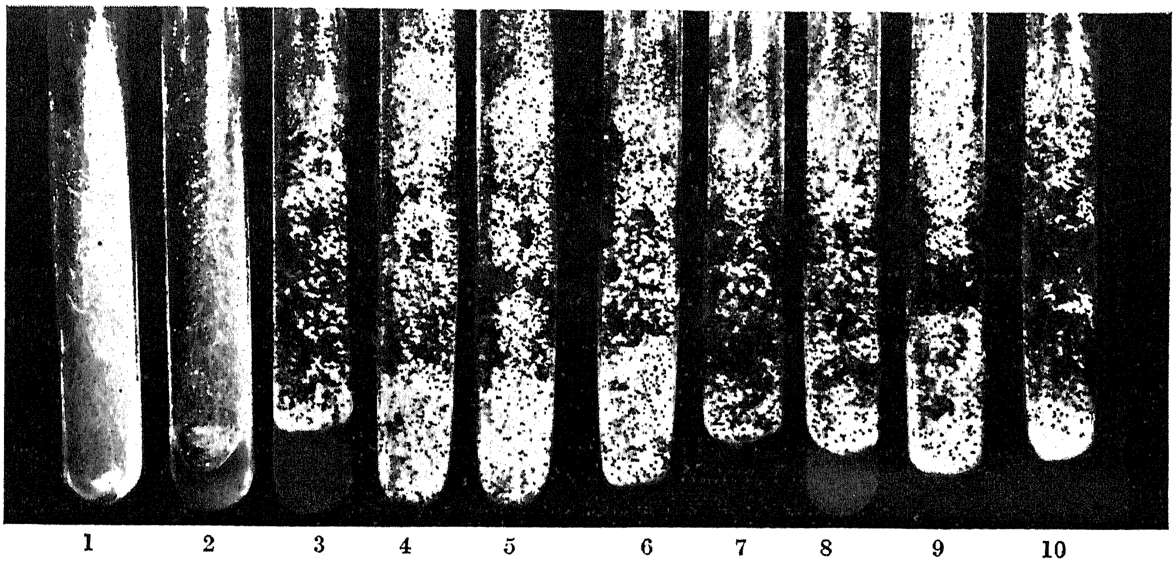

Plate showing the effect of excluding a single vitamin from a vitamin mixture (+All) on growth of *Sclerotium oryzæ*.

1. Control. 2. Thiamine. 3. Biotin. 4. Pyridoxine. 5. Nicotinic acid. 6. Inositol. 7. Pantothenic acid. 8. *p*-Amino benzoic acid. 9. Riboflavin. 10. + All vitamins.