STUDIES ON THE PHYSIOLOGY OF *HELMINTHOSPORIUM CARBONUM* ULLSTRUP, THE INCITANT OF CORN LEAF SPOT

by RAM DAYAL *and* M. M. JOSHI,* *Department of Plant Pathology, Banaras Hindu University, Varanasi 5*

(Communicated by R. K. Saksena, F.N.I.)

(*Received 15 January 1968; after revision 24 December 1968*)

The optimum temperature, *p*H and humidity for the growth of *Helminthosporium carbonum* Ullstrup were found to be 26 °C, *p*H 8 and 95 per cent RH respectively. Maltose was the most suitable carbon source for growth, and galactose for sporulation. Tyrosine proved to be the best nitrogen source. Nitrate nitrogen was utilized more efficiently than nitrite and ammonium nitrogen. Of the five hormones tried, none but 3-Indol propionic acid significantly increased the growth and sporulation of the fungus.

INTRODUCTION

Helminthosporium carbonum Ullstrup, the incitant jo cornleaf spot, has been found to occur at Varanasi in 1964. From the literature available, it appears that little is known about the physiology of this fungus in culture (Hale and Roane 1961). Therefore, a detailed study on this aspect was undertaken in the present investigation.

MATERIALS AND METHODS

Monosporic culture of *Helminthosporium carbonum* was obtained by single spore isolations from infected corn leaves collected at the College of Agriculture farm, Banaras Hindu University. Stock cultures were maintained on potato —dextrose agar. Asthana and Hawker's medium (Dextrose 5 g, KH_2PO_4 1·75 g, KNO_3 3·5 g, $MgSO_4.7H_2O$ 0·75 g and distilled water 1,000 ml) was used as basal medium throughout the investigation. Twenty-five millilitres of the medium, with the acidity usually adjusted to *p*H 7, were dispensed into 100 ml Pyrex Erlenmeyer flasks and autoclaved at 15 lb pressure for 15 minutes. For all the experiments conducted, inoculum of equal size (5 mm agar discs) was used from 5 to 10 days' old cultures. Unless otherwise indicated, the flasks were incubated at room temperature (24±6 °C). Triplicates were made in each treatment. Amount of growth was based on the oven-dry weight of the washed mycelial mass in each container. The tests on solid media were made in Petri plates (100 mm diameter) containing 20 ml of basal medium

* At present Senior Research Assistant, Department of Plant Pathology, U.P. Agricultural University, Pantnagar, Dist. Naini Tal (U.P.).

with 2 per cent agar. The degree of sporulation was measured by visual observation of the slides examined under microscope. The size of conidia is based on the measurements of 100 conidia and recorded in micron.

In studies to determine the influence of H-ion concentration of the medium, initial *p*H values of 3–10 were adjusted with phosphoric acid and sodium hydroxide before autoclaving. To study the effect of humidity, various humidities were maintained by the method suggested by Spencer (1928). Carbon and nitrogen nutrition of the fungus were studied by substituting the original compound, i.e. glucose and potassium nitrate, in the basal medium with different sources of carbon and nitrogen in such a way so as to supply the same amount of these constituents normally present in the basal medium. In the case of hormone trials 10 μg of each hormone was added to 100 ml of the basal medium and 25 ml of this medium was distributed into Erlenmeyer flasks.

Results and Discussion

Effect of temperature

Environmental factors greatly influence growth, sporulation and conidial size of *Helminthosporium carbonum* Ullstrup. The fungus grew within a temperature range of 18–34 °C, optimum being 26 °C (Table I). This temperature is favourable for sporulation as well as for the size of conidia. Chandwani and Munjal (1963), and Misra and Singh (1963) also observed that best growth of *Helminthosporium gramineum* and *H. turcicum* was obtained at 25° and 25–30 °C respectively.

Table I

Radial growth, sporulation and size of conidia of H. carbonum *at different temperatures*

Temperature (°C)	Radial growth* (mm)	Sporulation	Size of conidia (μ)
18	14·0	Good	59·95 × 14·16
22	26·5	Good	62·55 × 15·66
26	57·5	Excellent	69·95 × 15·99
30	30·0	Good	65·99 × 16·23
34	14·5	Fair	41·63 × 11·39

* Growth after 10 days.

Effect of hydrogen-ion concentration

The fungus could grow over a wide range of *p*H from 3 to 10; a *p*H range from 6 to 8 was more favourable optimum being *p*H 8 (Table II). Similar

results were obtained by Thind and Sandhu (1956) while working with *Gloeosporium psidii*. The optimum *p*H for growth seems to be best also for sporulation and size of conidia. During the growth period, the fungus considerably increased the initial *p*H of the medium at each level except *p*H 9 and 10, which is in conformity with the results obtained by Hale and Roane (1961) for *Helminthosporium carbonum* Race 1, and Chandwani and Munjal (1963) for *H. gramineum*.

TABLE II

Effect of p*H of the medium on the growth, sporulation and conidial size of* H. carbonum

Initial *p*H of the medium	Final *p*H of the medium	Dry wt. of fungal mat. (mg)	Sporulation	Size of conidia (μ)
3	6·0	19·5	Good	56·61 × 14·15
4	6·1	22·0	Good	63·27 × 16·65
5	6·7	29·3	Good	63·55 × 14·99
6	7·3	32·0	Good	63·71 × 11·65
7	7·6	34·0	Good	65·34 × 21·10
8	8·2	37·3	Good	73·26 × 16·65
9	9·0	34·3	Nil	–
10	10·0	32·6	Nil	–

Effect of relative humidity

The organism under investigation could tolerate a wide range of relative humidity from 55 to 100 per cent. The maximum growth was obtained at 95 per cent RH (Table III). Almost similar results were obtained by Misra

TABLE III

Radial growth, sporulation and size of conidia of H. carbonum *at different relative humidities*

Relative humidity (%)	Radial growth* (mm)	Sporulation	Size of conidia (μ)
100	56·5	Fair	78·2 × 13·99
98	60·75	Good	60·69 × 12·39
95	81·75	Good	55·81 × 12·39
92	74·50	Fair	49·93 × 10·74
88	53·83	Fair	49·90 × 10·72
81	49·50	Fair	44·39 × 10·72
65	49·0	Fair	36·79 × 9·99
55	44·5	Fair	36·63 × 9·99

* Growth after 6 days.

and Singh (1963) for *Helminthosporium turcicum*. There was not much influence of relative humidity on sporulation; however, good sporulation was observed at 95 and 98 per cent RH. Conidia attained maximum size at 100 per cent RH. Similar results were obtained by Klotz (1923), Ramakrishnan (1931), Chaudhury (1944) and Berger (1963) with some of the *Cercospora* spp. studied by them.

Effect of carbon source

Though, disaccharides in general appear to be good sources of carbon for the fungus, maltose was the best followed by galactose (Table IV). Glucose, the common carbon source for many fungi, supported only moderate growth of this fungus. Chlamydospores too were formed on glucose. Fructose seems to be the poorest source. These findings do not agree with those of Hale

TABLE IV

Effect of different sources of carbon on growth, sporulation and size of conidia of H. carbonum

Carbon sources	Dry wt. of fungal mat. (mg)	Sporulation	Size of conidia (μ)
Glucose ..	53·6	Good	41·62 × 10·72
Fructose ..	40·6	Poor	49·95 × 10·72
Galactose ..	78·3	Excellent	78·25 × 14·98
Sucrose ..	73·0	Fair	49·95 × 10·72
Lactose ..	73·0	Good	46·62 × 9·57
Maltose ..	85·0	Nil	–
Starch ..	47·0	Poor	36·63 × 9·99
Control ..	0·0	Nil	–

and Roane (1961) for *H. carbonum* Race 1, but are in conformity with observations reported by Converse (1953), and Chandwani and Munjal (1963) for *Helminthosporium gramineum*. The difference in the utilization of sugars by *H. carbonum* may be attributed to the behaviour of different isolates of the fungus. Starch was also utilized as a sole source of carbon and this suggests the possibility of production of amylase enzyme capable of hydrolysing it. Similar result was found with *H. gramineum* by Chandwani and Munjal (1963). No growth was observed in complete absence of carbon. The carbon source which is best for growth may not always prove good for sporulation. Excellent sporulation and largest conidia were observed on galactose while no sporulation occurred on maltose. Sucrose supported poor sporulation. Sucrose and maltose supported poor or no sporulation of *Leptomitus lacteus* (Schade 1940) and *Chytridium* sp. (Crasemann 1954) as well.

Effect of nitrogen source

Since the fungus under study showed very little growth and no sporulation in the absence of nitrogen, it is evident that nitrogen is essential for its growth and sporulation (Table V). Good utilization of nitrate nitrogen (given in the form of potassium and sodium nitrate) finds support from the results obtained by Thind and Sandhu (1956), and Misra and Thakur (1965) for *Gloeosporium psidii* and *G. musarum* respectively. Sodium nitrite was comparatively a poor source and in this respect the results were similar with that of Saksena and Kumar (1960) for some *Sphaeropsidales*. Ammonium sulphate proved to be the poorest source of nitrogen. This may be due to a sharp drift in *p*H towards acidic side. Similar results were obtained by Blank and Talley (1941) with *Phymatotrichum omnivorum*, Thind and Randhawa (1957) with *Colletotrichum capsici*, and Singh and Khanna (1966) with citrus isolate of *Alternaria tenuis*. Among the organic sources used, tyrosine supported best growth. Asparagine also proved to be a good source of nitrogen while methionine was poorest among all the organic sources used. The above findings agree with those of Converse (1953), and Chandwani and Munjal (1963) for *Helminthosporium gramineum*. All the nitrogen sources, except methionine, supported good sporulation. Only abundant chlamydospores were formed on ammonium sulphate and in the medium devoid of nitrogen, which were most unfavourable for growth. Maximum size of conidia was obtained on sodium nitrate.

TABLE V

Effect of different sources of nitrogen on growth, sporulation and conidial size of H. carbonum

Nitrogen source	Dry wt. of fungal mat. (mg)	Sporulation	Size of conidia (μ)
Sodium nitrite ..	29·0	Good	44·95 × 10·82
Sodium nitrate ..	69·0	Good	61·60 × 11·65
Potassium nitrate ..	60·0	Good	54·94 × 14·15
Ammonium sulphate	23·3	Nil	–
Asparagine ..	80·0	Good	59·94 × 14·98
Glycine	61·0	Good	48·28 × 15·81
Leucine ..	62·0	Good	59·94 × 14·98
Methionine ..	24·0	Fair	46·62 × 11·65
Tyrosine ..	89·5	Good	58·27 × 14·98
Control	12·0	Nil	–

Effect of hormones

Five growth regulators, namely 3-Indol propionic acid (IPA), Indol-3-yl-acetic acid (IAA), 1-Naphthyl acetic acid (NAA), Indolyl-3-butyricum acetic

acid (IBA) and 2, 4-Di chlorophenoxy acetic acid (2, 4-D), were tried for their effects on growth and sporulation.

The results (Table VI) indicate that except 3-Indol propionic acid none of the other hormones could influence the growth of the fungus; however, Indolyl-3-butyricum acetic acid considerably reduced mycelial output. Almost similar results were found by Thind and Sandhu (1956) for *Gloeosporium psidii*. The reduction by some hormones in the dry weight of this fungus may be attributed to concentration used in this study.

TABLE VI

Growth, sporulation and size of conidia of H. carbonum *in the presence of different growth regulators in basal medium*

Growth regulator	Dry wt. of fungal mat. (mg)	Sporulation	Size of conidia (μ)
IPA	40·0	Excellent	58·27 × 14·98
IAA	26·0	Nil	–
NAA	30·0	Good	58·27 × 13·32
IBA	16·3	Nil	–
2, 4-D	28·0	Nil	–
Control	28·6	Good	59·94 × 14·15

ACKNOWLEDGEMENTS

The authors acknowledge their thanks to the Director, Commonwealth Mycological Institute, Kew, Surrey, England, for confirmation of the fungus and to Dr. R. K. Saksena, D.Sc. (Paris), for communicating this paper.

REFERENCES

Berger, R. D. (1962). Relations of the environmental factors on growth and sporulation of *Cercospora zebrina*. *Phytopathology*, 53, 286–294.

Blank, L. M., and Talley, P. J. (1941). The carbon utilization and carbo-hydrase activity of *Phymatotrichum omnivorum*. *Am. J. Bot.*, 28, 564–569.

Chandwani, G. H., and Munjal, R. L. (1963). Studies on physiology of *Helminthosporium gramineum* Rab., the causal agent of stripe disease of barley. *Indian Phytopath.*, 16, 301–306.

Chaudhury, S. (1944). Physiology of *Cercospora sesami*. *J. Indian bot. Soc.*, 23, 91–107.

Converse, R. H. (1953). Influence of the nitrogen compounds on the growth of *Helminthosporium gramineum* Rab. in culture. *Mycologia*, 45, 335–344.

Crasemann, J. M. (1954). The nutrition of *Chytridium* and *Macrochytrium*. *Am. J. Bot.*, 41, 302–310.

Hale, M. G., and Roane, C. W. (1961). The nutrition of *Helminthosporium carbonum* Race 1 in relation to parasitism on corn. *Phytopathology*, 51, 235–240.

Klotz, L. J. (1923). A study on the early blight fungus *Cercospora apii* Fes. *Bull. Mich. agric. Coll. Exp. Stn.*, No. 63.

Misra, A. P., and Singh, S. P. (1963). Effect of temperature and humidity on the development of *Helminthosporium turcicum* Pass. *Indian Phytopath.*, 16, 158–163.

Misra, A. P., and Thakur, D. P. (1965). Nutritional requirements of *Gleosporium musarum*. *Indian Phytopath.*, **18**, 116–122.

* Ramakrishnan, T. S. (1931). A leaf spot disease of *Andropogan sorghum* caused by *Cercospora sorghi* E and E. *Mem. Dep. Agric. Indian bot. Ser.*, **8**, No. 9.

Saksena, R. K., and Kumar, D. (1960). Nitrogen requirements of some *Sphaeropsidales*. *Proc. natn. Acad. Sci. India*, B **31**, 376–384.

Schade, A. L. (1940). The nutrition of *Leptomitus*. *Am. J. Bot.*, **27**, 376–384.

Singh, R. S., and Khanna, R. N. (1966). Physiological studies on *Alternaria tenuis* Auct., the fungus causing a black core rot of mandarin oranges. *J. Indian bot. Soc.*, **45**, 277–286.

* Spencer, H. M. (1928). Laboratory methods for maintaining constant humidity. *Int. critical Tabl.*, **4**, 67-68.

Thind, K. S., and Randhawa, H. S. (1957). The influence of different sources of nitrogen on the growth and sporulation of *Colletotrichum capsici*. *Proc. natn. Acad. Sci. India*, B **27**, 47–52.

Thind, K. S., and Sandhu, R. S. (1956). Studies on the nutrition of *Gloeosporium psidii* (G. Del) Sacc., the incitant of the guava anthracnose. *Indian Phytopath.*, **9**, 207–208.

---

* Original not seen.