

RELATION OF NITROGEN TO GROWTH AND  
SPORULATION OF *FUSARIUM*  
*VASINFECTUM* ATK.

BY C. V. SUBRAMANIAN

*University Botany Laboratory, Madras*

AND

K. V. SRINIVASA PAI

*Chemistry Department, Madras Christian College, Tambaram*

(Received January 23, 1953)

(Communicated by Prof. T. S. Sadasivan, F.A.Sc.)

THE role of nitrogen in the physiology of fungi has received considerable attention from mycologists in the past (*see* Lilly and Barnett, 1951; Hawker, 1950) and the present communication is but an addition to the voluminous literature already extant. The work reported here deals with some aspects of a study of a vascular wilt-producing species of *Fusarium*, *viz.*, *F. vasinfectum* Atk., the fungus causing cotton wilt. It is admitted that plant diseases, and especially those of a soil-borne nature, require for their elucidation study of physiology of the host, the parasite or the pathogen, and the host-parasite relationship itself (Brown, Brooks and Bawden, 1948). Comparatively little has been attempted in the investigation of such problems in relation to cotton wilt. The effect of trace elements on the physiology of *F. vasinfectum* and some other species of *Fusarium* has recently been studied in this laboratory (Yogeswari, 1948; Sadasivan, 1951). Doubtless, major elements like N, P and K are equally important. The results of a preliminary investigation on the effect of four different nitrogen sources on the growth, sporulation, nitrogen accumulation, etc., of *F. vasinfectum* are presented in this paper.

MATERIALS AND METHODS

A culture of *Fusarium vasinfectum* Atk. obtained from the Government Mycologist, Coimbatore, was used. The identity of the fungus and also its pathogenicity on cotton plants have been confirmed by one of us (C. V. S.) after careful study.

For chemical analyses, the fungus was grown in 100 ml. of Richard's synthetic liquid medium (Rawlins, 1933, p. 85) in 250 ml. Pyrex Erlenmeyer flasks (three replicates each), the nitrogen source and the amount of nitrogen supplied being varied. Four different sources of nitrogen, *viz.*, potassium

nitrate, ammonium sulphate, ammonium nitrate and urea were used, each at four different levels, *viz.*, 28 mg., 70 mg., 140 mg. and 210 mg. per 100 ml. of medium (levels 1-4 respectively). ANALAR chemicals were used throughout the investigation. Standard mycological technique (Rawlins, 1933) was followed. The pH of the media was adjusted to 5.5 prior to autoclaving, using a Beckman electronic pH meter and N/10 HCl or N/10 NaOH as reagents. The following determinations were made after 21 days' growth of the fungus in the cultures, these being incubated at room temperature (28-31° C.):—

1. dry weight of the fungal mat;
2. pH of the filtrate;
3. total nitrogen content of the fungal mat;
4. residual nitrogen in the filtrate:  
(a) total; (b)  $\text{NO}_3\text{—N}$ ; (c)  $\text{NH}_4\text{—N}$ ;
- and 5. residual reducing sugars in the filtrate.

The above estimations were made using methods already described in detail elsewhere (Srinivasa Pai, 1953).

For assessing sporulation of the fungus, it was grown in the different media (6 replicates each) for 21 days in 10 ml. each of the medium in Pyrex test tubes. Spore counts were made using a Neuberg hæmacytometer. For statistical analysis spore counts per unit area were considered; spore numbers per ml. of the medium have been computed from these figures and they are represented in Fig. 1.

## RESULTS

The results are presented in Table I and Fig. 1. The statistical analyses of some of the data are given in Table II.

From the results presented, the following conclusions may be drawn:

### *Changes in pH* (Table I)

1. In media with ammonium sulphate, at all levels, the pH of the media was brought down to about 2 at the end of 21 days.
2. In media with potassium nitrate, ammonium nitrate and urea, at all levels, the pH was raised to about 6-7.2, except in the medium with urea level 1, where there was no change in pH.

### *Dry weight of fungal mat* (Tables I and II, Fig. 1)

Statistical analysis of the data indicate that:

1. The highest mat weight was produced at potassium nitrate level 3, followed by ammonium nitrate levels 3 and 4.

TABLE I

Showing final pH, mat weight, N in mat, % N in mat, residual N and residual sugar in medium, spore counts per unit area and spore numbers per ml. of medium after 21 days' growth of *Fusarium vasinfectum* in culture media supplied with different sources and levels of N

No.	Final pH	Mat wt. in mg.	Nitrogen in mat mg.	% N in mat	Residual N in media			Residual sugar in gm.	Spore counts/unit area	Spores per ml.
					NO <sub>3</sub> -N	NH <sub>4</sub> -N (in mg.)	Total			
1	6.7	418.7	16.38	3.9	4.14	3.60	8.25	..	675	10,800,000
2	6.75	514.4	20.43	3.9	37.32	6.48	44.46	..	612	9,792,000
3	7.0	714.2	32.19	4.5	100.20	2.86	102.50	..	586	9,376,000
4	7.2	421.4	17.03	4.0	175.00	4.23	183.30	..	710	12,860,000
5	2.1	211.1	19.69	9.3	..	9.05	9.38	0.25	217	3,472,000
6	2.0	241.1	23.30	9.6	..	44.94	45.50	0.44	121	1,936,000
7	2.0	181.1	17.28	9.5	..	118.20	120.20	0.74	170	2,936,000
8	2.1	164.4	16.67	10.1	..	189.48	191.90	1.06	200	3,136,000
9	5.5	445.5	26.30	5.9	..	1.86	1.86	..	416	6,656,000
10	6.5	418.6	31.99	7.6	..	5.12	31.40	0.17	685	10,960,000
11	6.9	413.0	30.76	7.4	..	5.94	108.90	0.19	900	14,400,000
12	7.1	424.0	41.14	9.7	..	5.98	168.50	0.40	653	10,448,000
13	6.9	423.6	13.65	3.2	3.16	8.48	12.86	..	252	4,016,000
14	7.0	506.7	17.36	3.4	14.28	34.20	51.86	..	262	4,192,000
15	7.1	602.8	22.23	3.6	47.94	68.98	112.84	..	137	2,112,000
16	7.1	627.3	24.54	3.9	84.10	98.40	182.40	..	108	1,728,000

Nos. 1-4 : KNO<sub>3</sub> at 28, 70, 140 and 210 mg./100 ml. respectively;  
 5-8 : (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at 28, 70, 140 and 210 mg./100 ml. respectively;  
 9-12 : Urea at 28, 70, 140 and 210 mg./100 ml. respectively;  
 13-16 : NH<sub>4</sub>NO<sub>3</sub> at 28, 70, 140 and 210 mg./100 ml. respectively.

Note : In analysing the data statistically (Table II), fractions of 0.5 and over have been taken as one; fractions less than 0.5 have been omitted.

2. The lowest mat weights were produced in the case of ammonium sulphate at all levels.

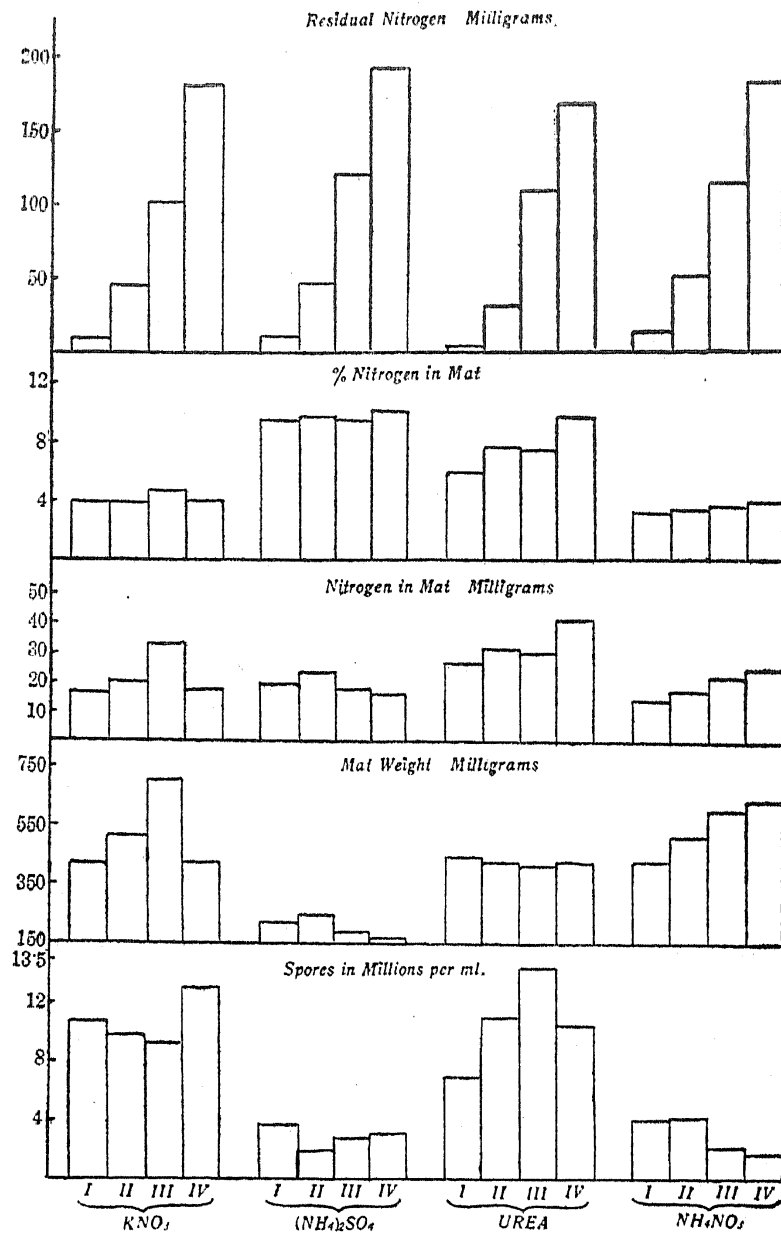
3. The differences between the mat weights in the other treatments were not significant.

*Accumulation of nitrogen in fungal mat (Tables I and II, Fig. 1)*

Statistical analysis of the data shows that:

1. Significantly the greatest amount of nitrogen was found in fungal mats raised in media with urea level 4 followed by potassium nitrate level 3 and urea levels 2 and 3.

2. The differences in the nitrogen content of the other treatments were not significant.



TEXT FIG. 1. Relation of nitrogen to growth and sporulation of *Fusarium vasinfectum* Atk.

*Percentage nitrogen in fungal mat* (Table I, Fig. 1)

The data show that:

1. The maximum percentage accumulation of nitrogen was in the case of ammonium sulphate at all levels and also in the case of urea level 4.
2. The minimum percentage accumulation of nitrogen was in the case of potassium nitrate and ammonium nitrate.

TABLE II

*Showing statistical analyses of some of the data presented in Table I  
(the treatment numbers used are the same)*

Mat weight :

Significance by F test : yes C.D. 1% : 8.4

Conclusion : 3, 16, 15, 2, 14, 9, 13, 12, 4, 1, 10, 11, 6, 5, 7, 8

Nitrogen in mat :

Significance by F test : yes C.D. 1% : 5

Conclusion : 12, 3, 10, 11, 9, 16, 6, 15, 2, 5, 4, 7, 8, 1, 13, 14

Total Residual Nitrogen (in Medium) :

Significance by F test : yes C.D. 1% : 4

Conclusion : 16, 4, 8, 12, 7, 15, 11, 3, 14, 6, 2, 10, 13, 5, 1, 9

Spores per unit area :

Significance by F test : yes C. D. : 7.5

Conclusion : 11, 4, 10, 1, 12, 2, 3, 9, 14, 13, 5, 8, 7, 15, 6, 16

3. Percentage accumulation of nitrogen was the same for a given salt, irrespective of the concentrations used, in the case of potassium nitrate, ammonium sulphate and ammonium nitrate. In the case of urea, however, there were increases in percentage accumulation of nitrogen in the fungal mat with increasing doses of the nitrogen source.

*Total residual nitrogen in medium* (Tables I and II, Fig. 1)

1. Residual nitrogen significantly increased from levels 1 to 4, in the case of all four sources, indicating that the greater the amount supplied the greater the amount left behind.

2. Comparing levels 1 and 2 for all four sources, it is found that significantly the lowest residual nitrogen was left behind in media with urea.

3. Comparing all four sources at level 3, it is found that significantly the greatest residual nitrogen was left behind in media with ammonium sulphate, while the lowest residual nitrogen was left behind in the medium with potassium nitrate.

4. Comparing all four sources at level 4, it is seen that the least residual nitrogen was left behind in the medium with urea; the differences between the other salts, viz., ammonium nitrate, potassium nitrate and ammonium sulphate, were not statistically significant.

5. Almost complete depletion of nitrogen appeared to have taken place only in the case of the medium with urea level 1.

*Residual reducing sugars in medium (Table I)*

No residual sugar could be detected in media with all levels of potassium nitrate and ammonium nitrate, and urea level 1. In the other media different amounts of sugars could be detected after 21 days' fungal growth.

*Sporulation (Tables I and II, Fig. 1)*

1. Significantly the greatest sporulation occurred in the medium with urea level 3.
2. In media with ammonium nitrate and ammonium sulphate, at all levels, significantly lower number of spores was produced than in media with all levels of urea and potassium nitrate.
3. Comparing urea and potassium nitrate, it is seen that significantly the lowest sporulation occurred in the medium with urea level 1. Sporulation at other levels was not statistically different in media supplied with these two sources.

## DISCUSSION

A comparison of the four different sources of nitrogen used in this investigation in relation to the growth, sporulation, etc., of *Fusarium vasinfectum* would be interesting.

The estimations of residual nitrogen at the end of 21 days' growth of the fungus show that there is probably an optimum amount of N required for growth of the fungus and additional supply of this element would not favour greater uptake of this element by the fungus, even if the C/N ratio is altered by varying the N content in the media. In other words, the utilization of N by the fungus is limited by certain factors, for instance the supply of carbon, and therefore increase in N in the medium without a proportionate or comparable increase in C would not encourage greater uptake of N. Inorganic salts of N (potassium nitrate, ammonium sulphate and ammonium nitrate) and also organic nitrogen (urea) had similar effects as far as uptake of N was concerned with increasing concentrations of the salts.

From the data presented in this paper, it is obvious that *F. vasinfectum* can utilize nitrates, ammonium salts and organic sources of N, and in so far as its ability to fix atmospheric N has not been demonstrated, it should be placed in Group II of the classification proposed by Robbins (1937).

The sporulation of the fungus and its growth, as indicated by its mat weight, differed with different sources and with different levels of these sources. Ammonium sulphate proved to be a poor source of N for vegetative growth and for sporulation of the fungus, although the percentage of N accumulated in the fungal mat was the highest when this salt was used.

The poor vegetative growth and sporulation of the fungus at all levels of ammonium sulphate is probably related to the very low final pH obtained in this case; it is also likely that the medium is made unfavourable for the growth of the fungus by the production of substances inhibiting its own growth. The high percentage of nitrogen contained in the mat raised in media with ammonium sulphate is attributable to the poor uptake and accumulation of substances other than N. Compared to ammonium sulphate, ammonium nitrate was a good enough source for vegetative growth, but not for sporulation. Nitrate (as potassium nitrate) and organic nitrogen (as urea) were both much better sources for vegetative growth as well as sporulation, and between them they appeared to be equally good in this respect, although in the medium with potassium nitrate level 3 significantly the greatest mat weight was produced and in the medium with urea level 3 there was significantly the maximum sporulation. Considering, however, the accumulation of N in the fungal mat, it is evident that there is a somewhat greater percentage of N in the mat in the case of urea than in the case of potassium nitrate; it is noteworthy that the high percentage N content in the mat in the case of urea is not the result of poor accumulation of substances other than N in the mat as in the case of ammonium sulphate. On the other hand, this situation is explained partly at least by the fact that there is greater uptake of this element in the case of urea, and hence also a greater depletion of N from the medium as shown by the data for residual N.

The data on residual sugar in the media after 21 days' growth of the fungus show the improbability of further growth of the fungus in the case of all levels of potassium nitrate and ammonium nitrate, and urea level 1, since there was no sugar left in the media. Just when complete depletion of sugar took place within the 21-day period is not clear from the present work since periodical estimations of residual nitrogen were not carried out. The earlier work of Srinivasa Pai (1953) indicates, however, that in the case of urea level 3, depletion of sugar from the medium would be complete after 18 days' growth of the fungus, and in the case of all levels of potassium nitrate at the end of 21 days. Srinivasa Pai did not include ammonium nitrate in his study. The presence of residual sugar in the case of all levels of ammonium sulphate and urea levels 2-4 indicates the possibility of further growth of the fungus; in any case, sugar is not a limiting factor for further growth.

Apart from the points mentioned above, no generalizations are possible, regarding the interrelationships of nitrogen uptake, nitrogen accumulation, sugar depletion, vegetative growth and sporulation of the fungus. Inverse correlation between vegetative growth and sporulation is often mentioned

in literature and, indeed, in the present work such a correlation is obvious in the case of ammonium nitrate where increasing doses of N increased vegetative growth but decreased sporulation; but this need not be a universal feature as is shown by the poor vegetative growth as well as poor sporulation when ammonium sulphate is used as the nitrogen source. Similarly, decrease in C/N ratio might increase the amount of N accumulated in the mat as in the case of ammonium nitrate (see Fig. 1); but this is related to the particular source of nitrogen used, since similar results are not obtained when the media are prepared with the same C/N ratios, but using different sources of N, like potassium nitrate, ammonium sulphate and urea.

It is admitted that the present work is of a preliminary nature. Only the effect of variations in nitrogen sources and levels has been studied, other conditions being kept constant. Variation in other factors, *e.g.*, the source and the amount of carbon supplied, would doubtless give a different picture, particularly of the relation of different sources and doses of N to the physiology of the fungus. The main purport of this paper is only to emphasize the complexity of the problem and the need for further detailed work. For, the application of inorganic and organic fertilizers with a view to enrich the nitrogen status of soils cannot but have its effects on soil-borne pathogens perennating in soils; these findings, moreover, may have implications in relation to host-fungus physiology as well, with which is linked up the vital question of host-resistance to soil-borne pathogens.

#### SUMMARY

The effect of four different nitrogen sources, *viz.*, potassium nitrate, ammonium sulphate, ammonium nitrate and urea, each at four different levels, *viz.*, 28 mg., 70 mg., 140 mg., and 210 mg. per 100 ml. of medium, on the growth, sporulation, nitrogen accumulation, and sugar and nitrogen depletion from media by *Fusarium vasinfectum* has been studied. The results are presented in detail and discussed. The main conclusions are summarised under "Results".

#### ACKNOWLEDGEMENTS

We are grateful to Prof. T. S. Sadasivan for suggestions and criticisms during the course of the investigation, and to Sri. S. Suryanarayanan for the statistical analyses of the data.

#### REFERENCES

- Brown, W., Brooks, F. T. and Bawden, F. C. "A discussion on the physiology of resistance to disease in plants" *Proc. Roy. Soc.*, 1948, 135 B, 171-95.  
Hawker, L. E. .. *Physiology of Fungi*, 360 pp., University of London Press Ltd., London, 1950.



*Relation of Nitrogen to Growth and Sporulation of F. vasinfectum* 157

- Lilly, V. G. and Barnett, H. L. . . . *Physiology of the Fungi*, 1st. Edn., 464 pp., McGraw Hill Book Co., Inc., New York, 1951.
- Rawlins, T. E. . . . *Phytopathological and Botanical Research Methods*, 156 pp., John Wiley & Sons, Inc., New York, 1933.
- Robbins, W. J. . . . "The assimilation by plants of various forms of nitrogen," *Amer. J. Bot.*, 1937, 24, 243-50.
- Sadasivan, T. S. . . . "Role of trace elements in control of root-infecting fungi," *Proc. Indian Acad. Sci.*, 1951, 33 B: 138-49.
- Srinivasa Pai, K. V. . . . "Carbon, nitrogen and pH relationships to growth of soil *Fusaria* in culture," *Proc. Indian Acad. Sci.*, 1953, 37 (4), B.
- Yogeswari, L. . . . "Trace element nutrition of fungi. I. The effect of boron, zinc and manganese on *Fusarium* species," *Proc. Indian Acad. Sci.*, 1948, 28 B: 177-201.