UTILIZATION OF VARIOUS FORMS OF NITROGEN BY *DRECHSLERA SOROKINIANA*, THE PATHOGEN CAUSING FOOT ROT DISEASE IN WHEAT*

By C. V. Subramanian, F.A.Sc.

(*Centre for Advanced Studies, University Botany Laboratory, Madras*)

AND

P. D. Tyagi

(*Punjab Agricultural University Sub-Station, Gurdaspur, Punjab*)

Received April 8, 1968

Abstract

Detailed study was made of the effect of (i) various inorganic and organic sources (potassium nitrate, ammonium sulphate, ammonium nitrate, asparagine, urea) and (ii) various levels of nitrogen (280, 700, 1,400 and 2,100 mg. N per litre) on growth and metabolism of *D. sorokiniana*. Observations on mat weight, spore numbers, shift in pH of the medium, nitrogen accumulated in the mat, residual nitrogen (total, nitrate and ammoniacal nitrogen) and residual sugar in the medium were made after incubation for varying periods. The results are presented in detail and discussed.

Introduction

The role of nitrogen in the physiology of fungi has received considerable attention from mycologists in the past (*see* Foster, 1949; Hawker, 1950; Lilly and Barnett, 1951; Cochrane, 1958). Nitrogen is used by fungi for functional as well as structural purposes. The form in which nitrogen is supplied has a profound effect on the growth of fungi and on the course of their metabolism. The study of nitrogen nutrition of plant pathogenic fungi is important not only because nitrogen is a major element for growth, sustenance and reproduction, but also because disease reaction in plants

* Memoir No. 57 from the Centre of Advanced Studies in Botany, University of Madras.

is the resultant of host-parasite metabolic activities in which nitrogen plays a major role. Accordingly, a detailed study of nitrogen nutrition of *Drechslera sorokiniana* using (i) various inorganic and organic sources and (ii) various levels of nitrogen was undertaken, and the results are presented in this paper.

MATERIAL AND METHODS

The studies were conducted with two highly pathogenic strains of *D. sorokiniana*, one of which was isolated by the authors from wheat roots and the other was obtained from Centraalbureau voor Schimmelcultures, Holland. However, in this paper the results are presented only for the strain isolated from wheat roots because a similar pattern of nitrogen utilization was observed for both the strains.

The fungus was grown in 50 ml. aliquots of Richard's synthetic liquid medium (potassium acid phosphate 5 gm., magnesium sulphate 2·5 gm., ferric chloride 0·02 gm., sucrose 50 gm., distilled water 1,000 ml.). The source and level of nitrogen were varied: Potassium nitrate, ammonium sulphate, ammonium nitrate, asparagine and urea were supplied at levels equivalent to 280 mg. (Level 1), 700 mg. (Level 2), 1,400 mg. (Level 3), and 2,100 mg. (Level 4) of nitrogen per litre of the culture medium. The pH of the media was adjusted initially to 5·5 by using N/10 NaOH or N/10 HCl with the help of an electronic pH meter (Adair Dutt & Co.).

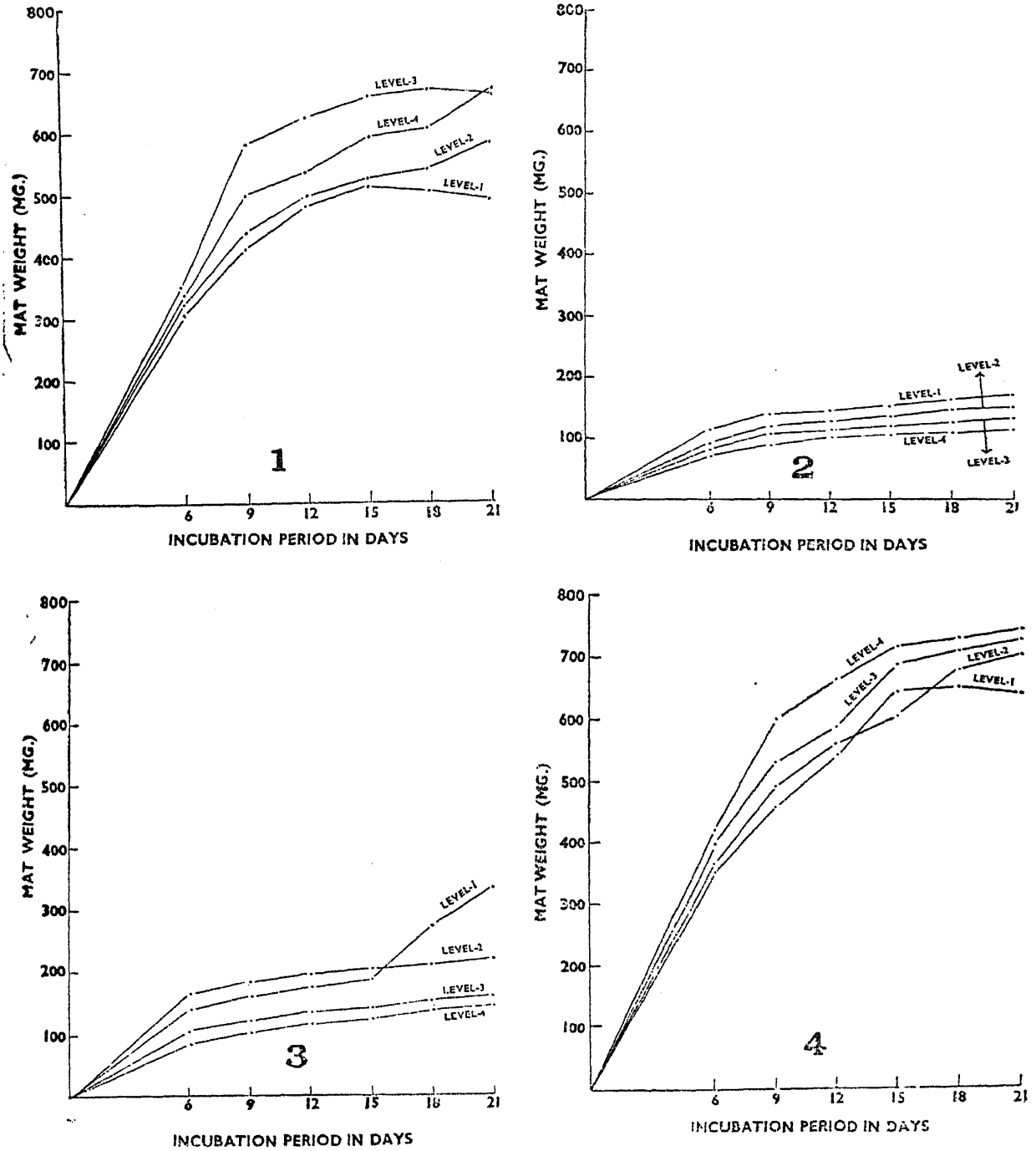
The stock cultures used for inoculation of media were 10–15 days old and maintained on oat meal agar slants at 25–28° C. A suspension of spores and mycelial fragments was obtained in sterile distilled water. The suspension was standardized with the help of a haemacytometer to provide approximately 10,000 spores and fragments per ml. One ml. of this suspension was added as inoculum to every 50 ml. of the synthetic medium used in the investigation (three replicates each). The flasks were incubated in a temperature-controlled room where the temperature ranged from 25 to 28° C. Standard mycological techniques were followed (Rawlins, 1933). The observations regarding mat weight, spore numbers, pH of the filtrate, nitrogen accumulated in the mat, residual nitrogen (total, nitrate and ammoniacal nitrogen separately) and residual sugar in the medium were made after incubation periods of 6, 9, 12, 15, 18 and 21 days. These estimations were made using methods described by Pai (1953) and Subramanian and Pai (1953).

Results and Discussion

The results are presented in Figs. 1–16 and Tables I–III.

Growth

The growth, as indicated by mat weight, differed with different sources and levels of nitrogen (Figs. 1–5). In general, higher mat weights were



Figs. 1–4. Fig. 1. Source of N-KNO_3. Fig. 2. Source of N-$(NH_4)_2SO_4$. Fig. 3. Source of N-NH_4NO_3. Fig. 4. Source of N-Asparagine.

obtained in media containing organic sources of nitrogen than in media containing inorganic sources. Amongst the sources employed, asparagine was the most favourable nitrogen source for growth. A close study of the

TABLE I

Showing residual sugar in media

Nitrogen source	Incubation in days	Residual sugar (gm.) Level 1	Level 2	Level 3	Level 4
Potassium nitrate	6	1·50	1·37	1·25	1·33
	9	1·06	0·96	0·47	0·75
	12	0·82	0·77	0·32	0·63
	15	0·72	0·67	0·21	0·43
	18	0·70	0·60	0·16	0·38
	21	0·68	0·46	0·14	0·18
Ammonium sulphate	6	2·00	2·06	2·06	2·12
	9	1·87	1·97	1·97	2·00
	12	1·80	1·92	1·95	1·95
	15	1·75	1·84	1·90	1·92
	18	1·75	1·80	1·87	1·90
	21	1·71	1·80	1·80	1·87
Ammonium nitrate	6	1·92	1·84	2·00	2·19
	9	1·84	1·79	1·95	2·06
	12	1·79	1·75	1·90	2·00
	15	1·71	1·75	1·84	1·95
	18	1·45	1·68	1·80	1·90
	21	1·29	1·65	1·80	1·84
Asparagine ..	6	1·33	1·29	1·25	1·10
	9	1·00	0·87	0·75	0·58
	12	0·71	0·67	0·58	0·30
	15	0·36	0·50	0·30	0·12
	18	0·36	0·18	0·14	0·05
	21	0·34	0·15	..	..
Urea ..	6	1·45	1·37	1·22	1·29
	9	1·22	1·00	0·42	0·66
	12	0·94	0·79	0·34	0·60
	15	0·81	0·60	0·22	0·40
	18	0·77	0·58	0·14	0·29
	21	0·77	0·38	0·05	0·15

data prcsented in Figs. 1–5 shows that the order for mat production with different sources was asparagine > urea > potassium nitrate > ammonium nitrate > ammonium sulphate.

TABLE II

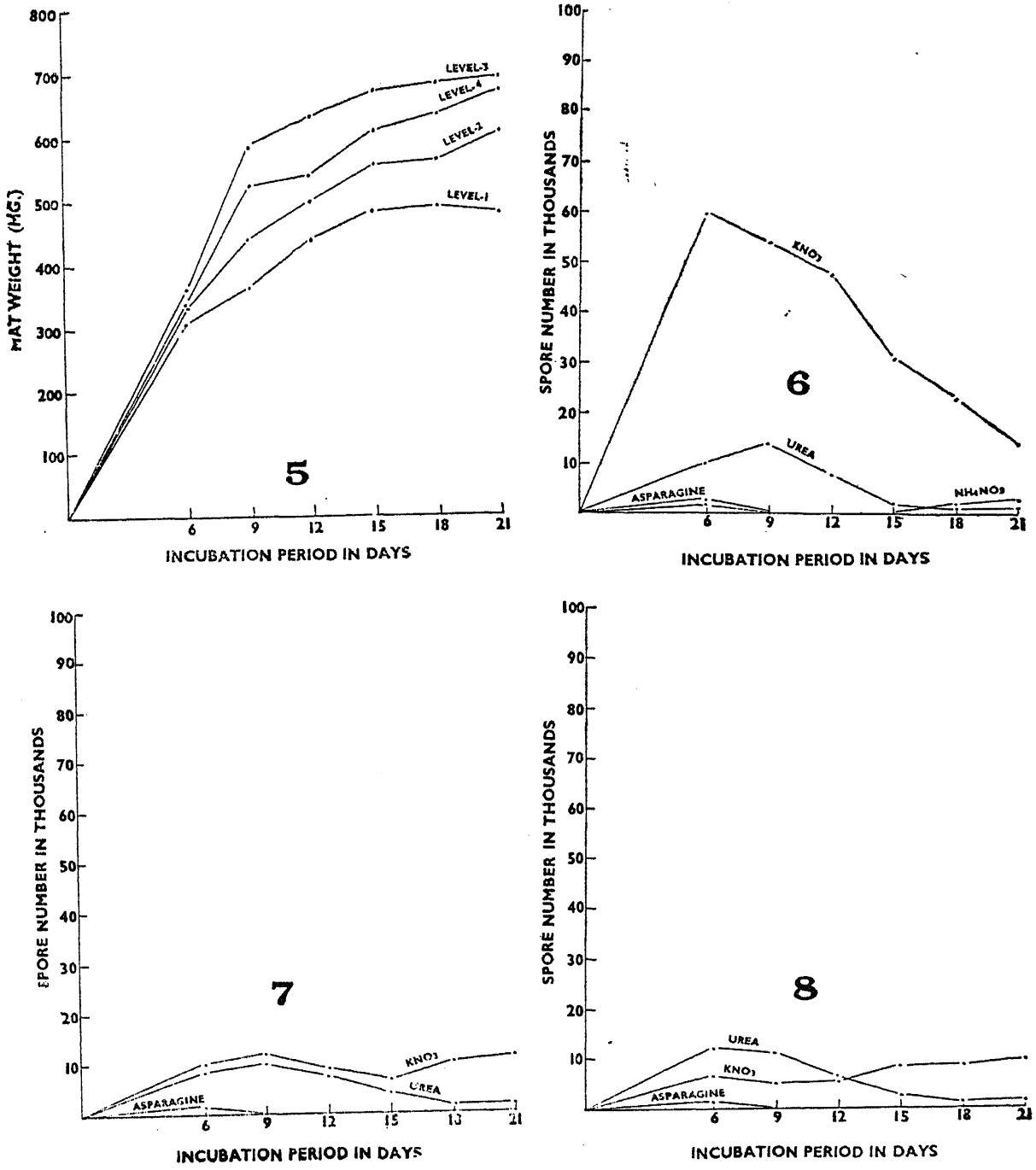
Showing percentage nitrogen in mat of Drechslera sorokiniana

Nitrogen source	Incubation in days	Percentage nitrogen Level 1	Level 2	Level 3	Level 4
Potassium nitrate	6	2·69	3·96	4·31	3·91
	9	2·88	3·70	4·61	4·94
	12	2·65	3·59	4·49	4·71
	15	2·64	4·66	4·49	4·37
	18	2·51	4·70	4·54	4·87
	21	2·47	4·96	4·75	4·36
Ammonium sulphate	6	6·11	7·23	7·25	8·20
	9	6·19	7·11	7·13	7·87
	12	6·11	7·27	7·56	7·82
	15	6·07	7·00	7·11	7·92
	18	5·76	6·76	6·89	7·77
	21	5·53	6·62	6·72	7·36
Ammonium nitrate	6	4·40	6·27	6·59	6·91
	9	4·49	6·31	6·76	6·93
	12	4·45	6·64	7·00	6·94
	15	4·44	6·86	7·00	6·94
	18	4·04	6·76	6·86	6·74
	21	3·64	6·50	6·73	6·65
Asparagine ..	6	2·21	5·17	5·31	5·35
	9	2·12	5·21	5·42	5·40
	12	2·10	5·19	5·46	5·48
	15	2·00	5·22	5·36	5·41
	18	2·00	4·82	5·47	5·45
	21	2·01	4·80	5·48	5·46
Urea ..	6	2·64	4·59	4·80	5·01
	9	2·87	4·04	4·61	5·08
	12	2·74	4·08	4·81	5·02
	15	2·78	4·53	4·86	4·64
	18	2·75	4·46	5·15	4·73
	21	2·71	4·86	5·42	4·83

This reveals the preference of *D. sorokiniana* for organic sources of nitrogen; further, the differences in growth with different sources of nitrogen could be of considerable significance in the ecology of the organism. It may be mentioned here that "*Helminthosporium*" spp. have been considered to be nitrate-utilizing fungi (Lilly and Barnett, 1951). From the present study it is evident that potassium nitrate supported maximum growth of *D. sorokiniana* amongst the inorganic nitrogen sources; however, organic sources of nitrogen were better than potassium nitrate.

The mat weight progressively increased with increasing concentration of nitrogen from levels 1–4 when asparagine was the source and from levels 1–3 when urea and potassium nitrate were the sources; on the other hand, the mat weight decreased from levels 1–4 when the source of nitrogen was ammonium nitrate or ammonium sulphate. In the case of *Fusarium vasinfectum* and *F. moniliforme* somewhat similar results have been reported by Pai (1953) in that the mat weights increased from levels 1–3 with sources of nitrogen such as potassium nitrate and peptone, notwithstanding the fact that neither nitrogen nor sugar was a limiting factor even at level 1. Similarly, in the case of present fungus, with sources such as asparagine with which increasing growth was obtained with increase in the nitrogen level, even at level 2 some residual nitrogen was left behind (Table III). The depletion of sugar was incomplete for a considerable period even at level 4 (Table I). Therefore, neither nitrogen nor sugar is a limiting factor for growth at these levels, but yet increase in the amount of nitrogen supplied in the medium resulted in higher mat weights. Some explanation is required as to why increases in nitrogen lead to better mat weights. There is no doubt that such increases in mat weight were accompanied by increasing uptake of nitrogen and sugar; however, the question remains to be answered as to why nitrogen and sugar should be utilized in highest amounts only at level 4 and not at level 2, as seen in the case of asparagine, when sufficient nitrogen and sugar was present even at level 2. The possible explanation is that the initial C/N ratio of the culture medium was responsible for this; the amount of N, C, P, K and Mg utilized will probably depend on this ratio and these elements would ultimately be responsible for the magnitude of growth. However, this ratio seems to be related to the particular source of nitrogen used, since similar results are not obtained when media are prepared with the same C/N ratio, but using different sources of nitrogen such as potassium nitrate, ammonium sulphate, asparagine etc. Similar conclusions can be drawn from the results reported by Pai (1953) for *Fusarium vasinfectum* and *F. moniliforme*, by Subramanian and Pai (1953) for *F. vasinfectum*, and by Sharma (1963) for *F. udum*.

It is likely that some of the environmental factors such as pH may play a part in altering this C/N ratio (at which optimum growth is obtained) for different sources of nitrogen; the shift in pH would depend on the nature of the nitrogen compound used in the medium. It should be noted that the initial pH was adjusted to the same level in all the media; however, the moment the fungus starts utilizing nitrogen and other compounds, the



FIGS. 5–8. Fig. 5. Source of N-Urea. Fig, 6, Level of N-1, Fig, 7, Level of N-2, Fig, 8, Level of N-3,

pH instantaneously changes in all the media; in some cases it would shift towards neutrality or alkalinity and in other cases towards acidity.

Besides nitrogen, determination of the amounts of carbon and inorganic ions accumulated in the mat at different levels of these sources would throw much light on the problem.

In general, growth increased with increasing period of incubation and maximum mat weights were obtained on the 21st day of incubation when final observations were taken. However, at level 1 of potassium nitrate, asparagine and urea, maximum growth was recorded between 12 and 15 days, the mat weight decreasing on further incubation. From the data obtained on residual nitrogen in the medium it is logical to believe that nitrogen had become a limiting factor at level 1 with these sources of nitrogen which resulted in decrease of mat weight with concurrent increase in total residual nitrogen of the medium (Table III). This is most probably due to autolysis of the fungal tissues.

Spore Numbers

In regard to spore numbers, it was seen that potassium nitrate appeared to be the source that favoured maximum sporulation followed by urea, sporulation being very poor with asparagine (Figs. 6–9). Spores were not detected with ammonium sulphate and also at levels 2, 3 and 4 of ammonium nitrate. Compared to asparagine, urea was a good enough source for sporulation. It is known that certain fungi sporulate better with simpler nitrogenous substances such as nitrates while others with comparatively complex nitrogenous compounds, *viz.*, asparagine, peptone, etc. Thus, on the basis of semi-quantitative data, nitrates have been reported to be good sources of nitrogen for sporulation of certain imperfect fungi, such as *Phyllosticta cycadina* (Tandon and Bilgrami, 1954), *Alternaria tenuis* (Grewal, 1955), *Bipolaris rostrata* (Aggarwal and Shinkhede, 1959) and *Sclerotium rolfsii* (Kodanda Pany and Apparao, 1963). A number of species of genera belonging to the Sphaeropsidales produce pycnidia most readily with potassium nitrate as the sole source of nitrogen and least readily with peptone, while albumin and asparagine are intermediate in effect (Leonian, 1924; Mix, 1933). It seems, therefore, that *D. sorokiniana* requires simpler nitrogenous compounds such as nitrates for good sporulation. On the other hand, in contrast to the present fungus, *Curvularia penniseti* (Aggarwal, 1958), *Fusarium udum* (Sharma, 1963) and a strain of *Bipolaris turcicum* (Malca and Ullstrup, 1962) sporulated profusely on media containing asparagine. However, asparagine has also been found

TABLE III

Showing residual nitrogen in media

Residual nitrogen (mg.)

Nitrogen source	Incubation in days	Level 1			Level 2			Level 3			Level 4		
		Total	NO_3-N	NH_3-N	Total	NO_3-N	NH_3-N	Total	NO_3-N	NH_3-N	Total	NO_3-N	NH_3-N
Potassium nitrate	6	5·54	5·25	..	21·14	19·67	..	52·57	48·72	..	90·37	88·62	..
	9	1·89	1·47	..	17·15	15·54	..	41·44	37·80	..	77·91	73·50	..
	12	1·10	0·87	..	15·40	12·60	..	38·92	36·61	..	76·86	73·08	..
	15	0·35	traces	..	9·45	8·68	..	38·15	35·56	0·56	76·44	72·87	0·77
	18	0·41	,,	0·35	8·61	6·37	0·49	37·10	33·25	0·84	73·71	68·74	0·91
	21	0·42	..	0·35	5·25	4·41	0·70	35·98	31·50	1·05	73·22	68·32	1·19
Ammonium sulphate	6	7·07	..	6·86	27·65	..	25·90	62·30	..	57·75	97·37	..	94·64
	9	4·84	..	3·99	25·34	..	23·94	60·97	..	57·15	96·81	..	93·38
	12	4·20	..	3·64	24·64	..	23·31	60·34	..	56·91	96·95	..	93·10
	15	4·20	..	3·50	24·22	..	22·47	60·20	..	56·91	95·83	..	91·77
	18	3·57	..	2·94	23·87	..	20·65	58·94	..	56·63	95·20	..	89·81
	21	3·36	..	2·94	23·38	..	20·37	58·66	..	56·49	94·85	..	89·60
Ammonium nitrate	6	7·63	4·90	2·66	23·80	15·40	8·19	61·81	32·55	28·70	98·42	51·73	45·64
	9	6·79	4·69	1·96	21·77	14·98	6·51	59·50	31·99	26·81	96·46	51·73	43·40
	12	5·95	4·34	1·40	20·44	14·56	5·46	58·17	31·64	26·11	95·20	51·45	41·02
	15	5·46	4·13	0·98	18·97	13·93	4·55	57·54	31·50	25·55	94·85	51·24	40·18
	18	2·66	2·38	..	18·83	13·93	4·48	56·70	31·50	24·50	92·81	50·61	39·06
	21	1·19	0·63	..	18·20	13·72	4·06	56·21	31·36	23·87	91·98	50·40	38·57
Asparagine	6	5·95	..	traces	15·12	..	traces	48·44	..	0·14	80·78	..	0·42
	9	4·06	..	0·14	8·47	..	0·21	39·48	..	0·28	69·86	..	0·49
	12	2·24	..	0·28	5·05	..	0·35	36·47	..	0·35	66·50	..	0·63
	15	traces	..	0·21	2·66	..	0·28	30·66	..	0·56	63·77	..	0·93
	18	,,	..	0·21	1·40	..	..	30·94	..	0·70	64·05	..	1·19
	21	0·91	..	0·77	traces	..	..	27·51	..	0·84	62·93	..	1·33
Urea	6	5·40	..	..	19·11	..	..	50·96	..	..	86·38	..	..
	9	1·18	..	..	16·17	..	..	48·02	..	..	76·51	..	..
	12	1·61	..	..	13·93	..	..	37·94	..	..	75·39	..	traces
	15	traces	..	..	8·82	..	..	35·70	..	0·28	73·99	..	0·35
	18	,,	..	..	8·05	..	0·42	33·46	..	0·63	72·12	..	0·70
	21	,,	..	traces	4·62	..	0·56	31·08	..	1·12	70·84	..	1·26

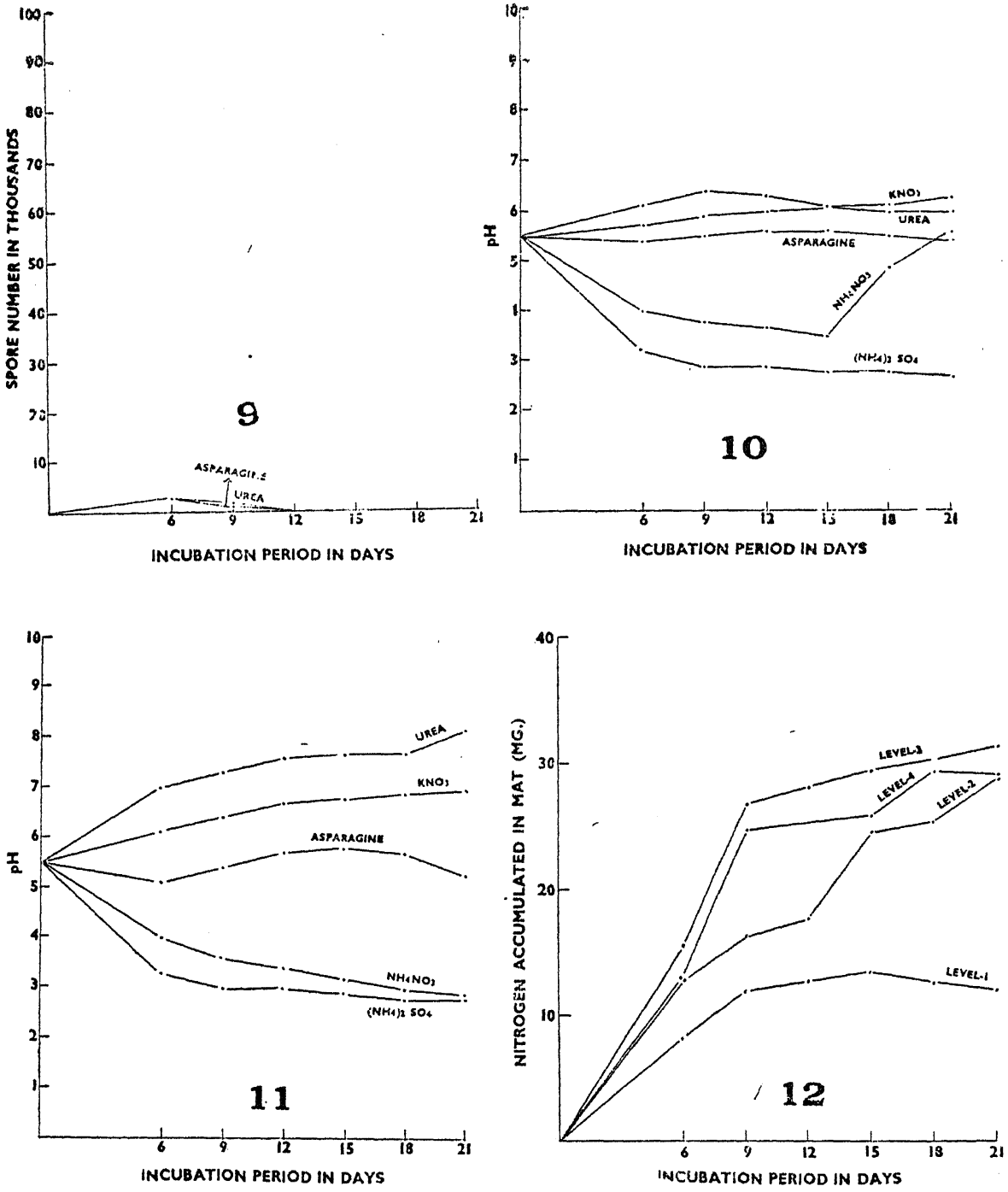
to depress sporulation in many cases (Mix, 1933; Bille-Hansen, 1953; Plunkett, 1953; Aschan, 1954) including *D. sorokiniana.* The adverse effect of asparagine on sporulation has been explained as being either due to ammonia accumulation or to the competition between vegetative growth and reproductive activity (Cochrane, 1958); in the case of *D. sorokiniana* the adverse effect of asparagine is probably due to the competition between vegetative growth and reproductive activity.

It is well known that spore production is possible only over a narrower range of pH than that permitting mycelial growth (Lilly and Barnett, 1951; Hawker, 1950, 1957; Cochrane, 1958). Thus, the absence of sporulation in ammonium sulphate and ammonium nitrate media, seen in the case of the present fungus also, is generally considered to be due to the fact that the pH of the medium is lowered to levels unfavourable for sporulation (Figs. 10–11). However, in the case of *D. sorokiniana* there appears to be some direct antagonism between the ammonium ion and sporulation, since the fungus did not sporulate even when the pH remained at high levels by the addition of succinic acid or calcium carbonate to ammonium sulphate and ammonium nitrate media. The fungus did not sporulate in media with ammonium acetate and ammonium oxalate also. It is, therefore, unlikely that the poor sporulation or absence of sporulation is purely a pH effect. It is interesting to mention here that the nitrate-ion has been stated to be a specific inhibitor of some phase in the sexual cycle of *Neurospora crassa* (*see* Hawker, 1957). Thus, it is probable that the ammonium-ion may be a specific inhibitor for the sporulation of *D. sorokiniana.*

High concentrations of nitrogen are known to suppress sporulation (Westergaard and Mitchell, 1947; Plunkett, 1953); this is evident from the data obtained with potassium nitrate where optimum sporulation is seen at level 1. However, this is not the case with organic sources of nitrogen, *viz.*, asparagine and urea. This leads one to conclude that, as in the case of growth, the optimum concentration of nitrogen for sporulation is not the same for all the sources of nitrogen. Hawker (1950) pointed out that the carbon-nitrogen ratio is usually less important than the actual concentration of these elements in determining sporulation in fungi. From the results obtained with *D. sorokiniana* it may be deduced that the concentration of nitrogen optimum for sporulation would differ for different sources of nitrogen.

Spore numbers at different periods of incubation did not follow a rigid pattern, spore numbers increasing or decreasing with incubation period. However, very often, spore numbers increased up to a certain incubation period and declined on further incubation. The data on hand do not permit much speculation regarding this behaviour; we do not know if there is a definite rhythm in sporulation as has been reported for some fungi (Ingold, 1960). Further, it is likely that after a certain incubation period the spores already formed may germinate and further formation of spores may be checked due to the accumulation of toxic substances in the medium with the result that the fungus may continue to grow in the vegetative form.

The spores already produced may not germinate if germination-inhibiting stale products accumulate in the medium. For these reasons the data on "spore numbers" are of limited value and can, at the most, be taken to indicate in a general way the favourable or unfavourable effects of the given sources for sporulation.



FIGS. 9-12. Fig. 9. Level of N-4. Fig. 10, Level of N-1, Fig. 11, Level of N-3, Fig. 12, Source of N-KNO_3.

Changes in pH

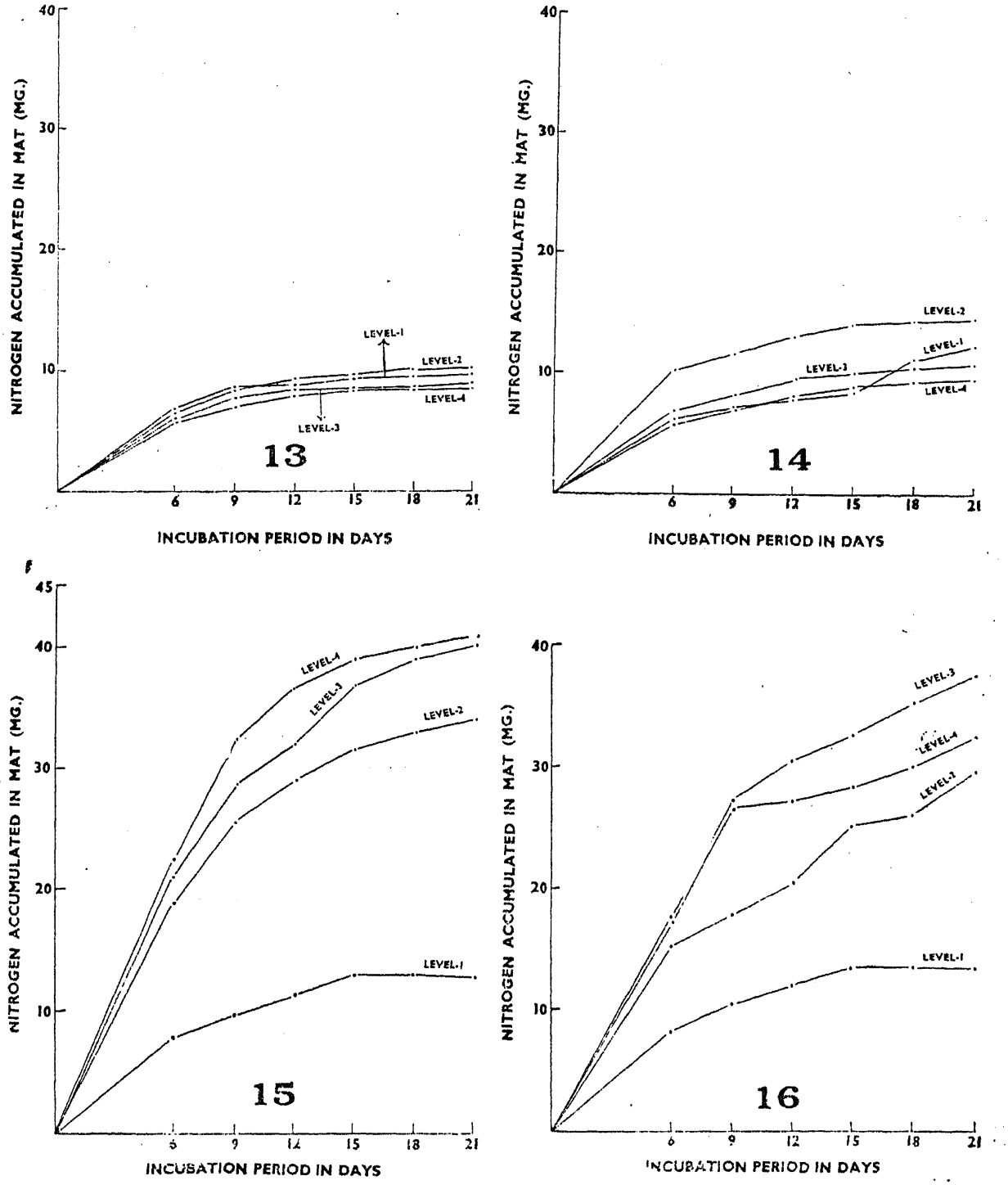
The pH of all media was initially adjusted to 5·5 which is quite suitable for growth of the fungus. The drift in pH of the media was influenced by the form and source of nitrogen metabolized by them (Figs. 10–11; data are presented only for levels 1 and 3, the pattern of drift in pH being same at levels 2, 3 and 4). When potassium nitrate or urea formed the source of nitrogen, the tendency was to lower the hydrogen-ion concentration, thereby raising the exponent pH towards neutrality or alkalinity. When ammonium sulphate or ammonium nitrate formed the source of nitrogen, the pH dropped and the media turned highly acidic. However, at level 1 of ammonium nitrate, there was a drop in pH in the early period of incubation followed by an increase in the later period of incubation. In the case of asparagine, which favoured maximum growth, the pH was generally maintained nearabout the original pH; at any rate, no extraordinary increase or decrease was observed.

In general, the pH changes at various levels of nitrogen were dependent upon the type of nitrogen compound present, the amount of nitrogen utilized and the extent of growth.

It has been found that, in general, media containing nitrate as nitrogen source become neutral or alkaline after growth of micro-organisms and media containing ammonium salts of strong acids turn acidic (Waksman, 1920; Brian *et al.*, 1947; Haskins and Weston, 1950; Brock, 1951; Pai, 1953; Subramanian and Pai, 1953; Apparao, 1954; Sharma, 1963). In the case of nitrates, increase towards neutrality or alkalinity is perhaps consequential on the differential removal of H-ions from the medium; an acid reaction of the medium, containing ammonium salts of inorganic acids, is perhaps due to the reaction $NH_4^+ \rightarrow NH_3 + H^+$ followed by the utilization of ammonia formed, thereby causing a drift towards acidity. At level 1 of ammonium nitrate, the pH drops in the early period of incubation because ammonium nitrogen is utilized in higher amounts than nitrate nitrogen. On the other hand, the pH rises in the later period of incubation because nitrate nitrogen is used rapidly with the exhaustion of ammonium nitrogen from the medium.

Although slackening of growth in ammonium sulphate and ammonium nitrate media due to high acidity was observed, yet the fungus made growth at very low pH (pH below 3). This shows that *D. sorokiniana* can survive under extreme conditions of pH in the substrate. It is, therefore, not surprising that Schaffnit and Meyer-Hermann (1930) placed this fungus

in their "Aestatic" group (those organisms that possess the ability to thrive over a wide range of soil pH) on the basis of their studies on pH optima for growth of a group of soil-borne fungi. The tolerance of the fungus to a wide range of pH is in keeping with its wide distribution in a variety of soils and also partly explains why it might be difficult to eradicate the fungus from soils.



FIGS. 13–16. Fig. 13. Source of N-$(NH_4)_2$ SO_4. Fig. 14. Source of N-NH_4NO_3. Fig. 15. Source of N-Asparagine. Fig. 16. Source of N-Urea.

Sugar Utilization

Sugar utilization was found to vary with the source of nitrogen present in the medium. It is evident from the data presented in Table I that the fungus utilized much of the sugar initially supplied in media containing asparagine, urea and potassium nitrate, but large amounts were left unutilized in ammonium nitrate and ammonium sulphate media. Generally, the order for sugar utilization with different sources of nitrogen was:

asparagine > urea > potassium nitrate > ammonium nitrate > ammonium sulphate.

With all sources and levels of nitrogen, except levels 3 and 4 of asparagine, residual sugar was detected in the medium even at the end of 21 days when final observations were taken. Although with organic sources of nitrogen such as asparagine and urea, sugar was completely depleted on the 21st day of incubation at the levels mentioned above, the mycelial dry weight of the fungus did not decrease; in fact, there was an increase in the mycelial weight. This indicates that the fungus, after the depletion of sugar supplied, probably utilized carbon from the asparagine or urea molecule for the building up of its mycelial mat. However, in the early period of incubation, the fungus did not seem to utilize carbon from the asparagine or urea molecule in preference to the readily available sugar, for, if that were the case, much more residual sugar should have been detected in asparagine or urea medium, but that is not the case.

Nitrogen Utilization

D. sorokiniana is unable to fix atmospheric nitrogen but can utilize nitrate, ammonium, and organic nitrogen. Thus, it would fit into Group II of Robbins' classification, although the groupings proposed by Robbins obviously need modifications in the light of our knowledge of nitrogen utilization by many fungi (Brian *et al.*, 1947; Apparao, 1956).

In the present investigation data were obtained on the nitrogen accumulated in the mycelial mat, residual nitrogen in the medium and the percentage nitrogen in the mat calculated on a dry weight basis for several sources and levels of nitrogen and at different incubation periods, and a discussion of the data along with what has already been covered under growth, pH changes and sugar utilization would be rewarding.

The determination of total nitrogen in mycelial mats revealed the fact that more nitrogen was accumulated in mats obtained from media contain-

ing organic sources of nitrogen than those containing inorganic sources (Figs. 12–16). Similar results were reported by Pai (1953) in the case of *Fusarium vasinfectum* and *F. moniliforme*. Generally, the order for nitrogen accumulation in mat was asparagine > urea > potassium nitrate > ammonium nitrate > ammonium sulphate. It will be recalled that the same order of efficiency for the different sources was seen for mat weights also; this indicates that accumulation of nitrogen was directly proportional to growth as determined by dry weights of the mats.

With a given source, the accumulation of nitrogen varied with the initial level of nitrogen. At level 1 in the case of asparagine, urea and potassium nitrate, almost the entire amount of nitrogen from the substrate was utilized and stored up in the fungal tissues; however, in the case of ammonium sulphate and ammonium nitrate at the same level only about 70–90 per cent of the nitrogen supplied was stored up in the mycelial mats. Moreover, synchronous with the fall in value of accumulated nitrogen in mat at level 1 of potassium nitrate, asparagine and urea after about the 15th day of incubation, increase in total residual nitrogen was detected in the medium. This was clearly due to autolysis of the fungus, since there was a fall in mat weight also. At levels 2, 3 and 4, all the nitrogen supplied was not utilized by the fungus even after 21 days, although the accumulation of nitrogen in the mat increased with the period of incubation with all the sources of nitrogen. In the case of ammonium sulphate and ammonium nitrate media, the accumulation of nitrogen in the mat increased up to level 2 after which there was a decrease; in the case of potassium nitrate medium, this increase in nitrogen accumulation was perceptible up to level 3. With urea, the accumulation of nitrogen in the mat increased up to level 3. Increase in the concentration of nitrogen in the form of asparagine (up to level 4) was accompanied by an increase in the amount of nitrogen in mycelial mat. Thus, the trend in accumulation of nitrogen followed usually the same pattern as that for growth for the respective sources and levels of nitrogen (*see* under growth). These observations are quite in keeping with the findings of Pai (1953) and Subramanian and Pai (1953) who found that the level of nitrogen at which maximum accumulation of nitrogen in mat took place varied with the source of nitrogen.

While discussing the methods of measuring growth, Cochrane (1958) wrote: "determination of growth by measurement of the total cellular nitrogen has been used only rarely in work with fungi. Although the chitin nitrogen complicates interpretation, this is the best method for determination of growth defined as synthesis of protoplasm, and deserves

wider use". Hence, in the present study, along with the dry weight of the mycelial mats, total cellular nitrogen was also determined throughout and indeed as may be seen from the data, this was usually in direct proportion to the dry weight of the fungus.

The *percentage* nitrogen content of the fungal mat calculated on its dry weight basis showed that the highest percentage was obtained in ammonium sulphate medium (Table II). In a general way, the pattern of percentage nitrogen content followed the order: ammonium sulphate > ammonium nitrate > asparagine and urea > potassium nitrate. Thus, it is evident that in media which turned highly acidic following growth of the fungus, the percentage nitrogen in the fungal mats was the greatest. A high percentage of nitrogen in mats obtained from ammonium salts media has been reported in many cases (Klotz, 1923; Tamiya, 1942; Subramanian and Pai, 1953; Pai, 1953); the cause for this is not clear. According to Cochrane (1958), either the low pH resulting from utilization of ammonium ion reduces autolytic loss of nitrogen, or the nitrate is simply less available to the cell for synthesis. Subramanian and Pai (1953), however, have attributed this to the poor uptake and accumulation of substances other than nitrogen in the mat.

The percentage nitrogen in mat was also affected by the concentration of nitrogen present in the medium. With all sources of nitrogen, the percentage nitrogen content in the mycelial mat was the lowest at level 1 as compared with other levels; increasing the concentration of nitrogen to level 2 resulted in about two-fold increase in the percentage nitrogen of the mat. In the case of many other fungi, an increase in the amount of available nitrogen was accompanied by a significant, as much as three-fold, increase in cell nitrogen (Heck, 1929; Hilpert *et al.*, 1937; Steinberg and Bowling, 1939; Tamiya, 1942).

In the present study the percentage nitrogen in mycelial mats at different periods of incubation did not follow a rigid pattern; it either increased or decreased slightly with the age of the culture. Pai (1953) reported a decrease in the percentage nitrogen of mat with increasing incubation periods in the case of *Fusarium vasinfectum*, although no rigid pattern was seen in the case of *Fusarium moniliforme*. On the other hand, in the case of *Aspergillus niger* the percentage nitrogen in the mat diminished with age in ammonium sulphate medium and increased in sodium nitrate medium; these results were explained on the basis of differential autolysis occurring in the two media, nitrogenous compounds being more rapidly lost in the acid medium, and carbonaceous materials in the alkaline (*see* Foster, 1949).

In contrast to this, Cochrane (1958), in an attempt to explain the high nitrogen content of the mycelium obtained from media containing ammonium salts, assumed that the autolysis of fungal cells is less in an acid medium. It is known that even during the accelerated growth phase certain nitrogenous and carbonaceous compounds are exuded into the medium although their amount may be small; however, the major escape of these substances takes place during the phase of autolysis (Cochrane, 1958). In the light of the results obtained with *D. sorokiniana*, where even in the absence of autolysis percentage nitrogen in mat slightly increased or decreased with the period of incubation, the authors feel that the percentage nitrogen in mat would depend on the differential escape of nitrogenous and carbonaceous compounds, independent of autolysis, into the medium ; the correct estimate of these substances may help in explaining the fluctuations as seen in the percentage nitrogen more precisely. In some cases the autolytic processes may proceed concomitantly with the growth of the fungus so that an actual increase in mycelial weight is observed despite unmistakable evidence of autolysis (Schmidt, 1936).

The analytical data for nitrogen left behind in the substrate showed that, in general, utilization of nitrogen was better with organic sources of nitrogen than inorganic, thus bringing out the superiority of organic sources of nitrogen in the building up of fungal tissues for *D. sorokiniana* (Table III). This was further substantiated by the mat weights which were higher with organic sources of nitrogen than with the inorganic. It is also evident from the data obtained with ammonium nitrate that the fungus utilizes ammoniacal nitrogen in preference to nitrate nitrogen at all the levels. The amount of nitrate nitrogen utilized at levels 3 and 4 was very much less than at levels 1 and 2.

Generally, in the case of level 1, most of the nitrogen supplied initially was depleted; on the other hand, for levels 2, 3 and 4 considerable residual nitrogen was detected, the highest level showing a higher amount of residual nitrogen than the immediately lower one and so on. The complete depletion of nitrogen for level 1 was due to the fact that the amount of nitrogen supplied initially was very small. Further, for level 1 after about the 15th day of incubation, increase in total residual nitrogen was noticed in media containing potassium nitrate, asparagine and urea; synchronous with increase in the residual nitrogen of the substrate, a decrease in the mat weight and the amount of accumulated nitrogen in the fungal mat was also noticed; this is a natural sequence of autolysis. Such increase in nitrogen content of

the substrate due to autolysis of fungal tissues has been observed by other workers (Klotz, 1923; Dietzel, Behrenbruch and Eucken, 1950; Pai, 1953).

In the present work, 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 nitrogen to the physiology of the fungus. The results presented in this paper emphasize the complexity of the problem and the need for further 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.

ACKNOWLEDGEMENTS

One of us (PDT) is thankful to the Ministry ot Education, Govt. of India, for the award of a research scholarship during the tenure of which this work was carried out.

REFERENCES

Aggarwal, G. P. .. "Nutritional studies on *Curvularia penniseti*. II. Carbon and nitrogen requirements," *Phyton B. Aires*, 1958, **12**, 143–51.

——— and Shinkhede, C. G. .. "Physiological studies of *Helminthosporium rostratum* Drechs.," *Ibid.*, 1959, **13**, 45–54.

Apparao, A. .. "The role of pH in nitrogen utilization of *Piricularia oryzae*," *Experientia*, 1956, **12**, 215.

Aschan, K. .. "The production of fruit-bodies in *Collybia velutipes*. I. Influence of different culture conditions," *Physiologia Pl.*, 1954, **7**, 571–91.

Bille-Hansen, E. .. "Fructification of a coprophilous *Coprinus* on synthetic medium," *Ibid.*, 1953, **6**, 523–28.

Brian, P. W., Curtis, P. J. and Hemming, H. G. "Glutinosin: a fungistatic metabolic product of the mould *Metarrhizium glutinosum* S. Pope," *Proc. R. Soc.*, 1947, **135 B**, 106–32.

Brock, T. D. .. "Studies on the nutrition of *Morchella esculenta* Fries," *Mycologia*, 1951, **43**, 402–22.

Cochrane, V. W. .. *Physiology of Fungi*, John Wiley & Sons, Inc., New York, 1958, p. 524.

Dietzel, E., Behrenbruch, H. and Eucken, M. "Stoffwechselversuche an actinomyceten," *Arch. Mikrobiol.*, 1950, **15**, 179–84, (cited from Cochrane, 1958).

Foster, J. W. .. *Chemical Activities of Fungi*, Academic Press, Inc., New York, 1949, p. 648.

Grewal, J. S. .. "Effect of nitrogen nutrition on growth and sporulation of *Alternaria tenuis* strain B causing core rot of apples," *Lloydia*, 1955, **18**, 74–81.

Haskins, R. H. and Weston, W. H. "Studies in the lower Chytridiales. 1. Factors affecting pigmentation, growth and metabolism of a strain of *Karlingia*," *Am. J. Bot.*, 1950, **37**, 739–50.

Hawker, L. E. .. *Physiology of Fungi*, University of London Press Ltd., London, 1950, p. 360.

———— .. *The Physiology of Reproduction in Fungi.* Cambridge University Press, Cambridge. 1957, p. 128.

Heck, A. F. .. "A study of the nature of the nitrogenous compounds in fungus tissue and their decomposition in the soil," *Soil Sci.*, 1929, **27**, 1–46.

Hilpert, R. S., Frieson, G. and Rossee, W. "Der Einfluss des Nahrbodens auf die chemische Zusammensetzung des *Aspergillus niger*," *Biochem. Z.*, 1937, **289**, 193–97.

Ingold, C. T. .. "Dispersal by air and water—The take-off," In *Plant Pathology*, 1960, **3**, 137–68.

Klotz, L. J. .. "Studies in the physiology of the fungi. XVI. Some aspects of nitrogen metabolism in Fungi," *Ann. Mo. bot. Gdn.*, 1923, **10**, 299–368.

Kodanda Pany, V. and Apparao, A. "Studies on the nutritional physiology of *Sclerotium rolfsii* Sacc." *Proc. Ind. Acad. Sci.*, 1963, **57** B, 326–338.

Leonian, L. H. .. "A study of factors promoting pycnidium formation in some Sphaeropsidales," *Am. J. Bot.*, 1924, **11**, 19–50.

Lilly, V. G. and Barnett, H. L. *Physiology of the Fungi*, 1st Edition, McGraw-Hill Book Co., New York, 1951, p. 464.

Malca, I. and Ullstrup, A. J. .. "Effects of carbon and nitrogen nutrition on growth and sporulation of two species of *Helminthosporium*," *Bull. Torrey bot. Club*, 1962, **89**, 240–49.

Mix, A. J. .. "Factors affecting the sporulation of *Phyllosticta solitaria* in artificial culture," *Phytopathology*, 1933, **23**, 503–24.

Pai, K. V. S. .. "Carbon, nitrogen and pH relationships to growth of soil fusaria in culture," *Proc. Ind. Acad. Sci.*, 1953, **37** B, 131–48.

Plunkett, B. E. .. "Nutritional and other aspects of fruit body production in pure cultures of *Collybia vellutipes* (Curt.) Fr.," *Ann. Bot.*, 1953, **17**, 193–216.

Rawlins, T. E. .. *Phytopathological and Botanical Research Methods*, John Wiley & Sons, Inc., New York, 1933, p. 156.

Schaffnit, E. and Meyer-Hermann, K. .. "Ueber den Einfluss der Bodenresktion auf die Lebensweise von Pilzparasiten und das Verhalten ihrer Wirtspflanzen". *Phytopath. Z.*, 1930, **2**, 99–166.

Schmidt, M. .. "The occurrence of chitin in micro-organisms," *Arch. Mikrobiol.*, 1936, **7**, 241–60.

Sharma, H. C. .. "Growth and sporulation of *Fusarium udum* in relation to C/N ratio and nitrogen sources," *Physiologia Pl.*, 1963, **16**, 276–80.

Steinberg, R. A. and Bowling, J. D. "Optimum solutions as physiological reference standards in estimating nitrogen utilization by *Aspergillus niger*," *J. agric. Res.*, 1939, **58**, 717–32.

Subramanian, C. V. and Pai, K. V. S. "Relation of nitrogen to growth and sporulation of *Fusarium vasinfectum* Atk.," *Proc. Ind. Acad. Sci.*, 1953, **37 B**, 149–57.

Tamiya, H. .. "Atmung, Garung und die sich daran beteligenden Enzyme von *Aspergillus*," *Adv. Enzymol.*, 1942, **2**, 193–238.

Tandon, R. N. and Bilgrami, K. S. "Effect of some nitrogen compounds on the growth and sporulation of *Phyllosticta cycadina*," *Proc. natn. Acad. Sci. India*, 1954, **24 B**, 191–96.

Waksman, S. A. .. "Studies in the metabolism of Actinomycetes. III. Nitrogen metabolism," *J. Bact.*, 1920, **5**, 1–30.

Westergaard, M. and Mitchell, H. K. "*Neurospora*. V. A synthetic medium favouring sexual reproduction," *Am. J. Bot.*, 1947, **34**, 573–77.