Arab Gulf J. scient. Res. **3** (2), pp. 633-649 (1985)

Effect of Pretreatment with Patoran and Diuron on Growth and Nitrogen Metabolism of *Cunninghamella echinulata*

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ABSTRACT. Fungal felts, produced by spore germination on patoran or diuron, seemed to be adapted to the continuous supply of large does of the herbicides (up to 160 μg/ml). A higher biomass yield followed continuous culturing of the mats of *Cunninghamella echinulata* on fresh herbicide-containing media, though this was smaller than in control samples. The effect was more substantial after patoran treatment as compared with diuron.

Patoran induced a consistent inhibition of respiratory carbon dioxide output even after the pretreated mycelia were transferred to a plain nutritive medium. Continuous supply of the herbicide was highly toxic. However, *C. echinulata* seemed more adapted to diuron.

The deleterious effects of pretreatment with the herbicides was more apparent on nitrogen metabolism; secretion of nitrogen from the mycelium increased with both treatments, particularly with the continuous supply of the herbicides. Recovery, after an initial herbicide treatment, seemed to induce better nitrogen accumulation than continuous supply of the herbicides. The lower residual nitrogen content of mycelia continuously supplied with the herbicides, as compared with their respective 'recovering' samples, indicates lesser tolerance of the former samples to the herbicides with prolonged exposure to the large doses.

Species of fungi differ widely in their sensitivity to toxic substances. They may be adapted, by repeated culturing on media containing low concentrations of the toxic substance, to tolerate a normally inhibitory concentration. The physiological basis of such acquired resistances has been attributed to alteration in distribution of surface charges leading to lower permeability to the drug (Von Brand *et al.* 1953, Pramer 1956). Recently, Subil and Takacsova (1978) and Colson *et al.* (1979) confirmed that the resistance of *Saccharomyces cerevisiae* to diuron, mucidin, antimycin and funiculosin was genetical; genetically-based tolerance has been observed by Khakimov and Kvitko (1978) with *Chlamydomonas reinhardii*.

However, there is no doubt that toxic substances do affect enzymes. Toxic substances interfering with protein metabolism are expected to inactivate a variety of enzymes. The action of 2:4-dichloro-6-(0-chloroaniline-o-)-s-triazine and its relatives (Wolf *et al.* 1955) is presumably of this type.

The effect of urea derivatives on plant metabolism has so far been studied in higher plants, whereas lower plants have seldom received much attention. Murray *et al.* (1969) noticed that *Aspergilli* were most sensitive to phenyl urea herbicides. They were most tolerant to fenuron and least to diuron. Harhash and Haikal (1974) recorded an alteration in nitrogen metabolism of *Fusarium oxysporum* following application of diuron to culture media. Haikal (1976) showed that *F. oxysporum* was sensitive to small doses of diuron, but tolerant to large doses of patoran. Ali (1982) reported that *Cunninghamella echinulata* was very highly tolerant to diuron (up to 160 μg/ml) but very sensitive to patoran (160 μg/ml arrested growth even after 8 days' incubation). The potency of patoran decreased with prolonged exposure of the fungal spores to the herbicide but the resistance of the fungus to diuron declined under the same experimental conditions.

The aim of this investigation is to throw some light on the mechanism of resistance of *C. echinulata* to diuron and patoran. This was performed by subjecting the mycelium (during its formation from a spore suspension) to large doses of the herbicides, then transferring such mycelia to plain media (recovery) or further supplementation of the herbicides in fresh media. During these variations in culture conditions the growth rates, respiration and nitrogen metabolism were monitored. *C. echinulata* represents the most common zygomycete, saprophytic, fast growing, soil-borne fungus in the cultivated lands in Egypt.

Material and Methods

The fungus, used in this investigation, *Cunninghamella echinulata*, was isolated from Egyptian garden soil and maintained in culture on modified Czapek Dox medium of the following composition per litre solution:

Sucrose	30 g	Magnesium sulphate	1 g
Potassium nitrate	4 g	Potassium chloride	0.5 g
Sodium dihydrogen phosphate	2 g	Ferrous sulphate	0.1 g
		Agar	15 g

1 ml of a very dense spore suspension of *C. echinulata* was inoculated into 250 ml-conical flasks containing 100 ml of the nutrient solution containing one of three levels of herbicides and a control. These levels were 80, 120 or 160 μg/ml active ingredient of either diuron (3-3:4-dichlorophenyl) 1:1-dimethyl urea) or patoran (3-(4-bromophenyl)-1-methyl-1-methoxy urea). The flasks were incubated, at 25°C, for 12 days before the fungal mats produced were thoroughly washed with

sterile distilled water, drained and further supplied with fresh media either free from, or containing, the same concentrations of the herbicides. Flasks were further incubated, at 25°C, for four days during which mycelial dry weight, at 80°C, and carbon dioxide output were measured and nitrogen analysis of the mats carried out after two days and at the end of the experimental period.

Nitrogen analysis of the media was carried out after centrifugation at 3000 rpm for 15 minutes at 4°C to remove any mycelial fragments or spores. Mycelial mats were extracted by borate buffer, at pH 8, and the residue, after extraction, was dissolved in 1*N* sodium hydroxide to determine true proteins. Another portion of the residue was wet digested before its total insoluble nitrogen content was assayed by the Berthelot reaction (Chaney and Marbach 1962). Amino acids were determined by the Russel (1944) method; peptides and proteins by the Folin phenol procedure (Lowry *et al.* 1951), whereas nitrates and nitrites were estimated by use of phenol disulphonic acid and diazotization with α-naphthyl-amine and coupling with sulphanilic acid, respectively (Paech and Tracey 1955). Carbon dioxide output was measured (Said and Naguib 1953) by passing a slow current of sterile, carbon dioxide-free air very close to the surface of the growing mats, to remove the carbon dioxide and collecting it in a standard volume of *0.5 N* sodium hydroxide. The carbonate formed was precipitated with barium chloride and the excess alkali was titrated, in a closed system, against standard acid.

The values in the graphs represent the mean of 5 replicate samples for each treatment, the standard errors being within the limits of 2-4%.

Results

Mycelial Dry Weight

Figure 1 shows that the initial dry weight of patoran-treated mycelia was far less than that of the control samples, the reduction being greater at the larger doses of the herbicide; this condition was less apparent with diuron supplementation.

Continuous supply of patoran resulted in a lower dry weight gain than supplementation with fresh plain media. The increase in dry weight, under both conditions, was far less than that of the control samples particularly towards the end of the experiment; larger doses were more effective than lower doses.

Similar trends were observed with diuron pre-treatment though recovery on plain medium after 12 days' incubation on 80 μg/ml diuron was stimulatory to dry weight gain.

Carbon Dioxide Output

Figure 2 shows that, compared to the controls, the carbon dioxide output, per biomass, of patoran-treated mycelia, floating over plain media, significantly de-

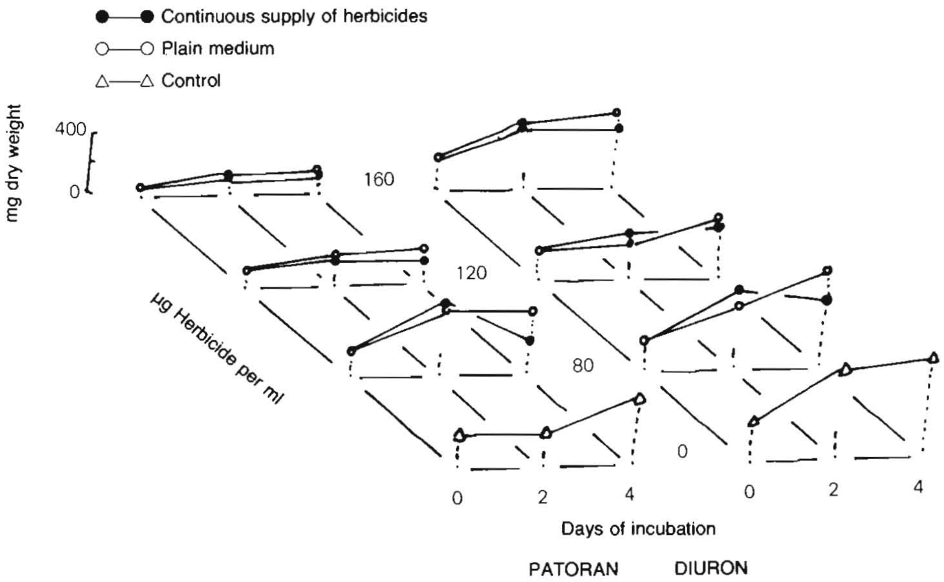

Fig. 1. Dry weight gain by mycelial felts of *Cunninghamella echinulata*, growing on various concentrations of either patoran or diuron, during 'recovery' on plain media or continuously supplied with the same doses of the herbicides for a further four days.

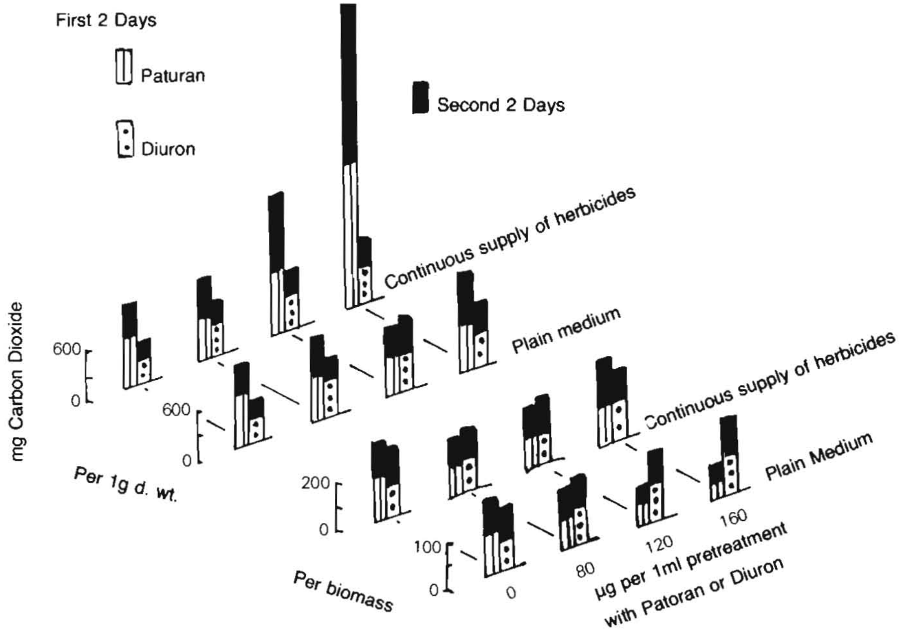

Fig. 2. Fluctuations in carbon dioxide output by patoran- and diuron-fed mucelial felts of *Cunninghamella echinulata* when further supplemented with the same doses of the herbicides or grown on plain media for four days.

creased with increased concentration during the first 12 days of incubation. This effect was more marked during the first than the second part of the final experiment period. On the other hand, diuron was stimulatory under the same conditions.

The continuous supply of patoran slightly suppressed carbon dioxide evolution but 160 µg/ml somewhat reversed this effect. Conversely, continuous supply of diuron had little effect except that the 160 µg/ml dose was slightly stimulatory.

Per unit dry weight, the transfer of patoran-treated mycelia to plain media promoted carbon dioxide output. On the other hand, recovery after diuron treatment was highly stimulatory. Continuous supply of patoran exerted almost no effect at 80 µg/ml level but the larger doses were remarkably stimulatory, particularly 160 µg/ml. Continuous administration of diuron, however, induced enhanced effects similar to those on plain media, with only minor differences found between concentrations.

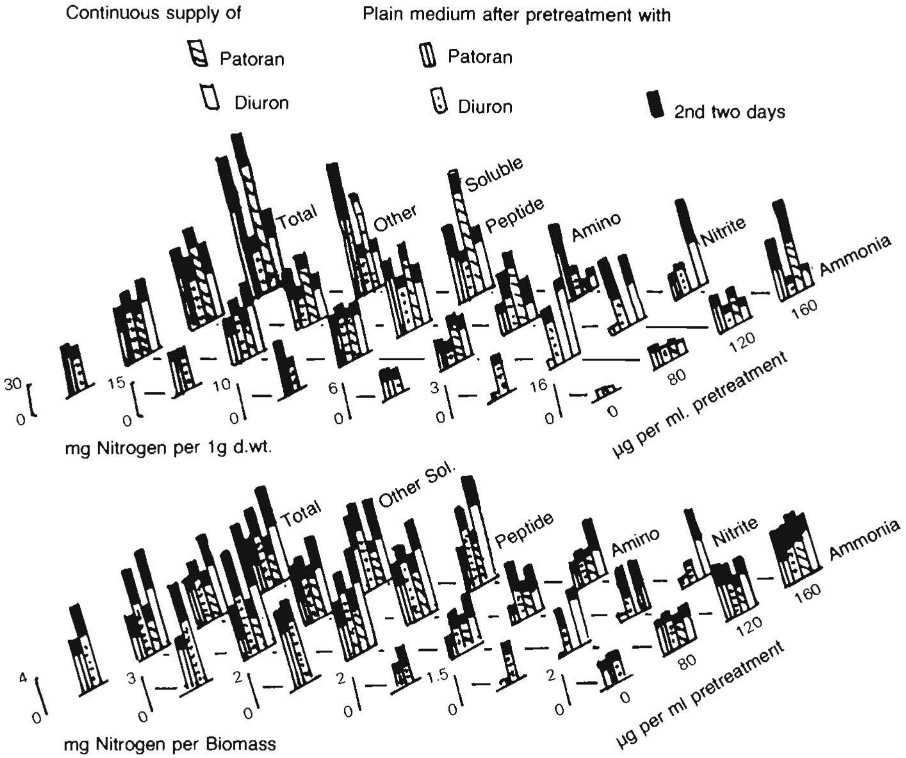

Fig. 3. Drifts in the various types of secreted nitrogen by patoran- and diuron-fed mycelial felts of *Cunninghamella echinulata* when further subjected to the same doses of the herbicides or returned to plain nutritive media for a further four days.

Nitrogen Secretion

Figure 3 shows that, under all experimental conditions, the fluctuations in secretion of total nitrogen as well as of various components, by patoran-treated mycelial felts, were small compared with the control mycelia. The same applies to diuron treatment though continuous supply of 80 μg/ml caused a slight increase, mostly in the 'other soluble' fraction. Futhermore, nitrite nitrogen disappeared from the media, following continuous supply of all levels of patoran used.

On a dry weight basis, nitrogen secretion increased strikingly with increasing concentration of the original patoran treatment and more so with continuous supply of the herbicide. All fractions showed the same changes but these were greatest in the ammonia and nitrite nitrogen fractions.

Diuron treatment also promoted nitrogen secretion, almost to the same extent, irrespective of the concentration of the original treatment, whether the mycelia were recovering on plain media or continuously supplied with the herbicide. All fractions were affected in the same way.

Nitrogen Content of the Mycelia

Tables 1-1 and 1-2 show that, under all experimental conditions, the total nitrogen content of the mycelial felts increased with progress of the experimental period. Compared with their respective initial values, the nitrogen gain was more pronounced under recovery conditions than under continuous supply of either herbicides but even so the total nitrogen of the mycelia was far less than in the untreated controls.

Under most conditions, the total insoluble nitrogen fraction of the recovering mycelia was higher than the total soluble nitrogen; this situation was reversed with continuous supply of both herbicides. Residual nitrogen formed the larger proportion of the insoluble component and further increased under recovery conditions compared with continuous supply of the herbicides. The amino and other soluble components were greater than the ammonia and peptides in the increased soluble nitrogen fraction of the variously treated mycelia.

Per unit dry weight, the initial total nitrogen content of the patoran-treated samples was far greater than that of the controls but the reverse was found under diuron treatment. Compared with the initial levels, continuous supply of patoran resulted in smaller nitrogen accumulation than during recovery of mycelia, except at 120 μg/ml, where there were minor differences between the two treatments. Similarly, recovery from diuron treatment induced higher nitrogen accumulation compared with continuous supply of the herbicide, except at 160 μg/ml, where the reverse was observed (Tables 2-1 and 2-2).

Compared with the control samples, the total insoluble nitrogen accumulation within patoran-treated mycelia was largely inhibited, particularly with continuous supply of the herbicide; this was alleviated with application of 160 μg/ml. The residual nitrogen was slightly, if at all, affected, except at 80 μg/ml, where continuous supply of the herbicide severely lowered the accumulation of this nitrogen component.

Under all levels of diuron pre-treatment, the total insoluble fraction predominated over the total soluble form more so under recovery conditions on plain nutrient. The residual nitrogen formed the major proportion.

Under patoran treatment, the other soluble and amino nitrogen formed the major components responsible for the observed fluctuations in the total soluble nitrogen, whereas ammonia and peptide also participated in the increased soluble nitrogen content of the recovering mycelia from 160 μg/ml treatment. Peptides were also prominent following continuous application of 120 μg/ml patoran.

The drop in total soluble nitrogen of diuron-treated mycelia was mainly attributable to the peptide component, whereas the amino and other soluble nitrogen fractions were involved to a lesser extent. Indeed, the other soluble nitrogen was hardly affected with continuous supply of 160 μg/ml.

Discussion

The results of this investigation clearly establish that the felts of *Cunninghamella echinulata*, produced by germination on various high levels of either patoran or diuron, were adapted to the presence of these herbicides in their media. This is clear from the progressive increase in dry weight when these mats were subject to the doses of the herbicides. An even higher biomass yield was obtained when the mats were cultured on fresh plain media. Under all conditions, the dry weight gain was less than that of the controls, except after treatment with 80 μg/ml, where the gain in biomass was almost restored to the control level; this was more so after diuron treatment.

These observations also lead to the suggestion that patoran and diuron induced intrinsic changes in the mycelial physiology that were not reversed by removal of the causal agents from the media, *i.e.* both herbicides induced metabolic and possibly genetical disorders; patoran had a greater effect than diuron in this respect.

Genetical control of resistance to various compounds has been demonstrated by several investigators. Thus Colson *et al.* (1977, 1979) confirmed the genetical control of resistance of *Saccharomyces cerevisiae* to diuron, mucidin and antimycin. Similarly, Khakimov and Kvitko (1978) proved that the sensitivity or resistance of *Chlamydomonas reinhardii* was genetically controlled.

Table 1-1. Effect of pretreatment with various concentrations of patoran on the nitrogen content of 12-days old mycelial felts of *Cunninghamella echinulata* supplemented with or without the same dose of the herbicide. (mg per mycelial growth ± standard deviation)

µg per ml		Days Incub-ation	Ammonia	Amino	Peptide	Other Soluble	Total Soluble	Alkali Soluble Proteins	Residnal Nitrogen	Total Insoluble	Total Nitrogen
Pre-treatment	Experi-mental										
0	Initials	0	0.5 ± 0.0	1.8 ± 0.1	1.3 ± 0.1	3.3 ± 0.1	6.9 ± 0.3	4.3 ± 0.2	8.9 ± 0.3	13.2 ± 0.5	20.1 ± 0.7
	0	1st 2	0.7 ± 0.0	4.4 ± 0.1	6.5 ± 0.2	6.7 ± 0.2	18.3 ± 0.6	8.2 ± 0.3	14.9 ± 0.5	23.1 ± 0.8	41.4 ± 1.3
		2nd 2	1.1 ± 0.1	1.4 ± 0.1	−1.8 ± 0.1	−0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	–	0.5 ± 0.0	0.9 ± 0.0
		Total 4	1.8 ± 0.2	5.8 ± 0.2	4.7 ± 0.2	6.4 ± 0.2	18.7 ± 0.3	8.7 ± 0.3	14.9 ± 0.5	23.6 ± 0.8	42.3 ± 1.3
80	Initials	0	0.4 ± 0.0	3.0 ± 0.1	1.2 ± 0.1	4.1 ± 0.2	8.7 ± 0.3	3.1 ± 0.1	8.1 ± 0.3	11.2 ± 0.4	19.9 ± 0.6
	0	1st 2	0.4 ± 0.0	3.2 ± 0.1	1.8 ± 0.1	4.2 ± 0.1	9.6 ± 0.3	3.2 ± 0.1	10.9 ± 0.4	14.1 ± 0.5	23.7 ± 0.8
		2nd 2	0.3 ± 0.0	0.5 ± 0.0	1.4 ± 0.1	0.9 ± 0.1	3.1 ± 0.1	2.4 ± 0.1	1.9 ± 0.1	4.3 ± 0.2	7.4 ± 0.3
		Total 4	0.7 ± 0.0	3.7 ± 0.1	3.2 ± 0.1	5.2 ± 0.2	12.7 ± 0.4	5.6 ± 0.4	12.8 ± 0.4	18.4 ± 0.6	31.1 ± 1.1
	80	1st 2	0.5 ± 0.0	3.2 ± 0.1	1.5 ± 0.1	6.3 ± 0.2	11.5 ± 0.4	3.7 ± 0.1	5.4 ± 0.2	9.1 ± 0.3	20.6 ± 0.8
		2nd 2	1.2 ± 0.1	3.3 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	5.4 ± 0.2	0.2 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	6.4 ± 0.3
		Total 4	1.7 ± 0.1	6.5 ± 0.2	1.8 ± 0.1	6.9 ± 0.2	16.9 ± 0.5	3.9 ± 0.2	6.2 ± 0.2	10.1 ± 0.4	27.0 ± 1.0
120	Initials	0	0.1 ± 0.2	1.1 ± 0.1	1.5 ± 0.1	3.0 ± 0.1	5.7 ± 0.2	3.5 ± 0.1	5.5 ± 0.2	9.0 ± 0.3	14.7 ± 0.5
	0	1st 2	0.7 ± 0.0	2.7 ± 0.1	1.6 ± 0.1	2.9 ± 0.1	7.9 ± 0.3	4.0 ± 0.1	8.1 ± 0.3	12.1 ± 0.4	20.0 ± 0.7
		2nd 2	–	2.8 ± 0.1	1.2 ± 0.1	0.7 ± 0.0	4.7 ± 0.2	0.4 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	5.5 ± 0.2
		Total 4	0.7 ± 0.0	5.5 ± 0.2	2.8 ± 0.1	3.6 ± 0.1	12.6 ± 0.4	4.4 ± 0.3	8.5 ± 0.3	12.9 ± 0.4	25.5 ± 0.8
	120	1st 2	0.5 ± 0.0	1.4 ± 0.1	2.1 ± 0.1	3.2 ± 0.1	7.2 ± 0.3	4.0 ± 0.2	6.8 ± 0.4	10.8 ± 0.4	18.0 ± 0.6
		2nd 2	0.1 ± 0.0	0.8 ± 0.0	1.8 ± 0.1	1.2 ± 0.1	3.9 ± 0.1	0.8 ± 0.0	0.3 ± 0.0	1.1 ± 0.1	5.0 ± 0.2
		Total 4	0.6 ± 0.0	2.2 ± 0.1	3.9 ± 0.1	4.3 ± 0.2	11.1 ± 0.4	4.8 ± 0.2	7.1 ± 0.2	11.9 ± 0.4	23.0 ± 0.8
160	Initials	0	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	1.6 ± 0.1	2.3 ± 0.1	4.2 ± 0.2	6.5 ± 0.3	8.1 ± 0.3
	0	1st 2	0.3 ± 0.0	1.4 ± 0.1	1.0 ± 0.1	2.1 ± 0.1	4.8 ± 0.2	2.6 ± 0.1	5.2 ± 0.2	7.8 ± 0.3	12.6 ± 0.4
		2nd 2	1.4 ± 0.1	2.3 ± 0.1	3.6 ± 0.1	2.5 ± 0.1	9.8 ± 0.3	0.4 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	13.0 ± 0.4
		Total 4	1.7 ± 0.1	3.7 ± 0.2	4.6 ± 0.2	4.6 ± 0.2	14.6 ± 0.5	3.0 ± 0.1	8.0 ± 0.3	11.0 ± 0.4	25.6 ± 0.8
	160	1st 2	0.2 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	2.9 ± 0.1	4.3 ± 0.2	2.6 ± 0.1	3.4 ± 0.1	6.0 ± 0.2	10.3 ± 0.4
		2nd 2	0.1 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.3 ± 0.1	0.6 ± 0.0	0.9 ± 0.0	1.5 ± 0.1	2.8 ± 0.1
		Total 4	0.3 ± 0.0	1.5 ± 0.1	0.8 ± 0.0	3.0 ± 0.1	5.6 ± 0.2	3.2 ± 0.1	4.3 ± 0.1	7.3 ± 0.2	13.1 ± 0.4

Table 1-2. Effect of pretreatment with various concentrations of diuron on the nitrogen content of 12-day old mycelial felts of *Cunninghamella echinulata* supplemented with or without the same dose of the herbicide. (mg per mycelial growth ± standard deviation)

μ per ml		Days Incuba-ation	Ammonia	Amino	Peptide	Other Soluble	Total Soluble	Alkali Soluble Proteins	Residual Nitrogen	Total Insoluble	Total Nitrogen
Pre-treatment	Experi-mental										
0	Initials	0	1.0 ± 0.1	1.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	5.1 ± 0.2	5.2 ± 0.2	17.2 ± 0.6	22.4 ± 0.8	27.5 ± 0.9
	0	1st 2	1.8 ± 0.1	4.4 ± 0.1	7.1 ± 0.2	11.5 ± 0.4	24.8 ± 0.8	7.3 ± 0.3	18.9 ± 0.6	26.2 ± 0.9	51.0 ± 1.7
		2nd 2	0.3 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	−1.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	1.0 ± 0.1	1.3 ± 0.1	1.5 ± 0.1
		Total 4	2.1 ± 0.1	5.1 ± 0.2	7.4 ± 0.2	10.4 ± 0.4	25.0 ± 0.8	7.6 ± 0.3	19.9 ± 0.7	27.5 ± 0.9	52.5 ± 1.7
80	Initials	0	1.0 ± 0.1	0.8 ± 0.1	0.4 ± 0.0	2.7 ± 0.1	4.9 ± 0.2	3.5 ± 0.1	7.5 ± 0.3	11.0 ± 0.4	15.9 ± 0.5
	0	1st 2	1.6 ± 0.1	3.2 ± 0.1	1.1 ± 0.1	3.7 ± 0.2	9.6 ± 0.3	5.3 ± 0.2	12.5 ± 0.4	17.4 ± 0.6	27.4 ± 0.9
		2nd 2	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	1.2 ± 0.1	2.5 ± 0.1	0.4 ± 0.1	2.9 ± 0.1	4.1 ± 0.2
		Total 4	2.0 ± 0.1	3.3 ± 0.1	1.2 ± 0.1	4.3 ± 0.2	10.8 ± 0.4	7.8 ± 0.3	12.9 ± 0.5	20.7 ± 0.7	31.5 ± 1.0
	80	1st 2	0.8 ± 0.1	2.3 ± 0.1	0.7 ± 0.0	2.5 ± 0.2	6.3 ± 0.2	5.9 ± 0.2	13.8 ± 0.5	19.7 ± 0.6	26.0 ± 0.9
		2nd 2	0.2 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	1.4 ± 0.1	0.5 ± 0.0	−1.6 ± 0.1	−1.1 ± 0.1	0.3 ± 0.0
		Total 4	1.0 ± 0.1	2.6 ± 0.1	1.4 ± 0.1	2.7 ± 0.1	7.7 ± 0.2	6.4 ± 0.2	12.2 ± 0.4	18.6 ± 0.6	26.3 ± 0.9
120	Initials	0	1.8 ± 0.1	2.5 ± 0.1	1.0 ± 0.1	3.8 ± 0.1	9.1 ± 0.3	3.0 ± 0.1	9.2 ± 0.3	12.2 ± 0.5	21.3 ± 0.7
	0	1st 2	1.6 ± 0.1	2.3 ± 0.1	1.0 ± 0.1	4.3 ± 0.2	9.2 ± 0.3	7.7 ± 0.3	15.4 ± 0.5	23.1 ± 0.8	32.3 ± 1.0
		2nd 2	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	2.1 ± 0.1	1.1 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	5.9 ± 0.2
		Total 4	2.1 ± 0.1	2.9 ± 0.1	1.6 ± 0.1	4.7 ± 0.2	11.3 ± 0.4	8.8 ± 0.3	18.1 ± 0.6	26.9 ± 0.9	38.2 ± 1.2
	120	1st 2	1.8 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	4.2 ± 0.2	11.3 ± 0.4	4.0 ± 0.1	14.6 ± 0.6	18.6 ± 0.6	29.9 ± 1.0
		2nd 2	1.1 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	3.0 ± 0.1	4.6 ± 0.2	2.1 ± 0.1	1.9 ± 0.1	4.0 ± 0.2	8.6 ± 0.3
		Total 4	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	7.2 ± 0.3	15.9 ± 0.2	6.1 ± 0.2	16.5 ± 0.5	22.6 ± 0.7	38.5 ± 1.2
160	Initials	0	0.8 ± 0.0	1.4 ± 0.1	0.6 ± 0.0	2.8 ± 0.1	5.6 ± 0.2	1.7 ± 0.1	10.2 ± 0.4	11.9 ± 0.4	17.5 ± 0.6
	0	1st 2	1.5 ± 0.1	3.0 ± 0.1	1.8 ± 0.1	5.4 ± 0.2	11.7 ± 0.4	5.5 ± 0.2	13.1 ± 0.4	18.6 ± 0.6	30.3 ± 1.0
		2nd 2	0.2 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	1.8 ± 0.1	0.8 ± 0.1	0.4 ± 0.0	1.2 ± 0.1	3.0 ± 0.1
		Total 4	1.7 ± 0.1	3.7 ± 0.2	2.4 ± 0.1	5.7 ± 0.1	13.5 ± 0.4	6.3 ± 0.2	13.5 ± 0.5	19.8 ± 0.7	33.3 ± 1.1
	160	1st 2	1.7 ± 0.1	2.5 ± 0.1	1.1 ± 0.1	9.3 ± 0.3	14.6 ± 0.5	4.9 ± 0.2	9.4 ± 0.3	14.3 ± 0.5	28.9 ± 1.0
		2nd 2	–	0.6 ± 0.0	0.5 ± 0.0	2.8 ± 0.1	3.9 ± 0.1	0.4 ± 0.0	1.1 ± 0.1	1.1 ± 0.1	5.4 ± 0.2
		Total 4	1.7 ± 0.1	3.1 ± 0.1	1.6 ± 0.1	12.1 ± 0.1	18.5 ± 0.2	5.3 ± 0.2	10.5 ± 0.4	15.8 ± 0.5	34.3 ± 0.6

Table 2-1. Effect of pretreatment with various concentrations of patoran on the nitrogen content of 12-days old mycelial felts of *Cunninghamella echnulata* supplemented with or without the same doses of the herbicide. (mg per 1 g dry weight ± standard deviation)

μ per ml		**Days Incuba-ation**	**Ammonia**	**Amino**	**Peptide**	**Other Soluble**	**Total Soluble**	**Alkali Soluble Proteins**	**Residual Nitrogen**	**Total Insoluble**	**Total Nitrogen**
Pre-treatment	**Experi-mental**										
0	Initials	0	2.1 ± 0.1	7.6 ± 0.3	5.6 ± 0.2	13.4 ± 0.5	28.7 ± 1.0	17.7 ± 0.7	40.0 ± 1.3	57.7 ± 1.9	86.4 ± 2.8
	0	1st 2	2.7 ± 0.1	16.9 ± 0.6	24.6 ± 0.8	26.2 ± 0.9	70.4 ± 2.3	32.0 ± 1.0	57.9 ± 1.9	89.9 ± 2.9	160.3 ± 5.9
		2nd 2	2.8 ± 0.1	3.7 ± 0.1	−4.7 ± 0.2	−0.8 ± 0.0	1.0 ± 0.1	1.3 ± 0.1	0.1 ± 0.0	1.4 ± 0.1	2.4 ± 0.1
		Total 4	5.5 ± 0.2	20.6 ± 0.7	19.9 ± 0.6	25.4 ± 0.9	71.4 ± 2.3	33.3 ± 1.1	58.0 ± 1.9	91.3 ± 3.0	162.7 ± 6.0
80	Initials	0	2.3 ± 0.1	16.7 ± 0.7	6.7 ± 0.3	22.4 ± 0.8	48.1 ± 1.6	17.0 ± 0.6	44.8 ± 1.5	61.8 ± 2.0	109.9 ± 3.4
	0	1st 2	2.0 ± 0.1	14.8 ± 0.5	8.2 ± 0.3	19.4 ± 0.6	44.4 ± 1.5	14.6 ± 0.5	50.4 ± 1.6	65.0 ± 2.0	109.4 ± 3.4
		2nd 2	1.3 ± 0.1	1.9 ± 0.1	5.4 ± 0.2	3.3 ± 0.1	11.9 ± 0.4	9.7 ± 0.3	7.6 ± 0.3	17.3 ± 0.7	29.2 ± 1.0
		Total 4	3.3 ± 0.1	16.7 ± 0.5	13.6 ± 0.4	22.7 ± 1.8	56.3 ± 1.8	24.3 ± 0.8	58.0 ± 1.9	82.3 ± 2.6	138.6 ± 5.3
	80	1st 2	2.5 ± 0.1	14.9 ± 0.5	6.9 ± 0.2	28.7 ± 0.9	53.0 ± 1.7	16.9 + 0.6	24.9 ± 0.8	41.8 ± 1.5	94.8 ± 4.0
		2nd 2	5.0 ± 0.2	14.0 ± 0.5	1.3 ± 0.1	2.4 ± 0.1	22.7 ± 0.7	0.9 ± 0.0	3.4 ± 0.1	4 3 ± 0.2	27.0 ± 1.0
		Total 4	7.5 ± 0.2	28.9 ± 0.9	8.2 ± 0.2	31.1 ± 1.0	75.7 ± 2.4	17.8 ± 0.6	28.3 ± 0.9	46.1 ± 1.5	121.8 ± 4.8
120	Initials	0	1.0 ± 0.0	8.3 ± 0.4	11.0 ± 0.4	21.3 ± 0.7	41.6 ± 1.4	24.5 ± 0.9	39.3 ± 1.3	63.8 ± 2.0	105.4 ± 3.4
	0	1st 2	4.1 ± 0.2	16.2 ± 0.5	9.8 ± 0.3	17.3 ± 0.6	47.4 ± 1.6	24.3 ± 0.8	48.6 ± 1.6	72.9 ± 2.4	120.3 ± 4.1
		2nd 2	–	13.7 ± 0.5	5.8 ± 0.2	3.6 ± 0.1	23.1 ± 0.7	1.6 ± 0.1	2.1 ± 0.1	3.7 ± 0.1	26.8 ± 0.9
		Total 4	4.1 ± 0.2	29.9 ± 1.0	15.6 ± 0.5	20.9 ± 0.7	70.5 ± 2.3	25.9 ± 0.8	50.7 ± 1.7	76.6 ± 2.5	147.1 ± 4.9
	120	1st 2	3.4 ± 0.1	10.0 ± 0.3	15.1 ± 0.5	15.3 ± 0.5	43.8 ± 1.5	28.4 ± 0.9	47.5 ± 1.5	75.9 ± 2.4	119.7 ± 4.0
		2nd 2	0.9 ± 0.0	5.2 ± 0.2	12.2 ± 0.4	8.2 ± 0.3	26.5 ± 0.9	5.6 ± 0.2	1.9 ± 0.1	7.5 ± 0.3	34.0 ± 1.2
		Total 4	4.3 ± 0.1	15.2 ± 0.5	27.3 ± 0.9	23.5 ± 0.8	70.3 ± 2.3	34.0 ± 1.1	49.4 ± 1.6	83.4 ± 2.7	153.7 ± 5.1
160	Initials	0	0.8 ± 0.0	3.1 ± 0.1	9.3 ± 0.4	7.6 ± 0.3	20.5 ± 0.7	31.8 ± 1.1	59.1 ± 1.9	90.0 ± 3.1	111.4 ± 4.1
	0	1st 2	3.0 ± 0.1	13.3 ± 0.4	10.2 ± 0.3	20.6 ± 0.7	47.1 ± 1.6	25.8 ± 0.8	49.0 ± 1.6	74.8 ± 2.4	121.9 ± 4.4
		2nd 2	10.6 ± 0.3	16.8 ± 0.5	26.7 ± 0.9	18.2 ± 0.6	72.3 ± 2.3	3.0 ± 0.1	20.3 ± 0.7	23.3 ± 0.9	95.6 ± 3.8
		Total 4	13.6 ± 0.4	30.1 ± 0.9	36.9 ± 1.2	38.8 ± 1.2	119.4 ± 3.8	28.8 ± 0.9	69.3 ± 2.3	98.1 ± 3.2	217.4 ± 8.1
	160	1st 2	3.1 ± 0.1	6.4 ± 0.2	8.9 ± 0.3	36.5 ± 1.2	54.9 ± 1.8	32.7 ± 1.1	43.2 ± 1.4	75.9 ± 2.4	130.8 ± 4.8
		2nd 2	0.6 ± 0.0	10.6 ± 0.4	1.5 ± 0.1	0.8 ± 0.1	13.5 ± 0.5	6.2 ± 0.2	9.4 ± 0.3	15.6 ± 0.6	29.1 ± 1.0
		Total 4	3.7 ± 0.1	17.0 ± 0.6	10.4 ± 0.3	37.3 ± 1.2	68.4 ± 2.4	38.9 ± 1.2	52.6 ± 1.7	91.5 ± 3.0	159.9 ± 5.7

Table 2-2. Effect of pretreatment with various concentrations of diuron on the nitrogen content of 12-days old mycelial felts of *Cunninghamella echnulata* supplemented with or without the same doses of the herbicide. (mg per g dry weight ± standard deviation)

μ per ml		Days Incubaation	Ammonia	Amino	Peptide	Other Soluble	Total Soluble	Alkali Soluble Proteins	Residual Nitrogen	Total Insoluble	Total Nitrogen
Pre-treatment	Experimental										
0	Initials	0	3.3 ± 0.1	3.7 ± 0.2	4.8 ± 0.2	3.7 ± 0.6	17.5 ± 0.6	16.1 ± 0.6	53.4 ± 0.8	69.5 ± 2.3	87.0 ± 2.9
	0	1st 2	3.9 ± 0.2	9.6 ± 0.3	15.4 ± 0.5	24.9 ± 0.8	53.8 ± 1.8	15.8 ± 0.5	41.0 ± 1.4	56.8 ± 1.9	110.6 ± 4.0
		2nd 2	0.5 ± 0.0	1.0 ± 0.1	0.5 ± 0.0	−1.8 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	1.6 ± 0.1	2.0 ± 0.1	2.2 ± 0.1
		Total 4	4.4 ± 0.2	10.6 ± 0.3	15.9 ± 0.5	23.1 ± 0.8	54.0 ± 1.8	16.2 ± 0.6	42.6 ± 1.4	58.8 ± 2.0	112.8 ± 4.0
80	Initials	0	3.5 ± 0.1	1.7 ± 0.1	0.9 ± 0.1	7.5 ± 0.3	13.6 ± 0.5	9.1 ± 0.4	21.7 ± 0.7	30.8 ± 1.0	44.4 ± 1.4
	0	1st 2	5.3 ± 0.2	11.1 ± 0.4	6.7 ± 0.2	12.7 ± 0.4	35.8 ± 1.2	18.2 ± 0.6	42.8 ± 1.2	61.0 ± 2.0	96.8 ± 3.5
		2nd 2	0.7 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.1 ± 0.1	2.1 ± 0.1	4.4 ± 0.2	0.8 ± 0.0	5.2 ± 0.2	7.3 ± 0.3
		Total 4	6.0 ± 0.2	11.2 ± 0.4	6.9 ± 0.3	13.8 ± 0.4	37.9 ± 1.3	22.6 ± 0.8	43.6 ± 1.4	66.2 ± 2.2	104.1 ± 3.7
	80	1st 2	2.4 ± 0.1	6.9 ± 0.2	2.1 ± 0.1	7.7 ± 0.3	19.1 ± 0.1	18.0 ± 0.6	42.1 ± 1.4	60.1 ± 2.0	79.2 ± 2.9
		2nd 2	0.4 ± 0.0	0.6 ± 0.0	1.5 ± 0.1	0.4 ± 0.0	2.9 ± 0.1	0.9 ± 0.1	−3.3 ± 0.1	−2.4 ± 0.1	0.5 ± 0.0
		Total 4	2.8 ± 0.1	7.5 ± 0.3	3.6 ± 0.1	8.1 ± 0.3	22.0 ± 0.3	18.9 ± 0.7	38.8 ± 1.3	57.7 ± 1.9	79.7 ± 2.9
120	Initials	0	4.5 ± 0.2	6.6 ± 0.3	2.8 ± 0.1	9.7 ± 0.3	23.6 ± 0.3	5.4 ± 0.2	21.2 ± 0.2	26.6 ± 0.9	50.2 ± 1.7
	0	1st 2	5.7 ± 0.2	8.0 ± 0.1	3.7 ± 0.1	15.0 ± 0.5	32.4 ± 1.1	26.9 ± 0.9	54.1 ± 1.8	81.0 ± 2.7	113.4 ± 4.3
		2nd 2	1.1 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	5.5 ± 0.2	3.3 ± 0.1	7.1 ± 0.3	10.4 ± 0.4	15.9 ± 0.5
		Total 4	6.8 ± 0.2	9.7 ± 0.3	5.3 ± 0.2	16.1 ± 0.5	37.9 ± 1.3	30.2 ± 1.0	61.2 ± 2.0	91.4 ± 3.0	129.3 ± 4.7
	120	1st 2	5.7 ± 0.2	8.5 ± 0.3	8.1 ± 0.3	13.3 ± 0.4	35.6 ± 1.2	12.6 ± 1.2	46.1 ± 1.5	58.7 ± 1.9	94.3 ± 3.4
		2nd 2	2.7 ± 0.1	0.8 ± 0.1	0.5 ± 0.0	7.8 ± 0.3	11.8 ± 0.4	5.3 ± 0.2	4.8 ± 0.2	10.1 ± 0.3	21.9 ± 0.8
		Total 4	8.4 ± 0.3	8.3 ± 0.3	8.6 ± 0.3	21.1 ± 0.7	47.4 ± 1.6	17.9 ± 0.6	50.9 ± 2.3	68.8 ± 2.3	116.2 ± 4.1
160	Initials	0	3.1 ± 0.1	5.4 ± 0.2	2.4 ± 0.1	10.8 ± 0.1	21.7 ± 0.7	6.4 ± 0.7	38.4 ± 1.3	44.8 ± 1.5	66.5 ± 2.2
	0	1st 2	4.3 ± 0.2	8.6 ± 0.3	5.3 ± 0.2	15.5 ± 0.5	33.7 ± 1.2	15.9 ± 0.5	37.8 ± 1.3	53.7 ± 1.8	87.4 ± 2.8
		2nd 2	0.5 ± 0.0	1.4 ± 0.1	1.3 ± 0.1	0.6 ± 0.0	3.8 ± 0.1	1.6 ± 0.1	0.8 ± 0.1	2.4 ± 0.1	6.2 ± 0.2
		Total 4	4.8 ± 0.2	10.0 ± 0.4	6.6 ± 0.2	16.1 ± 0.5	37.5 ± 1.3	17.5 ± 1.3	38.6 ± 1.2	56.1 ± 1.8	93.6 ± 2.9
	160	1st 2	4.9 ± 0.2	7.6 ± 0.3	9.3 ± 0.3	21.9 ± 0.8	43.7 ± 1.5	16.6 ± 0.6	28.2 ± 0.9	44.8 ± 1.5	88.5 ± 3.2
		2nd 2	0.1 ± 0.0	1.4 ± 0.1	2.8 ± 0.1	5.2 ± 0.2	9.5 ± 0.3	1.0 ± 0.1	2.6 ± 0.1	3.6 ± 0.1	13.1 ± 0.5
		Total 4	5.0 ± 0.2	9.0 ± 0.3	12.1 ± 0.4	27.1 ± 1.0	53.2 ± 1.8	17.6 ± 0.6	30.8 ± 1.0	48.4 ± 1.6	101.6 ± 3.6

Further evidence for the previous suggestion comes from the percentage gain in dry weight, which was comparatively low in all herbicide treatments, even during recovery on a plain media.

Such major cellular changes are reflected in the metabolic activities of the fungus. The carbon dioxide output, by recovering patoran-treated mycelia, decreased with increased doses originally applied. This indicates a permanent inhibitory effect of patoran on the respiratory mechanism which suggests that physiological and perhaps also genetical disorders are involved. Such disorders may be reflected in enzymatic activities including those of respiration.

On the other hand, the higher rate of respiration (per unit dry weight) of the continuously patoran-fed mycelia, coupled with low dry weight gain, indicates the inability of the fungus to adapt to prolonged exposure to large doses of this herbicide. In fact, it shows the very toxic effects of the herbicide under such conditions.

In this connection, it may be mentioned that Alexander (1968) showed that diuron induced changes in enzyme activities of sugar cane including abnormal enzyme synthesis and formation of substances which block the sites of herbicide activity. Bednarz (1981) reported that the toxic action of monuron, diuron, atrazine and 2:4-D on several green algae was irreversible even when they were transferred to media free from these substances. Neither *Chlorella pyrenoidosa* nor *Dictyosphaerium pulchellum* developed increased tolerance to diuron. Akinyemiju and Dickmann (1982) showed that clones of two cultivars from *Populus* sections *Aigeiros* and *Tacamahaca* differed in their response to simazine and diuron. Even the relatively more tolerant *Aigeiros* clones were severely damaged or killed by large doses of both herbicides.

Cunninghamella echinulata seemed, however, to be more adapted to diuron than patoran. This is mainly indicated from the carbon dioxide output where minor differences between the various treatments and controls were observed (per mycelial growth). The apparent differences between the treatments and control (per unit dry weight) might be attributed to the initially lower dry weight of the former while growing on the diuron media.

The deleterious effects of pre-treatment with either of the herbicides was more apparent on nitrogen metabolism where secretion of nitrogen increased in both cases; this was more marked with continuous supply of the herbicides. Patoran seemed to have a greater effect since secretion was increased by increasing the original dose; this was not observed with diuron treatment.

These observations further indicate that both herbicides increased the permeability of the fungus, diuron being less effective than patoran. This might be attributed to the possibility that di-substitution in the phenyl ring (3- and 4-positions) counteracted, to some extent, the toxic effect of the monosubstitution (4-position) on *Cunninghamella echinulata*. The reverse was observed by Haikal (1976) using *Fusarium oxysporum*. Von Brand *et al.* (1953) suggested that the resistant strains of *Trypanosoma gambiense* to arsenicals were less permeable to the drugs.

Such difference in response towards the original herbicide treatment was more apparent in the nitrogen gain by the mycelia. The percentage of nitrogen accumulation (with respect to corresponding initial values) was far less in patoran-treated than in diuron-treated mycelia. Diuron treatment increased this accumulation except at the larger doses where it dropped to control levels. These observations and conclusions add further support to our previous suggestions.

In spite of these observations, the net gain in total nitrogen in any of the treatments was, in most cases, lower than that of the samples originally cultured in herbicide-free media – an indication of the intrinsic effects of the herbicides. The lower residual nitrogen content of the mycelia continuously supplied with the herbicides, as compared with their respective 'recovering' samples, indicates the lower tolerance of the former samples to the herbicides with prolonged exposure to large doses. Structural changes in the mycelial walls might explain the increased permeability (nitrogen secretion) by such treated samples.

Similarly the lower alkali-soluble protein content of such treated samples reflects the inhibitory effect of the continuous supply of the herbicides on protein synthesis; this feature is further confirmed by the relatively higher soluble peptide content of such mycelia. This lends further support to the possible impairment of enzymatic activity by such treatments.

According to Pusztai and Vegh (1978), prolonged treatment of barley with nine substituted urea herbicides, including diuron and patoran, increased the frequency of chromosome aberrations. Tarchevskii *et al.* (1982) noticed that diuron and actinomycin D (inhibitors of photophosphorylation) and quercetin (inhibitor of energy transfer) delayed cellulose synthesis. Belova and Tarchevskii (1981) reported that diuron reduced the rate of albumin, globulin and gliadin synthesis without affecting the relative rate of protein synthesis in wheat. Peirson and Elliott (1981) showed that diuron blocked nitrite reduction by leaf tissues of *Phaseolus vulgaris*.

From the above discussion, one may reach the conclusion that large doses of both patoran and diuron caused physiological and perhaps also genetical disorders to the young active growing mycelia of *Cunninghamella echinulata* that continued even after the transfer of the mycelia to fresh herbicide-free media. Continuous supply of the herbicides intensified such disorders. Under all conditions diuron was less effective than patoran.

Such disorders manifested themselves in the lower dry weight gain by the treated mycelia, lower percentage accumulation of dry matter even during recovery on herbicide-free media, impairment of respiratory and nitrogen metabolism enzymes as well as increased permeability as a result of alterations in the chemical composition of the mycelial walls.

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(Received 26/03/1984;
in revised form 15/12/1984)

تأثير سابق المعاملة بجرعات كبيرة من الباتوران والديورون على الأيض النتروجينى لفطرة *الكاننجهاملا إكينولاتا*

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أثبتت نتائج هذا البحث أن الغزل الفطرى الناتج عن إنبات جراثيم فطرة الكاننجهاملا فى محاليل تغذية تحتوى على تركيزات عالية من الباتوران أو الديورون له القدرة على تحمل استمرار إمداده بنفس تركيزات هذه المبيدات العشبية. إلا أن نقل هذه الأغزال الفطرية إلى محاليل تغذية خالية من المبيدات يؤدى الى زيادة أكبر فى الكتلة الحية ولو أن هذه الزيادة أقل بكثير من تلك التى يحصل عليها الغزل الفطرى غير المعامل.

وقد لوحظ أن التأثير المثبط للباتوران على خروج ثانى أكسيد الكربون الناتج عن التنفس لازال واضحا ومستمرا حتى بعد انتقال الغزل الفطرى إلى أوساط تغذية خالية من المبيد، فى حين تلاشى هذا الأثر المثبط بعد المعاملة بالديورون.

وقد ظهر الأثر الضار لسابق المعاملة بالمبيدات واضحا على الأيض النتروجينى اذ ازداد معدل خروج المواد النتروجينية بعد كلا المعاملتين خاصه مع استمرار تزويد الغزل الفطرى بهذه المبيدات.

كما يبدو أن انتقال الغزل الفطرى إلى أوساط تغذية خالية من المبيدات يساعد على زيادة تراكم المواد النتروجينية داخل الغزل إذا قورن باستمرارية التغذية بالمبيدات .

ويعتبر نقص النتروجين المتبقى فى الغزل الفطرى المغذى باستمرار بالمبيدات عن مثيله النامى فى محاليل خالية من المبيدات دليلا على نقص قدرة تحمل العينات السابقة باستمرار تعرضها للجرعات الكبيره من المبيدات .