filled with a strongly electron dense material (fig. 6 and 7). The presence of dense, crystalline-like material within the cells and the endocytotic vesicles was indicative of cytosis. Within the capsule the parasite and encapsulated cells were disrupted. No collagen was seen in any section. No encapsulated metacercariae were found in the brain of experimentally infected fish that had been kept at 17–18 °C for up to 6 months. Around excized metacercariae from these fish was the normal number of the rounded cells always accompanying experimental and natural infections (fig. 1).

Discussion. Explanations drawing upon the supposed immunologically privileged nature of the brain for the survival, unencapsulated, of *D. phoxini* are clearly inadequate. Similarly, derived explanations for the survival of other unencapsulated parasites, as in the pericardium of *Xenopus*[13], must also be questioned.

The capsule is formed from a small number of overlapping cells identified from their ultrastructure as astrocytes. Whether the deposition of the electron dense intercellular matrix is the cause of the death of the parasites, or a consequence of its death, remains to be determined. Furthermore, the reasons for the lack of synchrony in the encapsulation of all parasites in one fish must be sought.

A mechanism does exist in *P. phoxinus* to wall off and kill *D. phoxini*. It is proposed that the vacuolated cells around the parasites, which have so often been noted, are part of the natural processes of repair in a brain damaged by the activity of the trematode parasites. In natural conditions, the repair process is not fully effective and as a direct consequence, the parasite survives. The parasites and fish from Wales used in these experiments would not naturally experience temperatures much, if at all, in excess of 20 °C. Therefore, the factor leading to encapsulation is high temperature during the complex development[9,14] of the metacercaria from the cercaria.

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A pesticide-resistant mutant of the N_2-fixing blue-green alga *Nostoc muscorum*

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Summary. The fungicide dithane Z-78 (zinc ethylene bisdithiocarbamate) has been found to be an inhibitor of growth and heterocyst differentiation in *Nostoc muscorum*. Its inhibitory effect has been reversed by exogenous glucose. A spontaneous mutant resistant to a toxic concentration of the fungicide grows in the presence of dithane at a dose normally applied in fields and does not require an exogenous carbon source for its growth.

The success of modern agriculture depends on the extensive use of pesticides[3] which usually kill their target organisms by being either an inhibitor of photosynthesis, or respiration, or growth[4]. The blue-green algae, besides being able to fix nitrogen, possess photosynthetic machinery identical to the chloroplasts of higher plants[5,6]. Pesticides are therefore expected to interfere with the photosynthetic machinery of naturally occurring blue-green algae. A majority of blue-green algae, which are heterocystous and filamentous, are of immense value in rice technology[7]. Heterocysts and their adjacent vegetative cells depend upon each other. The heterocysts supply fixed nitrogen to vegetative cells, and in return utilize their carbohydrates and energy for N_2 fixation[5]. Pesticides which inhibit photosynthesis thus indirectly affect the N_2-fixing machinery of blue-green algae. This prompted the authors to investigate which pesticides are capable of inhibiting the photosynthetic process in blue-green algae, and also to raise a mutant population of this group of nitrogen-fixers resistant to such pesticides.

The biological effects of different concentrations of dithane Z-78 (obtained from the Plant Protection Department, Govt. of India, Muzaffarpur), which was prepared by the method described previously[8], on growth and the frequency of heterocysts of *Nostoc muscorum* in N_2 and NO_3^- media were examined. Culture conditions, and growth and heterocyst measurement methods were the same as those described previously[8].

Experiments in general were done with 5 replicates and the results obtained were analyzed statistically using the calculation of the standard error[9] in order to assess the biological significance and reproducibility of the findings.

In contrast to the control, the dithane-supplemented cultures of *Nostoc muscorum* did not show significant growth in N_2 and NO_3^- media (fig. 1). In addition, dithane significantly inhibited the heterocyst frequency of the alga in N_2

Maximum heterocyst frequency* of parent *Nostoc muscorum* and its dithane-resistant mutant strain in N_2 medium containing or lacking glucose (500 ppm)

Dithane dose (ppm)	Heterocyst frequency (%) Parent		Mutant	
	Without glucose	With glucose	Without glucose	With glucose
0 (control)	5.35 ± 0.18	5.72 ± 0.17	5.28 ± 0.15	5.55 ± 0.05
10	2.25 ± 0.16	5.38 ± 0.13	5.28 ± 0.16	5.55 ± 0.08
25	1.76 ± 0.09	5.31 ± 0.16	5.28 ± 0.09	5.55 ± 0.12
50	0.0	5.15 ± 0.12	5.28 ± 0.12	5.55 ± 0.09
75	0.0	5.08 ± 0.08	5.28 ± 0.08	5.55 ± 0.11
100	0.0	5.02 ± 0.09	5.28 ± 0.10	5.55 ± 0.07

* The number of heterocysts per 100 vegetative cells each based on a random sampling of 12 filaments. The values are the means ± SE of 5 independent readings.

medium. The decrease in heterocyst frequency was concomitant with an increase in the concentration of dithane (table). Normally, *N. muscorum* differentiates 5–6% heterocysts within 20–24 h on their transfer from inorganic nitrogen-containing medium to nitrogen-free medium. On supplementation with 10 or 25 ppm dithane it formed heterocysts with almost ½ or ¼ the normal frequency, respectively. The alga showed complete suppression of heterocyst formation with 50, or 75 or 100 ppm dithane (table). However, the inhibitory effect on growth in N_2 and NO_3^- media (fig. 1) and on heterocyst formation in N_2 medium (table) were reversed after the supplementation of dithane-inhibited cultures by 500 ppm glucose. These effects of dithane are similar to that of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) on *N. muscorum*[10]. Dithane belongs to the carbamate group of pesticides and carbamates in general have been found to be inhibitors of photosynthesis in higher plant systems (mainly by preventing chloroplast electron flow through PS II)[11]. DCMU is a specific inhibitor of PS II function and its application to oxygenic photosynthetic organisms is known to result in abolition of photochemically generated reducing power ($NADPH_2$) without causing any adverse effect on the generation of ATP through cyclic photophosphorylation[12]. $NADPH_2$ is the major source of reductant for the nitrogenase reaction in the blue-green alga *Anabaena cylindrica* and it has been found that DCMU inhibits nitrogenase activity by inhibiting the generation of $NADPH_2$[13]. Moreover, photosynthetic assimilation of inorganic carbon (CO_2) is a reductive process occurring at the expense of photochemically generated reductant during oxygenic photosynthesis. DCMU-treatment, as expected, blocks CO_2 assimilation in such systems. Accordingly, while obligate photoautotrophs fail to recover from DCMU inhibition of growth in the presence of an organic carbon supplement like glucose, the photoheterotrophs show rapid recovery from DCMU inhibition of growth under similar conditions. This simple technique has recently been used in blue-green algae for classifying them into obligate photoautotrophs and photoheterotrophs[14,15].

Nostoc muscorum has been known to photoassimilate organic substrates like glucose, acetate and amino acids[8,16]. It has been reported previously that DCMU inhibition of both growth and heterocyst differentiation in *N. muscorum* is readily reversed by glucose[10]. This suggested that heterocyst differentiation requires a photosynthetically fixed carbon supply and that glucose (by feeding electrons during the light reactions) can effectively substitute for photosynthetically generated organic carbon in growth and differentiation[10]. The fact that the same organic substrate (glucose) reversed the inhibitory effects of dithane on growth and heterocyst differentiation in this alga quite effectively, suggests that dithane is like DCMU in its mode of action. Experiments are being carried out in order to examine whether or not dithane acts on the reducing side of PS II like DCMU, i.e., to investigate the binding of the inhibitor to DCMU binding protein.

It is probable that dithane inhibits PS II generated reducing power and thereby affects the rate of N_2 fixation either indirectly by affecting heterocyst frequency, or directly by inhibiting nitrogenase activity due to a poor supply of $NADPH_2$. These experiments helped to place dithane Z-78 in the category of photosynthetic inhibitors. It was then intended to isolate a dithane-resistant mutant of *N. muscorum*. For this, a rich population of an exponentially growing alga was harvested and spread over 100 ppm dithane-supplemented N_2 agar plates. A few mutant colonies were found after a fortnight of incubation in a growth chamber. A clonal culture was made from one of these colonies in fresh N_2 medium. This mutant, on characterization for growth and heterocyst frequency, was found to resist 100 ppm concentration of dithane which is considerably higher than that applied in fields (67.5 ppm). It is remarkable that this mutant showed a good growth (fig. 2) and heterocyst frequency (table) in the presence as well as the absence of glucose. However, a marginal variation in the pattern of growth in N_2 of NO_3^- medium (fig. 2) and in heterocyst frequency in N_2 medium (table) was observed on supplementation with glucose. This suggested that mutation for dithane-resistance did not create a permeability

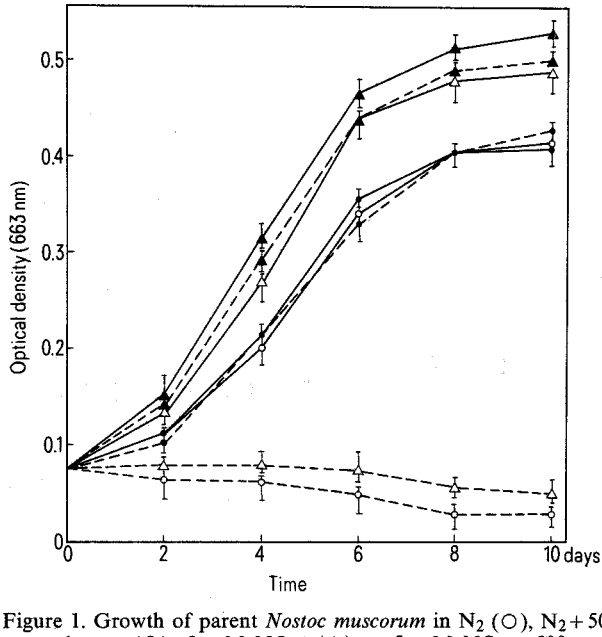

Figure 1. Growth of parent *Nostoc muscorum* in N_2 (○), N_2 + 500 ppm glucose (●), 5 mM NO_3^- (△) or 5 mM NO_3^- + 500 ppm glucose (▲) medium supplemented with zero (——) or 50 ppm (- - - -) concentration of dithane Z-78.

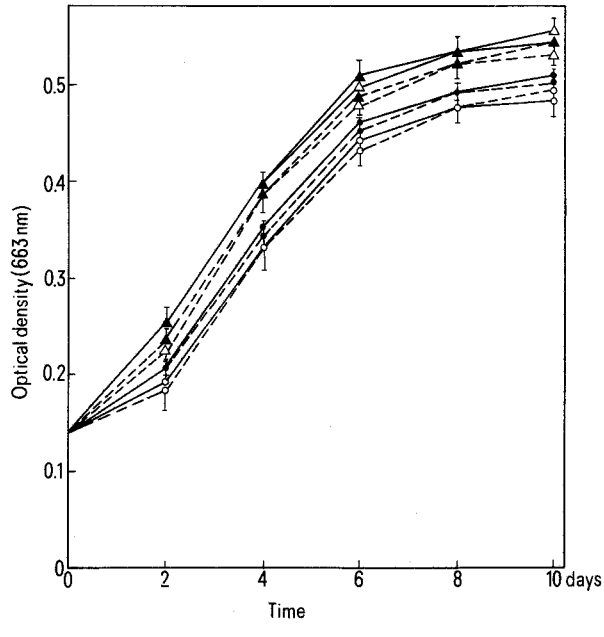

Figure 2. Growth of dithane-resistant mutant strain of *Nostoc muscorum* in N_2 (○), N_2 + 500 ppm glucose (●), 5 mM NO_3^- (△) or 5 mM NO_3^- + 500 ppm glucose (▲) medium supplemented with zero (——) or 100 ppm (- - -) concentration of dithane Z-78.

barrier for glucose, but probably formed a complete permeability barrier for dithane. Had there not been a permeability barrier for dithane, the dithane-resistant mutant would have been expected to be defective in PS II activity, leading to the development of an auxotrophy for a fixed carbon source, as in the previously reported methylamine-resistant mutant of *N. muscorum*[17]. Dithane-resistance was thus thought to be a case of permeation mutation. Further experiments to confirm the presence of a permeability barrier for dithane in the dithane-resistant mutant of *N. muscorum* are in progress. Experiments with glucose suggested the existance of an active glucose-transport system in *N. muscorum* as well as its dithane-resistant mutant strain.

The present findings give a clear indication that it would be possible to isolate mutant strains of blue-green algae resistant to other pesticides as well, which can grow, multiply and continue fixing nitrogen in fields treated with those pesticides.

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Chemical subdivisions within the genus *Arctostaphylos* based on flavanoid profiles[1]

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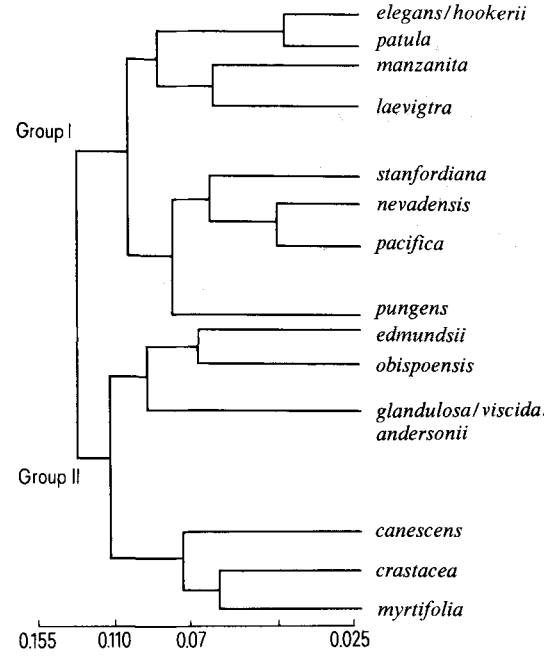
Summary. The flavonoid glycosides from 227 populations representing 20 species of *Arctostaphylos* have been identified. Certain glycosides are of values in subdividing the genus into discreet chemically related groups. A single linkage computer analysis shows the existance of subdivisions based both on oxidation levels of the flavonoids as well as glycoside variation. The ability to form 7-O-glycosides appears to be restricted and could be of future value in the identification of hybrids between those taxa capable of 7-O-glycoside synthesis and those unable to do so.

Arctostaphylos is a mainly North West American genus of woody plant sometimes divided into about 100 taxa[2]. Many of these taxa are described and delimited by a few trivial, if not questionable characters and subsequently the genus appears to be taxonomically difficult to evaluate. In all probability there are about 25–50 distinguishable taxa with definite delimitable characters.

Previous studies on *Arctostaphylos uva-ursi* (L.) Spreng. have indicated the usefulness of flavonoids as phytogeographic and taxonomic markers within the genus[3,4]. Furthermore, studies have been carried out on the distribution of flavonoid aglycones within the genus. Of 41 species, subspecies and varieties evaluated; 10/41 produced myricetin and quercetin derivatives; 21/41 produced myricetin, quercetin and kaempferol derivatives, and the remainder produced quercetin and kaempferol derivatives alone[5]. Several workers have suggested that levels of B-ring hydroxylation in flavonoids is indicative of primitive versus advanced phylogenetic standing[6]. It would appear therefore that within the genus *Arctostaphylos* there are both 'primitive' and 'advanced' taxa – those producing trihydroxy and dihydroxy derivatives but no monohydroxy flavonoids (i.e., kaempferol) and those not producing trihydroxy derivatives (i.e., myricetin).

As part of a continuing study of this genus a total of 227 populations representing the taxa in the table were evaluated for their flavonol glycoside profiles as examples of biochemically 'primitive' vs 'advanced' forms. Voucher specimens were deposited in the University of Alberta Vascular Plant Herbarium.



Phenogram of 20 taxa of *Arctostaphylos* on the basis of a single linkage analysis of their flavonoid-glycoside pattern.