ALGAL GROWTH INHIBITORS FROM DECOMPOSING NEW ZEALAND CROP PLANTS

EDWARD J. NORTON, PAUL A. BROADY & JOHN R. L. WALKER

Department of Plant and Microbial Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

(Received 22 November 1995; revised and accepted 17 December 1997)

ABSTRACT

Norton, E.J., Broady, P.A. & Walker, J.R.L. (1997). Algal growth inhibitors from decomposing New Zealand crop plants. *New Zealand Natural Sciences* 23 109-120.

The increasing use of decomposed barley straw as an algal control method in Europe has led to an interest in the use of decomposing straw materials for the control of nuisance growths of *Hydrodictyon reticulatum* (water net), an alga recently introduced to New Zealand. Antialgal activity was detected in liquor from anaerobically decomposing material including ryegrass (*Lolium* spp.), oat chaff, lucerne and red clover, but no activity was observed from barley straw with the assays used in this study. Isolation of the active compound(s) from ryegrass was attempted and two compounds with similar chemical properties were present in the active fractions. Efforts to separate these were unsuccessful. The active compound(s) was shown to be a non-phenolic, organic acid of molecular weight 650-700. The concentration required for activity was estimated to be about 0.1 g L^{-1} and the time required for the compound(s) to exert full effect was 6 h, with significant activity occurring in the first 2 h of exposure to algae. The compound(s) displayed strong activity against all Bacillariophyta and Cyanobacteria tested, plus most Xanthophyta and Chlorophyta including *H. reticulatum*, but did not inhibit representatives of the Rhodophyta or Eustigmatophyta. The antialgal effect was sensitive to pH with a reduction in activity as pH was increased from 3 to 7 and a loss of activity above pH 7. Implications of this for potential use of the compound(s) in the control of problem algae are discussed. It was concluded that the compound(s) displayed properties which were less than ideal for practical application but it would be worthwhile investigating other natural algicidal products (such as the other active straw materials) in an effort to find materials with more favourable properties for algal control applications.

KEYWORDS: Antialgal - ryegrass - barley straw.

INTRODUCTION

Nuisance growths of aquatic algae occur frequently throughout the world and the spread of problem algae to new locations is becoming an increasing concern for water-quality managers. In New Zealand the colonial green alga *Hydrodictyon reticulatum* L. Lagerheim (Chlorococcales, Chlorophyceae) was first noted in 1987 (Coffey & Miller 1988) and has since spread, forming seasonal nuisance growths in central and northern lakes and rivers of the North Island.

Options suggested for the control of *H. reticulatum* include the use of decomposing barley straw, a method which has aroused considerable research interest internationally. The antialgal capacity of barley straw decomposing in water has been established by field experiments (Welch *et al.* 1990, Ridge & Barrett 1992) and in the laboratory (Gibson *et al.* 1990). The field experiments were carried out in the Chesterfield Canal using local barley straw and antialgal activity was demonstrated for a range of eukaryotic algae and cyanobacteria, including both unicellular and filamentous forms (Gibson *et al.* 1990). These authors concluded that the antialgal effect was probably due to a substance(s) released from the rotted straw; these could have been of either plant or microbial origin and they suggested that aerobic microbial decomposition was essential in the release process. Subsequently Pillinger

et al. (1992) investigated the fungi growing on actively rotting straw and concluded that the consistent algal control shown by rotting barley straw could not be attributed to a unique mycoflora or a specific fungus. Pillinger *et al.* (1994) suggested that the straw itself was the source of algal inhibition and they proposed that oxidised lignin fractions were the primary source of algal inhibitors from barley straw. Whilst the identity of the antialgal compounds from barley straw still remains unproven, the effect is well-established and the method is now in widespread use (Newman & Barrett 1993, Ridge 1994).

Initial trials with barley straw in New Zealand yielded inconclusive results. Some inhibitory effect was observed against *H. reticulatum* in the field but consistent effects were not achieved with controlled experiments in the field or in the laboratory (Wells *et al.* 1994). Hawes and Smith (1992) reported antialgal activity on *H. reticulatum* grown in the presence of decomposing barley straw in laboratory experiments, but straw liquor added to *H. reticulatum* cultures did not cause inhibition. They suggested that in this case the inhibition was dependent on the physical presence of straw rather than a dissolved algicidal agent. These authors also noted antialgal activity from rotting *Typha orientalis* (raupo), a wetland plant common in NZ, which produces a large amount of leaf litter.

Because of the inconclusive nature of these preliminary results in New Zealand compared with the more positive findings from studies in the UK, it was decided to investigate a number of crop materials commonly available in NZ (including barley straw) and raupo, for release of antialgal compounds during degradation. A rapid bioassay was developed which was used to screen for antialgal activity from a number of NZ crop materials held under three types of degradation conditions. Attempts were made to isolate the active compound(s) from the liquor of anaerobically decomposed ryegrass hay (*Lolium* spp.) and a range of microalgae were tested for susceptibility to the partially purified extract. The concentration of active component(s) required for activity, the speed of inhibitor action, and the stability of the compound were estimated and results were used to assess the potential for application of the liquor as an algal control measure.

MATERIALS AND METHODS

DECOMPOSITION OF STRAW MATERIAL

A representative range of dried straw materials was gathered from farms in Canterbury, NZ. Straw types tested were; barley straw (*Hordeum* sp.), ryegrass hay (*Lolium* spp.), luceme (*Medicago sativa* L.), red clover hay (*Trifolium pratense* L.), oat chaff (*Avena sativa* L.), pea straw (*Pisum sativum* L.), ryegrass silage and raupo (*Typha orientalis* C.B. Presl). Silage was included after it became evident that anaerobically decomposing ryegrass was a source of antialgal activity. The materials were allowed to decompose in 1.5 L preserving jars filled with nutrient amended distilled water. Nutrients (0.1 g L^{-1} $(NH_4)_2SO_4$ and 0.2 g L^{-1} KH_2PO_4) were added to encourage bacterial growth and were based on media used for the enrichment culture of pseudomonads. Straw was added at the equivalent of 40 g L^{-1} (wet weight) which was the same as that used by Gibson *et al.* (1990). All straws were subjected to each of three separate treatments as follows:

1. *Aerobic treatment*: Jars were aerated with a continuous stream of air passed through cotton wool filters and the jars were sealed with cotton wool bungs and aluminium foil caps.
2. *Anaerobic treatment*: Jars were sealed with airtight lids and fitted with vacuum port bungs from which samples could be drawn by a syringe needle without contaminating the anaerobic environment in the jar.
3. *Sterile treatment*: Jars and their contents were autoclaved twice for 1 h and fitted with lids as for the anaerobic treatment.

All jars were incubated at temperatures ranging from 17-23°C. Samples of liquor (5 ml) were taken from the jars after 2, 3

and 4 months decomposition, freeze-dried, redissolved in 0.5 ml distilled H_2O and screened for antialgal activity.

SCREENING LIQUOR SAMPLES FOR ANTIALGAL ACTIVITY

Algae were cultured in liquid Bold's Basal Medium (BBM) (Nichols & Bold 1965) and an inoculum from logarithmic phase cultures was then filtered onto sterilised 45 mm cellulose acetate filters (0.45 μm pore size) using a weak vacuum. Filters were then placed aseptically onto surface dried, agarised (1% w/v) BBM plates, providing an even inoculum. Liquor samples (20 μl) to be tested for antialgal activity were applied to 6mm glass fibre antibiotic assay (AA) discs which were then placed onto the surface of the filters. Control discs were impregnated with sterilised, distilled water. Plates were then incubated at 20°C, under a 16:8 h light/dark cycle illuminated by cool white fluorescent lamps (standard conditions) and were checked daily for evidence of growth inhibition. Antialgal activity was usually visible within 2 days and was measured as the diameter of any zone of inhibition which developed after 5 days. Five freshwater algae were selected for their ability to grow rapidly and evenly over the filters and these were used during the initial screening of liquors. These were; *Golenkiniopsis* sp., *Chlorella vulgaris* (858), *Stichococcus* sp (392)., *Bracteacoccus minor* (686), and *Pseudococcomyxa simplex*, all from the culture collection of Dr Paul Broady, Plant and Microbial Sciences, University of Canterbury; strain number in parentheses. *Golenkiniopsis* sp. was isolated from Bromley Ponds, Christchurch and the remaining algae were isolated from Antarctic soils.

INITIAL PURIFICATION PROCEDURES

The attempts at purification of antialgal compound(s) are summarised in Fig. 1. Procedures were bioassay directed and *Golenkiniopsis* sp. was used as primary test organism. Crude ryegrass liquor which had been concentrated by freeze-drying (50:1 conc.) was extracted with ethyl acetate and the active organic layer then extracted with 1M $NaHCO_3$. The aqueous layer was adjusted with HCl to pH 3 before re-extraction with ethyl acetate which was evaporated off and the residue taken up in distilled H_2O (Fraction I). Activated charcoal powder R-G (Riedel-De Haen) was prewashed with methanol and acetone and rinsed with distilled H_2O then stirred into the aqueous Fraction I and the mixture poured into a column (15 mm x 120 mm) and eluted with distilled H_2O under vacuum. The eluant was evaporated and the residue taken up in distilled H_2O (Fraction II).

COLUMN CHROMATOGRAPHY

Glass columns (15 mm x 120 mm) were packed with either Sephadex G-15 or silica gel (10-40 μm particle size, Sigma No. S-6503) and connected to an ISCO UA-5 UV monitor (set at 280 nm) and Retriever II fraction collector. Fraction II was applied to the Sephadex column in sucrose solution and eluted with 0.1 % trifluoroacetic acid. Fractions from this column showing antialgal activity were pooled to give Fraction III. With the silica gel column, Fraction II was first bound to silica gel, applied dry, and eluted with tetrahydrofuran (THF) : glacial acetic acid : isopropanol (98.9 : 1 : 0.1); active fractions from this column were pooled to give Fraction IV.

SILICA GEL THIN LAYER CHROMATOGRAPHY (TLC)

Fraction II was chromatographed on silica gel TLC sheets and separated components were visualised under short (254 nm) and long (360 nm) wavelength UV light and with a number of spray reagents. Solvent trials indicated that THF : glacial acetic acid (99 :1) produced good mobility properties for the active zone.

BIOASSAY OF THIN LAYER CHROMATOGRAMS

Developed thin-layer chromatograms were oven-dried to remove traces of solvent and then cut to fit into 90 mm petri dishes. The bottom of each dish was covered with a thin layer of solidified (1% w/v) agarised BBM and the chromatograms were then placed face up, on top of the agar layer. Agarised (0.5%) BBM was prepared fresh and allowed to cool to approximately 37°C before adding an inoculum of *Golenkiniopsis*

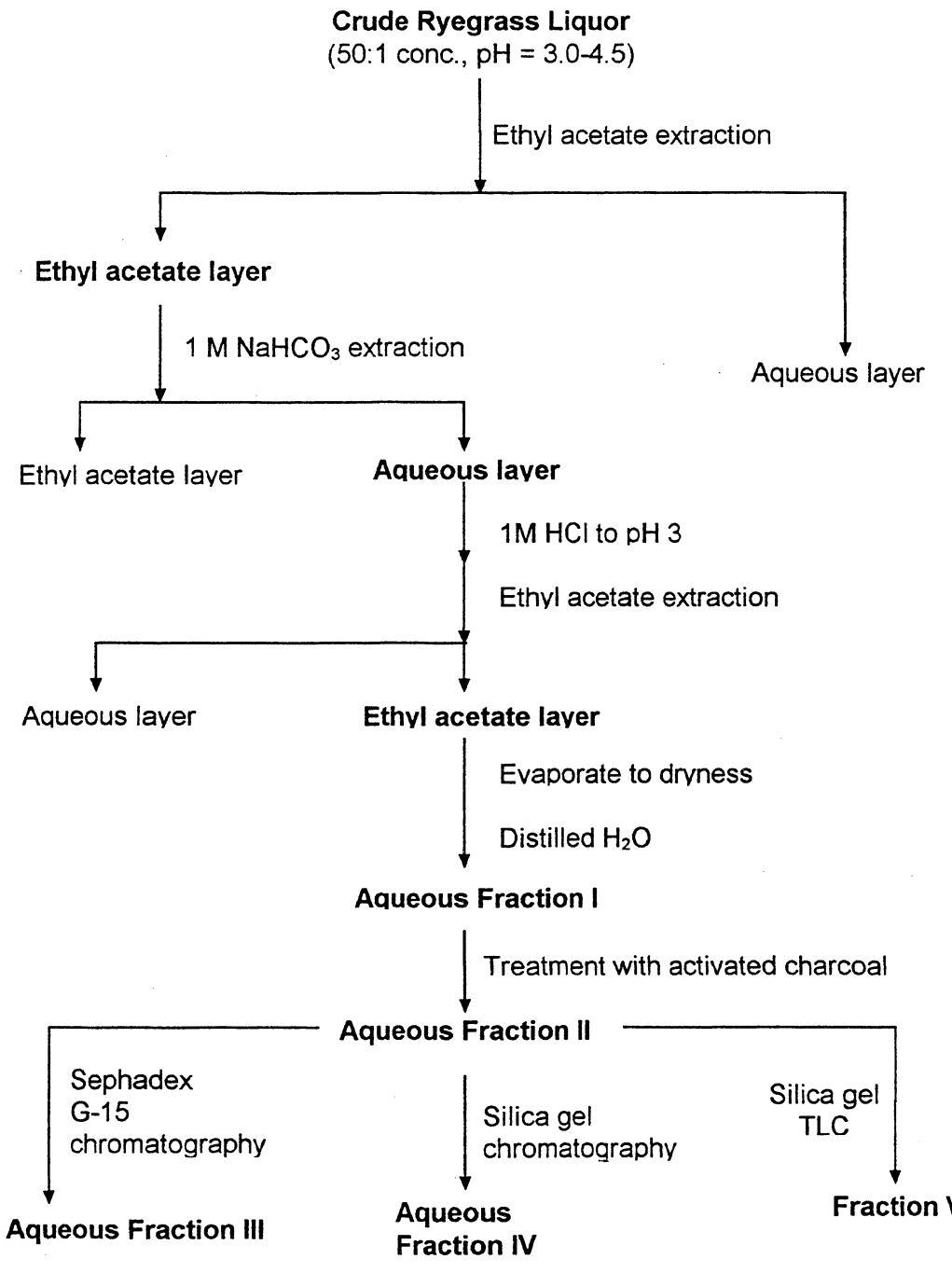

Figure 1. Scheme for attempted isolation of antialgal compound(s) from ryegrass liquor. Fractions I-V were partially purified and all produced the same single active zone in bioassays of TLC plates.

sp. grown to log phase in liquid BBM culture. This mixture was poured into the petri dishes, covering the chromatograms with a thin layer of algae-seeded agar which was then allowed to solidify. Plates were incubated under standard conditions and were checked daily for evidence of growth inhibition.

CHEMICAL CHARACTERISATION OF COMPOUNDS IN THE TLC ACTIVE ZONE

Chemical spray reagents were used to attempt to identify specific reactive groups present in the active zone of developed TLC plates. This was done in an attempt to define the type of compound(s) causing the observed activity. Reagents were made according to Smith (1969) and applied by aerosol spray to dried chromatograms. A crude estimate of molecular weight of the active compound(s) was obtained using elution data from a Sephadex G-15 column.

SCREENING A DIVERSE RANGE OF ALGAE

A range of algae and cyanobacteria (Table 1) was tested using the AA disc bioassay method. BG-11 medium (Rippka *et al.* 1979) was used for cyanobacteria whilst BBM was used for all other taxa. NaCl (4% w/v) was added to BBM for the Rhodophyta,

Table 1. The inhibitory effect of Fraction II. Data are means (± standard errors) of three replicate inhibition zones, dashes indicate no inhibition. Classification is after Lee (1989), Mattox and Stewart (1984) and Irvine and John (1984).

Taxa	Zone (mm)	Taxa	Zone (mm)
Cyanobacteria		Chaetophorales	
Nostocales		*Stigeoclonium* sp.	9.3 ± 0.3
Anabaena sp.	28.0 ± 2.1	Chlorosarcinales	
Calothrix sp.	20.0 ± 1.2	cf. *Tetracystis* sp.	-
Cylindrospermum sp.	19.0 ± 0.3	Pleurastrophyceae	
Tolypothrix sp.	10.0 ± 0.0	Pleurastrales	
Oscillatoriales		*Microthamnion* sp. (667)	-
Oscillatoria sp.	10.0 ± 0.3		
		Rhodophyta	
Chlorophyta		Porphyridiales	
Charophyceae		*Porphyridium purpureum*	-
Klebsormidiales			
Klebsormidium sp. (479)	-	**Bacillariophyta**	
Stichococcus, (392)	-	Pennales	
Zygnematales		cf. *Navicula*	>40 ± 0.0
Spirogyra sp.	10.0 ± 0.0	cf. *Nitzschia* sp.	17.7 ± 0.3
Chlorophyceae			
Volvocales		**Xanthophyta**	
cf. *Chlamydomonas* sp.	-	Mischococcales	
Chlorococcales		*Botrydiopsis* sp. (485)	-
Bracteacoccus minor (686)	6.5 ± 0.0	*Chlorellidium* sp. (597)	9.3 ± 1.7
cf. *Chlorella* sp.	-	Tribonematales	
cf. *Chlorella vulgaris* (858)	-	*Heterothrix* sp. (395)	6.5 ± 0.0
cf. *Chlorococcum* sp.	7.7 ± 0.3		
Golenkiniopsis sp.	14.8 ± 0.2	**Eustigmatophyta**	
Hydrodictyon reticulatum	9.5 ± 0.3	Eustigmatales	
Pseudococcomyxa simplex	-	*Eustigmatos* sp. (681)	-

and Si was added at 0.0028 g L^{-1} to BBM for the Bacillariophyta. All the cyanobacteria and two of the filamentous chlorophytes (*Spirogyra* sp. and *H. reticulatum*) required careful treatment to achieve an even inoculum on the cellulose acetate filters, since these algae did not form homogeneous unicellular suspensions in liquid culture. They were placed on filters either directly, using sterilised tweezers, or by filtration under gentle vacuum, using a sterilised needle to tease out the filaments and ensure an even distribution across the filter. Three replicate 20 µl aliquots of Fraction II at 5 g L^{-1} were assayed and results expressed as the mean diameter of the inhibition zone.

DETERMINATION OF INHIBITOR ACTIVITY AT DIFFERENT CONCENTRATIONS

Fraction II was evaporated to dryness in a rotary vacuum evaporator and the residue weighed and resuspended as a stock solution of 5 g L^{-1}. Culture flasks were prepared with 20 ml BBM plus Fraction II at 0 g L^{-1} (control), 0.005, 0.05, 0.1 and 0.25 g L^{-1} (final concentration) with three replicate flasks for each treatment. The flasks were autoclaved for 15 min at 1.1 kg cm^{-2}, a process which did not reduce the toxicity of extracts from ryegrass liquor. An inoculum of *Golenkiniopsis* sp. cells was added aseptically to each flask and all were incubated under standard conditions. Flasks were sampled at intervals (initially every 2 d) for up to 20 d, and cell counts were made using a haemocytometer.

DETERMINATION OF THE SPEED OF ACTION OF THE INHIBITOR

Culture flasks were prepared with 10 ml liquid BBM and Fraction II at 0 g L^{-1} (control) and 0.25 g L^{-1}. Flasks were autoclaved at 1.1 kg cm^{-2} for 15 min, cooled and at time zero, were seeded with an inoculum of *Golenkiniopsis* sp. to give a concentration of 500 cells ml^{-1}. Flasks were then incubated under standard conditions. At 0.5, 1, 3, 9 and 24 h the contents of three replicate flasks were filtered onto separate sterilised 45 mm cellulose acetate filters (0.45 µm pore size) and each filter was rinsed 3 times with 10 ml sterilised liquid BBM to remove any trace of the inhibitor test sample. Controls (no inhibitor) were filtered at time zero, and at the end of the 24 h experimental period. Filters were placed onto the surface of agarised BBM (1% w/v) plates and incubated under standard conditions for 7 d. Colonies which grew on the filters were counted using a stereo-microscope.

TESTING THE STABILITY OF THE ACTIVE COMPOUND(S)

Heat stability was determined by assaying Fraction II after exposure to a boiling water bath (15 min) and to autoclaving at 1.1 kg cm^{-2} for 15 min. Stability over a pH range (pH 3-7) was assessed by titrating Fractions I and II with $NaHCO_3$, followed by bioassays of three replicates at each pH. Hydrochloric acid ($1x10^{-2}$ M) was titrated to the same pH values and bioassayed as a control.

RESULTS

SCREENING FOR ANTIALGAL ACTIVITY

Of the eight straw types tested, four showed some antialgal activity when decomposed under anaerobic conditions (Table 2). No activity was observed from any straw type under sterile or aerobic decomposition conditions and no activity was produced by barley straw at any stage. Liquor from anaerobically decomposed ryegrass hay displayed the strongest activity and was chosen for further study.

ATTEMPTED ISOLATION OF ANTIALGAL COMPOUND(S)

Preliminary investigations demonstrated that antialgal activity from ryegrass liquor was not due to the general acidity of the anaerobic decomposition products but was the result of a discrete chemical(s) with antialgal properties (Norton, 1995). Unfortunately attempts to isolate the antialgal component(s) were unsuccessful. Active Fractions I-V (Fig. 1) were developed on silica gel thin layer chromatograms and bioassays of these showed an inhibition zone coinciding with a chromatogram region containing two unresolved spots. These compounds were visible after spraying with $FeCl_3$, as a

Table 2. Inhibition of algal growth by liquor from crop plants after 60 d decomposition under anaerobic conditions (*** = >20 mm zone; ** = 10-20 mm; * = <10 mm).

	Inhibition by liquor from decomposing crop plants			
Test alga	Lucerne	Ryegrass hay	Oat chaff	Silage
Chlorella vulgaris	*			
Sticchococcus sp.	*	**	**	
Bracteacoccus minor	*	***	***	*
Pseudococcomyxa simplex	*			
Golenkiniopsis sp.	*	***	**	**

yellow spot partially merged with a red band. All attempts to resolve these spots failed and consequent efforts to characterise the active compound(s) focussed on the impure Fraction II.

CHARACTERISATION OF THE ACTIVE COMPOUND(S)

Chemical spray reagents were used to define the type of compound(s) present in the active zone of developed TLC plates. The presence of acid group(s) was confirmed by positive colour reactions with pH indicators and with test reagents for acid groups ($FeCl_3$ and $Fe(CN)_3$). Resistance to heat denaturation eliminated the possibility of a protein and this was supported by a lack of reaction with isatin and Ehrlich reagent. The compound(s) was unlikely to have associated sugar groups since aniline phthalate and H_2SO_4 produced no reaction. 2:4-Dinitrophenylhydrazine (DNPH) reagent is specific for carbonyl groups which are commonly associated with keto-acids but this reagent also produced a negative result. Early suspicions that a phenolic acid might be responsible for the observed activity were rejected when diazotised *p*-nitraniline and Pauly reagent showed no reaction. A crude estimate of molecular weight (MW) of the active component(s) was calculated from elution characteristics of a Sephadex G-15 column (K_{av} x MW (theoretical max.); 0.46 x 1500 = 690). The estimate is therefore MW = 650-700, although this could be an underestimate due to the possibility of the active compound binding to the column matrix since it is recognised that Sephadex may be involved in ionic and aromatic interactions.

SCREENING A DIVERSE RANGE OF ALGAE

Algae varied in their susceptibility to Fraction II (Table 1). The two diatoms and five cyanobacteria tested were all strongly affected by the active liquor. Two of the three xanthophytes and six of the 14 chlorophytes (including *H. reticulatum*) were affected, while the single rhodophyte and eustigmatophyte were unaffected.

ACTIVE CONCENTRATION, SPEED OF ACTION AND STABILITY OF THE ACTIVE COMPOUND

The test alga *Golenkiniopsis* sp. was completely inhibited by Fraction II at 0.25 g L^{-1} and greater concentrations (Fig. 2). The cells never recovered. At 0.1 g L^{-1} growth was inhibited at first, but after 10 d growth began. Fraction II at 0.05 g L^{-1} or less did not inhibit the alga and growth rates at these concentrations were slightly higher than controls.

Fraction II exerted its maximum antialgal effect within the first 3 h of exposure and no cells were viable after 18 h exposure to 0.25 g L^{-1} (Fig. 3). The antialgal activity of Fraction II was stable to heat and autoclaving at 1.1 kg cm^{-2} but was not stable under pH adjustment (Table 3). Crude liquor and Fractions I and II displayed increasing loss of activity as pH was increased and this was accompanied by a colour change in Fraction II from clear (pH 3) to pink (pH 7).

DISCUSSION

Although four of the straw materials tested produced antialgal liquor, NZ barley straw did not cause inhibition of algae in bioassays used in this study. In the UK it was found that barley straw consistently

Table 3. The effect of pH (adjusted with 0.1 M $NaHCO_3$) on antialgal activity of crude liquor and Fractions I and II. Data are mean inhibition zone diameters (± standard errors) of three replicates. Dashes indicate no inhibition zone. Note: pH values refer to the test solutions and not the pH of the medium. Test solutions were then added (20 µl) to glass fibre discs and placed on 10 ml solidified BBM.

Liquor pH	Antialgal activity (mm)			
	HCl ($1x10^{-2}$ M)	Crude liquor	Fraction I	Fraction II
3.0	-	19.2 ± 0.7	18.3 ± 0.3	16.8 ± 0.2
4.0	-	15.3 ± 0.3	16.0 ± 0.6	13.7 ± 0.3
5.0	-	10.7 ± 0.3	14.2 ± 0.2	8.8 ± 0.6
6.0	-	9.0 ± 0.3	8.3 ± 0.3	-
7.0	-	-	6.2 ± 0.2	-

produced antialgal factor(s) when decomposition conditions were kept aerobic (Gibson *et al.* 1990). These authors used bioassays based on algae in liquid culture and they tested unrefined, unconcentrated straw liquor. In contrast, the present study employed bioassays on solidified agar and tested straw liquor which had been concentrated by freeze-drying. Unconcentrated barley straw liquor has been tested using the solidified agar assay and this was also inactive (Norton 1995). It is possible that the solidified agar assay resulted in loss of activity or did not detect antialgal activity present in barley straw liquor.

It is also possible that contrasting results from NZ and the UK may be explained by different straw varieties or decomposition conditions. Pillinger *et al.* (1992) concluded that the antialgal effects of barley straw are unlikely to be explained by antialgal properties of specific fungi, but it is possible that a microbial community is required which is not present on NZ straw. The antialgal activity from ryegrass was produced only under anaerobic conditions and aeration following anaerobic decomposition resulted in a gradual loss of activity (Norton 1995). Clearly this activity was produced by an entirely different set of processes to those for barley straw, and the antialgal factor(s) is unlikely to be the same as that responsible for algal control by barley straw in UK studies.

It has long been recognised that phytotoxic substances are present in most crop plants (Guenzi *et al.* 1967) and allelopathic effects have often been attributed to phenolic compounds. The phytotoxic effect of barley, oat and wheat-straw mulches in agriculture and forest plantations (Jobidon *et al.* 1989) has been attributed to five common phenolic acids (Guenzi & McCalla 1966). Phenolic compounds were also implicated in the allelopathic effect observed with grasses of old-field succession on certain nitrogen-fixing cyanobacteria (Parks & Rice 1969). More recently, a range of phenolic compounds have been shown to inhibit algae directly; these included various tannins (Hussein Ayoub 1988), a range of phenylpropanoids (Della Greca *et al.* 1989, 1992, Aliotta *et al.* 1992) and a number of common plant phenolic acids (Larson 1989). Pillinger *et al.* (1994) suggested that oxidised lignin fractions are the primary source of algal inhibitors from barley straw decomposed aerobically in water. Anaerobic degradation of straws and grasses is known to release phytotoxic acetic acid (Harper & Lynch 1982), lignin derived phenolics (Kivaisi *et al.* 1990) and simple phenolic acids.

Attempts to isolate and identify the active component(s) from ryegrass liquor were unsuccessful but results from chemical spray reactions suggest that the compound(s) was not a phenolic. It is proposed

that it is a non-phenolic organic acid of molecular weight approximately 650-700, is non-glycosidic and is neither a peptide nor an amino acid. It could be a free fatty acid although this was not investigated further. Free fatty acids have been reported to have strong antialgal properties (McCracken *et al.*, 1980).

Despite the lack of a purified extract, it was possible to obtain useful information on the inhibition characteristics of the active compound(s). Results from screening bioassays showed a limited spectrum of activity and it is possible that the inhibitor(s) targets an area of metabolism which is specific to the affected algae. However, organisms as taxonomically separate as the Cyanobacteria and the Bacillariophyta were strongly affected, while algae within the same division (Chlorophyta) were affected to differing degrees and it is therefore more likely that algae vary in their susceptibility to a more general mode of action, and thus the inhibitor concentration is an important factor.

Fraction II was algicidal to the test alga *Golenkiniopsis* sp. at a concentration of approximately 0.25 g L^{-1} (Figs. 2 and 3). Cells within zones of inhibition on plates of the AA disc bioassay never recovered to produce colonies and therefore activity in these zones may also be presumed algicidal. This is supported by microscope observations of chlorotic and ruptured cells taken from clear zones on the plate bioassay. By contrast Fraction II at 0.1 g L^{-1} in liquid culture inhibited growth initially but after 10 d some cells were still viable and began to grow (Fig. 2). It appears that at some critical concentration Fraction II is not lethal to all cells and instead has a growth inhibitive action. The possible disappearance of the active ingredient from Fraction II over time was not investigated.

An active concentration of approximately 0.1 g L^{-1} is relatively high and although this estimate is based on the measured weight of an impure fraction and is possibly too large, the true active concentration is likely to be too high for ease of use in algal control. By contrast the current recommended dose rate of barley straw for practical algal control is 0.005 g L^{-1}, which means the inhibitor must be active at exceedingly low concentration (Pillinger *et al.*, 1994). Nevertheless an inhibitor which is active only at high concentrations may be useful if it exerts its effect rapidly, before losses are incurred due to dilution, water movement and possibly degradation. The active component(s) of Fraction II (0.25 g L^{-1}) exerted its full effect within 6 h but significant activity took place in the first 2 h exposure.

The apparent inability of the active compound(s) to function at near neutral pH is a serious limitation in terms of its potential for use in algal growth control since there are few situations in which waters could be acidified to the levels that were reached (pH 4) in inhibition experiments with ryegrass liquor. Exceptions could include cooling towers or other industrial waters where the water is not discharged immediately into the environment. However in such cases, a variety of simple chemical control options exist and there is little need for control using a natural product. If the active compound from ryegrass liquor was to be used for algal control in open waterways, it would need to be stable in an active state at neutral pH. It might be possible to create derivatives which would be stable at higher pH, but this would require detailed study of structure/activity relationships using the purified active compound(s). It would be worthwhile to investigate other natural algicidal products, such as those in other active straw materials (Table 2) in an effort to find one with more favourable properties. In the UK, such properties are displayed by the barley straw algal control method and the reason for repeated failure of this method in New Zealand is still of great interest.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support of J. Healy of the Department of Plant and Microbial Sciences, University of Canterbury, Christchurch. Financial support was received (by EN) from the Agricultural and Marketing Research and Development Trust (AGMARDT), NZ and the National

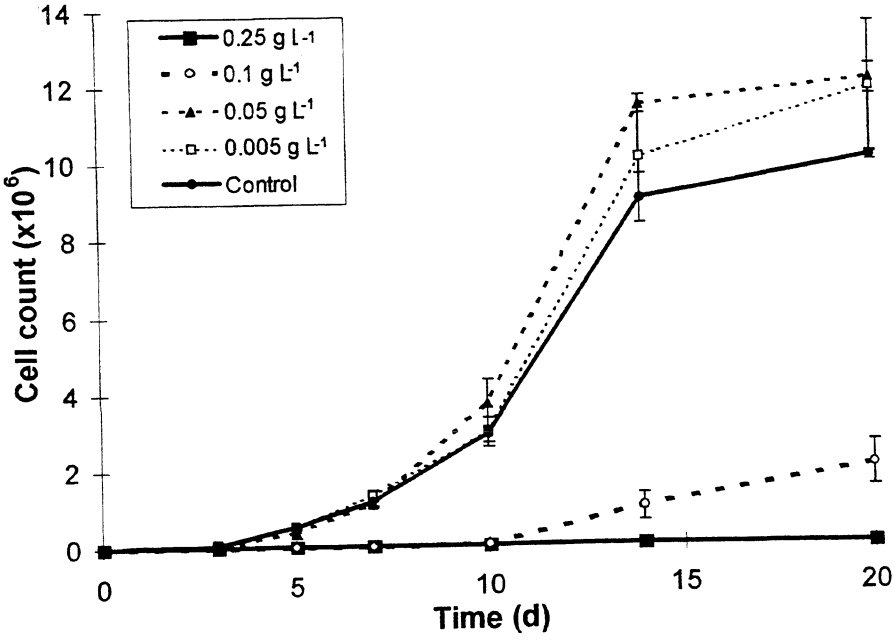

Figure. 2. The effect of Fraction II at a range of concentrations on the growth of *Golenkiniopsis* sp. The control shows normal growth with no addition of inhibitory fractions. Data points are means of three replicates and error bars indicate standard errors of means.

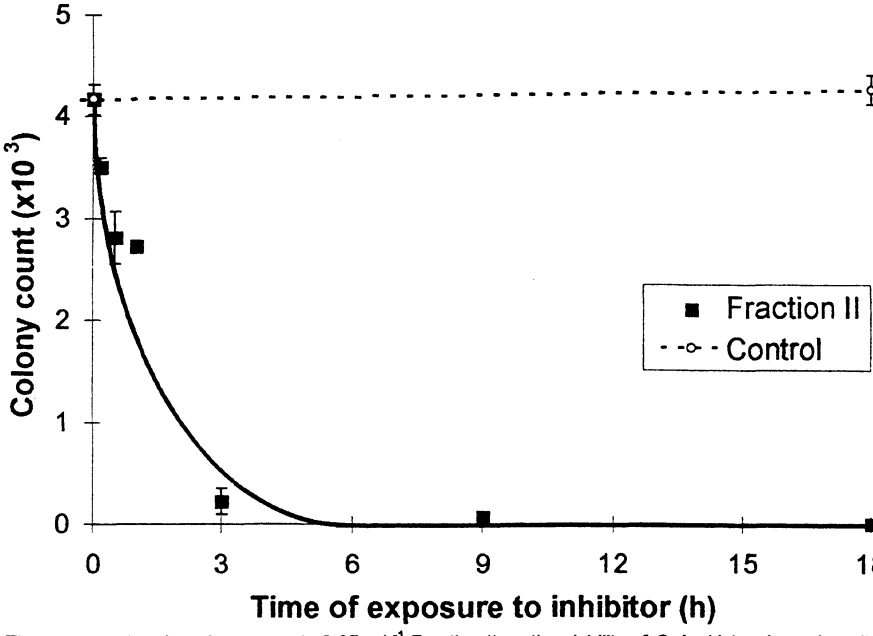

Figure. 3. The effect of duration of exposure to 0.25 g L^{-1} Fraction II on the viability of *Golenkiniopsis* sp. in culture. Viability was measured as the number of colonies formed by cells filtered from the test culture after different periods of exposure to inhibitor.

Institute of Water and Atmospheric Research (NIWA), Hamilton, NZ.

REFERENCES

Aliotta, G., Molinaro, A., Monaco, P., Pinto, G. & Previtera, L. (1992). Three biologically active phenylpropanoid glucosides from *Myriophyllum verticillatum*. *Phytochemistry* 31: 109-111.

Champ, W.S.T. & Caffrey, J.M. (1994). Preliminary results from barley straw trials in Irish watercourses. European Weeds Research Society 9th International Symposium on Aquatic Weeds (September 1994).

Coffey, B. & Miller, S.T. (1988). *Hydrodictyon reticulatum* L. Lagerheim chlorophyta: a new genus record from New Zealand. *New Zealand Journal of Botany* 26: 217-220.

Della Greca, M., Monaco, P., Previtera, L., Aliotta, G., Pinto, G. & Pollio, A. (1989). Allelochemical activity of phenylpropanes from *Acorus Gramineus*. *Phytochemistry* 28: 2319-2321.

Della Greca, M., Monaco, P., Pollio A. & Previtera, L. (1992). Structure-activity relationships of phenylpropanoids as growth inhibitors of the green alga *Selenastrum capricornutum*. *Phytochemistry* 31: 4119-4123.

Gibson, M.T., Welch, I.M., Barrett, P.R.F. & Ridge, I. (1990). Barley straw as an inhibitor of algal growth II: laboratory studies. *Journal of Applied Phycology* 2: 241-248.

Guenzi, W.D. & McCalla, T.M. (1966). Phenolic acids in oats, wheat, sorghum and corn residues and their phytotoxicity. *Agronomy Journal* 58: 303-304.

Guenzi, W.D., McCalla, T.M. & Norstadt, F.A. (1967). Presence and persistence of phytotoxic substances in wheat, oat, corn and sorghum residues. *Agronomy Journal* 59: 163-165.

Harper, S.H.T. & Lynch, J.M. (1982). The role of water-soluble components in phytotoxicity from decomposing straw. *Plant and Soil* 65: 11-17.

Hawes, I. & Smith, R. (1992). Assessment of the potential for water net (*Hydrodictyon reticulatum*) to grow in New Zealand wetlands and of barley straw as a possible control option. National Institute of Water and Atmospheric Research Ltd, NZ. Taupo Research Laboratory. File Report No. 140. P.O. Box 11-115 Hamilton, New Zealand.

Hussein Ayoub, S.M. (1988). Molluscicidal and algicidal properties of tannins. *Planta Medica* 54(6): 575-576.

Irvine, D.E.G. & John, D.M. (eds.) (1984). *Systematics of green algae*. Academic Press, London.

Jobidon, R., Thibault, J.R. & Fortin, J.A. (1989). Phytotoxic effect of barley, oat and wheat-straw mulches in Eastern Quebec Forest Plantations 2. Effects on nitrification and Black Spruce (*Picea mariana*) seedling growth. *Forest Ecology Management* 29: 295-310.

Kivaisi, A.K., Op den Camp, H.J.M., Lubberding, H.J., Boon, J.J. & Vogels, G.D. (1990). Generation of soluble lignin-derived compounds during degradation of barley straw in an artificial rumen reactor. *Applied Microbiology Biotechnology* 33: 93-98.

Larson, L.J. (1989). Effect of phenolic acids on growth of *Chlorella pyrenoidosa*. *Hydrobiologia* 183: 217-222.

Lee, R.E. (1989). *Phycology* (2nd edition). Cambridge University Press. Cambridge.

Mattox, K.R. & Stewart, K.D. (1984). Classification of the Green Algae: A Concept Based on Comparative Cytology. In: Irvine & John (eds.) *Systematics of Green Algae*. Academic Press. London.

McCracken, M.D., Middaugh, R.E. & Middaugh, R.S. (1980). A chemical characterisation of an algal inhibitor obtained from *Chlamydomonas*. *Hydrobiologia* 70: 271-276.

Newman, J.R. & Barrett, P.R.F. (1993). Control of *Microcystis aeruginosa* by decomposing barley straw. *Journal of Aquatic Plant Management*. (January special edition) 31: 203-206.

Nichols, H.W. & Bold, H.C. (1965). *Trichosarcina polymorpha* gen. et sp. nov.

Journal of Phycology 1:34-38.

Norton, E.J. (1995). *Algal growth inhibitors from decomposing plant materials*. A Masters thesis of the Department of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand.

Parks, J.M. & Rice, E.L. (1969). Effects of certain plants of old-field succession on the growth of blue-green algae. *Bulletin of the Torrey Botanical Club* 96: 345-360.

Pillinger, J.M., Cooper, J.A., Ridge, I. & Barrett, P.R.F. (1992). Barley straw as an inhibitor of algal growth III: the role of fungal decomposition. *Journal of Applied Phycology* 4: 353-355.

Pillinger, J.M., Cooper, J.A. & Ridge, I. (1994). The role of phenolic compounds in the antialgal activtity of barley straw. *Journal of Chemical Ecology* 20 (7).

Ridge, I. & Barrett, P.R.F. (1992). Algal control with barley straw. *Aspects of Applied Biology* 29: 457-462.

Ridge, I. (1994). Beating blanket weed. *The Garden* No. 597 (December)(letter).

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stanier, R.V. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111: 1-61.

Smith, I. (ed.) (1969). *Chromatographic and Electrophoretic Techniques*. William Heinemann Medical Books Ltd, London.

Welch, I.M., Barrett, P.R.F., Gibson M.T. & Ridge, I. (1990). Barley straw as an inhibitor of algal growth I: studies in the Chesterfield canal. *Journal of Applied Phycology* 2: 231-239.

Wells, R.D.S., Hall, J. & Hawes, I. (1994). Evaluation of barley straw as an inhibitor of waternet (*Hydrodictyon reticulatum*) growth. *Proceedings Plant Protection Society Conference, Waitangi, 1994*. 368-373.