Bull. Environ. Contam. Toxicol. (1995) 55:525–532

Environmental Contamination and Toxicology

Effects of Tributyltin and Formaldehyde on the Germination and Growth of *Phyllospora comosa* (Labillardiere) C. Agardh (Phaeophyta: Fucales)

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Received: 30 June 1994/Accepted: 14 February 1995

Toxicity testing on marine organisms is used in a biomonitoring role and as a management tool in evaluating the potential environmental impact of pollutants. Many of the biological toxicity tests used to date focus on short term mortality responses on adult animals. Such tests have by some workers (e.g. Anderson et al. 1991) been considered inadequate as they fail to consider more sensitive reproductive life stages or sub-lethal end points. More recent toxicity tests which have been developed address sub-lethal effects such as abnormal development, germination, reproduction and growth (e.g., Anderson and Hunt 1988). Tests involving macroalgal species have employed end points such as spore germination, sexual fusion and growth (e.g., Maruyama et al. 1991; Anderson et al. 1991). Tests that have been developed on Australian species have been primarily acute responses on invertebrate animal species (e.g. Ahsanullah et al. 1981). There has been no work published which addresses the effects of toxicants on Australian marine macroalgal species.

Phyllospora comosa (Labillardiere) C. Agardh is a large brown (fucoid) macroalgae. It is endemic to southeastern Australia where it forms dense stands in the upper sub-littoral zone. The reproductive and developmental biology of the species is well documented; it is easy to collect and is fertile all year (Burridge and Hallam 1993; Burridge et al. 1993). Burridge et al. (1995) investigated the acute toxic responses of 1-d-old zygotes and 7-d-old embryos of *P. comosa* to Tributyltin, 2,4 dichlorophenoxyacetic acid, 2,4 dichlorophenol and formaldehyde. The objectives of this study are to investigate the effects of Tributyltin and formaldehyde on germination and growth of *P. comosa* zygotes. Members of the order fucales have a very widespread distribution and share similar reproductive strategies. Methodologies developed in this study have the potential therefore for application on a much broader scale.

MATERIALS AND METHODS.

Healthy, mature specimens of *Phyllospora comosa* were collected at low tide from ocean beaches at Sorrento, Victoria. Plants were sexed according to Burridge (1990), healthy male and female receptacles were placed separately

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into 15-mL sea-water filled polythene vials and maintained on ice while being transported to the laboratory.

Gamete release and fertilisation was achieved following Burridge et al. (1993) and cultures were maintained according to Burridge et al. (1995). Stock toxicant solutions were prepared using fresh millipore filtered sea water (temperature: 15 $\pm$ 1 °C; salinity: 34 $\pm$ 2 ‰; pH: 8 $\pm$ 1; DO: 7 $\pm$ 1 mg/L) and serially diluted over the test concentration ranges. All glassware was washed according to Burridge et al. (1995). Whatman No.1 filter paper was used as a substratum to allow for zygote attachment.

Twelve hr after gamete mixing twenty five zygotes (Figure 1) were pipetted into 10-mL beakers containing the treatment solutions. Four replicates of 25 zygotes each were established for each treatment, sample size having been determined from power analysis (power = 0.86) (Zar 1984). Forty eight hr after dosing with the toxicant solutions the rate of germination success was determined based on the presence or absence of a developing germination rhizoid (Figure 2). For growth tests, culture containers were agitated after removal of receptacles, zygotes were left for approximately 24 hr to settle, adhere and germinate. Aluminium foil was placed over the bottom and sides of the toxicant culture chambers to eliminate uneven illumination. Parafilm was used to cover the containers which were incubated as previously. After 3 d the sea-water was decanted and toxicant solutions were added directly to the culture dishes. Three replicates were established for each treatment, and test solutions were changed at 3-d intervals. Ten juvenile plants were destructively sampled and their total lengths measured at 7, 14, 21 and 28 d (Figures 3, 4, 5 & 6) after addition of toxicant. Inhibition of growth involved an amorphous expansion of basal tissue and absence of differentiation that would normally lead to holdfast development (Figure 7). Growth data were analysed by two-factor analyses of variance to examine the effect of toxicant concentration and time of exposure whilst percentage germination data were normalized using an arcsine transformation and analysed using a single-factor analysis of variance (Zar 1984). Pair-wise comparisons on germination and growth data between the control and individual treatment groups were performed using Dunnett's tests (Zar 1984). For scanning electron microscopy (SEM), samples were prepared according to Burridge and Hallam (1993) and examined using a Hitachi 570 Scanning Electron Microscope.

RESULTS AND DISCUSSION

Zygotes exposed to formaldehyde concentrations of 0.1 mg/L and 1.0 mg/L exhibited germination rates of 66% and 41% respectively, while a 10 mg/L toxicant solution produced a germination rate of only 8% (Figure 8). Tests conducted with TBT resulted in zero germination in all test concentrations (10^{-6} mg/L and higher) and all toxicant treatments above 10^{-6} mg/L died within 24 hr. At 10^{-6} mg/L zygotes which had failed to germinate often exhibited apolar development. A cell wall developed and the zygotes underwent transverse division but failed to produce the apico-basal axis of development associated with

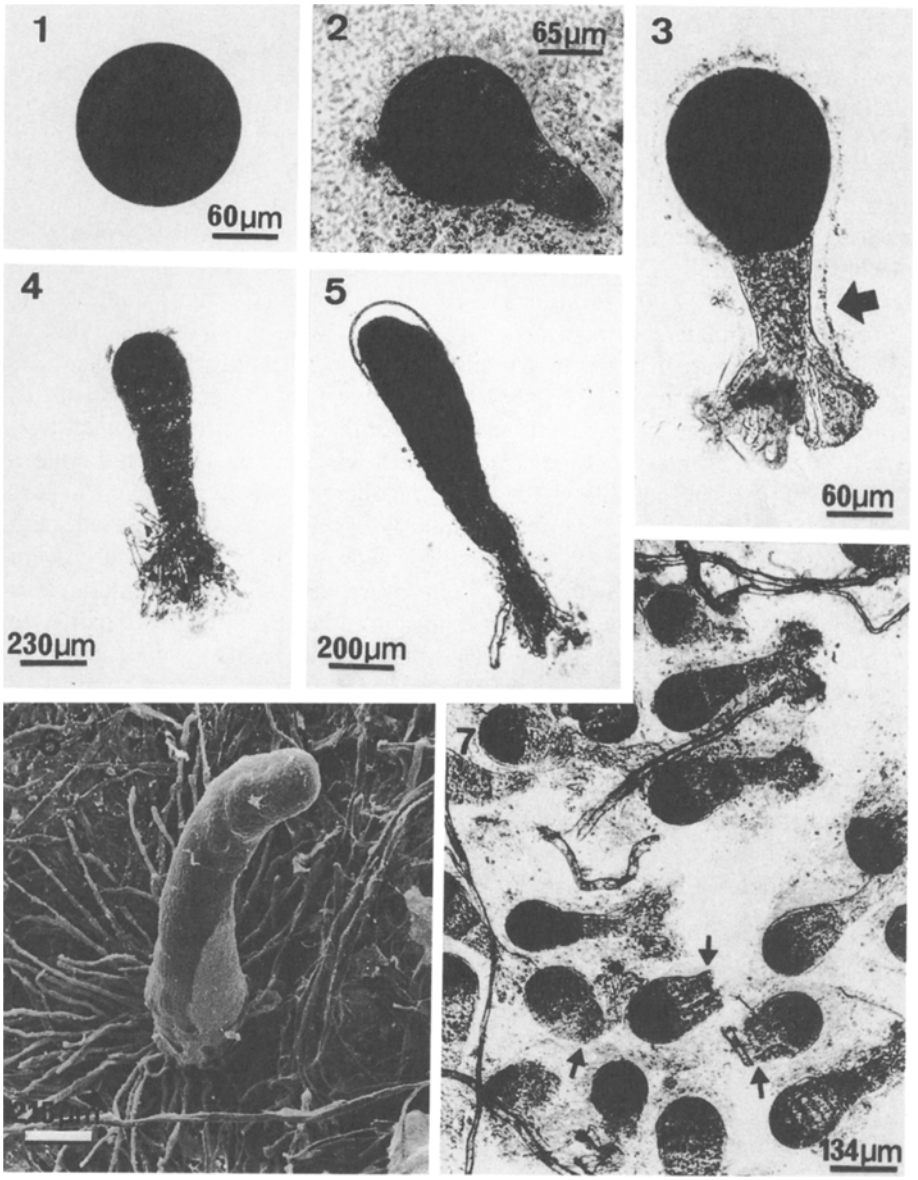

Figure 1. A 12-hr old zygote. Figure 2. A 36-hr old embryo showing development of the attachment rhizoid. Figure 3. A 7-d old germling. Note the differentiation into holdfast (arrow) and thallus. Figure 4. A 2-wk old germling. Figure 5. A 3-wk old germling. Figure 6. A scanning electron micrograph of a 4-wk old germling. Figure 7. 2-wk old germlings grown in 1 mg/L formaldehyde showing abnormal growth of the germling holdfast (arrows).

normal development.

Growth tests conducted over a 4-wk period produced very rapid, 100% mortality of all germlings exposed to TBT concentrations of 1.0 mg/L and higher. Exposure to dilutions of 10^{-3} to 0.1 mg/L and 10^{-4} mg/L produced a slower growth rate at 1 wk (relative to controls) (Figure 9) and complete (100%) mortality within 2 wk. Germlings cultured at 10^{-5}, 10^{-6} mg/L and controls exhibited a similar growth rate and mean length increased from 145 μm to 903 μm, 940 μm and 938 μm, respectively, at 2 wk. Treatments in 10^{-5} mg/L died between 2 and 3 wk, while germlings in 10^{-6} mg/L exhibited a significant decline in growth rate (relative to controls) between wk 2 and 4. When exposed to formaldehyde (Figure 10) germlings cultured at concentrations higher than 100 mg/L all died in the first wk. Embryos in 0.1 mg/L, 1.0 mg/L and 10 mg/L, exhibited growth rates to 2 wk significantly lower than the controls (Dunnett test, $P < 0.001$), and 10 mg/L cultures died between wk 2 and 3. At 4 wk the mean length of controls, 0.1 mg/L and 1.0 mg/L treatments were 1250 μm, 1150 μm, and 1160 μm respectively. Two-factor analysis of variance for all growth tests indicated that both increased concentration and duration of exposure were significant in reducing growth rate. There were also significant interactions between exposure time and toxicant concentration. Significantly, pair-wise comparisons using Dunnett's tests revealed that both toxicants have a significant negative effect on germination and growth even at the lowest concentrations used (10^{-6} mg/L for TBT and 0.1 mg/L for formaldehyde).

The inhibition of germination at very low toxicant concentrations suggests that the germination process is very sensitive to chemical exposure. This is consistent with work conducted on juvenile and larval stages of a number of animal species (e.g., Beaumont and Budd 1984; Fent and Hunn 1993). Stebbings (1985) has also indicated that larval stages are generally more sensitive to toxicants than adults by at least two orders of magnitude. Significantly, Maruyama et al. (1991) reported that adhesion of conchospores of the red algae *Porphyra yezoensis* Ueda was inhibited by a number of organotin compounds at concentrations in the low μg/L range and at concentrations appreciably higher than those which inhibited germination. High sensitivity at low concentrations also supports the findings of Burridge et al. (1995) where acute mortality tests showed unicellular *P. comosa* zygotes to be more sensitive than 7-d-old embryos [cf. Table 1 in Burridge et al. (1995)]. Further tests on *P. comosa* using even lower toxicant concentrations would elucidate the comparative sensitivity of juvenile and adult plants.

Apolar development at very low concentrations of TBT (following adhesion and development of a cell wall) suggests that the immediate effect of these compounds may be to inhibit development of the normal apico-basal axis associated with germination (Burridge and Hallam 1993). Further, mortality of undifferentiated zygotes at 72 hr in 10^{-6} and 10^{-5} mg/L (Burridge et al. 1995) may result from inhibition of polarisation. Whilst low concentrations of TBT inhibited germination at 10^{-6}-10^{-5} mg/L, adhesion to the substratum was

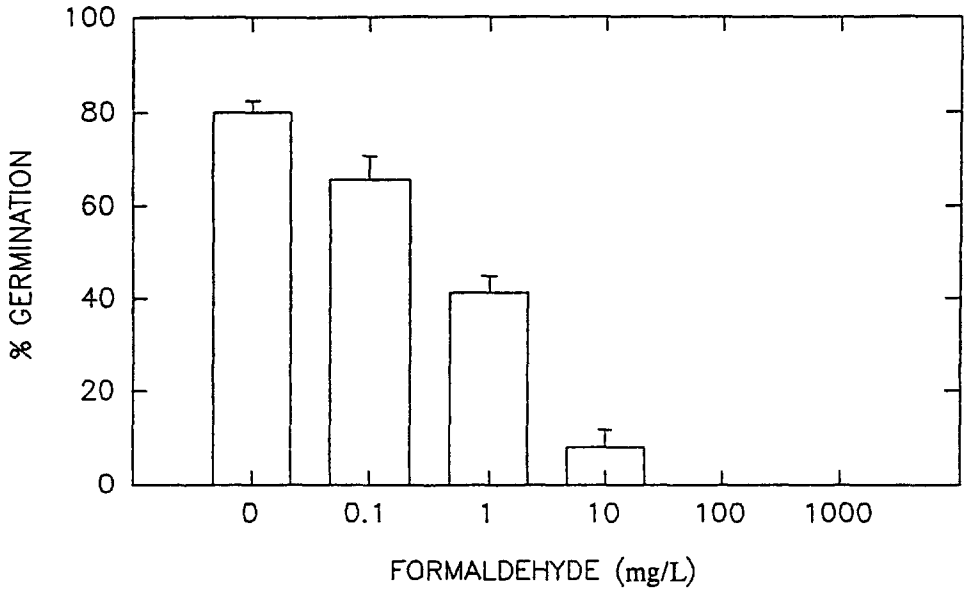

Figure 8. Germination of *P. comosa* zygotes in formaldehyde treatments.

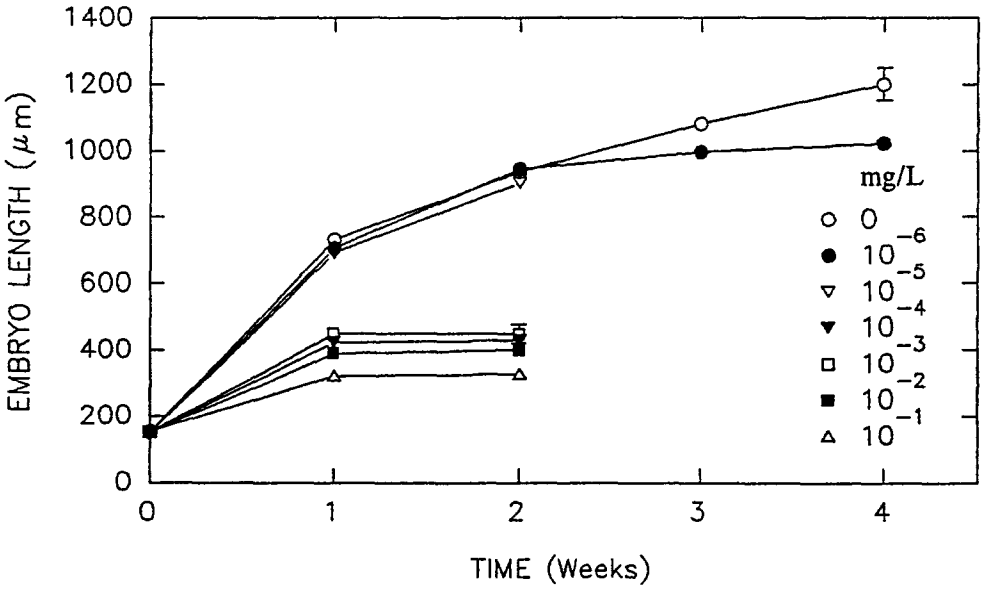

Figure 9. Growth of *P. comosa* germlings exposed to TBT at 3 days.

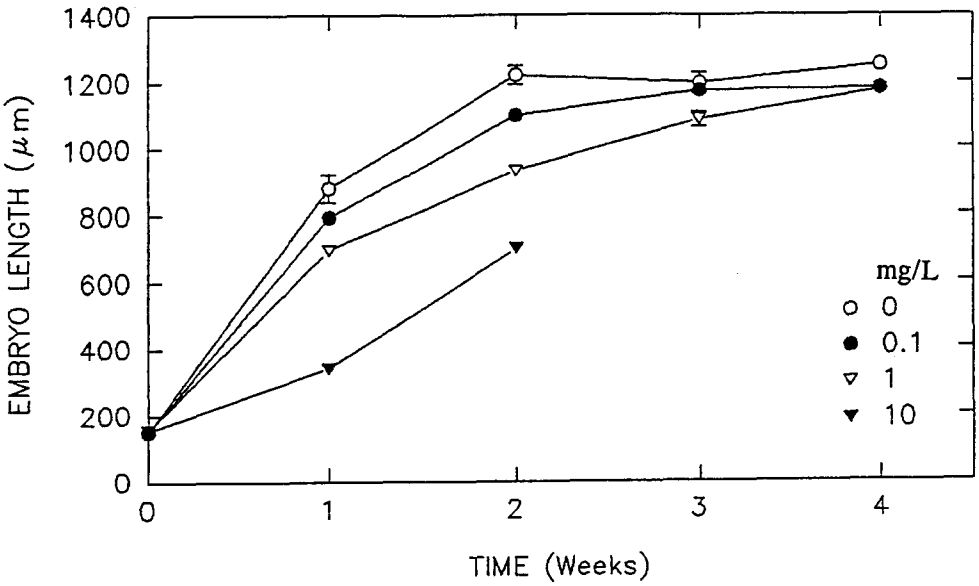

Figure 10. Growth of *P. comosa* germlings exposed to formaldehyde at 3 days.

unaffected, suggesting that very early development had not been impaired. Polar expression in *P. comosa* zygotes involves the accumulation of intracellular organelles and extracellular adhesive at the basal pole of the cell (Burridge and Hallam 1993). This also occurs during germination in most other fucoid species (e.g. Jaffe and Neuscheler 1969; Evans et al. 1982). TBT has been implicated as respiratory uncouplars through their ability to inhibit ATP production and metabolism (e.g. Fent and Hunn 1993; Kure and Depledge 1994). Polarisation and initial cell division of the zygotes would involve an increased respiratory demand (Burridge 1990) and uncoupling of oxidative phosphorylation may restrict development to substratum adhesion and the apolar mode of division observed. In the absence of polarisation and normal development, embryos may simply become non-viable at 72 to 96 hr. This is supported by the onset of necrosis in unfertilised eggs after 72 hr (Burridge et al. 1995). Inhibition of ATP production may also inhibit photosynthetic output, and inhibition of respiration and photosynthesis would almost certainly restrict growth rates of juvenile plants.

Zygotes are much less sensitive to formaldehyde which has been shown by other workers to have 96 hr LC_{50} values in the 10.0 to 1000.0 mg/L range and toxicity thresholds in the low mg/L concentration range (Verschueren 1983). Results here demonstrate that both germination and growth are affected by concentrations substantially lower than 10 mg/L and are in accordance with results of Burridge et al. (1995) where a greater sensitivity was also indicated for acute tests on *P. comosa* juveniles.

The results of this study indicate that germination and growth of fucoid zygotes may provide effective toxicity screening tests for marine pollutants. The results are also significant from a general ecological perspective as discharge criteria based solely on acute mortality tests may potentially result in substantial community impact through inhibition of germination and reduced population recruitment.

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