Didymo Chemical Testing – Oct 6-8, 2008

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Introduction

Cleaning of gear and equipment between rivers is now standard practice in New Zealand to prevent the spread of didymo and other aquatic pests. The currently recommended common disinfection products are household bleach and detergents, which have the disadvantages of, respectively, being corrosive to some materials, and requiring rinsing because they foam. Simon Gartenstein from Hydro Tasmania Consulting, provided NIWA with Phytoclean, a product routinely used for the control of *Phytophthora* for testing on *Didymosphenia geminata*, to establish its effectiveness in case of a *D. geminata* incursion into Tasmania, Australia.

Methods

Saturday 4 October 2008.

The Waitaki River, (2.5 hours south of Christchurch in the South Island of New Zealand), is a large river that is used for power generation. *D. geminata* is well established below the Waitaki Dam, down to the coast. The substrate consists of large boulders down to medium sized stones and cobbles. Rock samples were taken and placed in jars with river water and transported on ice back to Christchurch. Jars were placed in a 10ºC incubator with a 16 hr light, and 8 hr dark cycle. The rocks were left in the incubator undisturbed until the following Monday when testing took place. In the past we have noticed that there have been problems with the Neutral Red dye not being taken up by the cells when the samples have been chilled, and transported to the incubator. Testing is best done within 36-48 hours of removal of rocks from the river, as after this period the colonies begin to die off. Neutral Red is taken up by live cells, but not by dead cells. For more information about the Neutral Red technique, refer to Kilroy et al. (2007).

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New batches of dye were made up in filtered river water, and a set of control samples was checked. The cells stained well, with results ranging from 72 – 90% live cells when examined on a Leica compound microscope.

The concentration of the test product for the initial round of testing was based on levels recommended by the manufacturer and Hydro Tasmania Consulting for wash down of surfaces and equipment. The product was diluted using filtered river water.

For each test concentration, three replicates (small clumps of didymo about 1 cubic centimetre in volume) were randomly taken from different rocks and were chopped up roughly to ensure that the product and dye would penetrate to all cells. The clumps were then soaked in the diluted test product for 1 minute. They were then blotted, rinsed in filtered river water, blotted again to remove excess water, and placed in the dye solution for 15 mins. After this time slides were made up of the didymo by teasing small subsamples of the stained clumps apart and then random fields of view were checked for stained (live), unstained (dead), and empty cells. Empty cells are counted separately because some treatments result in increased numbers of cells without chloroplasts (e.g., when cells split). A minimum of 100 cells was counted on

each slide. The one minute exposure time was selected based on work carried out previously at NIWA in New Zealand.

Results

Table 1: Results of tests on Phytoclean using the initially recommended concentrations

Date	Treatment	Concentration	Exposure time	Replicate	Stained	Unstained	Empty	%live
6-Oct-08	control 1		na	1	106	12	3	90
	control 2		na	2	100	38	11	72
	control 3		na	3	107	22	5	83
	20ml/L	2%	1 min	1	0	100	2	0
	20ml/L	2%	1 min	2	0	104	7	0
	20ml/L	2%	1 min	3	0	100		0
	20ml/L	2%	1 min	1	0	101	7	0
	20ml/L	2%	1 min	2	0	106		0
	20ml/L	2%	1 min	3	0	100	5	0
	control 1		1 min	1	101	3	4	97
	control 2		1 min	2	100	23		81
	control 3		1 min	3	112	38	14	75
	15ml/L	1.50%	1 min	1	0	100	5	0
	15ml/L	1.50%	1 min	2	0	101		0
	15ml/L	1.50%	1 min	3	0	100	2	0
	10ml/L	1%	1 min	1	0	101		0
	10ml/L	1%	1 min	2	0	100		0
	10ml/L	1%	1 min	3	0	100		0
	2ml/L	0.20%	1 min	1	0	100	3	0
	2ml/L	0.20%	1 min	2	0	99	3	0
	2ml/L	0.20%	1 min	3	0	110	2	0

Phytoclean was effective in killing didymo cells at all concentrations tested (Table 1). There was an almost immediate colour change in the didymo samples when they were added to the diluted test product, which indicated a lethal effect. As the product was lethal at the 2% concentration, it was considered to be unnecessary to test any of the higher concentrations initially requested.

Reference

Kilroy, C.; Lagerstedt, A.; Davey, A.; Robinson, K. 2007. Studies on the survivability of the invasive diatom *Didymosphenia geminata* under a range of environmental and chemical conditions. NIWA Client Report CHC2006-116. For Biosecurity New Zealand. 110 p. (available from: http://www.biosecurity.govt.nz/files/pests/didymo/didymo-survival-dec-06-rev-may-07.pdf)