

# Testing "Check, Clean, Dry" decontamination procedures

Further tests on didymo and *Lindavia*

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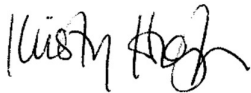


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## Executive summary

Check, Clean, Dry (CCD) procedures currently recommended by the Ministry for Primary Industries (MPI) to reduce the spread of nuisance aquatic organisms include treatment with dishwashing detergent (1-minute contact in a 5% solution). The recommendation was based on cell viability tests (carried out in 2006-07) on the nuisance alga (diatom) *Didymosphenia geminata* (didymo) using common brands of standard dishwashing detergent (Palmolive, Sunlight, and Down-to-Earth).

In 2017, to support a broader scope for CCD, MPI commissioned further testing on other freshwater organisms including the freshwater diatom *Lindavia intermedia* (*Lindavia*). *Lindavia* forms slime accumulations in lakes, which block water intakes and interfere with recreation. The 2017 tests showed that *Lindavia* was more resistant than didymo to decontamination using Sunlight dishwashing liquid.

MPI are in the process of considering further guidance to provide to the public on which detergent products are effective on algae because product selection may be based on their “environmentally-friendly” claims. The 2006-07 tests included selected environmentally friendly products. The results indicated that these detergents were not as effective in killing didymo cells as detergents that make no environmental claims.

In 2020, at MPI’s request, NIWA carried out further tests on both didymo and *Lindavia* using four detergents currently in use by different agencies, which state a range environmental claims in their marketing information. The products were: **Eco-store** detergent (provided by MPI), **Earthwise** dishwashing liquid (Lemon and Aloe Vera, used by at least one regional council), **Jasco** Citriclean (used by the Department of Conservation), and Lemon detergent from **Arnold** Products Limited (used by some regional councils). Tests were carried out using an established cell staining method to determine effectiveness (defined as the mean the percentage of non-viable cells in multiple replicates following treatment) of each product.

For didymo, Arnold was 100% effective, Eco-store and Jasco were ~95% effective, and Earthwise was ~85% effective. No product was more than 92% effective on *Lindavia* (range 44% to 92%). The same product (Earthwise) was least effective on both organisms.

In 2021, MPI requested that NIWA carry out additional tests with the same products to establish the contact times and concentrations required to ensure 100% effectiveness. Further tests on *Lindavia* using Sunlight were included because Sunlight was not 100% effective in the 2017 tests.

We carried out the tests in April and May 2021, starting with a concentration of 10% with a 1-minute contact time. Follow-up tests with increased concentration or contact time or combination of the two were required for Earthwise (on didymo) and for all four products on *Lindavia*.

Outcomes of the tests were:

- use of a 10% solution with a 10-minute contact time was 100% effective on both didymo and *Lindavia*, using all products tested;
- for didymo only, extending the contact time of a 5% solution to at least 10 minutes was 100% effective for all products.

As a result of the testing, our recommendation for amended CCD advice (on [Check, Clean, Dry: preventing didymo and other pests | MPI | NZ Government](#)) related to using dishwashing detergent for cleaning items that have been in contact with river or lake water to prevent the spread of nuisance aquatic organisms is to replace the current advice:

Dishwashing detergent or nappy cleaner	5% solution (500 mls diluted to 10 litres in water)	Soak or spray all surfaces for at least 1 minute
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with something similar to:

Dishwashing detergent or nappy cleaner	5% solution (500 mls diluted to 10 litres in water)*	Soak or spray all surfaces and allow to stay wet for at least 10 minutes
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\* Solutions of 10% are advised for lakes where *Lindavia* slime may be present.

# 1 Introduction

The “Check, Clean, Dry” message<sup>1</sup> was introduced by Biosecurity New Zealand (now part of the Ministry for Primary Industries, MPI) in 2005, following the discovery of the bloom-forming, non-indigenous diatom *Didymosphenia geminata* (didymo) in a Southland river in 2004. “Check, Clean, Dry” promotes prevention (or at least slowing) of the spread of nuisance or unwanted organisms between water bodies by decontaminating all items that could act as vectors. The methods underpinning the message were initially developed to help prevent the spread of didymo. Development included testing the effectiveness of readily available household cleaning products (e.g., bleach, dishwashing detergent) for decontaminating clothing and equipment that was likely to harbour live didymo cells and potentially spread them to other rivers. Effective methods were summarised by Kilroy et al. (2007).

The scope of Check, Clean, Dry now includes all aquatic nuisance organisms, not just didymo, and evidence to support this broader message has required further tests. In 2017, MPI commissioned NIWA to test the effectiveness of the original decontamination procedures on other freshwater organisms. The work included tests on several species of aquatic plants and introduced invertebrates (Burton 2017), and on the freshwater diatom *Lindavia intermedia* (hereafter *Lindavia*) (Kilroy and Robinson 2017). The accumulations of sticky slime produced by *Lindavia* in low-nutrient lakes can block water intakes and filters and is particularly problematic in Lakes Wānaka and Wakatipu, Otago. The slime also interferes with recreational activities such as swimming and angling. The 2017 tests found that *Lindavia* was more resistant than didymo to decontamination with the recommended 5% detergent (dishwashing detergent) for 1 minute. It was suggested that resistance to decontamination was caused by slime around the *Lindavia* cells, forming a protective layer.

Use of dishwashing detergents for carrying out Check Clean Dry procedures is popular because these products are inexpensive, readily available, easy to use in the field, non-damaging on other materials and safe to handle. Previous dishwashing detergent tests on didymo used the widely available products Palmolive, Sunlight and Down-to-Earth (Kilroy et al. 2007), and tests on *Lindavia* used Sunlight (Kilroy and Robinson 2017). These brands make no environmental claims. Environmental concerns have encouraged stakeholders to select alternative products based on their “environmentally-friendly” claims. However, initial tests on didymo in 2006-07 using selected environmentally friendly products indicated that these detergents were not as effective in killing didymo cells as detergents that make no environmental claims (Kilroy et al. 2007). With wide uptake of the Check, Clean, Dry message, MPI wished to provide further guidance to the public on which detergent products are effective.

In early 2020, MPI commissioned NIWA to carry out tests on both didymo and *Lindavia* using four additional detergents currently in use by various organisations. The products were an Eco-store brand detergent (packaged into sachets and provided by MPI), Earthwise dishwashing liquid (Lemon and Aloe Vera, a common supermarket brand, used by at least one regional council), Jasco Citriclean (used by the Department of Conservation), and Lemon detergent dishwash liquid from Arnold Products Limited (used by some regional councils). Tests were carried out following recommended Check, Clean, Dry methods (i.e., exposure to a 5% v/v solution for 1 minute).

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<sup>1</sup> See [Check, Clean, Dry: preventing didymo and other pests | MPI | NZ Government](#)

The outcomes of the 2020 tests were:

- for didymo, Arnold Lemon was 100% effective<sup>2</sup>, Eco-store detergent and Jasco Citriclean were ~ 95% effective, but Earthwise detergent was less than 75% effective;
- for *Lindavia*, none of the four products were 100% effective, with Earthwise detergent the least effective.

For reference, the summary results of the 2020 tests are included in this report as Appendix A. Recommendations were made to either increase the concentration used (especially of Earthwise detergent) or to extend the contact time to greater than 1 minute.

In early 2021, MPI requested that NIWA re-test the detergents that were not 100% effective in the earlier tests using higher product concentrations and longer contact times. The aim of the tests was to identify methods that were 100% effective. Also included in the tests was Sunlight dishwashing liquid (for *Lindavia* only) because Kilroy and Robinson (2017) found that treatment with 5% Sunlight for 1 minute was not 100% effective on *Lindavia*.

In this report we describe the methods and results of the follow-up tests carried out in April and May 2021. Note that the methods section is duplicated (with some modifications) from Kilroy and Robinson (2020) so that the present report can be understood without reference to the earlier reports.

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<sup>2</sup> Here percentage effective refers to the mean percentage of live cells observed in samples following treatment.



## 2 Methods

### 2.1 Products tested

The following brands of dishwashing detergent were tested. Abbreviations used in the text and tables are shown in square brackets.

- Eco-store brand detergent (sachets provided by MPI) [Eco-store (MPI sachet), Eco-store].
- Earthwise dishwashing liquid (Lemon and Aloe Vera) [Earthwise].
- Jasco Citriclean (provided by the Department of Conservation [Jasco Citriclean, Jasco]).
- Lemon detergent dishwash liquid from Arnold Products Limited [Arnold lemon detergent, Arnold].
- Sunlight dishwashing liquid [Sunlight].

Note that the marketing information on Jasco Citriclean and Arnold lemon detergent does not appear to make any specific claims that these products are “environmentally friendly”.

Part way through the trials, a new supply of the Jasco product was sent to us by Department of Conservation because they noted that re-stocked detergent from their supplier was a different colour from the original. The supplier assured them that the new product was exactly the same as the original (other than the colour). Nevertheless, extra tests on the new product (Jasco2) were run as a precaution.

### 2.2 Testing schedule and product preparation

MPI requested that we first test all the products using an increased concentration (10% rather than 5% solutions) but with the same contact time of 1 minute. In cases where the initial round of testing (hereafter Round 1) indicated <100% effective, subsequent rounds of tests were discussed with MPI and were determined to be:

- A. Round 2: testing of 5% (for didymo) and 10% solutions (for *Lindavia*) with a contact time of 10 minutes; and, if these tests were 100% effective,
- B. Round 3: re-testing using 5% solutions with a contact time of 5 minutes (for didymo) and 10 minutes (for *Lindavia*).

We tested:

- didymo viability following treatment with Eco-store, Earthwise, and Jasco;
- *Lindavia* viability following treatment with Eco-store, Earthwise, Jasco, Arnold, and Sunlight.

Ten percent solutions of all products were prepared by making up 20 ml of the product to 200 ml using distilled-grade water and 5% solutions by making up 10 ml of the product to 200 ml using distilled-grade water.

## 2.3 Laboratory facilities and permissions

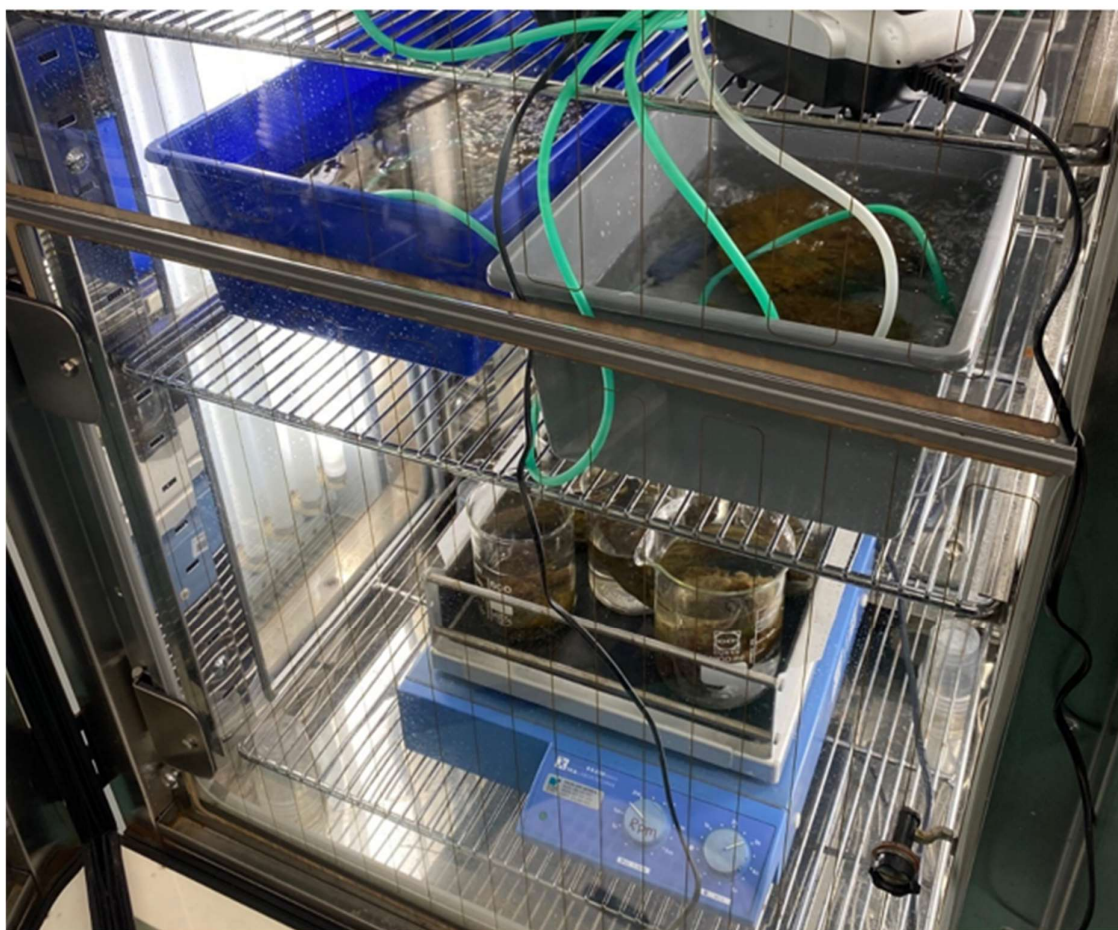
All the tests described below were carried out in the MPI-approved PC2 containment facility in the NIWA, Christchurch, laboratory (MPI-approved TF12982). Didymo is classed as an “unwanted organism” and NIWA holds permission from MPI to carry out experiments using live didymo cells (dated 6 May 2010) in the PC2 laboratory, with microscope scans carried out in an adjacent PC1-rated equipment room, following specified protocols. *Lindavia* is not classified as an unwanted organism, but the tests were carried out using the same protocols and facilities as used for didymo.

## 2.4 Sourcing and maintaining live material for testing

### 2.4.1 Didymo

Samples of didymo were collected on 12 and 28 April and 18 May 2021 from the Opuha River, Canterbury and on 13 April 2021 from the Selwyn River, Canterbury. We collected whole rocks, each with developing didymo colonies, and transported them directly to the PC2 laboratory. The samples collected on different dates were used in successive rounds of tests. See Table B-1 for details.

Each rock was transferred to a glass beaker or tray and covered with river water. The beakers were placed on a shaker table set at ~120 rpm to ensure water movement around the didymo colonies, to simulate a river environment. Air was bubbled through the water in trays holding larger rocks. Both the shaker table and trays were held in a controlled temperature cabinet at 5 – 8 °C and in a 12:12 light: dark cycle (Figure 2-1).



**Figure 2-1: Samples of didymo held in the controlled-temperature and light cabinet.** Samples in the trays have air bubbled through them; samples in beakers (lower shelf) are on a shaker table set at about 120 rpm.

Sub-samples of didymo were tested for viability within 12 h of collection. The tests were conducted on 14 April, 28 – 29 April and 19 May 2021.

#### 2.4.2 *Lindavia*

Samples of *Lindavia* (lake snow slime) were collected during routine monthly lake water quality monitoring runs by Otago Regional Council staff (contact: Tim Harrex). All samples were collected by towing a line underwater for 10 minutes, scraping the snagged material (slime) into a 1 litre container and topping up with lake water. Samples were couriered on ice to NIWA in Christchurch to arrive in the morning of the day following sampling.

Samples used were as follows:

22 April 2021	Lake Wakatipu
28 April 2021	Lake Dunstan at Clyde, Lake Dunstan at Cromwell
20 May 2021	Lake Hāwea
21 May 2021	Lake Wānaka, Lake Dunstan at Clyde, Lake Dunstan at Cromwell.

The *Lindavia* samples were transferred to open glass beakers which were maintained under the same conditions as didymo, except that the shaker table was not required (Figure 2-2). Sub-samples were tested for viability within 24 h of the sample arriving.



**Figure 2-2:** *Lindavia* sample collected from Lake Dunstan. Samples were transferred from screw-top containers to open beakers prior to sampling and testing.

## 2.5 General method for determining cell viability

### 2.5.1 Neutral Red assays

Cell viability after each treatment assay was determined using the Neutral Red (NR) staining technique developed for determining the viability of didymo cells and used successfully on *Lindavia* by Kilroy and Robinson (2017, 2020). Both didymo and *Lindavia* are diatoms, and we have already observed that NR staining is an effective technique for examining the viability of diatom taxa other than didymo (Kilroy et al. 2007).

Full details about NR staining are provided in Appendix 1 of Kilroy et al. (2007). In summary, NR stain is taken up by cells when they are alive, but not when they are dead. Live cells take up the stain in vacuoles in the cell. The acidic contents of the vacuoles in healthy cells cause NR to retain its red colour and also prevent the stain from leaching back into the cell. Viewed under a microscope at 200× or 400×, the vacuoles appear as deep crimson-purple spots or granules scattered or streaked

throughout the cell. When cells die their internal membranes lose integrity, NR cannot concentrate within vacuoles and NR generally appears straw-coloured.

Uptake of NR by cells does not always produce clear results because cells respond in various ways when they are compromised in some way. Therefore, some interpretation may be required when assessing the effectiveness of methods to kill cells. Observations on the shape, colour and arrangement of the chloroplasts are also used in the assessments. A treatment is judged to be 100% effective when *either* no stained cells are observed in complete microscope scans of at least three subsamples, *or* stained cells are seen, but the staining is abnormal or very faint and there are other signs of cell deterioration present, such as discoloration or shrinkage of chloroplasts.

For all tests we started with a stock solution of NR made up by dissolving 200 mg of the dye (a black metallic powder) in 200 mL of distilled-grade water (i.e., a 0.1% weight/volume (w/v) solution). This was further diluted with filtered river or distilled-grade water to a working solution of about 4% or 10% v/v for use with *didymo* and *Lindavia* respectively. For best results on *Lindavia* we found that it was necessary to add undiluted stock solution to each sample (see Section 2.6.2).

All microscope scans were carried out using a Leica DMLB compound microscope.

### 2.5.2 Method limitations with *Lindavia*

NR assays on *Lindavia* are more difficult than on *didymo* because (a) the cells are smaller and changes to chloroplast form are more difficult to identify; and (b) the amount of slime around the cells appears to prevent the stain penetrating to all cells especially in control samples. Therefore, we need to distinguish between cells that are unstained because they are non-viable and cells that are unstained because the stain did not reach them. This is especially critical in control samples. Following treatment with detergents, stain penetration appears to improve, which was evident from the presence of live (stained) cells within some treated samples. The problem caused by (b) was discussed in earlier reports (Kilroy and Robinson 2017, 2020), and method modifications to resolve the issue are described in Section 2.6.2 below.

## 2.6 Experimental procedure and cell counts

The tests were conducted at room temperature (18°C). All tests were carried out on a minimum of three subsamples of material. Control samples were tested at the start of the trials and after every 6-8 tests. In most cases, the products were tested in a random order unknown to the microscope operator to avoid any involuntary bias in the counts.

### 2.6.1 Procedure for didymo

The tests consisted of the following steps:

1. A small piece of mat (approx. 5 mm x 5 mm) was snipped from the top of a didymo colony. We selected pieces that appeared to be healthy and with plenty of cells (i.e., a pinkish to dark brown colour (not yellow or green), with individual cells just visible to the naked eye as dark specks). Each replicate was taken from a different colony or different rock.
2. The test piece was dropped into about 20 ml of the product solution in a 35 ml vial for exactly 1 minute.
3. The test piece was removed using forceps and rinsed by swirling the sample in two successive vials of distilled-grade water.
4. Rinsed test pieces were briefly blotted to remove excess water and then transferred to a vial of about 20 ml NR working solution. We allowed a staining time of 5 minutes.
5. Subsamples of stained test pieces were transferred to a glass microscope slide, teased apart so that as many cells as possible were in contact with the glass, and topped with a cover slip.
6. Microscope counts were conducted immediately using a Leica DMLB microscope. Slides were first scanned at 200 x. If stained cells were observed, counts were made on random fields of view at 400 x, working systematically across and down the cover slip so that no areas were viewed more than once. At least 100 cells were counted on each slide, and assessed as either stained or not stained, following Appendix 2 in Kilroy et al. (2007). We did not include empty cells or broken cells in the counts.
7. Control pieces were treated in the same way as treatment pieces except that the "treatment" was filtered river water.
8. Slides were numbered rather than labelled so that the microscope operator had no expectation of what the result should be.
9. Notes were made during the counts on the appearance of the cells, the appearance of the stain, any features of the stalks, and whether other algae were taking up or not taking up the stain.

### 2.6.2 Procedure for *Lindavia*

The treatment and staining method for *Lindavia* was similar to that for didymo with the following variations:

1. Each *Lindavia* sample comprised a very small amount of slime material (e.g., 1 mm x 2 mm), which typically contained thousands of cells.
2. Treatment and staining were carried out in 35 ml vials placed on a white tray so that the test pieces could be seen more clearly.
3. We modified the staining procedure to get the clearest results, because earlier tests showed that *Lindavia* cells take up stain more slowly than didymo cells. Initially we found that staining intensified if we allowed a 10 – 15 minutes delay between making the slides and doing the microscope counts. Later, we found that a better method was to use a more concentrated NR solution, and to use the NR stock solution directly rather than the NR working solution used in the didymo trials. The method used was to place 4 ml of lake water (filtered through a GFF filter to remove all suspended cells) into a clean vial, add the *Lindavia* sample, then add 5 drops of NR stock solution directly using a disposable pipette. Samples were gently swirled in the treatment and stain solutions to aid penetration of the solutions to as many cells as possible.
4. The microscope procedure was similar to that for didymo except that slides were first scanned at low power (40 x) to determine whether any portions of the sample had escaped staining. Stained parts of the sample typically had a pinkish background, whereas unstained parts (usually in the middle of dense areas of cells) were golden-brown. These unstained areas were relatively easy to see in controls, but less obvious in treatment samples.

## 3 Results

The results presented below are summarised in Table 3-1. Complete raw results, including comments on the appearance of many samples, are presented in Appendix B.

### 3.1 Didymo

#### 3.1.1 Round 1: 10% solutions with 1-minute contact time

Round 1 tests were carried out using material collected on 12 – 13 April and 28 April 2021. Control samples tested on 14 and 29 April 2021 returned an average of  $70 \pm 11$  % viable (stained) cells (mean  $\pm$  standard deviation), as indicated by NR staining and expected for healthy cells.

Treatment with both the Eco-store and Jasco products was 100% effective. No live (stained) didymo cells were observed in four replicate didymo samples. The Jasco2 product was also 100% effective on two additional samples tested on 29 April. However, Earthwise was not 100% effective (Table 3-1).

#### 3.1.2 Round 2: 5% solutions with 10-minute contact time

Treatment with Earthwise returned no viable cells from three replicate samples (Table 3-1).

#### 3.1.3 Round 3: 5% solutions with 5-minute (or less) contact time

Further tests were carried out in round 3 to check the effectiveness of a 5% treatment with an intermediate contact time. Two replicate samples treated with 5% Earthwise for 5 minutes returned ~2% viable cells in one of the replicates, suggesting that the longer contact time is necessary.

We also checked the effectiveness of the Jasco2 product at the originally recommended concentration (5%) with a 5-minute contact time. Three replicate samples returned no viable cells following this treatment. Finally, we re-tested the effectiveness of a 5% solution of Jasco2 with a 1-minute contact time as a check on the result in Kilroy and Robinson (2020). Consistent with the original results, viable cells were observed in both samples following treatment (1% and 3%) (Table 3-1 and see Table B-1 for details).

### 3.2 *Lindavia*

#### 3.2.1 Round 1: 10% solutions with 1-minute contact time

The Round 1 tests on *Lindavia* were carried out using a sample from Lake Wakatipu, collected on 22 April 2021 and two samples from Lake Dunstan (two sites) collected on 28 April 2021. The average percentage of stained cells in control samples was  $58 \pm 18$  %, with no consistent difference between lakes (five samples from each lake) (see Table B-2 for details).

Live (stained) cells were observed in the samples following treatment by all of the products. The average percentage of live (stained) cells varied from  $14 \pm 19$  % (Jasco) to  $32 \pm 25$  % (Sunlight). There was variability among replicates and in all cases at least one sample returned more than 40% live (stained) cells (Table 3-1, Table B-2).

#### 3.2.2 Round 2: 10% solutions with 10-minute contact time

Round 2 tests were carried out using a sample collected from Lake Hāwea on 19 May 2021 and samples collected from Lakes Wānaka and Dunstan (two sites) on 20 May 2021. The average percentage of stained cells in control samples was  $60 \pm 8$  % (Table 3-1, and Table B-2). The



treatments were 100% effective for all five products. No normally stained cells were observed in any of the replicate samples following treatment.

### 3.2.3 Round 3: 5% solutions with 10-minute contact time

Round 3 tests were carried out to determine whether increasing only the contact time but retaining the originally recommended 5% concentration would be sufficient for decontamination. The average percentage of stained cells in control samples was  $49 \pm 15$  % (Table 3-1). Complete tests (three replicates) were carried out using Eco-store, Jasco2 and Earthwise. In addition, as a rapid check, a single replicate was tested with Sunlight and two replicates with the Arnold product. Live (stained) cells were observed after treatment with Earthwise (on average 4% viable cells) and Arnold (average of 7% viable cells, in two replicates).

**Table 3-1: Summary results of CCD tests on didymo and *Lindavia* in April and May 2021.** Round 1 tests were carried out on all products. Rounds 2 and 3 were carried out depending on the results in Round 1. Shading is used to separate the results from Rounds 1, 2 and 3. Mean % viable = mean percentage of viable cells; \* = 1% concentration tested to verify initial result on Jasco in 2020 tests; \*\* = two replicates only; <sup>§</sup> = one replicate only. Refer to Appendix B for complete results.

Product	Round 1			Round 2			Round 3		
	Conc.	Contact time	Mean % viable	Conc.	Contact time	Mean % viable	Conc.	Contact time	Mean % viable
<b>Didymo</b>									
Controls			70			75			75
Eco-store (MPI sachet)	10%	1 min	0						
Earthwise Lemon and Aloe Vera	10%	1 min	2	5%	10 min	0	5%	5 min	2
Jasco Citriclean	10%	1 min	0				5%	5 min	0
Jasco2 Citriclean							1%*	5 min	2
<b><i>Lindavia</i></b>									
Controls			58			60			49
Eco-store (MPI sachet)	10%	1 min	26	10%	10 min	0	5%	10 min	0
Earthwise Lemon and Aloe Vera	10%	1 min	25	10%	10 min	0	5%	10 min	4
Jasco Citriclean	10%	1 min	14	10%	10 min	0	5%	10 min	0
Arnold Lemon detergent	10%	1 min	18	10%	10 min	0	5%	10 min	7**
Sunlight dishwashing liquid	10%	1 min	32	10%	10 min	0	5%	10 min	0 <sup>§</sup>

## 4 Discussion

### 4.1 Didymo

#### 4.1.1 Testing methods

As noted in previous reports, assays of didymo samples to determine percentage viable and non-viable cells are straightforward because of the large size of the cells (Figure 4-1), which make it relatively easy to observe NR staining.



**Figure 4-1: Stained didymo cell in a control sample.** Note the small paired (dividing) cell (*Encyonema minutum*) to the right of the didymo cell, with stained (red) granules.

#### 4.1.2 Effect of increasing concentration vs. contact time

We re-tested three detergent products that were not completely effective at a concentration of 5% with a 1-minute contact time. Tests on the Eco-store and Jasco<sup>3</sup> products confirmed that doubling concentration to 10% with the same 1 minute contact time was effective. However, Earthwise detergent still returned a few cells that appeared to be staining normally.

The relative ineffectiveness of the Earthwise product compared to the other products was seen in the presence of stained cells of other diatoms in three of the five replicate samples. We observed stained cells of the stalked diatom *Gomphoneis minuta*, the mucilage-producing diatom *Cymbella kappii*, and other small diatoms in at least one sample.

In the first part of the second round of tests, carried out using a sample collected on 27 April, we confirmed the 2020 tests results on 5% Jasco for 1 minute, in that an average of 2% cells still stained following treatment.

Because 10% is a high concentration and uses significant amounts of detergent, rather than extend the contact time with 10% Earthwise in the second round of tests, we first tried a longer contact time (10 minutes) with the original concentration (5%). Three replicates confirmed that this approach was effective.

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<sup>3</sup> We assume from the results that Jasco and Jasco2 had identical effects, and both are referred to as Jasco hereafter.

We continued to observe staining in other diatoms (e.g., *Encyonema minutum*) in Earthwise-treated samples (5% with 10-minute contact time) despite no viable didymo cells, suggesting that the large didymo cells were more susceptible to detergents than much smaller cells. *E. minutum* cells form mucilaginous masses and so could be enclosed in a much higher proportion of protective mucilage than didymo cells, which have at least 200 x the volume of *E. minutum* (Figure 4-1).

The final round of tests confirmed that if an extended contact time is used with a 5% solution of Earthwise detergent then the time should be at least 10 minutes. Consistent with the Earthwise results, a 5% solution of the Jasco product was also effective when the contact time was extended beyond 1 minute. However, 5 minutes was sufficient for Jasco, but was not completely effective for Earthwise (Table 3-1).

In summary, the tests for decontaminating didymo showed:

- For the Eco-store product (MPI sachets) and Jasco Citriclean (supplied by DOC) either increasing product concentration to 10% with the original contact time (1 minute), or increasing the contact time with the original concentration (5%) was effective;
- For Earthwise, only increasing the contact time (to 10 minutes) with the original concentration (5%) was effective. The response was marginal (i.e., extending the contact time to just 5 minutes was not long enough). Therefore, treatment with a 10% solution for 10 minutes may be the safest option.

## 4.2 *Lindavia*

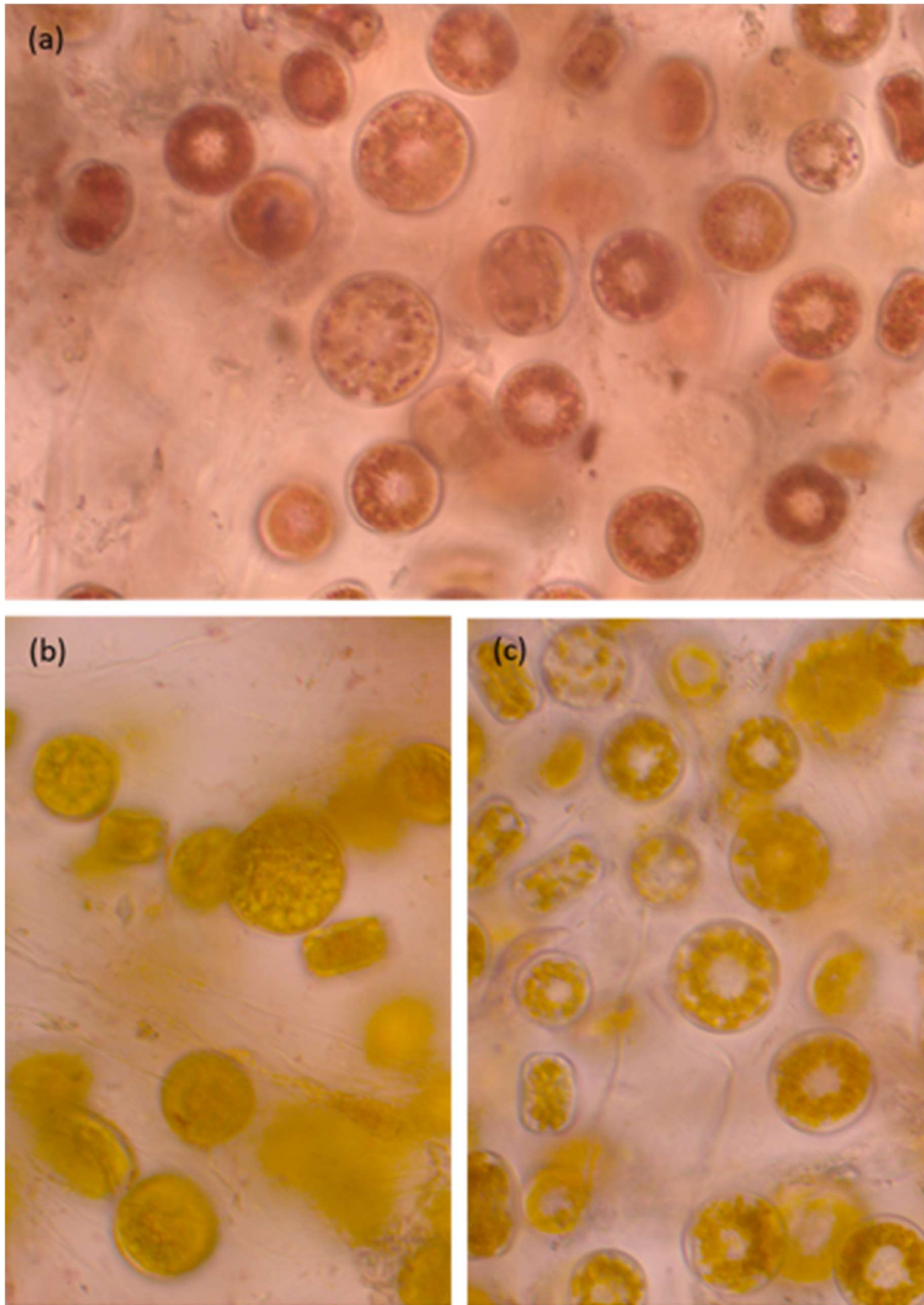
### 4.2.1 Testing methodology

The staining and cell counting methods used in the present tests were adjusted in light of problems encountered during the 2017 and 2020 tests, as described in Section 2.5.2. Nevertheless, patches of apparently unstained cells in control samples were a challenge for counting, especially when the microscope operator was not aware of the treatment on each sample. Also, we noticed that some samples appeared to be unhealthy when they arrived because it was difficult to find stained cells in the controls. These samples were not used for further tests. The patches of unstained cells resulted in percentages of viable (stained) cells always being lower in *Lindavia* control samples than in didymo control samples. Nevertheless, stained cells were relatively easy to distinguish in samples (Figure 4-2a) and the appearance of treated samples usually differed from that of control samples (Figure 4-2a,b,c).

### 4.2.2 Effect of increasing concentration vs. contact time

In the Round 1 tests, doubling the concentration of the detergents made little difference to the effectiveness of the treatments. Mean percentages of viable cells were higher overall in the 2021 tests than in 2020 for three of the four products re-tested (i.e., *less effective* at 10% than 5%). Only Earthwise returned a lower percentage, i.e., higher effectiveness on average than in 2020 from the tests on 5% solutions for 1 minute (56% viable reduced to 25% viable).

It is possible that the results were affected by the state of the samples (e.g., different amount of surrounding mucilage in the two years). The percentage viable counts in the controls were higher in 2020 than in 2021 (70% vs. 58% respectively), possibly indicating that more cells escaped staining in 2021, which could be explained by more mucilage in the sample. The material used in Round 1 in 2021 was prolific and very sticky, but we have no way of measuring the amount of mucilage.



**Figure 4-2: *Lindavia* cells after staining with Neutral Red.** (a) shows a control sample with stained cells (red granules); (b) cells after 10 minutes contact with 10% Jasco; (c) cells after 10 minutes contact with 10% Eco-store product (from MPI sachets). Note subtly greener appearance of cells in (b) compared to (c). The green colour change was much clearer on the whole samples.

Furthermore, Sunlight (which makes no environmental claims) returned the highest mean percentage of viable cells (32% viable cells across five replicates) after treatment for 1 minute with a 10% solution.

Regardless of the reasons for the differences and lack of differences of treatment with 5% and 10% solutions, extending the contact time to 10 minutes was effective for all detergents. The effect of longer contact was particularly clear with Jasco. All samples visibly changed colour during treatment, but the colour change (which caused the chloroplasts to turn from brownish to bright green, Figure 4-2b) only started to become visible after 5-6 mins in the treatment.

In the third round of trials, we repeated 10 min treatments on Earthwise, Eco-store and Jasco but using the original concentration of 5%. This was 100% effective on Eco-store and Jasco, but not on Earthwise.

Overall, the tests in 2021 showed that, when decontaminating for *Lindavia*:

- For the Eco-store product (MPI sachets), Jasco Citriclean (supplied by DOC) and possibly<sup>4</sup> Sunlight dishwashing liquid, increasing product concentration to 10% but retaining 1 minute contact time was not effective but increasing the contact time (to 10 minutes) using a 5% solution was effective;
- For Earthwise and the Arnold product, decontamination was effective only after increasing both the concentration from 5% to 10% and contact time from 1 minute to 10 minutes.

### 4.3 Implications of results

Currently the Check Clean Dry message regarding detergents is simple:

- use a 5% solution and ensure contact for at least 1 minute.

The 2020 and present tests confirmed the initial findings in 2007 that not all products are equally effective on didymo (i.e., “environmentally-friendly” products are less effective) (Kilroy et al. 2007). The 2017, 2020 and present tests showed that treatment varies across different algae (*Lindavia* is harder to deactivate than didymo). Ideally, Check Clean Dry would provide a consistent message that works for everything. The present tests showed that for both didymo and *Lindavia* the message could be:

- use a 10% solution and ensure contact for at least 10 minutes.

Users of CCD may consider that 10% solutions use a substantial amount of product, especially for large items. However, our tests showed that this concentration combined with at least 10 minutes contact is probably the best way to ensure effectiveness on *Lindavia* of products that make very clear environmental claims.

The fact that longer contact (10 minutes) with 5% solutions was almost certainly effective on didymo for all products, and close to being 100% effective on *Lindavia*, provides some flexibility around the message. Our observations suggested that using longer contact time definitely improves

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<sup>4</sup> “possibly” here because we tested only one replicate with 5% Sunlight with a 10-minute contact time.

effectiveness regardless of the concentration used (5% or 10%), and always works with didymo (provided the solution is at least 5%).

In practice, those using CCD would very likely always leave the solution in contact with the item being cleaned for more than 1 minute. For example, the usual procedure for waders is to thoroughly brush / scrub detergent solution into them and then leave them wet, which effectively extends the contact time. Usually it is not practical to rinse items (as we did in the laboratory tests) because, in a field situation, only river or lake water (probably contaminated) would be available as rinsing water.

The advantage of using household detergents rather than more aggressive cleaners such as bleach is that detergents are not damaging to materials and are generally not hazardous to people. Therefore, extending the contact time should not have any adverse effects.

## 5 Conclusions and recommendation

The outcome of these tests was that for both didymo and *Lindavia*, we identified combinations of concentrations and contact times that were 100% effective as decontamination methods.

Our overall recommendation is that, to accommodate for different effects of products and different effects on freshwater diatoms, the CCD advice for use of detergents to clean items after contact with lake or river water could be:

Clean items with a solution of detergent of at least 5% and ensure the detergent stays in contact with items for at least 10 minutes, with the caveat that if *Lindavia* is known to be present in the lake (or if in doubt) increase the concentration to 10%.

To translate this to the format used on the CCD website ([Check, Clean, Dry: preventing didymo and other pests | MPI | NZ Government](#)), consider replacing the following:

Dishwashing detergent or nappy cleaner	5% solution (500mls diluted to 10 litres in water)	Soak or spray all surfaces for at least 1 minute
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with something similar to:

Dishwashing detergent or nappy cleaner	5% solution (500mls diluted to 10 litres in water)*	Soak or spray all surfaces and allow to stay wet for at least 10 minutes
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\* Solutions of 10% are advised for lakes where *Lindavia* slime may be present.

## 6 Acknowledgements

This study was funded by the Ministry for Primary Industries. We thank Cassie Callard for facilitating the project, for providing sachets of the Eco-Store product, and for discussions over next steps during the course of the work. Thanks to Brenda Lawson, Department of Conservation, Turangi, for providing samples of Jasco Citriclean for testing (both the original version and the new version). We are very grateful to Tim Harrex (Otago Regional Council) for collecting samples of *Lindavia* for the tests during their routine lake water monitoring surveys in April and May 2021. We thank Kristy Hogsden for helpful review comments.



## 7 References

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## Appendix A Summary results of tests run in 2020

**Table A-1: Summary results of tests on *didymo* assessing effectiveness of four detergent products for decontamination .** Controls were run at the start of the trial and periodically throughout the trials.

Treatment	No. tests	Percentages of stained cells			
		Mean	Std Dev	Minimum	Maximum
Eco-store (MPI sachet)	5	5	6	0	14
Earthwise Lemon and Aloe Vera	5	26	17	10	55
Jasco Citriclean	4	1	1	0	3
Arnold Lemon detergent	3	0	0	0	0
Control (no treatment)	7	74	7	62	82

**Table A-2: Summary results of tests on *Lindavia* assessing effectiveness of four detergent products for decontamination.**

Treatment	No. tests	Percentages of live cells			
		Mean	Std Dev	Minimum	Maximum
Eco-store (MPI sachet)	5	15	9	0	21
Earthwise Lemon and Aloe Vera	5	56	29	22	90
Jasco Citriclean	5	8	6	1	16
Arnold Lemon detergent	5	17	17	1	36
Control (no treatment)	8	70	11	47	79

## Appendix B Raw data from all individual tests

**Table B-1: Results of tests on didymo.** Most samples were from the Opuha River. Results are listed in order of treatment, with control samples first. Slide order shows the order in which the tests were carried out. Conc. = concentration. Unst. = unstained. Under % live (cells), percentages have been rounded to the nearest 1% and calculations of standard deviations may differ slightly from those shown in the text. Under notes, all the other species listed as being stained are smaller diatoms.

Slide order	Date	Round	Treatment	Conc.	Exposure	Stained	Unst.	Total	% live	Notes
1	13-Apr-21	1	control			101	12	113	89	Healthy cells obviously stained and dead ones obvious
2	13-Apr-21	1	control			82	38	120	68	Healthy cells but not much stain
5	14-Apr-21	1	control			77	30	107	72	
6	14-Apr-21	1	control			81	29	110	74	Patches of unstained cells
17	14-Apr-21	1	control			55	37	92	60	Many unstained cells, stained cells in clumps
18	28-Apr-21	1	control			102	17	119	86	
3	14-Apr-21	1	Eco-store	10%	1min	0	100	100	0	No algae stained, chloroplasts have ragged edges
4	14-Apr-21	1	Eco-store	10%	1min	0	102	102	0	No algae stained, chloroplasts have ragged edges
13	14-Apr-21	1	Eco-store	10%	1min	0	120	120	0	
14	14-Apr-21	1	Eco-store	10%	1min	0	107	107	0	
9	14-Apr-21	1	Earthwise	10%	1min	3	99	102	3	Stained cells looked normal/ live
10	14-Apr-21	1	Earthwise	10%	1min	0	100	100	0	
15	14-Apr-21	1	Earthwise	10%	1min	2	105	107	2	Two cells seen lightly stained, looked 'normal'
16	14-Apr-21	1	Earthwise	10%	1min	0	104	104	0	
26	29-Apr-21	1	Earthwise	10%	1min	5	88	93	5	<i>Gomphoneis</i> , <i>Cymbella kappii</i> , etc. all staining normally as well as didymo
7	14-Apr-21	1	Jasco	10%	1min	0	104	104	0	
8	14-Apr-21	1	Jasco	10%	1min	0	105	105	0	Chloroplast edges smoother than in 4 but not normal
11	14-Apr-21	1	Jasco	10%	1min	0	102	102	0	Lots of other material present, not much didymo
12	14-Apr-21	1	Jasco	10%	1min	0	109	110	0	One didymo cell had dark stain throughout cell, counted as dead
19	28-Apr-21	1	Jasco2	10%	1min	0	100	100	0	Chloroplasts greenish, scanned whole slide, no stained cells seen

Slide order	Date	Round	Treatment	Conc.	Exposure	Stained	Unst.	Total	% live	Notes
20	28-Apr-21	1	Jasco2	10%	1min	0	100	100	0	
21	29-Apr-21	2	control			78	36	114	68	
23	29-Apr-21	2	control			59	48	107	55	Many <i>Encyonema minutum</i> also stained.
25	29-Apr-21	2	control			68	42	110	62	<i>Spirogyra</i> , other diatoms also staining
22	29-Apr-21	2	Jasco2	5%	1min	3	99	102	3	
24	29-Apr-21	2	Jasco2	5%	1min	1	100	101	1	
27	19-May-21	2	control			69	37	106	65	Not the best samples. Didn't count empty cells or those with hardly any chloroplast but still many unstained. Other small diatoms stained
28	19-May-21	2	control			95	16	111	86	Better sample
29	19-May-21	2	Earthwise	5%	10min	0	113	113	0	All didymo chloroplasts shrunken and sometimes "curly". Some cells took up stain but not in granules, more a continuous pink through cell
32	19-May-21	2	Earthwise	5%	10min	0	106	106	0	No stained cells seen
33	19-May-21	2	Earthwise	5%	10min	0	115	115	0	No normally stained cells seen. Some <i>Encyonema minutum</i> stained
35	19-May-21	2	Jasco2	5%	10min	0	105	105	0	Didymo cells still attached to stalks, but some split open. Green coloured, messy chloroplasts.
34	19-May-21	3	control			114	12	126	90	Most look healthy
39	19-May-21	3	control			63	41	104	61	Many didymo cells looked alive but not stained. Bad stain
40	19-May-21	3	control			81	28	109	74	Plenty of <i>A. min</i> stained
36	19-May-21	3	Jasco2	5%	5min	0	109	109	0	Greenish chloroplasts, all very dead. Stalks stained
37	19-May-21	3	Jasco2	5%	5min	0	109	109	0	No stained cells greenish chloroplasts
38	19-May-21	3	Jasco2	5%	5min	0	114	114	0	No stained anything except stalks
30	19-May-21	3	Earthwise	5%	5min	2	111	113	2	Two cells stained more or less normally. Otherwise as slide 29
31	19-May-21	3	Earthwise	5%	5min	0	112	112	0	A couple cells had granules but just a few at one end; shrunken chloroplasts. Counted as dead

**Table B-2: Results of tests on *Lindavia*.** Results are listed in order of treatment, with control samples first. Slide order shows the order in which the tests were carried out. Conc = concentration. Unst. = unstained. When samples from different lakes were available we tried to test samples from each lake. Under % live (cells), percentages have been rounded to the nearest 1% and calculations of standard deviations may differ slightly from those shown in the text. Under notes, all the other species listed as being stained are smaller diatoms.

Slide order	Date	Lake	Round	Treatment	Conc	Exposure	Stained	Unst.	Total	% live	Notes
1	23-Apr-21	Wakatipu	1	control			71	34	105	68	
2	23-Apr-21	Wakatipu	1	control			96	38	134	72	
7	23-Apr-21	Wakatipu	1	control			20	92	112	18	Maybe counted in a patch of cells the stain didn't reach?
12	23-Apr-21	Wakatipu	1	control			60	58	118	51	
17	23-Apr-21	Wakatipu	1	control			45	70	115	39	Stained streaks, large areas unstained (stain didn't reach it?)
20	29-Apr-21	Dunstan	1	control			84	27	111	76	NB changed staining technique – stronger stain, longer exposure (better results)
21	30-Apr-21	Dunstan	1	control			89	38	127	70	
22	30-Apr-21	Dunstan	1	control			86	45	131	66	
28	30-Apr-21	Dunstan	1	control			73	38	111	66	Unstained cells brown-coloured. Noted gradation from stained to unstained, so some judgement required
31	30-Apr-21	Dunstan	1	control			63	58	121	52	Count didn't include large patch of unstained cells that stain seemed to have missed
3	23-Apr-21	Wakatipu	1	MPI	10%	1 min	30	90	120	25	
8	23-Apr-21	Wakatipu	1	MPI	10%	1 min	11	92	103	11	
16	23-Apr-21	Wakatipu	1	MPI	10%	1 min	4	139	143	3	One stained streak. Stained cells looked normal
27	30-Apr-21	Dunstan	1	MPI	10%	1 min	81	45	126	64	Many cells looked alive; a few greenish coloured cells not stained
5	23-Apr-21	Wakatipu	1	Earthwise	10%	1 min	12	113	125	10	
14	23-Apr-21	Wakatipu	1	Earthwise	10%	1 min	15	115	130	12	
25	30-Apr-21	Dunstan	1	Earthwise	10%	1 min	64	53	117	55	Dead (unstained) cells more brown than greenish
9	23-Apr-21	Wakatipu	1	Jasco	10%	1 min	2	100	102	2	Some cells very green, non-normal shaped chloroplasts
10	23-Apr-21	Wakatipu	1	Jasco	10%	1 min	12	93	105	11	
15	23-Apr-21	Wakatipu	1	Jasco	10%	1 min	2	124	126	2	Whole slide looks greenish. A few cells stained but not normal, counted as dead. Two with "normal" stain. Stained pink centres counted as dead

Slide order	Date	Lake	Round	Treatment	Conc	Exposure	Stained	Unst.	Total	% live	Notes
24	30-Apr-21	Dunstan	1	Jasco	10%	1 min	57	76	133	43	Greenish chloroplasts in unstained cells; clumps of stained cells
6	23-Apr-21	Wakatipu	1	Arnold	10%	1 min	16	105	121	13	
13	23-Apr-21	Wakatipu	1	Arnold	10%	1 min	12	104	116	10	A few red-stained patches, mostly unstained background
23	30-Apr-21	Dunstan	1	Arnold	10%	1 min	21	23	44	48	Very few cells in sample. Material broke up.
30	30-Apr-21	Dunstan	1	Arnold	10%	1 min	1	109	110	1	Almost all cells with yellowish chloroplasts, unstained.
4	23-Apr-21	Wakatipu	1	Sunlight	10%	1 min	20	92	112	18	
11	23-Apr-21	Wakatipu	1	Sunlight	10%	1 min	64	47	111	58	
18	23-Apr-21	Wakatipu	1	Sunlight	10%	1 min	9	129	138	7	No stained streaks, stained cells looked "normal"
19	23-Apr-21	Wakatipu	1	Sunlight	10%	3 mins	5	157	162	3	
26	30-Apr-21	Dunstan	1	Sunlight	10%	1 min	72	58	130	55	Some unstained cells broken (squashed); stained cells all in a clump
29	30-Apr-21	Dunstan	1	Sunlight	10%	1 min	60	60	120	50	Some cells fully stained pink to bleached looking (counted as dead); some unstained with yellowish chloroplasts
32	21-May-21	Hāwea	2	control			81	47	128	63	Unstained patches. Only counted in stained patches
33	21-May-21	Hāwea	2	control			52	59	111	47	Didn't count in large area of unstained cells
39	21-May-21	Hāwea	2	control			75	47	122	61	Stained cells were coloured brownish green with clear red spots
40	21-May-21	Wānaka	2	control			76	38	114	67	Brown cells (normal looking) were unstained, but plenty of clearly stained cells
45	21-May-21	Wānaka	2	control			89	45	134	66	Plenty not staining - may not have left it long enough
59	22-May-21	Wānaka	2	control			71	60	131	54	Definite stained cells
46	22-May-21	Dunstan	3	control			47	87	134	35	Many not stained = doesn't look healthy
47	22-May-21	Dunstan	3	control			67	73	140	48	Similar to 46 above
34	21-May-21	Hāwea	2	MPI	10%	10 mins	0	125	125	0	Bubbles throughout. Big vacuoles in cells
41	21-May-21	Wānaka	2	MPI	10%	10 mins	0	110	110	0	Brown-yellow chloroplasts with "granules". Whole slide scan, no stained cells seen
49	22-May-21	Dunstan	2	MPI	10%	10 mins	0	115	115	0	
54	22-May-21	Dunstan	2	MPI	10%	10 mins	0	247	247	0	

Slide order	Date	Lake	Round	Treatment	Conc	Exposure	Stained	Unst.	Total	% live	Notes
37	21-May-21	Hāwea	2	Earthwise	10%	10 mins	0	114	114	0	Many cells with large vacuoles
44	21-May-21	Wānaka	2	Earthwise	10%	10 mins	0	135	135	0	Brownish cells
53	22-May-21	Dunstan	2	Earthwise	10%	10 mins	0	233	233	0	
36	21-May-21	Hāwea	2	Jasco	10%	10 mins	0	300	300	0	Clump is bright green. Whole slide scan, one partly stained cell but chloroplast green so doubt it is viable
43	21-May-21	Wānaka	2	Jasco	10%	10 mins	0	156	156	0	Green colour
52	22-May-21	Dunstan	2	Jasco	10%	10 mins	0	238	238	0	Green coloured chloroplasts
35	21-May-21	Hāwea	2	Arnold	10%	10 mins	0	135	135	0	Yellow-green cells, big vacuoles inside
42	21-May-21	Wānaka	2	Arnold	10%	10 mins	0	130	130	0	Whole slide scan, no stained cells seen
50	22-May-21	Dunstan	2	Arnold	10%	10 mins	0	120	120	0	Whole slide scan, no stained cells seen
38	21-May-21	Hāwea	2	Sunlight	10%	10 mins	0	100	100	0	Whole slide scan, no stained cells seen
48	22-May-21	Dunstan	2	Sunlight	10%	10 mins	0	130	130	0	Whole slide scan, no stained cells seen
55	22-May-21	Dunstan	2	Sunlight	10%	10 mins	0	194	194	0	Whole slide scan, no stained cells seen
51	22-May-21	Dunstan	3	control			53	67	120	44	Definitely plenty of live cells
56	22-May-21	Dunstan	3	control			62	65	127	49	Big patch of unstained cells not counted
66	22-May-21	Wānaka	3	control			90	17	107	84	Patches of stained cells
67	22-May-21	Wānaka	3	control			67	80	147	46	As 66 above
70	22-May-21	Dunstan	3	control			35	50	85	41	Definitely plenty of live cells
77	22-May-21	Dunstan	3	control			49	64	113	43	
57	22-May-21	Dunstan	3	MPI	5%	10 mins	0	172	172	0	Whole slide scan, no stained cells seen
60	22-May-21	Dunstan	3	MPI	5%	10 mins	0	1000	1000	0	Whole slide scan, no stained cells seen
62	22-May-21	Dunstan	3	MPI	5%	10 mins	0	500	500	0	Whole slide scan, no stained cells seen
64	22-May-21	Dunstan	3	MPI	5%	10 mins	0	500	500	0	Stain throughout mucilage. No stained cells seen
69	22-May-21	Dunstan	3	MPI	5%	10 mins	0	100	100	0	Whole slide scan, no stained cells seen

Slide order	Date	Lake	Round	Treatment	Conc	Exposure	Stained	Unst.	Total	% live	Notes
68	22-May-21	Dunstan	3	Earthwise	5%	10 mins	0	100	100	0	Whole slide scan, no stained cells seen
71	22-May-21	Dunstan	3	Earthwise	10%	1 min	28	95	123	23	Mostly unstained, but a couple of clumps stained normally
72	22-May-21	Dunstan	3	Earthwise	5%	10 mins	29	1000	1029	3	Some cells definitely stained
75	22-May-21	Dunstan	3	Earthwise	5%	10 mins	17	150	167	10	Some stained cells in a string of mucilage
58	22-May-21	Dunstan	3	Jasco	5%	10 mins	0	148	148	0	Greenish chloroplasts, no stained cells seen
61	22-May-21	Dunstan	3	Jasco	5%	10 mins	0	500	500	0	Greenish chloroplasts, no stained cells seen
63	22-May-21	Dunstan	3	Jasco	5%	10 mins	0	500	500	0	Most cells fully green; some have centre stained pinkish
65	22-May-21	Dunstan	3	Jasco	5%	10 mins	0	500	500	0	Two cells stained around outside, counted as dead. All chloroplasts greenish
73	22-May-21	Dunstan	3	Arnold	5%	10 mins	6	163	169	4	Some cells definitely stained
76	22-May-21	Dunstan	3	Arnold	5%	10 mins	17	162	179	9	Strings of mucilage with live and dead cells
74	22-May-21	Dunstan	3	Sunlight	5%	10 mins	0	1000	1000	0	Whole slide scan, no stained cells seen