Charophyte germination responses to herbicide application

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INTRODUCTION

Charophyte species form one of New Zealand’s prominent native submerged freshwater plant communities and are widely recognized as a beneficial component of lake ecosystems (Coffey and Clayton 1988). They are known as rapid colonizers and are usually the first aquatic plant species to re-establish after a disturbance event (Van den Berg et al. 1998, de Winton et al. 2000, van Donk and van de Bund 2002). New Zealand has four charophyte genera; Chara, Lamprothamnium, Tolypella, and Nitella (Wood and Mason 1977, de Winton et al. 2007), with Chara and Nitella species being the most common charophyte genera found in freshwater (Coffey and Clayton 1998).

The spread of tall-growing monospecific stands of aquatic invasive weed species has detrimental effects on native aquatic plants and biota. These effects include replacing native plants (de Winton and Clayton 1996, Champion et al. 2002) or displacing them to either deeper water where light is limited or to shallow areas where they are more exposed to wave action and desiccation (Closs et al. 2004). Displacement of native plants has detrimental impacts on native seed banks, thus limiting the emergence, growth, and reproduction of charophytes (de Winton and Clayton 1996, Bonis and Grillas 2002).

In New Zealand, the impact of invasive plants and the management objectives to remove or decrease weed beds of alien species have led to the development of aquatic control technologies, in particular the use of aquatic herbicides (Leonard and Creenland 1965, Clayton 1986, Wells et al. 1986, Clayton and Tanner 1988, Tanner et al. 1990, Wells and Clayton 1995, Hofstra and Clayton 2001b, Hofstra et al. 2001, Champion et al. 2002, Closs et al. 2004, Hofstra and Champion 2008, Clayton and Matheson 2010). For large-scale weed control in New Zealand, aquatic herbicides are the most widely used method (Champion et al. 2002), and diquat and endothall are the only aquatic herbicides registered for use on submerged invasive plants in standing and flowing water in New Zealand (Hofstra and Champion 2006). Diquat1 is formulated as dibromide salts (6,7-dihydrodipyrido[2,2,1-heptane-2,3-dicarboxylic acid) and is known to inhibit protein synthesis and cause disruption of membranes and respiration, resulting in wilting, desiccation, and collapse of the treated plants (Simsiman et al. 1976, Hofstra and Clayton 2001a, Hofstra et al. 2001, MacDonald et al. 2002, Hofstra and Champion 2008, Netherland 2009).

Fluridone2 is widely used throughout the United States for invasive weed management (Siemering et al. 2008) and, although not registered in New Zealand, it has been evaluated in herbicide efficacy trials (Wells et al. 1986, Hofstra and Clayton 2001b). Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), also known as a “bleaching herbicide,” is a systemic herbicide that inhibits the formation of carotenoid pigments essential for normal plant growth. The impact is observed in new shoot growth, which is often white, as the chlorophyll is destroyed by sunlight (Wells et al. 1986, Doong et al. 1993, Netherland 2009).

Chelated copper3 is a compound derived from a copper triethanolamine complex and copper hydroxide. Copper is a plant cell toxicant used as an algaecide for control of aquatic vascular plants as well as algae. Copper is a natural element and does not biodegrade in the water column but becomes biologically inactive by sorption to sediments and subsequent sedimentation. Copper in a chelated compound does not readily precipitate in the water column, allowing it to remain active for longer (Leslie 1990, Guha 1995, Durborow et al. 2007, Morris 2009, Netherland 2009).

Numerous field observations have demonstrated that the aquatic herbicides used in the present study are successful on target weeds such as Lagarosiphon major and that there is little to no impact (i.e., injury symptoms or biomass reduction) on established charophyte meadows (Leonard and Creenland 1965, Starling et al. 1974, Clayton 1986, Wells et al. 1986, Clayton and Tanner 1988, Hofstra and Clayton 2001a). However, their impact on critical early life stages of charophyte species has received relatively little attention. When oospores germinate, the initial primary protonema that emerges from the oospore is colorless and only becomes pigmented after cell division to form an intermodal cell with a nodal cell at both ends (Smith 1950, Bold and Wynne 1978). Early germing survival is dependent on the starch reserves accumulated in the oospores (de Winton et al. 2004) until the germling becomes pigmented; the germling then moves to a reliance on photosynthetic energy sources. These features of germlings and young charophyte plants may make them more susceptible to herbicide effects, especially diquat, endothall, and fluridone, which have modes of action that disrupt and inhibit photosynthesis (Netherland 2009).

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In this study, we hypothesised that charophyte germlings are a potentially vulnerable life cycle stage in herbicide treatments used for invasive weed control and could therefore represent a “bottleneck” in the charophyte life cycle under such treatments. Our main objective was to examine effects of selected herbicides (diquat, endothall, fluridone, and chelated copper) on charophyte germling growth.

MATERIALS AND METHODS

Seed bank material. Approximately 20 L of lake sediment containing oospores, hereafter referred to as seed bank material, was collected from Lake Tarawera (38°10’S, 176°23’E, depth 5 to 7 m), Lake Tikitapu (Blue Lake; 38°11’S, 176°19’E, depth 5 to 7 m), and Lake Rotoroa (Hamilton Lake; 37°47’S, 175°16’E, depth 1.5 m) to give a mixed age and composition of different Nitella and Chara species.

Each of the lake sediments was separately passed through a large garden sieve (12 mm mesh) into containers to remove any plant matter and large debris from the sediment. The sieved sediment from each lake was thoroughly homogenized (stirred for 5 minutes) before subsamples were taken to estimate the density and composition of oospores. Four subsamples of 10 mL (Lake Tarawera and Lake Tikitapu) or 100 mL (Lake Rotoroa) of sediment were passed sequentially through a sieve sequence of 500 µm and 250 µm mesh size. The contents of the sieves were placed into separate glass petri dishes and examined under a stereo microscope (Leica MZ 9.5, Leica Microsystems, Bio-Strategy Ltd). The oospores present were identified according to de Winton et al. (2007), counted, and recorded.

Once the oospore composition and concentration were determined for each lake, the three lake sediments were mixed together according to a predetermined mixing ratio to optimize the potential germination response. The mixing ratio was calculated based on 5% oospore germination as the lower range of published rates from natural sediment (de Winton et al. 2004). The goal was to have a mixing ratio that potentially yielded 100 germinating oospores per 120 mL of the mixed lake sediment. Once the lake sediments were mixed, they were stored at 2°C for approximately 5 months.

Experimental design. Mixed seed bank material (120 mL) was measured and placed into 130 mL plastic (HDPE) pots (Labserv®); a sufficient number of pots was prepared to ensure germling replicates for testing. The pots were placed into two water-filled storage containers (115 L, 79 by 55.5 by 39 cm). After 10 d, additional pots were filled with 120 mL of mixed seed bank material each, and three *L. major* apical shoots (15 cm) were planted into the sediment of each pot. The 10 d time delay was to minimize the experiment. Four days after treatment, the *L. major* shoot growth to ensure plants did not exceed the capacity of the jars used in the subsequent experiment, while allowing the pots of germlings time to germinate. The pots with *L. major* were placed into a third water-filled storage container (60 L, 63 by 44 by 34 cm). The three water containers were placed in a constant temperature room (20°C, 14 h:10 h light:dark cycle), with an average water temperature of 19.56°C (Onset HOBO® pendant temperature logger), 5.02 µmol m⁻² s⁻¹ photosynthetically active radiation (PAR; Onset HOBO® pendant light logger), and under constant aeration. The pots remained in the water-filled containers until widespread germination activity was noted (approximately 42 d). Sixty-five pots containing germlings and 25 pots of *L. major* were individually placed into 2 L glass jars (22.8 cm, 11.6 cm diameter; Arthur Holmes Ltd).

The jars with charophyte germlings received one of three treatment concentrations per herbicide; diquat (0.1, 1.05, or 2 ppm), endothall (0.1, 2.55, or 5 ppm), fluridone (0.005, 0.0775, or 0.15 ppm), and chelated copper (0.5, 0.75, or 1 ppm), as well as an untreated control. The jars with the *L. major* shoots were treated with one concentration (maximum label rate) per herbicide; diquat (2 ppm), endothall (5 ppm), fluridone (0.15 ppm), and chelated copper (1 ppm). Five replicates were utilized for each treatment and control. All treatments and the untreated controls were randomly assigned to the 2 L jars. The jars were set up under controlled photoperiod (14 h:10 h light:dark cycle), receiving an average of 36.6 µmol m⁻² s⁻¹ PAR (Onset HOBO® pendant light logger) at 20.34°C temperature (Onset HOBO® pendant temperature logger). After 24 h, aeration was started and maintained for the duration of the experiment.

Harvesting. Plants were harvested 14 d after treatment. Pots were removed from each jar and above-ground germling or *L. major* shoot height was measured. The germlings were removed and placed onto preweighed (+0.0001 g) foil sheets while *L. major* shoots were placed into separate brown paper bags. The foil sheets and paper bags were placed into an 80°C drying oven (Contherm Thermotec 2000 series oven, Bio-Strategy Ltd) and dried to constant weight. The foil sheets were weighed (+0.0001 g), initial foil weight deducted, and the charophyte biomass determined and recorded. The *L. major* shoots were weighed (+0.0001 g; without bag) and recorded.

An ANOVA (GenStat 12th Edition) and post-hoc test (Tukey HSD) was used to distinguish any significant differences (p ≤ 0.05) between the control and each of the treatment doses for each herbicide and algaecide.

RESULTS AND DISCUSSION

*Lagarosiphon major*. Over the 14 d experimental period, *L. major* shoots in the untreated control jars remained healthy and intact. Four days after treatment, the *L. major* shoots treated with diquat and endothall turned necrotic, and by day 12 there was total collapse of the shoots tissue (no possible recovery), although there were visible green charophytes growing in the *L. major* pots. *L. major* shoots treated with fluridone remained green with pink tips developing after 12 d of exposure. Four days after treatment, the *L. major* shoots treated with chelated copper showed darkening leaf color while the stem remained light green color. Over time, the darkened leaves became flaccid (day 12) and lost buoyancy (day 14) while the stems remained healthy and new lateral shoots began to grow.

There was no difference in *L. major* shoot height between the control, fluridone, and chelated copper treatments (Figure 1). In addition, there were no recoverable shoots in the diquat and endothall jars 14 d after treatment (Figure 1). Diquat and endothall achieved complete *L. major* control, and no biomass was recovered from these treatments. In compari-
son with the control (Tukey HSD test), *L. major* had a significantly lower biomass in the diquat, endothall, and chelated copper treatments while biomass of fluridone-treated *L. major* was similar (Figure 2).

All four treatments (diquat, endothall, fluridone, and chelated copper) severely affected *L. major* as expected. Diquat and endothall in particular had high efficacy on the *L. major* shoots causing total collapse of the shoots as observed in previous studies (Clayton 1986, Wells and Clayton 1993, Hofstra and Clayton 2001a). Fluridone impacted only *L. major* shoots as visual injury symptoms of chlorosis and purple coloration of new shoots were noted, while older plant tissue remained generally healthy as noted previously in research by Wells et al. (1986) and Hofstra and Clayton (2001b). The efficacy of fluridone on *L. major* biomass may have been limited by the duration of the experiment (approximately 2 weeks) because fluridone is a slow-acting herbicide that requires long exposures (45+ d) to be efficacious (Netherland 2009).

Charophyte germlings. Calculations of germination potential (Table 1) suggested oospore concentrations present in the experimental sediment were sufficient to enable germination and establishment of charophytes. With the exception of the germlings treated with chelated copper, germlings were present in the treatment pots throughout the 14 d experimental period, with noticeable oospore germination and new germlings emerging. The germlings treated with chelated copper were mostly brown and opaque with only a few new germlings beginning to emerge through the sediment by the conclusion of the experiment. These germlings in the chelated copper treatment (the positive control) were affected as expected, confirming charophyte susceptibility to algaecide effects under the experimental conditions used in this study.

Despite visual evidence of algaecidal effects in the chelated copper treatment, there was no overall difference in germling height between untreated germlings of the control and germlings in any of the herbicide or algaecide treatments by the end of the experiment (Figure 3). The charophyte biomass was lowest in the chelated copper treatments (Figure 4); however, there were no significant differences (p > 0.05, Tukey HSD test) in charophyte biomass between the untreated germlings and the treated germlings (Figure 4).

Although significant differences were apparent between some herbicide treatments (e.g., compared with the chelated copper treatment), visual injury symptoms associated with diquat, endothall, and fluridone were not detectable relative to the control treatment. Differences in charophyte biomass occur naturally as part of the germling response and growth. For example, chance timing of germination may drive the differences in the composition of germlings, such as the plants with earliest germination gaining a competitive advantage to resources such as light (Casanova and Brock 1990, de Winton et al. 2000). Moreover, the observed regeneration of new plants through germination of oospores still in the seed bank may have compensated for any herbicide impacts on the treated germlings.

Our results indicate that charophyte germlings were not susceptible to herbicide treatment, and that oospores were able to germinate continuously from sediments where they are present. This result has two important implications for field application of the herbicidal products for invasive weed management in lakes and waterways. First, if successive herbicide applications are carried out, germling establishment is still likely to be supported by continual germination from the sediment providing a large enough oospore population is present. Therefore the timing of herbicide applications for invasive aquatic plant management in lakes and waterways is not crucial for charophyte regeneration. Second, given the same level of target weed control (i.e., the level of target weed control is not compromised by product choice), the use of one product over another (with exception of copper algaecide) does not confer any advantage or benefit to native charophyte regeneration.

Further research in charophyte germling responses to herbicide application would involve repeating this experiment over a longer experimental timeframe.

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**SOURCES OF MATERIALS**

1 Reglone®, Syngenta Crop Protection Ltd., Auckland, New Zealand
2 Aquathol® K, United Phosphorous Inc, King of Prussia, PA
3 Sonar® AS, SePRO, Carmel, IN
4 K-Tea®, SePRO, Carmel, IN

**LITERATURE CITED**


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