PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Effect of hydrogen peroxide spraying on *Hydrocotyle ranunculoides* L.f. survival

An initial experiment

W. Joost van der Burg

Plant Research International, part of Wageningen UR
Business Unit Agrosystems Research
Concept, August 2010

invexo
minder invasieve planten en dieren

Interreg
Vlaanderen
Nederland
Europa investeert in uw regio

Europese Unie
Europees Fonds voor Regionale Ontwikkeling

Copies of this report can be ordered from the (first) author.

Plant Research International, part of Wageningen UR
Business Unit Agrosystems Research

Address	:	P.O. Box 616, 6700 AP Wageningen, the Netherlands
	:	Wageningen Campus, Droevendaalsesteeg 1, Wageningen, the Netherlands
Tel.	:	+31 317 48 08 42
Fax	:	+31 317 41 80 94
E-mail	:	info.pri@wur.nl
Internet	:	www.pri.wur.nl

Table of contents

1. Summary

This experiment was carried out in the framework of the Interreg project "Invasive exotic plants etc (INVEXO). Before a larger experiment was to be carried out, a first try-out of the principle was made.
The background of this pilot experiment was to find out if treatment in nature with hydrogen peroxide would be feasible as control measure.
The experiment was designed to 1. try to raise plants in containers, and 2. to observe the effect of hydrogen spraying on the plants. This would allow to determine the optimal plant growing conditions and to determine a range of doses that could be used in a possible larger experiment.
Five treatments were chosen *viz.* 0, 0.3, 3,10 and 30% hydrogen peroxide in water with four replications. A household plant sprayer was used to avoid corrosion of the normal experimental machinery that is normally used to apply herbicide under controlled conditions.
The initial plants were collected from the wild in early spring of that same growing season and raised in containers in the greenhouse. From the newly grown material small cuttings were taken and grown for 22 days. this was enough to cover the pot surfaces with leaves. Then the spraying was done using two sprays resulting leaf saturation (slightly dripping). The effects were almost immediate: after 3 hours the leaves treated with the 30% solution were heavily affected, getting transparent and curling upwards. In the 10% solution treatment about half of the leaf surfaces were affected. After 4 days no change in damage could be observed, except that the affected leaves were drying out while the non-affected leaves were growing as before. After 14 days the affected leaves were yellow and wilted and overgrown by healthy new leaves. Stolones and shoot tips, though all above-ground, were not affected in any treatment. The roots were immersed in the mud and not affected either.
In conclusion it may be say that only the highest concentration of 30% had a marked and direct effect, but still was not enough to kill the plant. The 10% had a clear effect as well but did not kill most of the leaves.
The higher concentrations are hazardous to people who will do the spraying, and are therefore not practical. The lower concentrations have little or no effect.

2. Introduction

This experiment was carried out in the framework of the Interreg project “Invasieve exoten in Vlaanderen en Zuid-Nederland” (Invasive exotics in Flanders and the South of the Netherlands) (INVEXO). In this project methods to control invasive plant (and animal) species are being investigated.
The background of the current pilot experiment was to find out if treatment in nature with hydrogen peroxide would be feasible as control measure. This experiment was carried out to determine if the principle had potential for further development.
Therefore, the experiment was designed to 1. try to raise plants in containers, and 2. to observe the effect of hydrogen spraying on the plants. This would allow to determine the optimal plant growing conditions and to determine a range of doses that could be used in a possible larger experiment.
It was decided to use a household plant sprayer instead of an experimental spraying machine, because of the corrisiveness of hydrogenperoxide which may damage the equipment, especially with the higher concentrations.
The initial plants were collected from the wild in Laarne, Belgium in early spring of 2010 and raised in containers in the greenhouse.

3. Materials and Methods

20 Black plastic pots of 20 cm diameter were filled for 3/4 with Lentse potgrond nr 4 and filled with water until over-saturation, resulting in liquid mud. In each pot, two cuttings with 2-3 internodes and a terminal tip were planted. Regularly, the growing stolones were guided back into the pot to get an even distribution of leaves over the surface. The plants were raised in a temperature-controlled greenhouse section at 20 °C during the day and 15 °C during the night. The day-night cylcle of the temparture was automatically adjusted by the level of light. The test was carried out in July and no additional lighting was used.
After 21 days the pots were all homogeneously covered with leaves and the treatments were carried out (Figure 1a and b).
Five hydrogen peroxide concentrations were prepared using a base solution of 30%: 0.0, 0.3, 3.0, 10 and 30%. These were applied on the replicates once with a household plant sprayer by pressing it two times. This resulted in saturation of the leaf surfaces. The treatments were replicated 5 times.
The effects were evaluated at regular intervals and each time photographs were taken.

Figure 1a. Plants 10 days after transplanting.

Figure 1b. Plants at 21 days after transplanting.

4. Results

The first evaluation was made 3 h after treatment. The lowest concentration, 0.3% did not show any signs of damage. The 3% concentration gave some faint brownish spots, The 10% concentration gave damage to approximately half of the leaf surfaces, while 30% gave a strong discoloration of the entire leaf surface (Figure 2b).

Figure 2a. Plants 3 h after treatment. Left to right: 0.0, 0.3, 3.0, 10 and 30% H_2O_2 solution.

Figure 2b. Plants 3 h after treatment with 30% H_2O_2.

After 6 h no major changes could be observed, indicating that the treatment has an immediate and no systemic effect, which is characteristic of a contact treatment. The affected areas were drying out resulting in the leaves curling upwards at the edges (Figure 3a). The leaves below the top layer who were not reached by the treatment, remained unchanged and healthy. The same applies to the stolones lying on the mud surface, irrespective of the treatment. The roots were likewise not effected.
After 24 h the leaves of the 30% treatment are clearly drying out and curling up more strongly, while the lighter treatments show their symptoms more clearly. The 3% treatment now shows the small spots more clearly (Figure 3b) and the 10% treatment now has also curled leaves, though less prominent than the 30% treatment. Still the stolones and young leaves are showing few symptoms and seem to be growing well.

Figure 3a. Plants 6 h after treatment with 30% H_2O_2.

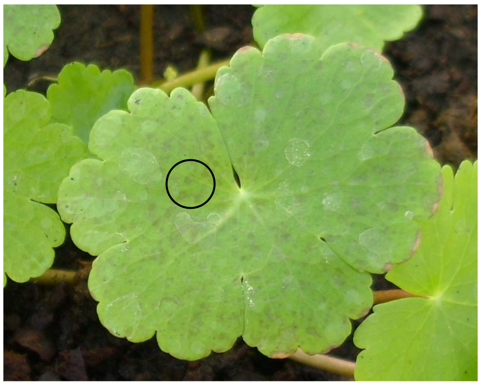

Figure 3b. Small brownish spots on the plants 24 h after the 3.0% H_2O_2 treatment.

After 5 days the affected leaves of the 10 and 30% treatments are becoming papery and dying (Figure 4a). The non-treated small leaves and stolones are growing as normal (Figure 4b). In the 30% treatment one third of the leaves is healthy and emerging above the dying leaves. In the 10% treatment two thirds are healthy, while of the 3% treatment only a small growth retardation can be observed and no dying leaves.

Figure 4a. Plants 5 days after treatment with 10% (left) and 30% H_2O_2.

Figure 4b. Plants 5 days after treatment with 30% H_2O_2 clearly showing recovering.

After 14 days, the affected leaves have become yellow and wilted, while the remainder has recovered (Figures 5a and b). The small brownish spots in the 3% treatment did not result in any dying of tissue.

Figure 5a. Plants 14 days after treatment. All plants growing vigorously. Plant in front: 30% treatment with 30% H_2O_2 solution. (The large container is not part of this experiment).

Figure 5b. Plants 14 days after treatment with 30% H_2O_2.

5. Conclusions and discussion

Plants of *Hydrocotyle ranunculoides* L.f. grow proliferically in greenhouses at temperatures between 15 and 20 °C. During the summer months of the North-Western Europe, no additional lighting is necessary.

Progressive damage was caused by the hydrogen peroxide treatment, with only slight symptoms with the 3% treatment, not resulting in any damage, damage of around 1/3 of the leaves fatally damaged with a 10% treatment, and approximately 2/3 of the leaves fatally damaged with a 30% solution. The remainder of the leaves was not or only slightly affected because they had been shielded by the taller leaves. Stolones or roots were not affected in any treatment. The two higher concentrations resulted in some growth retardation but after two weeks the plants were largely recovered and growing vigorously.

The higher concentrations are hazardous to people who will do the spraying, and are therefore not practical. Even higher concentrations are possible, but these solutions are even more dangerous to handle. The lower concentrations have little or no effect. Moreover, not the entire plant is affected, with essential elements such as roots, stolones and young and smaller leaves surviving the treatment. A second application after one week could kill off more of the leaves, but would still leave stolones and underground as well as underwater parts unaffected.

The application was a relatively large dose, resulting in almost complete wetting of the leaves. So increasing the dose does not seem an option. A separate dose-effect investigation could quantify this more clearly. Similary, repeated treatments could be carried out to study the effect of frequency.

Notwithstanding, the general conclusion is that application of spray technologies with the purpose of contact effects will be problematic or even impossible. This however, does not preclude the possible successful application of systemic herbicides. An option worth considering because the environmental hazard is relatively small and animal life on the plants and in the water is not affected.

Another idea to treat the water in which the plants grow with a solution of hydrogen peroxide could not be studied because we did not manage to get water standing over the soil. However, the likeliness that this would create a realistic opportunity is questionable: application of high volumes of hydrogen peroxide is costly, not without danger to those applying it, and will probably become too much diluted to have any effect. Moreover, the plants may still return from their base at the shores of the water courses. Finally, such treatments are not specific and will potentially kill the entire ecosystem locally.

The results however do suggest that a similar experiment with flame treatment would merit investigation: 1. the effect will be similar but with potentially more effect on the lower leaves (penetration of heat is more profound than of fluids); 2. because the leaves will probably wilt almost instantly, it is expected that the treatment can be repeated on the same day, which is of great logistic advantage; and 3. the environmental impact of the use of butane/propane or similar burners is less environmentally harmful.