

An experiment to test the potential of Silv-Ex® and Silv-Ex Plus® to eradicate
Phytophthora ramorum spores in water.

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Background:

Silv-Ex, as described by product literature (ANSUL Inc., Marnette, WI), is a foaming agent added to water to increase fuel penetration in fire fighting. It includes a “variety of synthetic ingredients including hydrocarbon surfactants” according to product literature. Since certain dispersal spores of *Phytophthora* species are susceptible to destruction by surfactants, these or other components of Silv-Ex’s chemistry may eliminate *Phytophthora* spores from water. This would alleviate the concern that *P. ramorum*, the causal agent of Sudden Oak Death, may be spread through firefighting efforts when infested water sources are drawn and applied to non-infested areas or plants.

Objective:

Test the effect of Silv-Ex and Silv-Ex Plus on *P. ramorum* sporangia, cysts and zoospores in aqueous solution.

Methods:

Products: The two marketed versions of Silv-Ex will be tested: “Silv-Ex” and “Silv-Ex Plus”. Communication from Steve Hansen, Tyco International Marketing Manager for the Americas, indicated that they have distinct chemistries.

Product concentration: According to product literature and personal communication, the products are typically used at between 0.1 and 1.0 % concentration in water (v/v). We therefore tested solutions of 0.1, 0.5 and 1.0 % concentrations. Untreated de-ionized (DI) water served as untreated control.

Inoculum: Sporangia were rinsed from 10-14 day old cultures of 5 *P. ramorum* isolates grown on clarified V8 agar. The suspension was divided in two parts, and one part was chilled in the refrigerator for one hour to induce zoospore release. After approximately ten minutes of returning the chilled suspension to room temperature, zoospores were observed and induced to encyst through agitation of the suspension. We determined cyst concentration with a haemocytometer to be 12.5×10^4 . Sporangia were counted in five 0.10 ml subsamples of the unchilled suspension, and found to be present at 900/ml.

Experimental Design and Methods:

The experimental treatments represented a factorial of the two product types, three concentrations, plus an untreated control for a total of 7 treatments. Each treatment was replicated eight times for a total of 56 experimental units.

We added 0.80 liters of water to each of 48 one-quart screw-cap wide-mouth glass mason jars and eight one-liter screw-cap wide-mouth plastic bottles. Using micropipeters, we added 8.1, 4.05, and 0.81 ml of Silv-Ex or Silv-Ex Plus to achieve 1.0, 0.5 and 0.1 % solution treatments, respectively. We mixed the products by shaking and inverting the capped bottle for approximately 20 seconds. We then added 0.80 ml of *P. ramorum* cysts suspension and 2.22 ml

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of sporangial suspension to the 800 ml treatments for 1×10^4 cysts and 2000 sporangia per container. The liquid in each container was agitated again. The solutions and control were then left overnight in randomized block arrangement in two different cabinets in the laboratory.

The next day, we added six Rhododendron leaf discs to each container to detect any infective spores, and to stimulate the release of new zoospores from cysts. The bottles were incubated for 6-8 days, again in randomized block arrangement, being agitated twice during this period to re-suspend cysts and sporangia. The leaf discs were then collected from each bottle, rinsed in two baths of DI water, and placed onto *Phytophthora* selective media. They were observed over three weeks for growth of *P. ramorum* colonies. A second set of baits were introduced one week after the removal of the first, and incubated for the same period of time. We cultured and evaluated these bait leaf discs as described for the first set.

Filtering the solution proved impractical due to the great viscosity of treatment solutions, even at 0.1%. Therefore, after re-suspending the inoculum by agitating the solution, a 10 ml subsample was placed directly onto *Phytophthora* selective media.

Cultures were examined to determine and quantify the growth of *P. ramorum* from bait leaf discs or solutions.

Results:

We observed a great amount of foam in all the Silv-Ex and Silv-Ex Plus solutions on the initial mixing, demonstrating the strong surfactant quality of the material even at the lowest concentration. The expectation therefore was that *Phytophthora* spores were unlikely to survive in any treatment solutions. Additionally, the bait discs sank to the bottom of the containers a day after introduction to the treated solutions, while they remained floating in controls for most or the entirety of the experiment.

In fact, there was no *P. ramorum* growth from direct culture of treatment solutions for either product, while the control had an average of 5.4 colony forming units (CFU) per ml (Figure 1). Results were similar with the infection of bait leaf discs. However, leaf discs in one replication of Silv-Ex Plus had 100% infection at 0.1% concentration, and 50% infection at 0.5 and 1.0%. In comparison, bait infection rates for controls were 100 % with nearly all replications, and consistently 0 for Silv-Ex treatments (Figure 2). The cause of *P. ramorum* viability in one of eight replications of Silv-Ex Plus treatments is uncertain, but since the mixtures show foaming as other treatments, we may deduce that *Phytophthora* spores remained viable and infective in this solution for sometime. The second set of baits, however, did not show any *P. ramorum* infection in any Silv-Ex solutions (Figure 3), while untreated water resulted in an average 3.6 of 6 baits infected per container. Since these were the same solutions tested earlier, this indicates that in Silv-Ex Plus solutions where *P. ramorum* was detected on baits the first time, those infections either failed to produce disseminating spores or that those spores did not survive in the solution more than one week.

Sporangia, zoospores and zoospore cysts are the dispersive propagules of *P. ramorum* and those most likely to occur in naturally infested waters. At present, we can conclude that these propagules are effectively eliminated from aqueous solutions of Silv-Ex, even at 0.1%

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concentration (v/v), and that the product minimizes the risk of spreading the pathogen through firefighting efforts that draw from infested water sources. Silv-Ex Plus is likely also as effective, though the results here are less absolute. The infection of some baits in treated solutions may indicate that both products may fail to eliminate the pathogen, at least for a time, under certain conditions, or at least in the presence of host tissue. The occurrence of infections with bait leaf tissue and not with direct plating may indicate that the pathogen found some protection inside host tissue. If so, the pathogen may survive in some host tissue debris in water drawn for fire control, even when treated with the tested products. Water treatment with these products would nonetheless minimize free inoculum in the water. Additionally, the failure of a second set of baits to detect the pathogen in these treatments indicates that the pathogen will not be able to produce disseminating spores from infected tissue in treated solutions. This additionally reduces the risk of substantial inoculum being dispersed through treated water.

In the light of this data, the treatment of potentially *P. ramorum*-infested water with Silv-Ex or Silv-Ex Plus substantially reduces, but does not eliminate, the concern for spreading the pathogen through fire-fighting efforts.

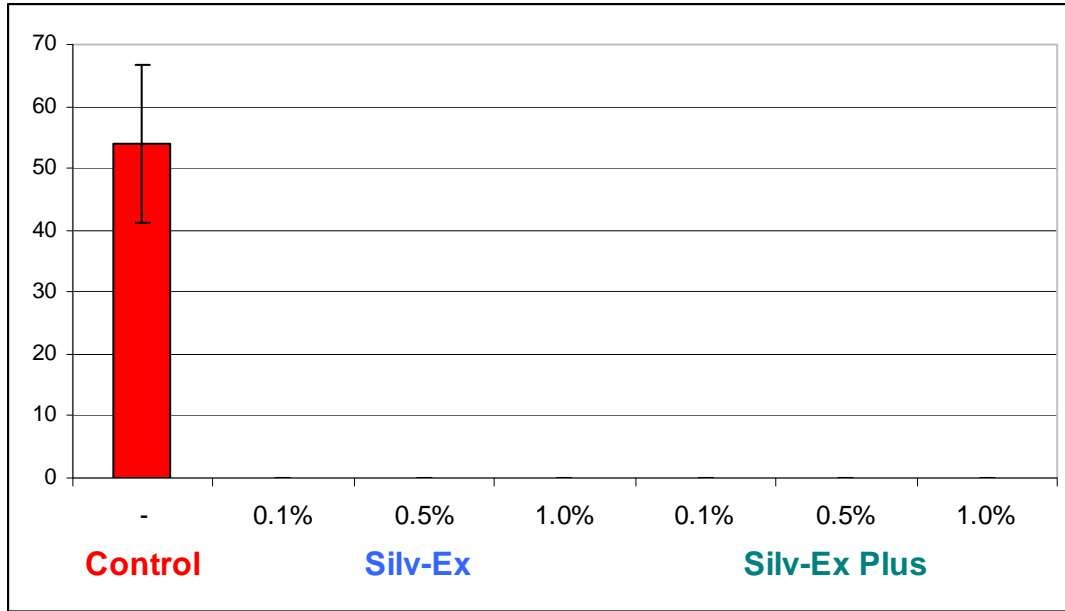


Figure 1. Average CFU of *P. ramorum* (Y-axis) from a 10 mL subsample of treatment solution (X-axis): control (DI water), Silv-Ex and Silv-Ex Plus at 0.1, 0.5 and 1.0 % concentration (v/v). Bar indicates standard error.

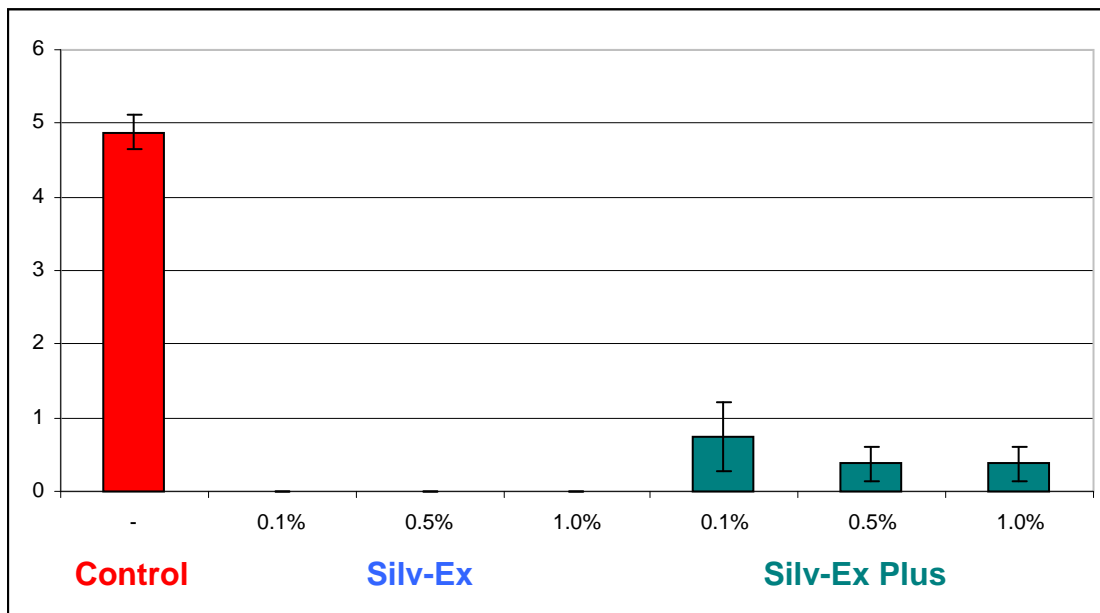


Figure 2. Average number of Rhododendron leaf bait discs out of six infected with *P. ramorum* (Y-axis) after 6-8 days incubation in inoculated treatment solutions prepared 1 day before: control (DI water), Silv-Ex and Silv-Ex Plus at 0.1, 0.5 and 1.0 % concentration (v/v). Bars indicate standard error.

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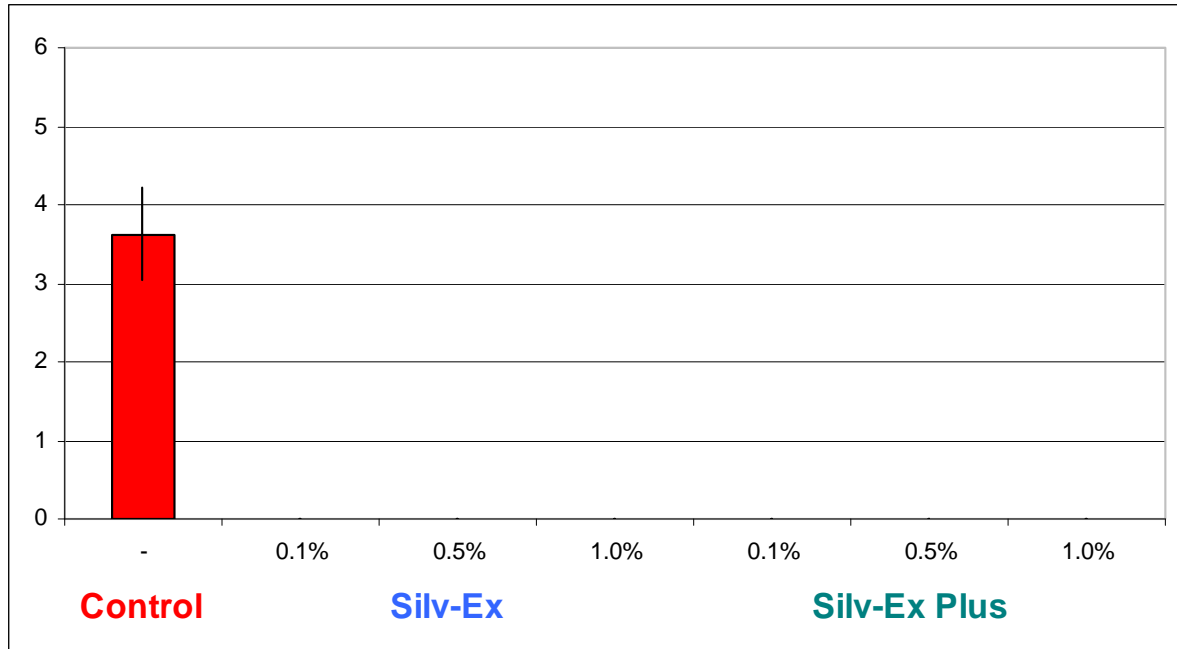


Figure 3. Average number of Rhododendron leaf bait discs out of six infected with *P. ramorum* (Y-axis) after 6-8 days incubation in inoculated treatment solutions prepared 2 weeks before and already baited once one week before: control (DI water), Silv-Ex and Silv-Ex Plus at 0.1, 0.5 and 1.0 % concentration (v/v). Bar indicates standard error.



Treatments pictured from left o right: Control, Silv-Ex 0.1 %, Silv-Ex 0.5 %, Silv-Ex 1.0 %, Silv-Ex Plus 0.1 %, Silv-Ex Plus 0.5 %, Silv-Ex Plus 1.0 %