Phytochemistry of Plants Associated with a 400-Year-Old Stand of Hemlock at Clear Lake Reserve, Ontario

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Abstract

Several species of higher plants and mushrooms have been surveyed growing under the canopy of old stands of hemlock surrounding Clear Lake near Minden, Ontario. Some of the hemlock seedlings growing on fallen trunks together with debris in which they were growing were brought to a greenhouse in pots, and some of them were transferred into sandy soil. The conditions of growth involved a gradient of shading. As an analysis of the debris revealed a low pH, the soil in all pots was kept at a pH of 5.5. The best growing seedlings were in the most shaded areas in pots filled with plant debris and the worst growth was in sandy soil in full sunlight. We have measured the concentration of phenolic compounds, which was found to be highest in dying plants in sandy soils in full sunlight, and lowest in shaded areas in pots filled with debris.

Introduction

Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. Ecology nowadays includes an increased amount of chemistry because communication between a plant and its environment depends to a large extent on secondary metabolites (Zobel and Brown, 1996), of which phenolics make up *ca*. 100,000 different structures (Harborne, 1994). These compounds are involved as well in allelopathy, dealing with the interactions between two plants, which process can change depending upon variations in the phytochemicals produced under particular environmental conditions (Zobel *et al*., 1999).

Natural conditions in the old growth of hemlock surrounding Clear Lake near Minden have been ideal for investigation of plant-plant interactions and as a source of plants used sometimes as reference samples for the model system because the chemistry of the environment in which they grow has been extensively studied over the past 30 years. Success of preserving 1500 km^2 of the Clear Lake Reserve a few years ago, is an example of how the public, foresters, academics and government can work together. Now we know that our research will continue to be relevant because the preserve is the common denominator for other studies. Since *Tsuga canadensis* has been used as a medicinal plant for at least 500 years (Erichson-Brown, 1979), we chose to investigate it as well as one of the macrophytes, *Eriocaulon*. (Fig. 1).

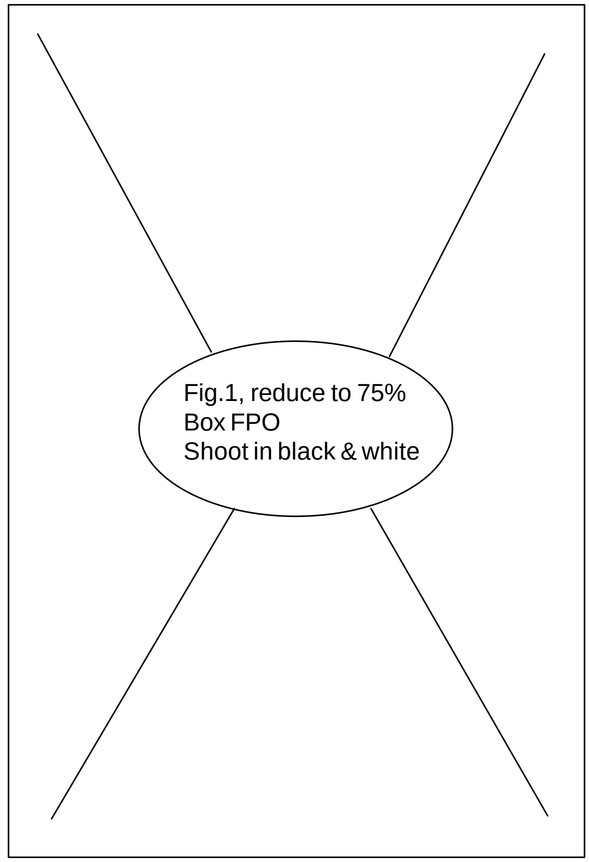

Figure 1.—*Eriocaulon* growing on the bottom of Clear Lake, with stones visible as low as 6 m from the surface, owing to clarity of the water.

Material and Methods

Seedlings of hemlock were removed from fallen, decaying logs of this species (Fig. 2), and transferred into pots containing sandy soil with the pH adjusted to 5.5, or, for comparison, 3.5, as this is almost the lowest pH of acid rain found in this region. Some plants in a Trent University garden were left in the full sun, some were placed in full shade, and the third group in partial shade under a maple tree. The samples were taken after one week, because from our previous experiment we had found that measurable results were obtained after this period (Zobel *et al*., 1995). The concentration of phenolic compounds was evaluated following the method of Harborne (1964, 1967), using a Nova spectrophotometer at 325 nm, because both flavonoids and coumarins absorb there.

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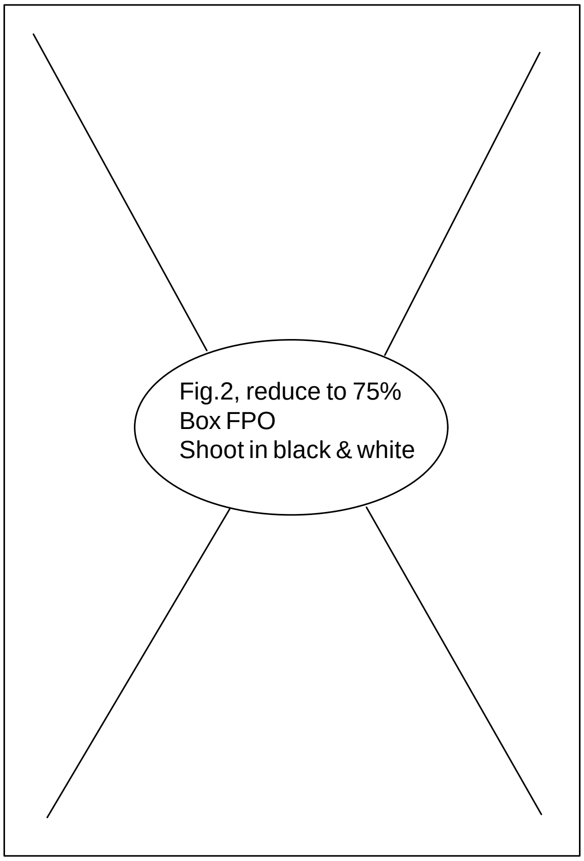

Figure 2.— Decaying stump of old hemlock provides a home to seedlings (arrow) of hemlock. Various age small hemlock trees are visible near the old ones (O).

Warburg apparatus was used for measurement of the oxygen released as an evaluation of catalase activity after *Eriocaulon* from Clear Lake had been exposed to 366nm UV itself or UV and HCl of pH 5.5 or UV and 100 ppm cadmium.

Results and Discussion

The reaction of hemlock seedlings to pH and shading is shown in Table 1. The greatest growth was observed in shaded areas when the pH was 5.5, and the seedlings grew better than under the same light conditions but in more acidic soil. The concentration of phenolics was inversely proportional to depth of shade, and the most phenolics were in dying plants grown in full light and pH 3.5. The worst growing seedlings had almost double the phenolics concentration of the best growing. Increased production of phenolics can be an indication of stress, the response to which is production of the phenolics (Zobel and Nighswander, 1990), but if the plant exceeds a certain threshold some cells inside the leaf would die because of precipitation of their proteins by the phenolics, forming microscopic necroses that, after further reaction, would cause death of the whole leaf. We observed a similar reaction of switching on defense-phenolic compounds both in pine (Zobel and Nighswander, 1991), and in rue shrubs (Zobel *et al*, 1993), when exposed to simulated acid rain or sprays of aluminum or sodium chloride, which would lead to death of the cells. We proposed a very simple test (Zobel, 1986; Zobel and Nighswander, 1991) which could be applied by foresters under field conditions, by performing a reaction for phenolic compounds on hand-cut cross-sections of pine and hemlock needles to locate necrosis and evaluate the stage of suffering of leaves from toxification.

Lakes are included in this 1500 km^2 area. Aquatic plants can be used as indicators of changes in environmental conditions, thus the reaction of our model system, *Eriocaulon*, was used for the evaluation of the plant's ability to scavenge free radicals, by measurement of catalase activity. The results are shown in Figs. 3-4, where we can see that differences were substantial between plants treated with normal light and 366nm UV. The reaction of the extract from the whole plant was different from that of the surrounding water, and roots and shoots, after being separated, showed differences in activity. The reaction of the compounds extruded to the surrounding water, which extensively absorbs UV, can be protective as a shield. This is what we observed under laboratory conditions, but under field conditions the extruded compounds would be quickly diluted. This suggests that there is still another explanation for the high concentration of extruded compounds —possibly the elimination of toxic compounds synthesized in the cells that, if retained within the vacuoles of these cells, might eventually precipitate the proteins, causing cell death. To explain the process, acidification of the lakes in this area should be closely monitored by forest managers, because leachate from forest soils and decaying plants, as well as exudate from living plants, is a substantial source of organic compounds and minerals usable by the fauna and flora in both forest and lake.

It is beneficial to leave behind undisturbed areas of forest larger than merely small clumps, because the smaller ones could be devastated by wind and the larger ones have the possibility of succession of one kind of forest by another, and continuous natural development of the old growth. The role of foresters should be to find such old growth areas, and recommend to governments protection of such areas. If the public is more aware of the process, the pressure on government could be exerted to designate more such areas as the Clear Lake Reserve.

Table 1.—Hemlock Seedling Growth and Phenolic Compound Concentration as Affected by pH and Light Conditions.

	Conditions					
pH	Full light growth	phenolics	Semi-shade growth	phenolics	Full shade growth	phenolics
		AU		AU		AU
5.5	++	100±18	+++	90±10	+++++	70±15
3.5	±	120±25	+	100±10	++	100±15

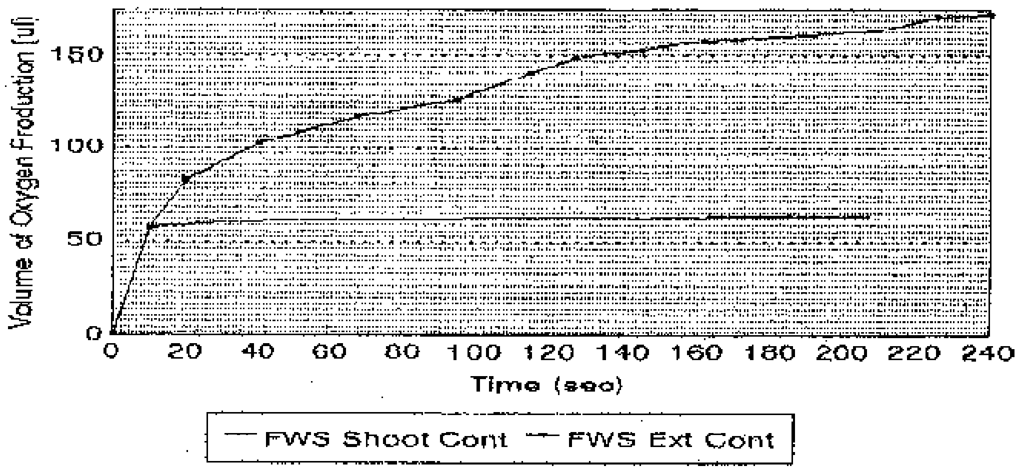

Figure 3.—Catalase-catalysed oxygen production during four hours after visible light treatment of *Eriocaulon*.

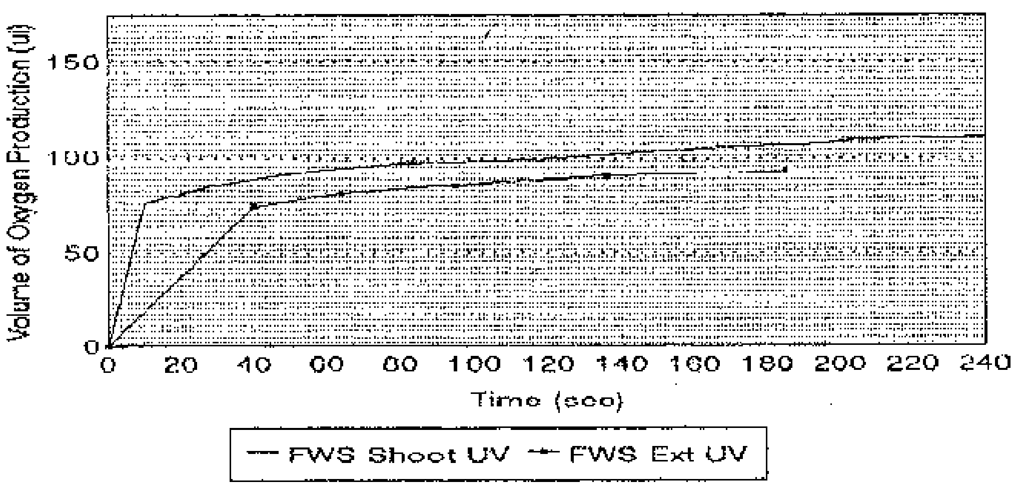

Figure 4.—Catalase-catalysed oxygen production during four hours after UV treatment of *Eriocaulon*.

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