Potentially Allelopathic Effects of Poison Hemlock (*Conium maculatum*) on Native Plant Revegetation at Wilder Ranch State Park

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Invasive exotics negatively impact native species composition in a variety of ways. Often these effects are indirect. For example, exotic species can reduce the fecundity of natives through modification of physical or chemical factors in the environment. These inducted effects mainly occur through either of two negative interference interactions. First, competition is a negative interference interaction in which resources are removed from the common environment by one organism, reducing the availability of that resource for another organism (Gliessman 1995). By competing with native plants for limited soil nutrients, moisture, light, etc, an exotic can reduce the supply of these resources and thus interfere with growth and establishment of other species.

However, suppression of growth cannot always be explained by competition (Altieri 1981). Amensalism is a second type of negative interference interaction. Whereas plant competition occurs through a reduction or removal of a growth factor needed by both plants, amensalism, and specifically allelopathy, occurs by the addition of a toxic factor to the environment (Altieri 1981, Gliessman 1995). Allelopathy is defined by the direct or indirect effect of one plant on another through the release of chemicals from the former into a common environment. Although still a mysterious area of chemical ecology, it is commonly believed that many plants are involved in such biochemical interactions.

Allelopathy assumes special significance when it involves exotic plants and issues of restoration. At Wilder Ranch State Park (WRSP), located near Santa Cruz on the central coast of California, wetland restoration and native plant revegetation strategies must take into account the presence of poison hemlock (*Conium maculatum*), an invasive exotic and highly toxic weed native to Eurasia. Considered "rather rare in California" in 1891 (Greene, qtd. in Robbins 1940), poison hemlock has since become highly invasive, spreading prolifically into disturbed areas along California's central coast (personal observation) as well as into the entire California floristic province (Hickman 1993). Most common in cool, moist regions, hemlock can be found throughout the United States excepting an area between central Montana and northeastern Minnesota (USDA 1971). Abroad, poison hemlock has become naturalized throughout many of the temperate regions of the world, including the Middle East (Ahmad et al. 1987), Australia (Auld and Medd 1987), and less successfully, in eastern Canada and British Columbia (Frankton and Mulligan 1970).

Through a series of both laboratory and field experiments at the Center for Agroecology at UC Santa Cruz (UCSC) and at WRSP, I sought to test the following hypotheses: first, that the proliferation of poison hemlock in natural areas such as Wilder Ranch is due in part to allelochernicals found in its leaves and stalks which inhibit the recruitment and survivorship of native species of the coastal bluff community; and second, that a particular management practice for hemlock at Wilder Ranch, which involves the cutting but not removal of hemlock just after flowering, merely increases the allelochernical content of the soil and resulting potential inhibition of native plant revegetation.

A leachate bioassay experiment was used as an initial screening technique of allelopathic potential; soil bioassays further examined this potential within soil collected from under a mature stand of poison hemlock; while the field experiment sought to examine potentially allelopathic interactions from an ecological perspective through the manipulation of hemlock vegetation. This research was based on my belief that any effective management program for exotic species must stem from as complete an understanding as possible of the interactions and mechanisms of successful establishment of these species within the community/ies they are affecting.

Materials and Methods

Initial Leachate Bioassays

Fresh leaves and canes from *C. maculatum*, as well as dried, previous season vegetation, were collected at WRSP. Extracts made from the freshly dried as well as previous season material were filtered under vacuum to obtain two leachates of 10% concentration. Forty grams of sand, a piece of Watman's filter paper, and 10 mL of the respective leachate or distilled water as a control were added to each Petri dish. Each of the three treatments was replicated three times. Ten seeds of the appropriate test species were placed in each dish after having been soaked for one hour in the leachate or control. The dishes were sealed with parafilm and incubated in darkness at 25'C for 72 hours. Root measurements and germination counts were taken after 72 hours or after germination took place.

Soil Bioassays

Soil to a depth of approximately one inch was collected from under a mature stand of *C. maculatum* in September and October of 1996 before the first seasonal rains at WRSP. On the same dates, soil was also collected from some distance outside the hen-dock stand. This second sample served as the "control" soil. This procedure was repeated again in February 1997 after a significant amount of seasonal rain in order to examine how rainfall would affect the potential allelochemical content of the hen-dock soil. All soil samples were sieved through a 5mm screen to remove large clods of dirt, roots and other vegetative material. Each glass Petri dish was filled with 40g of soil and a specified number of seeds of each test species. Fourteen mL of distilled water was added to each dish, which was then sealed with parafilm and either incubated in darkness at 25°C or subjected to a 12 hour photoperiod with natural temperature flux. The latter method, chosen when the prior was unsuccessful in achieving germination, involved placing the dishes by night in a glass-enclosed outer room and by day on inside windowsills which received partial sunlight.

After germination and growth occurred, germination counts, root, and shoot measurements were recorded. Seed from the eight native , test species and *C. maculatum* were collected at or near WRSP.

Field Experiment

The field experiment was set up in October 1996 on the top of a south facing slope at WRSP. An attempt was made to select a site which contained a well-established or "mature" stand of *C. maculatum* as well as a site near enough to possess similar abiotic conditions but with an absence of hemlock. Thus, nine $2m^2$ plots were located in a mature hemlock stand, while six $2m^2$ plots were located in a nearby grassland. A 50cm buffer zone was set between plots in each area. Various manipulations of hemlock vegetation on the soil surface constituted five different treatments, each with three replications.

Treatments within the hemlock stand were: (A) All hemlock vegetation removed from soil surface, (B) Hemlock vegetation cut and laid upon soil surface, (C) Control: hemlock vegetation (mostly withered standing canes by October) left undisturbed. Within the near-by grassland, treatments were: (D) All hemlock vegetation removed from Treatment A laid upon grassland surface, (E) Control: grassland left undisturbed. The dominant grassland species was Leymus triticoides.

The field experiment was composed of two parts. First, seed of yarrow (*Achillea millefolium*) and coast buckwheat (*Eriogonum latifolium*), two native species common to the coastal bluff area, was sown into subplots within each main treatment replication. Resulting influences of the different treatments on recruitment and survivorship were examined using a G-test, while height and mean number of leaves of these two species and of *C maculatum* were recorded through time. Second, a germinating weed seed bank analysis of vascular plants was performed in January 1997 in order to examine diversity and abundance of the weed seed bank in the different field treatments. For this analysis, five 10 cm rounds of soil were removed from each plot with a coffee can. After pooling the samples, all plant individuals were counted, identified, and weighed. Care was taken to remove soil from roots before weighing. Because the treatments within the hemlock stand showed variation between replications, the following dependent variables were analyzed using a two-way analysis of variance without replication: species richness, abundance, wet biomass, and the Shannon Index of diversity. The two independent variables for this test were treatment and distance from surrounding stand of hemlock.

Statistical Analysis:

Unless otherwise stated, all results were analyzed using a single factor analysis of variance, with nonparametric data being log-transformed prior to analysis and a significance level of P = 0.05.

Bioassay Results

The 10% new season *C. maculatum* leachate significantly inhibited both germination and root growth of the two crop and three native test species (See Fig. 1 for germination results). In some cases, inhibition was as high as 100% (*Eruca sativa, Nassella pulchra*). The one exception was the stimulation of germination of western dock (*Rumex occidentalis*). Although root growth of the two exotic grasses cultivated oat (*Avena sativa*) and ripgut grass (*Bromus diandrus*) was significantly lower under the 10% new season leachate, neither germination nor root growth was significantly affected under the 10% old season leachate.

The effects of soil collected before and after rainfall from under established *C. maculatum* stand on germination and growth of several native species were as follows. Although shoot growth remained for the most part unaffected in the different soil treatments, root growth was significantly inhibited in before rainfall hemlock soil for five of the eight native test species (Fig. 2). Similar results were obtained in hemlock soil collected after seasonal rains, with inhibition in some cases increased.

Although root lengths of yarrow (*Achillea millefohum*) and yellow bush lupine *(Lupinus arboreus*) were not significantly affected in the different soils before rainfall, root length in after rainfall hemlock soil showed significant inhibition. Effects on germination were dependent on species, with some species experiencing inhibition while others, most notably California figwort (*Scrophularia califomica*), experienced a higher germination in hen-dock soil. Results of the germination and growth of *S. califomica* in hemlock soil collected before rain were unique in that there was a significant stimulation of shoot and root growth as well as germination.

There were no significant effects on germination, root, or shoot growth of *C. maculatuin* in the different soils. Bioassay results of potential allelochemical effects of *C. maculatum* on itself are inconclusive at this point and require further study.

Field Experiment Results

Effects of manipulation of *C. maculatum* vegetation on recruitment and survivorship of two coastal natives and a weed seed bank were as follows. G-test results from the incorporated natives study indicated that recruitment and survivorship for *A. millefolium* (G = 761.85 and G = 26.01) and *E. latifolium* (G = 179.84 and G = 146.29) were dependent on treatment (at P = 0.01). The highest recruitment for both species occurred in the treatment where all hemlock vegetation was removed from the soil surface, while the lowest recruitment occurred in the treatment were all hemlock vegetation was cut and left to lie upon the soil surface (Fig. 3). Results of seedling survivorship followed a dissimilar pattern, with survivorship for *A. millefoliun* being highest in the cut and lay treatment, while for *E. latifolium* highest survivorship occurred in the control treatment of the hemlock stand plots.

Results of the germinating weed seed bank analysis did not indicate any significant differences in species richness, abundance, or Shannon Index of diversity in the different treatments. Effects on *C. maculatum* itself in the different treatments showed an interesting trend of highest mean seedling abundance, but correspondingly lowest mass per seedling (Fig. 4) and mean seedling height through time, in the removal treatment of the hemlock stand plots.

Results in the grassland plots were inconclusive due to the influence of the rhizomatous grass *Leymus triticoides*. Few seeds of *A. millefolium* (insufficient for statistical analysis), and none of *E. latifolium*, germinated in the grassland plots. Interestingly enough, there were no significant differences in weed diversity or abundance in the hemlock added versus control treatment of the grassland plots. Addition of *C. maculatum* litter had no effect on mean above-ground shoot abundance or mass of *L. triticoides*.

Discussion

The significant inhibition of root growth and germination of a majority of the species tested in the preliminary leachate bioassays indicate that freshly dried hemlock biomass is potentially allelopathic. However, the lack of significant results with previous season hen-dock biomass indicate that any potential allelochemicals do not remain persistent in hemlock vegetation itself, but may instead move fairly quickly into the soil.

In the soil bioassays, the significant inhibition of a majority of the native test species in hemlock soil collected before rainfall does indicate an allelochernical presence in soil under a well-established stand of *C maculatum*. Allelochernicals are commonly released to the surrounding environment through the processes of root exudation, litter decay, and/or precipitation from rainfall or fog. Although root exudation of allelochemicals was not addressed in this research, the allelopathic potential of leaves and stalks (present in a 10% leachate concentration) could very likely be the source for the inhibition present in the soil.

According to Fischer et al. (1994), winter rains often play a major role in the release and transport of allelochemicals. However, the strong level of inhibition experienced in soil collected before seasonal rains may implicate fog as a larger factor in allelochernical release in this case. The coastal location of WRSP is subject to intense summer fog, which could have worked to gradually leach toxins from vegetation into the soil. Results in soil collected after seasonal rainfall may indicate that there is some augmentation by winter rain; that hemlock allelochernicals may remain fairly persistent in soil from season to season; and/or that rainfall maintains a pattern of transport of these chemicals from newly emerging vegetation to replace subsequent leaching from the soil.

Stimulation caused by low quantities of allelochemicals is not unheard of (Rice, in Putnam and Tang 1986; Leather and Einhellig 1988; Gliessman 1995). The increased vigor in hemlock soil of a minority of species in the soil bioassays, most notably *Scrophularia califomica*, indicates that a few species are actually stimulated by a potential allelochernical effect.

The greater abundance, yet lower seedling height and mass of *C. maculatum* in the hemlock removal treatment of the field experiment, coupled with a greater mean height through time in the cut and lay treatment, indicates either an allelochernical or physical effect on its recruitment and vigor created by the removal and addition of hen-dock vegetation. It is possible that although the disturbance from vegetation removal stimulated seedling recruitment, increased exposure or density dependent factors lowered the overall vigor of hemlock seedlings in this treatment.

Management Implications

As to the second question posed in this research, does the increase in the amount of hemlock biomass in contact with the soil defeat the management goal of controlling the invasive effects of hemlock? In the field experiment, positive results of seedling recruitment of *A. millefolium* and *E. latifolium* under a hemlock removal regime, coupled with negative results when hemlock vegetation was cut and laid upon the soil surface, substantiate the hypothesis that the removal of hemlock vegetation would stimulate the recruitment of native species, while the cutting and laying of vegetation upon the soil would merely concentrate any potential allelochemicals from the vegetation into the soil. However, the low germination rates of both test species (30% and 10.7% for *A. millefolium* and *E. latifolium*, respectively, in the removal treatment) indicate that recruitment within the hemlock stand was generally unsuccessful, as these two species normally have high recruitment.

The lack of a corresponding pattern in survivorship and overall "vigor" of the two coastal natives in the different treatments substantiates the earlier hypothesis that allelochemicals present in hemlock vegetation do not long persist, and indicates that such differences from the manipulation of hen-dock vegetation were significant only at the onset of the field experiment. Combined with the insignificant effects of treatment on the germinating weed seed bank study, it appears that physical influences were important factors as well and became more significant through time.

From this field experiment it does appear that a management regime of poison hemlock such as that of WRSP has the potential to negatively affect the recruitment of other, native plants. However, effects on native plant survivorship and vigor are questionable at this time. A more accurate depiction of any potential allelochemical effects could be gathered from a repetition of the field experiment in June or July, at the height of hemlock's flowering period. A subsequent examination of seedling recruitment and survivorship in the fall, rather than setting up the experiment in October after the canes have already been significantly weathered, would allow results to be gathered following an entire season of summer fog to potentially leach allelochemicals from freshly cut plant material into the soil. More closely tailoring a field experiment to the phenology of hemlock and the timeline of a typical management plan such as mowing before seed set might shed more light on these issues.

Conclusions

My field observations of the prolific, early seedling vigor of *C. maculatum* in moist, disturbed areas lead me to believe that early competitive advantage is an important factor in its establishment. However, its relatively slow germination rate in the soil bioassays I performed, coupled with the high number of qualitatively unviable seeds (I have noticed an incredible loss of whole umbel heads to insect predation), indicate somewhat of a paradox in its establishment which could be explained in part by allelopathy. In this sense, even if it is not actively being stimulated by allelochernicals, the inhibition of recruitment of other plants through such a mechanism would reduce or even eliminate early competition, offering poison hemlock an advantage until its own recruitment and phenomenal burst of seedling growth.

In summary, I believe that *C. maculatum* is a weed that deserves increased attention and an active management plan. The methodology of such a plan should take into consideration the potentially allelopathic characteristics of this invasive plant. With vegetation that is allelopathic, the mowing or cutting but not removal of such vegetation may pose problems with native seed bank recruitment; similarly, the disturbance favored by hemlock for its own recruitment indicates that a removal regime must be comprehensive through time as well as space until the seed bank itself has been significantly reduced. A timed mowing regime just after flowering or before seed set is a common management practice used by many land managers; however, according to Huxtable (1993) the alkaloid content of this plant (which is most likely responsible for its potentially allelopathic properties) is concentrated in the flower heads.

A study by Fairbairn and Ali (1968) found that during fruit development of *C. maculatum*, plant-produced chemicals are converted "fairly rapidly into coniine and into other bound forms of the alkaloids" (also see Grieve 1931). Thus, a mowing regime at this stage in the life cycle may merely concentrate allelochemicals in the soil. One interesting management altemative involves the use of a biological control agent. There is some evidence that the leaf-tying moth, (*Agonopterix alstroemeriana*, Oecophoridae), can arrest the growth and seed production of poison hemlock through defoliation and its use of hemlock as a host plant (pers. comm. Ronnie Ryno; Savela 1997). Further research into this and other management altematives, coupled with increased attention and focus on the natural history and biochemical processes of poison hemlock, will greatly improve our understanding of its mechanisms of invasion and establishment and will provide the tools for its effective control. In our ongoing efforts to combat the advancement of invasive species, we must recognize the tendency for different factors to work together to interactively affect patterns of species establishment and persistence. The more we explore these factors, the greater will be our understanding of how to manage natural ecosystems to promote ecological integrity.

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