2413

Bioresponse of Nontarget Organisms Resulting from the Use of Chloropicrin To Control Laminated Root Rot in a Northwest Conifer Forest:

Part 2. Evaluation of Bioresponses

by

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Known impacts of chloropicrin

Most research has concentrated on the effects of combinations of methyl bromide and chloropicrin fumigants on disease-causing organisms. Little research has been reported on the response of saprophytic fungi to chloropicrin. Some evidence exists for the escape of some species of fungi during fumigation efforts thus positioning the survivors for rapid recolonization of the available substrate. The occurrence of *Trichoderma* spp. in roots of fumigated Douglas-fir stumps was noted during the evaluation of several fumigants for the control of laminated root rot (Thies and Nelson 1982). Following fumigation, increased numbers of *Trichoderma* spp. were found in some soils (Mughogho 1968). Combinations of methyl bromide and chloropicrin control a variety of soil-borne diseases in forest tree nurseries (Peterson and Smith 1975) and can reduce populations of various other fungi. These include VA mycorrhizal fungi (Jones and Hendrix 1987, McGraw and Hendrix 1986), ectomycorrhizal fungi (Trappe et al. 1984), as well as root disease-causing species of *Pythium, Rhizoctonia, Phoma* (Sumner et. al. 1985), *Helicobasidum momta*, (Sakuwa et al. 1984), *Verticillium albo-atrum*, and *Sclerotium* (Himelrick 1986).

Nematode populations are reduced by chloropicrin alone and in combination with methyl bromide or dazomet. Research has concentrated on the commercially important parasites of plant roots and no information was found on free-living fungal or bacterial feeding nematodes. Fumigation reduced the nematode root pathogens *Meloidogyne* spp. and *Paratrichodorus minor* (Sumner et al. 1985) and chloropicrin in combination with ethylene dibromide reduced *Belonolimus longicaudatus, Meloidogyne incognita*, and *Hoplolaimus galeatus* (Rhoades 1983).

As a measure of species richness, soil arthropods in conifer forests of the Pacific Northwest average nearly 200 species/m^2 (personal communications Andrew R. Moldenke, Oregon State University, Corvallis, OR). Thus soil arthropods may be an important and sensitive indicator to evaluate potential ecosystem effects of chloropicrin on nontarget soil organisms. Soil arthropods are sensitive indicators for soil moisture, successional stages, plant communities, and mycorrhizal biomass (Cromack et al. 1988, Moldenke and Fichter 1988). All arthropod species are presumed to be sensitive to chloropicrin, although differing feeding preference, microhabitat choices, and position in the food web will expose the diversity of species to different concentrations of fumigant.

Objectives

One objective of this study was to determine changes in diversity of specific nontarget organism components of a coastal ecosystem which occur as a result of application of chloropicrin to stumps to control laminated root rot. A major challenge was to evaluate the impact of chloropicrin on a range of organism groups. A sample scheme capable of distinguishing at least two levels of spatial variability was designed. Background variability of the organisms resulting from heterogeneity in soil microsites and seasonal shifts is a common difficulty in studies of soil organisms. Sampling intensity had to be great enough that treatment effects could be assessed, without increasing the work load beyond that of a limited budget.

The approach described here can be applied to any ecosystem, and to a number of situations where effects of pesticide application need to be assessed. With this approach, the effect of pesticide on plants, soil detrital foodweb organisms, mycorrhizal colonization of dominant plants, and survival of sensitive bioassay

plants can be interpreted in a biologically meaningful manner at a number of spatial scales. For example, the pesticide may reduce root rot, but destroy those organisms responsible for nutrient cycling, or could allow another pathogen to become a problem, exchanging one pathogen problem for another. Root-feeding nematodes might be advantaged, resulting in seedling death. Alternatively, greater mycorrhizal colonization of roots might occur, and benefit the survival of desired plant species. Before we continue with the use of pesticides, we should attempt to understand beneficial or detrimental interactions which may be produced. Our approach allows us to assess these possible beneficial or detrimental effects.

Experimental site

When the experimental site was cut early in the fall of 1988, see Part 1. of this study, p. 81) soil consisted of a moss (bryophyte) layer overlying poorly developed litter, and fermentation layers, with a 1-5 cm depth humus soil horizon resulting from the rapid decomposition rates in these systems. When soil development is limited, nutrient cycling is usually tightly coupled to organism dynamics (Read and Birch, 1988; Perry *et al.* 1989). When litter falls to the forest floor, it is rapidly decomposed, and the nutrients converted to microbial biomass. These nutrients are then released by arthropod, nematode and protozoan grazing of decomposers, resulting in high soil fertility and maximum nutrient availability to plants during times of rapid plant utilization (Coleman 1985). However, with clearcutting, much of the thin soil layer was destroyed and mineral soil revealed.

The location of each infected stump in the clearcut stand and three mature trees in adjacent uncut stands were mapped. Circular treatment plots 0.4 ha in size were established in the clearcut area and blocked into groups of four based on similar inoculum rating (see part 1 for explanation). Each of the four plots in each block received one of the following treatments: all stumps treated with 100% chloropicrin, only infected stumps treated with 100% chloropicrin, all stumps treated with 20% chloropicrin, and a control plot with no application chloropicrin. The 100% label dosage was 3.3 ml of chloropicrin per kilogram of stump and root biomass.

Choice of bio-response parameters

Bio-response assessment was initiated in the spring of 1989. Five major component groups of organisms were assessed; the above ground plant community, detrital foodweb organisms, mycorrhizal colonization of Douglas-fir roots, and the response of chloropicrin-sensitive plants. These assessments will continue until the fall of 1991.

Soil foodweb organisms respond more rapidly than plants to environmental change and disturbance. Responses of bacteria and fungi often reflect day-by-day fluctuations in temperature, moisture, grazing, and nutrient availability, and thus are not useful as measures of ecosystem response to disturbance. However, by examining changes in activity, in ratios of fungal to bacterial biomass, or important populations of microorganisms, longer term impacts on the system can be assessed.

Protozoa, nematodes and microarthropods are intimately involved in nutrient cycling (Coleman 1985) and thus are good indicators of ecosystem health (Ingham et al. 1985; Ingham and Horton, 1987). Mycorrhizal fungi are extremely important in survival of Douglas-fir; in field sites, Douglas-fir is not found without the symbiotic fungus (Trappe et al. 1984). Thus, monitoring mycorrhizal fungi can be extremely important in determining if pesticides have an effect.

Tomatoes and alfalfa were planted in the field sites. Tomato is extremely sensitive to chloropicrin (Rhoades 1983), and alfalfa is symbiotic with N-fixing rhizobium. Release of chloropicrin in field soils could be monitored with tomato, and natural populations of rhizobia could be assessed by examining the roots of the alfalfa.

Spatial scales

Within the 0.4 ha circular plots, responses were monitored on several spatial scales, as well as over time.

Aboveground higher plant responses were assessed each spring and fall by monitoring percent cover of each species present,

(1) in the entire plot,

(2) in six 2 m X 3 m plots arranged sequentially at 1, 2 and 3 meters from four individual stumps at the edge of the plot, and

(3) in six 1 meter square plots located between two stumps within 2 meters of each other.

Detrital foodweb responses (numbers and activity of bacteria, active and total fungal biomass, and numbers and community structure of protozoa, nematodes, and microarthropods) were monitored over time. Samples were taken mid-spring, early

summer, late summer, and mid-fall each year.

To determine if soil organism numbers, activity or community structure changed on a whole plot spatial scale, twelve random points were sampled in each of five plots per treatment on the four sample dates each year. The same four random sets of coordinates were designated in each third of the plot to maximize coverage of the area.

To determine if a localized effect of chloropicrin treatment occurred, soil samples were taken at 4 distances (0.5, 1.0, 1.5, and 2.0 m) along three equally spaced radii extending from tree stumps (five separate tree stumps per treatment). While soil samples for microarthropods were taken from each point, samples for the other organisms were bulked by distance. Since it was chloropicrin treatment, not soil heterogeneity, that was being assessed, averaging the differences resulting from soil microsite heterogeneity was acceptable.

On each sample day, the actual point from which soil was removed was repositioned from the original marker by pre-determined distances. A 7.5 cm diameter, 7.5 cm deep sample of soil was removed from each point for microarthropod estimates. An approximately 2 cm diameter, 5 cm deep soil sample was taken from each point, but soils from each of the three quadrants (i.e., 4 soil samples) were bulked for bacteria, fungi, nematodes, and protozoa assessments.

Active bacteria, active fungi, and total fungal biomass were assessed by the FDA method of Ingham and Klein (1984). Total bacterial numbers were assessed by FITC staining (Babiuk and Paul, 1970). Protozoa were determined by MPN and direct microscopic viewing (Darbyshire, et al. 1974). Nematodes were assessed by Baerman extraction and microscopic observation (Anderson and Coleman, 1977). Microarthropod numbers were assessed by high efficiency Tullgren extraction and observation (Merchant and Crossley 1970).

Chloropicrin-sensitive bioassay plants (tomatoes and alfalfa) were planted each year in the spring at 1 and 2 meters distance from stumps of five trees of each treatment type and were randomly placed in another five plots of each treatment. Survival was assessed on each sample date. In the second year, a ring of alfalfa was planted at 1 meter distance from each stump. All alfalfa plants were examined for N-fixing bacteria nodules on their roots at the end of the second year.

RESULTS

Chloropicrin application was not the only environmental variable to which these sites were responding. Two extremely important correlated variables were (1) removal of canopy cover and (2) compaction of the soil by heavy machinery. All the experimental sites were exposed to both, either of which may have ecosystem effects equal to or greater than the chloropicrin treatments. The three types of plant plots, the two types of detrital foodweb organism plots, placement of chloropicrin-senstitive plants, and placement of Douglas-fir seedlings to assess mycorrhizal colonization allowed assessment of bio-responses to these environmental variables.

In addition to compaction and canopy removal, we observed a gradient of organism numbers and community structure from north to south and east to west in this stand. The blocking initiated at the beginning of the study based on *Phellinus* inoculum density in stumps reflected changes in soil organism community structure, and was related to surface soil characteristics. We use these block effects as covariates in statistical analyses.

Pesticide application did not impact establishment, growth or survival of any plant species in the first year after application of chloropicrin.

In the first year, chloropicrin impacted soil bacteria, fungi, protozoa, and nematodes only in a few isolated points near stumps:

(1) area 8, point 2, 20% chloropicrin treatment,

(2) plot 2, 20% treatment, 2.0 distance,

(3) plot 602, 100% treatment, 2.0 distance, and

(4) plot 736, 100% treatment, 2.0 distance.

When these single points were averaged with all five replicates from a treatment, variance was significantly increased, but no significant treatment effect was observed.

In these isolated cases, reductions in numbers of organisms were considerable, from around 10^7, or 10 million total number of bacteria per gram soil to less than 100,000 per gram soil in impacted points. Fungi normally measured about 600 m of hyphae per g with between 10 and 50% of those hyphae active, dependent on season. In impacted areas, less than 5 m of hyphae per g were found, with no active hyphae present. Protozoa tended to number around 10,000 per g soil, but in impacted soils, were less than 10 per g. We have not finished assessing protozoan community structure, but no

immediately obvious changes were detected in the first year. In control soils, nematodes tended to number around 100 per 5 g soil, but in impacted soil, less than 10 per 5 g soil were found. However, no significant changes in nematode community structure were observed in the first year.

Microarthropod community structure has been assessed for the first year, and it is clear that though individual species respond both positively and negatively to a chloropicrin-fumigated environment, these responses were not of great enough magnitude to radically alter total densities or biomass either around individual trees, or in whole plots. However, in tree-centered samples, guild diversity was decreased in the 20% treatments, and comparisons of individual species showed that 15 of the commonest species decreased with increasing dosage, 8 increased with dosage, 2 were highest in 20%, and 3 were lowest in 20% chloropicrin treatments. The commonest species of springtail was unaffected by chloropicrin, whereas most oribatid species were affected, although increases and decreases tended to cancel out overall effects. Coupling these analyses with covariate information (removal of canopy cover, compaction, plant diversity, etc.), should lead to identification of a small set of indicator taxa that can be used for more efficient monitoring of similar situations.

In the second year, the areas impacted in the first year expanded, and four to five new points of impact were observed. The position of newly impacted points appear random. Complete analysis of variance has not been performed at this time, because not all samples have been completely analysed.

There was no difference between survival of tomato plants in year one in any treatment, but in year two, more tomato plants died in the 100% treatments than in other treatments. There was no significant difference in nodulation of alfalfa roots in any plot in any year.

CONCLUSIONS

In the first year after chloropicrin application, reductions in organism numbers in a few isolated points and changes in individual species of microarthropods were significant on the spatial scale of a single stump. These impacts could be important to a new seedling trying to obtain nutrients from the soil in an impacted area. On an larger spatial scale, such as the 0.4 ha plots, and certainly on an ecosystem-level, the impact was not significant, based on plant response (no impact on plant community structure or on chloropicrin-sensitive bioassay plants) or on numbers of bacteria, fungi, protozoa, nematodes or microarthropods.

Will the impact be detrimental or beneficial, in the long term? In the second year, numbers of plant-feeding nematodes have increased from barely detectable numbers present in soil to comprising up to 50% of the nematode population in some samples in the second year. This could be detrimental to Douglas-fir seedlings, but this is a stand-wide effect, not an effect of chloropicrin application. In fact, those points impacted by the chloropicrin have below-detection level numbers of nematodes, and so, chloropicrin application could be beneficial for Douglas-fir seedlings growing in those areas, because the plant-feeding nematodes have been negatively impacted in those places.

The increase in plant-feeding nematodes has coincided with increase in exotic weed species on these sites. Plant-feeding nematodes may be attacking the roots of these weedy species and thus be reducing competition between the weed species and Douglas-fir seedlings. In areas impacted by chloropicrin, it may be that the weeds don't suffer limitation by root-destroying nematodes and the Douglas-fir seedlings will experience increased competition. We will be able to assess this interaction by continued examination of the soil and the plant communities over time.

All of our information supports the conclusion that very little chloropicrin escaped from roots in the first year. Only at a few points, not significant on an ecosystem scale, were effects detected. In the second year, responses of soil organisms and chloropicrin-sensitive plants provided information that more chloropicrin escaped from roots, but still not enough to result in an effect on the plant community. Early warning that potential effects may occur is being provided by the soil organisms, but we don't have the database to tell us if this means an overall detrimental effect on the ecosystem, or an overall beneficial effect on the ecosystem.

We know that the fungus that causes laminated root rot is being killed in these stumps (Thies and Nelson, 1985). Within the stump, other organisms are being killed, and it is likely that wood decomposition is being slowed. Is that positive or negative? We don't know. What is the balance sheet going to indicate in ten years? Will the detrimental effects outweigh the positive?

Whatever happens in this particular ecosystem, however, the type of sampling being done,

.limited as it is, allows biologically meaningful conclusions to be made about the impact of this pesticide in this ecosystem. This approach of assessing bio-responses gives us the ability to make predictions about the possible trajectories this ecosystem may take. We have the means to make useful predictions, and by looking at this suite of organism responses, we can indicate problem areas before a situation may result in irreversible loss of a particular habitat or species.

So, is chloropicrin use detrimental? If the points where organism numbers have been negatively impacted don't spread farther than they have in the second year, and if the pathogens don't cause a problem as impacted areas are re-colonized, the liklihood is that the pesticide should continue to be registered for this use, with the clear explanation that higher doses, applied in a different manner, might be detrimental. However, impacted areas are likely to continue to expand, because not all the pesticide has volatilized from the stumps. How far will the affected areas expand? Will pathogens colonize the center of these impacted areas and cause problems? Will we select for worse disease problems by using this pesticide? Once all the chloropicrin is out of the stumps, how long before affected areas will be re-colonized by the normal organisms? Or will these areas be pushed into completely different ecosystem trajectories, similar to what has occurred in some plots in southern Oregon with completely different disturbances (Borchers and Perry, 1989)? Answers to these questions are not available. But continued monitoring in this system will allow these questions to be answered for this system. Extrapolations can be made to other systems with the understanding that differences between another system and this one must be understood in predicting possible impacts.

COOPERATION

The following organizations are cooperating in support of this study: Simpson Timber Co.; Great Lakes Chemical Co.; National Agricultural Pesticide Impact Assessment Program (NAPIAP), US Department of Agriculture; Pacific Northwest Research Station, US Department of Agriculture, Forest Service; and the departments of Forest Science, Botany and Plant Pathology, and Entomology, Oregon State University.

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