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Detection of Bromacil Herbicide in Ponderosa Pine

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Bromacil is a substituted uracil herbicide, 5-bromo-3-sec-butyl-6-methyluracil. It is a photosynthesis inhibitor, which apparently affects the Hill reaction in photosystem II (HILTON et al. 1964, HOFFMAN et al. 1964). It has a low oral toxicity and causes minimal effects on soil microorganisms (SHERMAN & KAPLAN 1975, WEED SCIENCE SOCIETY OF AMERICA 1974). Because it is readily absorbed through the root system of plants, bromacil usually is applied to the soil as an aqueous solution or suspension during or just before periods of active plant growth. It is not readily adsorbed onto soil particles and may be persistent in the soil for more than one growing season, having a half-life of five to six months (GARDINER et al. 1969, WEED SCIENCE SOCIETY OF AMERICA 1974).

Until recently, bromacil (Tradename - HYVAR) was used as part of a vegetation control program along roadways at the Los Alamos National Laboratory. There are about 180 miles of paved roads on the Laboratory property, and vegetation control is required for both safety and road surface maintenance. The prescribed method of application was to spray a four-foot wide strip of bromacil solution along the edges of roadways with a spray-bar. The application rate was 2.5-5.0 lb/acre. The application usually was made in the fall of the year so that melting snow in the spring would leach the bromacil into the soil, from which it would be taken up by the vegetation during the spring growing season. During the late spring and early summer of 1978, bromacil was determined to be the proximate cause of damage to numerous trees at

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substantial distances away from roadways at Los Alamos. This paper describes the investigation that was undertaken to determine the cause of the tree mortality.

MATERIALS AND METHODS

An initial visual appraisal of the dead and dying trees was made by personnel from the Environmental Surveillance Group at Los Alamos and by U.S. Forest Service insect and herbicide specialists from the Albuquerque office. Ponderosa pine needles were collected and analyzed for chloride content and for bromacil residues. Chloride analyses were performed by neutron activation analysis (NADKARNI & MORRISON 1973). Bromacil residues were determined by gas chromatography. The procedure used was a modification of that described by PEASE (1966). This procedure is described below.

Method - Bromacil concentrations were measured by gas chromatography-mass spectrometry using mass fragmentography to reduce any possible interferences from background. Three samples were analyzed: pine needles from affected trees, unaffected pine needles from trees where no spraying had occurred, and unaffected pine needles that were treated with bromacil in the laboratory. The extraction method was essentially that of PEASE (1966). One hundred grams of needles were homogenized with 500 mL of 1% NaOH solution for 5 min. The homogenized material was vacuum filtered through Whatman 42 paper. The retained solid was extracted a second time with 400 mL of 1% NaOH and vacuum filtered. The combined filtrates were neutralized with 10 N H_2SO_4 and extracted with 250 mL $CHCl_3$. The extraction was repeated three times, and the combined $CHCl_3$ portions taken to dryness at room temperature. The residue was transferred to a separatory funnel using 200 mL of 1 N NaOH and 200 mL n-hexane. After agitation, the hexane was separated and discarded. The NaOH layer was extracted twice with ethyl acetate. The residue from the ethyl acetate evaporation was transferred to a separatory funnel using 50 mL nitromethane followed by 50 mL hexane. After a second wash with 50 mL hexane, the nitromethane layer was evaporated nearly to dryness, transferred to a 5-mL volumetric flask and diluted to volume with nitromethane. This solution was used for analysis.

Equipment - All analyses were performed on a gas chromatograph-mass spectrometer operated in the full mass scan (30-350 amu) mode. Electron impact ionization (70 eV) was used. The chromatographic column was a 1.8 m x 2 mm ID glass column packed with 3% OV-101 on 100/120 mesh Chromosorb W HP, operated with 30 cc/min He flow, temperature programmed from 125 to 250°C at 16°C/min. The injection port was held at 280°C. A silicone membrane separator maintained at the GC oven temperature was used as an interface to the mass spectrometer. The mass spectrometer was operated with a transfer line temperature of 250°C, an ion source temperature of 145°C, and an analyzer temperature of 100°C. Mass chromatograms were reconstructed and chromatographic peak areas were determined using the standard program features of the 5934A data

system.

Interpretation - The possibility of interferences in gas chromatographic procedures can be reduced by using compound selective monitoring techniques such as mass fragmentography. A solution of bromacil in nitromethane was used to establish the bromacil retention time and mass spectrum. From this information, mass fragmentography using chromatogram reconstruction on the bromacil fragment ions at m/e 205, 207 and 260 appeared to uniquely identify and quantitate bromacil from the extraction procedure. These ions are the two strongest ions (205 and 207) in the bromacil mass spectrum and the molecular ion (260). The ratio of ion intensities and the gas chromatographic retention time serve as unique identification of bromacil, and the area of the peaks obtained from the reconstructed chromatograms serve to quantitate the bromacil. The reconstructed chromatograms of the unaffected needle sample, the affected needle sample, and the unaffected needle sample with added bromacil are shown in Figs. 1-3. The area of each mass chromatogram peak for the blank plus bromacil chromatogram was calculated and used to approximate the concentration of bromacil in the needle sample.

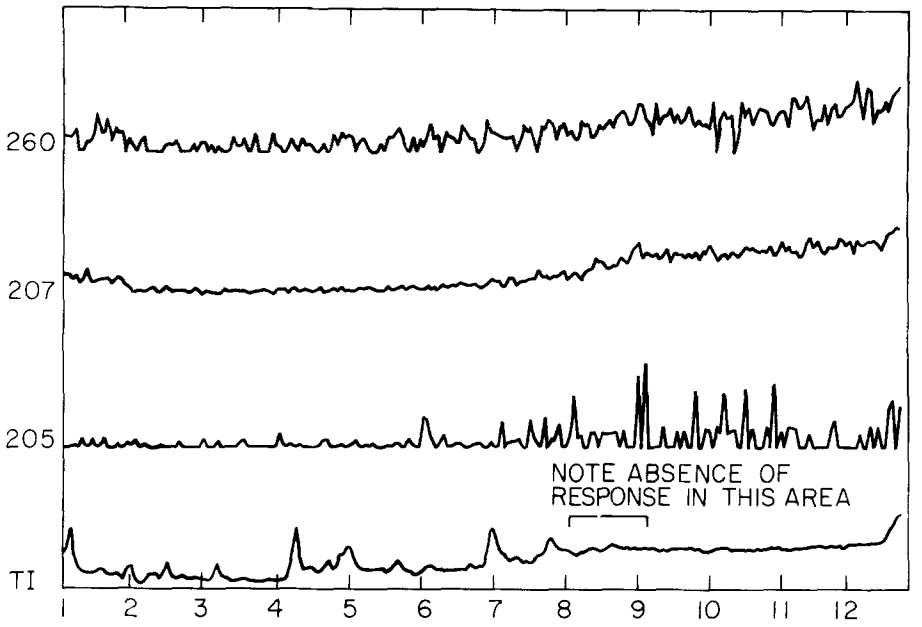

Fig. 1. Chromatograms for an unaffected pine needle sample analyzed for bromacil.

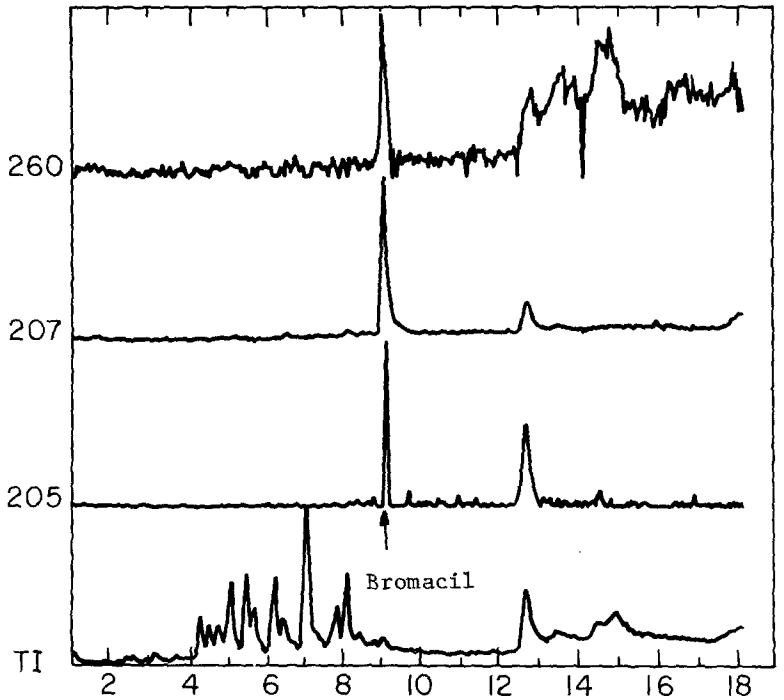

Fig. 2. Chromatograms for an affected pine needle sample analyzed for bromacil.

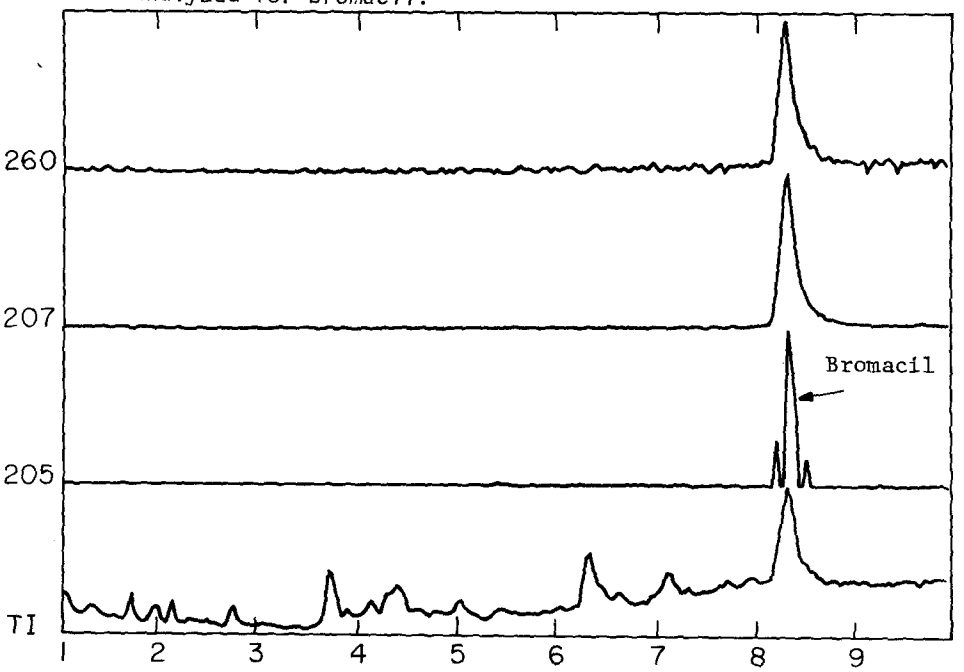

Fig. 3. Chromatograms for an unaffected pine needle sample fortified with bromacil.

RESULTS AND DISCUSSION

Visual estimates placed the vegetation damage at 2500-3000 trees killed or severely damaged. Although the foliage of some trees was not entirely brown, the percentage of dead needles on these trees was great enough to virtually assure the subsequent death of the tree. Those trees not killed outright will suffer several years of reduced growth. Affected trees included ponderosa pine, piñon pine, and juniper, but the ponderosa pine sustained most of the damage. Damage generally was confined to a 30 to 50 ft strip along each side of the road, although in drainage areas damage extended farther from the road. Examination of the affected area showed no evidence of insects as causal agents. The appearance of the needles on the damaged trees was not characteristic of either insect or salt damage. The needles were entirely brown, desiccated in appearance, and very brittle. Salt damage generally is characterized by chlorosis and eventual browning of the tips of the needles. Furthermore, chloride analyses performed on the foliage showed the chloride concentrations to be below those associated with salt damage in previous investigations in Los Alamos County (MENLOVE 1973, WALTERS 1977).

Results of bromacil analysis on random tree foliage samples showed that there were bromacil residues in the foliage of the affected trees. Bromacil concentrations in the foliage were estimated to be in the range of 30-50 ppm (fresh weight). Figure 2 shows the bromacil residue peak elicited from the foliage of damaged trees. Figure 1 shows the absence of this peak in unaffected trees, and Fig. 3 shows the reappearance of the peak in foliage of unaffected trees that was fortified with bromacil.

Bromacil is known to be mobile in the soil (ZANDVOORT et al. 1980). Nevertheless, the appearance of the bromacil residues in the foliage of some of the damaged trees was unexpected because of the distance of these trees from the zone of bromacil application. There probably are several reasons for this. One is that trees, particularly coniferous trees, are especially sensitive to bromacil. Manufacturers' literature and labels caution against "use on right-of-ways or other sites where marketable timber or other desirable trees or shrubs are immediately adjacent to the treated area." Los Alamos National Laboratory application instructions specified that bromacil was not to be applied within several feet of desired vegetation (trees). The bromacil, however, was more mobile than anticipated. The situation was aggravated by the unusual climatic conditions during the winter of 1977-1978. Little snow fell during this winter, and so the bromacil was not leached into the soil in the spring. Heavy rains in the fall and early spring washed the herbicide away from the pavement edge, accounting for the damage extending into drainage areas.

Dead trees also were found in areas upslope from the road. This probably occurred because tree roots grew into the ditches along the road as sources of water. Large pine trees can have roots that extend out to three times the drip line diameter. Individual tree root systems also can anastomose as the trees mature. These phenomena could explain the translocation of bromacil to trees located uphill and at some distance from the road.

Although wind drift during the application of the bromacil solution and weakening of trees by road salt could have played some role in the observed tree mortality, the major cause of damage appeared to be the unanticipated movement of the bromacil away from the roadside. The indication is that bromacil, already known to be a potent herbicide, should be used with even more caution and under more stringent application conditions than normally might be considered necessary. The problem at Los Alamos has been alleviated by more careful herbicide application procedures and by switching to an herbicide that is less toxic to coniferous vegetation.

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