United States
Department of
Agriculture

Forest Service

**Southern Forest
Experiment Station**

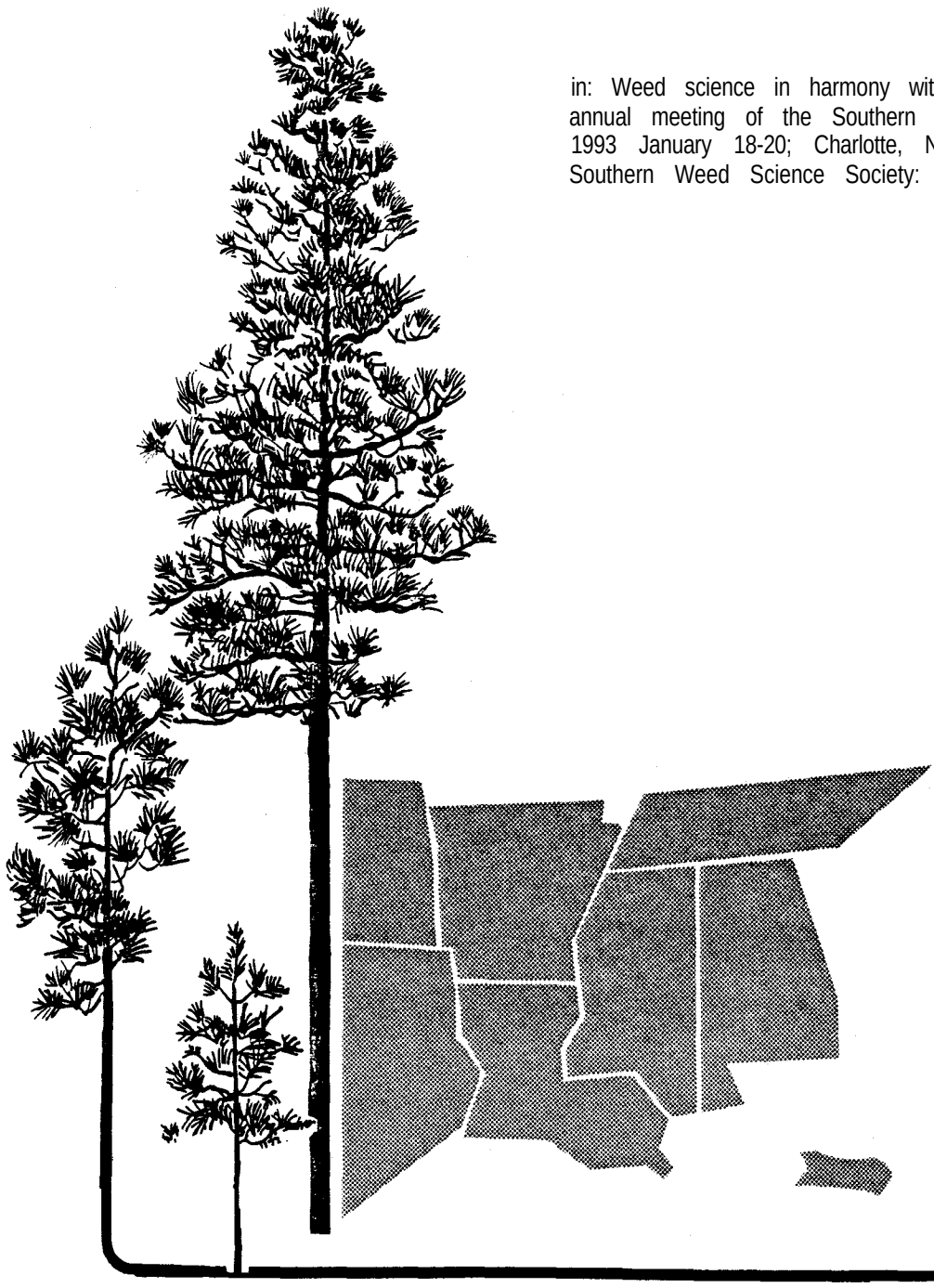
New Orleans,
Louisiana

Proceedings Reprint

A REVIEW OF PROMISING NEW IMMUNOASSAY TECHNOLOGY FOR MONITORING FOREST HERBICIDES

McMahon, C.K.

in: Weed science in harmony with the environment: 46th annual meeting of the Southern Weed Science Society; 1993 January 18-20; Charlotte, NC. Champaign, IL: Southern Weed Science Society: **220-222.**



Southern Forest Experiment Station

A REVIEW OF PROMISING NEW IMMUNOASSAY TECHNOLOGY FOR MONITORING FOREST HERBICIDES. C.K. McMahon, USDA Forest Service, Southern Forest Experiment Station, Auburn, AL 36849.

Rising costs of classical instrumental methods of chemical analysis coupled with an increasing need for environmental monitoring has lead to the development of highly sensitive, low-cost immunochemical methods of analysis for the detection of environmental contaminants. These methods known simply as immunoassays are chemical assays which use antibodies as reagents. A target compound (such as a pesticide) is detected by an antibody which binds only to that substance. The binding efficiency can be designed to permit measurements down below the picogram (10^{-12} gram) level. Some of the assays can be performed in a complex sample matrix with little or no sample preparation. The technique allows for a rapid analysis at a relatively low cost ($5-10 per sample) compared to classical chromatographic and spectrophotometric techniques ($40-200/sample). Allied instrumentation such as low-cost photometers as well as the associated training of technical staff is much less complex and costly than classical techniques.

Immunoassays can be either a qualitative (yes/no) or quantitative test. It is very rare for an immunoassay to give a false negative if procedures are carefully followed, however some false positive results do occur. Immunoassays are currently being used primarily as a screening technique that augments rather than replaces existing technology. In a screening application only the positive immunoassay results are confirmed with classical chemical assay methods leading to the obvious savings in time and cost.

Immunoassays are not an entirely new technology. These methods were first introduced in the 1960's as a preferred analytical method in clinical and forensic laboratories to detect a wide range of hormones, drugs, and viruses. What is new is the application of immunoassays to the field of environmental monitoring and the development and marketing of test kits for home and professional use. You can now buy an immunoassay test kit for pregnancy hormones at your local drug store and you can obtain test kits for selected pesticides from several vendors including Agri-Diagnostics Associates (Moorestown, N.J.); Enzytec Inc. (Kansas City, MO) Millipore Corp. (Bedford, MA); and Ohmicron Corp. (Newtown, PA).

The initial source of antibodies used in immunoassays is an animal such as a mouse, rabbit or guinea pig which can be injected and immunized with the target substance, thereby producing antibodies with the desired characteristics. This is not as easy as it sounds since animals of the same species can produce antibodies with different characteristics. On the other hand, over time one animal can yield enough antibodies for millions of tests. Polyclonal antibodies which are taken directly from the animal tend to recognize and bind to several of the molecular characteristics of the target compound; whereas, monoclonal

antibodies (developed from specific cells taken from the animal and grown in the laboratory) tend to recognize and bind to more specific structural sites. Thus polyclonal immunoassays are generally much more sensitive but less specific than monoclonal assays.

Until recently small molecules (such as most pesticides and other "environmental" chemicals) could not directly stimulate antibody production in an animal. To overcome this, research immunologists found that they could convert a small molecule into an immunogen (capable of producing antibodies) by covalently attaching the pesticide to a larger protein carrier macromolecule. Once the antibody with the desired characteristic is obtained the next step is to develop some way to quantify the antibody response using a radioisotopic, fluorescent, or enzyme "tag." The immunoassay method receiving the most attention today is know as ELISA (Enzyme-linked immunosorbent assay) in which the final step yields a visible color comparison between the unknown and a set of standards. This color response is read optically or with a simple photometer. From this rather complicated process, a method can be developed for a specific herbicide such as atrazine or for a family of herbicides such as the triazines. It takes a highly skilled multidisciplined team of scientists approximately 3 months to a year to develop a prototype immunoassay method, and often 2 years to develop a fully validated method, and a commercially available kit. The costly developmental process is currently a major drawback for developing immunoassay methods for limited pesticide markets such as forestry herbicides. The federal (EPA, FDA, USDA), state, and private institutions which are developing and evaluating immunoassays for environmental substances have given first priority to compounds which are widely used, have moderate to high toxicity and/or have been found as contaminants in national or regional water testing programs. Thus compounds such as pcb's, agrochemicals and home-use pesticides have been given priority.

There are several dozen reports in the literature describing immunoassays for pesticides. However, fewer than two dozen pesticide kits are commercially available. At the present time only ten ELISA kits are available for herbicides; alachlor, atrazine, cyanazine, 2,4-D, imazaquin, isoproturon, metolachlor, paraquat, trifluralin, and a kit for the triasine family. This is not an exciting list for those of us in forestry weed science. However, we have recently learned that prototype kits are now under development or being considered for glyphosate, imazapyr, metsulfuron, and triclopyr. A broadly reactive "uron screen" that will detect diuron, monuron, and linuron is also being developed. Commercially available kits for other herbicides of interest to the forestry weed science community (e.g. hexazinone) will only be developed if immunoassay vendors perceive a sufficient market to offset the high costs of development. It has been my observation that herbicide residue sampling and analysis is often missing or severely constrained from otherwise well-designed and well-executed forestry weed science projects simply because of the prohibitive cost of classical chemical assays. How many in this audience would

be willing to incorporate herbicide monitoring in your work if you new it would cost $20,000 rather than $200,000 in a comprehensive research study or $5,000 rather $50,000 in a comprehensive monitoring program? The need to monitor routinely for herbicides in an operational setting will undoubtedly increase, either forced by regulatory action or the result of voluntary stewardship practices. Other operational applications for tank mix monitoring, drift monitoring, buffer strip, and streamside management zone monitoring are also possibilities. I challenge you to make known your own views on how forestry weed science programs could benefit from this immunoassay technology.

The immunoassay experts are quick to acknowledge that immunoassay technology is no panacea and can be easily oversold. They also emphasize that immunoassays must be carried out and interpreted by personnel who are professionally trained in the chemical sciences in order to avoid mistaken interpretations and/or abuse. At the same time, it is readily apparent that immunoassay is rapidly becoming accepted as a powerful new technology for pesticide analysis, to augment rather than replace existing technology. The full potential for immunoassays has yet to be realized. In the next decade we are likely to see many exciting new developments such as immunoaffinity chromatography which exploits the interaction between an antibody and its antigen to purify and concentrate. the target substance from complex environmental matrices. Concepts for immunoprobes or immunosensors comprising an antibody-coated **solid** support are being explored to monitor pesticide levels by direct immersion in an aqueous solution with a real-time optical or electronic readout. Similar concepts are being developed for direct reading personal exposure monitors. Also possible are production of highly sensitive and selective antibodies using recombinant biotechnology techniques to overcome some of the current time and cost limitations in conventional animal antibody production. Finally, the exciting prospect of multianalyte (compound or class-specific) immunosensors capable of rapidly monitoring trace levels of several analytes could revolutionize pesticide analysis and environmental monitoring in the next decade.

Additional Reading

J.M. Van **Emon,** R.O. **Mumma,** eds; Immunochemical Methods for Environmental Analysis. ACS Symposium Series, No. 442. American Chemical Society, Washington, D.C. 1990.