Reusable Fiber Optic Immunosensor for Rapid Detection of Imazethapyr Herbicide

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Imazethapyr is the active ingredient of PURSUIT herbicide. This herbicide belongs to the imidazolinone class of compounds. Polyclonal antibodies have been prepared in rabbits and sheep which specifically recognize this class of compounds. Using immune sera, ELISAs have been developed for imazaquin, imazapyr, imazethapyr, and imazmethabenz methyl (the active ingredient for SCEPTER, ARSENAL, PURSUIT, and ASSERT herbicides, respectively) with sensitivity at low nanogram per milliliter levels. The quantitation of imidazolinones in soil requires a certain amount of sample pretreatment, thus, the throughput is not ideal. A simpler immunoassay method for screening large amounts of soil samples economically would be useful. Using the same polyclonal antibody, fluorescent immunoassay employing optical fiber and fluorescence (Block, M. J.; Hirschfeld, T. B. U.S. Patent 4,582,809, 1986) was developed to assay for imazethapyr. Purified sheep antibody was immobilized on quartz fibers. A mixture of fluorescein-labeled imazethapyr analog and free imazethapyr was presented to the fiber for direct competition of the antibody binding sites or displacement of a previously bound fluoresceinated imazethapyr analog on the surface of the fiber. The response time for the detection of imazethapyr ranged from seconds to minutes. The sensitivity of the assay was 1 nM (0.3 ng/mL). This binding of the fluorochrome to the fiber was reversible by washing with a phosphate buffer saline. Multiple measurements were easily processed with a single fiber over the course of several hours. Analysis of imazethapyr residue in soil can be accomplished by subjecting a clarified soil extract solution directly for analysis without further treatment. The cross-reactivity data indicated that the assay is apparently specific for the imidazolinone class of compounds.

INTRODUCTION

Imazethapyr (Figure 1) is the active ingredient of PURSUIT herbicide. This herbicide belongs to the imidazolinone class of compounds, which is effective in weed control by virtue of its ability to inhibit branched-chain amino acid biosynthesis (Shaner et al., 1984). The most effective use of imidazolinone herbicides depends on the knowledge of the metabolism of these compounds in different plants as well as the residual level of compound in the soil (Shaner et al., 1991). Therefore, monitoring the imazethapyr residue in soil before planting is very useful. A generic polyclonal antibody for the class of imidazolinone compounds has been generated (Wong et al., 1992). This antibody recognizes the entire class of imidazolinone compounds. Immunoassays have been reported for imazaquin (active ingredient for SCEPTER herbicide), imazapyr (active ingredient for ARSENAL herbicide), imazethapyr (active ingredient for PURSUIT), and imazamethabenz methyl (active ingredient for ASSERT herbicide). The sensitivity of these assays is at the nanogram level. Soil residue analysis for imazaquin by ELISA has been developed (Stocker et al., 1992). The method requires some pretreatment of the soil extract before processing by ELISA; thus, the throughput of this method is not ideal. Two possible paths can be taken to improve the existing immunoassay for soil sample analysis: (1) generation of anti-imazethapyr antibody with greater avidity than the existing ones, so that sample matrixes may be diluted before assay, or generation of monoclonal antibody which has minimal matrix cross-reactivity; (2) using the existing antibody but changing the format of the assay and the detection method so that the matrix effect can be eliminated. Since generation of antibody is time-consuming and does not guarantee success, we opted to investigate alternative assay systems to improve throughput. Recent reports suggested that

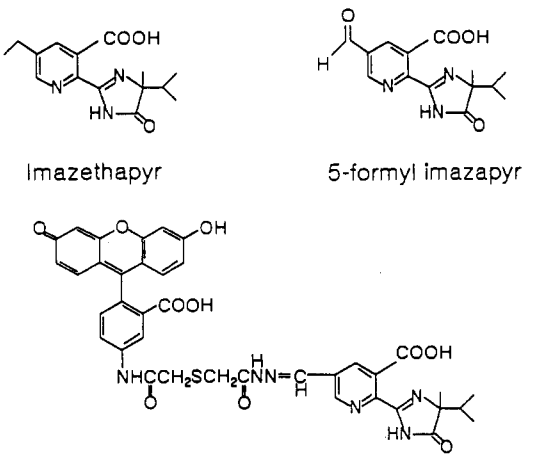

Figure 1. Structures of imazethapyr, 5-formylimazapyr, the analog compound for fluorescein label preparation, and fluorescein hydrazino methylene imazapyr (FHMI).

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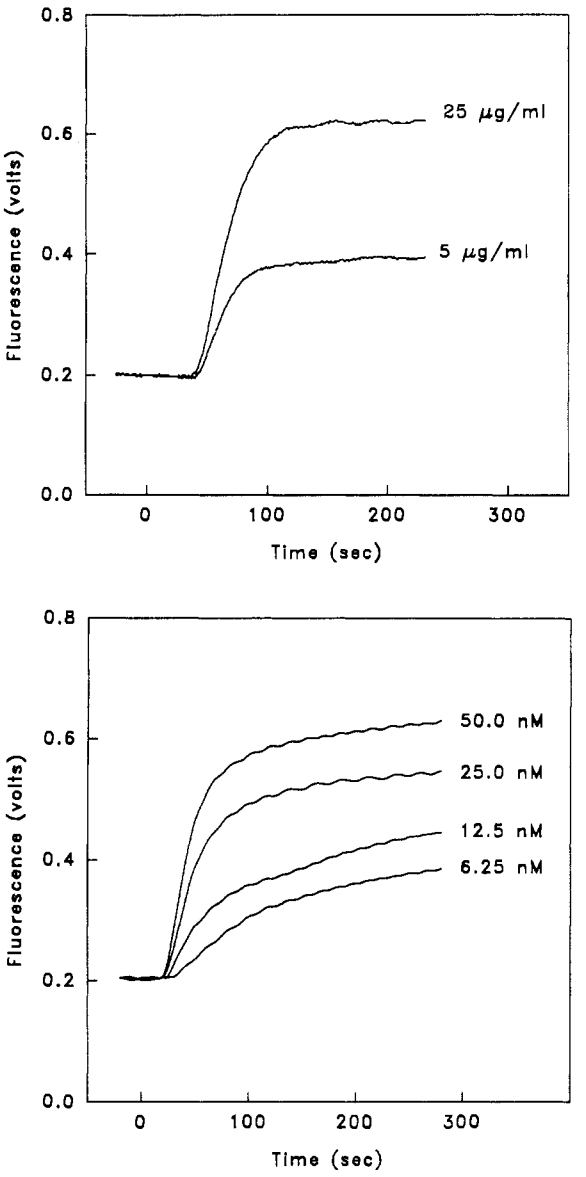

Figure 2. Dependence of signal on the concentration of antibody immobilized on the fiber. A solution of 25 nM FHMI in casein/PBS (0.1%) was perfused throughout (top). The bound fluorescent signal was correlated with the concentration of FHMI (bottom). Fibers were immobilized with a 25 μg/mL antibody. Solutions of FHMI varied from 6.25 to 50 nM in casein/PBS were perfused.

fiber optic evanescent immunosensor (Anis et al., 1992; Rogers et al., 1991) may fill this niche.

Biosensors are analytical devices by which biological molecules such as enzymes, antibodies, or receptors are used as a base to capture analytes whereby signals can be generated and detected. The early biosensors used enzymes to detect glucose or urea (Clark and Lyons, 1962; Guilbault and Montalvo, 1969). More recently, immunosensors were developed where the specificity and the high affinity of the antibody are utilized to capture analytes. The immunocomplexes thus formed have been measured by different means such as evanescent-excited fluorescence (Andrade, 1985), piezoelectric oscillation (Prusak-Sochaczewski, 1990; Radjakovic, 1989), and surface plasmon resonance (Fagerstam, 1990) or through a silicon sensor-based pH detector (Olson, 1990; Rogers, 1992). Biospecific receptors such as the nicotinic acetylcholine receptor have been used for biosensor construction (Eldefrawi et al., 1988; Gotoh et al., 1987; Taylor et al., 1988; Rogers et al., 1989, 1991).

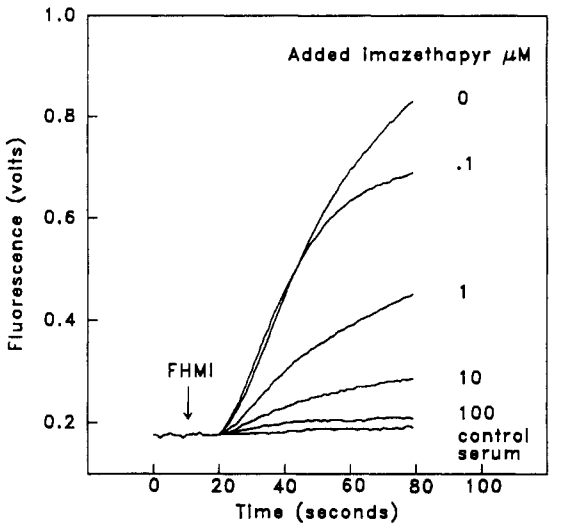

Figure 3. Inhibition of FHMI association by imazethapyr. Varying concentrations of imazethapyr were added to the perfusion solution (25 nM FHMI in casein/PBS). Each perfusion solution reacted with a new antibody coated fiber. All fibers were prepared on the same day.

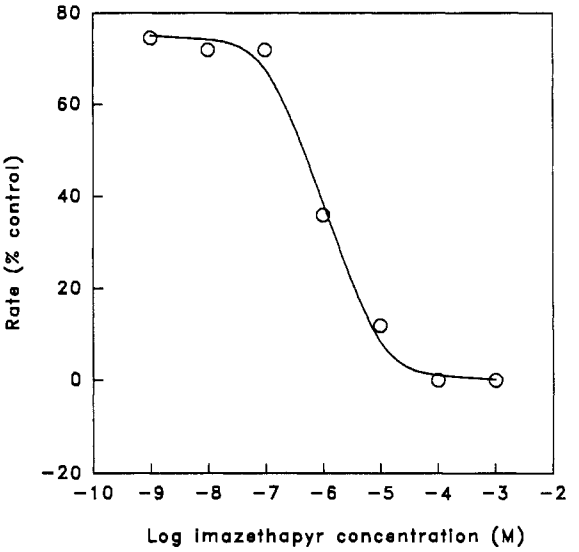

Figure 4. Dose–response curve of imazethapyr on FHMI binding. The rate of fluorescence change due to FHMI bound without any addition of imazethapyr in the perfusate was taken as 100%. Addition of imazethapyr to the perfusate decreased fluorescence to a new level, which is calculated as percent of the zero imazethapyr rate. The rate percent values on the y axis are plotted against the concentration of the imazethapyr on the x axis.

We used a fiber optic evanescent fluorosensor as described by Rogers et al. (1991) for the development of the imazethapyr immunosensing system. In this paper, we report the successful demonstration of an immunosensor using antibodies from a polyclonal sheep serum, immobilized on quartz fibers. Fluorescein hydrazide linked imazethapyr analog was bound to the antibody-coated fiber and generated an optical signal. The quantity of imazethapyr in solution can be determined either by displacing the bound fluorescein-linked imazethapyr analog, thus decreasing the baseline fluorescence signal previously established (i.e., dissociation), or by reducing the amplitude and/or rate of fluorescence uptake by the fiber (i.e., association). The sensor has a dynamic concentration range of 10^{-9}–10^{-3} M. This immunosensor system is fast, requires a small sample volume, and is quite transparent to sample matrix, thus requiring minimum

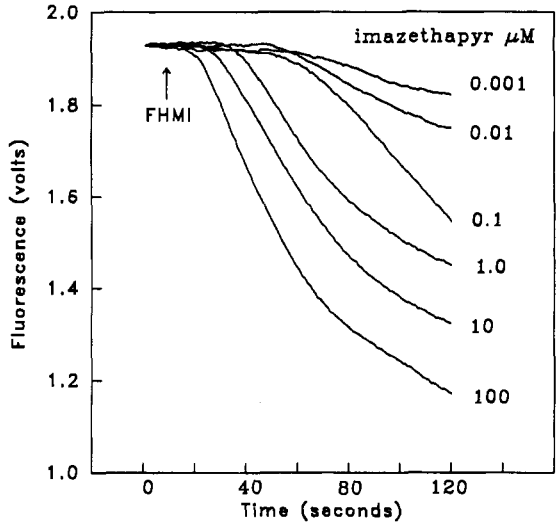

Figure 5. Displacement of bound FHMI by imazethapyr. A 25 nM FHMI in casein/PBS perfusion solution was used to establish a steady-state level of fluorescence. After the steady-state level was reached, the perfusate (FHMI, casein/PBS) was changed to contain different concentrations of imazethapyr (0.001–100 μM). The decrease in fluorescence is measured in seconds.

sample preparation. Another important feature of the sensor is that it is reusable.

MATERIALS AND METHODS

Materials. Analytical grade imazethapyr, imazapyr, imazaquin, imazamethabenz methyl, and 5-formylimazapyr were synthesized at the American Cyanamid Co. (Princeton, NJ). All other herbicides were commercial products. 5-(((2-(Carbohydrazino)methyl)thio)acetyl)aminofluorescein was obtained from Molecular Probes, Inc. (Eugene, OR). Protein A column was from Schleicher and Schuell (Keene, NH). All other chemicals were of the highest grade from J. T. Baker Co. (Phillipsburg, NJ) and Sigma Chemical Co. (St. Louis, MO).

Instrumentation. The portable fluorometer described by Rogers et al. (1989) and manufactured by ORD Corp. (North Salem, NH) was used to measure the fluorescence signal. Quartz fibers (60 mm × 1 mm diameter) with polished ends were purchased from ORD. The fibers were washed with ethanol and used without further treatment. The fiber optic immunosensor made use of the evanescent wave effect by exciting a fluorophore bound within the evanescent zone as described by Block et al. (1986). A portion of the resultant fluorophore emission trapped in the wave guide was transmitted back up the fiber and detected after transmission through 510 LP and 530/30 nm filters. The excitation light is filtered through a six-cavity dielectric filter (center wavelength, 485; fluorescence width half-maximal, 20) and reflected from a 510 nm long pass filter at 45°. The fluorescence is transmitted through the 510 nm long pass and further filtered by a 530/30 (Glass et al., 1982). The flow cell had a volume of 46 μL, which was exchanged every 12 s at a flow rate of 0.23 mL/min.

Antibody Production and Purification. Polyclonal antibody was raised in sheep using the immunogen described before (Wong et al., 1992). The IgG was purified from whole serum by passing through a protein A column following the manufacturer's recommendation of loading with 1.5 M glycine/3 M NaCl, pH 8.9, and eluting with 0.1 M sodium citrate buffer, pH 3.5.

Immobilization of Antibodies. The purified antibody was noncovalently immobilized to the quartz fibers by incubating the fibers overnight at 4 °C in an appropriately diluted antibody solution in phosphate buffer saline, pH 7.0 (PBS). The coated fibers were briefly rinsed with 5 mL of PBS to remove unbound material. This fiber was then mounted in the flow cell and perfused with PBS containing casein (0.1%) to reduce nonspecific binding.

Preparation of a Fluorescein-Linked Imazethapyr Analog. The fluorescent compound 5-(((2-(carbohydrazino)methyl)thio)acetyl)aminofluorescein (FL-hydrazide) was conjugated to 5-formylimazapyr (Figure 1) at equal molar ratio in pyridine at 40 °C. The reaction was monitored by thin-layer chromatography by using a solvent system of ethyl acetate/methanol (7:4). The reaction was complete when no indication of FL-hydrazide remained, usually requiring 24 h. The product, 2-{[5-carboxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin)-2-yl]-methylene}carbohydrazino-5-(((methyl)thio)acetyl)aminofluorescein (fluorescein hydrazino methylene imazapyr, designated FHMI, Figure 1), was stored at 4 °C and used without further purification.

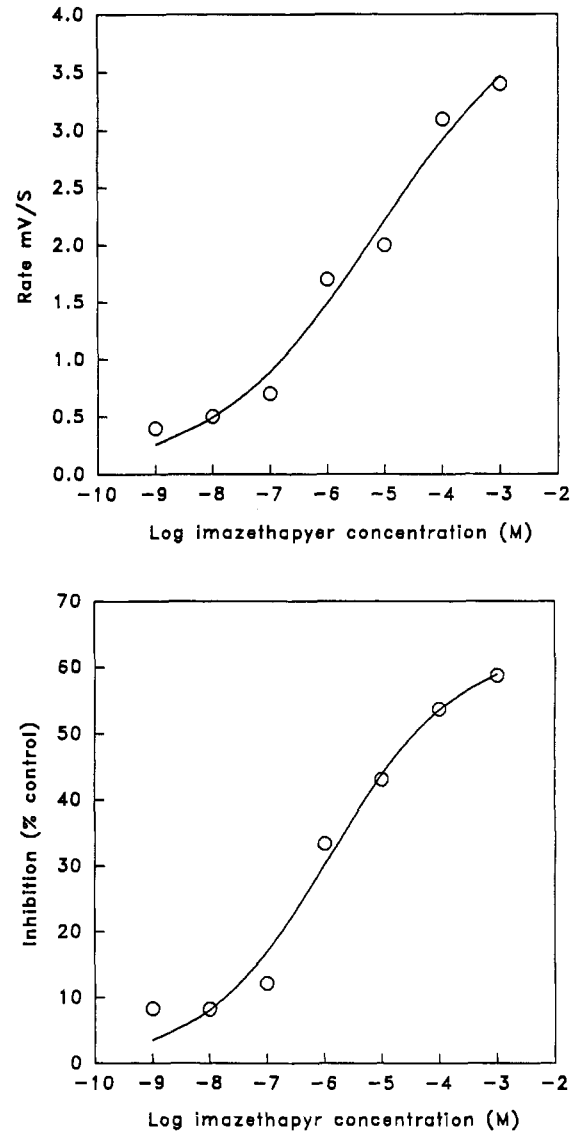

Figure 6. Dose–response relationship of imazethapyr on displacement of FHMI. The initial rate of fluorescence change was calculated for the data in Figure 5 and plotted against imazethapyr concentration (top). On the basis of the steady-state binding level as 100%, the corresponding new steady-state voltage levels resulting from FHMI displacement by different imazethapyr concentrations were calculated as percent inhibition (y axis) (bottom).

Fluorescence Measurement. After the antibody-coated fiber had been perfused with casein/PBS, the perfusate was changed to 50 nM FHMI (in casein/PBS). Binding of the FHMI to the antibody-coated fibers resulted in fluorescence (i.e., optical signal) that usually reached steady state in about 2 min. Competition between imazethapyr and FHMI was accomplished by introducing free imazethapyr into the perfusate solution containing FHMII in PBS/casein either to inhibit association or to displace bound FHMI (enhance dissociation).

Data Collection and Analysis. The optical signal was recorded on a X–Y recorder and simultaneously captured by an

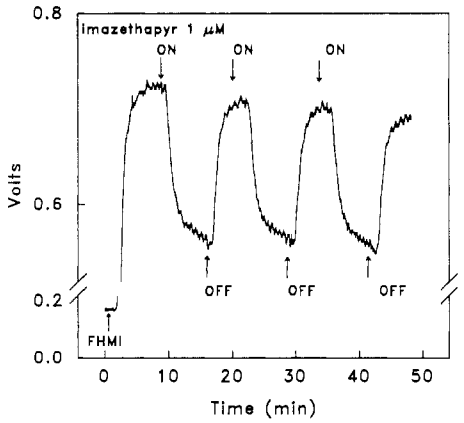

Figure 7. Reusability of the fiber sensor. A 25 nM FHMI in casein/PBS solution was perfused to reach a steady state of binding (about 5 min), at the point indicated as "ON"; 1 μM imazethapyr was introduced in the perfusate (FHMI, casein/PBS). As a considerable amount of FHMI was displaced, the perfusion solution was switched back to 25 nM FHMI casein/PBS (indicated as "OFF"). The ON and OFF process was repeated.

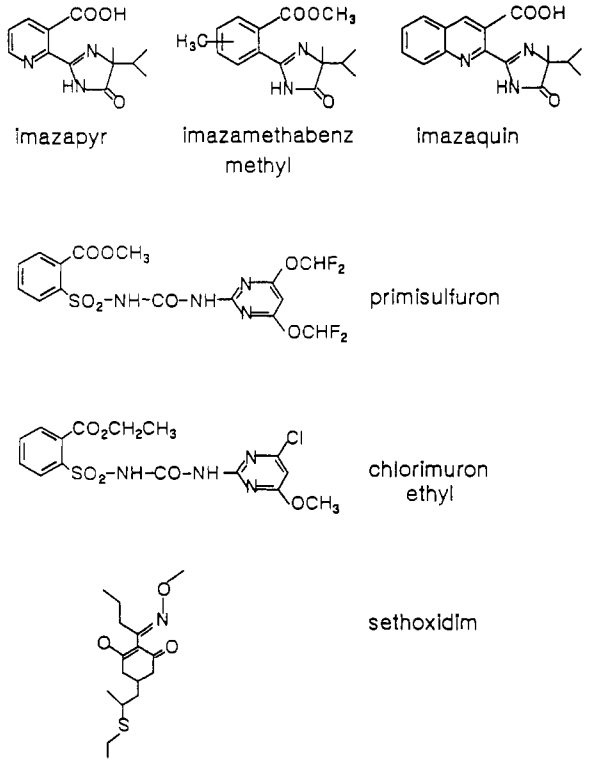

Figure 8. Chemical structures of three imidazolinone class compounds and three non-imidazolinone agrochemicals used to demonstrate the specificity of the fiber optic sensor.

IBM computer via A to D converter (DAC20, Keithly, MA). Signals were either analyzed on line or stored on magnetic media for further analysis with the aid of a software package (Asyst Software Technologies Inc., Rochester, NY).

RESULTS AND DISCUSSION

Determination of Antibody and FHMI Concentration Used. FHMI (50 nM) in PBS bound nonspecifically to quartz fibers as measured by total internal fluorescence. However, addition of 0.1% casein in the perfusate eliminated this nonspecific signal. When control sheep IgG was immobilized on the fiber, a very small baseline signal was observed when perfused with FHMI. When FHMI was added to a fiber coated with anti-imidazolinone antibody, a strong signal was generated. This indicated

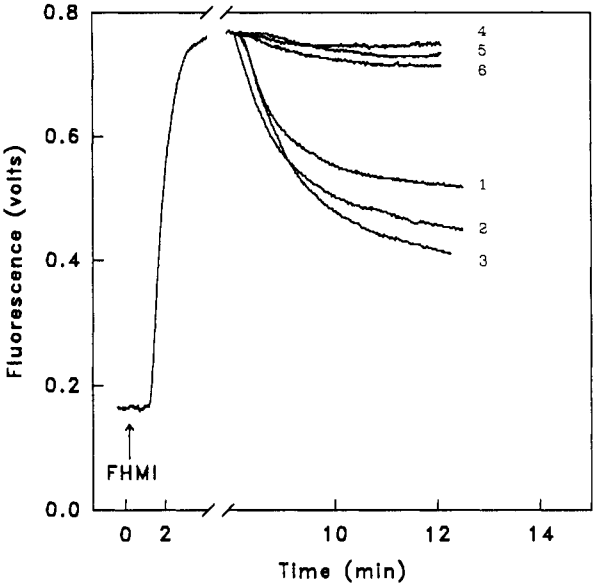

Figure 9. Displacement of FHMI by the imidazolinone and non-imidazolinone compounds. A 25 nM FHMI in casein/PBS solution was perfused to reach a steady state before a solution of 1 μM compound was added to the FHMI, casein/PBS. Plot 1, imazamethabenz methyl; 2, imazapyr; 3, imazaquin; 4, chlorimuron ethyl; 5, primisulfuron; 6, sethoxydim.

that the binding of the FHMI was specific for the antibody. Figure 2 shows the concentration-dependent signal generation by varying the amount of antibody immobilized on the fiber and demonstrates the dependence of signal on the FHMI concentration infused. The optimal concentration of 25 μg/mL antibody was used for immobilization, while 25 nM FHMI was used for all perfusions.

Inhibition of FHMI Association by Imazethapyr. Addition of imazethapyr in the perfusion solution (25 nM FHMI in 0.1% casein/PBS) decreased the fluorescent signals in a time- and concentration-dependent manner (Figure 3). The effective range of imazethapyr concentration was from 0.1 to 100 μM. The rate of association was calculated from the slope of the fluorescence signal plot during the initial 20-s segment of the response. Taking the rate of fluorescence signal uptake in the absence of imazethapyr as 100%, the percent rate of association of FHMI with varying concentration of imazethapyr was plotted against the concentrations of the imazethapyr used. The IC_{50} of this dose–response curve was 2 μM (Figure 4).

Displacement of Bound FHMI with Imazethapyr. Perfusion of the antibody-coated fiber with 25 nM FHMI in casein/PBS results in a time-dependent increase in total internal fluorescence transmitted through the fiber as a result of binding of FHMI to the antibody. Fluorescence reaches a steady-state level in approximately 2 min. If imazethapyr was added to the FHMI in casein/PBS perfusion solution after steady-state fluorescence was established, reduction of the fluorescence signal was almost immediate. A concentration-dependent displacement between 0.001 and 100 μM imazethapyr was observed (Figure 5). Both the initial rate of displacement and the new steady state (4–6 min) reached after the introduction of the free imazethapyr in the perfusate gave similar dose–response curves (Figure 6) with IC_{50} of 0.3 μM. It is interesting to note that greater sensitivity was observed with this displacement method.

Reusability of the Fiber Optic Sensor. One of the most significant features of this sensor is the ability to use the same sensor for multiple measurements without significant loss of sensitivity. This is due to reversible

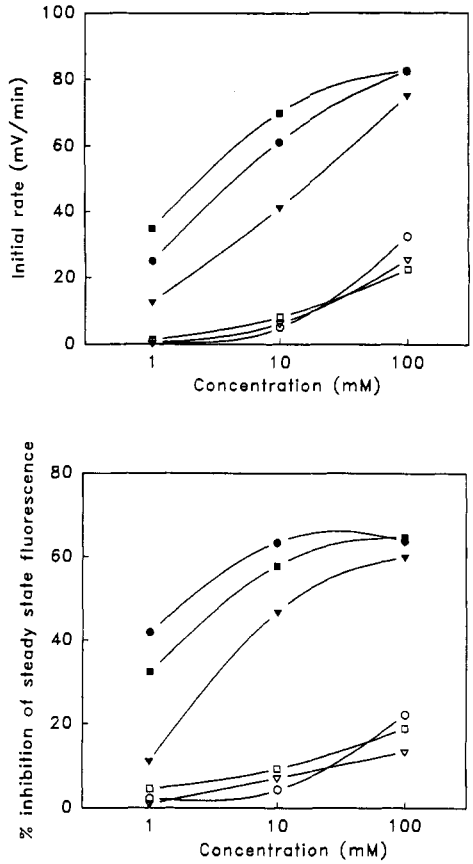

Figure 10. Concentration-dependent FHMI displacement by imidazolinone and non-imidazolinone compounds. Three concentrations (1, 10, and 100 μM) of each compound were used for displacement of signal from steady-state bound levels of fluorescence (top). The imidazolinones displaced the signal in a concentration-dependent manner, while the non-imidazolinone, even at 100 μM concentration, did not. Each symbol represents a mean of three measurements (SD + <10%) (bottom). ○, chlorimuron ethyl; ▽, primisulfuron; □, sethoxydim; ●, imazamethabenz methyl; ▼, imazapyr; ■, imazaquin.

binding of both FHMI and imazethapyr to the antibody coating the fiber. A single antibody-coated fiber was perfused until a steady-state equilibrium was reached. Then, a perfusate containing 1 μM free imazethapyr with 25 nM FHMI in casein/PBS was introduced. A reduction in fluorescent signal was observed almost immediately due to the displacement of FHMI from the fiber by free imazethapyr (Figure 7). When free imazethapyr was removed from the perfusate, the optical signal reversed to almost its original steady-state level. Although there was an apparent small decrease in the steady-state level and a small climb in the baseline fluorescence, these changes do not affect the rate of change of fluorescence signal which can be used to calculate the concentration of the analyte. The decrease in steady-state level may be due to the physical detachment of the bound antibody from the fiber upon long period of perfusion, thus reducing the overall signal at steady state. Covalently linking the antibody to the fibers may eliminate this phenomenon. The baseline shift, on the other hand, may be due to a small population of antibody in the polyclonal mix which binds the FHMI exceptionally tightly to allow the signal to return to the baseline upon washing with PBS. Perhaps a ligand-specific affinity chromatography method for antibody purification instead of the protein A method to remove this population of antibody may address this issue.

Specificity of the Fiber Optic Sensor. The effect of six agrochemicals (Figure 8) on the immunosensor was

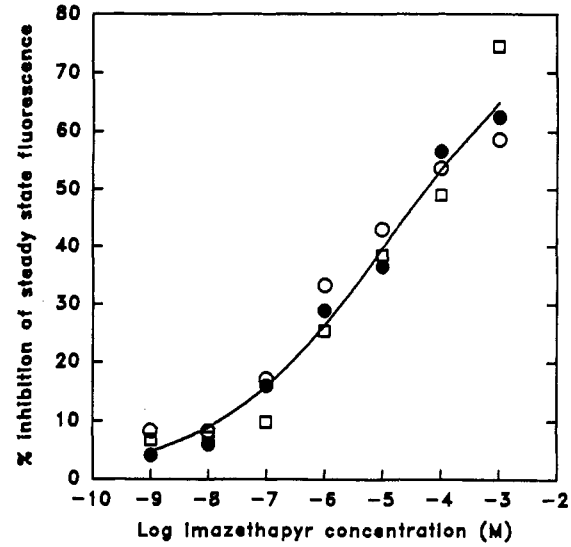

Figure 11. Matrix transparency of the fiber sensor. Soils Plano and Sassafras were extracted with water at 1:1 ratio (v/w). The extracts were centrifuged at 3000 rpm for 10 min. The clear supernatant was adjusted to neutral pH by the addition of $^1/_{10}$ the volume of 10× PBS buffer. After filtering through a 0.45-μm filter, the soil extraction solution was used in place of PBS to prepare the perfusion solutions. FHMI displacement format was used to obtain the dose responses with imazethapyr. The plot of percent inhibition of the two soil extracts (●, Plano; ○, Sassafras) are presented along with the PBS (□) dose response. Each symbol represents an average of two measurements.

investigated. Three of the compounds, imazapyr, imazaquin, and imazamethabenz methyl, belong to the same imidazolinone class. The others, chlorimuron ethyl, sethoxydim, and primisulfuron, are agrochemicals with different chemical structures. When 10 μM concentrations of the compounds were added to the FHMI in casein/PBS perfusion solution after a steady-state binding of the FHMI was established, the three imidazolinone compounds displaced FHMI from the quartz fiber, whereas the three unrelated chemicals did not (Figure 9). Varying the concentration of the compounds from 1 to 100 μM demonstrated that imazapyr, imazaquin, and imazmethabenz methyl displaced FHMI with IC_{50} values ranging from 2 to 20 μM, while chlorimuron ethyl, sethoxydim, and primisulfuron did not displace FHMI significantly even at 100 μM (Figure 10). These results indicate the generic nature and specificity of the polyclonal antibody for detecting imidazolinone herbicides (Wong et al., 1992).

Detection of Imazethapyr in Untreated Soil Extracts. Two types of soil (Plano and Sassafras) with different organic matters and clay content were extracted in water at a soil to water ratio of 1:1. After sedimenting the particles and filtering through a 0.45-μm filter, the extracts were neutralized with $^1/_{10}$ the volume of 10× PBS buffer and used as a substitute for PBS buffer in preparing the perfusates. The dose–response curves for imazethapyr obtained in PBS and the two soil extracts were superimposable (Figure 11). This indicates that the sensor system is quite transparent to matrix in the samples.

Conclusions. A fiber optic evanescent fluorosensor was successfully used for the detection of imazethapyr herbicide in buffered solution and in soil extracts. An IgG fraction of a polyclonal antibody was immobilized on quartz fiber, and direct competitive binding of FHMI and free imzethapyr was monitored by total internal fluorescence transmitted through the optic fiber. The dose–response curves obtained from either the binding or the displacement modes indicated that the assay sensitivity was better in the displacement mode (0.001 μM for

displacement and 0.1 μM for binding). Greater sensitivity may be achieved by fine tuning the amount of antibody immobilized, the concentration of FHMI used, and the signal capturing capability of the instrument.

The reversibility of binding of both FHMI and imazethapyr suggests that this immunosensor may be useful for large sample handling for screening purposes. Automation of the assay system can also be envisioned. Since the samples are perfused into the flow cell, a very short interaction time is allowed on the fiber probe. As a result, only the material with the highest affinity for the immobilized antibody will be bound to the sensor. Matrix materials, which may present problems in normal ELISA (Wong, unpublished data) where incubation time is prolonged, seem to have a minimal effect in this system. This evanescent-excited fluorescence sensor offers the advantage of speed, sensitivity, and matrix transparency. Its application in agrochemical monitoring will undoubtedly be expanded.

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LITERATURE CITED

Andrade, J. D.; Vanwagenen, R. A.; Gregonis, D. E.; Newby, K.; Lin, J. N. Remote Fiber-optic Biosensor based on Evanescent-excited Fluoroimmunoassay. Concept and Progress. *IEEE Trans. Electron Devices* **1985**, *32*, 1175–1179.

Anis, N. A.; Wright, J.; Rogers, K. R.; Thompson, R. G.; Valdes, J. J.; Eldefrawi, M. E. A Fiber-Optic Immunosensor for Detecting Parathion. *Anal. Lett.* **1992**, *25*, 627–635.

Block, M. J.; Hirschfeld, T. B. Apparatus Including Optical Fiber for Fluorescence Immunoassay. U.S. Pat. 4,582,809, 1986.

Clark, L.; Lyon, I. Glucose Enzyme Electrode. *Ann. N. Y. Acad. Sci.* **1962**, *102*, 582.

Eldefrawi, M. E.; Sherby, S. M.; Andreou, A. G.; Mansour, N. A.; Annau, Z.; Blum, N. A.; Valdes, J. J. Acetylcholine Receptor-based Biosensor. *Anal. Lett.* **1988**, *21*, 1665–1680.

Fagerstam, L. G. A Non-Label Technology for Real-Time Biospecific Interaction Analysis. In *Techniques in Protein Chemistry 11*; Villafranca, J. J., Ed.; Academic Press: New York, 1990.

Glass, T. R.; Lackie, S.; Hirschfeld, T. Effect of Numerical Aperture on Signal and Cylindrical Waveguide Evanescent Fluorosensor. *Appl. Opt.* **1987**, *26*, 2181–2187.

Gotoh, M. E.; Tarniya, E.; Kagawa, Y.; Karube, I. Acetylcholine Sensor Based on Ion Sensitive Field Effect Transistor and Acetylcholine Receptor. *Anal. Lett.* **1987**, *20*, 857–870.

Guilbault, G. G.; Montalvo, J. A Urea Specific Enzyme Electrode. *J. Am. Chem. Soc.* **1969**, *91*, 2164–2167.

Olson, J. D.; Panfili, P. R.; Armenta, R.; Femmel, M. B.; Merrick, H.; Gumprz, J.; Goltz, M.; Zuk, R. F. A Silicon Sensor-based Filtration Immunoassay Using Biotin-mediated Capture. *J. Immunol. Methods* **1990**, *134*, 71–79.

Prusak-Sochaczewski, E.; Luong, J. A New Approach to the Development of a Research PZ Crystal Biosensor. *Anal. Lett.* **1990**, *23*, 401–409.

Radjakovic, L.; Ghaemmaghami, V.; Thompson, M. Adsorption of Film Free and Antibody Coated Piezoelectric PZ Sensor. *Anal. Chim.* **1989**, *217*, 111–121.

Rogers, K. R.; Valdes, J. J.; Eldefrawi, M. E. Acetylcholine Receptor Fiber-optic Evanescent Fluorosensor. *Anal. Biochem.* **1989**, *182*, 353–359.

Rogers, K. R.; Cao, C. J.; Valdes, J. J.; Eldefrawi, A. T.; Eldefrawi, M. E. Acetylcholinesterase Fiber-Optic Biosensor for Detection of Acetylcholinesterases. *Fundam. Appl. Toxicol.* **1991**, *16*, 810–820.

Rogers, K. R.; Fernando, J. C.; Thompson, R. G.; Valdes, J. J.; Eldefrawi, M. E. Detection of Nicotinic Receptor Ligands with a Light Addressable Potentiometer Sensor. *Anal. Biochem.* **1992**, *202*, 111–116.

Shaner, D. L.; Mallipudi, N. M. Mechanism of Selectivity of the Imidazolinones. In *The Imidazolinone Herbicides*; Shaner, D. L., O'Conner, S. L., Eds.; CRC Press: Boca Raton, FL, 1991; Chapter 7.

Shaner, D. L.; Anderson, P. C.; Stidham, M. A. Imidazolinones: Potent Inhibitors of Acetohydroxyacid Synthetase. *Plant Physiol.* **1984**, *76*, 545–546.

Stocker, D. R.; Rittenburg, J. H.; Grothaus, G. D.; Wong, R. B.; Roman, Y. Development and Application of an Immunoassay System for Quantifying Imazaquin in Soil. *Abstracts of Papers*; 203rd National Meeting of the American Chemical Society, San Francisco, CA; American Chemical Society: Washington, DC, 1992; AGRO 210.

Taylor, R. F.; Marcentric, I. G.; Cook, E. J. An Acetylcholine Receptor-based Biosensor for the Detection of Cholinergic Agents. *Anal. Chim. Acta* **1988**, *213*, 131–138.

Wong, R. B.; Ahmed, Z. H. Development of an Enzyme-Linked Immunosorbent Assay (ELISA) for Imazaquin Herbicide. *J. Agric. Food Chem.* **1992**, *40*, 811–816.

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