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# Optical analytical methods for detection of pesticides[a]

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## Abstract

The global pesticide market has grown steadily since the 1940s, with the agricultural sector being the largest user of pesticides. The effect of pesticides on human health is manifested either through direct exposure to the material or indirect exposure to contaminated resources. Farmers and those dwelling near areas where pesticides are used may suffer from direct exposure, while the general population might be exposed indirectly, for example, by drinking contaminated water. Exposure to pesticides may cause a variety of symptoms, including headaches, dizziness, and vomiting, damage the nervous system, and even cause death. The risks involved in pesticide use include air pollution and soil and water contamination. The environmental implications of pesticide use include development of resistance among pests, a decline in biodiversity, interruption of the food chain, and disruption of the ecological balance. Pesticide use may also cause changes in physical parameters of the ecosystem. Effective activity of pesticides requires reaching proper leaf coverage. To prevent pest attacks due to insufficient leaf coverage, farmers tend to apply pesticides in excess. In view of the environmental and health implications of pesticide use, there is a clear need to limit pesticide application. Yet farmers lack the means to perform real-time *in situ* assessment of leaf coverage. Existing pesticide detection methods are complex, time-consuming, and unsuited to field application. Optical methods have the potential to provide quick assessments and can be used *in situ*. Several optical methods for detection of pesticides in general and on leaves in particular were developed. The findings indicated that the main problems in pesticide detection using fluorescence are the low autofluorescence of the pesticides and the nonreproducible spectral response of the leaves. These obstacles were solved by employing labeling agents. For example, rhodamine was suggested, mainly due to its excellent surface adhesion and its extremely high fluorescence quantum yield. The labeling agents were sprayed on leaves in the form of aerosols, thus creating a uniform layer of nanocrystals and microcrystals on the surface of the leaves. The effects of pesticides on the spectral characteristics of the labeling agents were examined using laser-induced fluorescence (LIF) spectroscopy. When pesticide droplets were applied to a pretreated leaf, two phenomena were observed. The first was a substantial fluorescence increase. The second was material-specific spectral shifting as a result of interaction between the labeling molecules and organic components in the pesticide droplet. It was possible to utilize these spectral shifts for quantification of the pesticide concentration in the droplet. These spectral shifts enabled detection of pesticides on plants, although they were not sufficient for providing quantitative information on the extent of pesticide coverage. To detect pesticide coverage, several imaging data techniques were applied, such as LIF scanning of the examined plant surface. This method revealed the droplet shape by scanning and recording the fluorescence intensity at many points on a grid. Since application of this method is expensive and time-consuming, a second technique was also developed: it requires only a UV source and a CCD camera and it enables direct imaging of the pesticides on plants. The data obtained included the droplet shape and its location on the plant. When pesticide identification was required, application of a special hyperspectral fluorescence imaging method was introduced. Fourier transform hyperspectral imaging analysis provided simultaneous full spectral resolution at each pixel, enabling identification of the pesticide and its mapping on the plant. In practice, test plants have to be pretreated with labeling material before pesticide application. The changes in the labeling compound fluorescence can then be used for detection of the pesticide on the plant and quantification of the overall coverage. Low-cost mapping of the pesticide microdroplets could be obtained using a CCD camera, while accurate information could be based on Fourier transform hyperspectral imaging. Since these methods provide immediate results, they may allow the farmer to estimate leaf coverage during pesticide application and adjust spraying accordingly.

**Keywords:** analysis; fluorescence; pesticides.

[a] **Dedication:** This review is dedicated to the late Professor Angelika Anders-von Ahlften of Leibniz University of Hanover (1949–2008). She initiated and promoted with much enthusiasm the research on pesticide detection on plants. She took an active part in this project, with the intention to contribute to reducing the global pesticide usage and protecting the environment. The authors and the whole community are missing her.

## Background

### Pesticides

In his book *Pesticide Pollution*, Agarwal (2009) defines *pesticides* as, "chemical compounds or mixture of substances

with diverse chemical nature and biological activity. They are specially designed and manufactured for their use to prevent, destroy, repel, attract, sterilize, simplify or mitigate any undesired life declared to be the pest (p. 19)."

Between 20% and 40% of the world's crops are lost to pests and diseases. Crops can be harmed at any stage of production, from seeds or bulbs, through harvesting, to product storage. Pesticides protect plants from pests, diseases, and weeds, promoting higher yields and increased profits. Indeed, the use of pesticides since the 1940s has been one of the major contributors to enhanced agricultural production. Hence, pesticides have become an integral part of modern agriculture and their use has grown rapidly. Today, there are 520 approved active ingredients contained in nearly 3000 products on the global market, and crops are treated with pesticides up to 15 times a year.

Pesticides are used in a variety of sectors as detailed in Table 1 (An Introduction to Plant Protection Products).

### Pesticide usage and market size

Since 1945, the global pesticide market has increased significantly, as shown in Figure 1 (Tilman et al. 2001).

According to the World Health Organization (2001), the value of pesticides on the global market was estimated as US$32 billion in 2000, with the share of developing countries being around 10%.

According to SBI (2008), a market research company (SBI 2008), the increase in pesticide sales in all major pesticide segments is expected to continue in the coming years. They report a record rise from US$41 billion in 2007 to US$52 billion in 2008 (Pimentel et al. 1992). The market continues to expand despite global economic instability, pressure from the media and environmental considerations. It is expected that the market will grow at an annual rate of 7%, to reach a value of US$73 billion in 2013.

In the USA, the world's leading pesticides market, trends are similar to those in the global market. According to an Environmental Protection Agency (EPA) report (Kiely et al. 2004), the amount of pesticides used in the agricultural sector accounts for the major part of the total amount used in the USA, with the two nonagricultural sectors (industry/commercial/government and home and garden) together accounting for less than 25% of total use.

### Classification of pesticides

Pesticides may be classified in different ways (Merrington et al. 2002, Agarwal 2009, EPA Web site: www.epa.gov):

- The most common pesticide classification is according to their biological activity and chemical structure. This class includes organophosphate, carbamate, organochlorine, and pyrethriod pesticides.
- Another common way to classify pesticides is according to the type of pests against which they are used. This class of pesticides includes insecticides, herbicides, rodenticides, fumigants, fungicides, mulluscicides, ascaricides, miticides, and nematocides.
- Pesticides may further be classified according the source from which they are obtained, i.e., chemical-based or biopesticides, including microbial pesticides, plant-incorporated protectants, and biochemical pesticides.
- The globally harmonized system of classification and labelling of chemicals (GHS) (United Nations Economic Commission for Europe) is an additional pesticide classification method based on pesticide toxicity, irritation, and sensitization hazards. This classification, which groups chemicals by types of hazard and other safety factors, includes four subgroups numbered 1–4, with subgroup 1 representing the highest toxicity and subgroup 4 the lowest.

### Environmental impact of pesticides

Although the use of pesticides has increased the yield of agricultural products, this progress had serious environmental implications. The largest volume of pesticide use is in developed countries; however, its use is also growing rapidly in developing countries, and pesticide sales in the latter countries reached US$3 billion at the beginning of the decade. The lack of proper pest management, particularly in Asia and Africa, has led to an increase in the quantity of pesticides used per area as well as the frequency of pesticide application, use of higher concentrations of pesticides, and application of more pesticide mixtures to combat pesticide resistance by pests (World Health Organization 2001). Pesticide use per area in 2004 stood at 2 kg/ha vs. 0.49 k/ha in 1961 (EPA Web site: www.epa.gov).

Environmental threats can derive from several factors, resulting in water and soil contamination. One factor is erroneous pest management involving excessive pesticide application. Another can result from disposal of wastewater from washing of equipment or disposal of pesticides or their recipients unsafely and illegally. Lastly, the storage of pesticides can involve potential hazards: according to the United Nations Food and Agriculture Organization, the accumulation of unused or obsolete stocks of pesticides presents a serious and growing threat to populations and the environment in Eastern Europe, Africa, Asia, the Middle East, and Latin America (Food and Agriculture Organization).

**Water, soil, and air pollution** Air pollution is closely related to pesticide application techniques. Pesticide application by aerial spraying produces more aerosols than hand-spraying. Since these aerosols contain the active ingredients of the pesticide, they can harm whomever or whatever comes in contact with them. Once in the atmosphere, pesticides are usually decomposed by visible radiation. Nevertheless, winds can carry them over distances of several kilometers, subsequently returning to the ground with rain. INRA readings have shown that rainwater and fog can contain 0.1 and 14 μg/l of pesticides, respectively (EPA Web site: www.epa.gov).

Pesticides contaminate soil, groundwater, and surface water as well as plants and animals. Decomposition by

**Table 1** Usages of pesticides.

| Sector | Pesticide | Usage |
|---|---|---|
| Agriculture and horticulture | Seed treatments | Presowing protection against disease and insects |
| | Herbicides | Protect yield and quality against competition from weeds |
| Agriculture and horticulture | Fungicides | Minimize yield loss and maintain crops free of harmful fungi and their toxins |
| | Insecticides | Protect crops from insect attack and insect-borne disease infestation |
| | Desiccants | Prepare crops for harvest to prevent yield loss |
| | Plant growth regulators | Optimize productive yield, improve harvest efficiency and keep crops healthy |
| Forestry | Herbicides | Control undergrowth and prevent stump regrowth |
| Amenity, sport, and recreation | Herbicides | Keep streets, pavements, and urban areas weed-free |
| | Fungicides | Maintain quality, weed-free playing surfaces |
| | | Keep grass healthy and disease-free |
| | Insecticides | Protect turf from insect attack |
| Transport and utilities | Herbicides | Maintain safe accessible transport routes, power transmission lines, and pipelines |
| Home and garden | Herbicides | Maintain weed-free gardens, paths, and driveways |
| | Fungicides | Protect plants from insect attack |
| | Insecticides | Keep garden plants disease-free |

microorganisms can take up to several years, depending largely on pesticide solubility, soil structure, and soil permeability. Pores in soil enable water containing pesticides to infiltrate the soil layers and possibly contaminate ground water. Monitoring of water borings as well as bodies of water in many countries has detected the presence of pesticide contamination (Wauchope 1978, Wynne 1986, Briggs and Council 1992, Gustafson 1993).

As stated above, contamination of one source may result in contamination of another source or affect it in other ways. For example, the presence of pesticide in water sources can lead to a decline in the concentration of dissolved oxygen. This is due to pesticide degradation by aerobic microorganisms, which increases oxygen consumption. The decline in dissolved oxygen concentrations causes changes in the local environment, thus creating a different environment than the one the organisms were accustomed to. Moreover, the lack of sufficient levels of oxygen in water bodies may cause extensive mortality among vulnerable organisms. Direct exposure of organisms to pesticides may also cause mortality, with the dead organisms in turn becoming a potential source of poisoning that can harm their natural predators. Both direct and secondary exposure can lead to the death of individual organisms and even to the disappearance of complete species, thus reducing biodiversity. In the natural equilibrium of an ecosystem, species function together interdependently and each has a certain role. If the role is essential to the ecosystem and another species cannot replace the lost species, the natural equilibrium is altered, with varying consequences, such as interruption of the food chain.

**Ecosystem changes** As demonstrated in the previous example, the ecosystem is a complex, interconnected web of organisms and elements in soil, water, and air, coexisting in a delicate balance. This balance, or equilibrium, may be disrupted by the use of pesticides. For example, pesticides may harm nontarget species such as worms, fungi, and bacteria that are essential to the ecosystem not only as a food source for other organisms but also as a soil conditioner.

Figure 2 presents a possible scenario in terms of the effect of pesticide application on pest populations. It describes how the level of pest population at first declines following the use of pesticides but begins to rise again as pests develop a resistance to the chemicals used and the population of beneficial predators declines. Once the use of pesticides is stopped due to unprofitable crop production, the pest population rises dramatically and then slowly drops to a new, sometimes higher, level of equilibrium (Wilson and Tisdell 2001, Zhu et al. 2005).

**Health impact** Pesticides can affect human health by direct exposure to the material or indirectly by exposure to contaminated resources (Wilson and Tisdell 2001, Blair and Freeman 2009).

Direct exposure to pesticides affects the health of people working with pesticides, such as farmers or others in proximity to high concentrations of the materials, such as people who live near agricultural fields or who work in pesticide manufacturing plants (Alavanja et al. 2004, Jones et al. 2009). Exposure can be through inhalation, dermal contact, or oral exposure. The Pesticide Action Network (PAN) estimated that 3 million people around the world are poisoned each year by pesticides, and of these, 200,000 die as a result of the poisoning (Wilson and Tisdell 2001).

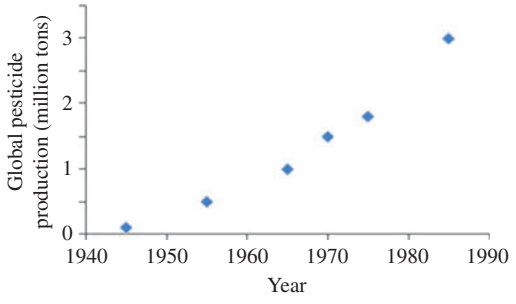


**Figure 1** Global pesticide production (Tilman et al. 2001).

Occupational injuries in agriculture are associated with various sources, such as mobile machines, farm animals, farm chemicals, and poisonous animals. Table 2 shows the incidence of pesticide poisoning as a percentage of overall injuries in the agricultural sector of Western countries (Litchfield 1999).

Table 2 shows that the percentage of such poisoning in California, USA, dropped by almost half during a period of roughly two decades. Interestingly, the percentage in the USA as a whole was 70% higher than that of California over a period of 4 years. Nevertheless, the situation in developing countries is far worse. Kesavachandran et al. state that while developing countries account for only about 20% of the world's use of agrochemicals, they suffer 99% of the deaths related to pesticide poisoning (Kesavachandran et al. 2009). Other reports support similar findings (Wilson and Tisdell 2001, CBS 2009).

Pesticide poisoning in developing countries occurs due to many reasons. Farmers are not proficient with the use of these chemicals, at times because they are illiterate and cannot read the instructions. Some do not take the necessary precautions since they are not fully aware of the adverse effect the material has on their health. Moreover, manual application (hand-spray) methods increase the likelihood of coming in direct contact with pesticides (Wilson and Tisdell 2001, Kesavachandran et al. 2009).

Many pesticides harm pests by damaging their nervous system. Since the human brain and the nervous system of pests share certain biochemical characteristics, pesticides may be neurotoxic to humans as well (Bjørling-Poulsen et al. 2008).

It is well established in the literature that constant exposure to high levels of pesticides is associated with several symptoms and abnormalities of the human nervous system (Alavanja et al. 2004). Most of the data refer to organophosphates, whose effects on the body have been studied extensively. The body may respond to exposure within a few minutes: common symptoms can include headaches, dizziness, nausea, and vomiting. More severe reactions may include muscle weakness, change in heartbeat, and even coma. A delayed response, occurring 1–4 days after exposure, may be muscle weakness. If breathing muscles are affected, the result may be fatal. Neurotoxic effects may appear even weeks after exposure and may lead to leg paralysis.

The effect of chronic exposure to low levels of pesticides is less clear. Reported symptoms include headaches, dizziness, weakness, difficulty in breathing, reduced motor functions, lack of concentration, and confusion. A study by the European Federation of Farm Worker Unions found that workers directly exposed to pesticides, even at low levels of exposure, commonly reported acute headaches, vomiting, stomach pains, and diarrhea. In France, one user in five experiences symptoms at least once a year.

Some suggest that exposure to pesticides may be linked to Parkinson disease and other neurological ailments. Although neurotoxic studies on low-level exposure are not always consistent, it is suggested that the central nervous system may be more vulnerable to pesticides than the peripheral nervous system. In Western countries, there is an increase in diseases such as asthma, some autoimmune diseases, and cancer. Researchers assume that this morbidity is related to changes in immune system functioning, partly attributed to exposure to chemicals, including pesticides (Corsini et al. 2008). Extensive research has been performed to identify a link between exposure to pesticides and different types of cancer (e.g., leukemia, ovarian and testicular cancer, cancer of the prostate, lung, liver, kidney, and breast). Alavanja et al. (2004) published an extensive review of works that examined the relationship between different types of pesticides and cancer morbidity. For each cancer type, the authors list the agricultural agents suspected of being a possible cause.

It is important to note that conclusions connecting pesticides to toxicity, especially in low-level cases, cannot be decisive (Alavanja et al. 2004, Corsini et al. 2008) Epidemiological studies have limited data on several parameters such as application methods, exposure levels, and duration. Indeed, only arsenic-containing insecticides were proved to be carcinogenic to humans (Alavanja et al. 2004).

## Analytical methods for pesticide detection

Many methods have been developed for detection of pesticide residues, each having its advantages and disadvantages. Separation methods, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC), are common methods for detection of traces. The most common pesticide detection methods are gas

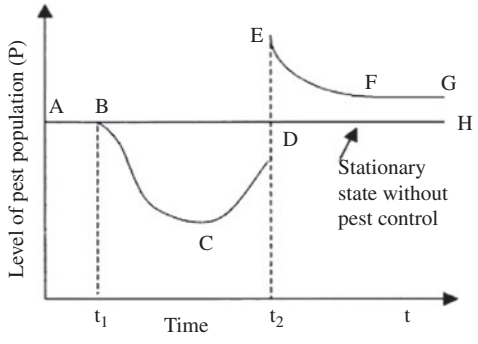


**Figure 2** Level of pest population (Wilson and Tisdell 2001).

**Table 2** Pesticide poisoning incidence from overall injuries in agriculture.

| Percentage of pesticide poisoning (%) | Country/region |
|---|---|
| 2.2 | Western Australia (1995–1996) |
| 2 | New York State, USA (1984–1996) |
| 3.9 | USA (1993) |
| 4 | California, USA (1965–1971) |
| 2.3 | California, USA (1989) |
| 1.3 | Sweden (1984–198) |
| <1 | UK (1970–1980) |
| <1 | UK (1995–1996) |

chromatography-mass spectrometry and high-performance liquid chromatography-ultraviolet/visible spectrometry (Coly and Aaron 1998a, 1998b). Their detection limits are relatively low, and with the addition of specific detectors, these methods are very selective. Nevertheless, the instruments also have several flaws: purchase and maintenance costs are high, a knowledgeable technician is required for operation, analysis is time consuming and requires meticulous preparation, and the instruments cannot be used in the field.

According to EPA (Environmental Protection Agency), a variety of analytical methods and procedures can be used to identify/quantify pesticides, including ion chromatography, atomic absorption, wet chemistry, and ultraviolet spectrophotometry.

Apart from the analytical methods developed for the detection of pesticides, fluorescence methods were also devised. Detection methods based on fluorescence have many advantages (Surette and Mallet 1975, Hjorth et al. 2006, Lakowicz Press). These methods are considered to be simple, rapid, selective, sensitive, and relatively low-cost; their main advantage is the possibility of performing the test in the field, yielding almost instantaneous results.

Although fluorescence-based methods for the detection of pesticides have many advantages, they are still limited since relatively few of these compounds are strongly fluorescent (Coly and Aaron 1998). Nevertheless, direct measurement of fluorescence is feasible, using a simple fluorimeter. White and Weissler (1972) (White and Weissler 1972) discussed the fluorimetric quantification of the residues of gibberellin sprays on cherries, as well as the quantification of chlorinated and phosphorothioate pesticides.

Due to the clear advantages of using fluorescent methods, considerable efforts were invested in bypassing the low natural fluorescence of pesticides by manipulating the materials to yield measurable fluorescence. One way involves mathematic manipulations. For example, Coly and Aaron (1998) indicated that the sensitivity and selectivity of fluorescence analysis can be improved by processing the first and/or second derivatives of the spectra.

Since only a small number of pesticides are naturally fluorescent, additional techniques have been developed to modify nonfluorogenic pesticides into fluorophores. These techniques include analyte treatment and other procedures, as will be detailed later.

Chemical derivatization, such as hydrolysis, heat treatment, complexation reactions, fluorogenic labeling, and solvent polarity effect enhance the quantum fluorescence yield (Coly and Aaron 1998). A specific modification method should be selected for each pesticide, according to its unique properties. Some of these methods, such as heating, cannot be used directly on the crops in the field due to the physical damage caused to the leaf tissues.

Pretreatment of some pesticides (such as carbaryl, benomyl, and azinphos-methyl) with aqueous alkaline solutions yields fluorescent anions (Coly and Aaron 1998). Garcia-Sanchez and Gallardo (1992) quantified the concentration of the insecticide azinphos-methyl in soil samples by alkaline hydrolysis, which resulted in fluorophore anthranilic acid. Mallet and Surette (1974) heated methabenzthiazuron, naptalam, thioquinone, and warfarin, all of which gave fluorescence upon treatment. Naturally, before the treatment, these analytes have a very weak fluorescence or no fluorescence at all.

Frei et al. (1971) used 0.05% solutions of flavones such as fisetin, kaempferol, quercetin, morin, and rutin (in ethanol) for quantitative determination of organothiophosphorus pesticides. They applied an *in situ* fluorimeter, after separation on silica gel layers. Fluorescent spots were obtained when a silica gel plate, previously exposed to the pesticides, was sprayed with the solutions after bromination (Panneton et al. 2006).

Another approach was the based on direct complexation reactions, where the analyte reacts with a ligand to form a strongly fluorescent complex. Blair et al. (1987) created complexes with flavonol and morin, which were detected using a microscope and HPLC. Also, indirect complexation reactions based on ligand exchange, as shown in Figure 3, were suggested (Coly and Aaron 1998). Bidleman et al. (1972) detected sulfur-containing organophosphorus insecticides by indirect complexation with calcein, calcein blue, and palladium(II) using a TLC plate.

Labeling pesticides with a fluorogenic compound is a very sensitive method of pesticide detection and is therefore widely used (Frei and Lawrence 1971). Fluorogenic labeling involves fixing a fluorophore molecule to a nonfluorescent analyte. Often, this method can be implemented *in situ*.

Lawrence et al. (1976) used dansyl chloride to detect organophosphorus and carbamate pesticides. The pesticides were hydrolyzed in sodium hydroxide to generate phenols. The resulting fluorescent derivatives were separated and quantitatively analyzed by *in situ* TLC and HPLC.

Photochemically induced fluorometry (PIF) was another method developed for pesticide detection. In this method, nonfluorescent pesticides are converted into fluorescent compounds using UV radiation for a fixed time duration (Coly and Aaron 1998). Patel et al. (1991) detected nitrogenous pesticides using flow-injection analysis and liquid chromatography with a PIF detector. The results indicated that, in all the tested nitrogenous pesticides, an improvement in fluorescence yield was recorded after UV irradiation and phototransformation. Since the reaction of the pesticide is induced by light, it can be performed *in situ*. The method is easily implemented because it does not require heating and the procedure takes only a short time. It also yields better substances for further analysis in comparison with other modification methods. The main disadvantage of this method is that a part of the products are not fluorescent and quantification of the traces is less accurate. In some cases, the fluorescent products are unstable and have a short life span, which reduces the accuracy of the analysis.

It is noteworthy that for in the above-mentioned approaches, a different specific procedure is needed for the detection of each pesticide or each pesticide family. In many cases, this approach is not acceptable and additional methods are needed.

## Common optical methods used

The methods used in optical detection of pesticides include traditional fluorimetry, laser-induced fluorescence (LIF),

hyperspectral imaging, and RGB imaging using a CCD camera. The main experimental setups are briefly described in the following:

### Laser-induced fluorescence

A simple LIF setup is described in Figure 4.

The excitation system was based on a nitrogen laser (MSG 801 SD, LTB, Berlin, Germany) operating at a wavelength of λ= 337 nm. Excitation and emission took place through quartz optical fibers.

For scanning measurements, the excitation fiber was extended by a lens system, which focused the beam onto the sample. The sample was placed on a sample holder located above an *xy*-table operated by micrometer screws, allowing resolution control of about 10 µm.

Detection was conducted by a spectrometer (MS 125) and recorded with a CCD camera (InstaSpec IV) (both from LOT-Oriel, Darmstadt, Germany). The CCD camera was cooled to -20°C to improve the signal-to-noise ratio. The CCD chip consisted of 1024×128 pixels. The spectrometer was equipped with a fixed grating of 150 lines/mm, which could map a spectral region of about 500 nm onto the CCD chip. This resulted in a resolution of about 0.5 nm/pixel.

### Hyperspectral imaging

Hyperspectral imaging was used for chemical mapping of plant surfaces, achieved using Fourier transform spectral imaging microscopy (FT-SIM). This experimental setup (shown in Figure 5) provides simultaneous fluorescence spectra at all pixels, thus allowing for two-dimensional chemical analysis. The pesticide-contaminated samples were examined by a UV fluorescence microscope coupled to a spectral imaging unit and a CCD camera. The microscope (Axiolab ABO100W/2; Carl Zeiss, Jena, Germany) was equipped with UV transparent objectives (Fluar and Ultrafuar) providing several magnifications (×10, ×20, and ×40). Samples were irradiated by a mercury lamp through the microscope objectives. Fluorescence was excited at 365 nm using a narrow-band filter (10 nm). A dichroic mirror placed in the optical path was used for cutting off the reflected light at wavelengths lower than 390 nm.

Microscope images were transferred to an imaging Fourier transform spectrometer and to a CCD camera for simultaneous recording of the fluorescence spectra at each image pixel. The electrothermally cooled CCD detector consisted of 480×640 pixels of 10×10 µm each (Hamamatsu 4880, Hamamatsu Photonics, Japan).

### Fluorimetry

The fluorimeter used was an Aminco-Bowman Series II luminescent spectrometer (Thermo Electron, Madison, WI, USA) equipped with two monochromators for scanning the excitation and emission wavelengths at 1-nm resolution. CW xenon lamp was used.

### Fluorescence imaging

Fluorescence microscopy was obtained by a fluorescence microscope (Carl Zeiss, Jena, Germany), equipped with a xenon lamp and a band-pass filter (transmission peak at 400 nm). The fluorescence images were recorded using a color CCD camera (Satisec Colour Camera SAT-11 SP, Taiwan).

Direct fluorescence imaging was obtained by excitation in the UV-C range (lamp) using a CCD digital camera (Canon PowerShot A60; Canon, USA).

## Sensitive fluorescence probes for pesticides

Nile red (NR) was tested as a fluorescence probe for pesticides such as methoxychlor (DDTD) in aqueous media (Hassoon and Schechter 1998). This compound is photochemically stable and has long-wavelength absorption band, which makes it a very good candidate for this purpose. Most pesticides absorb at shorter wavelengths and do not interfere with its absorption.

### Fluorescence spectra

The emission of the NR solutions was induced by excitation a 550-nm wavelength. The emission spectrum of 0.5 µM NR in aqueous solution has a low intensity peak centered at about 663 nm, as shown in Figure 6 (Hassoon and Schechter 1998). In the presence of DDTD, an enhancement of the fluorescence intensity and a blue shift of the NR fluorescence peak can be clearly observed. In addition, a second peak at

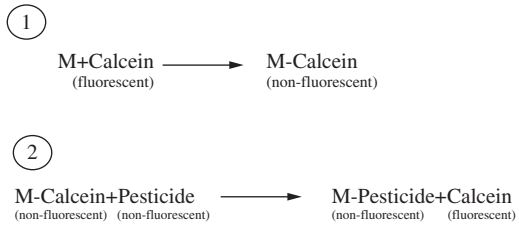


**Figure 3** Indirect complexation.

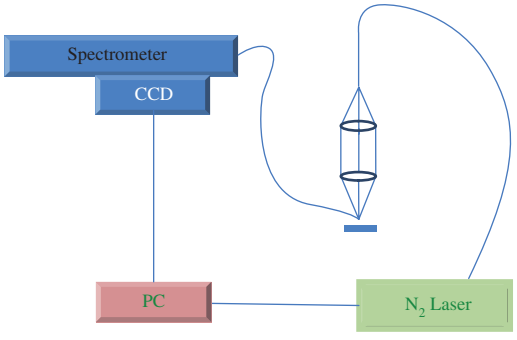


**Figure 4** Schematic experimental setup of LIF.

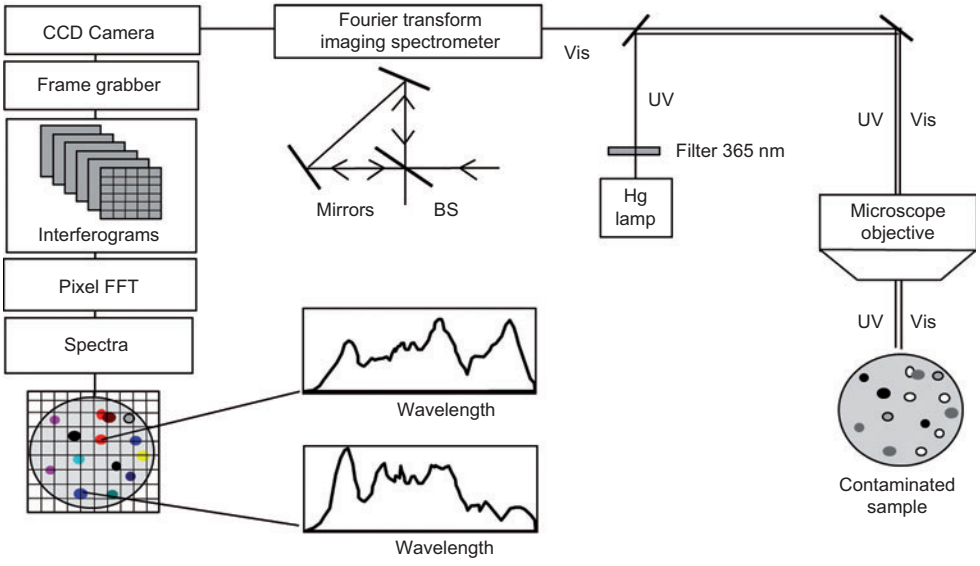


**Figure 5** Schematic experimental setup of FT-SIM plant analysis, providing simultaneous chemical analysis in each pixel of the examined surface.

~605 nm clearly showed up in the fluorescence spectrum and its intensity highly increased as a function of the DDTD concentration.

A strong interaction (probably a complexation reaction) between NR and DDTD-type compounds takes place. This is supported by the appearance of a new fluorescence peak at ~605 nm and by the changes observed in the absorption spectra.

The blue shift and the fluorescence intensity enhancement were explained by the hydrophobic interaction (Hassoon and Schechter 1998). The strong stabilization of NR molecules in hydrophobic environments leads to a solvation interaction with the hydrophobic DDTD molecules. It is assumed that the uncomplexed NR molecules in the water solution are stabilized by a layer of DDTD molecules. The NR molecules in the presence of DDTD environment are denoted in the following NR(DDTD).

## Quantification

Pesticide quantification in aqueous solutions can be based on each of the three effects upon NR fluorescence: The new peak of the produced complex, the enhancement of the original NR peak, and the wavelength shift of the later. All three possibilities were examined. For this purpose, the DDTD concentration was varied in the range of 1–7 ppm (4–20 µM), corresponding to a molar ratio (DDTD/NR) of 8:40. At lower concentrations, the peak at 605 nm was hardly observed.

**Fluorescence intensities** The relative fluorescence intensities of the two peaks, corresponding to the NR-DDTD complex and to the uncomplexed left NR, NR(DDTD), are shown in Figure 7 as a function of the pollutant concentration (Hassoon and Schechter 1998). The peak intensities were obtained by Lorentzian fittings. Although the absolute amount of NR(DDTD) in the solution slightly decreases with increasing DDTD concentration (due to the complex formation), its corresponding peak still increases linearly. This effect was attributed to the intensive fluorescence enhancement due to polarization, which is stronger than the fluorescence loss due to the complex formation (Hassoon and Schechter 1998).

Note that the fluorescence calibration plot of DDTD based on the solid complex (NR-DDTD) peak had a larger slope than that based on the fluorescence enhancement at the original wavelength (due to the uncomplexed NR). This suggests preferred application of the fluorescence peak at 605 nm for DDTD determination.

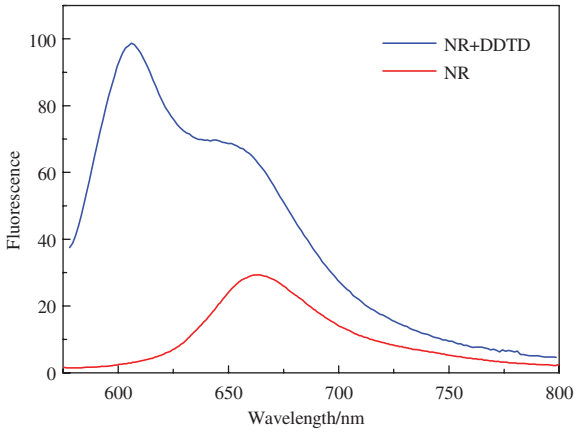


**Figure 6** Fluorescence spectra of aqueous solutions of 0.5 µM pure NR and in the presence of 10 µM DDTD (Hassoon and Schechter 1998).

**Wavelength shift** The blue shift of the NR peak was found in linear correlation with the DDTD concentration, as shown in Figure 8 (Hassoon and Schechter 1998). Thus, this effect can also be used for quantification of DDTD in the parts per million range.

### Selectivity

The selectivity of this method to dichlorodiphenyltrichloroethane was checked using several other pesticides (Hassoon and Schechter 1998): atrazine (6-chloro-*N*-ethyl-*N*-isopropyl-1,3,5-triazine-2,4-amine), 2,4-dichlorophenoxyacetic acid (2,4-D), and (c) pentachloronitrobenzene. The fluorescence effects found with dichlorodiphenyltrichloroethane were not observed for the other compounds. However, some quenching of the NR fluorescence by pentachloronitrobenzene was observed, and this was the only effect. Thus, a certain level of selectivity is ensured by the proposed method.

## *In situ* determination of pesticides on plants

Direct detection of certain solid pesticides on vegetables can be accomplished by measuring the specific fluorescence of a solid complex formed by the pesticides and NR sprayed over the sample. A hand-held fiber-optic spectrometer was developed and applied for detecting the NR-pesticide complex emission (Hassoon and Schechter 2000). The method was exemplified using two pesticides, applied to eggplant and analyzed as pure contaminants and in mixtures: methoxychlor and *N*-(phosphonomethyl) glycine monoisopropylamine.

In all studied cases, it was found that fluorescence spectra of pesticides measured directly on vegetables possess a certain background. This background is a result of reflected light and matrix emission. It affects the pesticide fluorescence mainly at low concentrations. Nevertheless, when a sample of the clean matrix is available, the background could be eliminated by subtraction (Hassoon and Schechter 2000).

### Fluorescence spectra

An example of the fluorescence emission spectrum, obtained from NR-methoxychlor complex on glass surface is shown in Figure 9 (Hassoon and Schechter 2000). It was measured using a hand-held fiber-optic spectrometer. The peak maximum at ~605 nm is similar to that of the complex emission in the aqueous solution. No fluorescence signal was observed from the methoxychlor spot alone before spraying with NR or from the sprayed NR itself. Additional measurements were carried out on other vegetable peels and green leaves, and the results were similar. Note, however, that optical filters were needed in some cases when the reflected light was too strong.

The successful measurements on glass implied possible direct measurements on plants. Emission spectra obtained from contaminated eggplant surface are shown in Figure 10 (Hassoon and Schechter 2000). It is interesting that the complex emission from the eggplant surface is at the same wavelength and is similar in its intensity to the one measured from contaminated glass. However, in this case, a weak emission at the same wavelength range was obtained after spraying with the NR solution with no pesticide contamination. This background emission was attributed to residual contamination or to the natural composition of the substrate. Nevertheless, this background emission can easily be subtracted.

An additional relatively strong reflection was observed in the range of 700–800 nm. It is a result of the room light reflected from the eggplant surface. Also, a wide and intense peak due to the NR-methoxychlor complex was observed at 605 nm. The increase of the emitted fluorescence from the methoxychlor-contaminated spot was utilized for quantitative

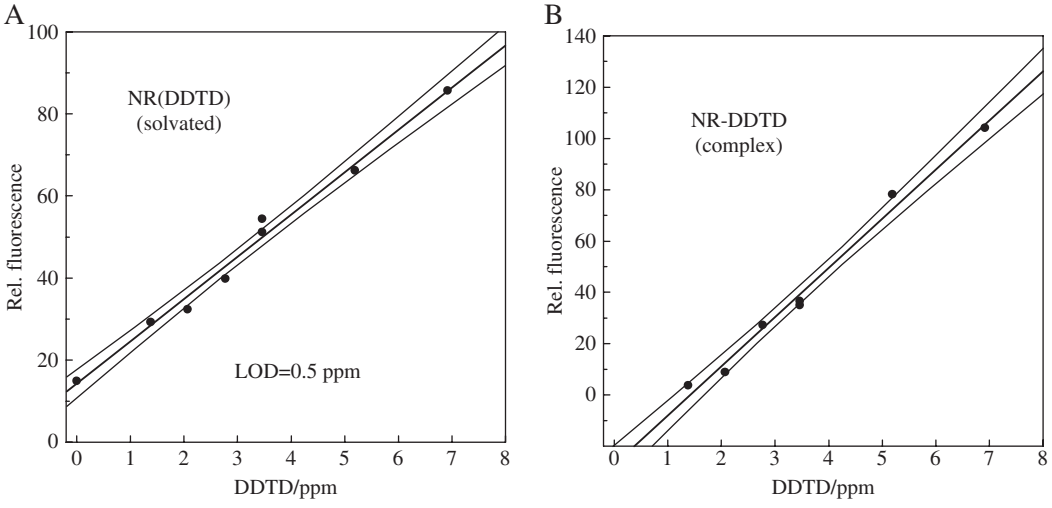


**Figure 7** Fluorescence relative intensity of (A) NR(DDTD) (DDTD-solvated NR) and (B) colloidal NR-DDTD complex as a function of DDTD concentration (Hassoon and Schechter 1998).

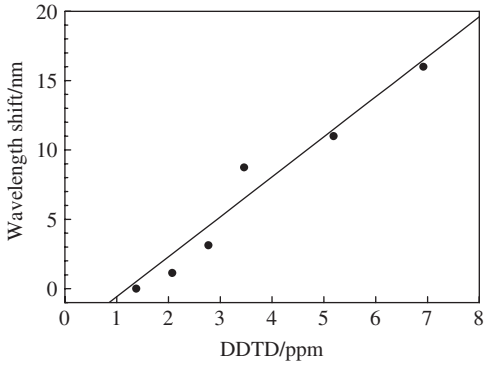


**Figure 8** Fluorescence spectral shift of NR as a function of DDTD concentration (Hassoon and Schechter 1998).

chemical analysis. Spot-to-spot spectral fluctuations were of the order of 5% (Hassoon and Schechter 2000).

### Quantification

For testing the quantification, the absolute quantity of pesticide applied to the plant surface was varied. Linear calibration curves were obtained in the range of 0–5 nmol (~0–1 μg). For example, Figure 11 shows the calibration plot of methoxychlor on eggplant peel surface (Hassoon and Schechter 2000). The plots of methoxychlor and *N*-(phosphonomethyl) glycine monoisopropylamine salt on glass are also shown for comparison. In all cases, the complex emission intensity linearly increased with the pesticides quantity in the examined spot. It was found that the slope of the calibration curve for the contaminated eggplant was slightly higher than that of the contaminated glass. This was attributed to the higher detection yield of the optical fiber spectrometer at the eggplant surface (Hassoon and Schechter 2000).

The limit of detection cased on 95% confidence intervals was 0.17 nmol for methoxychlor on eggplant and 0.32 nmol for *N*-(phosphonomethyl) glycine monoisopropylamine contamination. These values are rather low and indicate a good sensitivity. Such detection limits in the nanogram range indicate that the method is suitable for detection of these pesticides on vegetables, resulting from application by spraying.

### Probe selectivity

The above probe showed a certain level of selectivity to the tested pesticides. The interactions of NR-methoxychlor and of *N*-(phosphonomethyl) glycine monoisopropylamine salt are strong and significantly affect the NR fluorescence properties. The weak binding in molecules forming complexes is usually attributed to intercalations associated with π-stacking, hydrogen bonding, and electrostatic and/or hydrophobic interactions. Most probably, the hydrophobic interactions are dominant in the above complexes. This might explain structure-selectivity relationships, which are effective in analytical applications.

### Analysis of pesticide mixtures

The above method was also evaluated for analysis of pesticide mixtures (Hassoon and Schechter 2000). For this purpose, an eggplant was contaminated by the two pesticides at a series of concentrations. The measured spectra were decomposed in the following way: The spectral data of the pure components, in the range of 580–670 nm, defined two vectors, $\mathbf{V}_{MC}$ and $\mathbf{V}_{NPGM}$. The spectrum of each mixture was described in the form

$$\mathbf{V}_{mix}=\alpha\mathbf{V}_{MC}+\beta\mathbf{V}_{NPGM}+B$$

where α and β are fitting parameters related to the quantity of the two chemical species and B is a constant baseline. The parameters were calculated by the Levenberg-Marquardt optimization algorithm. The results indicated that this method allows for resolution of simple mixtures of pesticides. It works well as long as the concentration of the various components is comparable.

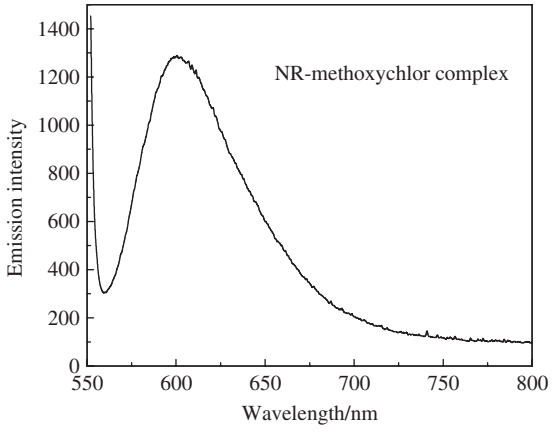


**Figure 9** Emission spectrum of the NR-methoxychlor solid complex (Hassoon and Schechter 2000).

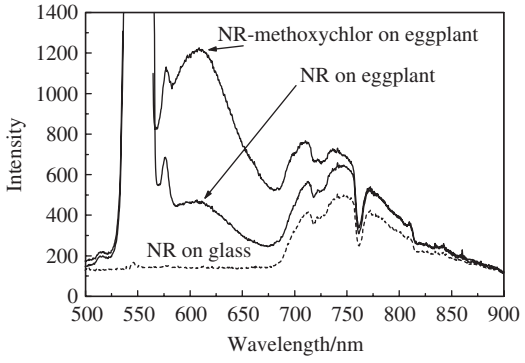


**Figure 10** Emission spectra obtained from solid NR on glass (no emission at 605 nm), from solid NR on eggplant (indicating the background emission) and from the NR-methoxychlor complex on eggplant. Analysis is based on the emission at 605 nm, whereas the room-light reflections are at 700–800 nm (Hassoon and Schechter 2000).

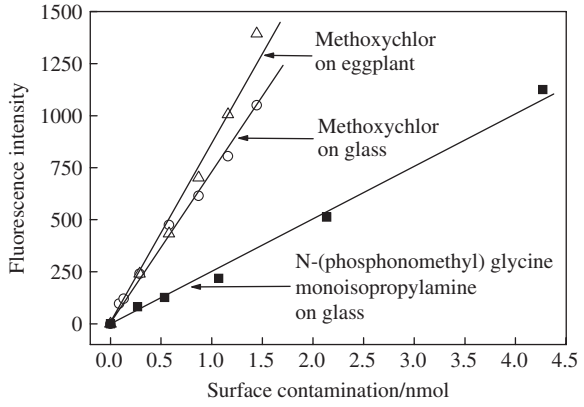


**Figure 11** Calibration plots for the two pesticides on glass and on eggplant surfaces (Hassoon and Schechter 2000).

## Fast optical assessment of pesticide coverage on plants

Based on the above findings, a more comprehensive attempt was carried out to establish possible assessment of pesticide coverage on plants using optical methods (Hake et al. 2007). Although pure chemicals were used in many previous investigations, in this study, all pesticides were prepared and treated according to the manufacturers' recommendations. Therefore, the feasibility of *in situ* assessment of pesticide coverage on plants could be tested. The plant contamination was simulated by application of a droplet of 1 µl of the pesticide solution.

### The studied pesticides

A variety of commercially available pesticides were examined. These include herbicides having different activity modes as well as insecticides, as shown in Table 3. All pesticides were prepared in the same matrix and conditions as when sold for agricultural applications. The Table provides also the pesticide trade name, active ingredient, and mode of action. Their chemical structure is shown in Figure 12.

### The studied plants

Two different leaf types were used for testing the analytical procedure: ivy leaf (*Hedera helix*) and lemon tree leaf. The leaves were chosen based on healthy appearance and similarity of physical characteristics (such as size and color). They were collected shortly before the experiment was conducted, sealed in plastic bags, and kept refrigerated. The leaves were washed in tap water and dried.

### Laser-induced autofluorescence spectra of pesticides

The autofluorescence of the pesticide solutions is shown in Figure 13 (Hake et al. 2007). One microliter of each pesticide solution was tested on a glass slide, using nitrogen laser as excitation source at 337 nm. The autofluorescence of water was the reference for all measurements. Note that the fluorescence intensity of Gesatop 50 is very high and it was divided by 20 to place it in a scale comparable to the other pesticides.

The results show that only *Gesatop* 50 exhibits significant autofluorescence. All the other materials yield a very low autofluorescence, which is not sufficient for their detection at low concentrations.

### Pesticide effect on chlorophyll fluorescence

Since the pesticide contamination spots showed no significant autofluorescence, another approach was attempted: It was based on following the changes in chlorophyll fluorescence in the presence of the pesticides. Chlorophyll has a well-known fluorescence spectrum when the molecule is excited at 430 nm (Peterson et al. 2001). One microliter of Romectin was applied on a lemon tree leaf and left to dry. Fluorescence spectra were measured at an excitation wavelength of 430 nm. The measurements were performed on the same leaf on different locations, before and after application of the pesticide contamination (Hake et al. 2007).

Figure 14 shows the thus measured fluorescence spectrum a lemon tree leaf and of a spot on the leaf contaminated by *Romectin*.

The results indicate a decrease in the leaf fluorescence intensity at the spot treated with the pesticide. However, this decrease

**Table 3** Tested pesticides, their active ingredient, and their mode of action.

| Mode of action | Active ingredient | Trade name |
|---|---|---|
| Inhibition of photosynthesis at PSII | Isoproturon | Arelon flüssig |
| Inhibition of carotenoid biosynthesis | Clomazone | Centium 36CS |
| Synthetic auxins (with indoleacetic acid-like action) | Dichlorprop-P | Duplosan DP |
| Inhibition of photosynthesis at PSII | Simazine | Gesatop 50 |
| Inhibition of ALS | Flupyrsulfuron methyl | Lexus |
| Inhibition of photosynthesis at PSII | Metobromuron | Patoran FL |
| PSI electron diversion | Deiquat | Reglone |
| Stimulation of GABA | Abamectin | Romectin |
| Inhibition of EPSP synthase | Glyphosate | Roundup UltraMax |

ALS, acetolactate synthase; EPSP, 5-enolpyruvylshikimate-3-phosphate; GABA, gamma-aminobutyric acid; PSII, photosystem II. Pesticides were supplied by Bayer, Germany.

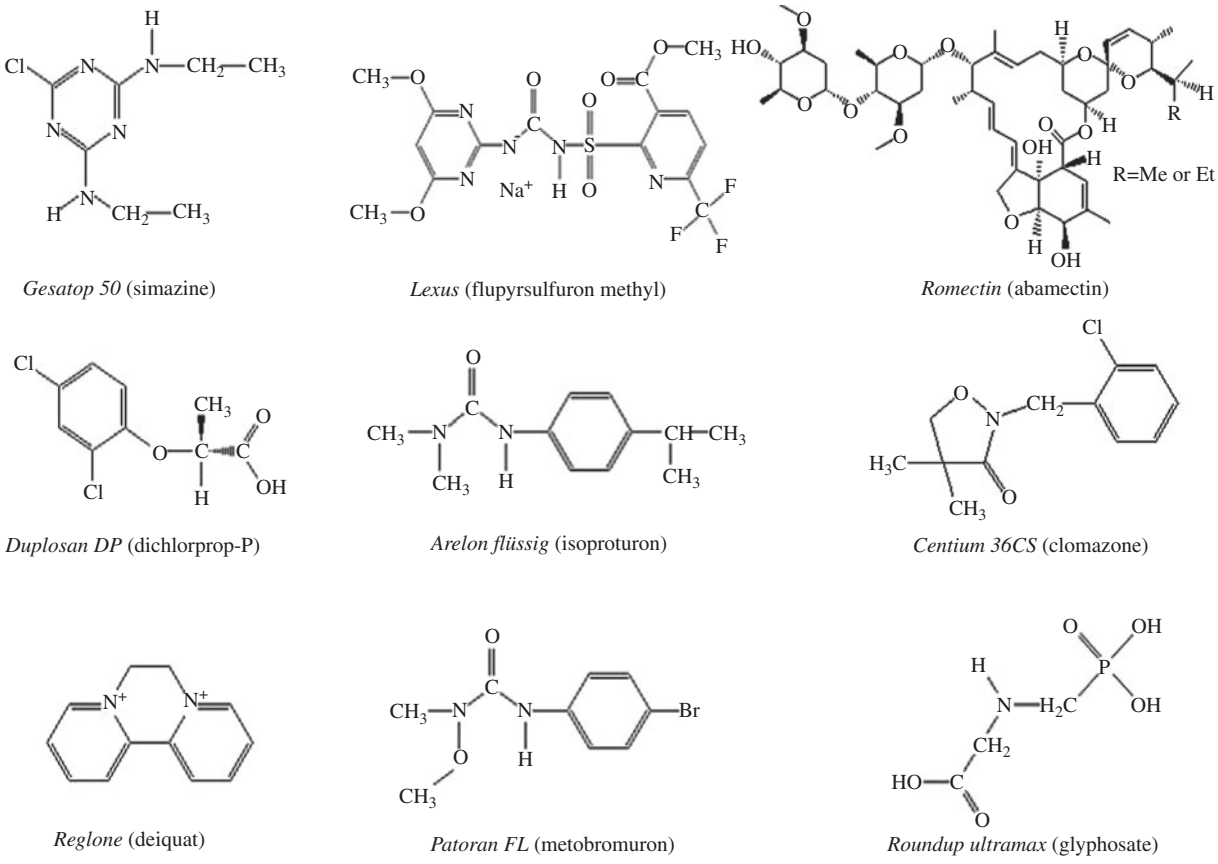


**Figure 12** Pesticide molecular structures.

(of ca. 15%) should be compared with point-to-point fluctuations of the natural leaf fluorescence, as shown in Figure 15 .

Probably, these fluctuations result from different biological factors affecting chlorophyll fluorescence. Additional fluctuations were found in relation to the leaf age, its location on the tree, exposure to sun, and nutrient content (Lichtenthaler et al. 1990, Artus et al. 1992, Gitelson et al. 2003, Porcar-Castell et al. 2008). Therefore, accurate detection of pesticides on leaves could not be based on their effect upon chlorophyll fluorescence.

A different approach was the application of a labeling agent that would block the fluorescence of the chlorophyll on the one hand and generate fluorescence in the presence of pesticides on the other. This agent needs to be sensitive to the various pesticides. It is also important that this material can be applied easily and uniformly on the leaf surface.

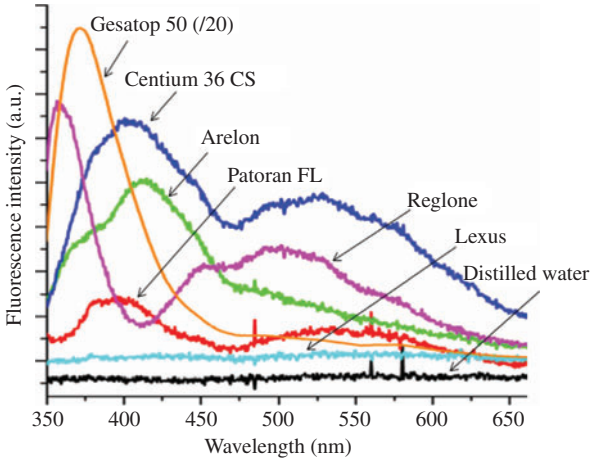


**Figure 13** Autofluorescence of pesticides: 1 µl of each pesticide solution was applied and the spectra were obtained using LIF system. Excitation at 337 nm was provided by a nitrogen laser (Hake et al. 2007).

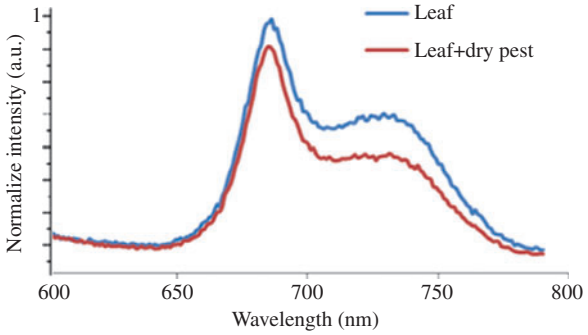


**Figure 14** Fluorescence spectra of untreated lemon leaf and lemon leaf contaminated with 1 µl of *Romectin* (excitation wavelength: 430 nm).

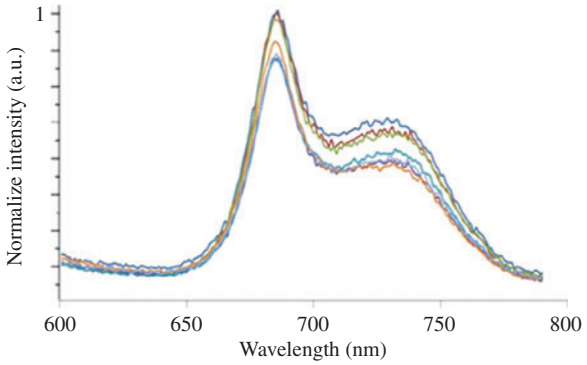


**Figure 15** Fluorescence spectra of an untreated leaf measured at different spots (excitation wavelength: 430 nm).

### Application of rhodamine dye as a fluorescence labeling material

Rhodamine 6G has many advantages as a labeling agent: it has an excellent adhesion to the leaf surface. When applying the solution of this dye on a leaf, it adhered well upon solvent evaporated. Another important advantage of rhodamine 6G is its low quantum yield in its solid phase, while possessing an excellent quantum yield in solution. There is a significant difference in the quantum yield of the dry and dissolved rhodamine 6G particles. Therefore, the presence of liquid pesticide on the leaf leads to significant changes in the fluorescence intensity. Additional advantages of rhodamine 6G include relatively low cost, availability, low toxicity, and its easy dissolution in various solvents.

The application of the labeling agent to plants was carried out in the following manner (Hake et al. 2007). Rhodamine 6G powder was dissolved in acetone at a concentration of $10^{-4}$ M. This solution was applied on the leaves using a hand-spray, such that by the time the rhodamine 6G aerosols reached the leaf surface, the acetone had already evaporated, resulting in a uniform layer of nano- and micro-rhodamine 6G crystals.

To test its potential of rhodamine crystals as a labeling agent, their fluorescence change upon contact with a droplet was measured.

**Fluorescence change of dissolved nanodye and microdye crystals** The dynamics of the fluorescence intensity of rhodamine 6G crystals during dissolution in a water droplet was studied. A single microscopic rhodamine 6G crystal was placed on the surface of a lemon tree leaf. A droplet of water was applied to the leaf next to the crystal. The rhodamine 6G crystal was pushed toward the drop, beginning to dissolve in the drop as it touched it. The process was monitored under a fluorescence microscope (Hake et al. 2007).

The series of images in Figure 16 show the dissolution process as a function of time. As the particle dissolved, the fluorescence intensity increased. In the first left image, the rhodamine 6G crystal (circled) is still dry and has a reddish color (the fluorescence of the solid material). Upon dissolving, a bright yellow intensity becomes evident as the reddish fraction decreases.

As can be seen, there is a distinct difference between the fluorescence intensity of dry and dissolved rhodamine 6G crystals. The fluorescence intensity of the dry rhodamine 6G nanocrystal and microcrystals is very low in comparison with that of the dissolved material.

### Effects of pesticides on rhodamine fluorescence

The pesticides affect both the intensity of the rhodamine dye and its emission wavelength, as described in the following:

**Effect of pesticides on fluorescence intensities** To investigate the effect on pesticides upon the rhodamine fluorescence intensity, several compounds were applied. One microliter of each pesticide solution was applied to an ivy leaf coated with rhodamine 6G in a uniform layer consisting of nanocrystals and microcrystals. A pure water droplet was

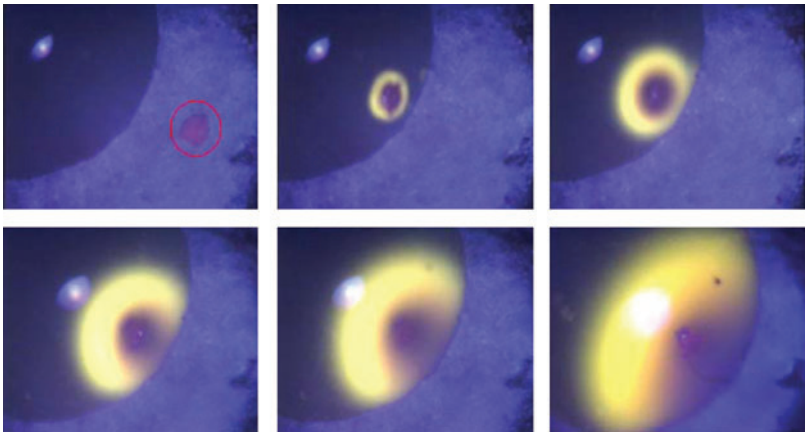

**Figure 16** Fluorescence microscope images monitoring the dissolution of rhodamine microcrystallite in a droplet of water on a lemon tree leaf (excitation at 365 nm) (Hake et al. 2007).

used as reference. Spectra were obtained using a LIF system equipped with nitrogen laser as an excitation source. The results are shown in Figure 17 (Hake et al. 2007).

The results show that the dry layer of rhodamine 6G nanocrystals and microcrystals had the lowest fluorescence intensity, whereas the water induced the highest intensity measured. The various pesticide solutions yielded spectra between these two extremes.

The unique composition of each pesticide solution led to a different fluorescence intensity of the labeling compound. The increased intensities (relative to dry rhodamine 6G) are dependent on the chemical composition of the pesticide solutions. Moreover, it was noted that the spectral peaks obtained in the presence of the pesticides were shifted relative to that obtained with water.

**Analysis of spectral shifts** The spectral shifts observed in the fluorescence of rhodamine 6G in the presence of various pesticides are of considerable importance. First, they may allow for identification of the applied pesticide. Second, the spectral differences between all pesticides and pure water are important for assessment of pesticide coverage, since they provide a clear distinction between pesticide presence and natural water droplets that may persist on the plant.

Figure 18 shows the peak obtained for a pesticide solution in comparison with the peak obtained from distilled water applied on a dry rhodamine 6G layer. It should be noted that the fluorescence intensities were normalized to facilitate comparison between the spectra. Application of a water droplet on the dry rhodamine 6G layer is characterized by a peak at 560 nm, whereas the spectrum resulting from the application of *Duplosan DP* is characterized by a peak at 578 nm.

In the cases where the pesticide caused a clear spectral shift, the presence of the pesticide could be detected and quantified, as shown in Figure 19 (Hake et al. 2007). For example, the actual rhodamine 6G spectral shift caused by the presence of Arelon flüssig was measured as a function of its concentration. Spectra were obtained using a LIF system with nitrogen laser as excitation source at 337 nm.

A clear relationship was found between the concentration of the pesticide solution and the spectral shift. The spectral shift increased with pesticide concentration until a plateau was reached. Therefore, the spectral shifts could be used for estimating the concentration of the pesticides (within a given dynamic range).

Nevertheless, not all pesticides induced a significant spectral shift. For example, the measurements indicated that application of Roundup UltraMax did not result in a quantifiable shift.

### Imaging of pesticide coverage on leaves

The spectral data allow for accurate detection of the pesticide and quantitative estimation of its presence on a leaf. However, such data obtained at a single spot are not adequate for the assessment of pesticide coverage. For this purpose, additional imaging data must be obtained.

Chemical imaging may be obtained in several experimental modes: point-by-point scanning the inspected area, obtaining spectral features at each point; direct imaging using a CCD camera, which provides fluorescence imaging at a given excitation wavelength; and hyperspectral imaging using Fourier transform spectral imaging analysis, which provides simultaneous full spectral resolution at each pixel. These different experimental modes are discussed in the following.

**Scanning data** The power of LIF scanning was demonstrated (Hake et al. 2007). A droplet of Roundup UltraMax was applied on an ivy leaf precoated with a dry rhodamine 6G layer. LIF scanning was performed along the diameter of this droplet. For this purpose, the sample was located on a sample holder operated by micrometer screws, which allowed scanning steps of 0.125 mm. Precise excitation was facilitated by a focused nitrogen laser beam. Excitation

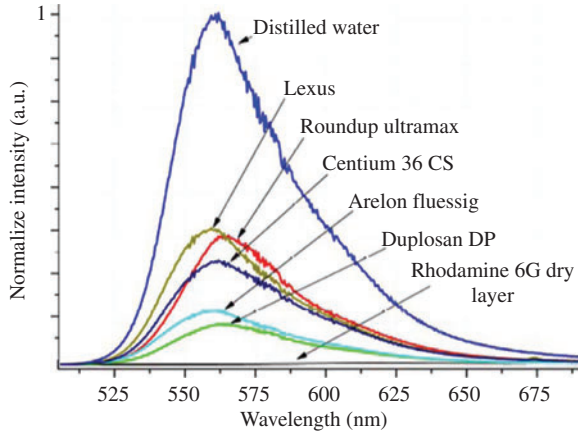


**Figure 17** Fluorescence emission spectra of pesticide micro droplets applied on rhodamine 6G layer on an ivy leaf; the spectra were obtained using a LIF system (excitation at 337 nm) (Hake et al. 2007).

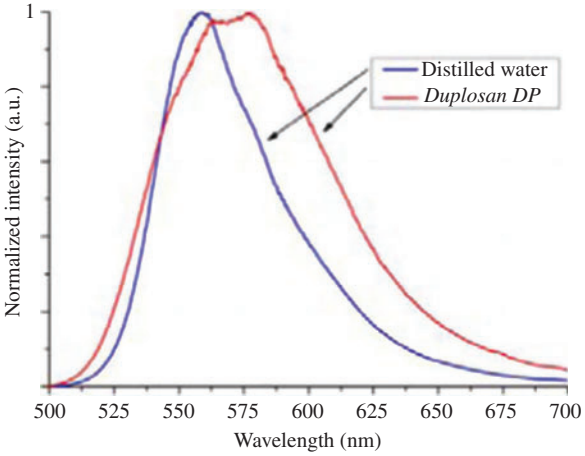


**Figure 18** Fluorescence intensity of rhodamine 6G layer on ivy leaf surface applied with distilled water and with *Duplosan DP*. Measurement was performed using a LIF system (excitation at 337 nm) (Hake et al. 2007).

wavelength was at 337 nm, and the emission was recorded at 561 nm.

Figure 20 shows the fluorescence intensity distribution along the scanning line for a specific emission wavelength. The droplet contour is clearly distinguished from the background of the dry rhodamine 6G crystal. Such scanning allows for precise two-dimensional definition of the pesticide droplet shape.

Extending the imaging measurements for a series of emission wavelengths is also possible and might be useful for handling pesticide mixtures. An example of such data is shown in Figure 21.

The above results allow for very precise definition of the shape of pesticide droplets. However, measurements using standard instrumentation are time-consuming due to the mechanical scanning procedure. Alternative scanning could be carried out using commercially available mirror-driven scanning techniques, where the excitation laser can scan the whole surface within a second. It should also be mentioned that this technique generates detailed spectral data, which are not required for the farmer in the field. For this purpose, simpler (and less expensive) imaging techniques should be applied.

**Direct imaging** The simplest imaging setup was obtained using a UV light source and a commercial CCD camera. The following experiment was conducted to demonstrate the power of such a simple setup (Hake et al. 2007): half an ivy leaf was dipped in an aqueous solution of rhodamine 6G, thus forming a labeling layer on the leaf surface. It was left to dry, leaving an almost homogeneous thin layer of rhodamine nanocrystals. The other half of the leaf remained untreated. Droplets of various pesticides were applied to both sides of the leaf. Imaging was performed using a commercial CCD camera and a UV light source at 360 nm (Figure 22).

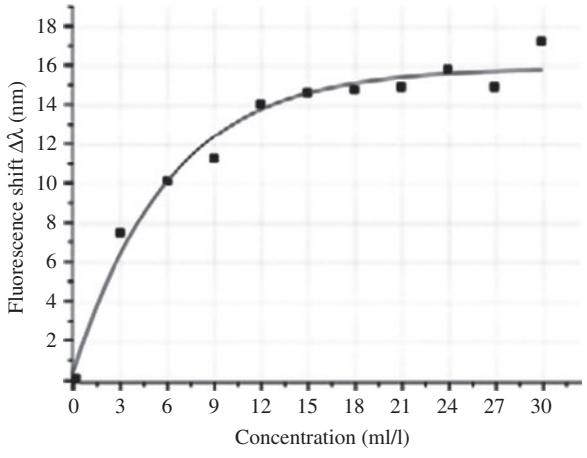


**Figure 19** Fluorescence shift as a function of *Arelon flüssig* concentration, conducted by applying pesticide droplets on an ivy leaf coated with a rhodamine 6G layer. Measurement was performed using a LIF system with nitrogen laser as excitation source at 337 nm (Hake et al. 2007).

The pesticide droplets on the right side of the leaf (which was pretreated with rhodamine 6G) was clearly visible, whereas those applied to the left side (untreated area) were not visible. It means that such a simple setup can be used for assessing the area covered by droplets. However, the method does not allow for distinguishing between the pesticide compounds or between pesticides and water droplets due to the lack of spectral data.

**Hyperspectral imaging** The imaging spectral information can be obtained using hyperspectral methods. Hyperspectral imaging, which provides full spectral resolution in each pixel, was obtained by a dedicated instrumental setup of Fourier transform imaging. Unlike the scanning procedure, this setup provides simultaneous spectroscopy without moving the sample or the detector. Therefore, it allows for quick mapping of pesticides on plant surfaces.

An example of the data obtained using such hyperspectral imaging is shown in Figure 23 (Hake et al. 2007). A lemon leaf was precoated with a rhodamine 6G and a Romectin droplet was applied. The method provides full simultaneous spectral resolution in all pixels, so the measured spectra can be compared with library spectra and allow for identification.

The spectra at four example points are indicated in Figure 24. Note the much higher intensity in points a and b, which represent the pesticide-contaminated area. Points c and d are out of the pesticide spotted area, thus possessing much weaker emission.

Generally, this method can provide accurate coverage mapping. Moreover, hyperspectral imaging has the potential to provide also pesticide identification, based on the full spectral features.

## Conclusions

This review was focused on studies about optical detection of pesticides on plant surfaces. In addition, the feasibility of assessing pesticide coverage on leaves using fluorescence methods was addressed. Since many of the pesticides exhibit low autofluorescence, reliable results require additional

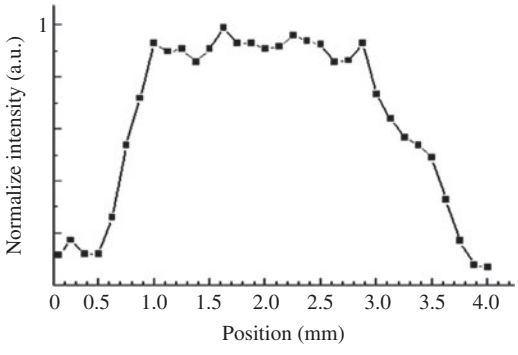


**Figure 20** Fluorescence intensity as function of position along the diameter of a *Roundup UltraMax* droplet on a leaf precoated with a rhodamine 6G layer (Hake et al. 2007).

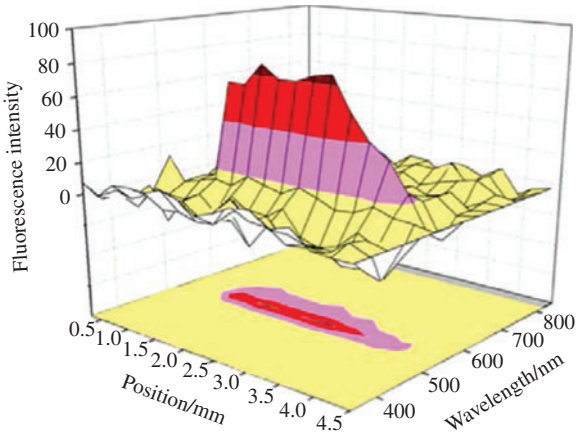


**Figure 21** Three-dimensional description of the fluorescence spectra along the droplet diameter. The *Roundup UltraMax* droplet was applied on a leaf precoated with a rhodamine 6G layer (Hake et al. 2007).

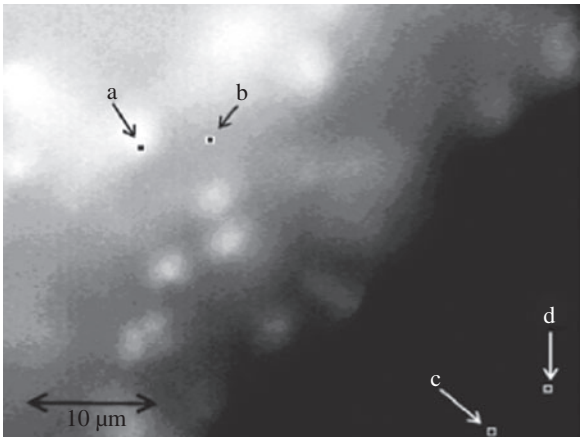


**Figure 23** Fourier transform hyperspectral imaging of a lemon leaf surface pretreated with rhodamine 6G crystals after application of a droplet of *Romectin* (upper left: for example, points a and b) and with no pesticide (lower right: for example, points c and d) (Hake et al. 2007).

intervention. Moreover, detection of the pesticides based on their effect upon the leaf fluorescence was not successful. The spectral data of leaves were not reproducible due to the chlorophyll content and other bioemitting molecules. This obstacle was overcome using a labeling agent. Several such agents have been considered and examined. It was found that rhodamine 6G nanocrystals and microcrystals can be used as an indicator of pesticide presence. Quantitative coverage assessment was possible using leaf pretreatment, which included the creation of a uniform layer of rhodamine 6G nanocrystals and microcrystals.

When pesticide droplets were applied to the pretreated leaf, two phenomena were observed. The first is a substantial increase in the dye fluorescence compared with its solid-state emission. This change was attributed to the dissolution of the crystallites. The second is material-specific fluorescence shifting, resulting from the interaction of dye molecules and organic components in the pesticide droplet. This information enables the determination of pesticide coverage extent and distinguishing between different pesticides. In many cases, the spectral shifts can also be quantified and used for analytical purposes.

Three imaging techniques were suggested for analysis of pesticide coverage. The first was scanning using a LIF system, enabling definition of the droplet shape in two dimensions, in addition to spectral data. Since this process is time-consuming, a second technique was suggested. It consisted of a UV source and a commercial CCD camera. This technique allows for direct imaging of the pesticide on the leaf. The obtained data included droplet shape and its location on the leaf. When spectral data are needed (e.g., when pesticide identification is required), hyperspectral imaging should

**Figure 22** CCD imaging of applied pesticide solutions droplets on an ivy leaf partly coated with rhodamine 6G nano-crystallite layer (excitation wavelength: 360 nm).

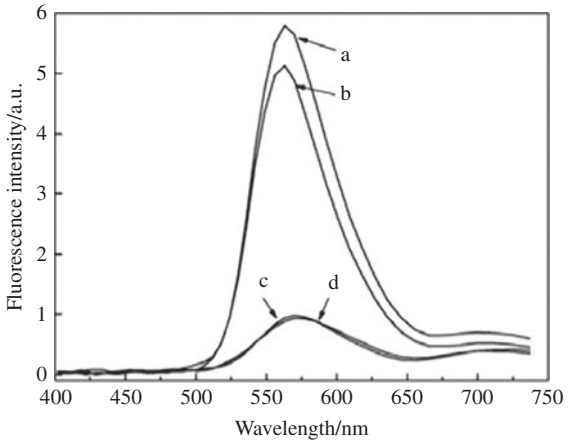


**Figure 24** Fourier transform hyperspectral imaging analysis of a lemon leaf surface pretreated with rhodamine 6G crystals and contaminated by a droplet of *Romectin*. Points a and b represent the spectra at the contaminated part, whereas points c and d represent clean sites (Hake et al. 2007).

be utilized. Hyperspectral imaging using Fourier transform spectral imaging analysis provided simultaneous full spectral resolution at each pixel, thus enabling accurate mapping of the leaf.

The detection on tiny or twisted leaves may require additional engineering efforts. In the case of leaves with nonflat surface, quantification could be very difficult, since observation of fluorescence would depend on the viewing angle.

The dedicated imaging instrumentation was based on either direct CCD imaging or on LIF, which proves full spectral features. The former is a low-cost technology and can easily be implemented in the form of a hand-held instrument. It is suitable for field operation and may provide quantitative information on pesticide coverage. However, chemical identification would require detailed spectral information, which calls for more complicated instrumentation.

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