Biological Determination of Photosynthetic Inhibitors in Soils and Water and Application of Bioassays to Herbicide Investigations

Wilfried Pestemer

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Unkrautforschung, Messeweg 11-12, D-3300 Braunschweig

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Bioassays, Photosynthetic Inhibitors, Fate and Activity of Herbicides

Bioassay techniques and procedures for the determination of photosynthetic inhibitors in situ, in vivo, and in vitro are reviewed.

Examples of bioassays for herbicide development and research are given.

Bioassay techniques used in herbicide investigations are based on the response of a living organism to different concentrations of a chemical.

Bioassays for determination of herbicides in soils and water can be classified in different ways:

- Location: Greenhouse-, growth chamber-, and laboratory tests.
- 2. Medium: Treatment in substrate-free medium (Hydroculture with nutrient solution or soil water extract).
 - Treatment in sorption-free medium (Hydroponic culture in vermiculite, perlite, cellulose, agaragar, quartz-sand or other supports). Treatment in sorptive medium (soils with surface treatment, uniform incorporation, or treatment of layers with a herbicide).
- 3. Time consumption: Short-time-bioassays (minutes some days) with algae, chloroplasts, mito-chondria, plant tissues, leaf discs, CO₂- or O₂-measurement. Long-time-bioassays (growth tests during 3 30 days) with whole plants.

Dose-response relation of bioassays depends strongly on the age and stage of development of the indicator plant and on growth conditions (environment). Results are estimated visually (symptoms like necrosis, chlorosis, epinasty and deformations) or by objective measurements expressed direct or as percentage of the control plant by the ED₅₀-(median effective dose), GR₅₀- (median growth reduction), EC₅₀- (median effective concentration) or ID₅₀-value (median inhibition dose). For quantifying the results appropriate controls and standards must be included in each experiment.

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For photosynthetic inhibitors (ureas, diazines, triazines, triazines, triazinones, uracils, some amides and carbamates and dipyridylium salts) many methods are outlined:

A lot of short-time-bioassays based on the effect of herbicides on the oxygen development produced by photosynthesis in Chlorella sp. [1] and estimation of this oxygen by the bioluminescense system of photobacteria [2], a biological oxygen monitor [3] or by Warburg apparatus [4]. The growth reduction of microalgae in nutrient solution [5, 6] or on agar-plates [7] and the creeping movement of blue algae [8] are described as rapid and inexpensive methods for determining the inhibitory effect of herbicides. The chlorophyll production of Chlorella pyrenoidosa [9] and the measurement of culture turbidity or extractable pigments [10, 11] are usable methods. Further on different Lemna sp. are very susceptible to photosynthetic inhibitors. Evaluation is based on the number of individual plants, their fresh or dry matter [12, 13], their bleaching effect [14] and their nitrate reductase activity and chlorophyll content [15]. Other shorttime-bioassays are based on tissue culture systems [16, 17], the inhibition of chlorophyll formation in etiolated and excised tissues [18], the different accumulation of leaf tissue starch [19], the light nitrite reduction of cucumber leaf discs [20] and on the observation that leaf discs floated on liquid medium sink in a few hours if photosynthesis was inhibited [21].

Long-time-bioassays with whole plants (so-called growth tests) are probably the most commonly used ones in herbicide research. The response of the test plant is closely related to that of a crop or weed in the field. There are numerous plant species used for bioassays and referred in some compilations [22 –



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25]. Photosynthetic inhibitors do not affect germination or seedling growth during the first 3-8 days after sowing, retardation of growth occurs only after food reserves in the endosperm were consumed. Therefore the content of food reserve can be determined in a dark germination test and substracted from all yield results to give the matter yield under herbicide stress [24]. Another technique is to use pregrown selected uniform plants in a system involving nutrient solution or soil [26]. Bioassays are suitable for estimating the biological activity of herbicides. Chemical and physical procedures are only used to identify substances. Comparative studies of instrumental and bioassay methods are listed by Hurle [22]. They showed in some cases satisfactory agreement, but residues determined chemically were sometimes higher or lower. Various factors could explain this anomalous results [27].

The bioassays described above have been used to investigate many scientific and practical aspects of herbicide behaviour in the environment. Application of bioassays for herbicide development (screening) and research (see [28, 29] for review) include inherent herbicidal activity of a compound, its physiological selectivity, mode of action and sites of uptake, leaching and sorption properties, volatility and photodecomposition, dependence on soil moisture and temperature. The results of these investigations give a good survey of phytotoxicity, plant availability, persistence and residue situation as to the behaviour of herbicides in the environment and form the basis for their responsible use in practise.

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