

## The role of a multi-technique approach for tracking nanoherbicides in plant systems

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Abstract – About 1.7 Mt of herbicides per year are used worldwide, but only around 0.1% reach the target-site<sup>1</sup>. Nanotechnology has become a viable tool to increase the toxicity of herbicide to the target weeds, reducing the environmental impacts of herbicide use<sup>2</sup>. Research regarding metallic and polymeric-based nanoparticles as herbicide carriers has been pushed forward, with the goal to improve the herbicide delivery or tune the herbicide-plant interaction<sup>3,4</sup>. Tracking nanoherbicides in plant systems is still a challenge due to the low official and reproducible methods, the high cost of techniques and the need of specific tracers as indicators. The objective of this work was to highlight the need of integrated techniques to unveil the effect of NPs on herbicide mode of action. In general, the studies with plants use NPs as models, and the effective tracking of pesticide-loaded NPs is not deeply investigated mainly for polymeric nanosystems. For example, radiometric techniques allowed to identify the behavior of <sup>14</sup>C-nanoatrazine in plants, and <sup>14</sup>C-nanometribuzin in soil and plants<sup>3,5</sup> using quantitative and qualitative approaches. These works showed how polymeric nanosystems can change the herbicide interaction, uptake and translocation pathways in plants, highlighting radioactivity labelling (<sup>14</sup>C) as a useful tool for this perspective. Confocal microscopic (CfM) with the nanosystem labeled with a fluorescent marker can be essential for understanding NPs behavior in plants, because radiometric techniques are restricted to mapping the active ingredient behavior<sup>6,7</sup>. CfM was able to study the movement of atrazine-polymeric NPs via root and leaves<sup>8</sup>. Fluorescent labeling also allows a quantitative and qualitative approach, but it is restricted to the nanosystem and does not determine any information concerning the active ingredient<sup>7</sup>. Concerning metal-based NPs, ICP-MS can quantify the NPs inside plant tissues<sup>9</sup>, but it is not able to investigate qualitatively the distribution patterns. When it is integrated with CfM or X-ray techniques, a completely quantitative and qualitative analysis is obtained<sup>10</sup>. Integration of Raman Spectroscopy<sup>11</sup>, X-ray diffraction<sup>10</sup>, X-ray absorption near-edge structure (XANES)<sup>12</sup> and microscopy<sup>10</sup>, can be suitable for NP detection and quantification of NPs in plant systems. Moreover, tracking nanoherbicides needs an interdisciplinary approach to determine the integrated behavior of pesticides and NPs in plants, as well as the effect of NPs in herbicide mode of action. This work highlights the importance of understanding NP-herbicides-plants interaction to track their fate, to unveil their interaction with plants in a micro and macro scale, leading to optimize their effectiveness and minimize potential negative environmental impacts.

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