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The interactions between surfactants and the epicuticular wax on soybean or weed leaves: Maximal crop protection with minimal wax solubilization

Tatiana Cardoso Damato [a], Letícia D.M. Carrasco [b], Ana Maria Carmona-Ribeiro [b], Ricardo Vagner Luiz [c], Roberta Godoy [c], Denise F.S. Petri [a,*]

[a] Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, 05508-000, São Paulo, SP, Brazil
[b] Department of Biochemistry, Institute of Chemistry, University of São Paulo, Brazil
[c] Dow Brasil Sudeste Indl Ltda, São Paulo, SP, Brazil

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A B S T R A C T

Surfactants are commonly added to aqueous herbicide formulations to reduce the surface tension, facilitating their application. In the present study the interaction between the surface of freshly harvested soybean leaves and pure water, solutions of cationic (CTAB), anionic (SDS) and uncharged (ethoxylated sorbitan monolaurate 20 EO – ESM20) surfactants and a commercial herbicide formulation (CF) was investigated by means of contact angle measurements and scanning electron microscopy (SEM). The wettability and the hysteresis in the contact angle values determined for soybean leaves were the largest for the surfactant with the highest critical micelle concentration. The epicuticular wax on soybean leaves presented rosette-like clusters of platelets, as observed by SEM. After interaction with the surfactants or CF, most of the wax platelet rosettes disappeared from the surface, indicating that the epicuticular wax was dissolved by the surfactant. The wax contained long hydrocarbon and long chain esters, as qualitatively analyzed by gas-chromatography coupled with mass spectroscopy. The wax extracted from soybean leaves presented no significant biocidal activity against *Micrococcus luteus*, *Staphylococcus aureus* or *Escherichia coli*. Its protective role against pathogens depends solely on the tridimensional arrangement on the surface, which impairs the physical attachment of microorganisms to the surface. For comparison, the wettability and morphology of *Eleusine indica*, a common weed, was investigated. The weed leaves presented smooth surfaces and a much lower contact angle values. Therefore, surfactants should be added to the herbicide formulations with the compromise to protect the crop adequately and to minimize the solubilization of protective epicuticular wax layer on soybean leaves.

1. Introduction

Soybean is a cheap source for edible oil and protein. The United States Department of Agriculture (USDA) estimates that the Global Soybean Production 2016/2017 will be 324.2 million metric tons (http://www.globalsoybeanproduction.com). USA, Brazil and Argentina are responsible for approximately 80% of total production. Particularly, in Brazil over the last 20 years, the production increased 6 times whereas the area dedicated to soybean plantations increased less than 3 times, evidencing an efficiency gain. One factor that helped to improve harvesting efficiency was the development of herbicide formulations, which are mainly composed of herbicide, adjuvants and water. Generally, the herbicide is provided together with the genetically modified soybean seeds, so that it acts selectively on the metabolic pathway of weed and are harmless to soybean. Surfactants are adjuvants added to the formulation in order to reduce the interfacial tension between leaf surface and formulation, improving the spreading of formulation droplets on the leaves (Tominack, 2000). One important consequence of enlarging the droplet-wetted area is the reduction in the evaporation time, making the spray application process more efficient. These trends were observed for spray application of four

* Corresponding author.
E-mail address: dfsp@iq.usp.br (D.F.S. Petri).

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different adjuvants (crop oil concentrate, modified seed oil, nonionic surfactants and oil surfactant blend) on four different soybean plant surfaces, namely, abaxial and adaxial leaflet surfaces, petiole and basal stem (Gimenes et al., 2013).

The effect of surfactants on the absorption capacity of herbicides by plants leaves was (probably) first investigated by Freed and Montgomery (1958). They observed that uncharged and negatively charged surfactants favored the absorption and translocation of 3-amino-1,2,4-triazole (herbicide) by Black Valentine beans leaves because (i) they reduced the surface tension of formulations and (ii) there were favorable interaction between surfactant and herbicide, however, they suggested some direct effect on the leaves surface and/or underlying layers. More recent studies reported by Basu and coworkers (Basu et al., 2002) compared the effect of cationic, anionic and neutral surfactants on the wetting capacity of formulations on leaves for different plants; regardless of the type of plant, the neutral surfactant provided the best wettability and herbicide retention on leaf, reducing wastage. The effect of nonionic surfactant concentration on the wetted area on leaf surfaces by spray droplets upon application of formulations is well reported in the literature (Xu et al., 2011; Gimenes et al., 2013); in comparison to pure water, the wetted area considerably enlarged with the increase of surfactant concentration up to a critical value, but for concentrations higher than the critical value the wetted area no longer increased.

In the case of herbicide formulations, in the ideal situation, the interaction between the formulation droplets with the plant surface of interest is minimal and with the weed surface is maximal. The wetting study reported by Basu and coworkers (Basu et al., 2002) referred to the interaction between surfactants and cauliflower, cabbage and spinach leaves, and French bean, wheat, and Bengal gram seeds. To the best of our knowledge there is no literature report demonstrating the effects of water, solutions of surfactants and commercial herbicide formulation on the microrelief structure and wettability of soybean (*Glycine max*) and a common weed (*Eleusine indica*) leaves. Thus the present study aims to contribute to the current understanding about such effects by means of scanning electron microscopy (SEM) and static contact angle measurements. Cetyltrimethylammonium bromide (CTAB), a cationic surfactant, ethoxylated sorbitan monolaurate 20 EO (ESM20), a neutral surfactant, and sodium dodecyl sulfate (SDS), an anionic surfactant, were chosen as surfactants. The commercial herbicide formulation contained ionic and nonionic surfactants. Generally, the herbicide formulations are applied at two different growth stages of soybean, namely, at the seeding and at V2 stage of plant development. Particularly at V2, herbicide spraying reaches not only weeds (target), but also soybean leaves. Considering that the epicuticular wax microcrystals protect the soybean leaves against water loss (Kim et al., 2009) and pathogens (Furtado et al., 2009), the issues raised by the present study focused on (i) how the surfactants and herbicide formulation affect the wax microcrystals structural features and (ii) which type of surfactant presents the highest affinity for the surface of soybean and weed leaves. In order to gain insight about the chemical composition and antimicrobial properties of soybean leaves wax, the wax microcrystals were extracted, characterized by gas chromatography coupled with mass spectrometry (GC-MS) and tested against gram positive and gram negative bacteria. The main goal is to gain fundamental knowledge about the interaction between soybean and weed leaves surface and surfactants and herbicide formulations to achieve high crop protection using the minimum of adjuvants and to minimize damages to the wax layer.

1.1. Background

The wetting property of a liquid on a surface might be expressed by the contact angle (θ) of a droplet of this liquid on the surface. For an ideal surface (perfectly smooth and chemically homogeneous) θ results from the equilibrium of three forces, namely, the liquid–air (γ_L), liquid–solid (γ_{SL}), and solid–air (γ_S) interfacial tensions, as proposed by Young (Berg, 2010), and schematically represented in Fig. 1a:

$$\gamma_S = \gamma_{SL} + \gamma_L \cos\theta \tag{1}$$

In the case of water, low values of θ indicate that the liquid wets well the surface, whereas high values of θ indicate weak wettability. The work of adhesion (W_{SL}) between the liquid and the surface can be determined by the Young-Dupré equation (Berg, 2010; Chaudhury, 1996; Kosaka et al., 2009):

$$W_{SL} = \gamma_L(1 + cos\theta) \tag{2}$$

The addition of surfactants to herbicides formulations is mainly due to the very low wettability (high θ values) of many plants, which is caused by the hydrophobic nature of the epicuticular wax microcrystals, surface roughness and by the hierarchical structures present on leaves (Taylor, 2011). The well-known "Lotus effect" exhibited by the leaves of lotus (*Nelumbo nucifera*) is the maximization of such features, making them self-cleaning surfaces. Micro- and nanostructures of wax crystals impair the adhesion of dust particles on the surface of lotus leaves; the dust particles picked up by water droplets are repelled from the surface and roll away (Barthlott and Neinhuis, 1997; Koch et al., 2008). The poor wetting is not only due to the wax microcrystals but also to the surface roughness. The air entrapped among the microcrystals is also hydrophobic, so that the water droplet set on the leaf surface probes a surface composed of wax, leaf components and air, causing superhydrophobicity as predicted by Cassie-Baxter model (Berg, 2010) and schematically represented in Fig. 1b. In this model the effective contact angle (θ_{CB}) results from the contribution of the interaction between water and each component on the surface:

$$cos\theta_{CB} = f_1 cos\theta_1 + f_2 cos\theta_2 \tag{3}$$

where f_1 and f_2 are the area fractions occupied by component 1 and 2 on the surface, respectively, and θ_1 and θ_2 are the contact angle values for component 1 and 2 in contact with water, respectively. If one of the components is air with θ value of 180°, the larger the area fraction with air, the more hydrophobic is the surface.

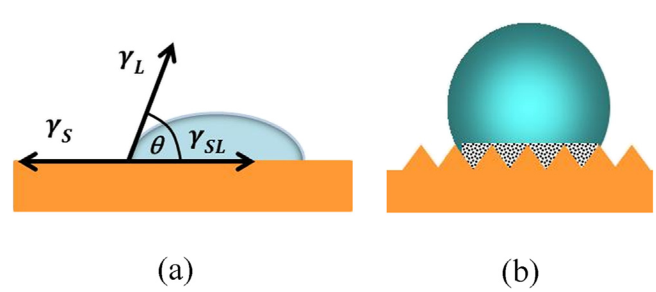

Fig. 1. Schematic representation of a droplet of a liquid on (a) an ideal surface along with the interfacial forces and equilibrium contact angle and (b) a rough hydrophobic surface, with air among the micrometric structures.