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Herbicide effects on cuticle ultrastructure in *Eleusine indica* and *Portulaca oleracea*

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Key words: quizalofop, lactofen, cuticle, *E. indica, P. oleracea*

ABSTRACT: *Eleusine indica* and *Portulaca oleracea* are two common weeds in peanut crops in southern Córdoba. Two chemicals are frequently used to control them, quizalofop for grasses and lactofen for dicots. The objective is to study the effects of quizalofop and lactofen on cuticle ultrastructure in *E. indica* and *P. oleracea*, respectively. In the lab, quizalofop was applied on *E. indica* and lactofen on *P. oleracea*. Three plant categories were analyzed in each species: 3, 1-2, and no tiller in *E. indica*, and 8, 6, and 2 nomophylls in *P. oleracea*. Leaf samples from both species were collected at 7 and 16 days post-application and were treated for scanning electron microscopy. *E. indica* cuticle treated with lethal dose shows areas where epicuticular waxes disappear, specially in the youngest individuals. These areas are located predominantly on periclinal walls of typical epidermic cells and subsidiary cells. On the other hand, *P. oleracea* shows cuticle discontinuities that may be caused by lactofen entry. They are smaller and less frequent in plants having 8 or more nomophylls. The remaining waxes act as a herbicide accumulation compartment and, therefore, would partially prevent the active ingredient entry to epidermic cells.

Introduction

In southern Córdoba, *Eleusine indica* (L.) Gaertner "goosegrass" (Poaceae) and *Portulaca oleracea* L. "common purslane" (Portulacaceae) are two common weeds in peanut crops. It is well known the interference that occurs when *E. indica* grows in association with *Arachis hypogaea*. Peanut subterranean parts intermix with the weed roots, representing a major problem at harvest (Rainero and Rodríguez, 1993, 1994; Rodríguez and Rainero, 1995). The other important weed in this area, *P. oleracea*, is very competitive and host of nematodes, virus, and harmful insects for different crops including peanut (Norris, 1997).

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These weeds are usually controlled with herbicides. Two chemicals are frequently used in this region, quizalofop for grasses and lactofen for dicots. The first one belongs to the aryloxyphenoxypropanoate group (APP), known for inhibiting *de novo* synthesis of fatty acids catalized by acetyl CoA carboxylase (ACCase) and subsequent fatty acid biosynthesis in plastids (Cobb, 1992; Devine and Shimabukuro, 1994). On the other hand, lactofen is a diphenyl ether that inhibits the enzyme protoporphyrinogen oxidase (PPG-oxidase), present in the tetrapyrrole synthesis pathway (Hart *et al*., 1995; Vidal, 1997; Dayan *et al*., 1998; Warabi *et al*., 2002).

When herbicides are applied on plants, they must cross the cuticle in order to reach its site of action. The cuticle is an extracellular lipid layer with a thickness varying from 0.5 to 15 µm. Generally, it is composed of cutin and embedded waxes with epicuticular waxes on the outer surface (Chamel, 1986; Chamel and Vitton, 1996). Its ultrastructure varies from amorphous to highly crystalline deposits. Cuticular waxes are composed of complex mixtures with long-chain aliphatic and cyclic components. They represent the main barrier that controls diffusion rate of lipophilic compounds because of their physical structure (Santier and Chamel, 1998). Cutin, on the other hand, is a polyester of hydroxylated fatty acids and does not constitute a limiting factor for diffusing substances because its components leave spaces between chains big enough to be crossed by all kinds of molecules (Franke, 1969; Kerstiens, 1996). Structural differences between waxes and cutin are responsible for absorption properties of each species cuticle (Schreiber, 1995). Regarding to this issue, leaf age is an important factor that determines diffusion because there is an increase in deposition of different cuticular chemical compounds as plants grow up which, in turn, inhibits absorption (Hull *et al*., 1975).

Cuticle penetration is merely a physical process, that is usually explained by the "two-pathway hypothesis". According to this point of view, one path goes through the amorphous fase of cuticular waxes and is exclusive for lipophilic solutes, whereas the second path goes through water-filled molecular pores and is utilized by inorganic ions and hydrophilic organic compounds (Chamel, 1986; Santier and Chamel, 1998; Schreiber *et al.*, 2001; Beattie and Marcell, 2002; Schreiber, 2002).

Even though many studies have been carried out in the world to understand weed-herbicide interaction, in southern Córdoba *E. indica* and *P. oleracea* survive herbicide control (Malpassi, 2004 a, b). Therefore, there is a need to know in detail their characteristics, specially cuticular ones at the ultramicroscopic level, to be able to control them effectively. The objective is to study the

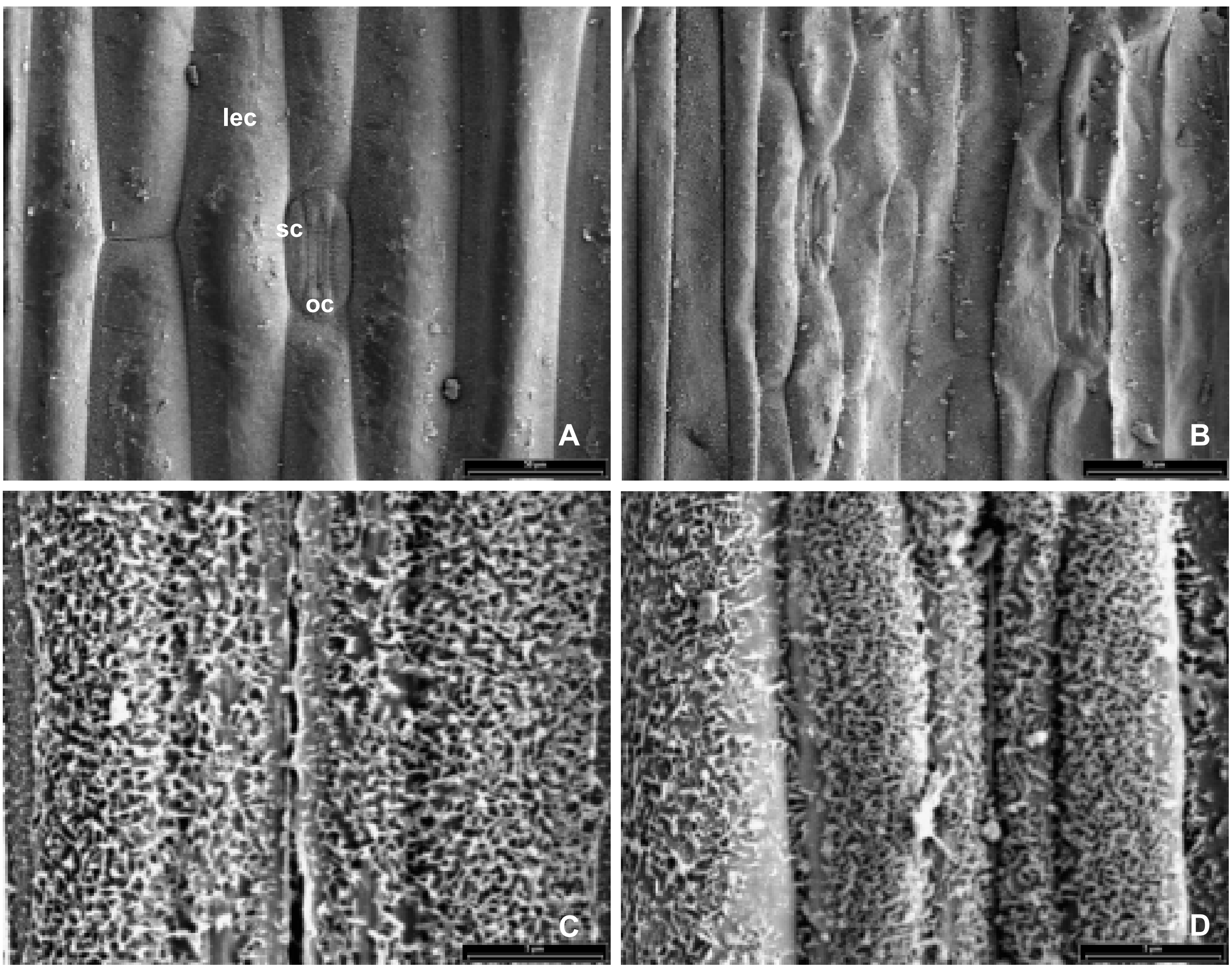

FIGURE 1. Leaf epidermis of *Eleusine indica*. A. Control: stoma (SEM, scale bar = 50 µm). B. Treated plant with 3 tillers at seven days post-application (SEM, scale bar = 50 µm). C. Control plant showing epicuticular waxes (SEM, scale bar = 5 µm). D. Treated plant with 1-2 tillers at seven days post-application (SEM, scale bar = 5 µm). epw = epicuticular waxes, lec = long epidermic cell, oc = occlusive cell, sc = subsidiary cell.

effects of quizalofop p tefuril and lactofen herbicides on cuticle ultrastructure in *E. indica* and *P. oleracea*, respectively.

Materials and Methods

The study was carried out in the lab of the National University of Río Cuarto (UNRC). *Eleusine indica* (L.) Gaertner "goosegrass" and *Portulaca oleracea* L. "common purslane" were seeded in pots containing soil from the experimental field of UNRC. These pots were at 25°C approximately, receiving sunlight during 12 h. They were periodically irrigated in order to keep the soil at field capacity and prevent the plants from suffering water stress.

Three treatments of quizalofop p tefuril (commercial name: LÓGICO from BAYER): 1. control or C (0 ml.ha^{-1}), 2. lethal dose or LD (300 ml.ha^{-1}), and 3. sublethal dose or SLD (75 ml.ha^{-1}) were applied on well-hydrated plants of *E. indica* that fell in one of the following categories: 0, 1-2, and 3 tillers. On the other hand, three treatments of lactofen 24 (diphenyl ether) [1-(carboethoxy) ethyl 5 (2–chloro-4 (trifluromethyl–2 nitrobenzoate)] (commercial name: COBRA from BAYER): 1. control or C (0 ml.ha^{-1}), 2. lethal dose or LD (300 ml.ha^{-1}), and 3. sublethal dose or SLD (100 ml.ha^{-1}) were applied on well-hydrated *P. oleracea* plants that fell in one of the following categories: 2, 6, or 8 nomophylls. All doses of both herbicides correspond to commercial products. They were applied till dripping with a plot pulverizer equipment which had an application pressure of

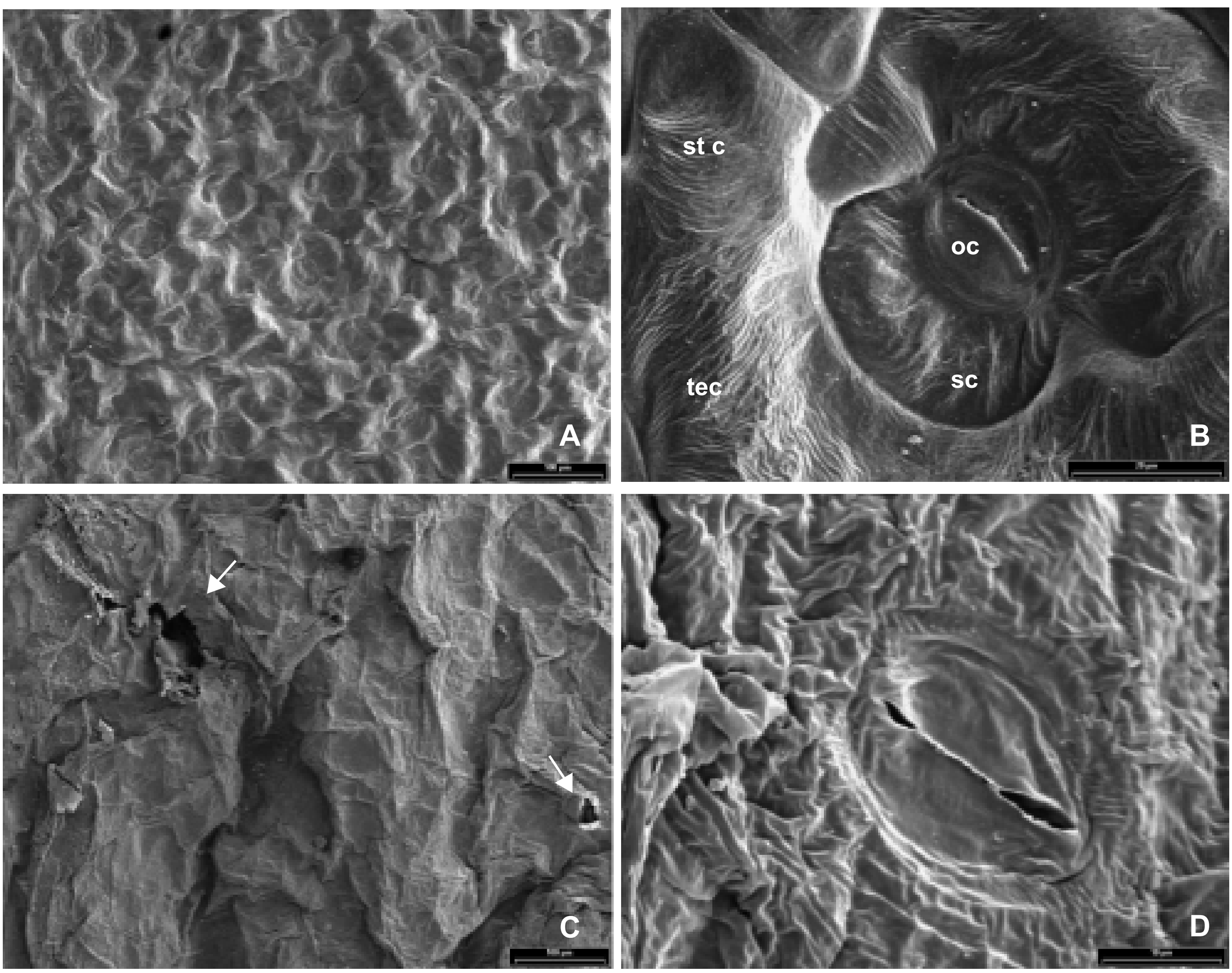

FIGURE 2. Leaf epidermis of *Portulaca oleracea*. A. Cuticular superficial view (SEM, scale bar = 100 μm). B. Striated cuticle showing a stomatal complex (SEM, scale bar = 20 μm). C. Cuticle from a plant with two nomophylls treated with lethal dose seven days after application (SEM, scale bar = 100 μm). D. Stoma from the same plant (SEM, scale bar = 10 μm). Arrows indicate cuticle cracks. tec = typical epidermic cell, oc = occlusive cell, sc = subsidiary cell, st c = striated cuticle.

50 lb at 0.50 m from the plant. Leaf samples from both species were collected at 7 and 16 days after herbicide application. They were fixed in glutaraldehyde 4% in sodium cacodilate 0.1 M pH 7.4 and were processed to observe epidermis with scanning and transmission electron microscope (Roland and Vian, 1991).

Results

Eleusine indica cuticle shows a soft relief that reflects epidermic cell turgidity and stomata at the same level of typical cells (Fig. 1A). Besides these specialized cells, trichomes and prickles are also observed. At higher magnification (5,400 x), epicuticular waxes with tubular form are uniformly distributed (Fig. 1C). The cuticle is thin, from 0.1 to 0.4 µm depending on the size of the analyzed plant, and shows the epicuticular waxes and a polylamellate region of cutin and intracuticular waxes in its transversal ultrastructure (Fig. 1E). After seven days from quizalofop application, the cuticle acquires a more undulated and folded aspect than in the control (Fig. 1B). These differences are due to plasmolysis of subjacent epidermic cells, causing the overlapped aspect of the cuticle. Stomatal cells are also plasmolysed, so the cuticle that covers them repeats the folded and overlapped aspect that is observed in the rest of the epidermis. The cuticle that covers trichomes and prickles does not show modifications. Differences in epicuticular waxes can also be observed. After seven days from herbicide application, less wax on stomatal subsidiary cells is detected as well as on periclinal walls of typical epidermic cells (Fig. 1D, 1F). It does not happen the same situation on occlusive cells. After 16 days from application, the cuticle shows less epicuticular waxes and its aspect is more undulated in all analyzed plants.

The cuticle of *Portulaca oleracea* control shows an undulated relief with peaks and valleys (Fig. 2A). Stomata are located in depressions, so they are slightly sunken (Fig. 2B). They are accompanied by two sub-

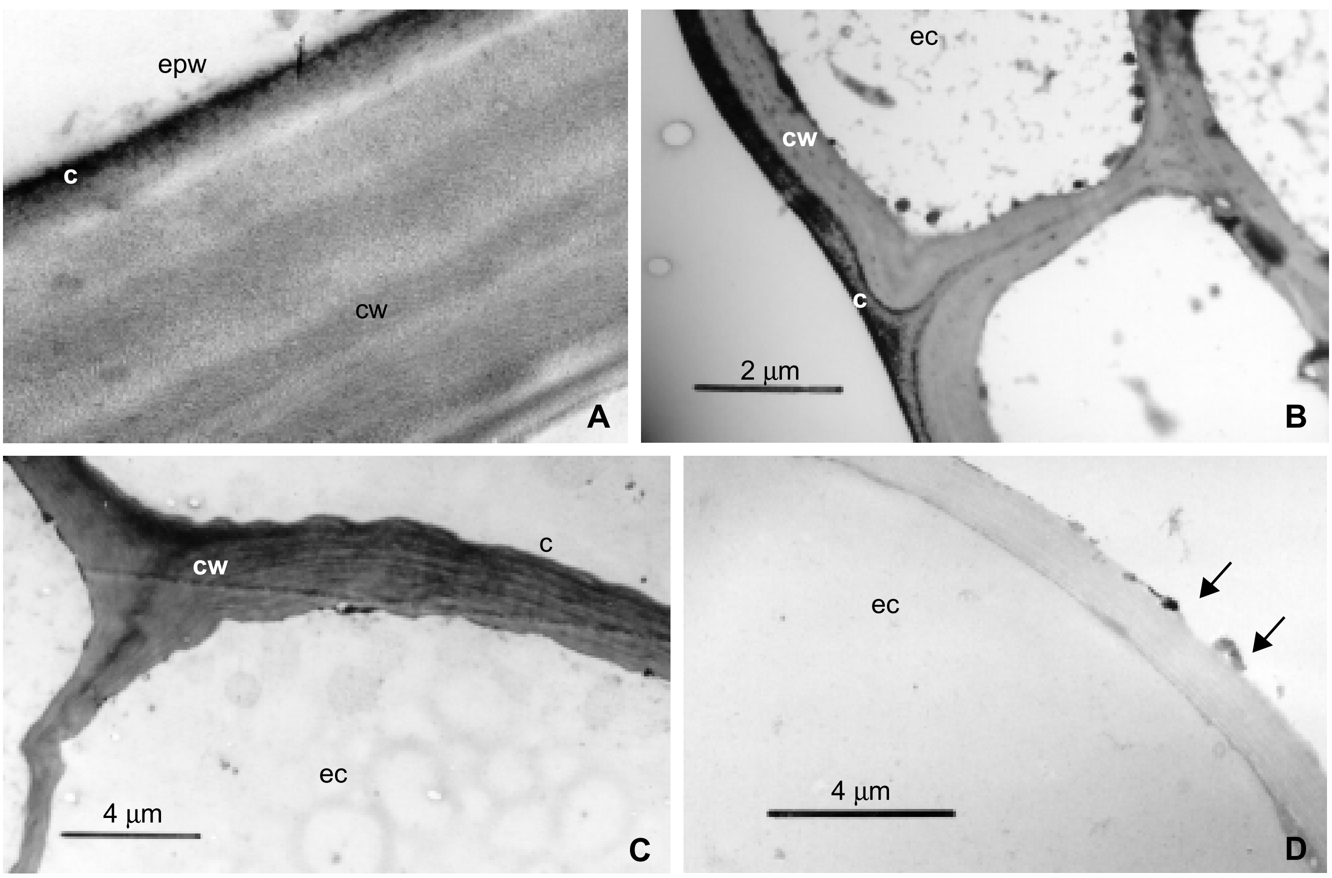

FIGURE 3. A. Transection of control epidermis of *Eleusine indica* (TEM, X 20,000). B. Treated plant of *Eleusine indica* with three tillers 16 days post-application (TEM, scale bar = 2 µm). C. Control cuticle of *Portulaca oleracea* (TEM, scale bar = 3 µm). D. Cuticle from a plant of *Portulaca oleracea* with eight nomophylls treated with lethal dose seven days after application (TEM, scale bar = 4 µm). Arrows indicate cuticle cracks. c = cuticle, cw = cell wall, ec = epidermic cell, epw = epicuticular waxes.

sidiary cells, one bigger than the other, that are placed parallel to occlusive cells. The cuticle transections observed with transmission electron microscopy (TEM) show a relatively thin continuous cuticle, 0.2 to 0.5 µm thick depending on the plant category analyzed (Fig. 2 E). Epicuticular waxes are not observed with SEM or TEM, although the cuticle has striated appearance with SEM (Fig. 2B, 2E). When lactofen is applied, some cuticular changes can be observed. Seven days after application, the cuticle is more undulated and folded than in the control (Fig. 2A, 2C). As it occurs in *E. indica*, these aspect differences are due to plasmolysed subjacent epidermic cells. Moreover, some fractures or cracks can be observed in it, regardless the analyzed plant category, but are bigger and more frequent in the youngest plants (Fig. 2C, 2F). Stomatal cells are also plasmolysed, as the rest of typical epidermic cells and, consequently, the cuticle that covers them reflects the situation (Fig. 2D).

Discussion

Eleusine indica cuticle treated with lethal dose of quizalofop shows areas where epicuticular waxes disappear, specially in the youngest individuals. These areas are located predominantly on periclinal walls of typical epidermic cells and stomatal subsidiary cells. According to bibliography, the highest absorption of substances occurs in these areas (Franke, 1969; Kerstiens, 1996). In previous experiments, it has been observed that individuals that have no more than two tillers die due to herbicide action, but those which have three or more survive (Malpassi, 2004a). These last ones show a cuticle 0.2 µm thicker with more epicuticular wax deposition than the first ones. These waxes act as an important accumulation compartment of agrochemicals and, consequently, represent the major obstacle for diffusing lipophilic substances because they retain them in their structure (Schreiber, 1995; Kerstiens, 1996; Santier and Chamel, 1998; Beattie and Marcell, 2002). If quizalofop cannot dissolve waxes in older plants, they would be able to retain more substances than younger ones.

Therefore, there are two important factors for *E. indica* plant survival after herbicide application. Firstly, the cuticle of individuals with more than two tillers have epicuticular waxes that this herbicide would not dissolve. They act as a herbicide accumulation compartment and, therefore, would partially prevent the active ingredient entry to epidermic cells and, ultimately, to the phloem (Chamel and Vitton, 1996). Secondly, basal bud survival in plants having three or more tillers that cannot be reached by the herbicide because of its scarce concentration in meristematic tissues caused by the minimized phloem transport (Devine and Shimabukuro, 1994; Malpassi, 2004a). On the other hand, sublethal dose allows all plant categories to survive. In this situation, the active ingredient would not be enough concentrated in the cuticle during a critical period of time to dissolve waxes, so more herbicide would be trapped in its structure.

P. oleracea shows a different pattern. Cuticle discontinuities are observed and may be caused by lactofen entry. This herbicide molecule is so big and has so many free electrons that can provoke instability in those molecule unions that limit cuticular pores. Therefore, they can break and release electrons which, in turn, cause other types of unions that, consequently, enlarge the pore and allow herbicide entry (Hart *et al.*, 1995). Once this molecule entries epidermic cells, causes their plasmolysis. When these cells lose water, they shrink and generate mechanical forces that would finally break the cuticle, specially in the most fragile areas (modified pores). In the future, it would be interesting to analyze herbicide pathway across the cuticle during the first 24 h after application with electron microscopy, to determine the precise moment these discontinuities appear. If the first herbicidal molecules are able to enlarge the pores, those which come after them would find a direct entry to the cell wall which does not constitute an obstacle for diffusing substances.

P. oleracea with 8 nomophylls or more also show two strategies that would allow them to survive lactofen lethal dose. The first one is that their cuticle is thicker, therefore, it is not so vulnerable to be modified by herbicide action and acts as a more efficient retention compartment for lipophilic substances (Hull *et al.*, 1975; Chamel, 1986; Bukovac *et al.*, 1990; Chamel and Vitton, 1996). This situation would, consequently, cause that less herbicide penetrates weed tissues. The second one is accessory bud presence, hard to reach for a diffusing herbicide as lactofen (Malpassi, 2004 a, b). On the other hand, all plant categories survive when the sublethal dose is applied. The active ingredient is more diluted, so it is less probable that it can modify cuticle structure. Therefore, lactofen is retained in the wax network inhibiting herbicide penetration to weed cells. It is even less probable that the scarce herbicide quantity reaches target tissues. As a consequence, there is higher bud proportion not affected in all plant categories.

Eleusine indica and *Portulaca oleracea* cuticle characteristics constitute an additional important factor, besides bud placement, for herbicide survival. Plant age, apparently, is determinant of the amount of herbicide in target tissues because of differences the cuticle shows as plants grow up.

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