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The phenotype of grape leaves caused by acetochlor or fluoroglycofen, and effects of latter herbicide on grape leaves

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ABSTRACT

Fluoroglycofen and acetochlor are two different herbicides used in vineyards to eradicate weeds. This present study first characterized the effects of these chemicals on phenotype of grape leaves. Results showed that acetochlor caused the middle- and upper-node grape leaves become yellow at 60th day after treatment, while fluoroglycofen caused the ones became dark green. Then the effects of fluoroglycofen on photosynthetic pigments and chloroplast ultrastructure were characterized. Results showed that fluoroglycofen increased the chlorophyll and carotenoid contents by different extent in different node leaves, while it did not affect the net photosynthesis rate significantly. Chloroplast ultrastructure analysis showed that the gap between thylakoids layers in few chloroplasts of middle-node leaves increased, which was also observed in ones of upper-node leaves; the number and size of chloroplast increased. Analysis on the deformed leaves of grapevines treated with 375 g ai ha^{-1} fluoroglycofen showed that the starch grain per cell was much more and larger than that in the same size control leaves; the dark green and yellow parts had more or fewer chloroplast than the control, respectively, but both with more grana per chloroplast and less layers per granum. Chloroplasts went larger and round. Taken together, these results suggested that fluoroglycofen caused the grape leaves become dark green, which might be associated with the changes of chloroplast; the growth inhibition in the second year might be due to accumulation of starch.

1. Introduction

Due to labor shortage and lack of knowledge on grass cover in orchards, herbicides application becomes increasingly universal in recent years. Just because of that, there are more and more reports about herbicide phytotoxicity. For grapevine, application of classical herbicides including 2,4-D [1,2], glyphosate [3], chlorsulfuron [4], diuron [5], trichloroacetate [6], flazasulfuron [7] and flumioxazin [8–10] bring negative effects on its growth and development.

It is well known that herbicides absorbed from spray drift induce abnormal growth through morphological, anatomical, and cytological effects, which vary depending upon the type of the herbicide and plant species [11]. For instance, in soybean leaves, the nonauxinic herbicide, trifluralin caused palisade cells to be compact, short, and separated [12], whereas glyphosate that acts as 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) inhibitor inhibited formation of intercellular air spaces in Eucalyptus leaves [13]. The auxinic herbicide, 2,4-D induced elongation of mesophyll cells in grass leaves [14] and injured membrane systems of the mesophyll cells in *Phaseolous vulgaris* [15]. Grapevines (*Vitis vinifera* L.) are a broad-leaved perennial crop, and its roots were succulent. There was only studies suggested that vines develop abnormal and malformed leaves and this occurs frequently due to phytotoxicity of vapors and spray drifts of phenoxy herbicides [16–18]. Bondada [2] suggested that the 2,4-D injured leaves of grapevine showed severe abnormalities in their external and internal organization of cells.

Fluoroglycofen [O-[5-(2-chloro-α,α,α-trifluoro-of-toluene-oxy)-2-nitro-benzoyl]-hydroxy ethyl] is a post-emergence herbicide applied to control broad leaf weed and grasses, which belongs to diphenylethers herbicide. Many studies have demonstrated that diphenylethers herbicide that acts as protoporphyrinogenoxidase inhibitor, such as lactofen can inhibit soybean plant growth [19], flumioxazin reduce chlorophyll contents and photosynthesis in alga or grapevine [8,20]. The previous reports have showed that the diphenylethers herbicides can cause rapid photobleaching and desiccation of green plant tissues [21]. But in our previous investigation, in Qufu, Shandong China, we observed in two of

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vineyards which were sprayed with paraquat, acetochlor and fluoroglycofen on the ground perennially, the grape leaves grown dark and round. Then, our research results showed that 22, 460 g ai ha^{-1} acetochlor caused the chlorophyll content decrease after the grapevines were treated for 30 days, while 187.5 g ai ha^{-1} fluoroglycofen caused the content increase [22]; but we did not find the one which caused the grape leaves grown dark green. In this present study, we selected the grapevines grown in sand, in order to study effects of acetochlor and fluoroglycofen on the phenotype of grape leaves, and the mechanism of dark green.

2. Materials and methods

2.1. Plant and growth conditions

Experiment 1 was conducted in a greenhouse at the Shandong Agriculture University, China. One-year old grapevines (*V. vinifera* × *Vitis labrusca* cv. Kyoho) were grown in plastic pots (25 cm in diameter) containing sand in a greenhouse operating at photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}, relative humidity of 75–80% and a photoperiod of 14/10 h light/dark at 25 °C. When the shoots had ten leaves, 11, 230 g ai ha^{-1} acetochlor (0.070 g ai per pot, T1) or 187.5 g ai ha^{-1} fluoroglycofen (0.00115 g ai per pot, T2) were sprayed on the sand. Simultaneously, control (CK) one was sprayed with water. The experiment was performed twice replications, each replication containing six plants in each treatment group (n = 12). Once grapevines had been exposed to herbicides for 60 d, the leaf phenotype was yellow and dark green respectively in T1 and T2 groups. Then the physiological indices were analyzed on the leaves at the upper-node (13/14), middle-node (8/9) and bottom-node (3/4) in T2 grapevines. During the experiment, the plants were irrigated with equal volume of Hoagland nutrient solution (guarantee the amount of liquid not flowing out of the pot) once every two days.

Experiment 2 was conducted in a greenhouse at the Shandong Agriculture University, China. One-year old grapevines (*V. vinifera* × *V. labrusca* cv. Kyoho) were grown in plastic pots (25 cm in diameter) containing garden earth, sand and matrix soil (2:1:1) in a greenhouse operating at photosynthetic photon flux density of 300 μmol m^{-2} s^{-1}, relative humidity of 75–80% and a photoperiod of 14/10 h light/dark at 25 °C. When the shoots had ten leaves, three concentrations of fluoroglycofen were sprayed on the soil in 2010, while the control one was sprayed with water. The experiment was performed twice replications and each replication containing three plants in each treatment group. In 2011, the highest concentration groups (375 g ai ha^{-1} fluoroglycofen, 0.00230 g ai per pot) caused the leaves deformed. The chloroplast ultrastructure of deformed leaves and the same size ones in control were analyzed.

2.2. Gas exchange measurements

Measurements of the net photosynthetic rate (Pn) and the stomatal conductance (Gs) were made on the upper-node (13–14), middle-node (8–9) and bottom-node (3–4) leaves of grape seedlings using an open system (Ciras-2, PP Systems, Hitchin, UK). The light-saturating photosynthetic rate was made at a CO_2 concentration of 360 L L^{-1} and at a temperature of 25 °C with relative humidity 80% and saturating light (1000 mol m^{-2} s^{-1}).

2.3. Measurements of pigment content

After herbicide treatment for 60 d, the different node leaves were harvested, and the chlorophyll and carotenoid content was determined spectrophotometrically in 80% acetone with a double beam spectrophotometer Unicam UV 550 (ThermoSpectronic, Cambridge, UK) according to Lichtenthaler [23].

2.4. Electron microscopy observations

Leaf samples were fixed for 12 h in a 3.5% (v/v) glutaraldehyde solution, washed for 2 h with 0.1 M phosphate buffer (pH 7.2), post-fixed for 6 h with 1% OsO_4 (w/v) solution, washed for 2 h with 0.1 M phosphate buffer (pH 7.2), dehydrated using a graded ethanol series (45%, 50%, 70%, 95% and 100%), and embedded in spur epoxy resin. The samples were cut using a LKB chip cutter (LKB, Sweden). Ultra-thin sections (0.7 mm) were stained with uranyl acetate and lead citrate and then observed using a JEM-1200EX transmission electronic microscope (Jeol Ltd, Tokyo, Japan).

2.5. Statistical analysis

Statistical analyses were performed by analysis of variance (ANOVA) using SPSS version 13.0 (SPSS, Chicago, USA) and comparisons between the mean values were made by least significant difference (LSD) at a 0.05 probability level.

3. Results

3.1. Effects of fluoroglycofen and acetochlor on leaf phenotype in Kyoho grapevines

At the 60th day, compared with the control, the upper- and middle-node leaves became yellow in acetochlor treated grapevines (Fig. 1b and e), however the ones in fluoroglycofen treated grapevines became dark-green (Fig. 1c and f). Especially, no matter in acetochlor or fluoroglycofen treated groups, the symptoms of upper-node leaves were more significant.

3.2. Effects of fluoroglycofen on CO_2 assimilation and stomatal conductance in grape leaves

The net photosynthesis rate (Pn) in upper- and middle-node leaves of fluoroglycofen treated grapevines was lower than that of control, while the one in bottom-node leaves was higher (Table 1); however, the differences between fluoroglycofen treated grapevines and control was not significant. The stomatal conductance (Gs) decreased by 30.9% in upper node leaves; however it increased by 14.3% and 78.2% in middle and bottom node leaves, respectively; but there was only significant difference in the bottom node leaves.

3.3. Effects of fluoroglycofen on chlorophyll and carotenoid contents in grape leaves

As shown in Table 2, 187.5 g ai ha^{-1} fluoroglycofen significantly increased the chlorophyll and carotenoid contents of grape leaves in the 60th day after treatment. The chlorophyll a, b and carotenoid content in bottom node leaves increased most by 45.3%, 68.2% and 71.4%, respectively; while the increase extent was higher in upper node leaves than in middle ones. Fluoroglycofen obviously enhanced the ratio of chlorophyll a–b in upper node leaves, however, it had no significant effects on the ratio in middle and bottom node leaves.

3.4. Effects of fluoroglycofen on chloroplast ultrastructure in grape leaves

In the 60th day after fluoroglycofen treatment, the cell membrane and chloroplast membrane was integrated, while the