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Plant Growth Responses of Apple and Pear Trees to Doses of Glyphosate[1]

Respostas de Crescimento de Plantas de Maçã e Pera a Doses de Glyphosate

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ABSTRACT - Glyphosate is commonly used for intra-row weed management in perennial plantations, where unintended crop exposure to this herbicide can cause growth reduction. The objective of this research was to analyze the initial plant growth behavior of young apple and pear plants exposed to glyphosate. Glyphosate was sprayed on 2-year-old 'Gala' apple and 'Abbè Fetel' pear plants at doses from 18 to 720 g per hectare of acid equivalent (a.e.). The plant height of neither species was not significantly reduced (less than 1%) by any glyphosate dose at 240 days after spraying, whereas the stem diameter and the dry mass of stem and leaves were reduced by 720 g a.e. ha^{-1}. The glyphosate dose required to reduce the aboveground dry mass by 50% was 162 and 148 g a.e. ha^{-1} for apple and pear, respectively. Aboveground dry mass was reduced 2% and 6% for apple and pear plants, respectively, at 720 g a.e. ha^{-1}. Hormesis was not observed in either species at doses down to 18 g a.e. ha^{-1}. Both species showed low susceptibility to glyphosate; however apple was less susceptible than pear.

Keywords: *N*-phosphonomethyl-glycine, *Malus domestica*, *Pyrus communis*, spray drift.

RESUMO - Glyphosate é comumente usado para manejo de plantas daninhas na linha de plantio de culturas perenes, onde a exposição não intencional da cultura a esse herbicida pode causar redução do crescimento. O objetivo desta pesquisa foi analisar o comportamento do crescimento inicial de plantas jovens de maçã e pera expostas ao glyphosate. Glyphosate foi pulverizado sobre plantas de maçã 'Gala' e pera 'Abbè Fetel', de dois anos de idade, em doses de 18 a 720 g por hectare de equivalente ácido (e.a.). A altura de plantas de ambas as espécies não foi significativamente reduzida (menos de 1%) aos 240 dias após a aplicação, enquanto o diâmetro do caule, assim como a massa seca de caule e folhas, foi reduzido devido à exposição ao glyphosate em doses maiores que 720 g e.a. ha^{-1}. A dose de glyphosate requerida para reduzir a massa seca da parte aérea em 50% foi de 162 e 148 g e.a. ha^{-1}, para maçã e pera, respectivamente. A massa seca da parte aérea foi reduzida em 2% e 7% para plantas de maçã e pera, respectivamente, em 720 g e.a. ha^{-1}. Hormese não foi observada nas espécies em doses até 18 g e.a. ha^{-1}. Ambas as espécies foram pouco suscetíveis ao glyphosate; contudo, a maçã foi menos suscetível que a pera.

Palavras-chave: *N*-phosphonomethyl-glycine, *Malus domestica*, *Pyrus communis*, deriva.

INTRODUCTION

Apple (*Malus domestica*) and pear (*Pyrus communis*, *Pyrus callereana*, etc.) trees are two fruits most commonly grown in temperate regions worldwide. In Southern Brazil, 'Gala' and 'Fuji' apples are the most commonly grown cultivars (Silveira et al., 2013), whereas European pears (*P. communis*), as 'Abbè Fetel', are a relatively small crop with the potential for expansion (Rufato et al., 2012; Machado et al., 2013). Brazil is one of the main countries importing pears from around the world, whereas it exports apples. Any

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management strategy that can improve crop production is important for both apple and pear plantations.

Weed management is an important issue for the production of perennial crops, including fruits such as apple and pear (Bremer Neto et al., 2008; Azevedo et al., 2012; Miñarro, 2012; Lisek, 2014). Brazilian growers commonly use inter-row mechanical weeding associated with intra-row chemical weed control. Herbicides can reach the fruit plants both directly by accidental application and indirectly by spray drift, as reported on other perennial crops, such as citrus, eucalyptus, and coffee, where similar weed management is used (Tuffi Santos et al., 2006; Gravena et al., 2009; Machado et al., 2010; Carvalho and Alves, 2012; Carvalho et al., 2012a, 2013a, 2015; Silva et al., 2015).

The main herbicide used in Brazilian fruit plantations is glyphosate (*N*-(phosphonomethyl) glycine). Glyphosate kills plants by inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19) of the shikimate pathway (Duke et al., 2003). Inhibition of EPSPS reduces in the aromatic amino acids phenylalanine, tyrosine, and tryptophan that are needed for protein synthesis. Products of the these amino acids such as lignin, alkaloids, flavonoids, and benzoic acids necessary for cell wall development, defense against pathogens, and many other processes are also reduced. Inhibition of EPSPS leads to high levels of shikimate accumulation due, in part, to unregulated flow of carbon into the shikimate pathway. This drains carbon from other pathways, leading to metabolic dysfunction. Glyphosate preferentially translocates to metabolic sinks, such as meristems and expanding cells, where it slows or stops plant growth (Gougler and Geiger, 1981; Duke, 1988; Duke et al., 2003).

There are many indirect effects of glyphosate, such as decreased levels of the activity of the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) (Ahsan et al., 2008) and disorganization of the photosynthetic apparatus (María et al., 2005). Other general consequences of the exposure to glyphosate are chlorosis and plant growth reduction, mainly in metabolically active tissues, such as immature leaves, sprouts, floral buds, and root tips (Duke, 1988; Duke et al., 2003). At high glyphosate doses, these symptoms are followed by plant death. Although glyphosate can damage woody plants (Schrübbers et al., 2014), the doses recommended for weed management are generally not lethal.

Hormesis has been associated with low doses of glyphosate, particularly in woody plants such as coffee, eucalypts, and *Pinus caribea* (Velini et al., 2008; Carvalho et al., 2013a; Belz and Duke, 2014). Thus, low doses of glyphosate in plantation culture of some species of woody plants might be harmless or even beneficial. The mechanism of glyphosate hormesis is unknown (Belz and Duke, 2014).

We found no literature on the effects of glyphosate on apple or pear trees. We hypothesized that young apple and pear trees will have different levels of susceptibility to glyphosate and that very low doses would produce a hormetic effect. Therefore, the objective of this study was to evaluate the initial growth responses of young apple and pear trees to glyphosate at a range of doses, from low doses that could be expected from drift to recommended doses for weed control.

MATERIAL AND METHODS

Plant material

Two-year-old saplings of ‘Gala’ apple grafted onto apple clonal rootstocks and European pear ‘Abbè Fetel’ grafted onto quince (*Cydonia oblonga*) rootstocks were used in this research.

Growing conditions

The experiment was performed from September, 2012, until June, 2013, under subtropical climatic conditions, at a latitude of 27º49’0”S, a longitude of 50º19’35”W, and an altitude of 930 m.

Plants of both species were grown in 20-L pots filled with a mixture of an aluminic humic Cambisol soil and an organic substrate in a 3:1 (v:v) proportion. Soil substrate was fertilized by using 5 g of NPK (5-20-20) per pot at the time of transplanting and adding 5 g of urea (48% of N) per pot 30 days after transplanting (at the time of glyphosate spraying).

The pots were maintained under field conditions and the substrate was watered just when no rainfall occurred during the experimental period.

The plantings of both species were maintained under a period of adaptation for 30 days before being exposed to glyphosate.

Herbicide information and spraying conditions

We used a glyphosate-based herbicide, containing 48% of active ingredient of an isopropylamine salt of *N*-(phosphonomethyl)-glycine, corresponding to 36% of acid equivalent (ae) (Roundup Original®, Monsanto, Brazil). The field dose recommended to control several weed species is 720 g a.e. ha^{-1}, corresponding to 2 L ha^{-1} of the labeled herbicide.

The herbicide was sprayed directly onto the aboveground parts of both apple and pear saplings by using a CO_2^- pressurized sprayer with flat plan nozzles (TeeJet, 80.02, USA), pressure of 1.90 kgf cm^{-2}, and volume of application of 200 L ha^{-1}. At the time of spraying, there were no clouds, the air temperature was 18 °C, the air humidity was 79%, and the wind was less than 4.5 km h^{-1}.

Experimental design

The treatments consisted of spraying glyphosate onto the apple and pear saplings at the doses of 0, 18, 36, 72, 180, 360, and 720 g a.e. ha^{-1}, corresponding to 0, 0.05, 0.1, 0.2, 0.5, 1, and 2 L ha^{-1}. The experiment was arranged in a completely randomized design with 10 replicates, for each fruit species.

Plant growth measurements

Evaluations of plant growth characteristics were performed during the eight months after glyphosate spraying. Plant height and stem diameter were evaluated once at each month after glyphosate spraying with a yardstick and a digital caliper, respectively. The plant height was measure from the soil surface to the top of main stem of the plant. The stem diameter was considered as a mean between the rootstock diameter (at 5 cm above soil surface) and the scion diameter (at 5 cm above the grafting).

The plants were cut close to the soil surface at eight months after glyphosate spraying and the leaves were separated from the stem. The stem and leaves of each plant were weighed after being dried for 96 h in a forced air oven at 60 °C.

Statistical analysis

The plant height and stem diameter data were analyzed by ANOVA under a factorial scheme of 7 x 8 (seven glyphosate doses and eight sampling times), considering the sampling times in a split-pot design. Once the interaction between glyphosate doses and sampling times was not significant (P > 0.05), we opted to analyze the mean value of the eight sampling times by non-linear regression. The dry mass data was also analyzed by non-linear regression.

For the regression analyses, the following non-linear model was used:

$$y = c + \frac{d - c}{1 + \left(\frac{x}{g}\right)^b}$$

where: y represents the value of the characteristic studied; c and d represents the lower and upper curve asymptotes, respectively; b represents the slope of the curve; g represents the inflection point of the curve; and x represents the glyphosate dose.

RESULTS AND DISCUSSION

General statistical results. ANOVA indicated no interaction between glyphosate doses and sampling times (P > 0.05) on plant height and stem diameter for both apple and pear. Thus, we analyzed the significance of the glyphosate doses on the mean value (among the eight sampling times) of plant height and stem diameter for both fruits. In this way, there was no significance of glyphosate doses on plant height just for apple, while a significant result

(P < 0.05) was observed on plant height of pear and on stem diameter of both apple and pear. In addition, we also found significant results (P < 0.01) of glyphosate doses on dry mass of stem, leaves, and aboveground plant parts for both fruits. Therefore, we performed the regression analysis for all characteristics showing significant results of ANOVA, where the glyphosate doses represented the independent variable and the plant characteristics were the dependent variables.

'Gala' apple. The plant height of apple varied from 131.4 cm (720 g a.e. ha^{-1}) up to 134.0 cm (untreated control) (Figure 1), showing no significant difference between glyphosate doses. At 72 g a.e. ha^{-1}, stem diameter, stem dry mass, and leaf dry mass reduced by 7% (Figure 1), 4%, and 10% (Figure 2), respectively, in comparison to the untreated control. Thus, low glyphosate doses affected significantly apple growth. At 720 g a.e. ha^{-1}, the highest reduction reached 22%, 22%, and 48% for stem diameter, and dry mass of stem and leaves, respectively (Figures 1 and 2). Therefore, labeled glyphosate field doses affected strongly the plant growth of apple. In spite of significant effects of glyphosate on the characteristics cited above, apple trees survived after 240 days of herbicide application, and the reduction of the aboveground dry mass reached just 2% at 720 g a.e. ha^{-1} (Figure 3), indicating a relative tolerance of this species to glyphosate.

'Abbè Fetel' pear. The plant height of pear decreased from 132.8 cm (untreated control) down to 126.9 cm (720 g a.e. ha^{-1}) (Figure 1). At 72 g a.e. ha^{-1}, stem diameter, stem dry mass, and leaf dry mass reduced by 9% (Figure 1), 7%, and 25% (Figure 2), respectively, in comparison to the untreated control. Thus, low glyphosate doses significantly affected pear

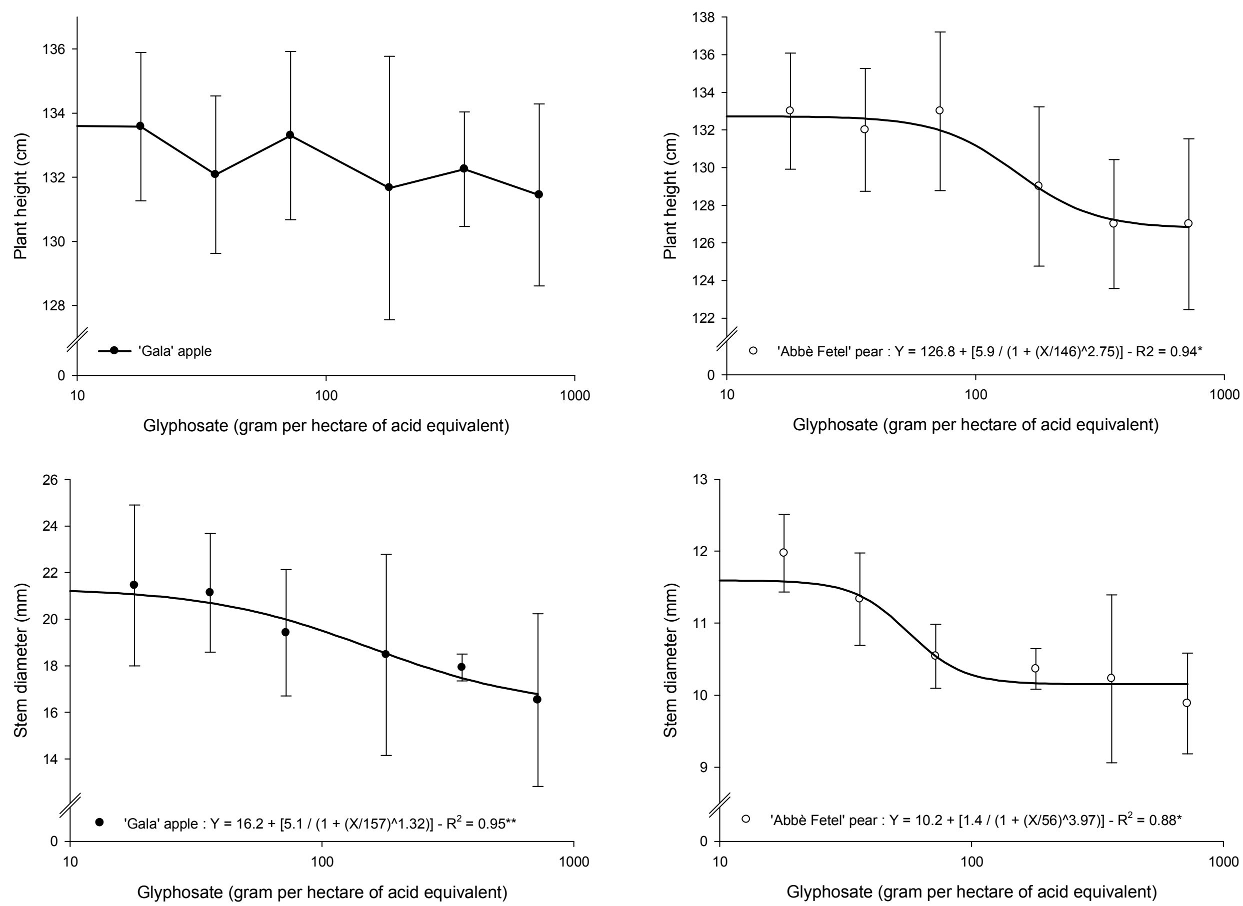

Figure 1 - Plant height and stem diameter (average data of monthly evaluations during 240 days after herbicide spraying) of 'Gala' apple and 'Abbè Fetel' pear exposed to a range doses of glyphosate. Vertical bars indicate the standard error of 10 replicates.

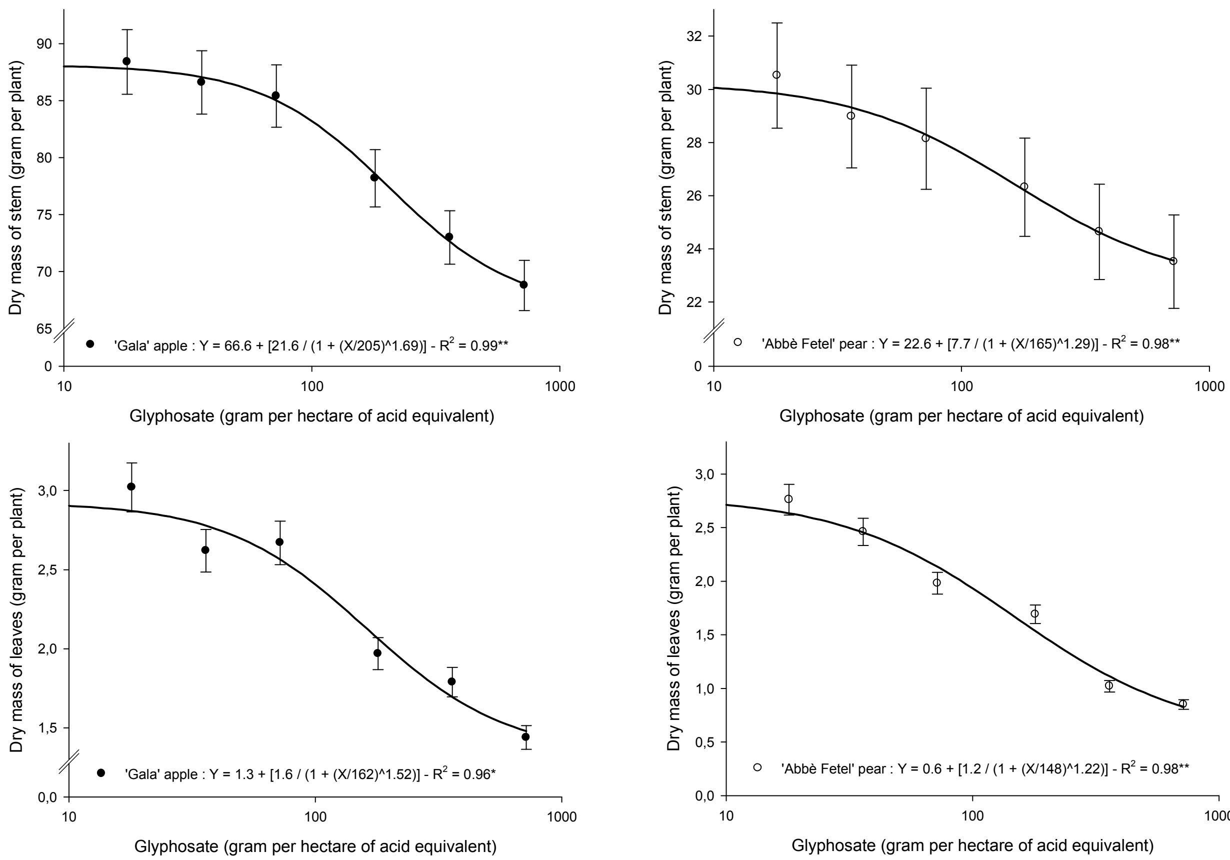

Figure 2 - Dry mass of stem and dry mass of leaves of 'Gala' apple and 'Abbè Fetel' pear exposed to a range doses of glyphosate, at 240 days after herbicide spraying. Vertical bars indicate the standard error of 10 replicates.

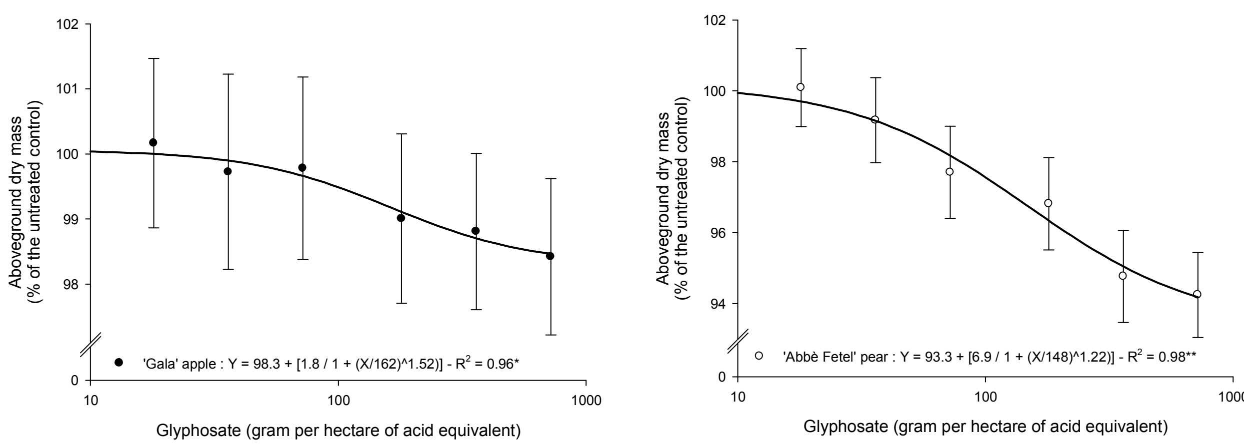

Figure 3 - Aboveground dry mass, as a percentage of the untreated control, of 'Gala' apple and 'Abbè Fetel' pear exposed to a range doses of glyphosate, at 240 days after herbicide spraying. Vertical bars indicate the standard error of 10 replicates.

growth. At 720 g a.e. ha^{-1}, the highest reduction reached 5%, 13%, 22%, and 72% for plant height, stem diameter, and dry mass of stem and leaves, respectively (Figures and 2). Therefore, labeled glyphosate field doses affected strongly the plant growth of pear. In spite of significant effects of glyphosate on the characteristics cited above, pear trees survived after 240 days of herbicide application and the reduction of the aboveground dry mass reached just 6% at 720 g a.e. ha^{-1} (Figure 3), indicating a relative tolerance of this species to glyphosate.

We have found no literature on the effects of glyphosate on the growth of young apple and pear plants, but such information is available for other tree crops. Hormetic effects of low doses of glyphosate were observed in young plants of eucalyptus, pine (Velini et al., 2008), and coffee (Carvalho and Alves, 2012; Carvalho et al., 2012a, 2013a). On the other hand, Tuffi Santos et al. (2006) and França et al. (2010) found no hormesis with glyphosate on coffee and eucalyptus plants, respectively. However, the hormetic effect of low doses of glyphosate is dependent on the plant species (Tuffi Santos et al., 2006), the growing conditions (Belz and Cedergreen, 2010), the plant growth stage at the moment of the exposure to the herbicide (Velini et al., 2008; Carvalho et al., 2013a), and the end point measured and the time of its measurement after treatment (Belz et al., 2011).

The lack of glyphosate-caused hormesis was a surprise because it was observed in several tree species, as we have cited above. However, Belz and Cedergreen (2010) concluded that the parthenin hormesis in plants depends on the growing conditions and, additionally, Carvalho et al. (2013a) verified that it is also dependent on the plant growth stage. Thus, with different experimental parameters, we may have observed hormetic effects if apple and pear plants were exposed to low doses of glyphosate. Low doses of a combination of simazine plus amitrole were reported to stimulate plant growth and nitrogen content of both peach and apple trees (Ries et al., 1963), but these herbicides have entirely different modes of action than glyphosate.

Glyphosate is a post-emergent acid herbicide (pka 0.3, 2.6, 5.6, and 10.3) with high water solubility (15,700 mg L^{-1} at 25 °C and pH 7) and low lipophilicity (log P < -2.77) (Kegley et al., 2010). In a water solution, the anionic forms of the molecules of glyphosate are found in higher levels than the non-ionic forms. So, the glyphosate absorption through the plant cuticles is not fast and occurs mainly through the polar pathway (through the cutin matrix). Glyphosate also shows no significant soil activity due to its very high soil adsorption (discussed in detail by Duke et al. (2012), so that it is not used as a pre-emergent herbicide. On the other hand, glyphosate translocation in susceptible plants occurs through the xylem (migration to the tips of the treated leaves) and the phloem (migration to untreated leaves, the rest of shoot, and roots), reaching the meristematic tissues (Duke, 1988; Duke et al., 2003; Gougler and Geiger, 1981), where it accumulates to toxic levels, causing cell death.

Differences in the response of plants to the exposure to glyphosate can be derived from differences in spray retention or/and drop contact angle in the leaves (Chachalis et al., 2001), (Norsworthy et al., 2001; Michitte et al., 2007), composition of the leaf epicuticular wax (Michitte et al., 2004; Nandula et al., 2008; Guimarães et al., 2009; Hatterman-Valenti et al., 2011), herbicide absorption or/and translocation (Dinelli et al., 2008; Guimarães et al., 2009; Ge et al., 2010; Carvalho et al., 2012b), and herbicide degradation (Rojano-Delgado et al., 2010, 2012; Carvalho et al., 2012b, 2013b). Thus, any difference in the herbicide absorption, translocation, or/and metabolism can influence the plant response to glyphosate, culminating in a higher or lower susceptibility to this herbicide.

Our results show relatively high tolerance of both 'Gala' apple and 'Abbè fetel' pear to glyphosate. However, our data indicate that a glyphosate drift or even an accidental application of glyphosate directly to the shoot of both species can reduce the initial plant growth, particularly of pear plants. Thus, special care must be employed in using glyphosate for weed management in apple and pear orchards. Growers should be alert to the correct use and maintenance of the equipment and machinery, the choice of specific nozzles, the calibration of sprayers, the application under adequate environmental conditions, and so on.

Our results indicate that 'Gala' apple grafted onto apple clonal rootstocks and 'Abbè Fetel' pear grafted onto quince rootstocks are tolerant to glyphosate at doses up to 720 g a.e. ha^{-1}. However, early plant growth of both species can be reduced by glyphosate exposure. Growth of 'Abbè Fetel' pear and 'Gala' apple is reduced with glyphosate exposures higher than 18 and 72 g a.e. ha^{-1}, respectively. 'Abbè Fetel' pear is slightly more susceptible to glyphosate than 'Gala' apple at doses higher than 36 g a.e. ha^{-1}

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