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The Use of Glyphosate in Sugarcane: A Brazilian Experience

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Additional information is available at the end of the chapter

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1. Introduction

In Brazil, sugarcane (*Saccharum* spp.) fields are renewed in intervals of five to six profitable crops on average. With each harvest, sugarcane displays a decrease in productivity due to diverse factors. Genetic, phytosanitary and edaphoclimatic issues are the main factors contributing to the degeneration that necessitates the renewal of sugarcane fields with more productive cultivars. After the last economical harvest, the ratoon crop is destroyed using mechanical or chemical processes or a combination of both. Chemical destruction is more practical and causes less impact on soil structure and quality due to less soil disturbance. Glyphosate is the most widely used non-selective herbicide in the chemical eradication of ratoon crops because there is a broad spectrum of plants susceptible to glyphosate. Glyphosate's mechanism of action is through inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a precursor of the aromatic amino acids phenylalanine, tyrosine and tryptophan, which are essential for protein synthesis. The recommended dosage of glyphosate for the eradication of plants is 1440 to 2880 g acid equivalents (a. e.) ha^{-1} [1,2]. However, sugarcane cultivars present varying degrees of susceptibility and require different amounts of herbicide for the complete death of the plant.

In Brazil, sugarcane cultivars commercially released by genetic improvement programmes are not characterised in terms of their susceptibility to glyphosate. Nevertheless, knowledge of the degree of cultivar tolerance to glyphosate can generate savings for producers and benefit to the environment through the reduction of the quantity of applied herbicide. Literature studies of cultivar responses to herbicides, especially glyphosate, are supported solely by phytotech-

nical observations, such as plant height, girth and mass gain. However, these characteristics are greatly affected by the environment and require longer periods of evaluation and data collection, such as the 12-month studies of [3] and [4].

The use of isoenzymatic markers allows for the prompt analysis of results with larger sample numbers while using a relatively simple and inexpensive technique that can substitute or reduce field experimentation. However, the choice of the correct enzymes to analyse is critical to the success of this technique and obtaining robust results.

2. Sugarcane crops

Sugarcane probably originated in New Guinea, and from there, it was introduced to India, where the oldest evidence of its existence has been recorded [5]. Officially, Martins Afonso de Souza brought the first sugarcane plant to Brazil in 1532 and started its cultivation in the Captaincy of São Vicente (Capitania de São Vicente). This transfer was the beginning of an industry that found in Brazil, among other nations that would later initiate production, its most fertile ground for rapid expansion and perpetuation for an almost uninterrupted 500 years. Starting in the 1970s, sugarcane farming became increasingly important for Brazil as the agro-industrial sector was tapped to contribute to a solution to the emerging energy crisis because of the potential for energy production from sugarcane as a renewable source [6]. Growth in the sugarcane-ethanol sector is important for the Brazilian economy in that the sector's growth entails both the creation of jobs and of 100% national renewable energy.

According to taxonomic classification, sugarcane belongs to the *Poaceae* family and the *Saccharum* genus. Sugarcane is a semi-perennial plant requiring a tropical or sub-tropical climate [7]. With a C4 metabolism, sugarcane is classified as having among the highest rates of photosynthetic efficiency and a high efficiency for water usage [8]. The sugarcane plant is divided into aerial (culm, leaves and inflorescences) and underground parts (roots and rhizomes). The culms are cylindrical and are composed of nodes and internodes; these parts are defined as the aboveground portion that supports the leaves and inflorescences [9]. According to [10], each node has one alternating bud and a root system. Inflorescences are panicles with a hermaphrodite flower containing one ovule; the pistils terminate in purple or reddish stigmae that characterise the flower's plumose panicle [9]. The root system is fasciculated and serves to support, as well as to absorb and transport water and nutrients [8]. Sugarcane tillering influences the sugarcane handling system because each tiller behaves as an independent plant with individual organs, such as roots, leaves and fruits [11].

The most appropriate agricultural conditions for sugarcane propagation are found between the 30° north and 30° south latitudes, which are characteristic of tropical and subtropical regions. Outside of these latitudes, lower temperatures limit the growth and development of the plant [12]. According to [10], the optimal temperature range for the growth of this crop is between 20 and 35 °C with an ideal photoperiod of 10 to 14 hours [12] and an annual rainfall ranging between 1,000 and 1,600 mm, preferably with abundant rain during the vegetative

growth period and a dry period during maturation, which favours increased sucrose accumulation [13].

In Brazilian regions where it is traditional to grow sugarcane, planting may occur at different times of the year, as long as the producer possesses an irrigation system and cultivars that are adapted to each season [14]. Traditionally, in south-central Brazil, there are two cycles for planting: "cane of the year" and "year and a half". In "cane of the year", planting is performed between September and November, and the cane is harvested after 12 months [14]. This type of plantation addresses the demand for raw materials in the spring cycle (at the end of the harvest). In "year and a half" cane, planting is performed between January and April-May. In contrast to cane of year, this cycle allows for harvest during the autumn season (the beginning of harvest). Additionally, several producing units have practiced winter planting, particularly June through July, using rescue irrigation, and these units have obtained great productivity compared to "year and a half" cane planting.

Currently, Brazil is the largest producer of sugarcane in the world followed by India, China and Thailand [15]. The national production is estimated as 641.982 million tons with an average productivity of approximately 76.4 t ha^{-1} [16].The national sugar-energy industry sector accounted for 1,283,258 jobs up to 2008 with 37.5% occupied by plant growth, 44.8% in the production and refining of sugar and 17.7% in the production of ethanol. This sector also accounted for approximately 3.85 million people that are employed indirectly [17]. The production and processing of sugarcane is currently managed by the private sector in Brazil, which achieves the lowest cost for production worldwide for both sugar and ethanol, emerging as a highly competitive segment in international markets [18].

3. Characteristics of the herbicide glyphosate

Glyphosate was commercially released in 1974 under the trade name Roundup initially in the USA for industrial purposes, in the United Kingdom for use in wheat crops and in Malaysia for use in rubber trees. Currently, the molecule is registered in more than 130 countries for the control of over 300 species of weeds in over 100 types of crops [19], making it the most widely used herbicide [20]. Worldwide, there are numerous registered trademarks of the herbicide, which, according to [50], number more than 150. In Brazil, glyphosate is also registered for the eradication of sugarcane ratoon crops [21].

The molecule belongs to the glycine-derived chemical group (Group G), and its mechanism of action consists of inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimic acid pathway [22], which is found only in microorganisms and plants [23]. The molecular formula of glyphosate is $HO_2CCH_2NHCH_2PO(OH)_2$. Glyphosate has a solubility in water of 15,700 mg L^{-1} at 25 °C and pH 7, a density of 1.74 g mL^{-1}, a vapour pressure of 2.45 x 10^{-8} Pa (45 °C), pK$_a$ values of 2.6, 5.6 and 10.3 (acid) and a k$_{ow}$ between 0.0006 and 0.0017. In the soil, glyphosate is strongly adsorbed to colloids, and its leachability is notably low. The compound has an average K$_{oc}$ of 24,000 mL g^{-1}, and its volatilisation and photodegradation are negligible [22]. The half-life of the molecule in the environment depends on the surrounding

soil texture and microbial activity and may vary from a few days to several years [24]. In roots, the absorption is slow due to the low diffusion and high adsorption to the soil, which also favours microbial action in the transformation of the molecule into its main metabolite, aminomethylphosphonic acid [25; 26]. Glyphosate is absorbed through leaf cuticles, and its translocation occurs mainly via the cellular symplast to the leaves and apical meristem, as well as to underground organs [22]. According to [27], glyphosate absorption depends on such factors as the age of the plant, environmental conditions, surfactants and herbicide concentration in the soil milieu.

As an herbicide, glyphosate is among the less hazardous agro-toxins used in agriculture [28]. Glyphosate-based herbicides, when used according to their respective guidelines, display low toxicity and are safe to humans [30].

Often, the glyphosate molecule is not efficient in penetrating waxy cuticles. Therefore, commercial formulations contain surfactants capable of reducing surface tension in herbicide droplets, thus increasing their penetration in leaves [20]. However, these surfactants are more toxic than the glyphosate molecule [29]. For example, polyoxyethylene amine, the predominant surfactant in Roundup® [30], has been classified as moderately to highly toxic in laboratory tests [31]. Glyphosate is a unique molecule, and although it is considered to be of low toxicity, its unrestrained use can affect the environment through direct or indirect effects on non-target organisms [32].

In plants, the EPSPS enzyme catalyses a reaction between shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) to produce 5-enolpyruvylshikimate-3-phosphate and inorganic phosphate. Glyphosate binds to the catalytic site of EPSPS and to the S3P substrate to form the EPSP synthase-S3P-glyphosate complex [33]. The relevance of the shikimate pathway is such that approximately 35% of all plant mass is related to derivatives from this pathway; moreover, 20% of all of the carbon fixed during photosynthesis also travels through this metabolic pathway [34].

According [35], the inhibition of amino acids compromises the production of carotenoids and chlorophyll, thus causing irreversible cellular damage. Therefore, the translocation of the herbicide throughout the entirety of the plant causes plant death in a few days or weeks (Figure 1). The inhibition of EPSPS leads to the accumulation of high levels of shikimate in vacuoles, which is intensified by the loss of control of the carbon flow across this pathway [36]. Thus, there is an obstruction in the production of the aromatic amino acids phenylalanine, tyrosine and tryptophan, which are essential for protein synthesis and serve as precursors for secondary metabolites that are important for plant growth [37], resulting in the slow development of symptoms [33].

4. Reforming sugarcane fields and the use of glyphosate

With each harvest cut, sugarcane sprouts new tillers that develop into culms [38]. Nevertheless, ratoon-crop productivity gradually diminishes with an increasing number of cuts [39], thereby

requiring the renewal of the field. In the state of São Paulo, the average productivity of sugarcane fields is approximately 80 to 85 t ha^{-1}, considering the longevity of ratoons to be between five and six cuts [40]. However, in the region of Ribeirão Preto, SP, ratoon crops after the sixth cut are no longer economically viable, and renewal of the sugarcane field is necessary [41]. Degeneration after successive years of production makes the renewal of sugarcane fields essential. The causes of degeneration are diverse and involve a combination of genetic, physiological, phytosanitary, edaphoclimatic and phytotechnical factors. The factors impacting degeneration may also be linked to characteristics of the growing environment, such as a decrease in soil fertility [42]. Another cause for degeneration can be soil compaction and consequent difficulties in root development, as proposed by [43]. The authors note that compacted soil still presents difficulties for root development, even if its humidity levels are close to the soil's capacity.

[44] found that degeneration is linked to the health of the plants. Sugarcane-field longevity may also be affected by competition with weeds [45], nematode infestations [46] and uprooting of tufts during mechanical harvesting [47].

The fact that various cuts are performed from a single plantation allows for the formation of a significant number of root systems, which often make the elimination of the ratoon crop difficult, especially if the eradication is performed mechanically, which may also compromise the settlement of the next plantation. At the time of crop renewal, the ratoon crop is first eliminated through desiccating herbicides, specifically glyphosate, and after plant death, eradication is later completed using mechanical destruction of the crop [48].

Glyphosate is the most widely used herbicide for the chemical eradication of sugarcane ratoons due to its ease of use, low cost and absence of residual effects on the soil, which allows for repeated plantation in the same area, as is often practiced by farmers [49, 50]. Tolerance to glyphosate is highly prevalent in cultivars, and while certain cultivars are eradicated with a dose of 1080 g a. e. ha^{-1}, others require a dose of 2520 g a. e. ha^{-1}. According to [1,2], the minimal lethal dose for sugarcane is 1440 g a. e. ha^{-1}.

The progression of symptoms caused by a glyphosate application occurs in a gradual fashion (Figure 2) until the eradication of the plants [23, 36]. The authors noted that glyphosate-induced damage develops slowly until complete death in contrast to the effects of other herbicides. According to these authors, molecular stability inside the plant allows for the occurrence of irreversible effects on processes that control both annual and perennial plants (Figures 3 and 4).

After the application of glyphosate in the eradication of sugarcane crops, there was a stunting of plant growth, with treated plants retaining the same size up to 45 days after herbicide application [51]. The negative effect on growth was evaluated by measuring the plant height, with the treated plants maintaining similar average height values throughout the evaluation period in contrast to controls, which were able to maintain vigorous vegetative growth. The growth stunting was due to the indirect influence of glyphosate on the regulators of plant growth, such as indole-3-acetic acid. This hormone is fundamental for cellular elongation, apical dominance and stem and root growth and is dependent on the shikimate pathway, being inhibited when there is a disruption of EPSPS [36].
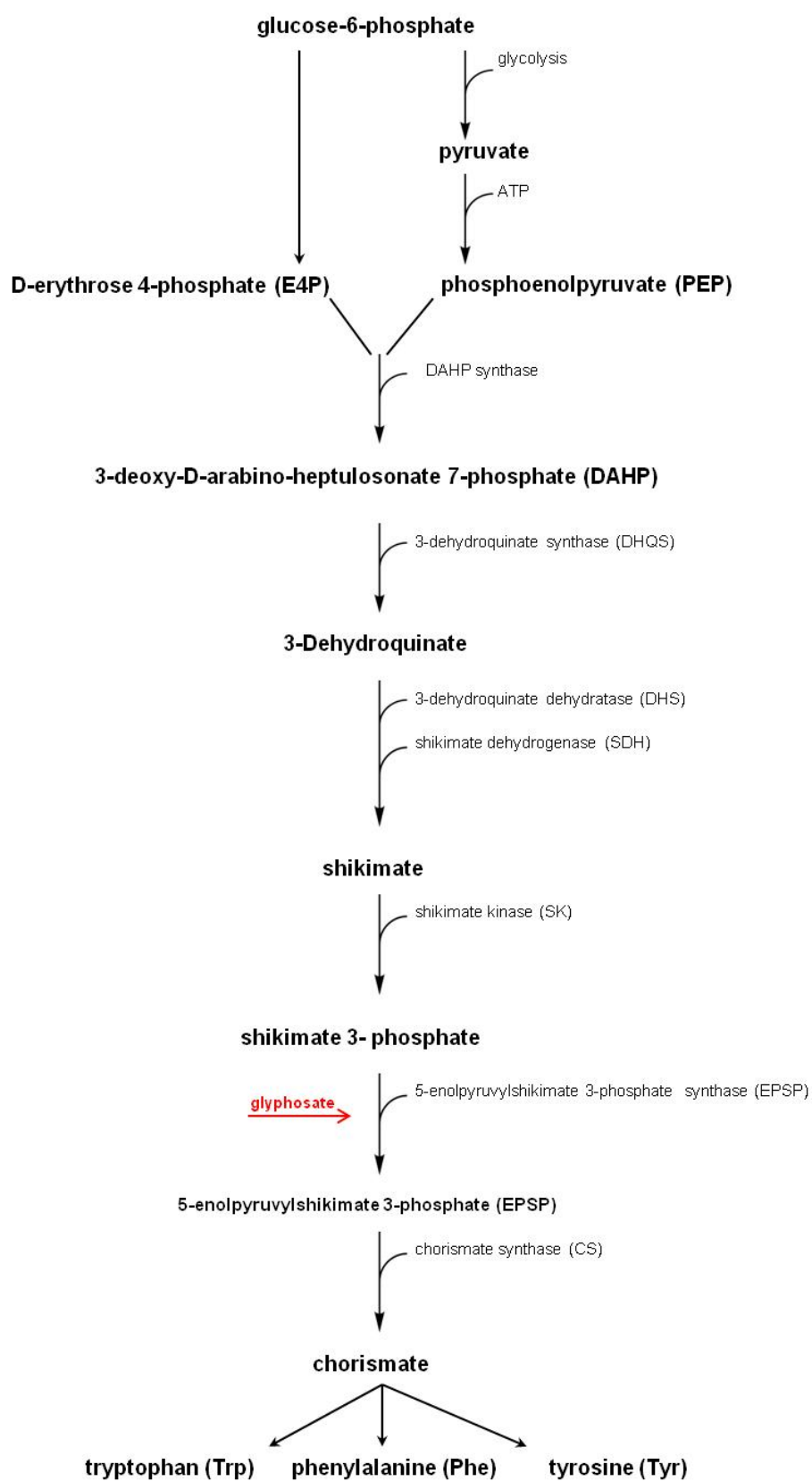
glucose-6-phosphate

glycolysis

pyruvate

ATP

D-erythrose 4-phosphate (E4P)   **phosphoenolpyruvate (PEP)**

DAHP synthase

3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP)

3-dehydroquinate synthase (DHQS)

3-Dehydroquinate

3-dehydroquinate dehydratase (DHS)

shikimate dehydrogenase (SDH)

shikimate

shikimate kinase (SK)

shikimate 3- phosphate

glyphosate   5-enolpyruvylshikimate 3-phosphate synthase (EPSP)

5-enolpyruvylshikimate 3-phosphate (EPSP)

chorismate synthase (CS)

chorismate

tryptophan (Trp)   **phenylalanine (Phe)**   **tyrosine (Tyr)**

Figure 1. The shikimate pathway and the action of glyphosate on plants.

Figure 2. Sugarcane plants ten days after the application of glyphosate (2880 g a.e. ha^{-1}).

The varying tolerance of sugarcane cultivars to glyphosate was studied [52], who found different sensitivities among cultivars. The authors also classified the genotypes IAC86-2210, IAC83-1313, IAC82-2045, PO83-698 and IAC83-4157 as susceptible to glyphosate, IAC86-3154, IAC87-3184, RB72454 and SP80-1842 as of intermediate susceptibility, and IAC82-3092, IAC87-3396 and RB806043 as tolerant. Nevertheless, complete death, even in the less susceptible cultivars, occurred after 45 days following application. A plant's inherent tolerance is related to the plant's capability for absorption, translocation, metabolism and/or elimination of a herbicide [53]. In [54] also noted that differences in absorption depend primarily on morpho-anatomical characteristics of the species and that in the aerial parts of the plant, absorption is highly influenced by the presence or absence of cuticles. The physicochemical content of the leaf surface is another form of plant resistance to glyphosate [55]. According to these authors, leaves with flat cuticle surfaces and without large quantities of wax can better retain applied droplets. After penetration, the herbicide can then be metabolised into secondary compounds without herbicidal activity, or its potency might be enhanced [56].

The plant's development stage is another factor that should be considered in the eradication of cultivars because plants must be 40 to 80 cm tall at the time of glyphosate application [2], and the total leaf area must be sufficient to intercept the herbicide. The inherent resistance to glyphosate is greater in taller plants [57]. After the formation of culms, plants become more tolerant to the herbicide [58].

A relationship between plant size and glyphosate efficacy was also observed [59] while studying *Conyza bonariensis*. The authors observed that herbicidal efficacy was greater when

the plants presented up to two pairs of leaves. Nevertheless, in more advanced stages of development, it was necessary to increase herbicide dosage by up to fivefold.

Glyphosate applied 40 days after the last harvest caused the highest percentage of dead tillers, and also, genotypes IAC87-3184, RB835489 and SP87-344 displayed high to intermediate sensitivity, while IAC91-5155 was considered tolerant to the herbicide [49]. The most effective application time for eradication was 65 days after cane harvesting [60]. The same authors reported that a dose of 960 g a. e. ha^{-1} eradicated the majority of cultivars, except for Co997, which needed 1920 g a. e. ha^{-1} of the herbicide.
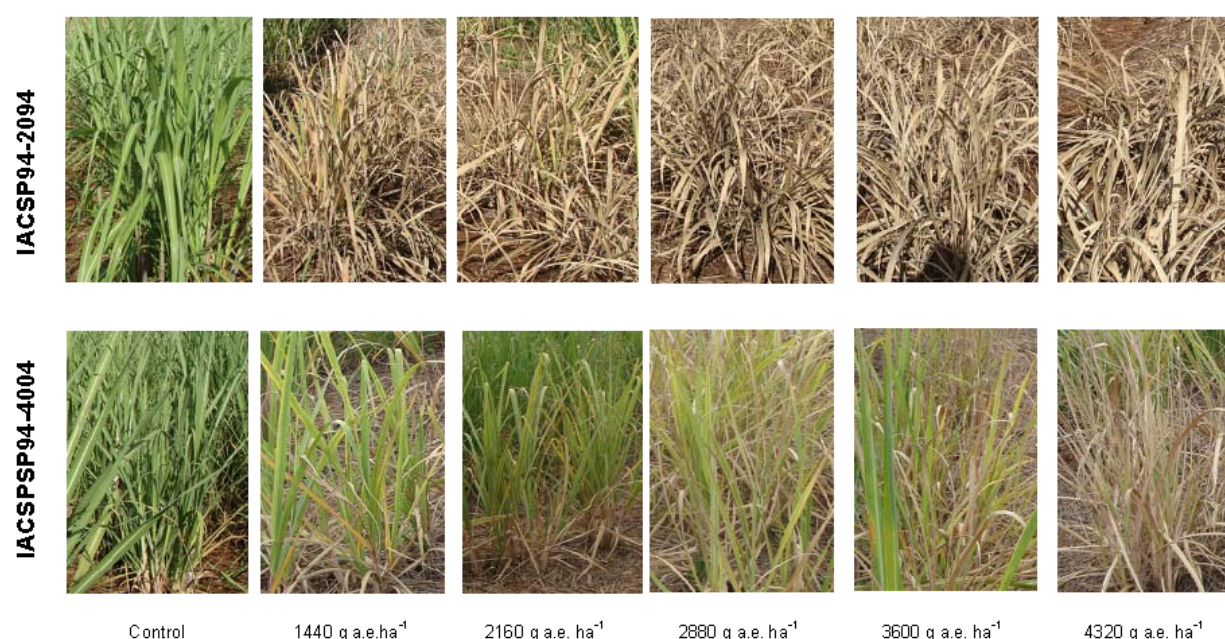

Figure 3. Intoxication symptoms caused by glyphosate rates in sugarcane cultivars (IACSP94-2094-4004 and IACSP94-4004) to 27 days after application. Instituto Agronômico de Campinas -IAC, 2012.

The development of transgenic sugarcane plants, particularly those with tolerance to glyph-osate, will most certainly change the way in which sugarcane is eradicated. The use of glyphosate will reduce the costs associated with the control of weeds across cycles; however, during the period of cane eradication, the herbicide will now have a limited impact due to the tolerance introduced to the cultivars. In this case, eradication may have to be performed mechanically, which will have a negative impact on soil conservation and might stimulate weed germination from soil propagule banks. From this perspective, it is important to emphasise that research aimed at sugarcane plants tolerant to glyphosate should also consider the use of herbicides in the eradication of future cultivars.

In the eradication of ratoon crops, glyphosate is used to eradicate the crop and also to control emerged weeds. However, when a sugarcane field also possess weeds that are hard to control, such as *Cynodon dactylon* and *Cyperus rotundus*, the use of higher doses of residual herbicides after the application of glyphosate is adopted in a process known as "disinfestation".
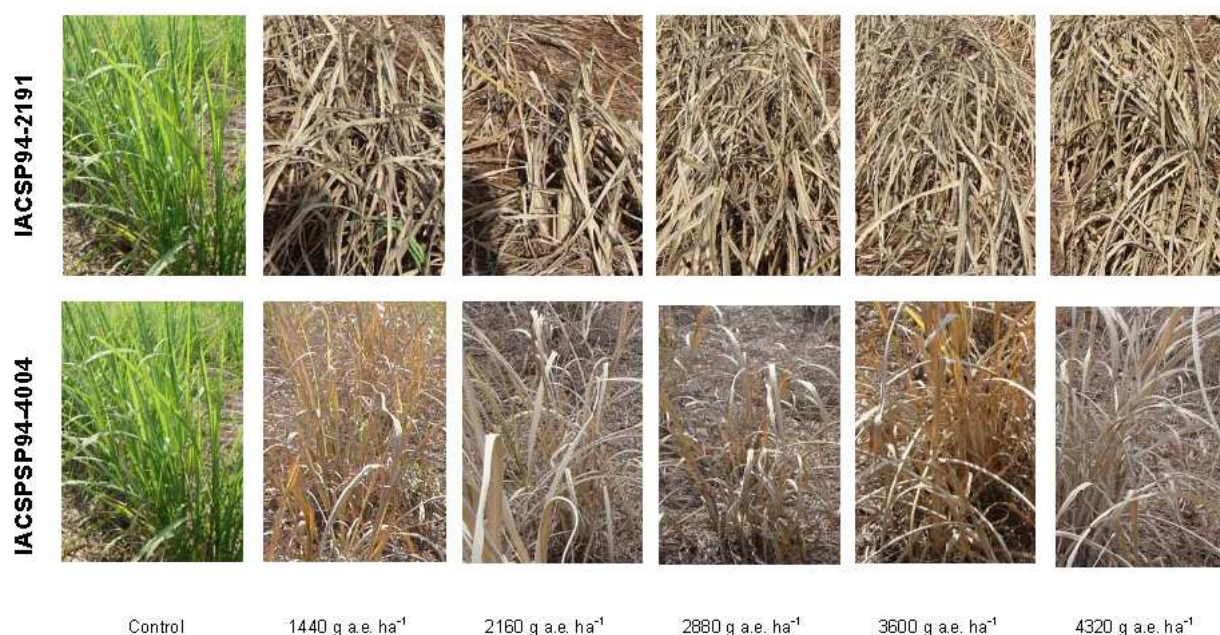
Figure 4. Intoxication symptoms caused by glyphosate rates in sugarcane cultivars (IACSP94-2094-4004 and IACSP94-2191) to 45 days after application. Instituto Agronômico de Campinas - IAC, 2012.

The aggressive biological nature of hard to control weed species requires that handling start with the desiccation of the plants to optimise the use of glyphosate in the eradication of ratoon while also being able to introduce residual herbicides at higher doses. In these instances, the use of glyphosate in crop eradication serves the dual role desiccating the ratoons and controlling problematic weeds.

In the time period following eradication but before planting sugarcane, the producer should formulate a strategy and opt for techniques that ensure higher sustainability of the system. These methods include such techniques as crop rotation or planting green-manure crops, although these may still compromise the techniques' sustainability if installed in fields that have been infested with "difficult-to-control weeds" or previously treated with residual herbicides for "disinfestation". The producer should carefully plan to use techniques that generate the most effective soil preparation and handling of weeds while simultaneously ensuring that after the treatments, the soil remains prepared for a new sugarcane plantation.

5. The shikimate pathway and isoenzymatic markers

The shikimate pathway is found only in plants and microorganisms and is completely absent in mammals, birds, reptiles, fish and insects. These organisms extract the aromatic compounds necessary for survival and reproduction from their diet, while plants must produce such compounds because they do not have alternative means to obtain the compounds [23].The shikimate pathway is initiated with the reaction of PEP and erythrose 4-phosphate, a reaction

catalysed by the enzyme DAHP synthase (3-deoxy-D-arabino-heptulosonate-7-phosphate synthase [61]. The resulting product is the seven-carbon acyclic intermediate 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP).

DAHP is converted to cyclic form through catalysis by 3-dehydroquinate synthase in the presence of NAD^+ as a coenzyme. In this process, 3-dehydroshikimate dehydratase dehydrates the cyclic form of DAHP. Next, shikimate dehydrogenase, in the presence of $NADP^+$ (oxidised NADPH), reduces the cyclic and dehydrated DAHP to shikimate. The molecule is later phosphorylated by the SP3 kinase, which converts a molecule of ATP to ADP. The phosphorylated shikimate subsequently reacts with one molecule of PEP in the presence of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, resulting in the production of EPSP. The shikimate pathway terminates with the production of chorismate (chorismic acid) through the dephosphorylation of EPSP by chorismate synthase.

Other metabolites essential to plant life may be produced from chorismate, including the amino acids tryptophan, phenylalanine and tyrosine, as well as vitamin K, ubiquinone and tetrahydrofolate [62, 23]. The amino acid phenylalanine is a precursor not only of proteins but also of other secondary products, such as phenolic compounds, anthocyanins, lignin and promoters and growth inhibitors. Tryptophan is also a precursor to indole-3-acetic acid, which is responsible for apical dominance and is vital for cellular growth and several other regulatory processes. Therefore, inhibitors of the shikimate metabolic pathway represent a strategic alternative in the development of herbicides with low environmental impact, such as glyphosate [23]. In this context, it could be interesting to use protein electrophoresis as a tool to study the eradication of plants by glyphosate using the isoenzymes involved in the metabolic pathway of shikimic acid.

Isoenzymes are the multiple molecular forms of enzymes that perform the same or similar catalytic activities. These enzymes are coded by one or more genes and may play an important role in survival across diverse environments. Isoenzymes are directly affected by both biotic and abiotic stressors [63]. The band intensity and isoenzymatic profile are plant-, tissue- and development stage-specific [64]. Some factors that affect plant metabolism, such as mineral nutrition, low temperature and diseases, among others, influence the activity of isoenzymes, specifically, esterases, peroxidases, phosphatases and phenolases, which in turn generate different expression patterns and levels of activity.Isoenzymatic patterns were used as tools by [65] who concluded that the enzymatic system of malate dehydrogenase is an efficient marker for aerobic respiration in pepper seeds during the maturation period. In Serbia [66] (the Vojvodina region), observed that the shikimate dehydrogenase system is also an efficient isoenzymatic marker for the study of genetic variability and polymorphisms in different almond genotypes.

In [67] was used the same technique in soy cultivars and registered a difference in electrophoretograms in terms of peroxidase activity. In [68] used isoenzymatic markers to identify species of lettuce nematodes, and [69] used the method "in vitro" in sugarcane to observe varietal differences among doses of glyphosate.

6. Esterase isoenzymes in abiotic stress

Enzymatic activity is influenced by stress factors, such as non-optimal temperature or nutrient levels and infection by pathogens. These stress responses subsequently lead to gene activation and, as a consequence, to the emergence of several molecular forms [70]. Because of the involvement of isoenzymes in changes to metabolism and defence mechanisms in plants, studies involving isoenzymes can be used in cases of both biotic and abiotic stress [63]. The authors report that polymorphisms displayed by isoenzymes are intermediate products of gene expression and are closer to the final phenotypical expression than those of DNA polymorphisms.

Esterases are isoenzymes comprising a group of genetically distinct enzymes that are found across a large spectrum of living organisms and that play a large variety of roles; nevertheless, esterases display a common trait of catalysing the hydrolysis of esters, peptides, amides and halide bonds [71]. Esterases can be found as both monomers and dimers [72, 73]. Esterases are significantly linked to lipid metabolism, such as that of membrane phospholipids, due to catalysis of ester hydrolysis [74].

In polyacrylamide gel electrophoresis assays, the α-esterase isoenzyme is detected using naphthyl ester substrates and histochemical stains. Enzyme isoforms with an affinity to hydrolyse α-naphthyl acetate are identified on the gel as black bands derived from the precipitation of α-naphthol, which results from the hydrolysis of α-naphthyl with the Fast blue RR salt stain [75].

Esterase isoenzymes have also been extensively explored in studies of genetic diversity due to their high rate of polymorphisms [76, 77]. In [78] the authors reported that sugarcane cultivars could be identified using esterase isoenzymes, and [79] in studying the parameters for sugarcane differentiation, observed that the electrophoretic profile of esterases is maintained in plants of varying physiological ages, as long as the growth environment is controlled.

Esterase isoenzymes are among the most widely used enzymes in the evaluation of enzymatic alteration in plants that are affected by parasitic nematodes across various pathosystems [80]. In [75] was studied esterase polymorphisms in 16 cultivars of soy that underwent or were spared treatment with glyphosate. The authors observed variation in the sensitivity to α-esterase isoenzymes of the different cultivars and also found that sensitivity did not seem to be connected with the homozygous RR status of the genetically modified plants.

7. Practical results of the isoenzymatic profiles of shikimate dehydrogenase and α-esterase in sugarcane

The tolerance of sugarcane cultivars to chemical eradication using varying doses of glyphosate was investigated [51] using phytotechnical parameters and isoenzymatic markers. The author hypothesised that the study of isoenzymatic profiles of shikimate dehydrogenase and α-esterase could optimise phytotechnical fieldwork observations regarding herbicidal tolerance.

Shikimate dehydrogenase was selected because it is involved in the shikimic acid pathway, which is affected by glyphosate action, and α-esterase was chosen because it is associated with oxidative stress. The isoenzymatic profiles of shikimate dehydrogenase and α-esterase were studied in sugarcane cultivars IACSP94-2094, IACSP94-2101, IACSP93-3046, IACSP94-4004, IAC86-2480 and RB72454 at 8, 24, 48, 72 and 144 hours after the application of glyphosate at doses of 0, 1440, 2160, 2880, 3600 and 4320 g a. e. ha^{-1}. The results showed that the bands for shikimate dehydrogenase tended to position near the cathode (at the top of the gel), while α-esterases were positioned closer to the anode, due to a greater migration during the gel run. The enzymatic system of shikimate dehydrogenase presented bands that were less sharp and also had a lower number of bands (three). There were no observed polymorphisms among cultivars, regardless of whether the data were analysed according to herbicide dosages or in relation to controls. Therefore, the isoforms remained constant among the different cultivars and treatments. In [81] was studied 20 enzymatic systems in the identification of sugarcane cultivars and also did not obtain any promising results using shikimate dehydrogenase. In [82] was studied populations of *Stryphnodendron adstringens*, known in Brazil as *barbatimão*, and also did not find polymorphisms for shikimate dehydrogenase.

The enzymatic system of α-esterase was specific for each studied cultivar, allowing for cultivar identification based on this biochemical marker [51]. This observation corroborates the findings [83], who created an analytical key for sugarcane cultivars and found a different pattern of α-esterase in each of the ten cultivars studied. A large number of bands of the α-esterase enzymatic complex were found with varied band intensity and thickness. The characteristics of this complex can be related to the degree of ploidy of the plant species; sugarcane is polyploid [70, 84].

Cultivars of variety IACSP93-3046 and RB72454 did not present differences in terms of bands owing to the application of glyphosate. These cultivars were considered susceptible to glyphosate based on field studies reporting a percentage of tiller death of 93.16% and 94.25% respectively. Moreover, marked toxic effects were rated as high as 94% for IACSP93-3046 and 95.5 % for RB72454 [51].

Sugarcane cultivar IACSP94-4004 was the only cultivar to show an alteration in its band pattern due to the application of glyphosate. Across all of the treatments in which the herbicide was applied, there were two additional bands that were not present in the controls and that were present from the first assessment 8 hours after the application (HAA) of herbicide to the last assessment at 144 HAA. The appearance of additional bands may be due to the expression of genes from this enzymatic system in response to stress caused by glyphosate treatment, thereby demonstrating that the cultivar response to the herbicide is directly linked to the genotype of each cultivar variety. In fact, cultivar IACSP94-4004 was relatively tolerant to the field experiments, as it was the cultivar to show the lowest average (percentage) of tiller death at 45 HAA (80.15 %), the symptoms of toxicity in this cultivar were less pronounced with an average of 82.5 %, and only glyphosate doses of 3600 g a. e. ha^{-1} caused symptoms similar to those caused by the highest dose of 4320 g a. e. ha^{-1}.

Evaluation of the isoenzymatic system of esterases has been used in other studies to characterise cultivar tolerance. Nevertheless, in the evaluation of sugarcane eradication,

the isoenzymatic profiles of shikimate dehydrogenase and α-esterase did not constitute a reliable tool [51].

8. The use of glyphosate in sugarcane as a ripener

Maturation is one of the most important aspects of sugarcane crops because maturation is directly related to the optimal time-point for harvest/industrial transformation.For the plant to enter the maturation process naturally, one or more sources of stress are necessary, with a gradual reduction in photoperiod, temperature or precipitation being the most effective stimulants [85]. In Brazil, more specifically in the south-central region, the maturation process is initiated in April/May when the climate becomes colder and drier. However, even under favourable conditions, sugarcane maturation may also be induced in responsive cultivars as a strategy to produce high-quality raw material across all of the different phases of harvesting.

To guarantee that maturation is complete, uniform, early and programmed and to avoid undesirable flowering, the sugarcane-ethanol industry has been adopting the use of ripeners (growth regulators) in sugarcane. Ripeners are chemical compounds that induce the translo-cation and storage of sugars, mainly sucrose, in the culm. Therefore, the goal of the ripeners is both to advance and maintain natural maturation and to provide high-quality raw materials for early industrial transformation, as well as to aid in the handling of cultivars [85].

The same authors state that to artificially induce maturation, growth regulators are applied by aircraft eight to ten months after the last harvest, that is, during the plants' vegetative state. In practice, the months of February and March or October are the periods during which farmers aim to apply the enhancers because they can anticipate the beginning or the end of the harvest.

There are two basic types of ripeners for sugarcane fields, "non-stressors" and "stressors". Non-stressor ripeners do not diminish the plants' growth rate, and their action induces the release of ethylene, the compound responsible for maturation that helps in the accumulation of sucrose in sugarcane culms. Stressor compounds, such as glyphosate, are growth inhibitors that markedly decrease the sugarcane growth rate, making the plants accumulate sucrose instead of expending it as an energy source for growth. This reduction in growth rate forces the plant to mature [85]. In [86] sugarcane plants with stagnated growth stop sprouting new leaves, and as a consequence, the reduced number of phytochromes in chloroplasts becomes insufficient to detect the photoperiod and thus stimulate the transition of the apical bud from vegetative to reproductive.

The effect of glyphosate, after it is applied to sugarcane, has a rapid onset, allowing for an increase of sucrose accumulation in 30 to 40 days after application. The glyphosate dose used is normally 0.3 to 0.4 l ha^{-1} and may reach a maximum of 0.6 – 0.8 l ha^{-1}, leading to differences in maturation speed as a function of dosage. Harvest should be performed when the highest levels of sucrose are reached [12].

9. Conclusions

Glyphosate is a high-efficiency molecule in the sugar-ethanol industrial sector in Brazil. Glyphosate can be used as an herbicide in the control of weeds and the chemical eradication of sugarcane crops, and when applied in low dosages, glyphosate can be used as a ripener.

Independently of where the product is used in the production system, the key factor is the adequacy of the dosage. If cultivar tolerance is known, it is possible to adjust the dose of glyphosate to eradicate sugarcane ratoon crops. Research has shown that a dose of 2 kg ha^{-1} (1440 g a. e. ha^{-1}) of the commercial product Roundup WG eradicates 92.76% of the tillers of the IACSP94-2094 cultivar but only 40.3% of the IACSP94-4004 cultivar. For the most effective eradication of the IACSP94-4004 cultivar, 4 kg ha^{1} (2880 g a. e. ha^{-1}) of chemical is needed. This finding demonstrates that knowledge of plant tolerance can be a valuable tool to adjust glyphosate doses to the appropriate concentrations for ratoon-crop eradication.

Reducing the quantity of applied glyphosate is possible, as long as the crop is sensitive. Environmental and economic benefits can also be obtained by applying lower quantities of the herbicide. Information on cultivar tolerance also allows producers to know when to use higher herbicide concentrations than those recommended in the literature. However, if the producer applies a lower dose than is necessary, the ratoon crop will not be completely eradicated. As a consequence, there will be additional expenditure on a additional herbicide application or on mechanical eradication, which is highly problematic because it causes the greatest disruption to the soil and later leads to the presence of crop stubble in the new sugarcane field.

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