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TESE

Metabolismo e Fisiologia de *Conyza sumatrensis* resistente a herbicidas

Jéssica Ferreira Lourenço Leal

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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METABOLISMO E FISIOLOGIA DE *Conyza sumatrensis* RESISTENTE A HERBICIDAS

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Sob a Orientação da Professora
Camila Ferreira de Pinho

e Coorientação do Professor
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Tese submetida como requisito parcial para obtenção do grau de **Doutora em Fitotecnia,** no Programa de Pós-Graduação em Fitotecnia, Área de Concentração em Produção Vegetal.

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RESUMO GERAL

LEAL, Jéssica Ferreira Lourenço. **Metabolismo e Fisiologia de *Conyza sumatrensis* resistente a herbicidas.** 2022. 114f. Tese (Doutorado em fitotecnia); Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2022.

A *Conyza* spp. é considerada uma das principais plantas daninhas do Brasil e tem sido frequentemente associada a casos de resistência a herbicidas. O cenário é pior em casos de resistência múltipla a herbicidas. Os objetivos deste estudo foram entender o metabolismo e fisiologia de *Conyza sumatrensis* resistente a herbicidas e o tipo de herança genética para resistência ao paraquat e diquat. No Capítulo I foi observado que herbicidas inibidores do FSI e FSII mostram rápidas alterações no índice de desempenho fotossintético mesmo antes de serem observados danos visuais de crescimento e desenvolvimento. A técnica de fluorescência da clorofila *a* demonstram claramente um potencial para rastrear rapidamente as perturbações metabólicas em *Conyza* sob aplicação dos herbicidas metribuzin e paraquat. No Capítulo II foi descrito que os sintomas observados no biótipo resistente ao 2,4-D foram necrose nas folhas em 30 minutos, com o restabelecimento do crescimento normal dentro de 1 a 2 semanas após o tratamento com 2,4-D. O biótipo resistente a 2,4-D mostra um rápido dano fotossintético e aumento no conteúdo de H_2O_2 em comparação ao biótipo suscetível. Além disso, a atividade da enzima antioxidante basal é maior no biótipo resistente. No Capítulo III, foi sugerido que o biotipo de buva com múltipla resistência aos herbicidas 2,4-D, paraquat, saflufenacil, glifosato e diuron pode também apresentar resistência aos herbicidas inibidores ALS clorimuron-etílico, imazapique + imazapir e etoxissulfurom. Estudos serão desenvolvidos para confirmar a hipótese através de dose-resposta. O capítulo IV confirmou a resistência de *C. sumatrensis* ao diquat com fator de resistência de 25,6 e 6,35 para LD_{50} e GR_{50}, respectivamente, em comparação com o biótipo suscetível. O biótipo resistente ao paraquat não induz as enzimas antioxidantes, como um possível mecanismo de resistência ao paraquat, mas mostra rápida recuperação dos parâmetros fotossintéticos e crescimento contínuo quando submetido ao paraquat, enquanto o biótipo suscetível não sobrevive a aplicação do herbicida paraquat e morre. No Capítulo V conclui-se que a resistência ao paraquat em F2 e F3 foi baseada em um modelo de um único gene dominante (3:1), enquanto os resultados do diquat foram baseados em dois genes segregados independentemente (15:1) em *Conyza sumatrensis*.

Palavras-chave: Resistência Múltipla. c. Fluorescência da Clorofila *a*.

GENERAL ABSTRACT

LEAL, Jéssica Ferreira Lourenço. **Physiological and Metabolism Responses of *Conyza sumatrensis* to Herbicides.** 2022. 114f. Dissertation (Ph.D in plant science); Agronomy Institute, Federal Rural University of Rio de Janeiro, Seropédica, RJ, 2022.

Conyza spp. is considered one of the most important weeds in Brazil and has been frequently associated with cases of herbicide resistance. The scenario is even worse when multiple herbicide resistance is involved. In this work, we studied the metabolic and physiological responses of resistant and susceptible *Conyza sumatrensis* to herbicides and identified the kind of genetic inheritance of paraquat and diquat resistance in *Conyza sumatrensis.* In Chapter I, it was found that both PSII- and PSI -inhibiting herbicides show a rapid negative effect on photosynthetic energy dynamics that can be monitored prior to the onset of a visual effect of the herbicide, demonstrating the potential use of chlorophyll fluorescence to rapidly investigate herbicide-induced metabolic disorders. In chapter II, it was described that the resistant biotype showed necrosis in leaves about 30 minutes after application and plants recovered from leaf damage 1 to 2 weeks after 2,4-D application. The 2,4-D-resistant biotype exhibited rapid photosynthetic damage and increased H_2O_2 content compared with the susceptible biotype. In addition, basal antioxidant enzyme activity is higher in the resistant biotype. In chapter III, it was suggested that the biotype with multiple resistance to the herbicides 2,4-D, paraquat, saflufenacil, glyphosate, and diuron may also have resistance to the herbicide inhibitors ALS chlorimuron-ethyl, imazapique + imazapyr, and ethoxysulfuron. Studies are being developed to confirm this hypothesis. Chapter IV confirms resistance of *C. sumatrensis* to diquat by the dose-response test with a resistance factor of 26-fold and 6-fold for LD_{50} and GR_{50} values, respectively, compared to the susceptible biotype. The paraquat-resistant biotype does not induce antioxidant enzymes, which is a possible mechanism for resistance to paraquat, but shows rapid recovery of photosynthesis and continued growth when exposed to paraquat. In Chapter V, it was demonstrated that paraquat resistance in F2 and F3 is based on a model with a single dominant gene (3:1), while the diquat results are based on two independently segregated genes (15:1) in *C.sumatrensis.*

Keywords: Multiple resistance. Genetic inheritance. Chlorophyll Fluorescence.

SUMÁRIO

1 INTRODUÇÃO GERAL

O Brasil é um dos maiores produtores agrícolas do mundo, devido à sua extensa área agricultável e clima favorável a produção. No entanto, as plantas daninhas causam graves perdas devido à competição com a cultura de interesse por recursos essenciais do meio (SWANTON et al., 2015; GHARDE et al., 2018). A buva (*Conyza* spp.) é considerada uma das principais plantas daninhas do Brasil e têm sido frequentemente associadas a casos de resistência a herbicidas. É uma planta daninha cosmopolita, onde a perturbação mínima do solo associada ao pousio proporcionou um nicho favorável para a adaptação ecológica da espécie. Essas espécies produzem muitas sementes que podem se espalhar por longas distâncias (WU et al., 2007; SAVAGE et al. 2014).

O controle químico de plantas daninhas é o principal método utilizado na agricultura moderna, contudo o uso dos mesmos herbicidas por longos períodos exerce pressão de seleção de plantas daninhas resistentes a herbicidas (DÉLYE et al. 2013; POWLES; YU 2010). Atualmente, existem 106 casos de *Conyza* spp. resistentes a herbicidas no mundo (HEAP, 2022). No Brasil, biótipos de *Conyza* spp. foram reportados como resistentes ao glifosato, clorimuron, paraquat e saflufenacil, e múltiplas resistências aos herbicidas glifosato e clorimuron; clorimuron, glifosato e paraquat e o último caso mais alarmante foi ao glifosato, paraquat, saflufenacil, diuron e 2,4-D (HEAP, 2022; PINHO et al., 2019).

O manejo de plantas daninhas resistentes a herbicidas é um dos maiores desafios agrícolas. Por isso, a rápida detecção e entendimento sobre a dinâmica de dissipação de energia fotossintética em plantas resistentes é extremamente importante para o manejo proativo. Os herbicidas de forma direta ou indireta afetam a fotossíntese das plantas, metabolismo primário, afetando a estabilidade do aparelho fotossintético, resultando em mudanças na indução de fluorescência de clorofila *a* (DAYAN; ZACCARO, 2012; SOUSA et al., 2014). Além disso a maioria das perturbações causadas pelo tratamento de herbicidas nas plantas está relacionada à geração de EROS e consequente estresse oxidativo. Portanto, a compreensão do comportamento fotossintético diferencial de *Conyza* spp. é importante para o conhecimento das respostas metabólicas dessas plantas em situações de estresse.

A evolução da resistência a herbicidas em plantas daninhas é função de vários determinantes, incluindo taxa de mutação, frequência inicial do gene de resistência, fluxo gênico, pressão de seleção e modo de herança (MAXWELL; MORTIMER 1994). Uma vez que a resistência a herbicidas tenha evoluído, os alelos de resistência podem se espalhar por meio do movimento de sementes e pólen (GHANIZADEH et al., 2019). Compreender a base genética da resistência a herbicidas em plantas daninhas é fundamental para desenvolver modelos para prever, monitorar e gerenciar a resistência de plantas daninhas.

Os objetivos deste estudo foram entender o metabolismo e fisiologia de *Conyza sumatrensis* resistente a herbicidas e o tipo de herança genética para resistência ao paraquat e diquat.

2 REVISÃO BIBLIOGRÁFICA GERAL

2.1 Plantas Daninhas e Resistência a Herbicidas

O Brasil está entre os maiores produtores agrícolas mundiais de soja, milho, cana-de-açúcar, café, laranja, dentre outras culturas. Contudo, diversos fatores podem vir a interferir no desenvolvimento e produtividade das culturas, dentre eles doenças e plantas daninhas.

Plantas daninhas são quaisquer plantas que ocorrem onde não são desejadas (PITELLI et al., 1987), interferindo nas atividades do homem, e que quando presentes em agroecossistemas interferem com as culturas ocasionando danos econômicos. As plantas daninhas são dotadas de características peculiares e que interferem na estratégia de seu manejo. A presença dessas plantas reduz a eficiência agrícola, aumenta os custos de produção e diminui a qualidade do produto final, reduzindo seu valor comercial e ainda dificulta ou até impede a colheita (VASCONCELOS et al., 2012; SWANTON et al., 2015; GHARDE et al., 2018).

Os métodos normalmente utilizados para controlar as plantas daninhas são o mecânico, o físico, o biológico, o químico e o cultural, sendo o químico o mais empregado. Isso se dá devido à economia, rendimento operacional elevado e controle rápido das plantas daninhas (BUENO et al., 2013). Entretanto, o uso de herbicidas de forma intensiva, é um dos fatores que levam a pressão de seleção de biótipos resistentes. Considera-se biótipo, como um grupo de indivíduos com carga genética semelhante, porém pouco diferenciado da maioria dos indivíduos da população (KISSMANN, 1996). No caso de plantas daninhas resistentes a herbicidas essa diferenciação geralmente caracteriza o biótipo resistente.

A resistência de plantas daninhas aos herbicidas é um tópico de crescente preocupação na agricultura. A *Weed Science Society of America* (1998), define resistência como a ocorrência natural da habilidade hereditária de alguns biótipos, dentro de uma população, sobreviver e se reproduzir após o tratamento com um herbicida que seria letal aos demais indivíduos desta população. Diante do C_{50} (dose de herbicida que proporciona 50% de controle) e GR_{50} (dose de herbicida que causa 50% de redução de massa), calcula-se o fator de resistência (F = R/S) que expressa o número de vezes em que a dose necessária para controlar 50% da população resistente é superior à dose que controla 50% da população suscetível (CHRISTOFFOLETI, 2004).

A resistência de planta daninhas onera ainda mais o custo de produção. Estudos realizado em 2017, nas principais regiões produtoras do Brasil, avaliou que os custos de produção em lavouras de soja com plantas daninhas resistentes ao glifosato podem subir, em média, de 42% a 222%, principalmente pelo aumento de gastos com herbicidas e pela perda de produtividade da cultura. Em áreas com infestações isoladas de buva e de azevém os valores sobem, em média, entre 42% e 48% respectivamente. O cenário é ainda pior em casos de infestações de buva e capim-amargoso na mesma área de produção com aumento médio de 222% (ADEGAS et al., 2017).

No mundo estão registrados 512 casos de plantas daninhas resistentes a herbicidas (HEAP, 2022), sendo que 53 desses casos ocorrem no Brasil. O cenário é ainda pior devido aos casos de resistência múltipla. No Brasil há 18 casos (HEAP, 2022), dentre eles merece destaque a buva (*Conyza* spp).

2.2 *Conyza* spp (buva)

A buva vem causando grandes problemas no sistema de produção agrícola, principalmente devido ao fato de sua boa adaptabilidade ao sistema de plantio direto. Dentre do gênero *Conyza* as espécies que vêm sendo mais estudadas devido ao seu caráter invasivo são *Conyza canadensis*, *C. bonariensis* e *C. sumatrensis*. Contudo, a identificação das espécies

tem sido desafiadora para a ciência das plantas daninhas, principalmente após a comprovação de hibridação entre as espécies (MAROCHIO et al., 2017).

Considerada uma planta daninha dicotiledônea, com ciclo anual, pertence à família *Asteraceae* e encontra-se disseminada globalmente em climas temperados e zonas subtropicais (THEABAUD; ABBOTT, 1995). As espécies de *Conyza* possuem propagação unicamente através de semente. São plantas muito prolíficas, com produções de sementes maiores que 375.000 por planta em *Conyza bonariensis*, 200000 em *C. canadensis* e mais de 60000 em *C. sumatrensis* (GREEN, 2010). As sementes não têm dormência e germinam prontamente quando em condições favoráveis de temperatura, luz e umidade.

A emergência dessa planta daninha concentra-se no período do final do outono e início primavera, que coincide com o período de pousio ou plantio de inverno (WU et al., 2007; SAVAGE et al., 2014) o que favorece o estabelecimento dessa planta em áreas produtivas quando não há manejo outonal. Por isso, as recomendações de manejo de áreas infestadas com buva resistente visam o rápido controle, de modo que os biótipos não cheguem à produção de sementes, visando o controle dessa planta daninha em estádio fenológico inicial quando há mais opções de manejo.

Atualmente, no Brasil, a buva é uma das principais plantas daninhas nos sistemas produtivos, podendo reduzir a produtividade da soja em até 48% dependendo da densidade de plantas por metro quadrado (GAZZIERO et al., 2010; BLAINSKI et al., 2015). Para Trezzi (2015) plantas de *Conyza bonariensis* podem reduzir o rendimento de soja em 36%, 12% e 1,0%, quando estabelecidas em 81, 38 e 0 dias antes da semeadura da soja, respectivamente.

No Brasil, o primeiro relato de *Conyza* spp resistente ao glifosato foi no Rio Grande do Sul em 2005 (LAMEGO; VIDAL, 2008). Após a resistência de buva ao glifosato, os herbicidas inibidores da ALS (inibidor acetolactato sintase) passaram a ser empregados amplamente para controle dessa espécie na cultura da soja. Como resultado da alta pressão de seleção exercida pelos herbicidas inibidores da ALS, em 2011, foram identificados biótipos de buva com resistência múltipla ao glifosato e aos inibidores da ALS (SANTOS et al., 2014). O caso se agravou em 2016 e 2017, quando foi registrado resistência da buva ao herbicida paraquat (inibidor do fotossistema I - FSI) e saflufenacil (inibidor da protoporfirinogênio oxidase - Protox) e resistência múltipla aos herbicidas clorimurom (ALS), glifosato (EPSPs) e paraquat (FSI). O último caso alarmante foi diagnosticado pelo nosso grupo de pesquisa Plantas Daninhas e Pesticidas no Ambiente-UFRRJ, onde se detectou um biótipo de buva com resistência a cinco mecanismo de ação: FSI, Protox, FSII (fotossistema II), Mimetizador de auxina e EPSPs (PINHO et al., 2019; HEAP, 2022).

2.3 Mecanismos de Resistência de Plantas Daninhas

As plantas daninhas podem sobreviver à aplicação de herbicidas mediante um ou mais mecanismos de resistência. Eles são divididos em mecanismos de resistência ligados ao sítio-alvo (*TSR- Target-site resistance*) as quais incluem aumento da expressão da proteína alvo ou alterações estruturais no sítio de ligação do herbicida; ou mecanismos de resistência fora do alvo (*NTSR- Non-target-site resistance*), que estão associados à absorção ou translocação diferencial e/ou compartimentalização em vacúolo/paredes ou outras organelas e metabolização do herbicida (DÉLYE et al., 2013).

2.3.1 Herbicida paraquat

Estudos demonstram que o fator de resistência de buva ao paraquat varia de 9 a 352,5 vezes quando comparados com populações S (suscetíveis) (VANGESSEL et al., 2006; EUBANK et al., 2012; MORETTI et al., 2015). O paraquat e o diquat são herbicidas não

seletivos de contato e ação rápida, que atuam no cloroplasto desviando os elétrons do fotossistema I (FSI), o que resulta em um grave dano oxidativo aos tecidos. Há 72 casos de relato de resistência ao paraquat e 10 ao diquat no mundo, sendo 21 desses casos relacionados ao gênero *Conyza* (HEAP, 2022).

Os níveis de resistência ao paraquat entre os biótipos R e S variam de 10 a 30 vezes no estádio de roseta vegetativa para 100 vezes ou mais após 10 semanas, quando as plantas estão entrando em seu ciclo reprodutivo (YE; GRESSEL, 2000). Estes resultados demonstram a dificuldade de controle de buva em estágios fenológicos avançados.

A compreensão atual do mecanismo molecular da resistência do paraquat em plantas superiores inclui a translocação reduzida devido ao sequestro de paraquat no vacúolo e aumento da atividade de enzimas antioxidativas (SZIGETI, 2005; HAWKES, 2014; MORETTI; HANSON, 2017). A atividade aumentada de APX (ascorbato peroxidase) e GR (Glutationa redutase) está correlacionada com a resistência a paraquat em *Conyza* spp. (YE; GRESSEL, 2000; CHIANG et al., 2008). A atividade dessas enzimas também foi relatada como um mecanismo de resistência ao paraquat em *Mazus pumilus* (TSUJI et al., 2013).

2.3.2 Herbicida saflufenacil

O saflufenacil é um dos herbicidas utilizados no manejo de *Conyza* spp. resistentes ao glifosato e paraquat e tem sido amplamente utilizado como estratégia de controle dessas plantas (MELLENDORF et al., 2013; WAGGONER et al., 2013; MORETTI et al., 2015). No entanto, biótipos de plantas daninhas do Brasil apresentaram resistência ao saflufenacil (HEAP, 2022). Este herbicida pertencente à família química da pirimidinodiona, é um herbicida inibidor da Protox, está enzima catalisa a oxidação do protoporfirinogênio IX em protoporfirina IX pelo oxigênio molecular, e a inibição da enzima Protox resulta em um acúmulo da protoporfirina IX, que na presença de luz, gera grandes quantidades de oxigênio singleto que atacam as membranas de lipídios e proteínas, levando à morte da planta (DAYAN; DUKE, 2010).

No mundo, foram identificados 38 casos de plantas daninhas resistentes aos herbicidas inibidores da Protox, sendo três biótipos com resistentes ao herbicida saflufenacil. No Brasil, foi relatado resistência aos herbicidas inibidores da PROTOX em *Euphorbia heterophylla* em 2004 e *Conyza sumatrensis* em 2017 (HEAP, 2022). O mecanismo de resistência aos herbicidas Protox, em diferentes espécies, foi atribuído a uma mutação no sítio-alvo PPX2 (Protox 2), caracterizada por uma deleção do Gly-210 devido à perda de três nucleotídeos consecutivos; também foram relatadas a substituição de arginina-128 para glicina ou metionina (R128G ou R128M) (PATZOLDT et al., 2006; SALAS et al., 2016; SALAS et al., 2017), substituição de arginina-98 para leucina (MENDES et al., 2020) e ainda substituições de glicina para alanina (G399A), glicina para ácido glutâmico (G114E) e serina para isoleucina (S149I) (RANGANI et al., 2018; MONTGOMERY et al., 2021). Mais recentemente, uma mutação de ponto único na PPO1 levou a uma alteração do aminoácido alanina para treonina (A212T) (BI et al. 2020). No*n-target site* mediado pelas enzimas P450 e GST como mecanismo de resistência em *Amaranthus palmeri* aos herbicidas inibidores da Protox.

2.3.3 Herbicida diuron

Uma abordagem comum para gerenciar plantas daninhas resistentes a herbicidas é mudar para herbicidas alternativos ou misturas de herbicidas que ainda controlam efetivamente as populações resistentes, ou como parte do programa que integra os herbicidas pré-emergentes junto com a aplicação de pós-emergentes (BECKIE et al., 2006). Os herbicidas inibidores de Fotossistema II (FSII), são amplamente utilizados no manejo de plantas daninhas. Estes bloqueiam a transferência de elétron da QA (plastoquinona QA) para QB-plastoquinona,

resultando na interrupção da transferência de elétrons (POWLES; YU 2010). A transferência de elétrons interrompida causa estresse oxidativo pela produção de espécies reativas de oxigênio, que causam rápida degradação celular. Os herbicidas deste mecanismo de ação são divididos em 3 grandes grupos: C1 (Triazinas), C2 (Uréias e Amidas) e C3 (Nitrilas e outros).

Atualmente existem 10 casos de plantas daninhas resistentes ao herbicida diuron no mundo. O primeiro caso foi relatado em 1987, em *Chloris barbata* nos Estados Unidos. Em 2002, a resistência cruzada aos inibidores de PSII, incluindo atrazina, simazina e diuron, foi relatada em *Conyza canadensis* presente nos campos de mirtilo nos Estados Unidos (HEAP, 2022). E em 2017, o primeiro caso de *Conyza sumatrensis* resistente ao diuron foi relatado no Brasil (PINHO et al, 2019).

Até o momento, o sequenciamento do gene *psbA* indicou as mutações Ser-264-Gly; Ser-264-Thr; Val-219-Ile; Asn-266-Thr; Ala-251-Val; Ala-251-Thr; Phe-255-Ile, Leu-218-Val e Val-219-Ile responsáveis pela resistência aos inibidores de FSII em diferentes culturas (MENGISTU et al., 2000; PERRY et al., 2012). Além disso há relato de mecanismos NTRS como redução da absorção/translocação e aumento do metabolismo dos herbicidas em plantas daninhas resistentes aos herbicidas inibidores do FSII (DE PRADO et al. 1997; PRESTON et al. 1996; PRESTON 2003).

2.3.4 Herbicida 2,4-D

O herbicida 2,4-D é utilizado para controle de plantas daninhas de folha larga, comumente em mistura de tanque com o glifosato no manejo de dessecação. Os primeiros casos de resistência ao 2,4-D no mundo, foram relatados em 1957, em cenoura silvestre (*Daucus carota* L.) (SWITZER, 1957) e trapoeraba (*Commelina diffusa*) (HILTON, 1957). Atualmente, existem 82 casos de plantas daninhas resistentes aos herbicidas mimetizadores de auxinas e 47 desses são ao herbicida 2,4-D (HEAP, 2022). No começo deste estudo, relatamos o primeiro caso de *Conyza sumatrensis* resistente ao 2,4-D no mundo (PINHO et al, 2019).

A sinalização de auxina envolve quatro classes principais de proteínas: transportadores de auxina (efluxo (PIN e ABCB) e influxo (AUX/LAX)), repressores transcricionais (Aux/IAAs) (*Auxin/Indole-3-acetic Acid*), fatores de resposta de auxina (ARFs) (*Auxin Response Factor*) e o complexo ubiquitina ligase E3 Skp1-Cullin-F-box TIR1/AFB (F-box *Transport Inhibitor Response 1/Auxin signaling F-Box protein*) (SCFTIR1/AFB). A auxina é transportada dentro e entre as células via transportadores PIN, ABCB e AUX/LAX e interage com o complexo SCFTIR1/AFB, que após a criação do complexo SCF-auxina-Aux/IAA, causa ubiquitinação de repressores transcricionais Aux/IAA permitindo a indução de genes de resposta de auxina. Portanto, a percepção da auxina é governada por uma molécula de ligação de auxina ao complexo co-receptor SCFTIR1/AFB, que medeia a ubiquitinação de uma família de reguladores transcricionais, as proteínas Aux/IAA (TODD et al., 2020).

Evidências sugerem que os herbicidas auxínicos desencadeiam a morte da planta aumentando rapidamente o acúmulo de ABA através da expressão de genes responsivos a auxina como o NCED (dioxigenase 9-cis-epoxicarotenóide), resultando em regulação negativa de genes associados à fotossíntese (GAINES, 2020). O acúmulo de etileno também pode contribuir para morte da planta inibindo a fotossíntese e produzindo H_2O_2 e espécies reativas de oxigênio.

Os mecanismos de resistência de plantas daninhas a herbicidas auxínicos são relatados como redução na absorção e/ou translocação (WEINBERG et al., 2006; GOGGIN et al., 2016; BUSI et al., 2018) e metabolismo diferencial (BUSI et al., 2018; TORRA et al., 2021).

Estudos demostram que mutações nos receptores de auxina AFB5, T1R1, SGT1b conferem resistência a algumas auxinas sintéticas (TODD et al., 2020), assim como mutação

na Aux/IAA16 na posição Gly127Asn (GWPPV para NWPPV) (LECLERE et al., 2018) e alterações nos ARF5, ARF7, ARF19 (ativadores transcriptacionais) podem levar a resistência (TODD et al., 2020). E ainda, mutações que reduzem a atividade ou perda de função de AUX1 / LAX são suficientes para conferir resistência a herbicidas auxinicos como o 2,4-D (TODD et al., 2020).

2.3.5 Herbicida glifosato

O glifosato é um dos herbicidas mais utilizados no mundo e seu uso intensivo resultou na evolução de plantas resistentes em todo o mundo (POWLES, 2008; BECKIE; HARKER, 2017). Glifosato é um inibidor da via do chiquimato, essencial para a produção dos aminoácidos aromáticos essenciais fenilalanina, triptofano e tirosina. A 5-enolpiruvilshiquimato-3-fosfato sintase (EPSPs) é a enzima-alvo para o glifosato e converte shiquimato-3-fosfato e fosfoenolpiruvato em 5-enolpiruvil-shikimato-3-fosfato. A inibição da via do chiquimato resulta em acúmulo de ácido chiquímico, seguido por clorose e morte de plantas (STEINRÜCKEN; AMRHEIN, 1980).

Biotipos resistentes ao glifosato de *C. bonariensis*, *C. canadensis* e *C. sumatrensis* são relatados em muitos países, inclusive no Brasil. Existem atualmente 343 casos de plantas daninhas resistentes ao glifosato no mundo (HEAP, 2022). O primeiro caso de *Conyza* spp. resistente ao glifosato foi observado nos Estados Unidos, em 2000. No Brasil, *C. bonariensis* e *C. canadensis* foram reportadas como resistentes ao glifosato em 2005 e *C. sumatrensis* em 2010 (HEAP, 2022).

Mecanismos de resistência ao glifosato incluem mutação no sítio-alvo, duplicação do gene do sítio-alvo, sequestro do vacúolo, captação celular limitada e rápida necrose (SAMMONS; GAINES, 2014). Mecanismos de resistência ao glifosato incluem mutações em Pro-106 (ou seja, Pro-106-Ala, Pro-106-Ser, Pro-106-Thr, Pro-106-Leu) e uma substituição de aminoácidos dupla (Thr -102-Ile + Pro-106-Ser) (DÉLYE et al., 2004; SAMMONS; GAINES, 2014; AMARO-BLANCO et al., 2018; PAGE et al., 2018), superexpressão do gene EPSPs e transportadores ABC (TANI et al; 2015) e redução da translocação e absorção do herbicida (FERREIRA et al., 2008; NOL et al., 2012; GONZALEZ-TORRALVA et al., 2014, 2017), muitas das vezes ocasionados pelo armazenamento do herbicida no vacúolo ou outros compartimentos celulares (DALAZEN et al., 2019). Enzimas do ciclo de Calvin e proteínas de função desconhecida também são identificadas como possíveis candidatas envolvidas na resistência do glifosato em *Conyza canadensis* (GONZALEZ-TORRALVA et al., 2017).

De forma geral a resistência a herbicidas pode estar correlacionada a um ou mais mecanismos de resistência. Estudos mostram que a resistência causada por mecanismos não relacionados ao local de ação, principalmente causada pelo incremento de metabolização, e sistema antioxidante enzimático são altamente problemáticas devido aos maiores riscos de resistência múltipla, sendo um novo desafio para o manejo de plantas daninhas.

2.4 Herança Genética

A evolução da resistência a herbicidas em plantas daninhas é função de vários determinantes, incluindo taxa de mutação, frequência inicial do gene de resistência, fluxo gênico, pressão de seleção e o modo de herança (MAXWELL; MORTIMER 1994).

O fluxo gênico é o movimento de genes resistentes entre e dentro de populações de plantas. Isso pode ocorrer através do movimento de pólen ou sementes, em casos de heranças genéticas nucleares, dispersas por mecanismos como vento, animais, água e implementos agrícolas (MAXWELL; MORTIMER 1994). O fluxo gênico pelo movimento de sementes é uma das maneiras comuns pelas quais as plantas daninhas resistentes são transferidas entre os

campos. A dispersão de sementes tem o potencial de impactar o movimento de genes de resistência em uma escala muito maior do que o fluxo de pólen. Isso é um problema para espécies altamente proliferas como o gênero *Conyza*, onde uma única planta pode produzir mais de 200.000 sementes (GREEN, 2010).

Além disso, o fluxo gênico entre espécies de plantas daninhas pode ocorrer através do polén, ocasionando impacto na disseminação de alelos de resistência a herbicidas (MAITY et al., 2022). Uma vez que a resistência da planta daninha tenha evoluído, os alelos de resistência podem se espalhar facilmente (GHANIZADEH et al., 2019).

Informações sobre o fluxo gênico através da dispersão de pólen para plantas autopolinizadas, como *Conyza* spp. não são bem disseminadas porque a taxa de cruzamento dessas espécies é baixa (HUANG et al., 2015). Porém, Soares et al., (2015) relataram a ocorrência de hibridização entre espécies do gênero *Conyza*, e uma alogamia média de 4% é descrita (HENRY et al., 2008). Isso pode produzir um impacto na disseminação de alelos de resistência a herbicidas através das espécies (MAITY et al., 2022).

Na grande maioria dos casos, a resistência a herbicidas é caracterizada por um único gene presente no núcleo, que apresenta dominância completa ou incompleta (GHANIZADEH et al., 2019), o que leva a rápida evolução da resistência. Ainda há resistência de plantas daninhas a herbicidas ligada a efeitos multigênico (PRESTON, 2003) e herança materna (citoplasmática) (GHANIZADEH et al., 2019).

Se a resistência for controlada por um único gene, ela será representada por uma proporção mendeliana típica de 3:1 para fenótipo resistente (3) e suscetível (1). No entanto, se a geração F2 não exibir uma proporção mendeliana ou 3:1 (R:S) para resistência, isso é uma indicação de que a resistência pode ser controlada por múltiplos genes (PRESTON et al., 2009). Como por exemplo, quando dois genes dominantes têm o mesmo efeito em uma característica, indicando efeito duplicado, isso leva a uma razão de segregação de 15:1 (WEINBERG et al., 2006).

Compreender a base genética da resistência a herbicidas em plantas daninhas é fundamental para desenvolver modelos para prever, monitorar e gerenciar a resistência de plantas daninhas a herbicidas.

2.5 Estresse Fisiológico Ocasionado por Herbicidas

Os herbicidas de forma direta ou indireta afetam a fotossíntese das plantas, metabolismo primário, afetando a estabilidade do aparelho fotossintético, resultando em mudanças na indução de fluorescência de clorofila a (DAYAN; ZACCARO, 2012; SOUSA et al., 2014).
Além disso a maioria das perturbações causadas pelo tratamento de herbicidas nas plantas está relacionada à geração de EROS e consequente estresse oxidativo.

2.6 Fotossíntese

A fotossíntese é o processo fisiológico fundamental do metabolismo das plantas. Durante a fotossíntese, a planta utiliza a energia solar para oxidar a água e produzir poder redutor de alta energia ATP e NADPH, utilizados para a síntese de açúcares nas reações de fixação do carbono (TAIZ et al., 2017). A energia luminosa absorvida pelos pigmentos fotossintéticos pode ser dissipada na forma de fluorescência da clorofila *a* ou na forma de calor ou ainda direcionada a fase fotoquímica (KRAUSE; WEIS,1991; BUCHANAN et al., 2015; TAIZ et al., 2017).

Na fotossíntese, o complexo antena, formado pelos pigmentos, coleta a luz e transferem a energia, por ressonância, para o centro de reação (CR) (P680 para o PSII; P700 para o PSI). A principal função do complexo antena é transferir energia de excitação para os centros de

reação. A água é oxidada no complexo de evolução do oxigênio, formado por íons de manganês, no lado do lume do tilacoide, liberando prótons resultantes da oxidação da água e contribuindo com o gradiente eletroquímico de H+. Os elétrons são direcionados a cadeia de transporte de elétrons para o processo de oxi-redução (VINYARD et al., 2013; BUCHANAN et al., 2015; TAIZ et al., 2017). Portanto a energia absorvida da luz é utilizada para impulsionar a transferência de elétrons por uma série de componentes que atuam como doadores e aceptores de elétrons.

As reações no centro de reação do Fotossistema II (FSII) iniciam com a excitação de P680, que rapidamente perde um elétron para reduzir o aceptor primário – feofitina, que transfere o elétron de forma sequencial as plastoquinonas QA e QB (OKAMURA et al., 2000; VINYARD et al., 2013). Depois de receber dois elétrons, QB-2 capta dois prótons do estroma e se converte em plastohidroquinona, sendo sua forma protonada o plastoquinol (PQH2). O PQH2 então transfere seus elétrons para o complexo citocromo b6f (Cyt b6f), e os H+ são liberados no lado do lume do tilacoide (incrementando o gradiente eletroquímico). Os elétrons, por sua vez são repassados do Cytb6f ao FSI por intermédio da proteína plastocianina (PC) (PRIBIL et al., 2014; BUCHANAN et al., 2015; TAIZ et al., 2017).

O fotossistema I (FSI) é o outro complexo envolvido na fase fotoquímica da fotossíntese. Os elétrons são transferidos da PC para o P700 e daí para um monômero de clorofila (A0), para uma filoquinona (A1), para os centros ferro-enxofre (Fe-S), e finalmente para a proteína ferro-sulfurosa hidrossolúvel, a ferredoxina (Fd) (TAIZ et al., 2017). A flavoproteína associada à membrana, ferredoxina-NADP redutase (FRN), reduz o NADP+ a NADPH, completando assim o transporte acíclico de elétrons (KARPLUS et al.,1991). O gradiente eletroquímico de H+ (força motriz de prótons) gerado através da membrana do tilacoide é utilizada para sintetizar ATP via ATP sintase (STOCK et al., 2000; CROFTS et al., 2004). Os produtos gerados na fase fotoquímica (NADPH e ATP), são críticos para a produção de carboidratos a partir de CO_2, que ocorre na fase bioquímica (BENSON, 2004).

2.6.1 Fluorescência da clorofila *a*

A fluorescência ocorre quando a clorofila excitada é de-excitada pela reemissão de um fóton em um comprimento de onda maior e menos energético ao absorvido e, assim retorna ao seu estado basal. A clorofila excitada também pode dissipar a energia em forma de calor, sem emissão de um fóton, ou ainda transferir a energia para o processo fotoquímico, no qual desencadeará reações de oxidação-redução. Esses processos estão em competição direta pela energia de excitação, desse modo qualquer aumento em um processo de um irá causar o decréscimo dos outros (STRASSER et al., 1995; BARBAGALLO et al. 2003; STRASSER et al., 2004; TSIMILLI-MICHAEL; STRASSER 2008).

A fração de radiação absorvida e dissipada varia de acordo com o estado metabólico, e fornece a base para o entendimento dos processos fotossintéticos das plantas. Embora a fluorescência da clorofila *a* represente apenas uma pequena fração da energia absorvida, aproximadamente 0,5–10%, esta fornece um indicador eficaz sobre o desempenho fotossintético das plantas (KAUTSKY et al., 1931; STRASSER; STRASSER, 1995; STRASSER et al., 2004; YUSUF et al., 2010; DAYAN; ZACCARO, 2012). A intensidade de fluorescência é inversamente proporcional a energia utilizada para a fotossíntese ou emissão de calor (KRAUSE; WEIS, 1991), visto que esses são processos competitivos, podendo esta ser usada para monitorar o funcionamento do aparato fotossintético das plantas (STRASSER et al., 2004; STIRBET; GOVINDJEE, 2012).

A fluorescência da clorofila *a* é uma maneira simples, não invasiva, não destrutiva, rápida e relativamente fácil de determinar a eficiência e a atividade da cadeia de transporte de

elétrons fotossintética (KALAJI et al., 2011), de plantas sobre quaisquer estresses. É uma técnica capaz de fornecer informações quanto a perturbações no metabolismo vegetal induzidas por herbicidas, por exemplo, mesmo que não haja quaisquer danos visuais nas plantas (BARBAGALLO et al., 2003; OUKARROUM et al., 2007; DAYAN; ZACCARO, 2012; TROPALDI et al., 2015; ZHANG et al., 2016).

Quando uma folha fotossintetizante adaptada ao escuro é iluminada com um pulso de luz saturante, a intensidade de fluorescência pode atingir seu pico máximo num curto período (1s), exibindo transitórios característicos conhecidos como efeito Kautsky) (KAUTSKY; HIRSCH, 1931). Kautsky e Hirsch (1931), notaram que numa transição de escuro para a luz a intensidade de fluorescência transiente ou polifásica inicialmente aumenta, seguida por um declínio até chegar a um nível constante. Esta cinética exibe a sequência de passos, definidos como O, J, I e P, os quais refletem o sucessivo preenchimento do pool dos aceptores de elétrons do fotossistema II (YUSUF, et al., 2010) (Figura 1). A análise dos transientes do OJIP permite entender melhor as relações entre a estrutura e função do aparelho fotossintético e avaliar rapidamente a viabilidade da planta.

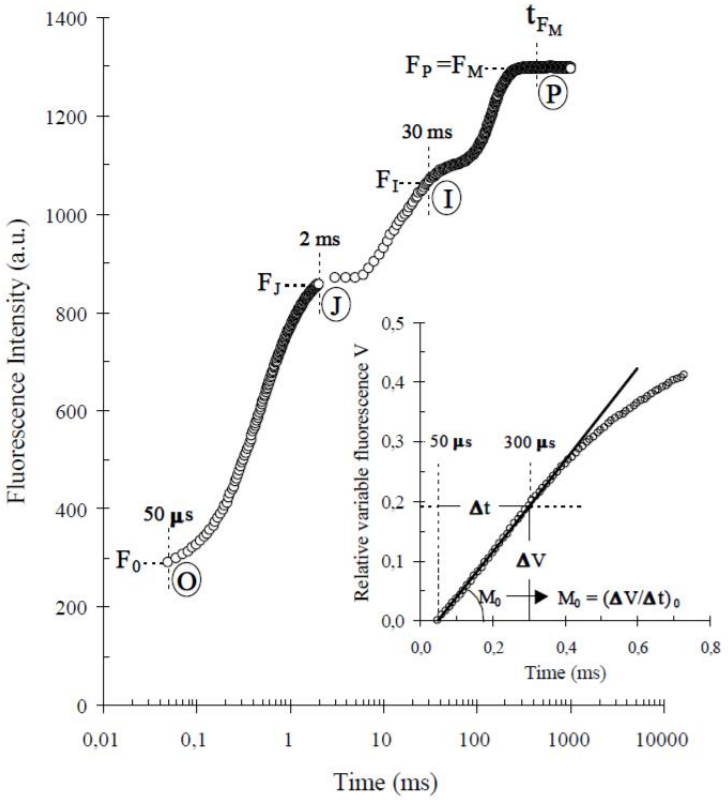

Figura 1. Fluorescência polifásica (O-J-I-P), exibida por plantas superiores. O transiente é plotado em uma escala de tempo logarítmica de 50 ms a 1 s. A intensidade de fluorescência F0 (a 50 ms); as intensidades de fluorescência FJ (a 2 ms) e FI (a 30 ms); a intensidade máxima de fluorescência, FP = FM. (Fonte: TSIMILLI-MICHAEL et al., 2000).

A cinética da emissão de fluorescência (efeito Kautsky) é caracterizada por duas fases: Rápida (até 1 s) e lenta (até alguns minutos). A fase rápida é rotulada como OJIP (STRASSER et al., 1995; STIRBET; GOVINDJEE, 2012), onde O é a origem (a 20 ou 50 µs), J (a 2 ms) e I (30 ms), são passos intermediários, e P é o pico máximo (a cerca de 300 ms) (STRASSER et al., 2000; TSIMILLI-MICHAEL et al., 2008) (Figura 2). A fase lenta é chamada PSMT

(PAPAGEORGIOU; GOVINDJEE, 1998), onde S representa o estado semi-estacionário, M para um máximo, e T para um nível de estado terminal constante. A cinética de emissão da fluorescência transiente ou polifásica permite o cálculo de parâmetros de fluorescência que caracterizam o funcionamento do aparelho fotossintético (YUSUF et al., 2010).

Na fase rápida da fluorescência transiente (OJIP) duas intensidades têm grande importância e estão relacionados ao estado redox do lado aceptor do FSII, que são a fluorescência mínima (F0) e a máxima (FM). O parâmetro F0 (fluorescência mínima ou O) é conseguido através da adaptação da amostra ao escuro (15-30 min até uma hora), caracterizada quando todos os centros de FSII estão abertos (oxidados), e a inibição não fotoquímica (qN) está ausente, é um indicador de perdas de energia durante a excitação de transferência de energia na antena e da antena para o centro de reação de FSII. A fluorescência máxima (FM ou P) é obtida por um pulso de luz saturante (~3.000 μmol fótons m^{-2} s^{-1}) é o nível máximo de fluorescência da clorofila quando todos os centros de reação do FSII estão fechados (reduzidos), ou seja, quando todas as Q_A estão reduzidas (STRASSER; STIRBET, 2001; STRASSER et al., 2004). O declínio na FM indica que o objeto fotossintetizante sofre estresse, o que significa que os receptores de elétrons do FSII não podem ser totalmente reduzidos.

A diferença entre a fluorescência proveniente de centros de reação fechado e aberto é chamada de fluorescência variável, ou Fv (FV= FM-F0), e é uma medida relativa dos centros de reação fotoquimicamente ativos. O valor do FV diminui sob a influência de fatores de estresse. Há evidências substanciais de que o parâmetro FV/FM, ou seja, a razão (FM-F0)/FM medida em plantas adaptadas ao escuro, reflete a eficiência do potencial quântico do FSII e pode ser usado como um indicador confiável da atividade fotoquímica do aparelho fotossintético. Para a maioria das plantas totalmente desenvolvidas sob condições sem estresse, o valor máximo de FV/FM é igual a 0,8 (KRAUSE; WEIS,1991; FARALONI et al., 2011; KALAJI et al., 2011; GUIDI et al., 2019).

A fase OI da cinética, revela mudanças no processo entre o éxciton capturado pelos CRs até a redução da PQ (YUSUF et al., 2010). A fase JI está envolvida com a redução dos transportadores de elétrons do intersistema da cadeia de transporte de elétrons (plastoquinona, Cytb6f e plastocianina); enquanto na fase IP são refletidas as reduções dos receptores de elétrons do lado aceptor do FSI, ou seja, ferredoxina (Fd), outros intermediários e NADP+ (SCHANSKER et al., 2005; YUSUF et al., 2010).

A parte lenta é mais difícil de interpretar, pois ocorre um aumento do número de processos envolvidos durante esta fase, como por exemplo, extinção de fluorescência não-fotoquímica, síntese de ATP, ciclo de Calvin, entre outros (STIRBET; GOVINDJEE, 2011).

Para comparar as amostras obtidas nas análises das transientes, é necessário normalizar os dados para fluorescência variável relativa (W): $W_{OJ} = (Ft - F0)/(FJ - F0)$, $W_{OI} = (Ft - F0)/(FI - F0)$ and $W_{IP} = (Ft - FI)/(FP - FI)$, e realizar a subtração da cinética como $\Delta W = W_{tratamento} - W_{controle}$ (YUSUF et al., 2010). Estas subtrações da cinética revelam bandas que geralmente são escondidas entre as etapas O, J, I e P dos transientes primários (STRASSER et al., 2004; YUSUF et al., 2010). A diferença cinética ΔW_{OJ} revela a banda "K" (a cerca de 300ms) relacionada com a dissociação do complexo de evolução do oxigênio, enquanto a ΔW_{OK} revela a banda "L" (em cerca de 150ms) que é um indicador da conectividade energética ou do grau de agrupamento das unidades do FSII (STRASSER; STIRBET et al., 1998). As bandas são indicadores de distúrbios fisiológicos antes do aparecimento de sinais visíveis de estresse (OUKARROUM et al., 2007).

O estudo da cinética da fluorescência transiente da clorofila *a* pode ser avaliado através de análises multiparamétricas, conhecidas como Teste-JIP (STRASSER; STRASSER, 1995). Esses parâmetros calculados quantificam o fluxo de energia que passa pelos fotossistemas e

avaliam o desempenho fotossintético das plantas. O teste JIP fornece informações adequadas sobre a conformação, estrutura e função do aparato fotossintético em qualquer estado fisiológico, podendo ser utilizado para estudar ponto a ponto as mudanças no comportamento deste aparato, através das alterações dos parâmetros obtidos (STRASSER; STRASSER, 1995; STRASSER et al., 2004; STIRBET; GOVINDJEE, 2011). Dentre os parâmetros que são derivados a partir deste teste encontram-se os que quantificam o fluxo de energia através do centro de reação (CR) (ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC e RE_0/RC); os rendimentos energéticos (φP0, φE0, φD0 e φR0), as eficiências (ψE_0, ψR_0 e δR_0) e os índices de performance fotossintética (PI_{ABS} e PI_{TOTAL}) (Tabela 1) (STRASSER; STRASSER, 1995; STRASSER et al., 2004). Todos esses fluxos de energia estão interligados e são dependentes de propriedades estruturais e atividade fotossintética da amostra biológica.

Tabela 1. Principais parâmetros do Teste JIP (STRASSER et al., 2004 e adaptado de YUSUF et al., 2010). Seropédica-RJ, 2022

Parâmetros de fluorescência calculados a partir dos dados primários obtidos	
$F_V = F_M - F_0$	Fluorescência variável;
F_V/F_M	Rendimento quântico máximo do FSII;
V_t	Fluorescência variável relativa em um tempo "t"
V_j	Fluorescência variável relativa em relação ao nível J;
V_i	Fluorescência variável relativa em relação ao nível I;
$M_0 = 4(F_{300\mu s} - F_0)/(F_M - F_0)$	Declive inicial aproximado (em ms^{-1}) da fluorescência transiente V= f (t);
$S_s = V_J/M_0$	Área total normalizada complementar correspondente apenas a fase OJ (reflete um único volume de eventos de redução de Q_A);
$S_m = (Area)/(F_M - F_0)$	Área total normalizada complementar acima da curva OJIP (reflete múltiplos eventos de redução Q_A);
$N = S_m/S_s$	Número total de elétrons transferidos para a cadeia de transporte de elétrons entre o tempo de 0 et (necessário para atingir F_M).
Atividade específica por centro de reação (RC)	
$ABS/RC = M_0 (1/V_J) (1/\varphi Po)$	Medida do tamanho aparente do sistema antena ou o fluxo de absorção por RC;
$TR_0/RC = M_0 (1/V_J)$	Máxima taxa pela qual um éxciton é capturado pelo RC resultando em uma redução da plastoquinona (Q_A^-);
$ET_0/RC = M_0 (1/V_J) \Psi_0$	Reoxidação da Q_A^- via transporte de elétrons em um RC ativo
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Razão de dissipação total de energia de excitação não capturada do total de RC, sendo a dissipação neste caso à perda de energia na forma de calor;
RE_0/RC	Redução do aceptor final de elétrons no lado do aceptor de elétrons do FSI por RC.
Rendimentos energéticos ou taxas de fluxo	
$\varphi P_0 = TR_0/ABS = F_V/F_M$	Rendimento quântico máximo fotoquímico;
$\varphi E_0 = ET_0/ABS$	Rendimento quântico de transporte de elétrons de Q_A^- para o intersistema de aceptores de elétrons;
$\varphi D_0 = 1 - \varphi Po = (F_0/F_M)$	Rendimento quântico para dissipação de energia;
$\varphi R_0 = RE_0/ABS$	Rendimento quântico de transporte de elétrons de Q_A^- para o aceptor final de elétrons do FSI.
Eficiências	
$\psi E_0 = ET_0/TR_0$	Eficiência com que um éxciton capturado no RC pode mover um elétron de Q_A^- para o intersistema de aceptores de elétrons;

$\psi R_0 = RE_0/TR_0$	Eficiência com que um éxciton capturado no RC pode mover um elétron dentro da cadeia de transporte de elétrons de Q_A^- para os aceptores finais de elétrons do FSI;
$\delta R_0 = RE_0/ET_0$	Eficiência com que um elétron pode mover o intersistema de aceptores de elétrons reduzidos no intersistema para o aceptor final de elétrons do FSI.
Índices de desempenho	
$PI_{ABS} = \frac{RC}{ABS} \times \left(\frac{\varphi_{P0}}{1-\varphi_{P0}}\right) \times \left(\frac{\Psi_0}{1-\Psi_0}\right) = \frac{RC}{ABS} \times \frac{TR_0}{DI_0} \times \frac{ET_0}{1-ET_0}$	Índice de desempenho fotossintético (conservação de energia a partir do éxciton para a redução dos aceptores de elétrons do intersistema)
$PI_{total} = PI_{ABS} \times (\frac{\delta_0}{1-\delta_0})$	Índice de desempenho fotossintético total (conservação de energia a partir de éxciton para a redução de aceptores finais do FSI)

Os rendimentos quânticos representam o número de elétrons transferidos em uma determinada etapa do estágio de fotossíntese em proporção ao número de fótons absorvidos pelo FSII. As eficiências refletem a probabilidade da transferência de elétron através da cadeia de transporte de elétrons. Os índices de performance fotossintética (PI_{ABS} e PI_{TOTAL}) descrevem o funcionamento da cadeia de transporte de elétrons desde a absorção do fóton até a redução dos aceptores finais do FSI (STRASSER et al., 2000; TSMILLI-MICHAEL; STRASSER, 2008), dependem da densidade do centro de reação, da eficiência de captura e da eficiência do transporte de elétrons. Consequentemente, alterações em qualquer um destes componentes em decorrência de um estresse pode ser facilmente observado por esses parâmetros. Dessa forma, a fluorescência da clorofila *a* tem um potencial de ser aplicado na compreensão de ensaios de herbicidas e diagnósticos de suscetibilidade e resistência de plantas daninhas a herbicidas (ZHANG et al., 2016).

2.6.2 Espécies reativas de oxigênio (EROS) e sistema antioxidante

A maioria das perturbações causadas pelo tratamento de herbicidas nas plantas está relacionada à geração de EROS e consequente estresse oxidativo nas plantas (COBB et al., 2010). Dos 21 mecanismos de ação de herbicidas descritos, aproximadamente 43% causam produção direta de EROS e outros 29% estão indiretamente envolvidos no aumento de EROS nas plantas (COBB et al., 2010; CAVERZAN et al., 2019). O tempo de resposta para a ocorrência de estresse oxidativo e danos visíveis nas plantas varia com o modo de ação do herbicida, tipo de herbicida e formulação, espécies de plantas, estágio de desenvolvimento e condições ambientais (COBB et al., 2010; CAVERZAN et al., 2019).

A saturação da cadeia transportadora de elétrons da fotossíntese, ocasionada por algum estresse, pode sobrecarregar a energia em clorofilas e carotenoides, levando à formação de EROS que causam danos as proteínas, membranas e DNA (HUGIE et al., 2008; BAKER, 2008), podendo levar a planta a morte. Em baixas concentrações, as EROS são conhecidas como moléculas sinalizadoras que ativam múltiplas respostas de defesa. Quando o um aumento de EROS é maior que a capacidade antioxidante da célula, caracteriza-se o processo de estresse oxidativo (PANDHAIR; SEKHON, 2006). Portanto o nível e o tipo das EROS são fatores determinantes para o tipo de resposta.

As EROS são normalmente referidas como subprodutos de reações redox que se apresentam tanto como radicais livres, como na forma molecular de um não radical (KOVALCHUK, 2010). As EROS são produzidas em vários locais: cloroplastos, mitocôndrias,

peroxissomos, membranas plasmáticas, reticulo endoplasmático e na parede celular. Na presença de luz, cloroplastos e peroxissomos são as principais fontes de produção de EROS, enquanto a mitocôndria é o principal produtor de EROS em condições de pouca luz. As formas de EROS podem ser geradas por excitação (transferência de energia), formando oxigênio singleto ($^1O_2$), ou por reações de transferências de elétrons, resultando em o radical aniônico superóxido (O_2•-), peróxido de hidrogênio (H_2O_2) ou radical hidroxila (OH•). O oxigênio singleto é menos reativo do que o radical OH•, contudo mais reativo do que o O2•- e o H_2O_2 (BARBOSA et al., 2014).

O superóxido (O_2•-) geralmente é a primeira EROS a ser formado. O radical superóxido é formado principalmente no FSI localizado no tilacoide durante fluxo de elétrons na cadeia de transporte de elétrons não-cíclica (ETC). Porém também pode ser formado em outros compartimentos como mitocôndrias e peroxissomos. Ocasionalmente, o O_2 reage com os elétrons liberados pela CTE produzindo $O^{\bullet-}_2$, podendo causar peroxidação de lipídeos no ambiente celular e nas membranas celulares. A dismutação do superóxido a H_2O_2 é muito rápida e pode ocorrer tanto de forma espontânea como catalisada pela enzima superóxido dismutase (BHATTACHARJEE, 2010).

O oxigênio singleto ($^1O_2$) é formado quando a energia armazenada na clorofila em seu estado tripleto não é dissipada, sendo então transferida para o O_2 (BHATTACHARJEE, 2010). Essa EROS pode-se difundir a distâncias significativas a partir do seu sítio de produção e pode ser extinto transferindo sua energia de excitação para outras moléculas (β-caroteno, tocoferol, dentre outras), e retornando ao estado fundamental ou por reações de oxidação com outras moléculas (lipídios, proteínas, aminoácidos, ácidos nucleicos e carboidratos) causando danos às células (TRIANTAPHYLIDÈS; HAVAUX, 2009).

O peroxido de hidrogênio (H_2O_2) em baixas concentrações, atua como molécula envolvida na sinalização a vários estresses bióticos e abióticos e, em altas concentrações, leva a morte programada da célula. É uma EROS capaz de atravessar membranas celulares, podendo migrar para compartimentos diferentes das células. O H_2O_2 tem uma ação deletéria, porque participa da reação formadora de radical hidroxila (OH•), o oxidante mais reativo na família das EROS. Os peroxissomos são organelas que realizam reações de oxidação de substratos orgânicos, resultando na produção de H_2O_2 e são considerados os principais sítios intracelulares de geração dessa ERO (KARUPPANAPANDIAN et al., 2011; SHARMA et al.,2012).

O radical hidroxila (OH*) apresenta uma meia-vida muito curta, pois reage muito rapidamente com moléculas biológicas, sequestrando aleatoriamente um átomo de hidrogênio. É considerada a mais oxidante dentre as EROS (MYLONA; POLIDOROS, 2010). O superóxido pode doar elétrons ao Fe^{3+} formando Fe^{2+} que, por sua vez, reduz o H_2O_2 e forma OH• e OH-. O conjunto de reações através das quais o O_2 •-, o H_2O_2 e o Fe^{2+} rapidamente geram OH• é conhecido como "reação de Haber-Weiss", enquanto a reação final a oxidação do H_2O_2 pelo Fe^{2+}, é denominada reação de Fenton (GIL; TUTEJA, 2010). O OH• é uma molécula altamente nociva em sistemas vivos. Por isso a sua formação pela redução de íons metálicos na presença do O_2 •- deve ser evitada. As enzimas do sistema antioxidante não eliminam o OH• diretamente, de modo que a regulação de seus precursores, O_2 •- e H_2O_2, é o passo fundamental na prevenção dos riscos do OH•, reunindo a ação das enzimas antioxidantes (MYLONA; POLIDOROS, 2010).

Para reduzir os efeitos nocivos das EROS, as plantas protegem suas células e compartimentos sub-celulares pela indução do sistema de defesa antioxidante. Isso ocorre principalmente pela ação de enzimas antioxidantes que estão presentes em diferentes compartimentos celulares (cloroplastos, mitocôndrias, peroxissomos e citosol), como catalase (CAT), superóxido dismutase (SOD), ascorbato peroxidase (APX), glutationa peroxidase

(GPX), glutationa redutase (GR), polifenol oxidase (PPO), guaiacol peroxidase (GPOD), monodesidroascorbato redutase (MDHAR) e dehidroascorbato redutase (DHAR), dentre outras; e moléculas não enzimáticas, tais como ácido ascórbico, glutationa, carotenoides e antocianinas (MITTLER 2002; GILL et al., 2011) (Figura 2). Este sistema antioxidante detoxifica essas formas de EROS, e atua associado ao mecanismo que dissipa o excesso de energia absorvida antes da formação de oxigênio singleto, formado pelas xantofilas.

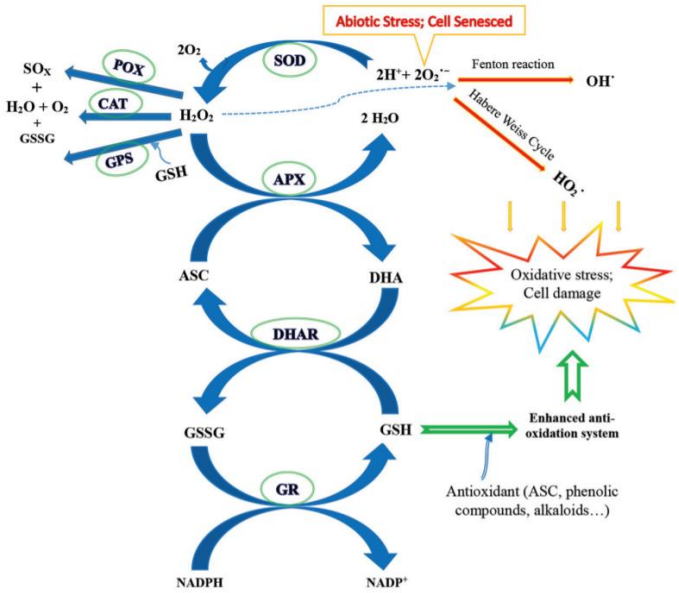

Figura 2. Esquema diagramático dos sistemas de defesa antioxidante. Ascorbato (ASC), glutationa (GSH), compostos fenólicos e alcalóides, GSSG, glutationa oxidada; DHA, desidroascorbato, superóxido dismutase (SOD), ascorbato peroxidase (APX), desidroascorbato redutase (DHAR), glutationa redutase (GR), peroxidase (POX), catalase (CAT) e glutationa peroxidase (GPX). EROs, incluindo radicais superóxido ($O\cdot^{-2}$), peróxido de hidrogênio (H_2O_2), radicais hidroxila (OH·) e radicais perihidroxila (HO·2), se acumulam quando as plantas sofrem estresse abiótico ou são senescidas pela reação de Fenton e / ou pelo mecanismo de Habere Weiss. Fonte: (GILL; TUTEJA, 2010; XU et al., 2015).

O mais importante mecanismo de detoxificação das EROS inclui a atividade das enzimas superóxido dismutase (SOD), ascorbato peroxidase (APX) e catalase (CAT). As SODs são consideradas a primeira linha de defesa contra as EROS, catalisam a dismutação de dois radicais O_2*-, gerando H_2O_2 e O_2. Dessa forma agem indiretamente na redução do risco de formação do OH* a partir do O2*- (MITTLER, 2002; APEL; HIRT, 2004). São classificadas de acordo com seus cofatores metálicos: cobre e zinco (Cu/Zn-SOD), manganês (Mn-SOD) e ferro (Fe-SOD) (GILL; TUJETA, 2010). Em geral, as plantas contêm uma Mn-SOD localizada na matriz mitocondrial e uma Cu/Zn-SOD citosólica, com Fe-SOD e/ou Cu/Zn-SOD, presentes no estroma do cloroplasto. O número de isoenzimas de cada tipo de SOD varia muito de planta para planta, assim como a abundância relativa de cada enzima. Essas enzimas participam da modulação do nível de H_2O_2 em cloroplastos, mitocôndrias, citosol e peroxissomos (MITTLER, 2002; BHATTACHARJEE, 2010).

A APX e a CAT são as enzimas mais importantes dentre os componentes de desintoxicação do H_2O_2. A catalase (CAT) é uma das principais enzimas constitutivas que atuam na eliminação do H_2O_2 gerado durante a fotorrespiração e a β-oxidação dos ácidos graxos, sendo encontrada nos peroxissomos, glioxissomos e mitocôndrias e converte duas

moléculas de H_2O_2 a H_2O e oxigênio molecular (MITTLER, 2002). As plantas possuem várias isoformas de CAT, as quais podem dismutar diretamente o H_2O_2 ou oxidar substratos, tais como metanol, etanol, formaldeído e ácido fórmico. Como a CAT opera sem agente redutor, ela fornece às plantas uma forma energeticamente eficiente para remoção do H_2O_2 (SHARMA et al., 2012). A atividade da CAT é efetiva, principalmente, em concentrações relativamente altas de H_2O_2, por isso são consideradas indispensáveis para a detoxificação de EROS, especialmente em condições de estresse severo. No entanto, esta enzima tem menor afinidade pelo H_2O_2 do que as enzimas Peroxidases.

A ascorbato peroxidase (APX) é uma enzima induzida em plantas e possui função de proteção antioxidativa, sendo um componente integral do ciclo Ascorbato-Glutationa (ASC-GSH) (FOYER; NOCTOR, 2011). A atividade de peroxidases pode aumentar em plantas submetidas a diversos tipos de estresse e pode ser tomada como marcador bioquímico de estresse resultante tanto de fatores bióticos como abióticos. Em plantas, o mais importante redutor para o H_2O_2 é o ascorbato, sendo que a APX, no primeiro passo do ciclo ascorbato-glutationa, usa duas moléculas de ascorbato para reduzir o peróxido de hidrogênio à água, com a geração concomitante de duas moléculas de monodehidroascorbato (MDHA) (FOYER; NOCTOR, 2011). A GPX é menos específica ao substrato doador de elétrons, decompõe H_2O_2 pela oxidação de co-substratos como compostos fenólicos ou ascorbato.

O Guaiacol peroxidase (GPX) é uma enzima que elimina o excesso de H_2O_2 tanto durante o metabolismo normal quanto durante o estresse. Ela desempenha um papel vital na biossíntese de lignina, bem como defende contra o estresse biótico degradando o ácido indol-acético (IAA), utilizando H_2O_2 no processo. A GPX prefere compostos aromáticos como guaiacol e piragalol (ASADA, 1999) como doadores de elétrons. Como a GPX é ativa intracelularmente (citosol e vacúolo), na parede celular e extracelularmente, é considerada enzima chave na remoção de H_2O_2.

A monodehidroascorbato redutase (MDHAR) é responsável pela regeneração do ácido ascórbico (AA) a partir de MDHA de vida curta, usando o NADPH como agente redutor, acabando por repor o pool de AA celular. Uma vez que regenera AA, é co-localizada com o APX nos peroxissomas e mitocôndrias, onde a APX retira H_2O_2 e oxida o AA no processo (MITTLER, 2002). A dehidroascorbato redutase (DHAR) reduz o desidroascorbato (DHA) para AA usando glutationa reduzida (GSH) como doadora de elétrons (ELTAYEB et al., 2007) . Isso faz com que seja outro agente, além do MDHAR, que regenera o pool de AA do celular. A DHAR é encontrada abundantemente em sementes, raízes e brotos verdes e estiolados.

A glutationa redutase (GR) é uma enzima responsável por catalizar a redução da GSSG (glutationa oxidada) para GSH (glutationa reduzida) e para isso usa NADPH como redutor. A GSH é utilizada para regenerar AA a partir de MDHA e DHA e, como resultado, é convertida em sua forma oxidada (GSSG). A GSH é uma molécula importante dentro do sistema celular, participando inclusive do ciclo ascorbato-glutationa (NOCTOR et al., 2002).

As glutationas S-transferases (GST) são enzimas que catalisam a conjugação de GSH à substratos hidrofóbicos eletrofílicos, como xenobióticos (REZAEI et al., 2013). São proteínas encontradas preferencialmente no citoplasma.

No sistema antioxidante não enzimático estão incluídos principalmente os compostos fenólicos, que são sintetizados pelas plantas em resposta a estresse. Antioxidantes não enzimáticos são encontrados em todos os compartimentos celulares e os mais importantes são o ácido ascórbico (vitamina C) e a glutationa (GSH), os quais têm propriedades hidrofílicas. O ácido ascórbico, é um dos mais importantes antioxidantes não enzimáticos e pode inativar várias

EROS. Juntamente com a glutationa (GSH), participa do Ciclo do Ascorbato-Glutationa, no qual o H_2O_2 é eliminado pela APX mediante a peroxidação do ácido ascórbico.

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3 CAPÍTULO I

PHOTOSYSTEM II- AND PHOTOSYSTEM I-HERBICIDE INHIBITOR-DRIVEN CHANGES IN THE DYNAMICS OF PHOTOSYNTHETIC ENERGY DISSIPATION OF *Conyza* spp.

Submitted: Acta Physiologiae Plantarum

3.1 RESUMO

A compreensão do comportamento fotossintético diferencial de *Conyza* spp. sobre os herbicidas inibidores do fotossistema é importante para o conhecimento das respostas metabólicas dessas plantas em situações de estresse. O objetivo deste estudo foi avaliar a dinâmica da dissipação de energia fotossintética de *Conyza* spp. mediante aplicação de herbicidas inibidores de FSII e inibidores de FSI. Os tratamentos incluíram a aplicação de metribuzin, paraquat e uma testemunha não tratada. A fluorescência transiente da clorofila *a* foi realizada 0,5, 1, 2, 4, 8, 24, 48 e 96 horas após a aplicação (HAA) e a análise das trocas gasosas foi realizada 2, 4 e 96 HAA, quando também foi feita análise de controle visual. Aos 7DAA e 2HAA foi observado danos visuais nas plantas sob aplicação dos herbicidas metribuzin e paraquat respectivamente, enquanto mudanças na dinâmica da dissipação de energia fotossintética foram observadas desde às 0,5 HAA. Após 0,5-1 HAA, as plantas apresentaram redução na reoxidação de QA- (quinona) por centro de reação ativo, redução no rendimento de transporte de elétrons de QA- para o intersistema e redução no rendimento de transporte de elétrons de QA- para o aceptor final de elétrons-PSI, para ambos os herbicidas, isso ocasionou declínio em 90% do índice de desempenho fotossintético e aumento nas perdas de energia na forma de calor e fluorescência. Na análise das trocas gasosas, observou-se redução na taxa fotossintética, na taxa de transpiração e na condutância estomática após 2 HAA para ambos os herbicidas. Essas diminuições no índice de desempenho fotossintético nas plantas tratadas com metribuzin e paraquat mesmo antes de serem observados danos perceptíveis no crescimento e desenvolvimento demonstram claramente o potencial do uso da fluorescência da clorofila *a* para rastrear rapidamente as perturbações metabólicas na buva sob aplicação dos herbicidas metribuzin e paraquat.

Palavras-chave: Fluorescência da Clorofila *a*. Fotossíntese. Herbicida. Metribuzin. Paraquat

3.2 ABSTRACT

This work aimed to evaluate the dynamic dissipation of photosynthetic energy of fleabane upon PSII- and PSI-inhibitor (photosystem I and II) herbicides application. Treatments were comprised of the application of metribuzin (PSII) and paraquat (PSI), following recommended doses for fleabane (*Conyza* spp.) plants (12- to 15- cm tall) and an untreated check. Chlorophyll a transient fluorescence was performed at 0.5, 1, 2, 4, 8, 24, 48 and 96 hours after application (HAA) and gas exchange analysis was performed at 2, 4 and 96 HAA, besides visual control analysis performed up to 28 days after application (DAA). The visual control was observed at 7DAA and 2HAA by applying PSII- and PSI-inhibitor, respectively, while changes in the dynamic dissipation of photosynthetic energy were observed at 0.5 HAA. At 0.5 to 1 HAA, plants showed a decrease in QA- (quinone) re-oxidation per reaction center, reduction in electron transport yield from QA- to the end electron acceptor of the PSI, for both herbicides. Moreover, plants showed a decline of 90% in the performance index and a higher increase in energy dissipation as heat and fluorescence. Fleabane also showed a great decrease in net assimilation rate (CO2), transpiration rate and stomatal conductance upon 1 HAA, for both herbicides. Therefore, both PSII- and PSI-inhibiting herbicides show a rapidly negative effect on photosynthetic energy dynamics that can be monitored before the appearance of any visual effect of herbicide which demonstrates the potential use of chlorophyll fluorescence to rapidly screen metabolic perturbations caused by herbicides.

Key words: Chlorophyll a Fluorescence. Photosynthesis. Herbicide. Metribuzin. Paraquat

3.3 INTRODUCTION

Losses in agricultural production due to weeds is of utmost importance in agricultural systems. The estimates obtained showed potential yield losses of 50% promoted by weed in corn and soybean production worldwide (SOLTANI et al., 2016; GHARDE et al., 2018). In Brazil, fleabane (*Conyza* spp. L.) was reported among the most troublesome weeds that can decrease the average production in soybean by 50% (BLAINSKI et al., 2015). The invasiveness of fleabane is associated with its high fecundity, high potential of both seeds production and dispersion level, staggering emergence, resistance to herbicides, and adaptation to a no-till farming system (WU et al., 2007; SAVAGE et al., 2014; ALCÁNTARA-DE LA CRUZ et al., 2020).

To fleabane control, several post-emergence herbicides with different modes of action are available. Among them, metribuzin and paraquat (PSII- and PSI-inhibitor) are commonly used in the early post-emergence control or associated/sequential to another herbicide during burndown. In Brazil, there is a report of *Conyza sumatresis* resistant PSII- (diuron) and PSI- (paraquat) inhibiting herbicides (PINHO et al., 2019). Therefore, it is crucial to understand the photosynthetic behavior of fleabane to herbicides with mode of action on photosystem since they represent the most important way to manage weed such as fleabane in crop systems.

Changes in the electron flow in the electron transport chain (ETC) of photosynthesis are associated with two groups of herbicides. The first one consists of a triazine, such as metribuzin (4-amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one), that targets the D1 protein in PSII by competing with the binding of plastoquinone at its QB-binding site. The second one consists of a bipyrilidium group, of which paraquat (1-methyl-4-(1-methylpyridin-1-ium-4-yl) pyridin-1-ium; chloride) is the best known. Paraquat acts as an electron acceptor by diverting them from PSI, which can lead to reactive oxygen species (ROS) formation that further causes visual symptoms in leaves due to cell injuries (TREBST, 2007; DUKE; DAYAN, 2011; DAYAN; ZACCARO, 2012; SOUZA et al., 2014b). Photosynthetic inhibition lead to plant starvation due to the loss of fixed carbon, a result of the blockage in electron transfer, preventing the conversion of light energy into electrochemical energy. Changes in normal ETC flow induced by herbicide can be monitored by chlorophyll a fluorescence measurement (STRASSER; STRASSER, 1995; STRASSER et al., 2004; DAYAN; ZACCARO, 2012; TROPALDI et al., 2015), before any visual effects appearance (BARBAGALLO et al., 2003; OUKARROUM et al., 2007).

Chlorophyll fluorescence provides a very sensitive method to evaluate overall changes in the status of plant bioenergetics (STRASSER et al., 2004; TSIMILLI-MICHAEL; STRASSER, 2008; KALAJI et al., 2014). Measures of changes in chlorophyll fluorescence have been used in photosynthesis research and have the potential to be applied for herbicide assays and diagnostics of herbicide resistance, which can be used for the study of the effect of PSII- and PSI-inhibiting herbicides as well as herbicides with other modes of action (DAYAN; ZACCARO 2012; SOUZA et al. 2014; TROPALDI et al. 2015; ZHANG et al. 2016).

Besides the knowledge regarding the mode of action of herbicides on plants, including photosynthetic-inhibiting herbicides, a little is known for specific herbicides on ETC of plants, especially in weed. Moreover, there are no studies of the dynamic dissipation of photosynthetic energy processes in fleabane plants. Therefore, this study aimed to evaluate the dynamics energy dissipation of the photosynthetic ETC of *Conyza* spp. upon PSII- (metribuzin) and PSI-inhibitor (paraquat) herbicide application.

3.4 MATERIAL AND METHODS

Plant Material and Growth Conditions

The experiment was carried out in a greenhouse under natural light and temperature conditions. Seeds of fleabane (*Conyza* spp. L.) were sown in a 1-L polyethylene pot with Planosol soil (SANTOS et al., 2018) containing sandy loam soil (18% clay, 5% silt, 77% sand). After emergence, seedlings were thinned to keep only one plant per plot. Experimental units were arranged in a randomized complete block design with four replicates. Treatments were comprised of application of paraquat (400 g a.i. ha^{-1} + 0,1% non-ionic surfactant; PSI-inhibitor), metribuzin (480 g a.e. ha^{-1}) (PSII-inhibitor) and an untreated check. Herbicide applications were initiated when plants reached 12- to 15-cm tall (growth stage). All treatments were sprayed using a CO_2-pressurized backpack sprayer equipped with four TeeJet XR110015 flat-fan nozzles (TeeJet Technologies, Springfield, IL, USA), which delivered 150 L ha^{-1} of spray solution at 280 kPa and a ground speed of 4.53 km hr^{-1}.

Visual Control Analysis

At 0.5, 1, 2, 4, 8, 24, 48 and 96 hours after application (HAA) and at 7, 14, 21 e 28 days after application (DAA) the visual assessment of *Conyza* spp. was performed. It was used at a scale of 0-100%, where visual control of 0% represents the absence of symptoms and 100% represents the death of the plant (FRANS et al., 1986).

Chlorophyll *a* Fluorescence Transients Analysis

Chlorophyll *a* fluorescence transient was measured at 0,5, 1, 2, 4, 8, 24, 48 and 96 HAA in dark-adapted leaves using a Handy-PEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments Ltd, UK). Intact young leaves with a fully expanded first leaf still attached to the plant were kept in dark for at least 20 min in specially provided clips to conduct measurements. The polyphasic fluorescence rise, OJIP, was induced by one saturating red-light flash (peak at 650 nm) with 3.000 μmol photons m^{-2} s^{-1} and measured during the first second of illumination (STRASSER; TSIMILLI-MICHAEL, 2004). Initial fluorescence was considered the measurements at 50 μs (F_0). The JIP-test was also normalized and subtracted to reflect the OJIP-phases as relative variable fluorescence: $W_t = (F_t - F_0)/(F_J - F_0)$, $W_{OI} = (F_t - F_0)/(F_I - F_0)$ and $W_{IP} = (F_t - F_I)/(F_P - F_I)$ (STRASSER et al., 2004; TSIMILLI-MICHAEL; STRASSER, 2008).

Gas Exchange Analysis

Gas exchange was measured in the first fully expanded leaf using a portable infra-red CO_2 analyser (model LI-6400XT LI-COR, Inc., Lincoln, NE, USA). The measurements were taken 1, 4 and 96 HAA, with an in-chamber CO_2 concentration of 380 μmol mol^{-1} and a natural photon flow density (~1.300 ± 50 μmol photons m^{-2} s^{-1}. Net assimilation rate (A, μmol m^{-2} $s^{-1)}$, transpiration rate (E, mmol m^{-2} $s^{-1)}$ and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) were evaluated.

Statistical Analysis

The data of visual control and gas exchange analysis were checked for normality and ANOVA was performed. When F was significant, the means were separated at $p \leq 0.05$ and adjusted using Fisher's Protected LSD, by SAS 9.0 statistical software program (SAS Institute Inc. Cary, NC, USA) and all graphs were designed using the Sigma Plot software 12.5.

3.5 RESULTS

The PSII-inhibitor (metribuzin) provided ≤ 10% of visual control of fleabane up to 7DAA, ≥ 95% visual control at 14DAA and fully controlled with 21 DAA (Fig. 1A). On the other hand, PSI-inhibitor (paraquat) showed visual control symptoms with 2 HAA, reaching a percentage of ≥ 80% of control at 8 HAA and fully control with 48 HAA (Fig. 1B). However, before the appearance of control symptoms, changes in the dynamics dissipation of photosynthetic energy were observed with 0.5 to 1 HAA, for both herbicides (Fig. 2A-L). The PSII-inhibitor appears to rapidly decline the flow of electrons on ETC, compared to PSI-inhibitor upon herbicide application (Fig. 2A-F), however, paraquat takes a short-time compared to metribuzin to completely impairs the ETC (Fig. 2G-L).

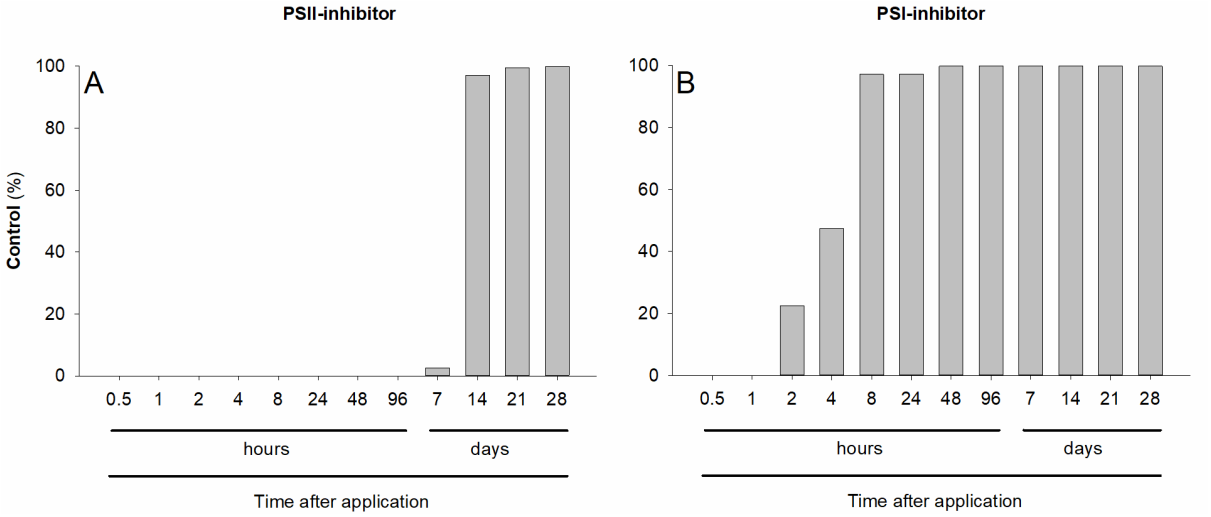

Figure 1. Visual control of fleabane plants subjected to PSII- and PSI-inhibitor herbicides (metribuzin (A) and paraquat (B), respectively).

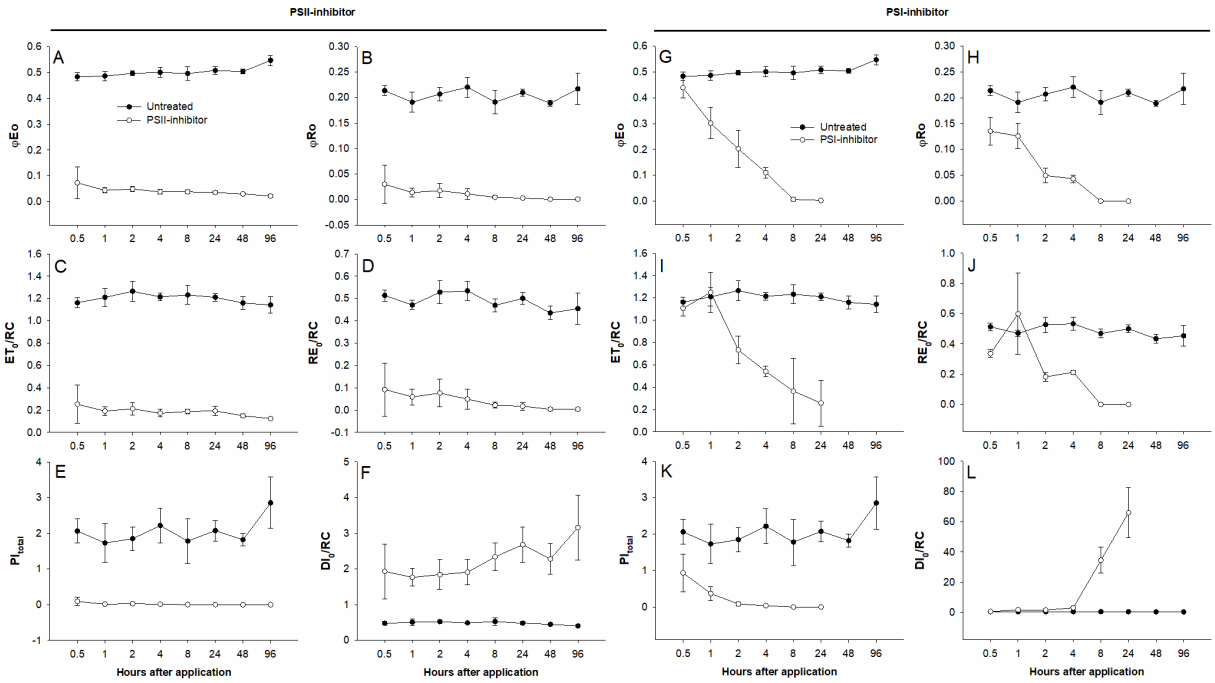

Figure 2. Chlorophyll *a* fluorescence (JIP-test parameters) of fleabane plants subjected to PSII- and PSI-inhibitor herbicides (metribuzin and paraquat, respectively). DI_0/RC – The flux of energy dissipated (heat) in process other than trapping per active photosystem II (PSII) per RC; ET_0/RC – The flux of electrons transferred from quinone A (Q_A^-) to plastoquinone (PQ) per active PSII per RC; RE_0/RC - Electron flux reducing end electron acceptors at the PSI acceptor side, per RC; φEo - Quantum yield for electron transport (ET); φRo - Quantum yield for reduction of end electron acceptors at the PSI acceptor side; PI_{total} - Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors.

Chlorophyll *a* fluorescence analysis of fleabane plants, treated with PSII-inhibitor showed a rapid decrease at 0.5 HAA and maintained low up to 96 HAA regarding electron transport flux (further than Q_A^-) per reaction center (ET_0/RC; Fig. 2C) and the quantum yield for electron transport (φ_{Eo}; Fig. 2A), which limited both the electron flux reducing (RE_0/RC; Fig. 2D) and quantum yield for reduction (φ_{Ro}; Fig. 2B) end electron acceptor at the PSI acceptor side per RC. Additionally, there was an increase in the flux of energy dissipated (heat) in the process other than trapping per active PSII (DI_0/RC; Fig. 2F) and decrease in the performance index for energy conservation from exciton to the reduction of PSI end acceptors (PI_{total}; Fig. 2E). All the above-described parameters were similar in plants subjected to PSI inhibitor, however with a progressive decline starting at 1 to 2 HAA and a complete impairment of the photosynthetic ETC within 24 HAA (Fig. 2G-L).

PSII-inhibitor also increased apparent antenna size of an active PSII (ABS/RC), which increased the maximum trapped exciton flux per active PSII (TR_0/RC), strongly increasing the DI_0/RC and decreasing the maximum quantum yield for primary photochemistry (φ_{Po}) and efficiency with which an electron from the intersystem electron carrier moves to reduce end electron acceptors at the PSI acceptor side (δ_{Ro}), with similar results since 1 HAA up to 96 HAA (Fig. 3A-C). Similar results were observed when PSI-inhibitor was applied, however, the worse effects were showed by plants with 4 HAA (Fig. 4A and B).

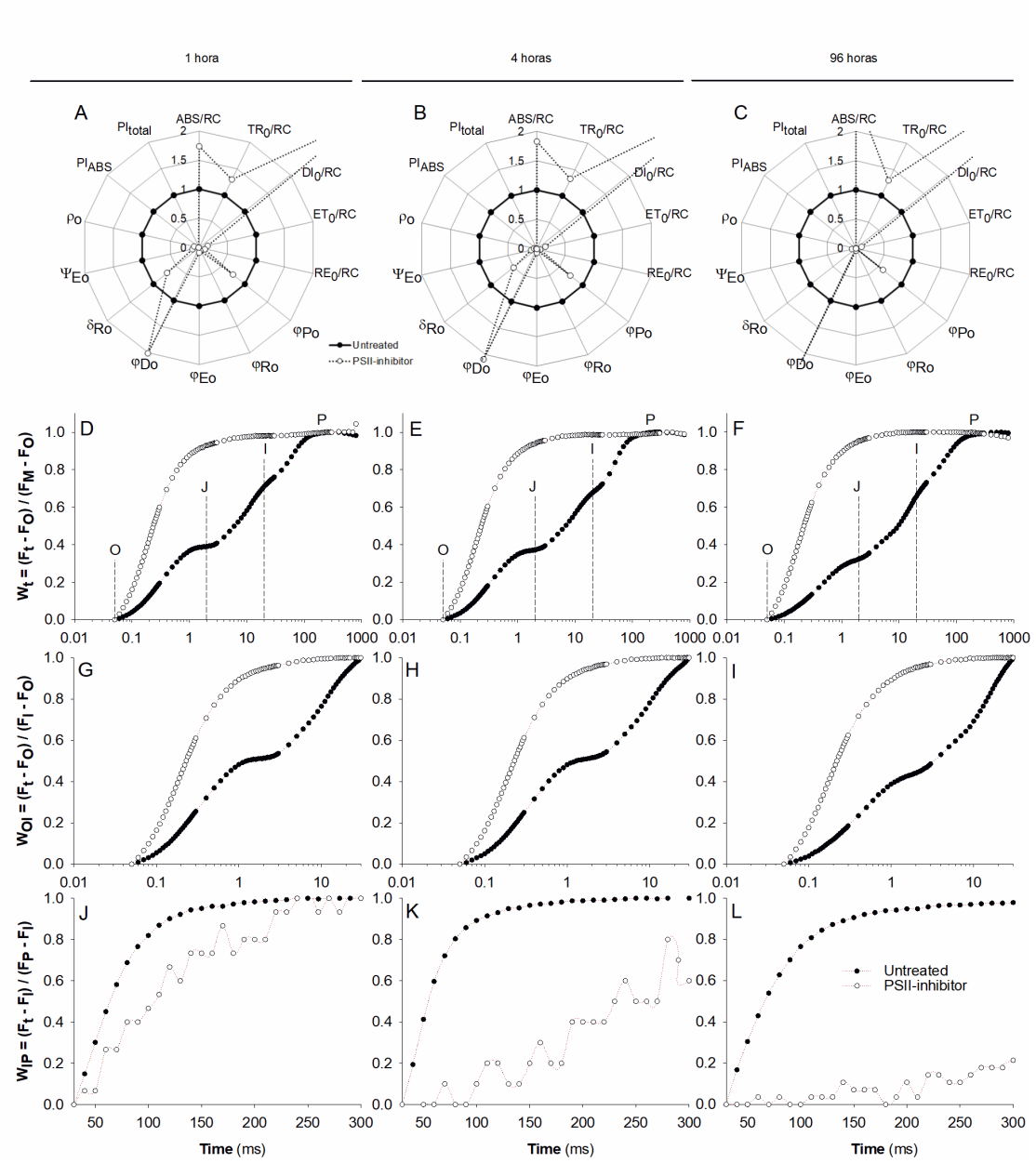

Figure 3. Chlorophyll *a* fluorescence of fleabane plants subjected to PSII-inhibitor herbicide (metribuzin). Photosynthetic parameters deduced by JIP-test analysis of fluorescence transients normalized using as reference the control (A, B and C). Relative variable fluorescence between the steps O and P (W_t; D, E and F) on logarithmic time; Relative variable fluorescence between the steps O and I (W_{OI}; G, H and I) on logarithmic time scale; Relative variable fluorescence between the steps I and P (W_{IP}; J, K and L).

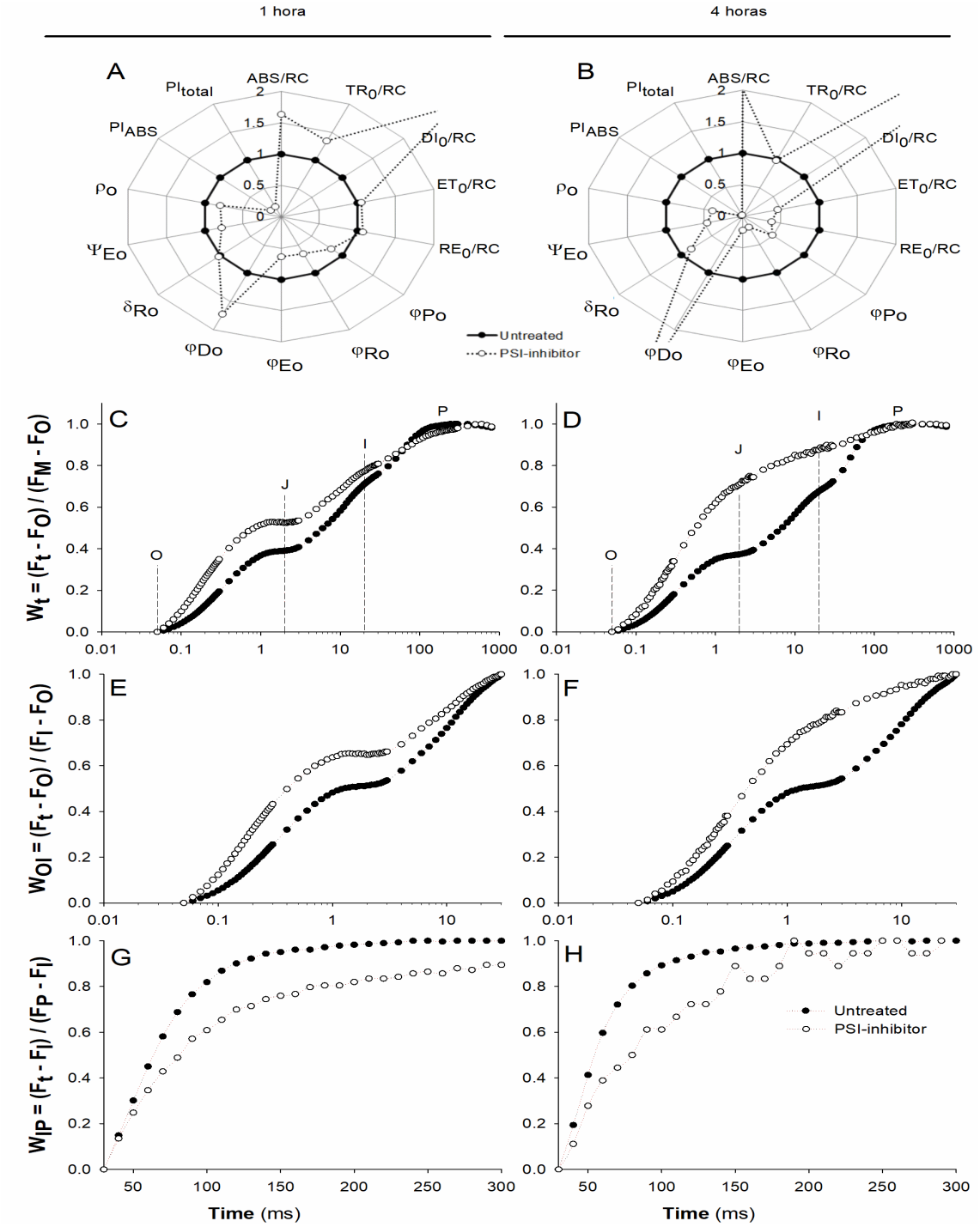

Figure 4. Chlorophyll *a* fluorescence of fleabane plants subjected to PSI-inhibitor herbicide (paraquat). Photosynthetic parameters deduced by JIP-test analysis of fluorescence transients normalized using as reference the control (A and B). Relative variable fluorescence between the steps O and P (W_t; C and D) on logarithmic time; Relative variable fluorescence between the steps O and I (W_{OI}; E and F) on logarithmic time scale; Relative variable fluorescence between the steps I and P (W_{IP}; G and H).

Plants showed an increase in the fluorescence emission with loss of the typical polyphasic Chl *a* fluorescence OJIP (W_t) transients, as marked in the plot (on logarithmic time scale), the

most induction of fluorescence at J-step, since 1 HAA for PSII-inhibitor (Fig. 3D-F) and 4 HAA for PSI-inhibitor (Fig. 4C and D). In addition, similar results were observed in the sequence of events from exciton trapping by PSII up to plastoquinone (PQ) reduction (W_{OI}) for PSII-inhibitor (Fig. 3G-I) and PSI-inhibitor (Fig. 4E and F). Moreover, decreases occurred in the PSI-driven electron transfer to the end electron acceptors on the PSI acceptor side, from PQH_2 (W_{IP}), with strong decreases in plants subjected to PSII-inhibitor with 4 HAA (Fig. 3J-L). Plants subjected to PSI-inhibitor showed more slight decreases up to 4 HAA (Fig. 4G and H).

At 1 HAA, plants stress mediated by metribuzin (Fig. 5A) and paraquat (Fig. 5D) induced reduction in more than 95% of photosynthesis rate and the plants showed a reduction in stomatal conductance and transpiration rate in relation untreated plants. This behavior was maintained up to 96 HAA for PSII-inhibitor, while after 4 HAA plants subjected to PSI-inhibitor did not withstand the stress imposed by the paraquat treatments and died (Fig. 5).

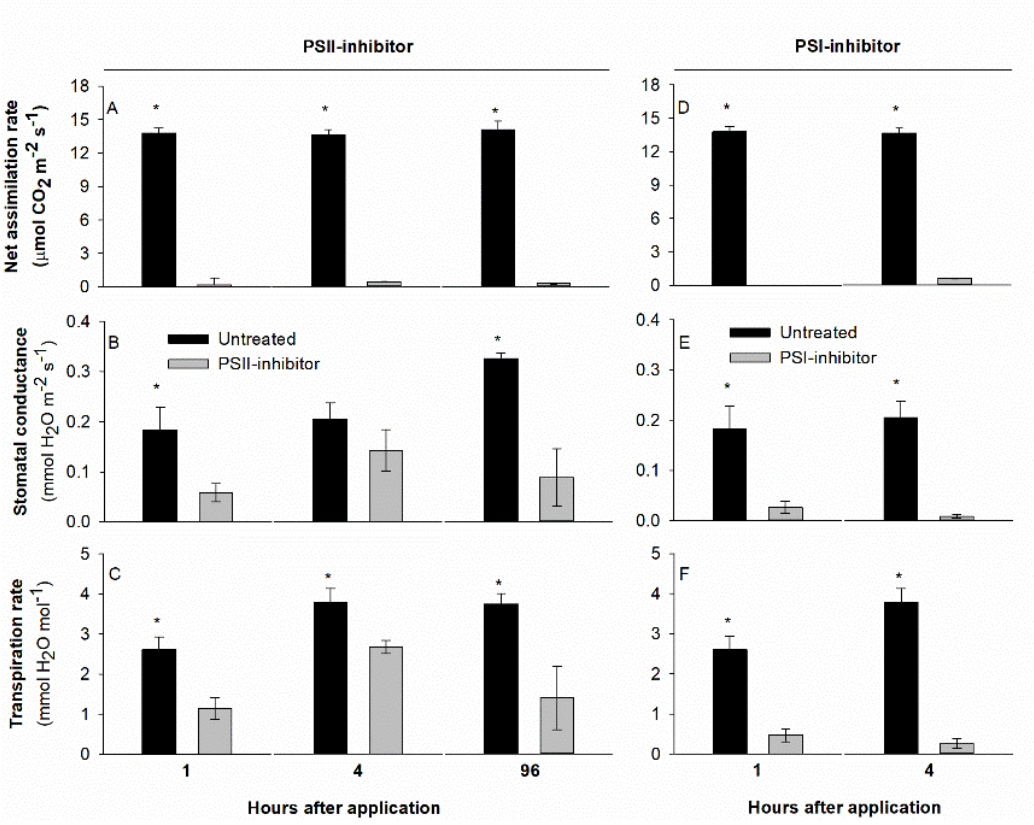

Figure 5. Gas Exchange of fleabane plants subjected to PSII- and PSI-inhibitor herbicides (metribuzin and paraquat, respectively). Values represent the mean ± standard deviation (SD). Asterisk indicates a significant difference by Fisher's Protected LSD ($p \leq 0.05$) between untreated and PSII- or PSI-inhibitor in each time of measurement, independently.

3.6 DISCUSSION

Chlorophyll *a* fluorescence is a potential stress indicator that can be indicative of physiological disturbances caused by herbicides of a different mode of action (DAYAN; ZACCARO, 2012; ZHANG ET al., 2016). Therefore, it is an important signal from weeds in response to herbicide treatment that can be detected before the appearance of any visual symptoms, especially those herbicides that directly affects both PSII and PSI, by leading to an imbalance or impairment of ETC. Besides that, Chl *a* fluorescence also can be implied to detect changes or disorders of herbicide assays and diagnoses of herbicide resistance in plants that could provide information for the management of weeds. (DAYAN; ZACCARO, 2012; KAISER et al., 2013; SOUZA et al., 2014a; ZHANG et al., 2016; LEAL et al., 2020).

Our results indicated that the visual control (visual symptoms) was observed firstly in plants that were applied PSI- and later PSII-inhibitor, at 2HAA and 7DAA, respectively. However, changes in the dynamic dissipation of photosynthetic energy were observed right after a few mins of the application of both herbicides, at 0.5 HAA. At this time, an impairment in ETC was evidenced by a decrease in Q_A^- re-oxidation per reaction center, reduction in electron transport yield from Q_A^- to the end electron acceptor of the PSI, for both herbicides. Moreover, the decline of 90% in the performance index resulted in a higher increase in energy dissipation as heat and fluorescence. The impairment of ETC affected the reducing energy power leading to a great decrease in net assimilation rate (CO_2), transpiration rate and stomatal conductance upon 1 HAA, for both herbicides. Moreover, PSII-inhibitor impaired completely the photosynthetic process after 96HAA while PSI-inhibitor occurred faster, within 24HAA.

PSII-inhibitor herbicide impairs electron transport chain by competitively associating with the plastoquinone B (Q_B)-binding site of the D1 subunit of PSII, and therefore, blocks photosynthetic electron flow from Q_A to Q_B, greatly reducing the production reducing power energy and adenylate energy charge which reflects in limitations of CO_2 carboxylation by Rubisco (HESS, 2000). Also, as a result of the blockage at the level of the electron transport chain (ETC), the plant is unable to re-oxidize Q_A, which generates over-reduction of ETC, thus producing reactive oxygen species (ROS). When ROS production exceeds the scavenger capacity of photo-protective components such as enzymatic and non-enzymatic compounds, severe damage of proteins, lipids, and pigments, and eventually cell membrane destruction lead to visual symptoms that later cause plant death (HESS, 2000; SHERWANI; KHAN, 2015).

The blockage by metribuzin (PSII-inhibiting herbicide) in the electron flow causes the re-emission of the absorbed energy as non-photochemical quenching (fluorescence and heat) rather than photochemical quenching one (MULLER et al., 2001). The changes in the photochemical process rapidly affect CO_2 assimilation, in a few minutes after the herbicide application. Thus, making the Chl *a* fluorescence an efficient technique for understanding the dynamics of the energy dissipation of *Conyza* spp. upon metribuzin application even before the appearance of any visual symptoms. Besides, herbicides-induced perturbations in metabolism, even in metabolic reactions not directly associated with the photosynthetic process, can be detected from the changes in fluorescence parameters at the beginning of the herbicide application (BARBAGALLO et al., 2003; OUKARROUM et al., 2007).

The impairment of the electron flow by the fast competing of herbicide with the binding of plastoquinone at its Q_B-binding site on PSII (TREBST, 2007; DUKE; DAYAN, 2011; DAYAN; ZACCARO, 2012), (0.5-1 HAA) increase exciton captured by RCs until the reduction of PQ (TR_0/RC, W_{OI} phase) and lead to a great decrease in the re-oxidation of Q_A^- (ET_0/RC), as well as in the transport of electrons from Q_A^- to the intersystem (ψEo, φEo) and the final electron acceptor of the PSI (φRo and W_{IP} phase), declining the reducing energy

power. Another evidence of the interruption of the electron flow is the intensity of the fluorescence levels in J-step which demonstrates that most of the Q_A is completely reduced (STRASSER et al., 1995). This results in a decrease in the photosynthetic performance (PI_{total} and φPo) of the plants, and loss of energy by fluorescence (Wt) and heat (DI_0 / RC and φDo).

Not all herbicides have the same impact on ETC. The herbicides diuron and atrazine completely block the electron flow after 3 hours of application, while the bentazon herbicide causes an initial decrease of 80% in the electron flow at the first hours and recovers afterward (DAYAN; ZACCARO, 2012; LIMA et al., 2018), proving that the herbicide bentazon does not cause a permanent change in the activity of photosystems (MACEDO et al., 2008) as observed in fleabane when applied the herbicide metribuzin, with the interruption of the electron flow throughout the evaluation periods.

Plants that suffer from the action of PSII- and PSI-inhibiting herbicides have extremely reduced carbohydrate production due to the drastic decline net assimilation rate (POWLES; YU, 2010) since the blockage in ETC lead to a reduction in NADPH and ATP, necessary for CO_2 fixation. This decline in CO_2 assimilation occurs after 2 HAA and is correlated to the blocking of electron flow from Q_A, which reflected in the reductions in electron acceptor on the accepting side of the PSI, that is ferredoxin (Fd), other intermediates and $NADP^+$ (W_{IP}) (SCHANSKER et al., 2005). Besides the reduction in CO_2 assimilation, the stomatal conductance and transpiration rate are also limited due to a decline in CO_2 carboxylation by Rubisco, leading to stomatal closure (SILVA et al., 2010). It is noted that the reduction of the stomatal opening causes a reduction in transpiration rate (MEDEIROS et al., 2019).

The death of plants is not only a result of the reduction in carbohydrates production. As energy is continuously absorbed by the light harvest complex of PSII and is not used in photochemical quenching, as evidenced by ET_0/RC, ψEo, φEo, φRo and W_{IP}, the over-reduction in ETC leads to the formation of ROS that causes the death of the cell and consequently the whole plant (BAKER, 2008).

Paraquat (PSI-inhibitor) is one of the most used herbicides globally in agriculture. This herbicide is also known to act by diverting electrons from the iron-sulfur centers in the ETC of the PSI. Besides fleabane suffers by starvation of loss of fixed carbon within a few hours after application of paraquat, the main effect, especially those related to visual symptoms results from toxicity due to the impairment in the ETC, that prevents the conversion of light energy into electrochemical one and a result of ROS production (DYER; WELLER, 2005). The reduced paraquat species react with oxygen to form the radical superoxide anion ($O_2^{\bullet -}$), which generates hydroxyl radicals either directly or hydrogen peroxide intermediate. This ROS disrupts the unsaturated fatty acids, chlorophyll, lipids, and proteins in the cell membrane, quickly leading to plant death. This happens within a few hours after the treatment in a condition of bright sunlight, with complete desiccation occurring after a few days (BROMILOW, 2004; BROWN et al., 2004; GRILLO et al., 2014; TREBST, 2007; DUKE; DAYAN 2011; DAYAN; ZACCARO 2012). Therefore, paraquat herbicide (PSI-inhibitor) acts more quickly on plant control than metribuzin herbicide. The visual control upon paraquat application appeared after 2 HAA and completely controlled fleabane within 24 HAA while the first symptoms of metribuzin appeared with 7 DAA.

Besides the faster control of fleabane by paraquat compared to metribuzin, the PSII-inhibitor impairs more rapid the ETC than paraquat in a few hours after application. This can be explained by the mechanism of action of each herbicide; while the PSII-inhibitor causes the complete blockage at the level of the electron transport chain, the PSI-inhibitor diverts electrons from the iron-sulfur centers in the PSI, which maintain, at least for a few hours, the ETC working. Taking this, the death of fleabane in a few days upon paraquat application may be

related much more to the burst oxidative that causes severe damages to cell (24 HAA), while the death of plants upon metribuzin seems to be more related to carbon starvation (7 DAA), besides ROS production along this time. The rapid effects of metribuzin on plant metabolism is supported by the reduction of ≥35% in electron transport from Q_A^- to the final electron acceptor of the PSI and reduction of the final electron acceptor on the electron acceptor side of the PSI within 0.5HAA while it occurs within 1 to 2 HAA for paraquat.

PSI-inhibitor increases fluorescence at 1 to 2 HAA, which also leads to an increase in energy dissipation in the form of heat (DI_0/RC and φDo), and consequently a decrease in photosynthetic performance (PI_{total}). As the absorbed energy by the plant has three main destinations that occur in direct competition: photochemical (energy production and reducing power), heat and fluorescence (KRAUSE; WEIS, 1991), so that any increase in the efficiency of one will cause a decrease in the performance of the others (Strasser et al. 2004). The increase in the exciton captured by the RCs until the reduction of plastoquinone (PQ) (W_{OI}, TR_0/RC) and the reduction in the efficiency with which an exciton captured in the RC can move an electron from Q_A^- to the intersystem and the final electron acceptor on the PSI side (ψEo, φRo and IP-phase), increases the size of the antenna system (ABS/RC), probably due to the need for plants to increase their capacity to absorb energy. As ETC is impaired the absorbed energy is then dissipated much more as heat and fluorescence, which decreases the performance indexes of plants (SOUSA et al., 2014a). Then, the changes in the flow of electrons in the ETC triggers reductions in carbon fixation rates of fleabane plants (FOYER et al., 1994).

The chlorophyll fluorescence and gas exchange measurements allowed to describe the effects on the photosynthetic energy dynamics upon PSII- and PSI-inhibitor herbicide application (metribuzin and paraquat, respectively), providing adequate information on the conformation, structure and function of the photosynthetic apparatus (STIRBET; GOVINDJEE, 2011). Therefore, both herbicides show a rapidly negative effect on photosynthetic energy dynamics that can be monitored before the appearance of any visual effect of herbicide which demonstrates the potential use of chlorophyll fluorescence to rapidly screen metabolic perturbations caused by herbicides. Besides, PSII-inhibitor impairs in a few minutes the ETC of fleabane and takes a few days to lead plants to death, probably due to carbon starvation while PSI-inhibitor takes a few hours to impairs ETC and lead plants to death within a day, probably due to oxidative damages.

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4 CAPÍTULO II

RAPID PHOTOSYNTHETIC AND PHYSIOLOGICAL RESPONSE OF 2,4-D–RESISTANT SUMATRAN FLEABANE (*Conyza sumatrensis*) TO 2,4-D AS A SURVIVAL STRATEGY

LEAL, J. F. L.; SOUZA, A. DOS S, BORELLA, J.; ARAUJO, A. L. S.; LANGARO, A. C.; ALVES, M. M.; FERREIRA, L. J. S.; MORRAN, S.; ZOBIOLE, L. H. S.; LUCIO, F. R.; MACHADO, A. F. L.; GAINES, T. A.; PINHO, C. F. Rapid photosynthetic and physiological response of 2,4-D–resistant Sumatran fleabane (*Conyza sumatrensis)* to 2,4-D as a survival strategy. **Weed Science**, v. 70, 2022. doi: 10.1017/wsc.2022.10

4.1 RESUMO

Neste trabalho, avaliamos as rápidas respostas metabólicas e fisiológicas, mediadas por estresse oxidativo, induzidas pelo tempo, em buva[*(Conyza sumatrensis* (Retz.) E.Walker)] resistente e suscetível ao herbicida 2,4-D sob condições naturais, condições variávies de temperatura e luminosidade. Na condição natural, foram avaliados controle, fluorescência de clorofila *a* e sistema antioxidante do biótipo resistente e suscetível ao 2,4-D (1005 g e.a. ha-1). Em outro ensaio, os biotipos foram submetidos a temperatura (15 ° C vs 25 ° C) e claro e escuro, e foram avaliados danos mediados pelo estresse oxidativo. Os dados foram ajustados usando LSD-Fisher $p \leq 0,05$. Os sintomas observados no biótipo resistente ao 2,4-D foram necrose nas folhas em 30 minutos, com o restabelecimento do crescimento normal dentro de 1 a 2 semanas após o tratamento com 2,4-D. As atividades da enzima antioxidante basal no biótipo resistente aumentou em comparação com a suscetível. O biótipo resistente apresentou grande redução na cadeia de transporte de elétrons fotossintético a 1 HAA, enquanto o biotipo suscetível só apresentou essas alterações metabólicas após 4HAA. Após 1 a 2 semanas da aplicação do 2,4-D, o biótipo resistente se recuperou do dano e o biótipo suscetível morreu. A produção de H_2O_2 é responsiva à temperatura e aumenta mais rapidamente no biótipo resistente a 2,4-D do que no suscetível nas temperaturas de 15 ° C e 25 ° C, com maior aumento a 25 ° C. Por outro lado, no biotipo resistente a 2,4-D, a produção de H_2O_2 não depende da luz, com aumentos mesmo em condições escuras. O biótipo resistente a 2,4-D mostra um rápido dano fotossintético e aumento no conteúdo de H_2O_2 em comparação ao biótipo suscetível. Além disso, a atividade da enzima antioxidante basal é maior no biótipo resistente.

Palavras-chave: Buva. Rápida resposta. Fluorescência da clorofila *a*. Enzimas antioxidantes. Dano oxidativo.

4.2 ABSTRACT

In this work, we evaluated the short time-induced oxidative stress–mediated rapid metabolic and physiological responses of resistant and susceptible Sumatran fleabane [*Conyza sumatrensis* (Retz.) E. Walker; syn.: *Erigeron sumatrensis* Retz.] to 2,4-D herbicide. Under fixed conditions (25 C and 65 ± 5% relative humidity), we assayed injury symptoms, chlorophyll a fluorescence, and antioxidative systems of biotypes both resistant and susceptible to 2,4-D (1,005 g a.e. ha−1). Under 15 versus 25 C temperatures and light and dark conditions, oxidative stress–mediated damage was assayed on plants that received 2,4-D herbicide applications. The injury symptoms observed in the 2,4-D–resistant biotype were rapid necrosis in leaves within 30 min, with the reestablishment of normal growth within 1 to 2 wk after 2,4-D treatment. The basal antioxidant enzyme activities of superoxide dismutase, catalase, and ascorbate peroxidase were greater in the resistant than in the susceptible biotype, although the activities of all enzymes generally did not differ between untreated and treated in the resistant biotype. The resistant biotype showed great reduction (at 1 and 4 h after application) in the photosynthetic electron transport chain performance index, while these metabolic changes were only detected after 4 h in the susceptible biotype. The resistant biotype recovered from the foliar damage 1 to 2 wk after 2,4-D application, while the susceptible biotype was controlled. The production of H_2O_2 was responsive to temperature and increased more rapidly in the 2,4-D–resistant biotype than in the susceptible one at both 15 and 25 C; however, there was a greater increase at 25 C in the resistant biotype. H_2O_2 production was not light dependent in 2,4-D–resistant *C. sumatrensis*, with increases even under dark conditions. The 2,4-D–resistant biotype showed rapid photosynthetic damage, possibly due to the rapid necrosis and leaf disruption, and increased H_2O_2 content compared with the susceptible biotype.

Key-words: Sumatran Fleabane. Rapid response. Chlorophyll *a* fluorescence. Antioxidative enzymes. Oxidative damage

4.3 INTRODUCTION

Conyza species have high fecundity, high potential for dispersion by seed, staggered emergence, adaptation to no-till farming systems, and resistance to different site-of-action herbicides, which gives these species high invasive potential worldwide (WU et al., 2007; SAVAGE et al. 2014). Currently, the management of *Conyza* spp. has become more difficult due to the increase in herbicide-resistant biotypes (HEAP, 2021). There are 106 cases of *Conyza* spp. resistant to herbicides globally (HEAP, 2021). In Brazil, the first report of herbicide-resistant *Conyza* spp. occurred in 2005, when failures were observed after glyphosate application (MOREIRA et al., 2007).

In 2017, Sumatran fleabane [*Conyza sumatrensis* (Retz.) E. Walker; syn.: *Erigeron sumatrensis* Retz.] was first reported with multiple resistance to five herbicide (paraquat, saflufenacil, diuron, 2,4-D, and glyphosate) sites of action (PINHO et al., 2019) and subsequently to diquat (LEAL et al., 2022). The resistant biotype shows different responses to each herbicide application, and in this report, we demonstrate a rapid resistance response to 2,4-D herbicide in this biotype that is not seen when the other five herbicides (or site of action) are applied. This was the first case in the world reported for *Conyza* with an extremely complex and atypical response to 2,4-D. This resistant biotype exhibits a differential response to 2,4-D application compared with the susceptible biotype, with a symptom of necrotic in leaves within 30 to 60 min following 2,4-D application and normal growth resuming within 1 to 2 wk after 2,4-D treatment, resulting in failed control. A rapid response as part of an evolved resistance mechanism to herbicide treatment was also reported by Moretti et al. (2018) in giant ragweed (*Ambrosia trifida* L.), with the resistant biotype surviving glyphosate application by using rapid necrosis as an adaptation strategy to survive herbicide application. In addition, Harre et al. (2017), documented the involvement of H_2O_2 (hydrogen peroxide) in the rapid response of A. trifida resistant to glyphosate, similar to the rapid response of *C. sumatrensis*.

Necrotic symptoms may be a result of an oxidative burst mediated by increased reactive oxygen species (ROS) (GILL; TUTEJA, 2010; PEER et al., 2013; SONG, 2014). Along with ROS production, changes in antioxidant enzymatic systems and photosynthetic capacity can also occur to support the rapid response as a survival strategy (HARRE et al., 2018). Photosynthetic traits, such as chlorophyll (Chl) a fluorescence could be used to monitor cases of resistance involving physiological changes in 2,4-D–resistant plants. The use of Chl *a* fluorescence to monitor resistance has been reported in *C. sumatrensis* upon paraquat application, with resistant biotypes surviving and showing recovery of the dynamic electron transport chain energy fluxes within a day, while susceptible plants rapidly show great disorder in the photosynthetic apparatus and die within hours after paraquat application (LEAL et al., 2022). As well, Brunharo and Hanson (2017) reported that in tall windmill grass (*Chloris elata*) resistant biotypes, the herbicide affects photosynthetic performance until the molecules are trapped by the mechanism of action operating in plant cells. Here, we propose an opposite response to 2,4-D application compared with paraquat. Photosynthetic performance declines more rapidly in the resistant than in the susceptible biotype during a period of rapid necrosis induction that occurs within a few days. In contrast, the decline in Chl *a* fluorescence takes longer in susceptible biotypes, until the plant dies. Chl *a* fluorescence technique can show these differences in photosynthetic performance between the biotypes to rapidly detect herbicide stress (DAYAN; ZACCARO, 2012; HASSANNEJAD et al., 2020). Moreover, plants treated with paraquat start to show photosynthetic recovery after 24 h, while with 2,4-D it may happen in about 40 d, after plant regrowth.

The conditions of herbicide application, such as suboptimal and light conditions, can also influence plant metabolism, growth, or development by altering homeostatic balance (KRANNER et al., 2010). These factors cause changes in chlorophyll fluorescence induction and responses in the defense system. The herbicide stress affects the stability of the photosynthetic apparatus and indirectly affects chlorophyll fluorescence induction (DAYAN; ZACCARO 2012; KALAJI et al., 2014). Metabolic perturbation induced by herbicides, even herbicides not directly associated with photosynthetic metabolism, can be detected from changes in fluorescence parameters, even before any visual effects appear (BARBAGALLO et al., 2003; OUKARROUM et al., 2007; CAVERZAN et al., 2019). Thus, fluorescence is a promising technique to describe the differential photosynthetic response due to any source of stress (STIRBET; GOVINDJEE, 2011; DAYAN; ZACCARO, 2012; GUIDI et al., 2019).

The exposure of plants to herbicides may cause oxidative stress, leading to the generation of ROS, such as superoxide radicals ($O2.^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH) (CAVERZAN et al., 2019). In response to the damage caused by ROS, the antioxidative enzyme system (including superoxide dismutase [SOD], catalase [CAT], peroxidases, ascorbate peroxidase [APX], and glutathione reductase) (GILL; TUTEJA, 2010) may be differentially expressed/activated in resistant and susceptible biotypes (BENEDETTI et al., 2020). The herbicide can also differentially modulate the induction of enzymatic antioxidant systems in resistant and susceptible biotypes (CAVERZAN et al., 2019).

We hypothesize that 2,4-D–resistant *C. sumatrensis* induces rapid response (rapid necrosis), possibly as a mechanism to avoid 2,4-D translocation. The rapid response may be associated with a higher production of H_2O_2 and differential antioxidative enzymes. The rapid response can be affected by temperature and light and dark conditions as well. In this work, we evaluated the short timeinduced oxidative stress–mediated rapid metabolic and physiological responses of *C. sumatrensis* biotypes resistant and susceptible to 2,4-D under fixed (simulated natural growth conditions) and variable (temperature and light) conditions.

4.4 MATERIALS AND METHODS

Plant Material

Seeds of a 2,4-D–susceptible biotype and a 2,4-D–resistant *C. sumatrensis* with multiple resistance to six herbicides (paraquat, diquat, saflufenacil, diuron, 2,4-D, and glyphosate) (PINHO et al., 2019; LEAL et al., 2022) of *C. sumatrensis* were originally collected from a site at Assis Chateaubriand-Paraná, Brazil. Two experimental approaches (fixed: simulated natural growth conditions; and variable: temperature and light conditions) were conducted to evaluate the rapid metabolic and physiological responses of these plants to 2,4-D application. All the experiments described were independently conducted twice.

Experimental Setup under Fixed Conditions

The first trial was conducted in a greenhouse with temperature conditions of 25 ± 5 C and 65 ± 5% relative humidity. Seeds from both 2,4-D–resistant and 2,4-D–susceptible biotypes were sown in 2.5-dm^{-3} pots filled with potting mix soil. After germination, the seedlings were thinned to one plant per pot. To promote active growth and avoid nutritional deficiencies, the soil was fertilized with N, P, and K (5-20-20) weekly and irrigated daily. Experimental units were arranged as a randomized complete block design with four replications. The treatments were 2,4-D–resistant biotype and 2,4-D–susceptible biotype with and without application of 2,4-D-amine herbicide (1005 g a.i. ha) (DMA® 806 BR SL, Corteva Agrisciences, São Paulo, SP, Brazil), without adjuvants. When plants reached 10-cm height, the 2,4-D herbicide was sprayed at 1,005 g a.e. ha−1 using a CO2-pressurized backpack sprayer with four XR-110015 flat-fan nozzles (TeeJet® Technologies, Wheaton, IL, USA), delivering 150 L ha^{-1} at 240 kPa.

After herbicide application, the injury symptoms were assessed as a percentage of visual injury using a scale from 0% to 100%, with 0% indicating no symptoms and 100% indicating plant death (FRANS et al., 1986). The injury was recorded at 1, 4, 8, and 24 h after application (HAA) and 2, 3, 7, 14, 21, 35, and 42 d after application (DAA). Chl *a* fluorescence transients were also measured at 1, 4, and 48 HAA and 42 DAA. Subsequently, at 1, 4, and 8 HAA, the fully expanded first leaf was harvested from a different plant each time. Leaves were removed by clipping the base of the leaf at the end of the petiole and were immediately frozen in liquid nitrogen and temporarily stored at −80 C until an analysis of enzymatic activity. After removal of leaves, plants were discarded to avoid influencing other analyses. In addition, the analysis performed at 42 DAA was only done for resistant plants upon regrowth.

Enzyme activity measurements

To measure the enzymatic activities, the fully expanded first leaf (last mature leaf) was collected (±0.2 g) and crushed to a powder using liquid N2 in porcelain mortars, containing 5% polyvinyl polypyrrolidone and homogenized in 100 mM potassium phosphate buffer, pH 7.8, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 20 mM ascorbic acid, 5 mM dithiothreitol, 5 mM β-mercaptoethanol, and 0.01% Triton X-100. The homogenate was centrifuged at 12,000 × g for 20 min at 4 C, and the supernatant was used as crude enzyme extract. An aliquot of the extract was used to determine protein content as described by Bradford (1976), using bovine serum albumin as standard. The supernatant was then used as an enzyme extract to assay SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and APX (EC 1.11.1.11).

Total SOD activity was measured as described by Giannopolitis and Ries (1977). SOD activity was measured in a 200-µl reaction mixture containing 75 µM p-nitro blue tetrazolium chloride (NBT), 2 µM riboflavin, 14 mM methionine, 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.8), and 5 µl of enzyme extract. The samples were placed under

fluorescent lamps at 4,000 lx for 5 min, and absorbance at 560 nm was recorded.One unit of SOD activity was equal to the amount of enzyme necessary to cause 50% inhibition of NBT reduction at 560 nm.

The catalase activity was determined according to Azevedo-Neto et al. (2006). CAT activity was assayed in a 200-µl reaction mixture containing 100 mM potassium phosphate buffer (pH 7.0), 12.5 mM H_2O_2, and 10 µl of enzyme extract. The reaction was initiated by adding H_2O_2 last. Catalase activity was determined by monitoring H_2O_2 consumption and measuring a decline in the absorbance at 240 nm and calculated using a molar extinction coefficient of 39.4 M−1 cm−1.

APX activity was determined by the method of Nakano and Asada (1981). The activity was assayed in a 200-µl reaction mixture containing 100 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2, and 10 µl of enzyme extract. The reaction was initiated by adding H_2O_2 last. The activity of APX was observed at 290 nm and calculated using a molar extinction coefficient of 2.8 mM−1 cm−1.

Chl *a* Fluorescence Transients Analysis

Chl *a* fluorescence transients were measured in dark-adapted leaves using a Handy-PEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments, King's Lynn, Norfolk, UK). The last fully expanded leaves were kept in the dark for 20 min in specially provided clips to conduct measurements. The polyphasic fluorescence rise, OJIP, was induced by one saturating red-light flash (peak at 650 nm) with 3,000 µmol photons m−2 s−1 and measured during the first second of illumination (10 µs to 1 s). The OJIP fluorescence transients are based on the polyphasic fast fluorescence rise from the lowest intensity FO (minimum fluorescence, the O level) to the highest intensity FM (maximum fluorescence,the P level) with two intermediate steps labeled J and I (STRASSER et al. 2004). The fluorescence intensities were determined at 50, 100, and 300 µs (F50µs, F100µs and F300µs, respectively), 2 and 30 ms (F2ms- FJ and F30ms -FI), and at FM using the JIP test parameters (for analysis of chlorophyll a fluorescence, see (STRASSER et al., 2004; TSIMILLI-MICHAEL; STRASSER, 2008)). The intensity measured at 50 µs was considered the initial fluorescence (F0). The plotted fluorescence values were the average of eight measurements of each treatment. The JIP test was also applied to analyze and compare the OJIP transients using the untreated treatment (normalizations) as the reference and subtraction of values to compare the samples for the events reflected in the OP (Wt), OI (WOI), and IP (WIP) phases.The transients were normalized as relative variable fluorescence:Wt = (Ft − F0)/(FJ − F0), WOI = (Ft − F0)/(FI − F0) and WIP =(Ft − FI)/(FP − FI).

Experimental Setup under Variable Conditions: 15 C versus 25 C

The *C. sumatrensis* resistant and susceptible biotypes were germinated in commercial soil potting media in a greenhouse. Environmental conditions inside the greenhouse were set up for 25 C, 65 ± 5% relative humidity, and 12 h light d^{-1}. The experiments were conducted using 10-cm plants. The plants were acclimated to each environmental condition (15 C or 25 C) for 3 d before the herbicide application. Treatments included no herbicide treatment (untreated) and 2,4-D application at 1,005 g a.e. ha^{-1} with six biological replicates per treatment.

Both biotypes were placed in a chamber with a constant temperature of 25 or 15 C for 3 d before herbicide treatment and were kept at this temperature until the end of the experiment. The plants were watered as scheduled, light intensity was 520 µmol photons m−2 s−1 (photosynthetically active radiation [PAR]) of 12 h d−1, and relative humidity was 60% in both chambers. The H_2O_2 production was evaluated at 0.5, 1.5, and 3 HAA.

Experimental Setup under Variable Conditions: Light versus Dark Conditions

Plants were acclimated into a chamber under low light (PAR = 330 μmol photons m^{-2} s^{-1}) conditions for 3 d before the herbicide treatment. Afterward, both biotypes were sprayed with 2,4-D at 1,005 g ha−1 and maintained in the chamber in complete darkness for 24 HAA or light intensity (PAR-520 μmol photons m^{-2} s^{-1}) of 12 h d^{-1}. Both chambers were maintained at 60% relative humidity and 25 C temperature

The H_2O_2 Content

After 24 h under light or dark acclimation, the H_2O_2 content was evaluated at 0.5, 1.5, and 3 HAA. The production of H_2O_2 was indirectly measured by staining leaf disks in solutions containing 3,3 0-diaminobendizine (DAB) (THORDAL-CHRISTENSEN et al., 1997; QUEIROZ et al., 2020; TAKANO et al., 2021). The DAB solution contained 0.1 g DAB solubilized in 200 ml of water with pH 3.8. Twenty-four leaf disks from control and treated leaves in each condition were placed in 20-ml glass tubes containing staining solution. The samples were then shaken under 20-Hg vacuum for 1 h. Leaf disks were washed in distilled water and boiled in 70% (v/v) ethanol solution with solution replaced every 20 min, repeated four times. Leaf disks were then stored in 70% (v/v) ethanol solution for 12 h and scanned. The levels of H_2O_2 were quantified using Photoshop software (Adobe Systems) to measure the color intensity in each leaf disk, removing background levels. The data were represented as relative intensity of treated samples compared with control samples (treated intensity − control intensity).

Statistical analysis

For analysis of enzymatic activity and ROS measurement, the data were submitted to ANOVA ($P \leq 0.05$), and when statistical significance was identified, means were separated and adjusted using Fisher's protected LSD, $P \leq 0.05$. Statistical analyses were performed using SAS v. 9.0 Statistical Software Program (SAS Institute, Cary, NC, USA).

4.5 RESULTS AND DISCUSSION

Injury Symptoms

The 2,4-D–resistant biotype showed a rapid necrotic response following 2,4-D treatment, as all leaves (young and old) developed necrotic spots that spread across the leaf within 30 to 60 min after application (Figure 1; Supplementary Material S1). At 8 HAA, visible necrosis occurred in all leaves that received herbicide. However, the meristems were not affected by 2,4-D, and the resistant biotype survived through continued growth from the apical and axillary meristems after 1 to 2 wk of growth following application (Figure 1). In contrast, the 2,4-D–susceptible biotype developed typical symptoms of auxin herbicide exposure after 6 to 24 h, such as epinasty and stem-thickening symptoms. The susceptible biotype showed control above 80% within 72 HAA and death of all plants within 7 to 14 DAA (Figure 1).

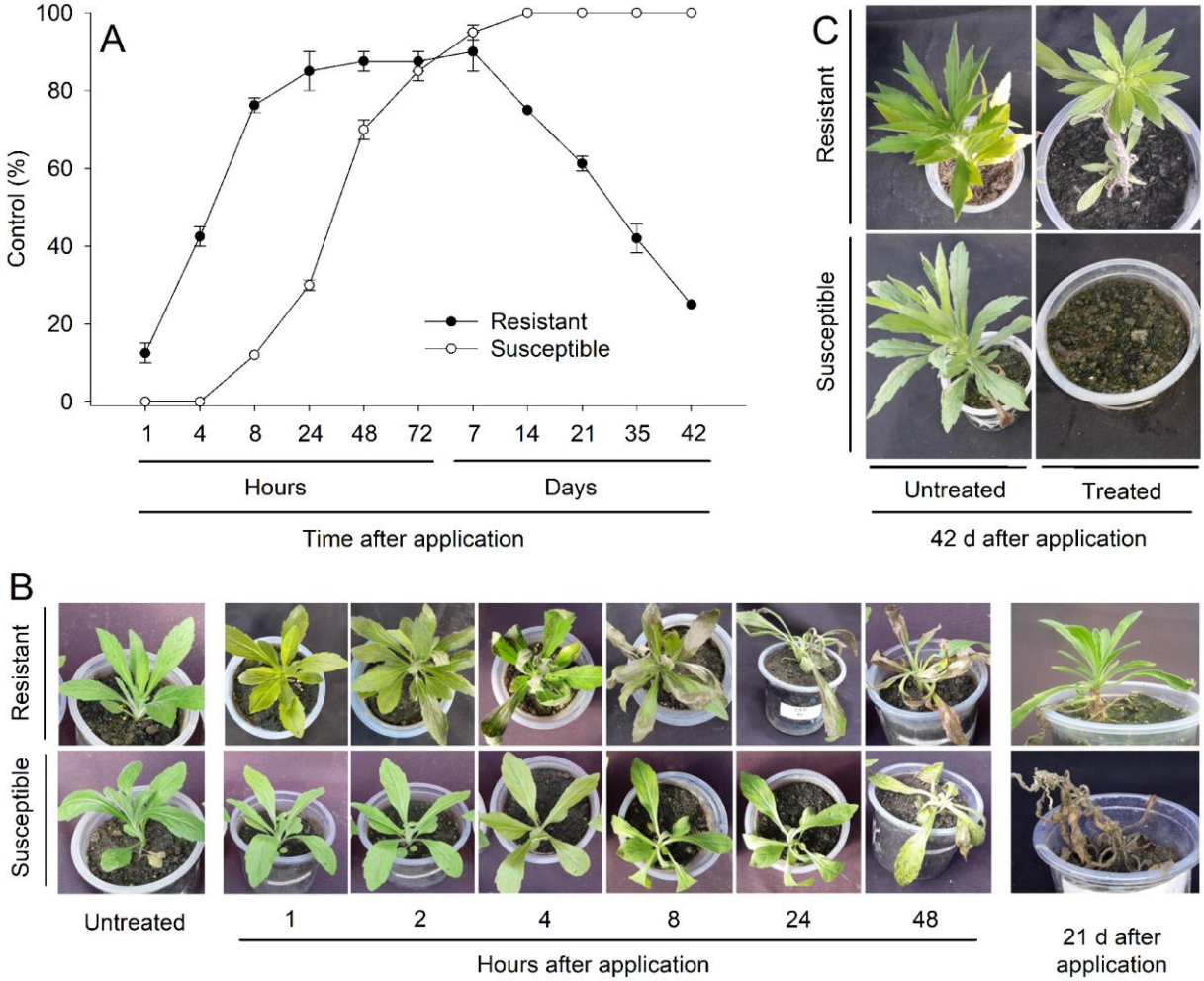

Figure 1. Visual control (A) and injury symptoms (B and C) of Sumantran fleabane (*Conyza sumatrensis*) 2,4-D-resistant and -susceptible biotypes treated with 2,4-D (1.0 kg a.e. ha^{-1}).

Rapid physiological responses of *C. sumatrensis* to 2,4-D were also reported by Queiroz et al. (2020) in a 2,4-D–resistant biotype. However, the results showed differences in relation to the times symptoms appeared and plant regrowth. Queiroz et al. (2020) showed rapid necrosis symptoms within 2 h after 2,4-D application, and total necrosis of leaves after 1 d, which may have been modulated by light and temperature conditions during the experiment. The symptoms

were observed in the mature leaves; the meristems and young leaves had no rapid necrosis. In addition, at 21 DAA, plants reestablished growth from only the axillary meristems and not the apical meristem.

The herbicide 2,4-D kills plants by altering the plasticity of the cell walls, influencing the amount of protein production, and increasing ethylene hormone concentration in the tissues (GROSSMANN, 2000; SANDALIO et al., 2016). It can be absorbed through roots, stems, and leaves and is translocated to the meristems of the plants, which leads to plant death (GROSSMANN, 2000). However, the resistant biotypes showed a rapid cell death that might limit the translocation of herbicide to other parts of the plant to ensure the resistant biotype's survival. A rapid response as part of evolved resistance to herbicide treatment, with resistant *A. trifida* plants having decreased glyphosate translocation, was also reported by Moretti et al. (2018). This mechanism was reported as rapid necrosis and is an adaptation strategy to survive herbicide application. In addition, Harre et al. (2017) documented the involvement of H_2O_2 in the rapid response of *A. trifida* resistant to glyphosate. The production of H_2O_2 increases more rapidly during the first hours, which leads to leaf tissue necrosis in the resistant compared with the late response of the susceptible biotype. Thus, the resistant biotype is not inherently more tolerant to oxidative stress (HARRE et al., 2018).

Here, the 2,4-D–resistant biotypes displayed symptoms similar to those identified in *A. trifida* resistant to glyphosate, defined as apoptosis-like programmed cell death (PCD) or hypersensitive response (LESPÉRANCE, 2015; VAN HORN et al., 2018; HARRE et al., 2018; MORETTI et al., 2018).

Chlorophyll *a* fluorescence transients analysis

Chl *a* fluorescence analysis, normalized as the relative variable fluorescence curve (Wt) and the calculation of the parameters of the JIP test, provides detailed information on the structure and function of the photosynthetic apparatus (STRASSER; STRASSER, 1995). The photosynthetic performance is related to energy conservation from excitation captured to the reduction of the electron acceptor of intersystem (PIABS) and photosystem I (PSI; PItotal). At 1 HAA, the 2,4-D–resistant biotype showed a decline of 20% in photosynthetic performance (performance index PIABS and PItotal) (Figure 2A), indicating a loss of photochemical efficiency by the plants (TREBST, 2007). At this time point, no variations in photosynthetic parameters were observed in relation to the 2,4-D–susceptible biotype (Figure 2A), indicating that the first physiological symptoms can be rapidly detected by Chl *a* fluorescence in the resistant biotype.

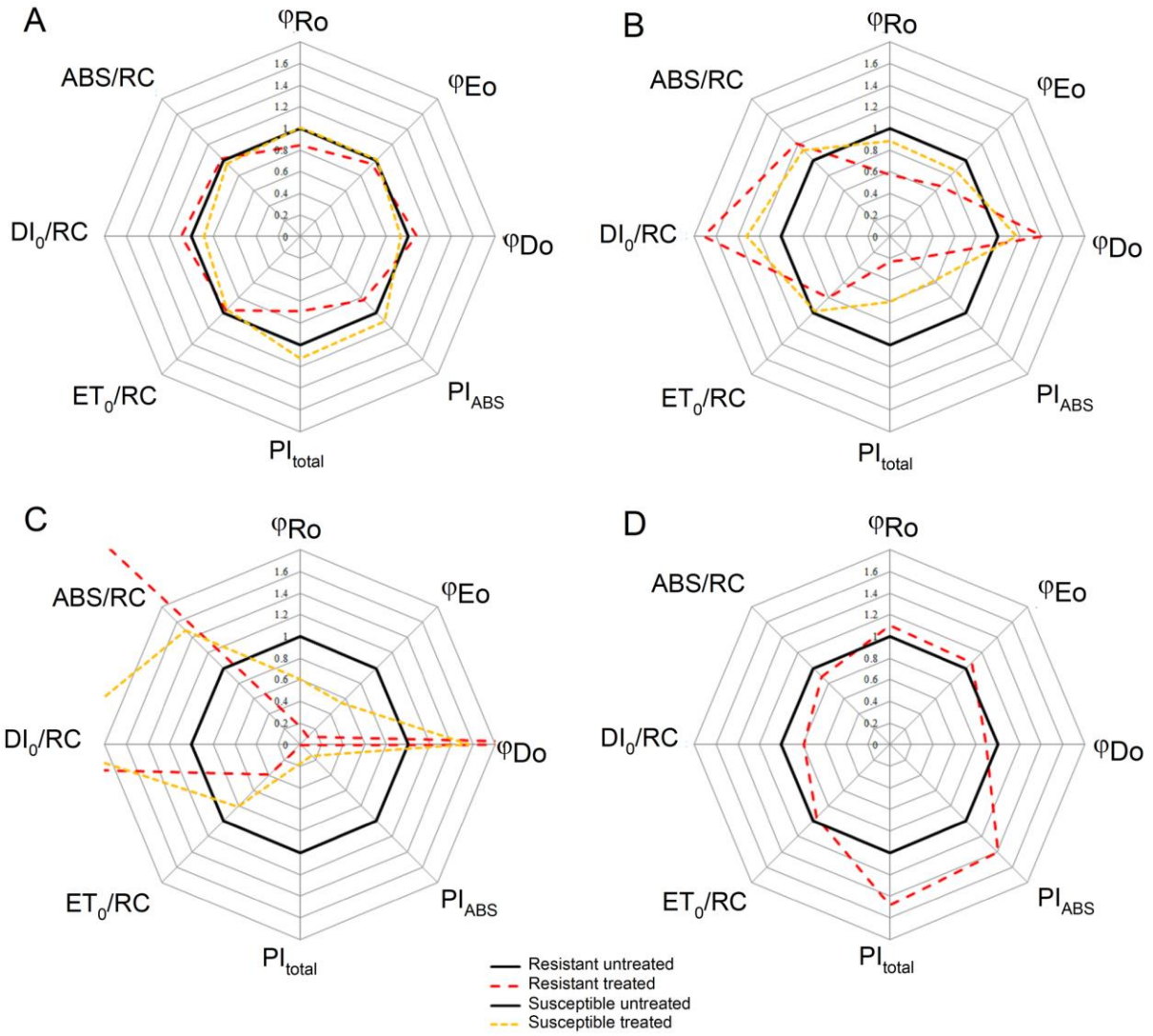

Figure 2: Chlorophyll *a* fluorescence transient of dark-adapted leaves of 2,4-D-susceptible and 2,4-D-resistant biotypes of Sumantran fleabane (*Erigeron sumatrensis*) at 1 h after application (HAA) (A), 4 HAA (B), 48 HAA (C), and 42 d after application (D). Among the parameters selected by the highlighted JIP-test: φRo - Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE). φEo - Quantum yield for electron transport (ET); φDo - Maximum quantum yield of non-photochemical de-excitation; PI_{ABS} - Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{total} - Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors. DI_0/RC - Dissipation of an active RC; ABS/RC- a measure of the apparent size of the antenna system; TR_0/RC - Maximum trapping rate per RC.

At 4 HAA, the 2,4-D–resistant biotype and the 2,4-D–susceptible biotype showed a slight variation in the relative variable fluorescence (Wt) (Figure 3A). There was an increase in the excitation captured by the reaction centers (RCs) until the reduction of plastoquinone (PQ) as observed in the OI phase (Figure 3D) in both biotypes. However, there was a reduction of the electron transfer from PQ to the final electron acceptor of the PSI, as highlighted in the IP phase (Figure 3G) for the 2,4-D–resistant biotype. Furthermore, the 2,4-D–resistant biotype presented a decrease of 20% in QA− reoxidation per RC (ET0/RC) and a decrease of 30% in

the quantum yield of electron transport from QA− to the electron acceptor intersystem (ϕE0 parameter) and 40% electron transport quantum yield of QA− for the final electron acceptor of the PSI (ϕR0 parameter) (Figure 2B). In addition, a decline of 80% in the photosynthetic performance and an increase of 70% in energy dissipation as heat per active RC (DI0/RC and ϕD0) were observed (Figure 2B). However, the 2,4-D–susceptible biotype showed a subtle variation in these parameters. Photosynthetic damage was rapidly observed in the resistant biotype compared with the susceptible biotype due to the differential physiological response of the 2,4-D–resistant biotype.

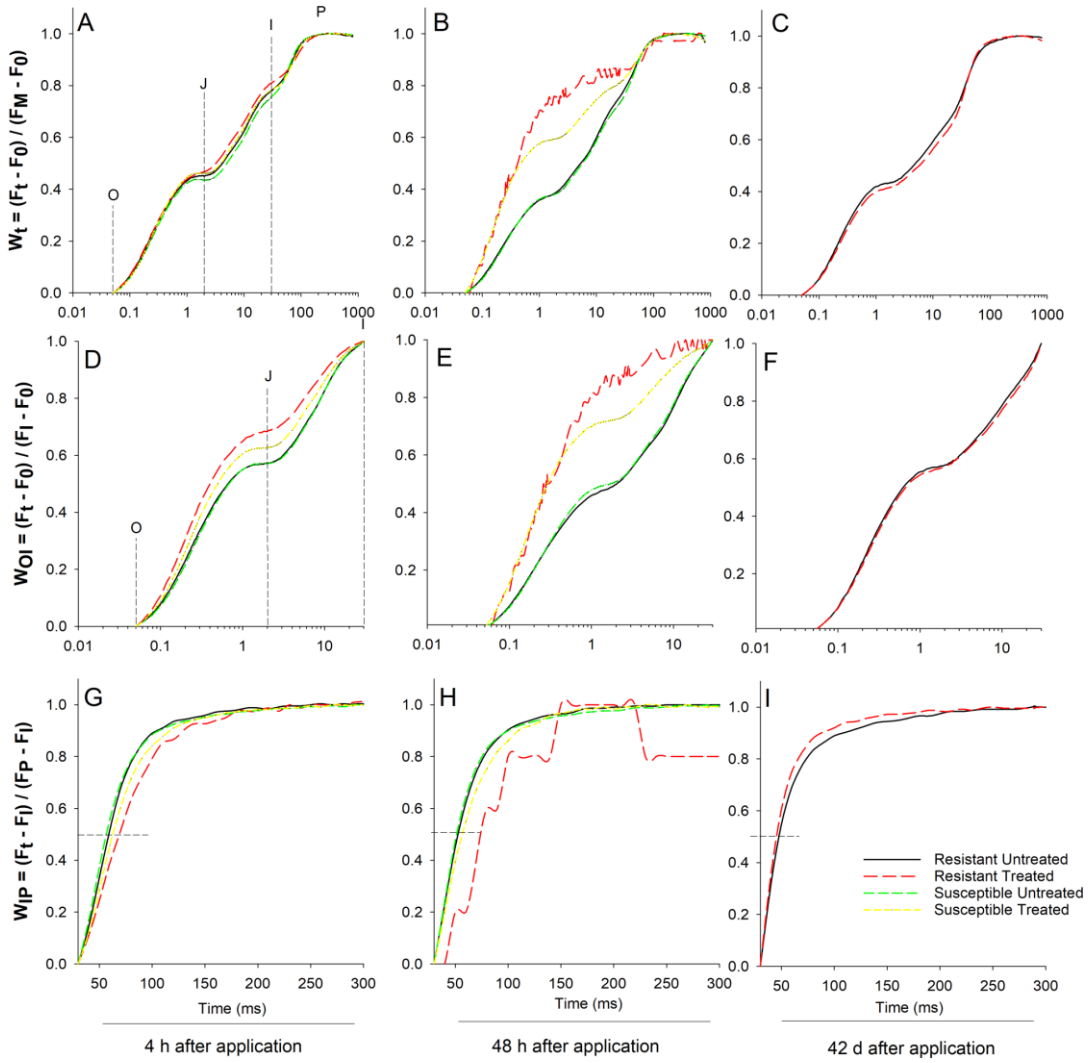

Figure 3. Chl *a* fluorescence transient of dark-adapted leaves of 2,4-D-susceptible and 2,4-D-resistant Sumantran fleabane (*Erigeron sumatrensis*) biotypes at 4 h after application, (A, D, G), 48 h after application (B, E, H), and 42 d after application (C,F, I). Data correspond to the relative variable fluorescence between the steps O and P (Wt) (A, B, C); between the steps O and I (WOI) (D, E, F) and between the steps I and P (WIP) (H, I, J) on a logarithmic time scale. Data correspond to the photosynthetic parameters deduced by the JIP-test analysis of the fluorescence transients normalized using the reference the control (n = 8).

At 48 HAA, both biotypes showed an increase in the relative variable fluorescence (Figure 3B) and energy dissipation as heat (Figure 2C). PIABS and PItotal declined 100% and 80% for 2,4-D–resistant and2,4-D–susceptible biotypes, respectively (Figure 2C). The ϕE0

declined 40% for the 2,4-D–susceptible biotype and 80% for the 2,4-D–resistant biotype (Figure 2C). In addition, there was a reduction of electron transfer from PQ to the final electron acceptor of the PSI, as highlighted in the IP phase (Figure 3H) and ϕR0 parameter (Figure 2C), for both biotypes, which was more pronounced in the resistant biotype.

The resistant biotype survived the 2,4-D application through regrowth from the apical meristem with 1 to 2 wk after application, and by 42 DAA, this biotype showed normal photochemical activity in new leaves (upon regrowth) when compared with untreated resistant plants (Figures 2 and 3).

Antioxidant Enzyme Activities

The basal activity levels of 2,4-D–resistant and 2,4-D–susceptible biotype leaves of untreated plants were different for all antioxidant enzymes (Figure 4). The resistant biotype constitutively expressed greater antioxidant enzyme activities for SOD, CAT, and APX in untreated leaves compared with the susceptible biotype. This finding is quite intriguing and needs to be elucidated by molecular analysis. However, so far, the resistant biotype shows resistance to six herbicides with different sites of action; this adaptation is to be expected as the genes coding for antioxidant enzymes are constitutively expressed as induced by the herbicide. This finding may also be supported by reports of antioxidative enzymes operating as a response mechanism in resistant biotypes to herbicides, such as paraquat (YE; GRESSEL, 2000; HARRE et al., 2018). Studies reported that hairy fleabane [*Conyza bonariensis* (L.) Cronquist; syn. *Erigeron canadensis* L.] resistant to paraquat constitutively showed more SOD and APX activities before paraquat treatment compared with a susceptible biotype (SHAALTIEL, GRESSEL 1986; YE, GRESSEL 2000). Although the differences in basal enzymes between the resistant and susceptible biotypes have not been elucidated, we suggest that these differences may be constitutively incorporated upon the development of a mechanism of resistance to maintain the normal growth of plants.

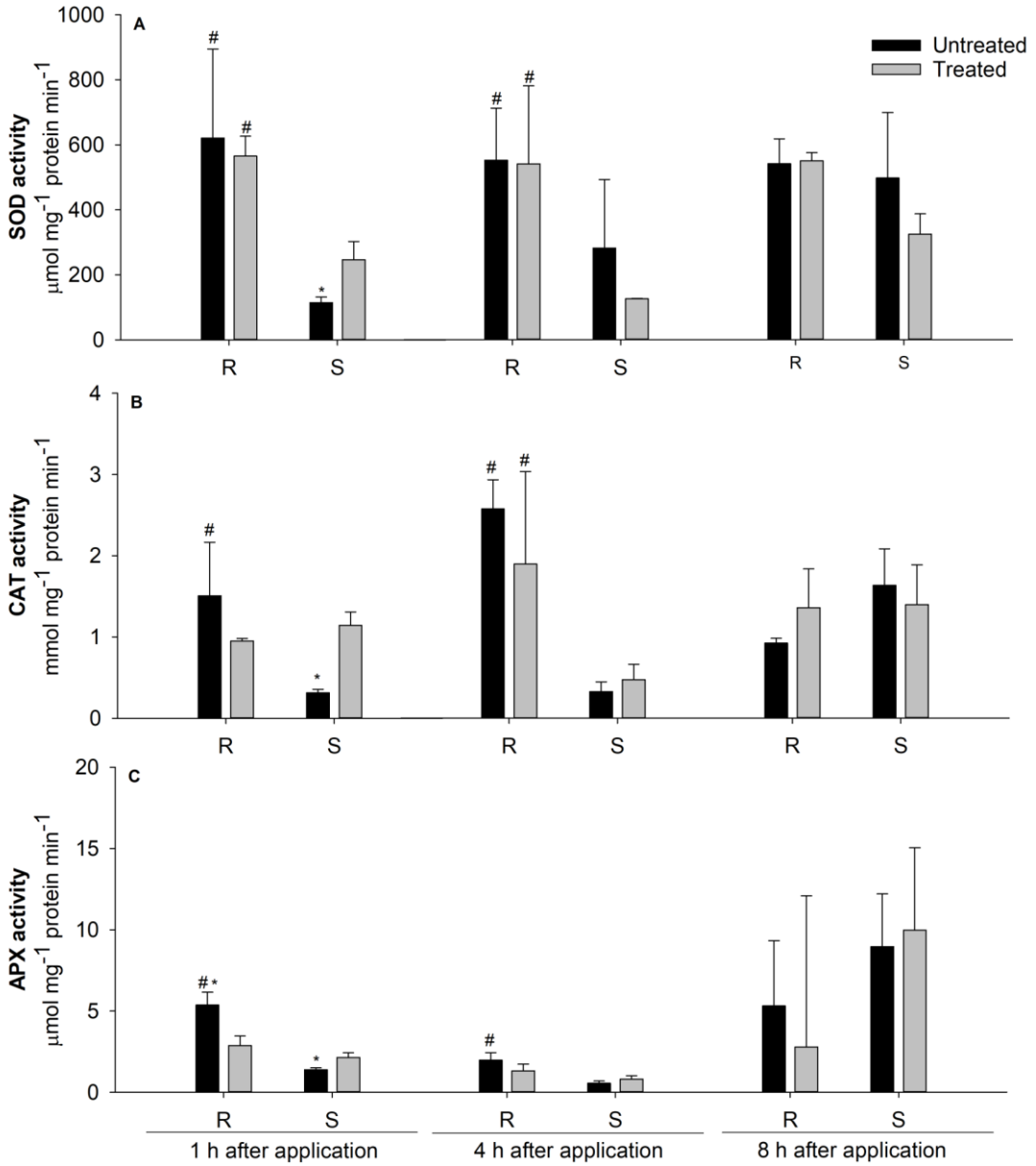

Figure 4. Change in superoxide dismutase (SOD) (A), catalase (CAT) (B), and ascorbate peroxidase (APX) (C) enzymatic activities in 2,4-D–resistant and 2,4-D–susceptible *Conyza sumatrensis* biotypes at 1, 4, and 8 h after application of 2,4-D herbicide (1,005 g a.e. ha−1). The treatment effects were separated at $P \leq 0.05$ and adjusted using Fisher's protected LSD. Symbols above error bars: *compared between treatment (untreated and treated); #compared between biotype (resistant and susceptible). Values represent the means ± SD

In addition, it is not necessarily an increase in the activity of the enzymes upon herbicide application compared with basal ones. This can be observed in our study and can be related to the resistance mechanism of action operating in the biotype to counteract the herbicide in the tissues. Antioxidant enzyme activities were also reported to be similar between the rapid responses of glyphosate susceptible and glyphosate-resistant *A. trifida* biotypes following glyphosate treatment (HARRE et al., 2018). There is also a difference in tissue damage between juvenile and mature leaves of the resistant biotype, which has a differential and transiently increases in antioxidant enzyme expression in juvenile leaves. However, considering the overall

induction of antioxidative enzymes, juvenile leaves induced lower expression than mature leaves (HARRE et al., 2018). Constitutively, levels of antioxidant enzymes can be also a response to the resistant and susceptible biotypes of *Conyza*.

The activities of all enzymes did not differ between untreated and treated 2,4-D–resistant biotypes at 1, 4, and 8 HAA (Figure 4). However, at 1 HAA, the antioxidant enzyme activities were significantly higher in the treated 2,4-D–susceptible biotype than in the untreated (Figure 4). SOD is one of the most important enzymes used against oxidative stress in plant defense systems (GIANNOPOLITIS; RIES, 1977). The increase in SOD activity might be due to increased production of the superoxide radical, since this is the first enzyme to act on the antioxidant system, initiating the dismutation of the superoxide radical into H_2O_2 (AZEVEDO-NETO et al., 2006). The increases in CAT and APX activity in the leaves might be related to high levels of H_2O_2 originating from the conversion of the superoxide radical through SOD-mediated reactions, as CAT and APX enzymes help to overcome the damage to tissue metabolism by reducing toxic levels of H_2O_2 (MITTLER, 2002; APEL; HIRT, 2004).

The enzymatic activity may influence the photosynthetic behavior of the susceptible biotype, as the enzymes were able to detoxify the ROS and no damage was found at 1 HAA in the photosynthetic apparatus. Otherwise, at 1 HAA, the resistant biotype showed a decline in photosynthetic activity and did not show enzymatic changes between treated and untreated. The increase in enzymatic activity at the first hours in the susceptible biotype may be related to an attempt to cope with the increase in ROS production over time, which fails and leads to plant death. On the other hand, the resistant biotype rapidly induces ROS production itself to trigger PCD and avoid herbicide translocation with no need to induce antioxidant enzymes. It is also noteworthy that there are other enzymatic and non-enzymatic compounds not measured here that may be involved in this mechanism of detoxification: enzymes such as glutathione peroxidase, glutathione reductase, guaiacol peroxidase, glutathione-S-transferase, peroxiredoxin, monodehydroascorbate reductase, and dehydroascorbate reductase and non-enzymatic molecules such as ascorbate, glutathione, tocopherol, phenolics, proline, and others (GILL; TUTEJA, 2010; IRATO; SANTOVITO, 2021). It is also worth mentioning that quantifying the dynamic expression of antioxidant genes as well as changes in enzymatic activity and compounds as a means of coping with ROS is also important to elucidate basal and herbicide responses of resistant and susceptible biotypes.

15 °C versus 25 °C

The temperature becomes a relevant factor in Brazil; this biotype is widespread in the country, and when present in the south (a colder region), visual symptoms are observed only at 2 to 3 HAA, while in hotter regions, such as the midwestern part of the country, the symptoms are often observed at 30 min after application. This information is very relevant, considering the widespread dissemination of this plant worldwide.

The interaction between both temperature treatment and biotypes was significant ($P < 0.05$) (Figure 5). The accumulation of H_2O_2 in the resistant biotype was higher at all time points compared with the susceptible biotype. Higher H_2O_2 accumulation was associated with the rapid physiological response observed in this biotype, as higher H_2O_2 levels lead to oxidative damage of cells (Figure 5) (GILL; TUTEJA, 2010) as a mechanism to induce PCD to avoid herbicide translocation. H_2O_2 is one of the main ROS compounds generated outside and within the cell in response to stresses, and it is also induced on the application of exogenous auxins in plants (PEER et al., 2013; SONG, 2014 PETERSON et al., 2016). Auxin is involved in the regulation of several processes, such as cell viability, cell cycle progression, and PCD, which depend on ROS signaling (XIA et al., 2005). The accumulation of H_2O_2 before tissue death is

a response to an induced oxidative burst involved in the signaling of apoptosis-like PCD (LAM et al., 2001). The symptoms observed in *C. sumatrensis* were similar to those reported in the rapid response of glyphosate-resistant *A. trifida*, defined as apoptosis-like PCD (LESPÉRANCE, 2015).

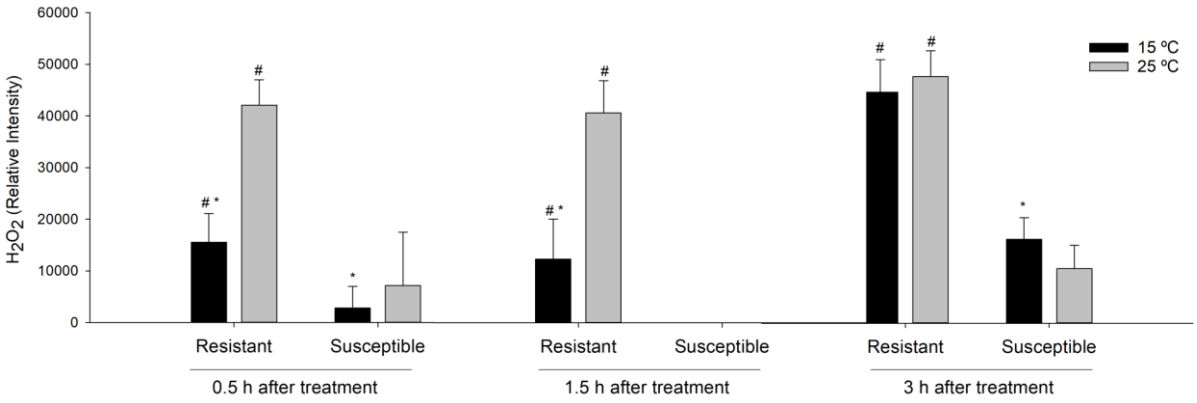

Figure 5. Changes in hydrogen peroxide (H_2O_2) in 2,4-D-resistant and 2,4-D-susceptible Sumantran fleabane (*Conyza sumatrensis*) biotypes following treatment with 2,4-D herbicide (1005 g a.e. ha $^{-1}$) at 0.5; 1.5 h, and 3 h after application (HAA) at 15 and 25°C. The treatment effects were separated at $p \leq 0.05$ and adjusted using Fisher's Protected LSD. * compared between temperature (15 and 25°C); # compared between biotype (resistant and susceptible).

Although there was no increase in the activity of antioxidant enzymes between the treated and untreated 2,4-D–resistant biotypes (Figure 4), there was an accumulation of H_2O_2 in the cell induced by herbicide spray (Figure 5). The overproduction of ROS leads to oxidative damage (GILL; TUTEJA, 2010) and maintaining antioxidant enzymes at basal levels or even at a reduction is crucial for a plant to sustain ROS accumulation and induce PCD, a mechanism to avoid herbicide translocation. The temperature effect appears to modulate the velocity of the beginning of the necrosis, as a higher concentration of H_2O_2 was detected at 0.5 HAA in the 2,4-D–resistant biotype compared with the susceptible biotype at both 15 and 25 C (Figure 5). However, within 30 min and 1.5 HAA, the resistant biotype showed higher H_2O_2 concentrations at 25 C compared with 15 C, while no differences were observed at 3 HAA (Figure 5).

These results reflect those of Derr and Serensits (2016), who also found that herbicide applications at warm temperatures generally cause faster injury symptom development than at cold temperatures. The lower temperature reduces metabolism rates, absorption, and translocation, leading to a delay in initial weed injury (DERR, SERENSITS, 2016; GANIE et al., 2017).

H_2O_2 is produced in plant cells under normal conditions associated with stress signaling (APEL; HIRT, 2004; CAVERZAN ET AL., 2019) and in repsonse to herbicides, as shown in this study. Accumulation of H_2O_2 has been reported in an *A. trifida* rapid response biotype at 0.5 h after glyphosate treatment under 25 °C conditions (HARRE et al., 2018). ROS accumulation occurred before tissue death in *A. trifida*, and this rapid response had not been previously associated with glyphosate application in plants. The rapid response in *C. sumatrensis* following 2,4-D treatment in this study induced the generation of H_2O_2 that may be associated with stress signaling and rapid cell death in the 2,4-D–resistant biotype (Figure 5).

Light versus Dark Conditions

The interaction between light treatment and biotypes was not significant ($P < 0.05$) at 0.5 and 1.5 HAA. For this reason, the results are shown separately, with one graph for biotypes (susceptible and resistant) and another for light and dark conditions. The 2,4-D– resistant biotype showed higher production of H_2O_2 than the susceptible biotype at 0.5 and 1.5 h after light treatment (Figure 6A). Under light and dark conditions, there was higher variation in the results when data was plotted together, showing no differences (Figure 6B).

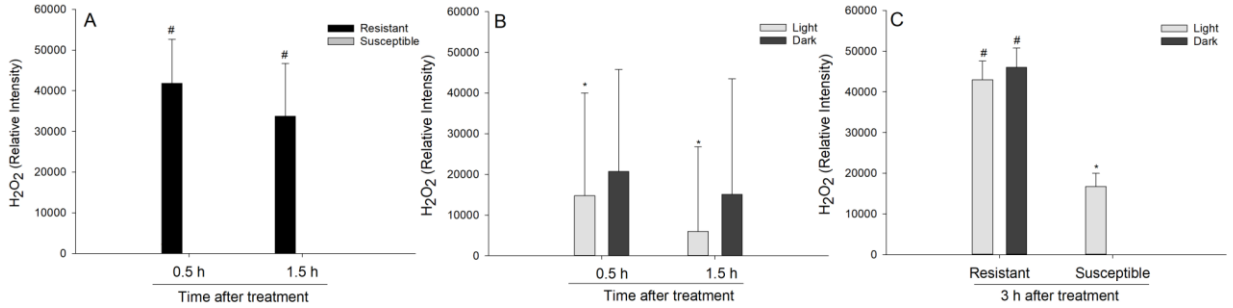

Figure 6. Changes in hydrogen peroxide (H_2O_2) in 2,4-D-resistant and 2,4-D-susceptible Sumantran fleabane (*Conyza sumatrensis*) biotypes following treatment with 2,4-D herbicide (1005 g a.e. ha^{-1}) at 0.5; 1.5 h and 3 h after application (HAA) at 15 and 25°C. The treatment effects were separated at $p \leq 0.05$ and adjusted using Fisher's Protected LSD. * compared between light and darkness; # compared between biotype (resistant and susceptible).

Interaction between light treatment and biotypes was significant at 3 HAA. The 2,4-D–resistant biotype produced similar levels of H_2O_2 under dark and light conditions. Higher H_2O_2 levels were observed in the 2,4–D resistant biotype than in the susceptible biotype under both dark and light conditions (Figure 6C).

The phytotoxicity of 2,4-D in part involves oxidative stress caused by the overproduction of ROS (GROSSMANN, 2000; Queiroz et al. (2020) reported that the 2,4- D–resistant *C. sumatrensis* biotype Marpr9-rn had rapid necrotic symptoms that began at approximately 2 h after herbicide application, while the evolution of H_2O_2 started at 15 min after application. Rapid necrosis in response to to 2,4-D at higher light intensities was observed. However, after 60 min, there were no differences between the low and high light conditions (29 and 848 μmol m−2 s−1, respectively) (QUEIROZ et al., 2020). Harre et al. (2018) observed that light was necessary to induce the rapid response in *A. trifida* following glyphosate treatment. In this study, the rapid metabolic and physiological response of *C. sumatrensis* resistant to 2,4-D did not show light dependence for the production of ROS (Figure 6).

The *C. sumatrensis* 2,4-D–resistant biotype showed rapid photosynthetic damage after 2,4-D treatment compared with the susceptible biotype. The antioxidant enzyme activities were higher in the resistant biotype. Temperature effects appeared to modulate the speed of initiation of the rapid necrosis process. The symptoms occurred faster in the 2,4-D–resistant biotype under higher temperatures. Production of H_2O_2 in the 2,4-D–resistant biotype was not light dependent. 2,4-D may induce a rapid response by interrupting auxin translocation, including 2,4-D, to the whole plant, as both auxins and 2,4-D are transported via polar mechanisms through the same transporters. Auxin accumulation may lead to induction of ethylene and ROS production that induce PCD of tissues affected by herbicide application, avoiding plant death and allowing regrowth after a few days from lateral meristems not affected directly or indirectly

(translocation) by the herbicide. In addition, the rapid response seems to be a mechanism operating only in response to systemic herbicides, as is the case in 2,4-D translocation being blocked in *Conyza*. Although we suggest the actual mechanism of the plant's response to 2,4-D remains to be elucidated in the resistant biotype, and molecular approaches may be a useful tool to understand the metabolic mechanism(s). Our understanding of the basal antioxidant responses of resistant and susceptible biotypes also needs to be improved.

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5 CAPÍTULO III

O USO DE HERBICIDAS INIBIDORES DA ALS PARA CONTROLE DE BUVA COM MÚLTIPA RESISTÊNCIA A HERBICIDAS

5.1 RESUMO

A buva (*Conyza* spp.) é considerada uma das principais plantas daninhas do Brasil e têm sido frequentemente associadas a casos de resistência a herbicidas. O cenário é agravado em casos de resistência múltipla. O objetivo do trabalho foi propor novas alternativas de manejo químico para o biotipo de buva com resistência múltipla aos herbicidas 2,4-D, glifosato, diuron, paraquat e saflufenacil, O ensaio foi disposto em blocos ao acaso com quatro repetições em esquema fatorial, sendo o fator A os biotipos de buva suscetível e outro com comprovada resistência aos herbicidas paraquat, saflufenacil, diuron, 2,4-D e glifosato; e o fator B os herbicidas cloransulam-metílico, metsulfurom-metílico, clorimurom-etílico, imazapique + imazapir e etoxissulfurom e testemunha sem aplicação. Aos 14 e 35 dias após aplicação (DAA) foi realizada a avaliação visual e avaliações da fluorescência transiente da clorofila *a*. Os dados foram submetidos a ANOVA ($p \leq 0,05$) e quando significativos ao teste de Tukey ($p \leq 0,05$). Houve interação entre os fatores biotipos (resistente e suscetível) e os herbicidas. Para o biotipo suscetível, os herbicidas cloransulam-metílico, metsulfurom-metílico, clorimurom-etílico, imazapique + imazapir e etoxissulfurom demostraram 100% de controle aos 35DAA. No entanto, o biotipo resistente apresentou baixo percentual de controle (<20%) quando submetido aos herbicidas clorimurom-etílico, imazapique + imazapir e etoxissulfurom e controle satisfatório quando submetido os herbicidas cloransulam-metílico e metsulfurom-metílico. Os resultados sugerem que o biotipo de buva com múltipla resistência aos herbicidas 2,4-D, paraquat, saflufenacil, glifosato e diuron pode também apresentar resistência aos herbicidas inibidores ALS clorimuron-etílico, imazapique + imazapir e etoxissulfurom. Estudos serão desenvolvidos para confirmar a hipótese através de dose-resposta.

Palavras-chave: *Conyza sumatrensis.* Alternativas de controle. Herbicidas Pós-emergentes.

5.2 ABSTRACT

The *Conyza* spp. is considered one of the main weeds in Brazil and has been frequently associated with cases of resistance to herbicides. The scenario is worse in cases of multiple herbicide resistance. The objective of the work was to propose new chemical management alternatives for the biotype of *Conyza* spp. with multiple resistance to the herbicides 2,4-D, glyphosate, diuron, paraquat and saflufenacil. The test was described in randomized blocks with four repetitions in a factorial scheme, with factor A being susceptible biotype and another with proven resistance to the herbicides paraquat, saflufenacil, diuron, 2,4-D and glyphosate and factor B was the chloransulam-methyl, metsulfurom-methyl, chlorimurom-ethyl, imazapique + imazapir and ethoxysulfuron herbicides and control without application. At 14 and 35 days after application (DAA), visual assessment and transient fluorescence of chlorophyll *a* were performed. The data were discovered using ANOVA ($p \leq 0.05$) and when proving the Tukey test ($p \leq 0.05$). There was interaction between biotype factors (resistant and susceptible) and herbicides. For the susceptible biotype, the herbicides chloransulam-methyl, metsulfuron-methyl, chlorimurom-ethyl, imazapique + imazapyr and ethoxysulfuron showed 100% of control at 35DAA. However, the resistant biotype has a low percentage of control (<20%) when subjected to the herbicides chlorimurom-ethyl, imazapique + imazapir and ethoxysulfuron and satisfactory control when submitted to the herbicides chloransulam-methyl and metsulfurom-methyl. The results obtained this study suggesting that the multiple resistance biotype to the herbicides 2,4-D, paraquat, saflufenacil, glyphosate and diuron can also show resistance to the herbicides inhibitors ALS chlorimuron-ethyl, imazapique + imazapyr and ethoxysulfuron. The studies will be developed to confirm the hypothesis.

Keywords: *Conyza sumatrensis*. Management. Post-emergent Herbicides.

5.3 INTRODUÇÃO

A competição de plantas daninhas com culturas de interesse pode levar a uma perda média de 15 % da produção mundial de grãos (UNIVERSO AGRO, 2018). Esse cenário pode ser ainda pior quando há infestações de plantas daninhas resistentes a herbicidas (ADEGAS et al., 2017). No Brasil, infestações de buva (*Conyza* spp.) podem reduzir a produtividade da soja em até 48% (GAZZIERO et al., 2010; BLAINSKI et al., 2015).

A buva é uma planta daninha de folha larga com ciclo anual, pertence à família *Asteraceae* e encontra-se disseminada globalmente em climas temperados e zonas subtropicais (THEABAUD; ABBOTT, 1995). No Brasil é considerada uma das principais plantas daninhas em lavouras de soja, fato que se dá principalmente devido a sua boa adaptabilidade nos sistemas conservacionistas do solo, capacidade de autopolinização e grande produção de sementes que são facilmente dispersas pelo vento e pela água (SAVAGE et al., 2014).

As espécies de *Conyza* spp. possuem reprodução autógama e a propagação se dá unicamente através de sementes. São plantas muito prolíficas, com produções de sementes maiores que 375.000 por planta em *Conyza bonariensis*, 200.000 em *C. canadensis* e mais de 60.000 em *C. sumatrensis* (GREEN, 2010). A emergência dessa planta daninha concentra-se no período do final do outono e início primavera, que coincide com o período de pousio ou plantio de inverno o que favorece o estabelecimento dessa planta em áreas produtivas quando não há manejo ortogonal.

A habilidade de autopolinização da espécie aliada à grande produção de sementes facilmente dispersáveis são fatores que podem contribuir para a boa adaptabilidade ecológica, para a sobrevivência de biótipos resistentes de buva e para as altas infestações nos sistemas conservacionistas de solo (MOREIRA et al., 2007).

No mundo existem relatos de biótipos de *Conyza* spp resistentes a diversos herbicidas, entre eles: glifosato, paraquat, diquat, atrazina, simazina, clorimurom, clorsulfurom, cloransulam-metílico, imazapir, metribuzim, piritiobaque, sulfometurom, diurom, saflufenacil, iodosulfurom, metsulfurom, dentre outros (HEAP, 2022).

No Brasil, o primeiro relato de *Conyza* spp resistente foi ao herbicida glifosato em 2005 (LAMEGO; VIDAL, 2008) e o último caso alarmante foi a resistência múltipla aos herbicidas 2,4-D, glifosato, diuron, paraquat e saflufenacil (PINHO et al., 2019).

Tendo em vista o diagnóstico de resistência múltipla de buva no Brasil, é necessário traçar estratégias proativas de manejo de resistência, prevendo o comportamento de outros herbicidas no controle da espécie. Diante do exposto o objetivo do trabalho foi avaliar a eficácia de controle de herbicidas inibidores da ALS no controle do biotipo de buva com resistência múltipla aos herbicidas 2,4-D, glifosato, diuron, paraquat e saflufenacil.

5.4 MATERIAL E MÉTODOS

Os experimentos foram conduzidos em casa vegetação na Universidade Federal Rural do Rio de Janeiro, localizada em Seropédica, Estado do Rio de Janeiro, Brasil (-22°45'39''S, -43°42'00''N, a 25 metros de altitude).

As sementes de buva (*Conyza sumatrensis*) foram semeadas em bandejas preenchidas com substrato comercial. Posteriormente foram transplantadas para vasos de polietileno com capacidade de 300 mL preenchidos com substrato. Procedeu-se a adubação semanal com nitrogênio, fósforo e potássio (NPK, 5-20-20) e irrigação diária de modo a manter o solo na capacidade de campo.

O ensaio foi disposto em blocos ao acaso com quatro repetições em esquema fatorial, sendo o fator A os biotipos de buva suscetível e outro com comprovada resistência aos herbicidas paraquat, saflufenacil, diuron, 2,4-D e glifosato (PINHO et al., 2019) e o fator B os herbicidas inibidores da enzima ALS (Tabela 1) e testemunha sem aplicação.

Tabela 1. Ingredientes ativos utilizados para avaliação do controle de *Conyza sumatrensis.*

Tratamentos	**Dose**
Inibidores da ALS	
Clorimuron etílico[1]	20 g ia. ha^{-1}
Cloransulam metílico	40 g ia. ha^{-1}
Metsulfuron metílico[2]	4 g ia. ha^{-1}
Imazapique + Imazapir	78,8+26,2 g ia. ha^{-1}
Etoxissulfuron[3]	80 g ia. ha^{-1}

Adição de adjuvantes a calda: [1]0,05% de óleo mineral; [2]0,5% de óleo mineral; [3]0,2% óleo metilado de soja

As aplicações foram realizadas quando as plantas se encontravam com 12 a 15cm, para isso foi utilizado um pulverizador costal pressurizado a CO_2 com quatro pontas XR-110015 (TeeJet Technologies, Wheaton, IL), fornecendo 150 L ha^{-1} de calda numa pressão de 40 psi.

Aos 14 e 35 dias após aplicação (DAA) foi realizado a avaliações visuais de controle utilizando uma escala de 0 a 100%, onde 0% ausência de controle e 100% morte das plantas. Aos 35 DAA as plantas foram coletadas e secas em estufa a 70ºC, por 72 horas, e em seguida pesadas para mensurar a massa seca da parte aérea (MSPA).

As avaliações da fluorescência transiente da clorofila *a* foram realizadas aos 14 e 35 DAA utilizando um fluorômetro portátil Handy-PEA. As análises foram feitas apenas no biotipo resistente visto que o suscetível apresentava alto percentual de controle no dia da avaliação, impossibilitando o procedimento da análise. Os clipes utilizados para estas medições foram colocados no terço médio de folhas jovens completamente expandidas (área de 4 mm de diâmetro da folha) durante 20 min. Após esse período a amostra foi submetida a um pulso de luz saturante numa intensidade de 3.000 μmol m^{-2} s^{-1} e a partir da curva de emissão de fluorescência transiente obtida após o pulso, procedeu-se o cálculo dos parâmetros estabelecidos pelo Teste JIP (STRASSER; STRASSER, 1995). Os valores de fluorescência plotados foram a média de oito medições de cada tratamento.

Os dados foram submetidos a ANOVA ($p \leq 0,05$) e quando significativos ao teste de Tukey ($p \leq 0,05$).

5.5 RESULTADOS E DISCUSSÃO

Houve interação entre os fatores biotipos (resistente e suscetível) e os herbicidas. Para o biotipo suscetível, os herbicidas cloransulam-metílico, metsulfurom-metílico, clorimurom-etílico, imazapique + imazapir e etoxissulfurom demostraram 93-98% de controle aos 14DAA e aproximadamente 100% aos 35DAA. No entanto o biótipo resistente apresentou baixo percentual de controle (<20%) quando submetido aos herbicidas clorimurom-etílico, imazapique + imazapir e etoxissulfurom e controle satisfatório quando submetido aos herbicidas cloransulam-metílico e metsulfurom-metílico (Figura 1 e 2).

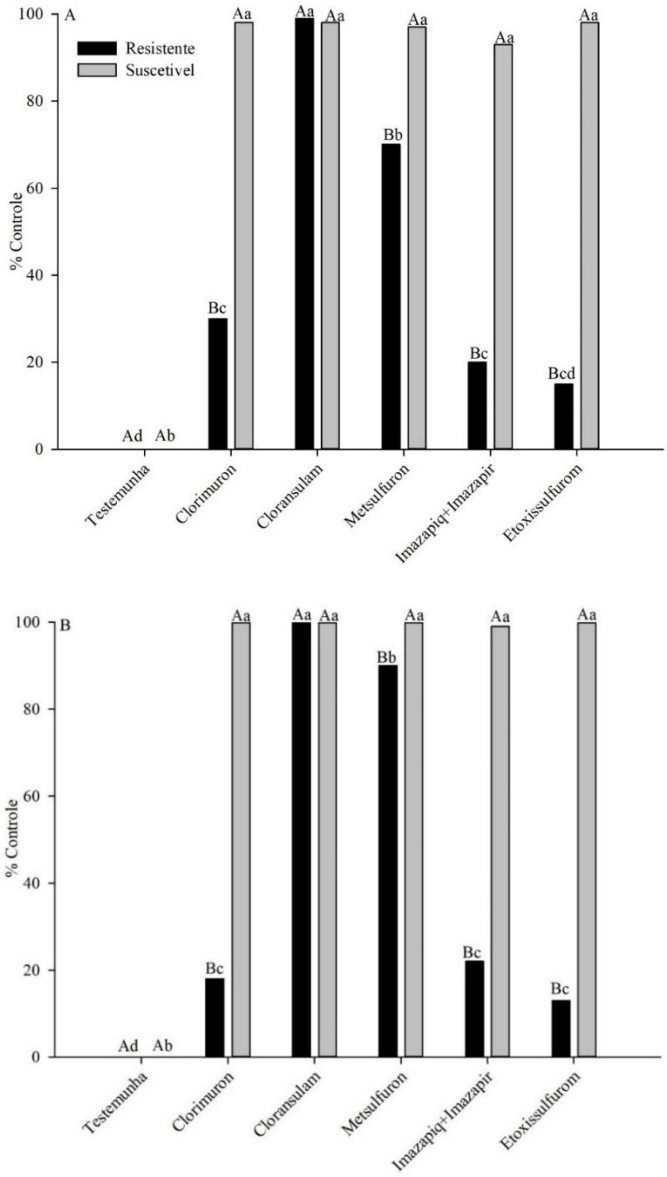

Figura 1: Porcentagem de controle aos 14 dias após aplicação (DAA) (A) e aos 35 DAA (B) de plantas de buva no estádio de 12-15 cm, submetidas aos herbicidas cloransulam-metílico, metsulfurom-metílico, clorimurom-etílico, imazapique + imazapir e etoxissulfurom. A comparação entre biotipos (R e S) são representadas pelas letras maiúsculas e a comparação entre herbicidas pelas letras minúsculas. Letras iguais não diferem entre si pelo teste de Tukey a 5% de probabilidade. Seropédica-RJ, 2021.

Com relação à redução de massa seca, o biotipo resistente quando submetido aos herbicidas clorimuron, imazapique + imazapir e etoxissulfurom apresentou comportamento semelhante a testemunha sem aplicação. Enquanto que o biotipo suscetivel quando submetido aos herbicidas cloransulam-metílico, metsulfurom-metílico, clorimurom-etílico e etoxissulfurom apresentou 100% de redução de massa seca (Figura 2).

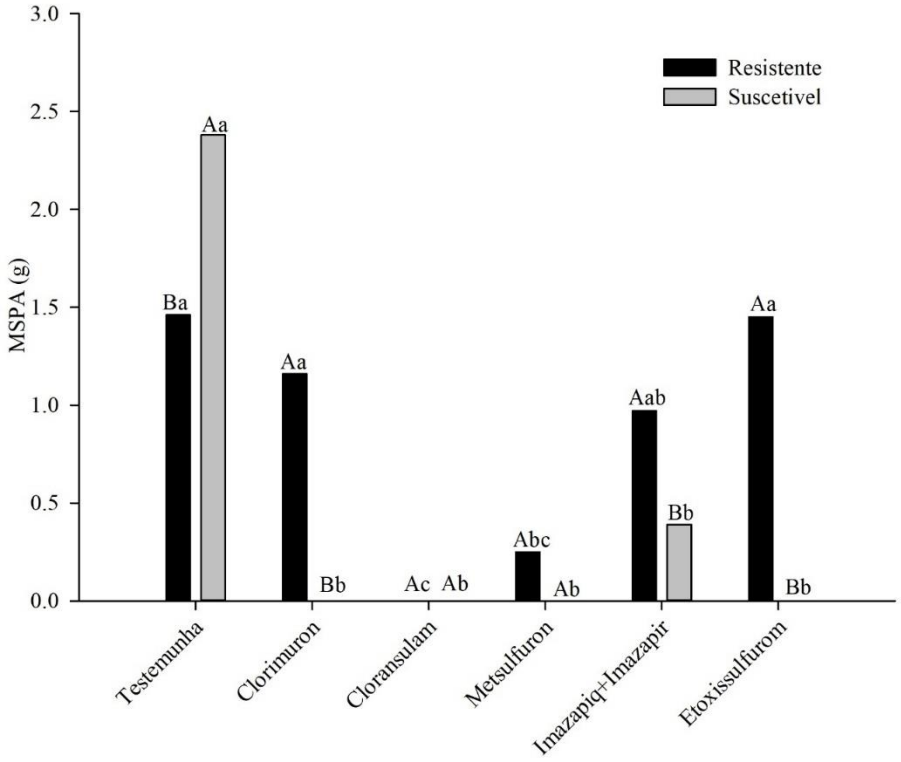

Figura 2: Massa seca de parte aérea (MSPA) de plantas de buva no estádio de 12-15 cm, submetidas aos herbicidas cloransulam-metílico, metsulfurom-metílico, clorimurom-etílico, imazapique + imazapir e etoxissulfurom aos 35 dias após aplicação. A comparação entre biotipos (R e S) são representadas pelas letras maiúsculas e a comparação entre herbicidas pelas letras minúsculas. Letras iguais não diferem entre si pelo teste de Tukey a 5% de probabilidade. Seropédica-RJ, 2021

Os resultados sugerem que o biotipo de buva com múltipla resistência aos herbicidas 2,4-D, paraquat, saflufenacil, glifosato e diuron pode também apresentar resistência aos herbicidas inibidores ALS clorimuron, imazapique + imazapir e etoxissulfurom. Estudos de dose-resposta serão desenvolvidos para confirmar a hipótese.

Casos de resistência de buva aos herbicidas inibidores da ALS vêm crescendo no Brasil (SANTOS et al., 2015; ALBRECHT et al., 2020). Após a resistência de buva ao glifosato, o uso de herbicidas inibidores da ALS passou a ser amplamente empregado para controle dessa espécie no sistema de produção da cultura da soja. Como resultado da alta pressão de seleção exercida pelos herbicidas inibidores da ALS, em 2011, foram identificados biótipos de buva com resistência simples ao clorimuron e resistência múltipla ao glifosato e aos inibidores da

ALS (HEAP, 2021). Em 2017, foi relatado a resistência múltipla de *C. sumatrensis* aos herbicidas inibidores da ALS, FSI e EPSPs (ALBRECHT et al., 2020; HEAP, 2021).

Os mecanismos de resistência de plantas daninhas aos herbicidas inibidores da ALS são amplamente descritos na literatura. Mecanismos de resistência *target site* e *non-target site* são relatados em plantas daninhas resistente aos herbicidas inibidores da ALS. A resistência aos inibidores de ALS pode ser causada pela mutação do gene ALS, que resulta na alteração de um único resíduo de aminoácido no sítio de ligação do herbicida ou ainda por metabolismo do herbicida ou redução na absorção e translocação (TRANEL, 2002; WHITE et al., 2002; REY-CABALLERO et al., 2017; GAINES et al., 2020).

Uma ferramenta que vem sendo utilizada para detecção de estresses ocasionados por herbicidas é a fluorescência da clorofila *a* (ZHANG et al., 2016). Os herbicidas de forma direta ou indireta afetam a fotossíntese das plantas, metabolismo primário, e consequentemente afeta a estabilidade do aparelho fotossintético, resultando em mudanças na indução de fluorescência de clorofila *a* (DAYAN; ZACCARO, 2012; SOUSA et al., 2014). Sendo assim, a fluorescência da clorofila *a* pode ser usada para detectar qualquer perturbação no metabolismo da planta, e se aplica a herbicidas de diferentes mecanismos de ação, incluindo herbicidas inibidores da ALS (DAYAN; ZACCARO, 2012).

A análise de fluorescência da clorofila *a* mostrou que o cloransulam-metílico e metsulfurom-metílico foram os herbicidas que ocasionaram maior dano fotossintético as plantas de buva resistente, corroborando com os dados observados no percentual de controle e massa seca. Aos 14 DAA os tratamentos cloransulam-metílico e metsulfurom-metílico diminuiram em aproximadamnte 40% o rendimento quântico de transporte de elétrons de QA- para o intersistema de aceptores de elétrons (φE_0) e o rendimento quântico de transporte de elétrons de QA- para o aceptor final de elétrons do FSI (φR_0), consequentemente foi observado declínio de 80% no desempenho fotossintético (PI_{ABS} e PI_{TOTAL}) e aumento de mais de 80% de energia perdida na forma de calor (DI_0/RC e φD0). Enquanto clorimurom-etílico, imazapique + imazapir e etoxissulfurom apresentaram sutil redução nos parâmetros φE_0 e φR_0, ocasionando redução de 40%, 40% e 20% no desempenho fotossintético (Figura 3).

Aos 35 dias, o herbicida metsulfurom-metílico levou a 20% de redução nos parâmetros φE_0 e φR_0 e acentuada redução (60%) no desempenho fotossintético, aumento de 80% na dissipação de energia na forma de calor (Figura 3). Esses resultados confirmam a sensibilidade do biotipo frente ao herbicida que ocasionou 90% de controle aos 35DAA (Figura 1). Os herbicidas clorimurom-etílico e etoxissulfurom mostraram recuperação em todos os parâmetros fotossintéticos, com exceção do desempenho fotossintético que apresentou redução de 40%. Enquanto as plantas sob aplicação da mistura comercial imazapique + imazapir mostrou completa estabilização dos parâmetros fotossintéticos (Figura 3). O herbicida cloransulam controlou 100% as plantas e por isso não há dados de fluorescência aos 35DAA (Figura 1).

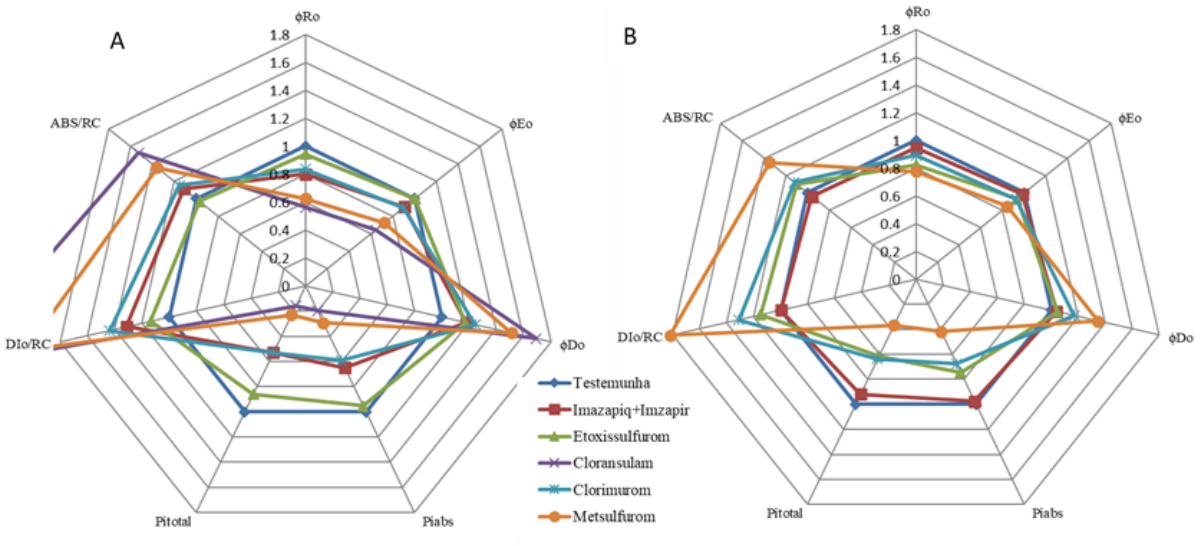

Figura 3. Intensidade de fluorescência da clorofila a obtida para a espécie *Conyza sumatrensis* em estádio de 12-15 cm, aos 14 DAA (A) e 35 DAA (B). Parâmetros do Teste JIP obtidos após aplicação dos tratamentos com herbicidas inibidores da ALS, expresso em relação aos valores do tratamento testemunha (valor transformado para 1). Seropédica-RJ, 2021.

5.6 CONCLUSÃO

Os herbicidas cloransulam-metílico, metsulfurom-metílico, clorimuron-etílico, imazapique + imazapir, etoxissulfurom apresentaram controle satisfatório do biotipo de buva suscetível a herbicidas avaliado. Enquanto para o biotipo resistente apenas os herbicidas cloransulam-metílico e metsulfurom-metílico demostraram controle satisfatório.

Os resultados sugerem que o biotipo de buva com múltipla resistência aos herbicidas 2,4-D, paraquat, saflufenacil, glifosato e diuron pode também apresentar resistência aos herbicidas inibidores ALS clorimuron-etílico, imazapique + imazapir e etoxissulfurom, devendo ser conduzidos novos estudos para confirmar a hipótese.

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6 CAPÍTULO IV

SUMATRAN FLEABANE (*Conyza sumatrensis*) RESISTANT TO PSI-INHIBITING HERBICIDES AND PHYSIOLOGICAL RESPONSES TO PARAQUAT

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6.1 RESUMO

O manejo de plantas daninhas resistentes a herbicidas é um dos maiores desafios agrícolas. Por isso, a rápida detecção e entendimento sobre a dinâmica de dissipação de energia fotossintética em plantas resistentes é extremamente importante para o manejo proativo. O objetivo deste estudo foi avaliar a resistência cruzada a herbicidas inibidores de PSI (paraquat e diquat) de *Conyza sumatrensis* e sua resposta fisiológica à aplicação de paraquat. A pesquisa foi realizada com dois biótipos de *C. sumatrensis,* um suscetível e outro com resistência múltipla a herbicidas de cinco mecanismos de ação. Um ensaio de dose-resposta foi realizado para avaliar a resistência cruzada ao diquat em um biótipo de *C. sumatrensis* previamente reportado resistente ao paraquat. As atividades enzimáticas da superóxido dismutase (SOD), catalase (CAT) e ascorbato peroxidase (APX), teor de peróxido de hidrogênio (H_2O_2) e fluorescência da clorofila *a* foram medidas em ambos os biótipos após a aplicação do paraquat. O ensaio de dose-resposta confirmou a resistência a *C. sumatrensis* ao diquat com fator de resistência de 25,6 vezes e 6,35 vezes para os valores LD_{50} e GR_{50}, respectivamente, em comparação com o biótipo suscetível. O acúmulo de H_2O_2 ocorre mais rapidamente no biótipo suscetível ao paraquat do que no resistente. O biótipo resistente ao paraquat tratado não mostrou aumento nas atividades de CAT, SOD e APX em comparação com o biótipo resistente não tratado. Além disso, a atividade de SOD e APX foi maior no biótipo sensível ao paraquat tratado em comparação com o não tratado, 5 horas após a aplicação (HAA). A fluorescência da clorofila *a* forneceu informações adequadas sobre o processo fotossintético que pode ser usado para diagnosticar a resistência ao paraquat 24 HAA. Há resistência ao diquat no biótipo de *C. sumatrensis* resistente ao paraquat. O biótipo resistente ao paraquat não induz as enzimas antioxidantes, como um possível mecanismo de resistência ao paraquat, mas mostra rápida recuperação dos parâmetros fotossintéticos e crescimento contínuo quando submetido ao paraquat, enquanto o biótipo suscetível não sobrevive a aplicação do herbicida paraquat.

Palavras-chave: Enzimas antioxidantes. Fluorescência da clorofila *a*, Dano oxidativo, *Conyza sumatrensis*

6.2 ABSTRACT

Herbicide-resistant weed management is one of the greatest agricultural challenges in crop production. Thus, the quick identification of herbicide-resistant weeds is extremely important for management. This study aimed to evaluate resistance to PSI-inhibiting herbicides (diquat) and physiological response to paraquat application in Sumatran fleabane [*Conyza sumatrensis* (Retz.) E. Walker; syn.: *Erigeron sumatrensis* Retz.]. The research was conducted with two *C. sumatrensis* biotypes, one susceptible and the other with multiple resistance to herbicides from five different modes of action (glyphosate, paraquat, diuron, saflufenacil, and 2,4-D). A dose–response assay was carried out to evaluate herbicide resistance to diquat in the paraquat-resistant *C. sumatrensis* biotype. The enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), hydrogen peroxide (H_2O_2) content, and chlorophyll a (Chl *a*) fluorescence were measured in both biotypes after paraquat (400 g a.i. ha^{-1}) application. The dose–response assay confirmed resistance of *C. sumatrensis* to diquat with resistance factor levels of 26-fold and 6-fold for LD_{50} and GR_{50} values, respectively, compared with the susceptible biotype. Accumulation of H_2O_2 occurred more rapidly in the paraquat-susceptible biotype than in the resistant one. Paraquat treatment caused an increase in SOD and APX activity in the susceptible biotype, but antioxidant enzyme activities were unaffected by paraquat in the resistant one at 5 h after application (HAA). Chl *a* fluorescence increased across the first 4 HAA in both resistant and susceptible biotypes. However, at 24 HAA, the resistant biotype showed a decline in fluorescence close to untreated plants, while the susceptible biotype died, confirming resistance to diquat in the paraquat-resistant *C. sumatrensis* biotype. The paraquat-resistant biotype does not induce antioxidative enzymes, as a possible mechanism of resistance to paraquat, but shows rapid recovery of photosynthesis and continuous growth when subjected to paraquat, while the paraquat-susceptible biotype does not survive.

Keywords: Antioxidative enzymes, Chlorophyll *a* fluorescence, Oxidative damage, *Erigeron sumatrensis*.

6.3 INTRODUCTION

Herbicides have been the most effective tool for weed control worldwide. However, weed resistance to herbicides in the field is expanding and increasing the cost of crop production and weed management (NORSWORTHY et al., 2012). In Brazil, *Conyza* spp. represent one of the greatest concerns in agricultural production, particularly due to the development of resistance to multiple herbicides. The first report of Conyza spp. resistance was to glyphosate in 2005 (HEAP, 2021), and more recently in 2017, there was a report of Sumatran fleabane [*Conyza sumatrensis* (Retz.) E. Walker; syn.: *Erigeron sumatrensis* Retz.] having multiple resistance to five different herbicides (glyphosate, paraquat, diuron, saflufenacil, and 2,4-D) (PINHO et al., 2019). Early diagnosis of herbicide resistance is essential for weed management.

In Brazil, there have been no reports of cross-resistance of photosystem I (PSI)-inhibiting herbicides (paraquat and diquat) in *Conyza* spp. Until now, all reports were about only paraquat resistance. Paraquat and diquat are PSI inhibitors that act as electron acceptors by diverting electrons from PSI to molecular oxygen, leading to reactive oxygen species (ROS) production (HESS, 2000). High ROS production induces rapid cell death in plants within a few hours after herbicide application (HAWKES, 2014). These herbicides are nonselective and are used in preplant burndown of many cropping systems due to their rapid action (Bromilow 2004).

The paraquat-resistance mechanism is usually due to reduced translocation attributed to vacuole sequestration (HAWKES 2014; MORETTI; HANSON 2017). Non–target site resistance occurs through mechanisms that reduce the number of herbicide molecules that reach the herbicide target site. Mechanisms that trap the herbicide in source leaves through sequestration within vacuoles or alter the activity of active membrane transporters will reduce the total amount of herbicide translocated, thus conferring resistance to plants (GAINES et al., 2020). Furthermore, the protective antioxidative system can also contribute to resistance by detoxifying ROS production (HAWKES, 2014; PYON et al., 2004; YE; GRESSEL, 2000). In plants, as in other organisms, the antioxidative system is composed of an efficient set of enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GILL; TUTEJA, 2010). This antioxidative system can be activated/induced in response to stressful conditions caused by increased ROS in cells (FOYER et al. 1994; GILL; TUTEJA 2010). Like ROS production, changes in photosynthetic capacity in response to herbicide stress can also be measured (DAYAN; ZACCARO, 2012; KALAJI et al., 2014; LEAL et al., 2020; STRASSER; STRASSER, 1995; STRASSER et al., 2004) and can be useful to identify resistant and susceptible biotypes (BRUNHARO et al., 2016), including in *Conyza* spp. biotypes.

Changes in chlorophyll fluorescence occur due to impairment in the electron flow in the photosynthetic electron transport chain (ETC) caused by herbicides (DAYAN; ZACCARO, 2012), including paraquat. The chlorophyll fluorescence technique has been applied in herbicide assays and diagnostics of resistance to PSI-inhibiting herbicides, as well as herbicides with other modes of action in several weed species (DAYAN; ZACCARO, 2012). Chlorophyll fluorescence data enable the description of the effects on photosynthetic energy dynamics after PSI-inhibiting herbicide application, providing information on the conformation, structure, and function of the photosynthetic apparatus (KALAJI et al., 2014; STRASSER et al., 1995).

The chlorophyll a (Chl *a*) fluorescence technique can be used to quickly screen for resistance to PSI-inhibiting herbicides (DAYAN; ZACCARO, 2012; HASSANNEJAD et al., 2020), because susceptible plants rapidly show great disorder in the photosynthetic apparatus and die within hours after herbicide application, while resistant plants survive and show

recovery of the dynamic energy fluxes of ETC within a day, depending on the herbicide mode of action. Brunharo et al. (2016) reported that in resistant biotypes, paraquat affects photosynthetic performance until the molecules are trapped by the mechanism of action operating in plant cells. Therefore, this study aimed to evaluate *C. sumatrensis* resistance to PSI-inhibiting herbicide (diquat) and to characterize the physiological response (Chl *a* fluorescence and antioxidative enzyme activity) to paraquat application.

6.4 MATERIAL AND METHODS

The research was conducted with two Sumatran Fleabane (*Conyza sumatrensis)* biotypes, one susceptible and the other with multiple resistance to five mode of action herbicides (2,4-D, paraquat, diuron, saflufenacil, and glyphosate) (PINHO et al., 2019). Both biotypes were originally collected from a site at Assis Chateaubriand-Paraná, Brazil (24.282611°S, 53.513°W). The seeds of both biotypes were sown in 2.5-dm^{-3} pots filled with commercial substrate and kept in greenhouses. After emergence, the seedlings were thinned to 1 plant per pot and left to grow to 10 cm in height. Herbicides (described in the following section) were applied using a CO_2-pressurized backpack sprayer with four XR-110020 flat-fan nozzles (TeeJet®, Spraying Systems Co., Wheaton, IL, USA), spraying 150 L ha^{-1} at 240 kPa.

***Conyza Sumatrensis* Control Assays**

The experiments were complete randomized blocks with a five by two factorial scheme and four replications. Factor A was represented by the following herbicides in recommended field doses: paraquat (400 g a.i. ha^{-1}; Gramoxone®, 200 g a.i. L^{-1}, Syngenta Brazil, São Paulo, SP, Brazil) plus 0.1% (v/v) nonionic surfactant; diquat (400 g a.i. ha^{-1}; Reglone®, 200 g a.i. L^{-1}, Syngenta Brazil) plus 0.5% (v/v) nonionic surfactant; diuron (1,600 g a.i. ha^{-1}; Diuron Nortox®, 800 g a.i. kg^{-1}, Nortox, Arapongas, PR, Brazil); and paraquat + diuron (400 + 200 g a.i. ha^{-1}; Gramocil®, 200 þ100 g a.i. L^{-1}, Syngenta Brazil) plus 0.1% (v/v) nonionic surfactant; and an untreated check. Factor B was the susceptible and resistant biotypes. At 42 d after application (DAA), the aerial parts of the plants were harvested, separated into paper bags, and dried in a forced-air circulation oven (60 ± 5 C) until a constant mass was obtained, after which dry mass was determined using an analytical balance.

Dose-Response Curve Assays

A dose–response assay was carried out in a randomized block design with four replications. The treatments consisted of doses of diquat herbicide at 1/32×, 1/16×, 1/8×, 1/4×, 1/2×, 1× (400 g a.i. ha^{-1}), 2×, 4×, 8×, 16×, and 32× plus 0.1% (v/v) nonionic surfactant sprayed on both biotypes (susceptible and suspected diquat resistant) and included an untreated check (without herbicide). Plant injury (LD) and reduction in plant growth (GR) were measured at 42 DAA using log-logistic models proposed by Streibig (1988) and Seefeldt et al. (1995) (Equation 1):

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)^{c}\right]}$$

where: *y* is the response based on dry mass, *a* is the amplitude between the maximum and minimum points of the variable, *x* is the dose of the herbicide (g a.i. ha^{-1}), *b* is the herbicide dose giving a 50% response (GR_{50} and LD_{50}) and *c* is the inflection point around *b*

The inverse equation was used to calculate the GR_{50} and LD_{50} (Equation 2):

$$:x = b\left(\left|\frac{a}{y} - 1\right|\right)^{\frac{1}{c}}$$

Resistance index (RF= R/S) was calculated based on the values of LD_{50} (plant injury) and GR_{50} (dry mass).

Physiological Response Assays

A second experiment was carried out in a randomized block design with four replications. The herbicide paraquat at 400 g a.i. ha^{-1} plus 0.1% (v/v) nonionic surfactant was sprayed on susceptible and resistant biotypes. Subsequently, at 1, 3, and 5 h after application (HAA), the leaves were harvested by clipping the base of the leaf at the end of the petiole, immediately frozen in liquid nitrogen, and temporarily stored at −80 C until analyses of enzyme activity and hydrogen peroxide were performed.

The activity of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and APX (EC 1.11.1.11) was measured in leaves (±0.2 g) of *C. sumatrensis*. In order to perform the analysis, the tissues were ground using liquid N2 in porcelain mortars (by replicates) with 5% polyvinyl polypyrrolidone and a mix of 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid, 20 mM ascorbic acid, 5 mM dithiothreitol, 5 mM β-mercaptoetanol, and 0.01% Triton X-100. The homogenate was centrifuged at 12,000 × g for 20 min at 4 C, and the supernatant was used as a crude enzyme extract. An aliquot of the crude extract was used to determine protein content as described by Bradford (1976) using bovine serum albumin as the standard. Total SOD activity was measured as described by Giannopolitis and Ries (1977), the CAT activity according to Azevedo-Neto et al. (2006), and APX activity according to Nakano and Asada (1981).

Hydrogen peroxide content was estimated based on Velikova et al. (2000). A solution of 0.1% trichloroacetic acid was added to leaf tissue (±0.2 g) and ground using liquid N_2 in porcelain mortars. Samples were then centrifuged at 12,000 × g for 20 min at 4 °C. An aliquot of the supernatant was added to 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide to determine the H_2O_2 content. Absorbance was measured at 390 nm. The H_2O_2 content was given on a standard curve prepared with known H_2O_2 concentrations.

The Chl *a* fluorescence transient was measured in dark-adapted leaves of resistant and susceptible biotypes at 1, 4, and 24 h and 14 d after paraquat application, using a Handy-PEA fluorimeter (Hansatech Instruments Ltd, King's Lynn, Norfolk, UK), as described by Strasser and Strasser (1995) and Strasser et al. (2004). The plotted fluorescence values were the means of eight measurements of each treatment. The JIP test was also applied to analyze and compare the OJIP transients using normalizations and subtractions to compare the samples for the events reflected in the OJ, OI, and IP phases, as described by Yusuf et al. (2010). The OJIP fluorescence transients were based on the polyphasic fast fluorescence rise from the lowest intensity FO (minimum fluorescence, the O level) to the highest intensity FM (maximum fluorescence, the P level) with two intermediate steps labeled J and I (Strasser et al. 2004). The transients were normalized as relative variable fluorescence: $Wt = (Ft - F0)/(FJ - F0)$, $WOI = (Ft - F0)/(FI - F0)$ and $WIP = (Ft - FI)/(FP - FI)$, as described by Yusuf et al. (2010).

Statistical Analysis

The control assay data, enzymatic activity, and hydrogen peroxide measurements were checked for normality (Shapiro-Wilk) and homogeneity (Bartlett) of variance, and then ANOVA was performed. Data were analyzed using the GLM procedure to evaluate the differences between treatments. When F was significant ($P \leq 0.05$), the averages were separated and adjusted using Fisher's protected LSD ($P \leq 0.05$). The dose–response data of plant injury (LD_{50}) and reduction in plant growth (GR_{50}) were estimated using the three-parameter log-logistic equation proposed by Streibig (1988) and Seefeldt et al. (1995). Statistical analyses were performed using SAS v. 9.0 statistical software (SAS Institute, Cary, NC).

6.5 RESULTS AND DISCUSSION

Control Assay

The control assays at recommended field doses showed that the susceptible biotype was effectively controlled by paraquat, diquat, diuron, and paraquat + diuron (Figure 1A). However, the resistant biotype was significantly less affected by all herbicides (Figure 1A). The dry-mass analysis of the *C. sumatrensis* resistant biotype when paraquat or diuron was used was similar to paraquat + diuron (Figure 1A). A previous report by our group confirmed that the *C. sumatrensis* biotype showed resistance to paraquat (resistance index of LD50 = 25.51 and GR_{50} = 51.83) and diuron (resistance index of LD_{50} = 7.29 and GR_{50} = 5.05) (PINHO et al., 2019). However, in this study, we first report the behavior of this biotype with paraquat and diuron in a tank mix, and this is the first report of *C. suma*trensis resistance to diquat in Brazil, as diquat did not control the resistant biotype in our study (Figure 1A and B).

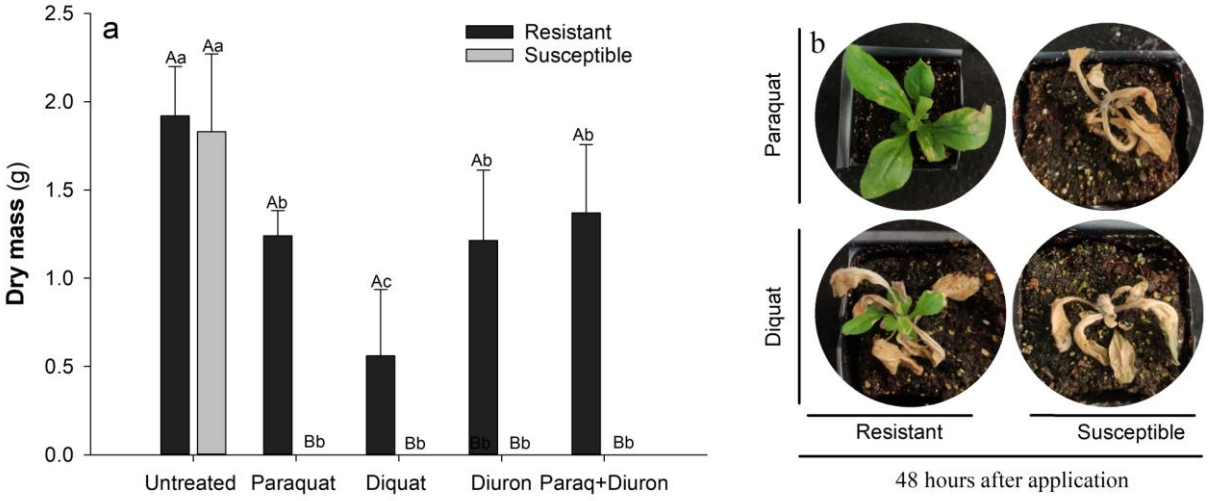

Figure 1. Dry mass (g) of resistant and susceptible *Conyza sumatrensis* biotypes in response to the application of paraquat, diquat, diuron, and paraquat þ diuron herbicides at 42 d after application (A) and comparation of resistant and susceptible *Conyza sumatrensis* biotypes in response to the application of paraquat and diquat at 48 h after application (B). Means followed by the same uppercase letters between biotypes and lowercase letters among herbicides do not differ statistically from each other at $P \leq 0.05$ by LSD test

The symptoms observed in resistant plants after the application of diquat were completely different from those seen with paraquat (Supplementary Video S1). Whereas plants showed complete desiccation and regrowth in the axillary meristem a few days after diquat application, paraquat resulted in only a few necrotic spots on leaves (Figure 1B).

Diquat Dose-Response

Based on LD_{50} and GR_{50} values, the resistance index to diquat was 26-fold and 6-fold, respectively, when compared with the susceptible biotype (Table 1; Figure 2). The dose–response curve confirmed resistance to the PSI-inhibiting herbicide diquat in the previously reported multiple-resistant *C. sumatrensis* biotype.

Populations resistant to paraquat are usually also resistant to diquat, another bipyridilium herbicide, but at reduced levels (PRESTON, 1994), as observed in the present study. *Conyza sumatrensis*, hairy fleabane [*Conyza bonariensis* (L.) Cronquist; syn.: *Erigeron*

bonariensis L.], and horseweed [*Conyza canadensis* (L.) Cronquist; syn.: *Erigeron canadensis* L.] biotypes with resistance to diquat and paraquat have been reported in Japan and Canada (HEAP, 2021). This is the first case of *C. sumatrensis* with resistance to diquat, as well as paraquat, in Brazil.

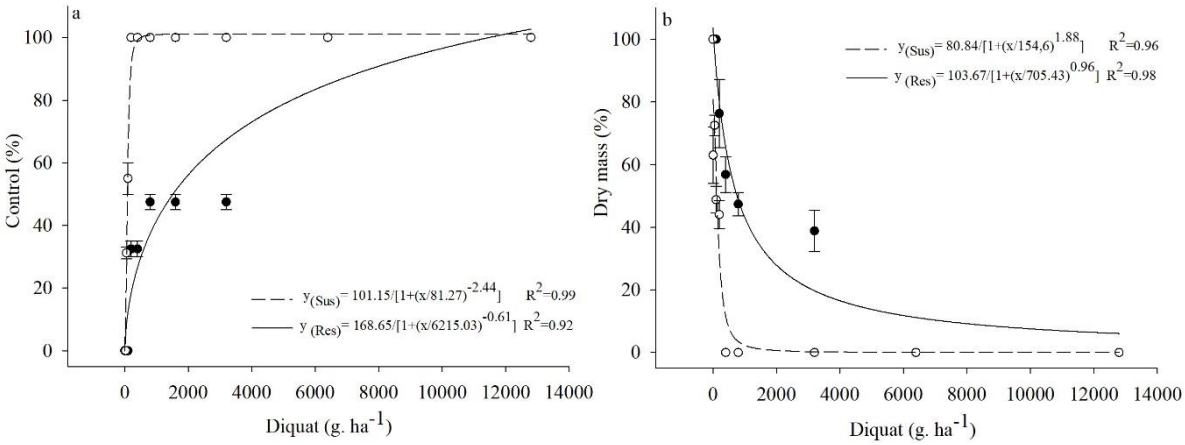

Figure 2. Diquat dose–response curves for multiple-resistant (2,4-D, paraquat, diuron, saflufenacil and glyphosate) (Res) and susceptible (Sus) *C. sumatrensis* biotypes. Plant dry mass (A) and percent of control (B) were obtained at 42 days after application. Vertical bars represent the standard error of the mean.

Table 1. Parameter estimates of diquat dose–response curves indicating the herbicide dose that led to a 50% reduction of dry mass (GR_{50}) and 50% reduction of control (LD_{50}) for multiple-resistant and susceptible Conyza sumatrensis biotypes determined at 42 DAA.

Values	Resistant	Susceptible	RF[b]
[a]LD_{50}	1522.66	60.75	25.06
[a]GR_{50}	759.41	119.56	6.35

[a]LD_{50}: herbicide dose causing 50% control of plants; $_{GR50:}$ herbicide dose causing 50% growth reduction of plants.
[b]Resistance levels were indicated by the resistance factor (RF). RF (resistance factor) = R/S.

Physiological Responses of *Conyza sumatrensis* Biotype Resistant to Paraquat

The paraquat-susceptible biotype developed typical symptoms within the first few hours after spraying. Among the symptoms quickly exhibited by plants in response to PSI inhibitors are the appearance of brown, desiccated, or chlorotic tissue and, eventually, complete necrosis of the leaf (HAWKES, 2014). At 3 and 5 HAA, the paraquat-susceptible biotype was injured within a range of 70% to 80%, with 100% control at 24 HAA (Figure 3). However, the paraquat-resistant biotype showed ≤20% injury from 3 to 48 HAA, resulting in only a few leaves with necrotic spots (Figure 3).

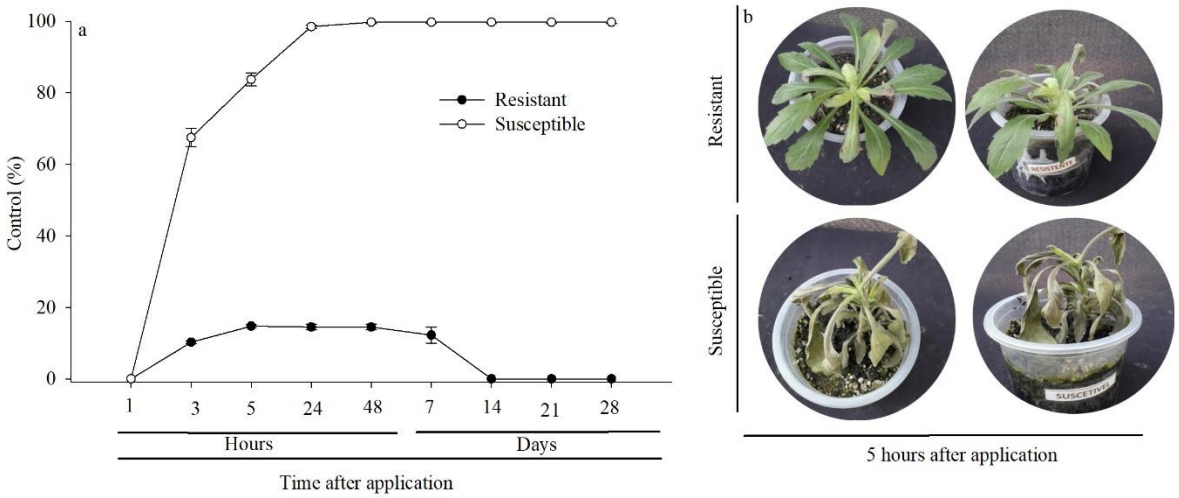

Figure 3. Control (A) and symptoms observed (B) in paraquat-resistant and -susceptible *C. sumatrensis* biotypes treated with paraquat.

Normally, PSI-inhibiting herbicides cause the death of plants within a few days after application due to oxidative damage induced by ROS. Paraquat exerts phytotoxic effects by generating superoxide radicals, which in turn produce different ROS–H_2O_2 and hydroxyl radicals (BROMILOW, 2004).

The accumulation of H_2O_2 in the paraquat-susceptible biotype was higher at all time points in comparison to the paraquat resistant biotype (Figure 4). Also, at 3 and 5 HAA, there was accumulation of H_2O_2 in leaves of the paraquat-susceptible biotype compared with the untreated check, while whereas the paraquat-resistant biotype showed a difference between treated and untreated plants only at 5 HAA (Figure 4). In the paraquat-susceptible biotype, ROS have been identified as the main cause of plant death within a few hours after herbicide application (HESS, 2000), whereas the paraquat-resistant biotype showed lower H_2O_2 concentration and less visible injury in the present study.

The overproduction of ROS in plants is generated by several abiotic or biotic stresses (CAVERZAN et al., 2019; GILL; TUTEJA, 2010), such as those caused by paraquat reported in this study. When there is an imbalance between ROS production and the detoxification capacity of the enzymatic antioxidant system, oxidative damage occurs (GILL; TUTEJA, 2010). Overproduction of ROS in plants causes damage to DNA, proteins, and lipids, which can lead to cell death (CAVERZAN et al., 2019).

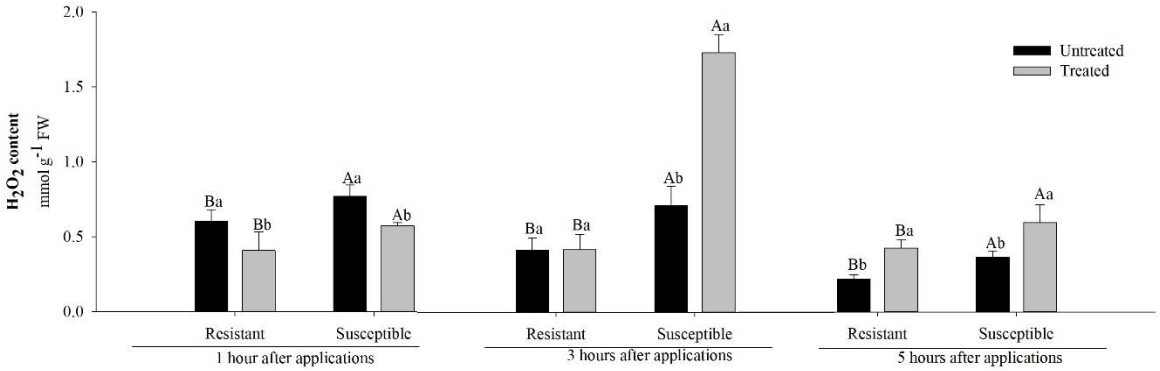

Figure 4. Changes in hydrogen peroxide (H_2O_2) concentration in paraquat-resistant and paraquat-susceptible *Conyza sumatrensis* biotypes in response to paraquat at 1, 3, and 5 h after application. Means followed by the same lowercase letter between untreated and treated and uppercase letters between biotypes do not differ statistically from each other at $P \leq 0.05$ by LSD test at each time poin.

Most of the plant distress caused by herbicides is related to ROS generation and consequent oxidative stress (CAVERZAN et al., 2019). However, some reports have demonstrated the involvement of antioxidant systems in ROS detoxification in herbicide-resistant weeds (CAVERZAN et al., 2019; HAWKES, 2014; PIASECKI et al., 2019; PYON et al., 2004; YE; GRESSEL, 2000).

The paraquat-resistant biotype did not show an increase in the activity of CAT, SOD, and APX compared with the untreated check (Figure 5). Moreover, SOD and APX activity at 5 HAA was significantly higher in the paraquat-susceptible biotype compared with the untreated control. In this biotype, the increase observed in SOD at 5 HAA is more likely a response to the superoxide initially produced by paraquat, as reported by Niwa et al. (1990). The increase in APX may be a response to H_2O_2 produced after paraquat application. SOD enzymes are at the frontline in the defense against ROS, promoting the dismutation of the superoxide radical into H_2O_2 (AZEVEDO NETO et al., 2006; GILL; TUTEJA, 2010). APX and CAT play a key role in catalyzing the conversion of H_2O_2 to H_2O (APEL; HIRT, 2004).

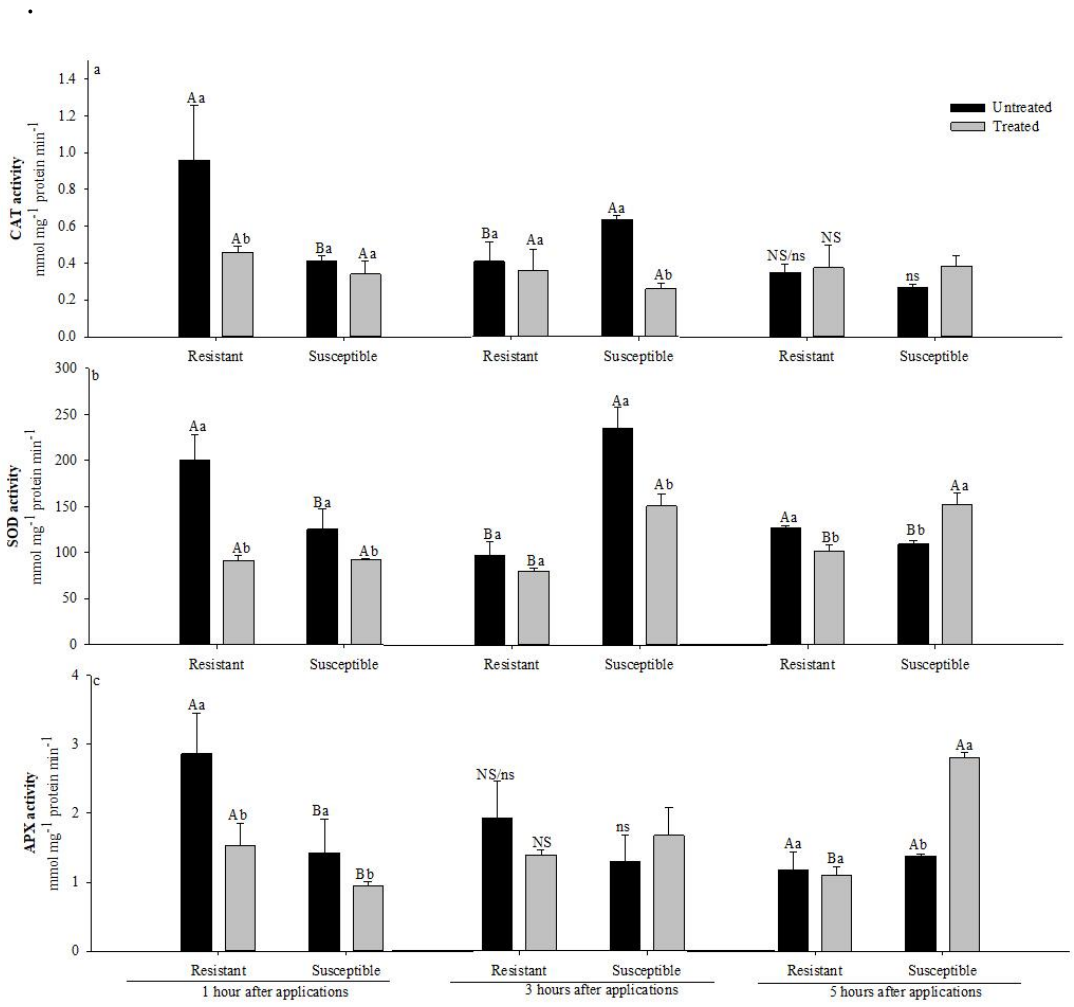

Figure 5. Change in catalase (CAT; A), superoxide dismutase (SOD; B), and ascorbate peroxidase (APX; C) activity in paraquat-resistant and paraquat-susceptible *Conyza sumatrensis* biotypes following treatment with paraquat herbicide at 1, 3, and 5 h after application. Means followed by the same uppercase letters between biotypes and lowercase letters between untreated and treated do not differ statistically from each other at $P \leq 0.05$ by LSD test at each time point.

The high activity of CAT, APX, and SOD may be corelated with the mechanism of resistance to paraquat (PYON et al., 2004; YE; GRESSEL; 2000). However, this experiment suggests that CAT, APX, and SOD may not provide antioxidative protection against oxidative damage caused by paraquat to the paraquatresistant *C. sumatrensis* biotype, because the activity of these enzymes decreased or showed no difference in comparison with the untreated control. This result suggests that CAT, APX, and SOD are not involved in this *C. sumatrensis* biotype's resistance to paraquat. The antioxidant enzyme activity (SOD and APX) was greater in the paraquat-susceptible biotype than in the paraquat-resistant biotype at 5 HAA. However, the paraquat-resistant biotype maintained other oxidative stress coping mechanisms, so antioxidant enzyme activity may not be directly involved in paraquat's mechanism of action, as reported in studies of Tsuji et al. (2013).

Paraquat acts as an electron acceptor by diverting them from PSI, leading to changes in the electron flow in the ETC of photosynthesis (BROMILOW, 2004), which can be monitored

by Chl *a* fluorescence measurement (STRASSER et al., 1995, 2004). Chl *a* fluorescence transient analysis provides detailed information on the structure and function of the photosynthetic apparatus from light absorption and electron transport to energy production (STRASSER et al., 1995). After application of paraquat herbicide, there was a decrease in quantum yield (a measure of the efficiency of photon emission as defined by the ratio of the number of photons emitted to the number of photons absorbed) of electron transport from quinine A (QA−) to the terminal electron acceptor of the PSI (ϕRo) (Figure 6A), and in the IP phase (Figure 7I), WIP shows that PSI-driven electron transfer occurs to the end electron acceptors on the PSI acceptor side, starting at PQH_2 (plastoquinol).

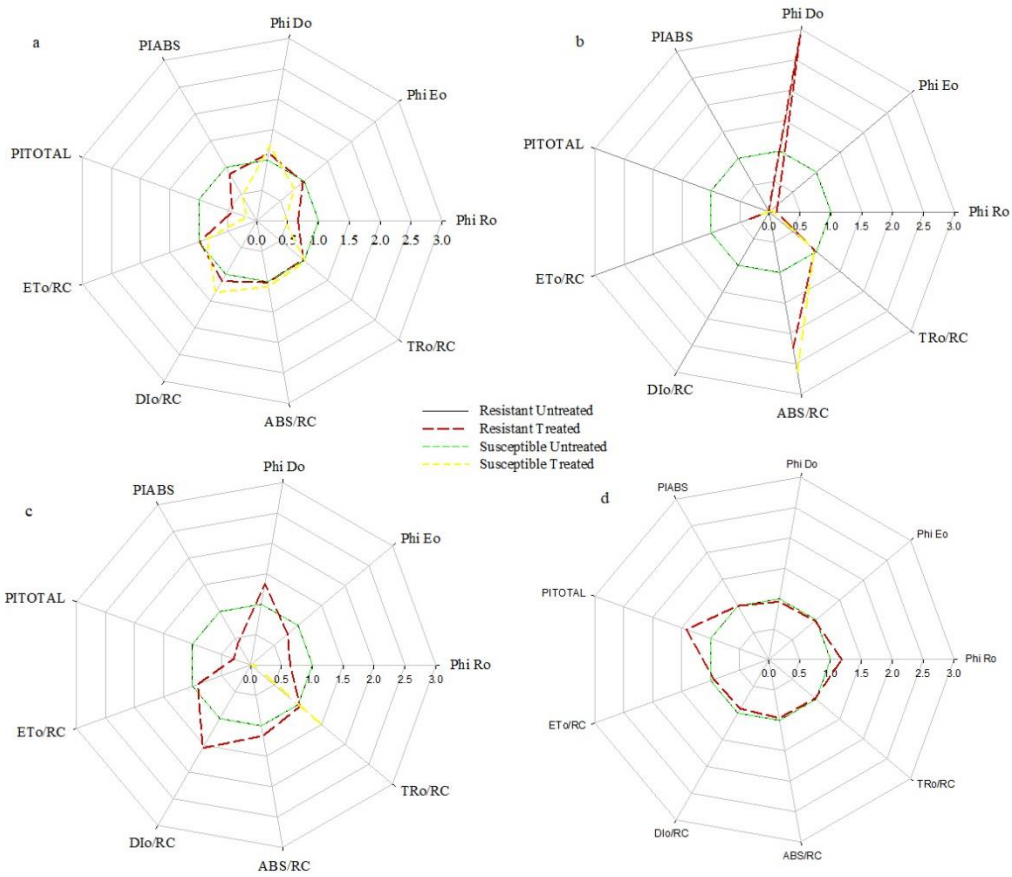

Figure 6. Chlorophyll a fluorescence transient of paraquat-resistant and paraquat-susceptible *Conyza sumatrensis* biotypes treated with paraquat at 1 h after application (HAA) (A), 4 HAA (B), 24 HAA (C), and 14 d after application (D). Data correspond to nine structural and functional photosynthetic parameters (average values of 10 replicates) derived by the JIP test from the fluorescence transients. For each parameter and for both biotypes, the values were normalized, using the control as reference (untreated, black lines), presented in the panels by a regular polygon (all parameters equal to unity). The deviation of the behavior pattern (treated biotypes) from the regular polygon demonstrates the fractional impact compared with the untreated control. TR0/RC, maximum trapping rate per reaction center (RC); ABS/RC, absorption flux (of antenna chlorophyll) per RC; DI0/RC, dissipation of an active RC; ET0/RC, electron transport of an active RC; PIABS, performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PItotal, performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors; ϕDo, maximum quantum yield of nonphotochemical de-excitation; ϕEo, quantum

yield for electron transport (ET); ϕRo, quantum yield for reduction of end electron acceptors at the PSI acceptor side.

A decline in photosynthetic total performance index (PItotal: performance index [potential] for energy conservation from the exciton to the reduction of PSI end acceptors) and an increase both in energy dissipation flux as heat (DI0/RC [reaction center]) (Figure 6A) and relative variable fluorescence (Figure 7A) were observed for both biotypes at 1 HAA. The photosynthetic performance index (PIABS: performance index [potential] for energy conservation from exciton to the reduction of intersystem electron acceptors) decreased 60% in the paraquat-susceptible biotype compared with the paraquat-resistant biotype, indicating that PIABS was a good parameter for detecting stress caused by paraquat. A previous study showed the same results in common cocklebur (*Xanthium strumarium* L.) (HASSANNEJAD et al., 2020). The photosynthetic performance index can be a very suitable and sensitive parameter for investigating a plant's overall photosynthetic efficiency under different abiotic stresses (KALAJI et al., 2014; STRASSER et al., 1995, 2004). Szigeti et al. (1996) observed differences in chlorophyll fluorescence characteristics of paraquat-sensitive *C. canadensis* biotypes using a multichannel chlorophyll spectrofluorometer.

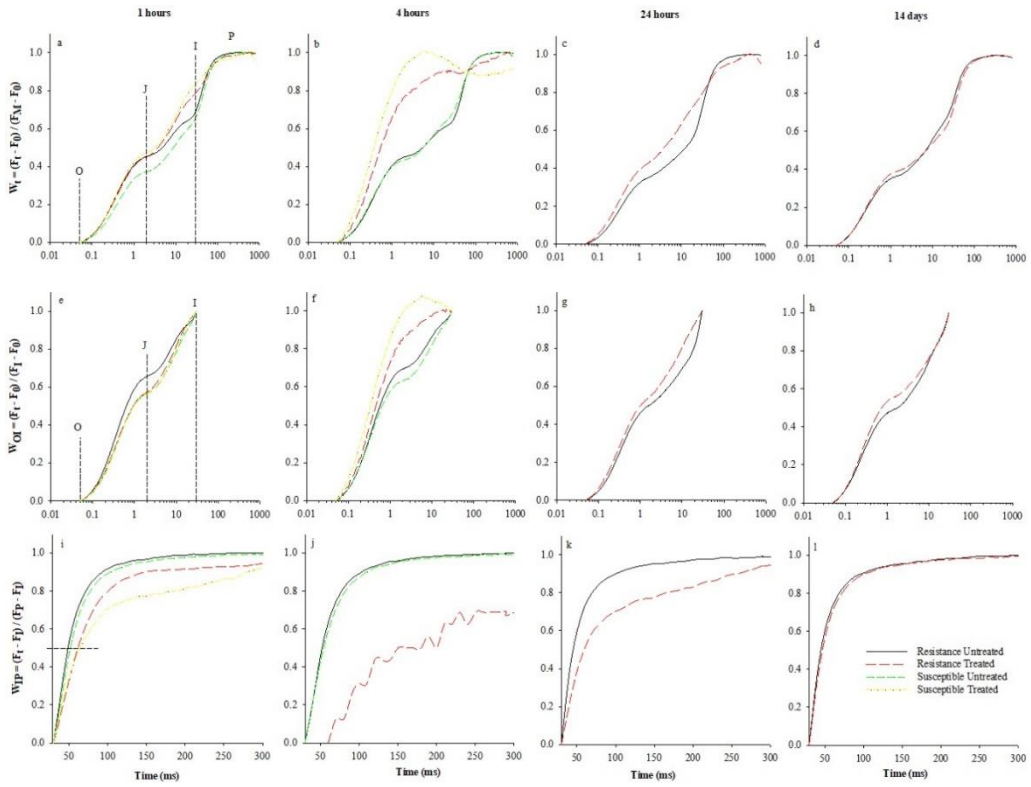

Figure 7. Chlorophyll a fluorescence transient of paraquat-resistant and paraquat-susceptible *Conyza sumatrensis* biotypes at 1 h after application (HAA) (A, E, and I), 4 HAA (B, F,and J), 24 HAA (C, G, and K), and 14 d after application (D, H, and L) of paraquat. Data correspond to: the photosynthetic parameters deduced by the JIP test analysis of the fluorescence transients normalized using the reference the control (Wt) (A–D); and the relative variable fluorescence between steps O and I (WOI) (E–H) and steps I and P (WIP) (I–L) on a logarithmic timescale.

At 4 HAA, there was an increase in the excitation captured by the RCs until the reduction of plastoquinone (PQ) (OI phase; WOI presented as relative variable fluorescence

show the sequence of events from exciton trapping [energy captured by the RC capable to drive photochemistry reactions] by photosystem II (PSII) up to PQ reduction]) in both biotypes (Figure 7F). Nevertheless, the intensity of fluorescence levels in J step (parameters derived from the OJIP steps; Figure 7B) demonstrated the interruption of the electron flow due to PSI-inhibiting herbicide, which suggests that most of the QA is completely reduced (STRASSER et al., 1995) in both biotypes. Decrease of 100% in quantum yield of electron transport from QA^- to the intersystem electron acceptors (ϕEo), ϕRo (Figure 6B), and IP phase (Figure 7J) were detected, resulting in a 100% reduction of photosynthetic performance. Furthermore, all energy was dissipated as heat (ϕDo) (Figure 6B) and variable fluorescence (Figure 7B).

Besides the decrease in functionality (PI) in the resistant biotype, the structural components of photosystems might not be affected, allowing the biotype to recover within 24 HAA. Although the mechanism of resistance in this biotype remains to be elucidated, it is possible to suggest that vacuolar sequestration of paraquat is mediated by transporters that can operate to compartmentalize paraquat in the cell, precluding its action on PSI (BRUNHARO; HANSON, 2017). On the other hand, besides losses of functionality in the susceptible biotype, the increase in ROS probably affected structural components leading to death in this biotype within 24 HAA (Figures 6C and 7G–K).

In addition to the recovery of the functionality of the photosynthetic ETC in the paraquat-resistant biotype within 24 HAA (Figures 6C and 7G–K), photosynthesis was completely stabilized within 14 DAA (Figures 6D and 7D–I). Chl *a* fluorescence transient analysis could be applied to monitor for paraquat-resistant biotypes, as shown in this study. To determine whether paraquat was active within the plant cells, the photosynthetic performance was assessed after paraquat application using the parameters of fluorescence. In addition, the maximum quantum yield of PSII (Fv/Fm) has been reported to monitor paraquat effects on plants until paraquat is transiently in cells, which leads to a decline in Fv/Fm up to 5 HAA. The recovery of the photosynthetic performance was reported within 48 h, whereas Fv/Fm values in susceptible plants dropped to zero by 48 HAA. Therefore, paraquat reaches the chloroplasts of the resistant biotype, as indicated by the transitory inhibition of photosynthetic activity in the resistant biotype's leaves (BRUNHARO et al., 2016). Moreover, an alternative hypothesis of exclusion of paraquat from leaf cells has been proposed in hare barley (*Hordeum murinum* L.) (PRESTON, 1994). Paraquat exclusion would explain less damage to leaf cells and hence less translocation of paraquat (PRESTON et al., 2005).

Although the proper mechanism of *C. sumatrensis* resistance remains to be elucidated, the use of the Chl *a* fluorescence technique has been used in several studies regarding the effects of PSI-inhibiting herbicides on weeds (DAYAN; ZACCARO, 2012) and could provide information or early diagnosis of herbicide resistance and management of weeds, as Chl *a* fluorescence is a quick, easy, and nondestructive tool that can be also applied in the field. In addition, molecular analysis can provide insights into resistance mechanisms, especially those related to non-target sites, to understand how plants reduce the number of herbicide molecules that reach the herbicide target site.

Dose–response experiments confirmed cross resistance of an *C. sumatrensis* biotype to PSI-inhibiting herbicides (paraquat and diquat). In the paraquat-susceptible biotype, H_2O_2 accumulation led to plant death within a few hours after herbicide treatment, whereas the paraquat-resistant biotype showed lower H_2O_2 concentration, which resulted in the appearance of few symptoms. Paraquat application did not induce antioxidative enzymes in the paraquat-resistant biotype. However, the paraquat-resistant biotype showed a fast recovery of photosynthetic parameters, as well as continuous growth when subjected to paraquat, while the paraquat-susceptible biotype did not survive. Fluorescence parameters provided information

about the photosynthetic apparatus, enabling diagnosis of the paraquat-resistant biotype. Our results showed that it is possible to detect recovery of the paraquat-resistant biotype at 24 HAA, while the paraquat-susceptible biotype did not recover from the stress caused by paraquat.

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7 CAPÍTULO V

INHERITANCE OF PARAQUAT AND DIQUAT RESISTANCE IN *Conyza sumatrensis* FROM BRAZIL

7.1 RESUMO

O fluxo gênico é o movimento de genes entre e dentro de populações de plantas. Isso pode ocorrer através do movimento do pólen ou das sementes por mecanismos como vento, animais, água e implementos agrícolas. Compreender a base genética da resistência a herbicidas em pantas daninhas é fundamental para desenvolver modelos para prever, monitorar e gerenciar a resistência de planta daninhas a herbicidas. Os objetivos deste estudo foram identificar o tipo de herança genética para resistência ao paraquat e diquat em *Conyza sumatrensis*. O parental R usado nesse estudo, apresenta resistência confirmada aos herbicidas 2,4-D, saflufenacil, glyphosate, diuron, diquat e paraquat. As gerações F1 foram derivadas de cruzamentos parentais R (resistente) e S (suscetível), incluindo dois cruzamentos S♂ x R♀ (C4 e C5) e os três cruzamentos recíprocos R♂ x S♀ (C7, C8 e C9). A progênie F1 foi tratada com 1005 g a.e. ha^{-1} de 2,4-D quando as plantas tinham 10 a 12 cm de altura. Toda a progênie F1 sobreviveu a aplicação de 2,4-D, exceto o parental S, e foi autopolinizada para produzir a progênie F2. A progênie F2 e os parentais foram germinados e transplantados, e quando chegou no estádio de 10 a 12 cm de altura o C4-F2; C5-F2; C7-F2; C8-F2 e C9-F2 receberam a aplicação do paraquat nas doses de 0,25x, 0,5x, 1x (400 g a.e. ha^{-1}) e 2x), e C5-F2; C7-F2; C8-F2 receberam o diquat nas doses de 0,25x, 0,5x, 1x (400 g a.e. ha^{-1}). Ambos os herbicidas foram associados a 0,5% de tensoativo não iônico e o ensaio foi realizado com 32-48 repetições por tratamento. A resposta da progênie F2 foi classificada como plantas sobreviventes (resistente) ou plantas mortas (suscetível) aos 21 dias após a aplicação dos herbicidas. Em paralelo, vinte e nove indivíduos C9_F2 (sem aplicação de herbicida) foram autopolinizados para produzir a progênie C9_F3. Os experimentos com as 29 linhagens (F3) e parentais R e S foram conduzidos com 16 repetições por tratamento. Quando as plantas chegaram no estádio proposto, procedeu-se á aplicação do paraquat (0,5x e 1x). Um teste de ajuste qui-quadrado ($\chi 2$) foi usado para comparar as plantas sobreviventes/mortas observadas e esperadas com base no modelo de gene dominante (3:1) para o paraquat e dois genes dominantes e independentes (15:1) para o diquat. O modelo foi rejeitado se $p \leq 0,05$. A resistência ao paraquat em F2 e F3 foi baseada em um modelo de único gene dominante nas doses usadas. Enquanto os resultados do diquat foram baseados em dois genes dominantes e independentes (15:1). Nossos resultados ressaltam a necessidade e urgência de adotar estratégias integradas para controlar *Conyza sumatrensis* e inibir a evolução de novos casos de resistências.

Palavras-chave: Segregação, resistência múltipla, qui-quadrado, herdabilidade

7.2 ABSTRACT

Gene flow is the movement of genes between and within plant populations. This can occur through pollen or seed movement by mechanisms such as wind, animals, water and agricultural implements. Understanding the genetic basis of herbicide resistance in weeds is key to developing models for predicting, monitoring, and managing weed resistance. The aims of this study were to identify the type of genetic inheritance to paraquat and diquat resistance in *Conyza sumatrensis*. One susceptible (S) and resistant (R) biotypes of *Conyza sumatrensis* were used in this study. The multiple-resistant parental was confirmed to 2,4-D, saflufenacil, glyphosate, diuron, diquat and paraquat herbicides. F1 families were derived from R and S parental crosses, including two S♂ x R♀ (C4 and C5) and the three reciprocal crosses R♂ x S♀ (C7, C8 and C9) crosses. The F1 progeny were treated with 1005 g a.e. ha^{-1} of 2,4-D when plants were 10 to 12 cm tall. All F1 progeny survived with 2,4-D application, except S parental, and it was self-pollination to produce F2 progeny. The F2 progeny and parentals were germinated on the surface of soil, and it were transplanted. The doses 0, 0.25x, 0.5x, 1x (400 g a.e. ha^{-1}) and 2x for paraquat were applied in F2 progeny (C4-F2; C5-F2; C7-F2; C8-F2 and C9-F2), and the doses 0, 0.25x, 0.5x, 1x (400 g a.e. ha^{-1}) for diquat were applied in F2 progeny (C5-F2; C7-F2; C8-F2). Paraquat and diquat dose-response studies were conducted separately with R and S (parental). Both herbicides were mixed with 0.5% non-ionic surfactant. It was carried on with 32-48 replications per treatment. The response of F2 progeny was rated as survival (resistant) or dead (susceptible) at 21days after herbicides applications. Twenty-nine F2 individuals from C9_F2 (without herbicide application) were self-pollinated to produce C9_F3 progeny. The greenhouse experiments were carried on with 29 lines (F3) with 16 replications per treatment and R and S (parental) check. The doses were paraquat (0.5x and 1x) conducted separately with R and S (parental) as described previously. A chi-square goodness-of-fit test ($\chi 2$) was used to compare the observed and expected plant survival based on single dominant gene model by 3:1 (paraquat), two dominant genes (15:1) (diquat). The model was rejected if $P \leq 0.05$. The paraquat resistance in F2 and F3 was based on a single-dominant gene model at the herbicide rates used. While the diquat results was based on a two-dominant gene and two genes model (15:1) at the herbicide rates used. Our findings underscore the necessity and urgency of adopting integrated strategies to control *Conyza sumatrensis* and to inhibit the evolution of new herbicide-resistant.

Keywords: Segregation, multiple resistance, chi-square goodness-of-fit, heritability

7.3 INTRODUCTION

The first reported case of herbicide resistance in *Conyza* spp. was to paraquat in Japan in 1980 (HEAP, 2022). Today, there are 72 reported cases of resistance weeds to paraquat and 10 to diquat in the world, and 21 of these cases is related to the *Conyza* genus (HEAP, 2022).

The evolution of herbicide resistance in weeds is a function of several determinants, including mutation rate, initial frequency of the resistance gene, gene flow, selection pressure and the mode of inheritance (MAXWELL; MORTIMER, 1994).

Once a resistant weed has evolved, resistance alleles can spread via seed and pollen movement (GHANIZADEH et al., 2019). Thus, pollen can be transported long distances and could be passed and the gene flow between weed species may produce an impact on dissemination of herbicide resistance alleles (MAITY et al., 2022). Information on gene flow through pollen dispersion for self-pollinating plants, as *Conyza* spp. is lacking because the outcrossing ratio is low (HUANG et al., 2015). Although the gene flow through pollination is low compared with seed dispersion, gene flow from herbicide-resistant weeds through pollination has the potential to produce multiple herbicide-resistant weeds.

Conyza genus has been appointed as a complex polyploid, but differential ploidy may not be a restrictive factor for changes of alleles between *Conyza* plants (SOARES et al., 2015). Soares et al., (2015) reported the occurrence of hybridization between species from *Conyza* genus, and Henry et al., (2008) and Zelaya et al., (2007) reported an average 4% allogamy in *Conyza*.

Herbicide resistance in the majority of characterized by semi-dominance or dominance of inherited herbicide resistant alleles (GHANIZADEH et al., 2019). More complex scenarios include additive gene effects, where a single allele modulates the overall level of resistance. However, maternal (cytoplasmic) inheritance has also been documented for triazine-resistant weeds with the target-enzyme mutation mechanism of resistance (GHANIZADEH et al., 2019).

If resistance is controlled by a single gene, it will be represented by a typical Mendelian ratio of 3:1 for resistant to susceptible phenotype, or a 1:2:1 ratio for resistant, segregation R/S, and susceptible phenotypes, respectively (GHANIZADEH et al., 2019). However, if the F2 generation does not exhibit a Mendelian ratio of either a 1:2:1 (R:R/S:S) or 3:1 (R:S) for resistance, that is an indication that resistance may be controlled by multiple genes (PRESTON et al., 2009). When two dominant genes have the same effect on a trait, indicating duplicate effect, it leads to a segregation ratio of 15:1 (WEINBERG et al., 2006).

In majority of the studied examples, paraquat resistance is determined by single, nuclear-encoded genes with partial or full dominance, with a few examples of recessive, multigenic or cytoplasmic control (YU et al., 2009). A single, dominant, nuclear gene was suggested to be responsible for paraquat resistance in *Conyza* (SHAALTIEL et al., 1988) and in *Lolium rigidum* (YU et al., 2009)

Understanding the genetic basis of herbicide resistance in weeds is key to developing models for predicting, monitoring, and managing weed resistance. The aims of this study were to identify the type of genetic inheritance to paraquat and diquat resistance in *Conyza sumatrensis*.

7.4 MATERIAL AND METHODS

One susceptible (S) and resistant (R) biotypes of *Conyza sumatrensis* were used in this study. The multiple-resistant parental was confirmed by Pinho et al., (2019) to 2,4-D, saflufenacil, glyphosate, diuron and paraquat herbicides and by Leal et al., (2022) to diquat. It was original collection site from Assis Chateaubriand-Paraná, Brazil (24°16'53.8"S 53°30'47.5"W). Seeds were germinated on the surface of soil and it was transplanted in plastic pots containing soil mix. All plants were kept under greenhouse conditions at 24–28°C temperature, 80% relative humidity and 15-h photoperiod. The plants were watered daily and fertilizer two times on week until reproductive stage.

Progeny F1

The crosses between R and S parents were made following by Okada and Jasieniuk, (2014), where the capitula of plants serving as female parents were emasculated by removing the disk florets before anthesis. Emasculated capitula were covered with glass bags with non-emasculated capitula removed from the capitulescence. Capitula of plants serving as male parents were covered with glass bags before anthesis. Controlled pollination was made 1 to 2 d after emasculation by touching the capitula of the female parent and the male parent to transfer pollen. Glass bags were placed back on the female capitula until seed set, and the male capitula were discarded.

F1 progeny were derived from R and S parental crosses, including two S♂ x R♀ (it was termed C4 and C5) and the three reciprocal crosses R♂ x S♀ (it was termed C7, C8 and C9) crosses (Figure 1). The parental plants (R and S) were self-pollinated by placing glass bags over capitula before anthesis and leaving the bags in place until seed set.

The F1 progeny were treated with 1005 g a.e. ha^{-1} of 2,4-D when plants were 10-12 cm tall. Plants from F1 progeny were compared to the parental R and S parents and visually classified as survived and dead at 45 days after treatment. All F1 progeny survived with 2,4-D application, except S parental, and it was self-pollination to produce F2 progeny (Figure 1).

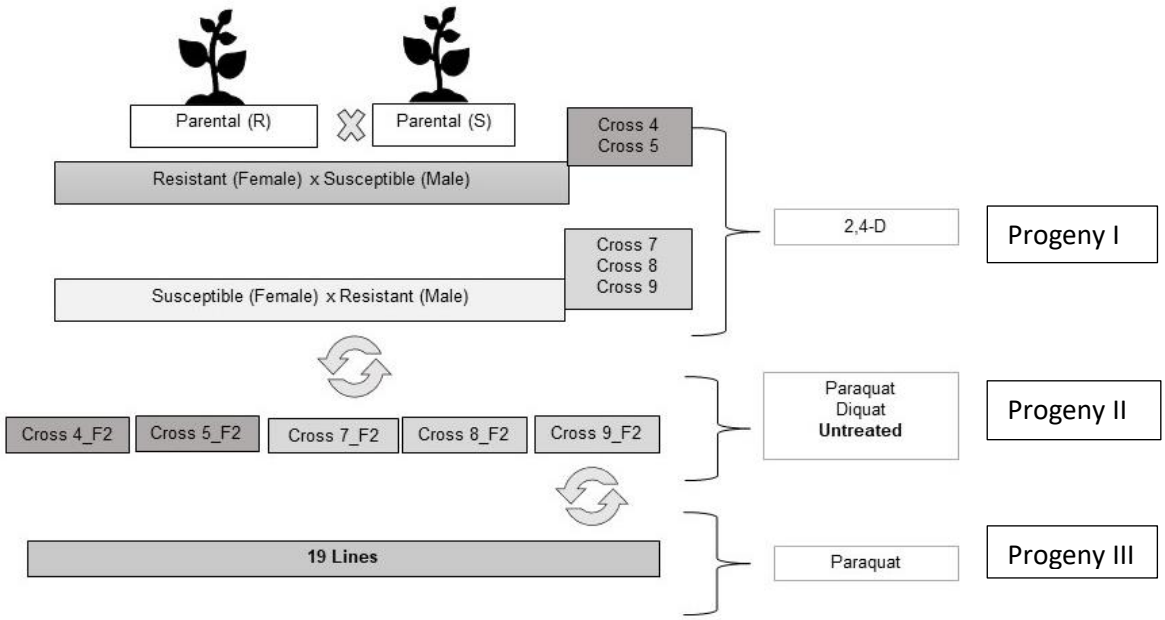

Figure 1. Description of progeny were derived from R and S parental crosses.

Progeny F2

The progeny C4-F2; C5-F2; C7-F2; C8-F2 and C9-F2 were germinated on the surface of soil and it were transplanted, as described above. The greenhouse experiments were carried on with 32-48 replications per treatment.

The doses 0, 0.25x, 0.5x, 1x (400 g a.e. ha^{-1}) and 2x for paraquat were applied in F2 progeny (C4-F2; C5-F2; C7-F2; C8-F2 and C9-F2), and the doses 0, 0.25x, 0.5x, 1x (400 g a.e. ha^{-1}) for diquat were applied in F2 progeny (C5-F2; C7-F2; C8-F2). Paraquat and diquat dose-response studies were conducted separately with R and S (parental). Both herbicides were mixed with 0.5% non-ionic surfactant. When plants reached 10-12-cm height, the herbicide treatments were sprayed using a CO2-pressurized backpack sprayer with four XR-110015 flat-fan nozzles (TeeJet® Technologies, Wheaton, IL, USA), delivering 150 L ha^{-1} at 240 kPa.

The response of F2 progeny was rated as survival (resistant) or dead (susceptible) at 21days after herbicides applications.

Progeny F3

Twenty-nine F2 (C9_F2) plants were grown untreated and seed from individual plants harvested separately to produce F3 progeny (Figure 1). The greenhouse experiments were set in completely randomized design with 29 lines (F3) with 16 replications per treatment and R and S (parental) check. The doses were paraquat (0.5x and 1x) conducted separately with R and S (parental) as described previously (Figure 1).

Statistical Analysis

Plants from F2 and F3 progeny for paraquat and F2 for diquat were compared to the R and S parentals and visually scored for survival or dead at 21days after treatment (DAT). A chi-square goodness-of-fit test ($\chi 2$) was used to compare the observed and expected plant survival based on single dominant gene model by 3:1 for paraquat and two-dominant gene and two genes model (15:1) for diquat. The model was rejected if $p \leq 0.05$.

7.5 RESULTS AND DISCUSSION

Inheritance of Paraquat Resistance

Chi square tests performed for goodness of fit to a 3:1 (F2) and 1:2:1 (F3) segregation suggests that the observed frequencies after paraquat treatment were in accordance with expected frequencies, which proved that it is a single dominant gene model for paraquat in *Conyza sumatrensis* (Table 1 and 2). It was done by summing 0.75 × survival of the resistant population and 0.25 × survival of the susceptible population (Figure 2 and 3).

Table 1. Chi-square analysis for goodness of fit of the observed segregation of paraquat resistance in F2 after application of the 0.25D, 0.5D, 1D (400 g a.e. ha^{-1}) and 2D dose. Expected survival was based on a single-dominant gene model at the herbicide rates used.

F2_Inheritance Model 3:1							
Dose	Cross	Observed Survival	Observed Dead	Total	Expected Survival	X^2	P
0.25D	C4	28	10	38	28.5	0.035	0.851
	C5	27	5	32	24	1.5	0.221
	C7	24	8	32	24	0	1
	C8	22	10	32	24	0.667	0.414
	C9	41	7	48	36	2.778	0.096
	R	48	0	48	36	-	-
	S	30	18	48	36	-	-
0.5D	C4	32	6	38	28.5	1.719	0.189
	C5	28	4	32	24	2.667	0.103
	C7	29	3	32	24	4.167	0.042
	C8	26	6	32	24	0.667	0.414
	C9	37	11	48	36	0.111	0.739
	R	48	0	48	36	-	-
	S	15	33	48	36	-	-
1D	C4	32	6	38	28.5	1.712	0.190
	C5	25	7	32	24	0.167	0.683
	C7	23	9	32	24	0.167	0.683
	C8	24	8	32	24	0	1
	C9	36	12	48	36	0	1
	R	48	0	48	36	-	-
	S	10	38	48	36	-	-
2D	C4	26	9	35	26.25	0.009	0.922
	C5	26	6	32	24	0.667	0.414
	C7	21	11	32	24	1.5	0.221
	C8	26	6	32	24	0.667	0.414
	C9	31	17	48	36	2.78	0.096
	R	32	0	32	24	-	-
	S	0	32	32	24	-	-

* x^2 and probability that a single gene confers resistance (i.e., survival–dead rates are identical to those obtained by assuming that 25% of the F2 population is susceptible, 75% is resistant). Cross C4 and C5 (S♂ x R♀); C7, C8 and C9 (R ♂ x S♀)

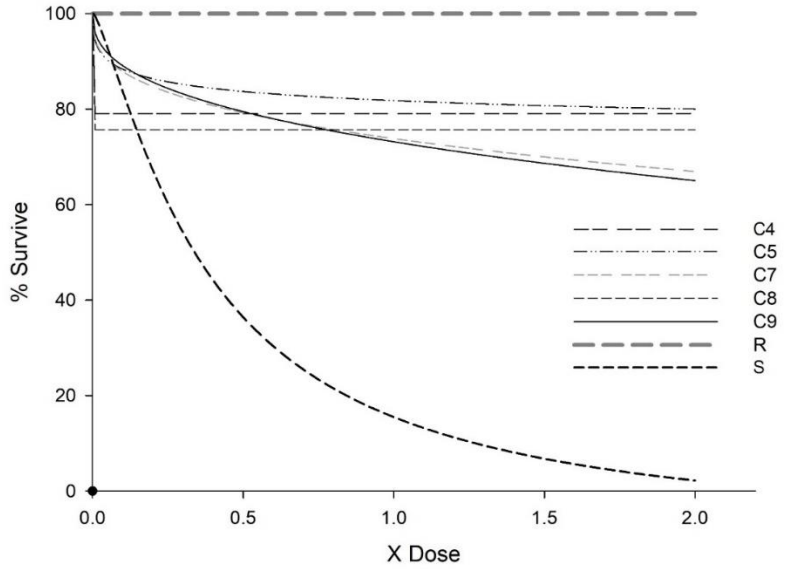

Figure 2: Percentual of survival plants (C4, C5, C7, C8, C9, R, S) in F2 after paraquat application of the 0.25D, 0.5D, 1D (400 g a.e. ha^{-1}) and 2D dose. The data were analyzed using a nonlinear regression model with the dose–response curve based on the four-parameter.

Table 2. Chi-square analysis for goodness of fit of the observed segregation of paraquat resistance in F3 families derived from 29 plants generated from C9_F2 self-pollinations. It was observed after application of the 0.5D and 1D (400 g a.e. ha^{-1}) dose. Expected survival was based on a 1:2:1 model.

F3_Segregation 1:2:1				
0.5D				
Alelo	Observed Survival	Expected Survival	X^2	p-value
AA	8	7.25		
Aa	12	14.5	0.931	0.628
aa	9	7.25		
1D				
Alelo	Observed Survival	Expected Survival	X^2	p-value
AA	7	7.25		
Aa	13	14.5	0.5862	0.7459
aa	9	7.25		

* x^2 and probability that a single gene confers resistance (i.e., survival–mortality rates are identical to those obtained by assuming that 25% of the F3 population is susceptible, 50% R/S, and 25% resistant). AA- Lines were like resistant biotype; Aa- intermediate; aa- like susceptible

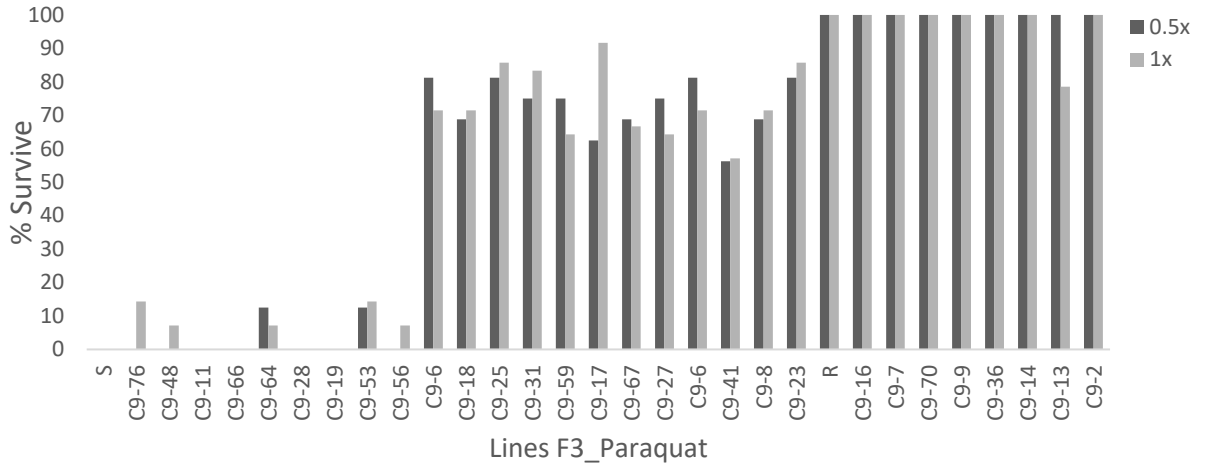

Figure 3. Frequencies of survival plants after 0.5D and 1D (400 g a.e. ha^{-1}) paraquat treatment within 29 lines of *Conyza sumatrensis*. C9 (R ♂ x S♀).

The F2 progeny segregated 3:1 for resistance(R): susceptible (S) at 0.25D, 0.5D, 1D and 2D doses of paraquat (Table 1), also the F3 progeny segregated 1:2:1 (Resistant, R/S, and Susceptible phenotypes) at 0.5D and 1D paraquat doses (Table 2), which is expected for a single dominant gene model. Out of the 29 lines in F3, seven produced only resistant progeny, 13 showed segregation for resistance and susceptibility and the remaining 9 gave only susceptible progeny at 400 g a.e. ha^{-1} of paraquat.

The Susceptible individuals and S parental had 100% mortality with 800 g a.e. ha^{-1} (2x Dose) while the R parental and R individuals had no mortality at this rate (Figure 2). Susceptible individuals become completely necrotic within 24 hrs and did not recover, while resistant individuals showed ≤10-15% injury from 3 to 48 HAA, resulting in only a few leaves with necrotic spots.

Similar results from the reciprocal crosses (C4 and C5 (S♂ x R♀); C7, C8 and C9 (R♂ x S♀)) in F2 progenies indicated nuclear inherited resistance alleles. As a result, nuclear inheritance allows seed and pollen movement carrying herbicide resistance alleles (BUSI et al., 2011).

Gene flow is the movement of resistant genes between and within plant populations. This can occur through pollen or seed movement by mechanisms such as wind, animals, water and agricultural implements (MAXWELL; MORTIMER, 1994). Gene flow by seed movement is one of the common ways through which resistant weeds are transferred between fields. Seed dispersal has the potential to impact resistance gene movement on a much larger scale than pollen flow. It is a greater problem for *Conyza* genus, because a single plant can produce more than 200,000 seeds (GREEN, 2010). Also, there is reported an average 4% allogamy in *Conyza* genus (ZELAYA et al., 2007; HENRY et al., 2008; SOARES et al., 2015) and it could produce an impact on dissemination of herbicide resistance alleles through species (MAITY et al., 2022).

It is challenging to mitigate the spread of herbicide resistance, particularly when resistance is usually endowed by a single dominant or semidominant nuclear gene (RICHTER; POWLES, 1993).

Inheritance of Diquat Resistance

When two dominant genes have the same effect on a trait, indicating a duplicate effect, it leads to a segregation ratio of 15:1 by summing 0.9375 × survival of the resistant population and 0.0625 × survival of the susceptible population. In this case, chi-square analysis was based on a two-dominant gene and two genes model (15:1) for diquat at 0.25D, 0.5D and 1D (400 g a.e. ha^{-1}) dose, which proved that it is a two-dominant gene and two genes model to diquat in *Conyza sumatrensis* (Table 3; Figure 4) and it is expected for two genes segregated independently.

Table 3. Chi-square analysis for goodness of fit of the observed segregation of diquat resistance in F2 after application of the 0.25D, 0.5D and 1D (400 g a.e. ha^{-1}) dose. Expected survival was based on a two-dominant gene and two genes model (15:1) at the herbicide rates used.

F2_Inheritance Model 15:1							
Dose	Cross	Observed Survival	Observed Dead	Total	Expected Survival	X^2	P
0.25D	C5	24	4	28	26.25	3.086	0.0789
	C7	32	0	32	30	2.13	0.1441
	C8	27	0	27	25.3	1.8	0.1797
	R	31	0	31	29.06	-	-
	S	0	32	32	30	-	-
0.5D	C5	25	2	27	25.31	0.0617	0.8038
	C7	32	0	32	30	2.13	0.1441
	C8	31	0	31	29.06	2.067	0.1506
	R	32	0	32	30	-	-
	S	0	32	32	30	-	-
1D	C5	28	3	31	29.06	0.6215	0.4305
	C7	32	0	32	30	2.13	0.1441
	C8	21	10	31	29.06	35.78	0.0000
	R	32	0	32	30	-	-
	S	0	32	32	30	-	-

*Cross C5 (S♂ x R♀); C7 and C8 (R ♂ x S♀)

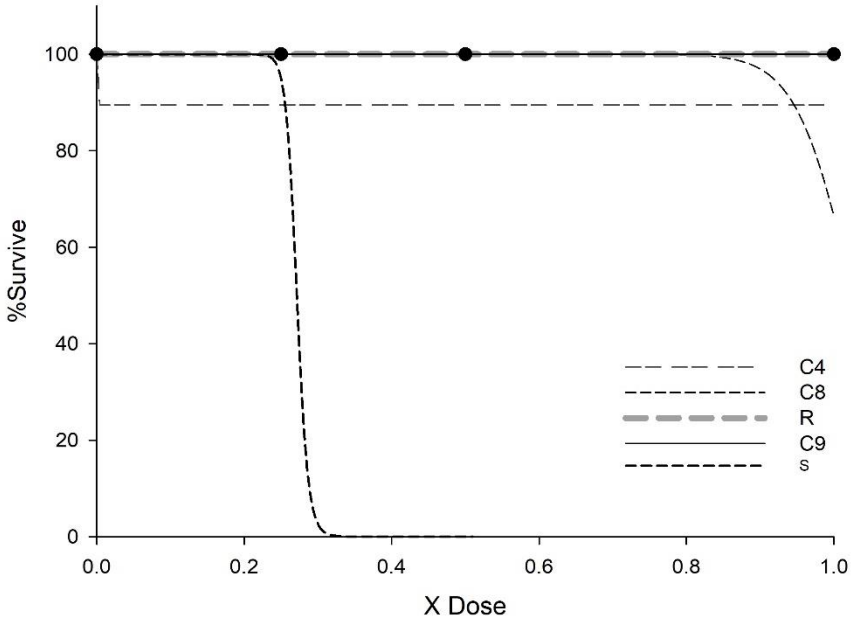

Figure 4: Percentual of survival plants (C4, C8, C9, R, S) in F2 after diquat application of the 0.25D, 0.5D, 1D (400 g a.e. ha^{-1}) dose. The data were analyzed using a nonlinear regression model with the dose–response curve based on the four-parameter.

The Susceptible individuals and S parental had 100% mortality with 100 g a.e. ha^{-1} (0.25x Dose) while the R parental and R individuals had no mortality at this rate (Figure 4). The symptoms observed in resistant plants after the application of diquat were completely different from those seen with paraquat. Whereas plants showed complete desiccation and regrowth in the axillary meristem a few days after diquat application, paraquat resulted in only a few necrotic spots on leaves (Leal et al., 2022).

Therefore, knowledge of the paraquat and diquat inheritance resistance in *Conyza sumatrensis* may help in the development of possible management strategies and in the judicious use of herbicides, as well as in preventing the spread of resistance.

7.6 CONCLUSION

The paraquat resistance in F2 and F3 was based on a single-dominant gene model (3:1), while the diquat results was based on two genes segregated independently (15:1) in *Conyza sumatrensis*.

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8 CONCLUSÕES GERAIS

I. Herbicidas inibidores do FSI e FSII mostram rápidas alterações no índice de desempenho fotossintético mesmo quando não são observados danos visuais de crescimento e desenvolvimento, a técnica de fluorescência da clorofila *a* demonstrou claramente um potencial para rastrear rapidamente as perturbações metabólicas em *Conyza* sob aplicação dos herbicidas metribuzin e paraquat.

II. Os sintomas observados no biótipo resistente ao 2,4-D foram necrose nas folhas em 30 minutos, com o restabelecimento do crescimento normal dentro de 1 a 2 semanas após o tratamento com 2,4-D. O biótipo resistente a 2,4-D mostra um rápido dano fotossintético e aumento no conteúdo de H_2O_2 em comparação ao biótipo suscetível. Além disso, a atividade da enzima antioxidante basal é maior no biótipo resistente.

III. Os resultados sugerem que o biotipo de buva com múltipla resistência aos herbicidas 2,4-D, paraquat, saflufenacil, glifosato e diuron pode também apresentar resistência aos herbicidas inibidores ALS clorimuron-etílico, imazapique + imazapir e etoxissulfurom. Estudos serão desenvolvidos para confirmar a hipótese através de dose-resposta.

IV. O ensaio de dose-resposta confirmou a resistência de *C. sumatrensis* ao diquat com fator de resistência de 25,6 e 6,35 para LD_{50} e GR_{50}, respectivamente, em comparação com o biótipo suscetível. O biótipo resistente ao paraquat não induz as enzimas antioxidantes, como um possível mecanismo de resistência ao paraquat, mas mostra rápida recuperação dos parâmetros fotossintéticos e crescimento contínuo quando submetido ao paraquat, enquanto o biótipo suscetível não sobrevive a aplicação do herbicida paraquat e morre.

V. A resistência ao paraquat em F2 e F3 foi baseada em um modelo de um único gene dominante (3:1), enquanto os resultados do diquat foram baseados em dois genes segregados independentemente (15:1) em *Conyza sumatrensis.*