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Thesis

Florpyrauxifen-benzyl selectivity to rice

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This thesis is dedicated to my parents, Adriana and Eduardo.

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Constitución 1991, Colombia, Titulo II, Capítulo 1, Artículo 12: Nadie será sometido a desaparición forzada, a torturas ni a tratos o penas crueles, inhumanas o degradantes.

Traducción de los Wayú: Pedazo Diez-Dos: Nadie podrá llevar por encima de su corazón a nadie ni hacerle mal a su persona, aunque piense y diga diferente.

Jaime Garzón

Abstract

Velásquez-Rodríguez, Juan Camilo**. Florpyrauxifen-benzyl selectivity to rice.** Advisor: Luis Antonio de Avila. 2021. 61 f. Thesis (Masters in Crop Protection, concentration area: Herbology) - Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, 2021.

Florpyrauxifen-benzyl is a new auxinic herbicide developed for selective weed control in rice. Herbicide selectivity in rice can be affected by environmental conditions and crop practices. This thesis aims to evaluate the effect of environmental conditions and P450 inhibitors on florpyrauxifen-benzyl selectivity in rice. In chapter I, field experiments were carried out at the Embrapa Clima Temperado experimental station RS, Brazil in 2019/20 and repeated in 2020/21 growing season, aiming at evaluating the crop injury and yield response of rice to florpyrauxifen-benzyl doses affected by planting time, and growth stage at herbicide application. In chapter II, two dose-response experiments were carried out under greenhouse conditions at the Federal University of Pelotas, Pelotas RS, Brazil, evaluating the addition of P450 inhibitors and two rice cultivars' responses to florpyrauxifen-benzyl. Lastly, in chapter III, two growth chamber experiments were carried out to evaluate plant injury, and *CYP71A21*, *OsGSTL3, OsGTy,* and *OsWALK21.2* gene expression of rice plants sprayed with florpyrauxifen-benzyl in variable temperature conditions at the time of application. Rice plant injury due to florpyrauxifen-benzyl in early and medium planting time was greater than at late planting time. Herbicide applications at V_2 with 60 g ai ha^{-1} of florpyrauxifen-benzyl reached higher injury than at V_6 or R_0 through the evaluation period with no yield reduction. The average in doses that cause 50% of plant injury (ED_{50}) and growth reduction (GR_{50}) were >6-fold, >4-fold, and 2.7-fold times the label rate (30 g ai ha^{-1}) when applied florpyrauxifen-benzyl alone, with malathion, and with dietholate followed by piperonyl butoxide, respectively. 'Pampeira' cultivar showed lower ED_{50} and GR_{50} than IRGA 424 RI. Rice plant injury was greater in treatments where temperature increase from 28/25ºC to 38/36ºC rather than those that decrease from 28/25ºC to 18/15ºC for the 24 hours after application. Temperature changes suggest downregulation of *CYP71A21*, *OsGSTL3,* and *OsWALK21.2*. Results from these experiments suggest florpyrauxifen-benzyl injuries to rice do not cause lasting negative effects. Spraying malathion before florpyrauxifen-benzyl does not reduce the selectivity; however, the addition of two P450 inhibitors (dietholate and piperonyl butoxide) decreases the doses that cause 50% of growth reduction, but it seems to not compromise the crop selectivity. Pampeira cultivar showed less tolerance to florpyrauxifen-benzyl but doses required to GR_{50} was 2.9 times? the label rate. High temperature before or after florpyrauxifen-benzyl spraying time can increase rice plant injury due, in part, to the reduction of plants herbicide detoxification.

Keywords: Herbicide tolerance. Herbicide metabolism. Rinskor. *Oryza sativa*.

Resumo

Velásquez-Rodríguez, Juan Camilo**. Seletividade do florpyrauxifen-benzyl ao arroz.** Orientador: Luis Antonio de Avila. 2021. 61 f. Dissertação (Mestrado Fitossanidade, área de concentração: herbología) – Facultade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2021.

Florpyrauxifen-benzyl é um novo herbicida auxínico desenvolvido para o controle seletivo de plantas daninhas em arroz. A seletividade em arroz pode ser afetada pelas condições ambientais e prática de lavoura. Esta dissertação tem o objetivo de avaliar o efeito das condições ambientais, inibidores da P450 e temperatura na seletividade do florpyrauxifen-benzyl em arroz. Para atingir esse objetivo, uma série de experimentos foi realizada e a dissertação foi dividida em capítulos. No capítulo I, experimentos de campo foram conduzidos na estação experimental da Embrapa Clima Temperado RS, Brasil, na safra 2019/20 e repetido em 2020/21, com o objetivo de avaliar a fitotoxicidade à cultura e a resposta da produtividade do arroz às doses de florpyrauxifen-benzyl, afetadas pela época de plantio e estádios de crescimento na aplicação do herbicida. No capítulo II, dois experimentos de dose resposta foram conduzidos em casa de vegetação na Universidade Federal de Pelotas RS, Brasil, avaliando a adição de inibidores da P450 e a resposta de duas cultivares de arroz ao florpyrauxifen-benzyl. Por último, no capítulo III dois experimentos em câmara de crescimento foram realizados para avaliar a fitotoxicidade e expressão gênica de *CYP71A21*, *OsGSTL3, OsGTy* e *OsWALK21.2* em plantas de arroz pulverizado com florpyrauxifen-benzyl em condições de temperatura variável no momento da aplicação. A fitotoxicidade à planta de arroz devido ao florpyrauxifen-benzyl no plantio precoce e médio foram maiores do que aqueles causados no plantio tardio. As aplicações de herbicida feitas em V_2 com 60 g ia ha^{-1} de florpyrauxifen-benzyl atingem maior fitotoxicidade do que aquelas feitas em V_6 ou R_0, através das avaliações. No entanto, não houve redução de rendimento. As doses médias que causam 50% dos danos às plantas (ED_{50}) e redução do crescimento (GR_{50}) foram >6 vezes, >4 vezes e 2,7 vezes as doses recomendadas no rótulo (30 g ia ha^{-1}) quando aplicado apenas florpyrauxifen-benzyl, com malathion e com dietholate seguido de piperonyl butoxide, respectivamente. A cultivar pampeira apresentou menor ED_{50} e GR_{50} que IRGA 424 RI. A fitotoxicidade do arroz foi maior nos tratamentos em que a temperatura aumentou de 28 / 25ºC para 38 / 36ºC do que naqueles que diminuíram de 28 / 25ºC para 18 / 15ºC nas 24 horas após a aplicação. Mudanças na temperatura sugerem regulação negativa do *CYP71A21*, *OsGSTL3 e OsWALK21.2*. Os resultados desses experimentos sugerem que os danos do florpyrauxifen-benzyl ao arroz não causam efeitos negativos duradouros. Pulverizar malathion antes do florpyrauxifeno-benzil não reduz o GR_{50} e o ED_{50}; entretanto, a adição de dois inibidores (dietholate and piperonyl butoxide) diminui a doses que causam redução de 50% no crescimento, más parece não comprometer a seletividade da cultura. A cultivar pampeira mostrou-se menos tolerante ao florpyrauxifen-benzyl do que a cultivar IRGA 424 RI, más a doses requerida para GR_{50} foi 2.9 vesses a doses comercial. Um aumento na temperatura antes ou depois do momento de aplicação do florpyrauxifen-benzyl pode aumentar o dano à planta de arroz devido, em parte, à redução da desintoxicação do herbicida da planta.

Palavras-chave: Herbicidas auxínicos. Metabolismo de herbicidas. Rinskor. *Oryza sativa.*

Summary

1 General Introduction

Rice (*Oryza sativa.* L.) is one of the most important global cereal, being the third place in land use and providing 21% of the total calories ingested by humanity (AWIKA, 2011; FAO, 2013; IRRI, 2019). Averaged over the last five years, it has been produced 755 million tons of rice worldwide, in approximately 160 million hectares (FAOSTAT, 2021). Several researchers have estimated yield losses in rice production areas due to weeds, and values vary from 10% to 100% depending on weed species, weed density, rice variety, and management systems (BRIM-DEFOREST; AL-KHATIB; FISCHER, 2017). Additionally, 52 weed-resistant unique cases in rice crop areas have been reported worldwide, limiting the strategies to prevent rice yield losses (HEAP, 2021). In this context, florpyrauxifen-benzyl, a new synthetic auxin herbicide (SAHs) (WSSA, HRAC Group 4), has been developed for selective post-emergent use in irrigated rice (DUY et al., 2018; EPP et al., 2016).

Florpyrauxifen-benzyl mimics indole-3-acetic acid (IAA), a phytohormone endogeny produced by plants involved in many vital processes such as growth, cell division, and expansion (EPP et al., 2016; MILLER; NORSWORTHY, 2018a). Florpyrauxifen-benzyl site of action is related to the liberation of gene expression associated with ethylene and abscisic acid, which decontrol metabolic routes such as photosynthesis, cell division, and development (GAINES, 2020). Previous researches have investigated the effects of florpyrauxifen-benzyl on subsequent crops, control of *Echinochloa* spp., and the influence of soil moisture on weed control (MILLER; NORSWORTHY, 2018a, 2018b, 2018c). Other recent research has investigated rice cultivar's response to florpyrauxifen-benzyl regarding the temperature at spraying time, growth stage application, and in tank-mix with imazethapyr and malathion (WRIGHT et al., 2020a, 2020b). However, because of the novelty of this active ingredient, there are many issues to investigate regarding the rice selectivity, such as applications at rice initial reproductive stage, changes in temperature after spraying, or quantify the effect of inhibitors on the dose of florpyrauxifen-benzyl.

Selectivity in weed management refers to the capacity of a specific herbicide to eliminate weeds in a crop without affecting product yield or quality. This term may be confused with crop tolerance, which refers to the ability of a plant or population to continue growth or function when the crop is exposed to a potentially harmful agent, thus, both definitions allow us to understand the plant-herbicide interaction (BESTE, 1983; CARVALHO et al., 2009). This approach is widely used to control weeds in rice crops (e.g., ACCase inhibitors: cyhalofop-butyl; PSII electron disruptors: propanil), and in many cases, it is highly dependent on the rice ability to degrade the herbicide (CARVALHO et al., 2009). Visible injuries can determine the level of crop tolerance of rice to triclopyr, another auxin herbicide; however, crop injury may not lead to yield losses in field experiments (PANTONE; BAKER, 1992). Therefore, it is possible that rice exposed to florpyrauxifen-benzyl shows lasting adverse effects on yield with no apparent injury.

Like many other herbicides, florpyrauxifen-benzyl efficiency depends on the abiotic factors (e.g., temperature, relative humidity, and wind) and biotic factors such as crop selectivity, weed resistance, and insect pest interaction (BUSI et al., 2018). Previous rice crop observations have described leaf malformations, stem curling, chlorosis, heigh and tillers number reduction, and shoot dry weight reduction as common symptomology of florpyrauxifen-benzyl in rice (WRIGHT et al., 2020a). However, the specific enzymes or gene expression related to florpyrauxifen-benzyl metabolism in rice has not been fully described.

Metabolic degradation has been determined as the principal process of how plants dissipate pesticides, followed by growth dilution and volatilization (76%, 21%, and 3%, respectively) (JACOBSEN; FANTKE; TRAPP, 2015). Cytochrome P450 monooxygenases (CYP450s), glutathione-s-transferase (GST), and glucosyltransferase (GT) are involved in herbicide detoxification (VIJAY et al., 2019). Cytochrome P450 enzyme activity is pH- and temperature-dependent; For example, rimsulfuron (acetolactate synthase inhibitor) hydroxylation rate in maize (Km: 95 μM) was temperature-dependent when the herbicide half-life into the leaf increased from 1.3 to 2.7 hours from 30ºC to 10ºC, respectively (KOEPPE et al., 2000). Thus, florpyrauxifen-benzyl selectivity to rice may vary to the extent that environmental factors affect metabolic degradation.

Cytochrome P450 superfamily plays vital roles in plants (e.g., growth regulation, xenobiotic degradation, and cell division), and 10 CYP families encode for 356 isoenzymes in rice (XU; WANG; GUO, 2015). Synthetic auxin herbicides such as 2,4-D and dicamba have been reported to be hydroxylated by P450s in *Papaver rhoeas* L. and *Parthenium hysterophorus* L.. Additionally, inhibition of P450 reduced the resistance ratio to these herbicides but did not affect resistance to picloram (MORA et al., 2019; TORRA et al., 2017). Thus, P450 inhibition depend on several conditions including the isoenzyme expressed by plant species, the herbicide metabolism and the specific interaction of inhibitor with the enzyme.

Considering the selective mechanism as a natural tolerance in rice, a recent report on SAHs weed resistance proposed that there are seven mechanisms, three of them are related to metabolic process such as; loss of the first step (P450 monooxygenation activity), lack of pro-herbicide bio-activation by loss of esterase activity and, specific auxin transport proteins (TODD et al., 2020). The selectivity mechanism to 2,4-DB has been reported as lack of esterification, which does not allow the activation of 2,4-DB to 2,4-D, permitting the selective use of SAHs in a broadleaf crop such as alfalfa (*Medicago sativa* L.) (LINSCOTT; HAGIN; DAWSON, 1966). Likewise, florpyrauxifen-benzyl ester, to become toxic for plants, has to be transformed to the acid form by de-esterification (MILLER; NORSWORTHY, 2018b). Therefore, if the rice selectivity to florpyrauxifen-benzyl is related to those mechanisms, the environmental factors can affect the rice response due to the relationship between degradation by metabolism, temperature, and inhibitors.

Planting time and growth stage at the spraying moment are additional elements intrinsic in abiotic factors which can affect selectivity. Rio Grande do Sul state is responsible for 70% of rice production in Brazil (IRGA, 2018). From the total rice area growing in Rio Grande do Sul, approximately 1.1 million hectares, the planting generally begins on the 14th of September, on 25th of October reaching 50%, and before 15th of November climbing to 90% (IRGA, 2017). The south of Brazil has opted to drive early planting time due to coinciding the reproductive stage with the cooler night temperatures and the maximum solar radiation period (SOSBAI, 2018). Those factors decrease transpiration rates at night and improve nitrogen assimilation efficiency during panicle initiation and grain filling, resulting in yield increase (SOSBAI, 2018). However, early planting time exposes rice seedlings to cold stresses, which reduce germination and delay crop establishment primarily due to low air and soil temperature (MERTZ et al., 2009; SHIVRAIN et al., 2009). Hence, rice selectivity to florpyrauxifen-benzyl can be affected by planting time due to environmental factors variation across planting times.

There are several reports of rice crop response to florpyrauxifen-benzyl in field experiments worldwide. However, due to the novelty of this herbicide, the majority of those are part of conference proceedings. In Australia, the maximum visual injury reported was 16% using 60 g ai ha^{-1}, but this did not affect rice grain yield (WELLS;

TAYLOR, 2016). In Siri Lanka, rice grain yield showed no significant differences with untreated plots, indicating high levels of selectivity (HAPUKOTUWA et al., 2018). In Italy, visual injury was no more than 8% at BBCH12-21 stage; with no grain yield reduction (VALLE et al., 2012). Field studies in Brazil reported, on average, 4% crop injury ranging from 0 to 30% at a rate of 40 g ai ha^{-1} (BUNDT et al., 2017a, 2017b; RUBIN et al., 2017a, 2017b). However, the impact on the grain yield has not been reported in these studies.

Considering the novelty of florpyrauxifen-benzyl in Brazil and the variation in the response of rice, it was hypothesized that (I) early planting time and application of florpyrauxifen-benzyl at the early rice stage increase rice plant injury resulting in yield losses; (II) the rice tolerance to florpyrauxifen-benzyl, in terms of effective doses, would decrease by the addition of P450 inhibitors insecticides and would differ across cultivars, (III) the changes in temperatures after application time would decrease rice tolerance to florpyrauxifen-benzyl doses, promoting plant injury, decreasing growth, and affecting gene expression of *CYP71A21, OsGSTL3, OsGTy1 OsGTy2,* and *WALK21.2.*

Therefore, the objectives of this research were to (I) evaluate the effect of planting time, plant growth stage at spraying time, and florpyrauxifen-benzyl doses on rice crop injury and yield components, (II) evaluate rice response to florpyrauxifen-benzyl doses applied after P450 inhibitor treatment, and to different rice cultivars, and (III) evaluate the response of rice to florpyrauxifen-benzyl doses under varying air temperature after application, and determine whether the expression of *CYP71A21, OsGSTL3, OsGTy1 OsGTy2,* and *WALK21.2* genes would differ across temperatures conditions after spraying time.

2 Chapter I – Florpyrauxifen-benzyl selectivity in rice, as affected by planting time, rice stage and application rate.

2.1 Introduction

Rice (*Oryza sativa*. L.) is one of the most important food sources, providing 21% of the calories ingested worldwide (AWIKA, 2011). Currently, the importance of rice has been highlighted because of an increase in food demand and supply changes due to the COVID-19 pandemic outbreak (URIOSTE DAZA et al., 2020). Producing 10.2 million tons, on average, in the last five years, Brazil is the top rice producer in the Americas, and Rio Grande do Sul state was responsible, on average, for 77% of this production (CONAB, 2020). In approximately 1.1 million hectares of the total rice area grown in the state of Rio Grande do Sul, planting generally begins in September 14, reaching 50% by October 25, and 90% before November 15 (IRGA, 2017). The south of Brazil has opted to drive early planting time to match the reproductive stage with the cooler night temperatures and the maximum solar radiation period (SOSBAI, 2018). However, early planting time exposes rice seedlings to cold stresses, which reduce germination and delay crop establishment, mainly due to low air and soil temperatures (MERTZ et al., 2009; SHIVRAIN et al., 2009).

Weeds are the most important biotic factor causing severe yield losses in rice. Although weeds can cause up to 100% of yield losses in rice, their potential has been averaged in 37%, of which currently 10% is the actual yield loss (OERKE, 2006). The most important weeds in southern Brazil rice areas include the weedy rice complex (*Oryza sativa*), the *Echinochloa* spp. complex, and the *Cyperus* spp. complex. These species are difficult to control mainly due to their tolerance to hypoxia (KRAEHMER et al., 2016). Historically, southern Brazil has adopted technologies as Clearfield®, which allows the use of imidazoline herbicides for selective control of weedy rice (ZISKA et al., 2015). However, weeds have evolved resistance to several active ingredients in Brazil, with 52 unique resistance cases to date (HEAP, 2021; VARGAS et al., 2016). Recently, Corteva Agriscience has discovered, and commercialized florpyrauxifen-benzyl, a new auxin herbicide (WSSA Group 4), for selective use in rice with a broad spectrum of weed control even those with confirmed resistance to other auxin herbicides such as quinclorar-resistant *Echinochloa crus-galli* (L.) Beauv. (EPP et al., 2016; RYAN MILLER; NORSWORTHY; SCOTT, 2018). Therefore, florpyrauxifen-benzyl is a viable tool that provide efficient weed management for rice production in Brazil.

The symptomology and crop response to florpyrauxifen-benzyl in the field include leaf malformations, stem curling, and stunted plants (WRIGHT et al., 2020b). In Italy, the effect of florpyrauxifen-benzyl has been tested on the different growth stages of rice (BBCH12-21, BBCH22-32, and BBCH37-45), and the highest injury level was 8% at BBCH12-21 (VALLE et al., 2012). Additionally, even with two sequential applications of 60 g ai ha^{-1} did not cause more than 10% of visual injury; however, grain yield was not assessed (VALLE et al., 2012). In Brazil, the efficacy of this herbicide has been tested to control several weed species and crop response evaluated indirectly. These studies report, on average, 4% of crop injury ranging from 0 to 30% at 40 g ai ha^{-1} applied between 3-leaf and 4-leaf stage, but the effect on grain yield has not been reported (BUNDT et al., 2017a, 2017b; RUBIN et al., 2017a, 2017b).

Most of those studies evaluated the impact of florpyrauxifen-benzyl on crop injury when the herbicide was sprayed at the vegetative stage, but not at an early reproductive stage and its subsequent effect on crop yield. In addition, these studies have not considered the effect of planting time and variations in weather conditions. Planting time and spraying time are related to the environmental variations that affect rice selectivity to herbicides due to reduced rice metabolism (MARTINI et al., 2015). In plants, pesticides are dissipated by metabolism, growth dilution, and evaporation (76%, 21%, and 3%, respectively) following absorption; all of these processes depend on environmental conditions that affect plant physiology (JACOBSEN; FANTKE; TRAPP, 2015). Therefore, it is hypothesized that early planting time, and application of florpyrauxifen-benzyl at a late rice stage and at higher doses can increase plant injury and result in yield losses. The objective of this study was to evaluate the effect of planting time, plant growth stage at spraying time, and herbicide doses on florpyrauxifen-benzyl safety to rice.

2.2 Materials and Methods

A field experiment was carried out at the Embrapa Clima Temperado experimental station, Capão de Leão, RS in 2019/2020 and repeated in 2020/2021 growing seasons. The soil in the area was sandy-loam. Pampa CL cultivar was sowed at a density of 250 plants m^{-2} (90 kg ha^{-1}), and fertilized with 350 kg ha^{-1} of NPK (5-20-20) at the time of planting. Nitrogen was applied 50% before flooding (V_3-V_4) and 50%

at the panicle initiation stage (R_0), totalizing 150 kg ha^{-1}. KCl was also applied at 45 kg ha^{-1} at the R_0 stage.

The experiment was conducted in a randomized block design and factorial arrangement with four replications. Factor A included three sowing dates (2019 growing season: September 30, October 25, and November 11; 2020 growing season: September 25, October 20, and November 10) corresponding to early, medium and late planting time, respectively. Factor B consisted of three growth stage when herbicide was applied (when 50% of the plants reach: V_2: collar formation on leaf two on the main stem; V_6: collar formation on leaf six on the main stem and R0: Panicle development initiated). Factor C comprised three doses of florpyrauxifen-benzyl (0, 30 and 60 g ai ha^{-1}, corresponding to untreated check, label rate and maximum label rate recommended by season), we considered the maximum label rate per season in order to evaluate the response simulating issues such as e.g., nozzel overlapping or overdoses at tank mix in commercial field conditions. The experimental units consisted of plots of 2 m wide and 5 m long (10 m^2).

The weed control program for the experiment consisted of a burndown application with glyphosate (enolpyruvyl shikimate phosphate synthase inhibitor) at 1,440 g ae ha^{-1} 30 days prior to planting. A complementary burndown plus a pre-emergence herbicide was applied when rice was at the spiking stage (S_3) (COUNCE; KEISLING; MITCHELL, 2000). In this application, glyphosate (1,440 g ae ha^{-1}) was tank-mixed with imazapic + imazapyr (acetolactate synthase inhibitor) at 24,5 and 73,5 g ai ha^{-1}, respectively. Additionally, in postemergence, imazapic + imazapyr (24,5 and 73,5 g ai ha^{-1}) was sprayed to keep plots free of weeds. The addition of acetolactate synthase inhibitor have been reported to be safe to rice when applied with florpyrauxifen-benzyl (WRIGHT et al., 2020b) thus we decided to used. Florpyrauxifen-benzyl applications were performed for each stage of the application corresponding to each treatment. All herbicide applications were carried out using a backpack sprayer with a four-nozzle boom (Tee-Jet AIXR110015) calibrated to deliver 150 L ha^{-1} spraying solution. All application was performed with wind speed below 4 Km h^{-1}.

For 2019/2020 season was necessary to perform seeds treatment using Vitavax@-Thiram 200 SC (formulate mix of carboxina plus tiram, 200 g L^{-1} each one) applying at rate of 50 g ai in 100 kg of rice seeds. Additionally, it was necessary to apply the fungicide Bim$^{@}$ 750 BR, (triciclazol 750 g kg^{-1}) at 150 g ai ha^{-1} and insecticide Safety (etofenproxi 300 g L^{-1}) at 90 g ai ha^{-1} in V_4-V_5 stages. Any of these practices were not necessary for 2020/2021 season due to less impact of pest through the experimental conditions.

Rice plant injury was evaluated visually where “0%” correspond to the absence of symptoms and “100%” plant death. Plant injury was determined at 3, 7, 14, 21, 28, 35, and 42 days after application (DAA). Rice grain yield was obtained by harvesting panicles in the central area of 3-m^2. In addition, collecting panicles in a linear meter, tiller number, grains per panicle, grain biomass, vain grains per panicle, and unfilled grains per panicle in each experimental unit were determined and, based on unfilled grains per panicle divide by grains per panicle was calculated the spikelet sterility percentage. The grain yield was adjusted to 13% of humidity.

Normality and homogeneity of variance were analyzed by the Shapiro Wilk test and transformation were not necessary for rice grain yield and yield components (tiller number, grains per panicle, grain biomass, vain grains per panicle, and unfilled grains per panicle and spikelet sterility percentage). Whereas due to the high numbers of “0” on plant injury response there was necessary to convert in a scale of 0.001 to 0.999 proportional to the visual injury scale (0% to 100%) previously described. Variables were analyzed in a mixed model operating the year and planting time as a random factor using the function *lmer* in the package *lme4.*R (BATES et al., 2020). A chi-squared distribution was considered for all response variables. Analysis of deviance type II Wald chi-square test was performed to determine the effect of each factor and its interactions. The analysis of deviance showed that there were no significative differences between seasons (runs of the experiment); thus, the variance of each experiment was included in the means analysis. Planting time was operated as a random factor, and spraying time and rates were considered as fixed factors. Mean comparisons and aggrupation were made by the method contrast mean of Kenward-roger (*p-value* <0,05). All analyzes were carried out at the R® version 3.5.2 GUI 1.70 statistic ambient (R CORE TEAM, 2019). We performed a mixed model instead the common ones (linear models) in order to consider fix and random effects, this analysis were performed as Oliveira, (2021) proposal.

2.3 Results and discussion

In general, there was a significant p-value at least in one of double interaction for plant injury through evaluation time (Table 1). The means were compared considering the significative interactions; the triple-interaction was significative at 7 and

21 DAA. The double interaction of spraying time and rates were significant at all evaluations times. Therefore, for 7, 14, 28, 35, and 42 DAA, the aggrupation considers differences within each planting time.

Maximum plant injuries were observed at high doses (60 g ai ha^{-1}), sprayed at V_6 for early planting time 7 DAA (27.4%), sprayed at V_2 for late planting time 14 DAA (34.8%), and sprayed at V_2 for medium planting time 21 DAA (34.4%) (Table 1). Regarding the planting timing, injuries in late planting time showed lower values than early and medium planting time. Moreover, at late planting time, plants seem to have a better recovery from the injury than early or medium planting time. Herbicide applications at R_0 did not show differences in plant injury compared to the non-treated in neither planting time nor florpyrauxifen-benzyl rate. In contrast, applications made at V_2 spraying time showed more significant plant injuries than V_6. Mostly, injuries were higher at double the label rate (60 g ai ha^{-1}) than at label rate (30 g ai ha^{-1}). Likewise, plant injuries at 42 DAA for label rates were greater at V_2 than at V_6, suggesting better plant recovery for applications at V_6.

Our results are similar to greenhouse experiments, where the rice injury caused by florpyrauxifen-benzyl (30 g ai ha^{-1}) was higher at 1-leaf (15%) than at 5-leaf rice (4%) (WRIGHT et al., 2020a). Rising the rate of florpyrauxifen-benzyl and the growth stage of spraying was similar to the observed for triclopyr (another auxin herbicide). The increased triclopyr rate raise the rice crop injury in different cultivars, being higher when sprayed at 0.8 than 0.6 Kg ai ha^{-1} and exposed greater injury when sprayed at the early stage of growth (V_2: 65-45% V_4: 40-15%) than late stages (panicle initiation: 0%) (PANTONE; BAKER, 1992). However, those results are contrary to observe in a medium-grain rice cultivar sprayed with florpyrauxifen-benzyl in field conditions, where it has been reported greater injury (13%) for applications in 5-leaf rice than at 1-leaf (<3%) (WRIGHT et al., 2020b). The variation of rice responses between field and controlled-conditions experiments justified the hypothesis that the environmental conditions significantly affect rice response to florpyrauxifen-benzyl; likewise, these authors observed differences across cultivars.

Plant injuries observed were similar to those observed in previous studies in Brazil, reporting rice injury ranging from 0 - 30% at 30 DAA using doses of 40 g ai ha^{-1} (BUNDT et al., 2017a, 2017b; RUBIN et al., 2017a). Nonetheless, those injuries were higher than the reported at similar doses in experiments from other rice-producing countries, 9.6% for Italy (VALLE et al., 2012), and 16% in Australia (WELLS; TAYLOR, 2016). This result suggests that the environmental conditions of each rice production area may be involved in rice crop response to the herbicide.

The injuries observed in this study did not cause lasting effects and did not affect rice yield (Table 1 and Table 2). Pantone and Baker (1992) report that panicle initiation applications of triclopyr (other synthetic auxin herbicide) do not cause visual injury in rice, but it causes significant yield losses. However, the applications made at the early reproductive stage (R_0) did not show more than 3.8% of injury for all planting times, and there was no impact on grain yield (Table 1 and Table 2). Other studies carried out in the USA and Italy also have observed that rice injuries do not result in lasting negative effects on yield (VALLE et al., 2012; WRIGHT et al., 2020b). Then it is possible to conclude that the use of florpyrauxifen-benzyl surrounding panicle initiation is safe in terms of grain yield; however, it is not label recommended due to the weeds stage, it probably presents losses in control efficacy (ARENA et al., 2018).

Table 1. Rice crop injury as affected by planting time, and florpyrauxifen-benzyl application time, and rate.

Spraying time	Rate[1]	Rice Plant Injury (%)					
		Early planting time (September 30, 2019/September 25, 2020)					
		7 DAA[2]	14 DAA	21 DAA	28 DAA	35 DAA	42 DAA
non-treated		0 f[3]	0 e	0 g	0 d	0 c	0 c
V_2	30	5.6 de	14.1 d	18.1 cd	11.3 b	5.9 b	5.8 ab
	60	6.6 cde	26.4 b	23.3 bc	22.9 a	12.3 a	7.5 a
V_6	30	21.4 b	18.3 cd	8.8 ef	4.3 cd	1.3 c	1.3 c
	60	27.4 a	27.4 ab	24.1 bc	8.8 bc	5.6 bc	5.6 bc
R_0	30	0.0 f	0.6 e	0.0 g	0.0 d	0.0 c	0.0 c
	60	1.9 ef	1.9 e	1.3 g	0.6 d	0.0 c	0.0 c
		Medium planting time (October 25, 2019/October 20, 2020)					
non-treated		-	0 c	-	0 c	0 d	0 d
V_2	30	7.4 cd	16.1 b	19.4 bcd	11.6 ab	14.1 a	11.0 a
	60	7.5 cd	24.1 a	34.4 a	17.1 a	12.3 a	9.1 abc
V_6	30	11.0 c	6.3 c	8.8 ef	6.9 bc	5.6 cd	5.6 cd
	60	19.9 b	11.9 bc	15.0 de	11.9 ab	10.6 ab	10.6 ab
R_0	30	0.0 f	3.8 c	3.8 fg	3.8 c	1.3 d	0.6 d
	60	0.0 f	1.9 c	1.9 g	1.9 c	0.6 d	0.0 d
		Late planting time (November 11, 2019/November 10, 2020)					
non-treated		-	0 d	-	0 c	0 c	0 d
V_2	30	1.6 ef	15.6 bc	16.3 d	9.4 a	6.3 ab	5.6 bc
	60	4.5 def	34.8 a	25.8 b	7.5 ab	6.9 ab	6.9 ab
V_6	30	0.0 f	6.3 cd	6.3 fg	4.4 bc	3.8 b	1.9 c
	60	0.0 f	8.1 c	6.3 fg	4.4 bc	3.1 b	2.5 d
R_0	30	0.0 f	0.0 d	1.3 fg	1.3 c	0.6 c	0.0 d
	60	0.0 f	0.0 d	1.9 fg	1.3 c	0.6 c	0.6 d
		Pr(>Chisq)[4]					
PT x ST		2.21E-12	0.0003	0.0279	0.0796	0.2248	0.1942
PT x Rate		1.18E-05	0.3934	0.2667	0.0218	0.0389	0.1692
ST x Rate		3.34E-10	4.71E-12	7.54E-15	2.28E-07	3.01E-06	9.01E-06
PT x ST x Rate		7.08E-06	0.0579	0.0266	0.2382	0.3376	0.5393

[1]Florpyrauxifen-benzyl g a.i ha^{-1}.

[2]Abrebiations: DDA, days after application; Growth stage spraying when 50% of crop reach V_2: collar formation on leaf two on the main stem; V_6: collar formation on leaf six on the main stem R_0: Panicle development has initiated. PT: Planting time. ST: Spraying time.

[3]Mean aggrupation with different letters means significant differences for fixed effects into each planting time by the Kenward-roger method and across random factor (PT) when necessary (confidence level 95%).

[4]Analysis of deviance type II Wald chi-square test.

Field conditions affect crop response to herbicides to the extent that the environmental variation affects the plant degradation product of less metabolic rate, less growth dilution, and herbicide evaporation (JACOBSEN; FANTKE; TRAPP, 2015). In this study, to find explicative variables for the interaction of rice plants, planting time, plant growth stage, and florpyrauxifen-benzyl doses, it was considered the daily radiation, rainfall, and temperature taken from Embrapa Clima Temperado Station (Figure 1).

In this experiment, the entrance of water to the field was made at V_4, which means that V_2 treatments were sprayed before the irrigation; thus, rainfall defined the soil humidity at these treatments' spraying time. Although the label recommendation is to spray prior to flooding (ANONYMOUS, 2021), the time until flooding for V_2 application stages were longer than the V_6 and R_0, where soil moisture were applied after flooding initiation then the soil moisture was considered to be close to field capacity and the entrance of water was performed 48 hours after spraying. Five days before spraying V_2, early planting time accumulate more rainfall millimeter than medium and late planting (20.6, 4 and 0 mm, respectively for 2019 and 87, 0.2, 0.6 mm, respectively for 2020) (Figure 1). Increased the absorption from 54% to 97% and translocation from 11% to 27% of florpyrauxifen-benzyl in *Echinochloa crus-galli* have been reported when the soil moisture changes from 0.25 to two times the field capacity, respectively (MILLER; NORSWORTHY, 2018b). Consequently, in addition to the early growth plants, this variable can explain the high values on injuries and less recovery for treatment sprayed in V_2 at early planting time (Table 1), considering that there can be more absorption and translocation consequence of soil humidity as described before.

At V_2 applications, it was observed that solar radiation averaged over five days before treatment was lower for early planting time (665.6 and 702.8 umol m^{-2} s^{-1} for 2019 and 2020, respectively), compared to medium (1401.9 and 946.1 umol m^{-2} s^{-1} for 2019 and 2020, respectively) and late planting time (1574.2 and 1306.1 umol m^{-2} s^{-1} for 2019 and 2020, respectively) (Figure 1). Likewise, solar radiation was lower over the five days after V_2 treatments at early (1126.6 and 1179.5 umol m^{-2} s^{-1} for 2019 and 2020, respectively) than medium (1206.7 and 1306.1 umol m^{-2} s^{-1} for 2019 and 2020, respectively) and late planting time (1359.2 and 1048,7 umol m^{-2} s^{-1} for 2019 and 2020, respectively) (Figure 1). This suggests that applications made at V_2 for early planting time presented low solar radiation as a product of less solar incidence because early time in the spring season (the 1st to 5th of November). This variable can explain in part the greatest injury observed for applications at V_2 growth stage in early planting time since less radiation represents less photosynthetically activity reducing the availability of ATP necessary for secondary metabolism as pesticide detoxification additional to another process as translocation (JACOBSEN; FANTKE; TRAPP, 2015; VIJAY et al., 2019). However, further studies must be done to prove this hypothesis.

The temperature has been described to reduce the metabolic activity in plants and consequently decrease the herbicide detoxification due to less plant degradation and less growth dilution (JACOBSEN; FANTKE; TRAPP, 2015; MARTINI et al., 2015). Additional greater injury recovery has been reported for florpyrauxifen-benzyl at warmer (32/24 °C, day/nighttime) temperatures rather than the coolness (24/17 °C day/nighttime) (WRIGHT et al., 2020a). Averaged over the years and spraying time, the temperature five days after spraying was lesser for early planting time (20.6 °C) than medium and late planting time (22.3 °C, 23.9 °C, respectively) (Figure 1). Into each planting time for V_2 treatments, the temperature five days after spraying was 18.3 °C, 20.3 °C, and 23.4 °C for early, medium, and late planting time, respectively (Figure 1). As the temperature after spraying is lower on average for the V_2 treatments at early and medium compared to late planting time, this variable may explain the higher injuries and the less injury recovery observed on those treatments (Table 1). Thus, the florpyrauxifen-benzyl selectivity to rice can vary to the extent that temperature affects metabolic degradation.

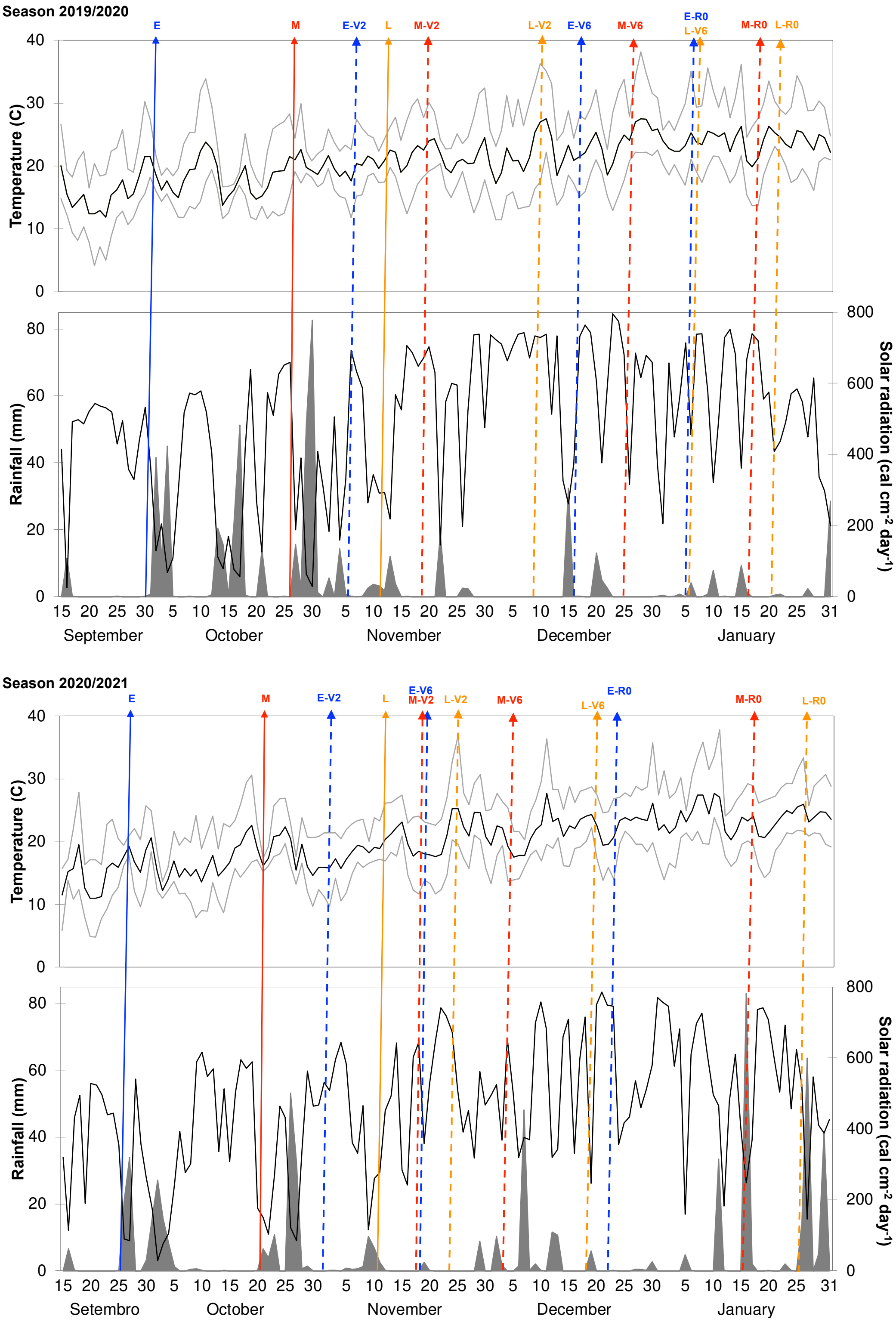

Figure 1. Daily mean, maximum and minimum air temperature, rainfall (gray shading), and solar radiation throughout the experiment. The continuous line represents planting date and discontinue line represents spraying dates. Planting time abbreviations E: Early planting time (blue), M: Medium planting time (red), and L: Late planting time (orange). Spraying time abbreviations V_2: collar formation on leaf two on the main stem; V_6: collar formation on leaf six on the main stem, and R_0: Panicle development has initiated. (Source: Embrapa Clima Temperado weather station)

The tiller number, the number of grain panicle^{-1}, the number of unfilled grains panicle^{-1}, grain yield, and sterility were not affected by any treatments (Table 2). At season 2019/2020, thousand-grain mass showed differences for florpyrauxifen-benzyl rate and spraying time interaction (*Pr(>Chisq):* 0.002). Being greater at V_6 x 60 g a.i ha^{-1} (27.3 g); however, this difference was not detected at season 2020/2021 (26.1 g). Likewise, at season 2019/2020, the early planting time showed greater grain yield (9,337 kg ha^{-1}) than the medium and late planting time (6.885 and 6,887 kg ha^{-1}, respectively); but it was not observed considering data for the two seasons evaluated. Thus, the yield variability for the first season of the experiment could be due to some differential management between planting times but there was no effect of florpyrauxifen-benzyl on grain yield and yield components.

Table 2. Yield components and analysis of deviance type II Wald chi-square test of rice as affected by planting time (two seasons 2019/2020; 30/September, 25/October and 11/November; and 2020/2021; and 25/September 20/October 10/November), spraying time (V_2, V_6, and R_0) and florpyrauxifen-benzyl rates (0, 30 and 60 g ai ha^{-1}).

Treatments	Tiller number	Number of grains per panicle[1]	1000 grain mass (g)	Unfilled grains panicle[1]	Grain yield (kg ha^{-1})	Spikelet sterility (%)
V_2 x 0	4.7^{ns}	106.0^{ns}	26.5^{ns}	11.8^{ns}	8,635.0^{ns}	10.9^{ns}
V_2 x 30	5.7	104.1	26.2	12.5	8,220.0	11.3
V_2 x 60	5.0	110.8	26.2	13.3	8,582.0	12.3
V_6 x 0	5.0	103.3	26.0	12.4	8,406.0	11.4
V_6 x 30	5.7	106.5	26.5	12.1	8,277.0	11.4
V_6 x 60	5.7	103.3	26.7	10.4	8,227.0	9.7
R_0 x 0	5.9	102.8	26.2	11.3	8,356.0	10.4
R_0 x 30	4.8	106.5	26.4	12.0	8,746.0	11.1
R_0 x 60	5.3	104.9	26.4	11.3	8,145.0	10.8
Factor			Pr(>Chisq)[1]			
Block	0.1800	0.1458	0.1254	0.1499	0.0452	0.1345
Planting time (PT)	0.3134	0.2185	0.2549	0.5756	0.2518	0.3517
Spraying time (ST)	0.5818	0.6454	0.8780	0.4770	0.7302	0.5597
Rate	0.8522	0.7281	0.6305	0.8324	0.7989	0.8738
PT x ST	0.5238	0.6427	0.5596	0.3530	0.2946	0.3639
PT x Rate	0.1067	0.2724	0.3856	0.8304	0.5863	0.8701
ST x Rate	0.1230	0.6984	0.2879	0.6109	0.4621	0.5237
PT x ST x Rate	0.7983	0.6513	0.5344	0.9932	0.7485	0.9852

^{ns}: non-significant.
[1]Analysis of deviance type II Wald chi-square test.

In average the 1000-grain mass Pampa CL cultivar has been reported in 25.6 g (MAGALHÃES JR et al., 2017). At season 2019/2020, the double doses of florpyrauxifen-benzyl (60 g ai ha^{-1}) applied at V_6, showed higher 1000-grain mass than the other left treatments. This result can be supported due to parthenocarpy, which means that grain starts filling without been fertilized; this has been reported in addition to exogeny auxins in rice (ZHAO et al., 2013). The V_6 growth stage is close to reproductive initiation; thus, rice plant exposure to florpyrauxifen-benzyl before another development, pollen fertility, or fertilization may result in parthenocarpy, then seeds would have had more time to fill grains result in more grain mass. However, more studies have to be made to prove this hypothesis.

In general, the earliest planting time, the early growth stages, and rising doses of florpyrauxifen-benzyl suggest promoting injuries to rice plants. However, there was no detected adverse lasting effect on rice yield or yield components. Early and medium planting promoted the injuries to florpyrauxifen-benzyl in rice more than the late planting time; additionally, better plant recovery was detected for late planting time. The environmental observations suggested that low radiation and low temperature surrounding the application time were related to increased injuries. The R_0 applications did not show injuries in any planting time or doses, and Applications made at V_2, and V_6 showed more significant injuries than at R_0. Then based on the rainfall averaged five days before spraying, the greater soil moisture seems to promote the injuries the treatment V2. Lastly, the rising in doses of florpyrauxifen-benzyl increases the rice injuries but does not affect yield.

2.4 Conclusion

The highest crop injury was promoted by florpyrauxifen-benzyl application in early followed by the medium. The application in late planting time promoted faster recovery. Low solar radiation and low temperature surrounding the florpyrauxifen-benzyl application was suggest to be related to the promote the injury observed at early and medium time.

Florpyrauxifen-benzyl applications at V_2 and V_6 promoted greater rice plant injury than applications at R_0. It was suggested high soil moisture before spraying increase the injuries.

An increase in florpyrauxifen-benzyl doses suggests greater injuries especially when it was sprayed at V_2 and V_6 but no effect on yield.

The rice yield and yield components were not affected by the florpyrauxifen-benzyl application in any doses, application stage, and planting time.

3 Chapter II - Selectivity of florpyrauxifen-benzyl on rice, as affected by P450 inhibitors, and the tolerance of two cultivars.

3.1 Introduction

The capacity of a specific method to eliminate weeds in a crop without affecting product yield or quality is considered as the selectivity, and tolerance of specific methods varies among conditions such as crop cultivar and the addition of others pest management products (CARVALHO et al., 2009). Several herbicides with different modes of action have been used as selective weed control in rice (e.g., ACCase inhibitors: cyhalofop-butyl; PSII electron disruptors: propanil; ALS inhibitors: bispyribac-sodium; Synthetic auxins: 2,4-D), and degradation to non-toxic metabolites is the primary selective mechanism (SINGH et al., 2008; USUI, 2001). Activation by hydroxylation, conjugation, and compartmentalization are the three steps to herbicide degradation in plants, and the activity of the enzymes involved conditioned the rice tolerance to herbicides (VIJAY et al., 2019). Specific cases of selective mechanisms in rice have been described; one example of this is the lacked esterification activity providing the selectivity to cyhalofop-butyl (RUIZ-SANTAELLA; HEREDIA; DE PRADO, 2006). Another is the aryl-acyl-amidase activity that gives rice the capacity to detoxify propanil (USUI, 2001). Florpyrauxifen-benzyl is a new Synthetic auxin herbicide (SAHs) disclosed to selective control of broad-spectrum of weeds in rice (DUY et al., 2018; EPP et al., 2016). However, because of the novelty of this herbicide, the selective mechanism has not been fully described.

Monooxygenation mediated by the cytochrome P450 enzyme (CYP450) is the most common first step of herbicide degradation, followed by glutathione-s-transferase (GST) conjugation and, less frequently the glycosyltransferase (GTs) conjugation (VIJAY et al., 2019). Organo-phosphate insecticides as malathion, insecticide synergist as piperonyl butoxide (PBO), and herbicide safener as dietholate have been described as P450 inhibitors, and its use changes the crop tolerance to herbicides (KOEPPE et al., 2000; OLIVEIRA et al., 2018). Dietholate is a broadly used safener in rice seed traded against clomazone injuries (SANCHOTENE et al., 2010). The inhibition mechanism of herbicide synergist has been described as interact or compete for the binding site of different P450 enzymes (BUSI; GAINES; POWLES, 2017). Additional SAHs such as 2,4-D and dicamba have been reported to be hydroxylated by P450 (MORA et al., 2019; TORRA et al., 2017).

All those agrochemicals can inhibit the CYP P450; however, due to the high diversity of P450 and plant-specificity is extremely difficult to consider that those chemicals inhibited all the P450 isoenzymes (XU; WANG; GUO, 2015). Moreover, although there is a report of malathion effect mixed with florpyrauxifen-benzyl on rice response (WRIGHT et al., 2020b), there has not been reported the level of doses in which the addition of inhibitors affects the rice response or the effect of other inhibitors as PBO or dietholate on rice selectivity. Hence elucidating the interaction of those agrochemicals applied in rice with florpyrauxifen-benzyl remains to be explored.

New rice cultivars with high yield potential have been part of the current concern of rice production worldwide; this is the case of BRS Pampeira, a new non-Clearfield® cultivar developed to perform high yields in the Rio Grande do Sul state, Brazil (MAGALHÃES JR et al., 2017). Response to florpyrauxifen-benzyl among cultivars has been described for cultivars from the USA, but it has not been described for Brazilian cultivars (WRIGHT et al., 2020b). Thus, it is essential to provide farmers the confidence to use this herbicide in the new cultivar. Moreover, consequently increasing the use of new tools in integral weed management.

Therefore, this study hypothesizes that the rice tolerance to florpyrauxifen-benzyl would decrease by adding P450 inhibitors and would differ across cultivars. Hence the objective of this study was to evaluate the rice response to florpyrauxifen-benzyl as affected by P450 inhibitor treatments and determine whether rice tolerance would differ across cultivars.

3.2 Materials and Methods

Two independent experiments were carried out; the first evaluate the addition of P450 inhibitors and the second the cultivar response. Both experiments were carried out in a greenhouse at the Federal University of Pelotas (UFPel), the first was repeated twice in November 2019 and April 2020 and the second was repeat simultaneously in April 2020. Four rice plants of cultivar IRGA 424 RI and Pampeira were established in 0.5-liters pots that were previously filled with rice paddy soil (Albaqualf) collected from the 0-20 cm soil profile nearby rice field. Each pot with four plants was considered the experimental unit. IRGA 424 RI plants were used to evaluate the additions of inhibitors, and plants for Pampeira and IRGA 424 RI were used to evaluate cultivars' response.

For both experiments, the inhibitor experiment and the cultivar respond experiment, a randomized block design in a factorial scheme was performed (8 x 3 and

8 x 2, respectively) with four and three replications, respectively. Eight florpyrauxifen-benzyl doses, 0; 15; 30; 60; 120; 240; 480 and 960 g a.i. ha^{-1} was applied for both experiments and considered as “factor A” these doses were considered taking into account the commercial dose (30 g ai ha^{-1}). The factor B for P450 inhibitor experiment consisted in three treatments: inhibitor-non-treated control, malathion (1 kg ai ha^{-1}), and dietholate, follow by piperonyl butoxide (PBO) (10 g a.i. seed-kg^{-1} and 4.2 Kg ai ha^{-1}, respectively). The decision to add two inhibitors (dietholate follow by piperonyl butoxide) was because a preliminary test that did not show inhibitor effects individually. Dietholate was applied as a seed treatment while malathion and piperonyl butoxide were sprayed one hour before florpyrauxifen-benzyl applications. The factor B for the cultivars' response experiment consisted in two cultivars, IRGA 424 RI and Pampeira. The greenhouse conditions were 17-25 ºC and relative humidity 70 ± 10% averaged over experiments runs.

Herbicide applications were made when rice plants had three leaves (V_3). A water sheet around five centimeters above the soil ground level was maintained after herbicide application until the end of the experiments. The application of herbicide and inhibitors was made with a 30 psi CO_2 constant pressure backpack sprayer equipped with a two meters spray bar and four flat jet nozzles (Tee-Jet AIXR110015) at 0.5 m apart from each other and was set to distribute a spray volume of 150 L ha^{-1}.

Plant injury was evaluated as a visual variable where “0%” correspond to the absence of symptoms and “100%” to dead plant, symptoms based on chlorosis, wilting, epinasty, leaf malformation, tissue swelling, and stunted growth. Plant injury was determined at 3, 7, 14, 21, and 28 days after application (DAA). At 28 days after application, the shoot dry weight was determined. The 50% growth reduction dose values (GR_{50}) from shoot dry weight and 50% plant injury (ED_{50}) with their corresponding parameters were calculated for each treatment using a logistic model (Equation 1) function of the *drc* package in R® version 3.5.2 GUI 1.70 statistic ambient (R CORE TEAM, 2019; RITZ et al., 2015);

$$y = f(x) = C + \frac{D - C}{1 + \exp\left(b(\log(x) - \log(e))\right)}$$

Equation (1)

where, *C* represents the lowest limit *D* represent the upper limit; *b* describes the slope of curve around the *e* (ED_{50} or GR_{50}); and the values of *e* corresponded to the rate that reduces to 50% of the response variable *y*, and *x* is the florpyrauxifen-benzyl dose in g a.i. ha^{-1}.

Florpyrauxifen-benzyl *e* values indices were compared using *EDcomp* function of the *drc* package. *EDcomp* function compares using t-student test, where $p<0.05$ indicates significant differences between *e* indices. This same function was operated to compare the curves between runs. Data showed that there were no differences between runs; therefore, the data was gathered. Confidence intervals were calculated using *confint* function. Using the calculate ED_{50} of the check without inhibitor and treatments was estimate an inhibition ratio, as follow: Inhibition ratio = (ED_{50} check without inhibitor – ED_{50} inhibition treatment) / ED_{50} check without inhibitor * 100.

3.3 Results and Discussion

3.3.1 Effect of P450 inhibitors on rice response to florpyrauxifen-benzyl

Generally, the efficient doses of florpyrauxifen-benzyl for rice plant injury (ED_{50}) through time were numerically lesser when plants were treated with inhibitors than plants without inhibitors (Table 1). Likewise, the dietholate followed by PBO treatments showed lower ED_{50} in comparison with both check without inhibitor and with malathion (Table 1). However, the *p-value* of the *t-test* only detected significant differences for ED_{50} at 21 DAA between check without inhibitor and dietholate followed by PBO treatment. Although at 28 DAA, there was not detected significance, this treatment reaches almost 40% of inhibition with a p-value = 0.06.

The ED_{50} variations described above could be due to rice recovery variations over time. This fact supports the hypothesis that P450 could be involved in a certain level of the rice tolerance mediated by metabolism. Similar results have been described when the resistant ratio for *Parthenium hysterophorus* L. to others SAHs (2,4-D and dicamba) decreases but not as much as the susceptible type when applicated with malathion (MORA et al., 2019). Also, this could be observed when the ED_{50} plant injury inhibition ratio was numerically higher averaged over time for the treatment with dietholate followed by PBO compared with malathion alone (averaged over time 47.4%, 25.9%, respectively).

Table 1. Parameters estimate of the dose response curve of rice plant injury evaluated at three, seven, 14, 21 and 28 days after florpyrauxifen-benzyl treatment as affected by P450 inhibitors applied one hour before for malathion and Piperonyl butoxide, and at seed treatment for dietholate.

Treatments	b[2]	SE[3]	Rice plant injury (%)		ED_{50}[5]	CI 95%	Inhibition ratio (%)[6]	*p-value*[7]
			d[4]	SE	g ai ha^{-1}			
			3 DAA[1]					
Check without inhibitor	-1.1	(0.8)	38.8	(30.0)	285.2	(0-651.1)	0.0	
Malathion	-3.8	(0.9)	40.7	(1.7)	111.4	(105.9-105.9)	60.9	0.238
Dietholate fb PBO[8]	-1.9	(0.5)	49.4	(3.1)	64.4	(56.9-71.8)	77.4	0.556
			7 DAA					
Check without inhibitor	-2.0	(1.2)	64.5	(9.4)	119.6	(89.7-149.6)	0.0	
Malathion	-4.4	(0.5)	81.9	(1.6)	109.5	(107.3-111.8)	8.4	0.684
Dietholate fb PBO	-4.9	(1.1)	84.5	(2.3)	82.6	(80.3-85.0)	30.9	0.172
			14 DAA					
Check without inhibitor	-1.2	(0.4)	83.5	(24.4)	252.0	(87.8-416.1)	0.0	
Malathion	-2.8	(0.3)	88.8	(2.3)	134.2	(128.5-140.0)	46.7	0.079
Dietholate fb PBO	-4.3	(0.8)	87.3	(2.3)	88.3	(86.0- 90.7)	64.9	0.189
			21 DAA					
Check without inhibitor	-4.7	(2.0)	56.4	(3.2)	116.5	(108.2-124.9)	0.0	
Malathion	-2.5	(0.3)	87.9	(2.6)	148.2	(140.4-155.9)	0.0	0.051
Dietholate fb PBO	-3.5	(0.8)	88.5	(2.5)	88.2	(85.9-91.0)	24.3	0.000
			28 DAA					
Check without inhibitor	-1.6	(0.5)	80.9	(8.5)	139.4	(110.7-168.1)	0.0	
Malathion	-2.8	(0.3)	91.6	(2.2)	120.4	(115.9-124.8)	13.6	0.461
Dietholate fb PBO	-2.3	(0.4)	92.4	(3.0)	84.6	(80.7-88.4)	39.3	0.062

[1]Abbreviation: DAA. days after application.
[2]Slope around ED_{50}.
[3]SE: stand error
[4]Upper limit for all plants.
[5]Doses of florpyrauxifen-benzyl (g a.i. ha^{-1}) causes 50% of crop injury.
[6](ED_{50} check without inhibitor – ED_{50} inhibition treatment) / ED50 check without inhibitor *100.
[7]Florpyrauxifen-benzyl vs. inhibition treatment fb florpyrauxifen-benzyl on rice crop injury t-statics comparison of ED_{50}. *p-value*>0.05 means non-significand difference between treatments.
[8]Piperonyl butoxide.

Based on the confidence interval of GR_{50} calculate from dry shoot weight collected 28 DAA, there was a significant difference between check without inhibitor and with dietholate follow by PBO inhibitor, whereas for the treatment with malathion did not show difference (Figure 1). This fact confirms the result observed on ED_{50} where there was an effect of dietholate follow by PBO on florpyrauxifen-benzyl application at 21 and 28 DAA. The dietholate followed by PBO showed the lowest GR_{50} with a 63% inhibition ratio than florpyrauxifen-benzyl without inhibitor; however, as observed with ED_{50}, the dose to reach GR_{50} was 2.9-fold times the recommended label rate (89.6 g ai ha^{-1}) (Table 2). This means that the use of florpyrauxifen-benzyl remained safe to the plant even applied with two inhibitors and at the maximum recommended label rate per season (60 g ai ha^{-1}).

Table 2. Parameters estimate of the dose response curve of rice dry shoot weight evaluated at 28 days after florpyrauxifen-benzyl treatment as affected by P450 inhibitors applied one hour before for malathion and piperonyl butoxide (PBO), and at seed treatment for dietholate.

Treatments	b[1]	SE[2]	Dry shoot weight (g plant^{-1})		GR$_{50}$[4]	CI[5] 95%	Inhibition ratio (%)[6]	*p-value*[7]
			d[3]	SE	g ai ha^{-1}			
Check without inhibitor	1.4	(0.3)	4.4	(0.2)	216.9	(144.5-274.7)		
Malathion	2.0	(0.4)	4.0	(0.2)	243.0	(186.9-289.6)	0.0	0.573
Dietholate fb PBO	1.1	(0.2)	3.5	(0.2)	89.6	(59.5-125.4)	63.1	0.031

[1]Slope around GR50.
[2]SE: standard error.
[3]Upper limits for all plants.
[4]Doses of florpyrauxifen-benzyl (g a.i. ha^{-1}) causes 50% of grow reduction.
[5]CI: confidence interval.
[6](GR$_{50}$ check without inhibitor – GR$_{50}$ inhibition treatment) / GR$_{50}$ check without inhibitor *100.
[7] Florpyrauxifen-benzyl vs. inhibition treatment fb florpyrauxifen-benzyl on rice dry shoot weight *t-statics* comparison of GR$_{50}$. *p-value*>0.05 means non-significand difference between treatments.

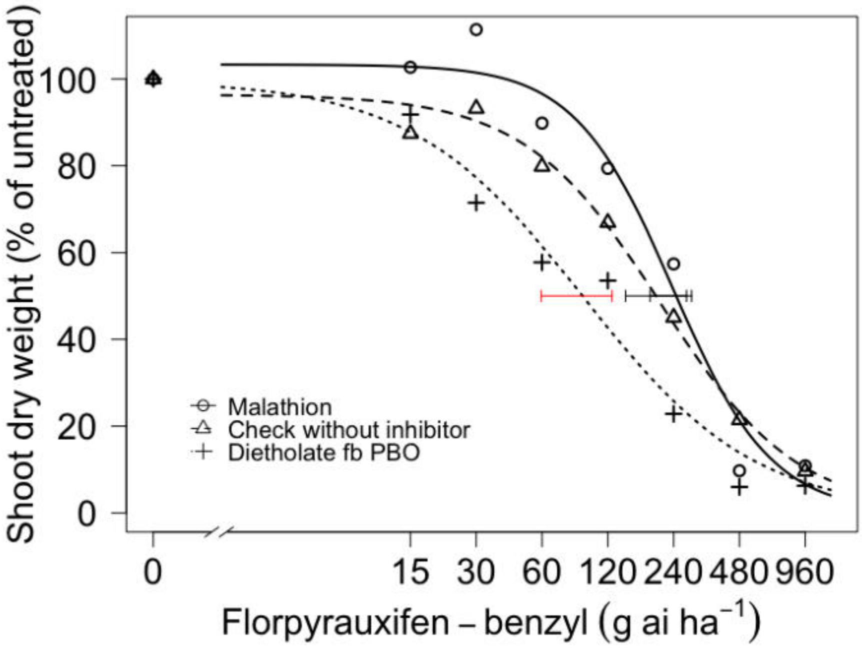

Figure 1. Modeled dose-respond of rice growth reduction sprayed with florpyrauxifen-benzyl and applied with two P450 inhibitor treatments (malathion (1.000 g ai ha^{-1}) and dietholate (10 g ai seed-Kg^{-1}) followed by (fb) piperonyl butoxide (PBO) (4.200 g ai ha^{-1})). Dietholate were applied as a seed treatment while malathion and piperonyl butoxide were applied one hour before florpyrauxifen-benzyl spraying. Confidential interval is estimated at the 50% of growth reduction, using *confint* function in drc R-package.

Our result is similar to those observed by (WRIGHT et al., 2020b) on field experiments carried out in Arkansas rice areas; they report 2% of rice injury when applied florpyrauxifen-benzyl (30 and 60 g ai ha^{-1}) plus malathion (700 g ai ha^{-1}). Quinclorac-resistant accessions of Barnyardgrass (*Echinochloa crus-galli*) have been reported to be controlled with florpyrauxifen-benzyl, and it was considered that ED_{50} and GR_{50} were lesser than the recommended doses (30 g ai ha^{-1}) (MILLER; NORSWORTHY; SCOTT, 2018). The ED_{50} and GR_{50} values reported were 13.8 g ai ha^{-1} (SE: 2.2) and 20.2 g ai ha^{-1} (SE: 0.8) of florpyrauxifen-benzyl, respectively (MILLER; NORSWORTHY; SCOTT, 2018). The ED_{50} and GR_{50} values for rice in this study were all above those reported, which means that the control of troublesome species as Barnyardgrass remain below the selectivity doses (30 g ai ha^{-1} maximum 60 g ai ha^{-1} per season). Thus, this implies to farmers the confidence to use florpyrauxifen-benzyl mixed with any of those P450 inhibitors at label recommended doses for Barnyardgrass control.

Two outcomes can be inferred from these results; first, the safe use of florpyrauxifen-benzyl when applied with P450 inhibitors since the average ED_{50} plant injury values through time were >4-fold and 2.7-fold times the label recommended rate of florpyrauxifen-benzyl when applied with malathion and dietholate followed by PBO, respectively. Therefore, given the importance of these inhibitors, where one of these is widely used as an insecticide and the other is widely used as a safener to clomazone injury in rice this result brings important information for farmers and technicians about the use of florpyrauxifen-benzyl (MOGHBELLI et al., 2020; SANCHOTENE et al., 2010).

Secondly, the P450 monooxygenase seems to be linked with florpyrauxifen-benzyl metabolisms in plants at a certain level since adding two inhibitors reduces the rice GR_{50} significantly; however, considering that the addition of malathion does not compromise rice selectivity, further studies must be done to elucidate the role of P450 in florpyrauxifen-benzyl metabolism in rice. Contrary to (WRIGHT et al., 2020b) conclusion, which suggests that there is no dependency of P450 on the degradation of this herbicide in rice. We considered that there could be more interaction of florpyrauxifen-benzyl with P450 inhibitors in plants since there have been reported 356 CYP genes encoding for P450 enzymes on rice (XU; WANG; GUO, 2015). Hence, using those insecticides could selectively inhibit part of those CYP isoenzymes but not whole, which means that another group of P450 could be involved. An example of this is when triazole, imidazole, and pyrimidine derivatives are inhibited selectively by cytochrome P450 (DONALDSON; LUSTER, 1991).

3.3.2 Rice cultivar response to florpyrauxifen-benzyl application

The response of both IRGA 424 RI and Pampeira to florpyrauxifen-benzyl doses at 28 DAA are observed in Figure 2. The confidential intervals at 50% of plant injury and growth reduction do not intersect between cultivars, suggesting a significant difference (Figure 2). The rice cultivar Pampeira was more sensitive than IRGA 424 RI to the dose rising of florpyrauxifen-benzyl in terms of both plant injuries and shoot dry weight reduction. This result supports the hypothesis that cultivars show different levels of tolerance.

At commercial doses of florpyrauxifen-benzyl (30 g ai ha^{-1}), the plant injury of Pampeira was 20% which was higher compared with the IRGA 424 RI. Likewise, at this same dose, the growth reduction for IRGA 424 RI was lesser than Pampeira (Figure 2). This indicates that even at commercial doses of florpyrauxifen-benzyl is probably to observe differences in sensitivity among cultivars. Therefore, this information implies that framers must expect different levels of tolerance among cultivars to florpyrauxifen-benzyl even at commercial doses; however, as observed in the results of the previously chapter these injuries do not cause lasting effect on yield.

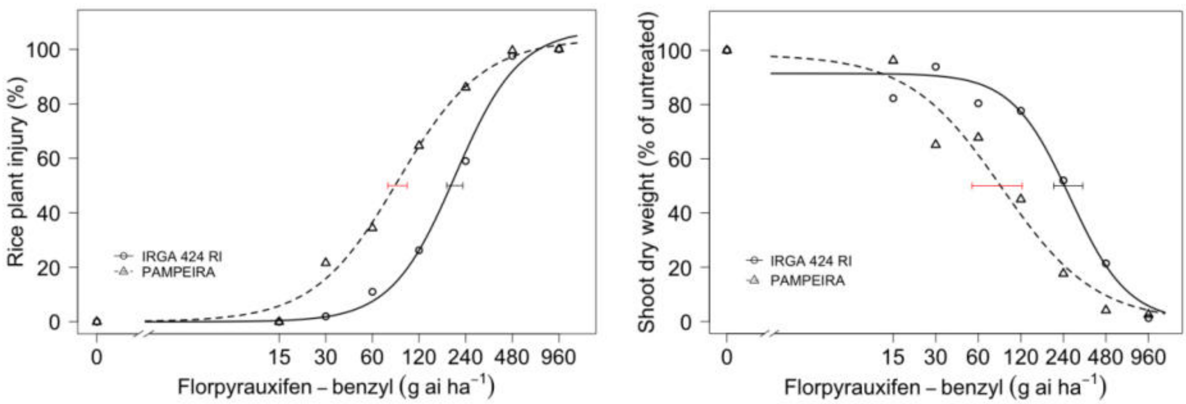

Figure 2. Rice cultivar PAMPEIRA e IRGA 424 RI response to florpyrauxifen-benzyl. Left related of plant injury and right relative to the reduction of the dry shoot weight. The confidential interval was estimated at 50% of plant injury and growth reduction, respectively.

The ED_{50} and GR_{50} values calculated from the log-logistic model showed that Pampeira was 2.3-fold and 3.0- fold times lesser than the IRGA 424 RI, respectively (Table 3). *T-tests* were used to analyze the differences between cultivars for the ED_{50} and GR_{50} values; as observed at the *p-value*, there was a significant differential response of rice cultivars to florpyrauxifen-benzyl being less tolerant Pampeira than

IRGA 424 RI (Table 3). The Pampeira cultivar ED_{50} and GR_{50} minimum values from the confidence interval were 75.4 g ai ha^{-1} and 54.2 g ai ha^{-1}, respectively (Table 3). Considering the recommended doses of florpyrauxifen-benzyl (30 g ai ha^{-1}) and the maximum per season (60 g ai ha^{-1}) (ARENA et al., 2018). This result suggests that use the maximum label recommended per season of florpyrauxifen-benzyl represents a risk in terms of GR_{50} for Pampeira since minimum values in the confidential interval overlap doses under 60 g ai ha^{-1}. Thus, rice producers in southern Brazil must be considered this less tolerance of Pampeira and avoid using the maximum label doses.

Table 3. Parameters estimate of the dose response curve of rice plant injury and rice dry shoot weight evaluated at 28 days after florpyrauxifen-benzyl treatment to two rice cultivars.

Cultivar	b[1]	SE[2]	Rice plant injury (%) 28 DAA		ED_{50}[4]	CI 95%[5]	*p-value*[6]
			d[3]	SE	g ai ha^{-1}		
IRGA 424 RI	-2.1	(0.2)	100	(3.7)	205.5	(181.2-229.8)	
Pampeira	-1.6	(0.1)	100	(3.2)	88.0	(75.4-100.5)	0.000
			Dry shoot weight (g)		GR_{50}[5]	CI 95%	
IRGA 424 RI	2.1	(0.5)	4.6	(0.2)	267.2	(204.9-329.4)	
Pampeira	1.3	(0.2)	4.5	(0.3)	88.3	(54.2-122.5)	0.004

[1]Slope around ED50 and GR50.
[2]SE: standard error.
[3]Upper limit for all plants.
[4]Doses of florpyrauxifen-benzyl (g a.i. ha^{-1}) causes 50% of plant injury and growth reduction.
[5]Confidencial interval
[6]IRGA 424 RI vs. PAMPEIRA on plant injury and rice dry shoot weight *t-statics* comparison of ED50 and GR_{50}. *p-value* > 0.05 means non-significand difference between treatments.

Doses response of florpyrauxifen-benzyl to rice cultivars has not been currently reported. However, it has been reported response of cultivars to subsequent applications (WRIGHT et al., 2020a). These authors found that long rice grain hybrid CL745 was more susceptible to the sequential application of florpyrauxifen-benzyl than the long and medium grain variety CL111 and CL272. The hybrid CL745 injuries at two sequential doses of 30 g ai ha^{-1} (total 60 g ai ha^{-1}) were between 46%, and 64% at 60 g ai ha^{-1} (total 120 g ai ha^{-1}) when applications were made four days apart. Thus, a similar result has been reported regarding differential rice cultivars response to florpyrauxifen-benzyl and the values of injuries are similar as the observed for Pampeira at 120 g ai ha^{-1} (Figure 2). These results, and the observed in this study, support the hypothesis that there is a differential response of rice across cultivars to florpyrauxifen-benzyl doses.

It was necessary 20.2 g ai ha^{-1} of florpyrauxifen-benzyl to reduce biomass to 50% of Barnyardgrass (*Echinochloa* spp.), one of the most troublesome weeds in rice crops (MILLER; NORSWORTHY; SCOTT, 2018). This value is below the observed in this study which means that for both cultivars, IRGA 424 RI and Pampeira, it is possible to use florpyrauxifen-benzyl to control important weeds in field conditions.

3.4 Conclusion

There was no effect of malathion on rice tolerance to florpyrauxifen-benzyl, whereas the effect of dietholate fb PBO decrease the doses required to 50% injuries and growth reduction suggesting that P450 may play a role on florpyrauxifen-benzyl selectivity in rice.

There was a significant difference in tolerance between cultivars, being IRGA 424 RI more tolerant than Pampeira.

4 Chapter III – Effect of temperature regime on florpyrauxifen-benzyl selectivity in rice and on the expression of target candidate genes

4.1 Introduction

Herbicide crop tolerance, in many cases, is highly dependent on the crop's ability to degrade the herbicide (CARVALHO et al., 2009). Researches considered that visible injuries could determine herbicides' crop tolerance level; however, not always injuries couple with yield losses in field experiments (PANTONE; BAKER, 1992). Degradation by metabolism is the principal process of how plants dissipate pesticides, followed by growth dilution and volatilization (76%, 21%, and 3%, respectively) (JACOBSEN; FANTKE; TRAPP, 2015). Cytochrome P450 (CYP450) is a widely known enzyme related to the metabolism of several herbicides in plants, conferring in many cases resistance in weeds and tolerance in crops (DIMAANO et al., 2020). Several conditions, like air temperature, herbicide rate, growth stage, and abiotic interaction, can change crops' tolerance to herbicides. One example of this is the metabolization of rimsulfuron (ALS inhibitor) in maize mediated by CYP450, which was temperature-dependent when the half-life increased from 1.3 to 2.7 hours from 30ºC to 10ºC, respectively (KOEPPE et al., 2000).

Florpyrauxifen-benzyl is a new auxin herbicide release to control important agronomical weeds in rice (DUY et al., 2018). Previous rice crop observations have described leaf malformations, stem curling, chlorosis, heigh and tillers number reduction, and shoot dry weight reduction as common symptomatology of florpyrauxifen-benzyl in rice (WRIGHT et al., 2020a). Research of florpyrauxifen-benzyl has been focusing on explore weed control, the influence of soil moisture, and cultivar response to tank mix with imidazolinones and malathion (MILLER; NORSWORTHY, 2018b; WRIGHT et al., 2020b); however, because the novelty of this herbicide, the specific enzymes involved in the degradation processes have not been fully described.

Changes in temperature after spraying are crucial to avoid crop injuries. An example of this can be observed in weed control experiments with *Brachypodium hybridum* P. Beauv., where warm temperatures (28/34 °C night/day) for at least 48 hours after spraying are crucial to increase tolerance to pinoxaden, an ACCase inhibitor herbicide (MATZRAFI et al., 2016). Recently in a rice cultivar response experiment to florpyrauxifen-benzyl carried out in a growth chamber, it has been reported that injuries were promoted by warm growth temperatures (32/24 °C day/nighttime) than low (27/18 °C) (WRIGHT et al., 2020a). However, this study does not explore the changes in temperature after treatment. Moreover, there are no reports of gene expression related to the florpyrauxifen-benzyl degradation in rice.

The natural tolerance of auxin herbicides in crops is mainly due to non-target sites, and this means that the active ingredient is metabolized before reaching the target site rather than modify or overexpress the target site (BUSI et al., 2018). Gene expression on recurrent selection experiment of *Echinochloa colona* L. applying florpyrauxifen-benzyl indicates that CYP450 is upregulated when plants had grown at 45 °C compared to those which growth at 30 °C (BENEDETTI et al., 2020a). Glutathione s-transferase (GST) is another enzyme close related to herbicide degradation (VIJAY et al., 2019). In transgenic rice, to express *OsGSTL2* gene, it was observed increase in rice tolerance to glyphosate and chlorsulfuron (HU, 2014). However, regulation of genes is not a cheap process; consequently, the upregulation of one gene may represent the downregulation of others or energy diminish (VILA-AIUB; YU; POWLES, 2019). Thus, other components as transcription factor related to plant stress (*OsGTy*) (FANG et al., 2010), or enzymes responsive to cell membrane damage/signaling (*WALK21.2*) (MALUKANI et al., 2019) may be affected by temperature and herbicide, and consequently condition the rice response to florpyrauxifen-benzyl.

Therefore, it was hypothesized that the exchange in temperatures after application time would decrease the rice tolerance to florpyrauxifen-benzyl doses, promoting plant injury, and gene expression of *CYP71A21, OsGSTL3, OsGTy1 OsGTy2,* and *WALK21.2*. Then, the objectives of this study were to (I) evaluate the response of rice to florpyrauxifen-benzyl doses under exchanges in air temperatures after application time, and (II) determine whether the expression of *CYP71A21, OsGSTL3, OsGTy1 OsGTy2,* and *WALK21.2* genes on rice leaf treated with florpyrauxifen-benzyl doses would differ across temperatures conditions after spraying time.

4.2 Materials and Methods

4.2.1 Temperature surrounding time application experiment (whole plant experiment)

The experiments were carried out in a growth chamber at the Federal University of Pelotas (UFPel), and it was repeated twice in 2019 and 2020. Nine rice plants of cultivar IRGA 424 RI were established in five-liter pots previously filled with sieved paddy soil (Albaqualf) collected from the 0 – 20 cm soil profile nearby rice field.

The experiment was organized in a complete randomized block design in a factorial arrangement with four replications. Factor A consisted of three rates of florpyrauxifen-benzyl: 0, 30, and 60 g a.i. ha^{-1} (30 g a.i. ha^{-1} corresponds to the recommended dose). Factor B corresponded to six temperatures regarding conditions at initial growing, for 24 hours after application, and to the end of the experiment (Table 1). The temperatures were chosen according to the minimum, optimal and maximum rice conditions (18/15, 28/25, and 38/36 °C day/night) (SÁNCHEZ; RASMUSSEN; PORTER, 2014). The growth chamber was programmed to maintain a controlled day/night temperature according to the treatments previously described under controlled conditions with a photoperiod of 12 hours (900 µmol m^{-2} s^{-1}) and a constant relative humidity of 70 (± 5) %.

Table 1. Temperature regime "Factor B".

Temperature treatment	Initial growing (until 3 leaves)[1]	After application (for 24 hours)[1]	Experimental to the end (28DAA)[1,2]
T1 (all medium)	28/25°C	28/25°C	28/25°C
T2 (med-low-med)	28/25°C	18/15°C	28/25°C
T3 (high-low-med)	38/36°C	18/15°C	28/25°C
T4 (med-high-high)	28/25°C	38/36°C	38/36°C
T5 (med-high-med)	28/25°C	38/36°C	28/25°C
T6 (med-high-low)	28/25°C	38/36°C	18/15°C

[1]Temperature day/night
[2]DDA, days after application

Herbicide applications were carried out using a backpack sprayer with a four-nozzle boom (Tee-Jet AIXR110015) calibrated to deliver 150 L ha^{-1} spraying solution. Application conditions were air temperature 26 °C, humidity 65%, and wind velocity 4 km h^{-1}. Herbicide applications were sprayed when rice reach the three-leaf stage (V_3). The water sheet was settled at around five centimeters above the soil ground level 24 hours after herbicide application until the end of the experiment.

Visual evaluation of plant injury was assessed to each plant where "0%" correspond to the absence of symptoms and "100%" to dead plant. Plant injury was determined at three, seven, 14, 21, and 28 days after application. The plant height and tillers were determined at three, seven, 14, 21, and 28 days after application, and at

28 days after application shoot dry weight was determined. Shoot dry weight values are reported relative to the nontreated plants. Normality and homogeneity of variance were analyzed by the Shapiro Wilk test. Variables were analyzed in a mixed model operating each run as a random factor using the function *lmer* in the package *lme4.*R (BATES et al., 2020). A chi-squared distribution was considered for all response variables. III Analysis of Variance with Satterthwaite's method was done to determine each factor's effect and its interactions. The variance analysis showed no difference in the two runs of the experiments; thus, the variance of each experiment was included in the analysis. Mean comparisons and aggrupation were made by the method contrast mean of Kenward-roger (*p-value* <0,05). All analyzes were carried out at the R® version 3.5.2 GUI 1.70 statistic ambient (R CORE TEAM, 2019).

4.2.2 Gene expression experiment

4.2.2.1 Plant materials, growth conditions and treatments.

Four commercial rice seeds (*Oryza sativa*. cv IRGA424 RI) were planted at 1 cm depth in each 500 g pots, previously filled with soil as described before. All pots were incubated in a growth chamber at the Federal University of Pelotas, Brazil. The initial growth conditions were 28/25 ºC (day/night temperature) for treatment T1, T2, and T4. For T3, the initial conditions were 38/36 ºC. Same florpyrauxifen-benzyl doses previously described were sprayed when rice reach the three-leaf stage (V_3). Herbicide was sprayed with the same conditions as described above. The growth conditions for the first six hours after spraying were equal to the initial growth conditions. After that time, pots corresponding to each treatment were transferred to specific temperature conditions; T1 continue 28/25 °C, T2 and T3 decrease to low 18/15 °C, and T4 transferred to high 38/36 °C. To total, four temperature treatments were established in three doses and three evaluation times (6, 12, and 24 hours after spraying), each with two biological replicates. The experiment was considered as a factorial experiment in randomized block design.

Approximal eight grams of leaf tissue were collected at each evaluation time from both biological replicates. Plant material was collected manually using esterized gloves and scissors. The collected material was mixed into each treatment and evaluation time then placed in aluminum foil envelopes, kept in liquid nitrogen until it was stored in an ultra-freezer at -80 °C. The whole material was freezing with liquid nitrogen and macerate using laboratory mortar and pestle, and then the material was stored in microtubes of 1.5 mL at -80 °C.

4.2.2.2 RNA extraction and cDNA synthesis

Total RNA was extracted from two grams of leaf tissue using PureLink Plant RNA Reagent (Invitrogen®) following the protocol described by the manufacturer. Quantity and purity of the RNA was verified by spectrophotometry in Nanuvue (GE Helthcare) and integrity by agarose gel electrophoresis. Each sample (1 µg) was treated with DNase I Amplification Grade (Invitrogen®) and were converted into cDNA using oligo(dT) and SuperScript III first-strand system kit (Invitrogen®).

4.2.2.3 Gene expression quantification by RT-qPCR

The RT-qPCR experiment was performed following to the MIQE guidelines (BUSTIN et al., 2009) using oligonucleotides for the reference and target genes (Table 2). To determine amplification efficiency and specificity of each oligonucleotide, validation experiments were performed using four cDNA dilutions. Oligonucleotides that were 90-110% efficient and with only one peak in the dissociation curve were used.

Gene expression assay was performed in LightCycler® 480 Instrument II (Roche) thermocycler using three biological and three technical replicates. Reactions were performed using cDNA 1 µL in 1:25 dilution (determined during validation experiments), UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen) 11.0 µL, ROX Reference Dye (Invitrogen) 0.25 µL, 10X PCR Buffer 2.0 µL, MgCl 50 mM 1.5 µL, Platinum™ Taq DNA Polymerase 0.05 µL, dNTPs 0.2 µL, SYBR Green I (Invitrogen) 3.0 µL and oligonucleotide 10 mM 1.5 µL of each forward and reverse in a 20 µL of final volume reaction. Negative control reactions without cDNA were also performed for each oligonucleotide pair. The PCR condition was of initial denaturation at 95 °C for 5 minutes, 45 cycles of 95°C for 20 seconds, 60 °C for 15 seconds and 72 °C for 20 seconds. Reactions were performed in LightCycler® 480 Multiwell Plates 96 (Roche).

Target gene expression quantification was conducted using the ΔΔCT method (LIVAK; SCHMITTGEN, 2001) using as baseline the expression of T1 treatment (28/25°C along the experiment) without herbicide application normalized respect to *Os18S*, *OsEF1α* and *OsUBQ5* reference genes (JAIN et al., 2006). Gene expression data was converted in Log_2-fold change. The mean and confidential interval at 95% of three technical replicates were used to compared treatments.

Table 2: Oligonucleotides used in this study for RT-qPCR assay.

Gene	ID	Oligonucleotide - (5'-3')		Reference
Cytochrome P450 CYP71A21	*OsCYP71A21*	Foward	TGTGACAATGATCTTCTACGAGGT	Hirose et al. (2007)
		Reverse	TCCATCTCTTTGTATGTTTTCCAA	
Wall-associated kinase like 21.2	*OsWAKL21.2*	Foward	GCCACTTTCCCGCTAAGAAGAG	Malukani et al. (2019)
		Reverse	CGCCAAGACACCTCCAACTATG	
Transcription factor from GTy family	*OsGTy-1*	Foward	ATCTGGTGGTCCAACTGCTGA	Fang et al. (2010)
		Reverse	CGGCGTTTTTCAAGTCGAAT	
Transcription factor from GTy family	*OsGTy-2*	Foward	TTGTCACCTCATGCTCGAATG	Fang et al. (2010)
		Reverse	GGAAAGGGTTTGTTGATATCCAAC	
Glutathione S-Transferase	*OsGSTL3*	Foward	CAAGATGAAGCAGGCAGAG	Zhang et al. (2014)
		Reverse	GCACACCAACACCAACTT	
18S ribosomal RNA	*Os18S*	Foward	CTACGTCCCTGCCCTTTGTACA	Jain et al. (2006)
		Reverse	ACACTTCACCGGACCATTCAA	
Elongation Factor 1-α	*OsEF1α*	Foward	TTTCACTCTTGGTGTGAAGCAGAT	Jain et al. (2006)
		Reverse	GACTTCCTTCACGATTTCATCGTAA	
Ubiquitin 5	*OsUBQ5*	Foward	ACCACTTCGACCGCCACTACT	Jain et al. (2006)
		Reverse	ACGCCTAAGCCTGCTGGTT	

4.3 Results and Discussion

4.3.1 Rice response to florpyrauxifen-benzyl, as affected by temperature after the application time.

There was no interaction between fixed factors for plant injury and physiological responses (tillers, height, and dry shoot weight) (Table 3). However, for plant injury, it was operated an untreated level into Factor A to explore differences among temperature (Table 3). The double rate of florpyrauxifen-benzyl was significant at three and seven days after spraying (Table 3). And plant injury compared with the untreated in all temperatures was significantly different over time.

Generally, injuries through evaluations were lesser for treatments where temperatures keep all medium or decrease until low for 24 hours after spraying (T1, T2, and T3) than those where temperature increase from medium to high for 24 hours after spraying (T4, T5, T6) (Table 3). There was an exception for T5 at seven and 14 DAA where injuries were not different to the untreated and similar to treatments where temperature decrease from medium to low for 24 hours after spraying. However, at 21 and 28 DAA injuries were similar to those where temperature increased from medium to high 24 hours after application (Table 3). Thereby, results in this research suggest that the increase in air temperature 24 hours after spraying (from 28/25ºC to 38/36ºC) promoted the rice injury to florpyrauxifen-benzyl.

Warm temperatures and increased doses of florpyrauxifen-benzyl have been reported to exacerbate the injuries in rice, and the differential response seems to vary among cultivars. In a rice tolerance experiment to florpyrauxifen-benzyl rates conducted in a growth chamber at warm and low temperatures (32/24 ºC and 27/18 ºC day/nighttime), it has been reported that the long-grain pure-line (CL111) was more tolerant over warm temperature conditions than the medium-grain pure-line CL272 with 18% of plant injury at 60 g a.i. ha^{-1}, and long-grain hybrid CLXL745 with 25% of plant injury at 60 g a.i. ha^{-1} (WRIGHT et al., 2020a). The cultivar IRGA 424 RI used in this experiment correspond to a medium non-hybrid cultivar; thus, the injury increases in treatments where temperature increase after spraying couple with the reported for medium-grain pure-line CL272 cultivar or long-grain hybrid CLXL745 at 32/34 ºC. Results herein and in the Wright et al. (2020a) suggest that increase in the rate of florpyrauxifen-benzyl represent an increase in rice plant injuries.

The differential tolerance of rice cultivars to herbicides has been reported, and for an instant, this is considered a result of differential crop metabolism rate among cultivars. One example of this has been reported for triclopyr, also a synthetic auxin herbicide, where rice cultivars Mars and Tebonnet (15% and 16% of the injury, respectively) were more tolerant than Lemont (25% of injury) (PANTONE; BAKER, 1992). Another example is the differential tolerance to imazamox among Clearfield® rice cultivars (BOND; WALKER, 2011). Hence it is likely that florpyrauxifen-benzyl is metabolized in rice plants, and then temperature changes after treatment affect this metabolism rate causing the injuries observed in this study.

Visible injuries can determine herbicides' crop tolerance level; however, not always injuries couple with yield losses in field experiments. One example of this is when triclopyr applications at early rice reproductive stages (R0) do not show plant injuries, but they represent a significative yield loss (PANTONE; BAKER, 1992). However, this is not the case of florpyrauxifen-benzyl because applications in rice made at 1-, 3- and 5- leaf stage showed plant injuries, but it does not represent a significant yield loss (WRIGHT et al., 2020b). In the first chapter of this research, florpyrauxifen-benzyl does not have lasting effects on yield. Therefore, the injuries observed in these results do not represent adverse effects on yield.

Tillers, height and dry shoot biomass did not show significant differences between non-treated, commercial, and two times the commercial rate of florpyrauxifen-benzyl (Table 4 and Table 5). The effect for the main factor temperature was individually significant through all evaluations time ($p < 0.05$), whereas the florpyrauxifen-benzyl rate was not significant (Table 4 and Table 5). Based on these results, it can be inferred that florpyrauxifen-benzyl does not reduce those variables. The greatest shoot dry biomass was for T5 (4.4 g $plant^{-1}$), and the lowest was for T6 (0.6 g $plant^{-1}$). However, the percentage related to the untreated was not different (Table 4). Thus, it was considered that the effect on growth observed in this study was more related to the temperature treatment than the herbicide rate. Height and tillers number of rice after florpyrauxifen-benzyl spraying (30 and 60 g ai ha^{-1}) has been described to not be affected compared to the untreated in field experiments in Arkansas, USA (WRIGHT et al., 2020b).

Table 3. Effect of three florpyrauxifen-benzyl rates and six temperature treatments on rice plant injury at 3, 7, 14, 21 and 28 days after application.

Treatments	Rice plant injury (% of untreated)				
	3 DAA	7 DAA	14DAA	21DAA	28DAA
Rate					
0	0.0 c	0.0 c	0.0 ns	0.0 ns	0.0 ns
30	5.3 b	6.4 b	9.4	7.8	5.3
60	8.9 a	9.0 a	11.9	9.0	8.4
Temperature					
Untreated	0.0 c	0.0 d	0.0 d	0.0 d	0.0 c
T1 (all medium)	4.9 b	8.4 bc	6.9 c	7.2 c	2.0 c
T2 (med-low-med)	3.8 bc	5.5 cd	5.7 c	2.1 d	1.8 c
T3 (high-low-med)	3.8 bc	5.3 cd	9.4 c	4.4 cd	2.9 c
T4 (med-high-high)	9.4 a	13.0 a	20.8 a	11.3 b	9.0 b
T5 (med-high-med)	11.8 a	3.9 d	6.8 c	10.9 b	9.7 b
T6 (med-high-low)	8.9 a	10.1 ab	14.3 b	14.4 a	15.7 a
			p-value		
Run	0.9990	0.9994	0.9982	0.9911	0.9998
Rate	0.0150	0.0148	0.0675	0.1248	0.0653
Temperature	0.0013	0.0070	0.0000	0.0000	0.0000
Temperature x Rate	0.8553	0.4189	0.4063	0.2370	0.2974

[1]DAA: Days after application.
[2]Means were grouped by the method of Kenward-roger (confidence level 95%). Means followed by the same letter within a column are not different.
[3]Rate in g ai ha^{-1}.
[4]Changes in temperature treatments at Initial growing (until 3 leaves after application) - after application (for 24 hours) - experimental to the end (28DAA). High (38/36 C); Med: medium (28/25 C); and low (18/15 C).

In a rice cultivar tolerance experiment, long-grain cultivar (CL111) showed that neither florpyrauxifen-benzyl rate nor high temperature (32/24ºC) reduce the number of tillers and biomass, evaluated 28 days after application (WRIGHT et al., 2020a). However, medium-grain cultivar (CL272) showed a reduction in at least 20% of the height and 13% of biomass regardless of the temperature growth conditions (32/24ºC or 28/17ºC, day/night temperatures) (WRIGHT et al., 2020a). And a long-hybrid cultivar (CLXL745) showed a reduction in height at 28/17ºC conditions regardless of the florpyrauxifen-benzyl rate (WRIGHT et al., 2020a). In this experiment, a reduction in these variables was not observed due to florpyrauxifen-benzyl, which means that IRGA 424 RI, a medium non-hybrid cultivar, has similar tolerance to other cultivars as CL111, extensive used in the USA.

Table 4. Effect of three florpyrauxifen-benzyl rates and six temperature treatments on rice tiller number, and shoot dry weight at 3, 7, 14, 21, and 28 days after application.

Treatments	Rice plant tillers (number of tillers plants^{-1})					Shoot biomass (% of untreated)
	3 DAA[1]	7 DAA	14 DAA	21 DAA	28 DAA	28 DAA
Rate						
Non-treated	1.9 ns	2.8 ns	5.6 ns	7.7 ns	8.9 ns	100.0ns
30	1.7	2.7	5.8	7.9	8.7	100.0
60	1.5	2.7	5.8	8.0	9.5	94.0
Temperature						
T1 (all medium)	1.6 a[2]	2.8 b	6.1 bc	8.1 b	8.8 c	100.0ns
T2 (med-low-med)	1.3 a	2.3 bc	5.9 bc	7.5 b	8.8 c	102.0
T3 (high-low-med)	1.1 a	2.2 cd	5.3 c	10.2 a	11.2 ab	101.0
T4 (med-high-high)	1.5 a	3.6 a	8.3 a	10.5 a	11.8 a	100.0
T5 (med-high-med)	1.5 a	3.6 a	6.7 b	8.5 b	10.4 b	90.0
T6 (med-high-low)	1.4 a	1.7 d	2.1 d	2.3 c	3.1 d	98.0
			p-value			
Run	0.9974	0.9987	0.9974	0.9989	0.9991	0.9979
Rate	0.7557	0.7924	0.9947	0.9366	0.4267	0.4279
Temperature	0.0063	0.0057	5.98E-05	1.83E-09	6.62E-13	3.77E-10
Rate x Temperature	0.8721	0.9713	0.8781	0.9851	0.8804	0.9225

[1]DAA: Days after application.
[2]Means were grouped by the method of Kenward-roger (confidence level 95%). Means followed by the same letter within a column are not different.

Table 5. Effect of three florpyrauxifen-benzyl rates and six temperature treatments on rice height at 3, 7, 14, 21, and 28 days after application.

Treatments	Rice plant height (cm)				
	3 DAA[1]	7 DAA	14 DAA	21 DAA	28 DAA
Rate					
Non-treated	33.6 ns	37.5 ns	44.4 ns	48.9 ns	52.4 ns
30	33.4	37.9	44.0	48.2	51.8
60	33.3	37.2	44.4	48.8	53.3
Temperature					
T1 (all medium)	33.7 ab	36.5 b	45.4 ab	50.0 b	53.3 b
T2 (med-low-med)	32.4 b	36.2 b	46.3 ab	51.9 ab	56.2 ab
T3 (high-low-med)	29.8 c	34.4 b	44.1 b	51.8 ab	54.9 b
T4 (med-high-high)	34.1 ab	40.5 a	45.8 ab	49.5 b	55.8 ab
T5 (med-high-med)	34.7 ab	41.6 a	48.3 a	53.3 a	59.2 a
T6 (med-high-low)	35.9 a	36.0 b	35.6 c	35.5 c	35.5 c
			p-value		
Run	0.9955	0.9896	0.9994	0.9977	0.9955
Rate	0.3744	0.6868	0.4650	0.5001	0.1135
Temperature	0.0490	5.44E-07	1.29E-11	6.11E-13	8.47E-13
Rate x Temperature	0.6256	0.3664	0.6141	0.3008	0.2592

[1]DAA: Days after application.
[2]Means were grouped by the method of Kenward-roger (confidence level 95%). Means followed by the same letter within a column are not different.

4.3.2 The gen response of rice to florpyrauxifen-benzyl, as affected by temperature after the application time.

4.3.2.1 Cytochrome P450 monooxygenase

CYP71A21 is a gene encoding for a P450 monooxygenase enzyme in rice seedling, which has been described as responsive to herbicides, including auxins (HIROSE et al., 2007). Six hours after the spraying at commercial and double doses, *CYP71A21* expression was lesser than the untreated for rice growth at 28/25 ºC, whereas it was greater than untreated for rice growth at 38/35 ºC (Table 5). At 12 hours, the treatment, which continues at medium temperature (28/25 ºC), showed greater expression of *CYP71A21* after spraying florpyrauxifen-benzyl, form -0.6-fold to 1.6 and 1.8-fold for untreated, 30 and 60 g ai ha^{-1}, respectively. At 24 hours after treatment, all *CYP71A21* gene expression was downregulated. This result suggests that change in temperature after treatment reduce the response in expression of *CYP71A21* in rice and consequently affect the tolerance of rice to florpyrauxifen-benzyl. This result is supported by the rice plant injury observed in table 3 where the increase in temperature from medium to high reach the highest injuries than the treatment at constant temperature (28/25 ºC). However, the downregulation occurred regardless the sense of temperature change from medium to high, high to low and medium to low.

Relative expression of P450 has been described in rice weed Junglerice (*Echinochloa colona*) regarding the florpyrauxifen-benzyl spraying, temperature, and water stress in the second generation after sublethal doses (BENEDETTI et al., 2020a, 2020b). *CYP72A14* increases expression in Junglerice treated with florpyrauxifen-benzyl (4.2-fold) compared with the untreated (-0.3-fold), this expression was greater induced when plants growth at optimal conditions (30 ºC: 4.2-fold) and at drought stress (4.2-fold) than at heat stress (45 ºC: 3.2-fold) and well-watering (1.7-fold) (BENEDETTI et al., 2020a, 2020b). Contrary, *CYP72A15* was greater induce at 45 ºC rather than at 30 ºC (4.5-fold and 0.1-fold, respectively). The spraying of florpyrauxifen-benzyl decreased *CYP709B1* and *CYP709B2* expressions from 6.1- and 8.4-fold to 4.9- and 2.0-fold, respectively (BENEDETTI et al., 2020a). These results and the observed in this research demonstrated that CYPs act differently in response to the application of florpyrauxifen, mainly in adverse environmental conditions.

4.3.2.2 Glutathione S-transferase

OsGSTL3 gene encodes glutathione s-transferase, a widely known enzyme that confers herbicide tolerance in rice (HU, 2014). Six hours after florpyrauxifen-benzyl spraying, it was observed an overexpression for double doses, whereas the high to low treatment (T3) showed a slight upregulation at commercial doses. At 12 hours after spraying, it was observed an increase in expression of *OsGSTL3* for treatments in constant temperature (T1: all medium, 28/25 ºC) at commercial doses compared to untreated, and treatment where temperature change from medium to low (28/25 ºC to 18/15 ºC) showed downregulation at double of commercial doses. The rest of the treatments at 12 and 24 hours after spraying showed downregulation. Generally, *OsGSTL3* seems to be more downregulated at 24 hours after spraying for treatment, where temperature increase from medium to high (28/25 ºC to 38/35 ºC) compared with treatments where the temperature was kept constant (28/25 ºC) or decrease from medium to low or high to low.

Many examples of GST expression have been described providing plants the ability to tolerate herbicides. Organ-specific expression of *AtGSTU19* confer tolerance of chloroacetamide in *Arabidopsis sp.* (DERIDDER; GOLDSBROUGH, 2006). Upregulation of *SbGSTF1* and *SbGSTF2* is induced by fluxofenim, a safener used in seed treatment in Sorghum (BAEK et al., 2019). Likewise, *OsGSTL2* transgenic rice improves the glyphosate and chlorsulfuron tolerance in rice plants (HU, 2014). Herein it was observed that there was a differential profile in the expression of *OsGSTL3* product of temperature changes over time in rice, but it cannot be proved if there was an overexpression cause by the florpyrauxifen-benzyl spraying. This because the increase in florpyrauxifen-benzyl rate does not show a consistent increase in expression on *OsGSTL3*.

4.3.2.3 GT transcription factors

OsGTy1 and *OsGTy2* are two genes encoding transcription factors in rice (FANG et al., 2010). *OsGTy1* has been reported to be induced by highly salinity stress, drought, abscisic acid, and salicylic acid (FANG et al., 2010). Likewise, in addition to salt and abscisic acid, *OsGTy2* is responsive to cold stress (FANG et al., 2010). Hence *OsGTy1* and *OsGTy2* serve as reporter genes regarding rice plants' status in the function of common stress that could be present through the experiment. Additional to rice response to stresses, those genes are considered to have no significant increase in the function of phytohormone addition, such as indole acetic acid (FANG et al., 2010). *OsGTy1* values three hours after stress have been reported; over 10-fold were considered for salt stress, around 4-fold for drought stress and abscisic acid, and over 6-fold for salicylic acid. Likewise, *OsGTy2* values over 3-fold have been described for cold stress.

Untreated plants showed stable relative expression over the time and temperature treatments from 0.2 to 2.2-fold for *OsGTy1* and from 2.1- to 3.4-fold for *OsGTy2*. At commercial doses, there were few cases where *OsGTy1* relative expression slightly increased. At six hours after spraying florpyrauxifen-benzyl, *OsGTy1* decrease the expression compared to the untreated, whereas *OsGTy2* expression is similar between sprayed and untreated (Table 5). *OsGTy1* for T3 at six hours after spraying florpyrauxifen-benzyl showed an increase compared to the untreated for both doses. However, *OsGTy2* for T3 at six hours showed downregulation in the untreated and a slight increase at double doses of florpyrauxifen-benzyl. This response is expected since *OsGTy2* responds to cold stress, and in this case, the T3 is the only treatment where temperature started high (38/36 ºC).

The highest value of *OsGTy2* at untreated conditions was for T2 at 12 hours (3.4-fold changes); this can be explained since in this treatment, temperature decrease from medium (28/25 ºC) to low (18/15 ºC). The maximum value of relative expression of Os*GTy2* was observed for T3 at commercial doses of florpyrauxifen-benzyl (4.1-fold changes); because this is the most extreme treatment regarding the temperature change (38/36 ºC to 18/15 ºC), this response is expected. Treatments where temperature change from medium to high (28/25 ºC to 38/36 ºC) showed an increase in *OsGTy2* expression between 12 and 24 hours after spraying. Whereas *OsGTy1* showed an increase in commercial doses and double doses. Therefore, the expression seen for *OsGTy1* and *OsGTy2* indicates that rice plants are not affected by florpyrauxifen-benzyl; thus, rice plants can respond to external stresses like drought or salinity; additionally, it was observed the effect for the temperature treatment.

4.3.2.4 *OsWAKL21.2*

Wall associate kinases (WAKs) are enzymes localized through the cell wall and membrane, and they are capable of sending signals into the cytoplasm (KOHORN, 2016). WAKs have an essential role in physiological prosses such as cell elongation, pollen development, and abiotic and biotic stress tolerance (KOHORN, 2016). *OsWAKL21.2* encodes to a WAK, which has a dual function as a receptor or co-receptor of wall damage and a receptor of biotic stress (MALUKANI et al., 2019). *OsWAKL21.2* overexpression in rice induces tolerance to cell wall degradation products and immune response to lipases/esterases (MALUKANI et al., 2019). Florpyrauxifen-benzyl is an auxinic herbicide; hence, it can affect the cell wall since one of the physiological consequences of spraying them in plants is decontrolled the cell elongation process as a result of enzyme activation at the cell membrane (BUSI et al., 2018). Thus, the *OsWAKL21.2* gene expression helps us evaluate the rice consequences of florpyrauxifen-benzyl on the cell wall. Additionally, this gene provides us as a reporter of the rice capacity to respond to abiotic and biotic factors.

Generally, the untreated plants showed positive values of *OsWAKL21.2*. for all temperature treatments at 6 and 12 hours after treatment (Table 5). However, at 24 hours was observed decreasing in expression for treatments where temperature change from high to low and medium to high (T3: 38/35 ºC to 18/15 ºC, and T4: 28/25 ºC to 38/35 ºC, respectively) compared to all medium temperature and medium to low (T1: 28/25 C and T2: 28/25 C to 18/15 C, respectively) (Table 5). The addition of florpyrauxifen-benzyl suggests a decrease in the expression of *OsWAKL21.2*. It was a fact when *OsWAKL21.2* at six hours after florpyrauxifen-benzyl spraying, showed a decrease in the expression compared to untreated (Table 5). Thus, it can be inferred that rice loses part of the capacity to express *OsWAKL21.2* after sprayed florpyrauxifen-benzyl at high to low and medium to high treatments.

In summary, the rice leaf expression of *CYP71A21* and *OsGSTL3* seems to be affected the majority by the temperature treatments than the florpyrauxifen-benzyl doses. This result implies that the addition of other pesticides which need these genes to degrade could be affected. On the other hand, the genes responsive to additional abiotic factors (*OsGTy1* and *OsGTy2*) such as drought or salinity were no affected by neither temperature nor the florpyrauxifen-benzyl doses. Finally, *OsWAKL21.2*, a gene responsive to cell wall damage and membrane signaling, indicates a reduction over florpyrauxifen-benzyl doses in high to low and medium to high treatments this result is linked with plant injuries observed previously. Thus, the rice crop tolerance to florpyrauxifen-benzyl depends in part on the loss of rice capacity to express *OsWAKL21.2* and high temperatures before or after spraying exacerbate this fact.

Table 5. Relative mRNA abundance (Log_2-fold change of gene expression) in rice (*Oryza sativa* cv IRGA 424 RI) leaves. mRNA abundance of each gene from untreated plants served as the baseline for determining relative RNA levels. The color scale below the heatmap shows the expression level.

Florpyrauxifen-benzyl		Log2-fold change of gene expression									
		T1 all medium			T2 med-low		T3 high-low			T4 med-high	
Gene	Doses	6 HAS*	12 HAS	24 HAS	12 HAS	24 HAS	6 HAS	12 HAS	24 HAS	12 HAS	24 HAS
CYP71A21	0	3.4(0.32)	-0.6(0.02)	-3.1(0.17)	1.9(1.06)	-1.9(0.14)	-0.8(0.35)	-4.3(0.81)	-4.4(1.66)	-3.4(1.32)	-3.0(0.46)
	30	-0.9(0.07)	1.6(0.21)	-2.1(0.84)	-0.1(0.08)	-4.0(0.63)	2.5(0.25)	-7.6(1.82)	-5.1(1.22)	-4.6(1.94)	-2.7(0.91)
	60	0.7(0.13)	1.8(0.55)	-4.3(1.00)	-4.9(1.32)	-3.0(1.28)	0.6(0.83)	-5.0(1.12)	-3.4(0.64)	-3.3(0.66)	-4.6(0.81)
OsGSTL3	0	0.20(0.37)	0.3(1.15)	-0.8(0.44)	0.6(0.02)	-2.2(0.32)	-2.3(0.87)	-0.5(0.68)	-3.3(0.33)	-1.0(0.67)	-6.1(1.91)
	30	-0.7(0.18)	1.1(0.96)	-2.4(1.31)	0.4(0.50)	-3.3(1.12)	0.9(0.00)	-4.0(1.35)	-3.6(1.79)	-1.6(0.30)	-5.4(1.65)
	60	2.9(1.01)	0.2(0.18)	-3.0(0.66)	-2.7(0.59)	-2.7(1.13)	-0.9(0.16)	-2.0(0.88)	-3.5(0.85)	-1.5(0.62)	-6.2(1.37)
OsGTy1	0	2.0(0.32)	1.4(0.32)	0.8(0.47)	2.2(0.34)	2.2(0.41)	1.7(0.31)	0.2(0.41)	1.0(0.40)	1.8(0.52)	1.3(0.33)
	30	0.5(0.25)	2.1(0.29)	1.0(0.31)	2.5(0.54)	3.3(0.64)	2.1(0.32)	0.1(0.20)	2.1(0.07)	0.3(0.31)	1.3(0.48)
	60	1.3(0.39)	1.4(0.26)	3.0(0.18)	-0.2(0.22)	1.9(0.35)	2.9(0.03)	0.3(0.37)	1.7(0.46)	1.4(0.49)	2.1(0.55)
OsGTy2	0	1.4(0.49)	1.3(0.29)	2.7(0.14)	3.4(0.61)	2.4(0.51)	-2.1(0.95)	1.8(0.97)	1.9(0.22)	1.5(0.41)	2.1(0.33)
	30	1.5(0.38)	0.9(0.45)	1.3(0.79)	3.0(0.17)	1.8(0.11)	0.0(0.13)	-2.9(0.95)	4.1(0.98)	1.6(0.40)	2.9(0.15)
	60	1.4(0.46)	2.6(0.17)	2.2(1.18)	-3.0(1.35)	1.8(0.42)	0.8(0.59)	-0.8(0.18)	1.6(0.02)	1.7(0.76)	1.9(0.38)
WALK21.2	0	6.9(0.16)	6.6(0.81)	4.9(0.04)	3.1(0.10)	4.5(0.20)	5.0(0.41)	4.6(0.48)	-1.0(0.98)	5.7(0.92)	1.7(0.73)
	30	3.4(0.28)	5.4(0.89)	2.7(0.70)	5.6(1.16)	0.5(0.01)	0.1(0.00)	-1.2(0.09)	-2.0(0.51)	3.5(0.77)	2.6(0.34)
	60	2.9(0.90)	5.4(0.39)	3.3(0.78)	5.0(0.32)	2.4(0.65)	1.7(0.64)	0.0(0.56)	3.0(0.36)	3.6(1.20)	0.6(0.33)
Scale											
Expression scale (Log2-fold change)					<-8	-8 to -4	-4 to -2	-2 to 0	0 to 2	2 to 4	>4

* HAS = hours after herbicide spraying.

2.4 Conclusion

Florpyrauxifen-benzyl injury in rice plant was affected by temperature after application. High temperatures after herbicide application promoted the highest injury in rice. Rice growth, e.g., tillers, plant height, and shoot dry weight of rice, were no affected by the florpyrauxifen-benzyl application.

Rice leaf *CYP71A21* and *OsGSTL3* genes were downregulated when the temperature comes from high to low and medium to high.

The expression of *OsGTy1* and *OsGTy2* on rice leaf was no affected by neither temperatures nor the florpyrauxifen-benzyl doses.

Downregulation of *OsWAKL21.2* on rice leaf was detected over florpyrauxifen-benzyl doses when the temperature comes from high to low and medium to high, associating the plant injury described in the whole plant experiment.

5 Final Remarks

New alternatives of weed control and selectivity to crop have a pivotal matter for industry and growers. Hence this study contributed to the knowledge about the performance of florpyrauxifen-benzyl selectivity to rice in field conditions and controlled conditions, under different air temperature conditions and relating with the cultural practices as interaction with insecticides and response to different cultivars. Briefly, results showed that the early rice stages, environmental conditions of early planting time, and a rate increase of florpyrauxifen-benzyl promote rice injuries; however, there was no lasting adverse effect on grain yield or quality (Chapter I). The addition of malathion does not compromise the rice selectivity to florpyrauxifen-benzyl, and the addition of two inhibitors of P450 decrease the doses to reach 50% of growth reduction; however, it kept over the commercial recommendation doses, and the tolerance level is lesser for Pampeira cultivar than IRGA 424 RI (Chapter II). Finally, high temperatures before or after spraying may condition the rice selectivity to florpyrauxifen-benzyl due to decreased rice capacity to respond to herbicide degradation and biotic and abiotic stresses (Chapter III).

Consequently, all results provide farmers and agronomists the confidence to use this herbicide in their producing areas and increase the use of new tools in integral weed management.

On the other hand, auxin herbicides as florpyrauxifen-benzyl have been studied due to their particular site of action and unclear weed resistant mechanism. Thus, this study contributes to expanding the knowledge of this group of herbicides. Moreover, brings basic results to hint about the florpyrauxifen-benzyl metabolic route and potential rice selective mechanism.

Overall, these results bring additional questions regarding the rice response to florpyrauxifen-benzyl. Some of those questions are; whether others P450 is related or not with the florpyrauxifen-benzyl metabolism? What is happening with the metabolites of florpyrauxifen-benzyl after absorption in rice? What are the enzymes related to the metabolism of florpyrauxifen-benzyl? Is there an AUXIN transporter’s related in rice selectivity to florpyrauxifen-benzyl?

6 Vitae

I am a Colombian citizen raised in a middle-income family from a small city called Fusagasugá, 50 km south of Bogotá. As a young child, I use to help my father and my mother in the bakery. This experience allows me to understand the importance of cooperative and hardworking and build solid principles as honesty and commitment.

I got my bachelor's degree in Agronomy at the National University of Colombia. I am currently doing a postgraduate master's degree in weed science at the Federal University of Pelotas (UFPel), Brazil. My interest in agronomy started as a child, having as a model my father, who encouraged me to love agriculture while working on his small farm in the Andes. During my undergraduate program in Agronomy, weed science was my favorite subject. In the end, I decide to work on my Master's degree in this area.

Since 2017, I have been doing experimental research in weed science as part of my final research project at the undergraduate level, and now with the Master's research project. The undergraduate research thesis was entitled "Alternative post-emergence control on Colombian weedy rice morphotypes and genotypes". The results showed a weedy rice morphotype resistant to ALS-inhibitor herbicides (manuscript in the writing process). This research taught me the basic steps to detect and determine weed resistance and the importance of identifying resistance in the field.

My Master's project, entitled "Selectivity of florpyrauxifeno-benzyl to rice", aims to evaluate the selectivity of the herbicide florpyrauxifeno-benzyl to rice under varying abiotic conditions. Also, I have assisted in other projects as an intern, determining the resistance of weeds in Colombian accesses of *Eleusine indica* to glyphosate and identifying symptoms of herbicides in Brazilian crops. I also had the opportunity to participate in two international congresses in Brazil (2018) and Costa Rica (2019). These experiences allowed me to recognize the problems of weed science from a global and holistic perspective and improve my communication skills.

My interest is to continue pursuing the study of herbicide physiology and improve the understanding of weed resistance, growing my capacity and experience to launch a professional career in research. I hope to return all the knowledge acquired by becoming a high-caliber researcher and/or professor applying my skills and expertise in Colombia. I am a person who is not afraid of hard work, with powerful motivation to learn and grow as a professional.

7 References

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