ABSORPTION, TRANSLOCATION AND METABOLISM OF BISPYRIBAC-SODIUM ON RICE SEEDLINGS UNDER COLD STRESS

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Abstract: Rice production is highly affected by weed competition. The efficacy of chemical weed control and crop safety, as well its crop selectivity, is a function of absorption, translocation and metabolism of herbicides. This study investigates the effect of cold stress 22/16C (day/night) on absorption, translocation and metabolism of ^{14}C-bispyribac-sodium on rice seedlings. Maximum ^{14}C-bispyribac-sodium absorption occurred at 24 HAT and was stimulated by the warm 30/22C (day/night) temperature. A large amount of total absorbed herbicide was retained on the treated leaf, indicating that bispyribac-sodium had lower translocation to other plant parts. Injury on rice plants was enhanced by exposure to cold, emphasizing the negative effect on herbicide metabolism. Cold-grown plants had higher recovery of the parent herbicide than plants grown at a warm temperature. Moreover, warm grown plants showed a greater amount of metabolite B compared to cold grown plants

Keywords: herbicide selectivity, injury, low temperature, P450-monooxygenase.

INTRODUCTION

In Brazil, the rice planting time has being pushed to early spring, being one of the main management practices to achieve high yield. This practice promotes the improvement of nitrogen assimilation in plants grown under great solar radiation during December/January on the reproductive stage of rice, especially on microsporogenesis and panicle initiation (YOSHIDA, 1981). However, has been observed in the South and Southwest areas (Campanha) of RS, that the cold stress promoted by early planting may decrease bispyribac-sodium selectivity, increasing injury on rice.

Herbicide efficacy on weed control as well the selectivity on crops is a function of their absorption, translocation, and metabolism (OLIVEIRA JR., 2011). These factors are highly correlated with environmental conditions such as low temperatures. The cold stress affects the whole plant metabolism which by turn can indirectly cause negative effect on plant protection mechanisms, turning them more vulnerable to other stress agents (LYCAN & HART, 2006). Therefore, an understanding of the response caused by cold stress on absorption, translocation and metabolism may explain the increase of bispyribac-sodium toxicity on seedlings of early planted rice. The objective of this study was to evaluate the effect of cold stress on absorption, translocation and metabolism of bispyribac-sodium on rice seedlings.

MATERIAL AND METHODS

The experiments were conducted twice in 2013 in a growth chamber at Altheimer Lab – Crop, Soil and Environmental Sciences Department of University of Arkansas, Fayetteville, US. The plots consisted of 0.2- L plastic pots filled with washed sand. The experiments were arranged in a completely randomized scheme, using a factorial treatments with four replications. Factor A was composed of rice cultivars: IRGA 424 - cold tolerant; and Epagri 109 - cold susceptible; factor B consisted of two temperatures: 16/22 C and 25/30 C (night/day) simulating the early (cold) and late (warm) planting time. The factor C varied according the experiment: plant compartment and sampling times in hours for translocation, absorption and metabolism experiments, respectively.

When the plants presented three fully expanded leaves, four 1 µL droplets of ^{14}C-labeled-bispyribac-sodium working solution were applied on the adaxial side, totaling 1.68 and 5 kBq in each plant, for absorption/translocation and metabolism experiments, respectively. Once the droplets had dried, the plots were transferred to two different growth chambers set for each temperature (cold and warm) and 12-hour photoperiod (500 µmol m^{-2} s^{-1} of photon flux).

For the absorption evaluation, treated leaves were cut at 6, 12, 24 and 72 HAT (hours after application) and washed-off. The rinsate from each sample was mixed individually with 10 mL of scintillation cocktail and quantified on a liquid scintillation spectrometer (LSS). Absorption of ^{14}C-bispyribac-sodium was calculated by subtracting the amount of radioactivity in the leaf rinsate from the total applied.

For the translocation experiment, at 72 HAT plants were fractioned into treated leaf (TL), leaf above TL, leaf below TL, and roots, and the nutrient solution from each plant was taken. After cutting, the plant portions were dried at 60 C for 48 hours and then oxidized in a biological oxidizer, determining the amount translocated to each plant compartment.

For metabolism experiment, after harvest, the labeled herbicide was extracted and normal-phase thin-layer chromatography (TLC) was used to separate ^{14}C-labeled bispyribac-sodium metabolites. Silica gel plates were used as the stationary phase, and a mixture consisting of chloroform, methanol, glacial acetic acid and water (85:25:2:2, v/v/v/v) was used as the mobile phase. The radioactive positions and relative mobility (Rf) of ^{14}C-labeled bispyribac-sodium metabolites were determined by autoradiography. Thereafter, the spots were scraped into a 20 mL vial, scintillation cocktail was added and the radioactivity was quantified using the liquid scintillation spectrometer (LSS). ^{14}C-labeled bispyribac-sodium parent molecule was identified through comparison with standard Rf values. Metabolite data consisted of the sum percentage of each polar metabolite identified (below parent), and the sum percentage of all the metabolites less polar than parent herbicide (above parent). All the calculations were performed in relation to total radioactivity recovered from plant extraction.

Data were initially tested for normality and homogeneity of variance, transformed when needed, and submitted to analysis of variance and the interaction among treatment factors of each experiment was investigated.

RESULTS AND DISCUSSION

Foliar absorption of ^{14}C-bispyribac-sodium increased with time until 72 HAT; however, it was not statistically different from 24 HAT (Figure 1A). Warm temperatures promoted higher absorption compared to cold temperatures (Figure 1B). Moreover, the cold-tolerant IRGA 424 cultivar showed greater absorption compared to the cold susceptible Epagri 109 (Figure 1B, Table 1). This response is probably correlated with an increase in solute diffusion under higher temperatures, promoted by an alteration in fatty acid cuticle state (BAUR; SCHONHERR, 1995).

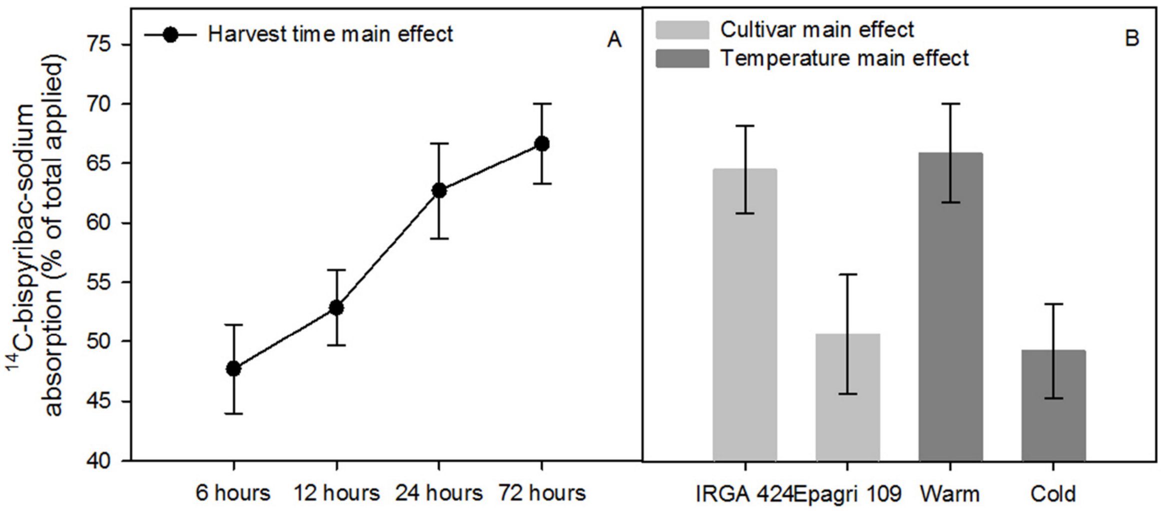

Figure 1. ^{14}C-bispyribac-sodium absorption by rice seedlings in different times (A); cultivar and temperature main effect (B) (95% confidence intervals).

For ^{14}C-bispyribac-sodium translocation, a significant interaction was observed between cultivars and plant compartment (Table 1). Both cultivars retained a greater percentage of absorbed ^{14}C in treated leaves than in other plant sections, averaging 70.2%, indicating that the herbicide has limited translocation to other plant parts and was not affected by temperature.

Table 1. ^{14}C-bispyribac-sodium translocation in two rice cultivars, averaged over warm and cold temperatures, at 72 hours after herbicide application (HAT)

Compartments	^{14}C-bispyribac-sodium in each plant compartment at 72 HAT(% of total absorbed)	
	IRGA 424	Epagri 109
Above treated leaf	2.20 bA[1]	1.80 bA
Below treated leaf	1.51 bA	1.22 bA
Root	0.93 bA	0.74 bA
Nutrient solution	0.05 bA	0.13 bA
Treated leaf	73.42 aA	66.98 aB
Warm[2]	15.11 [ns]	
Cold[3]	14.62	

[1] Means not followed by same lowercase (plant compartment) differ by Tukey's test (p≤0,05); and uppercase letters (cultivars) differ by the F test (p ≤ 0.05); [2] 22/30C night/day; [3] 16/22C night/day; [ns] means are not significantly different between temperature treatments, according to the F test (p≤0.05).

Concerning the the metabolism experiment, was observed that cold-exposed plants, in general, showed greater parental molecule (Figure 2A). In addition, warm-exposed plants demonstrated a greater amount of metabolite B (Figure 2B), reflecting the lower amount of the parental molecule. These results support the hypothesis of decrease on bispyribac-sodium selectivity observed in cold-exposed rice plants. For the other metabolites, no significant effect was observed.

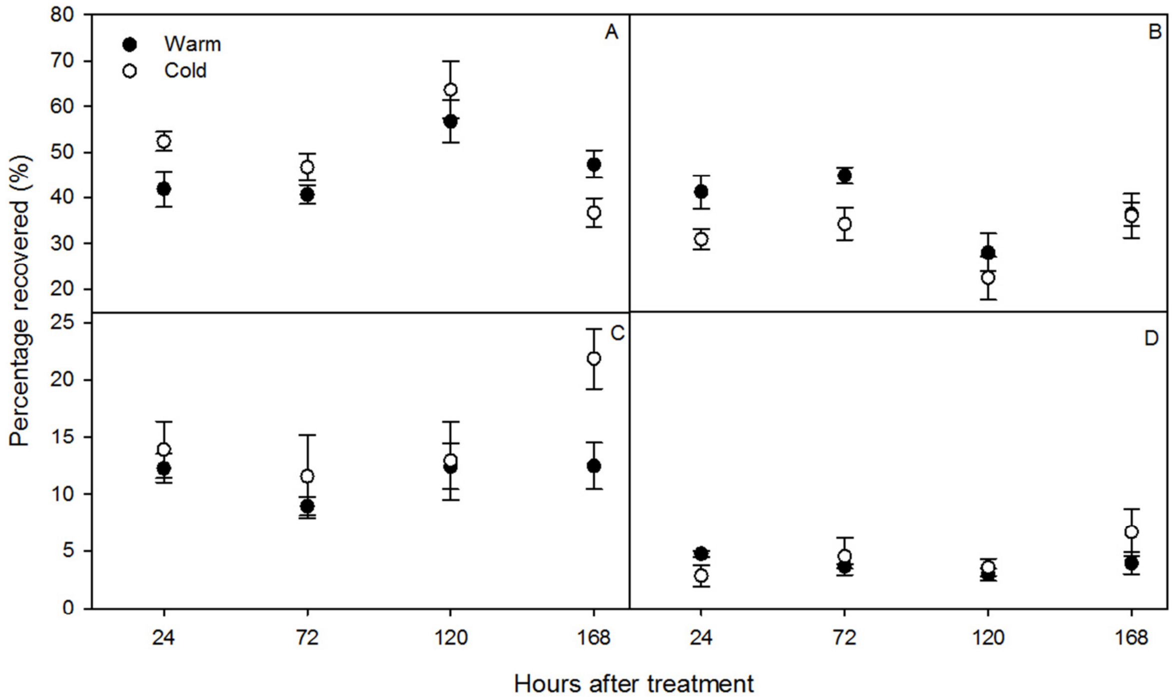

Figure 2. TLC separation: ^{14}C-bispyribac-sodium parental molecule (A), metabolite A (B), metabolite B (C) and metabolite C (D) under cold and warm temperature (95% confidence intervals).

A possible explanation for herbicide metabolism reduction is the effect of cooler/cold temperatures on plant membranes. It is very likely that cold stress promotes membrane rigidification (MURATA; LOS, 1997) causing lesions and decreasing their cryostability, which by turn, decreases the activity of membrane-bound enzymes, such as P450's (NAIR et al., 2009). Moreover, a P450's usually consists of two essential elements, a cytochrome P450 substrate-binding component and cytochrome P450 reductase component NAPH dependent (DURST; NELSON, 1995). Thus, photosynthesis impairment caused by abiotic stresses (UEMURA et al., 1995) such as cold, decreases the energy available as NADPH+ for non-essential metabolic functions, such as detoxification.

CONCLUSIONS

Maximum ^{14}C-bispyribac-sodium absorption occurred at 24 HAT and was stimulated by warmer temperature. Bispyribac-sodium presented lower translocation and was not affected by temperature. Lower temperature affects the herbicide metabolism with higher parental molecule amount observed at cold temperatures. Moreover, plants grown at warm temperatures had higher amounts of metabolite B than plants grown under cold temperatures.

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