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CAN THE JOIN USE OF AN ENZYME INHIBITOR AND AN INHIBITOR OF THE SYNTHESIS OF THIS ENZYME'S COENZYME BE A UNIVERSAL ALGORITHM FOR CREATING ANTI-RESISTANCE HERBICIDE COMPOSITIONS?

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Based on an analysis of factors that determine the effectiveness of herbicides, a hypothesis was proposed, that the join use of an enzyme inhibitor and an inhibitor of the synthesis of this enzyme's coenzyme should result in a synergistic interaction. To test this hypothesis, a field experiment was conducted in winter wheat crops to study the interaction effect when using a mixture of the herbicide diflufenican, an inhibitor of phytoene desaturase, and herbicide tolpyralate, an inhibitor of hydroxyphenylpyruvate dioxygenase, as the latter is involved in the synthesis of plastoquinone, which is a coenzyme for phytoene desaturase. It was found, that interaction between tolpyralate and diflufenican was additive with respect to broadleaf species, and synergistic with respect to grass species. It was concluded, that although the join use of an enzyme inhibitor and an inhibitor of the synthesis of this enzyme's coenzyme does not absolutely guarantee a synergistic interaction, the absence of antagonism and presence of additive or synergistic interactions for plant species with contrasting levels of resistance to the individual components indicates the potential for creating anti-resistance herbicide compositions using this algorithm.

Key words: herbicides, resistance, interaction.

The emergence of herbicide-resistant weed biotypes is a consequence of the selection pressure created by herbicides [1]. It is evident that to combat resistance, this pressure must be reduced, particularly through the integrated use of herbicides with different mechanisms of phytotoxicity [2]. However, the current range of herbicides limits the possibilities for creating effective anti-resistance formulations. One of the requirements for selecting components of such formulations is the nature of their interaction. To achieve high protection efficiency, the interaction of the components must be synergistic. At the same time, synergistic interaction has only been achieved in a formulation designed to protect maize crops [3]. In formulations developed for protecting winter wheat and sunflower crops, the interaction was merely additive [4, 5]. Therefore, it is generally accepted that solving the resistance problem requires the development of new her-

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bicides with mechanisms of action different from those currently available. However, this task has not yet been successfully addressed [6, 7]. A possible factor is the uncertainty of selection criteria for potential action sites that would ensure the high effectiveness of new herbicides. Various approaches for selecting such sites are proposed, but their validity has not yet been experimentally confirmed [8, 9].

Studies using proteomics and metabolomics methods to examine the effects of herbicides on plants have shown that inhibition of certain enzymes by herbicides leads to increased expression of genes encoding the action sites of these herbicides. This increase occurs particularly under the influence of herbicides that inhibit acetolactate synthase (ALS) [10], acetyl-CoA carboxylase (ACC) [11, 12], 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) [10, 13, 14], and protoporphyrinogen oxidase (PPO) [15]. These data indicate that plants have negative feedback systems aimed at compensating for the effects of herbicide exposure. At the same time, an analysis of the mechanisms of action of the most effective herbicide classes revealed that peculiarities of these herbicides is lack or disruption of these feedback systems [16].

Specifically, it has been established that inhibition of ALS enzyme activity by some herbicides is accompanied by damage to the ALS coenzyme thiamine diphosphate. At the same time plants lack a mechanism for restoring the thiamine diphosphate pool [17]. Similarly, the absence of negative feedback is also observed with herbicides that inhibit hydroxyphenyl-pyruvate dioxygenase (HPPD). This enzyme is involved in the synthesis of plastoquinone, which serves as a coenzyme for phytoene desaturase, which is a key enzyme in the biosynthesis of carotenoids [18]. In this case, exposure to HPPD inhibitors does not alter the expression of the gene encoding this enzyme [11]. It is likely that because the role of coenzymes in enzymatic reactions is limited to their reversible transformation, plants do not require strict control over their pools. As a result, plants lack the feedback mechanism necessary to compensate for coenzyme losses caused by herbicides.

Thus, enzymes involved in coenzyme synthesis are promising herbicide action sites. Since combating resistance requires not only the creation of new herbicides but also the development of anti-resistance herbicide compositions based on them, the question arises: what kind of interaction might occur when an enzyme inhibitor is combined with an inhibitor of that enzyme's coenzyme synthesis? The high efficacy of ALS-inhibiting herbicides, where enzyme activity is simultaneously inhibited and the coenzyme pool is reduced [17], suggests that such combinations may result in synergistic interactions. In this case, the parallel search for enzyme inhibitors and inhibitors of the synthesis of their coenzymes could be a universal approach for discovering new herbicides and developing anti-resistance herbicide compositions based on them.

This hypothesis can be tested by studying the interaction effect when a mixture of a phytoene desaturase inhibitor herbicide and an HPPD inhibitor is applied, since HPPD inhibition blocks plastoquinone synthesis, which is a coenzyme for phytoene desaturase. Until now, such mixtures have not been studied because herbicides from these classes are selective for different crops: phytoene desaturase inhibitors, such as diflufenican, are used in winter wheat crops, while HPPD inhibitor herbicides until recently have been used exclusively in maize crops. However, the new HPPD inhibitor herbicide, tolpyralate, has proven to be selective for winter wheat.

So the aim of our work was to study the interaction effect in a mixture of the phytoene desaturase inhibitor diflufenican with the HPPD inhibitor tolpyralate in field experiment in winter wheat crops.

Materials and methods

Experiments were conducted in the fields of the research farm of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (Glevakha village, Fastiv district, Kyiv region, 50°16′N, 30°18′E). The experiments were carried out in winter wheat crops of the Snihurka variety (predecessor mustard).

The following herbicides were used in the studies: Diflufenican (diflufenican, 500 g/L), and SL-573 100OD (tolpyralate, 100 g/L). Herbicide treatment was carried out in spring at the stage of tillering in winter wheat (BBCH 24). Spraying with herbicides was carried out using a knapsack rod sprayer with compressed air, pressure 0,4 MPa, rod length — 3 m, number of nozzles — 6, distance to the target — 50 cm, speed — 5 km/h, working fluid consumption — 300 L/ha. The area of the experimental plot was 15 m^2 (3×5 m), experiments were performed in four replications, the plots were placed randomly. Each experiment included a control variant (without herbicides).

Weed accountings were performed before herbicide treatment, 28 and 70 days after treatment (DAT). The effectiveness of herbicides was assessed for each weed species separately by reducing the number in treated areas compared to control [19], taking into account the visual assessment of the degree of suppression of herbicide-treated plants compared to the condition of these plants in control (weight and linear dimensions, leaf chlorosis, etc.). The degree of visual suppression was expressed as a percentage: 0 % — no signs of herbicide action, 100 % — complete death of weeds of this species. The effectiveness of weed control was calculated by formula:

$$E\ (\%) = 100 - B_2\ K_1\ (1 - E_B\ /\ 100)\ 100\ /\ (B_1\ K_2) \qquad (1),$$

where E (%) — the effectiveness of controlling of a particular species of weeds, taking into account the level of weediness and visual assessment of herbicide action; K_1 — the number of weeds per 1 m^2 in the first accounting in control plot (initial weediness), K_2 — the number of weeds per 1 m^2 in the control plot during the second (third) accounting, B_1 — the number of weeds per 1 m^2 in the first accounting in the treated plot (initial weediness), B_2 — the number of weeds per 1 m^2 in the second (or third) accounting at the treated plot, E_B — visual assessment of the herbicide action (the level of damage or the degree of suppression of weed plants expressed (in %) visually compared to plants of the same species in the control plot).

The effect of herbicide interaction in mixtures was determined by the Colby method [20] by comparing the actual and expected inhibitory effect of the herbicide mixture. The expected effect of the mixture was calculated by formula:

$$E_{1,2} = E_1 + E_2(100 - E_1)/100 \quad (2),$$

where $E_{1,2}$ — the expected effectiveness of the mixture of herbicides, E_1 and E_2 — the values of the effectiveness, respectively 1 and 2 components of the herbicides mixture.

The crop selectivity of herbicides was assessed by biometric measurements and phenological observations carried out in 7 days after herbicide treatment, and at each weed accounting.

Statistical processing of the results was performed by analysis of variance (ANOVA) using the Tukey (HSR) test. The results were presented as mean and standard errors (x±SE). Differences between data were considered significant at $p \leq 0.05$.

Results and discussion

The experiment was established on April 11, 2024. At the time of treatment was sunny weather, air temperature — 20 °C, wind — 4 m/s. The surface of the soil was wet, and surface of leaves was dry. The scheme of the experiment is given in Table 1.

An inspection of the crops seven days after treatment, and during subsequent weed assessments showed that when used separately, the herbicides tolpyralate and diflufenican had little to no negative impact on the cultivated plants. However, when the mixture of tolpyralate and diflufenican was applied, distinct signs of phytotoxicity were observed on the leaves in the form of yellow spots at both seven and fourteen days after treatment. By 28 days after treatment, the phytotoxic effects on winter wheat had significantly diminished, and by the end of the wheat growing season, the symptoms had practically disappeared (Table 2).

A pre-treatment assessment revealed that winter wheat field was infested with annual broadleaf weeds, including scentless chamomile (*Matricaria inodora* L.) (3 plants/m^2), cornflower (*Centaurea cyanus* L.)

TABLE 1. Scheme of field experiment

Treatment		Application rate	
Herbicide	Active substance	By herbicide	By active substance
Control	—	—	—
SL-573 100OD + Trend 90	tolpyralate	0.2 L/ha + 0,2 L/ha	20 g/ha
Diflufenican	diflufenican	0,2 L/ha	100 g/ha
SL-573 100OD + Diflufenican + + Trend 90	tolpyralate + + diflufenican	0.2 L/ha + 0,2 L/ha + + 0,2 L/ha	20 g/ha + 100 g/ha

TABLE 2. Phytotoxic effect (%) of herbicides on winter wheat 7, 14, 28 and 70 days after treatment (DAT)

Treatment	7 DAT	14 DAT	28 DAT	70 DAT
SL-573 100OD + Trend 90	0.8±0.5	0	0	0
Diflufenican	0.5±0.2	0	0	0
SL-573 100OD + Diflufenican + Trend 90	8±2	10±1	3±1	0

(2 plants/m^2), and field pansy (*Viola arvensis* L.) (10 plants/m^2). During the final assessment, the field pansy had completed its growth cycle, but the appearance of the annual grass weed common windgrass (*Apera spica-venti* (L.) Pal. Beauv.) (2 plants/m^2) was noted.

The values of the effectiveness of controlling dicotyledonous weeds with tolpiralate and diflufenican were close to 50 % (Table 3), which allows the correct application of the Colby method for evaluating the interaction effect. Calculations of the expected effect of the mixture of tolpyralate and diflufenican indicated that the expected effect on broadleaf weeds did not significantly differ from the actual effect, indicating an additive interaction between the herbicides. At the same time, despite the practically negligible effect of tolpyralate and diflufenican on the grass weed common windgrass, the effectiveness of controlling this weed with the mixture turned out to be quite high, which indicates a synergistic interaction (Table 4).

Thus, the conducted experiment did not provide definitive results regarding the potential of creating anti-resistance herbicide compositions by join use of enzymes inhibitors and inhibitors of the synthesis of coenzymes of these enzymes. On the one hand, it was established that creating herbicide compositions according to this algorithm does not guarantee synergistic interaction, as the interaction between tolpyralate and diflufenican concerning broadleaf weeds was additive. However, on the other hand, the interaction concerning grass weeds proved to be synergistic. The increased phytotoxic effect of the mixture on winter wheat also indicates a synergistic enhancement for grasses when tolpyralate is combined with diflufenican. Therefore, the nature of the interaction may vary for different plant species.

Nevertheless, the fact that no antagonism was observed, even in very contrasting plant species in terms of resistance to the components, and that

TABLE 3. Efficacy of herbicide control (%) at 28 and 70 days after treatment (DAT)

Treatment	28 DAT			70 DAT		
	Matricaria inodora	*Centaurea cyanus*	*Viola arvensis*	*Matricaria inodora*	*Centaurea cyanus*	*Apera spica-venti*
SL-573 100OD + + Trend 90	45±3	35±5	60±8	60±5	60±3	10±2
Diflufenican	40±5	15±3	75±5	30±5	20±5	5±1
SL-573 100OD + + Diflufenican + + Trend 90	65±6	45±5	85±5	75±5	65±3	85±5

TABLE 4. Actual and expected effects (%) of the mixture of herbicides tolpiralate and diflufenican on dicotyledonous and grass weeds 28 and 70 days after treatment (DAT)

Effect	28 DAT			70 DAT		
	Matricaria inodora	*Centaurea cyanus*	*Viola arvensis*	*Matricaria inodora*	*Centaurea cyanus*	*Apera spica-venti*
Actual	65±6	45±5	85±5	75±5	65±3	85±3
Expected	67±10	45±10	90±10	72±10	68±8	15±5

interaction was either synergistic or additive, supports a positive assessment of the prospects for the joint application of enzyme inhibitors and inhibitors of the synthesis of coenzymes for creating anti-resistance herbicide compositions.

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ЧИ МОЖЕ СПІЛЬНЕ ЗАСТОСУВАННЯ ІНГІБІТОРА ФЕРМЕНТУ ТА ІНГІБІТОРА СИНТЕЗУ КОФЕРМЕНТУ ЦЬОГО ФЕРМЕНТУ БУТИ УНІВЕРСАЛЬНИМ АЛГОРИТМОМ СТВОРЕННЯ АНТИРЕЗИСТЕНТНИХ ГЕРБІЦИДНИХ КОМПОЗИЦІЙ?

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На підставі аналізу чинників, які детермінують ефективність дії гербіцидів, було висловлено гіпотезу, що при спільному застосуванні інгібітора певного ферменту та інгібітора синтезу коферменту цього ферменту взаємодія має бути синергічною. З метою перевірки цієї гіпотези в умовах польового досліду в посіві озимої пшениці вивчали ефект взаємодії під час застосування суміші гербіциду інгібітора фітоендесатурази дифлуфенікану з інгібітором гідроксифенілпіруватдіоксигенази толпіралатом, оскільки останній бере участь у синтезі пластохінону, який є коферментом фітоендесатурази. Було встановлено, що у суміші толпіралату з дифлуфеніканом взаємодія адитивна щодо дводольних видів і синергічна щодо злакових видів рослин. Зроблено висновок, що хоча спільне застосування інгібітора ферменту та інгібітора синтезу коферменту цього ферменту не є абсолютною гарантією синергічної взаємодії, відсутність антагонізму та наявність адитивної або синергічної взаємодії щодо контрастних за ступенем стійкості до дії окремих компонентів видів рослин, є свідченням перспективності створення антирезистентних композицій гербіцидів за цим алгоритмом.

Ключові слова: гербіциди, резистентність, взаємодія.

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