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INTERACTION EFFECT IN THE TANK MIXTURES OF HERBICIDES DIFLUFENICAN, METRIBUZIN AND CARFENTRAZONE

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The search for new combinations of herbicides with different phytotoxic mechanisms, which spectra intersect, is one of the ways to prevent the emergence and spread of herbicide-resistant weed biotypes. A limiting factor for the complex application of certain herbicides is the effect of their interaction, as it is known that the antagonistic nature of the interaction is more common than synergistic or additive. Consequently, the aim of our work was to study in greenhouse conditions the effects of interaction under the complexation of herbicides of three different classes. In mixtures, there were used inhibitor of carotenoid biosynthesis diflufenican, inhibitor of electron transport in photosystem II of chloroplasts metribuzin and inhibitor of enzyme protoporphyrinogen oxidase (on the way of synthesis of chlorophyll) carfentrazone. This study was conducted for determine the possibility of using mixtures of these herbicides by application at autumn in winter wheat crops. The results of the research showed that only in the mixture of carfentrazone with metribuzin the interaction has signs of antagonism, and in other binary combinations, as well as in the ternary mixture, the interaction is additive. Taking into account the spectra of studied herbicides action and the results of their interaction effects determination, it was concluded that for autumn application in winter wheat crops are promising a mixture of diflufenican with metribuzin and triple mixture with carfenuzrazone.

Key words: herbicides, anti-resistant mixtures of herbicides, interaction effect, antagonism, additivity, synergism.

The main issues in chemical weed control are the spread of herbicide-resistant weed biotypes caused by permanent use of herbicides, and the selective pressure caused by them [1—3]. There are currently 508 herbicide-resistant biotypes among 266 weed species worldwide. Among them, the most common are biotypes that have a resistance to the most effective herbicides — inhibitors of the enzyme acetolactate synthase (ALS) [4]. In the Kherson region of Ukraine the emergence of resistant to ALS inhibitors barnyardgrass biotype (*Echinochloa cruss-galli* var. cruss-galli) was detected [5].

The only way to prevent the emergence of resistance today is the rotation of herbicides with different mechanisms of phytotoxicity in crops, and

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the complex use of herbicides to protect individual crops [6—8]. Herbicide complexation is widely used to improve weed control. However, before now in the develop of herbicidal compositions there are mainly used herbicides that complement each other in the spectrum of action. In order to ensure a synergistic increase in the efficiency of weed control during complexation, active substances with one phytotoxicity mechanism were widely used [9]. At the same time, to prevent resistance needed mixtures which the components differ in the mechanism of phytotoxic action, but the spectra of their action must intersect significantly [8]. In addition, a necessary requirement is the additive or synergistic nature of the interaction of the components of these mixtures [10].

Due to global climate change, rising average temperatures, particularly in the autumn and winter, the control of weeds in winter wheat crops in the autumn is especially important. The difference between autumn and spring herbicide application is that in autumn the treatment of winter wheat should be carried out at earlier stages of crop development. ALS inhibitor herbicides are highly selective for wheat, so in Ukraine it is recommended to use several herbicides of this class for autumn application, in particular Logran 75 WG (triasulfuron, 750 g/kg). However, the widespread use of ALS inhibitor herbicides in winter wheat and other crops has already led to the emergence of weed biotypes resistant to ALS inhibitors. In addition, winter wheat crops can be infested with predecessor crops, in particular sunflower or winter oilseed rape, especially hybrids resistant to herbicides of ALS inhibitors.

Recently, preparations based on the herbicide diflufenican have been developed for autumn application in winter wheat crops, the action of which is due to the inhibition of the activity of enzymes of the carotenoid biosynthesis pathway — phytoendesaturases. Company Corteva is testing a complex herbicide in which diflufenican is used with ALS inhibitors florasulam and penoxulam. Currently, Bayer has registered a complex herbicide Checker Xtend 39 WG in Ukraine, the active ingredients of which are diflufenican and ALS inhibitors amidosulfuron and iodsulfuron [11]. Complexation of diflufenican with ALS inhibitors certainly reduces, but does not completely rule out the possibility of herbicide resistance with this mechanism of action. Therefore, the urgent task is to find partners for the diflufenican among selective for winter wheat active substances of herbicides with different from the inhibition of ALS phytotoxicity mechanisms.

In Ukraine, the herbicides metribuzin (Zenkor Liquid), which belongs to the inhibitors of electron transport in photosystem II (PSII) of chloroplasts, and the inhibitor of the enzyme protoporphyrinogen oxidase (PPO) [12] carfentrazone (Aurora) are allowed for use in winter wheat crops. The possibility of complex application of diflufenican with metribuzin and carfentrazone depends on the nature of the interaction of these herbicides. It is known that the phytotoxic effect of all three classes of herbicides is due to the disorganization of photosynthesis and mediated by the formation of reactive oxygen species (ROS) [14]. Since the action of diflufenican is to inhibit the synthesis of carotenoids, one of the physiological functions of which is to protect the photosynthetic apparatus from ROS damage [15], it can be expected that the interaction of diflufenican with metribuzin and carfentrazone should be synergistic or at least additive. Confirmation of the validity of this assumption is the data on the synergistic interaction of herbicides of carotenoid biosynthesis inhibitors from the class of hydroxyphenylpyruvate dioxygenase (HPPD) with herbicides inhibitors of electron transport in PSII [16—20]. This possibility is also confirmed by the fact that the addition of carfentrazone to the HPPD inhibitor mesotrione increased the effectiveness of weed control [19, 21]. High weed control, suggesting synergism or additivity, has also been observed when another PPO inhibitor, flutiacet, was used in complex with mesotrione and inhibitor of electron transport metribuzin (MTZ) [22, 23]. At the same time, it should be taken into account that the mechanism of inhibition of carotenoid synthesis by diflufenican is due to the inhibition of phytoendesaturases and is different from the inhibition of HPPD. In addition, due to the limited selectivity of metribuzin and carfentrazone, the application rates of these herbicides in winter wheat crops are quite low. At the same time, it is known that the nature of the components of herbicidal mixtures interaction may depend on the level of phytotoxic action, and hence on the rate of application of these components [9].

Thus, the available information does not allow us to unambiguously determine what the interaction may be in mixtures of diflufenican, metribuzin and carfentrazone. The appropriateness of using any combination of these active substances obviously depends on the selectivity of each of these mixtures for crop and the nature of the interaction, which determines the effectiveness of this mixture weed control. It is obvious that these characteristics, first of all selectivity concerning winter wheat, can be reliably checked only in field experiments. However, before conducting field trials, it is advisable to check the nature of the interaction of different combinations of these active substances on model objects in controlled greenhouse conditions which was the aim of this study.

Materials and methods

Investigations of the effect of herbicides interaction in mixtures were performed in the greenhouse conditions. The sunflower (*Helianthus annuus* L., hybrid P64LE99, Pioneer) was selected as a model of annual dicotyledonous weeds, resistant to herbicides of ALS inhibitors, because spontaneous sowing of sunflower is often problem in Ukraine, in particular in the wheat crops.

Plants were grown in plastic pots with an area of 0.015 m^2, which contained 1 kg of soil (a mixture of soil and sand in a ratio of 3 : 1) under greenhouse conditions and in a miniphytotron under fluorescent lamps (light/dark 16/8 h). For investigation the following herbicides were used: Zencor Liquid (metribuzin, 600 g/l, Bayer); Aurora 40 VG (carfentrazone, 400 g/l, FMC); Diflufenican (diflufenican, 500 g/l). Treatment with herbicides was carried out by spraying the plants with herbicide solutions at the stage of the first pair of true leaves. Herbicides were applied in the recommended norms: carfentrazone — 16 g/ha, diflufenican — 100 g/ha, metribuzin — 240 g/ha, calculated on the area of pots.

The phytotoxic effect of herbicides was determined by the effect on the induction of chlorophyll fluorescence and chlorophyll content in the leaves. Induction of chlorophyll fluorescence was measured using a Junior-PAM fluorimeter (WALZ, Germany) and the maximum quantum yield of photosynthesis was determined by the ratio of variable fluorescence to maximum (F_v/F_m), estimated after adaptation of leaves in the dark for 20 minutes [24]. Variable fluorescence was determined by formula (1):

$$F_v = F_m - F_o \qquad (1),$$

where F_o is the minimum level of fluorescence excited by low-intensity light, at which the reaction centers of photosystem PSII remain open. F_m is the maximum level of fluorescence that is excited by a saturating pulse (0.6 s) of high-intensity light, which leads to the closure of all PSII reaction centers.

The content of photosynthetic pigments was determined by spectrophotometric method after extraction of plant material samples in DMSO at 67 °C for 3 h [25].

The inhibitory effect of herbicides was expressed as a percentage and calculated by formula (2):

$$I_e (\%) = 100 - F_e \cdot 100/F_c \qquad (2),$$

where I_e is the inhibitory effect, F_e and F_c are the values of the measured parameters in the experimental and control variants. The effect of herbicide interaction in mixtures was determined by the Colby method [26] by comparing the actual and expected inhibitory effect of the herbicide mixture. The expected effect of the mixture was calculated by formula (3):

$$I^e_{1,2} = I_1 + I_2(100 - I_2)/100 \qquad (3),$$

where $I^e_{1,2}$ is the expected inhibitory effect of the mixture of herbicides, I_1 and I_2 are the values of the inhibitory effect, respectively 1 and 2 components of the mixture of herbicides.

The experiment was performed in four replicates and was reproduced independently few times. Mathematical analysis of the results of study was performed using package «Microsoft Excel». The graphs show average values and standard errors.

Results and discussion

The efficiency of the photosynthesis process is undoubtedly an important indicator of plant viability, so the determination of fluorescence induction is used to assess not only damage of weeds but also herbicides selectivity [27, 28]. Value of the F_v/F_m varies from 0.75 to 0.85 in different non-stressed plants, while a decrease in this parameter indicates inhibition of electron transport in plant chloroplasts [24]. In control sunflower plants, the value of F_v/F_m was 0.8. On the 2nd day after treatment (DAT) of plants, there was a sharp decrease in the maximum quantum yield of photosynthesis under the action of MTZ, while the effect of carfentrazone and diflufenican on the value of the parameter F_v/F_m was quite weak (Fig. 1). This result is quite expected, as the action of MTZ is directly related to the blocking of electron transport in PSII of chloroplasts. The effect of carfentrazone and diflufenican on electron transport is not direct, but due to damage to chloroplasts by ROS, formed in result of inhibition of PPO and synthesis of carotenoids by these herbicides.

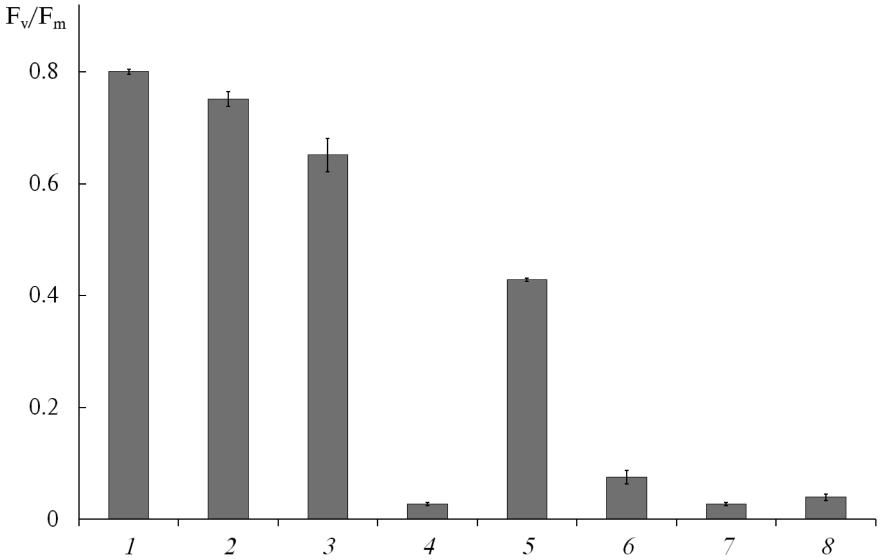

Fig. 1. Maximum quantum yield of photosynthesis (F_v/F_m) in sunflower leaves (model of annual dicotyledonous weeds) under the effect of herbicides carfentrazone, diflufenican and MTZ on the 2nd day after treatment.

Variants: *1* — control; *2* — carfentrazone (16 g/ha); *3* — diflufenican (100 g/ha); *4* — MTZ (240 g/ha); *5* — carfentrazone + diflufenican; *6* — carfentrazone + MTZ; *7* — diflufenican + MTZ; *8* — carfentrazone + diflufenican + MTZ. Error bars denote standard error

Thus, the change in the maximum quantum yield of photosynthesis is a specific criterion of phytotoxicity for MTZ, and changes in this parameter can be judged on how the partners in the complex affect its phytotoxic effect. Under the action of a mixture of MTZ with diflufenican, and a ternary mixture when carfentrazone was added to diflufenican and MTZ, the value of the F_v/F_m parameter did not differ significantly from the variant with the use of MTZ separately (see Fig. 1). It follows that in these variants the action of MTZ did not change, so the interaction was additive. At the same time, in the mixture of carfentrazone with MTZ there was a tendency to reduce the inhibitory effect on electron transport, compared with the action of MTZ alone, which may be evidence of antagonistic interaction.

An integral and most reliable criterion for assessing the phytotoxic effect of herbicides, for which phytotoxic activity is due to the effect on the process of photosynthesis, is the effect on the chlorophyll content [9, 29, 30]. It turned out that the fastest effect on this parameter had carfentrazone, because on the 3rd DAT significantly exceeded the effect of diflufenican and MTZ (Fig. 2). However, on the 6th DAT, the effect of MTZ significantly exceeded the effect of carfentrazone. On the 20th DAT, diflufenican effect was insignificant, and MTZ significantly exceeded the effect of carfentrazone.

Determination of herbicides effect on chlorophyll content confirmed the conclusion that in the mixture of carfentrazone with MTZ the interaction is antagonistic, because on the 20th DAT the expected effect significantly exceeded the actual effect of the mixture (Fig. 3). In other combinations, the expected effect was slightly higher than the actual one, but this

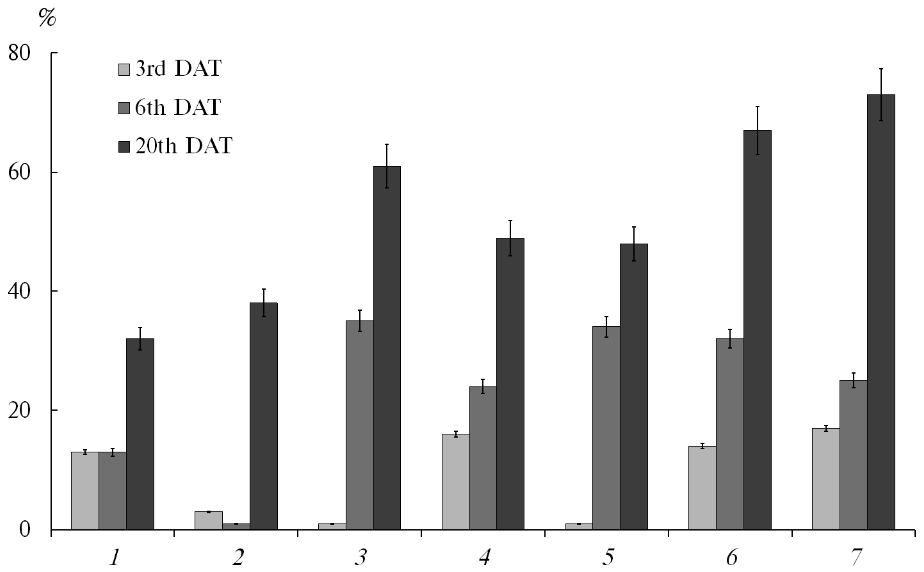

Fig. 2. Inhibitory effect (%) of herbicides and their mixtures on chlorophyll content in sunflower leaves (model of annual dicotyledonous weeds) on 3rd, 6th and 20th day after treatment.

Variants: *1* — carfentrazone (16 g/ha); *2* — diflufenican (100 g/ha); *3* — MTZ (240 g/ha); *4* — carfentrazone + diflufenican; *5* — carfentrazone + MTZ; *6* — diflufenican + MTZ; *7* — carfentrazone + + diflufenican + MTZ. Error bars denote relative standard error

difference was not significant. Therefore, it can be assumed that in binary mixtures of diflufenican with MTZ and carfentrazone, as well as in the ternary mixture of diflufenican with MTZ and carfentrazone, the interaction is additive.

Thus, the obtained data did not confirm the assumption of a possible synergistic increase of phytotoxic action under the complex application of the herbicides which interfere on photosynthesis by different ways. This

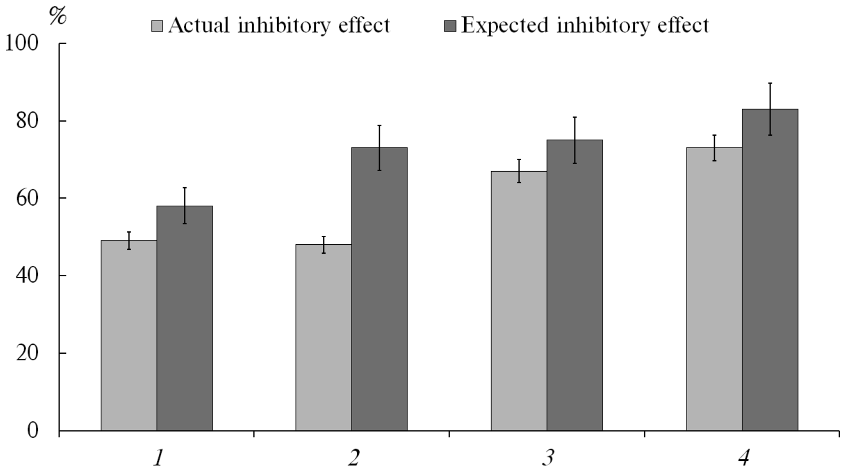

Fig. 3. Actual and expected inhibitory effect of herbicide mixtures on the chlorophyll content in sunflower leaves (model of annual dicotyledonous weeds) on 20th day after treatment.

Variants: *1* — carfentrazone + diflufenican; *2* — carfentrazone + MTZ; *3* — diflufenican + MTZ; *4* — carfentrazone + diflufenican + MTZ. Error bars denote relative standard error assumption was based on data of synergism in mixtures of HPPD inhibitors, which are also inhibitors of carotenoid biosynthesis, with inhibitors of electron transport [16—20] and PPO inhibitors [19, 21, 22]. Another nature of the interaction in mixtures of diflufenican with MTZ may be due to differences in the mechanisms of blocking the carotenoids biosynthesis by HPPD inhibitors and inhibitors of phytoendesaturases. Inhibition of carotenoid biosynthesis by HPPD inhibitors is due to the fact that blocking the activity of this enzyme leads to inhibition of the synthesis of plastoquinone, which is a cofactor of carotenoid synthesis [31].

Thus, the mechanism of synergism in mixtures of HPPD inhibitors with inhibitors of electron transport can be determined by two factors. At first, blocking plastoquinone biosynthesis may enhance the inhibition of electron transport by herbicides inhibitors of electron transport, the site of action of which is the site of binding of plastoquinone to D1 protein [14]. The second factor of synergism may be that the action of HPPD inhibitors reduces the antioxidant protection system of plants. In addition to inhibiting the carotenoids biosynthesis, the action of HPPD leads to inhibition of the synthesis of tocopherol, which is a classic antioxidant that can block the development of lipid peroxidation reactions [19]. In contrast to HPPD, phytoendesaturase inhibitors are direct inhibitors of the carotenoid biosynthesis pathway [32]. Thus, the synergistic enhancement of the phytotoxic effect of electron transport inhibitors in mixtures with phytoendesaturase inhibitors may be due to only one factor — a decrease in carotenoids content and a corresponding decrease in the activity of antioxidant protection.

However, the effect of diflufenican on sunflower plants — the model of dicotyledonous weeds in this study — was insignificant. This action probably did not lead to a significant decrease in the activity of antioxidant protection, and thus in the mixture of diflufenican with MTZ the interaction was additive. It is known that the nature of herbicidal mixtures components interaction may depend on the magnitude of the phytotoxic action of these components. In this case, for more resistant plant species with a correspondingly small level of phytotoxic action is more likely additive and even antagonistic interaction, and with increasing application rate and the corresponding increase in phytotoxic action the probability of additive or even synergistic interaction increases [9]. Therefore, it is possible that for more sensitive to diflufenican plant species, its interaction with MTZ will be synergistic.

Probably, the dependence of interaction effect nature on the phytotoxic effect level of mixture components can explain the antagonistic interaction in a mixture of MTZ with carfentrazone. The effect of carfentrazone on sunflower, although developing rapidly, was even smaller than the effect of diflufenican. Thus, in a mixture of carfentrazone with MTZ most likely occurred cross-adaptation, when under the action of two stressors the effect of the less strong of them reduces the effect of the next stressor [33, 34].

Thus, investigation of the interaction effects in mixtures of herbicides has shown that signs of antagonism were observed only when using a mixture of carfentrazone with MTZ. The phytotoxic effect of carfentrazone and diflufenican mixture was rather weak and inferior to that of MTZ alone. In addition, carfentrazone controls only dicotyledonous weed species, so the intersection of diflufenican spectra with carfentrazone is insufficient to effectively prevent resistance. The binary mixture of diflufenican with MTZ has an additive interaction and in all its characteristics (difference of the sites of action and intersection of the spectra of controlled weed species) satisfies the requirements for anti-resistant mixtures of herbicides. The addition of carfentrazone as a third component to a mixture of diflufenican with MTZ increased the inhibitory effect on chlorophyll content. Since a mixture containing three components with different mechanisms of action is more effective than a binary mixture from the point of view of combating resistance, the addition of carfentrazone to diflufenican and MTZ is quite expedient.

Summarizing the obtained data, it can be concluded that a mixture of diflufenican with MTZ and a triple mixture with the addition of carfentrazone to diflufenican and MTZ are promising for use as anti-resistance compositions when used in autumn in winter wheat crops. Final conclusions about the effectiveness of these mixtures can be made in a result of field trials.

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ЕФЕКТ ВЗАЄМОДІЇ ПРИ ЗАСТОСУВАННІ СУМІШЕЙ ГЕРБІЦИДІВ ДИФЛУФЕНІКАНУ, МЕТРИБУЗИНУ ТА КАРФЕНТРАЗОНУ

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Пошук нових комбінацій гербіцидів із різними механізмами фітотоксичності, спектри дії яких перетинаються, є одним із напрямів запобігання виникненню й поширенню резистентних до гербіцидів біотипів бур'янів. Лімітуючим чинником для комплексного застосування певних гербіцидів є ефект їх взаємодії, оскільки відомо, що антагоністичний характер взаємодії більш поширений, ніж синергічний чи адитивний. У зв'язку з цим, метою нашої роботи було вивчення в умовах вегетаційного досліду ефектів взаємодії при комплексуванні гербіцидів трьох різних класів — інгібітора біосинтезу каротиноїдів дифлуфенікану, інгібітора транспорту електронів у фотосистемі II (ФС II) хлоропластів метрибузину та інгібітора ферменту на шляху синтезу хлорофілу протопорфіриногеноксидази (ПРОТО) карфентразону для визначення можливості застосування сумішей цих гербіцидів восени в посівах озимої пшениці. Результати досліджень показали, що лише у суміші карфентразону з метрибузином взаємодія має ознаки антагонізму, а в інших бінарних комбінаціях, а також у потрійній суміші взаємодія є адитивною. Отже, врахувавши спектри дії досліджуваних гербіцидів і результати визначення ефектів їх взаємодії, зроблено висновок, що для запобігання виникненню резистентних біотипів бур'янів при осінньому застосуванні в посівах озимої пшениці перспективними є суміш дифлуфенікану з метрибузином та потрійна суміш із додаванням карфентразону до метрибузину та дифлуфенікану.

Ключові слова: гербіциди, антирезистентні суміші гербіцидів, ефект взаємодії, антагонізм, адитивність, синергізм.