



The effect of sodium nitroprusside on the action of herbicides, protoporphyrinogen oxidase inhibitors, and synthetic auxins

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To determine the factors that influence the nature of the effect of the nitric oxide donor sodium nitroprusside on the phytotoxicity of herbicides, we studied the dynamics of nitric oxide and hydrogen peroxide formation when sodium nitroprusside is used separately and in combination with herbicides. Three herbicides with different mechanisms of action were used for the studies: the protoporphyrinogen oxidase inhibitor carfentrazone, the synthetic auxin 2,4-D, the effect of which was increased when used together with sodium nitroprusside, and the carotenoid synthesis inhibitor aclonifen, the effect of which was accelerated, but not increased, when used together with sodium nitroprusside. Oilseed radish plants (*Raphanus sativus* var. *oleiformis*), which are sensitive to the action of the studied herbicides, were used as the model organism. It was found that treatment of plants with sodium nitroprusside, carfentrazone, and 2,4-D led to an increase in nitric oxide content in plants on the 1st and 4th days after treatment, while on the 8th day, the nitric oxide content in treated plants did not differ from the control. Treatment of plants with aclonifen did not affect the nitric oxide content in plants over the 8-day period following treatment. When sodium nitroprusside was used in combination with carfentrazone and 2,4-D, the increase in nitric oxide content was more pronounced and prolonged compared to the effect of the herbicides alone. When sodium nitroprusside was applied together with aclonifen, an increase in nitric oxide was also observed compared to the control; however, this increase was smaller in both magnitude and duration compared to the application of the nitric oxide donor alone. Treatment of plants with sodium nitroprusside resulted in a decrease in hydrogen peroxide content, while herbicide treatment led to an increase in hydrogen peroxide content in plants. When herbicides were applied in combination with sodium nitroprusside, an increase in hydrogen peroxide content was observed compared to the effect of the herbicides alone. At the same time, this increase was most pronounced for carfentrazone, and less intense and shorter-lived for 2,4-D and aclonifen. Since the content of hydrogen peroxide in plants decreased under the action of sodium nitroprusside alone, it is likely that the increase in hydrogen peroxide content during the combined application of herbicides with sodium nitroprusside is a consequence of the increased nitric oxide levels. It was concluded that the increase in induced nitric oxide formation is a key factor in the enhanced phytotoxic effect of the herbicides carfentrazone and 2,4-D when used in combination with sodium nitroprusside. The possibility of increasing the phytotoxic effect of individual herbicides through joint use with nitric oxide donors can be utilized to enhance the effectiveness of crop protection against weeds.

Keywords: carfentrazone; 2,4-D; aclonifen; nitric oxide; hydrogen peroxide; nitric oxide donor; phytotoxic action.

Introduction

Today, the modification of herbicide phytotoxicity is achieved by influencing the concentration of the active ingredient at the site of action. Increasing the phytotoxic effect of herbicides on weeds is accomplished through the use of adjuvants that enhance herbicide uptake by plants (Zhang & Yang, 2021). To improve the resistance of cultivated plants to herbicides, antidotes (safeners) are used, which selectively accelerate herbicide detoxification in cultivated plants without affecting this process in weed species (Kraehmer et al., 2014). However, at present, herbicide resistance can be increased through the use of antidotes only in cultivated plants from the grass family, such as wheat, barley, corn, and sorghum (Zhao & Fu, 2023). The development of antidotes to protect dicotyledonous crops remains an ongoing area of research (Pingarron-Cardenas et al., 2024). Therefore, developing alternative methods to modify the phytotoxic action of herbicides is an urgent task. The ability to modify the selective phytotoxicity of herbicides is particularly important due to the rapid spread of weed resistance to herbicides (Heap, January 17, 2025, www.weedscience.org). To prevent the emergence of resistance in weeds, it is essential to have a wide range of herbicides with different mechanisms of phytotoxicity, selective for certain crop. Additionally, methods to enhance the phytotoxic effect of herbicides are needed to improve the control of already existing resistant weed biotypes (Beckie, 2006; Norsworthy et al., 2012).

Based on the concept that plant cell death induced by herbicides occurs through the induction of programmed cell death (PCD) (Mor-

derer, 2023), it is logical to assume that modifying the phytotoxic action can be achieved by influencing the course of the herbicide-induced PCD process. One of the compounds capable of affecting the PCD process is nitric oxide (De Michele et al., 2009; Wang et al., 2013; Li et al., 2021). Importantly, the nitric oxide content in plants can be easily modified using donors of this compound, particularly sodium nitroprusside.

Nitric oxide is a gaseous signaling molecule that plays a key role in regulating a variety of physiological processes (Domingos et al., 2015). Nitric oxide can act as an activator of adaptive processes that enhance plant resistance to various stressors (Sami et al., 2018; Hancock, 2020; Parveen et al., 2022). The use of nitric oxide donors has enhanced plant resistance to drought (Ziogas et al., 2015; Al-Ashkar et al., 2024; Kaya et al., 2024), high temperatures (Parankusam et al., 2017; Siddiqui et al., 2017), salinity (Ramadan et al., 2019), heavy metal pollution (Singh et al., 2008), and mineral nutrient deficiencies (Kumar et al., 2010). At the same time, high concentrations of nitric oxide can lead to plant death (Sung & Hong, 2010; Timilsina et al., 2020), particularly by inducing DNA fragmentation (Wany et al., 2017; Lv et al., 2022).

The use of sodium nitroprusside and other nitric oxide donors has reduced the phytotoxic action of herbicides, particularly by mitigating herbicide-induced oxidative stress. Treatment with sodium nitroprusside reduced damage to the unicellular green alga *Chlorella vulgaris* caused by the herbicides atrazine and glufosinate, whose action disrupts photosynthesis and is mediated by the formation of reactive oxygen species (ROS). At the same time, treatment with sodium nit-

ropresside enhanced the expression of photosynthetic genes (*psbC*, *psaB*, *chlB*, and *rbcl*) (Qian et al., 2009). Pre-treatment of seeds with sodium nitroprusside increased the content of photosynthetic pigments in rice plants (*Oryza sativa* L.) exposed to the herbicide clomazone, an inhibitor of carotenoid synthesis that, in turn, increases ROS content in plants (Silva et al., 2019). Sodium nitroprusside and another nitric oxide donor, S-nitroso-N-acetylpenicillamine, reduced the phytotoxic action of the herbicides diquat and paraquat on potato plants (*Solanum tuberosum* L.). These herbicides act as electron scavengers from the natural acceptor in photosystem I of chloroplasts and are typical prooxidants (Beligni & Lamattina, 2002). Treatment with sodium nitroprusside reduced membrane damage, hydrogen peroxide content, and total phenolic content in arugula (*Eruca sativa* Mill.) plants exposed to the herbicide paraquat (Cüce & Muslu, 2022). Pre-treatment of soybean plants (*Glycine max* L.) with sodium nitroprusside reduced the oxidative stress effects induced by the herbicide lactofen, which inhibits the chlorophyll biosynthesis enzyme protoporphyrinogen oxidase (PPO) (Ferreira et al., 2010). The use of sodium nitroprusside also allowed for influencing the rate of herbicide detoxification in plants. Specifically, treatment with sodium nitroprusside stimulated the detoxification of the herbicide glyphosate, reducing stress levels and improving tomato growth in the presence of glyphosate residues in the soil (Soares et al., 2021).

Besides protecting crops from the effects of herbicides, it is of great importance to develop methods for enhancing the effectiveness of herbicide-based weed control through the stimulation of herbicide-induced reactions. A series of experiments was conducted to investigate the potential of enhancing the phytotoxic effect of herbicides through their combined use with sodium nitroprusside (Sychuk et al., 2013; Sychuk, 2015). Since the role of nitric oxide in the induction of programmed cell death may be due to its interaction with ROS (Li et al., 2021; Mandal et al., 2022), the effect of sodium nitroprusside on the phytotoxic effect of acetyl-CoA carboxylase (ACC) inhibitor herbicides, the action of which is mediated by the formation of ROS, was first investigated. When plants were pretreated with sodium nitroprusside, the phytotoxic effect of ACC inhibitor herbicides increased; however, when treated simultaneously, the effect decreased (Sychuk et al., 2013). When studying the effect of sodium nitroprusside on the action of herbicides that inhibit acetolactate synthase (ALS), where ROS formation is a secondary effect, the same pattern was observed: pretreatment with sodium nitroprusside led to an increase in the phytotoxic effect, while simultaneous treatment resulted in a decrease (Sychuk, 2015). At the same time, a field experiment revealed that co-treatment with sodium nitroprusside enhanced the phytotoxic effect of the PPO inhibitor herbicide saflufenacil (Sychuk, 2015).

It is obvious that from a practical point of view, only simultaneous treatment with herbicides and a nitric oxide donor is appropriate to increase phytotoxic action. Therefore, it was important to determine which herbicides could be more effective when used in combination with sodium nitroprusside. Considering that ACC and ALS inhibitors are characterized by a slow development of phytotoxic action, while PPO inhibitors, on the contrary, exhibit a high rate of development (Barker et al., 2023), it was suggested that during simultaneous treatment, the effect of sodium nitroprusside on herbicide action is determined by the rate of development of the herbicide's phytotoxic action. To test this hypothesis, the effect of sodium nitroprusside on the phytotoxicity of herbicides, which differ in the extent of ROS involvement and the rate of development of their phytotoxic effect, was studied (Ponomareva et al., 2022; Ponomareva & Yukhymuk, 2023). Three classes of herbicides were selected for the experiments: the PPO inhibitor carfentrazone, whose action is mediated by ROS formation and is characterized by rapid development (Barker et al., 2023); the synthetic auxin 2,4-D, where ROS formation is a secondary consequence of oxidative stress, with the primary action stemming from the disruption of the phytohormonal regulation system (Traxler et al., 2023), but still exhibiting a high rate of phytotoxic effect development; and aclonifen, a carotenoid biosynthesis inhibitor, which involves ROS in its phytotoxic action but has a slower development compared to PPO inhibitors and synthetic auxins (Barker

et al., 2023). Oilseed radish plants (*Raphanus sativus* var. *oleiformis*) were used as a model for annual weeds that are sensitive to the effects of the herbicides being studied. When applied separately, the inhibitory effect of carfentrazone on oilseed radish plants was less pronounced than that of 2,4-D. At the same time, when used in combination with sodium nitroprusside, the phytotoxic effect of carfentrazone was enhanced more than that of 2,4-D (Ponomareva et al., 2022). The combined use of aclonifen with sodium nitroprusside only accelerated the development of the phytotoxic action, without enhancing its effect (Ponomareva & Yukhymuk, 2023). When inactivated sodium nitroprusside and urea were used together with herbicides, at a concentration equivalent in nitrogen content to sodium nitroprusside, the phytotoxic effect of the herbicides remained unchanged. This is evidence that the enhanced phytotoxic effect of herbicides when used in combination with sodium nitroprusside is due to its ability to act as a nitric oxide donor (Ponomareva et al., 2022).

The results obtained confirmed the hypothesis that the rate of phytotoxic development is the main factor determining the nature of the influence of sodium nitroprusside on herbicide activity. However, it remained unclear how the rate of development influences the sensitivity of the herbicide's phytotoxic action to the effects of the nitric oxide donor. It is known that after herbicide application, an increase in the content of ROS, particularly hydrogen peroxide, is observed in plants after a certain period of time. The intensity of ROS formation and the duration of the period during which this increase is observed depend on the mechanism of the herbicide's phytotoxic action (Traxler et al., 2023). It is likely that the application of herbicides with certain modes of action may also lead to an increase in nitric oxide levels in plants. It is logical to assume that whether the effect of herbicides on nitric oxide and ROS levels will be enhanced when applied in combination with a nitric oxide donor depends on how closely the dynamics of changes in nitric oxide and ROS content induced by the herbicide coincide with those caused by the nitric oxide donor. Accordingly, how the nitric oxide donor affects the herbicide-induced changes in nitric oxide and ROS levels in plants may determine how the donor will influence the phytotoxic action of the herbicide.

Therefore, the aim of the study was to identify the factors that determine the nature of the nitric oxide donor's influence on the action of herbicides with different mechanisms of phytotoxicity. In this regard, the objective of the study was to compare the dynamics of changes in nitric oxide and ROS levels, particularly hydrogen peroxide, induced by the nitric oxide donor and herbicides with different mechanisms of phytotoxicity: the protoporphyrinogen oxidase inhibitor carfentrazone, the synthetic auxin 2,4-D, and the carotenoid biosynthesis inhibitor aclonifen, applied separately and in combination with sodium nitroprusside.

Material and methods

The vegetative experiment was set up according to the scheme presented in Table 1. The oilseed radish (*Raphanus sativus* var. *oleiformis*) was used as a model for annual dicotyledonous weeds. The plants were grown in containers with 1.3 kg of a soil-sand mixture (3:1 ratio), with 20 plants per container, on the institute's vegetative plot under natural light conditions. The plants were treated at the 2-leaf stage by spraying solutions of sodium nitroprusside, herbicides, and a mixture of herbicides with sodium nitroprusside. The experiments were conducted with four repetitions. Each experiment included a control group without treatment.

Table 1
Scheme of experiment

Treatment	Application rate
Control	Without treatment (processing)
Sodium nitroprusside	0.45 kg/ha
Carfentrazone	0.02 kg/ha
Carfentrazone + sodium nitroprusside	0.02 kg/ha + 0.45 kg/ha
Aclonifen	0.6 kg/ha
Aclonifen + sodium nitroprusside	0.6 kg/ha + 0.45 kg/ha
2,4-D	0.36 kg/ha
2,4-D + sodium nitroprusside	0.36 kg/ha + 0.45 kg/ha

The determination of the inhibitory effect of herbicides and the levels of nitric oxide and hydrogen peroxide (H₂O₂) in plant leaves was carried out 1, 4, and 8 days after treatment. The inhibitory effect of herbicides was calculated as the difference between 100% and the percentage of fresh weight of the aboveground part of herbicide-treated plants compared to the control.

The content of nitric oxide was determined using a method based on the conversion of the nitric oxide present in plants to nitrite, with the concentration of the latter established using the Griess reaction (Zhou et al., 2005). The plant material was ground in a cooled mortar in a 50 mM acetate buffer (pH 3.6) with the addition of 2% zinc acetate (Carlo Erba, France), and then centrifuged for 15 minutes at 8000 g at 4 °C. Activated charcoal (Newish Industrial and Trading Company Limited, China) was added to the supernatant, followed by filtration. The content of nitric oxide in the filtrate was determined using the Griess reagent by measuring the light absorption of the colored complex at a wavelength of 530 nm. The content of nitric oxide was calculated by comparison with the standard curve obtained from calibration solutions of sodium nitrite (NaNO₂) in acetate buffer and expressed in nmol/g of fresh weight.

The content of H₂O₂ was determined using the ferrous thiocyanate method (Sagisaka, 1976). A 0.3 g sample of plant material was ground in a cooled mortar in 5% trichloroacetic acid (Nile Chemicals, India), and then centrifuged for 10 minutes at 8000 g at 4 °C. The concentration of H₂O₂ in the supernatant was determined using Mora's salt (Alpharus LLC, Ukraine) and ammonium thiocyanate (Henan Yindu Environmental Protection Technology Co., LTD, China) by measuring the light absorption of the colored complex at a wavelength of 480 nm. The content of H₂O₂ was calculated in μmol/g of leaf fresh weight based on a calibration curve constructed from hydrogen peroxide solutions of known concentration. All analytical measurements were performed in triplicate.

The nitric oxide donor used was sodium nitroprusside (Na₂[Fe(NO)(CN)₅]) (Merck KGaA, Germany). The following herbicide formulations were used: Aurora WG (water-soluble granules) (carfentrazone, 400 g/kg) by FMC; Esteron 600 EC (emulsion concentrate) (ethylhexyl ester of 2,4-D, 600 g/L) by Dow AgroSciences; Challenge SC (suspension concentrate) (aclonifen, 0.6 kg/L) by Bayer CropScience.

The statistical analysis of the obtained results was performed using analysis of variance, and the data in the tables are presented as mean ± standard deviation (x ± SD). Differences between groups were considered significant using the Tukey test with P < 0.05.

Results

Determination of the mass of fresh weight of the above-ground part of oilseed radish plants 1, 4 and 8 days after treatment with so-

dium nitroprusside, herbicides and a mixture of herbicides with sodium nitroprusside showed that sodium nitroprusside slightly but significantly stimulated, and herbicides suppressed, the accumulation of fresh weight of plants (Table 2).

Table 2

The fresh weight (mg) of the aboveground part of oilseed radish plants (model for dicotyledonous weeds) under the influence of a nitric oxide donor and herbicides on the 1st, 4th, and 8th days after treatment (x ± SD, n = 12)

Treatment (rate by active ingredient)	1st day	4th day	8th day
Untreated	1170 ± 14 ^h	1375 ± 12 ^d	1673 ± 16 ^b
Sodium nitroprusside	1203 ± 15 ^g	1538 ± 14 ^c	1784 ± 30 ^a
Carfentrazone	1088 ± 12 ^k	1150 ± 11 ^{hi}	1322 ± 18 ^e
Carfentrazone + sodium nitroprusside	1045 ± 10 ^{kl}	901 ± 16 ^o	906 ± 20 ^o
Aclonifen	1132 ± 14 ^{ij}	1142 ± 8 ^{hij}	1045 ± 12 ^{kim}
Aclonifen + sodium nitroprusside	1073 ± 12 ^k	855 ± 13 ^p	1013 ± 18 ^{mm}
2,4-D	1116 ± 11 ^{jk}	1168 ± 12 ^h	1242 ± 16 ^f
2,4-D + sodium nitroprusside	1090 ± 13 ^k	1008 ± 16 ⁿ	854 ± 24 ^p

Note: the mean values that do not share the same letters are significantly different according to the Tukey test (P < 0.05), with all samples being compared to each other.

When herbicides were applied separately, the strongest inhibitory effect was observed with carfentrazone 1 day after treatment (Fig. 1). On the 4th day, the inhibitory effect was almost the same for all herbicides, while on the 8th day, the greatest inhibition of biomass accumulation was caused by aclonifen, and the least by carfentrazone. When applied in combination with sodium nitroprusside, the inhibitory effect of carfentrazone on the 1st day was the same as when applied separately, while on the 4th and 8th days, the inhibitory effect increased more than twofold, from 16.7% to 34.8% and from 21.2% to 45.5%, respectively. Similarly, when applied in combination with sodium nitroprusside, the inhibitory effect of 2,4-D on the 1st day was the same as when applied separately, while on the 4th and 8th days, it significantly increased, from 15.2% to 26.8% and from 25.4% to 49.1%, respectively. The increase in the effect of 2,4-D when applied in combination with sodium nitroprusside was somewhat smaller than the increase in the effect of carfentrazone. When applied in combination with sodium nitroprusside, the effect of aclonifen significantly increased on the 1st and 4th days, while on the 8th day, it was similar to the effect of aclonifen alone.

The determination of nitric oxide content showed that the effect of sodium nitroprusside on the content of this compound in plants is quite short-lived (Table 3). A significant increase in nitric oxide content compared to the control was observed on the 1st and 4th days after treatment, while on the 8th day, the nitric oxide content in the treated plants did not significantly differ from the control.

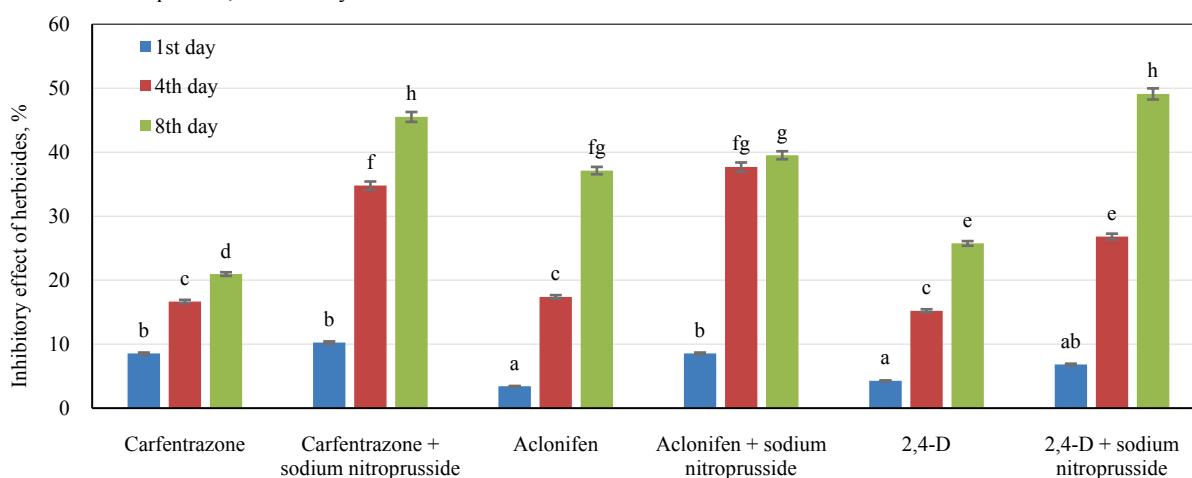


Fig. 1. Inhibitory effect (%) of herbicides on the accumulation of fresh weight in oilseed radish plants (model for annual dicotyledonous weeds) when applied separately and in combination with sodium nitroprusside: mean values that do not share the same letters are significantly different according to the Tukey test (P < 0.05, n = 12), with all samples being compared to each other

The dynamics of nitric oxide content changes under the effect of carfentrazone alone were similar to the effect of sodium nitroprusside: nitric oxide content increased on the 1st and 4th days after treatment and was almost indistinguishable from the control on the 8th day. When carfentrazone was applied in combination with sodium nitroprusside, nitric oxide content significantly increased on the 1st and 4th days after treatment compared to the effect of these substances applied separately, while on the 8th day, it did not differ from the control. The increase in nitric oxide content under the effect of 2,4-D alone was less intense and shorter-lived than under the effect of carfentrazone. Under the effect of 2,4-D, a significant increase in nitric oxide content compared to the control was observed only on the 1st day after treatment, and it was lower than under the effect of carfentrazone. When 2,4-D was applied in combination with sodium nitroprusside, a significant increase in nitric oxide content compared to the control was observed on the 1st and 4th days, and compared to the application of sodium nitroprusside alone – only on the 1st day after treatment. When aclonifen was applied alone, no significant changes in nitric oxide content compared to the control were observed. When aclonifen was applied in combination with sodium nitroprusside, an increase in nitric oxide content compared to the control was observed only on the 1st day after treatment and was similar to the effect of sodium nitroprusside alone. On the 4th and 8th days, nitric oxide content decreased to the control level and, on the 4th day, was lower than under the effect of sodium nitroprusside alone.

Table 3

Nitric oxide content (nmol/g fresh weight) in the leaves of oilseed radish plants (model for annual dicotyledonous weeds) on the 1st, 4th, and 8th days after treatment with sodium nitroprusside, herbicides, and herbicide mixtures with sodium nitroprusside ($x \pm SD$, $n = 12$)

Treatment (rate by active ingredient)	1st day	4th days	8th days
Untreated	35.00 ± 0.67 ^{ab}	35.13 ± 0.37 ^{ab}	34.30 ± 0.32 ^a
Sodium nitroprusside	57.00 ± 0.81 ^e	42.15 ± 0.79 ^{de}	36.35 ± 0.52 ^{abc}
Carfentrazone	52.60 ± 0.50 ^f	39.65 ± 0.61 ^{cd}	36.10 ± 0.88 ^{abc}
Carfentrazone + sodium nitroprusside	79.15 ± 1.11 ^j	61.98 ± 0.56 ^h	36.35 ± 1.04 ^{abc}
Aclonifen	36.13 ± 0.82 ^{abc}	36.75 ± 0.72 ^{abc}	34.33 ± 0.39 ^a
Aclonifen + sodium nitroprusside	57.23 ± 1.72 ^e	37.58 ± 0.81 ^{abc}	35.00 ± 0.61 ^{ab}
2,4-D	43.95 ± 0.74 ^c	38.65 ± 0.86 ^{bcd}	36.00 ± 0.35 ^{abc}
2,4-D + sodium nitroprusside	66.80 ± 1.05 ⁱ	44.33 ± 0.87 ^c	35.15 ± 1.11 ^{ab}

Note: see Table 2.

Treatment with sodium nitroprusside alone led to a decrease, whereas herbicide treatment, on the contrary, caused an increase in hydrogen peroxide content in the plants (Table 4).

Table 4

Hydrogen peroxide (H₂O₂) content (μmol/g fresh weight) in the leaves of oilseed radish plants (model for annual dicotyledonous weeds) on the 1st, 4th, and 8th days after treatment with the nitric oxide donor sodium nitroprusside and herbicides, applied separately or in combination with herbicides and the nitric oxide donor ($x \pm SD$, $n = 12$)

Treatment (rate by active ingredient)	1st day	4th day	8th day
Untreated	0.235 ± 0.006 ^{bc}	0.233 ± 0.012 ^b	0.228 ± 0.006 ^b
Sodium nitroprusside	0.185 ± 0.006 ^a	0.203 ± 0.026 ^a	0.203 ± 0.018 ^a
Carfentrazone	0.400 ± 0.008 ^{gh}	0.365 ± 0.018 ^{fg}	0.275 ± 0.010 ^{cd}
Carfentrazone + sodium nitroprusside	0.550 ± 0.008 ⁱ	0.565 ± 0.010 ^j	0.420 ± 0.018 ^h
Aclonifen	0.313 ± 0.006 ^c	0.293 ± 0.012 ^{bc}	0.258 ± 0.006 ^c
Aclonifen + sodium nitroprusside	0.350 ± 0.008 ^f	0.355 ± 0.012 ^{fg}	0.270 ± 0.012 ^{cd}
2,4-D	0.380 ± 0.008 ^g	0.353 ± 0.006 ^f	0.363 ± 0.006 ^{fg}
2,4-D + sodium nitroprusside	0.388 ± 0.010 ^g	0.400 ± 0.008 ^{gh}	0.283 ± 0.004 ^{cd}

Note: see Table 2.

Under the action of carfentrazone, an increase in hydrogen peroxide content was observed on the 1st and 4th days compared to the

control, while on the 8th day its content decreased but still significantly exceeded the control levels. When carfentrazone was applied in combination with sodium nitroprusside, hydrogen peroxide content throughout the entire observation period exceeded its levels in the carfentrazone-only treatment. Under the action of 2,4-D, an increase in hydrogen peroxide content was also observed compared to the control. On the 1st and 4th days, hydrogen peroxide content under the action of 2,4-D did not differ, while on the 8th day, it significantly exceeded the levels observed with carfentrazone treatment. When 2,4-D was applied in combination with sodium nitroprusside, hydrogen peroxide content compared to the separate application of 2,4-D did not change on the 1st day, increased on the 4th day, and decreased on the 8th day after treatment. Thus, although the hydrogen peroxide content under the action of 2,4-D did not differ on days 1 and 4 when applied separately, and even exceeded the carfentrazone treatment on day 8, when applied in combination with sodium nitroprusside, the increase in hydrogen peroxide content was greater for carfentrazone than for 2,4-D throughout the observation period. When aclonifen was applied separately, the hydrogen peroxide content exceeded the control, but this increase was lower than that observed under carfentrazone treatment on days 1 and 4, and lower than the effect of 2,4-D throughout the observation period. When aclonifen was applied in combination with sodium nitroprusside, the hydrogen peroxide content increased on days 1 and 4, and on day 8, it did not differ from the treatment with aclonifen alone.

Discussion

Nitric oxide is small in size and can easily diffuse through biological membranes, allowing it to effectively perform signaling functions and participate in various physiological processes in plant organisms (Soares et al., 2021). In this regard, nitric oxide donors, such as sodium nitroprusside, are used to influence specific plant characteristics. In particular, there is evidence that sodium nitroprusside treatment increases the content of secondary metabolites in ginseng roots (Zhang et al., 2025). The most well-studied aspect is the involvement of nitric oxide in the activation of plant protective systems in response to various stressors (Hancock, 2020; Parveen et al., 2022). The use of sodium nitroprusside increased the drought resistance of tomato plants. With pre-treatment using sodium nitroprusside, tomatoes under temperature stress showed increased accumulation of biomass and photosynthetic pigments (Kaya et al., 2024). The use of sodium nitroprusside reduced the negative impact of stressors, such as salinization (Ramadan et al., 2019; Sheikhalipour et al., 2024), heavy metal pollution (Niyofasha, et al., 2023), mineral nutrient deficiency (Kumar et al., 2010). At the same time, the use of nitric oxide donors to modify the phytotoxic effects of herbicides remains less explored. Basically, the available data suggest the possibility of reducing the phytotoxic effects of herbicides through pre-treatment of plants with a nitric oxide donor. There is a possibility of reducing the effects of the herbicides atrazine and glufosinate on the unicellular green algae *Chlorella* through pre-treatment with sodium nitroprusside. In this case, treatment with sodium nitroprusside also increased the expression of photosynthetic genes (psbC, psaB, chlB и rbcL) (Qian et al., 2009). Pre-treatment of rice seeds with sodium nitroprusside increased the content of photosynthetic pigments under the action of the clomazon herbicide (Silva et al., 2019). Sodium nitroprusside and S-nitroso-N-acetylpenicillamine reduced the phytotoxic effects of the herbicides diquat and paraquat on potato plants (Beligni & Lamattina, 2002). In arugula plants, treatment with sodium nitroprusside resulted in a reduction of membrane damage, hydrogen peroxide content, and total phenol content under the action of the paraquat herbicide (Cüce & Muslu, 2022). The use of sodium nitroprusside has also affected the rate of herbicide detoxification in plants. Treatment with sodium nitroprusside stimulated the detoxification of the herbicide glyphosate, thereby reducing stress levels and improving tomato growth in the presence of residual amounts of this non-selective herbicide in the soil (Soares et al., 2021). At the same time, there is data suggesting that high concentrations of nitric oxide can cause plant death (Sung & Hong, 2010; Timilsina et al., 2020), in particular, this can lead to

DNA fragmentation (Wany et al., 2017; Lv et al., 2022). However, the question of using nitric oxide donors to enhance the phytotoxic action of herbicides has remained unresolved.

The results obtained in our work confirmed the findings of previous studies (Ponomareva et al., 2022; Ponomareva & Yukhymuk, 2023), where the nature of the effect of sodium nitroprusside on herbicide action with different mechanisms of phytotoxicity was established. It was confirmed that the action of the protoporphyrinogen oxidase inhibitor carfentrazone increases when applied in combination with sodium nitroprusside, while the action of the synthetic auxin 2,4-D also increases, but to a lesser extent than that of carfentrazone. For the carotenoid biosynthesis inhibitor aclonifen, there is an acceleration of development, but not an increase in inhibitory action. The study of nitric oxide and hydrogen peroxide content in plants over 8 days after treatment with sodium nitroprusside and the investigated herbicides separately, as well as herbicides in combination with sodium nitroprusside, showed that the characteristics of the herbicides' effects with different mechanisms of phytotoxicity on the content of these compounds in plants, and accordingly, the effects of sodium nitroprusside on herbicide-induced changes in nitric oxide and hydrogen peroxide levels, may be the reason for the different nature of sodium nitroprusside's impact on the herbicides' inhibitory action. It is evident that the difference in the action of carfentrazone and 2,4-D compared to aclonifen lies in the fact that the development of the effects of carfentrazone and 2,4-D is accompanied by the formation of nitric oxide, whereas no such effect is observed with aclonifen, at least during the period when an increase in nitric oxide content is observed upon the application of sodium nitroprusside. Coincidence in time of the effects of sodium nitroprusside, carfentrazone, and 2,4-D on the increase in nitric oxide content in plants leads to a significant increase in nitric oxide levels when these herbicides are applied in combination with sodium nitroprusside, compared to their effects when applied separately. Under the influence of all studied herbicides, the hydrogen peroxide content increased compared to the control group. When herbicides were applied in combination with sodium nitroprusside, there was an increase in the hydrogen peroxide content compared to the effect of the herbicides applied separately. At the same time, the greatest increase was observed for carfentrazone, while the increase was less intense and shorter in duration for 2,4-D and aclonifen. Since the hydrogen peroxide content in the plants decreased with sodium nitroprusside alone, it is likely that the increase in hydrogen peroxide content upon combined application of herbicides with sodium nitroprusside is a result of the increased nitric oxide content. Therefore, it can be concluded that the increased induction of nitric oxide formation is the primary factor responsible for the enhanced phytotoxic action of carfentrazone and 2,4-D when applied in combination with sodium nitroprusside.

The basis for choosing nitric oxide donors as compounds capable of enhancing the phytotoxic effect of herbicides was the data on the participation of nitric oxide in the induction of PCD (De Michele et al., 2009; Wang et al., 2013; Li et al., 2021). The results we obtained, which demonstrated that the enhanced inhibitory action of herbicides with certain mechanisms of phytotoxicity when used in combination with a nitric oxide donor is accompanied by an increase in the content of this compound, generally support the hypothesis of the involvement of PCD in the herbicide-induced pathogenesis. However, they cannot be considered as sufficient evidence for this hypothesis. While there is clear evidence that the reduction in the phytotoxic effect of herbicides (Silva et al. 2019; Beligni & Lamattina, 2002) and including the protoporphyrinogen oxygen inhibitor lactofen (Ferreira et al., 2010) upon pretreatment with a nitric oxide donor is due to the stimulation of the plant antioxidant defense system by nitric oxide, the nature of the processes that cause the increase in the phytotoxic effect of herbicides due to an increase in the induced content of nitric oxide remains unclear. It is well-established that the elevation of nitric oxide levels, induced by its donor, accelerates the process of PCD in *Arabidopsis* cells (*Arabidopsis thaliana* (L.) Heynh.) upon pathogen infection (Clarke et al., 2000). An increase in nitric oxide content accelerates the DNA degradation characteristic of PCD under hypoxia in wheat roots (Wany et al., 2017), as well as

during the hypersensitive response in *Arabidopsis* (Lv et al., 2022). It is also known that nitric oxide plays a crucial role in peroxide-dependent PCD in rice leaf cells (*Oryza sativa* L.) (Lin et al., 2012). Although autophagy is considered one of the types of PCD, short-term induction of autophagy can enhance the plant's resistance to herbicide treatment (Zhao et al., 2018). At the same time, nitric oxide can inhibit autophagy, which in turn leads to the acceleration of PCD initiation (Filomeni et al., 2015). Thus, the use of a nitric oxide donor, which leads to an increase in nitric oxide levels in plants upon herbicide treatment, can directly accelerate PCD or promote the initiation of this process through the inhibition of autophagy. However, this issue requires further investigation.

Conclusions

Studies have shown that sodium nitroprusside can be used to enhance the phytotoxic action of PPO inhibitor herbicides and synthetic auxins. The results obtained indicate that the enhancement of the phytotoxic effect of herbicides when used together with sodium nitroprusside is achieved by intensification and prolongation of the herbicide-initiated increase in the content of nitric oxide in plants. This occurs when the herbicide's mechanism of action foresees an increase in nitric oxide in treated plants, and the dynamics of this increase coincide with the effects of sodium nitroprusside. The possibility of increasing the phytotoxic effect of individual herbicides through joint use with a nitric oxide donor can later be used to increase the effectiveness of crop protection against weeds.

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