

EFFECT OF PROPAQUIZAFOP TREATMENT ON PIGMENTS CONTENT AND GROWTH OF SWEET MAIZE SEEDLINGS

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ABSTRACT

Background. We examined the response of sweet maize seedlings (*Zea mays* var. *saccharata* L., cv. *Złota Karłowa*), as a non-target plant, to various doses of propaquizafop ((R)-2-[4-[(6-chloro-2-quinoxalinyloxy)phenoxy]-propionic acid 2-[[[(1-methylethylidene)amino]oxy]ethyl ester) applied to the root zone or on shoots.

Material and methods. The herbicide at concentrations of 0.56; 5.63 or 56.3 μM was used in a study on seedlings grown in hydroponic cultures in controlled light and temperature conditions. In the experiment the roots of maize seedlings were exposed to propaquizafop for 7 days. Also, seedling shoots were exposed by their immersing for 30 seconds in the investigated propaquizafop concentrations. We assessed the impact of propaquizafop on the seedlings elongation of primary roots and shoots. The content of anthocyanins in epicotyls and photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total carotenoids) in leaves were also measured.

Results. A weaker effect on shoot growth was found when the roots of sweet maize seedlings were exposed to propaquizafop than was the case in the application of the herbicide to shoot. Application of the herbicide to the root zone also had little effect on the levels of chlorophylls and carotenoids, or on the ratios between them, in the leaves. The presumed responsibility for this effect is a weak uptake of the herbicide by the roots and / or its transport to the leaves of the maize seedlings.

Conclusion. Propaquizafop has a smaller effect on sweet maize seedlings when applied to the root zone than it has after foliar exposition. A decline of anthocyanin content under the influence of low doses of propaquizafop suggests that it has a greater effect on the metabolism of phenylpropanoids than other inhibitors of acetyl coenzyme A carboxylase have.

Key words: anthocyanins, carotenoids, chlorophyll, herbicide, mode of use

INTRODUCTION

Aryloxyphenoxypropionates (AOPP) and cyclohexanediones are graminicides, post-emergence herbicides that cause growth inhibition of grass weeds. Together they account for approx. 10% of the current global herbicide market (Harwood, 1999). AOPP include the following chiral active ingredients: diclofop-methyl, fluazifop-*p*-butyl, haloxyfop-methyl, fenoxaprop-*p*-ethyl, quizalofop-ethyl, fenoxaprop-*p*-ethyl and propaquizafop

(PROP). The mechanism that suppresses the growth of grasses with these kinds of herbicides involves inhibition of the plastid acetyl coenzyme A carboxylase (ACCase), a key enzyme in fatty acid biosynthesis (Kunimitsu *et al.*, 1988; Liu *et al.*, 2009). This is accompanied by the oxidative membrane catabolism due to free radical lipid peroxidation (Shimabukuro and Hoffer, 1995; De Prado *et al.*, 1999). Graminicides are very effective against monocotyledonous species, exerting their herbicidal action in meristematic zones,

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and they are widely used in dicotyledonous crops. Experiments conducted with chickpea provide additional evidence that dicot chloroplasts contain a graminicide-tolerant, multi-subunit ACCase, which explains the lack of effect of AOPP, clethodim and cycloxydim on *de novo* fatty acid biosynthesis in dicots (Giménez-Espinosa *et al.*, 1999).

Graminicides induce the accumulation of reactive oxygen species (ROS), which is accompanied by lipid peroxidation (Banaś *et al.*, 1993; Luo *et al.*, 2004; Janicka *et al.*, 2008). Graminicide treatment has also been shown to cause an increase in phenylalanine ammonia lyase (PAL) activity with an accumulation of anthocyanins and other phenylpropanoids (Belkebir and Benhassaine-Kesri, 2013). Similarly, in a previous study of ours, high concentrations of fluazifop-*p*-butyl applied on leaves caused an increased accumulation of anthocyanin in the epicotyls of maize (Horbowicz *et al.*, 2013). Anthocyanin accumulation is probably a part of plant response to abiotic stress caused by oxidative stress (Banaś *et al.*, 1993; Shimabukuro and Hoffer, 1995; Chalker-Scott, 1999).

Numerous herbicides act as photosynthesis inhibitors (Ma *et al.*, 2002). However, there is limited information on the influence of graminicides on the content of photosynthetic pigments within plant tissue. Clethodim (cyclohexanedione ACCase inhibitor) applied on leaves caused browning or reddening of maize leaves (Radwan and Soltan, 2012). The rate of photosynthesis was significantly lowered due to the reduction of photosynthetic pigments. Mentioned authors concluded that clethodim leads to physiological, biochemical and metabolic processes that results in morphological changes in maize leaves. With increasing fluazifop concentration, a declining leaf pigment content and leaf chlorosis appeared after 7–10 day treatment of peanut leaves (Fayez *et al.*, 2014). In rape leaves subjected to sethoxydim herbicide (ACCase inhibitor), a reduction in growth rate and chlorophylls has been observed (Belkebir and Benhassaine-Kesri, 2013). In our previous study, an increase of chlorophyll and carotenoid content within maize leaves was observable under the lowest dose of fluazifop-*P*-butyl (0.65 μM), but a decrease of chlorophyll and carotenoid content was observed under the higher concentrations (6.5 and 65 μM) (Horbowicz *et al.*, 2013). As far as we know information available on the effect of propaquizafop on the growth and metabolism of maize seedlings.

Propaquizafop ((R)-2-[4-[(6-chloro-2-quinoxalinyloxy)phenoxy]-propionic acid 2-[[[(1-methylethylidene)amino]oxy]ethyl ester) is an active ingredient in commercial post-emergence herbicides Agil 100EC, Galeon 100EC, Falcon, Shogun, Zealot and others. The active ingredient is used in doses from 60 to 120 g per hectare. PROP has a broad spectrum of activity and is used for post-emergence control of annual and perennial monocotyledonous weeds, such as: *Avena* spp., *Echinochloa crus-galli*, *Lolium multiflorum*, *Apera spica-venti*, *Poa annua*, *Bromus* spp., *Agropyron repens*, and *Sorghum* spp. Herbicides containing PROP are used in the cultivation of dicotyledonous crops: potatoes, sugar beet and a variety of vegetables.

The currently described research relates to the effects of two modes of application of PROP – to the root zone and to shoots. The paper aims at quantifying the phytotoxicity of PROP to non-target sweet maize plants, as these plants are highly sensitive to various abiotic stressors, particularly herbicides. The study was focused on the following problems:

- (1) Which mode of herbicide use inhibits the growth of sweet maize seedlings the most?
- (2) Are photosynthetic pigments and anthocyanins altered by the herbicide?

MATERIAL AND METHODS

Sweet maize seeds (*Zea mays* var. *saccharata* L., cv. Złota Karłowa; PNOS, Ożarów Mazowiecki, Poland) were used in the experiment. Initially, the maize seeds were subjected to a soaking by leaving it for a day in distilled water. Germination of the soaked seeds was carried out by placing them between two layers of wet filter paper, which were then rolled up and inserted into a beaker containing distilled water (Horbowicz *et al.*, 2013). Germination was carried out in darkness at $24 \pm 1^\circ\text{C}$ over five days.

Following the germination process uniform seedlings were chosen and transferred to a 1/5–strength Hoagland nutrient solution in growth room conditions. Culture conditions were as follows: 16 h photoperiod at $22 \pm 2^\circ\text{C}/16 \pm 2^\circ\text{C}$ day/night. Light ($100\text{--}120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was provided by 400 W high-pressure sodium lamps. On the next day growth in such condition the effect of various concentrations of propaquizafop (0.56; 5.63 or 56.3 μM) added to the nutrient solution (root zone) was evaluated. In another part of the

experiment the maize seedlings were treated by immersing their shoots (epicotyls and leaves) for 30 seconds in solutions with the same herbicide concentrations, i.e. 0.56; 5.63 or 56.3 μM . Control seedlings were grown in 1/5–strength Hoagland nutrient solution only.

Estimation of maize seedlings elongation

Before using PROP and for 7-days after end of treatment the lengths of the upper parts (shoots) and the primary root of the maize seedlings were measured. The difference between the length before and after applying the herbicide was considered seedlings' elongation. For these measurements, 20–30 seedlings were taken for one replicate and for each dose of herbicide and for the control plants.

Assay of the pigments

For the analysis of anthocyanins, 1-cm fragments from the middle part of the epicotyl were taken. For the analysis of chlorophylls and carotenoids the middle parts of leaf fragments were collected. Fragments of 10 seedlings were taken for each dose of herbicide and for the control plants. The chemical analyses were carried out in 4–5 replicates.

The content of chlorophyll *a* and *b* and total carotenoids were analyzed spectrophotometrically by the method of Lichtenthaler and Wellburn (1985). Total anthocyanins were determined using the method described by Mancinelli (1984). For one replicate, randomly selected (200–400 mg) tissue of leaves or epicotyls was taken. In the case of carotenoids and chlorophylls determination, an 80% acetone solution in the ratio of 1 mg of plant tissue to 50 μl of the solvent was used. For the extraction of anthocyanins a 1% solution of HCl in methanol, in the ratio of 1 mg of plant tissue to 40 μl of the solvent, was applied.

The analyses of pigments were carried out in 4–5 replicates.

RESULTS AND DISCUSSION

Effect of propaquizafop applied to the root zone and on shoots on the growth of sweet maize seedlings

The obtained results indicate that the lower doses of PROP (0.56 and 5.63 μM) applied to the root zone

inhibit the elongation of the upper parts (shoots) and roots of maize seedlings, although the inhibition has not been proven statistically (Fig.1). This inhibition reached 25 and 40% relative to the control, respectively. A statistically significant inhibition (50%) of shoot growth was observed with the high dose of PROP (56.3 μM) applied to the root zone. PROP applied to the root zone caused much stronger inhibition of the root growth of maize seedlings (Fig. 1). The lowest PROP concentration (0.56 μM) resulted in a 66% decrease in elongation of the primary root, while the middle (5.63 μM) caused 78% and the highest (56.3 μM) 85% reduction in root elongation.

A much greater inhibitory effect on shoot growth was caused by PROP applied to the aerial parts of maize seedlings. Even the lowest PROP concentration (0.56 μM) resulted in a more than 50% reduction in shoot elongation and they reached only 35% of the control length (Fig. 2). Higher PROP concentrations almost completely inhibited the elongation of shoots of maize seedlings. PROP foliarly applied had less effect on root growth. The low concentration level inhibited the growth of the primary root at 25%, while the middle and the highest concentrations inhibited 62 and 69% of root elongation, respectively (Fig. 2).

The observed lower inhibitory effect on root growth than on shoots when PROP was applied to shoots is a different response of maize to that found when fluazifop-P-butyl had been applied in a previous experiment. A higher sensitivity of maize seedling roots as compared to the shoots has been noted for fluazifop-P-butyl herbicide (Horbowicz *et al.*, 2013). Significantly higher shoot growth inhibition under PROP foliarly applied suggests that there is slight uptake and/or transport of this herbicide from shoot to root in maize seedlings. According to Giménez-Espinosa *et al.* (1999) absorption and translocation of PROP by chickpea tissues is limited. The herbicide applied as ethyl ester was rapidly metabolized to the acid form followed by a slow conversion of the acid to a polar metabolite. The mentioned authors suggested that this was probably the main reason why only *circa* 4% of foliarly-applied [^{14}C] propaquizafop was translocated within 72 h after application.

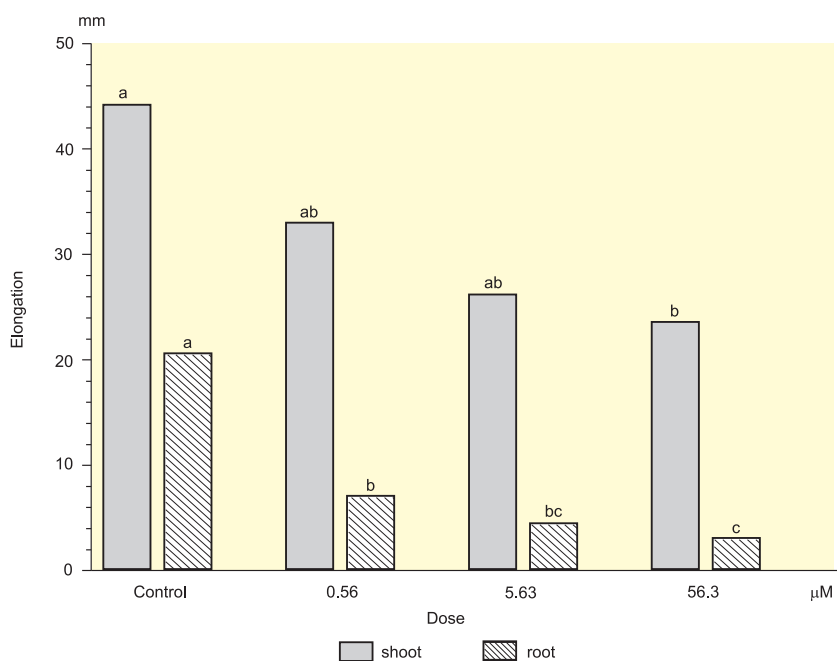


Fig. 1. The influence of propaquizafop dose applied to the root zone on the elongation of shoot and primary root of sweet maize seedlings. Mean results represent by bars marked by the various letters are significantly different at $P < 0.05$ according to the Newman–Keuls test

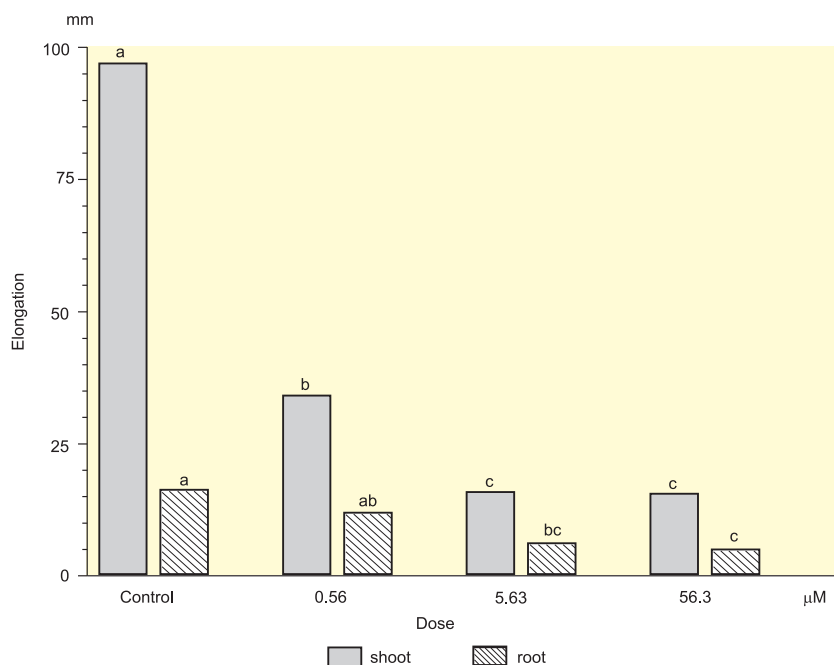


Fig. 2. The influence of propaquizafop dose applied on shoots on the elongation of primary root and shoot of sweet maize seedlings. Mean results represent by bars marked by the various letters are significantly different at $P < 0.05$ according to the Newman–Keuls test

Effect of propaquizafop on the level of photosynthetic pigments in the leaves of sweet maize seedlings

Irrespective of the PROP dose applied to the root zone, it increased the level of chlorophylls and total carotenoids in the leaves of sweet maize seedlings compared to the control (Table 1). However, only in the case of chlorophyll *a* at the lowest (0.56 μM) and the highest (56.3 μM) concentration of PROP was there a significant increase in the content of this pigment. The lowest dose of PROP applied on shoots did not affect the content of chlorophyll *a* and *b*, and total carotenoids. However, its higher concentration (5.63 and 56.3 μM) greatly reduced the levels of chlorophylls and carotenoids. In earlier study, clethodim applied on leaves significantly reduced the content of both chlorophylls, and the reduction was found to be dose-dependent (Radwan and Soltan, 2012). These authors noted that higher doses of clethodim led to a yellow appearance of the leaves. The ACCase enzyme is in part responsible for synthesizing precursors of carotenoids and the phytol group for chlorophylls and thus inhibition of the enzyme by an AOPP like PROP causes a decrease in pigments content (Bartley and Scolnik, 1995). In a previous study, an increase of chlorophyll and carotenoid content was noticed in maize leaves under the lowest dose of fluzifop-P-butyl (0.65 μM) applied on leaves, while a decrease of pigment content under the higher concentrations (6.5 and 65 μM) was observed (Horbowicz *et al.*, 2013).

The decline of photosynthetic pigments caused by ACCase inhibitors can be explained by overproduction of reactive oxygen species within plant cells, which can degrade the chlorophylls (Banaś *et al.*, 1993). In rape leaves subjected to sethoxydim herbicide (ACCase inhibitor), a reduction in growth rate and chlorophylls has been observed (Belkebir and Benhassaine-Kesri, 2013). These mentioned researchers have suggested that injuries visible on peanut leaves subjected to treatment with Fusilade are due to enhanced lipid peroxidation and photosynthetic pigment degradation.

Nowadays, it is commonly believed that the impact of various stress factors on the level of photosynthetic pigments should be assessed by the relative ratios of their content i.e. chlorophyll *a*/chlorophyll *b*, and chlorophyll *a*/total carotenoids

(Larcher, 2003; Lichtenthaler and Babani, 2004). Therefore, in addition to absolute pigments content their relationship was calculated (Table 1). The results obtained showed no effect on the photosynthetic dyes in leaves of sweet maize when the herbicide was applied to the roots. It confirms the phenomenon of higher herbicidal efficacy when PROP is applied to shoots rather than to roots.

The absence of an effect on chlorophyll and carotenoids in the case of PROP applied to the root zone is probably due to the low uptake of the herbicide by roots and/or poor translocation from the root to the shoot. Root exposure for one week to the PROP present in the nutrient medium did not significantly affect the photosynthetic pigments in maize leaves.

Effect of propaquizafop on the level of anthocyanins in the epicotyl of sweet maize seedlings

Whether applied to shoots or roots PROP reduced the accumulation of anthocyanins in the epicotyls of maize seedlings (Table 2). Even the lowest concentration of the herbicide (0.56 μM ; $5.6 \cdot 10^{-7}$ M) caused a significant decrease in the anthocyanin content regardless of the method of its application. The results obtained are different from the previously reported effect of fluzifop-P-butyl on the level of anthocyanins in the same cultivar of maize (Horbowicz *et al.*, 2013). In that experiment it was shown that high concentrations of fluzifop-*p*-butyl applied on leaves increased the accumulation of anthocyanin in the epicotyls of maize seedlings. Also, according to Belkebir and Benhassaine-Kesri (2013) graminicide treatment caused an increase in phenylalanine ammonia lyase activity and in the accumulation of anthocyanins. However, these studies did not deal with the effect of PROP on maize seedlings. Oxidative stress stimulates the production of free-radicals and is a signal to a plant for the production of antioxidants, which include flavonoids (Banaś *et al.*, 1993; Shimabukuro and Hoffer, 1996; Shimabukuro *et al.*, 2001). The decreasing anthocyanin content with the use of PROP suggests that not all graminicides similarly affect the metabolism of phenylpropanoids.

Table 1. The influence of the mode of propaquizafop use on photosynthetic pigments in leaves of sweet maize seedlings. Mean results± standard deviation marked by the various letters are significantly different at $P < 0.05$ according to the Newman–Keuls test

Object (dose of herbicide, μM)	Application to roots zone	Application on shoots
Total chlorophylls ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)		
Control (0)	1241±94 a	1378±61 a
Propaquizafop (0.56)	1556±70 a	1324±190 a
Propaquizafop (5.63)	1359±95 a	269±33 b
Propaquizafop (56.3)	1554±70 a	110±33 c
Chlorophyll <i>a</i> ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)		
Control (0)	922±37 b	1023±36 a
Propaquizafop (0.56)	1159±86 a	961±95 a
Propaquizafop (5.63)	1013±41 ab	186±20 b
Propaquizafop (56.3)	1154±69 a	74.0±20.2 c
Chlorophyll <i>b</i> ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)		
Control (0)	320±38 a	355±25 a
Propaquizafop (0.56)	397±35 a	363±85 a
Propaquizafop (5.63)	346±21 a	83.5±9.1 b
Propaquizafop (56.3)	400±34 a	35.6±12.6 b
Total carotenoids ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)		
Control (0)	188.2±16.1 a	182.0±4.5 a
Propaquizafop (0.56)	221.7±11.3 a	169.6±16.6 a
Propaquizafop (5.63)	200.2±11.5 a	59.1±6.1 b
Propaquizafop (56.3)	221.0±13.3 a	45.6±3.0 b
Chlorophyll <i>a/b</i> ratio		
Control (0)	2.96±0.06 a	2.89±0.10 a
Propaquizafop (0.56)	2.92±0.04 a	2.79±0.31 ab
Propaquizafop (5.63)	2.92±0.07 a	2.24±0.12 b
Propaquizafop (56.3)	2.88±0.04 a	2.14±0.27 ab
Chlorophyll <i>a</i> /total carotenoids ratio		
Control (0)	4.90±0.12 a	5.62±0.07 a
Propaquizafop (0.56)	5.23±0.12 a	5.67±0.36 a
Propaquizafop (5.63)	5.05±0.12 a	3.19±0.38 b
Propaquizafop (56.3)	5.22±0.02 a	1.61±0.20 b

Table 2. The influence of the mode of propaquizafop use on the content of anthocyanins in the epicotyl of sweet maize seedlings. Mean results± standard deviation marked by the various letters are significantly different at $P < 0.05$ according to the Newman–Keuls test

Object (dose of herbicide, μM)	Application to roots zone	Application on shoots
Total anthocyanins ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight)		
Control (0)	294.3±15.1 a	491.0±18.0 a
Propaquizafop (0.56)	185.0±7.0 b	218.0±28.2 b
Propaquizafop (5.63)	185.3±2.9 b	149.7±5.8 c
Propaquizafop (56.3)	142.0±6.6 c	204.7±14.5 b

CONCLUSIONS

Propaquizafop has a weak effect on the growth of sweet maize shoots when it is applied to the root zone, while its application to shoots clearly decreased shoot growth. The herbicide when applied to the root zone did not have an impact on either the content of chlorophyll and carotenoids or on the ratios chlorophyll *a*/chlorophyll *b* and chlorophyll *a*/total carotenoids in the leaves of sweet maize seedlings. This is probably caused by poor uptake and/or translocation of the herbicide. The decline of anthocyanin content under the influence of propaquizafop suggests that the formulation has a greater effect on the metabolism of phenylpropanoids than other acetyl coenzyme A carboxylase inhibitors. A low concentration (below 10^{-6} M) of propaquizafop was enough to reduce the anthocyanin content in maize seedlings. Analysis of the anthocyanin content in maize seedlings could be a quick preliminary test for the presence of harmful propaquizafop residues in the soil. However, this hypothesis requires further detailed studies.

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WPŁYW TRAKTOWANIA PROPACHIZAFOPEM NA ZAWARTOŚĆ BARWNIKÓW I WZROST SIEWEK KUKURYDZY CUKROWEJ

Streszczenie

Badano reakcję siewek kukurydzy cukrowej (*Zea mays* var. *Saccharata* L., Złota Karłowa), jako rośliny niebędącej celem zwalczania, na różne dawki propachizafopu zastosowanego do strefy korzeniowej lub na pęd. Herbicyd stosowano w stężeniach 0,56; 5,63 lub 56,3 μM , na siewki rosące hydroponicznie w kontrolowanych warunkach światła i temperatury. W eksperymentach siewki kukurydzy ekspozowano na propaquizafop przez 7 dni. Aby ocenić wpływ propaquizafopu na siewki, mierzono wydłużenie korzenia głównego i pędu, a także zawartości antocyjanów w epikotylach i barwników fotosyntetycznych (chlorofile, karotenoidy) w liściach. Propaquizafop stosowany do strefy korzeniowej (pożywka) wywierał słabszy wpływ na wzrost pędów kukurydzy i niewielki wpływ na poziom chlorofilu i karotenoidów w liściach siewek kukurydzy niż zastosowany dolistnie. Efekty te są prawdopodobnie spowodowane niewielkim poborem przez korzenie i/lub translokacją herbicydu w siewce kukurydzy. Spadek zawartości antocyjanów pod wpływem małych dawek propaquizafopu sugeruje, że ma on większy wpływ na metabolizm fenylopropanoidów niż inne inhibitory karboksylazy acetylokoenzymu A.

Słowa kluczowe: antocyjany, chlorofil, herbicyd, karotenoidy, metoda stosowania