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Effect of chlorophenoxy herbicides residues on probing behavior of cereal aphid

Wpływ pozostałości herbicydów chlorofenoksyoctowych
na żerowanie mszyc zbożowych

Summary. Chlorophenoxyacetic herbicides such as 4-chloro-2-methylphenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D) are used for cereal protection. However, some of them penetrate the tissues of protected plants and also get in contact with cereal pathogens. The up-to-date studies concerned their effectiveness in combating the weeds without paying attention to the herbivores occurring in cereal agrocenoses. Therefore, the aim of the present study was to determine the effect of chlorophenoxyacetic herbicides on the probing behavior of grain aphid, *Sitobion avenae* (F.), bird cherry-oat aphid, *Rhopalosiphum padi* (L.) and rose-grain aphid, *Metopolophium dirhodum* (Walk.). The experiment was carried out on wingless females feeding on winter wheat seedlings of Tonacja cv. using the electronic penetration graph (EPG) method. Tested herbicide preparations exerted an influence on the probing behavior of all aphids examined. The inhibitory effect of MCPA and 2,4-D on the uptake of phloem juice was found. It has been shown that chlorophenoxyacetic herbicides are not only effective in combating the weeds of cereal crops, but can also have positive side-effects in the form of limiting the cereal aphids population.

Key words: 2,4-D, MCPA, EPG, winter wheat

INTRODUCTION

Herbicides effectiveness depends on their dose, type of active substance, weather and soil conditions as well as weed infestation [Pawłowska et al. 1995]. A great interest in this group of compounds results from low costs of their use, low toxicity to mammals and a short period of retention in the soil [Zimdahl 1999, Krzyżanowski and Leszczyński 2007, Jakubiak 2017]. In Siedlce, chlorophenoxyacetic acids such as 4-chloro-2-

-methylphenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D) belong to the most commonly used plant protection agents [Krzyżanowski and Leszczyński 2006] in the form of sodium, potassium, dimethylamine salts and esters [Praczyk and Skrzypczak 2004, Tomlin 2009]. Numerous authors, like Pernak et al. [2011], Polit et al. [2014] or Jakubiak [2017] indicate a new form of herbicides – their modification to ionic liquids.

The aphid probing leads to a reduction in the plant assimilation rate, changes in respiration and transpiration. Numerous viruses that cause viral plant diseases have also been found in the saliva of aphids. The female founders of aphid families uptake the largest amounts of food [Smolarz 1969]. The winged maid of the mealy plum aphid takes an hourly quantity of juice amounting to 80% of her body's weight, while 6–19 times more within a day, which is equal to the products of a day-long photosynthesis of 0.3–7.5 cm^2 of a leaf area [Cichocka et al. 1998].

An electrical penetration graphs (EPG) record of aphid feeding developed by McLean and Kinsey [1964], and modified by Tjallingi [1985], provides information on the penetration of aphids into the plant tissues in the form of generated penetration charts. At the moment when the aphid rostrum touches the plant skin – salivary glands secret the saliva and mouth apparatus of an insect can penetrate deep into the plant tissue [Tjallingii 1988, Goszczyński and Cichocka 1990, Krzyżanowski 2017]. Saliva is released throughout the period of rostrum penetration, forming a salivary sheath in their path. Substances contained in the aphid saliva can block the transmission of juice through the sieves, and water through the vessels, causing an excessive growth and division of cells, enlargement of nuclei, increase in the content of nucleic acids, and degradation of chloroplasts [Cichocka et al. 1998]. Application of the EPG method allowed to observe the behavior of pests before and during penetration, to follow the time of feeding on the plant, the period after which they reach the phloem and the effect of treatments applied [Harrewijn 1990].

The aim of the study was to determine the effect of chlorophenoxyacetic herbicides, such as MCPA and 2,4-D, on the behavior of cereal aphids *Rhopalosiphum padi* (L.), *Metoplophium dirhodum* (Walker) and *Sitobion avenae* (F.) during feeding.

MATERIAL AND METHODS

Laboratory experiment. Five-day-old winter wheat seedlings of Tonacja cv. were used in the experiment. Kernels were sown into plastic pots, which were filled with a universal garden substrate; the pots were not additionally fertilized. Plant growth took place at 22–25°C with regular irrigation and photoperiod 16 hours a day / 8 hours a night.

Tests of Chwastox D 179 SL (MCPA) and Aminopielik D 450 SL (2,4-D) effects on the cereal aphids were carried out under conditions of the pot experiment. For each preparation, tests were performed applying the dose recommended by the producer to control weeds in cereal crops. Chlorophenoxyacetic herbicides were sprayed 2 hours before the aphids were transferred.

Preparation of insects for probing recording. From the parent colony of aphids (carried out on winter wheat of Tonacja cv. by Department of Biochemistry and Molecular Biology Siedlce University of Natural Sciences and Humanities), adult, wingless females were chosen and placed on the vacuum adapter under the stereoscopic microscope so that they were facing their dorsal side towards the lens. Then, using a preparative needle, a drop of silver paint (Demetron) was applied to the dorsal side of the insect and an EPG electrode (gold wire – Ø 20 µm and 2 cm long) was immersed in it. After the paint had dried, the vacuum in the adapter circuit was reduced and insects were transferred onto the test plants.

Characteristics of the EPG method and recording. The DC EPG system was used in the experiment. EPG recording was carried out using the STYLET computer program. In order to record, after fastening, the electrodes were placed in the probe sockets and applied to the tested plants. After combining the insect with the plant, a second electrode was placed in the pot to close the electrical circuit. Each channel had properly adjusted voltage in order to record correctly. Behavior of wingless females of *R. padi*, *M. dirhodum* and *S. avenae* during feeding on control and herbicide-treated plants was tested during 4-hour recording for 10 different aphids of each species, probing on 10 different plants. The resulting records processing was carried out using the PROBE 3.4 software.

In the analysis of EPG recording, following stages corresponding to the penetration of plant tissues and the lack of penetration were distinguished: np – the aphids stylets were outside the plant tissues, a moment when the insect rostrum has no contact with the plant; C – the stylets penetrated the epidermis and mesophyll; E1 – the aphid salivation into phloem sieve elements; E2 – the stylet passively ingesting phloem sap; F – mechanical movements of rostrum; and G – the aphid actively ingesting xylem sap.

Statistical analysis. The aphid probing results were analyzed by means of variance analysis. Significance of statistical differences between mean values was assessed applying Duncan's multiple range test at $p \leq 0.01$.

RESULTS

The comparative studies of models based on data presented by Tjallingi [1988] showed that on the control seedlings of wheat, the duration of pathway (C) took the most time in aphid activity, as much as 34% for *M. dirhodum* and the least 17% for *R. padi*, of the entire four-hour experiment (Fig. 1). The xylem phase was recorded for all species of aphids feeding on control seedlings of Tonacja cv. without added herbicides. Aphid salivation into phloem sieve elements and phloem sap ingestion were 40% for *R. pani*, while for *S. avenae* and *M. dirhodum* 29% and 18%, respectively, of the entire time (Fig. 1). The remaining time of recording was occupied by the non-penetration phase, i.e. up to 49% for *M. dirhodum*, 43% for *S. avenae* and the shortest time recorded for *R. padi*.

After the application of chlorophenoxyacetic herbicides, it was found that the shortest period of non-penetration was demonstrated by grain aphid. Moreover, the aphid punctured the substrate more frequently (Tables 1–3). Among other species, bird cherry-oat aphid showed a fivefold extension of the non-penetration period, while rose-grain aphid up to tenfold, relative to the grain aphid (Tables 1–3). Penetration of the epidermis and mesophyll (model C) was longer in the case of aphids feeding on plants with the applied preparation Chwastox D 179 SL. At the presence of the preparation, the contact of rostrum with epidermis and mesophyll cells for *S. avenae* aphid was as long as 109 minutes (Table 3), and for *R. padi* about 81 minutes (Table 1). Only in the case of rose--grain aphid, the contact of rostrum with epidermis and mesophyll cells increased by two times after the application of Aminopielik D 450 SL, in relation to feeding on plants treated with Chwastox D 179 SL (Table 2).

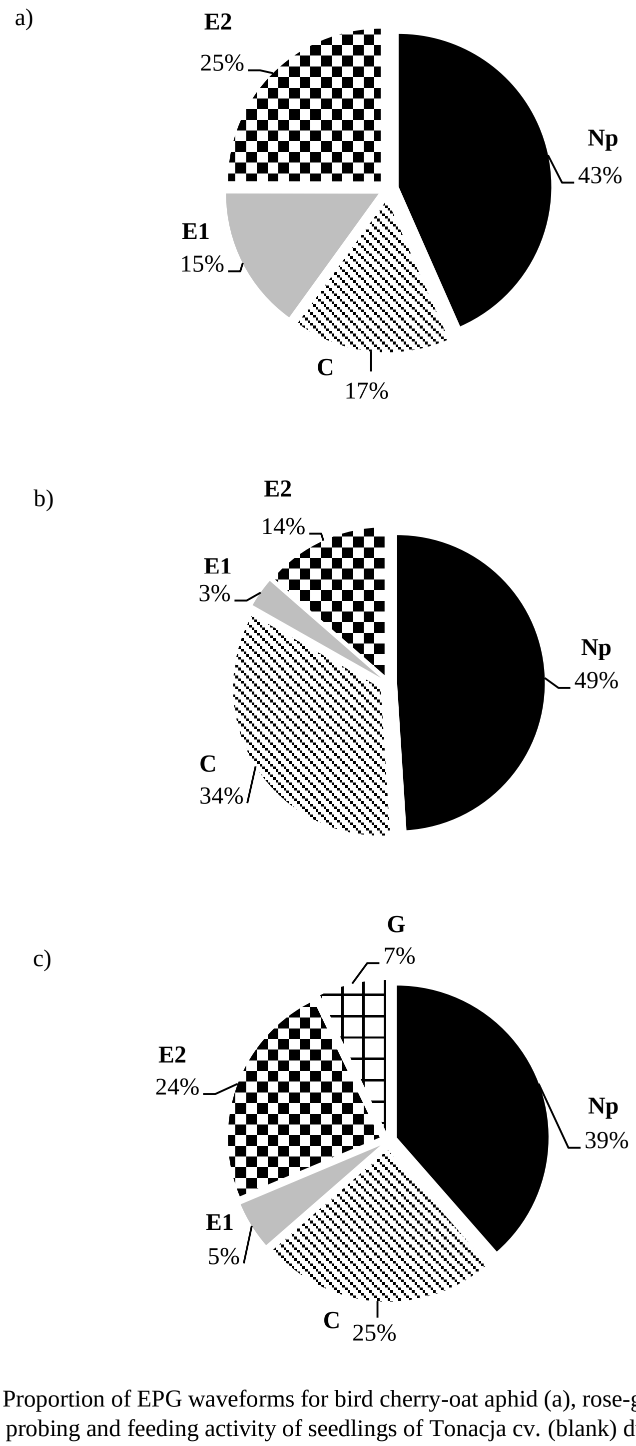

Fig. 1. Proportion of EPG waveforms for bird cherry-oat aphid (a), rose-grain aphid (b), cereal aphid (c) probing and feeding activity of seedlings of Tonacja cv. (blank) during 4-hours recording

Table 1. Parameters of EPG analysis of bird cherry-oat aphid (*Rhopalosiphum padi*) probing behaviour on winter wheat seeds (blank) and after chlorophenoxy herbicides treatment: Chwastox D 179 SL (MCPA) and Aminopielik D 450 SL (2,4-D)

EPG parameters		Control	MCPA	2,4-D
Total duration of probing (C + E1 + E2 + G)	Min	211.16 a ±25.40	81.63 b ±79.80	17.31 c ± 1.63
Total pathway duration (Np + C)	Min	149.36 a ±22.40	239.91 a ±39.90	150.85 a ±47.58
Total duration of phloem phase (E1 + E2)	Min	166.00 a ±13.75	0.00 b ±0.00	0.00 b ±0.00
Total number of probes	#	9.36 a ±1.15	7.30 a ±1.60	4.30 b ±1.10
Total duration of no probing (Np)	Min	104.13 a ±21.10	158.35 a ±79.60	133.53 a ±83.53
Mean duration of no probing phase (Np)	Min	34.71 a ±8.43	39.58 a ±8.30	58.06 a ±9.55
Total duration of pathway (C)	Min	45.23 a ±23.56	81.63 a ±29.80	17.31 b ±11.63
Number of duration of pathway (C)	#	3.67 a ±2.08	3.33 a ±1.53	2.00 a ±1.00
Time of the first pathway phase (C)	Min	29.68 a ±1.45	34.08 a ±44.06	13.26 b ±14.73

EPG; recording time 4 hours, n = 10 − number of replicates, means ±SD, Duncan test – letters at means indicate significant differences for $p < 0.01$

Table 2. Parameters of EPG analysis of rose-grain aphid (*Metopolophium dirhodum*) probing behaviour on winter wheat seeds (blank) and after chlorophenoxy herbicides treatment: Chwastox D 179 SL (MCPA) and Aminopielik D 450 SL (2,4-D)

EPG parameters		Control	MCPA	2,4-D
Total duration of probing (C + E1 + E2 + G)	Min	82.13 a ±4.22	23.78 b ±3.50	35.77 b ±3.10
Total pathway duration (Np + C)	Min	198.80 a ±4.60	238.70 a ±17.50	224.10 a ±34.70
Total duration of phloem phase (E1 + E2)	Min	35.03 a ±0.50	0.00 b ±0.00	0.00 b ±0.00
Total number of probes	#	39.00 a ±4.00	3.30 b ±1.50	3.30 b ±1.20
Total duration of no probing (Np)	Min	117.57 a ±5.11	214.92 a ±34.20	188.34 a ±33.85
Mean duration of no probing phase (Np)	Min	5.89 b ±0.12	107.4 a ±0.30	94.17 a ±0.20
Total duration of pathway (C)	Min	82.13 a ±4.25	23.78 b ±3.50	35.77 b ±3.10
Number of duration of pathway (C)	#	16.33 a ±1.81	1.33 b ±0.63	1.33 b ±0.60
Time of the first pathway phase (C)	Min	44.78 a ±7.20	23.78 a ±3.50	30.89 a ±2.91

EPG; recording time 4 hours, n = 10 − number of replicates, means ±SD, Duncan test – letters at means indicate significant differences for $p < 0.01$

Studied cereal aphids exposed to chlorophenoxyacetic herbicides did not contact with the phloem elements and did not obtain the E1 and E2 phases indicative of salivary secretion and phloem sap ingestion, as opposed to control seedlings (Fig. 1).

DISCUSSION

Chlorophenoxyacetic herbicides showed negative effect on the probing of tested aphid species *R. padi*, *M. dirhodum* and *S. avenae*. Aphids did not feed on plants treated with herbicides containing the active substances MCPA and 2,4-D. Change in the behavior of cereal aphids was probably related to the change in nutritional properties of plants treated with those herbicides. Sempruch et al. [2010] found that the population of grain aphid *S. avenae* increased only at the beginning of the growing season as a result of wheat spraying with Chwastox D 179 SL on control plots. In addition, they showed a decrease in total and soluble nitrogen content as well as an increase in the protein nitrogen concentration in tissues of winter triticale of Bogo cv. Only at the stage of wax maturity, the concentration of all analyzed nitrogen forms in plants treated with herbicide did not differ from the control. Similarly, Ostapczuk et al. [1997] found that MCPA causes some decrease in the content of protein amino acids in winter wheat of Arda and Juma cv. Research by Ciepiela and Sempruch [2001], after application of Chwastox D 179 SL in the cultivation of triticale of Bogo and Moniko cv., revealed a reduction in *S. avenae* population.

Chlorophenoxyacetic herbicides exert an unfavorable effect on all cereal agrocenosis, also on the host plant. Jakubiak [2017] reported that the standard preparations caused transient phytotoxic symptoms on tissues of winter wheat at the level of 2–3%, while the use of ionic liquids caused similar effects within the range of 9–14%. The author observed a significant decrease in the yield of winter wheat of Ludwig cv. compared to the control. Grygiel et al. [2012] note that the level of herbicide residues in crops depends on the ability of a given species and variety to collect and metabolize the active substance. The atmospheric conditions and the applied dose play an important role, because the higher the herbicide dose, usually the higher the residue level and after exceeding the limit dose, i.e. concentration of the preparation that can be metabolized by the plant, a very drastic increase in residues can be observed, thus various changes within a plant that feeds herbivores.

Plants providing a food for insects affect both their behavior and biology. Poor plant settlement, slower development and lower fertility may be the result of incomplete satisfaction of nutritional needs of herbivorous insects or adverse effects of nutrients on their biology [Goławska 2005]. Ciszewska [1977] and Zwolińska-Śniatałowa [1980] showed in most plants treated with systemic herbicides an increased content of total nitrogen, an increase in protein content and tendency to accumulation of amino acids, with large changes in the proportion of their exogenous and endogenous groups. Furthermore, plants treated with herbicides contained more iron, magnesium, potassium, phosphorus and zinc, and decreased levels of calcium and molybdenum. Research carried out by Krzyżanowski et al. [2007] on the effect of chlorophenoxyacetic herbicides on *S. avenae* population showed their adverse effect. Aminopielik D 450 SL containing 450 g l^{-1} of active substance 2,4-D caused 50% mortality of grain aphid population already at 40%

of the dose recommended for weed application in cereal agrocenoses. On the other hand, Chwastox D 179 SL caused 50% mortality of *S. avenae* at much higher concentrations constituting about 80–90% of the dose recommended by the manufacturer.

CONCLUSIONS

1. After application of chlorophenoxyacetic herbicides, the shortest period of non-penetration was demonstrated by grain aphid, while the longest – rose-grain aphid.

2. Epidermis and mesophyll penetration was longer in the case of aphids on plants with Chwastox D 179 SL applied. Only rose-grain aphid showed twice as long contact of rostrum with epidermis and mesophyll cells after application of Aminopielik D 450 SL.

3. Cereal aphids exposed to chlorophenoxyacetic herbicides did not contact with the phloem elements and did not obtain the E1 and E2 phases.

Summing up, it should be noted that the use of herbicides to control weeds may contribute to the reduction of aphid feeding in cereal crops, which is important for agricultural practice, especially in the context of Integrated Plants Protection.

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Streszczenie. Herbicydy chlorofenoksyoctowe, takie jak kwas 4-chloro-2-metylofenoksyoctowy (MCPA) i kwas 2,4-dichlorofenoksyoctowy (2,4-D) są wykorzystywane w ochronie zbóż. Jednak część z nich wnika do tkanek roślin chronionych, a także ma kontakt z patogenami roślin zbożowych. Prowadzone dotychczas badania dotyczyły ich skuteczności w zwalczaniu chwastów, bez zwracania uwagi na fitofagi występujące w agrocenozach zbożowych. Dlatego celem niniejszej pracy było określenie wpływu herbicydów chlorofenoksyoctowych na zachowanie podczas żerowania mszycy zbożowej, *Sitobion avenae* (F.), mszycy czeremchowo-zbożowej, *Rhopalosiphum padi* (L.) i mszycy różano-trawowej, *Metopolophium dirhodum* (Walk.). Eksperyment przeprowadzono na samicach bezskrzydłych żerujących na siewkach pszenicy ozimej odmiany Tonacja metodą elektronicznych zapisów żerowania (electrical penetration graph – EPG). Badane preparaty herbicydowe wywierały wpływ na zachowanie podczas żerowania wszystkich badanych mszyc. Modyfikowały znacząco czas trwania poszczególnych modeli EPG, całkowitą długość żerowania oraz częstotliwość występowania poszczególnych modeli. Stwierdzono hamujący wpływ MCPA i 2,4-D na pobieranie soku floemowego. Wykazano, że herbicydy chlorofenoksyoctowe nie tylko są skuteczne w zwalczaniu chwastów w uprawach zbożowych, lecz także mogą wywierać pozytywne efekty uboczne w postaci ograniczania liczebności populacji mszyc zbożowych.

Słowa kluczowe: 2,4-D, MCPA, EPG, pszenica ozima

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