



RESPONSE OF GAS EXCHANGE TO LEAF PIERCING EXPLAINED BY PIECEWISE LINEAR REGRESSION FOR TWO DEVELOPMENTAL FORMS OF RAPE PLANT (*BRASSICA NAPUS L. SSP. OLEIFERA METZG*)

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Oilseed rape (*Brassica napus* L. ssp. *oleifera* Metzg) was the subject of the study in two forms: winter cv. 'Muller' (at the rosette stage – the first internode BBCH 30 – 31) and spring cv. 'Feliks' (at the yellow bud stage BBCH 59). The main gas-exchange parameters, net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured on leaves prior to the piercing and immediately after the short-term piercing. The effect of mechanical wounding revealed different progress of the gas exchange process for the two forms. Piecewise linear regression with the breakpoint estimation showed that the plants at the same age but at a different vegetal stage, manage mechanical leaf-piercing differently. The differences concerned the stomatal conductance and transpiration changes since for rosette leaves the process consisted of five intervals with a uniform direction, while for stem leaves - of five intervals with a fluctuating direction. These parameters got stabilized within a similar time (220 mins) for both forms. The process of net photosynthetic rate was altered by the plant stages. 'Muller' plants at the rosette stage demonstrated dependence of P_N on time in *log*-linear progression: $y (P_N) = 8.01 + 2.73 \log_{10}(x t_2)$; $7 < t_2 < 220$; $R^2 = 0.96$. For stem leaves of 'Feliks' plants the process of transpiration, in terms of directions, was convergent with the process of photosynthesis. Those two processes were synchronized from 1st to 114th min of the test ($r = 0.85$; $p < 0.001$) in plants at the rosette stage and from 26th to 148th min in stem leaves ($r = 0.95$; $p < 0.001$).

Keywords: leaf piercing response, oilseed rape, net photosynthetic rate, transpiration, stomatal conductance, concentration of intercellular CO_2

Abbreviations:

C_i – intercellular CO_2 concentration
CCI – chlorophyll content index
 E – transpiration rate
 g_s – stomatal conductance
LA – leaf area
 P_N – net photosynthetic rate

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INTRODUCTION

Biomass production mostly depends on the level of gas exchange between the plant and the environment. Stressful environments, including drought, salinity, and unfavorable temperatures, considerably hamper the process of photosynthesis in most plants by altering the ultrastructure of the organelles and concentration of various pigments and metabolites including enzymes involved in this process as well as stomatal regulation (Ashraf and Harris, 2013). Plant respiration can be disturbed by biotic and abiotic stressors (Roitsch, 1999). The appearance of the stress factor caused by, e.g., hailstorm (Muro et al., 1998; Tartachnyk and Blanke, 2002) or an invasion of plant-eating pests (Hawkins et al., 1987; Holman and Oosterhuis, 1999; Gomez et al., 2004) results in stomata closing, decreasing the intensity of photosynthesis, sometimes also increasing evaporation from leaves. Changes in the intensity of gas exchange occur immediately after the induction of stress reaction. The response to stress is detectable by the net photosynthetic rate as well as transpiration and mostly depends on the plant species and the size of the leaves left on the plant after defoliation as well as the presence or lack of the apical bud (Evans, 1991; Wang et al., 1997; Roitsch, 1999). Despite many various types of damage triggered by biotic factors (insects, viruses, fungi), for various host plants the mechanism of regulation is the same and it involves a decrease in the transcription of nuclear genes encoding the components of photosynthesis. Biotic leaf wounding causes almost complete inhibition of genes taking part in the process of photosynthesis. It is seen mostly for the genes connected with the synthesis of pigment and transport of electrons (Bilgin et al., 2010). After infection caused by pathogens or plant-eating insects the effort of the plant to decrease the amount of photosynthetic protein is necessary to support the defense induction. The leaf nitrogen is involved in; much of it is found in photosynthetic proteins, primarily in RuBisCO, and if limited, it can even lead to its use with RuBisCO (Paul and Foyer, 2001). The infestation of scale insects decreases the chlorophyll and carotenoid content as well as the value of three indicators of photosynthetic activity. The reactions depend on the specific properties of plants and abundance of insects feeding on them (Golan et al., 2015). The research into rape (*Brassica napus* var. *oleifera*) performed so far demonstrated that beetles feeding on pollen (*Meligethes aeneus*) decrease the activity of the photosynthetic apparatus or increase transpiration, through a clear decrease in photosynthesis in unprotected plants, high stomatal conductance and poor fixation of CO₂. It is also known, as for that species, that the wounding compensation methods

are high effective (Axelsen and Nielsen, 1990) and mostly result from its high genetic potential which facilitates the formation of a huge number of flower buds, namely about 4-5 thousand per plant. The rape plants artificially deprived of inflorescence on the main shoot demonstrate a photosynthesis compensation capacity, optimizing the parameters of gas exchange; they decrease stomatal conductance.

The effect of mechanical stress on leaves is ambiguous for photosynthesis when the following conditions are considered: the plant species and age, as well as the stress type and its duration (Biddington, 1984). It was confirmed by Blamowski et al. (2003) for two species representing the genus *Brassica*: radish and spring rape at the rosette stage, exposed to the same wounding, i.e., the oldest leaves removal or defoliation of the youngest ones with the apical stem. Stress affects the course of gas exchange, distribution of assimilates and plant growth. Moreover, each interference in the relationship and cooperation of the organs providing the source of recipients of organic compounds also disturbs the production and activity of growth regulators. The compounds can regulate the distribution of assimilates, growth and gas exchange by affecting the biosynthesis and activity of enzymes or the absorption of gases (Starck and Ubysz, 1976; Pinto, 1980).

The aim of the present research was to determine the effect of mechanical wounding caused by short-term piercing of leaves in rape in its two developmental forms: the winter form at the rosette stage – the first internode (BBCH 30–31) and spring rape plant at the yellow bud stage (BBCH 59), on the progress of variation in the processes of assimilation and transpiration as well as stomatal conductance and the content of intercellular CO₂. We hypothesized that the leaves of the two cultivars (spring / winter form) belonged to *B. napus* sp., and because they came from plants at the same age but at different developmental stages (generative / vegetative), they represented various course of gas exchanges after a short leaf piercing.

MATERIAL AND METHODS

PLANTS AND TREATMENT

Oilseed rape (*Brassica napus* L. ssp. *oleifera* Metzg) was the subject of the study in two forms: winter cv. 'Muller' and spring cv. 'Felix'. Seeds obtained from a breeding company were germinated and grown in peat-filled pots (15 cm × 15 cm × 20 cm) in a greenhouse at 9/3°C (day/night environment) during the spring of 2014. Watering was applied to maintain the moisture at 65% relative water content. After 21 days, these plants at 4–5 leaf stage (BBCH 14–15) were transferred

to a chamber at 20/11°C (day/night) for 10 days, received 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light for 14 h per day, the relative humidity was 50%. Fertigation was applied according to the scheme with macronutrients (g dm^{-1} of nutrient solution): 2.0 $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (calcium nitrate tetrahydrate), 1.5 KNO_3 (potassium nitrate), 0.75 $(\text{NH}_4)_2\text{SO}_4$ (ammonium sulfate), 0.55 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (magnesium sulfate heptahydrate), 0.35 KH_2PO_4 (monopotassium phosphate) and for micronutrients (g dm^{-1} of nutrient solution): 0.33 Cu IDHA, 2.0 Mn IDHA, 0.57 Zn IDHA, 0.28 H_3BO_3 , EC = 2.6 and pH = 5.6. Application of nutrients was done twice a day with two emitters per pot dropping 100 ml for 60 s (Ferdiga system). Rape plants in 60 pots (30 per each cultivar) were cultivated until they reached BBCH 30–31 (rosette – the first internode) – *cv.* ‘Muller’, and BBCH 59 (the first petals ‘yellow bud’) – *cv.* ‘Feliks’ plants. All plants were the same age, however, their varied development was due to the fact that *cv.* ‘Muller’, as a winter form, remained at the rosette (vegetative) stage, while *cv.* ‘Feliks’, as a spring form, started the generative stage. The treated, fully expanded third leaves were chosen with the uniform area and CCI (measured by the chlorophyll meter CCM-200 plus, Opti-Sciences, Inc., USA) – Table 1. Wounding by piercing of the total leaf area with the pins having a diameter of 1 mm and a density of 10 punches per 1 cm^2 lasted 3 seconds.

MEASUREMENTS

The main gas-exchange parameters, net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured prior to the piercing and immediately after the piercing at the center of the wounded area, the leaves were fitted into a 6.25 cm^2 clamp-on Plant Leaf Chamber (PLC Broad with mixed Red/Blue LED array). Gas exchange measurements lasted 220 mins. This was performed using a portable open infrared CO_2 gas analyzer (LC-Pro+, ADC BioScientific Ltd, Hoddesdon, UK) between 10:00–14:00 h. The system allowed for an automated microclimate control in the PLC. The conditions were stable in PLC and amounted to light 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CO_2 concentration 360 ± 5 ppm; temperature $22 \pm 1^\circ\text{C}$,

water vapor pressure 10 ± 1 mbar (relative humidity approx. 40%). The rate of air flow through the LCpro+ chamber was approximately 200 $\text{ml} \cdot \text{min}^{-1}$. These conditions provided the strongest response of plants to leaf piercing. In the growth chamber where the gas-exchange parameters were measured oxygen concentration was ambient (21%).

DATA ANALYSIS

The analysis of piecewise linear regression with the breakpoint estimation was calculated to explain the time-relation changes of the parameters g_s , C_i , P_N , and E . The time intervals were estimated by non-linear methods according to Quasi-Newton and the lost function based on the least squares were proceeded (Haelterman et al., 2009). The relationships between the parameters were computed using the simple coefficient of correlation (r by Pearson). The results were processed using STATISTICA data analysis software system version 12.0 (StatSoft; Tulsa, Oklahoma, USA).

RESULTS

The characteristics of leaves are presented in Table 1. Both cultivars represented uniform LA ($F_{(1;58)} = 2.59$, $p = 0.12$), and the CCI was also statistically insignificant ($F_{(1;58)} = 3.44$, $p = 0.07$). The response to leaf piercing with pins was noticeable in both cultivars, however, its pattern varied in terms of the intensity of gas exchange parameters in time and in relationships between them.

STOMATAL CONDUCTANCE AND INTERCELLULAR CARBON DIOXIDE CONCENTRATION AS RESPONSE TO LEAF PIERCING

Stomatal conductance before piercing amounted to 0.280 $\text{mol m}^{-2}\text{s}^{-1}$ in ‘Muller’ winter rape plans and 0.390 $\text{mol m}^{-2}\text{s}^{-1}$ in ‘Felix’ spring rape (Table 2). Upon leaf piercing, g_{s1} decreased in ‘Muller’ by 0.01 $\text{mol m}^{-2}\text{s}^{-1}$ within 1 min, i.e., by 26% over 5.7 mins and in ‘Feliks’ it decreased by 0.006 $\text{mol m}^{-2}\text{s}^{-1}$, i.e., by 7% over 5.9 mins (Table 2). After the first interval g_{s2} increased

TABLE 1. The characteristics of leaf of rape cultivars.

Characteristic	Cultivar/stage		$F_{(1;58)}$	p
	‘Muller’ BBCH 30–31	‘Feliks’ BBCH 59		
LA ($c = 0.8$)	49.6 ± 2.47	44.2 ± 2.30	2.59	0.12
CCI	21.13 ± 1.36	17.65 ± 1.29	3.44	0.07

TABLE 2. Progress of stomatal conductance [g_s] changes after leaf piercing of two rape cultivars according to the piecewise linear regressions of the time and with confident limits (CL).

Break point of time [min]	95 % CL of time	g_s [mol m ⁻² s ⁻¹]	95 % CL of g_s	Relative stepwise change [%]
cv 'Muller' BBCH 30-31				
Starting = 0.0	–	0.280	–	100
5.7	5.5–5.9	0.207	0.190–0.220	- 26
27.4	25.3–29.5	0.242	0.238–0.246	+ 17
58.5	54.7–62.4	0.246	0.243–0.250	+ 1.7
116.0	110–122	0.294	0.289–0.300	+ 20
Ending = 220	–	0.354	–	+ 20
cv 'Feliks' BBCH 59				
Starting = 0.0	–	0.390	–	100
5.9	5.0–6.8	0.361	0.359–0.363	- 7
23.6	20.9–26.5	0.384	0.376–0.391	+ 6
63.4	60.4–66.4	0.265	0.261–0.269	- 29
155.0	149–161	0.408	0.407–0.409	+ 48
Ending = 220	–	0.421	–	+ 4

by 0.002 mol m⁻²s⁻¹ per 1 min (17%) over 6 – 27.4 mins in 'Muller' and by 0.0013 mol m⁻²s⁻¹ (6%) over 6 – 23.6 mins in 'Feliks' (Figs. 1a, 1b). Over the next interval there was seen a diametrically opposite reaction of g_s between winter and spring plants; 'Muller' was going through a 30 mins period of uniform level g_{s3} of 0.246 mol m⁻²s⁻¹, whereas in 'Feliks' g_{s3} was decreasing for 40 mins, every min-

ute by 0.0028 mol m⁻²s⁻¹, i.e., by 29%, to the level of 0.265 mol m⁻²s⁻¹ (Table 2). Only about an hour after piercing, the plants of both rape plant forms started the period of g_{s4} stabilization (Figs. 1a, 1b). In the 'Muller' plants, between 59th and 116th min, g_{s4} increased by 0.001 mol m⁻²s⁻¹, i.e., by 20%, and during successive 100 mins (g_{s5}) also by 20%, at the rate of 0.0004 mol m⁻²s⁻¹. In the 'Feliks' plants,

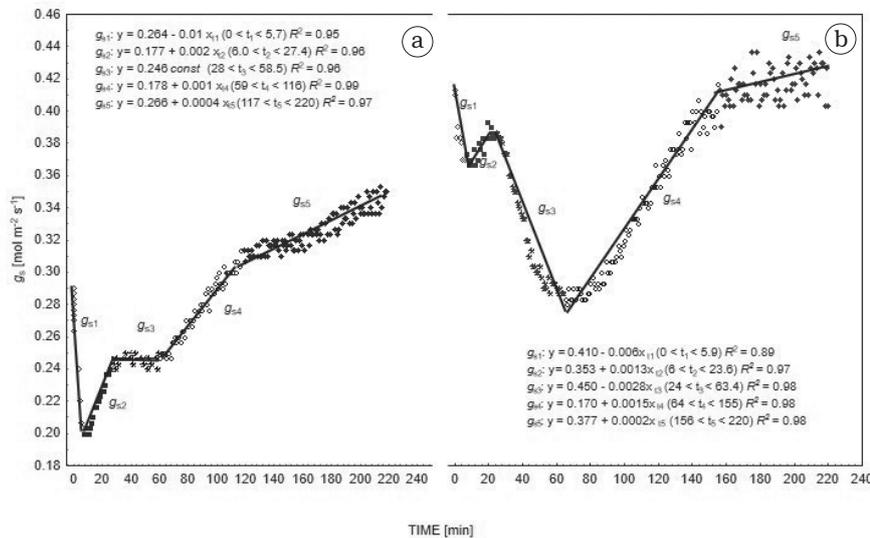


Fig. 1. Piecewise linear regressions for stomatal conductance after leaf piercing of rape plant. The confident limits (95% CL) of the linear parameters, the duration of each interval (t) and coefficients of determination (R^2); **(a)** cv. 'Muller', **(b)** cv. 'Felix'.

between 63rd and 159th min, g_{s4} increased by 48% at the rate of 0.0015 mol m⁻²s⁻¹ per 1 min, and for successive 65 mins (g_{s5}) – by 4% at the rate of 0.0002 mol m⁻²s⁻¹ per 1 min (Table 2, Figs. 1a, 1b).

Prior to the piercing, the concentrations of intercellular CO₂ (C_i) were 211 and 214 μmol mol⁻¹ for ‘Muller’ and ‘Feliks’, respectively (Table 4). Two minutes after piercing C_{i1} increased by 22 μmol mol⁻¹ (11%) in ‘Feliks’ and by 47 μmol mol⁻¹ (22%) in ‘Muller’ over 3 mins (Table 4). An hour after the piercing, C_i displayed a similar tendency to increase together with the g_s increase in both cultivars. After the first interval of growth there followed two periods of decrease in C_{i2} and C_{i3} : C_{i2} at a higher rate (-1.77 and -1.40 μmol mol⁻¹ for ‘Muller’ and ‘Feliks’, respectively) and C_{i3} – at a slower rate (-0.42 and -0.18 μmol mol⁻¹ for ‘Muller’ and ‘Feliks’, respectively). Such response lasted up to 46–64 mins after piercing, which was followed by the stage of C_{i4} growth in both cultivars at the same rate (by 0.11 μmol mol⁻¹ every minute) taking from 46th to 114th min in ‘Muller’ and from 63.5th to 146th min in ‘Feliks’, and then the C_{i5} period of stabilization (by 0.02 μmol mol⁻¹ in ‘Feliks’ and 0.05 μmol mol⁻¹ in ‘Muller’, every minute) – from 146th to 220th min and from 114th to 220th min of the test, respectively (Table 4, Figs. 2a, 2b).

Changes in g_s in 5 time sequences can be interpreted by the relationships between C_i and transpiration (E), and net photosynthetic rate (P_N), calculated as r -Pearson correlation coefficient and b coefficient of regression (Table 3). Provided that the power and direction of the correlations between g_s vs. E and

P_N were similar for both rape cultivars after piercing stress in 5 time intervals and throughout the test, the relationship between g_s and C_i in the case of ‘Muller’ was different from that in ‘Feliks’. In ‘Feliks’ it was a strong positive correlation between C_i with g_s ($r = 0.86$), while in ‘Muller’ – negative correlation with $r = -0.81$, which means that the leaves at the rosette stage retained CO₂ during the decrease in $g_{s1,2}$ over 1st–23rd min, while the leaves at the flowering stage were losing intercellular CO₂ with a decrease in $g_{s1,3}$ (from 1st to 6th min and from 24th to 63rd min) (Table 3, Figs. 2a, 2b). That proves the different initial response to the piercing. An hour after piercing the tendency in both cultivars got leveled off and resulted in an increase in C_{i4} by 10.2 (‘Muller’) and 7.78 (‘Feliks’) mol m⁻²s⁻¹ with an increase in g_{s4} by 0.1 mol. After 2.5 h, however, the tendency was maintained only in ‘Feliks’. In that time segment the reactions between g_s vs. P_N and E got much weaker and between 7th and 155th min they showed very strong positive correlations, especially in ‘Feliks’ (Table 3).

TRANSPIRATION AND NET PHOTOSYNTHETIC RATE RESPONSE TO LEAF PIERCING

At the first stage after piercing (up to 7th min) the leaves in ‘Muller’ reacted with a strong decrease in P_{N1} (-35%) and E_1 (-22%) – (Tables 5, 6), which was, at the same time, correlated with an increase in C_{i1} (Table 7). Then P_{N2} was increasing in that cultivar in \log -linear progression as a time function, giving two segments of the rate; up to 47th min by 0.055 μmol m⁻² s⁻¹ and from 48th to 220th

TABLE 3. Pearson’s coefficients of correlation (r) and slopes (b) between g_s vs. C_i , E and P_N parameters in time intervals for cv. ‘Muller’ and cv. ‘Feliks’ rape plants after leaf piercing

Interval [min]	Coefficient	C_i		E		P_N	
		‘Muller’	‘Feliks’	‘Muller’	‘Feliks’	‘Muller’	‘Feliks’
1-6	r	-0.81*	0.86*	0.99***	0.98***	0.91**	-0.28 ^{ns}
	b	-49.6	2.27	0.90	0.77	0.85	-
7-23	r	-0.90***	-0.21 ^{ns}	0.99***	0.90***	0.97***	0.85***
	b	-20.0	-	0.64	0.44	0.49	0.35
24-63	r	-0.23 ^{ns}	0.90***	0.55***	0.99***	0.48**	0.96***
	b	-	7.57	0.48	0.47	0.56	1.74
64-155	r	0.82***	0.91***	0.96***	0.99***	0.83***	0.96***
	b	10.2	7.78	0.45	0.33	0.17	1.85
156-220	r	0.21 ^{ns}	0.80***	0.24 ^{ns}	0.32**	0.37**	0.10 ^{ns}
	b	-	12.9	-	0.15	0.13	-
1-220	r	0.20*	0.90***	0.95***	0.74***	0.86***	0.95***
	b	2.50	7.87	0.49	0.23	0.26	1.67

r – correlation coefficient by Pearson, b – linear coefficient of regression, *significance at $p = 0.05$, ** $p = 0.01$, *** $p = 0.001$, ns – not significant

TABLE 4. Progress of intercellular CO₂ concentration [C_i] changes after leaf piercing of two rape cultivars according to the piecewise linear regressions of the time and with confident limits (CL).

Break point of time [min]	95% CL of time	C _i [μmol mol ⁻¹]	95% CL of C _i	Relative stepwise change [%]
cv. 'Muller' BBCH 30-31				
Starting = 0.0	–	211	194–229	100
3.0	0–3	258	255–262	+ 22
10.7	9–12	239	237–242	- 7
46.3	44–49	226	224–227	- 5
114	98–131	233	231–234	+ 3
Ending = 220	–	238	–	+ 2
cv. 'Feliks' BBCH 59				
Starting = 0.0	–	214	213–215	100
2.0	0–2	238	236–241	+ 11
8.8	8–9.6	226	225–227	- 5
63.5	58.3–8.6	217	216–218	- 4
146	137–155	226	225–227	+ 4
Ending = 220	–	229	–	+ 1.1

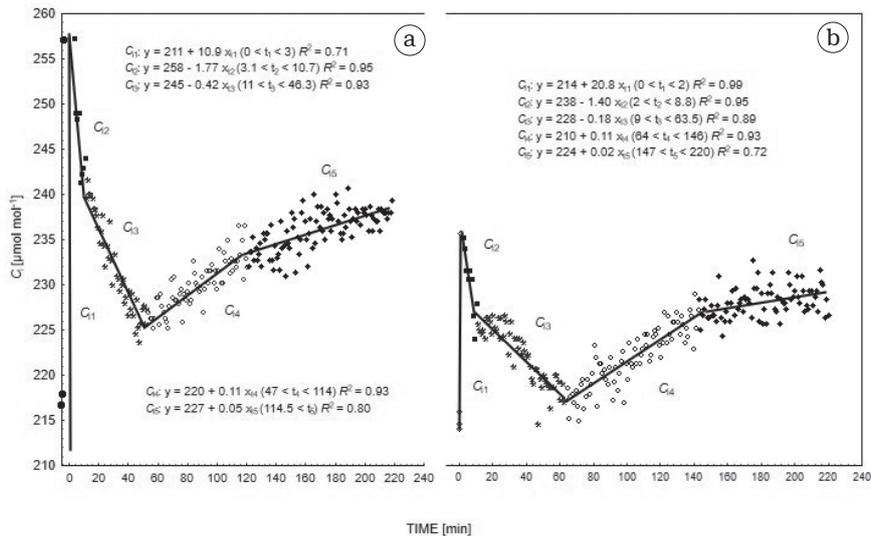


Fig. 2. Piecewise linear regressions for concentration of intercellular CO₂ after leaf piercing of rape plant. The confident limits (95% CL) of the linear parameters, the duration of each interval (t) and coefficients of determination (R²); (a) cv. 'Muller', (b) cv. 'Felix'.

min by 0.006 μmol m⁻² s⁻¹ (Fig. 3a). The increase of P_{N2} was accompanied by an increase in C₁₄ only between 60th and 114th min; however, throughout the test the tendency was slightly negatively correlated (r = -0.25) (Table 7). In 'Muller' there was found a very strong association of the increasing E₁₋₄ to increasing P_{N2} up to 114th min of the test, after which both processes no longer showed a lin-

ear dependence. The very pattern of E₃ in 'Muller' was convergent with the g_{s3} pattern, with the phase of 'dormancy' between 28th and 59th min, and two growth intervals, namely a rapid increase between 7th and 28th min by 0.017 mmol m⁻² s⁻¹ per min and 0.005 mmol m⁻² s⁻¹ slower over 60th–114th min after piercing (Fig. 4a, Table 6). The P_N and E changes in 'Feliks' showed a completely differ-

TABLE 5. Progress of net photosynthetic rate [P_N] changes after leaf piercing of two rape cultivars according to the piecewise linear regressions of the time and with confident limits (CL).

Break point of time [min]	P_N [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	95% CL of P_N	Relative stepwise change [%]
<i>cv. 'Muller' BBCH 30-31</i>			
Starting = 0.0	15.7	-	100
7.0	10.2	9.5-10.9	- 35
47.0	12.7	12.1-13.3	+ 25
Ending = 220	14.4	13.9-14.9	+ 13
<i>cv. 'Feliks' BBCH 59</i>			
Starting = 0.0	19.27	19.2-19.3	100
1.0	16.07	15.9-16.3	- 17
25.5	17.35	17.1-17.6	+ 8
74.0	14.94	14.7-15.2	- 14
148.0	17.83	17.6-18.1	+ 19
Ending = 220	17.88	-	+ 0.3

TABLE 6. Progress of transpiration rate [E] changes after leaf piercing of two rape cultivars according to the piecewise linear regressions of the time and with confident limits (CL).

Break point of time [min]	95% CL of time	E [$\text{mmol m}^{-2} \text{s}^{-1}$]	95% CL of E	Relative stepwise change [%]
<i>cv. 'Muller' BBCH 30-31</i>				
Starting = 0.0	-	3.58	-	100
6.6	6.2-6.9	2.79	2.75-2.84	- 22
27.5	27.3-28.1	3.15	3.14-3.16	+ 13
59.5	55.4-63.5	3.17	3.17-3.17	+0.6
114.0	110-118	3.48	3.46-3.50	+10
Ending = 220	-	3.56	-	+2
<i>cv. 'Feliks' BBCH 59</i>				
Starting = 0.0	-	4.05	-	100
9.0	6.5-10.6	3.82	3.79-3.88	- 5.2
19.3	16.5-22.0	3.93	3.91-3.95	+ 2.9
65.5	47.5-83.4	3.34	3.11-3.57	- 15
139.4	134-144	3.72	3.68-3.74	+ 11
Ending = 220	-	3.63	-	- 2.4

ent pattern after piercing. In the first minute after piercing there was recorded a decrease in P_{N1} from 19.27 to 16.07 $\mu\text{mol m}^{-2} \text{s}^{-1}$, i.e., by 17% (Table 5) and within 9 mins the decrease in the E_1 from 4.05 to 3.82 $\text{mmol m}^{-2} \text{s}^{-1}$, i.e., by 5.2% (Table 6). Then, both processes started to increase; P_{N2} for 24 mins at the rate of 0.05 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and E_2 for 10 mins at the rate of 0.009 $\text{mmol m}^{-2} \text{s}^{-1}$ (Figs. 3b, 4b).

At that time there was reported a significant negative dependence between a decrease in P_{N1} and an increase in C_{11} (Table 7). At the third stage, which took place from 26th to 74th min there occurred a decrease in P_{N3} by 0.04 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and E_3 by 0.013 $\text{mmol m}^{-2} \text{s}^{-1}$. Directional convergence of those two processes was confirmed by the coefficient of correlation $r = 0.95$. At the same time the

TABLE 7. Pearson's coefficients of correlation (r) and slopes (b) between P_N vs. C_i and E parameters in time intervals for cv. 'Muller' and cv. 'Feliks' rape plants after leaf piercing.

Coefficient	'Muller'			'Feliks'		
	Interval [min]	P_N vs. C_i	P_N vs. E	Interval [min]	P_N vs. C_i	P_N vs. E
r	1-6	-0.97***	0.93***	1-25	-0.84***	-0.1ns
b		-0.15	9.52		-0.11	-
r	7-27	-0.96***	0.97***	26-74	0.76**	0.95**
b		-0.22	7.36		0.16	3.65
r	28-59	-0.90***	0.50**	75-148	0.75**	0.95**
b		-0.11	7.46		0.19	5.36
r	60-114	0.46***	0.87***	149-220	-0.37**	-0.19ns
b		0.08	3.46		-0.05	-
r	115-220	-0.55***	0.00ns	1-220	0.73***	0.65***
b		-0.07	-		0.15	3.64
r	1-220	-0.25**	0.93***			
b		-0.06	5.39			

r - correlation coefficient by Pearson, b - linear coefficient of regression, * significance at $p = 0.05$, ** $p = 0.01$, *** $p = 0.001$, ns-not significant

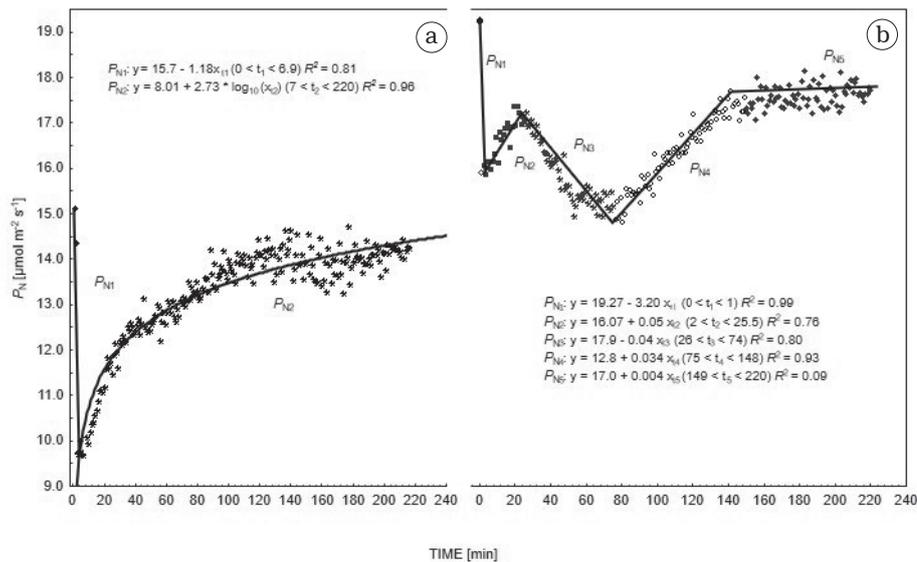


Fig. 3. Piecewise linear regressions and \log -time regression for net photosynthetic rate after leaf piercing of rape plant, the duration of each interval (t) and coefficients of determination (R^2); (a) cv. 'Muller', (b) cv. 'Felix'.

dependence between C_{13} and P_{N3} got reversed. At the successive stage there occurred rapid increasing in P_{N4} (by 19%) (Table 5) and E_4 (by 11%) (Table 6), which lasted to 148th min and demonstrated strongly convergent processes. After that, to the end of the test, there was observed stabilization of both processes with non-significant correlation between

them, an increase in P_{N5} of $0.004 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a slow decrease in E_5 by $0.0016 \text{ mmol m}^{-2} \text{s}^{-1}$ (Figs. 3b, 4b). In stem leaves in 'Feliks' the pattern of E was convergent in terms of the directions to the one observed for P_N . Those two processes were synchronized from 1st to 114th min of the test in plants at the rosette stage and from 26th to 148th min of

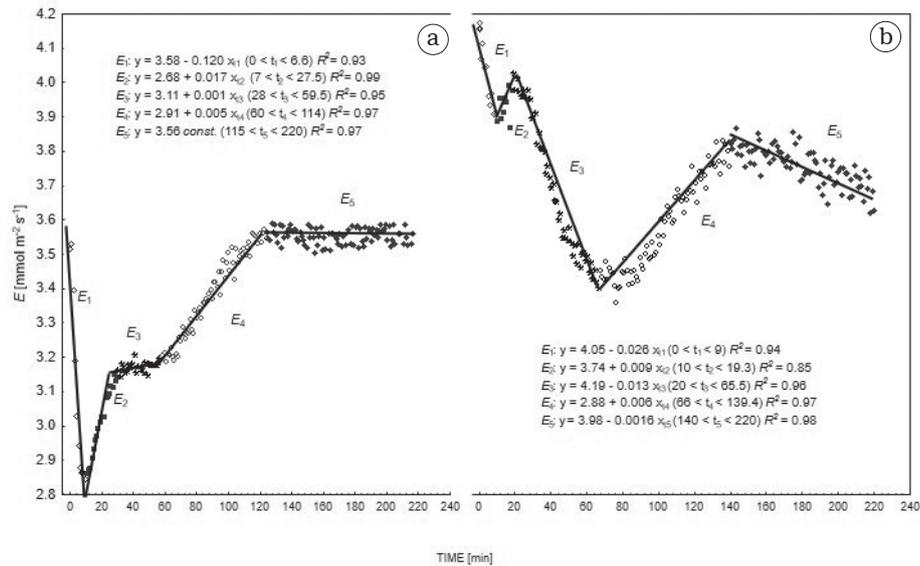


Fig. 4. Piecewise linear regressions for transpiration after leaf piercing of rape plant. The confident limits (95% CL) of the linear parameters, the duration of each interval (t) and coefficients of determination (R^2); (a) cv. 'Muller', (b) cv. 'Felix'.

the test for stem leaves (Table 7). Besides, throughout the time interval (1st–220th min) for 'Muller' the positive correlation (r) between P_N and E was very high 0.93 ($p < 0.001$) and for 'Felix' – it was high $r = 0.65$ ($p < 0.001$).

DISCUSSION

Gas exchange regulation is of great importance for water balance and the uptake of CO_2 . Controlling g_s is a complex process which depends on the water potential in leaves and on the intensity of transpiration as well as on such factors as the gradients of CO_2 concentration or the quality and intensity of light (Mott and Parkhurst, 1991; Sperry and Pockman, 1993; Messinger et al., 2006; Shimazaki et al., 2007).

Our research demonstrated that the plants of the same species, but at two BBCH stages, display different g_s changes after leaf piercing. Blamowski et al. (2003) studied two species of the *Brassica* genus; radish and spring rape, both at the rosette stage, exposed to wounding: the oldest leaves removal or defoliation of the youngest ones with the apical stem. The oldest leaves removal resulted in the same reaction in both species; it increased the intensity of transpiration; however, it did not affect the intensity of assimilation. The defoliation of the youngest leaves with the apical meristem showed a completely different effect in radish and spring rape plants. In the case of radish, the authors do not relate any effect on the photosynthesis to the

fact that at the rosette stage there occurs a very active acceptor of nutrient compounds (hypocotyl) which absorbs large amounts of carbon compounds supplied from leaves. This prevented the inhibition of photosynthesis, whereas in rape plants the defoliation of the youngest leaves with the apical meristem significantly decreased the intensity of assimilation, due to the accompanying increase of intercellular CO_2 concentration and slight fluctuations of the value of g_s , which pointed to non-stomatal limiting of photosynthesis. Koziółek et al. (2013) also described various stomatal conductance changes in leaves of shy plant (*Mimosa pudica*) due to abiotic factors, e.g., after thermal and light stimulation. The reaction after such stimulation on one part of the leaf showed a variable g_s pattern for the neighboring leaf part in the system of open and rolled pinnules after touching. For the open leaf at the first g_s stage it was a rapid growth about 40 s after thermal stimulation and taking about 1.7 mins, where g_s reached the maximum value of $0.180 \text{ mol m}^{-2}\text{s}^{-1}$, after which it decreased to $0.050 \text{ mol m}^{-2}\text{s}^{-1}$ and reached the period of stabilization after about 33 mins, at the level slightly lower than the initial state. This process is very similar to that in 'Felix' spring rape plant analyzed here after leaf piercing (Fig. 1b). Meanwhile, g_s in closed leaflets of shy plant, due to thermal and light stress, changed similarly to the one described here for 'Muller' winter rape plants. At the initial value g_s $0.150 \text{ mol m}^{-2}\text{s}^{-1}$ 8.3 min after induction there was a 3 fold decrease to $0.048 \text{ mol m}^{-2}\text{s}^{-1}$, then a slow increase started and after about 28.3 mins – g_s stabilization which,

finally, achieved the value higher than before the stress (Koziołek et al., 2013). The mechanical stress due to cutting of the main vein in sunflower (*Heliathus annuus*) leaf, disturbed the processes of P_N , E and stomatal conductance since it increased g_s by 0.22–0.23 mol m⁻²s⁻¹. The increase was compliant with the increase in E , which shows that the decrease in P_N observed was not an effect of limiting the release of CO₂, which can occur upon decreased g_s (Hanson et al., 2013). A decrease in P_N and increase in E were noted immediately after the cutting of the main vein. Photosynthesis reached the minimum value, on average, within 64 s after cutting-in, whereas the transpiration ratio assumed the highest value much later ($p = 0.0006$), on average within 143 s after cutting-in (Henson et al., 2013). In our research, in pierced ‘Muller’ leaves E first decreased by 22% and after 7 mins it started increasing rapidly to 27th min (+ 13%), then it became slower for 30 mins (+ 0.6%), and then increased by another 10% within 114 mins after piercing and it got stabilized to the initial value before the test. The plants of tomato *Lycopersicon esculentum* Mill. responded to chilling, as the stress factor, with stomatal conductance decreased by 32.2%, as compared with the control plants (Artuso et al., 2000). In reference to the stress-inducing factors of biotic origin, the reaction of g_s in leaves is differently described by researchers. As reported by Nabity et al. (2013) for wild tobacco (*Nicotiana attenuata*) plants damaged by tobacco hornworm (goliath worm) (*Manduca sexta* L.) and Aldea et al. (2005) for soybean (*Glycine max* L., cv. Pioneer 93B15) plants damaged by Japanese beetles (*Popillia japonica*) and corn caterpillars (*Helicoverpa zea* Bodie), the stress induced by plant-eating insects did not have a significant effect on g_s on wounded plants. Other biotic factors, e.g., viral infections, can cause changes in g_s , as demonstrated for mustard plants (*Brassica juncea* var. *tsatsai*) infected with turnip virus (Guo et al., 2005). No such reactions to mechanically-induced stress in other plants have been recorded. Nabity et al. (2013) found that the wild-type plants *Nicotiana attenuata* demonstrated a slight increase in C_i (by 1%), while the modified plants – a 3.8% decrease due to *Manduca sexta* L. insects feeding. Guo et al. (2005), on the other hand, report on C_i in leaves of the control plants and those infected with turnip mosaic virus in *Brassica juncea* var. *tsatsai*, being almost identical. Only Artuso et al. (2000) demonstrated that thermal stress (chilling) resulted in a decrease in the concentration of intercellular CO₂ in *Lycopersicon esculentum* Mill. (by 6.4%); however, the decrease was non-significant. According to Hinckley and Braatne (1994), stomatal conductance is inversely correlated with the concentration of carbon dioxide in leaves if tissues are adequately irri-

gated. Nardini et al. (2003) show linear dependence between gas exchange and water vapor conductance and the parameters are closely correlated with each other ($r^2 = 0.987$, $p < 0.01$). We found strong positive correlation between g_s and C_i ($r = 0.86$) for stem leaf wounded (‘Feliks’), while in ‘Muller’ – they negatively correlated with $r = -0.81$, which means that the leaves at the rosette stage retained CO₂ during the decrease in g_s over 1st–23rd min, while the leaves at the flowering stage were losing intercellular CO₂ with a decrease in g_s (from 1st to 6th min and from 24th to 63rd min). Photosynthesis and transpiration are, most frequently, correlated with each other due to the fact that stomata determine the conductance of water vapor and carbon dioxide (Farquhar and Sharkey, 1982). Mechanical leaf wounding in tomato resulted in an increase in the photosynthesis rate 1–5 mins after wounding (Herde et al., 1999). In our research, after the period of a rapid decrease, increasing P_N took place from 2nd to 7th min, earlier in rosette leaves and later in stem leaves. However, the latter did not reach the stabilization of P_N yet, showing successive decrease of P_N by 14% after 25 mins and only after 75 mins P_N started to get stabilized. Unlike ‘Muller’ plants at the rosette stage, there was demonstrated dependence of P_N on time in *log*-linear progression: $y (P_N) = 8.01 + 2.73 \log_{10} (x t_2)$; $7 < t_2 < 220$; $R^2 = 0.96$. The research reported by Hanson et al. (2013) shows that the response to the stress of cutting-in the midvein in sunflower was a fast decrease in P_N by an average of $8.5 \pm 4.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, i.e., by 40%, as compared with the status before the stress. Here, that decrease amounted to 35% at the rosette stage and to 31% in stem leaves, however, in the latter the decrease occurred in two-stages; 17% in the range from piercing to 1st min and 14% from 26th to 74th min. Midvein cutting-in in sunflower leaf also increased the process of E by $1.3 \pm 1.0 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Hanson et al., 2013). As reported by Artuso et al. (2000), processes P_N and E in tomato demonstrated a considerable decrease due to chilling stress by 37.7% and 29.5%, respectively, however, the authors do not describe how they were synchronized. Although genetic differences in photosynthetic capacity exist at intraspecific and interspecific levels, P_N is considered as one of the potential, physiological, selection criteria for stress tolerance (Ashraf, 2004). The reactions of plants to the effect of biotic stresses, mostly triggered by insects or viral pathogens, visible through changes in P_N and E of leaves, were studied at many stages. Infecting mustard plants with turnip mosaic virus appeared an essential factor decreasing the P_N by 52%, while for E at the initial stage of infection it did not matter, after which there was observed an increase in E and, at the final stage, a decrease, which was in no way related to the pattern of chang-

es in P_N (Guo et al., 2005). Comparisons of the reaction of tobacco to two types of stress were investigated by Hlaváčková et al. (2002). The mechanical wounding of *Nicotiana benthamiana* leaf surface significantly decreased the process of P_N measured after 11 days by $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ and additional infection of those leaves with virus PPV (*plum pox potyvirus*) – by $4 \mu\text{mol m}^{-2} \text{s}^{-1}$, which, in relative values, accounted for 34% and 62%, as compared with the control (unwounded) leaves. Thirty-nine days after inoculation/mechanical wounding, a decrease in P_N was, in both cases, similar and it accounted for 40%. For that reason the cited authors' claim that P_N and E are not synchronized after stress does not get confirmation; the authors state that at minimum P_N , E was maximum. In our research those two processes were synchronized from 1st to 114th min of the test in plants at the rosette stage and from 26th to 148th minute of the test in plants at the stem stage. Besides, throughout all time intervals (1st–220th min) for 'Muller' the positive correlation (r) between P_N and E was very high 0.93 ($p < 0.001$) and for 'Feliks' – high 0.65 ($p < 0.001$).

We conclude that the only convergent process in both rape plant forms was observed for the concentration of intercellular CO_2 . Here, after a rapid increase in C_1 (by 11–22%) taking 2–3 minutes after piercing, there occurred two stages of decrease and two stages of increase and in both leaf types the C_1 level at the end of the test exceeded the initial level by an average of 15–27 $\mu\text{mol mol}^{-1}$.

AUTHORS' CONTRIBUTIONS

AW-P is the author of the concept, statistical analyses, wrote the manuscript, WK designed the research, collected data, wrote the manuscript, AN collected data, obtained funding, wrote the manuscript, MK collected data, MT-S prepared plants.

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