Pollen – mediated intraspecific gene flow in winter oilseed rape (*Brassica napus* L. var. *oleifera*)

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The cultivation of genetically modified herbicide resistant oilseed rape (*Brassica napus* L. var. *oleifera*) has increased over the past few years, especially in North America and Asia.

In Europe, according to the principles of coexistence established by European Commission "farmers have the ability to make a practical choice between conventional, organic and GM-crop production, in compliance with the legal obligations for labeling and / or purity standards".

In the case of oilseed rape important obstacles concerning the integration of GM cultivars into the cropping system occur.

The most important are the flow of transgene-containing pollen to the surroundings and oilseed rape volunteers. The question of gene flow is particularly relevant to oilseed rape because this species is partially allogamous (20–40% of outcrossing), produces a huge quantity of pollen, 5×10^{12} pollen grain per ha. Pollen is dispersed by wind, hive bees, bumble bees and other insects.

The extent of pollen-mediated gene flow in oilseed rape is strongly dependent on climatic conditions (e.g. wind speed, wind direction) as well as its pollen characteristics. Rapeseed pollen is relatively large (32–33 μm), heavy and sticky, with viability estimates ranging from 1 to 5 days under natural conditions [1]. Mesquida and Renard [2] detected rapeseed pollen 32 m from oilseed rape plot, but noted that the concentration of pollen collected decreased rapidly with the distance from the pollen source.

Some studies have indicated that viable pollen can be found 1,5 km from the pollen source [3]. In discontinuous pollen-dispersal experiments, cross-hybridization rate was estimated to be 0,0156% and 0,0038% at 200 m and 400 m, respectively [4] whereas in a continuous pollen dispersal experiment, the frequency decreased sharply to 0,02% at 12 m and was only 0,00033% at 47 m from the central plot [5]. No obvious directional effects were detected that could be ascribed to wind or insect activity.

Transfer by seed drop at harvest can be very high and very important to coexistence over time. It is due to the phenomenon of secondary dormancy of oilseed rape seeds.

Pollen-mediated intraspecific gene flow in winter oilseed rape was investigated in Polish environmental conditions. Erucic acid was used as the genetic marker of cross pollination.

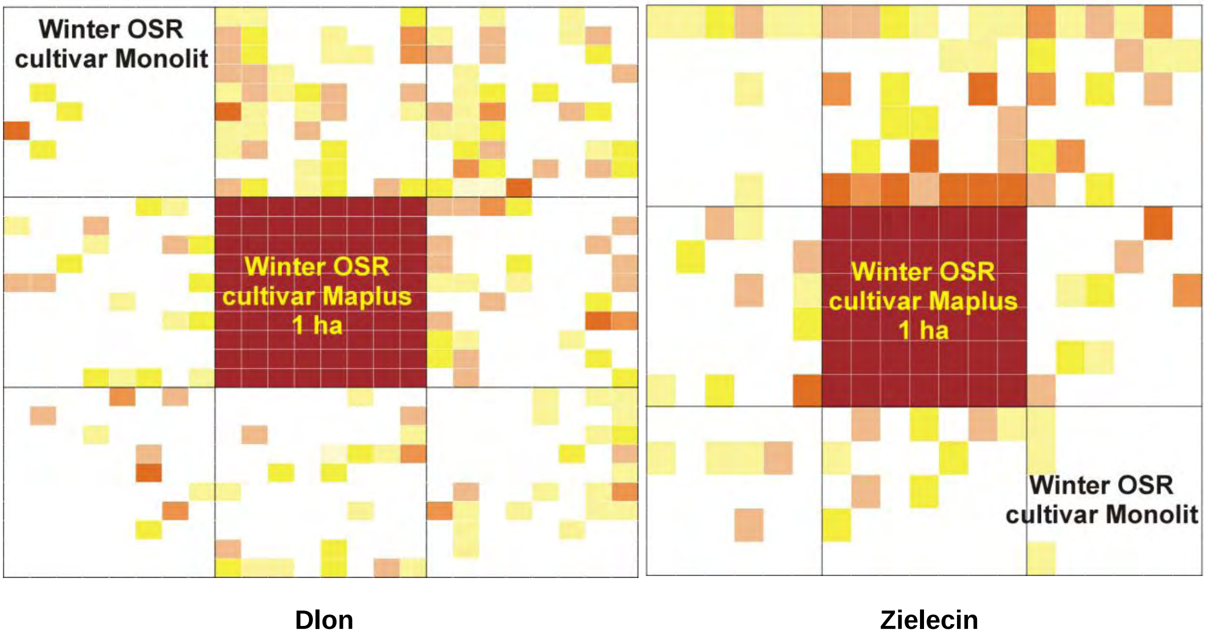
Material and Methods

The investigations were conducted in season 2008/2009 in two environments with different climatic and soil conditions – Zielecin (N 52^{o}10' E 16^{o}22') and Dlon (N 51^{o}46' E 17^{o}14'). The acreage of the field trial in each locality was about 9 ha.

Gene flow was investigated using a winter OSR cultivar Monolit – without erucic acid, selected as one DH line developed from isolated microspore culture and test system based on the winter OSR cultivar Maplus – the source of erucic acid, containing over 57% erucic acid. A field design involved. the pollen donor cv. Maplus (1 ha) surrounded by the pollen recipient cv. Monolit (8 ha) (Fig. 1).

During the period of flowering of winter oilseed rape (typically May), meteorological data (wind speed, wind frequency direction) were recorded by Institute of Meteorology and Water Management by on-site weather stations.

Each plantation was divided into small plots and at the harvest samples of seeds were taken from each plot to estimate the frequency of pollen dispersal at each compass distance. In all 1920 individual samples fatty acids composition was examined using gas chromatography [6].



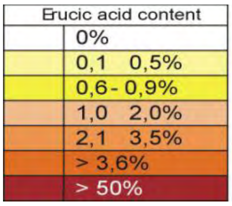
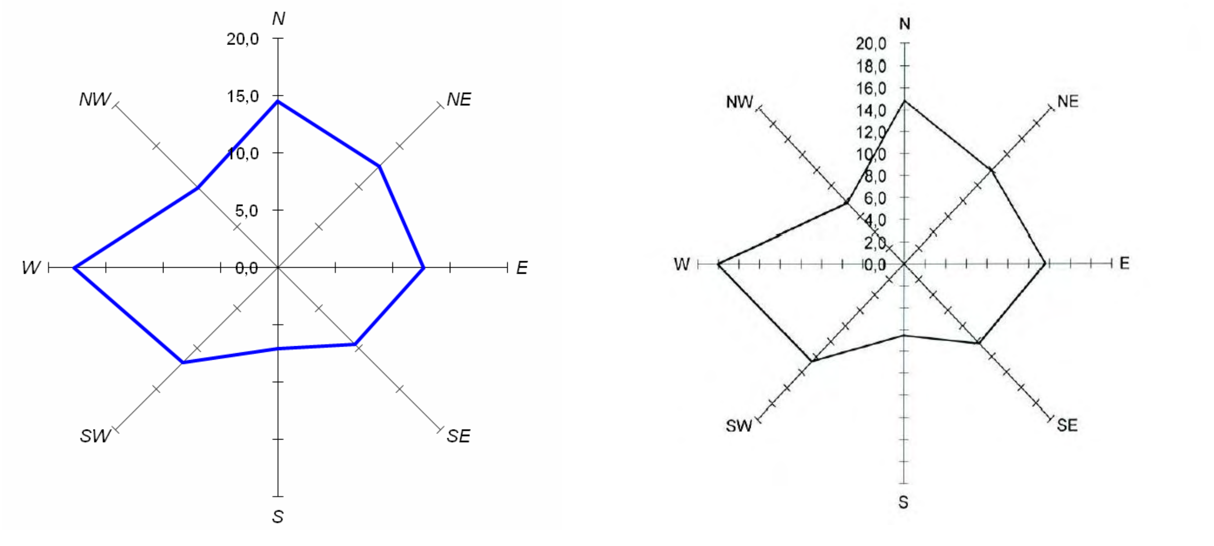


Fig. 1. Field design and results of pollen flow at Dlon and Zielecin

Results

Variability of erucic acid content in samples taken from plots with cultivar Monolit was very high and different in both localities. In Dlon for 619 individual samples of seeds erucic acid content ranged from 0,1 to 7,4%, in Zielecin for 1301 individual samples of seeds from 0,1 to 19,2% (Fig. 1).

At each experimental site, there was a mean wind direction during the flowering period. Wind frequency direction and wind speed recorded at each site are shown in Figure 2. During this flowering period in 2009 wind came from various directions but predominant wind direction at both localities was recorded from westerly, southwesterly and southerly direction. Predominant direction of pollen flow was consistent with predominant wind direction at both localities.



Dlon **Zielecin**

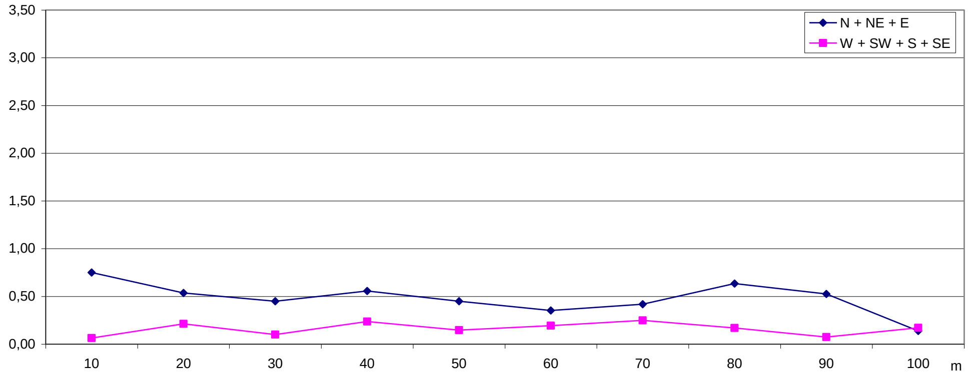
Fig. 2. Wind speed and frequency direction recorded during the flowering period at two experimental sites in 2009 at Dlon and at Zielecin

Table 1. Average of erucic acid content (%) in samples of seeds taken at different distances from the edge of Maplus field at Dlon and Zielecin in 2009

Compass direction	Distance from the edge of Maplus field at Dlon									
	10 m	20 m	30 m	40 m	50 m	60 m	70 m	80 m	90 m	100 m
North	0,70	0,16	0,29	0,25	0,74	0,73	0,40	0,60	0,59	0,16
Northeast	0,80	1,03	0,68	1,20	0,61	0,29	0,22	0,59	0,46	0,11
East	0,75	0,41	0,38	0,22	0,00	0,04	0,64	0,71	0,00	0,00
Southeast	0,00	0,00	0,00	0,00	0,08	0,21	0,39	0,46	0,01	0,00
South	0,01	0,25	0,25	0,46	0,16	0,06	0,05	0,00	0,21	0,51
Southwest	0,00	0,33	0,00	0,44	0,16	0,36	0,38	0,06	0,00	0,00
West	0,24	0,27	0,15	0,04	0,19	0,14	0,17	0,16	0,00	0,00
Northwest	0,00	0,00	0,00	0,00	0,00	0,08	0,12	0,33	0,00	0,00

Compass direction	Distance from the edge of Maplus field at Zielecin					
	15 m	30 m	45 m	60 m	75 m	90 m
North	7,54	0,79	0,36	1,26	0,11	0,73
Northeast	1,20	1,33	0,18	0,46	0,38	0,66
East	0,17	0,18	0,15	0,22	0,60	0,45
Southeast	0,10	0,07	0,00	0,00	0,03	0,00
South	0,50	0,16	0,24	0,10	0,00	0,00
Southwest	0,00	0,00	0,27	0,40	0,15	0,08
West	1,28	0,00	0,27	0,40	0,15	0,08
Northwest	0,00	0,00	0,08	0,07	0,00	0,26

Erucic acid [%] Dlon



Zielecin

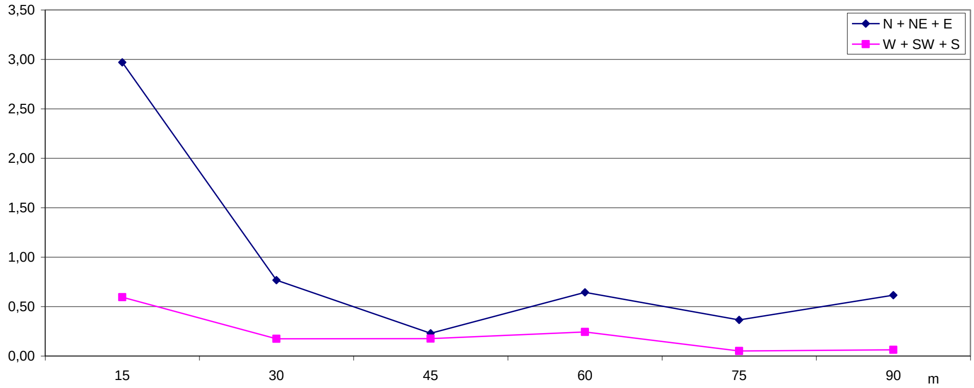


Fig. 3. The relationship between the distance from the source of erucic acid and the level of contamination with erucic acid at field experiment at Dlon and Zielecin in 2009

The distance of pollen flow was different in each locality.

Average of erucic acid content in seeds taken at different distances from the edge of Maplus field are shown in Table 1. The observed difference in the mean outcrossing rate in oilseed rape may be explained by different environmental conditions in both localities. The relationship between the distance from the source of erucic acid and the level of contamination with erucic acid at Monolit field are shown in Fig. 3. The largest flow of pollen is the case for northerly, northeasterly and easterly direction (N + NE + E). However, the results of the present study also show that the highest outcrossing rate has been generally found in the first 40 m from the edge of Maplus field, at each site a decrease in outcrossing with increasing distance was observed.

Conclusion

The obtained results indicate that gene flow through pollen and winds in oilseed rape is significant for short distance-up to about 40 m.

Problems of coexistence GM and non-GM oilseed rape can be reduced by management of cross-pollination between fields, through spatial separation and use of buffer or discard zones where crops are in close proximity. The most important problem for coexistence according to another investigation can be due to volunteers and ferals populations [7].

Acknowledgments

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References

1. Treu R., Emberlin J. 2000. Pollen dispersal in the crops Maize (*Zea mays*), Oil seed rape (*Brassica napus* ssp. *oleifera)*, Potatoes (*Solanum tuberosum*), Suger beet (*Beta vulgaris* ssp.*vulgaris*) and Wheat (*Triticum aestivum*). A Raport for the Soil Association from the National Pollen Research Unit.
2. Mesquida J., Renard M. 1982. Étude de la dispersion du pollen par le vent et de l'importance de la pollinisation anémophile chez le colza (*Brassica napus* ssp. *oleifera* Metzger). Apidologie, 13: 353-366.
3. Timmons A.M., O'Brajen E.T., Charters Y.M., Dubbels S.J., Wilkinson M.J. 1995. Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera*. Euphytica, 85: 417-423.
4. Scheffler J.A., Parkinson R., Dale P. 1995. Evaluating the effectiveness of isolation distance for field plot of oilseed rape (*Brassica napus*) using a herbicide-resistance transgene as a selectable marker. Plant Breeding, 114: 317-321.
5. Scheffler J.A., Parkinson R., Dale P., 1993. Frequency and distribution of pollen dispersal from transgenic oilseed rape (*Brassica napus).* Transgenic Research, 2: 356-364.
6. Byczyńska B., Krzymański J. 1969. A fast method for obtaining of fatty acids methyl esters to be analysed by gas chromatography method (in Polish). Tłuszcze Jadalne, XIII: 108-114.
7. Bartkowiak-Broda I., Wałkowski T., Popławska W., Ogrodowczyk M., Liersch A. 2008. Influence of volunteers and oilseed rape-like plants on the quality of winter oilseed rape yield (in Polish). Oilseed Crops Tom XXIX/2: 185-198.