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Regionalisation of flora elements in field boundaries sensitive to hybridisation with genetically modified oilseed rape

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Abstract *Background, aim, and scope* Gene flow via pollen dispersal to neighbouring non-genetically modified (GM) and organic fields or to biotopes containing the same crop species and/or their wild relatives are among the most debated potential environmental risks of GM crops. These crosses permit ingression of GM traits and may produce viable progeny. Current GM crop monitoring plans and concepts have not considered this a critical issue. In the present study, we develop a methodology for the regionalisation of the hybridisation risk of GM oilseed rape (OSR) (Brassica napus L.) with respect to related hybridisation partners (both OSR and related species) as well as neighbouring arable fields and biotopes. This methodology should constitute an important component of future spatial GM crop monitoring designs.

Materials and methods A vegetation database containing occurrence frequencies of OSR crossing partners in Brandenburg state was analysed, and literature surveys were performed on OSR outcrossing proofs with regard to different wild species, the viability of progeny and the potential establishment of crosses. We aggregated detailed biotope maps for the entire Brandenburg state in order to differentiate the nine main biotope groups relevant as habitats for OSR and hybridising Brassicaceae. We determined the types and areas of biotopes neighbouring all arable fields with an outside buffer of 50 m, and then ascertained whether the biotope composition outside the buffers was significantly different from that of the buffers. We then overlayed our buffering results with an ecoregion map of Brandenburg to upscale our results to larger regions.

Results Brassica rapa presented the highest potential for hybridisation, reproduction and persistence in this environment, but Raphanus raphanistrum, Brassica oleracea, Hirschfeldia incana, Sinapis arvensis and Diplotaxis muralis are also significant potential crossing partners for OSR. The highest average frequency of species occurring in biotopes applies to arable lands, settlements and industrial areas, disturbed areas, road verges and gardens, which together cover 84.2 % of the total area and 74.6 % of the neighbouring biotopes. Related species occurring most often in Brandenburg are Descurainia sophia, feral OSR, Sinapis arvensis, Diplotaxis tenuifolia and Diplotaxis muralis. All biotopes relevant to OSR-related species are present in all Brandenburg ecoregions, but there are differences in the proportion of each biotope, especially hedgerows, arable land, gardens and road verges. The Uckermark and Oder valley can be considered slightly more critical.

Discussion Hybridisation and persistence of GM OSR depends on (a) the related species' potential to hybridise and produce viable progeny, (b) the frequency of hybridisation partners at different biotope types, and (c) the frequency of directly neighbouring arable fields with sensitive biotopes. Integration of these factors gives the following rank order of hybridisation risks for different biotopes in the agro-environment: disturbed areas > arable land > road verges > settlements and industrial areas > gardens. Extrapolation of local relevée and biotope results to larger areas such as the Brandenburg state was shown to be feasible, and may also be done nationwide and EU-wide with suitable biotope datasets.

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Conclusions Cultivation of GM OSR in Brandenburg carries a considerable potential of hybridisation with related species and feral OSR in biotopes neighbouring arable fields. The methodology presented here is suitable to link spatially limited but highly detailed datasets on the occurrence of potential hybridisation partners for GM OSR with regional datasets and to extrapolate hybridisation risks, and therefore could serve as a monitoring instrument.

Recommendations and perspectives We suggest that populations of related species and the potential spread of GM traits should be monitored using a targeted approach. While further standardisation will be required, this methodology should be included as a regular component of GM crop monitoring.

Keywords Brassica napus L. · Environmental effects · Field boundaries · Genetically modified crop · GM oilseed rape (OSR) · Hybridisation · Oilseed rape · Spatial range · Wild relatives

1 Background, aim and scope

The cultivation area of genetically modified (GM) crops has dramatically increased over the past two decades and in 2008 covered 125 million hectares (James 2008). Oilseed rape (OSR) (*Brassica napus L.*) and its canola cultivars cover 5 % of the global biotech area. The potential adverse agronomic and environmental effects linked to both the genetic modifications and changes in agricultural practices have been the subject of intense debate in recent years (Champion et al. 2003; European Commission 2003; Graef et al. 2007). Among the potential environmental risks debated, gene flow via pollen dispersal to neighbouring fields and biotopes that may contain the same crop species and/or their wild relatives rank very high (Devos et al. 2004, 2005). As progeny from these crosses may be viable, in this way the GM traits can ingress into non-GM, organic fields and the environment (Beckie et al. 2006; Legere 2005). Detrimental effects on agriculture and/or the environment can occur if the progeny with the GM trait develops a selective advantage, spreads through cultivated fields and the environment, or if other long-term and/or cumulative effects occur triggering yet unknown environmental hazards. The longest persistence of GM OSR offspring known to date was reported by Warwick et al. (2007), who monitored persisting transgenic *Brassica rapa* hybrids over a 6-year period, in the absence of herbicide selection pressure and in spite of reduced fitness associated with its hybridisation.

The hybridisation risk for OSR is considerably higher than that of other crops for the following reasons:

a) High cross-pollination rate by wind and insects, predominantly bees (Mesquida et al. 1988). The range of honey-bees and other pollinators typically covers up to a few kilometres (Osborne et al. 2007; Steffan-Dewenter and Kuhn 2003).
b) Large pollen distribution range due to small pollen size. The majority of OSR pollen is deposited less than 100 m from the pollen source. The crossing rate decreases rapidly between 10 and 50 m from the pollen source (Ramsay et al. 2003), but low frequencies of cross-pollination have also been recorded at distances of 4 km from the source (Rieger et al., 2002; Squire et al., 2003).
c) Common feral oilseed rape populations either in subsequent crops or as volunteers outside cropped areas (Champolivier et al. 1999; Crawley and Brown 2004; Devos et al. 2004).
d) Numerous wild relative species exist and regularly occur in middle Europe, increasing outcrossing probabilities (OECD 1997).
e) OSR can establish on anthropogenic and ruderal sites and form persistent feral populations (Chèvre et al. 1997; Wilkinson et al. 2000; Yoshimura et al. 2006) that may act as both pollen donator and receptor.

On the basis of the aforementioned scientific findings a risk assessment of GM OSR as required by the European Community (2001) prior to its commercial release will likely conclude that OSR cultivation might lead to environmental harm.

Various studies on OSR pollen and seed dispersal show that the main influential factors are environmental and topographical conditions (Kuparinen et al. 2007; Squire et al. 2003) as well as farming practices (Gruber et al. 2004) and transportation activities (Kawata et al. 2009; Yoshimura et al. 2006). Moreover, the GM OSR hybridisation risk depends on the spatial arrangement of the GM and non-GM OSR fields as well as on the types of neighbouring biotopes to the introduced OSR pollen. Fields, field margins and various types of neighbouring biotopes, for instance, can serve as habitats for species hybridising with OSR (Menzel 2006; Wilkinson et al. 2000). The literature indicates that hybridisation partners like *Brassica rapa*, *Sinapis arvensis* and *Raphanus raphanistrum* are more frequent on disturbed ruderal sites than in other biotopes (Jørgensen et al. 2006).

The focus of our study was to develop a methodology for the regionalisation of the GM OSR hybridisation risk with respect to the potential presence of related hybridisation partners (both OSR and related species) and to neighbouring arable fields and biotopes within a 50 m distance, where most of the pollination occurs (Ramsay et al. 2003). This methodology was intended to be a contribution to the mandatory monitoring of GM crops (European Community 2001). As a case study we chose the Federal State of Brandenburg, Germany. We used data from plant species surveys that we had carried out over a few years in different Brandenburg biotopes. Our surveys did not include fields with present OSR cultivation. We also aimed to test whether our approach was suitable for predicting higher and lower hybridisation risk areas in different ecoregions, as well as whether our results could be extrapolated to other OSR cropping regions.

2 Materials and methods

To assess the frequency of potential OSR crossing partners, we combined data from vegetation monitoring surveys in different biotopes and areas of Brandenburg with that reported by Menzel and Born (2004), Menzel (2006) and with regional species occurrence given by the national open source database Floraweb (2009) to create a database of OSR crossing partners dating from 1996 to 2007. Additionally, we performed a literature survey on OSR outcrossing proofs with regard to different wild species, the viability of progeny and their potential establishment.

We used detailed digital biotope maps available for Brandenburg that were established based on field-work and CIR (colour infrared) imagery. The original fine-scale classification of 30 biotope types in Brandenburg was transformed and aggregated to differentiate the nine main biotope groups that we had surveyed. We discarded biotopes such as water bodies, forestland, heathland and intensive grassland, as these were determined by our vegetation database to be not relevant as habitats for OSR or hybridising Brassicaceae.

We used Arc/Info GIS to determine the types of biotopes neighbouring all arable fields in Brandenburg with an outside buffer of 50 m (Fig. 1). This distance was selected based on literature showing that the crossing rate decreases rapidly between 10 and 50 m from the pollen source (Downey 1999; Ramsay et al. 2003). It was also selected because of the highly detailed biotope map and because a 100 m or 200 m

Fig. 1 Section of biotope map (North Brandenburg) showing buffering strip results

distance would have added only very little information on the hybridisation potential. After clipping the buffers, the type and area of different biotopes were analysed. Overlapping of buffer strips was allowed.

To integrate the differences in species occurrence in different neighbouring (buffered) biotopes, the biotopes providing habitat for at least one potential outcrossing partner within the buffer zones of single arable fields were classified by combin-

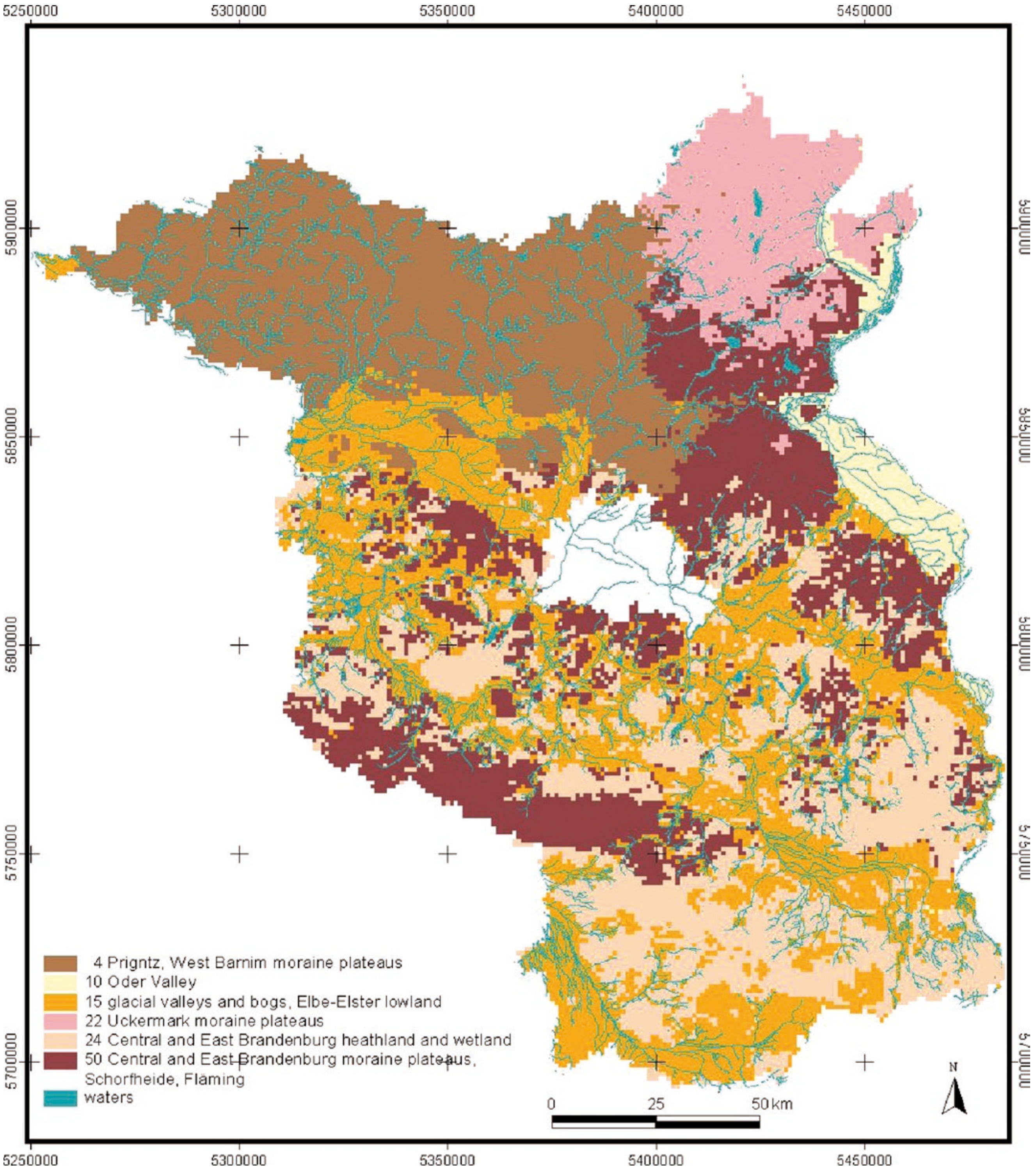

Fig. 2 Ecoregion map of Brandenburg (Graef et al. 2005, modified)

ing different multi-variate statistical procedures: cluster analysis (TWINSPAN), correspondence (CA) and canonical correspondence analysis (CCA) (CANOCO 4.5 – ter Braak and Šmilauer 2002), as well as discriminant analysis (SPSS 11.5). TWINSPAN provided a parallel classification of both the single fields and the biotope types included in their buffer zones and indicated the biotope types responsible for singular divisions between clusters (indicators). Discriminant analysis was used to countercheck the clustering results and to alleviate the main limitation of the TWINSPAN procedure, i.e., the request for interval-scaled input data. Canonical correspondence analysis provided additional information regarding the interrelation of different biotope types on the clustering result.

The next step was to overlay our buffering results with an ecoregion map of Brandenburg (Graef et al. 2005) that encompasses six major landscape classes on a 1 × 1 km grid (Fig. 2). The aims of overlaying were to classify biotope and OSR crossing partner composition and to possibly extrapolate our results to larger regions.

3 Results

3.1 Review and identification of plant species most sensitive to hybridisation with OSR pollen

We first reviewed and identified those species most sensitive to hybridisation with OSR pollen. Hybrid combinations of *Brassica napus* and wild relatives especially of the genera *Brassica*, *Diplotaxis*, *Erucastrum*, *Hirschfeldia*, *Rapistrum*, *Raphanus* and *Sinapis* (Table 1), which are close relatives of *Brassica napus* have been produced in a number of studies involving experimental hybridisation, spontaneous natural hybridisation and artificial in vitro techniques (Table 1, Fitzjohn et al. 2007). Hybridisability of OSR with the genus *Descurainia* is still uncertain, but there are some hints in the literature that some species can act as interspecific transfer bridges between *Brassica napus* and *Descurainia* (Brown et al. 1995).

Once successfully hybridised, the proportion of hybrids producing self-fertile seeds, i.e., the viability of the F1 progeny, is often low (Brown and Brown 1996, see Table 1). OSR hybrids with *Brassica rapa* have the highest potential to reproduce and persist in the environment, followed by hybrids with *Raphanus raphanistrum*, *Brassica oleracea*, *Hirschfeldia incana*, *Sinapis arvensis* and *Diplotaxis muralis* (see Table 1). The species *Brassica nigra*, *Raphanus sativus*, *Sinapis alba and Rapistrum rugosum* either poorly hybridise or have poorly viable progeny. For some OSR hybrid combinations little or no data is available. OSR crossing experiments using OSR as the pollen donor are more important because of the large OSR pollen production capacity and its dispersal in the environment. However, experiments using OSR as the female parent are also relevant because cultivated or feral OSR may also be pollinated by wild relatives; a considerable portion of the seeds are spilt during harvest and some of the hybrid seeds may germinate as feral or volunteer OSR in successive years. Table 1

Table 1 Review of hybridisation of *Brassica napus* with related wild species (Geneera 2004; Fitzjohn et al. 2007, modified)

	Success of experim. Hybridisation (M/F)	Median rate of hybridisation (M/F)	Success of natural hybridisation/No. trials	Success of hybrid backcross production[a] (M/F)	Success of hybrid F2 production[a] (M/F)	Additional references[b]
Brassica rapa	55:8/84:0	0.44/2.29	yes/19	5:4/13:0	43:3/35:0	1, 2, 3, 4, 19, 26
Raphanus raphanistrum	0:4/3:2	0/0[c]	yes/6	-/1:2	1:0/12:2	5, 6, 7, 8, 9
Diplotaxis muralis	3:0/1:1	0.19/0[3]	–	–	3:0/–	10, 11, 12
Diplotaxis tenuifolia	0:3/1:1	0/0[c]	–	–	–	11, 13, 14, 27
Brassica nigra	2:2/4:2	0[c]/0[c]	no/4	–	1:0/2:0	9, 16, 17, 19
Brassica oleracea	3:11/9:17	0[c]/0[c]	yes, no/1, 1	2:0/17:0	3:0/20:1	9, 16, 18
Erucastrum gallicum	0:1/1:0	0/0[c]	no/1	–/0:1	–/2:0	20, 21, 28
Hirschfeldia incana	1:2/1:2	0[c]/0[c]	yes/4	–	1:0/3:0	9, 15, 22
Raphanus sativus	1:5/1:2	0[c]/0[c]	no/1	–/0:4	–/1:0	9, 23
Rapistrum rugosum	–/1:0	–/0.71	–	–	–	9, 24
Sinapis alba	0:6/1:2	0/0[c]	no/1	–	1:0/–	9, 14, 22
Sinapis arvensis	1:10/5:8	0[c]/0[c]	yes, no/1, 6	0:1/5:3	0:2/5:3	9, 15, 19, 25

[a] No. of hybridisation trials where OSR was the male (M) or female (F) parent (successes:failures) (Fitzjohn et al. 2007).

[b] References: 1: Jorgensen et al. (2006); 2: Mikkelsen et al. (1996); 3: Metz et al. (1997); 4: Scott and Wilkinson (1998); 5: Eber et al. (1994); 6: Kerlan et al. (1993); 7: Darmency et al. (1998); 8: Rieger et al. (2001); 9: Scheffler and Dale (1994); 10: Salisbury (1991); 11: Ringdahl et al. (1987); 12: Birjal and Sharma (1996); 13: Salisbury (1989); 14: Klewer et al. (2003); 15: Plümper (1995); 16: Siemens (2002); 17: Bing et al. (1991); 18: Ford et al. (2006); 19: Brown and Brown (1996); 20: Warwick et al. (2003); 21: Lefol et al. (1997); 22: Raybould and Gray (1993); 23: Lelivelt and Krens (1992); 24: Heyn (1977); 25: Luo et al. (2000); 26: Warwick et al. (2007); 27: Pascher et al. (2000).

[c] At least one case of hybrid production was reported.

also shows that fertilisation and reproducibility of progeny differ greatly between species, depending on the parental combination.

Important criteria for the selection of potential receptors for successful hybridisation, apart from genetic compatibility, are dispersion options such as accessibility for pollen, the timely concurrence of flowering, the pollen maturity period and the type of pollination (presence of wind and/or insects). Table 1 presents the species that should be considered as potential hybridisation partners in Brandenburg state given current data. The listed species will be analysed and discussed in the next steps.

3.2 Habitat associations for relevant hybridisation partners of OSR in the State Brandenburg

The probability of occurrence of OSR crossing partners in Brandenburg biotopes is shown in Table 2. Biotopes not relevant as habitats for OSR and *Brassicaceae* hybrids are not shown. The habitat associations of particular species and biotopes are based on the relationships determined by the vegetation surveys. The regional frequency, as shown in the last column of Table 2, was used to weight the values for particular species. For this purpose, the frequencies for single biotopes were multiplied with a constant factor for each species to reach comparable sum values (column: sum), as given by Floraweb for the regional occurrence. Deviations from this procedure are due to (a) no values given in the regional database (Floraweb), (b) a great number of potential biotopes or (c) underestimation of species in the regional database most likely due to misclassification.

The areas of the biotope types relevant for species hybridising with OSR differ greatly (see Table 2). Overall, there is high variability between different species and different biotopes, with some species occurring very frequently in many different biotopes and others that are restricted to only a few specific biotopes. Biotopes with the highest average species frequency (total frequency/No. of species) are arable lands (13.5 %), settlements and industrial areas (11.6 %), disturbed areas (10.5 %), road verges (10.2 %) and gardens (7.7 %). They encompass a large share of the biotope types (84.2 %) that have a high probability of OSR-related species occurrence. More intensively utilised biotope types like meadows or plantations or dry grassland biotopes have lower OSR-related species probabilities. Road verges constitute a special case not only because of the large number of crossing partners, but also because of the roads‘ function as OSR transportation and dispersal routes (Yoshimura et al. 2006). The most frequent species in Brandenburg state are *Descurainia sophia* (100 % coverage), feral OSR (100 % coverage), *Sinapis arvensis* (98 % coverage), *Diplotaxis tenuifolia* (70 % coverage) and *Diplotaxis muralis* (51 %

Table 2 Occurrence of species hybridising with OSR in biotope types of Brandenburg (water and forest biotopes irrelevant for these species)

	Meadows	**Dry grass-lands**	**Dis-turbed areas**	**Hedgerows, plantations**	**Arable land**	**Gar-dens**	**Specific biotopes**	**Settlements, industrial areas**	**Road verges**	**Sum**	**% of occur-rence accord. to Floraweb (2009)**
Total biotope area (% total area)	9.0	0.5	10.6	6.0	36.1	10.3	0.4	24.2	2.9		
Brassica rapa	–	–	1	0	0	–	1	1	1	4	0
Raphanus raphanistrum	0.3	0.3	2	0.3	5	5	–	–		12.9	15
Diplotaxis muralis	–	1	10		3	1	1	15	20	51	50
Diplotaxis tenuifolia	–	1	20	1	3	2	1	25	20	73	70
Brassica nigra	–	–	3	–	–	–	–	–	–	3	3
Brassica oleracea	–	–	1	–	1	5	1	–	1	9	5
Descurainia sophia[b]	–	–	25	5	30	10	5	15	20	100	100
Erucastrum gallicum	–	–	5	0.5	–	–	–	–	5	10.5	10
Hirschfeldia incana	–	–	5	–	–	–	–	–	5	10	10
Raphanus sativus	–	–	15	–	15	30	3	10	10	83	–
Rapistrum rugosum	–	1	5	0.3	1	1	1	1	5	15.3	10
Sinapis alba	–	–	10	1	15	10	1	2	5	43	–
Sinapis arvensis	1	1	20	1	30	5	5	15	20	98	95
feral OSR	–	–	25	–	45	–	–	20	10	100	100
No. of species	2	5	14	8	11	9	9	9	12		
Frequency	1.3	4.3	147	9.1	148	69	19	104	122		
Average frequency[a]	0.7	0.9	10.5	1.1	13.5	7.7	2.1	11.6	10.2		

[a] Frequency/No. of species

[b] *Descurainia sophia* is included because it possibly forms gene transfer bridges from OSR to other genera

coverage). Some species with comparably higher progeny viability (*Brassica rapa*, *Raphanus raphanistrum*, *Brassica oleracea* and *Hirschfeldia incana* [see Table 1]) were found less frequently (4–12.9 % coverage). As expected, feral OSR often occurs on arable land, disturbed areas, settlements, industrial areas and road verges. The species *Raphanus sativus* and *Sinapis alba* were frequently found, though no records existed in the nationwide database Floraweb (2009).

3.3 Frequency of OSR-related wild species in biotopes neighbouring arable fields

To analyse the spatial potential for OSR hybridisation with related wild species close to arable fields, we determined relevant biotopes in 50 m buffers around all fields in Brandenburg (see Fig. 1; Table 3). The results show that most biotopes in the buffers were relevant habitat types for OSR-related wild species. The biotopes with a high average frequency (>10 %) of OSR-related species (arable lands, settlements and industrial areas, disturbed areas, road verges and gardens) comprised 74.6 % of the buffered areas. The share of relevant biotope types adjacent to arable fields is also proportional to the composition of relevant biotopes in the whole of Brandenburg (see Table 2).

Table 3 Composition of biotopes containing OSR-related wild species within a 50 m buffer around arable fields (Brandenburg state)

Relevant biotope types	Buffered area (ha)	% Total buffered area
Meadows	9,928	9.0
Dry grasslands	1,077	1.0
Disturbed areas	12,272	11.1
Hedgerows, plantations	6,221	5.6
Arable land	39,598	35.7
Gardens	10,686	9.6
Specific biotopes	248	0.2
Settlements and industrial areas	26,643	24.0
Road verges	4,193	3.8

The cluster analysis of biotope type combinations within the 50 m buffers of all arable fields showed five different biotope combinations (clusters) in Brandenburg (Table 4). The discriminant analysis resulted in 80.4 % of all buffers being correctly classified. The proportion of each biotope type within the region was highly variable. With regard to biotope types having a high frequency of OSR-related wild species (see Tables 1 and 2), cluster 2 (settlements and industrial areas, gardens, arable land and disturbed areas), cluster 5 (disturbed areas, road verges, arable land and some settlements and industrial areas) and cluster 4 (arable land and to a less extent settlements and industrial areas) are of great importance. Combined, they cover 83.2 % of the area and include all species hybridising with OSR. Cluster 2 is the most frequent combination (53.3 %), followed by cluster 4 (25.1 %).

Combining the clustering results (Table 4) with the frequency of occurrence of the different potential OSR hybridisation partners (see Table 2) allowed us to (a) assess the regional outcrossing risk for GM OSR and (b) target GMO monitoring to the most sensitive biotopes and wild relative species. In this regard, despite the fact that eight out of 14 potential hybridisation partners occur in regions dominated by biotope combinations described in cluster 1, they have a low hybridisation risk because their frequency of occurrence is considered low. Cluster 5 carries the highest hybridisation risk because most hybridisation partners occur there at high frequencies. Monitoring in regions dominated by cluster 5 should focus on disturbed areas, road verges, gardens and plantations. Cluster 3 has a medium hybridisation risk, and monitoring in these regions should focus on settlements and

Table 4 Cluster analysis and clusters of biotope types with OSR-related wild species in 50 m buffers adjacent to arable fields (*italic* highlights biotopes with higher percentages of OSR-related wild species)

Biotope types in the 50-m buffer area	Cluster 1 hedgerows, plantations (% area)	Cluster 2 settlements and gardens (% area)	Cluster 3 meadows and settlements (% area)	Cluster 4 arable land (% area)	Cluster 5 Disturbed areas, road verges, arable land (% area)
Meadows	0–1	0–22	*20–100*	0–1	0–4
Dry grasslands	0–2	0–1	0–1	0–1	0–1
Disturbed areas	0–35	0–28	0–20	0–10	*0–100*
Hedgerows, plantations	*0–100*	0–2	0–2	0–2	0–2
Arable land	0–33	*0–60*	*0–66*	*48–100*	*0–78*
Gardens	0–2	*0–70*	0–19	0–2	0–2
Specific biotopes	0–2	0–1	0–1	0–1	0–1
Settlements and industrial areas	0–2	*0–100*	*0–65*	0–30	0–31
Road verges	0–2	0–2	0–2	0–2	*0–78*
% relevant area buffered	8.3	53.3	8.6	25.1	4.8

industrial areas as well as arable land. Meadows are of minor importance with regard to biotope types and frequency of OSR-related wild species. Cluster 4 is dominated by agricultural land bordering other arable land. Here, monitoring the GM OSR and potential GM hybrids is of the highest importance.

3.4 Biotope sensitivity to GM OSR hybridisation in different ecoregions of Brandenburg

Cross-tabulations of (a) the biotopes relevant to OSR hybridising partners and (b) the aforementioned five clusters with the ecoregion map of Brandenburg (Graef et al. 2005) were carried out to upscale local biotope composition results to larger areas (Table 5). Our results show that all relevant biotope types are present in all Brandenburg ecoregions. There were differences between ecoregions in the proportion of each biotope, especially hedgerows, arable land, gardens and road verges. Biotope type areas were weighted with the number of present hybridising species (see Table 2). Biotopes with the highest frequency (11–14 species) of OSR-related wild species (arable land, disturbed areas and road verges) predominate in (a) glacial valleys, bogs and the Elbe-Elster lowland, (b) the Uckermark moraine plateaus, and (c) Central and East Brandenburg heathland and wetland. Other biotopes with medium-high frequency (8–9 species) of OSR-related wild species predominate in (a) Oder valley, (b) Central and East Brandenburg heathland and wetland, and (c) Schorfheide and Fläming. Few differences were observed between ecoregions in non-relevant areas such as meadows and dry grasslands. Weighting biotope areas with the number of hybridising species (see Table 5) provides a more focused picture of the ecoregions that are critical for monitoring. Hence, the Oder valley and the Uckermark moraine plateaus have the largest areas of relevant biotopes with the highest numbers of hybridising species. Inclusion of all biotopes present in Brandenburg reveals an even greater ecoregion-specific biotope composition (data not shown).

The aim of clustering buffered biotopes was to upscale the biotope sensitivity to the area of Brandenburg State, in order to determine regional differences in monitoring requirements and to compare the regionalisation results with the ecoregion classification scheme. The proportions of biotope buffer clusters differ only slightly between ecoregion types (data not shown). Cluster 2 (settlements and gardens), for instance, covers a greater share of the Oder valley and Uckermark moraine plateaus than in other ecoregions. Cluster 4 (arable land) predominates in the Prignitz, West Barnim moraine plateaus and the Uckermark moraine plateaus. Altogether, the results of the cross-tabulation with the biotope clusters are similar to those found with the biotope groups, thus indicating that biotopes neighbouring arable fields have a composition similar to that of the more distant biotopes.

Our analysis of the location and composition of biotopes containing OSR-related wild species adjacent to arable fields clearly indicates that some regions in Brandenburg must be considered more critical for monitoring. This is also shown in Fig. 3 using the Arc/Info density function. Those areas and regions with a higher frequency of critical biotopes are highlighted and thus may serve as a basis for a more focused monitoring.

4 Discussion

The objective of the present paper was to develop a methodology to regionalise the hybridisation risk of GM OSR

Table 5 Cross-tabulation of biotopes relevant for OSR hybridising partners with ecoregions in Brandenburg

Ecoregion in Brandenburg	**Total area in Brandenburg (%)**	**Meadows**	**Dry grasslands**	**Disturbed areas**	**Hedgerows, plantations**	**Arable land**	**Gardens**	**Specific biotopes**	**Settlements and industrial area**	**Road verges**
		% ecoregion area; in brackets: % ecoregion area weighted with No. of hybridising species (Table 2)								
Prignitz, West Barnim moraine plateaus	19.0	10.2 (20.4)	0.2 (0.9)	9.4 (132.0)	7.9 (62.8)	37.5 (412.5)	9.3 (83.9)	0.2 (1.8)	22.8 (205.6)	2.5 (29.5)
Oder valley	4.1	10.3 (20.6)	0.4 (2.1)	13.2 (185.3)	1.2 (9.4)	26.1 (287.6)	20.8 (187.0)	0.3 (2.3)	26.9 (242.3)	0.8 (9.0)
Glacial valleys and bogs, Elbe-Elster lowland	26.3	9.1 (18.1)	0.2 (1.1)	11.2 (157.4)	5.8 (46.5)	36.2 (398.2)	10.4 (93.9)	0.3 (3.0)	23.7 (213.3)	3.0 (36.0)
Uckermark moraine plateaus	8.6	10.0 (19.9)	1.5 (7.6)	13.1 (184.0)	2.1 (16.6)	37.9 (417.2)	8.0 (71.6)	0.1 (0.9)	25.6 (230.7)	1.7 (20.2)
Central and East Brandenburg heathland and wetland	19.5	7.7 (15.3)	0.3 (1.4)	9.6 (134.5)	8.3 (66.6)	36.9 (405.7)	9.7 (87.2)	0.5 (4.1)	23.1 (208.0)	4.0 (48.2)
Schorfheide, Fläming	22.6	8.5 (17.0)	1.0 (5.1)	10.0 (140.4)	5.1 (40.6)	35.2 (387.4)	10.5 (94.2)	0.8 (6.9)	25.8 (231.8)	3.2 (37.9)

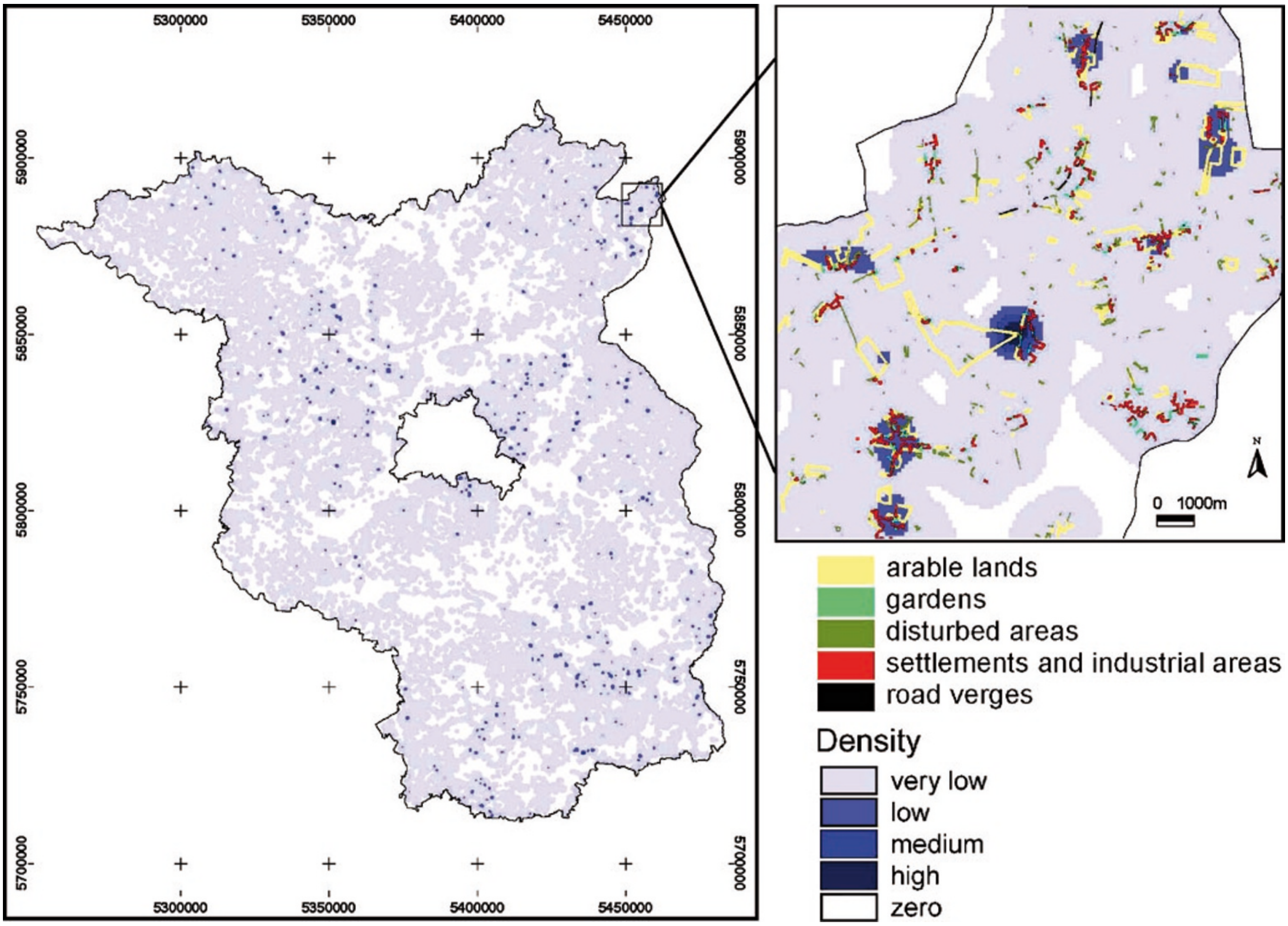

Fig. 3 Oilseed rape hybridisation potential in Brandenburg: Density of biotopes with highest frequency of OSR wild relatives (Arc/Info density function, pixel size 100 m, search radius 1 km)

with respect to the potential presence of related species. This methodology can be used to focus GMO monitoring schemes on the most sensitive landscape elements. For this purpose, the likelihood that different landscape elements contained OSR and related species first had to be assessed.

The risk of GM OSR hybridisation and persistence depends on (a) the related species‘ potential to hybridise and produce viable progeny (see Table 1), (b) the presence of hybridisation partners (see Table 2, both OSR and related species), and (c) the presence of neighbouring arable fields and relevant biotopes (see Tables 3 and 4). Integrating the related species‘ potential to hybridise and produce viable progeny, the frequency of related species and the frequency of neighbouring sites with OSR wild relatives, we arrive at the following ranking for hybridisation risk in the agro-environment: Disturbed areas > arable land > road verges > settlements and industrial areas > gardens.

These results correspond to those of Jørgensen et al. (2006), Scheffler and Dale (1994) and Wilkinson et al. (2000). To identify the relevant biotopes neighbouring OSR fields, we chose a distance of only 50 m even though OSR pollen has been reported to be carried across much larger distances (Champolivier et al. 1999; Rieger et al. 2002) depending on local environmental conditions (Middelhof et al. 2009; Schmidt and Schröder 2009). However, the crossing rate rapidly decreases within a 50 m distance from the OSR pollen source (Ramsay et al. 2003) and because of the high detail of the biotope maps, larger distances would not have added information on the hybridisation potential. Hence, our results on occurring related species and relevant biotopes should be considered as an underestimate of the overall hybridisation potential.

Our aim was to test whether our approach was suitable for identifying higher and lower risk ecoregions. This information would aid the design of monitoring schemes that focus on, for instance, the GM OSR hybrids that are most likely to persist, the most sensitive biotopes in the region, and those areas and fields where OSR is presently grown. Our approach was shown to be feasible because biotope composition and the frequency of wild relatives differ between ecoregions (see Table 5) and throughout Brandenburg state (see Fig. 3). Ecoregions more critical for monitoring are the Oder valley and the Uckermark moraine plateaus.

The cluster analysis of biotopes neighbouring arable fields yielded more precise spatial differentiation than the cross-tabulation of biotopes with the ecoregions, but only at a spatial scale below that of ecoregions. At larger spatial scales, ecoregions provide adequate information relative to the presented buffer method. Ecoregions can therefore be used for regionalisation of monitoring needs at larger scales like the state of Brandenburg. Greater differentiation between ecoregions would be expected if biotope classifications included water regime and trophic levels or if a higher number of more detailed ecoregion types had been selected for overlay and extrapolation (Graef et al. 2005). If comparable biotope datasets at different scales were available, upscaling or extrapolation of our local relevée and biotope results to even larger areas would be possible, as demonstrated by Metzger et al. (2005). Nationwide spatial biotope datasets are scarce and EU-wide data do not yet exist. On the basis of our findings and existing data, we show that all Brandenburg ecoregions contain biotopes with OSR-related wild species, some with a high frequency. Thus, the potential for OSR to hybridise with feral OSR and related wild species exists throughout Brandenburg.

Hybridisation is not limited to direct interactions between OSR and other plants. Hybrids may also cross with other species that are not directly hybridising with OSR. Through these transfer bridges, transgenes may ingress to other Brassicaceae (Brown et al. 1995; Pascher et al. 2000). In this study, however, we did not consider any distantly related Brassicaceae except for *Descurainia sophia*.

Our results should be considered in the context of the European Directive 2001/18/EC (European Community 2001). This regulatory framework requires an environmental risk assessment and a monitoring plan to detect additional potential adverse environmental effects before GM crops can be commercially cultivated (Züghart et al. 2008). Our results show that GM OSR is likely to hybridise with related species throughout many biotopes and in all ecoregions of Brandenburg. Most of the related species and their genera occur throughout Germany (Floraweb 2009); many are also found in the main OSR cultivation areas of Europe, Asia and North America (Warwick et al. 2009). Our results therefore also apply to a more global context. The likelihood that GM OSR hybrid progeny will persist in the environment depends on many factors, for example its viability, selective advantage of the GM trait and local environmental conditions (Breckling et al. 2009). Transgenic OSR hybrids persist in arable lands for years in Canada (Beckie et al. 2006; Warwick et al. 2007). However, no GM OSR hybrids have yet been reported to spread into the environment. Still, our results, together with the data from other studies, suggest that the environmental risk of cultivating GM OSR in the EU must be considered very carefully. For regions where GM OSR has already been approved and cultivated, we suggest that populations of related species and the potential spread of GM traits be monitored using a targeted approach as presented above.

5 Conclusions

Our case study, combined with results of other studies, shows that if GM OSR is cultivated in Brandenburg, the risk of hybridisation with related species and feral OSR in biotopes of neighbouring arable fields will be considerable. We developed a methodology suitable to link spatially limited but highly detailed datasets on the occurrence of potential hybridisation partners for GM OSR with regional datasets. The hybridisation risk can be extrapolated based on this analysis, such that GM OSR monitoring can in the future focus on relevant biotopes for OSR and its wild relatives. Our results show that the following biotopes contain the largest numbers of OSR-related species, including those species with high hybridisation rates and high viability of progeny: disturbed areas, arable land, road verges, settlements, industrial areas and gardens. These biotopes have a variable distribution throughout the ecoregions of Brandenburg, suggesting that the hybridisation risk differs to some extent across large regions. Even though GM OSR hybrids have not yet been reported to spread into the environment, the potential cultivation of GM OSR in the EU should be considered very carefully. The results also apply to a more global context because the surveyed OSR-related species and their genera occur throughout large regions of the Northern hemisphere.

6 Recommendations and perspectives

We recommend application of the methodology presented here and further development of a harmonised approach across different states and regions to monitor the potential ingression of transgenes into GM crop relatives. Although the availability, quality and scale of biotope maps as well as floristic data varies between EU states, we are convinced that developing a standardised procedure for spatial monitoring design is possible and important to produce comparable data across state boundaries. Other GM crop monitoring guidelines have already been developed, for instance, by The Association of German Engineers.

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