Europ. J. Agronomy 32 (2010) 121–126

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

You reap what you sow – or do you? – volunteers in organic row-sown and broadcast-sown oilseed rape fields

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ARTICLE INFO

Article history:
Received 18 September 2008
Received in revised form 10 August 2009
Accepted 1 September 2009

Keywords:
Brassica napus
Seed dormancy
Organic farming
Seed persistence
Genetically modified crop
ISSR

ABSTRACT

The frequency and origin of oilseed rape volunteers in organic row and broadcast-sown oilseed rape fields was evaluated using DNA markers (ISSR) for analysis of potential volunteers in nine organic oilseed rape fields with different history. Using the software AFLPOP, the potential volunteers were assigned to variety by comparing their ISSR fingerprints with those of present and previous varieties. Results showed that two of the five row-sown fields, had high numbers of volunteer in the row (5 and 9 volunteers m^{-2}), and as inter-row volunteers were also present (0.9 and 3.6 volunteers m^{-2}), the recommended inter-row hoeing should not be neglected. Potential volunteers were also identified in the broadcast-sown fields, but their identity could not be proven. In a scenario where GM oilseed rape is cultivated previous to the organic oilseed rape, the present results indicate that the frequency of GM volunteers would exceed the threshold of 0.9%, and thus GM labelling would be demanded. Some of the volunteers belonged to varieties cultivated 8–11 years previously, indicating a long persistence of volunteer populations. What you reap might be added in the past – with consequences for organic farmers' production and earning.

1. Introduction

Genetically modified (GM) oilseed rape (OSR) will most likely be cultivated in Denmark as well as in the rest of the EU countries in the medium term. Increasing effort is therefore directed towards identifying the problems with coexistence between GM and non-GM OSR (organic and conventional OSR) to limit that the transgenes disperse among fields. The processes of transgene dispersal are similar to non-GM gene escapes from fields in the agricultural landscape (Chandler and Dunwell, 2008). The spread of GM OSR might affect the purity and quality of the harvest of non-GM OSR.

To ensure products with a low level of GM, an EU threshold of less than 0.9% has been set for GM in non-GM food and feed (EC Regulation No, 1830/2003). However, recent studies based on GENESYS-modelling (Colbach et al., 2001a,b, 2008; Fargue et al., 2005, 2006) show that in many cases, it is difficult to meet the EU threshold due to impurities from volunteers if GM varieties previously have been grown in the field (Colbach et al., 2008). The modelling by Colbach et al. (2008) indicate that it might be problematic to grow organic OSR (or non-GM OSR) in fields where GM OSR has been grown previously, because if the threshold is exceeded, the product has to be labelled as GM OSR, thus probably bring in less.

OSR pods shatter very easily (Lutman et al., 2003) and a significant seed loss occurs in the field due to natural shedding and crop disturbance by harvesting machinery (Price et al., 1996). In winter OSR, direct cutting is found to give significantly lower losses of seeds than swathing and picking up (Price et al., 1996). The 'normal' seed losses during ripening and harvest are found to be about 6000 seeds/m^2 (Lutman and Lopez-Granados, 1998), a number that can be more than double if the harvest is delayed (Price et al., 1996) or due to climate events such as heavy storms. This 'normal' seed loss is the major source of the oilseed rape seed-bank.

OSR is able to establish long-lived seed-banks due to induced seed dormancy in the deeper soil layers (Pekrun et al., 1997; Gruber et al., 2004), when the seeds are incorporated into the soil by post-harvest tilling. The number of incorporated seeds is reduced by leaving the seeds to germinate on the soil surface before deep soil tilling (Lutman et al., 2003; Rasmussen, 2004). However, after 2 years there might be up to 800 seeds m^{-2} in the seed-bank, which is about 10 times as much as usually sown (Tolstrup et al., 2007). At the time during the conventional Danish crop rotation, when the oilseed rape seed-bank is assumed to be most depleted, 50–100 seeds/m^{-2} were found (Jørgensen et al., 2007). The persistence of incorporated OSR seeds differs among varieties due to the fact that different genotypes have different potentials for dormancy (Gruber et al., 2004). The depth of burial is also important because

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1161-0301/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.eja.2009.09.001

seeds incorporated in deeper layers are better preserved (Lutman et al., 2003).

In correspondence with the relatively high number of seeds found in Danish seed-banks, high frequencies of flowering OSR volunteers are observed in many Danish crops e.g. barley, pea and oilseed rape (Tolstrup et al., 2003; Jørgensen et al., 2007). Some of the volunteers set seeds that replenish the seed-bank (Jørgensen et al., 2007; Gruber and Claupein, 2007, 2008). A cropping interval of at least 4 years between OSR cultivation is recommended to minimize the dissemination of pests and diseases, but also reduces the amount of viable OSR seeds in the soil seed-bank. Recent studies show that the seed persistence of OSR in the seed-bank of arable fields is up to 17 years (Jørgensen et al., 2007; Messéan et al., 2007; D'Hertefeldt et al., 2008), so likely the soil seed-bank is not drained by the 4 years of cropping interval.

In conventional fields, volunteers of herbicide-susceptible OSR are most often controlled by the use of herbicides, but in organic fields this is not an option, and once emerged the volunteers can only be controlled mechanically.

Organic OSR fields constitute approximately 0.6% (800/125600 ha) of the total OSR area in Denmark (Tolstrup et al., 2007), but are believed to expand here and elsewhere, as the demand for organic oil and feed increases. Compared to spring oilseed rape winter oilseed rape is preferred by the organic farmers, one reason being its competitiveness against weeds, but it has the potential to become a weed problem itself in subsequent crops. The preceding crops to oilseed rape are often clover or clover-grass that produces a supply of N to the organic oilseed rape, but manure still has to be added. After thorough tilling, sowing of organic winter OSR takes place in mid August and is performed in two ways: with a distance between rows of 50 cm (row-sown) and of 12 cm (broadcast-sown). Row-sowing is recommended because it allows inter-row weed control (hoeing) and requires less seed to be sown. Hoeing is performed just after germination of the crop and often repeated in the spring. In row-sown fields the obtained OSR density is 35–40 plants m^{-2}. In comparison the OSR density aimed at is doubled in broadcast-sown fields. Experiments in conventional OSR cropping systems show that the yield is only marginally influenced by row distance if weeds are controlled (Danish Agricultural Advisory Service, DAAS). Harvest takes place from mid-July until early August. It is strongly recommended to remove the straw, or if it is not removed then to cut and roll the straw to promote germination of spilled seeds.

The aim of the present study was to study the origin and abundance of oilseed rape volunteers in organic OSR fields under natural conditions. Using molecular markers, we identified potential volunteers in organic OSR fields with different history and cultivation practices. For example the selection of fields comprised both row-sow and broad-cast sown fields, and comparing volunteer frequencies under these two types of practice would indicate if inter-row weeding could limit the number of GM volunteers below the threshold of 0.9% in row-sown fields. Potential volunteers were assigned to variety, by comparing their anchored ISSR (InterSimple Sequence Repeat) polymorphisms with those from a collection of reference varieties. The age of the volunteer populations was estimated by combining the information on current and previously cultivated varieties of OSR in the fields of collection.

2. Materials and methods

2.1. Plant material

Nine organic OSR fields in Denmark were investigated in 2005 or 2006; five row-sown and four broadcast-sown fields (Table 1). Four of the nine organic OSR fields (fields A, B, H, I) were located at Zealand. The remaining five fields (fields C, D, E, F, G) were located at the south-eastern part of Jutland. Though regional climatic differences in a given year can occur (mainly in relation to mm rainfall), the general climatic conditions among farms are assumed to have been rather similar, and likewise with the general OSR cultivation practice. Farmers do not register their cultivation practice in details, but only small differences in agricultural management (e.g. sowing dates, manure supply, inter-row distances) are likely. The general practice of cultivating organic oilseed rape is described in Section 1, the Introduction.

In the five row-sown fields, potential volunteer OSR was localized by focusing on individuals growing between the rows. A month after sowing, but before the first hoeing (ultimo September) OSR inter-row plants (potential volunteers) were counted in 50 random subplots of 0.7 m^2 (2 m × 0.35 m) in each field and the average density of potential volunteers was calculated (Table 1). Up to 20 individuals were randomly collected between the rows for ISSR analysis, together with approximately the same number of individuals in the row (Table 2). In the broadcast-sown fields it was difficult to distinguish the rows clearly, and instead 34–40 individuals were collected randomly in each field. In addition, seed samples of the certified seed lots cultivated in the nine fields (varieties 'Caracas' and 'Canberra') were included as references. For information about possible sources of volunteer impurities in the analyzed fields, farmers were interviewed about previously cultivated varieties of *Brassica napus* (Table 1). Seed lots of previously cultivated varieties were included in the analysis as well as certified seed lots of four varieties commonly cultivated from 1985 to 2000. The varieties were 'Bristol', 'Cannon', 'Capitol', 'Cerex', 'Express', 'Global', 'Korina', 'Olivia', 'Starlight' and 'Tarok'. The seed lots of these varieties were the original variety 'standard identifies' obtained from the Danish variety testing authority (The Danish Plant Directorate). To verify that the collected plants (four-leaf stage) belonged to *B. napus*, samples of *Brassica rapa* and *Sinapis arvensis* from Zealand (own collections) were included in the ISSR analysis. These two species can occur as weeds in OSR fields, and their seedlings can

Table 1
Characteristics of the nine analyzed organic fields with winter oilseed rape.

Field	Broad/row-sown	Variety	Field size, ha	Collection year	Number of plants collected inter-row, m^{-2} (±STEDV)	Previously cultivated varieties
A	Broadcast-sown	Caracas (w)[a]	17	2006		1995: 'Express' (w); 1987: 'Korina' (w); 1982: 'Olivia' (?)
B	Broadcast-sown	Canberra (w)	18	2006		1996: 'Canberra' (w); 1992: 'Canberra' (w)
C	Broadcast-sown	Caracas (w)[a]	6	2006		None since 1976, before that no knowledge
D	Broadcast-sown	Caracas (w)	7	2006		None since 2001, before that no knowledge
E	Row-sown	Caracas (w)	5	2006	0.61 (±1.04)	Unknown varieties were cultivated before 1996
F	Row-sown	Caracas (w)	4.4	2006	1.20 (±1.79)	None since 1988, before that no knowledge
G	Row-sown	Caracas (w)	13	2006	0	None since 2000, before that no knowledge
H	Row-sown	Caracas (w)	38	2005	3.60 (±3.34)	2000: 'Bristol' (w); 1994: 'Starlight' (s)
I	Row-sown	Canberra (w)	13.3	2005	0.91 (±1.76)	1997: 'Capitol'

Nomenclature: STEDV = standard deviation; (w) = winter variety; (s) = spring variety

[a] Mixed with seeds from earlier productions of Caracas and/or Canberra.