# Uncleaned crop seed sowing as a tool to conserve *Bromus grossus* and restore species-rich arable-dependent plant communities

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## SUMMARY

Segetal plants, which grow preferentially or exclusively in cereal fields, experienced a strong decline during the last century. Among them, *Bromus grossus* received particular attention, as it is highly threatened in Europe. Its decline is thought to be due to crop seed cleaning among other causes. Re-establishing the sowing of uncleaned crop seeds should therefore be considered as a tool for the conservation of this species. In this study, we aimed to evaluate (i) how the conservation of *B. grossus* relies on transfer in uncleaned crop seed, (ii) how this practice may help to restore new populations of this species, and (iii) the contribution of this practice to the dispersal of other segetal plants. From 2012 to 2016, we monitored eight fields from three farms in Southern Belgium where uncleaned spelt seed containing *B. grossus* was sown. We found that *B. grossus* grew in the year following seed sowing, but disappeared in the second year in most cases. This highlights the extreme dependence of *B. grossus* upon uncleaned spelt-seed sowing. We also showed that, through associated management practices, *B. grossus* acted as an 'umbrella species' to other arable-dependent plants. Transfer of uncleaned seed led to an increase in species richness in an experimental field from 12 species in 2015 to 43 species in 2017. Based on the germination of uncleaned seeds in a greenhouse, we concluded that it was likely to account for the dispersal of at least nine species, and possibly 15 others.

## BACKGROUND

Among the flora associated with arable fields are segetal plant species, which grow preferentially or exclusively in cereal fields (Rotchés-Ribalta *et al.* 2016). Segetal plants have undergone a strong decline during the last century due to changes in agricultural practices, and are now highly threatened throughout Europe (Storkey *et al.* 2012).

Among threatened segetal species, whiskered brome *Bromus grossus* Desf. ex DC. (Poaceae) has received particular attention. *B. grossus* is a 1-1.4 m high grass that grows preferentially in spelt *Triticum spelta* wheat crops. *B. grossus* is an anecophyte, and has no known natural habitat; it is only found in cultivated fields. Its life cycle mimics that of spelt, with germination occurring in autumn, flowering in early summer, and seeds that remain attached to the panicle at harvest time (end July–early August), so the spikelets are harvested with the spelt.

*B. grossus* is endemic to Europe, where it grows in only two EU countries (Belgium and Germany) and is threatened in both of them according to the latest reporting on the Habitat Directive for the period 2007-2012 (European Topic Centre on Biological Diversity 2014). Outside EU member states, it is also present in Switzerland, where it is endangered (Käsermann 1999). It was previously known in France, Luxembourg, Italy, Austria and former Czechoslovakia (Smith 1973). It is therefore considered the most threatened arable plant in Europe (Storkey *et al.* 2012). The species is listed in Annex II and IV of the European Habitats Directive 92/43/CEE and needs special conservation efforts in the EU countries where it still occurs.

The main causes of segetal plant declines have been the increase in herbicide and fertilizer use, as well as increasing field sizes leading to a decrease in density of field margins, which are refuge areas for segetal plants (Albrecht *et al.* 2016). Improved seed-cleaning processes may have also played a major role in this decline, especially for "crop mimic" species with large seeds, such as *B. grossus* and *Agrostemma githago* L.. These species are thought to depend on regular, inadvertant reintroduction alongside crop seed (Albrecht *et al.* 2016), but the extent and frequency of reintroduction that is required is not known. Other segetal species may also have been affected by seed-cleaning, as uncleaned seed may act as a more general dispersal pathway. Restoring dispersal of segetal plants through human activities, including dedicated management, is key for their long-term conservation (Bonn 2004, Mayer & Albrecht 2008). However, the re-establishment of suitable conditions at the field level, for example through organic agriculture and agri-environmental schemes (AES), may not be sufficient to recover populations of the most threatened species (Lang *et al*. 2016, Lemoine *et al*. 2018). The conservation programme for the legally-protected *B. grossus* therefore offers an opportunity for the long-term conservation of other segetal species which, although highly threatened, have no legal status in Wallonia, South Belgium. To assess how *B. grossus* may act as an 'umbrella species' to other segetal plants, we need to evaluate how uncleaned crop seed sowing for *B. grossus* conservation contributes to the dispersal of other arable plants.

In this study, we aimed to evaluate (i) how the conservation of *B. grossus* relies on uncleaned crop seed sowing, (ii) how this practice may help restore new populations of this species, and (iii) the contribution of this practice to the dispersal of other segetal plants.

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ISSN 1758-2067

## ACTION

***Bromus grossus* conservation program and monitoring:** Although *B. grossus* was considered extinct in Belgium, it was rediscovered in 2010-2012 in a few fields in the vicinity of Musson, Luxemburg Province (Delescaille *et al*. 2011). As soon as the plant was rediscovered, local farmers were encouraged to enter a conservation programme. This consisted of the conservation of a proportion of *B. grossus*-contaminated spelt, by sowing it from one field to another within the farm. No pesticides or herbicides were allowed in the years when uncleaned spelt was sown in a field. The financial incentive to support the project was provided through an AES dedicated to threatened arable plant conservation (Lemoine *et al*. 2018). The AES only included field margins (12 m width), and was paid at a rate of 1250 €/ha of margin. In practice, however, most farmers sowed entire fields with *B. grossus*-contaminated spelt.

The results described here were collected from the first three farms that entered this conservation programme in 2013. These were used to validate the efficacy of the programme prescriptions and to test some introduction and management options for *B. grossus*. All seed transfers within and between farms are shown in Figure 1. Apart from farm 2 in 2012, all seed management used normal farming machinery (cereal harvesters and seed drills).

Farm 1 was considered the "reference" farm, as the highest number of *B. grossus* plants were recorded there during the period 2010-2012. Monitoring of this farm therefore described the dynamics of a pre-existing population. However, it cannot be considered a perfect reference site, as inclusion in the *B. grossus* conservation programme in 2013 approximately coincided with the farm's conversion to organic farming. Four fields from this farm were monitored.

At farm 2, *B. grossus* was initially introduced in July 2012 through hand-collected seeds from farm 1. Seeds were mixed with pure spelt seeds and sowed with a standard cereal seed drill in autumn 2012. This resulted in a *B. grossus* seeding density of approximately 1 kg/ha. Three fields from this farm were monitored.

At farm 3, *B. grossus* was initially introduced in 2012 by direct re-sowing of uncleaned spelt seeds harvested from farm 1. At this farm, in 2013-2014 we tested whether storage alters *B. grossus* growing potential, by storing uncleaned spelt seeds containing *B. grossus* for one year in a barn, and then sowing it the next year (Figure 1). This management option may be useful if farmers are not able to dedicate fields for *B. grossus* cultivation every year. Storage may allow farmers to combine *B. grossus* conservation and the upkeep of crop rotations in dedicated fields. One field from farm 3 was monitored.

In the three farms, *B. grossus* densities were recorded from 2013 to 2016 (eight fields in total). Monitoring began in different years in different fields, starting in the first year that uncleaned spelt seeds were sown during the 2013-2016 period (as the fields to be used were not all known at the start of the study). Recording consisted of counting *B.grossus* panicles in six 1 x 1 m plots in each field, in each recording year. Counts from the six plots were averaged to give an average density for each field. All fields were located in the extreme southeast of Belgium (latitude range: 49°30'32'' – 49°34'33'' N,

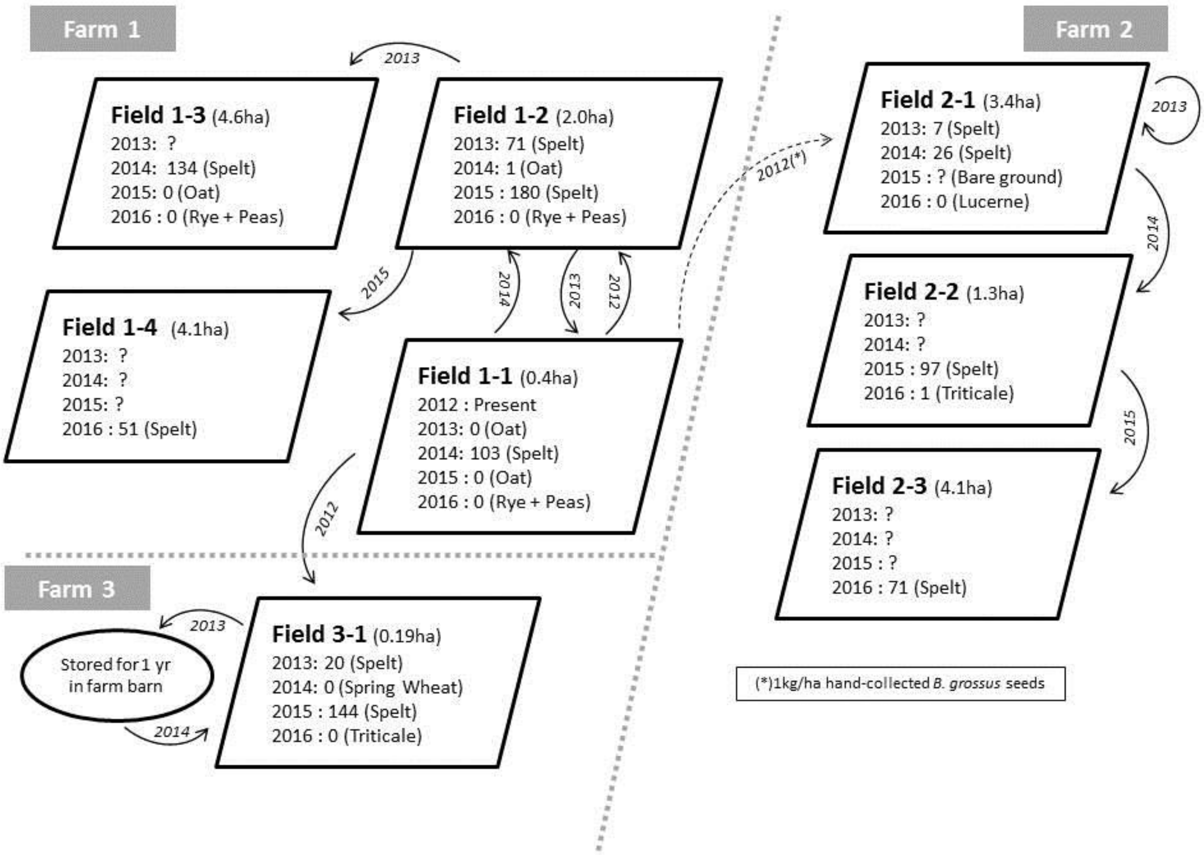


**Figure 1**. Schematic view of monitored fields and spelt seeds transfers. Figures represent *Bromus grossus* densities in each field and in each year from 2013 to 2016, as mean panicles/m². Arrows indicated uncleaned spelt seed transfer. Transfers occurred in the autumn of the indicated year. The crop in each field in each year is shown in parentheses. Question marks indicate fields were not monitored in that year.



ISSN 1758-2067

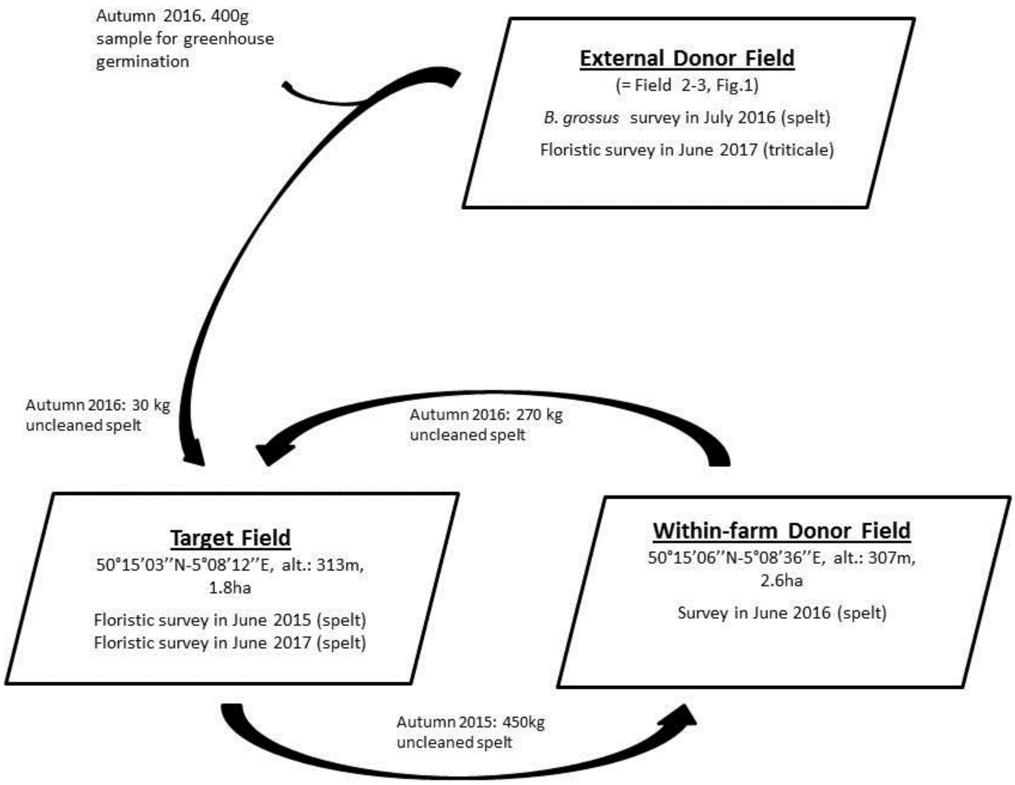


**Figure 2.** Schematic representation of transfers of uncleaned crop seed, as part of the *B. grossus* conservation programme, to restore species-rich segetal plant communities, and the surveys carried out to monitor the impact.

longitude range: 5°28'33''– 5°38'56'' E, elevation range: 266 m -323 m).

**Seed transfer evaluation:** In 2016-2017 we carried out further studies in order to evaluate how uncleaned seed transfer can contribute to the wider restoration of species-rich arable plant communities, and to determine which species are likely to be transferred. We took advantage of the fact that a farm previously committed to AES for segetal species conservation, and therefore monitored for it, entered the *B. grossus* conservation programme in 2016.

In autumn 2016, we procured approximately 30 kg of uncleaned spelt seed, harvested from field 2-3 (Figure 1), here called the 'external donor field' (Figure 2). We collected the seed from the farm barn approximately two weeks after the spelt was harvested. A floristic survey of the external donor field was carried out in June 2017, the year following seed transfer (Figure 2).

In autumn 2016, seeds from the 'external donor field' were sown in a 'target field', located on a different farm. The target field species composition was surveyed in June 2015, before the seed transfer when the field was under spelt cultivation. As the target field size was 1.8 ha, 30 kg seeds were insufficient to sow the entire field at a standard 170 kg/ha sowing density. Therefore, we blended the uncleaned seed with 270 kg seeds harvested in a nearby field, belonging to the same farmer as the target field, referred to here as 'within-farm donor field' (Figure 2). The within-farm donor field had itself been sowed in autumn 2015 using seeds harvested in the target field (Figure 2). The within-farm donor field species composition was surveyed in June 2016.

Of the 30 kg seed procured from the external donor field, 400 g was sampled to determine seed composition. The sample was first hand sorted into three categories: (i) spelt seeds, (ii) *B.grossus* seeds, and (iii) 'other' seeds. In November 2016, the 'other' seeds were sown in 30 x 25 x 7 cm containers filled with potting soil. Containers were then put to germinate in an unheated greenhouse and regularly watered until the end of seedling emergence in July 2017. All emergent seedlings were identified, counted and removed. Unidentifiable seedlings were transferred to separate containers and grown until identification was possible.

In June 2017 (after seed transfer), a second floristic survey was carried out in the target field to determine differences from 2015 (before seed transfer). We thereafter classified the species recorded in all species surveys (target field 2015, target field 2017, external donor field 2016, within-farm donor field 2016 and species emerged in greenhouse) according to the likelihood that they were transferred with uncleaned spelt seeds. We categorized species into five likelihoods using the procedure shown in Figure 3: (i) evident transfer, (ii) possible transfer, (iii) failed transfer, (iv) unknown, (v) unexplained advent. We considered a transfer as evident for species that appeared in the target field between 2015 and 2017 and were also recorded in the greenhouse experiment. We considered it as possible transfer either when they appeared in the target field while the species was not recorded in the greenhouse, or when they were recorded in greenhouse but were already present in target field in 2015 (Figure 3). In this latter case, the transfer may have reinforced an existing population. We then tested, using a chi-square test, whether transfer likelihood (evident OR possible vs. failed) differed between five categories of species: (i) threatened segetal species, (ii) other segetal species, (iii) other annual species, (iv) other perennial species and (v) pernicious species. Segetal status was established based on the habitat descriptions in Lambinon *et al.* (2004), and by comparison



ISSN 1758-2067

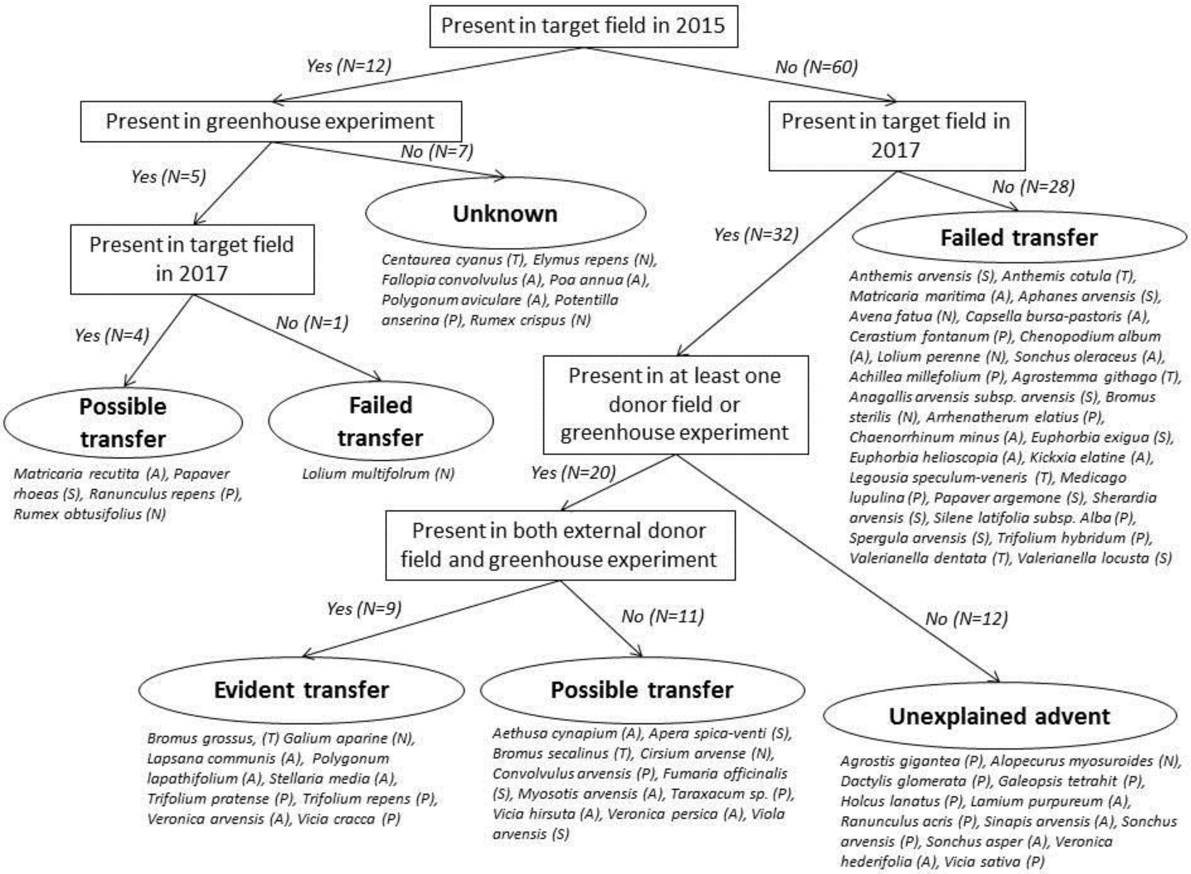


**Figure 3.** Decision tree used to determine species transfer likelihood with uncleaned spelt-seeds, and the species that fell within each likelihood category. Species were classified as: threatened segetals (T), other segetals (S), other annuals (A), other perennials (P), pernicious (N).

with neighbouring countries' segetal species lists (Cambecèdes *et al.* 2012 for France, Hofmeister & Garve 1998 for Germany). Conservation status was taken from the Walloon Red List for plant species (Saintenoy-Simon *et al.* 2006). Pernicious species were discriminated according to Storkey & Westbury (2007).

All floristic surveys were made according to the AES monitoring protocol, which consists of slowly walking along all field edges approximately 2 m inside the field margin, recording all observed species.

## CONSEQUENCES

**Transfer of *B. grossus*:** Monitoring revealed that uncleaned seed sowing was an essential measure for the conservation of *B. grossus* conservation, as populations of the species were observed only after fields were seeded with uncleaned spelt (Figure 1). Generally, *B. grossus* disappeared from the field in the year following introduction with uncleaned seeds. Occasionally, low densities were still observed in the following year, especially when winter cereals were grown, as observed in Field 2-2 in 2016 (Figure 1). Conversely, seed path monitoring (i.e. following the sequence of fields that were sown sequentially with uncleaned seeds) revealed that *B. grossus* densities tended to increase from year to year (Table 1). In autumn 2015, we recommended dilution of uncleaned spelt seeds with cleaned ones (a 25% proportion of uncleaned seeds was recommended) resulting in lower *B. grossus* densities in 2016 (Table 1). This recommendation was aimed at controlling *B. grossus* densities, as it is highly competitive with spelt and may therefore impact yield at high density.

**Impacts on other species:** A total of 72 species were identified across all surveys (both field and greenhouse). Species richness in the target field increased from 12 species in 2015 to 43 species in 2017 after the transfer of seed from the donor field. One of the 12 species present in 2015 (*Lolium multiflorum*) was not found again in 2017. Nineteen of the 32 additional species occurred in donor fields and were likely to have been successfully transferred with the uncleaned seed, the remaining 12 did not and were thus classified "unexplained advent" (Figure 3).

**Table 1**. Seed path monitoring results, i.e. following the sequence of fields that were sequentially sown with uncleaned seeds. Field numbers are provided in Figure 1. *B. grossus* densities are given in panicles/m².

| Farm | | Year | | | |
|---|---|---|---|---|---|
| | | 2013 | 2014 | 2015 | 2016* |
| 1 | Field | 1-2 | 1-1 | 1-2 | 1-4 |
| | *B. grossus* density | 71 | 103 | 180 | 51 |
| 2 | Field | 2-1 | 2-1 | 2-2 | 2-3 |
| | *B. grossus* density | 7 | 26 | 97 | 71 |
| 3 | Field | 3-1 | Stored | 3-1 | Stored |
| | *B. grossus* density | 20 | - | 144 | - |

**B. grossus* density decrease in 2016 was presumably due to the prescribed addition of clean seeds.



ISSN 1758-2067

**Table 2**. Number of seedlings of each taxon that emerged in the greenhouse experiment.

| Species | Emergence |
|---|---|
| *Anthemis sp.* | 3 |
| *Aphanes arvensis* | 6 |
| *Avena fatua* | 208 |
| *Bromus grossus* | 93 |
| *Capsella bursa-pastoris* | 1 |
| *Cerastium fontanum* | 211 |
| *Chenopodium album* | 2 |
| *Galium aparine* | 12 |
| *Lapsana communis* | 9 |
| *Lolium sp.* | 1,217 |
| *Matricaria sp.* | 481 |
| *Papaver rhoeas* | 313 |
| *Persicaria lapathifolia* | 131 |
| *Ranunculus repens* | 4 |
| *Rumex obtusifolius* | 709 |
| *Sonchus oleraceus* | 1 |
| *Stellaria media* | 36 |
| *Trifolium pratense* | 1 |
| *Trifolium repens* | 9 |
| *Triticum spelta* | 9 |
| *Veronica arvensis* | 54 |
| *Veronica persica* | 1 |
| *Vicia cracca* | 346 |
| **Total** | **3,857** |

The 400 g uncleaned seed sample taken from the external donor field yielded 306 g (76.5%) of spelt seeds, 50 g (12.5%) of *B. grossus* seeds and 44 g (11%) of other species seeds. Based on the mass of 300 *B. grossus* seeds (4.29 g), we estimated that the 30 kg of uncleaned spelt sown in the target field contained approximately 260,000 *B. grossus* seeds. In the greenhouse, the 44 g of other seeds produced 3,857 seedlings from 23 taxa (Table 2). This represented approximately 45% of the 49 species occurring at donor field. Only *Veronica persica* emerged in the greenhouse (one seedling) but was not observed in the field. The most abundant taxa germinating in the greenhouse were the pernicious *Lolium* sp. (1,217 seedlings) and *Rumex obtusifolius* (709 seedlings). The more common segetal *Papaver rhoeas* was also abundant (313 seedlings). A few (93) remaining *B. grossus* seedlings also emerged, which must have been missed during manual sorting.

Sowing uncleaned seeds was responsible for the transfer of nine species, including *B. grossus*, and possibly responsible for 15 other species (Figure 3). Among threatened species, transfer was shown to be possible for *Bromus secalinus*. We also concluded that transfer failed for 29 species, as they were present in at least one donor field but were not found in the target field. These include the threatened *Legousia speculum-veneris*, *Anthemis cotula*, *Valerianella dentata* and *A. githago*. Among species that failed to be transferred, some however appeared in the greenhouse sowing, such as *Anthemis* sp., *Lolium* sp. and *Matricaria* sp. The proportion of species that failed, or had evident or possible transfer did not differ between species categories ($\chi^2 = 1.87$, p = 0.76, Table 3).

## DISCUSSION

Sowing with uncleaned seed proved to be an essential management option to conserve *B. grossus*, as in almost every case *B. grossus* disappeared again the year following spelt cultivation. It sometimes persisted at the field margin or at low density within field when a winter cereal followed spelt (Figure 1). Little is known about soil seed bank persistence for this species, but the seed bank is known to be transient in the related species *B. secalinus* (Bonn 2004). The conservation of *B. grossus* at the field level is therefore likely to be impossible under normal farming practices, including organic farming, due to crop rotation. Therefore, *B. grossus* conservation needs to be planned at a multiple field level, or even at the farm level. However, when this is not possible, barn storage in some years is a viable alternative. In the case of excessive *B. grossus* densities developing, the dilution of uncleaned spelt seeds with cleaned ones can be retained as a management tool. This recommendation was aimed at avoiding greater yield losses, which are likely to discourage farmers from continuing with the conservation programme in the long term.

Through its legal status, *B. grossus* may act as an ‘umbrella species’ to other segetal plants. Uncleaned crop seed transfer associated with the *B. grossus* conservation programme helped the dispersal of several species. Incidentally, it therefore proved to be a good tool to restore species-rich arable fields. In our experiment, it more than doubled the species richness of the target field (from 12 to 43 species). This was however in a field which before the experiment was species poor. Also, among cereals, spelt is probably the most favourable for transferring seed, as it is harvested as a coated seed, such that the spikelets (or groups of spikelets) are harvested, and the grain is obtained through a further decortication or winnowing process. Compared to ‘naked grain’ cereals such as wheat, spelt spikelets are larger and have a lower density (coats being relatively light). This requires particular settings of the harvester machines, with the sieve open at its maximum and wind reduced. These settings make the harvester cleaning process rather inefficient. Therefore, we recommend paying special attention to the harvester settings if applying the crop seed sowing technique with another crop, notably wheat.

**Table 3**. Number of species in each of the transfer likelihood categories (Figure 3) for the five types of species considered.

| Transfer likelihood | Species categories | | | | |
|---|---|---|---|---|---|
| | Threatened | Segetal | Other annual | Other perrenial | Pernicious |
| Evident transfer | 1 | | 4 | 3 | 1 |
| Possible transfer | 1 | 4 | 5 | 3 | 2 |
| Failed transfer | 4 | 8 | 7 | 7 | 3 |
| Unexplained advent | | | 6 | 5 | 1 |
| Unknown | 1 | | 3 | 1 | 2 |



ISSN 1758-2067

Growing uncleaned seed in a greenhouse revealed that some pernicious weeds, such as *Lolium sp.* and *R. obtusifolius*, were the most abundant species in uncleaned crop seed. However, this did not result in a problematic situation in the target field. *R. obtusifolius* was already present before the experiment and its abundance in 2017 was rather low (data not shown) and *Lolium spp.* were not even recorded in 2017. These were not the only discrepancies between greenhouse and field survey. Several other species that emerged in greenhouse were not observed in the target field (*Anthemis arvensis*, *A. cotula*, *Matricaria maritima*, *Aphanes arvensis*, *Avena fatua*, *Capsella bursa-pastoris*, *Cerastium fontanum*, *Chenopodium album* and *Sonchus oleraceus*). This demonstrates that transfer with crop seed is not sufficient to ensure new species' establishment. Other filters may limit establishment, such as inadequate soil conditions in the target field or incorrect sowing depth (typically 3 cm for spelt). If sowing depth is the reason for establishment failure, we may however expect that some plants will appear in the following years when some seeds will be brought to the surface by tillage.

Another failure factor may be that seeds were not harvested in donor fields. There can be many reasons for this, including incompatible phenology, plant height and low seed terminal velocity that may lead to a species being discarded in the harvester grain separation mechanism. Species abundance may also impact the probability of dispersal with harvested crop seeds (Mayer & Albrecht 2008). In our study, this was probably the case for *A. githago*, which is well known as a seed contaminant which disperses through uncleaned crop seeds (Albrecht *et al*. 2016). At the external donor site, it was recorded at very low abundance (two or three individuals), therefore the probability that it occurred in transferred seeds was also very low. Low abundance may also reduce the detection probability during surveys.

In conclusion, crop seed transfer is a valuable management option for the conservation of *B. grossus*. It is also a simple approach to restore species-rich arable fields. It can be carried out by farmers, without any use of supplementary machinery or man-hours. It is however an imperfect tool, as not all species are likely to be dispersed this way. Other restoration techniques, such as soil transfer or sowing of threatened segetal plant seeds, may therefore be considered as complementary actions. These, however, require more time or equipment and may therefore be more appropriate on a site with long-term conservation objectives, such as a dedicated nature reserve.

## ACKNOWLEDGEMENTS

The authors acknowledge the farmers who participated in this study, and the anonymous reviewer for the constructive comments. Natagriwal is a non-for-profit association funded by the Walloon government.

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ISSN 1758-2067