# Persistence of Feral Populations of *Brassica napus* Originated from Spilled Seeds around the Kashima Seaport in Japan

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### Abstract

Concern has been raised about the importation and transportation of GM *B. napus*, because feral herbicide-tolerant GM *B. napus* plants have been found growing around some major seaports in Japan. We should monitor the persistence of these feral *B. napus* populations including the herbicide-tolerant GM cultivars to prevent the negative impact of GM *B. napus* on native plant communities. In this study, we examined the plant numbers and the persistence of the *B. napus* at 19 sites around the Kashima seaport in Japan once a month from July 2004 to December 2005. In the results, the plant numbers ranged from 0 to 202 depending on the sites and/or the seasons. Out of 19 sites, we observed the seed dispersals of *B. napus* plants at only four sites. Of these four sites, we finally confirmed the self-sustainment of *B. napus* populations at two sites. Many plants growing at most of the sites disappeared before flower budding due to frequent human disturbances.

Discipline: Weed control

Additional key words: genetically modified (GM) crop, oilseed rape, population persistence, selfsustainment

### Introduction

Concern has been raised in Japan about the importation and transportation of genetically modified (GM) Brassica napus L., because feral herbicide- (i.e. glyphosate- and glufosinate-) tolerant GM B. napus plants have been found growing around some major seaports where food and feed materials are imported from foreign countries<sup>1,13,16</sup>. Saji et al. and Nishizawa et al. suggested that feral GM B. napus plants occurring around the Kashima seaport in Ibaraki Prefecture are likely to be originated from imported seeds spilled during transportation<sup>13,16</sup>. In addition, Aono et al. reported that seeds with multiple-herbicide tolerance were detected from feral B. napus plants growing on roadside around the Yokkaichi seaport in Mie Prefecture, suggesting that hybridization might have occurred between two different kinds of feral GM B. napus cultivars at the seaport in Japan<sup>1</sup>.

Under the circumstances, there is a concern that these feral GM *B. napus* plants may spread in native plant communities and hybridize with the wild relatives.

Some previous studies showed that the herbicidetolerant GM cultivars did not have enhanced fitness compared with their host plants<sup>6,9,11</sup>. In contrast, Hancock suggested that novel transgenes that change the environmental tolerance of a species or alter its patterns of growth and development could result in dramatic adaptive shifts and have a major effect on fitness<sup>12</sup>. In the future, when the GM B. napus cultivars with such novel transgenes are imported and used in the food and feed industries in Japan, the feral plants originating from the spilled seeds of the cultivars may persist for a longer time and have a negative impact on native plant communities around the seaports in Japan. To prevent this negative impact, it is essential to continuously monitor the feral B. napus populations and detect any increase in their persistence as early as possible.

This paper reports the results obtained in the project on "Assurance of Safe Use of Genetically Modified Organisms" sponsored by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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Received 10 November 2009; accepted 27 July 2010.

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It is known that *B. napus* plants can grow in seminatural habitats, and the feral plants are often widespread at margins of fields, roadsides, or sometimes on waste ground in many countries where *B. napus* plants have been cultivated commercially on a large scale<sup>2,5,18</sup>. In these countries, the feral and volunteer plants originating from cultivated *B. napus* seeds have established around agricultural lands, and the population persistence of *B. napus* has been intensively examined<sup>3,4,7,11,14,15</sup>. In Japan, the cultivated acreage of *B. napus* is not so large and the feral plants have not been viewed as an agricultural problem. Therefore, the population persistence of *B. napus* has never been examined in Japan, although previous reports showed the distribution of feral *B. napus* plants over a large area<sup>13,16</sup>.

To estimate the population persistence of feral *B. napus*, we examined the number of growing *B. napus* plants and the presence or absence of self-sustainment at 19 sites around the Kashima seaport in Japan, where a large number of *B. napus* seeds were imported from foreign countries. In this study, we defined the self-sustainment of the populations as the occurrence of seedlings at the place where the maternal plants propagated their seeds.

### Methods

In April 2004, we performed a preliminary survey

of the distribution of flowering *B. napus* plants along main roads within a 5-km radius of the unloading point for *B. napus* seeds at the Kashima seaport (N  $35^{\circ}54'$ , E  $140^{\circ}40'$ ), Kamisu City, Ibaraki Prefecture, Japan. Then, we selected 19 permanent survey sites where sufficient numbers of plants for observation were growing (Fig. 1). We counted the number of *B. napus* plants within areas of about 30 m<sup>2</sup> at all the survey sites once a month from July 2004 to December 2005. The surveys were conducted twice a month on 13 and 30 July, 2004. We also labeled each plant and recorded their phenological stages (i.e., before or after flower budding or death) to know if they died before seed dispersal or not. Unfortunately, the survey at site 14 was discontinued because of land development after October 2005.

### **Results and discussion**

# 1. The number of growing plants and disappearance factors

The continuous growth of *B. napus* plants was recorded at all the sites except site 13, where plants occurred only in August 2004. The number of plants varied among the sites and the survey months, and ranged from 0 to 202 (Fig. 2). Sudden reductions in the number of plants were observed at most of the sites. Inter-specific competition with other species and frost-kill caused the



# Fig. 1. Location of sites surveyed (1~19; •) along road (gray heavy lines) and around main crossing within a 5-km radius of unloading point (thick arrow) of imported seeds of *B. napus* at the Kashima seaport

Sites 13 and 14 consisted of open space.

Sites 5 and 6 consisted of planted zone.

Sites 9, 10, 11, and 18 consisted of road pavement seam.

Site 12 consisted of open space, planted zone, and road pavement seam.

The other sites consisted of planted zone and road pavement seam.



Fig. 2. Plant numbers and phenology of feral B. napus in 19 sites around the Kashima seaport

The black area indicates the number of plants after flower budding. The gray areas show the number of plants before flower budding. \* The maximum value of the vertical axes in sites 11 and 15 was given in 200 plants. \*\* The maximum value of the vertical axis in site 6 was given in 100 plants. Site 13 was omitted, because the plants could not be continuously observed. The arrow indicates the disappearance factor of growing plants.

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disappearance of the plants at sites 6 and 16, respectively. The main factor behind the plant disappearance was human disturbance (e.g., mowing, spraying with herbicides, and pulling out the *B. napus* plants). Nishizawa et al. reported that plant numbers varied substantially along a 20-km section of Route 51 from 2005 to 2007: 2,162 plants in 2005, 4,066 in 2006, and only 278 in 2007<sup>13</sup>. They suggested that fewer plants were observed in 2007 probably due to roadwork<sup>13</sup>. Their suggestion was consistent with our observation that the main disappearance factor was human disturbance.

### 2. Self-sustainment of the populations

Many feral B. napus plants disappeared before flower budding without seed propagation at most of the sites because of frequent human disturbances, while matured seed dispersals were observed at four sites: 4, 6, 14, and 16 (Fig. 2). At site 6, seedlings were subsequently recognized at the places where seed dispersals occurred in the preceding surveys. There was a similar occurrence at site 14 in July 2005, although we could not count the exact number of seedlings because the site was surrounded by a fence for land development. The facts observed at sites 6 and 14 indicate the possibility of persistence by self-sustainment. At sites 4 and 16, some seedlings occurred at a place distant from the place of seed dispersal in the previous month. In the case of site 4, these seedlings may have mostly originated from spilled seeds from trucks because they occurred on the road pavement seam. Those at site 16, occurring on planted zone with sufficient soil, may have originated from spilled seeds and also a seed bank, which had accumulated many seeds propagated from maternal plants in the past. The repeated seed propagation of B. napus facilitates the development of a seed bank in the soil, which contributes to the persistence of feral populations because the period of seed dormancy of *B. napus* is generally long<sup>8,10,11</sup>, although our examination could not detect the contribution of the seed bank to population persistence. In sites 4, 7, 11, 15, 17, and 18, we frequently found many seedlings along the road pavement seam in the absence of maternal plants (Fig. 2). This fact indicated that the origin of the plants was the seeds spilled from trucks. This was consistent with previous studies<sup>13,17</sup>. Von der Lippe & Kowarik measured the deposition of seeds along roadsides in Germany and concluded that the spilled seed was the major pathway for recruitment of roadside populations of B. napus 17.

In our study, most of the *B. napus* plants growing at our survey sites disappeared before flower budding due to frequent human disturbances, although self-sustainment was confirmed at only two out of 19 sites. However, if the frequency of human disturbances decreases, the chance of self-sustainment will increase. Therefore, we should continue to monitor the number of maternal plants within feral *B. napus* populations and the frequency of human disturbance around the seaports in Japan. Such information based on continuous monitoring is useful for the effective implementation of risk-reduction measures against the negative impact of GM *B. napus*.

### Acknowledgments

We thank Mrs. Y. Nakazato and Mrs. A. Tomioka for their assistances in the analysis. We also thank two anonymous reviewers for their valuable comments on our manuscript.

### References

- 1. Aono, M. et al. (2006) Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. *Environ. Biosafety Res.*, **5**, 77–87.
- Beckie, H. J., Hall, L. M. & Warwick, S. I. (2001) Impact of herbicide resistant crops as weeds in Canada. *In* Proceedings of the Brighton Crop Protection Conference – Weeds. British Crop Protection Council, Farnham, Surrey, UK, 135–142.
- Claessen, D., Gilligan, C. A. & van den Bosch, F. (2005a) Which traits promote persistence of feral GM crops? Part 1: implications of environmental stochasticity. *Oikos*, 110, 20–29.
- 4. Claessen, D., Gilligan, C. A. & van den Bosch, F. (2005b) Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. *Oikos*, **110**, 30– 42.
- Crawley, M. J. & Brown, S. L. (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. *Proc. Soc Lon. B*, 259, 49–54.
- Crawley, M. J. et al. (2001) Transgenic crops in natural habitats. *Nature*, 409, 682–683.
- Garnier, A. & Lecomte, J. (2006) Using a spatial and stagestructured invasion model to assess the spread of feral populations of transgenic oilseed rape. *Ecol. Modeling*, 194, 141–149.
- Gruber, S., Pekrun, C. & Claupein, W. (2004a) Reducing oilseed rape (*Brassica napus*) volunteers by selecting genotypes with low seed persistence. *J. Plant Dis. Prot.*, Sonderheft 19, 151–159.
- Gruber, S., Pekrun, C. & Claupein, W. (2004b) Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. J. Agric. Sci., 142, 29–40.
- Gulden, R., Shirtliffe, S. J. & Gordon, T. A. (2003) Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. *Weed Sci.*, 51, 904–913.
- Hails, R. S. et al. (1997) Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparison of transgenic and non-transgenic lines of the crop. *Proc. R. Soc. Lond. B*, 264, 1–7.

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- Hancock, J. F. (2003) A framework for assessing the risk of transgenic crops. *BioScience*, 53, 512–519.
- Nishizawa, T. et al. (2009) Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadside: 3-year observations. *Environ. Biosafety Res.*, 8, 33–44.
- Pessel, F. D. et al. (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theor. Appl. Genet.*, 102, 841–846.
- 15. Pivard, S. et al. (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible

origin in a farmland area. J. Appl. Ecol., 45, 476-485.

- Saji, H. et al. (2005) Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. *Environ. Biosafety Res.*, 4, 217–222.
- 17. Von der Lippe, M. & Kowarik, I. (2007) Crop seed spillage along roads: a factor of uncertainty in the containment of GMO. *Ecography*, **30**, 491–504.
- Yoshimura, Y., Beckie, H. J. & Matsuo, K. (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environ. Biosafety Res.*, 5, 67–75.