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Weed Risk Assessment for *Brassica carinata* A. Braun (Brassicaceae) – Ethiopian mustard



Brassica carinata plants in a field trial in northern Florida [source: David Wright (2014)].

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Introduction Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as “any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment” (7 U.S.C. § 7701-7786, 2000). We use weed risk assessment (WRA)—specifically, the PPQ WRA model (Koop et al., 2012)—to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

Because the PPQ WRA model is geographically and climatically neutral, it can be used to evaluate the baseline invasive/weed potential of any plant species for the entire United States or for any area within it. As part of this analysis, we use a stochastic simulation to evaluate how much the uncertainty associated with the analysis affects the model outcomes. We also use GIS overlays to evaluate those areas of the United States that may be suitable for the establishment of the plant. For more information on the PPQ WRA process, please refer to the document, *Background information on the PPQ Weed Risk Assessment*, which is available upon request.

***Brassica carinata* A. Braun – Ethiopian mustard**

Species Family: Brassicaceae

Information Synonyms: *B. integrifolia* var. *carinata* (A. Braun) O. Schulz. (Clement and Foster, 1994); *B. integrifolia* auct. non (H. West) Rupr. (Stace, 2010); *Sinapsis integrifolia* H. West (MBG, 2014). Other synonyms listed on The Plant List (2014).

Common names: Abyssinian cabbage, Abyssinian mustard, Ethiopian kale, Ethiopian mustard, Ethiopian rape, and mustard collard (NGRP, 2014). Also African mustard (Rana, 2006).

Botanical description: *Brassica carinata* is an erect, annual herb growing from 30 to 200 cm tall (Alemayehu and Becker, 2002; APD, 2014; Mnzava and Schippers, 2007). It is usually branched with leaves arranged alternately on stems (Mnzava and Schippers, 2007). *Brassica carinata* (2N=34) is an amphidiploid (an allopolyploid behaving as a diploid) derived from an ancient cross between *B. oleracea* (2N=18) and *B. nigra* (2N=16) (Mabberley, 2008; NGRP, 2014; Stace, 2010). Hybridization occurred at least a few thousand years ago, since it has been cultivated in Ethiopia for at least that long (Alemayehu and Becker, 2002; Mnzava and Schippers, 2007).

Initiation: On August 4, 2014, the Environmental Protection Agency (EPA) contacted Jonathan Jones, USDA Biofuel Team Leader, with questions about the weed status of *B. carinata* (Lie, 2014). Later, we understood that EPA wanted to know whether or not this species should be regulated under their Renewable Fuel Standard as a potential invasive species (Lie, 2014). On October 6, the PPQ Weeds Cross-Functional Working Group requested that the PERAL Weed Team conduct a WRA of this species. Over the last 20 years, considerable interest has been shown in cultivating *B. carinata* as an oilseed crop for biofuel production and other uses (Babu et al., 2013; Getinet et al., 1996; Guerrero-Diaz et al., 2013; Newson et al., 2013; Pan, 2009; Warwick et al., 2006). This species has

great potential as a commercial crop due to increased drought and disease resistance, reduced shattering, and increased ability to compete with weeds relative to other oilseed brassicas (Marillia et al., 2014).

Taxonomic Scope of the analysis: For practical reasons, we are restricting our analysis to the original or wild form of *B. carinata* (genome BBCC) and the cultivars that have been developed or selected within the species. Numerous cultivars of this species are available (Mnzava and Schippers, 2007), and more biotypes are being selected in ongoing develop improved lines (e.g., Marillia et al., 2014; Taylor et al., 2010). Breeding efforts also include hybridization and backcrossing with *B. rapa* (Sheikh et al., 2014; Taylor et al., 2010), production of trigonomic hexaploids with *B. carinata* and *B. rapa* (e.g., Malek et al., 2013) and genetic engineering to introduce specific genes into the genome of *B. carinata* (Taylor et al., 2010). It is beyond the scope of this assessment to evaluate the weed risk associated with *B. carinata* cultivars derived from interspecific crossing, polyploidization, or with genetic engineering since we don't have enough information at the moment to evaluate how these changes may affect the weed potential of the plant.

Foreign distribution: Researchers believe that *B. carinata* originated in Ethiopia (Warwick et al., 2006). It has been introduced into and field-tested for biofuel production in Canada (Marillia et al., 2014), India (Malik, 1990), Italy (Bozzini et al., 2007), Pakistan (Zada et al., 2013b), and Spain (Velasco et al., 2003). It has also been introduced into Australia (Gunasinghe et al., 2013) and Pakistan (Zada et al., 2013a). Because of widespread interest in oilseed brassicas (Downey and Röbbelen, 1989), additional countries have also likely introduced it for biochemical analysis and/or breeding (e.g., Bangladesh: Malek et al., 2013).

U.S. distribution and status: *Brassica carinata* is already present in the United States but is not known to be naturalized here (Kartesz, 2014; NRCS, 2014) or in Canada (Brouillet et al., 2014). It was brought from Ethiopia in 1957 for leafy vegetable production (Stephens, 2009). In 1972, the Texas Agricultural Experiment Station released the cultivar TAMU Tex Sel, which never became popular (Stephens, 2009). *Brassica carinata* has been field tested in North Dakota, Montana, Florida, and Mississippi (Harrison, 2013; Marillia et al., 2014).

WRA area¹: Entire United States, including territories.

1. *Brassica carinata* analysis

Establishment/Spread Potential

We found no evidence that *Brassica carinata* naturalizes and spreads where it has been introduced. However, this species is able to germinate and grow as a contaminant from discarded bird seed and bird-seed screenings (Clement and Foster, 1994; Hanson and Mason, 1985). It also reportedly can “often escape” from cultivation in Africa (Mnzava and Schippers, 2007). Despite decades of some breeding and cultivation in the United States, and a longer history elsewhere, relatively little biological and ecological information has been published about this species. We did not find many traits contributing to invasive potential. *Brassica*

¹ “WRA area” is the area in relation to which the weed risk assessment is conducted [definition modified from that for “PRA area”] (IPPC, 2012).

carinata is self-compatible (PFAF, 2014; Séguin-Swartz et al., 2013), produces viable seeds (Cohen and Knowles, 1984; Séguin-Swartz et al., 2013) and is likely to disperse unintentionally via both agricultural activities (Gulden et al., 2003; Marillia et al., 2014; Wright, 2014) and as a contaminant in bird food (Clement and Foster, 1994; Hanson and Mason, 1985). We had a high amount of uncertainty for this risk element due to incomplete knowledge about its traits. We answered five questions in this risk element as unknown.

Risk score = 5 Uncertainty index = 0.24

Impact Potential We found no evidence that *B. carinata* affects natural and anthropogenic systems, although we did find one comment on an herbarium sheet that it is a garden weed (GBIF, 2014). We found no direct evidence of impacts in agricultural systems, although this species might harm animals. *Brassica carinata* seeds generally have a large amount of glucosinolate compounds (Getinet et al., 1996; Marillia et al., 2014) that could be problematic if animals are exposed to it in large quantities. Depending on the concentration and specific types of these compounds, glucosinolates can be toxic or anti-nutritional and can directly affect the thyroid (Assayed and Abd El-Aty, 2009). Those compounds have allelopathic effects on plants (reviewed in Earlywine et al., 2010; Gulden et al., 2008) and are being investigated as biofumigants against plant pests (Guerrero-Diaz et al., 2013). We had a very high amount of uncertainty for this risk element.

Risk score = 1.2 Uncertainty index = 0.26

Geographic Potential Based on three climatic variables, we estimate that about 13 percent of the United States is suitable for the establishment of *B. carinata* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes point-referenced localities and areas of occurrence. The map for *B. carinata* represents the joint distribution of Plant Hardiness Zones 9-12, areas with 0-100 inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical savanna, steppe, desert, Mediterranean, humid subtropical and marine west coast. Although we answered yes, we had high uncertainty about whether or not this species can grow without human assistance in desert areas or areas with 0-10 inches of annual precipitation. Some of the geo-referenced occurrences obtained from the Global Biodiversity Information Facility (GBIF, 2014) may have been derived from cultivated plants. Two researchers report that "[t]ruly wild types are not known" (Mnzava and Schippers, 2007). If seeds of *B. carinata* can withstand temperatures colder than those seen in hardiness zone 9, then it could establish further north than indicated in Fig. 1.

The area shown in Fig. 1 is an estimate as it only uses three climatic variables. Other environmental variables, such as soil and habitat type, may further limit the areas in which this species is likely to establish (i.e., naturalize). However, if it is cultivated as an annual and given suitable moisture, nutrients, light, and other growing requirements, it can likely be grown anywhere in the United States.

Entry Potential We did not assess the entry potential of *B. carinata* because it is already present in the United States. It was imported into the United States from Ethiopia in 1957 for leafy vegetable production (Stephens, 2009), has undergone cultivar development in Canada and presumably the United States (Taylor et al., 2010), and has been field tested in multiple states (see above) (Harrison, 2013; Marillia et al., 2014).



Figure 1. Predicted distribution of *Brassica carinata* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.

2. Results

Model Probabilities: P(Major Invader) = 9.7%
 P(Minor Invader) = 68.3%
 P(Non-Invader) = 22.0%

Risk Result = Evaluate Further

Secondary Screening = Evaluate Further

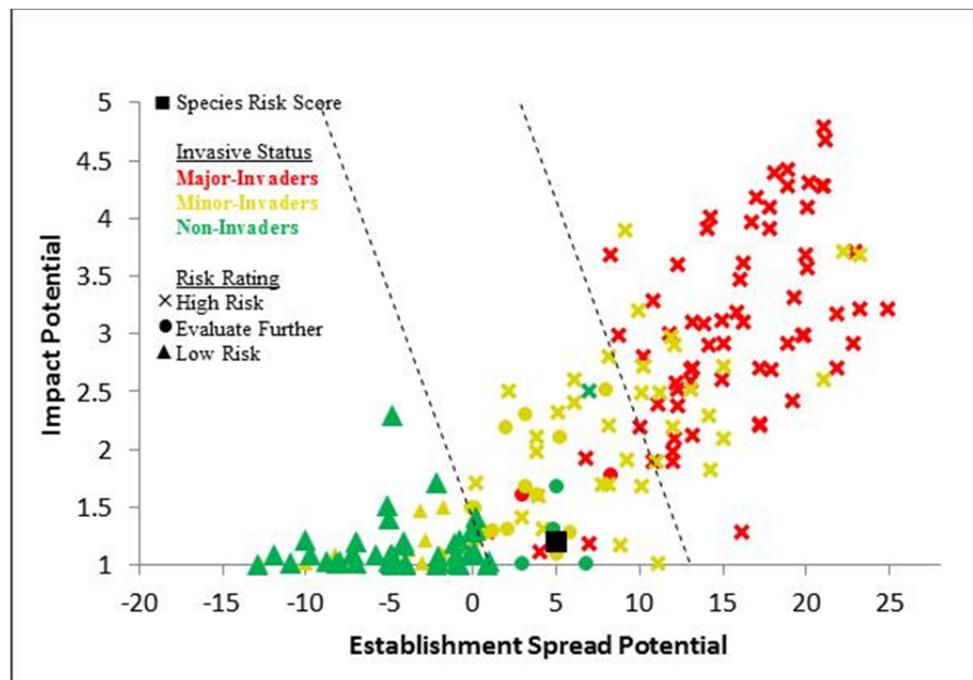


Figure 2. *Brassica carinata* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

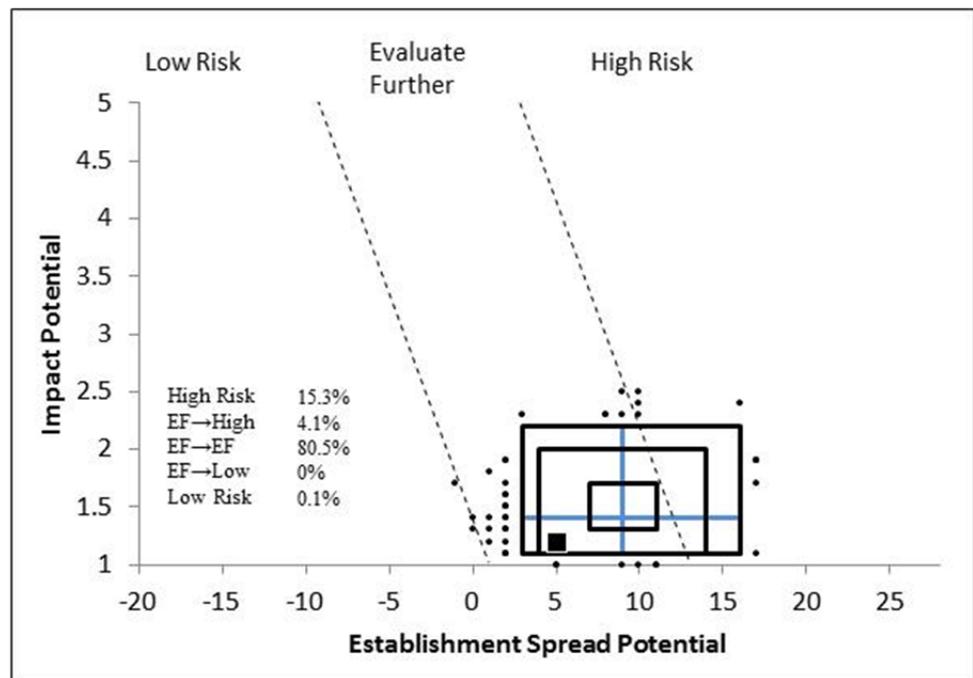


Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for *Brassica carinata*. The blue “+” symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *B. carinata* is Evaluate Further (Fig. 2). Due to the limited amount of biological and ecological information available for this species, we had a high level of uncertainty with the analysis. Our uncertainty analysis resulted in high risk outcomes about 20 percent of the time, while very few were low risk and 80.5 percent of the outcomes were Evaluate Further (Fig. 3). Additional information addressing unanswered questions would very likely shift the risk score, as well as reduce our uncertainty (Fig. 3). Overall, our analysis indicates that *B. carinata* poses a moderate weed risk potential. Its attributes are similar to other U.S. minor invaders (Fig. 2). Both of its parents, *B. oleracea* and *B. nigra*, are naturalized in the United States (Warwick, 1993+).

In this assessment we did not find any strong evidence that *B. carinata* is likely to become a major invader or weed. In Africa, "[t]ruly wild types are not known, but *Brassica carinata* often escapes from cultivation" (Mnzava and Schippers, 2007). Despite that, one breeder commented that it volunteers no more than canola, *B. napus* (Mayles, 2014). Another researcher indicated zero issues with it in northern Florida during three years of field trials (Wright, 2014). In Canadian field trials, volunteers were noted but not considered invasive (Mendenhall, 2013). It is not clear how this species will behave in the United States or if it will behave similarly in different regions. It is also not clear how continued breeding might affect its weed potential. Relative to other *Brassica* species, *B. carinata* has reduced seed pod shattering (Wright, 2014), which reduces its risk. However, relative to other oilseed brassicas, increased disease and drought resistance (Fredua-Agyeman et al., 2014; Warwick et al., 2006) and increased competitiveness with crop weeds (Mayles, 2014) increase its risk potential.

4. Literature Cited

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Appendix A. Weed risk assessment for *Brassica carinata* A. Braun (Brassicaceae). The following information came from the original risk assessment, which is available upon request (full responses and all guidance). We modified the information to fit on the page.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ESTABLISHMENT/SPREAD POTENTIAL			
ES-1 (Status/invasiveness outside its native range)	d - mod	0	<i>Brassica carinata</i> likely originated in Ethiopia a few thousand years ago (Mnzava and Schippers, 2007; Warwick et al., 2006). Its exact native distribution is not well understood because it has been cultivated for a long time in Africa; furthermore, it is often confused with <i>B. juncea</i> (Mnzava and Schippers, 2007). It is currently cultivated, native, and/or escaping from cultivation in many countries in Africa (Mnzava and Schippers, 2007). "Truly wild types are not known..." (Mnzava and Schippers, 2007). The NGRP (2014) reports <i>B. carinata</i> as naturalized in Ethiopia, but because this is where the species is believed to have originated (Warwick et al., 2006), we did not consider that evidence here. <i>Brassica carinata</i> is a casual species in the United Kingdom (Hanson and Mason, 1985; Stace, 2010), "sometimes appearing to be persistent, but probably repeatedly introduced" (Clement and Foster, 1994). It is reported as naturalized in Madagascar (Kull et al., 2012). This species has been grown since at least 1984 in Canada (Rakow and Getinet, 1998) and 1957 in the United States (Stephens, 2009) without evidence of naturalization in either country (Brouillet et al., 2014; Kartesz, 2014; Mendenhall, 2013; NRCS, 2014). One researcher stated that <i>B. carinata</i> does not volunteer any more than <i>B. napus</i> (Mayles, 2014), which without disturbance usually goes extinct in about three years (Hall et al., 2005). Third party evaluation of Canadian farms growing <i>B. carinata</i> stated that while the species may not be invasive in Canada, it does volunteer on site (Mendenhall, 2013). Based on the weight of this evidence, we answered "d" for casual/escaped with moderate uncertainty. Because the reference reporting it to be naturalized in Madagascar is merely a list of categorized species without any substantive description of behavior, we are unsure of its status in Madagascar. The alternate answers for the Monte Carlo simulation were both set as "e," which reflects a possible naturalized status in that country.
ES-2 (Is the species highly domesticated)	n - low	0	<i>Brassica carinata</i> (2N=34) is an amphidiploid (an allopolyploid behaving as a diploid) derived from an ancient cross between <i>B. oleracea</i> (2N=18) and <i>B. nigra</i> (2N=16) (Mabberley, 2008; Stace, 2010). Throughout most of Africa, where it is cultivated, it is used as leafy vegetable, but in Ethiopia, it is also grown for its seed oil (Mnzava and Schippers, 2007; NGRP, 2014; Taylor et al., 2010; Warwick et al., 2006). Grown as game-cover in England (Stace, 2010). Wild forms of <i>B. carinata</i> have not been reported but there are diverse ecotypes (Alemayehu and Becker, 2002). The species is currently being bred to improve a variety of traits including maturation time, yield, oil composition, disease resistance, etc. (e.g., Marillia et al., 2014; Taylor et al., 2010). Some breeding programs are crossing <i>B. carinata</i> with other oilseed brassicas to introduce desirable traits into <i>B. carinata</i> (Sheikh et al., 2014; Taylor et al., 2010). Because this evaluation is based on the species as a whole and not on any one cultivar, we

Question ID	Answer - Uncertainty	Score	Notes (and references)
			answered no with low uncertainty. Furthermore, breeding programs are focusing on crop improvement and not reduced weed potential.
ES-3 (Weedy congeners)	y - negl	1	<i>Brassica</i> is a relatively small genus with about 40 species (Mabberley, 2008). Seventeen of these have been reported as weeds, but five of them have been reported at least 55 or more times as weedy: <i>Brassica juncea</i> , <i>B. napus</i> , <i>B. nigra</i> , <i>B. oleracea</i> , and <i>B. rapa</i> (Randall, 2012). <i>Brassica rapa</i> subsp. <i>oleifera</i> (reported as <i>B. campestris</i>) is a serious or principal weed in 16 countries, including Canada and Mexico (Holm et al., 1979). <i>Brassica juncea</i> and <i>B. rapa</i> are principal weeds in Canada and Brazil, respectively (Holm et al., 1979). <i>Brassica kaber</i> competes with crops for water and nutrients, reducing yield in wheat by 16 percent and yield in spring rapeseed by 66 percent (Holm et al., 1997). <i>Brassica tournefortii</i> “forms dense stands, accumulates large quantities of dried plant material and thus increases fire hazard” (Weber, 2003).
ES-4 (Shade tolerant at some stage of its life cycle)	n - mod	0	We did not find any strong evidence that <i>B. carinata</i> is shade tolerant. An online factsheet states it can grow in partial shade or no shade but provided no supportive evidence (PFAF, 2014). One greenhouse study showed that plants lit from the sides, in addition to being lit from above, grew faster and produced more biomass than those lit from just above (Monti et al., 2009), suggesting that plants respond well to increasing light levels. Although none of this evidence is very conclusive, because this species is grown in open fields as an annual crop (Pan, 2009) like other <i>Brassica</i> species, we suspect it is not shade tolerant. It is noteworthy that <i>B. carinata</i> 's diploid parents, <i>B. oleracea</i> and <i>B. nigra</i> , occur in open habitats in the United States (Warwick, 1993+).
ES-5 (Climbing or smothering growth form)	n - negl	0	This species is an erect herbaceous annual, sometimes biennial or perennial, growing from about 30 to 200 cm in height (APD, 2014; Marillia et al., 2014; Mnzava and Schippers, 2007; Pan, 2009); it is not a vine and does not have a smothering growth form.
ES-6 (Forms dense thickets)	? - max	0	Unknown. We did not find very much information about this species' behavior outside of cultivation.
ES-7 (Aquatic)	n - negl	0	Not an aquatic plant; this species is a terrestrial herb (Getinet et al., 1996; Marillia et al., 2014; Mnzava and Schippers, 2007).
ES-8 (Grass)	n - negl	0	This species is not a grass; it is a mustard (NGRP, 2014).
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	We found no evidence that <i>B. carinata</i> fixes nitrogen. It is not a member of one of the plant families known to fix nitrogen (Martin and Dowd, 1990). Also, it is not a woody plant.
ES-10 (Does it produce viable seeds or spores)	y - negl	1	Propagated through seed production (Cohen and Knowles, 1984). In one field study, 98.5 percent of the seeds were viable (Séguin-Swartz et al., 2013).
ES-11 (Self-compatible or apomictic)	y - negl	1	<i>Brassica carinata</i> is autogamous (i.e., self-compatible; PFAF, 2014; Séguin-Swartz et al., 2013). It readily self-pollinates (Mnzava and Schippers, 2007). In a breeding program to introduce disease resistance from <i>B. carinata</i> to <i>B. napus</i> , hybrids of these two taxa were backcrossed twice to <i>B. napus</i> and the resulting progenies were self-pollinated for three generations (Fredua-Agyeman et al., 2014).
ES-12 (Requires special	n - negl	0	It readily self-pollinates without insect pollinators (Mnzava and

Question ID	Answer - Uncertainty	Score	Notes (and references)
pollinators)			Schippers, 2007). Pollinated by bees (PFAF, 2014). We expect that pollinators for <i>B. carinata</i> will be similar to the species that pollinate its close relatives. <i>Brassica nigra</i> , <i>B. rapa</i> , <i>B. napus</i> , and <i>B. oleracea</i> are pollinated by flies and bees, including honeybees (Bruinsma et al., 2008; Manning and Boland, 2000; Rader et al., 2013; Sharma et al., 2013; Stanley et al., 2013). <i>Brassica napus</i> is also probably pollinated to some degree by wind (Gulden et al., 2008; McCartney and Lacey, 1991). <i>Brassica rapa</i> and <i>B. napus</i> can be pollinated by bees and other non-specialist pollinators (Hall et al., 2005).
ES-13 (Minimum generation time)	b - negl	1	An annual plant, sometimes biennial or perennial (Babu et al., 2013; Mnzava and Schippers, 2007; Pan, 2009). Plants up to four years old have been recorded, but this is due to continual harvest of leaves under cultivation (Mnzava and Schippers, 2007). Under agronomic conditions plants are ready for harvest 148 to 172 days after planting (Alemayehu and Becker, 2002). In a Canadian agronomic study, <i>B. carinata</i> matured seeds in 101 to 111 days from planting (Getinet et al., 1996). In another study, plants matured in 94-110 days after planting (Warwick et al., 2006). In Africa, seed oil biotypes produce seeds in dry regions in about 4 months, whereas vegetable types produce seeds in about 5-6 months (Mnzava and Schippers, 2007). Alternate answers for the Monte Carlo simulation were "c" and "a."
ES-14 (Prolific reproduction)	? - max	0	We found no information on seed production in natural populations, but there was some information on agronomic yield. In a study of plant traits under agronomic conditions, plants produced an average of 270 pods (range = 179 to 352) and 3300 seeds (range = 1900 to 5200) per plant (Alemayehu and Becker, 2002). Assuming at least two plants per square meter and 98 percent seed viability (Séguin-Swartz et al., 2013), two plants alone would be sufficient to meet the threshold of 5000 seeds per square meter. In another study, seed yield was 597 and 1267 kg/ha in 1984 and 1985, respectively (Getinet et al., 1996), with thousand-seed weights ranging from 3.7 g to 4.6 g in 1984 and from 4.9 g to 5.7 g in 1985 (Getinet et al., 1996). Using the heaviest estimates of seed weight, this yield converts to 13,000 and 22,000 seeds per square meter. Other studies reported similar thousand-seed weights: 3.48 g (Alemayehu and Becker, 2002) and 2.0 to 3.9 g (Warwick et al., 2006). Therefore, under field production where water, herbicides, and fertilizers may be used to maximize yield, <i>B. carinata</i> is a prolific reproducer. However, because there is no information about seed production outside of cultivation, we answered unknown. Naturalized <i>B. rapa</i> produces fewer seeds than cultivated plants (Hall et al., 2005).
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - low	1	We found no direct evidence of unintentional dispersal by people for <i>B. carinata</i> . However, based on information from its close relative, <i>B. napus</i> (canola), we and another weed scientist (Johnson, 2014) believe unintentional dispersal is very likely to occur through regular agricultural activities. For example, in one study of yield loss due to unharvested seeds of canola (<i>B. napus</i>), the authors estimated that on average 3000 viable seeds per square meter were being added to the soil seed bank (Gulden et al., 2003). Even though <i>B. carinata</i> is the most shatter-resistant of all the brassicas (Marillia et al., 2014; Wright, 2014), some seeds are

Question ID	Answer - Uncertainty	Score	Notes (and references)
			still likely to be incorporated back into the soil (Wright, 2014). Combine harvesters may increase the spread of <i>B. napus</i> in fields (Gulden et al., 2008). Long-distance dispersal of volunteer <i>B. napus</i> to non-agricultural habitats such as railroads, roadways, and seaports is believed to occur through commercial seed movement (Gulden et al., 2008; Mizuguti et al., 2011). Seeds of <i>B. rapa</i> and <i>B. napus</i> are dispersed long distances through transport (Hall et al., 2005; Légère, 2005).
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	y - negl	2	In a study that germinated seeds in bird food and screenings from imported bird food, the authors found <i>B. carinata</i> as a contaminant (impurity) (Hanson and Mason, 1985). A bird-seed or oilseed alien (Clement and Foster, 1994). Bird-seed alien (Stace, 2010).
ES-17 (Number of natural dispersal vectors)	0	-4	Fruit and seed characters for ES-17a through ES-17e: Fruit 2.5 to 6 cm long (Stace, 2010). <i>Brassica carinata</i> 's fruit type is a silique, a long pod (less than 5 cm long) composed of two valves, with seeds in one row under each valve (Pan, 2009; Stace, 2010). Seeds are roughly spherical in shape (Fig. 2 in Marillia et al., 2014) and about 2 mm thick (Pan, 2009). <i>Brassica carinata</i> is relatively more resistant to seed shattering than <i>B. napus</i> (Alemayehu and Becker, 2002; Wright, 2014).
ES-17a (Wind dispersal)	n - negl		<i>Brassica carinata</i> does not possess adaptive features to aid in wind dispersal (Johnson, 2014). This statement is consistent with its biology. We found no information on seed dispersal of <i>B. carinata</i> . However, we suspect seed dispersal via wind is unlikely given fruit and seed morphology.
ES-17b (Water dispersal)	n - negl		<i>Brassica carinata</i> does not possess any adaptive features to aid in water dispersal (Johnson, 2014). An unpublished study found that only 5.5 percent of 1000 seeds floated, and that after 4 hours with periodic stirring every 30 minutes, only 0.2 percent of the seeds remained floating (Johnson, 2014). Given this evidence and the fact that this species grows in dry regions and is not restricted to riparian environments (Warwick et al., 2006), we answered no with negligible uncertainty.
ES-17c (Bird dispersal)	? - max		Unknown.
ES-17d (Animal external dispersal)	n - low		We found no evidence. Because fruit and seeds don't possess any obvious adaptations for attachment to animals, external dispersal seems unlikely.
ES-17e (Animal internal dispersal)	? - max		We found no information on seed dispersal of <i>B. carinata</i> . Sheep fed a diet including <i>B. napus</i> seeds were shown to pass germinable seeds up to five days after ingesting seeds (Stanton et al., 2003). Because internal animal dispersal may be occurring for a closely related congener, we answered unknown.
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	? - max	0	Little is known about seed dormancy and soil seed bank persistence of <i>B. carinata</i> (Johnson, 2014). One study found that seeds exhibit some primary dormancy for a few weeks after they mature (Tokumasu et al., 1985). But it is not clear how readily seeds may enter secondary dormancy, which is environmentally induced (e.g., seed burial, dry conditions), or how long they may persist in the soil. Through secondary dormancy, <i>B. rapa</i> and <i>B. napus</i> can survive for several years in the soil, but their seedbank declines very rapidly in agroecosystems (Hall et al., 2005). Seeds of <i>B. nigra</i> can survive in the soil for up to 40 years (Loewer,

Question ID	Answer - Uncertainty	Score	Notes (and references)
			2001). <i>Brassica kaber</i> seeds survive up to 26 years (Holm et al., 1997).
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	n - high	-1	We found no evidence of this, nor evidence of traits that would contribute to this kind of tolerance.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - high	0	We found no evidence that <i>B. carinata</i> is resistant to herbicides (e.g., Heap, 2014), but it may be able to acquire resistance through gene flow. <i>Brassica carinata</i> has been successfully hybridized with nine other related species, including <i>B. napus</i> (FitzJohn et al., 2007), which has been engineered for herbicide resistance (Schafer et al., 2011). A field study with four hectare plots of <i>B. carinata</i> and glyphosate-resistant <i>B. napus</i> planted next to each other demonstrated these species can cross with each other under field conditions, albeit at a low rate (0.002 percent and 0.005 of sampled <i>B. carinata</i> seeds; Séguin-Swartz et al., 2013). Hybrids were detected as far away as 150 meters from the common border (Séguin-Swartz et al., 2013). Pollen viability of hybrid plants was 14 percent and 8 percent for the two sites, and average seed set was 1.5 and 3.8 seeds per plant (Séguin-Swartz et al., 2013). Laboratory studies reported that <i>B. carinata</i> can cross with <i>B. napus</i> , but the resulting progeny are highly sterile (reviewed in Séguin-Swartz et al., 2013). A review of <i>B. carinata</i> concluded that while hybridization is possible, it is not likely to act as a vector for gene exchange (Taylor et al., 2010). Based on this evidence, we answered no with high uncertainty.
ES-21 (Number of cold hardiness zones suitable for its survival)	4	0	
ES-22 (Number of climate types suitable for its survival)	5	2	
ES-23 (Number of precipitation bands suitable for its survival)	9	1	
IMPACT POTENTIAL			
General Impacts			
Imp-G1 (Allelopathic)	y - high	0.1	<i>Brassica napus</i> and <i>B. rapa</i> produce allelopathic compounds with detrimental effects on crop yield, and seed germination of crops and other weeds (reviewed in Gulden et al., 2008). <i>Brassica</i> species in general can suppress weeds due to the presence of compounds known as glucosinolates (reviewed in Earlywine et al., 2010). <i>Brassica carinata</i> has a high glucosinolate concentration in oils extracted from its seeds (Getinet et al., 1996). One researcher commented that <i>B. carinata</i> competes well against weed species (Mayles, 2014), but did not clarify if this was due to plant morphology or allelopathy. This species has also shown some promise for use as a biofumigant against a common nematode species (Guerrero-Diaz et al., 2013). Consequently, we answered yes with high uncertainty.
Imp-G2 (Parasitic)	n - negl	0	We found no evidence. This species does not belong to a plant family known to contain parasitic species (Heide-Jorgensen, 2008; Nickrent, 2009).

Question ID	Answer - Uncertainty	Score	Notes (and references)
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	n - low	0	We found no evidence of this impact. Because this species has not been reported to be weedy in natural areas, and because the closely related <i>B. napus</i> and <i>B. rapa</i> are only a concern in agricultural fields (Gulden et al., 2008), we used low uncertainty for this question and the others in this subelement.
Imp-N2 (Change community structure)	n - low	0	We found no evidence.
Imp-N3 (Change community composition)	n - low	0	We found no evidence.
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	n - low	0	We found no evidence.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions)	n - low	0	We found no evidence.
Imp-N6 (Weed status in natural systems)	a - low	0	We found no evidence that this species is a weed of natural systems. Alternate answers for the Monte Carlo simulation were both "b."
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	n - low	0	We found no evidence. Because it has not been recorded as an anthropogenic weed, we used low uncertainty for this question, as well as Imp-A2 and Imp-A3.
Imp-A2 (Changes or limits recreational use of an area)	n - low	0	We found no evidence.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable plants and vegetation)	n - low	0	We found no evidence.
Imp-A4 (Weed status in anthropogenic systems)	a - high	0	This species occurs on trash dumps and waste ground in the London area (Clement and Foster, 1994) and elsewhere in the United Kingdom (Hanson and Mason, 1985). One herbarium record classified it as a garden weed (GBIF, 2014). Because one report of it behaving as a garden weed is not very strong evidence, particularly when the herbarium label provided no additional description, we answered no with high uncertainty. Alternate answers for the Monte Carlo simulation were both "b."
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - high	0	We found no evidence of this impact for <i>B. carinata</i> . <i>Brassica napus</i> and <i>B. rapa</i> , which are also grown as oilseed crops, are only a weed concern in agricultural fields (Gulden et al., 2008). "Herbicide-resistance and the stacking of genes in volunteer populations conferring resistance to multiple herbicides have contributed to increased difficulties in controlling volunteer <i>B. napus</i> in some crops. However, yield loss resulting from volunteer populations is not well documented in Canada" (Gulden et al., 2008). Because <i>B. carinata</i> 's closely related congeners may be problematic as volunteers, we used high uncertainty instead of moderate.
Imp-P2 (Lowers commodity value)	? - max	0	We found no direct evidence that <i>B. carinata</i> lowers commodity value. However, glucosinolate compounds (see Imp-P5) and their hydrolyzed products produce off-flavors in eggs from poultry fed <i>Brassica</i> seeds (Burrows and Tyrl, 2013). Similar off-flavors

Question ID	Answer - Uncertainty	Score	Notes (and references)
			develop in meat and milk from cattle fed Brassicaceae plants (Burrows and Tyrl, 2013). Because <i>B. carinata</i> seeds generally have a large amount of glucosinolate compounds (Getinet et al., 1996; Marillia et al., 2014), it is possible it may have a similar impact. Consequently, we answered unknown.
Imp-P3 (Is it likely to impact trade)	n - mod	0	We found no evidence of this impact for <i>B. carinata</i> . We note that European importers of Canadian mustard (<i>B. juncea</i>) require that shipments of seed be free of transgenes and that there is no outcrossing between genetically engineered canola (<i>B. napus</i>) and mustard (Séguin-Swartz et al., 2013).
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - mod	0	We found no evidence.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	y - low		Brassicaceae plants produce a group of compounds known as glucosinolates (GSLs), which, when broken down, yield isothiocyanates, nitriles, and other products (Assayed and Abd El-Aty, 2009; Halkier and Gershenzon, 2006). "Depending on the concentration and structural types of these compounds, their biological effects can be toxic, antinutritional or beneficial to health. Most serious economic problems in livestock seem to result from rapeseed meal; arising from GSLs or their breakdown products. In contrast, GSLs and their isothiocyanate (ITC) hydrolysis products are reportedly well-known protectors against carcinogenesis" (Assayed and Abd El-Aty, 2009). "One of the predominant rapeseed glucosinolates ... forms an oxazolidine-2-thione upon hydrolysis that causes goiter and has other harmful effects on animal nutrition" (Halkier and Gershenzon, 2006). Burrows and Tyrl (2013) review the harmful effects of these compounds, which include thyroid enlargement, embryonal death, growth retardation, liver hemorrhage, and digestive disturbances. <i>Brassica carinata</i> seeds generally have a large amount of glucosinolate compounds (Getinet et al., 1996; Marillia et al., 2014). "The goitrogenic nature of this glucosinolate poses a serious constraint to the commercial use of <i>B. carinata</i> meal in animal feed capacity" (Marillia et al., 2014). Although we found no direct evidence of toxicity resulting from consumption of <i>B. carinata</i> , we answered yes because the impact of these toxins at the genus and family level are well known (Burrows and Tyrl, 2013). We also note that breeders are aware of this problem and are trying to breed biotypes of <i>B. carinata</i> that have lower levels of glucosinolates so that animal meal can be made from seeds after the seed soil has been extracted (Getinet et al., 1996).
Imp-P6 (Weed status in production systems)	a - high	0	We found only one source stating <i>B. carinata</i> is a weed in agricultural systems, and that source only stated that it was a "weed of cultivation" (APD, 2014). This species has been present in the United States for about 40 years (Stephens, 2009) without demonstrating any weedy behavior. Furthermore, in the last 20 to 30 years it has been introduced to several countries for field trials as a potential biofuel plant (Bozzini et al., 2007; Gunasinghe et al., 2013; Malik, 1990; Velasco et al., 2003; Zada et al., 2013a; Zada et al., 2013b), and we found no evidence of weedy behavior. Without additional evidence or a more detailed explanation of why this species is a "weed of cultivation," we answered "a" with

Question ID	Answer - Uncertainty	Score	Notes (and references)
			high uncertainty. Alternate answers for the Monte Carlo simulation were both "b."
GEOGRAPHIC POTENTIAL			Unless otherwise indicated, the following evidence represents geographically referenced points obtained from the Global Biodiversity Information Facility (GBIF, 2014).
Plant hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z2 (Zone 2)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z3 (Zone 3)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z4 (Zone 4)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z5 (Zone 5)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z6 (Zone 6)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z7 (Zone 7)	n - negl	N/A	We found no evidence that it can establish in this zone.
Geo-Z8 (Zone 8)	n - high	N/A	Grown over the winter in Osimo, Italy (Raccuia et al., 2013), but an exact location was not given; Osimo encompasses both Zone 9 (coastal) and Zone 8 (inland). Because seedlings are not resistant to frost (Anonymous, 2014), we answered no with high uncertainty.
Geo-Z9 (Zone 9)	y - low	N/A	A few points in Ethiopia. Overwintered in Catania and Palozzola dello Stella, Italy (Raccuia et al., 2013). Several points around London and the southern United Kingdom (GBIF, 2014), but these are likely due to repeated introductions and not persistent populations (Clement and Foster, 1994).
Geo-Z10 (Zone 10)	y - negl	N/A	Many points in Ethiopia.
Geo-Z11 (Zone 11)	y - negl	N/A	Many points in Ethiopia. Also a few points in Eritrea, Tanzania, and Zambia.
Geo-Z12 (Zone 12)	y - high	N/A	Ethiopia.
Geo-Z13 (Zone 13)	n - high	N/A	One point near edge of this zone in Ethiopia.
Köppen -Geiger climate classes			
Geo-C1 (Tropical rainforest)	n - low	N/A	We found no evidence that it can establish in this climate class.
Geo-C2 (Tropical savanna)	y - low	N/A	Ethiopia. Two points in Tanzania.
Geo-C3 (Steppe)	y - negl	N/A	Many points in Ethiopia. Also Eritrea.
Geo-C4 (Desert)	y - high	N/A	Three accessions from the Bale province of Ethiopia that appear to be in desert (occurrence data; Alemayehu and Becker, 2002). We used high uncertainty as these may be under cultivation.
Geo-C5 (Mediterranean)	y - low	N/A	Some points in Ethiopia.
Geo-C6 (Humid subtropical)	y - low	N/A	Two points in Zambia. Also reported to be grown in this country (Mnzava and Schippers, 2007). Grown in Osimo and Palozzola dello Stella, Italy (Raccuia et al., 2013).
Geo-C7 (Marine west coast)	y - mod	N/A	Ethiopia. Several points in the southern portion of the United Kingdom, but these are likely due to repeated introductions and not persistent populations (Clement and Foster, 1994).
Geo-C8 (Humid cont. warm sum.)	n - mod	N/A	We found no evidence that it can establish in this climate class.
Geo-C9 (Humid cont. cool sum.)	n - low	N/A	We found no evidence that it can establish in this climate class.
Geo-C10 (Subarctic)	n - negl	N/A	We found no evidence that it can establish in this climate class.
Geo-C11 (Tundra)	n - negl	N/A	We found no evidence that it can establish in this climate class.
Geo-C12 (Icecap)	n - negl	N/A	We found no evidence that it can establish in this climate class.

Question ID	Answer - Uncertainty	Score	Notes (and references)
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	y - high	N/A	One point in Ethiopia. Three accessions from the Bale province of Ethiopia that appear to be in this precipitation band (occurrence data; Alemayehu and Becker, 2002). We used high uncertainty as these may be under cultivation.
Geo-R2 (10-20 inches; 25-51 cm)	y - low	N/A	Ethiopia. Grown in Tanzania, Zimbabwe, Zambia, and Malawi as a market crop (Mnzava and Schippers, 2007); this region receives from 10 to 70 inches of mean annual precipitation, but it is unknown whether these plants are irrigated or not.
Geo-R3 (20-30 inches; 51-76 cm)	y - negl	N/A	Ethiopia. Grown in Tanzania, Zimbabwe, Zambia, and Malawi as a market crop (Mnzava and Schippers, 2007); this region receives from 10 to 70 inches of mean annual precipitation, but it is unknown whether these plants are irrigated or not. Grown in Catania, Italy (Raccuia et al., 2013).
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	Ethiopia. Grown in Tanzania, Zimbabwe, Zambia, and Malawi as a market crop (Mnzava and Schippers, 2007); this region receives from 10 to 70 inches of mean annual precipitation, but it is unknown whether these plants are irrigated or not. Grown in Osimo, Italy (Raccuia et al., 2013). Found in waste dumps in the United Kingdom (Clement and Foster, 1994).
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	Ethiopia. Grown in Tanzania, Zimbabwe, Zambia, and Malawi as a market crop (Mnzava and Schippers, 2007); this region receives from 10 to 70 inches of mean annual precipitation, but it is unknown whether these plants are irrigated or not. Grown in Palozzola dello Stella, Italy (Raccuia et al., 2013).
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	Ethiopia. Grown in Tanzania, Zimbabwe, Zambia, and Malawi as a market crop (Mnzava and Schippers, 2007); this region receives from 10 to 70 inches of mean annual precipitation, but it is unknown whether these plants are irrigated or not.
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	Ethiopia. Grown in Tanzania, Zimbabwe, Zambia, and Malawi as a market crop (Mnzava and Schippers, 2007); this region receives from 10 to 70 inches of mean annual precipitation, but it is unknown whether these plants are irrigated or not.
Geo-R8 (70-80 inches; 178-203 cm)	y - low	N/A	Ethiopia.
Geo-R9 (80-90 inches; 203-229 cm)	y - mod	N/A	Ethiopia.
Geo-R10 (90-100 inches; 229-254 cm)	y - high	N/A	One point in Ethiopia.
Geo-R11 (100+ inches; 254+ cm)	n - high	N/A	We found no evidence that it can establish in this climate class.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	This species is already present in the United States. It was imported from Ethiopia in 1957 for leafy vegetable production (Stephens, 2009). In 1972, Texas Agricultural Experiment Station released the cultivar TAMU Tex Sel, which has never become very popular (Stephens, 2009). It has been field tested in North Dakota, Montana, Florida, and Mississippi (Harrison, 2013; Marillia et al., 2014).
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	Sold on the internet at Amazon.co.uk (Amazon, 2014). Now commonly planted as a game-cover crop in the United Kingdom

Question ID	Answer - Uncertainty	Score	Notes (and references)
			(PFAF, 2014).
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A	
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	A bird-seed or oil-seed alien (Clement and Foster, 1994). Bird-seed alien (Stace, 2010). A contaminant of imported bird seed (Hanson and Mason, 1985).
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	