



Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

**Environmental release of
Ceutorhynchus scrobicollis
(Coleoptera: Curculionidae) for
classical biological control of garlic
mustard, *Alliaria petiolata*
(Brassicaceae), in the contiguous
United States**

**Environmental Assessment
July 2025**

EAXX-005-32-24P-1743085515

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**Environmental Assessment
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- Addressed briefly, or left unaddressed, any issues or considerations that were, in the agency's judgment, comparatively not of a substantive nature;

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- Contains analysis that is adequate to inform and reasonably explain the responsible official's final decision regarding the proposed action or selected alternative.

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I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the root-mining weevil *Ceutorhynchus scrobicollis* (Nerenscheimer and Wagner) (Coleoptera: Curculionidae). *Ceutorhynchus scrobicollis* would be used for the classical biological control (biocontrol) of garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande (Brassicaceae)) in the contiguous United States.

Classical biological control of weeds is a control method where natural enemies from a foreign country (where the exotic target weed occurs) are used to reduce exotic weeds that have become established in the United States. Several different kinds of organisms have been used as biological control agents of weeds: insects, mites, nematodes, and plant pathogens. Efforts to study and release an organism for classical biological control of weeds consist of the following steps (TAG, 2016):

1. Foreign exploration in the weed's area of origin.
2. Host specificity studies.
3. Approval of the exotic agent by PPBP.
4. Release and establishment in areas of the United States invaded by the target weed.
5. Post-release monitoring.

This environmental assessment (EA) has been prepared, consistent with the National Environmental Policy Act of 1969 (NEPA) as amended and USDA's NEPA Implementing Regulations (Title 7 of the Code of Federal Regulations (CFR), part 1b). It examines the potential effects on the quality of the human environment that may be associated with the release of *C. scrobicollis* to control infestations of garlic mustard within the contiguous United States. This EA considers the potential effects of the proposed action and its alternatives, including no action.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities. The PPBP received a permit application requesting environmental release of *C. scrobicollis*, and the PPBP is proposing to issue permits for this action. Before permits are issued, the PPBP must analyze the potential impacts of the release of this agent into the contiguous United States.

The permit applicant's purpose for releasing *C. scrobicollis* is to reduce the spread of garlic mustard in the contiguous United States. Garlic mustard is an introduced European plant first recorded in North America in 1868 on Long Island, New York. By 2015, it had spread to 37 states and six Canadian provinces (Nuzzo, 1993b; USDA-NRCS, 2015). The spread across the continent continues and garlic mustard is one of the few introduced plant species that invades and dominates the understory of forested areas in North America. Sites dominated by garlic mustard frequently have lower native plant richness and cover (McCarthy, 1997; Anderson et al., 1996; White et al., 1993). Through abundant seed production as well as production of chemicals that inhibit growth of other plants, garlic mustard is able to rapidly colonize mesic (moist) forests and produce dense stands (Meekins and McCarthy, 2002), and it is more competitive than woody understory species (Meekins and McCarthy, 1999). Although herbicides have been shown to provide temporary, local reductions in garlic mustard populations, none have provided effective long-term control over large areas. In addition, abundance of garlic mustard in nearby unmanaged sites is a continued source for re-introduction of seeds. At present, natural area managers have no species-specific, long-term tool to manage garlic mustard. Therefore, the applicant has a need to release the root-mining weevil *C. scrobicollis*, a host-specific biological control organism, into the environment to reduce infestations of garlic mustard in the contiguous United States.

The following information regarding garlic mustard, *C. scrobicollis*, and the host specificity testing conducted is from a petition submitted to APHIS by the permit applicant (Van Riper et al., 2016).

II. Alternatives

This section will explain the two alternatives available to the PPBP—no action and issuance of permits for environmental release of *C. scrobicollis*. Although the PPBP's alternatives are limited to a decision on whether to issue permits for release of *C. scrobicollis*, other methods available for control of garlic mustard are also described. These control methods are not decisions to be made by the PPBP, and their use is likely to continue whether or not the PPBP issues permits for environmental release of *C. scrobicollis*, regardless of the ability of *C. scrobicollis* to reduce garlic mustard populations. These are methods presently being used to control garlic mustard by public and private concerns.

APHIS considered a third alternative, but it will not be analyzed further. Under this third alternative, the PPBP would have issued permits for the field release of *C. scrobicollis*; however, the permits would contain special provisions or requirements concerning release procedures or mitigating measures. No issues have been raised that would indicate special provisions or requirements are necessary.

A. No Action

Under the no action alternative, PPBP would not issue permits for the field release of *C. scrobicollis* for the biological control of garlic mustard. The release of this biological control agent would not take place. The following methods are presently being used to control garlic mustard; these methods will continue under the “No Action” alternative and will likely continue even if permits are issued for release of *C. scrobicollis*, depending on the efficacy of the organism to control garlic mustard.

1. Mechanical Control

Cutting flowering stems of garlic mustard at ground level provides effective control with minimal side effects. Cutting is most effective when plants are in full bloom or have developed siliques (seed pods) (Nuzzo, 1991) as plants cut earlier in the flowering period may produce new flowering stems. Pulling is effective if the upper half of the root is removed.

2. Chemical Control

Herbicides (glyphosate and triclopyr) are effective at controlling garlic mustard, but applications must be timed to the appropriate stage of growth (Becker et al., 2013). While some soil-applied herbicides can kill seedlings as they emerge (pre-emergence activity), none are known to provide total control. Therefore, the most effective are foliar applications of herbicides when garlic mustard has emerged and is actively growing. The proper timing of an application is specific to the active ingredient of the herbicide being used, but typical foliar applications are made to rosette plants in the fall or in the spring before bolting (elongation of shoots that will eventually flower and set seed). If desirable plants are present, herbicides with no residual activity are often preferred (e.g., glyphosate). These are applied when garlic mustard rosettes are present but desirable plants have not yet emerged (spring) or have gone dormant (fall). Because garlic mustard emerges earlier and goes dormant later than most desirable vegetation, it provides an application window for improved selectivity.

3. Cultural Control

Prescribed burning can maintain garlic mustard cover at low levels (Nuzzo et al., 1996) or have no effect (Luken and Shea, 2000), but fire does not automatically reduce abundance of garlic mustard (Nuzzo, 1991; Schwartz and Heim, 1996; Luken and Shea, 2000). Burning kills adult plants if the root crown is sufficiently heated. Fire management is only feasible in fire-tolerant communities with sufficient fuel to carry burns, and fire may alter composition of native herbaceous vegetation (Nuzzo, 1991; Nuzzo et al., 1996; Luken et al., 1997; Luken and Shea, 2000).

B. Issue Permits for Environmental Release of *Ceutorhynchus scrobicollis*

Under this alternative, the PPBP would issue permits for the field release of *C. scrobicollis* for the biological control of garlic mustard in the contiguous United States. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Biological Control Organism Information

1. Taxonomy and Description of *Ceutorhynchus scrobicollis*

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Curculionidae

Subfamily: Ceutorhynchinae

Tribe: Ceutorhynchini

Genus: *Ceutorhynchus*

Species: *C. scrobicollis* Nerenscheimer and Wagner

Common name(s): None

Synonym: None

The body length of *C. scrobicollis* adults ranges from 2.9 to 4.0 millimeters (mm). The head, thorax, abdomen, and legs are black. The elytrae (hardened forewings) are sparsely covered with

black hairs. The femora (segments of the insect leg) of the middle and hind legs have small teeth, and the claws are toothed. The first instar (developmental stage) larva (an active, immature stage of insects) has a white body with a distinctive dark brown head, while the second and third instar larvae have reddish brown heads.

2. Geographical Range of *Ceutorhynchus scrobicollis*

Native range: *Ceutorhynchus scrobicollis* is recorded from Austria (Colonnelli, 2004), Bosnia-Herzegovina (Colonnelli, 2004), Bulgaria (Colonnelli, 2004), Czech Republic (Colonnelli, 2004), Germany (Dieckmann, 1972), Italy (Abazzi and Osella, 1992), eastern France (Schott, 2000), Austria, and Hungary. The northernmost records are from Northern Germany and Poland, while the easternmost records are from the eastern Caucasus region. During field surveys, *C. scrobicollis* was found in northeastern Germany, in the vicinity of Berlin, and in eastern Austria near Vienna. *Ceutorhynchus scrobicollis* can be found in a wide range of habitats, such as roadsides, field edges, wastelands, and forests. *Ceutorhynchus scrobicollis* is present on *A. petiolata* growing on both sandy and loamy soils.

Expected, attainable range in North America: The native range of *C. scrobicollis* extends over a wide range of climatic conditions in Eurasia. The population used in host-specificity tests discussed later in this document and proposed for introduction into the contiguous United States originates from the area of Berlin, Germany. This area has a continental climate and an average minimum temperature of -3 °C (26.6 °F) in January, and an average maximum temperature of 24 °C (75.2 °F) in July, with a range of temperature from -22 °C to 37 °C (-7.6 to 98.6 °F). *Ceutorhynchus scrobicollis* is therefore thought to be well adapted to climatic conditions in most of the invasive range of garlic mustard in the United States.

CLIMEX software (version 4) was used to compare the climate of Berlin, Germany to North America and simulate an approximate map where climate conditions may be suitable for *C. scrobicollis* in the United States (Figure 1). CLIMEX is a software package for bioclimatic modeling that includes a 'Match Climates' tool for comparing the meteorological data of a 'home' location (Berlin) to an 'away' location or region (North America). The climate matching module allows the user to examine the similarities of two locations based on weighted factors like maximum and minimum temperature, average temperature, rainfall patterns, rainfall total, relative humidity, and soil moisture. These components (with the exception of soil moisture) contribute to the Composite Match Index (CMI), an indicator of how similar a location is to the 'home' location (Rafter et al., 2008). The CMI is a number

between 0 and 1 with 1 being a perfect match. Areas on the map (Figure 1), such as the Great Lakes basin, are very similar to Berlin's climate with a CMI greater than 0.7, while other regions like the southeastern United States and most of the west coast are a poor match with an index under 0.5. The midwestern and northeastern U.S. states had climates similar to Berlin, Germany.

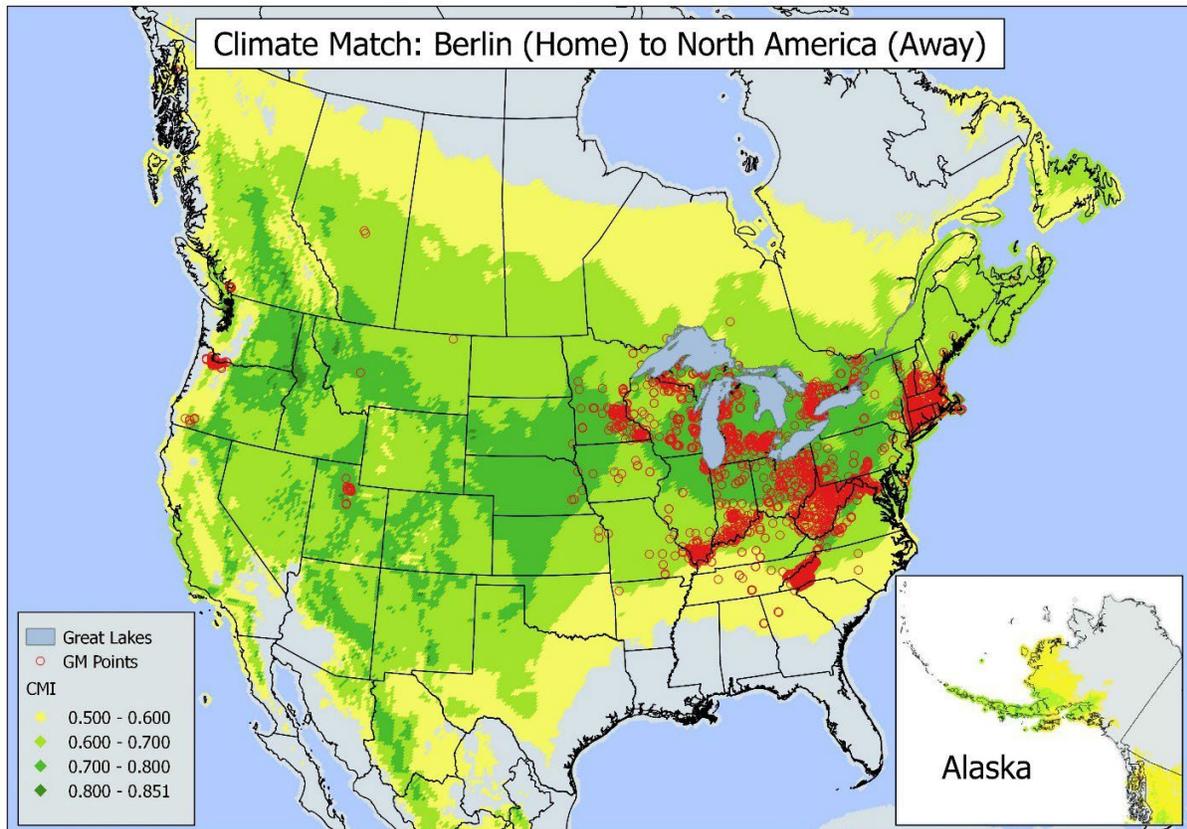


Figure 1. Climate matching for *Ceutorhynchus scrobicollis* in North America collected from Berlin, Germany. GM Points = garlic mustard points, CMI = Composite Match Index. Areas with CMI values from 0.0–0.5 are considered unsuitable, CMI values of 0.5–0.7 are interpreted as suitable but unlikely matches, and CMI values of approximately 0.7 or higher are usually interpreted as biologically significant.

Other areas of introduction: *Ceutorhynchus scrobicollis* has not been introduced elsewhere except into Canada. Canada approved the release of *C. scrobicollis* in June 2018 where it has been released in Ontario.

3. Life history of *Ceutorhynchus scrobicollis*

Life cycle. *Ceutorhynchus scrobicollis* has one generation per year. Adults oviposit (lay eggs) on garlic mustard rosettes from mid-September until the beginning of April of the following year. Larvae pass through three instars and due to repeated oviposition on the same plants, all three larval instars and eggs can be found at the same time in the same plants (Gerber et al., 2007b). Larvae mine leaf stalks (petioles), root-crowns, and shoot bases throughout the winter and early spring. Mature larvae leave the plants to pupate in the soil by early May. Adults emerge from early May to mid-June. After emergence, adults briefly feed on garlic mustard leaves, and then aestivate (become dormant) in summer. Characteristic feeding marks reappear on rosettes leaves from September onwards. In captivity, adults survived for more than one year and had a second, and in some cases even a third, oviposition period.

Oviposition behavior and fecundity (ability to produce offspring). Eggs are laid into petioles, leaves, and the growing tips (apical meristems) of garlic mustard rosettes. Females use their elongated rostrum or snout to bore holes into host plant tissue, deposit a single egg, and subsequently cover the opening with a secretion. Based on laboratory observations, females lay on average 231.2 ± 15.5 (standard error (SE)) eggs during their first oviposition period, and a similar number (243.6 ± 26.4 (SE) eggs) during their second oviposition period. Oviposition stops if average (mean) daily temperatures drop below -5°C (23°F).

III. Affected Environment

A. Taxonomy, Description, and Life History of Garlic Mustard

1. Taxonomy

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Capparales

Family: Brassicaceae

Tribe: Thlaspideae

Genus: *Alliaria* Heister ex Fabr

Species: *Alliaria petiolata* (Bieb.) Cavara & Grande

Common name: garlic mustard

2. Description

Garlic mustard is a cool season biennial plant (takes two years to complete its lifecycle) with a slender taproot. First year rosettes (Figure 2) have rounded, scallop-edged leaves that are dark green to purplish green. Second year bolting plants have heart shaped lower leaves and smaller triangular upper leaves in an alternate arrangement on the stem. Leaves have toothed margins. Plants usually produce a single unbranched flower stalk (Figure 3), although robust plants have been recorded with multiple branches and up to 12 separate flowering stalks. Flowers are produced in spring (usually April to May) in terminal racemes, and occasionally in short racemes (flower clusters with the separate flowers attached by short equal stalks at equal distances along a central stem). Some plants produce additional racemes in mid-summer. Flowers are typical of the mustard family, consisting of four white petals that narrow abruptly at the base, and six stamens, two short and four long. Flowers average 6 to 7 mm in diameter, with petals 3 to 6 mm long. Fruits are linear siliques, 2.5 to 6 centimeters (cm) long and 2 mm wide, held erect on short (5 mm), stout, widely divergent stalks. Siliques contain an average of 10 to 20 seeds. Mature seeds are black, cylindrical (3 mm x 1 mm) and range in weight from 1.6 to 2.8 milligrams (mg). Adult plants range in height from 0.05 meters (m) to 1.9 m, and average 1.0 m, at the time of flowering.



Figure 2. Garlic mustard rosette.



Figure 3. Garlic mustard flowering plant.

3. Life History

Garlic mustard is a biennial that spreads only by seed (Cavers et al., 1979). Each generation lives approximately 15 months, and the two age classes co-occur for approximately three months (April-June).

Up to 60 percent of seeds germinate sometime in March or April during the first spring after seed set, depending in the location (Baskin and Baskin, 1992). Seeds that fail to germinate the first spring may remain dormant in the seed bank for at least five years (Baskin and Baskin, 1992). Seedling density in heavily infested forests ranges from 830 to 1,800/square m (Anderson et al., 1996), and can be as high as 20,000/square m (Trimbur, 1973). Seedlings undergo high mortality, declining by 30 percent to more than 77 percent by late spring (Trimbur, 1973; Cavers et al., 1979; Byers and Quinn, 1998; Van Riper et al., 2010).

By June, seedlings develop the characteristic rosette of first year plants (Figure 2). First year rosettes are sensitive to summer drought (Mackenzie, 1995; Byers and Quinn, 1998) with 60 to 90 percent mortality by fall in drought years (Anderson et al., 1996; Byers and Quinn, 1998). By mid-fall, rosettes range from 4 to 10 cm in diameter (range 1 to 15 cm). Rosettes continue to grow in winter during snow-free periods when temperatures are above freezing (Cavers et al.,

1979; Anderson et al., 1996). Natural mortality continues throughout the winter (Nuzzo, 1993c) and total survival rate from seedling to adult stage varies from 1.4 to 45 percent (Cavers et al., 1979; Anderson et al., 1996; Byers and Quinn, 1998; Meekins and McCarthy, 2000; Van Riper et al., 2010). Mortality rates are strongly influenced by weather, with lower survival in years with dry summers (Meekins and McCarthy, 2000; Van Riper et al., 2010).

All plants that successfully overwinter bolt and flower the next spring, during April through June, depending upon the location. Garlic mustard flowers can be self- or cross-pollinated (Cavers et al., 1979; Anderson et al., 1996; Cruden et al., 1996). Flowers that are not insect pollinated self-pollinate (Cruden et al., 1996). Individual plants produce an average of 4 to 16 siliques, and each silique contains an average of 10 to 20 seeds. A silique is a long, narrow seedpod of many plants of the Brassicaceae. Seeds disperse in summer from June through August, depending upon the location, with seed production averaging 350 seeds, but occasionally as high as 7,900 seeds for robust plants (Nuzzo, 1993b). Seed production within dense patches of garlic mustard ranges from 3,607 to 45,018/square meter (Nuzzo, unpublished data; Trimbur, 1973; Anderson et al., 1996; Byers and Quinn, 1998) and has been estimated as high as 107,580/square meter (Cavers et al., 1979). At low density, garlic mustard seed production varies from 168 to 8,034/square meter (Meekins and McCarthy, 2000).

Garlic mustard spreads exclusively by seed (Cavers et al., 1979) with an average dispersal distance of 1.82 m (Biswas and Wagner, 2015). Anthropogenic (human-caused) distribution appears to be the primary dispersal mechanism (Lhotska, 1975; Nuzzo, 1993 a; b). Seeds are also widely dispersed in floodwaters and are likely dispersed by rodents and by white-tailed deer. Seeds require 50 to 105 days of cold stratification at 1 to 10°C to break dormancy (Baskin and Baskin, 1992). Garlic mustard seeds germinate in both light and dark after dormancy is broken (Bloom et al., 1990). Germination rates of 12 to 100 percent have been reported (Cavers et al., 1979; Roberts and Boddrell, 1983; Baskin and Baskin, 1992; Anderson et al., 1996; Byers and Quinn, 1998), but rates vary greatly within and among populations and habitats (Cavers et al., 1979; Byers and Quinn, 1998). Once established, garlic mustard becomes a permanent member of the community, steadily increasing in presence but with large annual fluctuations in cover and density (Byers and Quinn, 1998; Meekins and McCarthy, 1999; Nuzzo, 1999; Meekins and McCarthy, 2000).

B. Areas Affected by Garlic Mustard

1. Range of Garlic Mustard in North America

Garlic mustard was first recorded in North America in 1868 on Long Island, New York, and by 2015 had spread to 37 states and 6 Canadian provinces (Nuzzo, 1993b; USDA-NRCS, 2015) (Figure 4). This plant has spread exponentially since introduction (Nuzzo, 1993a; b). The current range in North America extends from Alaska, British Columbia (Cavers et al., 1979; White et al., 1993), Washington, and Oregon in the west, and in the east from New England (Gleason and Cronquist, 1991) and Ontario (Cavers et al., 1979) to Tennessee (Nuzzo, 1993b), Georgia and westward to Arkansas, Kansas, and South Dakota (herbarium specimens).

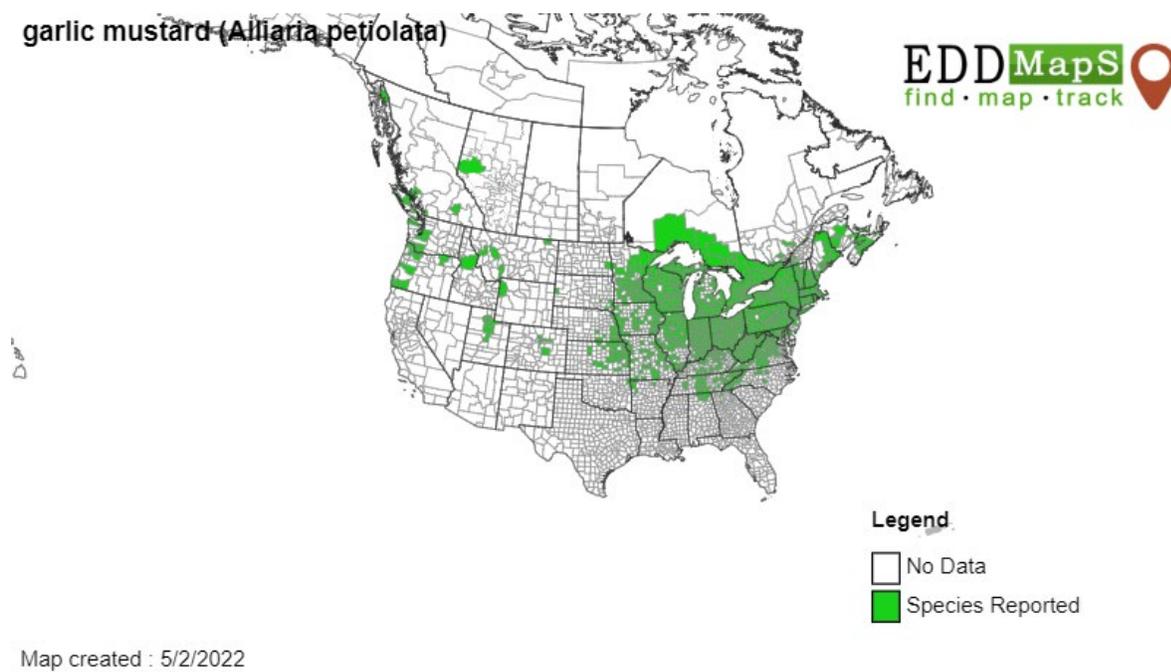


Figure 4. Distribution of garlic mustard in North America (EDDMapS. 2022).

In the United States, garlic mustard is most abundant in the New England and midwestern states, and populations are expanding in the northwestern states. Infrequent collections from mountain states indicate the plant may be a sporadic rather than established component of the regional flora, and/or in the process of becoming established in Utah (1971, 1983, 1984) and eastern Colorado (1952, 1958, 1966) (dates of herbarium collections). There are no herbarium records for garlic mustard in California (<https://ucjeps.berkeley.edu/consortium/>), August 2023), or Florida (<https://www.floridamuseum.ufl.edu/herbarium/collections/>, August 2023).

In Canada, garlic mustard is well established in Victoria and Vancouver, British Columbia (Cavers et al., 1979; White et al., 1993) and in the St. Lawrence Valley from Point Pelee in Ontario to Quebec City in Quebec (Cavers et al., 1979). Garlic mustard is especially abundant in southwestern Ontario, and near Toronto and Ottawa (White et al., 1993). White et al. (1993) recorded the plant as common in deciduous woods on the Canadian Shield, although Cavers et al. (1979) stated that the plant was noticeably absent from the region.

2. Native Range of Garlic Mustard

Garlic mustard is native to Europe (Tutin et al., 1964), and western Asia (Welk et al., 2002), occurring from England (Martin, 1982) east to Czechoslovakia (Lhotska, 1975), Iraq, Tadjikistan, and Nepal (Welk et al., 2002), and from Norway, Sweden, and Germany south to Italy. It is noticeably absent from Iceland, the Azores, Sardinia, and Spitsbergen (Tutin et al., 1964). Garlic mustard is also considered native to North Africa, India, and Sri Lanka (Cavers et al., 1979; Welk et al., 2002).

3. Range of Potential Spread in North America

Welk et al. (2002) predicts that garlic mustard will continue to expand its range through parts of North America with “deciduous wood vegetation, non-acidic soils and mesic habitat” with mean winter temperatures below 5° C and mean annual rainfall above 500 mm. Other climatic regions would less likely be invaded. Newly invaded regions are predicted to include parts of the Great Plains along riparian areas, including South Dakota and Nebraska, from northern Utah to southern Idaho, as well as isolated areas of Colorado.

4. Habitats Where Garlic Mustard is Found

In its native European range, garlic mustard is an edge species, growing along hedges and fencerows (Fitter et al., 1974; Martin, 1982; Durka et al., 2005; Stinson and Seidler, 2014) and in open woods (Wilmanns and Bogenrieder, 1988). It is disturbance adapted, is associated with calcareous soils (Clapham et al., 1962), and is frequently a component of ruderal communities (plants growing in waste areas or among refuse) (Swies and Kucharczyk, 1982/1983) and open, highly-disturbed forests (Klauck, 1986).

In North America, garlic mustard occupies similar habitat to its European range. It is most common in deciduous forests (Trimbur, 1973; Cavers et al., 1979; Nuzzo, 1993a,b; Byers and

Quinn 1998), and the partial shade characteristic of oak savanna, forest edges, hedgerows, shaded roadsides, and urban areas, and occasionally in full sun (Nuzzo, 1991). Garlic mustard is common in low-quality forests (Hawkes and Abrahamson, 1994; Schwartz and Heim, 1996), but readily invades high-quality forests. It grows on sand, loam, and clay soils, and on both limestone and sandstone substrates, but is rarely found on peat or muck soils. Growth of garlic mustard was reduced in more acidic soils (Anderson et al., 1996). It frequently grows in well-fertilized sites (Cavers et al., 1979), and is described as a nitrophile (a plant that prefers to grow in soil rich in nitrates) (Wilmanns and Bogenrieder, 1988).

Garlic mustard is also common in riparian habitats (wetland, adjacent to rivers and streams), particularly in the northeastern United States (Nuzzo, 1993a). Byers and Quinn (1998) reported that garlic mustard, once considered a plant of floodplains and moist woods in New Jersey, had become common in a wider range of habitats. In Minnesota, garlic mustard has invaded a variety of sites, including riparian habitats, oak savannah, and hardwood forests (Van Riper et al., 2010).

C. Plants Related to Garlic Mustard and Their Distribution

1. Native and Non-Native Relatives

According to a new tribal classification system based on molecular analyses, the genus *Alliaria* is now placed in the tribe Thlaspideae. There are 11 other genera in this tribe, with a native range restricted to southwestern Asia and Europe (Al-Shehbaz, 2012). The European species, *Noccaea caerulescens* (formerly *Thlaspi caerulescens*) (Koch and German, 2013) is in the tribe Noccaeeae. *Noccaea caerulescens* is able to hyperaccumulate both cadmium and zinc. This species is being studied in the United States for its ability to phytoextract heavy metals for use in environmental remediation (Wang et al., 2006). Two European *Thlaspi* species in the tribe Thlaspideae have naturalized in North America. *Thlaspi arvense*, field pennycress, is an invasive plant present in all states except Alabama and Hawaii (USDA-NRCS, 2023). Field pennycress is a weed in nursery, agronomic, and horticultural crops (Uva et al., 1997) and acts as a winter host of soybean cyst nematode (Venkatesh et al., 2000). *Thlaspi alliaceum*, roadside pennycress, is the second of the introduced *Thlaspi* species. This plant is present in Ohio, Indiana, Pennsylvania, Kentucky, Virginia, Tennessee, Maryland, Missouri, North Carolina, Georgia, Louisiana, New Jersey, and Delaware (USDA-NRCS, 2023). Another species from the tribe Thlaspideae, *Pachyphragma macrophylla*, is cultivated and sold as an ornamental ground cover in the United States.

North American species of *Thlaspi* have been re-assigned to the tribe Noccaeeae, genus *Noccaea*, as they form a group separate from some of the European species of *Thlaspi* (Koch and Al-Shehbaz, 2004). North American *Thlaspi* spp. (now *Noccaea*) native to North America are therefore not as closely related to *A. petiolata* as previously thought.

IV. Environmental Consequences

A. No Action

1. Impact of Garlic Mustard

a. Native plant and animal populations

Garlic mustard has long been suspected to have negative impacts on native species, particularly native plants. In a laboratory experiment (Meekins and McCarthy, 1999) documented that garlic mustard can outcompete chestnut oak (*Quercus prinus*) seedlings but was in turn outcompeted by seedlings of boxelder (*Acer negundo*) and the annual jewelweed (*Impatiens capensis*). In a field experiment, McCarthy (1997) found that removing garlic mustard resulted in greater relative cover of annual species. Nuzzo (1998) conducted an 8-year monitoring study in Illinois and found that in areas with garlic mustard, cover of native perennial herbaceous species declined significantly but species richness did not change. In Ohio, McCarthy (1997) found no correlation between species diversity and garlic mustard biomass, and determined that species richness was similar in plots with and without garlic mustard.

Roots of garlic mustard produce several allelopathic phytotoxic chemicals (chemicals produced by garlic mustard that inhibit neighboring plant growth), such as sinigrin and its breakdown product allyl isothiocyanate (AITC), and glucotopaeolin and its breakdown product benzyl isothiocyanate (BzITC) (Vaughn and Berhow, 1999). These phytochemicals are unique to garlic mustard and differ from chemical profiles of other native Brassicaceae species in North America, which suggests that these allelopathic phytochemicals are novel in garlic mustard's invaded range (Barto, 2010; Cantor, 2011).

Allelopathic chemicals produced by garlic mustard can negatively affect native plants by disrupting the association between certain beneficial fungi (arbuscular mycorrhizal fungi (AMF))

and roots of host plants (Roberts and Anderson, 2001; Stinson et al., 2006; Wolfe et al., 2008; Barto et al., 2010, 2011; Lankau, 2010). The majority of native plants growing in the forest understory require association with AMF for optimum growth and survival, especially in the seedling stage (Barlow, 2011). Garlic mustard does not require AMF for growth, and thus has a competitive advantage over species with disrupted AMF associations. As an example, when tree seedlings of native hardwoods, such as red maple, sugar maple, and white ash, were grown in soils with a history of garlic mustard, the seedlings had reduced AMF associations (Stinson et al., 2006). Species composition of AMF shifted in soils with a history of garlic mustard compared to control soils (Barlow, 2011). Effects of previous garlic mustard invasion altered AMF species richness and species composition for up to six years after garlic mustard was removed (Lankau, 2014).

Garlic mustard establishment and growth is also facilitated by nonnative earthworms (Nuzzo et al., 2009). Deer and earthworms individually impact non-native species and may also have interactive effects on garlic mustard (Davalos et al., 2015). Deer and non-native earthworms interact to facilitate garlic mustard establishment and growth (Davalos et al., 2015). Deer do not eat garlic mustard.

Garlic mustard flowers attract flies in the family Syrphidae, pollen-collecting beetles, and native bees away from native plant species that flower at the same time, and reduce seed set of species such as *Geranium maculatum* and *Trillium grandiflorum* (B. Blossey, unpublished data). No insects are known to depend on garlic mustard.

Garlic mustard threatens certain butterfly species including the rare Virginia white butterfly (*Pieris virginiensis*). Females of several native *Pieris* butterfly species (*Pieris napi oleracea*, *Pieris napi marginata*, and *P. virginiensis*) are attracted to garlic mustard because of the similarities between sinigrin in garlic mustard and chemicals in their original host plants *Cardamine concatenata* [*Dentaria laciniata*] and *Cardamine* [*Dentaria*] *diphylla* (Huang et al., 1994/1995). Frequent oviposition occurs on garlic mustard (Porter, 1994); however, most or all of the larvae die before completing development (Bowden, 1971; Courant et al., 1994; Huang et al., 1994/1995). *Pieris virginiensis* prefers garlic mustard for oviposition over its native host *Cardamine diphylla* and lays significantly more eggs garlic mustard in both lab and field settings (Davis and Cipollini, 2014). This strong preference suggests that sinigrin is not the only attractant and there may be other non-glucosinolate cues (Davis et al., 2015). The poor survival rate of *P. virginiensis* larvae on garlic mustard plants is likely due to the toxicity of the unique compound, alliarinoside (Davis et al., 2015). Thus, garlic mustard is a population sink for this

rare species (Porter, 1994; Haribal and Renwick, 1998; Davis and Cipollini, 2014).

b. Economic impacts

Garlic mustard is one of the few introduced herbaceous (non-woody) plant species that invades and dominates the understory of forested areas in North America. Garlic mustard-dominated sites frequently have low native herbaceous richness and cover, and it has been implicated as the cause of this low diversity (White et al., 1993; Anderson et al., 1996; McCarthy, 1997). The increasing abundance of the species triggers aggressive control attempts using hand weeding, fire, and herbicidal control. Treatment costs vary widely. In a survey of multiple Midwest states, herbicide applications to control garlic mustard ranged from \$67 to \$300 per acre and hand pulling costs ranged from \$100 to \$5,700 per acre. For example, a 20 acre patch of garlic mustard in Minnesota cost \$4,000 to treat with glyphosate herbicide, while a small patch (0.20 acres) in Maine cost \$1,300 to manually remove.

Control of garlic mustard is attempted on thousands of acres across many states each year. Evidence suggests that garlic mustard can limit tree growth and recruitment through interference with mycorrhizal associations of native trees, such as sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), and white ash (*Fraxinus americana*) (Stinson et al., 2006). Long-term losses in forest and timber productivity may have occurred, although at present there is not any quantitative assessment of the effect of garlic mustard invasion on forest productivity.

Garlic mustard invasion has been associated with increased nitrogen mineralization and low pH levels in sandy forest soils (Morris et al., 2012). This suggests that along with its ability to alter soil community and structure, garlic mustard colonization may be facilitated by soil acidification, especially in industrialized regions (Morris et al., 2012).

c. Beneficial Uses

Garlic mustard has little recorded beneficial value other than the occasional recipe suggesting use of young leaves in green salads for human consumption. All parts of the plant are edible. The species is not used as an ornamental and has no recreational value. Abundant seed set in mid-summer attracts deer mice (*Peromyscus maniculatus*), which consume and cache seeds (B. Blossey, unpublished data).

2. Impact from Use of Other Control Methods

The continued use of mechanical, chemical, and cultural controls at current levels would be a result if the “no action” alternative is chosen. These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *C. scrobicollis* to reduce garlic mustard populations in the contiguous United States.

a. Mechanical Control

Cutting flowering stems and pulling are very labor intensive. Viable seed may form on cut stems (Solis, 1998a,b; Frey, 2002) so all stems need to be removed from the site, adding to the labor costs. Garlic mustard frequently snaps off at or above the root crown when pulled, leaving buds which send up new flower stalks.

b. Chemical Control

Some herbicides have residual activity in the soil after an application to garlic mustard that may affect desirable vegetation through uptake by roots or emerging shoots. Large-scale repeated herbicide treatments are prohibitively expensive and time consuming, and can have impacts on nontarget plants.

c. Cultural Control

Fire does not automatically reduce abundance of garlic mustard (Nuzzo, 1991; Schwartz and Heim, 1996; Luken and Shea, 2000). Burning kills adult plants only if the root crown is sufficiently heated; a quick fire, or an incomplete fire, may remove rosette leaves but undamaged root crowns will subsequently produce flower stalks from adventitious buds (Nuzzo et al., 1996).

Burning may also enhance growth of seedlings that germinate after fire removes leaf litter (Nuzzo et al., 1996). Fire management is only feasible in fire-tolerant communities with sufficient fuel to carry burns, and fire may alter composition of native herbaceous vegetation (Nuzzo, 1991; Nuzzo et al., 1996; Luken et al., 1997; Luken and Shea, 2000).

B. Issue Permits for the Environmental Release of *Ceutorhynchus scrobicollis*

1. Impact of *C. scrobicollis* on Nontarget Plants

Host specificity of *C. scrobicollis* to garlic mustard has been demonstrated through scientific literature, field observations, and host range testing. If the candidate biological control agent only attacks one or a few plant species closely related to the target weed, it is considered to be very host-specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

a. Scientific Literature

Literature records for *C. scrobicollis*. Dieckmann (1972) describes garlic mustard as the only host for *C. scrobicollis* in its native range. In addition, *C. scrobicollis* has never been reported as a pest on commercially grown plants in the family Brassicaceae (the cabbage family) (Schwarz et al., 1990) confirming Dieckman's information.

Literature records on closely related species. European Ceutorhynchinae have been recorded from 26 different plant families (Wagner (1942) in Dieckmann (1972)). However, individual species usually are closely associated with specific host plants or genera and no polyphagous species (recorded from several different plant families) have been recorded in the Ceutorhynchinae (Dieckmann, 1972). Of the over 375 described *Ceutorhynchus* species, all except one (*C. linicola* developing in stem galls on *Linum perenne* and *L. usitatissimum*) are associated with Brassicaceae or the closely related Resedaceae (*C. resedae* Marsham and *C. landesi* Tempere on *Reseda* spp., and *C. debskii* Pic on *Ochradenus*). While most *Ceutorhynchus* spp. are oligophagous on several plant genera, some are recorded from only one or two species of Brassicaceae or are even described as from only one plant species.

b. Field Observations

None of the plant species closely related to garlic mustard (e.g., *Peltaria alliacea* and *Thlaspi arvense*) or of economic importance (e.g., *Brassica oleracea*) occur in the same habitat as *A. petiolata* and most of the other critical test plant species are of North American origin. Therefore, no field observations of potential *C. scrobicollis* attack on these species could be conducted. However, *C. scrobicollis* has never been found on any plant species other than garlic mustard by any of the three leading Curculionidae specialists (L. Behne, E. Colonnelli, and B.A. Korotyaev) nor has it been reared during extensive field surveys targeting three additional weeds in the family Brassicaceae, such as *Lepidium draba* (hoary cress) (Cripps et al., 2006), *Lepidium*

latifolium (perennial pepperweed) or *Isatis tinctoria* (dyer's woad). These data suggest that *C. scrobicollis* is monophagous (feeding on one or more plant species within a genus) in its native range as noted by Dieckmann (1972).

c. Host Range/Specificity Testing

Host range testing was conducted to determine the specificity of *C. scrobicollis* to garlic mustard and to determine if other plant species in the contiguous United States could be at risk of attack by *C. scrobicollis*. Plants closely related to the target plant are considered at higher risk of attack than more distantly related plants. See appendix 1 for full details of host specificity testing conducted for *C. scrobicollis*.

(1) Site of Field and Quarantine Studies and Population Studied

Most tests were conducted in the laboratory or common garden at CABI Switzerland, Delémont, Switzerland (47°21'N, 7°22'E). Additional tests were conducted in the quarantine facility of the University of Minnesota, St Paul, MN.

All host-specificity tests used *C. scrobicollis* originating from garlic mustard field sites in the vicinity of Berlin, Germany. According to molecular analysis of *C. scrobicollis* collected from eleven sites in Europe, all individuals from the Berlin region can be regarded as a single population, while weevils from more distant sites, such as Vienna, Austria, are significantly differentiated (Rauth et al., 2011). Field collected adults were used to establish a rearing colony at the CABI Switzerland Centre and both field collected and reared individuals were used to conduct host specificity tests. All *C. scrobicollis* sent to the quarantine facility in Minnesota for additional host specificity tests also originated from the Berlin area.

(2) Test Plant List

Test plant lists are generally established based on relatedness (phylogenetic relationships) between the target weed and other plant species (Wapshere, 1974). It is generally assumed that species closely related to the target are at greater risk of attack than species more distantly related. The test plant list consisted of 116 species and subspecies, with 85 in the family Brassicaceae, and the remaining 31 species in 22 different families. See appendix 1 for plant species tested for host specificity. Except for *Zea mays* (corn), *Triticum aestivum* (wheat), and *Glycine max* (soybean), which were included due to their economic importance, species in families other than Brassicaceae were chosen because they are native North American species

growing in the same habitat as garlic mustard. Biological control agents are expected to reach outbreak densities after their release in the invaded range, thereby considerably reducing or even locally eliminating the target weed. In such a case of successful control, plants growing in the same habitat as the target would be the first to encounter the temporarily high populations of biocontrol agents and any potential spill-over.

Summary and Interpretation of Host Range/Specificity Testing Results Arranged by Test Plant Category

See appendix 1 for complete descriptions and results of host specificity testing.

Category 1 – Genetic types of garlic mustard. For most host-specificity tests with *C. scrobicollis* at CABI Switzerland, two European and one U.S. population of garlic mustard were included. Tests in Minnesota were conducted using one North American population. The genetic variability of garlic mustard has been characterized using 27 native and 26 introduced populations (Durka et al., 2005). Overall, introduced populations were genetically less diverse. However, considerable variability was present, indicating that multiple introductions of garlic mustard into the United States have occurred. Most probable source regions include the British Isles and northern and central Europe. In a study investigating the palatability (being acceptable to the taste) of native European and invasive U.S. garlic mustard populations to *C. scrobicollis* weevils (Bossdorf et al., 2004), weevils had higher feeding rates on U.S. populations. However, in a subsequent experiment, larval development and number of offspring of *C. scrobicollis* did not differ between native and introduced populations. Thus, once released in North America, the researchers expect that *C. scrobicollis* will perform well on the invasive populations of garlic mustard (Van Riper et al., 2016).

Category 2 – North American species in the same genus as the target weed. The genus *Alliaria* does not naturally occur in North America (USDA-NRCS, 2023). The only species present is the target weed, *A. petiolata*. Therefore, no other *Alliaria* species were tested.

Category 3 – North American or introduced species in other genera in the plant family Brassicaceae, including economically and environmentally important plants. Eighty-five species, subspecies, and cultivars in the family Brassicaceae were tested. Twenty-five of these are of economic importance in the United States, either as oil seed crops (e.g., *Brassica nigra*, black mustard), as vegetables (e.g., *B. oleracea italica*, broccoli) or as ornamentals (e.g., *Hesperis matronalis*, dame's rocket, which is also considered invasive in the Northeast and

Midwestern United States). Three additional species are being studied as potential new crops (*Lesquerella fendleri*, *Physaria acutifolia*, and *Thlaspi arvense*). In addition to the six commercial *Brassica oleracea* species tested, several varieties were included because one *C. scrobicollis* adult emerged from *B. oleracea sabauda* during one of the test series. Forty of the 85 Brassicaceae tested are native to North America. If North American species were not available for testing, then European congeners were used instead as surrogates. For example, the European *Braya alpina* was used as a surrogate for the North American species *Braya glabella*.

Within the Brassicaceae, females accepted nearly half of the offered plant species in sequential no-choice tests for oviposition. While these results suggest a relatively unspecific oviposition behavior, the tests were conducted in confined, no-choice conditions using cut plant parts. Inflated host acceptance resulting from confined test conditions is a common and long recognized occurrence in host specificity investigations in many biocontrol programs (Cullen, 1990; Blossey et al., 1994; Marohasy, 1998; van Klinken, 2000; Briese, 2005). In nature, host selection consists of a hierarchical sequence of choice of habitat, approach of a plant from a distance, decision to stay on the plant, and the decision to oviposit; none of these behaviors are possible for females in confined conditions. In addition, offering cut plant pieces has the possibility to change plant chemistry and thus acceptance of test plants offered in cylinders. Thus, host specificity investigations use a sequence of increasingly realistic venues to assess host acceptance and the possibility for completion of larval development. Most plant species accepted in no-choice oviposition tests did not support larval development in subsequent tests and are therefore outside the physiological host range of *C. scrobicollis*.

Of 50 Brassicaceae exposed in no-choice larval development tests, five species allowed complete larval development, (the European *Peltaria alliacea*, *Thlaspi arvense*, and *Nasturtium officinale* (watercress), the native North American *Rorippa sinuata*, and the commercially grown *Brassica oleracea sabauda*). The remaining 45 species did not support larval development so were outside the physiological host range of *C. scrobicollis*.

In a no-choice larval development test, a single adult in a single replicate emerged from the commercially grown cabbage variety *Brassica oleracea sabauda*, while none of the other six *Brassica oleracea* varieties offered were attacked. Moreover, no attack occurred in subsequent tests under multiple-choice caged conditions. This suggests that *B. oleracea* and its various varieties are outside the physiological host range of *C. scrobicollis*. The researchers consider the emergence of a single adult a laboratory artifact. In addition, if commercially grown cabbage

varieties would be part of the normal host range of *C. scrobicollis*, the species would have been recorded as a pest in the European literature, which is not the case (Schwarz et al., 1990).

Three of the four remaining plant species that allowed development (wild-type *Nasturtium officinale*, *Peltaria alliacea*, and *Thlaspi arvense*) are of European origin. While *P. alliacea* is not present in North America, *N. officinale* and *T. arvense* are considered invasive in the United States (USDA-NRCS, 2023). Interestingly, according to the new phylogeny proposed for the Brassicaceae both *P. alliacea* and *T. arvense* are in the same tribe (Thlaspideae) as garlic mustard (Al-Shehbaz et al., 2006). Although the open-field test demonstrated that at least *P. alliacea* and *T. arvense* may act as alternative hosts for *C. scrobicollis*, none of the three plant species has ever been recorded as a host for *C. scrobicollis* in the literature or by curculionid taxonomists. This absence of attack in Europe may be due to *C. scrobicollis* habitat preferences. Typically, *N. officinale* grows in and along streams and ditches, and *T. arvense* in agricultural habitats; both quite different from the partial shade and forest understory communities where garlic mustard occurs. Habitat preferences of dispersing or foraging *C. scrobicollis* may therefore make it unlikely that *N. officinale* and *T. arvense* are encountered regularly in nature. However, *P. alliacea* does grow in forests and its distribution appears to overlap with that of *C. scrobicollis*. It therefore appears that it could be encountered, and it is not clear why *P. alliacea* is not a recorded field host of *C. scrobicollis*. It should also be noted that North American species of *Thlaspi* have been assigned to the tribe Noccaeeae, genus *Noccaea*, as they form a monophyletic group separate from most of the European species of *Thlaspi* (Koch and Al-Shehbaz, 2004). Therefore, North American *Thlaspi* spp. (now *Noccaea*) native to North America are not as closely related to garlic mustard as previously thought.

Nasturtium officinale is grown as a commercial crop in the United States. Wildtype *N. officinale* supported adult development in larval development tests when grown in dry soils. Because *N. officinale* is an obligate wetland plant (FWS, 1988), a second set of tests was conducted to determine whether *C. scrobicollis* could develop in *N. officinale* grown in water-saturated soils. This is important to know because *N. officinale* is cultivated as an annual crop and is grown in flooded fields. Because *C. scrobicollis* larvae pupate in the soil, it was important to determine whether this terrestrial species could complete its development in water-saturated soils, the conditions present when *N. officinale* is grown commercially.

In the second set of tests conducted at CABI Switzerland and the University of Minnesota, *C. scrobicollis* did not emerge from *N. officinale* grown in either saturated or “dry”, non-saturated conditions. The dry conditions were the same as those used in previous studies, where adults

emerged from *N. officinale*. The only difference between tests was that wildtype *N. officinale* was used for initial tests and commercial varieties were planted for the second set of tests. In addition, *C. scrobicollis* has not been collected from or reported as a pest of *N. officinale* in Europe, where it has been cultivated for more than a century.

Of the remaining test species, a single native North American species, *Rorippa sinuata*, allowed *C. scrobicollis* to complete larval development under no-choice conditions. However, the species was not attacked in single-choice tests when it was in the presence of garlic mustard. The species has a western distribution in North America and occurs in floodplains and wetlands; thus, it rarely overlaps with the range and habitats garlic mustard occupies in North America. In parts of its distribution the species is considered invasive (Stubbendieck et al., 1994). Under field conditions, risks to *R. sinuata* by *C. scrobicollis* are expected to be extremely low to non-existent (Van Riper et al., 2016).

Two other species in the tribe Cardamineae (*Cardamine bulbosa* and *Rorippa sessiliflora*) supported larval development to a certain extent under no-choice conditions. In single-choice larval development tests, where fertile females were given a choice of *C. bulbosa* or garlic mustard, *C. bulbosa* was not attacked and larvae were not recovered from *C. bulbosa* plants. One early instar larva was recovered from one replication of *R. sessiliflora*. No adults of *C. scrobicollis* emerged from either test plant. The researchers believe that under field conditions, risks to *C. bulbosa* by *C. scrobicollis* are extremely low to non-existent (Van Riper et al., 2016).

According to the new phylogeny (the evolutionary history of an organism) based on molecular analyses, the tribe Cardamineae is well separated from the tribe Thlaspideae to which garlic mustard belongs. However, based on traditional taxonomy, *Cardamine*, *Nasturtium*, and *Rorippa* were originally placed in the same tribe as garlic mustard (Arabideae) (Hegi, 1986). Independently of which taxonomy would be followed, these species appear to belong to a second group of less preferred hosts that may form part of the physiological, but not the ecological host range of *C. scrobicollis*.

Overall, *C. scrobicollis* can be considered a highly specialized herbivore. Its physiological host range includes a few additional species in the same tribe (Thlaspideae) as the primary host garlic mustard and the tribe Cardamineae. Most of these species either do not occur in North America or are themselves introduced and considered invasive. The results reported here, in combination with a lack of field records of plant use other than garlic mustard in Europe, indicate that the realized host range appears restricted to the target plant, garlic mustard.

Category 4 – Threatened and Endangered species in the Brassicaceae family. To better define the host range of *C. scrobicollis*, nine available threatened (T), endangered (E), and candidate (C) species were tested. For the remaining listed species for which seeds or plants were not available, surrogate species were tested. When selecting surrogates for testing, taxonomically related species were chosen with similar life histories, habitats, or ranges as the listed species.

With the selection of these Brassicaceae species, all T, E, C or surrogates from the Brassicaceae genera were tested, with the exception of *Warea*, *Leavenworthia*, and *Sibara*. *Ceutorhynchus scrobicollis* eggs were deposited into leaves or petioles of five out of nine T, E and C test species, which can be considered normal oviposition behavior for *C. scrobicollis*. Within the Brassicaceae, females accepted nearly half of the offered plant species in sequential no-choice tests for oviposition. While these results suggest a relatively unspecific oviposition behavior, tests were conducted in confined conditions using cut plant parts. Inflated host acceptance as a result of confined test conditions is a common and long recognized occurrence in host specificity investigations in many biocontrol programs (Cullen, 1990; Marohasy, 1998; van Klinken, 2000; Briese, 2005; Blossey et al., 1994). In nature, host selection consists of a hierarchical sequence of choice of habitat, approach of a plant from a distance, decision to stay on the plant, and the decision to oviposit; none of these behaviors are possible for females in confined conditions. In addition, offering cut plant pieces has the possibility to change plant chemistry and thus acceptance of test plants offered in cylinders. Thus, host specificity investigations use a sequence of increasingly realistic venues to assess host acceptance and the possibility for completion of larval development. Most plant species accepted in no-choice oviposition tests did not support larval development in subsequent tests and therefore are outside the physiological host range of *C. scrobicollis*.

Brassicaceae genera containing Threatened, Endangered, or Proposed Species:

Arabis. *Arabis macdonaldiana*, is listed as endangered and has been classified as a variety of *A. blepharophylla* by some taxonomists (Al-Shehbaz, 2010). *Arabis georgiana* (Georgia rockcress) is listed as a threatened species. *Arabis blepharophylla* was tested and *C. scrobicollis* did not oviposit on this species. Because *A. macdonaldiana* and *A. blepharophylla* are closely related, *A. blepharophylla* can serve as a viable surrogate for *A. macdonaldiana*. Another native, *Arabis alpina*, was tested and adults were able to oviposit but no larvae were present in no-choice larval development tests.

Boechera. Three listed endangered species in this genus are: *Boechera hoffmannii*, native to California, *Boechera perstellata*, and *Boechera serotina*, both native to the Appalachia region. These species were previously placed in the *Arabis* genus, but have been reclassified and placed in the *Boechera* genus according to the *Flora of North America* (Al-Shehbaz, 2010). *Boechera pusilla* is listed as a candidate species. In no-choice oviposition tests, no eggs were observed in *B. perstellata*. *Boechera serotina* was not tested because seeds of this species were not obtained.

With *B. hoffmannii*, a total of one egg and one dead first instar larva were found in shoots in a no-choice larval development test conducted at CABI Switzerland. No adult *C. scrobicollis* emerged from *B. hoffmannii*. At CABI's outdoor testing site in Switzerland, other *Ceutorhynchus* species can attack caged test plants, so it is necessary to run gene sequence tests to positively identify any eggs or larvae present in test plants. Unfortunately, it was not possible to identify the egg or larva due to problems obtaining gene sequences for these samples. However, in single-choice oviposition tests, *C. scrobicollis* females did not accept *B. hoffmannii* for oviposition when presented a choice between *B. hoffmannii* or garlic mustard. In two natives, *Boechera canadensis* and *Boechera laevigata*, *C. scrobicollis* eggs were recorded but no larvae developed in no-choice larval development tests.

Cardamine. *Cardamine micranthera* is listed as an endangered species and is native to Virginia and North Carolina. Seeds of *C. micranthera* were not obtained to test. However, six *Cardamine* species were tested, five of which are native to North America and no adults emerged in no-choice larval development tests. Early instar larvae were present in *C. bulbosa* in no-choice larval development tests. However, in single-choice larval development tests, when *C. scrobicollis* females were allowed a choice between *C. bulbosa* and garlic mustard, *C. bulbosa* was not attacked. The five native species tested (*Cardamine angustata*, *Cardamine bulbosa*, *Cardamine concatenata*, *Cardamine diphylla*, and *Cardamine pratensis*) all have overlapping ranges with *C. micranthera* and occupy similar habitats.

Caulanthus. *Caulanthus californicus* is listed as an endangered species. Seeds were not available for this species so a native surrogate was tested, *Caulanthus heterophyllus*. Both species of *Caulanthus* have overlapping ranges and are annual plants, thus, *C. heterophyllus* serves as an adequate surrogate for *C. californicus*. Oviposition in *C. heterophyllus* was recorded during sequential no-choice oviposition tests. One dead first instar larva was found in one replication in a no-choice larval development test and no adults emerged. However, 99 percent of eggs were deposited in garlic mustard leaves in single-choice oviposition tests when *C. scrobicollis* females were given a choice where to lay eggs.

Caulanthus californicus is confined to eight counties in southern California (FWS, ECOS, 2023). This species grows in a chaparral-plant community that is dominated by drought tolerant shrubs (Halsey, 2007). This Mediterranean climate is defined by hot, dry summers and cool, wet winters. The Ecoregions of southern California where *C. californicus* grows are; Central California Coastal Range, Great Valley, Sierra Nevada Foothills and Southern California Mountains and Valleys.

Garlic mustard seedlings and rosettes are not likely to survive the summer droughts of the chaparral, as summer drought can increase seedling and rosette mortality (Meekins and McCarthy, 2000). Welk et al. (2002) compared the frequency of garlic mustard in mapped geographical regions of Europe at different average monthly temperatures and precipitation. They reported that areas receiving less than 10 mm average rain from May through October had few to no populations of garlic mustard. In addition, Welk et al. (2002) reported that an average minimum May rain amount of 30 mm was required for flowering, combined with average temperatures ranging from 8 °C to 18 °C. Average precipitation and temperature for May at *C. californicus* sites are 4.6 mm and 21.4 °C respectively (NOAA.org Bakersfield Meadows Field Airport). Average rain from May through October is less than 10 mm. Thus, the area where *C. californicus* grows is too hot and dry to support garlic mustard growth and development and *A. petiolata* is currently not recorded in California (USDA-NRCS, 2023). In addition, there is not a good match between the temperate climate of the *C. scrobicollis* collection site in Europe and this southern California Mediterranean region (Figure 1).

Erysimum. There are three endangered species in this genus and all are native to California. *Erysimum menziesii*, an endangered species, was tested and no feeding or oviposition occurred in sequential no-choice oviposition tests. Two additional native *Erysimum* species were tested; *Erysimum asperum* and *Erysimum pumilum* and also no feeding or oviposition occurred. Test results indicate that these three *Erysimum* species are outside the physiological host range of *C. scrobicollis*. Eggs were found in *Erysimum linifolium*, a species native to Europe, but adults were not able to develop.

Eutrema. *Eutrema penlandii* (synonym *Eutrema edwardsi*) is a threatened species and is native to Colorado. *Ceutorhynchus scrobicollis* did not oviposit on *E. penlandii* (surrogate) in sequential no-choice oviposition tests.

Hesperidanthus. According to the *Flora of North America*, there are three threatened and

endangered species that have been moved from the genus *Schoenocrambe* to *Hesperidanthus* (Al-Shehbaz, 2010). These species are: *Hesperidanthus argillacea*, *Hesperidanthus barnebyi*, and *Hesperidanthus suffrutescens*. *Hesperidanthus linearifolia*, a species with a wide distribution, was tested as a surrogate and eggs were recorded in two of eight replications in sequential no-choice oviposition tests, but no larval development occurred in no-choice larval development tests. In single-choice oviposition tests, *C. scrobicollis* females did not lay eggs in *H. linearifolia* when presented a choice between *H. linearifolia* and garlic mustard. From these test results, we consider *Hesperidanthus* species not to be of risk to attack from *C. scrobicollis*.

Leavenworthia. There are two endangered and one threatened species in this genus; *Leavenworthia crassa*, *Leavenworthia exigua laciniata*, and *Leavenworthia texana*. *Leavenworthia texana* is native to eastern Texas, once again, an area with a low climate match for *C. scrobicollis* (Figure 1). *Leavenworthia texana* is only found in St. Augustine County, Texas, an area south of the current southerly range of garlic mustard. This region of Texas is unlikely to accumulate sufficient chill units to successfully vernalize garlic mustard rosettes in the winter. This would prevent rosettes from bolting, setting seed and establishing. With climate change predictions, garlic mustard range will only shift northward, further away from St. Augustine County, Texas.

Leavenworthia crassa occurs in five counties in Alabama. *Leavenworthia exigua laciniata* occurs in Kentucky. Unable to locate a source for these species, testing the surrogate *Leavenworthia torulosa* was proposed. However, after repeated attempts with multiple methods, seeds failed to germinate and the researchers were not able to test *L. torulosa*. *Leavenworthia crassa* and *L. exigua laciniata* may have overlapping geographic ranges with sites invaded with garlic mustard. However, all *Leavenworthia* species grow in open areas in full sunlight and are unlikely to overlap with sites invaded by garlic mustard, which favors shaded/semi-shaded sites. Additionally, habitats that support *Leavenworthia* species are extremely wet during the late winter and early spring and become extremely dry in summer (Rollins, 1963), in which *C. scrobicollis* pupae would not survive in the surface soil:litter interface. In the event that *Leavenworthia* plants were accepted as hosts, *C. scrobicollis* would not be able to complete its lifecycle.

Although *Leavenworthia* species were not directly tested, the researchers examined phylogenetic relationships among tested species and compared host range test results. The tribe that contains the host *Alliaria*, Thlaspideae, is in a different lineage and is distant from the Cardamineae tribe.

In Europe and western Asia where *C. scrobicollis* is native and Brassicaceae and *C. scrobicollis* have co-existed for centuries, there are no reports of *C. scrobicollis* using any species other than garlic mustard in extensive insect taxonomic surveys (Dieckmann, 1972; Freude et al., 1983; Rheinheimer and Hassler, 2010), including none reported in the Cardamineae tribe. Essentially, in its native range, *C. scrobicollis* has been in a natural, centuries-long open-field test and has failed to accept other species as its host.

Lepidium. There are two species listed as endangered and one as threatened in this genus, *Lepidium arbuscula* (native to Hawaii), *Lepidium barnebyanum*, and *Lepidium papilliferum*. In no-choice larval development tests conducted at CABI Switzerland, three larvae were found in one *L. barnebyanum* plant, one of which was identified as *C. scrobicollis* by molecular analysis. No adults emerged from the *L. barnebyanum* plants. There is a single population of *Lepidium barnebyanum* confined to Duchesne County, Utah. *Lepidium barnebyanum* is only found in a very specific habitat, marly shale barrens on three ridgelines in pockets of pinyon-juniper woodlands at a high elevation (6,200 to 6,500 ft.) (USDA-NRCS, 2015). Burls and McClaugherty (2008) found that the distribution of garlic mustard decreased as elevation increased, but this may be a function of rainfall washing away seed. Because *L. barnebyanum* grows only at a high elevation and in a different habitat from where garlic mustard occurs, there would be no natural pathway for *C. scrobicollis* to reach ridgeline populations of *L. barnebyanum*. The researchers do not plan to release *C. scrobicollis* in Utah where it is unlikely to disperse to or establish. *Lepidium papilliferum* occurs in Ada, Elmore, Gem, Payette, and Owyhee Counties in Idaho. Three additional *Lepidium* species were tested (*Lepidium draba*, *Lepidium squamatum*, and the North American *Lepidium virginicum*) and no adults developed on any *Lepidium* species. In addition, in single-choice oviposition tests, 95 percent of eggs were deposited on garlic mustard compared to *L. draba* or *L. virginicum* (Table 4).

Additional testing was conducted on *L. barnebyanum*. In these tests, *C. scrobicollis* did not negatively impact growth or development of *L. barnebyanum* in a growth chamber experiment. *Lepidium barnebyanum* plants were six months old at the beginning of the experiment. Plants were grown outside during fall in Minnesota to simulate plant conditions in its native range in Utah. After a week of exposure to *C. scrobicollis* adults, only a single suspect probe mark was observed on one of eight caged *L. barnebyanum* plants. However, it was atypical for a *C. scrobicollis* probing mark and was no longer visible when the experiment was terminated. The impact experiment was terminated when F1 *C. scrobicollis* offspring emerged from control garlic mustard plants in the same growth chamber, approximately three months after the initiation of the experiment. No larvae or larval tunneling were detected in any *L. barnebyanum*

plants. There were no differences in root or shoot dry weights, plant height or crown diameter between plants with *C. scrobicollis* added compared to control *L. barnebyanum* plants. Presence or absence of oviposition/larvae were not discernable with visual inspection, so roots and shoots were dissected under a scope to confirm that no larvae were present and no tunneling had occurred.

***Lesquerella*.** According to *The Flora of North America* (Al-Shehbaz, 2010), this genus no longer exists, and species have been placed in the *Physaria* or *Paysonia* genera.

***Nasturtium*.** The endangered *Nasturtium gambellii* (formerly *Rorippa gambellii*) was tested. In no-choice larval development tests conducted at CABI Switzerland and the University of Minnesota, *C. scrobicollis* was not able to complete development on *N. gambellii* growing in water saturated soil. In tests conducted in growth chambers at the University of Minnesota, four larvae were found in the upper part of stems of *N. gambellii*, which is not normal behavior. Larvae typically tunnel down the stem to the roots and crown, where they continue to develop to the third instar, then leave the crown to pupate in the soil. In contrast, when no-choice larval development tests were repeated in a more natural environment in a greenhouse at CABI Switzerland, no larvae were found.

In single-choice oviposition tests, when females were offered *N. gambellii* and garlic mustard, 98 percent of eggs were deposited in garlic mustard. *Nasturtium gambellii* is native to southern California, is an obligate wetland plant and grows in freshwater wetlands, a habitat where *C. scrobicollis* is unlikely to establish. Because third instar *C. scrobicollis* larvae exit garlic mustard plants to pupate in the soil, larvae would drown in wetland water saturated soils where *N. gambellii* grows (see results from tests with submerged *N. officinale*).

There is a poor match between the temperate climate of the *C. scrobicollis* collection site in Europe and this southern California Mediterranean region (Figure 1). The differences in climate and ecoregion between these two sites indicate that there is minimal risk of *C. scrobicollis* establishment in southern California. In addition, there are no natural pathways for *C. scrobicollis* to reach sites where *N. gambellii* grows as garlic mustard is not present in California. Also, the saturated or flooded soil where *N. gambellii* grows precludes the establishment of garlic mustard, which thrives in soils of temperate forests.

Additional testing was conducted on *N. gambellii*. In these tests, *C. scrobicollis* did not negatively impact growth or development of *N. gambellii* in a growth chamber experiment after two weeks

of exposure to *C. scrobicollis* adults. Adult mouthpart probing was not observed from *C. scrobicollis* but feeding from the flea beetle, *Phaedon armoraciae* was observed. The experiment was terminated when F1 *C. scrobicollis* adults emerged from control garlic mustard plants in the same growth chamber, approximately three months after the initiation of the experiment. There were no differences in shoot dry weights, plant heights, or percent plant cover between *N. gambellii* with *C. scrobicollis* exposure compared to control *N. gambellii* at the termination of the experiment. At the termination of the experiment, when shoots of *N. gambellii* were dissected, two second-instar larvae were found in one shoot of a single plant. The larvae were essentially “stuck” in the shoot, created a very short tunnel (2-cm length), did not continue to tunnel down to the crown and roots, and did not develop to the third instar. This behavior is atypical of *C. scrobicollis*. In garlic mustard, larvae tunnel from shoots into the crown and roots, where they develop to the third instar and exit the plant. Because *N. gambellii* grows in the water-saturated soils of aquatic systems, meaningful tests required plants be grown in saturated soil. This completely restricted *C. scrobicollis* larvae to the stems above the soil surface as they would drown if they tunneled into the roots or crown. It should be noted that in a similar no-choice development study that was conducted under more natural common garden conditions at the CABI facility at Delémont, Switzerland, no larvae were found in any *N. gambellii* plants.

***Noccaea*.** There is one endangered subspecies in this genus, *Noccaea fendleri* subsp. *californica* (previously *Thlaspi californicum*). *Noccaea fendleri* and *Noccaea fendleri* ssp. *siskiyouensis* (previously *Thlaspi montanum* var. *siskiyouensis*) were tested and no *C. scrobicollis* oviposition was recorded in either species.

***Paysonia*.** There are two species in this genus that are listed species. *Paysonia perforata* is an endangered species found in Tennessee. *Paysonia lyrata* is a threatened species with its native range in Alabama. *Paysonia densipila* was tested as a surrogate, with a range that overlaps with the other two *Paysonia* species. In sequential no-choice oviposition tests, two eggs were found in a single replication of *P. densipila*, but no larvae or adults developed in no-choice larval development tests. In single choice oviposition tests, *C. scrobicollis* females greatly preferred garlic mustard (88 percent of total eggs laid in garlic mustard) when presented a choice between *P. densipila* and garlic mustard. There is a poor match between the temperate climate of the *C. scrobicollis* collection site in Europe and the southeastern region of the United States (Figure 1).

***Physaria*.** In this genus there are six species listed as T or E, and one species under review as a candidate species. The threatened plant *Physaria douglasii* ssp. *tuplashensis* was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva

was present in one *P. douglasii* spp. *tuplashensis* replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, *C. scrobicollis* females did not lay eggs in *P. douglasii* spp. *tuplashensis* when offered together with garlic mustard. The native plant *Physaria acutifolia* and *Physaria fendleri* were tested, and neither species was accepted for oviposition by *C. scrobicollis*.

Physaria douglasii spp. *tuplashensis* is native to Washington and is restricted to a small area adjacent to the Columbia River. This species is restricted to dry, barren, nearly vertical exposures of caliche soil in sagebrush steppe habitat. The substrate is extremely alkaline and highly calcareous. Because garlic mustard is found in temperate deciduous forests, it is unlikely that garlic mustard would be able to establish in sites with desert caliche soils, so there would be no hosts available to support *C. scrobicollis* development.

Rorippa. *Rorippa subumbellata*, a candidate species native to California and Nevada, did not support larval development in no-choice larval development tests. There were no larvae or larval tunneling found in dissected plants. Sequential no-choice oviposition tests were not conducted with this species. *Rorippa gambellii* is an endangered species in California and would not be expected to support development of *C. scrobicollis* (see *N. gambellii* above).

Sibara. *Sibara filifolia*, an annual species, is listed as an endangered plant in California. This species only occurs on San Clemente Island, located 41 miles off of the coast of southern California. As there are no records of garlic mustard in California, there are no naturally occurring pathways for *C. scrobicollis* to reach San Clemente Island. The island location of *S. filifolia* provides a natural barrier to *C. scrobicollis*. Seeds of this species were not obtained to include in host-range testing.

Streptanthus. Two *Streptanthus* species are listed as endangered; *Streptanthus albidus* subsp. *albidus* (Metcalf Canyon jewelflower) and *Streptanthus niger* (Tiburon jewelflower). Both species are native to California and are serpentine endemic plants that have adapted to grow exclusively in heavy-metal rich serpentine soils (Kruckeberg, 1951; Cacho et al., 2014). *Streptanthus bracteatus* (bracted twistflower) is a species listed as threatened and is native to oak-juniper woodlands in Texas (Al-Shehbaz, 2010). The surrogate, *S. glandulosus* subsp. *glandulosus* (bristly jewelflower) was selected because it is also a Californian native and serpentine endemic, and has a wider distribution (Mayer and Beseda, 2010).

In host range testing, the surrogate, *Streptanthus glandulosus* subsp. *glandulosus*, was accepted

for oviposition in sequential no-choice oviposition tests, but larvae did not develop in no-choice larval development tests. Early instar larvae were found in *S. niger* plants in no-choice larval development tests, but no adults emerged. In single-choice oviposition tests, *C. scrobicollis* females preferred garlic mustard (81 percent of total eggs laid in garlic mustard) when presented a choice between *S. niger* and garlic mustard.

Streptanthus niger is an annual plant only found on two sites on the Tiburon Peninsula on the northern side of San Francisco Bay in California. This *Streptanthus* species is a serpentine endemic plant and accumulates heavy metals, such as nickel, but is not classified as a hyperaccumulator plant (Reeves et al., 1981). Research has shown that plants adapted to serpentine soils, with high heavy metal concentrations, may be toxic to herbivores. Insects feeding on serpentine endemic plants may be killed, even if the plant is not a hyperaccumulator species (Coleman et al., 2005; Cheryuiyot et al., 2013). Herbivores may also avoid or feed less on serpentine endemic plants with lower levels of accumulated heavy metals (Cheryuiyot et al., 2013). Because *S. niger* was grown in a standard growing medium for host specificity testing, there was no ability to test whether heavy metal concentration in this serpentine endemic species was toxic, sub-toxic or non-toxic to *C. scrobicollis*. In addition to classification as a serpentine endemic plant, there are several characteristics of *Streptanthus niger* which would support its placement as outside the ecological host range of *C. scrobicollis*. First, unlike the biennial garlic mustard, *S. niger* is an annual plant. Seeds germinate in March and April and plants flower and set seed in June. Thus, the phenology of *S. niger* does not synchronize with *C. scrobicollis*, where adult females actively lay eggs in fall and early spring. Secondly, climate matching between Tiburon Peninsula and the collection site of *C. scrobicollis* (Berlin, Germany) indicates that this California site would not be a good match for *C. scrobicollis* (Figure 1). The isolated location of *S. niger*, with populations growing on an open, barren, rocky peninsula is not likely to support garlic mustard, which is not known to be adapted to a serpentine soil type. In addition, there are no natural pathways for *C. scrobicollis* to reach the Tiburon Peninsula, because garlic mustard is not present in California to support populations of *C. scrobicollis*. These factors make it unlikely that either garlic mustard or *C. scrobicollis* could invade or establish at this site and the researchers conclude that *Streptanthus niger* is likely to be outside of the ecological host range of *C. scrobicollis*.

The researchers conducted additional testing on *S. niger* and the results are summarized here: Serpentine soils contain high concentrations of heavy metals. Plants adapted to serpentine soils such as *S. niger* may be toxic to herbivores. Insects feeding on serpentine plants may be killed, and herbivores may avoid or feed less on serpentine plants. The initial studies of *S. niger* were

conducted in non-serpentine soils. The researchers were fortunate to later acquire Henneke serpentine soil and conducted a series of additional studies examining the effect of *S. niger* grown in the Henneke serpentine soil on feeding, probing, oviposition, and larval development of *C. scrobicollis*. In a no-choice impact study on *S. niger*, aboveground biomass in the presence of *C. scrobicollis* did not differ compared to *S. niger* control plants. In no-choice tests on vegetative *S. niger* plants, probing by adult *C. scrobicollis* placed on *S. niger* did not differ when grown in serpentine compared to a non-serpentine silt loam. However, older *S. niger* leaves harvested from plants entering the reproductive phase (bud stage) had significantly fewer mouthpart probing marks and high adult mortality compared with leaves from plants grown in a non-serpentine silt loam soil. The effect of serpentine soil on growth and development of *S. niger* and garlic mustard was measured compared to a non-serpentine soil. Though model predictions show garlic mustard plants will not invade the habitat occupied by *S. niger*, garlic mustard grown in serpentine soil accumulated significantly less aboveground biomass than plants grown in non-serpentine soil. The reverse was found with *S. niger* plants, where they formed buds and flowers earlier and accumulated more aboveground biomass when growing in serpentine soil. One first generation adult *C. scrobicollis* was found on a *S. niger* plant in one replication of a single-choice test in containment. The adult did not survive after placement on a garlic mustard plant. This was an atypical event, as no other adults have ever emerged from 21 other *S. niger* plants in previous testing at CABI or Minnesota. Overriding choice and no-choice tests at CABI or the University of Minnesota is the fact that, absent co-occurrence of garlic mustard, *S. niger* cannot sustain multiple generations of *C. scrobicollis*. Garlic mustard would not co-exist with *S. niger* at its only known location on the Tiburon Peninsula because garlic mustard would not survive in serpentine soils in a Mediterranean climate. In addition, *C. scrobicollis* will not establish on *S. niger* because of the extremely low to non-existent emergence of adults.

There are additional reasons that would reduce the potential establishment of *C. scrobicollis* on *S. niger*:

- *Streptanthus niger* is a serpentine endemic species. *Streptanthus niger* is an annual plant only found on the Tiburon Peninsula on the northern side of San Francisco Bay in California. This *Streptanthus* species is adapted to grow on serpentine soils with high concentrations of heavy metals, such as nickel and chromium. Garlic mustard is not adapted to serpentine soil and when grown in serpentine soil it failed to thrive and accumulated significantly less aboveground biomass than plants grown in non-serpentine soil.

- *Difference in phenology (the timings of cyclical/seasonal biological events, such as egg laying, flowering, and hibernation) between S. niger and C. scrobicollis.* Unlike the biennial garlic mustard, *S. niger* is an annual plant. Seeds germinate in October and November with the onset of autumn rains and seeds germinate at once, rather than over a period of time. Rosettes develop over the winter and plants flower in May and June. Seed set occurs in June and July during the dry months. There is a gap between cohorts of *S. niger* in late summer to early fall. The previous generation has flowered and set seed and the next cohort of seedlings will not germinate until the onset of fall rains the following October or November. In contrast, *C. scrobicollis* aestivates (enters into a dormant period) during June, July, and August in the temperate regions of Northern and Central Europe. During this time, adults are largely inactive, but require occasional feeding on garlic mustard leaves to survive. In late summer/early fall, adults actively feed and oviposit. Thus, the lifecycles of *C. scrobicollis* and *S. niger* are not in synchrony. Adult *C. scrobicollis* require a food source during the summer when *S. niger* plants are senescing (deteriorating with age) before fall, during this gap between *S. niger* cohorts. During the summer and fall, *S. niger* plants would not be able to provide sufficient forage to sustain adult *C. scrobicollis*.
- *The Tiburon Peninsula has an inhospitable climate and habitat for both garlic mustard and C. scrobicollis.* Garlic mustard is native to the temperate forest understory or forest edge. The isolated location of *S. niger*, with populations growing on an open, barren, rocky peninsula is not likely to support garlic mustard. Comparing the suitability of the Henneke soil type on the Tiburon Peninsula for garlic mustard and *S. niger*, serpentine soil severely stunted the growth and development of garlic mustard, but *S. niger* thrived. No- to low-levels of summer rain would not support survival of garlic mustard seedlings. Winter temperatures would not be sufficiently cold for rosette vernalization (Katovich et al. unpublished data). Vernalization is the induction of the flowering process of the plant by its required exposure to long periods of cold during the winter. Climate matching between Tiburon Peninsula for garlic mustard and the range of *C. scrobicollis* in Europe demonstrates that this California site would not be a good match for *C. scrobicollis* as described in habitat suitability models.

The other endangered *Streptanthus* species, *S. albidus* ssp. *albidus*, is also a serpentine plant (Cacho et al., 2014) and it grows on outcrops with little soil development with a distribution limited to Santa Clara and Santa Cruz Counties, California. This species was not tested, but climate matching indicates that this California site would not be a good match for *C. scrobicollis*

(Figure 1). *Streptanthus albidus* ssp. *albidus* is also an annual species, so would have a similar lack of phenological synchrony with *C. scrobicollis*, as described for *S. niger*.

The species listed as threatened, *S. bracteatus* is native to oak-juniper woodlands in Texas (Al-Shehbaz, 2010) and was not tested, but climate matching indicates that this Texas site would not be a good match for *C. scrobicollis* (Figure 1).

Thelypodium. There are two federally listed *Thelypodium* species, *Thelypodium howellii* ssp. *spectabilis* (threatened) is native to Oregon and *Thelypodium stenopetalum* (endangered) is native to California. Unable to locate seed sources of these species, the researchers tested *Thelypodium laciniatum* and *Thelypodium milleflorum* as surrogates. Both of these surrogates have overlapping ranges and are biennial, like the listed species (*T. howellii spectabilis* is listed as a biennial/perennial). *Ceutorhynchus scrobicollis* laid eggs in *T. laciniatum* but not *T. milleflorum* in sequential no-choice oviposition tests. The researchers conclude that *T. milleflorum* is outside of the host range of *C. scrobicollis*. No larvae developed in *T. laciniatum* in no-choice larval development tests. In single-choice oviposition tests, *C. scrobicollis* females preferred garlic mustard (84 percent of total eggs laid in garlic mustard) when presented a choice between *T. laciniatum* and garlic mustard.

Thysanocarpus. *Thysanocarpus conchuliferus* is listed as endangered and is native to California. This species is only found on Santa Cruz Island, off the coast of southern California. The surrogate *Thysanocarpus curvipes* was tested because it has a similar life history and also grows on Santa Cruz Island. *Thysanocarpus curvipes* was not accepted for oviposition by *C. scrobicollis* in sequential no-choice oviposition tests. In addition, there are no natural pathways for *C. scrobicollis* to reach Santa Cruz Island as garlic mustard is not present in California.

Warea. There are four species in the genus *Warea* in North America. *Warea amplexifolia* and *Warea carteri* are listed as endangered and are native to Florida. Seed sources were unavailable for these species. A seed source for *Warea sessilifolia* was found but the seeds failed to germinate. According to climate matching (Figure 1), Florida's climate does not match well with Berlin, Germany, the source of *C. scrobicollis*, thus Florida may not be suitable for *C. scrobicollis* establishment. In addition, garlic mustard is not found in Florida.

Categories 5 and 6 – North American or introduced species in other orders or other families in the Capparales order that have some phylogenetic, morphological, or biochemical relationship to the target weed, including economically and environmentally

important plants. Glucosinolates (mustard oils), accompanied by the hydrolytic enzyme myrosinase, are a group of secondary compounds characteristic for members of the Brassicaceae. However, 15 additional families of dicotyledonous angiosperms (flowering plants with a pair of cotyledons or leaves in the embryo of the seed) contain glucosinolates (Fahey et al., 2001). Traditional classifications placed these families in many separate orders, but molecular analyses confirmed Dahlgren's view (Dahlgren, 1975; 1977 in Rodman et al., 1998), and placed all glucosinolate containing taxa, except for *Drypetes*, in the order Capparales (Brassicales) (Rodman et al., 1998; Ronse De Craene and Haston, 2006). Because it is impossible to test representatives of all 15 additional families in the Capparales, and because glucosinolates most likely constitute only one of several groups of secondary compounds influencing host acceptance of specialist *Brassica* insects, two species within these two categories were chosen: the ornamentals *Reseda lutea* L. (Resedaceae) and *Tropaeolum majus* L. (Tropaeolaceae). Both species are introduced in the United States. Neither species was accepted for oviposition by *C. scrobicollis* and are outside the host range of *C. scrobicollis*.

Category 7 – Any plant on which the biological control agent or its close relatives (within the same genus) have been previously recorded to feed and/or reproduce. Given the large number of highly specialized species in the genus *Ceutorhynchus*, it is not possible to test all recognized host plants of close relatives. Three other *Ceutorhynchus* species are currently being investigated as additional agents for garlic mustard as well as six other *Ceutorhynchus* potential biological control agents for three additional Brassicaceae weeds, *Lepidium draba* (hoary cress), *Lepidium latifolium* (perennial pepperweed), and *Isatis tinctoria* (dyer's woad). Most Brassicaceae species that were attacked by these *Ceutorhynchus* spp. during host-specificity tests were also tested with *C. scrobicollis*.

Category 8 – Plants in the family Brassicaceae or from other families growing in the same habitat as the target weed. These are the plants most likely encountered by introduced biocontrol agents. The researchers included 40 species commonly growing in the same forest communities as garlic mustard. Except for *Hesperis matronalis*, all of these are native to North America; 14 of these species are in the family Brassicaceae, and 26 are from 18 different plant families. *Ceutorhynchus scrobicollis* females only exhibit normal oviposition behavior on plant species in the Brassicaceae family. Thus, plant species outside of the Brassicaceae family that co-occur with garlic mustard in North America will not be at risk. Therefore, even if high populations of *C. scrobicollis* develop and locally eliminate garlic mustard, adult feeding on these plants is expected to be negligible.

Of the species tested within the family Brassicaceae that occur in the same habitat as garlic mustard only one species in each of the genera *Cardamine* and *Rorippa* allowed development of *C. scrobicollis* under no-choice conditions. No attack was observed in choice tests. The researchers believe that under field conditions risks to *Cardamine* and *Rorippa* spp. by *C. scrobicollis* are low.

2. Impact of *C. scrobicollis* on Garlic Mustard

At field sites, plants of garlic mustard attacked by *C. scrobicollis* can generally be easily spotted, because larval mining destroys the main shoot, leading to production of several weaker side shoots. In 2001 and 2002, two manipulative experiments were conducted to quantify the impact of *C. scrobicollis* on garlic mustard (Gerber et al., 2007a; b). *Ceutorhynchus scrobicollis* was released at different densities, at different time periods, and in combination with *C. alliariae* (another potential biocontrol agent) on individually potted garlic mustard plants in a common garden. Plant survival, plant height, biomass, and seed production of attacked plants were measured and compared to unattacked control plants.

Attack by *C. scrobicollis* significantly reduced rosette survival by up to 50 percent. Surviving plants produced more shoots but had reduced height and reduced shoot diameter (Figure 5). Overall, allocation to aboveground biomass was reduced in attacked plants compared to controls. Attack by *C. scrobicollis* delayed the onset of reproduction and in one of the experiments also significantly reduced seed production by 23 to 82 percent. In summary, attack by *C. scrobicollis* is expected to decrease fitness and competitive superiority of garlic mustard in the introduced range (Van Riper et al., 2016).

A model used to assess the effect of *C. scrobicollis* and three additional potential biocontrol agents on garlic mustard predicted that attack by *C. scrobicollis* should be particularly effective to control garlic mustard populations in North America because it affects the two factors having the greatest impact on population growth rate: rosette survival and fecundity (Davis et al., 2006). Model outcomes differed as garlic mustard demographic parameters were varied within ranges observed in North America, and in some cases a combination of agents will be necessary to suppress the most vigorous garlic mustard populations.

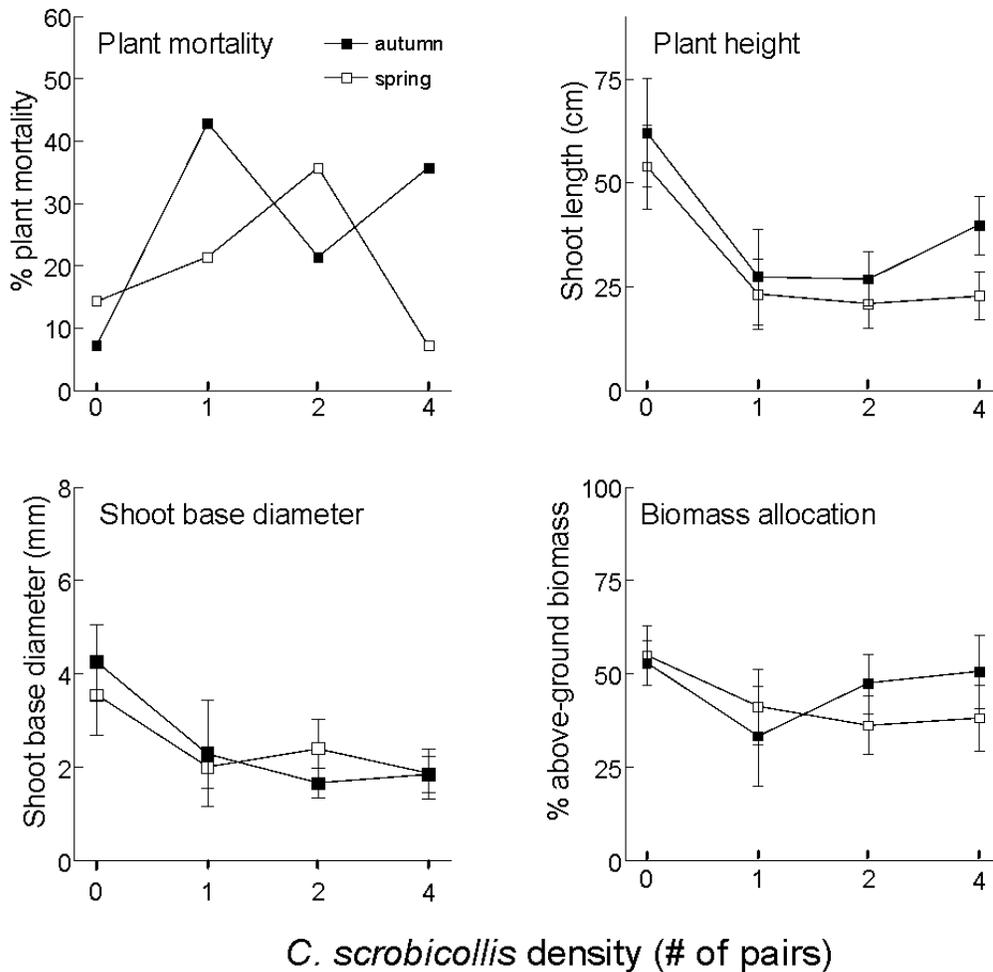


Figure 5. Effect of different densities of *Ceutorhynchus scrobicollis* and timing of attack on mortality, height, shoot base diameter, and aboveground biomass allocation of garlic mustard. Data given for growth are means (± 2 SE) of 8 to 13 plants each.

3. Wildlife

A reduction in garlic mustard abundance will not have negative impacts on wildlife because few species use this plant in North America. Potential reductions in garlic mustard flowering may affect native pollinators dependent on open flowers, but abundant native alternatives exist. However, reductions in garlic mustard abundance will remove an ecological trap for several

native butterfly species in the genus *Pieris*. At present, females lay eggs on garlic mustard but larvae fail to complete their development (Porter, 1994; Haribal and Renwick, 1998; Davis and Cipollini, 2014).

The availability of *C. scrobicollis* adults (and potentially larvae) may create additional food resources for generalist predators, such as spiders, carabids, birds, or salamanders. The potential magnitude of such effects small (and temporary if garlic mustard is successfully controlled). The introduced the European root-mining weevil, *Barypeithes pellucidus*, reaches abundances >100 per square meter in North America (Maerz et al., 2005), well beyond any imaginable abundance *C. scrobicollis* may achieve. Thus, effects of increased weevil abundance and increased prey supply as a result of the introduction of *C. scrobicollis* will be overwhelmed by other species.

4. Human Health

No impacts on human health are anticipated from the release of *C. scrobicollis* into the environment.

5. Economic Impacts

Potential economic gains from control of garlic mustard by *C. scrobicollis* would include reduction in the costs of herbicides, physical and mechanical control programs and maintenance of tourist income that might be lost if garlic mustard continues to invade forests and parks used for recreation. The ability of garlic mustard to interfere with seedling growth of many native tree species (Stinson et al., 2006) has the potential to greatly affect forest regeneration and thus income derived from forest products and timber sales.

6. Uncertainties Regarding the Environmental Release of *C. scrobicollis*

Once a biological control agent such as *C. scrobicollis* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (garlic mustard) to attack nontarget plants. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by *C. scrobicollis*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *C. scrobicollis* generally spread without

intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur, and in which the climate is suitable for reproduction and survival. However, significant non-target impacts on plant populations from previous releases of weed biological control agents are unusual (Suckling and Sforza, 2014). Host range testing and scientific literature have demonstrated that release of *C. scrobicollis* is not expected to attack nontarget plant species

In addition, this agent may not be successful in reducing garlic mustard populations in the contiguous United States. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on garlic mustard by *C. scrobicollis* will not be known until after release occurs and post-release monitoring has been conducted (see appendix 2 for release protocol and post-release monitoring plan). It is expected that *C. scrobicollis* will reduce the biomass, reproductive potential, and spread of garlic mustard.

Other private and public entities work to control garlic mustard in invaded areas in the contiguous United States using available mechanical, cultural, and chemical control methods. Release of *C. scrobicollis* is not expected to have any negative impacts in the contiguous United States because of its host specificity to garlic mustard. Reducing the reproductive potential, biomass, and spread of garlic mustard will have beneficial effects for Federal, State, local, and private weed management programs, and may result in a long-term, non-damaging method to assist in the control of garlic mustard.

The best result is that control of garlic mustard will be achieved and that the application of herbicides and other control measures will be greatly reduced. Direct management costs may be reduced with successful biological control. Natural resources would also be preserved as a result of avoiding the side effects associated with herbicide application.

7. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

APHIS has determined that release of *C. scrobicollis* into the environment may affect but is not

likely to adversely affect listed plants within the family Brassicaceae in the contiguous United States and will not adversely affect designated critical habitat of those species (Appendix 3), and may have completely beneficial effects to *Erythronium propullans*, Minnesota dwarf trout lily because garlic mustard invasion may be a severe threat to the viability of certain Minnesota dwarf trout lily populations in Minnesota (FWS, 2021).

An initial biological assessment was prepared and submitted to the U.S. Fish and Wildlife Service (FWS) (prepared by T.A. Willard, May 30, 2019). Revised versions were submitted to FWS in October 2022 and in February 2023 (USDA-APHIS, 2023) and are part of the administrative record for this EA. APHIS requested concurrence from the FWS on these determinations and received a concurrence letter dated September 18, 2023.

V. Other Issues

A. Protection of Children

Federal agencies also comply with Executive Order (EO) 13045, Protection of Children from Environmental Health Risks and Safety Risks. This EO requires each Federal agency, consistent with its mission, to identify and assess environmental health and safety risks that may disproportionately affect children and to ensure its policies, programs, activities, and standards address the potential for disproportionate risks to children. Consistent with EO 13045, APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No aspects of the proposed field release of *C. scrobicollis* could be identified that would have disproportionate effects on children.

B. Tribal Consultation and Coordination

Executive Order (EO) 13175, “Consultation and Coordination with Indian Tribal Governments,” was issued to ensure that there would be “meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications....”

APHIS is consulting and collaborating with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests in accordance with EO 13175. APHIS requested comments on the proposed action from 170 federally-recognized tribes in 23 states for a period starting from May 29, 2024 and ending

August 16, 2024. The Quawpaw Tribe of Indians in Oklahoma submitted a comment indicating that the proposed action would have no effect on their properties of cultural or sacred significance. APHIS also received a comment from the Leech Lake Band of Ojibwe in Minnesota requesting more information about *C. scrobicollis*. APHIS personnel met with the tribe's Division of Resource Management, Plant Program Director to discuss more information regarding the proposed environmental release. Following the meeting, the tribe Plant Program Director sent a letter of support for the proposed action, dated August 5, 2024.

VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *C. scrobicollis* on February 22, 2017. The TAG members that reviewed the release petition (16-02) (Van Riper et al., 2016) included the Environmental Protection Agency; United States Department of the Interior, Bureau of Land Management and U.S. Fish and Wildlife Service; U.S. Department of Agriculture, Forest Service, Agricultural Research Service, and National Institute of Food and Agriculture; and representatives from the National Plant Board, and Agriculture and Agri-Food Canada.

This EA was prepared by personnel at APHIS, Minnesota Department of Natural Resources, CABI Switzerland, and University of Minnesota. The addresses of participating APHIS units, cooperators, and consultants follow.

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Appendix 1. Host Specificity Testing

This information is from Van Riper et al. (2016). References cited in this appendix are included in section VII. References.

Sequential no-choice oviposition tests.

Material and Methods

All females were first placed in an oviposition test to ensure that they were laying eggs before inclusion in sequential no-choice oviposition tests. To determine whether any of the test species were accepted for oviposition under no-choice conditions, one pair of *C. scrobicollis* was placed into a transparent plastic cylinder (11 cm diameter, 15 cm high) or glass jar (0.47 l) and offered an excised test plant leaf for 2 to 4 days, followed by a garlic mustard leaf for 2 to 4 days. Test plant and garlic mustard leaves were then dissected and the number of *C. scrobicollis* eggs was recorded. Each exposure period was treated as one replicate. Replicates for test plants were only regarded as valid when females laid eggs into the test plant itself or a minimum of one egg onto the garlic mustard leaf that followed. If eggs were deposited in a test plant, then the test species was subsequently tested in no-choice larval development tests and for the most part, in single-choice oviposition tests.

Results

Of 116 test plant species and varieties (Table 1) offered in no-choice tests, 53 were not accepted for oviposition and were not tested further. Forty Brassicaceae species were accepted for oviposition (not including garlic mustard) (Table 1). Normal oviposition behavior, when eggs were inserted into leaves or petioles, only occurred on species in the Brassicaceae. On six test species in other plant families (the native North American *Aconitum noveboracense*, *Amphicarpaea bracteata*, *Hydrophyllum virginianum*, *Ranunculus hispidus*, *Phlox divaricata*, and *Viola sororia*) an occasional egg was laid on the leaf surface. However, these eggs desiccated, preventing further development.

Table 1. *Ceutorhynchus scrobicollis* sequential no-choice oviposition tests.

Family	Tribe	Species	Federal listing status ¹	Native to North America?	No. of replications	No. of replications with eggs	Total no. of eggs	Mean no. eggs per replication ± SE
Brassicaceae	Boechereae	<i>Boechera canadensis</i>	No	Yes	8	1	1	0.1
Brassicaceae	Boechereae	<i>Boechera laevigata</i>	No	Yes	10	4	13	1.3±0.7
Brassicaceae	Boechereae	<i>Boechera hoffmannii</i>	E	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Boechereae	<i>Boechera perstellata</i>	E	Yes	8	0	0	0.0
Brassicaceae	Camelineae	<i>Arabidopsis thaliana</i>	No	No	10	3	7	0.7±0.4
Brassicaceae	Camelineae	<i>Camelina sativa</i>	No	No	10	0	0	0.0
Brassicaceae	Camelineae	<i>Capsella bursa-pastoris</i>	No	No	10	0	0	0.0
Brassicaceae	Cardamineae	<i>Armoracia rusticana</i>	No	No	12	1	4	0.4
Brassicaceae	Cardamineae	<i>Barbarea vulgaris</i>	No	No	10	2	7	0.7±0.6
Brassicaceae	Cardamineae	<i>Cardamine angustata</i>	No	Yes	1	1	1	1.0
Brassicaceae	Cardamineae	<i>Cardamine bulbosa</i>	No	Yes	11	4	12	1.1±0.6
Brassicaceae	Cardamineae	<i>Cardamine concatenata</i>	No	Yes	11	3	4	0.4±0.2
Brassicaceae	Cardamineae	<i>Cardamine diphylla</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Cardamineae	<i>Cardamine heptaphylla</i>	No	No	4	0	0	0.0
Brassicaceae	Cardamineae	<i>Cardamine pratensis</i>	No	Yes	10	4	7	0.7±0.4
Brassicaceae	Cardamineae	<i>Iodanthus pinnatifidus</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Cardamineae	<i>Nasturtium gambellii</i>	E	Yes	4	1	1	0.25
Brassicaceae	Cardamineae	<i>Nasturtium officinale</i>	No	No	10	4	8	0.8±0.5

Family	Tribe	Species	Federal listing status ¹	Native to North America?	No. of replications	No. of replications with eggs	Total no. of eggs	Mean no. eggs per replication ± SE
Brassicaceae	Cardamineae	<i>Rorippa amphibia</i>	No	No	10	6	20	2.0±1.0
Brassicaceae	Cardamineae	<i>Rorippa sinuata</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Cardamineae	<i>Rorippa sessiliflora</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Cardamineae	<i>Rorippa subumbellata</i>	No	No	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Cardamineae	<i>Rorippa sylvestris</i>	No	No	12	1	1	1.0
Brassicaceae	Descurainieae	<i>Descurainia sophia</i>	No	No	10	0	0	0.0
Brassicaceae	Erysimeae	<i>Erysimum asperum</i>	No	Yes	10	0	0	0.0
Brassicaceae	Erysimeae	<i>Erysimum linifolium</i>	No	No	10	6	13	1.3±0.5
Brassicaceae	Erysimeae	<i>Erysimum menziesii</i>	E	Yes	9	0	0	0.0
Brassicaceae	Erysimeae	<i>Erysimum pumilum</i>	No	No	10	0	0	0.0
Brassicaceae	Lepidieae	<i>Lepidium draba</i>	No	No	22	5	11	0.5±0.3
Brassicaceae	Lepidieae	<i>Lepidium squamatum</i>	No	No	10	0	0	0.0
Brassicaceae	Lepidieae	<i>Lepidium virginicum</i>	No	Yes	10	5	9	0.9±0.4
Brassicaceae	Lepidieae	<i>Lepidium barnebyanum</i>	E	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Physarieae	<i>Paysonia densipila</i>	No	Yes	9	1	2	0.2 ± 0.2

Family	Tribe	Species	Federal listing status ¹	Native to North America?	No. of replications	No. of replications with eggs	Total no. of eggs	Mean no. eggs per replication \pm SE
Brassicaceae	Physarieae	<i>Physaria acutifolia</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Physarieae	<i>Physaria douglasii tuplashensis</i>	T	Yes	9	1	1	0.1 \pm 0.1
Brassicaceae	Physarieae	<i>Physaria fendleri</i>	No	Yes	10	0	0	0.0
Brassicaceae	Alysseae	<i>Alyssum saxatilis</i>	No	No	10	3	7	0.7 \pm 0.4
Brassicaceae	Alysseae	<i>Lobularia maritima</i>	No	No	11	0	0	0.0
Brassicaceae	Arabideae	<i>Arabis alpina</i>	No	Yes	10	2	2	0.2 \pm 0.1
Brassicaceae	Arabideae	<i>Arabis blepharophylla</i>	No	Yes	10	0	0	0.0
Brassicaceae	Arabideae	<i>Aubrieta columnae</i>	No	No	10	0	0	0.0
Brassicaceae	Arabideae	<i>Draba oligosperma</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Arabideae	<i>Draba ramosissima</i>	No	Yes	10	0	0	0.0
Brassicaceae	Brassiceae	<i>Brassica napus napus</i>	No	No	10	4	6	0.6 \pm 0.3
Brassicaceae	Brassiceae	<i>Brassica napus</i> 'Clearfield'	No	No	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Brassiceae	<i>Brassica nigra</i>	No	No	10	3	4	0.4 \pm 0.2
Brassicaceae	Brassiceae	<i>Brassica oleracea gemmifera</i>	No	No	10	0	0	0.0
Brassicaceae	Brassiceae	<i>Brassica oleracea gonglyodes</i> 'Cindy'	No	No	10	1	1	0.1
Brassicaceae	Brassiceae	<i>Brassica oleracea italica</i>	No	No	10	0	0	0.0

Family	Tribe	Species	Federal listing status ¹	Native to North America?	No. of replications	No. of replications with eggs	Total no. of eggs	Mean no. eggs per replication ± SE
Brassicaceae	Brassiceae	<i>Brassica oleracea italica</i> 'Coertal'	No	No	10	1	2	2.0
Brassicaceae	Brassiceae	<i>Brassica oleracea rubra</i> 'Ruby Perfection'	No	No	12	2	5	0.4±0.3
Brassicaceae	Brassiceae	<i>Brassica oleracea sabauda</i> 'Eiskönig'	No	No	11	0	0	0.0
Brassicaceae	Brassiceae	<i>Brassica oleracea sabauda</i> 'Paradisler'	No	No	27	8	21	0.8±0.3
Brassicaceae	Brassiceae	<i>Brassica oleracea sabellica</i>	No	No	11	3	4	0.4±0.2
Brassicaceae	Brassiceae	<i>Brassica oleracea sabellica</i> 'Frosty'	No	No	10	0	0	0.0
Brassicaceae	Brassiceae	<i>Brassica rapa rapa</i>	No	No	10	4	15	1.5±0.7
Brassicaceae	Brassiceae	<i>Cakile edentula</i>	No	Yes	12	11	39	3.3±0.7
Brassicaceae	Brassiceae	<i>Raphanus sativus</i>	No	No	10	2	2	0.2±0.1
Brassicaceae	Brassiceae	<i>Sinapis alba</i>	No	No	11	3	5	0.5±0.3
Brassicaceae	Cochlearieae	<i>Cochlearia officinalis</i>	No	No	10	0	0	0.0
Brassicaceae	Eutremeae	<i>Eutrema edwardsii</i>	T	Yes	8	0	0	0.0
Brassicaceae	Iberideae	<i>Iberis semperviens</i>	No	No	12	0	0	0.0
Brassicaceae	Noccaeae	<i>Noccaea fendleri</i> subsp. <i>siskiyouensis</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Noccaeae	<i>Noccaea fendleri</i>	No	Yes	10	0	0	0.0
Brassicaceae	Sisymbrieae	<i>Sisymbrium linifolium</i>	No	Yes	Refer to no-choice larval development tests	N/A	N/A	N/A
Brassicaceae	Sisymbrieae	<i>Sisymbrium irio</i>	No	No	10	0	0	0.0

Family	Tribe	Species	Federal listing status ¹	Native to North America?	No. of replications	No. of replications with eggs	Total no. of eggs	Mean no. eggs per replication ± SE
Brassicaceae	Thelypodieae	<i>Caulanthus heterophyllus</i>	No	Yes	2	1	1	0.5
Brassicaceae	Thelypodieae	<i>Hesperidanthus linearifolius</i>	No	Yes	8	2	4	0.5± 0.4
Brassicaceae	Thelypodieae	<i>Stanleya pinnata</i>	No	Yes	10	1	3	0.3
Brassicaceae	Thelypodieae	<i>Streptanthus glandulosus</i> subsp. <i>glandulosus</i>	No	Yes	7	1	1	0.14
Brassicaceae	Thelypodieae	<i>Streptanthus niger</i>	E	Yes	13	6	7	0.5 ± 0.2
Brassicaceae	Thelypodieae	<i>Thelypodium laciniatum</i>	No	Yes	9	4	10	1.1±0.5
Brassicaceae	Thelypodieae	<i>Thelypodium milleflorum</i>	No	Yes	9	0	0	0.0
Brassicaceae	Thelypodieae	<i>Thysanocarpus curvipes</i>	No	Yes	8	0	0	0.0
Brassicaceae	Thlaspideae	<i>Alliaria petiolata</i>	No	No	1110	1008	4208	3.8±0.1
Brassicaceae	Thlaspideae	<i>Peltaria alliacea</i>	No	No	10	10	81	8.1±0.8
Brassicaceae	Thlaspideae	<i>Thlaspi arvense</i>	No	No	20	17	57	2.8±0.6
Brassicaceae	Anchonieae	<i>Matthiola incana</i>	No	No	10	0	0	0.0
Brassicaceae	Euclidieusae	<i>Braya alpina</i>	No	No	9	1	1	0.1± 0.1
Brassicaceae	Hesperideae	<i>Hesperis matronalis</i>	No	No	10	0	0	0.0
Brassicaceae	Unassigned Genus	<i>Lunaria annua</i>	No	No	9	0	0	0.0
Apiaceae	Not reported	<i>Osmorhiza claytonii</i>	No	Yes	10	0	0	0.0
Araceae	Not reported	<i>Ariseama triphyllum</i>	No	Yes	8	0	0	0.0
Aristolochiaceae	Not reported	<i>Asarum canadense</i>	No	Yes	17	0	0	0.0
Asteraceae	Not reported	<i>Solidago flexicaullis</i>	No	Yes	10	0	0	0.0
Berberidaceae	Not reported	<i>Podophyllum peltatum</i>	No	Yes	2	0	0	0.0
Boraginaceae	Not reported	<i>Mertensia virginica</i>	No	Yes	10	0	0	0.0
Cyperaceae	Not reported	<i>Carex laxiflora</i>	No	Yes	8	0	0	0.0
Fabaceae	Not reported	<i>Amphicarpea bracteata</i> ³	No	Yes	10	3	1	0.1
Fumariaceae	Not reported	<i>Dicentra cucullaria</i>	No	Yes	4	0	0	0.0
Geraniaceae	Not reported	<i>Geranium maculatum</i>	No	Yes	10	0	0	0.0

Family	Tribe	Species	Federal listing status ¹	Native to North America?	No. of replications	No. of replications with eggs	Total no. of eggs	Mean no. eggs per replication ± SE
Hydrophyllaceae	Not reported	<i>Hydrophyllum virginianum</i> ³	No	Yes	12	1	1	1.0
Liliaceae	Not reported	<i>Allium canadense</i>	No	Yes	10	0	0	0.0
Liliaceae	Not reported	<i>Erythronium albidum</i>	No	Yes	7	0	0	0.0
Liliaceae	Not reported	<i>Erythronium americanum</i>	No	Yes	1	0	0	0.0
Liliaceae	Not reported	<i>Maianthemum (Smilacina) racemosum</i>	No	Yes	10	0	0	0.0
Papaveraceae	Not reported	<i>Sanguinaria canadensis</i>	No	Yes	10	0	0	0.0
Poaceae	Not reported	<i>Elymus hystrix (patula)</i>	No	Yes	3	0	0	0.0
Poaceae	Not reported	<i>Triticum aestivum</i>	No	No	10	0	0	0.0
Poaceae	Not reported	<i>Zea mays</i>	No	No	10	0	0	0.0
Polemoniaceae	Not reported	<i>Phlox divaricata</i> ³	No	Yes	10	1	1	1.0
Portulacaceae	Not reported	<i>Claytonia virginica</i>	No	Yes	10	0	0	0.0
Ranunculaceae	Not reported	<i>Aconitum novoboracense</i> ³	No	Yes	10	2	3	0.3±0.2
Ranunculaceae	Not reported	<i>Anemone canadensis</i>	No	Yes	6	0	0	0.0
Ranunculaceae	Not reported	<i>Isopyrum biternatum</i>	No	Yes	13	0	0	0.0
Ranunculaceae	Not reported	<i>Ranunculus hispidus</i> ³	No	Yes	9	2	3	0.3±0.2
Resedaceae	Not reported	<i>Reseda lutea</i>	No	No	10	0	0	0.0
Rubiaceae	Not reported	<i>Gallium aparine</i>	No	Yes	10	0	0	0.0
Tropaeolaceae	Not reported	<i>Tropaeolum majus</i>	No	No	10	0	0	0.0
Violaceae	Not reported	<i>Viola sororia</i> ³	No	Yes	10	1	1	1.0
Vitaceae	Not reported	<i>Parthenocissus quinquefolia</i>	No	Yes	10	0	0	0.0

¹E=endangered, T=threatened

²NR=Not Reported

³Eggs laid on external surface of plant. Not normal oviposition. Eggs dessicated.

No-choice development tests

Materials and Methods

No-choice development tests were conducted with plant species that had been accepted for oviposition in sequential no-choice oviposition tests. Two to three marked females and 1 to 2 marked males were released onto individually potted, gauze-covered rosettes of garlic mustard or test species. To verify that females were fertile, one pair was offered a cut leaf of garlic mustard in a cylinder for 2 to 3 days after which plant material was dissected for eggs. Only females that laid eggs were used for the tests. At each test, 2 to 13 garlic mustard plants were established at the same time as controls. After 2 to 4 weeks, weevils were retrieved and plants re-covered with gauze bags. Plants were either kept outdoors in garden beds or in a heated greenhouse (CABI-Switzerland) or under quarantine conditions (University of Minnesota). In quarantine, plants were maintained in a growth chamber at 15 °C with 9.5/14.5 hour day/night cycle. In late spring of the following year (CABI) or after 12 to 14 weeks (Minnesota), all plants were regularly searched for emerging adults until emergence ceased. After that, all plants were dissected and checked for larvae and larval mining.

Results

Of 85 test plant species and varieties offered, adults emerged from five species other than garlic mustard including, the European wildtype *Nasturtium officinale* (no adults emerged from commercial varieties of *N. officinale*), *Peltaria alliacea*, and *Thlaspi arvense*, and the native North American *Rorippa sinuata* (Table 2). In addition, one adult emerged from *Brassica oleracea sabauda* 'Paradisler'. Larvae were found in the native North American *Cardamine bulbosa* and a single early instar in *Rorippa sessiliflora*, but no *C. scrobicollis* adults emerged from these species. In tests conducted in growth chambers at the University of Minnesota, larvae were recorded in upper stems of *N. gambellii*, but not in roots or crowns of this obligate wetland species and no adults developed. This is not the usual larval behavior. Larvae normally tunnel down the stem to the roots and crown, where they continue to develop, then exit to pupate in the soil. In contrast, when tests were repeated at CABI Switzerland with *N. gambellii*, no larvae were found. Similarly, live larvae were recorded in *Streptanthus niger*, with no adult development. One larva was recorded in a single replication of *Lepidium barnebyanum* with no adult development.

Table 2. Results of no-choice development tests conducted between 1999 & 2015. *Ceutorhynchus scrobicollis*.

Family	Tribe	Species	Total no. of replications	No. of replications with adult emergence	Total no. of adults emerged	Mean no. adults emerged per replication \pm SE	No. plants with mining	No. larvae in test plant ¹
Brassicaceae	Boechereae	<i>Boechera canadensis</i>	11	0	0	0.0	0	0
Brassicaceae	Boechereae	<i>Boechera laevigata</i>	7	0	0	0.0	0	0
Brassicaceae	Boechereae	<i>Boechera hoffmannii</i>	7	0	0	0.0	0	0
Brassicaceae	Boechereae	<i>Boechera perstellata</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Camelineae	<i>Arabidopsis thaliana</i>	3	0	0	0.0	0	0
Brassicaceae	Camelineae	<i>Camelina sativa</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Camelineae	<i>Capsella bursa-pastoris</i>	5	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Armoracia rusticana</i>	10	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Barbarea vulgaris</i>	10	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Cardamine angustata</i>	5	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Cardamine bulbosa</i>	9	0	0	0.0	4	3
Brassicaceae	Cardamineae	<i>Cardamine concatenata</i>	9	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Cardamine diphylla</i>	5	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Cardamine pratensis</i>	7	0	0	0.0	0	0

Family	Tribe	Species	Total no. of replications	No. of replications with adult emergence	Total no. of adults emerged	Mean no. adults emerged per replication \pm SE	No. plants with mining	No. larvae in test plant ¹
Brassicaceae	Cardamineae	<i>Iodanthus pinnatifidus</i>	5	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Nasturtium gambellii</i> (UMN)	15	0	0	0.0	3	4
Brassicaceae	Cardamineae	<i>Nasturtium gambellii</i> (CABI)	6	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Nasturtium officinale</i> (commercial varieties)	9	0	0	0.0	5	2
Brassicaceae	Cardamineae	<i>Nasturtium officinale</i>	5	5	31	6.2	0	0
Brassicaceae	Cardamineae	<i>Rorippa amphibia</i>	7	0	0	0	0	0
Brassicaceae	Cardamineae	<i>Rorippa sinuata</i>	5	2	16	3.2	0	0
Brassicaceae	Cardamineae	<i>Rorippa sessiliflora</i>	4	0	0	0.0	1	1
Brassicaceae	Cardamineae	<i>Rorippa subumbellata</i>	6	0	0	0.0	0	0
Brassicaceae	Cardamineae	<i>Rorippa sylvestris</i>	7	0	0	0.0	0	0
Brassicaceae	Descurainieae	<i>Descurainia sophia</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Erysimeae	<i>Erysimum asperum</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Erysimeae	<i>Erysimum linifolium</i>	7	0	0	0.0	0	0
Brassicaceae	Erysimeae	<i>Erysimum menziesii</i>	No oviposition	N/A	N/A	N/A	N/A	N/A

Family	Tribe	Species	Total no. of replications	No. of replications with adult emergence	Total no. of adults emerged	Mean no. adults emerged per replication \pm SE	No. plants with mining	No. larvae in test plant ¹
Brassicaceae	Erysimeae	<i>Erysimum pumilum</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Lepidieae	<i>Lepidium draba</i>	14	0	0	0.0	0	0
Brassicaceae	Lepidieae	<i>Lepidium squamatum</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Lepidieae	<i>Lepidium virginicum</i>	7	0	0	0.0	0	0
Brassicaceae	Lepidieae	<i>Lepidium barnebyanum</i>	7	0	0	0.0	0	1
Brassicaceae	Physarieae	<i>Paysonia densipila</i>	8	0	0	0.0	0	0
Brassicaceae	Physarieae	<i>Physaria acutifolia</i>	5	0	0	0.0	0	0
Brassicaceae	Physarieae	<i>Physaria douglasii tuplashensis</i>	5	0	0	0.0	0	0
Brassicaceae	Physarieae	<i>Physaria fendleri</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Alysseae	<i>Alyssum saxatilis</i>	3	0	0	0.0	0	0
Brassicaceae	Alysseae	<i>Lobularia maritima</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Arabideae	<i>Arabis alpina</i>	7	0	0	0.0	0	0
Brassicaceae	Arabideae	<i>Arabis blepharophylla</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Arabideae	<i>Aubrieta columnae</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Arabideae	<i>Draba oligosperma</i>	4	0	0	0.0	0	0
Brassicaceae	Arabideae	<i>Draba ramosissima</i>	No oviposition	N/A	N/A	N/A	N/A	N/A

Family	Tribe	Species	Total no. of replications	No. of replications with adult emergence	Total no. of adults emerged	Mean no. adults emerged per replication \pm SE	No. plants with mining	No. larvae in test plant ¹
Brassicaceae	Brassiceae	<i>Brassica napus napus</i>	12	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica napus</i> 'Clearfield'	6	0	0	0	0	0
Brassicaceae	Brassiceae	<i>Brassica nigra</i>	9	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea capitata sabauda</i>	7	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea gemmifera</i>	5	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea gonglyodes</i> 'Cindy'	5	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea italica</i>	5	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea rubra</i> 'Ruby Perfection'	5	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea sabauda</i> 'Eiskönig'	7	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea sabauda</i> 'Paradisler'	5	1	1	0.2	0	0
Brassicaceae	Brassiceae	<i>Brassica oleracea sabellica</i>	5	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Brassica rapa rapa</i>	2	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Cakile edentula</i>	2	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Raphanus sativus</i>	5	0	0	0.0	0	0
Brassicaceae	Brassiceae	<i>Sinapis alba</i>	6	0	0	0.0	0	0

Family	Tribe	Species	Total no. of replications	No. of replications with adult emergence	Total no. of adults emerged	Mean no. adults emerged per replication \pm SE	No. plants with mining	No. larvae in test plant ¹
Brassicaceae	Cochlearieae	<i>Cochlearia officinalis</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Eutremeae	<i>Eutrema edwardsii</i> (<i>E. penlandii</i>)	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Iberideae	<i>Iberis semperviens</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Noccaeeae	<i>Noccaea fendleri</i> subsp. <i>siskiyouensis</i>	5	0	0	0.0	0	0
Brassicaceae	Noccaeeae	<i>Noccaea fendleri</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Sisymbrieae	<i>Sisymbrium linifolium</i>	5	0	0	0.0	0	0
Brassicaceae	Sisymbrieae	<i>Sisymbrium irio</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Thelypodieae	<i>Caulanthus heterophyllus</i>	10	0	0	0.0	1	0
Brassicaceae	Thelypodieae	<i>Hesperidanthus linearifolius</i>	5	0	0	0.0	0	0
Brassicaceae	Thelypodieae	<i>Stanleya pinnata</i>	4	0	0	0.0	0	0
Brassicaceae	Thelypodieae	<i>Streptanthus glandulosus</i> subsp. <i>glandulosus</i>	10	0	0	0.0	0	0
Brassicaceae	Thelypodieae	<i>Streptanthus niger</i> (UMN)	6	0	0	0.0	N/A	4
Brassicaceae	Thelypodieae	<i>Streptanthus niger</i> (CABI)	5	0	0	0.0	3	4

Family	Tribe	Species	Total no. of replications	No. of replications with adult emergence	Total no. of adults emerged	Mean no. adults emerged per replication \pm SE	No. plants with mining	No. larvae in test plant ¹
Brassicaceae	Thelypodieae	<i>Thelypodium laciniatum</i>	5	0	0	0.0	0	0
Brassicaceae	Thelypodieae	<i>Thelypodium milleflorum</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Thelypodieae	<i>Thysanocarpus curvipes</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Anchonieae	<i>Mattiola incana</i>	No oviposition	N/A	N/A	N/A	N/A	N/A
Brassicaceae	Euclideae	<i>Braya alpina</i>	5	0	0	0.0	0	0
Brassicaceae	Hesperideae	<i>Hesperis matronalis</i>	5	0	0	0.0	0	0
Hydrophyllaceae	Not reported	<i>Hydrophyllum virginianum</i>	5	0	0	0.0	0	0
Polemaniaceae	Not reported	<i>Phlox divaricata</i>	5	0	0	0.0	0	0
Resedaceae	Not reported	<i>Reseda lutea</i>	5	0	0	0.0	0	0
Violaceae	Not reported	<i>Viola sororia</i>	5	0	0	0.0	0	0

¹Number of live larvae recovered after dissection of test plant once adults had emerged from garlic mustard control plants. An indication that larvae in test plants were not able to complete development.

Single-choice oviposition and development tests

Materials and Methods

a. Single-choice oviposition tests were conducted with Brassicaceae species that were accepted for oviposition under no-choice conditions. Plant species in other families were tested if eggs were laid on the leaf surface, although this is not normal oviposition behavior. Prior to inclusion in tests, all *C. scrobicollis* females were placed in an oviposition test to ensure that they were laying eggs. One pair of *C. scrobicollis* was placed into a glass jar (0.47 l) and offered an excised test plant or garlic mustard leaf so that females were able to choose which species to accept for oviposition. After 2 to 4 days, leaves were dissected and the number of *C. scrobicollis* eggs recorded, along with presence/absence of feeding. Each exposure period was treated as one replicate. Replicates were only regarded as valid when females laid eggs into garlic mustard leaves or in test species. Tests were conducted under quarantine conditions at the University of Minnesota as described previously.

b. Single-choice larval development tests were established with *Rorippa sinuata* (N=5) and *Cardamine bulbosa* (N=5) because they supported adult and early instar larval development, respectively, under no-choice conditions (see Table 2). One test and one garlic mustard plant were transplanted together into a large pot (26 cm diameter) and two fertile females and one male were released. After 5 to 7 days, weevils were removed, and plants visually inspected. Plants were then re-potted individually into separate pots, covered with gauze bags and placed in a garden until adult emergence the following spring (CABI) or under quarantine conditions (University of Minnesota). In quarantine, plants were maintained in a growth chamber at 15 °C with 9.5/14.5 hour day/night cycle. In late spring of the following year (CABI) or after 12 to 14 weeks (Minnesota), all plants were regularly searched for emerging adults until emergence ceased. After that, all plants were dissected and checked for larvae and larval mining.

Results

a. Single-choice oviposition tests. Only species in the Brassicaceae family were accepted for oviposition in single-choice oviposition tests. Of 32 Brassicaceae species, 22 were accepted for oviposition when offered a choice between the test species and garlic mustard. However, the majority of eggs were deposited into garlic mustard leaves (Table 3), indicating that garlic mustard is the preferred host.

Table 3. Single-choice oviposition tests, *Ceutorhynchus scrobicollis*. CABI Switzerland and University of Minnesota 1999-2015.

Family	Pair	Species	No. of replications	Total no. of eggs*	Percent of all eggs laid**
Brassicaceae	1	<i>Boechera hoffmannii</i>	10	0	0.0
Brassicaceae	1	<i>Allaria petiolata</i>	10	30	100.0
Brassicaceae	2	<i>Armoracia rusticana</i>	5	7	21.0
Brassicaceae	2	<i>Allaria petiolata</i>	5	26	79.0
Brassicaceae	3	<i>Barbarea vulgaris</i>	5	10	22.0
Brassicaceae	3	<i>Allaria petiolata</i>	5	36	78.0
Brassicaceae	4	<i>Nasturtium officinale</i>	5	5	8.9
Brassicaceae	4	<i>Allaria petiolata</i>	5	51	91.1
Brassicaceae	5	<i>Nasturtium gambellii</i>	23	2	2.1
Brassicaceae	5	<i>Allaria petiolata</i>	23	94	97.9
Brassicaceae	6	<i>Rorippa amphibia</i>	11	5	6.7
Brassicaceae	6	<i>Allaria petiolata</i>	11	70	93.3
Brassicaceae	7	<i>Rorippa sylvestris</i>	5	2	9.5
Brassicaceae	7	<i>Allaria petiolata</i>	5	19	90.5
Brassicaceae	8	<i>Lepidium draba</i>	5	1	4.5
Brassicaceae	8	<i>Allaria petiolata</i>	5	21	95.5
Brassicaceae	9	<i>Lepidium virginicum</i>	5	1	3.6
Brassicaceae	9	<i>Allaria petiolata</i>	5	27	96.4
Brassicaceae	10	<i>Paysonia densipila</i>	11	8	11.9
Brassicaceae	10	<i>Allaria petiolata</i>	11	59	88.1
Brassicaceae	11	<i>Physaria douglasii tuplashensis</i>	9	0	0.0
Brassicaceae	11	<i>Allaria petiolata</i>	9	36	100.0
Brassicaceae	12	<i>Arabis alpina</i>	5	0	0.0
Brassicaceae	12	<i>Allaria petiolata</i>	5	33	100.0
Brassicaceae	13	<i>Brassica napus napus</i>	5	0	0.0
Brassicaceae	13	<i>Allaria petiolata</i>	5	29	100.0
Brassicaceae	14	<i>Brassica nigra</i>	5	18	40.9
Brassicaceae	14	<i>Allaria petiolata</i>	5	26	59.1

Family	Pair	Species	No. of replications	Total no. of eggs*	Percent of all eggs laid**
Brassicaceae	15	<i>Brassica oleracea capitata sabauda</i>	5	2	5.6
Brassicaceae	15	<i>Allaria petiolata</i>	5	34	94.4
Brassicaceae	16	<i>Brassica oleracea gonglyodes</i> 'Cindy'	5	0	0.0
Brassicaceae	16	<i>Allaria petiolata</i>	5	61	100.0
Brassicaceae	17	<i>Brassica oleracea var. capitata</i> 'Ruby Perfection'	5	0	0.0
Brassicaceae	17	<i>Allaria petiolata</i>	5	61	100.0
Brassicaceae	18	<i>Brassica oleracea sabauda</i> 'Eiskönig'	5	8	17.4
Brassicaceae	18	<i>Allaria petiolata</i>	5	38	82.6
Brassicaceae	19	<i>Brassica oleracea sabauda</i> 'Paradisler'	6	0	0.0
Brassicaceae	19	<i>Allaria petiolata</i>	6	43	100.0
Brassicaceae	20	<i>Brassica oleracea sabellica</i>	5	2	3.4
Brassicaceae	20	<i>Allaria petiolata</i>	5	57	96.6
Brassicaceae	21	<i>Brassica rapa rapa</i>	5	13	17.8
Brassicaceae	21	<i>Allaria petiolata</i>	5	60	82.2
Brassicaceae	22	<i>Cakile edentula</i>	5	19	37.3
Brassicaceae	22	<i>Allaria petiolata</i>	5	32	62.7
Brassicaceae	22	<i>Draba reptans</i>	5	2	4.5
Brassicaceae	22	<i>Allaria petiolata</i>	5	42	95.5
Brassicaceae	23	<i>Raphanus sativus</i>	5	0	0.0
Brassicaceae	23	<i>Allaria petiolata</i>	5	43	100.0
Brassicaceae	24	<i>Sinapis alba</i>	5	11	30.6
Brassicaceae	24	<i>Allaria petiolata</i>	5	25	69.4
Brassicaceae	25	<i>Thlaspi arvense</i>	5	41	75.9
Brassicaceae	25	<i>Allaria petiolata</i>	5	13	24.1
Brassicaceae	26	<i>Peltaria alliacea</i>	5	22	47.8
Brassicaceae	26	<i>Allaria petiolata</i>	5	24	52.2
Brassicaceae	27	<i>Caulanthus heterophyllus</i>	23	1	0.9

Family	Pair	Species	No. of replications	Total no. of eggs*	Percent of all eggs laid**
Brassicaceae	27	<i>Allaria petiolata</i>	23	112	99.1
Brassicaceae	28	<i>Hesperidanthus linearifolius</i>	10	0	0.0
Brassicaceae	28	<i>Allaria petiolata</i>	10	32	100.0
Brassicaceae	29	<i>Streptanthus niger</i>	10	12	19.0
Brassicaceae	29	<i>Allaria petiolata</i>	10	51	81.0
Brassicaceae	30	<i>Thelypodium laciniatum</i>	10	7	13.7
Brassicaceae	30	<i>Allaria petiolata</i>	10	44	86.3
Brassicaceae	31	<i>Braya alpina</i>	10	0	0.0
Brassicaceae	31	<i>Allaria petiolata</i>	10	36	100.0
Cyperaceae	32	<i>Carex laxiflora</i>	11	0	0.0
Brassicaceae	32	<i>Allaria petiolata</i>	11	59	100.0
Fabaceae	33	<i>Amphicarpaea bracteata</i>	9	0	0.0
Brassicaceae	33	<i>Allaria petiolata</i>	9	88	100.0
Hydrophyllaceae	34	<i>Hydrophyllum virginicum</i>	5	0	0.0
Brassicaceae	34	<i>Allaria petiolata</i>	5	33	100.0
Violaceae	35	<i>Viola sororia</i>	5	0	0.0
Brassicaceae	35	<i>Allaria petiolata</i>	5	63	100.0
Ranunculaceae	36	<i>Anemone canadensis</i>	11	0	0.0
Brassicaceae	36	<i>Allaria petiolata</i>	11	77	100.0
Ranunculaceae	37	<i>Aconitum novoboracense</i>	10	Start0	0.0
Brassicaceae	37	<i>Allaria petiolata</i>	10	73	100.0
Ranunculaceae	38	<i>Ranunculus hispidus</i>	8	0	0.0
Brassicaceae	38	<i>Allaria petiolata</i>	8	46	100.0
Polemoniaceae	39	<i>Phlox divaricata</i>	5	0	0.0
Brassicaceae	39	<i>Allaria petiolata</i>	5	45	100.0

* Total number of eggs laid on that test species (sum of all reps) compared to total laid on *A. petiolata* when *C. scrobicollis* was presented with equal numbers of both plant species at the same time.

** Percent of total number of eggs (sum of all reps of both species) that were laid on the test plant compared to the percentage laid on *A. petiolata* when *C. scrobicollis* was presented equal numbers of both plant species at the same time.

b. Single-choice larval development tests. No oviposition was detected on *Rorippa sinuata*, while eggs were found on most garlic mustard plants. Adults failed to emerge from any of the *R. sinuata* plants exposed, while an average of 6.4 ± 1.8 *C. scrobicollis* emerged from garlic mustard. No adults, larvae or larval tunneling were detected in *Cardamine bulbosa* plants upon dissection.

Multiple-choice field-cage test

Materials and methods

A multiple-choice field cage test was established to verify that commercially grown *Brassica oleracea* varieties are not at risk from *C. scrobicollis* attack (because one adult had emerged under no-choice conditions, see Table 2). Five individually potted plants of *Brassica oleracea sabellica*, *Brassica oleracea sabauda* “Eiskoenig”, *Barbarea vulgaris*, *Rorippa amphibia*, and garlic mustard (all Brassicaceae) were arranged in a Latin Square design in a gauze-covered field cage (2 x 2 x 1.6 m), and 24 fertile females and 12 males of *C. scrobicollis*, all marked, were released. After three weeks, all plants were removed, individually covered with gauze bags and placed in the CABI garden until the following spring. After adult emergence had ceased, roots were dissected for signs of larval mining.

Results

Mining and adult emergence only occurred on garlic mustard control plants (Table 4). Results show that the test plant species are not at risk from attack from *C. scrobicollis* under field conditions. These results help to define the ecological host range of *C. scrobicollis*, which is normally more restrictive than the physiological host range.

Table 4. *Ceutorhynchus scrobicollis* multiple choice field cage test, CABI, Delemont, Switzerland. 2001-2002.

Plant species	Number of replications	No. replications with mining	No. replications with emergence	Total number of adults	Mean adults replication \pm SE
<i>Alliaria petiolata</i>	5	5	5	95	19.0 \pm 2.3
<i>Barbarea vulgaris</i>	5	0	0	0	0.0
<i>Brassica oleracea sabauda</i> 'Eiskönig'	5	0	0	0	0.0
<i>Brassica oleracea sabellica</i>	5	0	0	0	0.0
<i>Rorippa amphibia</i>	5	Not investigated	0	0	0.0

Open-field test

Materials and Methods

An open-field test was conducted with the three European test plant species that had supported development under no-choice conditions (*Peltaria alliacea*, *Nasturtium officinale*, and *Thlaspi arvense* (Table 2)) at CABI Switzerland.

Eight wooden boxes (1 x 1 x 1.5 m) filled with sawdust were arranged in two rows in the Centre's garden, with a 2 meter distance between each box. To investigate the host choice behavior of *C. scrobicollis* in the presence and absence of its principal host, garlic mustard, the three test plants were exposed with (N = 4) or without (N = 4) garlic mustard plants. Two potted plants of each species were embedded in each box, and seven females and five males of *C. scrobicollis* were released. Because previous tests had shown that *C. scrobicollis* can complete development on all three test species, plants were not kept for adult emergence, but were dissected for eggs after four weeks. In addition, above-ground parts of all plants were dried to a constant weight at 80° C and weighed. Attack load (the number of eggs and first instar larvae/gram of dry biomass) was calculated to correct for differences in plant size.

Results

Eggs and first instar larvae were found in all test plant species under both test conditions, in the presence and absence of garlic mustard. With the exception of *N. officinale*, all replicates were attacked. Total and mean attack were always highest on *P. alliacea*, followed by *T. arvense* and the control, garlic mustard. However, when correcting for differences in biomass, attack (mean load) was highest on garlic mustard. Very few eggs were found on *N. officinale*. None of these plant species are native to the United States.

Appendix 2. Release Protocol and Post Release Monitoring for *Ceutorhynchus scrobicollis*.

References cited in this appendix are included in section VII. References.

1. Protocol for Releasing *Ceutorhynchus scrobicollis*

The identity of *C. scrobicollis* has been confirmed by European taxonomists and the researchers will continue to use individuals from the same populations that were tested for their host specificity for the introduction program. Morphological identification and separation of different *Ceutorhynchus* species is reliable. Collections and shipments of control agents will be organized and supervised by personnel of CABI Switzerland (Ghislaine Cortat, Harriet Hinz) with nearly a decade of experience working with *A. petiolata* and *C. scrobicollis*. Reference specimens will be sent to Dr. Boris Korotyayev (Russian Academy of Sciences, St. Petersburg, Russia) to confirm taxonomic identification and reference specimens were submitted to the High Security Containment Facility at the University of Minnesota.

General release protocol to ensure absence of natural enemies and cryptic or sibling species

All field-collected individuals will be checked for species identification using reliable morphological keys before being prepared for shipment. A sample of adults for each shipment and from each collection location will be sent to an insect pathology lab to assess prevalence of natural enemies or associated diseases. Specimens will be sent to quarantine facilities at the University of Minnesota. None of the field-collected adults will be directly field released in North America to avoid introduction of adult parasitoids. Instead, F1 adults or their offspring will be released.

Specific location of rearing or culturing facility

The facilities at the University of Minnesota, St. Paul, MN will be used for initial rearing. Methods to rear *C. scrobicollis* using potted plants have been successful. As control agents become available, an existing network of collaborators and established long-term monitoring sites will be utilized for initial field releases.

Initial sites and timing of releases

Initial field release of *C. scrobicollis* will be made in close vicinity to the rearing facilities in Minnesota. Releases of adults are anticipated ideally to occur in the fall or early spring but different timing and release procedures will be assessed by the researchers. At present, the number of adults that may be available for initial release is not known. At a minimum, the

establishment success will be assessed using a range of different adult densities, different timing of releases, and releases made in small or large field cages. Also, whether habitat types, population densities of the host plant, or local climate may affect population establishment and build-up will be assessed.

2. Post-release monitoring

Post-release monitoring will be conducted by the permittee and not by APHIS. APHIS does not have authority to require post-release monitoring, but the permit applicants have committed to the following post-release monitoring.

A standardized monitoring protocol, using permanent quadrats to assess the establishment and impact of *C. scrobicollis* against garlic mustard, has been in place since 2005 (at some sites). The protocol includes assessment of control agent populations, their feeding impact on garlic mustard survival and performance, as well as an assessment of associated plant communities. After a decade, some of these permanent quadrats appear to have been placed in an initial invasion and Garlic mustard has since moved to more suitable niches on the landscape. A sampling array of permanent quadrats will be established around a point of release for high-value monitoring sites where the original quadrats no longer have garlic mustard at levels to support establishment of *C. scrobicollis* but the surrounding habitat does. Several of the original monitoring sites will serve initially as controls, without release of insects. A priority will be placed on re-distribution of insects to collaborators based on their demonstrated ability to implement long-term monitoring at their release sites. Initial releases will focus on Minnesota and Wisconsin locations, with rearing and release protocols to allow second phase insects for release in Michigan, Illinois, Ohio, and Indiana where managers have expressed the need for biological control. Additional states in the Midwest, Northeast, and Northwest will follow in the third phase of rearing and release.

Groups to perform monitoring

The responsibility of monitoring will rest with local groups releasing *C. scrobicollis*. The standardized protocol is sufficiently easy to use to allow accurate data recording with minimal scientific expertise. The researcher anticipate conducting additional training workshops as control agents are made available. The researchers will require data be provided by cooperators receiving the initial releases to collect and analyze long-term impacts of release of *C. scrobicollis*.

Monitoring techniques to determine if the agents become established

Ceutorhynchus scrobicollis adults are small but visible with the naked eye. However, upon disturbance, adults fall off of plants into the leaf litter, making them difficult to find. Thus,

confirming *C. scrobicollis* establishment by first documenting the characteristic feeding marks of adults may initially be the most realistic approach. Once adult feeding is observed, leaf litter can be collected and placed into collection funnels to collect adults (Katovich, unpublished). The researchers are hesitant to do direct destructive sampling to confirm larval presence in the root crown in the initial years after releases have occurred. However, it is possible to collect and dissect plants after senescence, which will reveal characteristic feeding patterns in the crown and roots.

Monitoring techniques to determine the spread and impact on target and nontarget plants

Monitoring techniques to determine spread of control agents from the initial release sites will change as populations increase or rate of spread changes across sites and landscapes. Initial local spread will be recorded through either determination of adult presence and abundance, presence of feeding marks, or presence of attack in root crowns. As attack becomes more prevalent, GPS will be used to track insect dispersal fronts locally through a garlic mustard population. The established monitoring protocol already allows an assessment of spatial spread but it is anticipated that insects will disperse beyond the project transects within a few years. Regional spread beyond the initial release site would require field surveys.

This permanent monitoring protocol will allow an assessment of the impact on growth, abundance, and reproductive output of garlic mustard in response to control agents and their abundance (measured as the amount of leaf area removed). It will be particularly interesting to compare the development of native plant communities at control and release sites. The monitoring protocol does not directly include monitoring of non-target effects because there are no particular species at risk where releases will be made. The presence of many observers measuring impact of biocontrol agents on local garlic mustard populations will provide many field eyes to survey for potential non-target effects. Initially, there may be anecdotal observations of attack on native species. Suspected instances of a non-target attack will be identified and verified.

During and following post-release monitoring, if the permittee discovers, or APHIS otherwise becomes aware of, potential adverse effects of *C. scrobicollis* to federally listed species and/or critical habitats, APHIS will coordinate with the local U.S. Fish and Wildlife Service office(s) to determine what steps, if any, will be taken to avoid, minimize, or otherwise address the potential adverse effects to those listed species and/or critical habitats.

Appendix 3. Federally listed Brassicaceae plant species with may affect not likely to adversely affect determinations.

Species	Impacts/Effects
<i>Arabis georgiana</i> , Georgia rockcress	Georgia rockcress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Arabis blepharophylla</i> was tested as a surrogate for <i>A. georgiana</i> and <i>C. scrobicollis</i> did not oviposit on it. Another native, <i>Arabis alpina</i> , was tested and adults were able to oviposit but no larvae were present in no-choice larval development tests. <i>C. scrobicollis</i> will have no effect on the critical habitat of Georgia rockcress.
<i>Arabis macdonaldiana</i> , McDonald's rock-cress	McDonald's rock-cress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Arabis blepharophylla</i> was tested as a surrogate for <i>A. macdonaldiana</i> and <i>C. scrobicollis</i> did not oviposit on it. Because <i>A. macdonaldiana</i> and <i>A. blepharophylla</i> are closely related, <i>A. blepharophylla</i> can serve as a viable surrogate for <i>A. macdonaldiana</i> . Another native, <i>Arabis alpina</i> , was tested and adults were able to oviposit but no larvae were present in no-choice larval development tests.
<i>Arabis (Boechea) hoffmannii</i> , Hoffmann's rock-cress	Hoffmann's rock-cress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). This plant is now placed in the <i>Boechea</i> genus. In host specificity testing with <i>B. hoffmannii</i> , a total of one egg and one dead first instar larva were found in shoots in a no-choice larval development test conducted at CABI Switzerland. No adult <i>C. scrobicollis</i> emerged from <i>B. hoffmannii</i> . At CABI's outdoor testing site in Switzerland, other <i>Ceutorhynchus</i> species can attack caged test plants, so it is necessary to run gene sequence tests to positively identify any eggs or larvae present in test plants. Unfortunately, it was not possible to identify the egg or larva due to problems obtaining gene sequences for these samples; therefore, it was not possible to confirm that the larva was <i>C. scrobicollis</i> . However, in single-choice oviposition tests, <i>C. scrobicollis</i> females did not accept <i>B. hoffmannii</i> for oviposition when presented a choice between <i>B. hoffmannii</i> or garlic mustard. In two natives, <i>Boechea canadensis</i> and <i>Boechea laevigata</i> , <i>C. scrobicollis</i> eggs were recorded but no larvae developed in no-choice larval development tests.

Species	Impacts/Effects
<i>Arabis (Boechea) perstellata</i> , Braun's rock-cress	Braun's rock-cress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). This plant is now placed in the <i>Boechea</i> genus. In no-choice oviposition tests, no eggs were observed in <i>B. perstellata</i> . Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Braun's rock-cress.
<i>Arabis (Boechea) serotina</i> , Shale barren rock-cress	Shale barren rock-cress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Boechea serotina</i> was not tested because seeds of this species could not be obtained. However, in no-choice oviposition tests, no eggs were observed in <i>B. perstellata</i> . In two natives, <i>Boechea canadensis</i> and <i>Boechea laevigata</i> , <i>C. scrobicollis</i> eggs were recorded but no larvae developed in no-choice larval development tests. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not accept <i>B. hoffmanii</i> for oviposition when presented a choice between <i>B. hoffmanii</i> or garlic mustard.
<i>Cardamine micranthera</i> , Small-anthered bittercress	Small-anthered bittercress occurs in the same family as garlic mustard (Brassicaceae), and in one of the two tribes where some development of <i>C. scrobicollis</i> occurred (Cardamineae). <i>Cardamine micranthera</i> was not tested because seeds of this species could not be obtained. However, six <i>Cardamine</i> species were tested, five of which are native to North America, and no adults emerged in no-choice larval development tests, although oviposition occurred on five of the six species. Early instar larvae were present only in <i>C. bulbosa</i> in no-choice larval development tests. However, in single-choice larval development tests, when <i>C. scrobicollis</i> females were allowed a choice between <i>C. scrobicollis</i> and garlic mustard, <i>C. bulbosa</i> was not attacked.

Species	Impacts/Effects
<p><i>Caulanthus californicus</i>, California jewelflower</p>	<p>California jewelflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspidaceae and Cardamineae). Seeds were not available for this species so the native surrogate, <i>Caulanthus heterophyllus</i>, was tested. Both species of <i>Caulanthus</i> have overlapping ranges and are annual plants, thus, <i>C. heterophyllus</i> serves as an adequate surrogate for <i>C. californicus</i>. Oviposition in <i>C. heterophyllus</i> occurred during sequential no-choice oviposition tests. One dead first instar larva was found in one replication in a no-choice larval development test and no adults emerged. However, in single-choice oviposition tests, 99 percent of eggs were deposited in garlic mustard leaves, when <i>C. scrobicollis</i> females were given a choice where to lay eggs. Additionally, <i>Caulanthus californicus</i> is confined to 37 presumed extant occurrences: 5 occurrences in Kern County; 7 occurrences in Santa Barbara County; 22 occurrences in San Luis Obispo County; and 3 occurrences in Fresno County (FWS, 2020). This species grows in a chaparral-plant community that is dominated by drought tolerant shrubs (Halsey, 2007). This Mediterranean climate is defined by hot, dry summers and cool, wet winters. The Ecoregions of southern California where <i>C. californicus</i> grows are; Central California Coastal Range, Great Valley, Sierra Nevada Foothills and Southern California Mountains and Valleys. Garlic mustard seedlings and rosettes are not likely to survive the summer droughts of the chaparral, as summer drought can increase seedling and rosette mortality (Meekins and McCarthy, 2000). Welk et al. (2002) compared the frequency of garlic mustard in mapped geographical regions of Europe at different average monthly temperatures and precipitation. They reported that areas receiving less than 10 mm average precipitation from May through October had few to no populations of garlic mustard. In addition, Welk et al. (2002) reported that an average minimum May precipitation of 30 mm was required for flowering, combined with average temperatures ranging from 8 °C to 18 °C. Average precipitation and temperature for May at <i>C. californicus</i> sites are 4.6 mm and 21.4 °C respectively (NOAA.org Bakersfield Meadows Field Airport). Average precipitation from May through October is less than 10 mm. Thus, the area where <i>C. californicus</i> grows is too hot and dry to support garlic mustard growth and development and garlic mustard is currently not recorded in California. In addition, there is not a good match between the temperate climate of the <i>C. scrobicollis</i> collection site in Europe and this southern California Mediterranean region.</p>

Species	Impacts/Effects
<i>Erysimum capitatum</i> var. <i>angustatum</i> , Contra Costa wallflower	Contra Costa wallflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Erysimum menziesii</i> was tested and no feeding or oviposition occurred in sequential no-choice oviposition tests. Two additional native <i>Erysimum</i> species were tested; <i>Erysimum asperum</i> and <i>Erysimum pumilum</i> , and also no feeding or oviposition occurred. Eggs were found in <i>Erysimum linifolium</i> , a species native to Europe, but adults were not able to develop. In addition, garlic mustard is not reported to occur in California where this plant is native. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Contra Costa wallflower.
<i>Erysimum menziesii</i> , Menzies' wallflower	Menzies' wallflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Erysimum menziesii</i> was tested and no feeding or oviposition occurred in sequential no-choice oviposition tests. Two additional native <i>Erysimum</i> species were tested; <i>Erysimum asperum</i> and <i>Erysimum pumilum</i> and also no feeding or oviposition occurred. Eggs were found in <i>Erysimum linifolium</i> , a species native to Europe, but adults were not able to develop. In addition, garlic mustard is not reported to occur in California where this plant is native.
<i>Erysimum teretifolium</i> , Ben Lomond wallflower	Ben Lomond wallflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Erysimum menziesii</i> was tested and no feeding or oviposition occurred in sequential no-choice oviposition tests. Two additional native <i>Erysimum</i> species were tested; <i>Erysimum asperum</i> and <i>Erysimum pumilum</i> and also no feeding or oviposition occurred. Eggs were found in <i>Erysimum linifolium</i> , a species native to Europe, but adults were not able to develop. In addition, garlic mustard is not reported to occur in California where this plant is native.
<i>Eutrema penlandii</i> , Penland alpine fen mustard	Penland alpine fen mustard occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). <i>Ceutorhynchus scrobicollis</i> did not oviposit on <i>E. penlandii</i> (<i>edwardsii</i>) in sequential no-choice oviposition tests.

Species	Impacts/Effects
<p><i>Leavenworthia crassa</i>, Fleshy-fruit glade cress</p>	<p>Potential attack by <i>C. scrobicollis</i>. <i>Leavenworthia crassa</i> occurs in three counties in Alabama (Cullman, Lawrence and Morgan). Climate matching indicates a low match between the <i>C. scrobicollis</i> collection site in Europe and Alabama. Unable to locate a source for this species, the surrogate <i>Leavenworthia torulosa</i> was proposed for testing. However, after repeated attempts with multiple methods, seeds failed to germinate and it could not be tested.</p> <p><i>Leavenworthia crassa</i> may have an overlapping geographic range with sites invaded with garlic mustard. However, all <i>Leavenworthia</i> species grow in open areas in full sunlight and are unlikely to overlap with sites invaded by garlic mustard, which favors shaded/semi-shaded sites. Additionally, habitats that support <i>Leavenworthia</i> species are extremely wet during the late winter and early spring and become extremely dry in summer, in which <i>C. scrobicollis</i> pupae would not survive in the surface soil:litter interface. In the event that <i>Leavenworthia</i> plants were accepted as hosts, <i>C. scrobicollis</i> would not be able to complete its lifecycle on <i>L. crassa</i>.</p> <p>Although <i>Leavenworthia</i> species were not directly tested, the researchers examined phylogenetic relationships among tested species and compared host range test results. The tribe that contains the host Alliaria, Thlaspidaceae, is in a different lineage and is distant from the Cardamineae tribe. In Europe and western Asia where <i>C. scrobicollis</i> is native, Brassicaceae species and <i>C. scrobicollis</i> have co-existed for centuries, there are no reports of <i>C. scrobicollis</i> utilizing any species other than garlic mustard in extensive insect taxonomic surveys (Dieckmann, 1972; Freude et al., 1983; Rheinheimer and Hassler, 2010), including none reported in the Cardamineae tribe.</p> <p>Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of <i>L. crassa</i>.</p>

Species	Impacts/Effects
<p><i>Leavenworthia exigua laciniata</i>, Kentucky glade cress</p>	<p>Kentucky glade cress occurs in the same family as garlic mustard (Brassicaceae) and in one of the two tribes where some development of <i>C. scrobicollis</i> occurred (Cardamineae). This species was not tested in host specificity testing.</p> <p><i>Leavenworthia exigua laciniata</i> is native to Alabama, Georgia, Kentucky and Tennessee. Unable to locate a source for this species, the surrogate <i>Leavenworthia torulosa</i> was proposed for testing. However, after repeated attempts with multiple methods, seeds failed to germinate and it could not be tested.</p> <p><i>Leavenworthia exigua laciniata</i> may have an overlapping geographic range with sites invaded with garlic mustard. However, all <i>Leavenworthia</i> species grow in open areas in full sunlight and are unlikely to overlap with sites invaded by garlic mustard, which favors shaded/semi-shaded sites. Additionally, habitats that support <i>Leavenworthia</i> species are extremely wet during the late winter and early spring and become extremely dry in summer, in which <i>C. scrobicollis</i> pupae would not survive in the surface soil:litter interface. In the event that <i>Leavenworthia</i> plants were accepted as hosts, <i>C. scrobicollis</i> would not be able to complete its lifecycle on <i>L. exigua laciniata</i>.</p> <p>Although <i>Leavenworthia</i> species were not directly tested, the researchers examined phylogenetic relationships among tested species and compared host range test results. The tribe that contains the host <i>Alliaria</i>, Thlaspidaceae, is in a different lineage and is distant from the Cardamineae tribe. In Europe and western Asia where <i>C. scrobicollis</i> is native, Brassicaceae species and <i>C. scrobicollis</i> have co-existed for centuries, there are no reports of <i>C. scrobicollis</i> utilizing any species other than garlic mustard in extensive insect taxonomic surveys (Dieckmann, 1972; Freude et al., 1983; Rheinheimer and Hassler, 2010), including none reported in the Cardamineae tribe.</p> <p>Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of <i>L. exigua laciniata</i>.</p>

Species	Impacts/Effects
<p><i>Leavenworthia texana</i>, Texas golden gladeceess</p>	<p>Texas golden gladeceess occurs in the same family as garlic mustard (Brassicaceae) and in one of the two tribes where some development of <i>C. scrobicollis</i> occurred (Cardamineae). This species was not tested in host specificity testing.</p> <p>Texas golden gladeceess is native to eastern Texas, an area with a low climate match for <i>C. scrobicollis</i>. <i>Leavenworthia texana</i> is only found in St. Augustine County, Texas, an area south of the current southerly range of garlic mustard. This region of Texas is unlikely to accumulate sufficient chill units to successfully vernalize garlic mustard rosettes in the winter. This would prevent rosettes from bolting, setting seed and establishing. With climate change predictions, garlic mustard range will only shift northward, further away from St. Augustine County, Texas. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Texas golden gladeceess.</p>

Species	Impacts/Effects
<p><i>Lepidium barnebyanum</i>, Barneby ridge-cress</p>	<p>Barneby ridge-cress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). One larva was recorded in a single replication of <i>Lepidium barnebyanum</i> but no adult development occurred. Three additional <i>Lepidium</i> species, including the North American, <i>Lepidium virginicum</i>, were tested and no adults developed on any <i>Lepidium</i> species. In addition, in single-choice oviposition tests, 95 percent of eggs were deposited on garlic mustard compared to <i>L. draba</i> or <i>L. virginicum</i>. In additional testing conducted, in a single-choice oviposition test conducted in a growth chamber, no eggs were observed after <i>C. scrobicollis</i> was offered a choice between <i>L. barnebyanum</i> and garlic mustard.</p> <p>There is a single population of <i>Lepidium barnebyanum</i> confined to Duschense County, Utah. <i>Lepidium barnebyanum</i> is only found in a very specific habitat, marly shale barrens on three ridgelines in pockets of pinyon-juniper woodlands at a high elevation (6,200 to 6,500 ft.) (USDA- NRCS, 2015). Burls and McClougherty (2008) found that the distribution of garlic mustard decreased as elevation increased, but this may be a function of rainfall washing away seed. Because <i>L. barnebyanum</i> grows only at a high elevation and in a different habitat from where garlic mustard occurs, there would be no natural pathway for <i>C. scrobicollis</i> to reach ridgeline populations of <i>L. barnebyanum</i>. <i>C. scrobicollis</i> has not been reared during extensive field surveys targeting <i>Lepidium draba</i> (hoary cress) (Cripps et al., 2006) and <i>Lepidium latifolium</i> (perennial pepperweed).</p>
<p><i>Lepidium papilliferum</i>, Slickspot peppergrass</p>	<p>Slickspot peppergrass occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae). Four <i>Lepidium</i> species were tested (<i>Lepidium barnebyanum</i>, <i>Lepidium draba</i>, <i>Lepidium squamatum</i>, and the North American <i>Lepidium virginicum</i>) and no adults developed on any <i>Lepidium</i> species. In addition, in single-choice oviposition tests, 95 percent of eggs were deposited on garlic mustard compared to <i>L. draba</i> or <i>L. virginicum</i>, and no adults developed on any <i>Lepidium</i> species. <i>C. scrobicollis</i> has not been reared during extensive field surveys targeting <i>Lepidium draba</i> (hoary cress) (Cripps et al., 2006) and <i>Lepidium latifolium</i> (perennial pepperweed). Release of <i>C. scrobollis</i> will have no effect on the proposed critical habitat of slickspot peppergrass.</p>

Species	Impacts/Effects
<p><i>Paysonia (Lesquerella) lyrata</i>, Lyrate bladderpod</p>	<p>Lyrate bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Paysonia densipila</i> was tested as a surrogate, with a range that overlaps with lyrate bladderpod. In sequential no-choice oviposition tests, two eggs were found in a single replication of <i>P. densipila</i>, but no larvae or adults developed in no-choice larval development tests. In single choice oviposition tests, <i>C. scrobicollis</i> females laid 88 percent of total eggs laid in garlic mustard when presented a choice between <i>P. densipila</i> and garlic mustard. Also, there is a poor match between the temperate climate of the <i>C. scrobicollis</i> collection site in Europe and Alabama where this plant occurs.</p>
<p><i>Paysonia (Lesquerella) perforata</i>, Spring Creek bladderpod</p>	<p>Spring Creek bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Paysonia densipila</i> was tested as a surrogate, with a range that overlaps with this plant. In sequential no-choice oviposition tests, two eggs were found in a single replication of <i>P. densipila</i>, but no larvae or adults developed in no-choice larval development tests. In single choice oviposition tests, <i>C. scrobicollis</i> females laid 88 percent of total eggs laid in garlic mustard when presented a choice between <i>P. densipila</i> and garlic mustard. Also, there is a poor match between the temperate climate of the <i>C. scrobicollis</i> collection site in Europe and Tennessee where this plant occurs.</p>

Species	Impacts/Effects
<p><i>Physaria (Lesquerella) congesta</i>, Dudley Bluffs bladderpod</p>	<p>Dudley Bluffs bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested as a surrogate and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Dudley Bluffs bladderpod.</p>
<p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i>, White Bluffs bladderpod</p>	<p>White Bluffs bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>.</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> is native to Washington and is restricted to a small area adjacent to the Columbia River. This species is “restricted to dry, barren, nearly vertical exposures of caliche soil in sagebrush steppe habitat. The substrate is extremely alkaline and highly calcareous.” Because garlic mustard is found in temperate deciduous forests, it is unlikely that it would be able to establish in sites with desert caliche soils, so there would be no hosts available to support <i>C. scrobicollis</i> development. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of White Bluffs bladderpod.</p>

Species	Impacts/Effects
<p><i>Physaria filiformis</i>, Missouri bladderpod</p>	<p>Missouri bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>.</p>
<p><i>Physaria globosa</i>, Short's bladderpod</p>	<p>Short's bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Short's bladderpod.</p>

Species	Impacts/Effects
<p><i>Physaria (Lesquerella) kingii</i> ssp. <i>bernardina</i>, San Bernardino Mountains bladderpod</p>	<p>San Bernardino Mountains bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>. Garlic mustard does not occur in California. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of San Bernardino Mountains bladderpod.</p>
<p><i>Physaria obcordata</i>, Dudley Bluffs twinpod</p>	<p>Dudley Bluffs twinpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>.</p>

Species	Impacts/Effects
<p><i>Physaria pallida</i>, White bladderpod</p>	<p>White bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>. Garlic mustard does not occur in Texas.</p>
<p><i>Physaria thamnophila</i>, Zapata bladderpod</p>	<p>Zapata bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>. Garlic mustard does not occur in Texas. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Zapata bladderpod.</p>

Species	Impacts/Effects
<p><i>Physaria (Lesquerella) tumulosa</i>, Kodachrome bladderpod</p>	<p>Kodachrome bladderpod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Physaria douglasii</i> ssp. <i>tuplashensis</i> was tested and a single egg was found in one replication in a sequential no-choice oviposition test. One dead larva was present in one <i>P. douglasii</i> ssp. <i>tuplashensis</i> replication, but no adults emerged in no-choice larval development tests conducted in a growth chamber. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>P. douglasii</i> ssp. <i>tuplashensis</i> when offered together with garlic mustard. The natives <i>Physaria acutifolia</i> and <i>Physaria fendleri</i> were tested and neither species was accepted for oviposition by <i>C. scrobicollis</i>.</p>

<p><i>Nasturtium (Rorippa) gambellii</i>, Gambel's watercress</p>	<p>There is a potential for attack of Gambel's watercress by <i>C. scrobicollis</i> because it occurs in the tribe Cardamineae where some development of <i>C. scrobicollis</i> occurred. However, <i>C. scrobicollis</i> was not able to complete development on <i>N. gambellii</i> growing in water saturated soil. In tests conducted in growth chambers at the University of Minnesota, four larvae were found in the upper part of stems of <i>N. gambellii</i>, which is not normal behavior. Larvae typically tunnel down the stem to the roots and crown, where they continue to develop to the third instar, then leave the crown to pupate in the soil. In contrast, when no-choice larval development tests were repeated in a more natural environment in a greenhouse at CABI Switzerland, no larvae were found.</p> <p>In single-choice oviposition tests, when females were offered <i>N. gambellii</i> and garlic mustard, 98 percent of eggs were deposited in garlic mustard.</p> <p><i>Nasturtium gambellii</i> is native to southern California, is an obligate wetland plant, and grows in freshwater wetlands, a habitat where <i>C. scrobicollis</i> is unlikely to establish. Because third instar <i>C. scrobicollis</i> larvae exit garlic mustard plants to pupate in the soil, larvae would drown in wetland water saturated soils where <i>N. gambellii</i> grows.</p> <p>There is a poor match between the temperate climate of the <i>C. scrobicollis</i> collection site in Europe and this southern California Mediterranean. Therefore, it is unlikely that <i>C. scrobicollis</i> would establish in southern California. In addition, garlic mustard is not present in California.</p> <p>In additional studies conducted by the researcher, less than .5% of leaf tissue was affected by feeding. In choice tests with <i>N. gambellii</i> and garlic mustard, no oviposition or feeding observed.</p> <p><i>Ceutorhynchus scrobicollis</i> did not negatively impact growth or development of <i>N. gambellii</i> in a growth chamber experiment. After two weeks of exposure to <i>C. scrobicollis</i> adults, little to no exploratory probing was found, < 0.1% of leaf tissue. Feeding was also present from the leaf-defoliating beetle, <i>Phaedon armoraciae</i> (Coleoptera: Chrysomelidae) that was present in very low numbers at initiation of experiment, then increased in number after pots were placed in screened cages. The experiment was terminated when F1 <i>C. scrobicollis</i> offspring emerged from control garlic mustard plants in the same growth chamber, approximately three months</p>
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Species	Impacts/Effects
	<p>after the initiation of the experiment. There were no differences in shoot dry weights, plant heights, or cover between plants with <i>C. scrobicollis</i> exposure compared to control <i>N. gambellii</i> at the termination of the experiment. Percent plant cover was defined by percent of surface covered by leaves and stems, relative to bare soil in a visual estimation. At the termination of the experiment, when shoots of <i>N. gambellii</i> were dissected, two second-instar larvae were found in one shoot of a single plant. The larval tunneling caused by the larvae was approximately 2-cm in length including the larvae so the larvae essentially were stuck where they hatched in the shoot, unable to eat or develop. The larvae remained in the shoot and did not tunnel into root tissue or exit from the plant. In contrast, on garlic mustard control plants, larvae completed all three instars of development, pupated and F1 adults of <i>C. scrobicollis</i> were recovered. These results were similar to those in a previous no-choice larval development study with <i>N. gambellii</i> conducted in a growth chamber in Minnesota where larvae were “stuck” in <i>N. gambellii</i> stems and did not enter root/crown tissue. <i>Ceutorhynchus scrobicollis</i> third instar larvae enter the roots and crown of garlic mustard plants and exit to pupate in the soil. On <i>N. gambellii</i>, larvae failed to develop, and thus did not pupate or develop to adults. By including garlic mustard in the same growth chamber as <i>C. scrobicollis</i> we confirmed that chamber conditions supported <i>C. scrobicollis</i> development. However, <i>C. scrobicollis</i> caged on <i>N. gambellii</i> plants may also have been exposed cues from garlic mustard. In fact, in a similar no-choice development study conducted under more natural conditions in the field at the CABI facility in Delémont, Switzerland, no larvae at all were found in any <i>N. gambellii</i> plants.</p>
<p><i>Schoenocrambe</i> (<i>Hesperidanthus</i>) <i>argillacea</i>, Clay reed-mustard</p>	<p>Clay reed-mustard occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Hesperidanthus linearifolia</i>, a species with a wide distribution, was tested as a surrogate, and eggs were recorded in two of eight replications in sequential no-choice oviposition tests, but no larval development occurred in no-choice larval development tests. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>H. linearifolia</i> when presented a choice between <i>H. linearifolia</i> and garlic mustard.</p>

Species	Impacts/Effects
<p><i>Schoenocrambe</i> (<i>Hesperidanthus</i>) <i>barnebyi</i>, Barneby reed-mustard</p>	<p>Barneby reed-mustard occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p><i>Hesperidanthus linearifolia</i>, a species with a wide distribution, was tested as a surrogate, and eggs were recorded in two of eight replications in sequential no-choice oviposition tests, but no larval development occurred in no-choice larval development tests. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>H. linearifolia</i> when presented a choice between <i>H. linearifolia</i> and garlic mustard.</p>
<p><i>Schoenocrambe</i> (<i>Hesperidanthus</i>) <i>suffrutescens</i>, Shrubby reed-mustard</p>	<p>Shrubby reed-mustard occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>Potential attack by <i>C. scrobicollis</i>. <i>Hesperidanthus linearifolia</i>, a species with a wide distribution, was tested as a surrogate, and eggs were recorded in two of eight replications in sequential no-choice oviposition tests, but no larval development occurred in no-choice larval development tests. In single-choice oviposition tests, <i>C. scrobicollis</i> females did not lay eggs in <i>H. linearifolia</i> when presented a choice between <i>H. linearifolia</i> and garlic mustard.</p>
<p><i>Sibara filifolia</i>, Santa Cruz Island rockcress</p>	<p>Santa Cruz Island rockcress occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>This genus was not included in host specificity testing because seeds could not be obtained. However, <i>Sibara filifolia</i> is an annual species in California. This species only occurs on San Clemente Island, located 41 miles off of the coast of southern California. There are no records of garlic mustard in California, and the island location of <i>S. filifolia</i> provides a natural barrier to <i>C. scrobicollis</i>.</p>

Species	Impacts/Effects
<p><i>Streptanthus albidus</i> ssp. <i>albidus</i> (Researchers call it <i>S. glandulosus</i> ssp. <i>albidus</i>. They are the same.), Metcalf Canyon jewelflower</p>	<p>Metcalf Canyon jewelflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>In host range testing, the surrogate, <i>Streptanthus glandulosus</i> ssp. <i>glandulosus</i>, was accepted for oviposition in sequential no-choice oviposition tests, but larvae did not develop in no-choice larval development tests. Early instar larvae were found in <i>S. glandulosus</i> ssp. <i>niger</i> plants in no-choice larval development tests, but no adults emerged. In single-choice oviposition tests, <i>C. scrobicollis</i> females laid 81 percent of eggs in garlic mustard when presented a choice between <i>S. niger</i> and garlic mustard.</p> <p><i>S. albidus</i> ssp. <i>albidus</i> is a serpentine endemic (Cacho et al., 2014) and it grows on “outcrops with little soil development” with a distribution limited to Santa Clara County, California (www.calflora.org. Accessed June, 2016). This species was not tested, but climate matching indicates that this California site would not be a good match for <i>C. scrobicollis</i>. Garlic mustard does not occur in California.</p>
<p><i>Streptanthus bracteatus</i>, Bracted twistflower</p>	<p>Bracted twistflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>Early instar larvae were found in <i>S. glandulosus</i> ssp. <i>niger</i> plants in no-choice larval development tests, but no adults emerged. In single-choice oviposition tests, <i>C. scrobicollis</i> females laid 81 percent of eggs in garlic mustard when presented a choice between <i>S. niger</i> and garlic mustard. Less than 0.5% leaf feeding was recorded on this plant. In addition, this plant occurs in Texas and garlic mustard does not occur there. This plant is native to oak-juniper woodlands in Texas (Al-Shehbaz, 2010) and climate matching indicates that this Texas site would not be a good match for <i>C. scrobicollis</i>.</p>

Species	Impacts/Effects
<p><i>Streptanthus niger</i>, Tiburon jewelflower</p>	<p>Tiburon jewelflower occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>Early instar larvae were found in <i>S. glandulosus</i> ssp. <i>niger</i> plants in no-choice larval development tests, but no adults emerged. In single-choice oviposition tests, <i>C. scrobicollis</i> females laid 81 percent of eggs in garlic mustard when presented a choice between <i>S. niger</i> and garlic mustard. Less than 0.5% leaf feeding was recorded on this plant.</p> <p><i>Streptanthus glandulosus</i> ssp. <i>niger</i> is an annual plant only found on two sites on the Tiburon Peninsula on the northern side of San Francisco Bay in California. This <i>Streptanthus</i> subspecies is a serpentine endemic plant and accumulates heavy metals. In addition to classification as a serpentine endemic plant, climate matching between the Tiburon Peninsula and the collection site of <i>C. scrobicollis</i> (Berlin, Germany) indicates that this California site would not be a good match for <i>C. scrobicollis</i>. The isolated location of <i>S. glandulosus</i> ssp. <i>niger</i>, with populations growing on an open, barren, rocky peninsula is not likely to support garlic mustard, which is not adapted to a serpentine soil type. In addition, garlic mustard is not present in California to support populations of <i>C. scrobicollis</i>.</p>
<p><i>Thelypodium howellii</i> ssp. <i>spectabilis</i>, Howell's spectacular thelypody</p>	<p>Howell's spectacular thelypody occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>The surrogates <i>Thelypodium laciniatum</i> and <i>Thelypodium milleflorum</i> were tested. Both of these surrogates have overlapping ranges and are biennial, like <i>T. howellii spectabilis</i>. <i>Ceutorhynchus scrobicollis</i> laid eggs in <i>T. laciniatum</i> but not <i>T. milleflorum</i> in sequential no-choice oviposition tests. No larvae developed in <i>T. laciniatum</i> in no-choice larval development tests. In single-choice oviposition tests, <i>C. scrobicollis</i> females laid 84 percent of total eggs laid in garlic mustard when presented a choice between <i>T. laciniatum</i> and garlic mustard.</p>

Species	Impacts/Effects
<p><i>Thelypodium stenopetalum</i>, Slender-petaled mustard</p>	<p>Slender-petaled mustard occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>The surrogates <i>Thelypodium laciniatum</i> and <i>Thelypodium milleflorum</i> were tested. Both of these surrogates have overlapping ranges and are biennial, like <i>T. stenopetalum</i>. <i>Ceutorhynchus scrobicollis</i> laid eggs in <i>T. laciniatum</i> but not <i>T. milleflorum</i> in sequential no-choice oviposition tests. No larvae developed in <i>T. laciniatum</i> in no-choice larval development tests. In single-choice oviposition tests, <i>C. scrobicollis</i> females laid 84 percent of total eggs laid in garlic mustard when presented a choice between <i>T. laciniatum</i> and garlic mustard. In addition garlic mustard is not known to occur in California.</p>
<p><i>Thlaspi (Noccaea) californicum</i>, Kneeland Prairie penny-cress</p>	<p>Kneeland Prairie penny-cress occurs in the same family as garlic mustard (Brassicaceae), and in one of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae).</p> <p>In host-specificity tests, <i>Thlaspi arvense</i> supported limited adult development. However, this is a European species. Native <i>Thlaspi</i> have been moved to the genus <i>Noccaea</i>. <i>Noccaea fendleri</i> ssp. <i>californica</i> was previously <i>Thlaspi californicum</i>. <i>Noccaea fendleri</i> and <i>Noccaea fendleri</i> ssp. <i>siskiyouensis</i> (previously <i>Thlaspi montanum</i> var. <i>siskiyouensis</i>) were tested and no <i>C. scrobicollis</i> oviposition was recorded in either species. Release of <i>C. scrobicollis</i> will have no effect on the critical habitat of Kneeland Prairie penny-cress.</p>
<p><i>Thysanocarpus conchuliferus</i>, Santa Cruz Island fringe-pod</p>	<p>Santa Cruz Island fringe-pod occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>This species is only found on Santa Cruz Island, off the coast of southern California. The surrogate <i>Thysanocarpus curvipes</i> was tested, which has a similar life history and also grows on Santa Cruz Island. <i>Thysanocarpus curvipes</i> was not accepted for oviposition by <i>C. scrobicollis</i> in sequential no-choice oviposition tests. In addition, garlic mustard is not present in California.</p>

Species	Impacts/Effects
<i>Warea amplexifolia</i> , Wide-leaf warea	<p>Wide-leaf warea occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>A seed source was not found for this plant. Seeds of the surrogate <i>Warea sessilifolia</i> did not germinate and so it was not tested. However, according to climate matching, Florida's climate does not match well with Berlin, Germany, the source of <i>C. scrobicollis</i>, so Florida may not be suitable for <i>C. scrobicollis</i> establishment. In addition, garlic mustard is not found in Florida, so there are no natural pathways for <i>C. scrobicollis</i> to move from the invasive plant to <i>Warea</i> species.</p>
<i>Warea carteri</i> , Carter's mustard	<p>Carter's mustard occurs in the same family as garlic mustard (Brassicaceae), but not in either of the two tribes where some development of <i>C. scrobicollis</i> occurred (Thlaspideae and Cardamineae).</p> <p>A seed source was not found for this plant. Seeds of the surrogate <i>Warea sessilifolia</i> did not germinate and so was not tested. However, according to climate matching, Florida's climate does not match well with Berlin, Germany, the source of <i>C. scrobicollis</i>, so Florida may not be suitable for <i>C. scrobicollis</i> establishment. In addition, garlic mustard is not found in Florida, so there are no natural pathways for <i>C. scrobicollis</i> to move from the invasive plant to <i>Warea</i> species.</p>