Anthonomus kuscheli and Berberidicola exaratus would adversely affect the environment if feeding by larvae or adults significantly depressed the population dynamics of any native plant. Experiments to define the host range of these two weevils confirm that the risk of this outcome is not significant.

Field surveys and outdoor experiments were conducted in Chile (the home range of the control agents) in 2009/10 and 2010/11, and in containment in New Zealand. Plants to be tested were selected in accord with the well-established principle that the most likely alternative hosts for a specialist insect are those most closely related phylogenetically to the primary host (Briese, 2005; Sheppard et al., 2005).

The range of plants selected for testing in Chile was influenced by several factors:

- Berberidaceae and Ranunculaceae are the only families in the order Ranunculales represented in the New Zealand native flora (<u>http://nzflora.landcareresearch.co.nz/</u>).
- A There are no native species in the family Berberidaceae in New Zealand. The native flora is not closely related to the target plant
- No New Zealand species in the Ranunculaceae have fruits suitable for attack by Berberidicola exaratus (Webb et al.,1989).
- All New Zealand native species that form fruits resembling those of Darwin's barberry are too distantly related to Darwin's barberry to warrant testing
- A No New Zealand native species were available for testing in Chile

Some species tested in Chile were only available growing in the field, and so tests were conducted at a range of sites over two seasons. A total of 17 plant species belonging to families within the order Ranunculales were exposed to A. kuscheli and to B. exaratus (Tables 1 & 2). Greatest emphasis was given to testing other species of Berberis available in Chile, then species in the neighbouring sub-family, and finally species in related families within in the Order Ranunculales. There are many species of Ranunculus and Clematis in New Zealand native flora. Clematis montana and Ranunculus repens were selected as local surrogates for New Zealand species of the family Ranunculaceae. Adult weevils were added to cloth sleeves enclosing either the flower buds (A. kuscheli) or the developing fruits (B. exaratus) of test plants. To ensure that the weevils used were reproductively active at the time of the tests, weevils were also caged with the susceptible host, Darwin's barberry (controls). The flowering time of some test species did not coincide with that of Darwin's barberry, and as the true host was not present at all sites, controls for some tests were 5 - 10km distant from experiments. This meant that, by necessity, some experimental controls were not ideally placed in time or space. A conservative approach has been taken to interpretation of these data (Tables 1 & 2). Adults of both species were long-lived in tests. Plant parts in bags were examined several times through the season for damage and larval attack, and for new generation adults (A. kuscheli) or fallen mature larvae (B. exaratus). Methods and results are detailed in the reports of Norambuena & Escobar (2010) and Norambuena (2011).

Weevils were imported into New Zealand in early 2012, and were held in containment at Landcare Research, Lincoln. Additional tests on New Zealand test species were completed to enhance the data from trials in Chile. Individual weevils were placed with test plant material in petri dishes to see if they fed on those plants. Few plants had buds or fruit at that time of year, but several tests also examined the ability of weevils to lay eggs on New Zealand plants (Table 3).

Anthonomus kuscheli

Tests in Chile - Adult weevils fed on the leaves and/or buds of *B. darwinii*, and all other *Berberis* species (sub-family Berberidoideae). However, adults did not feed on foliage of *Nandina domestica* (sub-family Nandinoideae) or on four representative species of the related families Ranunculaceae, Lardizabalaceae or Papaveraceae (Table 1). A new generation of weevils was produced on *B. darwinii* and *B. thunbergii atropurpureum* but not on the other nine *Berberis* species tested. In fact, with the exception of *B. thunbergii*, no larvae were found in the buds of any plants other than the target weed, suggesting that adults only laid eggs on *B. darwinii*. Given the resistance of other *Berberis* species, the production of a new generation of weevils on this cultivar was anomalous and may be an artefact of heavy selection for ornamental values (see Section 6.4.1).

Tests in New Zealand – Adults fed actively on Darwin's barberry controls. There was no significant feeding on the foliage or buds of the four native and four exotic New Zealand species belonging to plant families related to the Berberidaceae that were presented in tests.

These data strongly indicate that adult *A. kuscheli* will not eat native plants. There is no significant risk that *A. kuscheli* will reproduce in any native plant species because weevils reproduced in only two of 7 well 'controlled' *Berberis* species tested, and in not at all in plants outside the sub-family Berberidoideae. There are no native species in this family. However, while it is possible that the foliage and buds of exotic species of *Berberis* may be damaged by transient adult weevils (see Section 6.4.1), these are not expected to support complete development (with the exception of *B. thunbergii atropurpureum*).

Berberidicola exaratus

Tests in Chile - Mature larvae of *B. exaratus* emerge from Darwin's barberry fruit and normally fall to the ground to pupate (Section 4.2). The remains of dead larvae in the cloth bags surrounding test plant foliage were taken as evidence of complete development on that host. Such larvae were found in seven of the eight well 'controlled' *Berberis* species tested (subfamily Berberidoideae). However, there was no sign of larval development on *Nandina domestica*, (subfamily Nandinoideae), or on more distantly related plant species outside the family Berberidaceae. Similarly, adults fed on the leaves and fruits of most *Berberis* spp. presented, but not on *Nandina domestica* (sub-family Nandinoideae) or on species from other families in the order Ranunculales (Table 1 and 3).

Tests in New Zealand – Fruits of *Berberis glaucocarpa* (a widespread weed in New Zealand) exposed to weevils produced larvae, but none were observed in native *Clematis paniculata* or in *Nandina domestica* As in Chile, adults did not feed significantly on the foliage of the seven species outside the sub-family Berberidoideae that were tested (Table 3).

From these results we conclude that *B. exaratus* is not specific to Darwin's barberry, and is able to complete development on several species within the genus *Berberis*. However, *Berberidicola exaratus* is unlikely to attack species outside the subfamily Berberidoideae, and will not reproduce on any New Zealand native plant species because:

- Species of the genus *Berberidicola* are only known to attack *Berberis* species (Clark & Burke,1989)
- ▲ B exaratus deposits eggs into feeding scars it creates on fruits. If there is no feeding on fruits, no eggs can be laid
- Species of the only family in the New Zealand native flora that is related to Darwin's barberry (Ranunculaceae) do not have the fruits necessary for larval development of *B. exaratus*
- A The species in the native flora that have fruits are only very distantly related to Berberis

Although no native plants will be attacked, it is possible that the foliage and buds of exotic species of *Berberis* may be damaged by transient adult weevils (see Section 6.4.1).

This interpretation of the data contained in reports by Norambeuna & Escobar (2010), Norambuena (2011) and Smith (2012) has been reviewed by Dr Q Paynter of Landcare Research, and his comments have been supplied to EPA.