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Host plant selection of *Chrysolina clathrata* (Coleoptera: Chrysomelidae) from Mpumalanga, South Africa

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Abstract Hyperaccumulated elements such as Ni may defend plants against some natural enemies whereas other enemies may circumvent this defense. The Ni hyperaccumulator *Berkheya coddii* Roessler (Asteraceae) is a host plant species for *Chrysolina clathrata* (Clark), which suffers no apparent harm by consuming its leaf tissue. Beetle specimens collected from *B. coddii* had a whole body Ni concentration of 260 μg/g dry weight, despite consuming leaf material containing 15 100 μg Ni/g. Two experiments were conducted with adults of this beetle species: a no-choice experiment and a choice experiment. In the no-choice experiment we offered beetles foliage of one of four species of *Berkheya*: *B. coddii*, *B. rehmannii* Thell. var. *rogersiana* Thell., *B. echinacea* (Harv.) O. Hoffm. ex Burtt Davey, and *B. insignis* (Harv.) Thell. The two former species are Ni hyperaccumulators (defined as having leaf Ni concentration > 1 000 μg/g) whereas the latter have low Ni levels (< 200 μg/g) in their leaves. Masses of beetles were monitored for 6 days. Choice experiments used growing stem tips from the same *Berkheya* species, placed into Petri dishes with five *Chrysolina* beetles in each, and the amount of feeding damage caused on each of the four species was recorded. Beetles in the no-choice experiment gained mass when offered *B. coddii*, maintained mass on leaves of the other Ni hyperaccumulator (*B. rehmannii* var. *rogersiana*), and lost mass when offered non-hyperaccumulator leaves. In the choice test, beetles strongly preferred *B. coddii* to other *Berkheya* species. We conclude that *C. clathrata* may be host-specific on *B. coddii*.

Key words *Berkheya coddii*, bioaccumulation, heavy metals, host plant specialist, nickel

Introduction

Serpentine (ultramafic) rocks contain relatively high amounts of magnesium, iron and other heavy metals and relatively low amounts of calcium, sodium and potassium, and the soils derived from them reflect this unusual chemistry (Alexander *et al.*, 2007). Serpentine soils form unique habitats that often differ markedly from habitats underlain by more widespread soil types (Brooks, 1987). Floristic comparisons between serpentine and non-serpentine soils (e.g., Jaffré *et al.*, 1998) have shown that serpentine soils often harbor high species diversity, in part due to high levels of endemism. Whereas the flora of serpentine habitats has been studied in many parts of the world (Brooks, 1987), their fauna has received little attention (Kruckeberg, 1984). We know of only a few surveys that specifically target the insect fauna of serpentine soils (e.g., Smith, 1981; Cañamero *et al.*, 2004). Clearly, much needs to be learned about the fauna of these unusual habitats.

One remarkable group of plants, many of which inhabit

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serpentine soils (Macnair, 2003), is metal hyperaccumulators. Hyperaccumulator plants take up strikingly large amounts of certain elements from the soil in which they grow. Most hyperaccumulator plants hyperaccumulate metals (Reeves & Baker, 2000), with particular metals having different concentration thresholds in plant tissues in order for those plants to be considered hyperaccumulators. For example, Reeves & Baker (2000) describe hyperaccumulators of Co, Cr, Cu, Ni and Pb as containing > 1 000 μg/g dry mass, whereas hyperaccumulators of Mn and Zn contain > 10 000 μg/g. The unifying feature of hyperaccumulators is their strikingly elevated concentration of an element that, in the tissues of most plants, is far less abundant.

The high concentration of a hyperaccumulated element in hyperaccumulator tissues may affect the suitability of those tissues as food sources for herbivores. The defense hypothesis of hyperaccumulation (Boyd & Martens, 1992) posits that the high elemental concentration in hyperaccumulator tissue provides a defensive benefit against plant natural enemies. To date, there is evidence that hyperaccumulated Cd, Ni, Se and Zn may provide defensive benefits against at least some natural enemies (Boyd, 2007). It is also clear that some plant enemies are not negatively affected by hyperaccumulated elements and these can attack hyperaccumulator plants. Surveys of Ni-hyperaccumulator plants in California, USA (Wall & Boyd, 2002), Mpumalanga, South Africa (Mesjasz-Przybylowicz & Przybylowicz, 2001; Boyd *et al.*, 2006a) and New Caledonia (Boyd *et al.*, 2006b) have documented insect species that feed on Ni-hyperaccumulators. However, for some insect species we know nothing beyond that they have been collected from hyperaccumulator plants, whereas in other cases we know only that feeding on hyperaccumulator tissue has been observed. For a few cases (e.g., Augustyniak *et al.*, 2002; Boyd *et al.*, 2004a; Wall & Boyd, 2006), choice and/or no-choice feeding trials have documented the host plant preference and suitability of hyperaccumulator tissues for some of these herbivores. For example, Augustyniak *et al.* (2002) conducted such studies with *Chrysolina pardalina* (Fabricius) (Coleoptera: Chrysomelidae), a herbivore of the Ni-hyperaccumulator *Berkheya coddii* Roessler (Asteraceae) in Mpumalanga, South Africa. Similar experiments by Wall & Boyd (2006) used *Melanotrichus boydi* Schwartz & Wall (Heteroptera: Miridae), a herbivore of the Ni hyperaccumulator *Streptanthus polygaloides* Gray (Brassicaceae) from California, USA (Schwartz & Wall, 2001).

A survey of insects from *B. coddii* in the vicinity of Badplaas (Lat. 25° 57' S, Long. 30° 33' E) in Mpumalanga Province, South Africa (Boyd *et al.*, 2006a), yielded beetle specimens identified as *Chrysolina clathrata* (Clark) that were apparently feeding without ill effect on leaves of *B. coddii*. *Berkheya coddii* is unusual amongst Ni hyperaccumulators because of its extremely high levels of Ni (up to 37 000 μg/g: Anderson *et al.*, 1997). As mentioned above, Augustyniak *et al.* (2002) have conducted extensive tests of host plant preference for *C. pardalina* and concluded that *C. pardalina* feeds specifically on *B. coddii* at all life-history stages. We decided to document the metal concentrations of adult *C. clathrata* and test host plant specificity by conducting no-choice and choice experiments involving *B. coddii*, a second Ni hyperaccumulator species, *B. rehmannii* Thell. var. *rogersiana* Thell. (hereafter called *B. rehmannii*) that hyperaccumulates Ni to lower levels (17 000 μg/g: Morrey *et al.*, 1992), and two non-hyperaccumulator species, *B. echinacea* (Harv.) O. Hoffm. ex Burtt Davey and *B. insignis* (Harv.) Thell. These *Berkheya* species all grow on serpentine soils located east of Badplaas.

Materials and methods

All beetles used in the experiments were adults collected from *B. coddii* plants growing on serpentine sites east of Badplaas. Voucher specimens of *C. clathrata* have been deposited at the Australian Museum, New South Wales, Sydney. Plants were identified by K. Balkwill (C.E. Moss Herbarium, School of Animal and Plant Sciences, University of the Witwatersrand).

Whole-body metal concentrations of Chrysolina clathrata

Beetles were killed immediately after collection and then dried at 65 °C for 72 h. Analyzing insects immediately after collection means that metal in the gut contents will be included in the measurements (Migula *et al.*, 2007), yet whole-body measurements that include gut contents are relevant measures of the metal dose received by a predator that consumes entire insect prey (Boyd, 2009). Four composite samples of dried beetles were analyzed for their whole-body metal concentrations (Wall & Boyd, 2002). Beetles were not rinsed with water before analysis so that metals would not be leached from their bodies. Samples were digested in borosilicate-glass test tubes using 3 - 5 mL of concentrated HNO_3 at 110 °C for 6 - 8 h. The residue was then dissolved in 3 - 5 mL of 1 mol/L HCl at 110 °C for 2 - 4 h. Solutions were diluted with distilled water to a volume of 25 mL and analyzed for Co, Cr, Cu, Mg, Mn, Pb and Zn with an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000, Franklin, MA, USA). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251, Wilmington, MA, USA).

Insect and plant materials for no-choice and choice experiments

After collection beetles were stored in containers with leafy *B. coddii* stem tips as a food source prior to their use in experiments. Stem tips of 5 cm in length, with their associated young leaves, were harvested from plants of the four *Berkheya* species growing in the field. Stem tips were used because field observations of beetles on *B. coddii* showed the greatest damage by beetles to these relatively young leaves. The collected stem tips were placed in plastic bags (a separate bag for each species) and transported to the laboratory, on ice in cooler boxes, where they were refrigerated at 4 °C until used for experiments. Stem tips were examined for herbivore damage and only those free of damage were used for feeding experiments.

No-choice experiment

In the no-choice experiment, stem tips were placed into 10 cm diameter Petri dishes (1 plant species per dish) so that there were 11 dishes of stem tips for each *Berkheya* species. Five beetles were weighed as a group and placed into each dish. The dishes were kept at room temperature (22 °C) and under fluorescent light at a photoperiod similar to that outdoors (14: 10 L: D) for 6 days. Remaining food was removed and fresh food provided at 2 and 4 days after the experiment began. The total mass of all living beetles in each dish was recorded at 3 and 6 days after the experiment began, along with counts of live beetles at each time. In the middle of the experiment (day 3), the plant material in each dish was examined for evidence of feeding. We recorded if feeding evidence (damage to leaves) was present or absent for the plant material in each Petri dish.

We calculated the mean mass per beetle in each Petri dish at the start of the experiment as well as at 3 and 6 days. Mean mass values were compared between days 0 and 6, using paired t-tests, to determine if beetles gained, maintained or lost mass for each plant species offered. We used contingency table analysis to determine if the frequency of damage to plant samples on day 3 of the experiment varied among plant species.

Choice experiment

One stem tip of each *Berkheya* species was placed into ten 10 cm diameter Petri dishes. We placed five beetles into each dish and stored them at room temperature. After 3 days, each *Berkheya* species in each dish was scored for amount of herbivore damage by estimating the amount of leaf material removed, approximated to the nearest 5%. Damage of less than 2.5% was assigned a numerical value of 1% so that it could be distinguished from cases in which 0% damage was observed. Data were log-transformed to meet statistical assumptions (Zar, 1996) of analysis of variance (ANOVA) and analyzed by one-way ANOVA using Fisher's protected least significant difference (PLSD) test for post-hoc mean separations (Abacus Concepts, 1998).

Plant analysis

Samples of plant material used for the above experiments were analyzed for their elemental composition. Three composite samples of stem tips from each *Berkheya* species were dried at 60 °C for 72 h, ground and analyzed for nine elements. Plant samples were dry-ashed at 485 °C, further oxidized with boiling 1 mol/L HNO_3, dissolved in 1 mol/L HCl, and analyzed for Ca, Cu, Fe, K, Mg, Mn, P and Zn using an inductively-coupled argon plasma spectrometer (Jarrell-Ash ICAP 9000, Franklin, MA, USA). Nickel was determined by analyzing the same extract with an atomic absorption spectrophotometer (Instrumentation Laboratory IL 251, Wilmington, MA, USA). Concentrations of each element were compared between *Berkheya* species by one-way ANOVA followed by Fisher's PLSD test for post-hoc mean separations (Abacus Concepts, 1998).

Results

Whole-body metal concentrations of Chrysolina clathrata

Mean whole body metal concentrations (μg/g dry mass) were (SE in parentheses, $n = 4$ for all values): Co, 2.6 (1.5); Cr, < 1; Cu, 96 (13); Fe, 88 (81); Mg, 1 400 (51); Mn, 64 (5.0); Ni, 260 (73); Pb, 3.4 (3.4) and Zn, 140 (13). Magnesium levels were greatest, with Ni as second greatest and then Zn. Little Co or Pb was present in these insect samples and Cr was below the detection limit.

No-choice experiment

Survival of beetles was high in all treatments, ranging from 96% for those offered *B. insignis* to 100% for those offered *B. coddii*. Beetles offered *B. coddii* gained mass (6.3%) after 6 days ($t = 3.6$, df = 10, $P = 0.000\,7$, Fig. 1), whereas those offered *B. rehmannii* did not show a significant mass change by that time ($t = 0.27$, df = 10, $P = 0.86$, Fig. 1). Beetles offered leaves of the non-hyperaccumulators lost mass (Fig. 1). Mass declined 7.3% for those offered *B. insignis* ($t = 4.0$, df = 10, $P = 0.003\,3$) and 17% for those

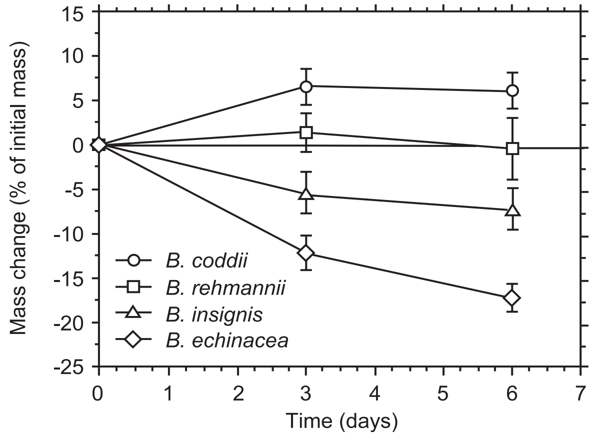

Fig. 1 Change in mean body mass (bars are ± SE, $n = 11$) of *Chrysolina clathrata* offered one of four species of *Berkheya* in a no-choice feeding trial. Paired *t*-tests (see text) contrasting day 0 and day 6 means for each *Berkheya* host-plant species showed that insects offered *B. coddii* gained mass, those offered *B. rehmannii* maintained mass, and those offered *B. insignis* or *B. echinacea* lost mass.

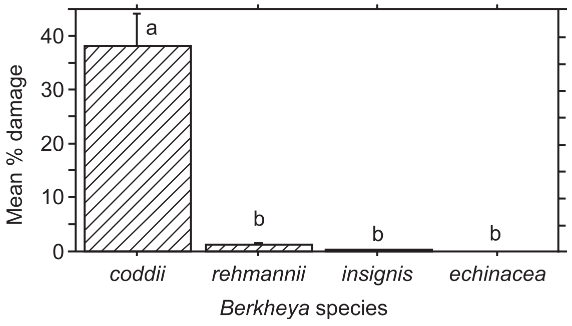

Fig. 2 Mean percent damage (estimated percentage of plant material consumed + SE) after 2 days by *Chrysolina clathrata* beetles offered a choice between stem tips from four species of *Berkheya*. Differing letters indicate means that differ significantly (Fisher's protected least significant difference test, $\alpha < 0.05$).

offered *B. echinacea* ($t = 9.1$, df = 10, $P < 0.000\,1$).

Damage frequency differed significantly among *Berkheya* species (contingency table analysis: $\chi^2 = 34$, df = 3, $P < 0.000\,1$). Damage was observed to plant material of both Ni hyperaccumulators (*B. coddii* and *B. rehmannii*) in all 11 Petri dishes, in 80% of dishes of *B. insignis*, and in none of the dishes containing *B. echinacea*. Subdividing the contingency table into pair-wise species comparisons revealed a significant difference in the damage frequency to *B. echinacea* compared to all other *Berkheya* species ($\chi^2 > 13$, df = 1, $P < 0.001$, in all comparisons). The difference in damage frequency between each of the two hyperaccumulators (*B. coddii* and *B. rehmannii*, with 100% damage) compared with *B. insignis* (with 80% damage) was marginally significant ($\chi^2 = 3.5$, df = 1, $P = 0.062$ in both cases).

Choice experiment

Beetles displayed a significant preference for *B. coddii* ($F_{3,36} = 77$, $P < 0.000\,1$). Almost 40% of the *B. coddii* material had been consumed after 2 days, compared to less than 2% of *B. rehmannii* and *B. insignis* (Fig. 2). We observed no evidence of feeding damage to *B. echinacea*.

Plant analysis

Elemental composition of plant material varied significantly among species for six of the nine elements we quantified (Table 1). The greatest variation was for Ni, which varied more than 200-fold between *B. echinacea* (with 72 μg/g) and *B. coddii* (with 15 100 μg/g). Significant differences among species were also found for Cu, Fe, Mg, P and Zn (Table 1). Of these elements, only P and Zn showed a pattern of difference among species similar to that of Ni, in which *B. coddii* differed from all other species and all other species did not significantly differ among one-another (Table 1). The results from multiple comparisons showed the Ni value for *B. rehmannii* did not differ significantly from Ni values for the non-hyperaccumulator *Berkheya* species (*B. echinata* and *B. insignis*): this probably is a statistical artifact due to the relatively extreme concentration of Ni in *B. coddii*, the small numbers of samples of each species ($n = 3$) and the large standard errors around the means (Table 1). When data from *B. coddii* were excluded and the statistical analysis re-done, Fisher's PLSD tests showed *B. rehmannii* differed in Ni concentration from each the two non-hyperaccumulator species at $P < 0.000\,1$.

Discussion

Chrysomelid beetles are a very large and diverse group and vary widely in feeding specificity (Jolivet & Hawkeswood, 1995), with some (including some species of *Chrysolina*) being quite host-specific (Daccordi, 2001). Augustyniak *et al.* (2002) showed that *Chrysolina pardalina* is a feeding specialist on *B. coddii* at all life-history stages. Our experiments showed that *C. clathrata* also may be host-specific on *B. coddii*. *Chrysolina clathrata* preferred *B. coddii* over the other three *Berkheya* species in the choice experiment, although it must be kept in mind that our experimental insects were originally collected from *B. coddii* and that

Table 1 Elemental analysis results (means, SE in parentheses) of samples of leafy stem tips for the four *Berkheya* species tested for possible host plant recognition by *Chrysolina clathrata*.

Element	*B. coddii*	*B. rehmannii*	*B. insignis*	*B. echinacea*
Ca	9 200 (1 990)	12 800 (527)	10 100 (2 670)	12 900 (607)
Cu	11.4^{c} (0.753)	15.4^{a} (-)	13.0^{bc} (1.06)	14.4^{ab} (0.572)
Fe	194^{b} (143)	202^{b} (7.37)	384^{a} (84.8)	145^{b} (9.02)
K	16 200 (2 850)	9 950 (781)	10 700 (3 370)	12 700 (770)
Mg	5 760^{a} (275)	5 010^{a} (446)	2 570^{b} (694)	2 800^{b} (86.9)
Mn	48.8 (10.2)	25.2 (1.02)	32.6 (9.86)	33.9 (0.752)
Ni	15 100^{a} (1 600)	2 110^{b} (169)	154^{b} (18.4)	72.3^{b} (18.6)
P	1 400^{a} (227)	711^{b} (42.7)	688^{b} (213)	584^{b} (42.0)
Zn	94.6^{a} (4.34)	25.8^{b} (1.32)	22.6^{b} (6.28)	25.7^{b} (1.52)

Means for an element with differing superscripts differ significantly (Fisher's PLSD test, $\alpha < 0.05$). Concentrations are expressed as μg/g dry mass: $n = 3$ for all data.

conditioning can influence host specificity tests. However, the no-choice experiment showed that either hyperaccumulator species appeared to be an acceptable host plant, as beetles maintained mass on both *B. coddii* and *B. rehmannii* (Fig. 1). *Chrysolina clathrata* did not feed on *B. echinacea*, possibly due to the trichomes present on leaves of this species. In the no-choice experiment, *Berkheya insignis* was a significantly less suitable host plant than *B. coddii*, in the short-term at least (judging by the loss of insect biomass: Fig. 1), but it was not apparent why *B. insignis* was a less suitable plant host.

Previous studies of other insects with a high Ni content have reported the bioaccumulation index (B) of Laskowski (1991), which compares the level of Ni in the insect against that in the tissue it consumes. We found that *C. clathrata* contained 265 μg Ni/g and its host plant 15 100 μg/g. Thus, the bioaccumulation index is very low (0.018). Given the extremely elevated Ni concentration of *B. coddii* leaves (15 100 μg/g: Table 1), the whole-body Ni concentration of *C. clathrata* (265 μg/g) suggests that tissue levels of Ni in the insect body are low. Because insects were killed immediately after they were collected, their guts probably contained high-Ni food. It would take a relatively small amount of food containing 15 100 μg Ni/g (only 1.8% of insect whole-body dry mass) to elevate the whole body concentration of a *C. clathrata* beetle from 0 to 265 μg/g. Such a contribution of gut contents to whole-body dry mass is certainly possible: Mesjasz-Przybylowicz *et al.* (2004) calculated that a single *C. pardalina* adult can consume up to 18.8 mg (dry mass) of *B. coddii* leaves per day and we measured the fresh mass of an adult *C. clathrata* at about 55 mg. Experiments with another herbivore of *B. coddii*, the grasshopper *Stenoscepa* sp. (Pyrgomorphidae), have documented the influence of gut contents on whole-body Ni concentrations (Boyd *et al.*, 2007). In that study, grasshoppers starved for 49 h had significantly lessened (73% less) whole-body Ni concentrations (950 μg/g) compared to those that had been fed continuously prior to being killed and analyzed (3 500 μg/g). However, note that even starved *Stenoscepa* sp. had relatively elevated whole-body Ni concentrations, implying elevated tissue-Ni levels for that Ni-tolerant insect. We conclude that tissue-Ni concentrations of *C. clathrata* are likely relatively low, but confirmation of this by tissue-level measurements, such as those done by Przybylowicz *et al.* (2003) with *C. pardalina*, is needed.

Our results are similar to studies of *C. pardalina* from Mpumalanga Province (Mesjasz-Przybylowicz & Przybylowicz, 2001; Augustyniak *et al.*, 2002; Mesjasz-Przybylowicz *et al.*, 2004). Our work with *C. clathrata*, and that of Augustyniak *et al.* (2002) with *C. pardalina*, showed that *B. coddii* was a preferred potential host plant of these beetles and that these beetles are remarkably tolerant of dietary Ni. However, Augustyniak *et al.* (2002) found that *C. pardalina* did not feed on *B. rehmannii* whereas we found that *C. clathrata* did accept that species as a host plant in our no-choice experiment (Fig. 1). It is not surprising that *C. clathrata* was able to use *B. rehmannii* as a host plant, since leaves of that species are chemically similar to leaves of *B. coddii* in that both contain hyperaccumulator concentrations of Ni (Table 1). Leaves of *B. rehmannii* and *B. coddii* may have other similar chemical constituents besides Ni that allow *C. clathrata* to accept them as possible host plants, but detailed chemical comparisons have yet to be made.

Our results are also similar to those of Mesjasz-Przybylowicz *et al.* (2004) in that both *C. clathrata* and *C. pardalina* contain elevated whole-body Ni levels. Reported concentrations of Ni in *C. pardalina* range widely: Mesjasz-Przybylowicz & Przybylowicz (2001) stated that adults of *C. pardalina* contained 2650 μg Ni/g whereas Mesjasz-Przybylowicz *et al.* (2002) reported adults containing 301 μg Ni/g. Our samples of *C. clathrata* contained a Ni concentration (265 μg Ni/g) that is similar to the latter report. It is unclear what factors underlie the wide range of Ni concentrations reported from *Chrysolina* beetles collected from *B. coddii* in Mpumalanga Province. Certainly, Ni concentrations in leaves of *B. coddii* vary greatly among sites (Augustyniak *et al.*, 2002), among leaves of different ages (Robinson *et al.*, 2003; Boyd *et al.*, 2004b), and among tissues within leaves (Robinson *et al.*, 2003). Thus, the Ni concentration of food ingested by these beetles can vary considerably. It is also possible that populations of beetles vary in their ability to eliminate Ni. The mechanisms that allow these beetles to feed on such high-Ni tissues are only just beginning to be investigated (Mesjasz-Przybylowicz *et al.*, 2004) and population- or species-level differences may exist.

It is interesting that two species of *Chrysolina* (*C. pardalina* and *C. clathrata*) have been reported as apparent specialists on *B. coddii*. A check of host records of specimens (E. Grobbelaar, personal communication, 2007) from the South African National Collection (SANC) revealed no specimens of *C. clathrata* in the collection. *Chrysolina pardalina* specimens were present, and were recorded from several species of *Berkheya* (all were species other than those included in our study) as well as sweet potato (*Ipomoea batatas* (L.) Lam., Convolvulaceae). Interestingly, there was one case in which three species of *Chrysolina*, *C. pardalina*, *C. tricolor* Vogel, and *C. vigintimaculata* Clark, were collected as adults and larvae from a single unidentified species of *Berkheya* in KwaZulu Natal (E. Grobbelaar, personal communication, 2007), showing that use of a single host-plant species by multiple *Chrysolina* species is not unprecedented. Additional specimen records revealed two other species of *Chrysolina*, *C. vulpina* Fabricius and *C. figurata* Clark, collected from species of *Berkheya*. In summary, a total of six *Chrysolina* species had been collected from at least five species of *Berkheya* (E. Grobbelaar, personal communication, 2007).

Additional host plant-herbivore studies involving *Chrysolina* and *Berkheya* are clearly needed. Recently, Bienkowski (2001) suggested the need for taxonomic revision of *Chrysolina* as well as studies of host plant associations, and Mesjasz-Przybylowicz & Przybylowicz (2001) agreed that the taxonomy of *Chrysolina* beetles in South Africa may need more careful study. Host-specific races or cryptic species of *Chrysolina* may exist, as well as undescribed species: new *Chrysolina* species from South Africa have been found with plant host specificity great enough to be useful to plant biological control efforts in Australia (Adair & Scott, 1997). These species may be an excellent target of studies using molecular systematic tools. Additional investigations of the taxonomy and host-plant relations of *Chrysolina*, especially those collected from *B. coddii*, are certainly warranted.

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