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THE NICKEL HYPERACCUMULATOR *STREPTANTHUS POLYGALOIDES* (BRASSICACEAE) IS ATTACKED BY THE PARASITIC PLANT *CUSCUTA CALIFORNICA* (CUSCUTACEAE)

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ABSTRACT

Metal hyperaccumulator plants can be defended from herbivore/pathogen attack by elevated tissue metal contents. We encountered the parasite *Cuscuta californica* Hook. & Arn. growing on a Ni hyperaccumulator (*Streptanthus polygaloides* A. Gray) and investigated this host/parasite relationship. Elemental levels in plant samples, and the Ni level in soil samples, were measured for two hosts: *S. polygaloides* and the nonhyperaccumulator *Lessingia nemaclada* E. Greene. Levels of 12 elements in parasitized and nonparasitized hosts, and corresponding *C. californica* samples, differed only for *S. polygaloides*, where significant differences were detected for six elements. Parasitized and nonparasitized *S. polygaloides* had similar amounts of Ni, K, P, Co and Pb, but parasitized plants had higher Ca than nonparasitized ones. *Cuscuta californica* had higher K and P, and lower Ni, Co and Pb than host *S. polygaloides. Cuscuta californica* parasitizing *S. polygaloides* contained 800 μg Ni/g, whereas that from *L. nemaclada* contained only 11 μg Ni/g. We concluded that hyperaccumulated Ni did not prevent attack of *S. polygaloides* by *C. californica*. We also tested the hypothesis that high-Ni *C. californica* was defended from generalist insect folivores. High- and low-Ni *C. californica* fed to neonate *Spodoptera exigua* larvae affected neither larval survival nor larval mass. We concluded that the elevated Ni content of *C. californica* parasitizing *S. polygaloides* did not benefit *C. californica* by defending it from a generalist herbivore.

Hyperaccumulators are plants that contain unusually elevated levels of metals in their tissues. Baker and Brooks (1989) reviewed hyperaccumulators of seven metals: Ni, Cu, Co, Zn, Pb, Cr and Mn. They define hyperaccumulators of Ni, Cu, Cr, Pb and Co as plants containing >1000 μg metal/g dry mass. Hyperaccumulators of Zn and Mn have >10,000 μg metal/g in their tissues. Nickel is the metal hyperaccumulated by the largest number of plant species, currently over 300 species (Brooks 1998), with each of the other metals hyperaccumulated by fewer than 30 species (Brooks 1998).

Although metal hyperaccumulation was defined over two decades ago by Brooks et al. (1977), the ecological function(s) of metal hyperaccumulation are only now being investigated. Boyd and Martens (1992) summarized five hypothetical (and not mutually exclusive) explanations for the hyperaccumulation of metals. These were: (1) metal tolerance/disposal; (2) drought resistance; (3) interference with neighboring plant species; (4) inadvertent uptake; and, (5) defense against herbivores/pathogens. Of these, the defense hypothesis has been supported by several recent experimental investigations. A harmful effect of hyperaccumulated Ni in plant tissues has been shown for lepidopteran folivores (Boyd and Martens 1994; Boyd and Moar 1999; Martens and Boyd 1994), a pathogenic bacterium (Boyd et al. 1994) and a pathogenic fungus (Boyd et al. 1994). In addition, Pollard and Baker (1997) demonstrated deterrence of herbivory by hyperaccumulated Zn for several types of invertebrate herbivores. For *Streptanthus polygaloides* A. Gray, hyperaccumulated Ni has been shown to cause acute toxicity in several invertebrate folivores (Boyd and Moar 1999; Martens and Boyd 1994) and negative effects on two plant pathogens (Boyd et al. 1994). Boyd (1998) reviewed the available experimental evidence and concluded that defense is a likely function of at least some hyperaccumulated metals.

Plant defense mediated by hyperaccumulated metals differs from other plant chemical defenses in at least two important ways. First, the toxic principle (metal ion) is translocated from the soil rather than constructed via biochemical pathways, and, second, metal-based defenses (due to their elemental nature) cannot be degraded by enzymatic attack. Hyperaccumulated metals thus constitute "elemental defenses" (Martens and Boyd 1994), and therefore differ from the more widespread secondary chemical defenses of plants.

As with other plant defenses, elemental defenses are not inviolate and are circumvented by some organisms. Investigations into how a defensive mechanism is circumvented help define the effectiveness and limitations of plant defense tactics (e.g., Becerra 1994). There is evidence that elemental defenses do not protect hyperaccumulators from some herbivores. For example, Boyd and Martens (1999) showed that the aphid *Acyrthosiphon pisum* was able to feed without apparent harm on Ni-hyperaccumulating plants of *Streptanthus polygaloides.*

As an additional case, we discovered a population of *S. polygaloides* that was parasitized by *Cuscuta californica* Hook. & Arn. var. *breviflora* Engelm. (Cuscutaceae). Parasitic plants attack many plant species (Kuijt 1969) and can have an important influence on host survival and reproductive success (Riches and Parker 1995; Pennings and Callaway 1996). They also may obtain defensive chemicals from their hosts that can defend the parasites from herbivory (e.g., Atsatt 1977; Schneider and Stermitz 1990). The research reported here examined the apparent failure of Ni hyperaccumulation to defend *S. polygaloides* against attack. We also hypothesized that, if *C. californica* plants attacking *S. polygaloides* contained elevated Ni contents, this Ni might defend *Cuscuta* against generalist folivores. Specific questions addressed are:

1. Does *C. californica* parasitizing *S. polygaloides* receive metal from its host?
2. Do parasitized *S. polygaloides* plants have elemental levels similar to nonparasitized plants?
3. Do the elemental parasite/host relationships of *C. californica* and *S. polygaloides* differ from those for a nonhyperaccumulator serpentine soil plant parasitized by *C. californica*?
4. What are the relationships between soil, host, and parasite Ni levels?
5. Does the elevated Ni content of *C. californica* parasitizing *S. polygaloides* benefit *C. californica* by protecting it from a generalist folivore?

METHODS

Study site. The study site is located in the Red Hills Management Area administered by the U. S. Department of Interior, Bureau of Land Management. Located in Tuolumne County, CA, this area is a large serpentine exposure that is occupied by serpentine chaparral, with the surrounding soils covered by oak savanna and oak woodland (Favre 1987). The serpentine chaparral is dominated by *Ceanothus cuneatus* (Hook.) Nutt. with a scattered overstory of *Pinus sabiniana* Douglas (Favre 1987).

Study species. Three plant species (two hosts, one parasite) were included in this study. One host species, *S. polygaloides* (Brassicaceae), is an annual Ni hyperaccumulator endemic to serpentine soils in the western foothills of the Sierra Nevada (Reeves et al. 1981; Kruckeberg and Reeves 1995). This species is a 2.5–9 dm-tall annual (Munz and Keck 1968) that hyperaccumulates Ni in all plant parts, ranging from a minimum of 1100 μg/g (dry mass) in fruits to as much as 16,400 μg/g in flowers (Reeves et al. 1981). The second host species, found in the Red Hills but not endemic to serpentine soils (Munz and Keck 1968), was *Lessingia nemaclada* E. Greene (Asteraceae). This species was selected for comparison because it also was parasitized by *C. californica* on the study site. This species, a 1–8 dm-tall annual common on dry gravelly slopes on the western side of the Sierra Nevada, ranges in elevation from 245–1070 m (Munz and Keck 1968). The third species is a parasitic member of the Cuscutaceae, *Cuscuta californica* var. *breviflora* (= *C. occidentalis* Millsp.). This taxon is reported from various hosts (*Grindelia* spp., *Solanum* spp., etc.) in many plant communities, ranging from the coast of California to Washington and Colorado (Munz and Keck 1968).

Plant and soil samples. We collected aboveground biomass samples of parasitized and nonparasitized *L. nemaclada* and *S. polygaloides* during early July 1995. The area chosen for study covered several hectares and contained a high density of plants of both host species. *Streptanthus polygaloides* plants selected for sampling were growing in a relatively dense stand that contained few *L. nemaclada* or other herbaceous associates. Patches of *C. californica*-parasitized *S. polygaloides* were selected and the aboveground material harvested and separated into *C. californica* and *S. polygaloides* components. Host density was great enough to allow a single *C. californica* stem to extend between a number of individual host plants. Care was taken to ensure that the *C. californica* sample did not include any host *S. polygaloides* biomass, but the host plant samples did contain some adhering *C. californica* stems. For each *C. californica*-parasitized *S. polygaloides* patch sampled, the nearest comparable nonparasitized patch was located and aboveground biomass harvested. All of the nonparasitized *S. polygaloides* patches were <2 m from the corresponding parasitized patch. For both parasitized and nonparasitized patches, samples from three local areas were composited into a single sample for analysis. A total of four sets of *S. polygaloides* composite samples were collected. We also collected soil samples to determine if soil Ni levels influenced *C. californica* presence or Ni levels in either host or parasite biomass. A soil sample was collected from each biomass sampling site from directly beneath the harvested host plants. Any litter present was removed to expose mineral soil, and a sample of the upper 5 cm of soil was removed. Soil samples were composited in the same manner as plant samples, to result in a soil sample that corresponded with each plant sample collected.

Lessingia nemaclada was sampled in a similar manner. Dense patches of *L. nemaclada* were located in two separate areas each ca. 200 m from the area in which *S. polygaloides* was sampled. Some *S. polygaloides* plants were present in both of the *L. nemaclada* sampling areas. *Lessingia nemaclada* plants were more widely dispersed than were *S. polygaloides* plants, so that each *L. nemaclada* sample was a composite of plants from many different locations in the same general area. Plants were classified as parasitized or nonparasitized and aboveground biomass was collected into separate composite samples. Two composite samples of parasitized *L. nemaclada,* and two composite samples of nonparasitized *L. nemaclada,* were collected from each of the two areas, for a total of four parasitized and four nonparasitized samples of *L. nemaclada.* As with *S. polygaloides,* soil samples were also collected. A soil sample was collected (in the same manner described above) from each location and soil samples were composited in parallel with the compositing of the biomass samples. Therefore, each *L. nemaclada* soil and biomass sample was composed of several subsamples from a particular location. Aboveground *C. californica*-parasitized material was separated into *C. californica* and *L. nemaclada* components. As with *S. polygaloides* samples, care was taken to ensure that the *C. californica* sample did not contain any host biomass, but the host plant samples contained some of the adhering *C. californica* stems.

Defense of C. californica *by Ni.* We hypothesized that the Ni content of *C. californica* parasitizing *S. polygaloides* may defend *C. californica* against polyphagous insect herbivores. We tested this hypothesis during the 1998 field season, using larvae of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Larvae of this insect were an appropriate selection as a representative polyphagous insect herbivore because of their broad host range (Metcalf and Metcalf 1993) and because *Spodoptera exigua* has been used as a "bioassay" herbivore in other research we have conducted on the Ni-based defense of *S. polygaloides* (Boyd and Moar 1999). We obtained larvae for this experiment from a laboratory colony established from insects collected from Alabama cotton fields and maintained on artificial diet (Chalfant 1975) at 28°C with a light-dark period of 12:12 h.

Neonate larvae were fed *C. californica* stems collected in 1998 from the Red Hills study site that parasitized either *S. polygaloides* or other herbaceous hosts. Four or five neonate larvae were placed on top of samples of freshly-collected *C. californica* stems within small plastic petri plates. A small piece of moistened sponge was included in each petri plate to help maintain the freshness of the plant material. Fifteen plates each of high-Ni *C. californica* (collected from *S. polygaloides*) and low-Ni *C. californica* (collected from herbaceous nonhyperaccumulator species) were started on June 15. Two days later, we began a second run using another hatching of neonates and a second set of 15 petri plates (each) for high- and low-Ni *C. californica.* Larvae were fed fresh field-collected *C. californica* stems every 3–5 days, at which time we removed from the petri plates any plant material that remained from the previous feeding. Larval survival was noted on days that larvae were fed. The experiment was conducted at room temperature and under ambient light conditions. Both runs were terminated on July 1, when fresh *C. californica* stems became difficult to find. Larvae still alive on July 1 were frozen, dried at 60°C for several days, weighed and the mean mass of the survivors in each petri dish was calculated. A sample of high- and low-Ni *C. californica* stems used to feed larvae was set aside for Ni analysis (procedure described below) midway through the feeding experiment.

Elemental analyses. Dried plant samples were ground and elemental analysis was performed for 12 elements for all plant samples except the *C. californica* samples from the *Spodoptera* feeding experiment, which were only analyzed for Ni content. Plant samples were dry-ashed at 485°C, further oxidized with boiling 1 *M* HNO_3, dissolved in boiling 1 *M* HCl, filtered, and analyzed for Ca, K, Mg, P, Cu, Fe, Mn, Cr, Pb, Co, and Zn using an inductively-coupled argon plasma spectrometer (ICAP 9000, Jarrell-Ash, Franklin, MA). Nickel was determined by analyzing the extract with an atomic absorption spectrophotometer (IL 251, Instrumentation Laboratory, Franklin, MA).

Soil samples were dried, ground and a subsample used to quantify extractable Ni. Soil samples were double-acid extracted using 20 mL of extractant (0.05 *M* HCl/0.025 *M* H_2SO_4) shaken with 5 g of dry soil for 5 min. The extract was analyzed for Ni using an atomic absorption spectrophotometer.

Data analyses. Concentrations of each element for each host species were analyzed by one-way Analysis of Variance (ANOVA) to determine whether elemental concentrations varied between nonparasitized host, parasitized host, and *C. californica* tissue samples. Fisher's Protected Least Significant Difference (PLSD) test was used for post-hoc means separation at $\propto = 0.05$ (Abacus Concepts 1992). Soil Ni data were analyzed by two-way ANOVA, with host species and the presence or absence of *C. californica* as main effects and including the interaction term. Regression analysis was used to further explore relationships between soil, host, and parasite Ni levels for each of the host species. For Ni data from host plants that were nonparasitized, host plant Ni content (dependent variable) was related to soil Ni (independent variable). Data sets from parasitized host plants were analyzed by examining the relationship between: (1) host plant Ni (dependent variable) and soil Ni content (independent variable), (2) *C. californica* Ni (dependent variable) and soil Ni (independent variable), and (3) *C. californica* Ni (dependent variable) and host plant Ni (independent variable).

The relationship between host and parasite was further explored by calculating quotients of elements in parasite versus host tissue (Pate 1995). The level of a given element in a *C. californica* sample was compared to that of the corresponding sample of its host plant by dividing the parasite's value by that of the host plant. Because concentrations of Co, Cr and Pb were very low (quantities were below our detection limit in several cases), quotients were not calculated for those elements. One-way ANOVAs tested the significance of host plant identity (*L. nemaclada* or *S. polygaloides*) on quotients for each element. Fisher's PLSD test was used for post-hoc means separation at ∝ = 0.05 (Abacus Concepts 1992). Quotients were transformed (arcsine square root transformation) prior to ANOVA to better meet assumptions underlying ANOVA (Zar 1996).

Data from the *Spodoptera* feeding experiment were analyzed to determine if larval survival or growth were influenced by the hyperaccumulation status of *C. californica*'s host plant. Survival data were analyzed via survival analysis, using the actuarial (life table) estimation method (Abacus Concepts 1994). Larval weight data, log-transformed to better fit ANOVA assumptions (Zar 1996), were analyzed by one-way ANOVA to determine if the Ni content of *C. californica* influenced larval growth.

RESULTS

Elemental contents of *Lessingia nemaclada* samples did not differ significantly between parasitized, nonparasitized, and *Cuscuta californica* samples (Table 1). This was not the case for samples from *Streptanthus polygaloides,* for which significant variation was detected for six of the 12 elements examined. This variation was due to differences in elemental contents between *S. polygaloides* and *C. californica,* rather than between parasitized and nonparasitized *S. polygaloides* plants. Parasitized and nonparasitized *S. polygaloides* had similar amounts of Ni, K, P, Co, and Pb (Table 1). Only Ca differed between these samples, being significantly higher in parasitized than in nonparasitized *S. polygaloides. Cuscuta californica* had higher K and P, and lower Ni, Co and Pb contents, than *S. polygaloides.* Calcium values for *C. californica* were similar to those for nonparasitized *S. polygaloides,* but significantly less than for parasitized *S. polygaloides* (Table 1).

Cuscuta californica parasitizing *S. polygaloides* contained a mean of 800 μg Ni/g, whereas *C. californica* parasitizing *L. nemaclada* had only 11.3μg Ni/g, a 70-fold difference (Table 1). Nickel contents of *C. californica* reflected the large difference in Ni between *S. polygaloides* and *L. nemaclada* (Table 1).

Two-way ANOVA of soil Ni values detected significant variation between host plant species (mean square = 4660, $F_{1,12}$ = 21.5, P = 0.0006), but *C. californica* infection (mean square = 452, $F_{1,12}$ = 2.08, P = 0.174) and the interaction term (mean square = 7.56, $F_{1,12}$ = 0.35, P = 0.855) were nonsignificant. Mean extractable Ni content of all eight *S. polygaloides* soil samples was 42 μg/g (SE = 3.9), whereas that for the eight *L. nemaclada* samples was almost two-fold greater (76 μg Ni/g, SE = 6.3).

There were no statistically significant linear regressions for *L. nemaclada* samples (Table 2). The same was found for *S. polygaloides* samples, with one exception: that between soil Ni and *C. californica* Ni for parasitized *S. polygaloides* plants (Table

TABLE 1. ELEMENTAL COMPOSITION OF PLANT SAMPLES, EXPRESSED AS μG/G DRY WEIGHT. Data are means (SE), n = 4. Plant material for *S. polygaloides* that varied significantly in composition for an element (as shown by ANOVA) is denoted by superscripts. Differing superscripts indicate significant post-hoc means separation (∝ = 0.05) by Fisher's PLSD test. No samples from *L. nemaclada* differed significantly in composition for any element measured.

	Plant material: *Streptanthus polygaloides*			Plant material: *Lessingia nemaclada*		
Element	Nonparasitized	Parasitized	*Cuscuta*	Nonparasitized	Parasitized	*Cuscuta*
Ni	2620^{a} (373)	3130^{a} (249)	800^{b} (153)	17.5 (4.2)	27.0 (8.4)	11.3 (1.8)
K	9140^{a} (2560)	6340^{a} (816)	$16{,}000^{b}$ (1058)	8060 (346)	9090 (640)	11,300 (3180)
P	1440^{a} (122)	1260^{a} (176)	3470^{b} (252)	866 (186)	973 (280)	1280 (422)
Ca	2420^{a} (437)	3540^{b} (359)	2140^{a} (216)	3430 (206)	3670 (319)	2170 (857)
Co	$2.3^{a,b}$ (0.88)	3.7^{b} (0.33)	0.69^{a} (0.34)	0.83 (0.53)	0.86 (0.42)	3.3 (3.2)
Pb	$4.4^{a,b}$ (2.0)	7.0^{b} (0.68)	0.61^{a} (0.36)	0.45 (0.45)	1.7 (0.7)	8.4 (8.4)
Mg	2050 (326)	2440 (281)	2050 (171)	2010 (218)	2930 (223)	2150 (442)
Fe	118 (36)	76.8 (10)	111 (38)	269 (77)	494 (132)	158 (49)
Zn	35.2 (5.9)	47.2 (4.0)	39.8 (3.2)	26.5 (2.2)	29.7 (3.6)	27.1 (8.4)
Mn	11.6 (1.7)	10.4 (1.2)	13.6 (2.2)	20.3 (2.1)	25.4 (4.0)	17.9 (7.0)
Cu	3.0 (0.68)	2.9 (0.90)	2.8 (0.80)	5.5 (0.66)	6.3 (1.6)	5.2 (1.5)
Cr	2.0 (0.95)	1.0 (0.36)	1.6 (1.4)	4.0 (1.6)	7.8 (3.5)	5.8 (4.9)

TABLE 2. RELATIONSHIPS BETWEEN HOST, PARASITE, AND SOIL NI CONTENTS AS REVEALED BY LINEAR REGRESSIONS. Regressions are presented between pairs of variables selected from each of the four datasets generated in the experiment. For all regressions, df = 1.

Dependent/Independent variables	Mean square	F	P
	L. nemaclada: No *Cuscuta* present		
L. nemaclada Ni/soil Ni	0.260	0.002	0.97
	L. nemaclada: Parasitized		
L. nemaclada Ni/soil Ni	322	1.24	0.38
C. californica Ni/soil Ni	9.98	1.42	0.36
C. californica Ni/*L. nemaclada* Ni	0.119	0.010	0.93
	S. polygaloides: No *Cuscuta* present		
S. polygaloides Ni/soil Ni	70,500	0.088	0.79
	S. polygaloides: Parasitized		
S. polygaloides Ni/soil Ni	119,000	1.49	0.35
C. californica Ni/soil Ni	264,000	33.2	0.029
C. californica Ni/*S. polygaloides* Ni	491,000	3.87	0.19

2). The regression for this analysis accounted for 94.3% of the variation in the data ($r^2 = 0.943$, $Y = 2502 - 47.3\ X$) and described an inverse relationship between these two variables. This inverse relationship was unanticipated, and may be a chance result stemming from small sample size.

Elemental parasite/host quotients for *C. californica* ranged widely, and for some elements varied due to host identity. Mean quotients varied from a low of 0.25 for Ni to a high of 2.8 for P, both with *S. polygaloides* as host (Table 3). Quotients for most elements did not vary due to host (Table 3). Exceptions were Ni, with a significantly higher quotient on *L. nemaclada* than on *S. polygaloides* (ANOVA: mean square = 0.238, $F_{1,6} = 6.65$, $P = 0.0418$), and K and P, both of which had significantly higher quotients on *S. polygaloides* (respective ANOVA's: mean square = 0.622, $F_{1,6} = 10.3$, $P = 0.0185$; and mean square = 0.645, $F_{1,5} = 9.61$, $P = 0.0268$). Quotients for Ca and Mg were <1 for both host species, whereas quotients for Fe, Mn, Cu and Zn showed no consistent trend relative to unity for either host (Table 3).

TABLE 3. ELEMENTAL QUOTIENTS FOR *CUSCUTA CALIFORNICA* VAR. *BREVIFLORA* PARASITIZING *LESSINGIA NEMACLADA* OR *STREPTANTHUS POLYGALOIDES*. Data are means (SE), n = 4. Differing superscripts for mean quotients of an element denote significantly differing means (Fisher's PLSD test, $\alpha = 0.05$).

Element	*Lessingia nemaclada*	*Streptanthus polygaloides*
Ni	0.60^a (0.13)	0.25^b (0.036)
K	1.2^a (0.35)	2.6^b (0.18)
P	1.4^a (0.38)	2.8^b (0.16)
Ca	0.59 (0.23)	0.61 (0.040)
Mg	0.72 (0.12)	0.87 (0.090)
Fe	0.33 (0.042)	1.6 (0.65)
Mn	0.71 (0.27)	1.4 (0.37)
Cu	0.91 (0.33)	1.3 (0.45)
Zn	1.0 (0.42)	0.85 (0.064)

Stems of high-Ni *C. californica* (*S. polygaloides* as host) and low-Ni *C. californica* (nonhyperaccumulators as hosts) were equivalent diets for *Spodoptera* larvae. For both runs, larval survival was initially greater for those fed high-Ni leaves. By the time the experiment was terminated, survival had dropped to similarly low levels (ca. 50%) for larvae fed *C. californica* from either type of host (Fig. 1). Survival analysis revealed no significant effect of *C. californica* host on larval survival (Mantel-Cox logrank test, $\chi^2 = 0.019$, df = 1, P = 0.89).

Larval growth also was unaffected by *C. californica* Ni content. Mean larval mass at the end of the experiment was similar for larvae fed high-Ni *C. californica* stems (6.8 ± 3.9 mg, mean ± SE, n = 29) and those fed low-Ni stems (6.6 ± 4.4 mg, mean ± SE, n = 28). ANOVA indicated no significant effect of *C. californica* host on mean larval mass (mean square = 0.001, $F_{1,55} = 0.011$, P =

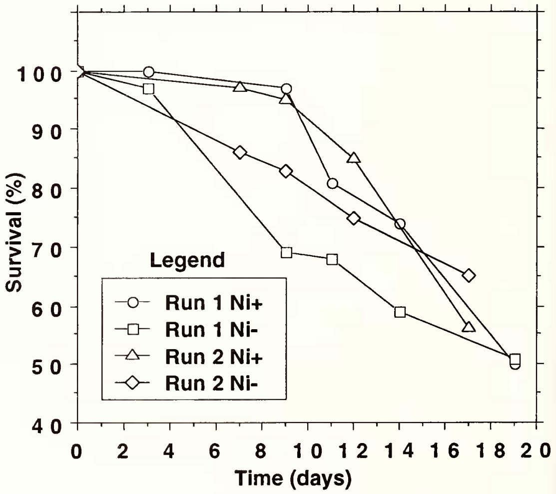

FIG. 1. Survival of *Spodoptera exigua* larvae fed *Cuscuta californica* collected from *S. polygaloides* (Ni+ *Cuscuta*) and *L. nemaclada* (Ni− *Cuscuta*).

0.92). *Cuscuta californica* stems used for this experiment had lower Ni contents than those collected in 1995. The 1998 sample of *C. californica* from *S. polygaloides* contained 470 μg Ni/g, whereas the mean for samples from 1995 was 800 μg/g (Table 1). The sample of *C. californica* collected from nonhyperaccumulator hosts in 1998 contained 28 μg Ni/g, versus 13 μg/g from *C. californica* parasitizing *L. nemaclada* in 1995 (Table 1).

DISCUSSION

Hyperaccumulated Ni did not prevent *Streptanthus polygaloides* from being attacked by *C. californica.* In the field, *C. californica* parasitizing *S. polygaloides* appeared as vigorous as nearby *C. californica* that used other herbaceous species as hosts. Because all plant defenses can be circumvented to some extent by some organisms (Levin 1976), this study helps to define the limitations of the Ni-based elemental defense of *S. polygaloides.* Whereas hyperaccumulated Ni negatively affects generalist folivores (Boyd and Martens 1994: Martens and Boyd 1994; Boyd and Moar 1999), organisms tapping vascular tissues are not similarly affected. Boyd and Martens (1999) showed that the pea aphid (*Acyrthosiphon pisum* [Harris]; Homoptera: Aphididae), a phloem-feeder as with many aphid species (Dixon 1985), was not harmed by feeding on Ni-hyperaccumulating *S. polygaloides* plants. Unlike aphids, *C. californica* may tap both phloem and xylem tissues (e.g., Lee and Lee 1989). The high Ni content of *C. californica* observed here, and the low Ni content of aphids feeding on high-Ni *S. polygaloides* reported by Boyd and Martens (1999), suggest that the Ni level in phloem fluid of *S. polygaloides* is relatively low and that the Ni present in *C. californica* arrives largely through the xylem connections. The latter idea is bolstered by the report of Kramer et al. (1996) that Ni in the Ni hyperaccumulator *Alyssum lesbiacum* is transported in xylem (as a histidine-Ni complex).

Our results show that this population of *C. californica* is remarkably Ni-tolerant, since these plants were able to withstand the elevated tissue Ni levels that we measured in *C. californica* parasitizing *S. polygaloides* (see Table 1). MacNicol and Beckett (1985) suggest that unadapted plants can withstand tissue Ni concentrations of approximately 10 μg/g without suffering a reduction in growth. Reeves (1992) pointed out that most plants growing on serpentine soils contain <100 μg Ni/g in their tissues. The mean value reported here for *C. californica* parasitizing *S. polygaloides,* 800 μg/g, therefore is exceptionally high relative to most serpentine species. Although the mean fell short of the 1000 μg/g level used to define hyperaccumulator status (Brooks 1987; Baker and Brooks 1989), one *C. californica* sample reached 1220 μg Ni/g. To our knowledge, these values are the highest Ni contents of any parasitic plant yet reported. It is unlikely that soil contamination contributed greatly to these Ni values. Reeves (1992) points out that high levels of Co (>50 μg/g) may indicate soil contamination. Levels of Co in our specimens were low, with means ranging from 0.69–3.7 μg/g (Table 1). Further evidence against soil contamination is shown by the low Cr values of our samples, which ranged from 1–8 μg/g (Table 1). Brooks et al. (1995) state that low Cr concentrations of plant samples imply lack of contamination by serpentine soils, as these soils often have elevated Cr concentrations.

Boyd and Martens (1998a) suggested that elemental defenses of plants may be circumvented in several ways by organisms that attack those plants: (1) by selective feeding on low-metal tissue; (2) by use of a generalist diet to dilute high-metal food; and, (3) by genuine tolerance of high metal levels. For *C. californica,* the relatively high Ni content when growing on *S. polygaloides* indicates that this population of *C. californica* possesses a high level of metal tolerance. Thus, *C. californica* is one of the first organisms documented to attack a metal-hyperaccumulator plant by way of physiological metal tolerance. However, we should note that the Ni content of *C. californica,* relative to its *S. polygaloides* host, was lower than for all other elements analyzed (see quotients in Table 3). The lessened concentrations of some heavy metals (Ni, Co, Pb) in *C. californica* parasitizing *S. polygaloides* (Table 1) implies discrimination against those elements during uptake by the parasite. We suggest that the high Ni content of *C. californica* from *S. polygaloides* results from its inability to be more effective in excluding metals from its tissues.

The high Ni levels found in *C. californica* tissue did not protect it from herbivory by *Spodoptera exigua* larvae. Neither acute toxicity nor sublethal (growth-reducing) effects were detected in our feeding experiment. This implies that *C. californica* does not receive a defensive benefit from the Ni it obtains from host *S. polygaloides.* This differs from some cases of chemical defense in insects, in which a chemical from a plant species is used by the insect in its own defense (see Duffey 1980), and from examples where parasitic plants obtain defensive chemicals from their hosts (e.g., Atsatt 1977; Schneider and Stermitz 1990; Marvier 1996). In this first test involving a Ni-based defense, we found no evidence of a protective benefit accruing to high-Ni *C. californica* plants.

To our knowledge, this report is the first definitive record of a parasitic plant obtaining high amounts of metal from a hyperaccumulator host. Reeves (1992) reported that the root parasite *Orobanche rechingeri* was believed to parasitize the Ni hyperaccumulator *Alyssum lesbiacum.* The parasite contained 600 μg Ni/g, but it was unclear if the Ni came from the host or directly from the serpentine soil. Because *C. californica* contacts soil only as a seedling and depends solely on its host for mineral nutrition during subsequent life history stages

(Kuijt 1969; Pate 1995), there is little doubt as to the source of the Ni found in *C. californica* tissues. Study of other parasite/hyperaccumulator interactions will determine if the case reported here is a representative outcome for parasite/hyperaccumulator interactions. Because parasitic plants are very widespread (Musselman and Press 1995) and occur on serpentine substrates (e.g., Kruckeberg 1984; Callizo 1992), it seems certain that more cases of parasitism of metal hyperaccumulators will be uncovered in the future.

Our results also have implications regarding the type of defense represented by metals. Plant defenses can be either constitutive or inducible (Karban and Baldwin 1997), and we know of little available evidence that bears on this point regarding metal-based elemental defenses (Boyd 1998; Boyd and Martens 1998b). To our knowledge, the only published information pertinent to this question is a study by de Varennes et al. (1996) using the Ni hyperaccumulator *Alyssum pintodasilvae* Dudley. They found that plants regrowing after an initial harvest of aboveground biomass contained 3.4–5 times more Zn than the first harvest of plant material. In our study of *S. polygaloides,* Ni levels did not vary significantly between parasitized and nonparasitized plants. Therefore, we conclude that the degree of Ni hyperaccumulation was not affected by *C. californica* attack and that Ni hyperaccumulation in *S. polygaloides* represents a constitutive, rather than inducible, defense.

Finally, this report has implications for applied uses of metal hyperaccumulators. Metal hyperaccumulators may be useful as "phytoextractors" of metals, either from metal-contaminated sites (McGrath et al. 1993) or as a way to "phytomine" metals from naturally metalliferous soils (Nicks and Chambers 1995). Parasitic plants, including some species of *Cuscuta* (Kuijt 1969), can become pests in agricultural situations (e.g., Riches and Parker 1995). Given the high levels of elemental defenses in a metal-hyperaccumulating "phytoextractor" plant, one might suspect that such plants would be well-defended against attack by insects, parasitic plants, etc. Our research shows that, at least in the case of *S. polygaloides,* plants hyperaccumulating Ni are not safe from attack by *C. californica.* Phytoextraction or phytomining industries must therefore be prepared to protect their metal-hyperaccumulating plants against parasitic plant pests using other techniques.

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