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Research

# Cadmium hyperaccumulation protects *Thlaspi caerulescens* from leaf feeding damage by thrips (*Frankliniella occidentalis*)

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## Summary

- Metal hyperaccumulation has been proposed as a plant defensive strategy. Here, we investigated whether cadmium (Cd) hyperaccumulation protected *Thlaspi caerulescens* from leaf feeding damage by thrips (*Frankliniella occidentalis*).
- Two ecotypes differing in Cd accumulation, Ganges (high) and Prayon (low), were grown in compost amended with 0–1000 mg Cd kg$^{-1}$ in two experiments under glasshouse conditions. $F_2$ and $F_3$ plants from the Prayon × Ganges crosses were grown with 5 mg Cd kg$^{-1}$. Plants were naturally colonized by thrips and the leaf feeding damage index (LFDI) was assessed.
- The LFDI decreased significantly with increasing Cd in both ecotypes, and correlated with shoot Cd concentration in a log-linear fashion. Prayon was more attractive to thrips than Ganges, but the ecotypic difference in the LFDI was largely accounted for by the shoot Cd concentration. In the $F_2$ and $F_3$ plants, the LFDI correlated significantly and negatively with shoot Cd, but not with shoot zinc (Zn) or sulphur (S) concentrations.
- We conclude that Cd hyperaccumulation deters thrips from feeding on *T. caerulescens* leaves, which may offer an adaptive benefit to the plant.

**Key words:** cadmium, *Frankliniella occidentalis*, herbivory, hyperaccumulation, *Thlaspi caerulescens*, thrips, zinc.

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## Introduction

Metal hyperaccumulation by higher plants is a rare and scientifically interesting phenomenon. Hyperaccumulators can accumulate metals or metalloids in the above-ground parts to concentrations 1–3 orders of magnitude higher than those in normal plants (Baker *et al.*, 2000). Approximately 400 taxa of hyperaccumulators have been reported, and the metals or metalloids accumulated include arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn) (Brooks, 1998; Baker *et al.*, 2000). Much progress has been made over the last decade in the understanding of the physiology and molecular mechanisms of metal hyperaccumulation (see reviews by Pollard *et al.*, 2002; Macnair, 2003; McGrath & Zhao, 2003). However, the evolutionary reason for metal hyperaccumulation remains unclear.

Boyd & Martens (1992) and Boyd (1998) discussed several hypotheses to explain how hyperaccumulation may have evolved. These hypotheses include: inadvertent uptake, metal tolerance/disposal, drought resistance, interference with other plants (elemental allelopathy), and defence against pathogens or herbivores. All except the first hypothesis have selective benefits accruing to the plant. There is little evidence linking hyperaccumulation with metal tolerance/disposal, elemental allelopathy or an enhanced drought resistance (see review by

Macnair, 2003). Of the other two hypotheses, the plant defence hypothesis has attracted a lot of attention in recent years and appears to be supported by some experimental data, whereas the inadvertent uptake hypothesis is essentially a non-adaptive hypothesis that is difficult to test experimentally.

So far, experimental studies regarding the plant defence hypothesis have focused on Ni, Zn and Se. Boyd and coworkers showed that Ni hyperaccumulated by several plant species was acutely toxic to a number of generalist herbivores (Boyd & Martens, 1994; Martens & Boyd, 1994; Boyd & Moar, 1999; Boyd *et al.*, 2002). For example, larvae of *Pieris rapae*, a generalist folivore, suffered 100% mortality after 12 days when fed high-Ni leaves of *Thlaspi montanum*, whereas those fed low-Ni leaves quadrupled in weight with a corresponding mortality of only 21% (Boyd & Martens, 1994). Further experiments using an artificial insect diet amended with Ni demonstrated that dietary Ni concentrations > 1000 mg kg$^{-1}$ were toxic to larvae of *Pieris rapae* (Boyd & Martens, 1994; Martens & Boyd, 1994). A defence is most effective if it deters attack rather than poisoning an attacking organism after damage has occurred (Boyd, 1998). Pollard & Baker (1997) investigated the feeding preferences of three species of herbivores towards low- or high-Zn leaves of the Zn-hyperaccumulator *Thlaspi caerulescens*. Locusts (*Schistocerca gregaria*), slugs (*Deroceras caruanae*) and caterpillars (*Pieris brassicae*) all showed significant preferences for plants with lower foliar Zn concentrations. In the studies mentioned above, variations in leaf metal concentrations were established by varying metal concentrations in the substrates. This approach has been criticized because of the possibility that plant properties other than the metal concentration could have been influenced by the treatments, thus complicating the interpretation of the herbivore feeding data (Macnair, 2003). Jhee *et al.* (1999) used genetic variation in leaf Zn concentration that existed among populations and amongst individual plants within the same population of *T. caerulescens*, while using uniform growing conditions for all plants. They found a highly significant avoidance of high-Zn leaves by *Pieris napi oleracea* larvae. Further evidence of a protective role of elemental accumulation against herbivory comes from the studies of Hanson *et al.* (2003, 2004), who showed that Se accumulation by *Brassica juncea* deterred feeding by caterpillars (*P. rapae*) and by phloem-feeding aphids (*Myzus persicae*). In particular, the protective effect of Se was evident at relatively low concentrations of Se in leaves, almost 2 orders of magnitude below the concentration used to define Se hyperaccumulation. In addition to the protective effect against herbivory, metal hyperaccumulation has been shown to protect plants from fungal diseases (Boyd *et al.*, 1994; Ghaderian *et al.*, 2000; Hanson *et al.*, 2003).

However, a number of studies have shown no defensive effects of metal hyperaccumulation. For example, neither Zn hyperaccumulation in *Arabidopsis halleri* (Huitson & Macnair, 2003) nor Se accumulation in *B. juncea* (Hanson *et al.*, 2003) deterred herbivory by snails. Also, Ni hyperaccumulation in *Streptanthus polygaloides* was not effective in defending plants against aphid attack, presumably because Ni concentrations in the phloem sap were not high enough to be toxic (Boyd & Martens, 1999). Surprisingly, elevated tissue Ni concentration in *S. polygaloides* enhanced infection of turnip mosaic virus instead of defending plants from the virus (Davis *et al.*, 2001). These results show that protective effects of metal hyperaccumulation are not absolute, but may be dependent on the ecological specifics of both the plant species and the herbivores or pathogens involved (Boyd, 2004).

Cadmium is a nonessential and toxic metal. Cd hyperaccumulation, defined as > 100 mg Cd kg$^{-1}$ dry weight (d. wt) in the shoots growing in the natural habit (Baker *et al.*, 2000), has been reported in *T. caerulescens* (Baker *et al.*, 1994; Robinson *et al.*, 1998; Reeves *et al.*, 2001), and occasionally also in *A. halleri* (Bert *et al.*, 2002). Interestingly, populations of *T. caerulescens* from southern France are capable of accumulating much more Cd in the shoots than those from other regions, and this superior Cd accumulation ability is apparently not related to Zn accumulation (Lombi *et al.*, 2000; Roosens *et al.*, 2003). Thus, a question arises as to why *T. caerulescens*, particularly its southern French populations, hyperaccumulates Cd. As discussed above, two possible explanations are inadvertent uptake and plant defence; the latter, which has not been tested for Cd in any plant species, is the subject of the present study. Specifically, we investigated whether Cd hyperaccumulation by *T. caerulescens* deters herbivory by western flower thrips (*Frankliniella occidentalis*). Thrips are a worldwide pest in many agricultural and horticultural crops, and *F. occidentalis* is one of the most common thrips species occurring in North American and Europe (Lewis, 1997). This species is highly polyphagous and known to feed on a wide range of plant species worldwide (Lewis, 1997; Mound, 1997). The insect feeds on both leaves and flowers through piercing and sucking, causing damage such as silver spots, necrosis, discolouration and growth distortions. Direct feeding damage caused by thrips pests is most harmful in dry climates and seasons when heavily attacked plants lose moisture rapidly. In warm regions and glasshouses, thrips can breed continuously, with up to 15 generations being completed each year (Lewis, 1997).

## Materials and Methods

### Effects of Cd on thrips colonization and leaf feeding damage on two contrasting ecotypes of *Thlaspi caerulescens*

Two ecotypes of *Thlaspi caerulescens* J. & C. Presl, Prayon (Belgium) and Ganges (Southern France), were used in two separate experiments. The two ecotypes hyperaccumulate Zn to similar degrees, but differ greatly in Cd accumulation (Lombi *et al.*, 2000; Lombi *et al.*, 2001). Seeds were sown in trays containing a general-purpose compost. Three-week-old seedlings were transferred to plastic pots each filled with 500 g of compost (two seedlings per pot). In experiment 1, the compost was amended with 0, 250, 500 or 1000 mg Cd $kg^{-1}$, and in experiment 2, with 0, 50, 100, 200 and 300 mg Cd $kg^{-1}$. Zn was added at 250 mg Zn $kg^{-1}$ in all treatments. Cd and Zn were added in a solution of $CdCl_2$ or $ZnCl_2$ and mixed thoroughly with the compost before transplanting. There were three and four replicates for each treatment in the first and second experiments, respectively. Pots were placed randomly on a bench located in the central area inside a glasshouse with the following conditions: 16 h day length with natural sunlight supplemented with SONT sodium-vapour lamps (Osram, Munich, Germany) to maintain a minimum intensity of 350 µmol photons $m^{-2}$ $s^{-1}$ over the waveband 300–1100 nm; temperature ranging from 15 to 30°C during the growth periods (experiment 1: from 29 September to 29 December, 2003; experiment 2: from 7 January to 17 April 2004). Plants were watered with deionized water throughout. The pots were rearranged randomly several times during the growth period. Plants were colonized by western flower thrips (*Frankliniella occidentalis* Pergande) from a background population inside the glasshouse.

At the end of the experiments, the above-ground parts of plants were cut and shaken to remove thrips, which were collected in a black plastic container filled with water. The number of thrips floating on the water was counted. The leaf feeding damage index (LFDI) was determined according to McKinney (1923). All leaves from each pot were evaluated visually and grouped into six classes with values of 0, 1, 3, 5, 7 and 9, which corresponded to percentages of total leaf area damaged by thrips feeding of 0, < 10%, 10–25%, 25–50%, 50–75% and > 75%, respectively. The LFDI values were calculated according to the following formula:

$$\text{LFDI (\%)} = 100 \times (\Sigma \text{class value} \times \text{corresponding leaf numbers})/(\text{total numbers of leaves} \times 9).$$

All leaves, petioles and stems were then rinsed with deionized water, blotted dry and dried at 60°C for 48 h. The dry weights were then recorded. Plant samples were ground and digested with a mixture of $HNO_3$ and $HClO_4$, and the concentrations of Cd, Zn, sulphur (S) and phosphorus (P) were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES; Fisons ARL Accuris, Ecublens, Switzerland). Shoot elemental concentrations are expressed on a dry weight basis.

### Variations in thrips colonization and leaf feeding damage in the $F_2$ and $F_3$ plants from an interecotypic cross of *T. caerulescens*

Our previous study showed a genetically determined variation in Cd accumulation in the $F_2$ plants from crosses between the Ganges and Prayon ecotypes of *T. caerulescens* (Zha *et al*., 2004). A pot experiment was carried out to investigate whether damage by thrips correlated with Cd accumulation in shoots in an $F_2$ population from the Prayon × Ganges (P × G) cross (experiment 3). Thirty 3- week-old $F_2$ seedlings were transferred to 30 pots, each containing 500 g of compost amended with 5 mg Cd $kg^{-1}$ and 250 mg Zn $kg^{-1}$. Twenty-four plants survived after transplanting. Plants were allowed to grow for a period of 48 days (from 15 April to 2 June 2004) under the same conditions as in experiments 1 and 2. The number of thrips, the LFDI and shoot metal concentrations were determined as described for experiments 1 and 2.

In a separate experiment (experiment 4), 300 $F_3$ seedlings derived from selfed $F_2$ plants of the Ganges × Prayon (G × P) and Prayon × Ganges (P × G) crosses were grown in pots each containing 350 g of compost amended with 5 mg Cd $kg^{-1}$ and 100 mg Zn $kg^{-1}$ for 2 months (from 8 March to 7 May 2004). We randomly selected 27 plants, which showed a large variation in thrips feeding damage, for the determination of the LFDI and shoot metal concentrations.

### Statistical analysis

Two-way analysis of variance (ANOVA) was used to test the statistical significance of the differences between the two ecotypes and Cd treatments in experiments 1 and 2. Correlation coefficients were calculated between the LFDI and shoot element concentrations in experiments 1–4 and the significance of the correlation was determined by *t*-test. Where appropriate, data were transformed logarithmically before statistical analysis to obtain homogeneity of variances. Statistical analyses were performed with the software Genstat® for Windows™ (VSN International, Hemel Hempstead, UK).

## Results

### Effects of Cd on thrips colonization and leaf feeding damage on two ecotypes of *T. caerulescens* differing in Cd accumulation

In experiment 1, the two ecotypes produced similar shoot biomass in the control treatment, but the biomass was greater in Ganges than in Prayon when Cd was added (Fig. 1a). Shoot biomass decreased significantly when Cd addition was > 250 mg $kg^{-1}$ for Prayon and > 500 mg $kg^{-1}$ for Ganges, respectively. Plants in these treatments were chlorotic, indicating Cd phytotoxicity. Shoot Cd concentration increased markedly with increasing Cd addition in both ecotypes (Fig. 1b). On average, shoot Cd concentration was significantly ($P < 0.05$; Table 1) larger in Ganges than in Prayon. By contrast, shoot Zn concentration was significantly ($P < 0.01$; Table 1) larger in Prayon than in Ganges. There were significant ($P < 0.001$) effects of ecotype and Cd treatment on both the number of thrips per plant and the LFDI (Table 1; Fig. 1c and d). Prayon had a significantly higher number of thrips and significantly higher LFDI than in Ganges, particularly in the 0 and 250 mg

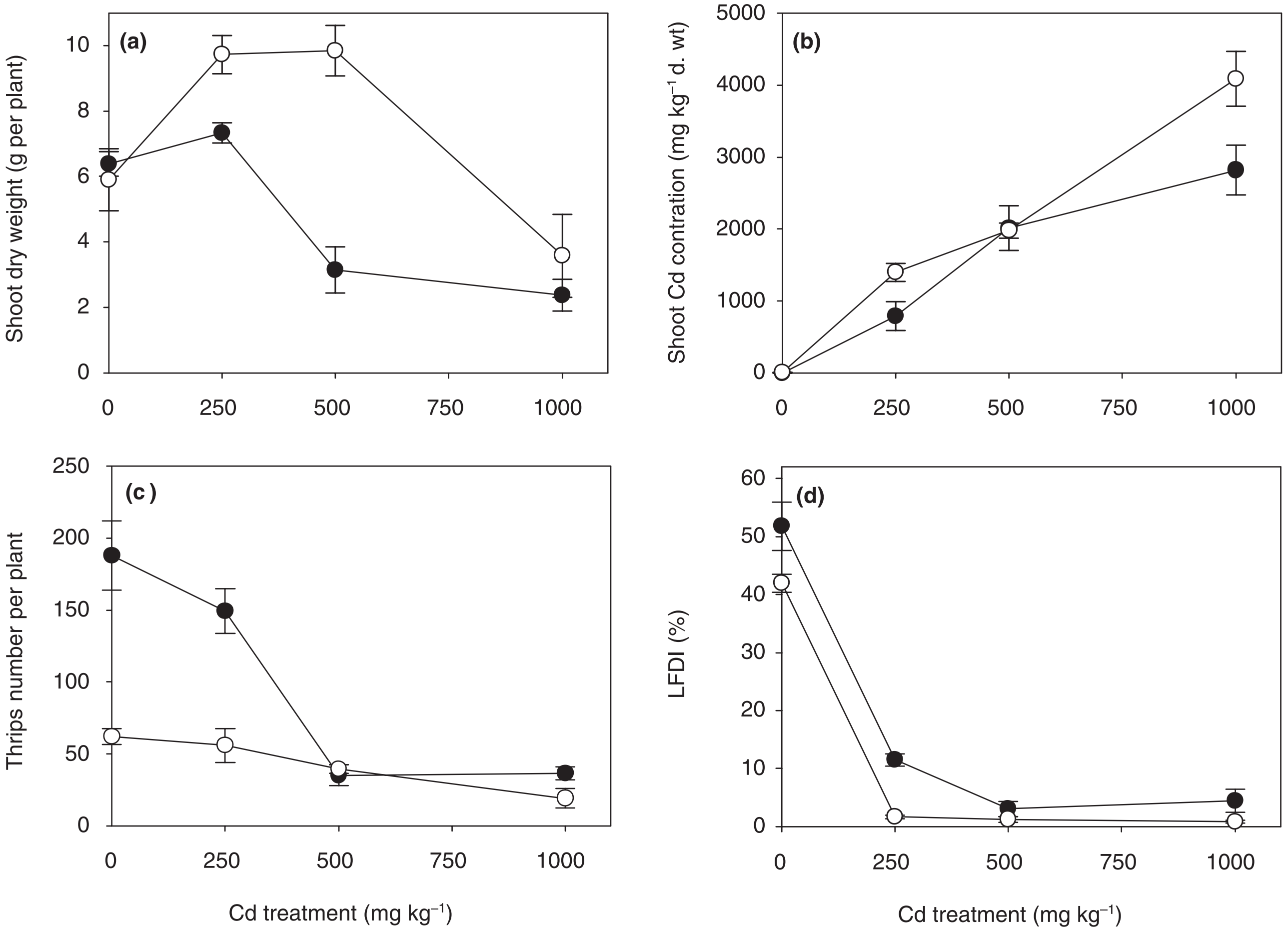

**Fig. 1** Effect of cadmium (Cd) treatments on shoot dry weight (a), shoot Cd concentration (b), number of thrips (*Frankliniella occidentalis*) per plant (c) and the leaf feeding damage index (LFDI) (d) of the Prayon (filled circles) and Ganges (open circles) ecotypes of *Thlaspi caerulescens* in experiment 1.

**Table 1** Ecotype means for thrips (*Frankliniella occidentalis*) number, the leaf feeding damage index (LFDI), shoot cadmium (Cd) and zinc (Zn) concentrations in *Thlaspi caerulescens* and significance of treatment effects in experiments 1 and 2

| | Experiment 1 | | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Thrips number per plant | LFDI (%) | Shoot Cd (mg kg$^{-1}$ d. wt) | Shoot Zn (mg kg$^{-1}$ d. wt) | Thrips number per plant | LFDI (%) | Shoot Cd (mg kg$^{-1}$ d. wt) | Shoot Zn (mg kg$^{-1}$ d. wt) |
| Ecotype | | | | | | | | |
| Prayon | 204 | 17.7 | 1407 | 2296 | 141 | 13.9 | 627 | 2614 |
| Ganges | 88 | 11.3 | 1869 | 1759 | 75 | 7.5 | 1418 | 2717 |
| ANOVA *F* prob. | | | | | | | | |
| Ecotype | *** | *** | * | ** | *** | *** | *** | NS |
| Cd | *** | *** | *** | *** | *** | *** | *** | NS |
| Ecotype × Cd | *** | NS | NS | *** | NS | NS | NS | ** |

ANOVA, analysis of variance; NS, not significant.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Cd kg$^{-1}$ treatments. Cd addition produced a greater reduction in the number of thrips in Prayon than in Ganges, resulting in significant ($P < 0.001$) ecotype–Cd interactions (Table 1). By contrast, Cd addition decreased the LFDI greatly in both ecotypes (Fig. 1d), and there were no significant interactions between ecotype and Cd (Table 1). The effect of Cd was most apparent from the first dose of Cd addition (250 mg Cd kg$^{-1}$), which was not phytotoxic to either ecotype.

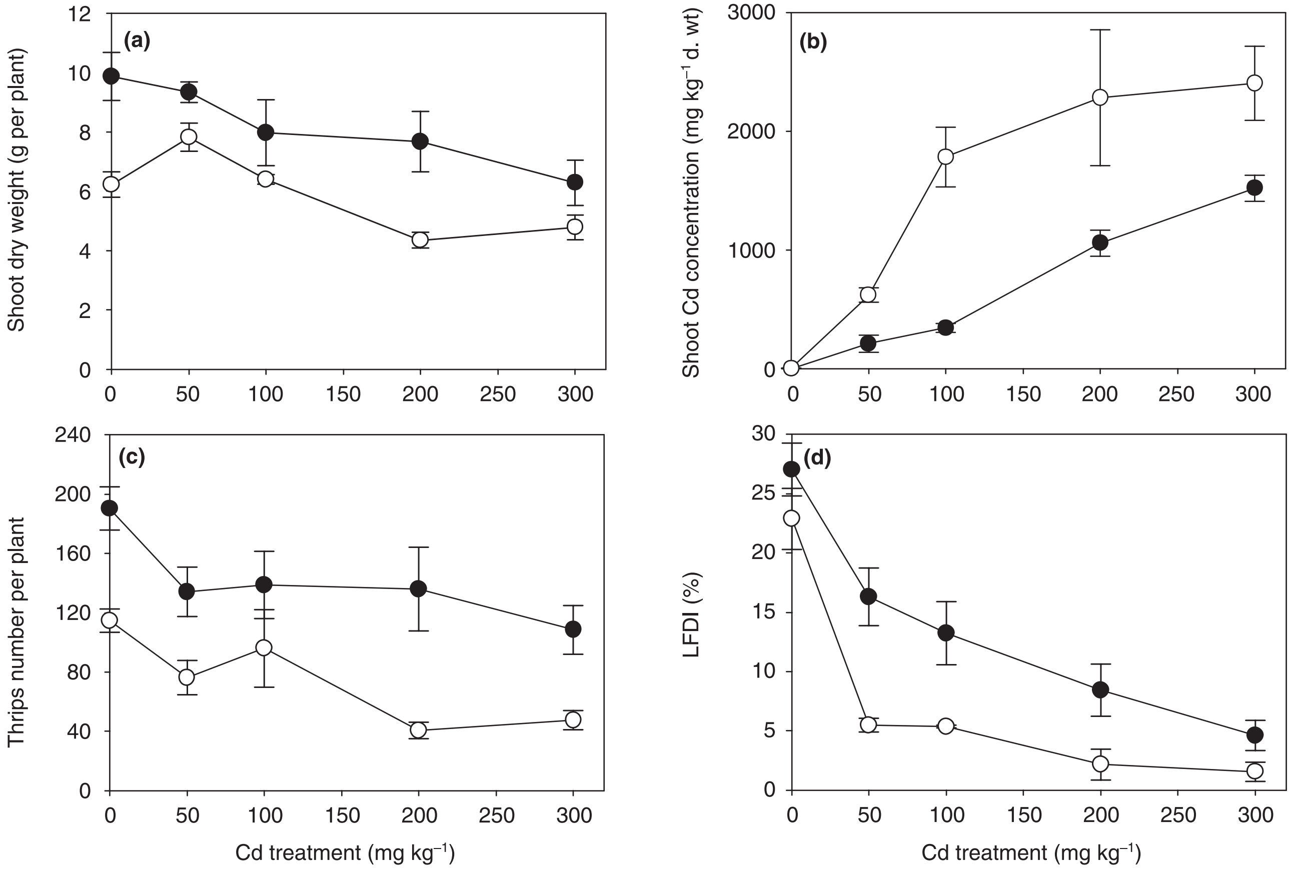

**Fig. 2** Effect of cadmium (Cd) treatments on shoot dry weight (a), shoot Cd concentration (b), number of thrips (*Frankliniella occidentalis*) per plant (c) and the leaf feeding damage index (LFDI) (d) of the Prayon (filled circles) and Ganges (open circles) ecotypes of *Thlaspi caerulescens* in experiment 2.

In experiment 2, the effects of Cd were investigated at a lower concentration range. Shoot biomass of Prayon was larger than that of Ganges (Fig. 2a). On average, shoot Cd concentration was more than 2-fold higher in Ganges than in Prayon (Fig. 2b; Table 1). The two ecotypes had similar mean concentrations of Zn in the shoots (Table 1). Similar to experiment 1, the number of thrips per plant and the LFDI were significantly ($P < 0.001$) higher in Prayon than in Ganges (Fig. 2c and d; Table 1). Cd additions decreased the number of thrips per plant and the LFDI significantly ($P < 0.001$) in both ecotypes, and there were no significant interactions between Cd treatment and ecotype.

Because the two ecotypes differed markedly in Cd accumulation, the ecotypic differences in colonization by thrips and feeding damage could be attributable to the difference in Cd accumulation. This was investigated by relating the number of thrips and the LFDI to the shoot Cd concentrations (Fig. 3). It is clear that Prayon tended to attract more thrips than Ganges in the low shoot Cd range (< 1000 mg kg$^{-1}$ d. wt). In both ecotypes, the number of thrips appeared to decrease once the shoot Cd concentration exceeded approximately 1000 mg kg$^{-1}$ d. wt (Fig. 3a and b). This pattern is more apparent in experiment 1 than in experiment 2, possibly because the former produced a wider range of shoot Cd concentrations than the latter. By contrast, the LFDI showed a log-linear relationship with shoot Cd concentration, i.e. a linear decrease in the LFDI with increasing $\log_{10}$(shoot Cd concentration) (Fig. 3c and d). A log-linear relationship is indicative of a potent effect of Cd in shoots at low concentrations, although caution is warranted here because of the gap in the range of shoot Cd concentrations between 10 and 100 mg Cd kg$^{-1}$ d. wt (Fig. 3c and d). The relationship between the LFDI and shoot Cd was similar for the two ecotypes in experiment 1. In experiment 2, the slope of the log-linear relationship was again similar for the two ecotypes, but the intercept was slightly larger for Prayon than for Ganges. The results suggest that Cd deterred thrips from feeding similarly in the two ecotypes, although Prayon was somewhat more susceptible to the feeding damage by thrips in experiment 2. According to the regression equations presented in Fig. 3(c and d), the concentrations of shoot Cd corresponding to a 50% reduction of the LFDI were 66 and 54 mg Cd kg$^{-1}$ d. wt for Prayon and Ganges, respectively, in experiment 1, and 148 and 74 mg Cd kg$^{-1}$ d. wt for Prayon and Ganges, respectively, in experiment 2.

Both ecotypes also accumulated large concentrations of Zn (1000–3500 mg kg$^{-1}$ d. wt), S (3500–13000 mg kg$^{-1}$ d. wt)

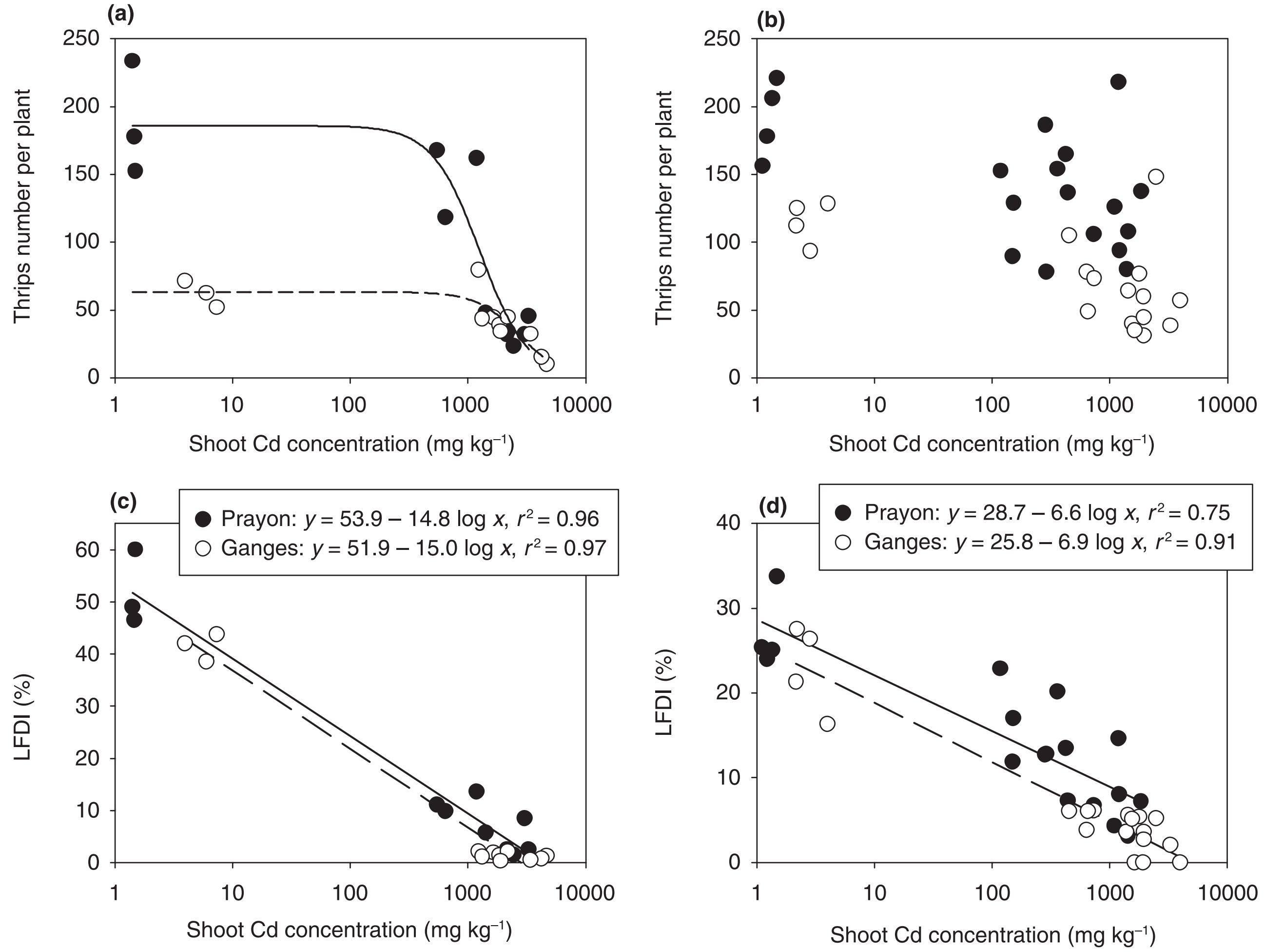

**Fig. 3** Relationships of shoot cadmium (Cd) concentration with thrips (*Frankliniella occidentalis*) number per plant in experiment 1 (a) and experiment 2 (b), and with the leaf feeding damage index (LFDI) in experiment 1 (c) and experiment 2 (d). Curves in (a) are the best fit of a logistic model. No fit was obtained in (b). Filled circles, Prayon; open circles, Ganges.

and P (1880–4600 mg kg$^{-1}$ d. wt) in the shoots. However, $\log_{10}$(shoot Zn concentration) correlated positively with the LFDI in experiment 1 ($r = 0.56$, $P < 0.01$), and in experiment 2 the correlation was not significant ($r = 0.05$, $P > 0.05$). There was also no significant correlation between $\log_{10}$(shoot S or P concentration) and the LFDI in either experiment (experiment 1: $r = -0.18$ and $-0.25$ for S and P, respectively, $P > 0.05$; experiment 2: $r = 0.04$ and $-0.10$ for S and P, respectively, $P > 0.05$).

## Experiments with $F_2$ and $F_3$ plants

In the experiment with the $F_2$ plants from the P × G cross (experiment 3), the concentrations of Cd and Zn in the shoots varied from 3 to 370 mg kg$^{-1}$ and from 201 to 6120 mg kg$^{-1}$, respectively. Colonization by thrips (0–41 per plant) and the LFDI (2–22%) were lower than in experiments 1 and 2. The LFDI was found to correlate negatively and significantly with either shoot Cd concentration ($r = -0.55$, $P < 0.01$) or $\log_{10}$(shoot Cd concentration) ($r = -0.47$, $P < 0.05$; Fig. 4a), but not significantly with either shoot Zn concentration ($r = -0.16$, $P > 0.05$) or $\log_{10}$(shoot Zn concentration) ($r = -0.16$, $P > 0.05$; Fig. 4b). Similarly, there was no significant correlation between the LFDI and shoot S or P concentration ($r = 0.30$ and 0.09 for S and P, respectively, $P > 0.05$).

In experiment 4, the concentrations of Cd and Zn in the shoots of the $F_3$ plants tested varied from 14 to 373 mg kg$^{-1}$ and from 921 to 6546 mg kg$^{-1}$, respectively, while the LFDI varied from 1 to 65%. The number of thrips was not counted in this experiment. Similar to experiment 3, there was a significant and negative correlation between the LFDI and either the shoot Cd concentration ($r = -0.42$, $P < 0.05$) or $\log_{10}$(shoot Cd concentration) ($r = -0.59$, $P < 0.01$; Fig. 5a). By contrast, there was no significant correlation between the LFDI and either the shoot Zn concentration ($r = -0.31$, $P > 0.05$) or $\log_{10}$(shoot Zn concentration) ($r = -0.35$, $P > 0.05$; Fig. 5b). The LFDI correlated with shoot S concentration significantly ($r = 0.55$, $P < 0.01$), but the correlation

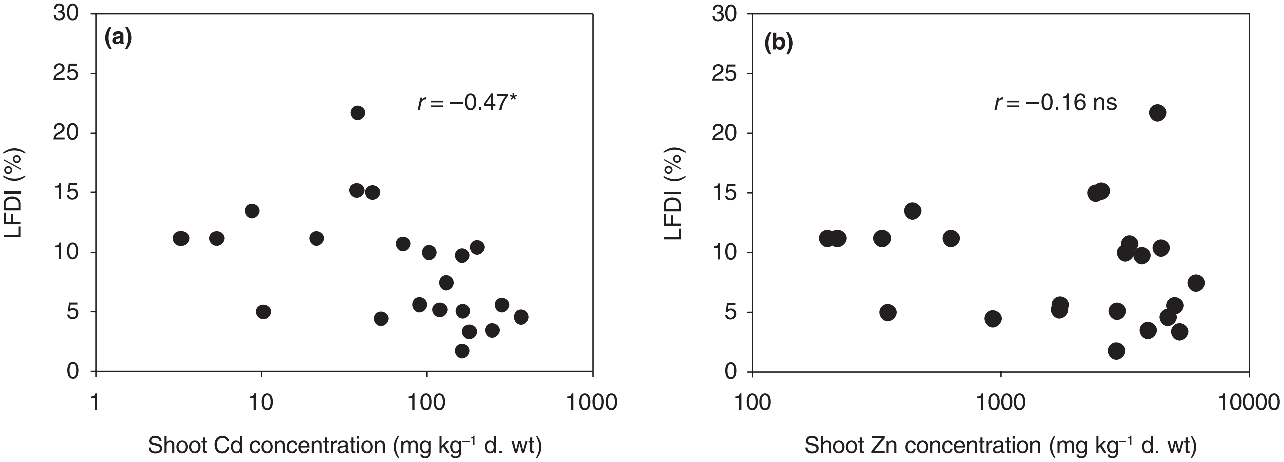

**Fig. 4** Relationships between the leaf feeding damage index (LFDI) and shoot cadmium (Cd) concentration (a) or shoot zinc (Zn) concentration (b) in the $F_2$ plants from a cross between the Ganges and Prayon ecotypes of *Thlaspi caerulescens* (experiment 3).

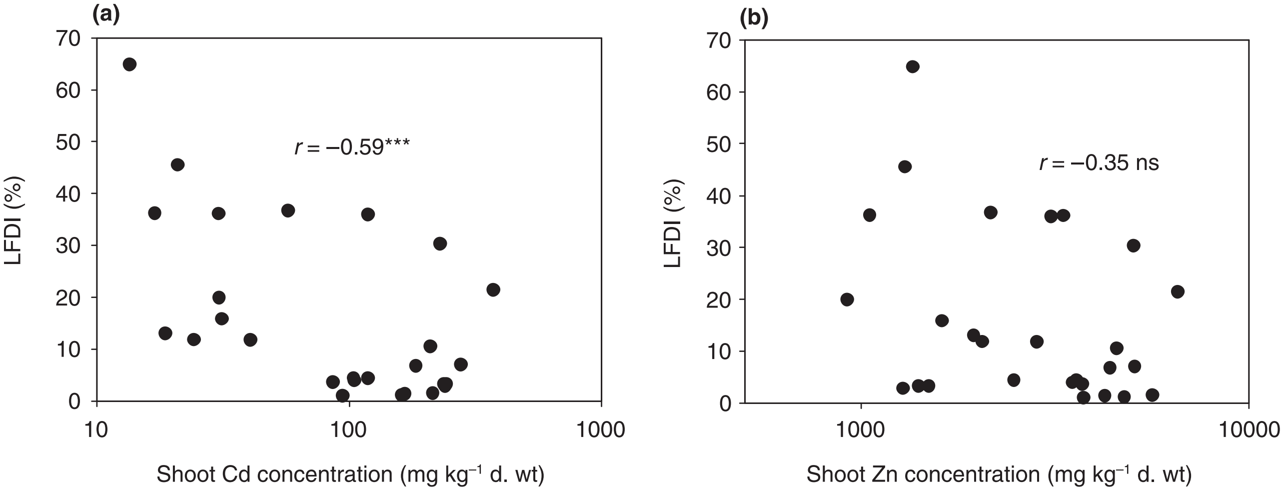

**Fig. 5** Relationships between the leaf feeding damage index (LFDI) and shoot cadmium (Cd) concentration (a) or shoot zinc (Zn) concentration (b) in the $F_3$ plants from crosses between the Ganges and Prayon ecotypes of *Thlaspi caerulescens* (experiment 4).

was positive (data not shown). There was no significant correlation between the LFDI and shoot P concentration ($r = 0.04$, $P > 0.05$).

## Discussion

Our results show that Cd hyperaccumulation in the shoots of *T. caerulescens* deterred thrips from damaging the leaves by feeding. This conclusion is based on three pieces of evidence. First, addition of Cd to the growth medium increased Cd accumulation by the plants, with a concurrent decrease in the LFDI (Figs 1 and 2). Secondly, the ecotypic difference in the LFDI appeared to be largely, if not entirely, accounted for by the difference in Cd accumulation ability (Fig. 3). Thirdly, there was a genetic correlation between Cd accumulation and the LFDI in the $F_2$ and $F_3$ populations from the crosses between the two contrasting ecotypes (Figs 4 and 5). The correlation was significant, although not particularly strong. By contrast, no significant negative correlations were observed between the LFDI and any other elements measured, including Zn, S and P.

The number of thrips per plant was also affected by Cd hyperaccumulation, but this was not as sensitive as the LFDI. Our experiments were carried out inside a heated glasshouse with a background population of thrips. Individual plant pots were not physically isolated from each other. Thus, thrips could choose on which plants to feed. The decreased thrips number per plant in some high-Cd treatments may result from (1) toxicity caused by feeding on high-Cd leaves, and/or (2) avoidance. We had no way of knowing which of the two, or both, was responsible. Feeding behaviour of thrips has been studied extensively. Before feeding, thrips do appear to examine the surface for appropriate chemical and physical cues and to test a small amount of sap (Kirk, 1997). Such behaviour may allow thrips to avoid feeding on high-Cd leaves, explaining the decreased LFDI. In *T. caerulescens* leaves, the majority (~70%) of Cd was found in the cell sap (Ma *et al.*, 2005). However, plant properties other than Cd concentration also appeared to play a significant role in attracting thrips. The Prayon ecotype was clearly more attractive to thrips than Ganges, as evidenced by the higher number of thrips per plant in the control treatments of experiments 1 and 2. The reasons for this difference are not known, but many factors could have influenced host selection by thrips, including leaf colour and shape, volatile compounds emitted or the thickness of leaf cuticle and waxes (Terry, 1997).

The interpolated leaf Cd concentration that was associated with a 50% reduction in the LFDI was in the range of 50–150 mg Cd $kg^{-1}$ d. wt. This range is comparable with the arbitrary value of 100 mg Cd $kg^{-1}$ d. wt commonly used to define Cd hyperaccumulation (Baker *et al.*, 2000). In comparison, populations of *T. caerulescens* growing on metalliferous soils in southern France (including the Ganges ecotype) generally contained several hundreds to 4000 mg Cd $kg^{-1}$ d. wt in shoots (Robinson *et al.*, 1998; Reeves *et al.*, 2001). Even the populations growing on nonmetalliferous soils in southern France were found to have elevated Cd concentrations in shoots (20–380 mg Cd $kg^{-1}$ d. wt) (Reeves *et al.*, 2001). Thus, it is probable that the extent of Cd accumulation in southern French populations, even on soils uncontaminated with Cd, could have played a protective role in nature against herbivores such as thrips.

In *T. caerulescens*, Zn accumulation to concentrations of 7430 and 14 000 mg $kg^{-1}$ d. wt was found to deter herbivory by caterpillars (*P. brassicae*), locusts (*S. gregaria*) and slugs (*D. caruanae*) (Pollard & Baker, 1997). In our study, experiments 1 and 2 were designed to test the effect of Cd, not Zn, and the shoot Zn concentrations were below 3500 mg $kg^{-1}$ d. wt. In the experiments with the $F_2$ and $F_3$ plants, variation in the LFDI was not significantly correlated with the variation in shoot Zn concentration (921–6546 mg $kg^{-1}$ d. wt). It may be that the concentrations of Zn reached in our experiments were not high enough to offer a protective role against thrips feeding damage. In their wild habitats, *T. caerulescens* populations from southern France contained 1000–53 000 mg Zn $kg^{-1}$ (mostly > 5000 mg Zn $kg^{-1}$ d. wt in the plant samples collected from metalliferous sites) (Reeves *et al.*, 2001). Whether Zn accumulation to the high concentrations observed in the field deters herbivory by thrips cannot be determined from the present study.

Plant secondary metabolites could be involved in deterrence of herbivory, a notable example being the S-containing compounds glucosinolates (Bennett & Wallsgrove, 1994; Noret *et al.*, 2005). Glucosinolates were not determined in our study; thus, their role in deterring thrips feeding could not be ascertained. Based on the analyses of Tolrà *et al.* (2001) and Noret *et al.* (2005) and the S concentrations measured in our experiments, glucosinolates could account for up to 50% of the total S in the leaves of *T. caerulescens*. In our study, there was no significant correlation (experiment 3) or a positive correlation (experiment 4) between the LFDI and shoot S concentration, indicating that a higher concentration of S was not associated with less feeding damage by thrips.

In conclusion, our study has provided evidence for a protective role of Cd hyperaccumulation in *T. caerulescens* against feeding damage by *F. occidentalis*, a generalist folivore. To our knowledge, this is the first report of a defensive role of Cd. If feeding damage by *F. occidentalis* (or similar folivores) is a selection pressure for plants growing in the natural habitat, Cd hyperaccumulation could offer an adaptive advantage. *F. occidentalis* is a cosmopolitan pest with a distribution worldwide, including southern France (Bournier, 1983), where the Ganges ecotype of *T. caerulescens* occurs. Although *F. occidentalis* may have originated from North America, another common thrips species, *Thrips tabaci*, probably originated in the Mediterranean (Mound, 1997). It would be interesting to examine whether Cd hyperaccumulation also protects *T. caerulescens* from feeding damage by other thrips species, such as *T. tabaci*.

The defence hypothesis may or may not be sufficient to explain the evolution of the large ecotypic difference in Cd hyperaccumulation observed amongst different populations. Our results do not rule out the alternative hypothesis, i.e. inadvertent uptake. Previous studies showed that Cd uptake by the low-Cd accumulating ecotype Prayon is possibly mediated by transporters of other metals, particularly Zn and Ca (Pence *et al.*, 2000; Lombi *et al.*, 2001; Zhao *et al.*, 2002), suggesting that Cd accumulation by the Prayon ecotype or similar populations is an inadvertent result of Zn or Ca uptake. This does not answer the question of why Zn hyperaccumulation occurs in the first place, which could still be explained by the plant defence hypothesis (Pollard & Baker, 1997; Jhee *et al.*, 1999). In the high-Cd accumulating ecotype Ganges, there is evidence for a high-affinity Cd uptake system differing from that for the uptake of Zn or Ca (Lombi *et al.*, 2001; Zhao *et al.*, 2002). However, this high-affinity system may also be an inadvertent result of the selection for the acquisition of other essential nutrients, such as Fe (Lombi *et al.*, 2002; Roosens *et al.*, 2003). If the inadvertent uptake hypothesis is indeed the evolutionary reason for Cd hyperaccumulation, one of the consequences may be enhanced defence against herbivory, as observed in the present study. This benefit could conceivably reinforce the selection for Cd hyperaccumulation.

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