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Does the Response of Insect Herbivores to Cadmium Depend on Their Feeding Strategy?

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4 1 DOES THE RESPONSE OF INSECT HERBIVORES TO CADMIUM DEPEND ON THEIR
5 2 FEEDING STRATEGY?
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16 11
17 12 **Abstract** – Phytoremediation has been proposed for the elimination of toxic metals in soil, yet little attention was
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19 13 given to the performance of insects that feed on contaminant-tolerant plants. We tested the performance of two
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21 14 herbivores with different feeding behaviors, the cabbage looper, *Trichoplusia ni*, and the green peach aphid, *Myzus*
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23 15 *persicae*, when reared on cadmium-tolerant *Brassica juncea* plants that contained different concentrations of
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25 16 cadmium. We also tested the performance of the aphid parasitoid *Aphidius colmani* developing in aphids reared on
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27 17 plants with different levels of cadmium. The hypothesis tested was the chewing insect would be more negatively
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29 18 affected than the sucking insect due to the localization of cadmium within the host plant, and that the aphid
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31 19 parasitoid would not be affected. We also compared the performance of *T. ni* on artificial diet with different levels of
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33 20 cadmium. Neither the phloem-feeding aphid nor its parasitoid was affected by cadmium in the host plant. In contrast
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35 21 the effects of cadmium on the foliage-feeding cabbage looper varied, with negative effects observed in experiments
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37 22 using artificial diet but not with natural host plants. The data obtained, together with the information available in the
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39 23 literature, would support the idea that the effects of toxic metals present in a host plant will be influenced by the
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41 24 herbivore's feeding strategy. However, a wide range of chewing and sucking species need to be tested to confirm
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43 25 this hypothesis.
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48 27 **Key words**– Cadmium, phytoremediation, feeding strategy, oviposition, *Myzus persicae*, *Trichoplusia ni*, *Aphidius*
49 28 *colmani*, *Brassica juncea*
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4 33 INTRODUCTION
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6 34
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8 35 High concentrations of metals in soils, originating from either natural or anthropogenic sources, may cause serious
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10 36 ecological problems due to their adverse effects on living organisms. Most current methods used for remediation of
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12 37 metal-contaminated soils are expensive and laborious, so there is an increasing interest in finding cheaper and more
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14 38 efficient means of improving soil quality. Phytoremediation, the use of plants to remove or chelate metals into less
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16 39 toxic forms, is a promising and cost effective method (Chaney et al., 1997; Cunningham et al., 1997).
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18 40
19 41 Phytoremediating plants often have high metal-tolerance (e.g. *Thlaspi caerulescens*; *Brassica juncea*), and
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21 42 are unaffected by the high concentrations of various contaminants that accumulate in their roots and shoots (Kumar
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23 43 et al., 1995; Jahangir et al., 2008). However, when these metals are incorporated into plant tissues they may be
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25 44 biotransferred up the food chain, with a potential of impacting species at higher trophic levels (Hanson et al., 2003;
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27 45 Boyd, 2004) and affecting tritrophic interactions (Butler and Trumble, 2008). While a few studies reported a positive
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29 46 effect of metals such as copper (Filser et al., 2000) or iron (Bahadorani and Hilliker, 2009) on the behavior of insect
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31 47 herbivores (feeding, taxis, and oviposition), generally, if an effect is discernable it is negative (Butler and Trumble,
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33 48 2008; Mogren and Trumble, 2010 and references therein).
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36 50 Effects observed will not only depend on both the type of metal and the concentrations found in different
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38 51 parts of the plant but also on the insect species that is exposed. For example, herbivores with chewing mouthparts
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40 52 that feed on leaves may be exposed to different levels of the metal than those with sucking mouthparts feeding in the
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42 53 vascular system. Furthermore, within a given species of herbivore it is important to consider how an individual
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44 54 could be exposed during different stages of the life cycle. For example, if a female can distinguish between plants
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46 55 with high and low concentrations of metals and avoid ovipositing on those with high levels, this would significantly
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48 56 reduce the probability of her offspring being exposed. Similarly, if during feeding larvae are able to detect high
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50 57 concentrations of metals, they could exhibit avoidance behavior and feed at more palatable sites.
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52 58
53 59 Cadmium (Cd) metal is extremely toxic to both plants and animals, including humans (Nordberg, 2009).
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55 60 The Brassicaceae family contains the most Cd-tolerant plant species (Prasad, 1995), and *B. juncea* (Indian mustard),
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57 61 has considerable potential for phytoremediation in soils with high concentrations of Cd (Kumar et al., 1995; Cui and
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59 62 Chen, 2011). We used this host plant to examine the impact of Cd on two herbivorous pests with different feeding
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61 63 strategies, testing the hypothesis that the foliage-feeding cabbage looper, *Trichoplusia ni*, would be affected
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4 61 significantly more than phloem-feeding green peach aphid, *Myzus persicae*, as the majority of Cd accumulates in
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6 62 leaf trichomes (Salt et al., 1995). We also carried out experiments to determine if females of either species could
7
8 63 differentiate between control and Cd-containing plants as a site for oviposition. Furthermore, we compared the
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10 64 success of a parasitoid, *Aphidius colmani*, when provided *M. persicae* that were feeding on Cd-treated and control
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12 65 plants.
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14 66 15 16 67 METHODS AND MATERIALS 17

18 68 *Plants*

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20 69 Seeds of *Brassica oleracea* L. (var. Capitata ‘Early Golden Acre’) and *B. juncea* (L.) Czern. (cv. AC Vulcan) were
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22 70 obtained from Agriculture and Agri-Food Canada in London ON and Saskatoon SK, respectively. Seeds were
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24 71 placed on top of wet filter paper in Petri plates, and germinated in the dark at room temperature. Once the radicles
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26 72 were > 8 mm long, seedlings were transferred into individual pots filled with moistened, autoclaved Promix. All
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28 73 plants were given distilled water daily, fertilized (20-20-20 all purpose fertilizer) once a week and kept at 60 % RH,
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30 74 with a 16:8 light:dark cycle and at 25:20 °C (day:night). Approximately 40 seeds were sown each week to provide a
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32 75 continuous supply of plant material. *Brassica oleracea* were used solely for insect rearing while *B. juncea* received
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34 76 one of three experimental treatments 3-4 weeks after sowing: one third received no Cd and served as control plants
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36 77 (C; control), while the remaining plants were watered once with CdCl₂ solutions to give 6 (LC; low cadmium) or 30
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38 78 (HC; high cadmium) mg Cd/kg of soil, which resulted in Cd concentrations that are 0.5 and 2.4 times the limit for
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40 79 agricultural soils, respectively (CCME, 2007). Furthermore, preliminary experiments found these soil concentrations
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42 80 resulted in 3 and 10 mg Cd/kg of leaf tissue, which caused sublethal effects in other herbivores (Quimby et al.,
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44 81 1979). Inflorescences were regularly removed, to ensure continued leaf production.
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47 48 49 83 *Aphids and aphid parasitoids*

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51 84 Ten green peach aphid adults from a laboratory colony were placed on five *B. juncea* plants from each of the three
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53 85 plant treatments (see above) and allowed to larviposit for 24 h. At this time the adults and additional neonates were
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55 86 removed to leave a constant density of 10 neonate nymphs/plant. The survival of the nymphs and the time to reach
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57 87 maturity (the production of nymphs) were determined. In addition, the proportion of individual nymphs that became
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59 88 alate adults was recorded.
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89 Five plants from each of the treatment groups were infested with a mixture of adult and nymph green peach
90 aphids and placed in individual cages. Three mated 1-day-old *Aphidius colemani* females were introduced and
91 allowed to parasitize aphids for 24 h. The parasitoids were removed and the plants examined daily for mummified
92 aphids. These were removed, and placed in individual gelatine capsules until adults emerged. The duration of larval
93 and pupal development, as well as the proportion of mummies that gave rise to adult parasitoids, were calculated.

94
95 ***Cabbage loopers***

96 *Insect colony* – Eggs were obtained from Insect Production Services, Natural Resources Canada (NRCAN). Upon
97 hatching, cabbage looper larvae were reared individually on pinto bean diet (modified after Shorey and Hale, 1965)
98 in 29.6 mL cups and the adults obtained were used to establish a colony. Adults were kept in 3.7 L glass jars, the
99 openings of which were covered with cheese cloth to provide a substrate for egg laying. Sugar solution (8%) was
100 provided for feeding. Eggs were surface-sterilized (10 min in 37% formaldehyde, followed by 10 min rinse with
101 water), and newly hatched larvae were reared on either artificial diet (approx. 30 mL Solo cups) or on fresh *B.*
102 *oleracea* leaves (in Petri plates). These individuals were subsequently transferred to modified pinto bean artificial
103 diet or plants containing Cd. For all treatments, pupae were surface-sterilized in the same manner as the eggs, sexed
104 and weighed. Developmental times and mortality for each stage were recorded.

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106 *Oviposition* – Fifteen newly emerged females were placed individually in cages with 2 males and once oviposition
107 was observed, females were transferred to mesh tents containing 8% sugar solution and three *B. juncea* plants (one
108 each of C, LC and HC) for 48 h. The plants were changed after 24 h allowing for determination of the total number
109 of eggs laid on treated and untreated plants during the two scotophases.

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111 *“No choice” feeding trails* – Twenty 3rd instar larvae were removed from their host plant (*B. oleracea*), starved for
112 24 h before the assay (to increase hunger levels), and placed in individual Petri dishes with either a control or Cd-
113 containing leaf disk of known area (measured using Image J software after pictures were taken). After 4 h, pictures
114 were taken, and the leaf surface area remaining was measured (Image J software) so that the area consumed and
115 number of feeding sites could be calculated.

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4 117 *Performance on artificial diet and B. juncea* – Newly hatched neonates from the colony were allowed to feed on
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6 118 either artificial diet or fresh *B. oleracea* leaves for 24 h before being assigned to different experimental treatments.
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8 119 There were six artificial diet treatments (0, 1, 2, 5, 10 and 20 mg Cd/kg diet) with four replicates (25 larvae each).
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10 120 There were three treatments for larvae reared on *B. juncea* host plants: C, LC, and HC soil treatments (25 larvae per
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12 121 treatment, repeated 5 times). Plants were replaced as they were defoliated. In both artificial diet and whole plant
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14 122 experiments, pupal mass, developmental times for both larvae and pupae were determined, and mortality was
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16 123 recorded daily.
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20 125 ***Cadmium content***
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22 126 The concentrations of Cd in mature, control and Cd-treated *B. juncea* leaves, 5 larvae from the rearing colony and 5
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24 127 larvae from each of control and Cd-treated *B. juncea*, as well as control and Cd-treated artificial diet were measured
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26 128 (n=3 for each treatment), using a modified United States Environmental Protection Agency (US EPA) test method
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28 129 SW-846 (US EPA, 2005). Oven-dried leaves and larvae were chopped into fine (1-2 mm) pieces using a razor blade
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30 130 while the dried diet was ground using a mortar and pestle. The three 0.1 g subsamples from each treatment were
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32 131 placed in individual 15 mL test tubes, 1 mL pure nitric acid (OmniTrace®, EM Science, USA) was added to each
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34 132 one, and tubes were covered with a glass marble and left overnight at room temperature to begin digestion of
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36 133 organic matter. The following day, the samples were heated to 100°C on a hot plate until the vapors became
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38 134 transparent, then cooled to room temperature and filtered (VWR, qualitative grade 413) into 50 mL sterile centrifuge
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40 135 tubes. Reverse osmosis water was used to rinse the test tubes and bring the volume to 25 mL. The samples were
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42 136 then analyzed for Cd content by inductivity-coupled plasma atomic emission spectrometry (ICP-AES). A certified
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44 137 standard reference material (SRM) from the National Institute of Standards and Technology (NIST 1573a, tomato
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46 138 leaves) and reagent blanks were used to assess accuracy of the sample digestion procedure for leaf and pinto bean
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48 139 diet. Digested SRM contained 92±3% of the certified amount of Cd and no Cd was detected in the reagent blanks.
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50 140 We did not assess the accuracy of the digestion procedure using an animal SRM for caterpillars, so it is possible that
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52 141 the values for Cd concentrations in looper larvae are underestimates. We did not measure Cd in aphids or parasitoids
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54 142 as this would have required > 400 individuals per experimental replicate in each treatment to obtain Cd
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56 143 concentrations above the detection limit of the ICP-AES (0.002 µg Cd/ml sample).
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4 145 **Statistical analysis**

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6 146 Data were analysed using SPSS® statistical software (Version 20.0) by *one-way ANOVA* followed by a *Tukey's post*
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8 147 *hoc test*, and in cases where data were non-parametric, GraphPad Prism (Version 4.0) was used to perform a
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10 148 *Kruskal-Wallis test* followed by *Dunn's multiple comparison*. Due to some differences between sexes in the
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12 149 developmental times of *T. ni* under the same concentrations of Cd, data for males and females were analysed
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14 150 separately. Oviposition data were arcsine transformed to ensure normality and equality of variance.
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20 153 RESULTS

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24 155 *Cd concentration* Leaves from control *B. juncea* had 0.6 ± 0.1 mg Cd/kg dry weight, while those from the LC and HL
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26 156 treatments contained 3.2 ± 0.6 mg/kg, and 8.0 ± 2.6 mg/kg dry weight, respectively. Larvae from the rearing colony
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28 157 had a dry weight Cd concentration of 0.4 ± 0.2 mg/kg, while those from the C, LC and HC plants had 1.3 ± 0.4 ,
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30 158 5.5 ± 1.0 , and 13.6 ± 0.6 mg/kg, respectively.
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34 160 *Aphids and parasitoids* The number of aphids reaching maturation ($H = 0.046$, $df = 2$, $P = 0.98$) and the age to the
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36 161 onset of reproduction ($H = 0.298$, $df = 2$, $P = 0.86$), as well number of nymphs per reproductive female ($H = 0.676$,
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38 162 $df = 2$, $P = 0.71$), did not differ among the three Cd treatments (Table 1). Furthermore, none of the individuals
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40 163 reared on either control or treated plants were alates. There were no significant differences in the (i) number of
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42 164 mummies produced ($H = 0.246$, $df = 2$, $P = 0.88$), (ii) times from parasitisation to mummification ($H = 0.100$, $df =$
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44 165 2 , $P = 0.95$) or from mummification to the emergence of adult parasitoids ($H = 1.999$, $df = 2$, $P = 0.37$), or (iii)
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46 166 proportion of mummies giving rise to adult parasitoids ($H = 1.220$, $df = 2$, $P = 0.54$) among the three Cd treatments
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48 167 (Table 1).
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52 169 *Cabbage loopers* The total number of eggs laid by mated *T. ni* females on control and Cd-containing plants did not
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54 170 differ ($F_{(2, 36)} = 2.63$, $P = 0.63$). Furthermore, there were no differences in either the areas of the C, LC, and HC *B.*
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56 171 *juncea* leaves consumed ($F_{(2, 87)} = 0.377$, $P = 0.69$) or the number of feeding sites ($F_{(2, 87)} = 0.092$, $P = 0.91$) by *T.*
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58 172 *ni* larvae in the no choice feeding trials.
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4 173 While the duration of larval development for both sexes was significantly shorter on HC plants than on
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6 174 controls (males: $F_{(2,168)} = 7.77$, $p < 0.01$; females: $F_{(2,163)} = 14.67$, $P < 0.001$; Fig. 1a, b), pupal development time
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8 175 did not differ among treatments for either males ($H = 2.301$, $df = 2$, $P = 0.32$ Fig. 1a) or females ($H = 5.801$, $df = 2$,
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10 176 $P = 0.06$; Fig. 1b). Male pupal mass did not differ among treatments ($H = 0.272$, $df = 2$, $P = 0.99$; Fig. 1a), while
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12 177 female pupae obtained from LC plants had approx. 10% higher mass compared to those from C or HC plants ($H =$
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14 178 7.130 , $df = 2$, $P < 0.05$; Fig. 1b). The proportion of larvae pupating ($F_{(2,12)} = 0.405$, $P = 0.68$) and pupae giving rise
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16 179 to adults ($F_{(2,12)} = 0.934$, $P = 0.42$) did not differ among treatments and controls (Fig. 2a).

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18 180 The developmental times of larvae ($H = 137.6$, $df = 5$, $P < 0.0001$ for males, and $H = 95.66$, $df = 5$, $P <$
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20 181 0.0001 for females) and pupae ($H = 38.00$, $df = 5$, $P < 0.0001$ for males, and $H = 43.28$, $df = 5$, $P < 0.0001$ for
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22 182 females) of both sexes were shortest on 1 mg Cd/kg diet, followed by the control, and longest on 2 and 20 mg Cd/
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24 183 kg diet (Fig. 3a, b). Both males ($H = 45.03$, $df = 5$, $P < 0.0001$) and females ($H = 32.40$, $df = 5$, $P < 0.0001$) reared
25
26 184 on 2 and 20 mg Cd/ kg diet had approx. 15% lower pupal masses, compared with individuals reared at all other
27
28 185 concentrations (Fig. 3a, b). While > 85% of larvae reared on control diet pupated, < 35% of those reared on 2 and 20
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30 186 mg Cd/ kg diet did ($H = 19.58$, $df = 5$, $P < 0.01$; Fig 2b). The proportion of pupae that produced adults did not differ
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32 187 among treatments, except for those from the 2 mg Cd/kg diet with about 50% ($H = 15.31$, $df = 5$, $P < 0.01$; Fig. 2b).

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DISCUSSION

Cadmium is primarily found in the epidermis of leaf tissue, with very little or none in phloem (Masjasz-Przybylowicz and Przybylowicz, 2011), thus a phloem-feeder, such as *M. persicae*, would ingest little Cd. Furthermore, aphids can excrete excess amounts of some metals (e.g. copper) in honeydew (Crawford et al., 1995), while others may accumulate in certain body regions and associate with specific organs of sap-feeding insects (Merrington et al., 2001; Migula et al., 2007). Even if present, these metals do not necessarily have adverse effects on life history traits; Crawford et al. (1995) found no negative effects of Cd on *Aphis fabae*, similar to the results of our study with *M. persicae*. Furthermore, we observed no alates (a winged form occurring when host plant quality is low) when the aphids were fed on Cd-containing plants. However, deviations from bilateral symmetry (Gorur, 2006), reduced reproductive potential and increased mortality (Gorur, 2007) were observed following accumulation of lead and copper in *Brevicoryne brassicae*. Thus, the impact on life history traits can vary depending on the metal

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201 and the species of sucking insect tested. If plants with high metal concentration are stressed, there might be
202 beneficial effects for the metal-tolerant herbivore, as their performance may be enhanced when feeding on these
203 stressed hosts (Koricheva et al., 1998). While some metals may be transferred up the food-chain (Vickerman and
204 Trumble, 2003), there is no evidence that this is the case for Cd (Zhang et al., 2009; Zhuang et al., 2009), which
205 would explain the absence of differences in the performance of *A. colemani*. However, negative impacts due to
206 synergistic effects of Cd with other compounds cannot be disregarded, as shown for Cd in combination with the
207 insecticide imidacloprid on *Aphidius ervi* parasitoid (Kramarz and Stark, 2003).

208 The choice of oviposition sites by a female determines the fitness of her offspring, so females might avoid
209 substrates that could prove toxic for their progeny (Mogren and Trumble, 2010). The few studies examining whether
210 females can detect metals in oviposition sites have given inconsistent results. *Drosophila melanogaster* females can
211 detect metals, and oviposit on substrates with concentrations that will enhance their offsprings' fitness (Bahadorani
212 and Hilliker, 2009), while females of the cabbage white butterfly, *Pieris rapae*, avoid ovipositing on plants with
213 high concentrations of selenium (Freeman et al., 2006). In contrast, females of humpbacked fly, *Megaselia scalaris*
214 (Trumble and Jensen, 2004), and the beet armyworm, *Spodoptera exigua* (Vickerman et al., 2002), did not avoid
215 ovipositing on artificial diet or plants with toxic amounts of chromium (VI) or selenium, respectively, similar to *T.*
216 *ni* females in this study. The diversity of responses observed in the different studies could be due to significant
217 interspecific differences in the presence of appropriate sensillae to detect different metals or possibly, in the cases
218 where no responses were observed, the concentrations present in the substrate were below detectable limits for the
219 species tested.

220 *Trichoplusia ni* larvae developed at a similar rate, and had similar performance on Cd-containing versus
221 control plants, indicating that they either do not have sensillae that detect this metal, or that the concentrations
222 present in the contaminated plants were not sufficiently high to modify their feeding behavior. Furthermore, there
223 was no evidence of post-ingestion associative learning (Hanson et al., 2003; Behmer et al., 2005), often observed in
224 feeding trial experiments where the consumption of metal-containing foliage has a negative effect on the growth and
225 development of the herbivore. The only significant difference observed was a 1-2 day shorter larval developmental
226 time for both sexes in the high Cd treatment, without a subsequent reduction in either pupal mass or adult
227 emergence. In fact female pupae were bigger on LC plants. These changes may be more accentuated under
228 fluctuating temperature conditions in the field and could actually benefit the herbivore, as a shorter larval period

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4 229 could reduce the exposure time to natural enemies attacking caterpillars (Dmitriew and Rowe, 2005; Muller and
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6 230 Arand, 2007), while larger pupal mass may result in higher fecundity (Gilbert, 1984; Honek et al., 2008). The better
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8 231 performance on low Cd plants is consistent with hormesis, a widely occurring toxicological phenomenon where low
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10 232 doses of a known toxin may actually be favourable (Calabrese and Blain, 2005), demonstrated in *Phormia regina*
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12 233 exposed to Cd (Nascarella et al., 2003). Furthermore, Cd can replace calcium in protein kinase C (Kiss and
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14 234 Osipenko, 1994), but can also be retained by cell lysosomes (Johnson and Foulkers, 1980; Lauverjat et al., 1989),
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16 235 which could explain no negative effect at low doses of Cd.

18 236 When *T. ni* larvae were reared on artificial diet the most detrimental effects were observed in the 2 and 20
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20 237 mg/kg treatments where not only was mortality high, but larval and pupal development times were longer.
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22 238 Therefore, it is possible that surviving individuals in these two treatments could have a greater probability of being
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24 239 attacked by natural enemies during larval development (but not during the pupal stage for while statistical
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26 240 differences were detected they were small and probably of little biological importance), and lower reproductive
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28 241 success as adults. These findings are consistent with other studies showing growth and development were negatively
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30 242 affected by Cd exposure in *Lymantria dispar* (Gintenveiter et al., 1993; Ilijin et al., 2010; Mircic et al., 2010), and
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32 243 by sewage sludge in *T. ni* (Larsen et al., 1994). Possible causes of insects' lower performance are reduced efficacy in
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34 244 obtaining nutrients, as the digestibility of plant material is reduced by the presence of Cd (Van Ooik et al., 2007),
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36 245 and disruption of metabolic processes (Janssens et al., 2009), as Cd affects protocerebral neurosecretory neurones
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38 246 (Ilijin et al., 2010) that affect growth and development. Additionally, metals can act as immunotoxins, affecting
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40 247 haemocyte morphology, which under natural conditions could result in increased probability of infections and attack
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42 248 by parasitoids (Borowska and Pyza, 2011), although this is not always the case (Popham et al., 2005).

44 249 We currently have no explanation for the absence of a normal dose-dependent response, with increasingly
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46 250 adverse effects with increasing Cd concentrations in the diet. However, we do believe the pattern is real as the same
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48 251 results were obtained in each of the four replicates. It should also be noted that in a study looking at Cd tolerance in
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50 252 two species of *Brassica*, using a hydroponic system, the observed reduction in root and stem dry mass was greater at
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52 253 low than medium doses of cadmium, when compared with controls (Gadapati and Macfie, 2006). Clearly, additional
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54 254 research is necessary to clarify this situation. However, the somewhat different findings obtained from the two sets
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56 255 of experiments (whole plants and artificial diet) suggest that caution should be taken when extrapolating the data
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58 256 from diets treated with metals to whole plants. Interestingly, the concentration of Cd in the 2 mg/kg diet was lower

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257 than the levels of Cd found in LC and HC plants, where possible hormesis was observed. It is also possible that the
258 presence of secondary plant compounds, such as isothiocyanates, in whole plants affect the uptake of Cd by
259 herbivores, a possibility that merits further investigation.

260 In conclusion, adult *T. ni* females did not discriminate between control and Cd-treated plants when
261 selecting host plants suggesting that they are unable to detect Cd, or that the concentrations of Cd in the plants used
262 in these experiments were below their detection limit. The phloem-feeding aphid and its parasitoid were unaffected
263 by plants that contained Cd while foliage-feeding loopers were affected but not always in a dose-dependent manner,
264 and the effects observed varied depending on whether the Cd was in an artificial diet or a treated host plant. In some
265 cases exposure to Cd could actually favor the herbivores, or be detrimental. The results obtained support our
266 hypothesis that the mode of feeding could influence the impact of Cd on herbivores. However, a broader range of
267 chewing and sucking species need to be tested to ensure difference are not just the result of interspecific tolerance to
268 Cd. From an applied perspective, our results suggest that the use of tolerant host plants for phytoremediation of
269 metals in the soil may be a source of pests, especially phloem-feeders, but could contribute to reduction in
270 populations of foliage-feeding insects in very heavily polluted areas. There is, however, no evidence to suggest that
271 the crops used for phytoremediation would be greater sources of infestation than other agricultural fields of crucifers
272 in the area.

273
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4 456 **Table 1** MEAN (\pm SE) PERCENT OF REPRODUCING FEMALES, AGE TO REPRODUCTION, AND
5 457 NUMBER OF NYMPHS PER REPRODUCTIVE FEMALE OF *Myzus persicae* AS WELL AS MEAN (\pm SE)
6 458 NUMBER OF MUMMIES, PERCENT MUMMIES EMERGING, TIME TO MUMMIFICATION, AND TIME TO
7 459 ADULT OF *Aphidius colemani*
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10 460 Note: Means followed by the same letter within each column are not significantly different based on Kruskal- Wallis
11 461 analysis ($\alpha = 0.05$).
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19 465 **Figure 1** Mean (\pm SE) pupal mass, larval, and development of *Trichoplusia ni* males (A) and females (B) after
20 466 feeding on Cd-containing *B. juncea* plants. C= control, LC = low Cd, HC = high Cd. Bars with the same letter are
21 467 not significantly different based on Tukey's post hoc test ($p < 0.05$) and Dunn's multiple comparison test ($p < 0.05$).
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23 468 **Figure 2** Mean (\pm SE) proportion of *T. ni* individuals reaching pupal (p) and adult (a) stages after feeding on Cd-
24 469 containing *B. juncea* plants (A) and Cd-containing artificial diet (B). C= control, LC = low Cd, HC = high Cd. Bars
25 470 with the same letter are not significantly different based on Dunn's multiple comparison test ($p < 0.05$).
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28 471 **Figure 3** Mean (\pm SE) pupal mass, larval, and pupal development of *Trichoplusia ni* males (A) and females (B) after
29 472 feeding on Cd-containing artificial diet. Cadmium concentrations are in mg/kg diet. Bars with the same letter are not
30 473 significantly different based on Tukey's post hoc test ($p < 0.05$) and Dunn's multiple comparison test ($p < 0.05$).
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Figure 1
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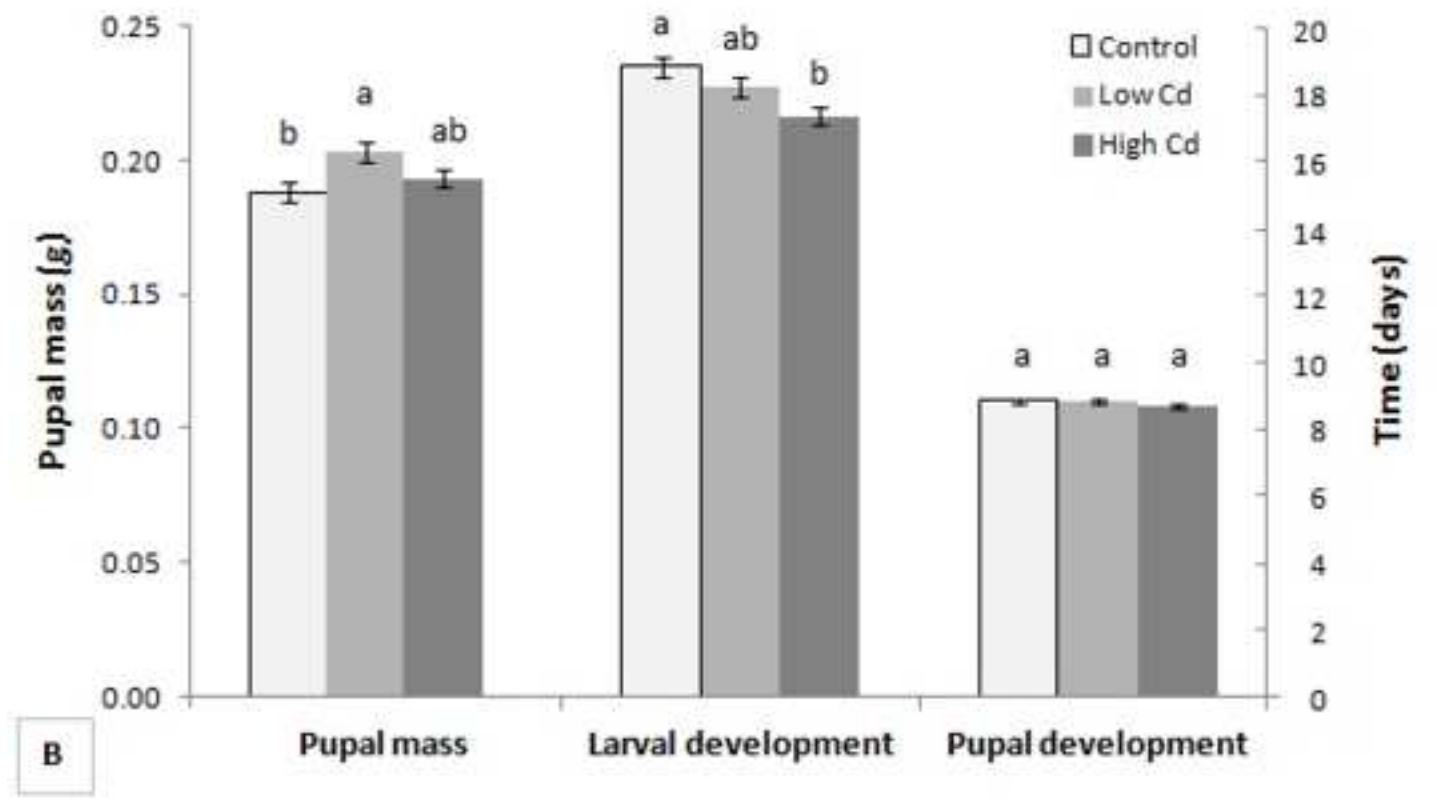
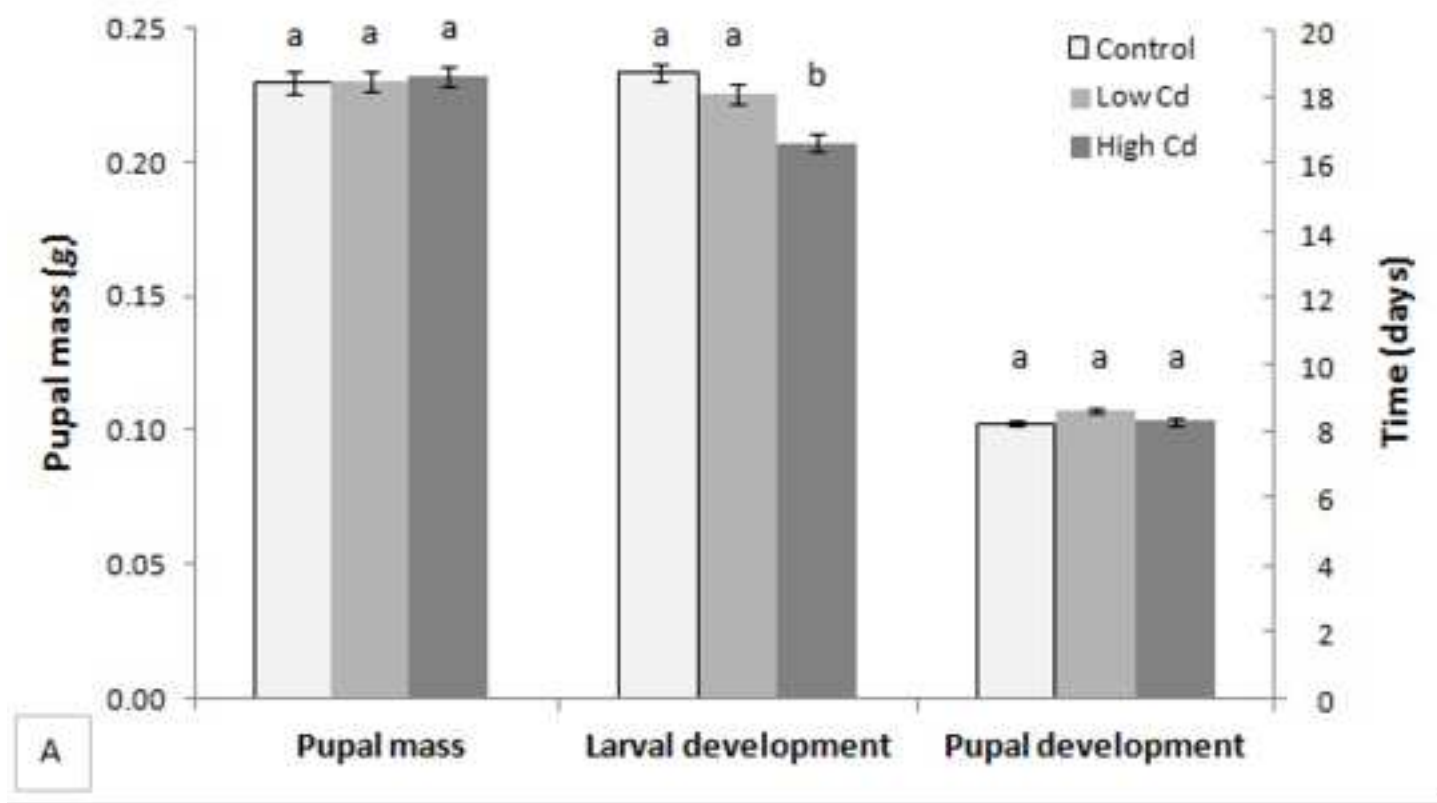


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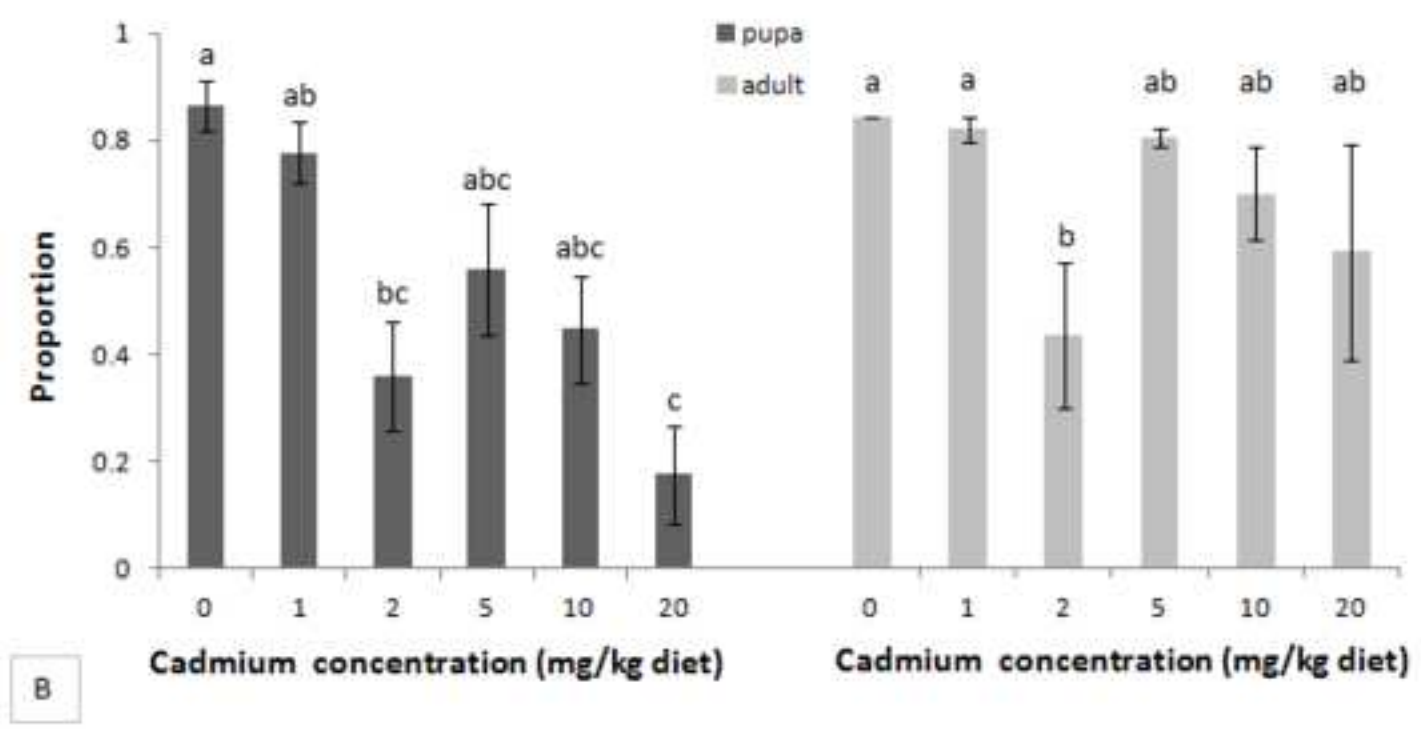
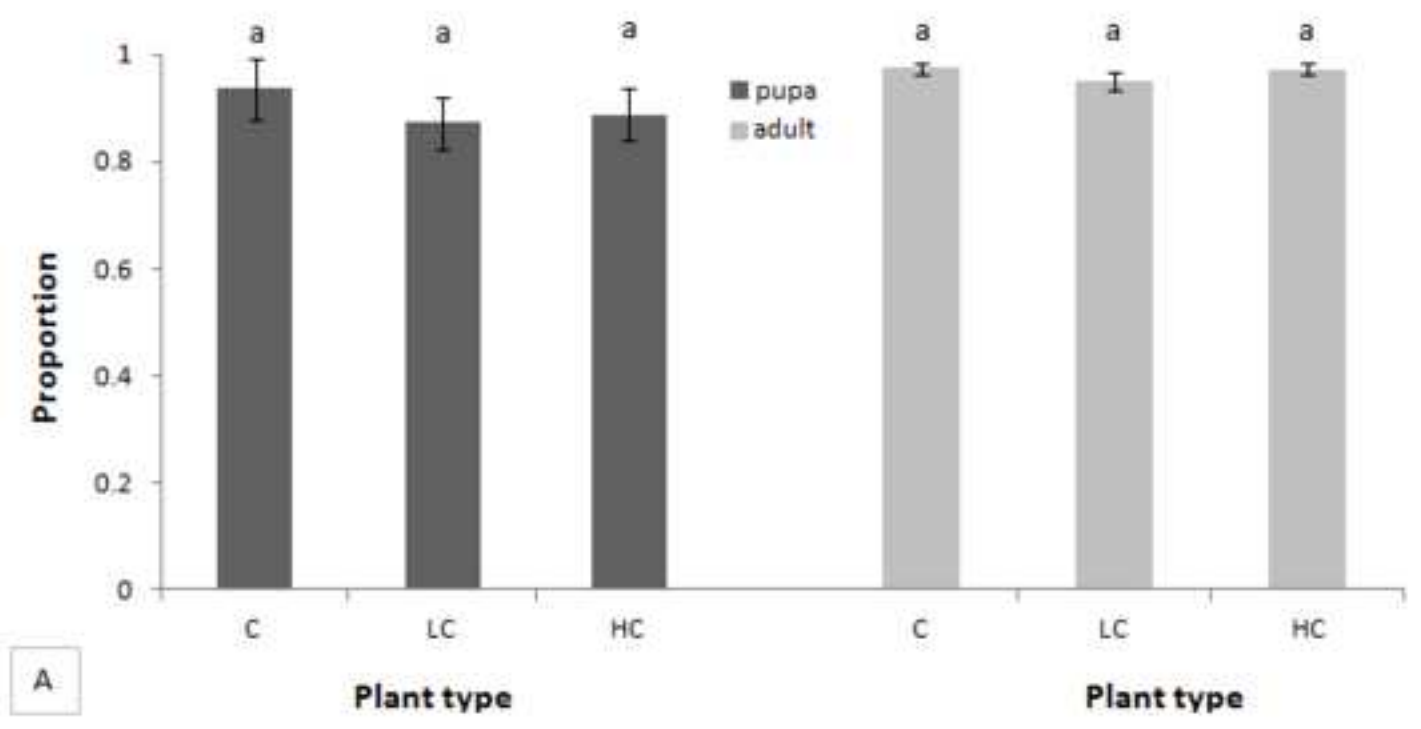


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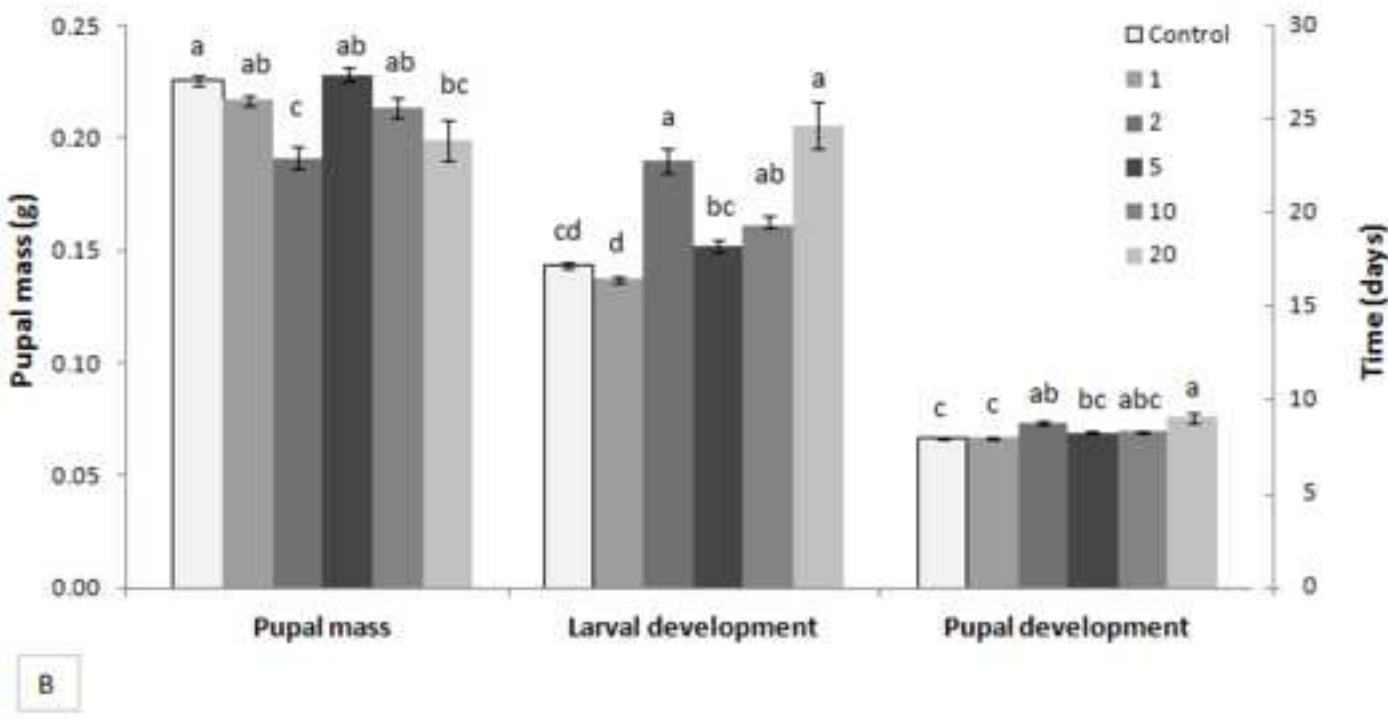
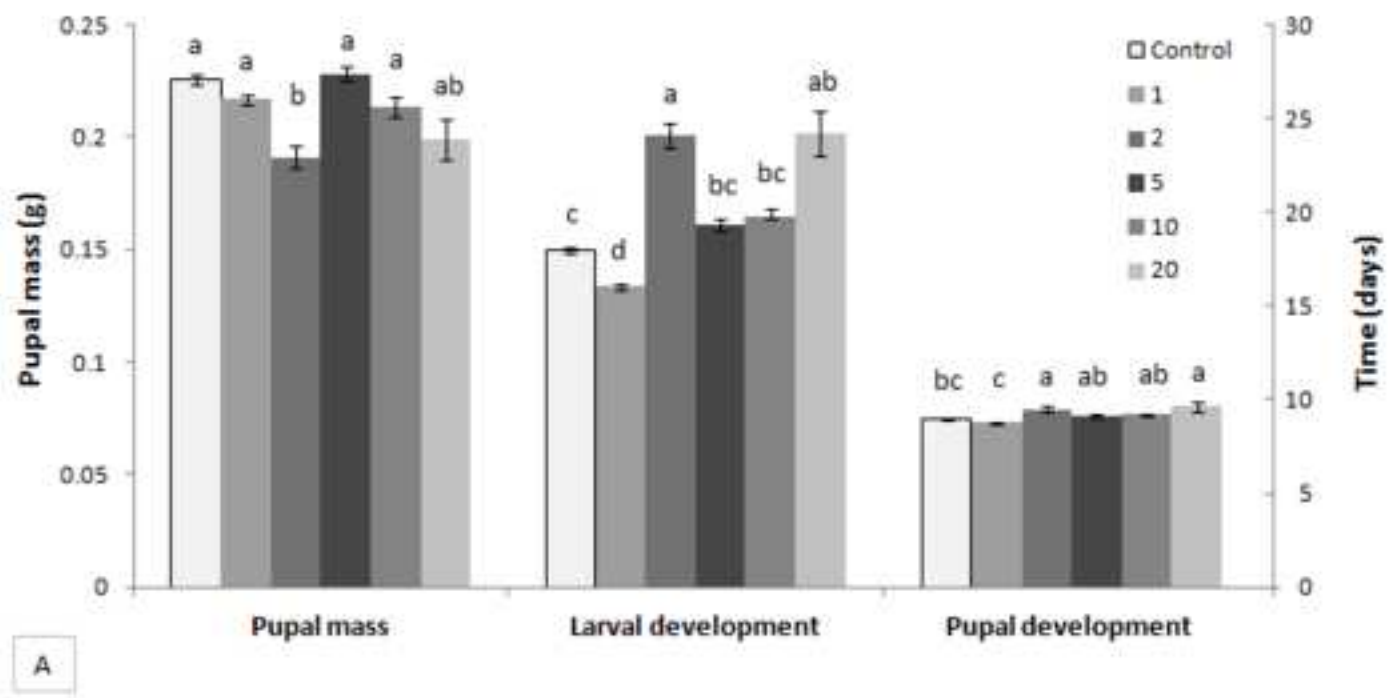


Table 1

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	Aphids			Parasitoids			
	Reproducing females (%)	Age to reproduction	Nymphs per reproductive female	Number of mummies	% emerging	Time to mummification	Time to adult
Control	64.0 ± 10.3 a	8.0 ± 0.4 a	2.52 ± 0.4 a	52.0 ± 12.1 a	38.7 ± 2.3 a	8.0 ± 0.1 a	12.4 ± 0.1 a
LC	60.0 ± 20.5 a	8.2 ± 0.3 a	2.14 ± 0.6 a	57.6 ± 11.7 a	40.1 ± 1.6 a	8.0 ± 0.1 a	12.6 ± 0.1 a
HC	62.0 ± 18.3 a	8.3 ± 0.4 a	2.12 ± 0.6 a	60.0 ± 11.2 a	39.2 ± 1.3 a	8.0 ± 0.1 a	12.5 ± 0.1 a