Uptake of dietary selenium by laboratory and field feeding *Podisus maculiventris* (Heteroptera: Pentatomidae)

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Toxicological, nutritional, and physiological roles of several trace elements have been documented for insects (Cohen 2004; Lavilla et al. 2010). Selenium (Se) has been of particular interest because it is an essential trace nutrient, an antioxidant, and exhibits a narrow range between concentrations that are either beneficial or toxic. Se is becoming more prevalent in the environment, in part, due to activities associated with industrialization. Insects acquire Se from water and food, where it can occur in a number of different forms (primarily sodium selenate, sodium selenite, selenomethionine, and selenocystine). The various forms of Se are known to be transferred between trophic levels from plants to insect herbivores and on to insect predators, although the bioavailability of each form at each trophic level is not fully understood. The chemical form of Se and its compartmentalization within the food (Trumble & Sorensen 2008) and the physiological state of the insect (Laskowski 1991) may affect the transfer of this trace element. Various outcomes have been demonstrated as a result of trace element transfer across trophic levels (Vickerman & Trumble 2003), e.g., diminished (biodiluted), retained (biotransferred), increased accumulation in the next trophic level (bioaccumulated), or consecutively increased across 3 or more trophic levels (biomagnified).

Several research groups have investigated the uptake of trace elements by a beneficial predator, the spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), and the effect(s) of dietary trace elements on life history parameters (Vickerman & Trumble 2003; Coudron et al. 2012; Cheruiyot et al. 2013). Two reports gave a partial perspective on the uptake of Se. Vickerman & Trumble (2003) reported *P. maculiventris* retained Se in an amount similar to that of the prey, a diet-reared caterpillar, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), when the prey was fed low amounts of Se, and in diminished amounts when the prey was fed high amounts of Se. Coudron et al. (2012) reported *P. maculiventris* retained Se in an amount similar to that of the prey, a diet-reared caterpillar, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), and in an increased amount when fed an artificial diet that contained more Se than the *T. ni* larvae but less Se than in the high doses that Vickerman & Trumble (2003) fed *S. exigua* larvae. Collectively, these findings demonstrated biotransfer without bioaccumulation occurred at low Se levels, bioaccumulation occurred as the Se levels increased, and biodilution occurred at high Se levels. This suggests an upper limit to the amount, or rate, at which *P. maculiventris* can accumulate Se and that this upper limit was correlated with diminished life history parameters (Vickerman & Trumble 2003), which in turn suggests a level of toxicity.

Here we report results of analysis of *P. maculiventris* reared on 3 types of food streams with 3 different outcomes of Se accumulation that expand our perspective of Se retention by an insect predator. Our findings confirm variable accumulation is possible within one insect. Furthermore, they provide new insight to previous findings and form the basis for proposing the quality of the food stream is an important consideration when investigating the biotransfer of trace elements across trophic levels. Additionally, this report provides the first evidence that *P. maculiventris* may be part of biomagnifying the concentration of Se across 3 trophic levels.

Four colonies of *P. maculiventris* that differed in their food stream and length of domestication were used for this study. One colony was composed of field-collected adults from alfalfa fields in Boone County, Missouri. These insects had fed almost exclusively on larvae of alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), which were infesting the stand of alfalfa and were the only prey observed in the field at the time of collection. Several nymphs and adults of *P. maculiventris* were observed actively feeding on *H. postica* at the time of capture. Adults were stored at −80 °C immediately after collection. Alfalfa weevils and alfalfa plants were collected at the same time and stored at −80 °C until Se analysis. Two outbred *P. maculiventris* colonies originated with adults collected from alfalfa fields in Boone County, Missouri, and then maintained for either approximately 30 or 300 generations in the laboratory in cardboard containers under constant long-day photoperiod (16:8 h L:D) at a temperature of 28 °C and 65% RH (Coudron et al. 2002). Coddled late 4th to early 5th instar *T. ni* larvae were provided 3 times per wk. The colony of *T. ni* had been maintained for more than 3 decades on a defined wheat germ–based diet with Wesson's Salt Mixture (MP Biomedicals, Santa Ana, California) under a photoperiod of 14:10 h L:D at 55% RH at 28 °C (Wilkinson et al. 1972). This diet was similar to the diet used to rear *S. exigua* (Vickerman & Trumble 2003) and contained 0.26 ± 0.01 µg Se/g dry weight (Popham & Shelby 2006). A 3rd outbred *P. maculiventris* colony that originated with adults collected from alfalfa fields in Boone County, Missouri, was reared on an artificial diet (Coudron et al. 2002) as the sole food source and maintained for 30 generations in the same conditions as the other domesticated colonies. The artificial diet contained bovine liver and whole egg as the 2 primary sources of Se. Instrumental neutron activation analysis was conducted at the University of Missouri Research Re-

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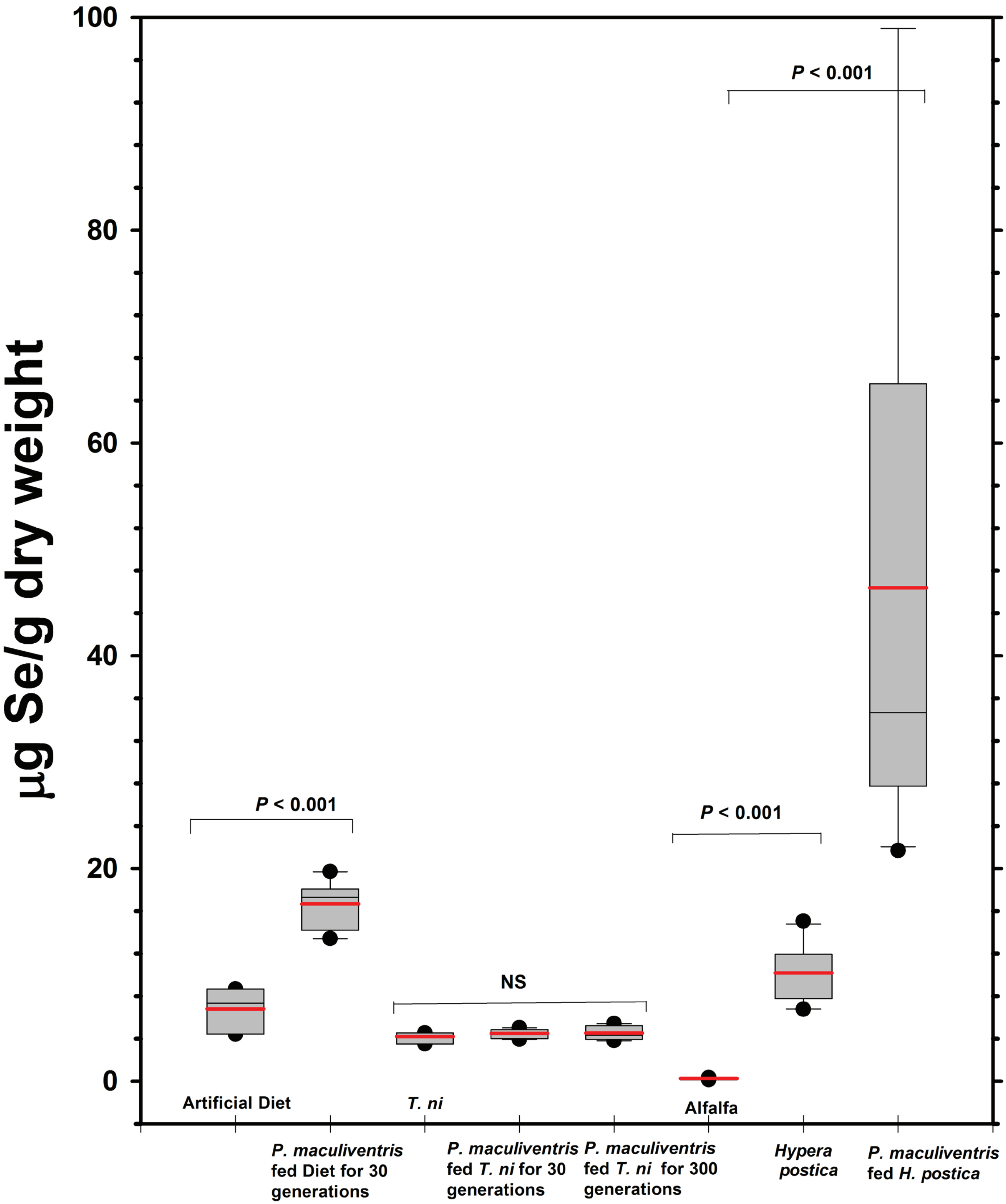

Fig. 1. Uptake of selenium (Se) from lower trophic levels measured in *Podisus maculiventris* adults following consumption of various diets or prey. Se levels measured in artificial diet, *P. maculiventris* fed on artificial diet, *Trichoplusia ni* prey, *P. maculiventris* fed on *T. ni* for 30 generations, *P. maculiventris* fed on *T. ni* for 300 generations, field-collected alfalfa foliage, field-collected *Hypera postica* prey, and field-collected *P. maculiventris* adults. Se concentrations were determined by neutron activation analysis and are expressed as µg Se/g dry weight. Tukey box plots with median (black line), mean (red line), the 25th and 75th percentiles (bottom and top of box, respectively), and the 5th and 95th percentiles (whiskers). Significant differences between means are shown as bars with *P* values above. NS = not significant.

actor, Columbia, Missouri, to measure the concentration of Se in whole bodies of adult females from the 4 colonies, and in the alfalfa foliage, the alfalfa weevil larvae, the *T. ni* larvae, and the artificial diet. Results are expressed as μg Se/g dry weight (Popham & Shelby 2006) (Fig. 1).

Podisus maculiventris females reared on the artificial diet as the sole food source contained significantly more Se (16.65 ± 0.78 μg Se/g dry weight; $n = 10$) than the diet (6.81 ± 1.25 μg Se/g dry weight; $n = 10$) (Mann Whitney U test; $P < 0.001$). This is interesting because previous work (Vickerman & Trumble 2003) had shown that *P. maculiventris* biodiluted Se when reared on *S. exigua* larvae that contained Se in the range of that in the artificial diet used in our study. Consequently, our findings suggest that it is not the concentration of Se in the food stream that is affecting the biotransfer. However, it is likely that the chemical form of Se in the artificial diet (the source of which was liver and hen egg) differed from that present in *S. exigua* larvae, or any other insect food source of *P. maculiventris*. The chemical form of Se is relevant because it may impact the way it is metabolized and stored by the insect.

Our results indicate *T. ni* larvae bioaccumulated Se from the artificial diet (Popham & Shelby 2006), and that females of *P. maculiventris* maintained on *T. ni* prey for 30 and 300 generations contained similar levels of Se (4.49 ± 0.15 and 4.53 ± 0.22 μg Se/g dry weight, for the colonies at generations 30 and 300, respectively; $n = 10$ per colony) as the *T. ni* prey (4.20 ± 0.34 μg Se/g dry weight; $n = 8$) (Mann Whitney U test; $P > 0.05$). This result indicates that *P. maculiventris* biotransferred but did not bioaccumulate Se from *T. ni* when the prey contained low levels, similar to previous reports for *S. exigua* and *T. ni* (Vickerman & Trumble 2003; Coudron et al. 2012). Additionally, we found no change in *P. maculiventris* Se content as a result of extended domestication (300 generations) when provided lepidopteran diet–reared *T. ni* as prey.

In contrast, the field-collected adult females contained much higher levels of Se (46.38 ± 8.21 μg Se/g dry weight; $n = 10$) than the females of colonies maintained in the laboratory, and had a much higher level than that found in the larval prey, *H. postica* (10.18 ± 0.82 μg Se/g dry weight; $n = 10$) (Mann Whitney U test; $P < 0.001$). Alfalfa leaves on which the weevils had been feeding contained only 0.26 ± 0.11 μg Se/g dry weight ($n = 5$), the lowest value measured in this study. Given the field environment, we cannot eliminate the possibility that other prey were consumed, although this seems unlikely. Assuming *H. postica* was the only prey (or the major food source), Se was biomagnified across the 3 trophic levels and was bioaccumulated at levels 4 times higher than when *P. maculiventris* was reared on *S. exigua*, in which case Se was biodiluted below the level in the food stream (Vickerman & Trumble 2003).

This report demonstrates that the biodilution observed previously with *S. exigua* was not due to an upper limit of accumulation of Se by *P. maculiventris* but rather may indicate a difference in the food quality between *S. exigua* and *H. postica* and a subsequent difference in physiological state (which we measure with fitness parameters and equate to health) of *P. maculiventris*. This conclusion is reasonable given that the developmental performance and the health of *P. maculiventris* have been shown to be affected by changes in the species of lepidopteran larval prey (de Clercq & Degheele 1993) and by the development stage of a coleopteran host (Morales-Ramos et al. 2016). Hence, it seems reasonable to propose that the food stream affects the health, which in turn may affect retention and accumulation of Se, even when the form and amount of Se are similar. Further, life history parameters have shown field-collected *P. maculiventris* to be more fit than prey-fed or diet-fed *P. maculiventris* (Coudron et al. 2002) and consequently in a better physiological state even though the Se content was 4 times higher than in the study where *P. maculiventris* biodiluted Se and demonstrated impaired fitness when reared on *S. exigua* (Vickerman & Trumble 2003). Consequently, it is unlikely that the elevated levels of Se in *S. exigua* represented an intrinsically toxic level.

Our results indicate that *P. maculiventris* is capable of retaining, bioaccumulating, and possibly biomagnifying dietary Se, as shown when the food source was *T. ni*, artificial diet, and alfalfa weevil, respectively. We also demonstrated that similar amounts of Se in different food streams can result in different amounts of Se retained by the predator. This finding demonstrates the existence of different accumulation scenarios, and plasticity within *P. maculiventris* adult females regarding physiological responses to dietary Se. The different responses by a single predator species to Se in 3 different food streams is particularly intriguing and highlights the complexity of responses that are possible as well as the challenge of understanding the factors influencing these outcomes. This report suggests caution in generalizing to other situations with differing arrangements of trophic levels and food streams.

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Summary

Podisus maculiventris (Say) (Hemiptera: Pentatomidae) is a generalist predator commercially available for augmentative biological control of pest insects in a variety of crop and orchard systems. It is exposed to a wide variety of micronutrients based on the soil type, plant, and insect prey items consumed. Bioaccumulation of selenium by adult females of *P. maculiventris* fed insectary-reared *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) larval prey was not statistically significant. However, adult females of *P. maculiventris* collected from the field that had been feeding primarily on alfalfa weevils (*Hypera postica* Gyllenhal; Coleoptera: Curculionidae), and those fed an artificial diet, showed significant bioaccumulation of selenium. This is the first report of biomagnification of a trace element across 3 trophic levels involving *P. maculiventris*.

Key Words: selenium; spined soldier bug; alfalfa weevil; alfalfa

Sumario

Podisus maculiventris (Say) (Hemiptera: Pentatomidae) es un predador generalista, el cual es comercialmente disponible para uso en control biológico aumentativo contra insectos plaga en una variedad de cultivos y sistemas de huertas. *Podisus maculiventris* es expuesto a una gran variedad de micronutrientes en su medio natural según el tipo de suelo, plantas e insectos presa consumidos. La bioacumulación de selenio por *P. maculiventris* alimentado a base de larvas de *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) criado en insectario no fue estadísticamente significativa. Sin embargo, *P. maculiventris* colectados en campo, consumiendo primariamente picudos de la alfalfa (*Hypera postica* Gyllenhal; Coleoptera: Curculionidae), así como los alimentados con dieta artificial mostraron bioacumulación significativa de selenio. Este es el primer reporte de bioacumulación de elementos traza a través de tres niveles tróficos incluyendo *P. maculiventris*.

Palabras Clave: selenio; chinche soldado vinculado; picudos de la alfalfa; alfalfa

References Cited

Cheruiyot DJ, Boyd RS, Coudron TA, Cobine PA. 2013. Biotransfer, bioaccumulation and effects of herbivore dietary Co, Cu, Ni, and Zn on growth and development of the insect predator *Podisus maculiventris* (Say). Journal of Chemical Ecology 39: 764–772.

Cohen A.C. 2004. Insect Diets. Science and Technology. CRC Press, Boca Raton, Florida.

Coudron TA, Wittmeyer J, Kim Y. 2002. Life history and cost analysis for continuous rearing of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on a zoophytophagous artificial diet. Journal of Economic Entomology 95: 1159–1168.

Coudron TA, Mitchell LC, Sun R, Robertson JD, Pham NV, Popham HJR. 2012. Dietary composition affects levels of trace elements in the predator *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Biological Control 61: 141–146.

De Clercq P, Degheele D. 1993. Quality of predatory bugs of the genus *Podisus* (Heteroptera: Pentatomidae) reared on natural and artificial diets, pp. 129–142 *In* Nicoli G, Benuzzi M, Leppla NC [eds.], Proceedings of the 7th Workshop of the IOBC Global Working Group "Quality Control of Mass Reared Arthropods. Rimini, Italy, 13–16 Sep 1993.

Laskowski R. 1991. Are the top carnivores endangered by heavy metal biomagnification? Oikos 60: 387–390.

Lavilla I, Rodriguez-Liñares G, Garrido J, Bendicho C. 2010. A biogeochemical approach to understanding the accumulation patterns of trace elements in three species of dragonfly larvae: evaluation as biomonitors. Journal of Environmental Monitoring 12: 724–730.

Morales-Ramos JA, Rojas MG, Shelby KS, Coudron TA. 2016. Nutritional value of pupae versus larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) as food for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). Journal of Economic Entomology 109: 564–571.

Popham HJ, Shelby KS. 2006. Uptake of dietary micronutrients from artificial diets by larval *Heliothis virescens*. Journal of Insect Physiology 52: 771–777.

Trumble J, Sorensen M. 2008. Selenium and the elemental defense hypothesis. New Phytologist 177: 569–572.

Vickerman DB, Trumble JT. 2003. Biotransfer of selenium: effects on an insect predator, *Podisus maculiventris*. Ecotoxicology 12: 497–504.

Wilkinson JD, Morrison RK, Peters PK. 1972. Effect of calco oil red N-1700 dye incorporated into a semiartificial diet of the imported cabbageworm, corn earworm, and cabbage looper. Journal of Economic Entomology 65: 264–268.