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Using elemental profiling to determine intrinsic markers to track the dispersal of *Prostephanus truncatus*, a pest of stored grain with alternative natural hosts

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Short title for running headlines

Elemental markers of dispersal by *P. truncatus*

Key words

Inductively Coupled Plasma Atomic Emission Spectroscopy, ICP-AES, elemental screening, chemoprints, biomarkers, larger grain borer, natal origin.

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Abstract

Detecting sources of insects attacking grain stores can help to develop more effective pest management models. This study considers combinations of chemical elements as intrinsic markers for tracing resource-use by *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) a pest of stored maize which occurs in natural environments where alternative hosts may support reservoirs of infestation.

P. truncatus were lab-reared on maize or field-caught in pheromone-baited flight-traps. Beetles and hosts were screened for multiple elements using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). For elements above detection limits we tested relationships between determinations for different host plants, and for beetles according to environment where captured.

An alternative host *Spondias purpurea* (Linnaeus) (Anacardaceae) contained more Al, B, Ca, Cu, Fe, Mg, Si and Sr, and less P and Zn than maize. Trends for P were consistent between maize and beetles infesting maize, but reversed for Ca and Mg. Elemental profiles of beetles were associated with environment, with significantly lower Al, Ca, Cu, Cr, Fe, P, S, Si, Sr, Ti and Zn determinations in maize-reared beetles than those captured in agricultural or natural environments. Additionally, Al, Ba, K, P, Sr and Ti determinations of field beetles captured in agricultural vs natural environments were significantly different. This suggests Al, Sr and Ti as candidate markers for environment, plus other possibilities likely since elemental concentrations (except B, Ba, Ni, and P) were significantly different in comparisons of all field beetles vs maize-reared beetles.

We present a robust practical solution which successfully identified combinations of elemental markers for remotely tracing resource-use and dispersal by *P. truncatus*. We discuss the application

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of chemical characterisation for identifying intrinsic markers of pests, particularly species with alternative hosts. We discuss how to manage the low replication and unbalanced sample sizes inherent in insect elemental screening, particularly when rarer elements are potential markers.

Introduction

Flight is the main dispersal mechanism of insect pests, with their establishment and spread dependent upon reaching suitable environments and hosts, and whilst many species are monitored for pest management purposes, their natal origin is unknown. Primary storage pests complete their life cycle inside intact cereals grains where their damage goes undetected, facilitating infestation by other pests (Munro, 1940). Infestation can be reduced through good hygiene and chemical or physical control with the solid structure of stores forming a barrier to pests. However, most small-scale tropical stores are open structure experiencing temperatures conducive to insect flight and reproduction, and may suffer high levels of infestation from incoming pests (Haines, 2000).

This study uses multiple elemental profiles to identify intrinsic markers of dispersal of the larger grain borer *Prostephanus truncatus* (Horn)(Coleoptera: Bostrichidae). Such analytical approaches have the potential to detect the assimilated diet of organisms, including evidence of natal diets in dispersing adults, in contrast to gut content analyses which reveal recent adult diet (Borgemeister et al. 1998a). This insect is native to Mesoamerica and an introduced pest of maize and dried cassava in Africa (Hodges et al., 1983; Hodges et al., 1985). It is frequently monitored using traps baited with synthetic analogues of its aggregation pheromone (Hodges et al., 1984) and a similar pheromone-trapping system exists for the lesser grain borer, Rhyzopertha dominica (F.) (Colepotera: Bostrichidae) (Williams et al., 1981). Such traps have provided insight into their distribution, activity and relative abundance (Cogburn et al., 1984; Dendy et al., 1989) with both species detected in/near grain stores as well as environments far from cereal production or storage (Borgemeister et al., 1998a; Mahroof et al., 2010; Nansen et al., 2002; Nansen & Meikle, 2003; Rees et al., 1990; Tigar et

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al., 1994). Systematic searching for *P. truncatus* around traps with high catches has rarely located insects suggesting that they are sparsely distributed inside diverse plant structures such as twigs, deadwood, roots and buried seeds (Nansen et al., 2004).

Most Bostrichidae are wood-borers requiring woody hosts (Lui et al., 2008) and the widespread occurrence of two bostrichid grain pests in natural environments suggests they may not depend solely upon stored grains. Evidence of *P. truncatus'* non-agricultural hosts include its occurrence in cerambycid-girdled twigs of *S. purpurea* (Linnaeus)(Anacardaceae) and *Bursera fagariodes* Engler (Burseraceae) in Mexican forests (Ramírez Martínez et al., 1994) and of *Lannea nigritana* (Sc. Elliot) Keay (Anacardaceae) in African forests (Borgemeister et al., 1998b), with the effects of twig-girdling thought to benefit cerambycid larvae and smaller wood-borers including *P. truncatus* (Calderón-Cortés et al. 2011; Forcella, 1982). Further signs of *P. truncatus'* host-flexibility include reproduction on *Delonix negra* (Bojer ex Hook) Raf. (Fabaceae), *Acacia polyacanthus* Willd (Fabaceae), *Commiphora rostrata* Engl. (Burseraceae), *Commiphora balensis* Engl. (Burseraceae) and *Euphorbia tirucalli* (Euphorbiaceae), plus boring or limited reproduction on 15 other woody species (Nang'ayo et al., 2002). It has been reared on Ficus and cassava roots and has limited reproduction on teak seeds, *Tectona grandis* Linn. F. (Lamiaceae) (Nansen et al., 2004). Whilst for *R. dominica,* alternative hosts include acorns of native North American oaks (Jia et al., 2008) with evidence of other non-grain hosts in natural habitats (Edde & Phillips, 2006).

Multi-elemental loadings of biological materials are commonly used to establish origin, and nutrient or contaminant levels in foods (Engström et al., 2004; Kelly et al., 2005) but rarely applied to insects, although used with varying degrees of success to trace host-use and natal origin of aphids, moths and weevils (Bowden et al., 1984; Bowden et al., 1985a; Bowden et al., 1985b; Burns et al., 1985; Sherlock et al., 1984; Sherlock et al., 1985; 1986). More recently, Tigar & Waldron (2003) proposed using elemental profiling to identify remote markers of *P. truncatus*, and Mahroof & Phillips (2012) applied the technique to *R. dominica* and found specific elements were associated with cereal-

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consumption or agricultural environments whilst others were indicative of natural host-consumption or non-agricultural environments.

This study uses ICP-AES to produce multiple elemental profiles of *P. truncatus* with the aim of identifying patterns of elements that can distinguish between insects according to their natal host. We explore elemental profiles of maize and a natural host *S. purpurea*, and of *P. truncatus* reared on maize and collected in Mexico from agricultural areas where maize was present and natural vegetation far from cereal production or storage. An intrinsic method to trace resource-use and origin of stored product and other pests routinely captured in biosecurity surveillance monitoring would increase our understanding of the role of natural reservoirs as sources of infestation, and thus help inform pest management.

Materials and Methods

Field and laboratory sampling

We collected maize grains and *S. purpurea* branches in Mexico, and captured *P. truncatus* in pheromone-baited flight-traps (lures supplied by AgriSense, UK) in August, a peak period of flight activity (Tigar et al., 1994). Traps were deployed for 48 hours to sample nearby insects based on knowledge of their likely dispersal towards pheromone-baits (Helbig et al., 1992). Trapping environments included arable areas where maize was grown and natural environments far from maize production and storage, further information is given in Table 1 which characterises samples for comparison and statistical analyses.

The laboratory-bred beetles (the maize category in Table 1) were a strain of *P. truncatus* collected in Tanzania and kept in culture since the 1980s (provided by the Natural Resources Institute, University of Greenwich, Chatham, Kent, UK and held under DEFRA licence at the University of West of

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Scotland). Insects were kept in honey jars in an incubator at 25˚C ± 0.5 ˚C and reared on Mexican maize through two generations from egg to adult before extraction and analysis (repeated attempts to rear *P. truncatus* on *S. purpurea* in the laboratory were unsuccessful). Beetles were euthanized by freezing immediately after field capture or removal from laboratory cultures, and defrosted before analysis.

Sample preparation and ICP-AES assays

All materials were rinsed in ultra-pure water and dried overnight at 40°C and homogenized by grinding in an agate pestle and mortar. Each *P. truncatus* determination required a bulk sample of 10 adults (approximately 10 mg). Insect samples were heated in a 20 minute microwave digestion programme reaching 600 W and the cooled digests were made up to 5 ml with ultra-pure water. For maize and *S. purpurea,* 0.2-0.3 g samples were mixed with 1 ml H_2O_2 and 3 ml c. HNO_3 in a PFM digestion bomb using the same digestion program as beetles. When cooled, the digests were made up to 25 ml with ultra-pure water.

The digests were screened for Al, B, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Si, Sr, Ti, V, Zn and Zr in a Perkin-Elmer Optima 3000 ICP Spectrometer under default conditions (Gal et al., 2008). Determinations for each analyte were means from four readings off a calibration curve, and those exceeding the calibration range were diluted as required. Detection Limits (DL) were established for rarer elements likely to be at low concentrations (see Table 2). We established reference samples for beetles and maize which were analysed in tandem with test samples and ICP-AES elemental standards for consistency of determination.

Data Analysis

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Multi-element loadings of *P. truncatus* were explored by classifying beetles according to site characteristics and proximity to maize as described in Table 1. Firstly, we placed them into three groups (maize, agriculture and natural) and compared loadings of elements between beetles in these groups. Then we combined all pheromone-trapped beetles (the agriculture and natural groups) into a single field class and compared their elemental loadings with those of maize-reared beetles. We also identified trends in elemental loadings of maize and *S. purpurea* and compared these with trends in *P. truncatus* according to environment of capture.

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For ease of visual interpretation, elemental determinations were grouped into low and high concentrations according to their relative values in insects and plant hosts. We used SYSTAT 13 with Exact tests (Systat Software Inc., 2009) to handle unequal replication and any missing values for determinations below detection limits (DL). The elemental data distributions were diverse with many skewed towards very low concentrations. As no single transformation could produce normal distributions of the data we performed non-parametric Kruskal Wallis (Mann-Whitney U) tests to examine differences between groups, with post-hoc Dwass-Steel-Chritchlow-Fligner tests to identify differences between pairs of groups. These make no assumptions about the normality of data distributions and hence are unlikely to produce significant results when there are no real differences between groups (Dytham, 2011).

Results

Elemental profiles and concentrations

Of the 20 elements detected Al, B, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Si, Sr, Ti, V, Zn and Zr, there were 14 above DL in all materials tested. Those below DL were Cr, Ni, Ti, V and Zr for maize and wood, and V and Zr for *P. truncatus*. Na concentrations in living organisms are often controlled by regulatory processes and are not considered further.

Comparison of elemental profiles for host plants

There were differences between elemental determinations of maize and wood, and results for low and high concentrations are shown in Figures 1 and 2 respectively. The S determinations were similar for both hosts, and apart from P and Zn which were at higher concentrations in maize than wood, most elements appear to be at higher concentrations in wood than in maize, including Ba and Sr which were below DLs in maize. There were significant differences for Al, B, Ca, Cu, Fe, Mg, P, Si, Sr and Zn between wood and maize determinations (Figures 1 and 2, and Table 3).

Elemental profiles of *P. truncatus* grouped by environment of capture and host availability

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There were differences in the concentration of some elements in *P. truncatus* classified by their environment of capture (agriculture, maize or natural). Figures 3 and 4 suggest that agriculture beetles contained more Al, B, Cr, Fe, Si, Ti, and Zn, and less Ni than maize or natural beetles. Whilst maize beetles appeared to have lower levels of Al, Ca, Cu, Fe, Mg, Mn, P, S, Si, Sr and Zn than either agriculture or natural beetles, with Ti below DLs. Elemental concentrations in agriculture and natural *P. truncatus* were similar, although agriculture beetles contained more Al, B, Cr, Fe, Si, Ti and Zn and less Ni than natural beetles. These differences were significant for Al, Ca, Cu, Cr, Fe, S, Si, Sr, Ti and Zn in a three-way KW comparison between agriculture, maize and natural groups, but not significant for B, Ba and Ni (Table 4). All pairwise comparisons between elemental determinations of maize against natural beetles, and agriculture versus maize beetles (except Ti) were significantly different at $P < 0.001$ (Table 4). However, only Al, Ba, K, P, Sr and Ti were significantly different in a pairwise comparison between agriculture and natural beetles (Table 4).

When *P. truncatus* were grouped according to those with and without known access to maize, the new field beetle group (all beetles caught in pheromone-baited traps) showed significant differences in the concentrations of most elements with the exception of B, Ba, Ni, and P compared with maize-reared beetles (Table 4).

Discussion

This study successfully demonstrates that concentrations of many chemical elements differ between cereals and a natural host of a grain pest, and between insects infesting maize or collected in environments where maize is present and those collected far from environments where only natural hosts are available. Therefore elemental screening of pests can identify potential intrinsic markers of dispersal between cereal infestations and natural reservoirs on alternative hosts. However, the elemental trends in host plants and insects differed, and those able to distinguish between insects reared on maize and others caught in environments without maize, were not the same as those that distinguished between maize and an alternative host. For *P. truncatus*, concentrations of Al, Ca, Cu, Cr, Fe, Si, Sr, Ti and Zn differed with their environment of capture, and Al, Sr and Ti were also significantly different when all field beetles were compared with those infesting maize suggesting their application as intrinsic markers. In addition, for the more refractory elements like Si, environmental associations with resistant mineral phases (quartz) probably restrict their wider biomarker application.

Mahroof & Phillips (2012) screened *R. dominca* and three hosts, acorns (*Quercus muhlenbergii* (Englelm)), wheat and maize, for 10 elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) and their mean ICP-AES determinations of maize for elements in common with this study are similar: Fe (20, 30 mg/kg), K (3600, 3800 mg/kg), P (2700, 3000 mg/kg) and S (800, 1000 mg/kg) (this study and Mahroof & Phillips (2012) respectively). They also found more P and Zn in maize than in a natural host, but trends for Fe and Mg in maize and natural foods were reversed. They saw no difference in Ca or Cu concentrations between maize and acorns, but distinguished wheat because it had more Ca and Mn than either acorns or maize. We screened a wider range of elements, and in addition found Ba and Sr were above DL in a candidate alternative host but not maize, and also detected more Al, Ca, Cu, Fe, Mg and Si, and less P and Zn in the alternative host than in maize.

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Five elements, Ca, K, Mg, P, S and Zn, were identified as likely markers for the environment of capture or known dietary history in both *P. truncatus* and *R. dominica,* with Al, B, Ba, Ca, Cu, Fe, K, Mn S, Sr, Zn and Si concentrations differing between maize-reared and field-captured *P. truncatus* suggesting they can distinguish between beetles that complete their life-cycle solely on maize from those that consume natural foods or mixed diets. It would be useful to test this experimentally and develop dispersal models for pests based upon unique suites of elements that vary with their natal hosts, and to investigate temporal changes in the elements present in insects and plants. A limitation of our study was that only one alternative host was profiled for a species which has many potential host plants (Nang'ayo et al., 2002). However, if elemental profiles of insects derive from the geochemistry of their environment we would expect to see chemical differences between those feeding on plants growing in natural environments and those infesting crops grown in soils that undergo regular cultivation and agrochemical regimes. In addition the interpretation of field-captured beetles was limited by lack of successful rearing of *P. truncatus* on *S. purpurea*, although other studies have also experienced negative or inconsistent results with *P. truncatus* on non-maize hosts that could not be controlled (Detmers et al., 1993; Nang'ayo et al., 2002, Nansen et al., 2004). *S. purpurea* is an appropriate model for alternative hosts as it is widely distributed in Mexico and a known host of *P. truncatus* in natural vegetation (Calderón-Cortés et al., 2011).

A number of studies using different analytical techniques have determined multiple chemical profiles of insects with the aim of tracking dispersal and movement between host plants and field locations. These include Energy Dispersive X-ray Spectrometry for aphids and moths (Bowden et al., 1984; Bowden et al., 1985b; Sherlock et al., 1986), and IPC-AES for cotton boll weevils (Burns et al., 1985) as well as *R. dominica* (Mahroof & Phillips, 2012). Technique, local geochemistry and the nature of materials tested can all influence the selection of particular elements as intrinsic markers, but multi-elemental screening shows potential for finding appropriate markers for each scenario. In the future, with recent improved detection and sensitivity of techniques, it will be possible to determine profiles for individual insects especially larger species. Also non-destructive methods like

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Laser Ablation can allow other analyses such as DNA-sequencing or stable isotope analysis to be completed on a single insect, increasing the data that can inform the origin of each individual. By comparison, a bulk sample as used here may miss differences between individuals, but can give an overall indication of assimilated diet by the population captured.

ICP-AES provides robust evidence for assessing intrinsic markers and identifying consistent trends in host materials and the herbivores consuming them. These can be tested in controlled field and laboratory feeding trails, and incorporated into multivariate predictive models in a similar way to the geospatial isoscape approach applied to stable isotope determinations (West et al., 2010), which can reveal assimilated and natal diet in holometabolous insects which switch between C3 and C4 plant hosts (Mahroof & Phillips, 2007). However, when screening for rare or trace elements which naturally exist at low concentrations in organisms, the data distributions are frequently left skewed and rarely conform to normal distributions, hence do not fit the assumptions of parametric techniques such as Linear Discriminant Analysis and Principal Components Analysis. In this study, as in many clinical trials and behavioural research, some data were based on small sample sizes or were imbalanced when a determination was below DLs. We addressed these using non-parametric tests in an exact inference method (Gibbons JD & Chakraborti S, 2003). Other chemical screening data of insect pests show similar data distributions, often with low or unequal replication (Burns et al., 1985; Peng et al., 2012), and in common with good practice in other studies we ensured consistency of chemical assays by comparing samples with laboratory standards and our reference materials. Nevertheless multi-elemental analyses are powerful tools for tracing dispersal of organisms particularly pests which survive in natural reservoirs as well as for elucidating the sources of invading organisms. Understanding the sources of pests will enable integrated pest management models to respond to changes in dispersal and new risks to stored commodities and crops. Future studies of pests and rare organisms will benefit from the increased accessibility of chemical screening and isotopic profiling as tools for studying the movement of animal pests as well as species of

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conservation concern, and for authenticating the origin of high value biological material including foodstuffs and organisms protected under CITIES.

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Table 1. Groups used to classify *P. truncatus* according to the characteristics of their collection sites and access to maize (n = number of determinations, each consisting of bulk samples of 10 beetles per determination).

Group for elemental comparison	**Definition and collection-site characteristics**
Maize (n=32)	Reared through two generations from egg to adult on maize
Agriculture (n=10)	Field-caught in pheromone-baited traps in open arable areas production, where maize was growing and approaching maturity
Natural (n=8)	Field-caught in pheromone-baited traps in areas of natural or semi-natural vegetation including dense deciduous and coniferous woodland, and semi-arid rangeland with sparse trees and shrubs. All at least 12 km from nearest dwellings, agriculture or maize stores
Field n=(18)	Combination of all field-caught in pheromone-baited traps (agriculture plus natural as defined above)

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Table 2. ICP-AES Detection Limits (DL) for elements most likely to occur at low concentrations. These were determined from the bulk reference samples of *P. truncatus* and maize (and incorporating material from all sources to be analysed) and extrapolated for wood from maize.

	Detection Limit (mg/kg)	
Element	***P. truncatus***	**Maize and wood**
Al	6	2.4
Ba	0.2	0.06
Cu	0.3	0.1
Cr	0.8	0.4
Fe	3	1.1
Mg	1	0.4
Mn	0.3	0.1
Ni	2.8	1.1
Sr	0.03	0.01
Ti	0.2	0.06
Zn	3.5	1.3

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Table 3. Results of pairwise comparisons between the elemental loadings of maize and wood, for elements above DLs in both plant hosts. All comparisons assume 1 df. (Results in bold were significantly different).

	Kruskal-Wallis (KW) test		
Element	Mann-Whitney U statistic	KW statistic (X^2 approximation)	p-value
Al	2	8.81	**0.003**
B	2	11.75	**0.001**
Ba	0	3.82	0.051
Ca	0	12.03	**0.001**
Cu	5	11.96	**0.001**
Fe	16	6.49	**0.011**
K	44	0.85	0.356
Mg	13	7.37	**0.007**
Mn	47	0.57	0.449
P	120	12.02	**0.001**
S	74	0.65	0.419
Si	6	9.78	**0.001**
Zn	120	12.02	**0.001**

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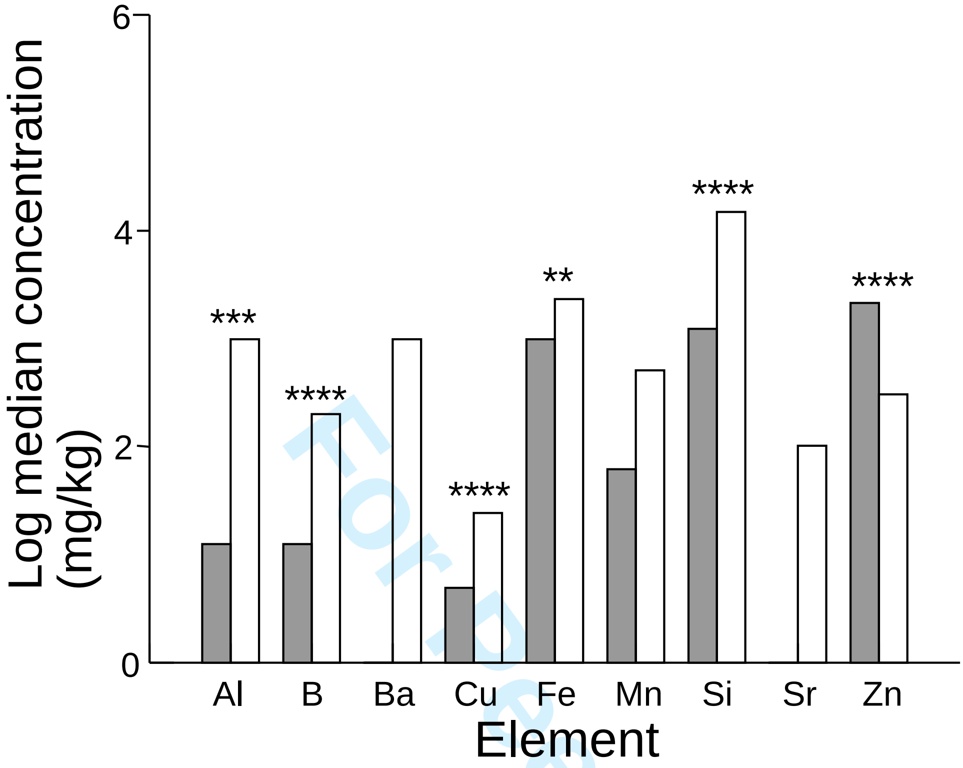
Table 4. Kruskal Wallis three-way comparison of beetles by agriculture, maize and natural groups, with post hoc Dwass-Steel-Chritchlow-Fligner pairwise comparisons between groups and Kruskal Wallis two-way comparison all field-caught and maize-reared beetles. (V and Zr were below DLs.) (Significant differences are in bold.)

Element	Three-way comparison agriculture*maize*natural		p-value for Dwass-Steel-Chritchlow-Fligner Test for Pairwise Comparisons			Two-way comparison field*maize	
	Kruskal-Wallis Test Statistic	p-value	agriculture * maize	agriculture * natural	maize * natural	Kruskal-Wallis Test Statistic	p-value
Al	27.09	**<0.001**	**<0.001**	**0.007**	**<0.001**	27.09	**<0.001**
B	1.35	0.51	**<0.001**	0.83	**<0.001**	0.72	0.4
Ba	0.37	0.83	**<0.001**	**0.003**	**<0.001**	0.34	0.56
Ca	27.59	**<0.001**	**<0.001**	0.97	**<0.001**	26.77	**<0.001**
Cr	6.27	**0.044**	**<0.001**	0.54	**<0.001**	5.6	**0.02**
Cu	14.41	**0.001**	**<0.001**	0.13	**<0.001**	14.35	**<0.001**
Fe	18.69	**<0.001**	**<0.001**	0.76	**<0.001**	17.68	**<0.001**
K	4.55	0.10	**<0.001**	**0.004**	**<0.001**	4.43	**0.04**
Mg	4.66	0.10	**<0.001**	0.81	**<0.001**	4.47	**0.03**
Mn	5.26	0.07	**<0.001**	0.56	**<0.001**	3.56	**0.06**
Ni	1.12	0.52	**<0.001**	0.08	**<0.001**	1.12	0.29
P	16.93	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1.77	0.18
S	1.94	0.38	**<0.001**	0.86	**<0.001**	16.93	**<0.001**
Si	9.95	**0.007**	**<0.001**	0.78	**<0.001**	9.69	**0.002**
Sr	16.56	**<0.001**	**<0.001**	**<0.001**	**<0.001**	15.51	**<0.001**
Ti	18.36	**<0.001**	0.90	**<0.001**	**<0.001**	17.86	**<0.001**
Zn	12.77	**0.004**	**<0.001**	0.43	**<0.001**	10.6	**0.001**



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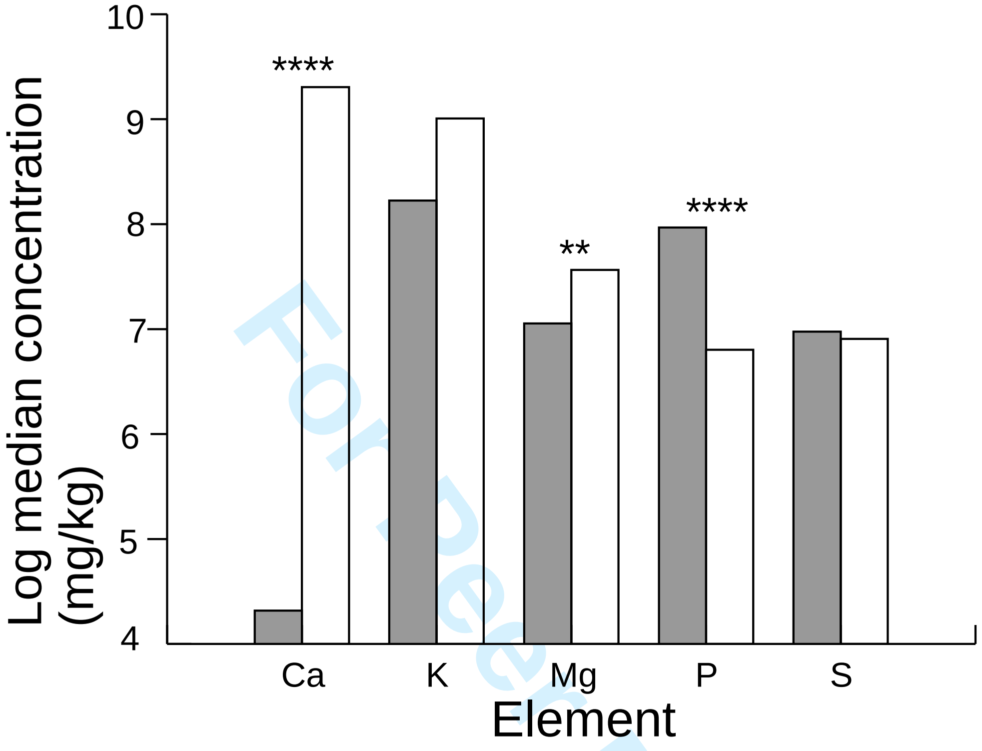

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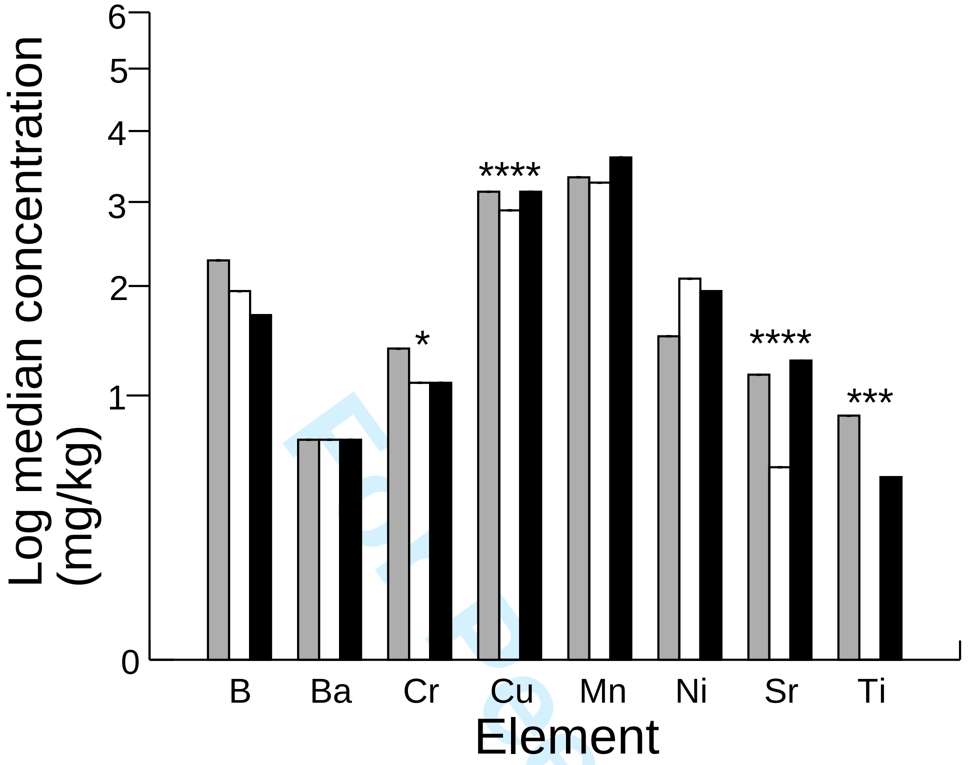

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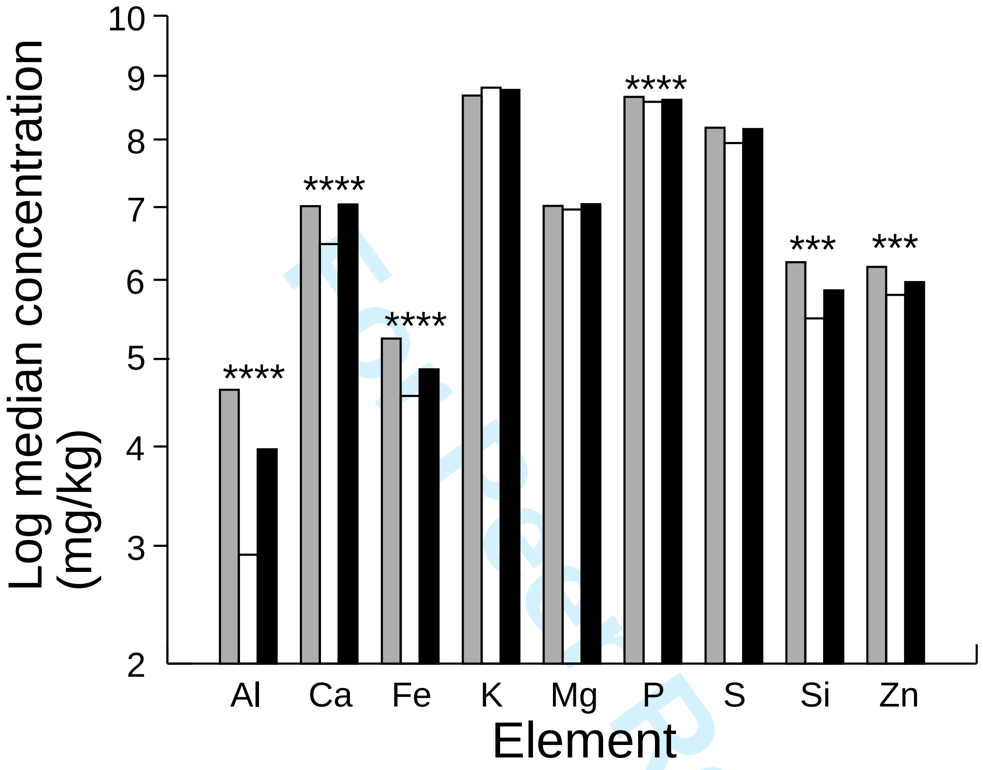

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