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

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17 **Short title for running headlines**

18 Elemental markers of dispersal by *P. truncatus*

19

20 **Key words**

21 Inductively Coupled Plasma Atomic Emission Spectroscopy, ICP-AES, elemental screening,

22 chemoprints, biomarkers, larger grain borer, natal origin.

23

24

25 **Abstract**

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

31
32 *P. truncatus* were lab-reared on maize or field-caught in pheromone-baited flight-traps. Beetles and
33 hosts were screened for multiple elements using Inductively Coupled Plasma Atomic Emission
34 Spectrometry (ICP-AES). For elements above detection limits we tested relationships between
35 determinations for different host plants, and for beetles according to environment where captured.

36
37 An alternative host *Spondias purpurea* (Linnaeus) (Anacardaceae) contained more Al, B, Ca, Cu, Fe,
38 Mg, Si and Sr, and less P and Zn than maize. Trends for P were consistent between maize and beetles
39 infesting maize, but reversed for Ca and Mg. Elemental profiles of beetles were associated with
40 environment, with significantly lower Al, Ca, Cu, Cr, Fe, P, S, Si, Sr, Ti and Zn determinations in maize-
41 reared beetles than those captured in agricultural or natural environments. Additionally, Al, Ba, K, P,
42 Sr and Ti determinations of field beetles captured in agricultural vs natural environments were
43 significantly different. This suggests Al, Sr and Ti as candidate markers for environment, plus other
44 possibilities likely since elemental concentrations (except B, Ba, Ni, and P) were significantly different
45 in comparisons of all field beetles vs maize-reared beetles.

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

1
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3 49 of chemical characterisation for identifying intrinsic markers of pests, particularly species with
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5 50 alternative hosts. We discuss how to manage the low replication and unbalanced sample sizes
6
7 51 inherent in insect elemental screening, particularly when rarer elements are potential markers.
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11 12 53 **Introduction**

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15 54 Flight is the main dispersal mechanism of insect pests, with their establishment and spread
16
17 55 dependent upon reaching suitable environments and hosts, and whilst many species are monitored
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19 56 for pest management purposes, their natal origin is unknown. Primary storage pests complete their
20
21 57 life cycle inside intact cereals grains where their damage goes undetected, facilitating infestation by
22
23 58 other pests (Munro, 1940). Infestation can be reduced through good hygiene and chemical or
24
25 59 physical control with the solid structure of stores forming a barrier to pests. However, most small-
26
27 60 scale tropical stores are open structure experiencing temperatures conducive to insect flight and
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29 61 reproduction, and may suffer high levels of infestation from incoming pests (Haines, 2000).
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33 62 This study uses multiple elemental profiles to identify intrinsic markers of dispersal of the larger
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35 63 grain borer *Prostephanus truncatus* (Horn)(Coleoptera: Bostrichidae). Such analytical approaches
36
37 64 have the potential to detect the assimilated diet of organisms, including evidence of natal diets in
38
39 65 dispersing adults, in contrast to gut content analyses which reveal recent adult diet (Borgemeister et
40
41 66 al. 1998a). This insect is native to Mesoamerica and an introduced pest of maize and dried cassava
42
43 67 in Africa (Hodges et al., 1983; Hodges et al., 1985). It is frequently monitored using traps baited with
44
45 68 synthetic analogues of its aggregation pheromone (Hodges et al., 1984) and a similar pheromone-
46
47 69 trapping system exists for the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera:
48
49 70 Bostrichidae) (Williams et al., 1981). Such traps have provided insight into their distribution, activity
50
51 71 and relative abundance (Cogburn et al., 1984; Dendy et al., 1989) with both species detected in/near
52
53 72 grain stores as well as environments far from cereal production or storage (Borgemeister et al.,
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55 73 1998a; Mahroof et al., 2010; Nansen et al., 2002; Nansen & Meikle, 2003; Rees et al., 1990; Tigar et
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3 74 al., 1994). Systematic searching for *P. truncatus* around traps with high catches has rarely located
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5 75 insects suggesting that they are sparsely distributed inside diverse plant structures such as twigs,
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7 76 deadwood, roots and buried seeds (Nansen et al., 2004).

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10 77 Most Bostrichidae are wood-borers requiring woody hosts (Lui et al., 2008) and the widespread
11
12 78 occurrence of two bostrichid grain pests in natural environments suggests they may not depend
13
14 79 solely upon stored grains. Evidence of *P. truncatus*' non-agricultural hosts include its occurrence in
15
16 80 cerambycid-girdled twigs of *S. purpurea* (Linnaeus)(Anacardaceae) and *Bursera fagaroides* Engler
17
18 81 (Burseraceae) in Mexican forests (Ramírez Martínez et al., 1994) and of *Lannea nigritana* (Sc. Elliot)
19
20 82 Keay (Anacardaceae) in African forests (Borgemeister et al., 1998b), with the effects of twig-girdling
21
22 83 thought to benefit cerambycid larvae and smaller wood-borers including *P. truncatus* (Calderón-
23
24 84 Cortés et al. 2011; Forcella, 1982). Further signs of *P. truncatus*' host-flexibility include reproduction
25
26 85 on *Delonix negra* (Bojer ex Hook) Raf. (Fabaceae), *Acacia polyacanthus* Willd (Fabaceae),
27
28 86 *Commiphora rostrata* Engl. (Burseraceae), *Commiphora balensis* Engl. (Burseraceae) and *Euphorbia*
29
30 87 *tirucalli* (Euphorbiaceae), plus boring or limited reproduction on 15 other woody species (Nang'ayo
31
32 88 et al., 2002). It has been reared on Ficus and cassava roots and has limited reproduction on teak
33
34 89 seeds, *Tectona grandis* Linn. F. (Lamiaceae) (Nansen et al., 2004). Whilst for *R. dominica*, alternative
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36 90 hosts include acorns of native North American oaks (Jia et al., 2008) with evidence of other non-
37
38 91 grain hosts in natural habitats (Edde & Phillips, 2006).

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43 92 Multi-elemental loadings of biological materials are commonly used to establish origin, and nutrient
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45 93 or contaminant levels in foods (Engström et al., 2004; Kelly et al., 2005) but rarely applied to insects,
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47 94 although used with varying degrees of success to trace host-use and natal origin of aphids, moths
48
49 95 and weevils (Bowden et al., 1984; Bowden et al., 1985a; Bowden et al., 1985b; Burns et al., 1985;
50
51 96 Sherlock et al., 1984; Sherlock et al., 1985; 1986). More recently, Tigar & Waldron (2003) proposed
52
53 97 using elemental profiling to identify remote markers of *P. truncatus*, and Mahroof & Phillips (2012)
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55 98 applied the technique to *R. dominica* and found specific elements were associated with cereal-

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3 99 consumption or agricultural environments whilst others were indicative of natural host-consumption
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5 100 or non-agricultural environments.
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8 101 This study uses ICP-AES to produce multiple elemental profiles of *P. truncatus* with the aim of
9
10 102 identifying patterns of elements that can distinguish between insects according to their natal host.
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12 103 We explore elemental profiles of maize and a natural host *S. purpurea*, and of *P. truncatus* reared on
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14 104 maize and collected in Mexico from agricultural areas where maize was present and natural
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16 105 vegetation far from cereal production or storage. An intrinsic method to trace resource-use and
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18 106 origin of stored product and other pests routinely captured in biosecurity surveillance monitoring
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20 107 would increase our understanding of the role of natural reservoirs as sources of infestation, and thus
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22 108 help inform pest management.
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25 26 109 **Materials and Methods**

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29 30 111 **Field and laboratory sampling**

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35 113 We collected maize grains and *S. purpurea* branches in Mexico, and captured *P. truncatus* in
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37 114 pheromone-baited flight-traps (lures supplied by AgriSense, UK) in August, a peak period of flight
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39 115 activity (Tigar et al., 1994). Traps were deployed for 48 hours to sample nearby insects based on
40
41 116 knowledge of their likely dispersal towards pheromone-baits (Helbig et al., 1992). Trapping
42
43 117 environments included arable areas where maize was grown and natural environments far from
44
45 118 maize production and storage, further information is given in Table 1 which characterises samples
46
47 119 for comparison and statistical analyses.
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52 121 The laboratory-bred beetles (the maize category in Table 1) were a strain of *P. truncatus* collected in
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54 122 Tanzania and kept in culture since the 1980s (provided by the Natural Resources Institute, University
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56 123 of Greenwich, Chatham, Kent, UK and held under DEFRA licence at the University of West of
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3 124 **Scotland**). Insects were kept in honey jars in an incubator at 25°C ± 0.5 °C and reared on Mexican
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5 125 maize through two generations **from egg to adult** before extraction **and analysis** (repeated attempts
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7 126 to rear *P. truncatus* on *S. purpurea* in the laboratory were unsuccessful). Beetles were euthanized by
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9 127 freezing **immediately after field capture or removal from laboratory cultures, and defrosted before**
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11 128 **analysis.**

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15 16 130 **Sample preparation and ICP-AES assays**

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19 131 All materials were rinsed in ultra-pure water and dried overnight at 40°C **and homogenized by**
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21 132 **grinding in an agate pestle and mortar. Each *P. truncatus* determination required a bulk sample of 10**
22
23 133 **adults (approximately 10 mg). Insect** samples were heated in a 20 minute microwave digestion
24
25 134 programme reaching 600 W and the cooled digests were made up to 5 ml with ultra-pure water. **For**
26
27 135 **maize and *S. purpurea*, 0.2-0.3 g** samples were mixed with 1 ml H₂O₂ and 3 ml c. HNO₃ in a PFM
28
29 136 digestion bomb using the same digestion program as beetles. When cooled, the digests were made
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31 137 up to 25 ml with ultra-pure water.

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35 138 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
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37 139 in a Perkin-Elmer Optima 3000 ICP Spectrometer under default conditions (Gal et al., 2008).

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39 140 Determinations for each analyte were means from four readings off a calibration curve, and those
40
41 141 exceeding the calibration range were diluted as required. Detection Limits (DL) were established for
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43 142 rarer elements likely to be at low concentrations (see Table 2). We established reference samples for
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45 143 beetles and maize which were analysed in tandem with test samples and ICP-AES elemental
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47 144 standards for consistency of determination.

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51 52 53 146 **Data Analysis**

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3 147 Multi-element loadings of *P. truncatus* were explored by classifying beetles according to site
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5 148 characteristics and proximity to maize as described in Table 1. Firstly, we placed them into three
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7 149 groups (maize, agriculture and natural) and compared loadings of elements between beetles in
8
9 150 these groups. Then we combined all pheromone-trapped beetles (the agriculture and natural
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11 151 groups) into a single field class and compared their elemental loadings with those of maize-reared
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13 152 beetles. We also identified trends in elemental loadings of maize and *S. purpurea* and compared
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15 153 these with trends in *P. truncatus* according to environment of capture.

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19 154 For ease of visual interpretation, elemental determinations were grouped into low and high
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21 155 concentrations according to their relative values in insects and plant hosts. We used SYSTAT 13 with
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23 156 Exact tests (Systat Software Inc., 2009) to handle unequal replication and any missing values for
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25 157 determinations below detection limits (DL). The elemental data distributions were diverse with
26
27 158 many skewed towards very low concentrations. As no single transformation could produce normal
28
29 159 distributions of the data we performed non-parametric Kruskal Wallis (Mann-Whitney U) tests to
30
31 160 examine differences between groups, with post-hoc Dwass-Steel-Chritchlow-Fligner tests to identify
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33 161 differences between pairs of groups. These make no assumptions about the normality of data
34
35 162 distributions and hence are unlikely to produce significant results when there are no real differences
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37 163 between groups (Dytham, 2011).

38 39 40 41 164 **Results**

42 43 44 165 **Elemental profiles and concentrations**

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47 166 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,
48
49 167 there were 14 above DL in all materials tested. Those below DL were Cr, Ni, Ti, V and Zr for maize
50
51 168 and wood, and V and Zr for *P. truncatus*. Na concentrations in living organisms are often controlled
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53 169 by regulatory processes and are not considered further.

54 55 56 57 170 **Comparison of elemental profiles for host plants**

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3 171 There were differences between elemental determinations of maize and wood, and results for low
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5 172 and high concentrations are shown in Figures 1 and 2 respectively. The S determinations were
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7 173 similar for both hosts, and apart from P and Zn which were at higher concentrations in maize than
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9 174 wood, most elements appear to be at higher concentrations in wood than in maize, including Ba and
10
11 175 Sr which were below DLs in maize. There were significant differences for Al, B, Ca, Cu, Fe, Mg, P, Si,
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13 176 Sr and Zn between wood and maize determinations (Figures 1 and 2, and Table 3).
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16 17 177 **Elemental profiles of *P. truncatus* grouped by environment of capture and host availability**

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20 178 There were differences in the concentration of some elements in *P. truncatus* classified by their
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22 179 environment of capture (agriculture, maize or natural). Figures 3 and 4 suggest that agriculture
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24 180 beetles contained more Al, B, Cr, Fe, Si, Ti, and Zn, and less Ni than maize or natural beetles. Whilst
25
26 181 maize beetles appeared to have lower levels of Al, Ca, Cu, Fe, Mg, Mn, P, S, Si, Sr and Zn than either
27
28 182 agriculture or natural beetles, with Ti below DLs. Elemental concentrations in agriculture and natural
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30 183 *P. truncatus* were similar, although agriculture beetles contained more Al, B, Cr, Fe, Si, Ti and Zn and
31
32 184 less Ni than natural beetles. These differences were significant for Al, Ca, Cu, Cr, Fe, S, Si, Sr, Ti and
33
34 185 Zn in a three-way KW comparison between agriculture, maize and natural groups, but not significant
35
36 186 for B, Ba and Ni (Table 4). All pairwise comparisons between elemental determinations of maize
37
38 187 against natural beetles, and agriculture versus maize beetles (except Ti) were significantly different
39
40 188 at $P < 0.001$ (Table 4). However, only Al, Ba, K, P, Sr and Ti were significantly different in a pairwise
41
42 189 comparison between agriculture and natural beetles (Table 4).
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49 191 When *P. truncatus* were grouped according to those with and without known access to maize, the
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51 192 new field beetle group (all beetles caught in pheromone-baited traps) showed significant differences
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53 193 in the concentrations of most elements with the exception of B, Ba, Ni, and P compared with maize-
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55 194 reared beetles (Table 4).
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195 **Discussion**

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

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

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3 219 Five elements, Ca, K, Mg, P, S and Zn, were identified as likely markers for the environment of
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5 220 capture or known dietary history in both *P. truncatus* and *R. dominica*, with Al, B, Ba, Ca, Cu, Fe, K,
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7 221 Mn, S, Sr, Zn and Si concentrations differing between maize-reared and field-captured *P. truncatus*
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9 222 suggesting they can distinguish between beetles that complete their life-cycle solely on maize from
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11 223 those that consume natural foods or mixed diets. It would be useful to test this experimentally and
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13 224 develop dispersal models for pests based upon unique suites of elements that vary with their natal
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15 225 hosts, and to investigate temporal changes in the elements present in insects and plants. A limitation
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17 226 of our study was that only one alternative host was profiled for a species which has many potential
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19 227 host plants (Nang'ayo et al., 2002). However, if elemental profiles of insects derive from the
20
21 228 geochemistry of their environment we would expect to see chemical differences between those
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23 229 feeding on plants growing in natural environments and those infesting crops grown in soils that
24
25 230 undergo regular cultivation and agrochemical regimes. In addition the interpretation of field-
26
27 231 captured beetles was limited by lack of successful rearing of *P. truncatus* on *S. purpurea*, although
28
29 232 other studies have also experienced negative or inconsistent results with *P. truncatus* on non-maize
30
31 233 hosts that could not be controlled (Detmers et al., 1993; Nang'ayo et al., 2002, Nansen et al., 2004).
32
33 234 *S. purpurea* is an appropriate model for alternative hosts as it is widely distributed in Mexico and a
34
35 235 known host of *P. truncatus* in natural vegetation (Calderón-Cortés et al., 2011).
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40 236 A number of studies using different analytical techniques have determined multiple chemical
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42 237 profiles of insects with the aim of tracking dispersal and movement between host plants and field
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44 238 locations. These include Energy Dispersive X-ray Spectrometry for aphids and moths (Bowden et al.,
45
46 239 1984; Bowden et al., 1985b; Sherlock et al., 1986), and IPC-AES for cotton boll weevils (Burns et al.,
47
48 240 1985) as well as *R. dominica* (Mahroof & Phillips, 2012). Technique, local geochemistry and the
49
50 241 nature of materials tested can all influence the selection of particular elements as intrinsic markers,
51
52 242 but multi-elemental screening shows potential for finding appropriate markers for each scenario. In
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54 243 the future, with recent improved detection and sensitivity of techniques, it will be possible to
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56 244 determine profiles for individual insects especially larger species. Also non-destructive methods like
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3 245 Laser Ablation can allow other analyses such as DNA-sequencing or stable isotope analysis to be
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5 246 completed on a single insect, increasing the data that can inform the origin of each individual. By
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7 247 comparison, a bulk sample as used here may miss differences between individuals, but can give an
8
9 248 overall indication of assimilated diet by the population captured.
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12 249 ICP-AES provides robust evidence for assessing intrinsic markers and identifying consistent trends in
13
14 250 host materials and the herbivores consuming them. These can be tested in controlled field and
15
16 251 laboratory feeding trails, and incorporated into multivariate predictive models in a similar way to the
17
18 252 geospatial isoscape approach applied to stable isotope determinations (West et al., 2010), which can
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20 253 reveal assimilated and natal diet in holometabolous insects which switch between C3 and C4 plant
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22 254 hosts (Mahroof & Phillips, 2007). However, when screening for rare or trace elements which
23
24 255 naturally exist at low concentrations in organisms, the data distributions are frequently left skewed
25
26 256 and rarely conform to normal distributions, hence do not fit the assumptions of parametric
27
28 257 techniques such as Linear Discriminant Analysis and Principal Components Analysis. In this study, as
29
30 258 in many clinical trials and behavioural research, some data were based on small sample sizes or were
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32 259 imbalanced when a determination was below DLs. We addressed these using non-parametric tests in
33
34 260 an exact inference method (Gibbons JD & Chakraborti S, 2003). Other chemical screening data of
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36 261 insect pests show similar data distributions, often with low or unequal replication (Burns et al., 1985;
37
38 262 Peng et al., 2012), and in common with good practice in other studies we ensured consistency of
39
40 263 chemical assays by comparing samples with laboratory standards and our reference materials.
41
42 264 Nevertheless multi-elemental analyses are powerful tools for tracing dispersal of organisms
43
44 265 particularly pests which survive in natural reservoirs as well as for elucidating the sources of invading
45
46 266 organisms. Understanding the sources of pests will enable integrated pest management models to
47
48 267 respond to changes in dispersal and new risks to stored commodities and crops. Future studies of
49
50 268 pests and rare organisms will benefit from the increased accessibility of chemical screening and
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52 269 isotopic profiling as tools for studying the movement of animal pests as well as species of
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3 270 conservation concern, and for authenticating the origin of high value biological material including
4
5 271 foodstuffs and organisms protected under CITIES.
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8 272 **Acknowledgements**

9
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24
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3 402 **Table 1.** Groups used to classify *P. truncatus* according to the characteristics of their collection sites
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5 403 and access to maize (n = number of determinations, each consisting of bulk samples of 10 beetles
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7 404 per determination).
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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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406 **Table 2.** ICP-AES Detection Limits (DL) for elements most likely to occur at low concentrations. These
407 were determined from the bulk reference samples of *P. truncatus* and maize (and incorporating
408 material from all sources to be analysed) and extrapolated for wood from maize.

409

Element	Detection Limit (mg/kg)	
	<i>P. truncatus</i>	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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3 411 Table 3. Results of pairwise comparisons between the elemental loadings of maize and wood, for
4 412 elements above DLs in both plant hosts. All comparisons assume 1 df. (Results in bold were
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6 413 significantly different).
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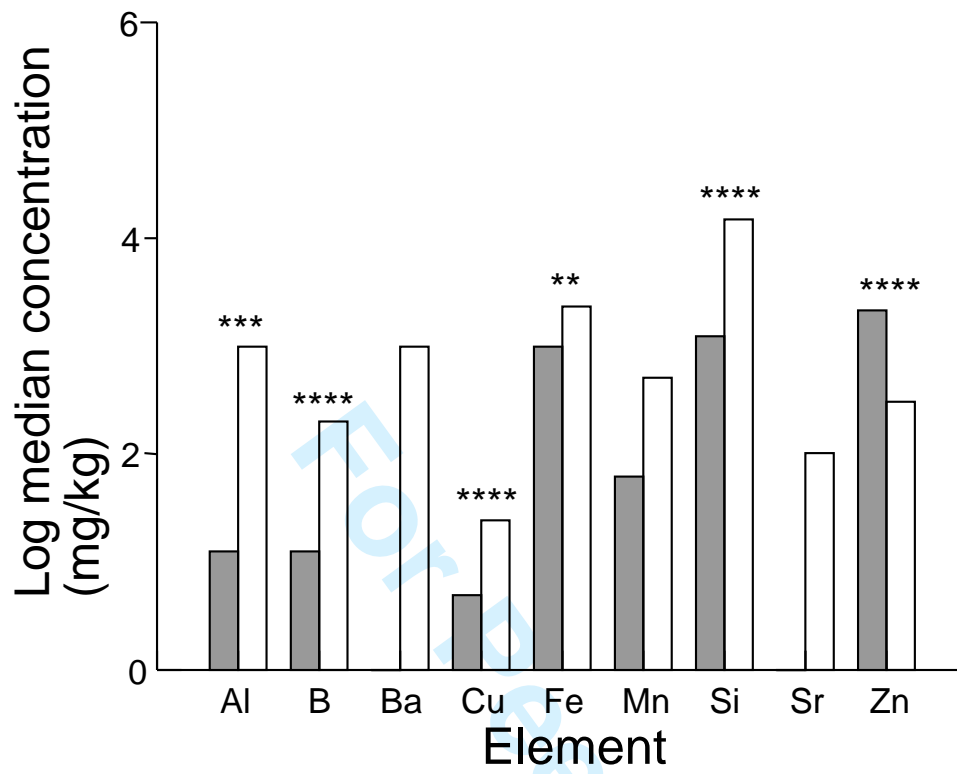
Element	Kruskal-Wallis (KW) test		
	Mann-Whitney U statistic	KW statistic (X^2 approximation)	p-value
Al	2	8.81	0.003
B	2	11.75	0.001
Ba	0	3.82	0.051
Ca	0	12.03	0.001
Cu	5	11.96	0.001
Fe	16	6.49	0.011
K	44	0.85	0.356
Mg	13	7.37	0.007
Mn	47	0.57	0.449
P	120	12.02	0.001
S	74	0.65	0.419
Si	6	9.78	0.001
Zn	120	12.02	0.001

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

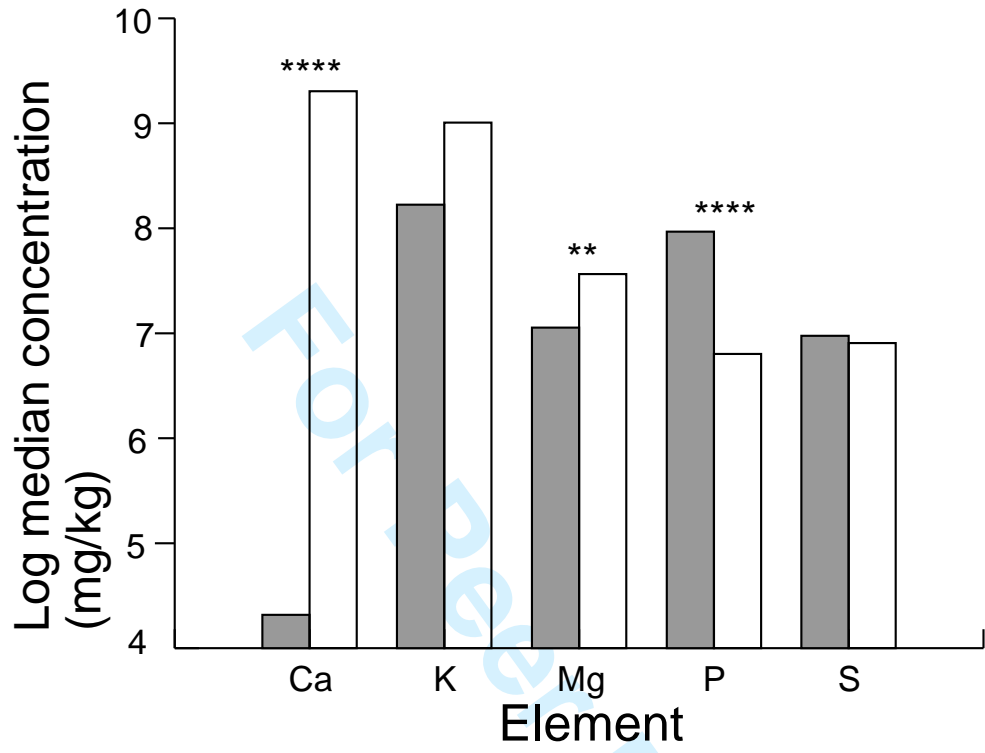
Element	Three-way comparison agriculture*maize*natural		p-value for Dwass-Steel-Chritchlow- Fligner Test for Pairwise Comparisons			Two-way comparison field*maize	
	Kruskal- Wallis Test Statistic	p-value	agriculture * maize	agriculture * natural	maize * natural	Kruskal- Wallis Test Statistic	p- value
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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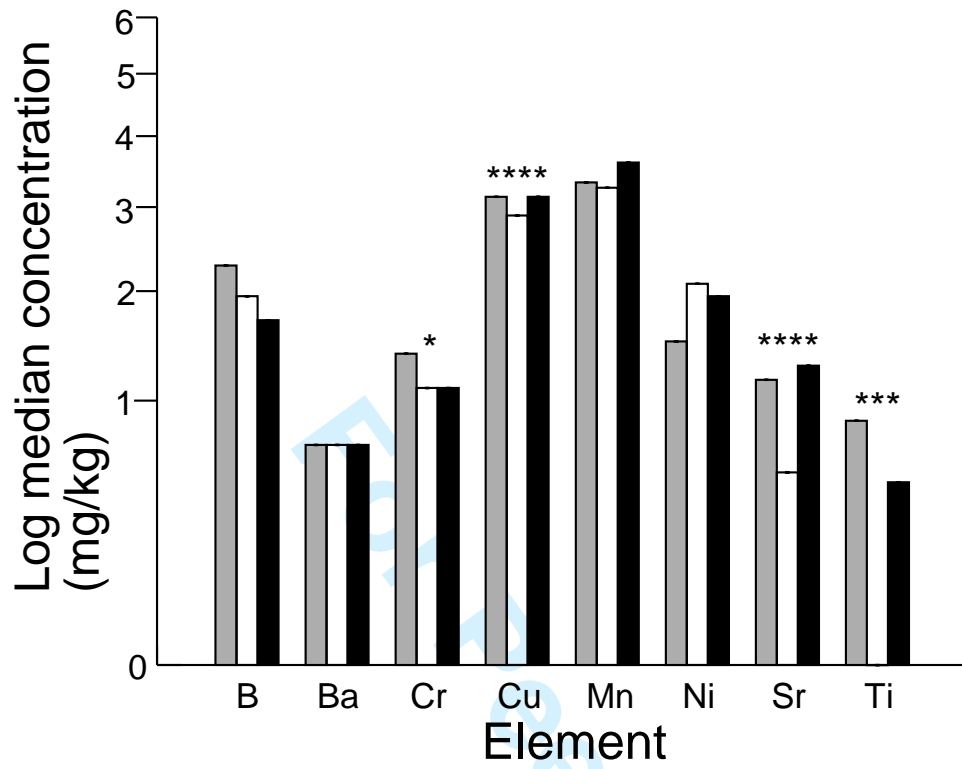


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