Use of macro and trace elements as biological markers in the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

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**A B S T R A C T**

The relationship between the occurrence and movements of *Rhyzopertha dominica* (F.), a serious pest of stored cereal grains, in distinctly different habitats is poorly known. Understanding the feeding ecology and dispersal patterns of *R. dominica* may likely help predict the abundance or movement of this pest in various habitats. Natal sites and movement of insects are studied using intrinsic methods involving elemental analyses. In this study, to understand the dietary history of *R. dominica*, trace and macro elements were used as potential markers. Insects reared on selected diets under laboratory conditions and adult beetles collected from the periphery of granaries or inside forested woodlands were used to determine 10 different elements in their body tissues. Quantities of 10 elements were also determined in respective laboratory hosts. The main discriminant elements for laboratory-reared *R. dominica* were Na, S and Zn, while significantly different elements were distinctive among field-collected beetles and included P, Ca, K, Zn, and Cu. The amount of Zn quantified in acorn-fed lab reared beetles was significantly lower than wheat- or corn-fed insects. Similarly, beetles captured in woodlands had significantly lower Zn in their body signatures, suggesting acorn seeds may have served as an alternate host in wooded habitats.

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1 Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a long-lived, internal grain feeding beetle that is a destructive pest of stored wheat and other cereal grains (Potter, 1935; Cotton, 1956; Cuperus et al., 1986; Fields and Phillips, 1994). *R. dominica* is highly polyphagous and has been recorded feeding on cereals, legumes, tubers, including packaging materials made from wood, leather, or sack (Potter, 1935). Adults and larvae of *R. dominica* are voracious feeders that cause damage to sound kernels. *R. dominica* is thought to have originated from the Indian sub-continent, but it is nearly cosmopolitan in its current distribution (Potter, 1935). *R. dominica* shows a widespread distribution on farms in all states of the US, and was first recorded in the US in 1861 (Schwardt, 1933; Subramanyam and Harein, 1989; Fields and Phillips, 1994).

*Rhyzopertha dominica* adults are known as strong fliers and they fly across different landscapes in search of food (Winterbottom, 1922; Hagstrum, 2001). Work by Cogburn (1988), and Edde and Phillips (2005) showed that *R. dominica* has been trapped in distinctly different habitats, such as those from the periphery of granaries or in woodlands considerable distances away from grain storages. The relationship between the abundance and occurrence of *R. dominica* in agricultural and wild habitats is poorly known. Wild habitats may serve as a temporary niche, alternate food sources, or overwintering sites for *R. dominica* in the absence of major human-stored hosts (Mahroof and Phillips, 2007).

Movement of insects between localities and ecosystems on a daily, seasonal, or annual basis represents a fundamental aspect of the ecology of populations and individuals. Understanding linkages between habitats used by insects throughout their life history is critical in terms of pest management. Pest management efforts for stored-product insects can be directed to either breeding sites, overwintering sites, or outside of the grain storage habitat as appropriate (Ramirez-Martinez et al., 1994). For example Hagstrum and Subramanyam (2006) indicated that knowledge of insect spatial distribution leads to improve pest suppression by treating isolated areas with a combination of an insect attractant and...
residual insecticide. The ability of *R. dominica* to reproduce in various habitats has been partially elucidated in previous work (e.g., Edde and Phillips, 2006a; Mahroof and Phillips, 2007) and would benefit from further investigations.

In the past, origins and movement of various animal species have been investigated using intrinsic methods of tissue assays that can be related to some geographical area (McLean et al., 1979; Hobson, 1999; Callaham et al., 2000). When populations vary in their intrinsic molecular compositions across areas, natal or sub-population may be inferred for individuals recovered elsewhere using various techniques. One such technique is the elemental profiling to track the dietary history of insects (Tigar and Waldron, 2002). Individual insects living in a particular environment may contain a combination and quantity of chemical elements characteristic to their environments. Populations of different origins may thus have different and characteristic elemental compositions. These variations in elemental compositions may enable researchers to separate or determine the geographical origins of the sub-populations (Bowden et al., 1984; Bowden et al., 1985a, b). Knowledge of the geochemistry of a locality where phytophagous insects occurs is important to differentiate their geographical origins. Analyses were done in the past (Bowden et al., 1984) for a series of elements, including S, P, Ca, K, Cl, Si, Al, Fe, and Mg and chemo-prints were directly related to the influence of environment on noctuid moth feeding on different host plants.

The essential elements required for plant growth that are ultimately found in tissues of phytophagous insects are segregated into two categories. They are the so-called macro elements and the trace elements. The distinction between macro and trace elements simply reflects the relative concentration found in tissues. Elements such as Mg, P, S, K, and Ca are referred as macro elements because they are required in large amounts (>10 mmol kg⁻¹ of tissue dry-weight). Mn, Fe, Cu and Zn are considered as trace elements and these elements are required in relatively small quantities (<10 mmol kg⁻¹ of tissue dry-weight) for plant growth (Hopkins and Huner, 2004).

The broad objective of the work described below was to demonstrate the utility of elemental analyses to determine the origin, and dispersal of *R. dominica* by relating elemental profiles to the resource use pattern and feeding ecology of the beetles. To determine whether *R. dominica* captured in non-agricultural woodlands can be distinguished from those captured around commercial granaries by their elemental profiles, we hypothesized that populations from different localities can be differentiated by variation in elemental composition, assuming that variation in elemental composition among individuals captured from a particular ecosystem is less than that between individuals captured from a different ecosystem. A specific objective was to rear *R. dominica* in the laboratory using selected diets and then compare elemental profiles of lab-reared beetles with those of feral *R. dominica* captured in agricultural and wooded habitats.

### 2. Materials and methods

#### 2.1. Laboratory-reared *R. dominica*

Eggs from adult *R. dominica* were collected from an established laboratory colony in the Department of Entomology and Plant Pathology at Oklahoma State University, Stillwater, OK, USA. About 500, 10-day-old adult *R. dominica* were sifted from the colony and transferred to transparent plastic boxes (31 × 17 × 8 cm), each containing a mixture of 50 g of whole kernels of hard red winter wheat, *Triticum aestivum* L., (Poaceae), and 10 g of insecticide-free bleached wheat flour. Adults were incubated at 29 °C, 60% r.h., under a photoperiod of L12:D12 for a 24 h ovipositional period. The contents of the boxes were sifted using a top-to-bottom series of sieves, 1400-μm mesh (USA No. 14), 850-μm mesh (USA No. 20) (Seedburo Equipment Co., Chicago, IL, USA) and 212-μm mesh (USA No. 70; Fisher Scientific, Madison, WI, USA) to collect the *R. dominica* eggs from the grain, grain dust and other insects.

Whole kernels of wheat, shelled yellow corn, *Zea mays* L. (Poaceae) and seeds, or “acorns”, of *Quercus muhlenbergii* Engelm, chinquapin oak (Fagaceae) were selected as experimental hosts for rearing larvae of *R. dominica* and subsequent elemental analyses. Wheat and corn were selected because these are the most common cereal grains grown and stored in large quantities in the southern USA. Chinquapin oak was selected because it was demonstrated to be a suitable larval food source for *R. dominica* (Edde and Phillips, 2006a; Mahroof and Phillips, 2007). Acorns from chinquapin oak were collected from wooded sites around Stillwater, OK. Wheat, corn and acorns were used for rearing *R. dominica* as described by Mahroof and Phillips (2007). Adults were separated from the experimental rearing diet two weeks after emergence, rinsed with double distilled water, air dried, transferred to an air-tight container, and stored in a freezer at −20 °C until chemical analyses. Un-infested samples of the three diets used for rearing *R. dominica* were also stored in the freezer for future analyses.

#### 2.2. Field-captured *R. dominica* adults

Four field sites were selected for the study, of which two sites were forested woodlands and the remaining two sites were agricultural grain storage areas. Pasture II (PII; 36°03′N; 097°10′W; ≈607 ha) and land adjacent to Lake Carl Blackwell (LCB; 36°07′N; 097°13′W; ≈268 ha) just west of Stillwater, OK served as the two forested woodland sites. Predominant plant species at both sites were *Q. muhlenbergii; Quercus stellata* Wangen., post oak; *Celtis occidentalis* L., hickberry (Ulmaceae); *Pinus taeda* L., loblolly pine (Pinaceae); *Cercis canadensis* L., eastern redbud (Leguminosae); and *Juniperus virginiana* L., eastern red cedar (*Cupressaceae*) (Edde and Phillips, 2006b). The surrounding area of the following two grain handling facilities used in this study, were considered as the two agricultural grain storage sites: the Stored Products Research and Education Center (SPREC) of Oklahoma State University near Stillwater (36°07′N; 097°08′W) and the Peavey Grain Co., Catosa, OK (36°19′N; 095°78′W), approximately 90 km to the east of Stillwater.

Four-unit Lindgren funnel traps (PheroTech, now Contech Enterprises, Delta, BC, Canada) were deployed in selected forested sites and periphery of the two grain storage facilities. Collection cups beneath funnel traps were baited with No-Pest strips (United Industries Corp., St. Louis, MO, USA) which release dichlorvos, a volatile insecticide intended to kill captured insects to prevent their escape from traps. All traps were baited with the synthetic *R. dominica* aggregation pheromone dominantalure 1 (DL1) and dominantalure 2 (DL2). Lures for funnel traps were prepared as described by Mahroof and Phillips (2007). Trapping was carried out during two periods in consecutive years, from July to October 2005 and May to August 2006. Traps were hung on polyvinylchloride pipe stands, 1.7 m above the ground with three traps per site and with a minimum spacing of 35 m between traps. Collection cups on the traps were emptied and trapped insects were collected on a weekly basis, and at the same time traps were serviced by replacing new fresh pheromone lures and new pieces of No-Pest strip. Trapped *R. dominica* were returned to the laboratory and stored in a freezer at −20 °C until chemical analyses.

#### 2.3. Elemental analyses for both laboratory-reared and field-captured *R. dominica*

Multiple chemical elements including macro elements such as P, Ca, K, Mg, Na and S and trace elements such as Fe, Zn, Cu and Mn...
were quantified for laboratory-reared insects, laboratory hosts and field-captured insects. The analyses were carried out at the Soil, Water and Forage Analytical Laboratory at Oklahoma State University, Stillwater, OK using Spectro Ciros Inductively Coupled Plasma-Atomic Emission Spectrometers (ICP-AES). Frozen whole insects were dried in a conventional oven at 60 °C for 48 h and ground to fine particles using a mortar and pestle. Approximately 100 mg of samples each were used for the laboratory-reared insects, field-captured insects and laboratory seed diets. Samples were placed in a digestion block and digested using concentrated trace metal grade nitric acid at 115 °C for 2.5 h and the clear digests were analyzed using the ICP-AES. All sample preparations were done in plastic materials to avoid metal contamination. Accuracy and precision of test results were assured through analyses of quality control samples from the North American Proficiency Testing Program (NAPT), one alfalfa check sample, and a method of blank sample. Element composition is reported on an "as is basis" such that no further drying of samples was done after submission to the analytical lab, and analyses were done at the same moisture contents as that of the samples submitted. The analytical description given above is related to the equipment available at the OSU Soil, Water and Forage Analytical Laboratory at the time of analysis. Elemental compositions for P, Ca, K, Mg, Na and S are reported as percentages whereas composition for Fe, Zn, Cu and Mn are reported as parts per million (PPM). The analyses for laboratory-reared insects, field- captured insects and laboratory diets were conducted three times.

2.4. Statistical analysis

Percentage or PPM data for element compositions were subjected to analysis of variance using the PROC GLM procedure of SAS 9.2 (SAS Institute, 2010). Least squares means were used for separating treatment means at α = 0.05. Elemental data for laboratory hosts, lab-reared R. dominica, and field-captured R. dominica were analyzed separately.

3. Results

3.1. Elemental analysis for laboratory hosts and lab-reared R. dominica

Composition for major and trace elements in wheat, corn and acorns used for rearing R. dominica in the laboratory showed significant differences among host seeds for nine of the ten elements (P < 0.01 for each element; Figs. 1A and 2A). Of these differences, for K and Fe, acorns (oak) had significantly higher elemental compositions when compared to wheat or corn and for Ca, acorns had significantly higher content than corn but not different from wheat. The concentration of Cu among the seed types was not significantly different. R. dominica reared on wheat, corn, and acorn also showed significant variation (P < 0.05) in the elemental compositions for P, Na, S and Zn in their body tissues. The P, S and Zn contents were significantly higher for R. dominica fed on wheat or corn, but individuals fed on acorns had significantly lower quantities of P, S, Ca and K in their body tissues (Figs. 1B and 2B). For Na, R. dominica fed on wheat or acorns showed significantly higher quantities in their body tissue than for individuals fed on corn. Elemental composition for Ca, K, Mg, Fe, Cu, and Mn did not vary among R. dominica fed on wheat, corn or acorn. When compared to the quantity of elements in the insect body to their respective hosts fed in the laboratory, except for K, all other macro and trace elements were highly enriched in adult R. dominica. This enrichment was noteworthy for Na, which was undetectable in the plant samples and clearly enriched in beetles (Fig. 1A vs. B). The enrichment from diet to insect bodies was considerably higher for trace elements than macro elements.

3.2. Elemental analysis for field-captured R. dominica

Elemental analyses for field-captured R. dominica showed significant differences for all the elements quantified except for S among the different sites (Figs. 3 and 4). Differences between the quantities of elements in adults captured between two agricultural sites (SPREC and Catoosa) were striking but the differences were minimal between two wooded sites (Fig. 3). Individuals captured in the periphery of the Catoosa grain elevator had significantly higher amounts of P, Ca and K when compared to the adults captured from the periphery of SPREC or two forested sites. There were no significant differences for the amount of S found in individuals originating from distinctly different habitats. For trace elements, significantly lower quantity of Zn, Cu, and Mn were found in individuals captured around Catoosa grain elevator when compared to SPREC (Fig. 4). Zn also seems to be the lowest enriched element in individuals from LCB or PII when compared to other trace elements from same sites.

Comparison of the laboratory-reared insects to field-caught insects shows that Zn may potentially be used as an indicator to determine whether R. dominica were found in agricultural or...
for field-captured R. dominica. Mean values were expressed in parts per million (PPM), each on a dry-weight basis. Abbreviations in the legend are defined as SPREC: Stored Products Research and Education Center; LCB: Lake Carl Blackwell; P II: Pasture II; see text for details of the locations. Means are based on \( n = 3 \). Mean values within an element for different field sites followed by different letters are significantly different \( (P < 0.05, \text{LS means}) \).

Fig. 2. Mean \( \pm \) SE values of Fe, Zn, Cu, and Mn for laboratory-reared R. dominica. Bar graphs represent mean values for host materials (A) and adult bodies of R. dominica fed on corresponding host materials (B), each on a dry-weight basis. Mean values were expressed in parts per million (PPM) and are based on \( n = 3 \). Mean values within an element for different hosts followed by different letters are significantly different \( (P < 0.05, \text{LS means}) \).

Fig. 3. Percentage mean \( \pm \) SE composition of P, Ca, K, Mg, Na, and S for field-captured R. dominica, each on a dry-weight basis. Abbreviations in the legend are defined as SPREC: Stored Products Research and Education Center; LCB: Lake Carl Blackwell; P II: Pasture II; see text for details of the locations. Means are based on \( n = 3 \). Mean values within an element for different field sites followed by different letters are significantly different \( (P < 0.05, \text{LS means}) \).

Fig. 4. Mean \( \pm \) SE values of Fe, Zn, Cu, and Mn for field-captured R. dominica. Mean values were expressed in parts per million (PPM), each on a dry-weight basis. Abbreviations in the legend are defined as SPREC: Stored Products Research and Education Center; LCB: Lake Carl Blackwell; P II: Pasture II; see text for details of the locations. Means are based on \( n = 3 \). Mean values within an element for different field sites followed by different letters are significantly different \( (P < 0.05, \text{LS means}) \).

4. Discussion

Several studies were carried out in the past to determine the elemental profiles of insects and use these elements as biomarkers for various indices. For example, to identify the survival and dispersal of entomophagous insects in the field (Jackson, 1991), to discriminate mass-reared sterilized males from feral males in sterile male-release studies (Burns et al., 1983), to use insects as contaminant biomonitors in support of general predictive models (Hare, 1992), and to relate the influence of environment on noctuid moth feeding on different host plants (Bowden et al., 1984). Individual insects utilizing a particular diet or environment may contain a combination and quantity of chemical elements characteristic to their diets or environments, and these characteristics may indicate different geographical origins of the sub-populations (Bowden et al., 1984). We used this principle to seek whether occurrence and abundance of R. dominica in different habitats such as stored-grain environments or wooded forest landscapes can be distinguished from each other. Data reported above clearly demonstrated that elemental profiles of adult R. dominica reflect the profiles of their host foods and such elemental profiles will differ among field populations that differ in geographic space and potential hosts available. Discerning the occurrence of R. dominica among different habitats is important because current pest management programs for R. dominica are designed for managing insects only in the grain, inside storage structures such as bulk grain bins (Reed et al., 1995; Sloderbeck et al., 2002). Elemental profiles may be useful in identifying habitats and geographic origins of R. dominica if the elemental profiles of host plants can be...
determined. Knowledge of spatial distribution of *R. dominica* aids in precognition targeted localized pest management approaches.

In the current study, among the ten elements compared, the main discriminant trace element for both laboratory-reared and field-collected *R. dominica* was Zn. When elemental compositions for laboratory-reared and field-captured adults were compared, the Zn concentration was highest in the wheat- or corn-fed *R. dominica*. Similarly, for field-captured *R. dominica*, adults collected around SPREC or the Catosa grain elevator had significantly higher concentration of Zn. Adults of *R. dominica* captured around the stored-grain environment likely may have fed on grain based products, which may potentially have contributed to the increased concentration of Zn in these adults. Analysis of laboratory acorn-fed *R. dominica* showed significantly lower amounts of Zn in their body tissues, as was the case with beetles captured in the two wooded sites, P II or LCB. We believe that the adults captured around wooded sites may possibly have fed on oak-based materials, a dominant plant species in these environments, and this hypothesis is supported by past research suggesting that oak is a suitable host for both development and pheromone production in *R. dominica* (Edde and Phillips, 2006a).

Our work in the past using stable carbon isotopes (Mahroof and Phillips, 2007) to identify prior food sources and habitats of *R. dominica* showed evidence for majority of insects in the agricultural field sites had $^{13}$C isotope values suggestive of development of these beetles on common hosts like wheat, a C 3 type plant. Our data also showed that *R. dominica* developed well on non-grain hosts such as acorn another C 3 type plant in forested sites. Current work further proved that elements like Zn may be used, to some extent, as biological markers to indicate the survival and development of *R. dominica* in wooded habitats.

Elemental composition has been used to delineate and predict origins and movements of insects (e.g., McLean et al., 1979), but conclusions that distinguish populations from different localities based on their elemental compositions must be carefully considered along with other information. Geographic locality, with its inherent differences in soil types, water chemistry and plant communities, is only a few of the potential causes of variability in elemental content found in insect bodies. Differences in elemental composition of insects may arise from differences between the sexes, differences in physiological state, and possibly from feeding on non-larval hosts by the dispersive adult stage, which may mask or alter any elemental pattern acquired during immature feeding. One limitation in the current study is that multiple insects were pooled for analyses and it is possible that single insect analysis may have provided a better insight of consistency or variation in elemental profiles within and between populations.

In summary, our current study in fact shows good potential for delineating the food sources of *R. dominica*, to confirm to some extent whether insects originate from agricultural or non-agricultural ecosystems, and that Zn may be a useful intrinsic elemental marker for future work. Thus, elemental markers may potentially be used as biological markers for delineating food sources and geographic origins of *R. dominica* for studies of population ecology and insights for pest management approaches.

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