Effects of Dietary Intake of Volcanic ash from Puyehue Cordon Caulle on *Tenebrio molitor* (Coleoptera: Tenebrionidae) Larvae Under Laboratory Conditions

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Effects of dietary intake of volcanic ash from Puyehue Cordon Caulle on *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae under laboratory conditions

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Abstract

The Puyehue Cordon Caulle volcanic outbreak from 2011 affected the Patagonian Region of Argentina. The insecticidal effect of volcanic ash, as well as other inert dusts, has been proven to occur by contact exposure, disturbing the water balance in insects. However, little has been studied on sub-lethal effects of volcanic ash or inert dusts on coleopterans through chronic dietary intake. The aim of this study was to gain further understanding on the impact of volcanism on tenebrionid larvae by studying the chronic dietary exposure of volcanic ash in laboratory bioassays. For this purpose, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), an insect pest of stored grain, was used as an experimental model organism. Larvae were fed with flour discs prepared with volcanic ash at 500, 1,000, 5,000, 30,000, and 50,000 ppm. Chronic dietary intake of volcanic ash was dose-dependent, with effects visible after long-term exposure (15 and 27 d). Mortality was observed at the two highest concentrations tested (30,000 and 50,000 ppm). At the lower concentrations (500, 1,000, and 5,000 ppm), sub-lethal effects were observed, including larval size decrease, weight loss, and reduction in the number of molts. Thus, chronic dietary intake of volcanic ash causes adverse effects on *T. molitor* larvae that leads to mortality at high concentrations and decrease of larval size at sub-lethal concentrations.

Key Words: Dietary toxicity; insecticidal activity; Puyehue-Caulle Range; volcanic dusts

Resumen

El complejo volcánico Puyehue-Cordón Caulle entró en erupción en 2011 y afectó la región Patagónica Argentina. El efecto insecticida de la ceniza volcánica, así como el de otros polvos inertes, se ha demostrado que ocurre por exposición por contacto, perturbando el balance hídrico en los insectos. Sin embargo, poco se ha estudiado sobre los efectos subletales de cenizas volcánicas o polvos inertes sobre coleópteros mediante la exposición alimentaria crónica. El objetivo principal del estudio fue obtener una mayor comprensión del impacto del vulcanismo sobre las larvas de tenebriónidos mediante el estudio de la exposición alimentaria crónica a ceniza volcánica en bioensayos de laboratorio. Para este propósito, se utilizó a *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) como organismo modelo experimental dado que es una plaga de granos almacenados. Las larvas fueron alimentadas con discos de harina preparados con ceniza volcánica a 500, 1,000, 5,000, 30,000, y 50,000 ppm. Se descubrió que la exposición alimentaria crónica de ceniza volcánica en larvas de *Tenebrio molitor* fue dosis-dependiente con efectos visibles después de una exposición a largo plazo (15 y 27 días). La mortalidad fue observada en dos de las concentraciones altas testeadas (30,000 y 50,000 ppm). A bajas concentraciones (500, 1,000, y 5,000 ppm), efectos sub-letales como la disminución del tamaño larval, pérdida de peso y reducción en el número de mudas fueron registrados. Por lo tanto, puede concluirse que la exposición alimentaria crónica a ceniza volcánica causa efectos adversos sobre las larvas de *T. molitor*, que conducen a mortalidad a altas concentraciones y a una disminución en el tamaño de las larvas a concentraciones sub-letales.

Palabras Clave: Toxicidad alimentaria; actividad insecticida; polvo volcánico; Puyehue-Cordón Caulle

Volcanic activity has very evident direct effects on arthropods due to the immediate and massive loss of habitat, environmental perturbations, and toxicity (Buteler et al. 2011). Volcanoes are a natural source of micro- and nanoparticles, which become electrically charged near the vent, mainly by fractoemission, triboelectrification, and further self-charging which lead to a wide array of particle sizes (Aplin et al. 2014). Moreover, nano-sized ash particles behave significantly different than bulk ash particles due to surface and quantum effects that influence the chemical reactivity of materials, as well as their mechanical, optical, electric, and magnetic properties (Roduner 2006). These traits may be responsible for the contact insecticidal toxicity of volcanic ash (Buteler et al. 2011), and its effect may be compared to that of other inert dusts like diatomaceous earth, which is used commercially as insecticide (Buteler et al. 2014).

The proposed mechanisms responsible for the insecticidal activity of inorganic dusts like volcanic ash include: (a) toxicity by ingestion, (b) dehydration, (c) chemical reactions at the insect cuticle, and (d) direct mechanical action (Chiu 1939; Edwards & Schwartz 1981). However, it has been shown that the main insecticidal mechanism of action of inorganic dusts is dehydration due to abrasive and sorptive effects on the insect surface (Ebeling 1971). Furthermore, mortality of insects exposed to inorganic dusts correlates inversely with particle size, and directly with the angular structure and material hardness (Alexander et al. 1944). Other phenomena, such as poisoning, may contribute to the

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insecticidal effect of inorganic powders in addition to contact toxicity, affecting generation time and survival (Ebeling 1971).

On 4 Jun 2011, after a dormant period of over 50 yr, the Puyehue Cordon Caulle Volcanic Complex in Chile (40.5234°S, 7.1464°W) erupted violently sending over 1,460 million cubic m of pyroclastic material into the atmosphere. The material was transported by the westerly winds that prevail in southern South America, affecting an area of over 7.5 million ha in the Argentine Patagonia (Sernageomin 2011). The pyroclastic flow ranged in size from volcanic bombs and lapilli, to volcanic ash (Heiken & Wohletz 1985). The distinct eruption stages and distance from the volcanic source affected the chemical composition of the ash mixture deposited in different areas. However, the ash in general was amorphous to the X-ray diffractometry, with a characteristic vesicular texture, composed principally of glass shards (SiO_2), alumina (Al_2O_3), and pumice ash stone and other minerals, potash feldspars, and plagioclases (Buteler et al. 2011).

The biotic effects caused by Puyehue Cordon Caulle ash fall were vast and highly varied, similar to those observed after the eruption of the Mt. St. Helens (1981), Hudson (Aug 1991) and El Chaitén (May 2008) volcanoes (Edwards & Schwartz 1981; Miserendino et al. 2012). Most areas have undergone changes in substrate condition and in ecosystem dynamics, species richness, and composition in terrestrial environments (Fernández-Arhex et al. 2016).

Previous studies on insecticidal activity of volcanic ash in laboratory exposure bioassays on *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and *Dichroplus vittigerum* (Blanchard) (Orthoptera: Acrididae) have shown the acute insecticidal effect of volcanic ash from Puyehue Cordon Caulle through contact toxicity (Buteler et al. 2011; Buteler et al. 2014; Fernández-Arhex et al. 2016). Kuriwada & Reddy (2016) found that the fallen ash adheres to the surface of plants and insects. The fallen ash has been reported to have reduced dehydration tolerance (Kuriwada & Reddy 2016) and mating behavior in the field cricket *Gryllus bimaculatus* (De Geer) (Orthoptera: Gryllidae). The present study investigated the potential for chronic dietary intake and sub-lethal effects of volcanic ash given that most studies have assessed contact toxicity. For this purpose, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), a pest of stored grain that is distributed throughout the world, was used as a biological model (Mewis & Ulrichs 2001). We conducted laboratory bioassays to determine the effect of dietary intake of volcanic ash from the 2011 eruption of Puyehue Cordon Caulle on *T. molitor* larvae, to obtain information on the mechanism of action of volcanic ash, and to understand its effect on insect larvae exposed to volcanic ash, and to inert dusts in general. Moreover, given that volcanic ash has a similar mode of action as insecticidal inert dusts (Chiu 1939; Ebeling 1971), the results obtained should provide useful information with regard to management of stored grain pests with inert dusts.

Materials and Methods

VOLCANIC ASH

Three random ash samples of volcanic eruption from Puyehue Cordon Caulle were collected from the ground in Collon Cura, Neuquén, Argentina (40.0400°S, 70.2405°W), during the first days of the eruption (15 Jun 2011). Volcanic ash used contains mainly silicon oxide (SiO_2) and alumina (Al_2O_3) (Fig. 1). Particle size distribution is bi-modal, composed of a majority of large particles approximately 50 to 60 µm in diameter, and smaller particles peaking at 0.5 µm in diameter (Buteler et al. 2014).

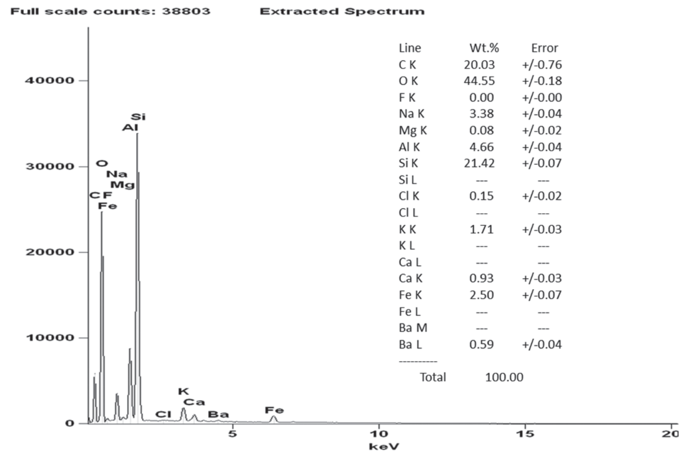

Fig. 1. Chemical composition of ash from Puyehue Cordon Caulle eruption collected in Collón Curá, Neuquén, Argentina (40.0400°S, 70.2405°W) 15 Jun 2011, determined by energy dispersive spectroscopy. Previously published in Buteler et al. (2011), Revista de la Sociedad Entomológica Argentina 70 (3–4), Figure 3, copyright RSEA, reproduced with permission.

TEST INSECTS

Tenebrio molitor L. 1758 (Coleoptera: Tenebrionidae) larvae were obtained from colonies with no history of exposure to insecticides, reared at the Laboratory of Environmental Toxicology IMBECU CONICET CCT-Mendoza on a mixture of wheat flour, broken peanut grains, and potatoes. The cultures were maintained in a rearing chamber in darkness at 27 ± 1 °C and 70 ± 5% RH. The desired RH was maintained by using a saturated solution of sodium chloride in water (Winston & Bates 1960).

DIETARY INTAKE BIOASSAY

The concentrations of volcanic ash used in the bioassay were obtained from preliminary tests carried out under controlled conditions. Chronic dietary intake of volcanic ash was tested on *T. molitor* larvae in a bioassay adapted from Talukder & Howse (1994). Larvae were fed flour discs containing 50,000 ppm of volcanic ash mixed with wheat flour and water. The flour discs were prepared with 9.5 g of white wheat flour, 0.5 g of volcanic ash, and 15 mL of distilled water (mQ quality) in a 100 mL Griffin glass beaker and mixed to homogeneity with a teflon-coated microbar. The flour discs containing 30,000 ppm were prepared with 0.3 g of volcanic ash mixed with 9.7 g of white wheat flour and 15 mL of distilled water. Using the same procedure, the mixtures of 5,000, 1,000 and 500 ppm were prepared with 0.05 g, 0.01 g and 0.005 g of volcanic ash mixed with 9.95 g, 9.99 g and 9.995 g of white wheat flour, following the order mentioned. The control discs were prepared with wheat flour and water. Aliquots of 100 µL of each suspension were placed on a Petri dish, and left to dry overnight at room temperature. Further, all disks were stabilized at 27 ± 1 °C and 70 ± 5% RH for 24 h. Each experiment consisted of 10 *T. molitor* larvae ranging from 12 to 16 mg. For this purpose, we used a precision electronic balance Chyo® model JK-180 with 1-mg readability to obtain the weight of each larvae. Larvae were distributed individually in the wells of a 72-well Tissue Culture Test Plate to avoid cannibalism. At the start of the bioassay, 3 disks, weighing between 80 and 100 mg, were rough milled and placed in each well of the Tissue Culture Test Plate to provide ad libitum food for each larva. Test plates were placed in an incubator at 27 ± 1 °C and 70 ± 5% RH in darkness. Body weight, molting, and mortality of larvae were recorded every 72 h for a 27-d incubation period. The endpoint of 27 d was chosen to allow for several molts, and enough time for the sub-lethal effects to be noticeable. Larval body length was measured using an electronic digital caliper Model CD-600 BS (Mitutoyo Corp., Kanogawa, Japan).

DATA ANALYSIS

Larval body weight and body length were measured in mg for each treatment at 3-d intervals up to 15 d for highest concentrations and 27 d for lower concentrations. An overall mean body weight was calculated for each treatment. Analysis of variance (ANOVA) was used to compare mean body weight among treatments, and final body length at the end of the bioassay. Statistical analyses were performed using software InfoStat version 2017 (http://www.infostat.com.ar/), with total mean body weight of larvae (mg), or final body length, as the response variable and treatment as primary effect. LS Means were compared using the Tukey procedure. Mortality data was expressed in percentage and molting in larvae was expressed as molting rate. Molting rate was calculated as the ratio of number of molts to the total number of incubation days.

Results

Continuous exposure of *T. molitor* larvae for 15 d to food treated with volcanic ash at 30,000 ppm and 50,000 ppm led to almost complete mortality, 70% and 100%, respectively. Larval body weight also was significantly different among treatments ($F = 93.67$; df = 2; $P < 0.0001$) (Fig. 2). At the lower concentrations of 500, 1,000, and 5,000 ppm, no mortality occurred, although treatment with volcanic ash reduced larval weight at all concentrations tested when compared to the control during the 27-d incubation period ($F = 133.97$; df = 3; $P < 0.0001$) (Figs. 3, 4). There also was a significant reduction in body length at the end of the bioassay among treatments ($F = 95.15$; df = 3; $P < 0.0001$) (Fig. 5) which was dose-dependent. Molting rate (MR) also was dose-dependent and inversely proportional to volcanic ash content in food (Fig. 6). The molting rate decreased as volcanic ash in food increased and was highest at the highest sub-lethal concentration tested (5,000 ppm). Larval growth and weight were inversely proportional to the concentration of volcanic ash in food.

Overall, treated larvae showed significant differences in body weight, in development (growth delay), and showed a reduced molting frequency compared with untreated larvae (controls) (Figs. 2–6).

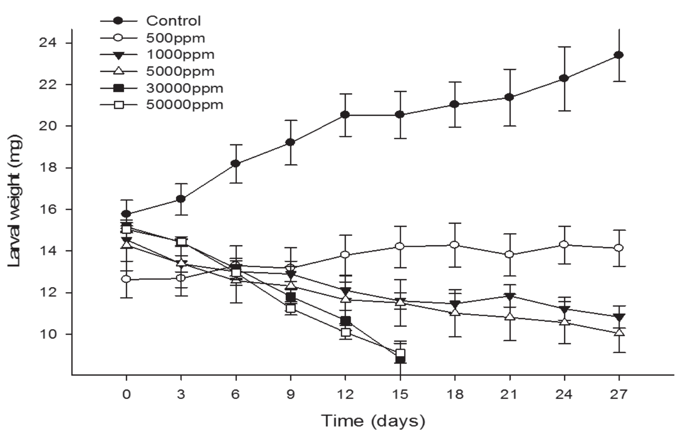

Fig. 2. Larval body weight of *Tenebrio molitor* larvae over the duration of the study, 27 d. Larvae were fed with flour disks treated with 500, 1,000, 5,000, 30,000, and 50,000 ppm of volcanic ash.

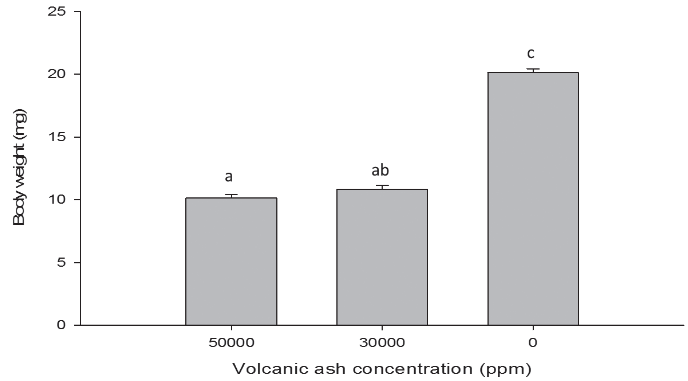

Fig. 3. Mean body weight of larvae (mg) fed 30,000 and 50,000 ppm of volcanic ash treated flour disks after 15 d. Bars with the same letter are not significantly different α = 0.05. Bioassay endpoint = 15 d, n = 10, substrate = treated and control insect food (ANOVA: $F = 93.67$; df = 2; $P < 0.0001$).

Discussion

Typically, bioassays testing insecticidal efficacy of inert dusts such as diatomaceous earth and volcanic ash, among others, evaluate product efficacy through contact toxicity as the main mode of action (Ebeling 1971; Buteler et al. 2014). However, results from the present study demonstrate that chronic dietary intake of volcanic ash contributes significantly to larval mortality in *T. molitor* and also leads to sub-lethal effects on growth and development at concentrations of 500, 1,000, and 5,000 ppm. At the highest concentrations tested, 30,000 and 50,000 ppm, mortality of *T. molitor* larvae started after 15 d and reached 70 and 100%, respectively.

There was an inversely proportional effect of volcanic ash concentration on body weight. These sub-lethal effects on body weight and reduced molting rate are distinctive symptoms of stress. Previous studies have shown volcanism results in reduced diversity of marine plankton invertebrates by eliminating most k-strategy species, and a reduction in specimen size in r-strategists (Keller & Abramovich 2009). Dwarfism or size decrease has been defined as a stress signal that leads organisms to optimize size and reproductive ability to survive. Thus, species that endure biotic or abiotic stress can exhibit reduction in body size to less than half their normal adult size (Twitchett 2001). In principle, biotic recovery would be expected to begin as soon as volcanic activity ends. However, the recovery of populations affected by volcanic ash is a complex process, due to the impact of this disturbance

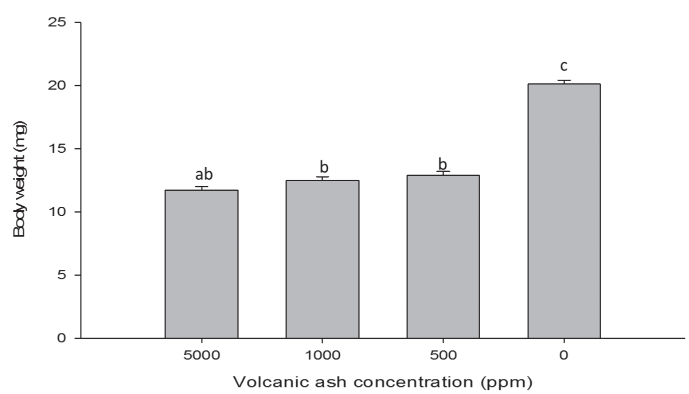

Fig. 4. Mean body weight of larvae (mg) fed on sub lethal concentrations (500, 1,000, 5,000 ppm) of volcanic ash treated flour disks. Bars with the same letter are not significantly different at α = 0.05. Bioassay endpoint = 27 d, n = 10, substrate = treated and control insect food (ANOVA: $F = 133.97$; df = 3; $P < 0.0001$).

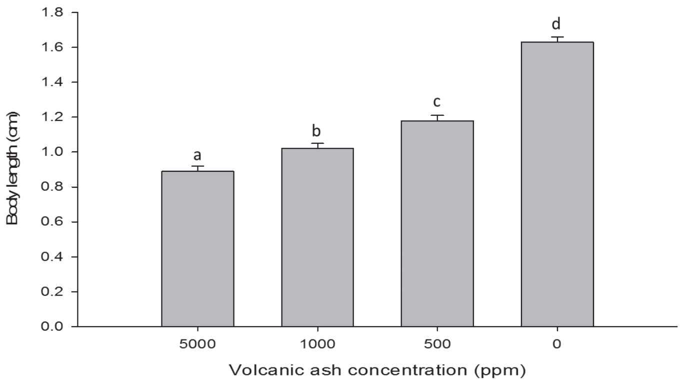

Fig. 5. Larval body length (cm) of *Tenebrio molitor* larvae fed on flour disks treated with sub-lethal concentrations (500, 1,000, 5,000 ppm) of volcanic ash. Bars with the same letter are not significantly different α = 0.05. Bioassay endpoint = 27 d, n = 10, substrate = treated and control insect food (ANOVA: F = 95.15; df = 3; P < 0.0001).

in the reproduction and development of surviving individuals. In desert areas, where dry ash persists, arthropod populations can be subjected to stress conditions for longer periods (Edwards & Schwartz 1981) because ash particles show greater insecticidal effect under low relative humidity environments. In contrast, ground-dwelling insects living in humid environments have a better chance to survive after ash fall given compaction of the ash with rain, which allows them to forage without picking up particles (Akre et al. 1981). However, ash also can be incorporated into the soil by rain, inhibiting feeding of soil insects, eventually resulting in mortality through chronic dietary exposure. Thus, there should be tangible long-term consequences due to volcanic ash on the surviving biota after ash fall out, especially in cases where adults of a species are epigeous and larvae hypogeous, showing vulnerability to ash during both phases of the insect life cycle.

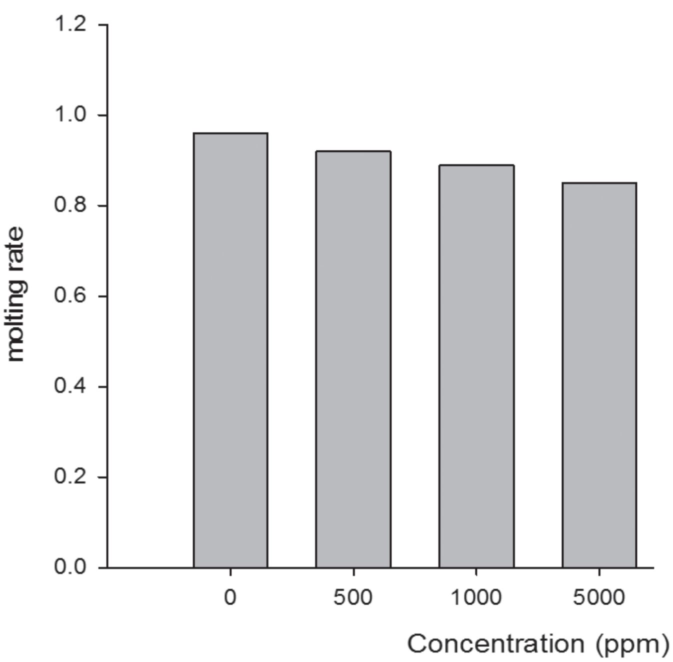

Fig. 6. Molting rate of *Tenebrio molitor* larvae feed on flour disks treated with sub-lethal concentrations (500, 1,000, 5,000 ppm) of volcanic ash. Molting rate = number of molts per incubation period of 27 d.

In conclusion, chronic dietary intake of volcanic ash, even in absence of contact toxicity, was observed as a mortality factor in *T. molitor* larvae fed on high doses of volcanic ash, and also as a major abiotic stressor at low doses. *Tenebrio molitor* larvae exposed to volcanic ash treated food at sub-lethal concentrations showed distinctive size decrease or dwarfing symptoms along with reduction in molting frequency. The extent of dwarfing caused by chronic dietary intake of volcanic ash to *T. molitor* larvae was dose-dependent and a good indicator of the degree of stress caused by volcanic ash on this species. The current bioassay results could be extrapolated to natural environments contaminated with ash due to volcanic activity, suggesting the potential of volcanic ash as an abiotic stressor to tenebrionid larvae.

Given that volcanic ash has a similar mode of action to other inert dusts, such as diatomaceous earth and nanoparticulate dusts used for insect pest control, it is expected that inert dusts also will cause mortality and sub-lethal effects through chronic dietary exposure, adding to the contact toxicity, which is typically the major factor considered when determining efficacy of inert dusts. Thus, these results have implications for the use of inert dusts for stored grain management given that sub-lethal effects through dietary exposure would lead to long term mortality, which should be taken into consideration.

Acknowledgments

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