



# Comparison of three different wood ashes and diatomaceous earth in controlling the maize weevil under laboratory conditions

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## ABSTRACT

We studied the insecticidal effects of ashes from three different tree species (common beech, Norway spruce, and black locust) on the mortality of *Sitophilus zeamais* adults under laboratory conditions. A diatomaceous earth formulation, SilicoSec<sup>®</sup> was used as a positive control. We studied the contact effects of ashes on adults and the insecticidal effect of ash mixed with wheat grain. The experiment was carried out at three different temperatures (15, 20 and 25 °C) and at two different relative humidity [r.h.] values (55% and 75%). The study on contact effects (immediate mortality and delayed mortality) was carried out in Petri dishes, where we applied ash in two concentrations, 10 and 20 g/m<sup>2</sup>. After 7 d there was more than 97% mortality for the beetles in all three wood ashes at 25 °C and 55% R.h., while after 14 d wood ash of Norway spruce showed the highest effectiveness (87%) at 20 °C and 55% R.h. 96% mortality was recorded at 20 °C and 55%, when SilicoSec<sup>®</sup> was applied. The experiment with the mixture of ashes and grains was performed by mixing 2.5 or 5 w% of ash with wheat. In both approaches, we established that lower R.h. in combination with higher temperatures led to higher adult mortality rates. Regardless of the approach, the preparation concentrations did not influence adult mortality. The most efficient ash was that of Norway spruce, yet we attribute its effectiveness not only to its highest content of SiO<sub>2</sub> (11.68%) but also to the other ingredients in ash, which can enhance its hygroscopic properties. Our research demonstrated that wood ash can efficiently protect stored crops from maize weevils. However, before introducing wood ash into the systems of protecting stored wheat grains against harmful insects, the influence of concentrations of ashes and their hygroscopic properties should be studied.

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## 1. Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is considered an insect pest, which has spread all over the world, and causes damage to maize grains and other species of stored cereals (Jean et al., 2015). Both adults and larvae feed on internally on grains and, although an infestation can start in the field, most damage occurs in storage (Suleiman et al., 2015). In stored maize, heavy infestations of these pests may cause weight losses of as much as 30–40%, although losses are commonly 4–5%. *S. zeamais* has been found to be amongst the most important pests of maize in the USA (Arbogast and Throne, 1997; Sedlacek et al.,

1998), Asia (Peng, 1998), and Europe (Trematerra et al., 1999). In the past, its suppression based on the use of DDT, malathion, pirimiphos-methyl, pyrethroids, etc. (Corrêa et al., 2011), which in many instances caused resistance and other negative effects (Jean et al., 2015). Recently, research in the field of alternative suppression methods has focused on the application of various essential oils (Tofel et al., 2016), as well as on use of different inert dusts, such as diatomaceous earth (Athanasios et al., 2011).

Inert dusts are all dry dusts that do not cause chemical reactions in nature. According to their chemical and physical composition and their level of activity, they are classified into several groups (Korunić, 1998; Liška et al., 2017), respectively non-silica dusts, group that contains sand, kaolin and wood ash; group with diatomaceous earths (or diatomite) mostly from amorphous hydrated silica, group with diatomaceous earths with high quality synthetic silicates and group with silica aerogels (Golob, 1997; Liška et al.,

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2017). The groups that are typically used locally (on small farms) and in less developed countries are kaolin and wood ash. The latter is known as one of the oldest insecticides (Panagiotakopulu et al., 1995), and its importance has increased in recent years, particularly due to the problems related to synthetic insecticides (Tofel et al., 2016; Guedes et al., 2017).

A recent study from Slovenia (Bohinc and Trdan, 2017) demonstrated the high insecticidal effectiveness of common beech ash against the granary weevil, *Sitophilus granarius* (L.) (Coleoptera:Curculionidae). According to the research, lower relative humidity level had negative impact on mortality after exposure intervals and also on progeny production. High efficacy of wood ash was demonstrated when wood ash was applied alone and at combined use with other products. Application of wood ash alone has been previously demonstrated by Akob and Ewete (2007) and Jean et al. (2015). Combined use of wood ash and other dusts as grain protectants has never been studied before research done by Bohinc and Trdan (2017). Most of research on this topic is focused on diatomaceous earth and zeolites (Korunić, 1998; Athanassiou et al., 2011, Eroglu et al., 2017). In this regard, numerous studies have proved that the insecticidal efficacy of diatomaceous earths (DE) differs according to its origin (Athanassiou et al., 2011), application dose rate, temperature and exposure intervals (Athanassiou et al., 2005), particle size (Rumbos et al., 2016), etc. According to Bohinc et al. (2018), the insecticidal efficacy of zeolites is also influenced by their origin. In contrast with the above, there are very few studies on the insecticidal effect of ashes in stored product protection (Akob and Ewete 2007; Bohinc and Trdan, 2017). Moreover, the chemical analysis of these materials has not been examined in detail, in terms of the physical and chemical parameters that affect their insecticidal efficacy. Jean et al. (2015) stated that future studies should focus the insecticidal properties of wood ashes from different woody plant species to determine the most efficient species.

The purpose of our research was to study the effects of wood ashes from three different tree species on adult mortality of the *S. zeamais*.

## 2. Materials and methods

### 2.1. Test insects and applied inert dusts

*S. zeamais* adults were reared at room temperature ( $22 \pm 2^\circ\text{C}$ ), and relative humidity (r.h) ( $55 \pm 5\%$ ) and continuous of darkness. The insects were maintained for eight months at the Laboratory of Entomology, Chair for Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management, Biotechnical Faculty, Ljubljana. *S. zeamais* were brought from the Pesticide and Environment Research Institute (Belgrade, Serbia) as part of a scientific cooperation between the institute and the Biotechnical Faculty (Ljubljana, Slovenia).

Our research was based on three different wood ashes and one DE formulation that was used as the positive control. We used wood from three different tree species, i.e., black locust (*Robinia pseudoacacia* L.), common beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karsten). The wood used for preparing the wood ash was acquired from the trees in the vicinity of the village Žlebe (latitude  $46^\circ 7' 14.13''\text{N}$ , longitude  $14^\circ 23' 41.45''\text{E}$ , 381 m above sea level). The wood was air-dried for several months in shadowed place. Diatomaceous earth (SilicoSec®, Biofa AG, Germany) served as the positive control. Wood ashes were obtained from different tree species, which were burned in wood-burning stove in January 2017. Each tree species wood was burned separately. Ashes were sieved through 1 mm mesh and packed into 1 l plastic jars with wide neck. Plastic jars were kept at room conditions in darkness,

until needed for bioassay.

### 2.2. Surface treatment (Bioassay 1)

The testing was carried out in plastic Petri dishes with a diameter of 13.5 cm and a surface area of  $153.5\text{ cm}^2$ . Three different wood ashes and SilicoSec® were applied at two different rates, 10 and  $20\text{ g/m}^2$ . We dispersed the inert dusts all over the Petri dish surface. Twenty *S. zeamais* adults of mixed sex and age were added to each Petri dish. Maize weevils were 2–4 weeks old.

Adult mortality was determined after the 1st, 2nd, 3rd, 4th and 7th day of application. Ten wheat grains (variety 'Olimpija') were also added to the Petri dish. Clean Petri dishes (without inert dust) served as the control treatment. After the 7th day, live adults were moved to untreated clean Petri dishes, containing only 20 grains of wheat. On the 8th, 9th, 10th, 11th and 14th, we evaluated delayed mortality. Bioassays were performed at three different temperatures ( $15$ ,  $20$  and  $25^\circ\text{C}$ ) and two different r.h. values ( $55$  and  $75\%$ ). Exposure studies were carried out at rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) at continuous darkness. All bioassays were repeated three times (three series of petri dishes) by preparing new treated and control petri dishes each time ( $3 \times 3$  petri dishes for each combination).

### 2.3. Admixture (Bioassay 2)

Plastic 1000 ml containers were filled with 270 g of winter wheat grains, variety 'Olimpija'. The efficacy of the inert dusts was tested at two different rates, 2.5 and 5% of dust per grain weight. The preparation of each individual treatment was conducted according to the process in Bohinc and Trdan (2017). Twenty *S. zeamais* adults were placed into 60-ml flasks. Untreated wheat served as control. Bioassays were performed at the same temperature and r.h. combinations as in the previous experiment. All bioassays were repeated three times. For each specific treatment exposed to specific parameters (temperature and r.h.), all 60-ml glass flasks were placed into 1000 ml vials. Three 60-ml glass flask were placed into one 1000 ml vial, and placed at the conditions mentioned above. After 56 d, the vials were opened, and then, the numbers of offspring (progeny) were counted. The number of progeny was expressed as the total number of alive adults per combination of specific temperature and r.h.

### 2.4. Geochemical analysis of wood ashes

Geochemical analysis of investigated wood ashes was carried out by inductively coupled plasma emission spectroscopy (ICP-ES) and mass spectroscopy (ICP-MS) at Bureau Veritas Mineral Laboratories Vancouver, Canada (Bureau Veritas, Mineral Laboratories, 2018). The analytical quality based on international standards. Prepared sample is mixed with  $\text{LiBO}_2/\text{Li}_2\text{B}_4\text{O}_7$  flux. Crucibles are fused in a furnace. The cooled bead is dissolved in ACS grade nitric acid and analyzed by ICP and/or ICP-MS. For the whole rock analysis by ICP-ES/MS 0.5 g of sample was leached in hot modified Aqua Regia (solution of equal parts HCl,  $\text{HNO}_3$  and  $\text{DI H}_2\text{O}$ ) for one hour in a heating block or hot water bath. Sample is made up to volume with dilute HCl (Table 1).

CRM standards used for the Leco (C & S) were GS311-1 and GS910-4, while in-house standard used for the LOI was Dolomite3 and SO-19. In-house standard used for the LF200 (trace element by fusion ICPMS and major elements by fusion ICP) was SO-19, and in-house standard and CRM used for AQ200 (trace elements by aqua regia ICPMS) were DS11 (inhouse) and Oreas 45Ea (CRM).

Equipment used were ELAN 9000 for the LF100 trace elements

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