Bulletin of Insectology ***70*** *(1): 17-25, 2017*
ISSN 1721-8861

Nanostructured alumina: biocidal properties and mechanism of action of a novel insecticide powder

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Abstract

Pest control using synthetic chemical pesticides is facing economic and environmental challenges worldwide. In this context, nanomaterials, emerge as potential tools that need to be investigated. The aim of this study was to examine the insecticidal effect and mode of action of nanostructured alumina (NSA) on *Sitophilus oryzae* (L.) (Coleoptera Dryophthoridae), an insect pest of stored products. Results demonstrated that the mechanism of insecticide action of NSA is based on physical phenomena rather than on biochemical mechanisms. Charged NSA particles attach to the insects cuticle due to triboelectric forces, sorbing its wax layer by surface area phenomena, resulting in insect dehydration. Moreover, intake of NSA particles was observed to be an additional mortality factor in exposed insects. Results show that NSA could provide an alternative to conventional synthetic organic insecticides and warrant further research on the development of enhanced NSA derived products.

Key words: nanoinsecticide, biological efficacy, mode of action, *Sitophilus oryzae*.

Introduction

The rapid incorporation of nanotechnology in various fields such as medicine, engineering, electronics and agriculture proves its enormous potential for developing new products. Nanomaterials are likely to have a key role in the evolution of agriculture, due to the wide spectrum of possibilities for the development of new technologies and strategies for pest control (Benelli, 2016), enhancement of productivity using encapsulation techniques for slow release of pesticides (Adak *et al*., 2012), nutrients (Ni *et al*., 2011), nanoparticle-mediated DNA transfer in plants (Sekhon, 2014), and for precision farming (Rai and Ingle, 2012). Dust nanoinsecticides show a great potential for insect pest control in stored products with a lower impact on the environment, than traditional products (Stadler *et al*., 2010a; Debnath *et al*., 2011; Kah and Hofmann, 2014; Buteler *et al*., 2015). Thus, nanotechnology applied to food production represents the beginning of a new advanced agriculture, or at least a new challenge for agriculture (NNCO, 2006).

Reducing the particle size of a substance results in increased surface/volume ratio per unit weight, which generally correlates with an increase in toxicity of the material (Paull and Lyons, 2008), feature that has been used by some researchers to control various microorganisms and insects by applying nanoparticles. For example, it has been found that silver-based nanomaterials have pediculocidal and larvicidal effect (Jayaseelan *et al*., 2011; Arjunan *et al*., 2012) as well as antimicrobial properties (Chopra, 2007; Baek and An, 2011), and can be incorporated into textile products (Kulthong *et al*., 2010; Petkova *et al*., 2014). Nanostructured alumina (NSA), characterized by large aggregates of 40-60 nm particles with a large specific surface area (14 $m^2 \times g^{-1}$, Mimani and Patil, 2001), was recently discovered as a contact insecticide (Stadler *et al*., 2010a). NSA is supposed to have a detrimental effect on insect water balance, similarly to microparticulate insecticidal inert dusts (IDs) in general (Stadler *et al*., 2010b; Buteler *et al*., 2015). The discovery of NSA's insecticidal activity opens new frontiers in pest management with IDs since the efficacy of NSA similar (Athanassiou *et al*., 2006; 2008; 2014) or greater than that obtained through some commercial diatomaceous earth (DE) based IDs (Stadler *et al*., 2010a). Also, long term treatments with NSA at low concentrations (62.5 ppm) were more effective in reducing rice weevils, *Sitophilus oryzae* (L.) (Coleoptera Dryophthoridae), progeny (F1) than commercially available microparticulate IDs for different humidity levels (Stadler *et al*., 2012).

Although dehydration appears to be the main cause of mortality, it cannot be assumed as the only one, for it is found that at sub-lethal concentrations, IDs exert further noxious effects on the insect (Subramanyam and Roesli, 2000; Kabir *et al*., 2011; Buteler *et al*., 2011; Buteler *et al*., 2014). Moreover, the striking differences observed in efficacy of insecticide dusts from different sources, among different insect species, and bioassay conditions (Arthur, 2000; Athanassiou *et al*., 2007; 2016; Kavallieratos *et al*., 2005; 2007a; 2007b; 2012; Shah and Kahn, 2014) mineral composition and type of formulation (Subramanyam and Roesli, 2000; Kavallieratos *et al*., 2005; 2007a; 2007b; 2012; 2015; Athanassiou *et al*., 2006; 2008; 2011; 2014; Iatrou *et al*., 2010) suggest a complex action mechanism involving several phenomena. Thus, investigations in this direction are necessary in order to understand all the factors involved in IDs efficacy and to determine if nanoparticulate materials have analogous insecticide mechanism of action as mi-

croparticulate materials.

The objective of this work was to gain further understanding on the mode of insecticide action of NSA by studying the effect of NSA and a microparticulate insecticide powder, DE, on the insect integument and its water balance, as well as on the insect's digestive system through laboratory bioassays. An insect pest of stored products was used as an experimental model organism.

Materials and methods

Test insects and bioassay conditions

Adults of the rice weevil, *S. oryzae* were used for the experiments. A laboratory strain of *S. oryzae*, without a history of exposure to insecticides (Strain LTA-IMBECU; insects obtained in 2005 from a type culture at the Faculty of Agricultural Sciences - University Buenos Aires) was reared in 250 mL flasks on wheat kernels var. Baguette 501 (NIDERA). Insects were reared in incubators, in continuous darkness, at 27 ± 1 °C and the relative humidity was maintained at 70 ± 5% RH, by using a reservoir with a sodium chloride saturated solution (Winston and Bates, 1960). Temperature and humidity inside the chambers were monitored with HOBO data recorders (Onset Computer, Bourne, MA, USA). Adults used in all experiments were 1-2 weeks old, of unknown sex and mating status.

Chemicals

Nanostructured alumina (NSA) was obtained by glycine-nitrate combustion synthesis technique using a redox mixture, with glycine as fuel and aluminum nitrate nonahydrate as oxidizer (Toniolo *et al*., 2005; Mimani and Patil, 2001). The bulk density of the powder was measured at 0.108 g/cm^3. NSA used was synthesized by the same procedure as reported by (Stadler *et al*., 2012). Particle size analysis conducted by the authors indicated evident platelet morphology where the thickness of the platelets was observed at 45 nm. NSA presented a bimodal size distribution with a high load of particles at 1.5 μm with a nanosized distribution of smaller particle diameters at 350 nm. Based on electro-magnetic properties, NSA tend to cluster and may behave as larger particles, depending on the surface reactivity, shape and size of the agglomerate (Karasev *et al*., 2004).

Diatomaceous earth (DE) was obtained from fossilized sedimentary deposits of single celled phytoplankton microalgae (diatoms) from San Juan-Argentina, which contains over 85% amorphous SiO_2; ≈4% Al_2O_3; ≈1% Fe_2O_3; ≈1% CaO and ≈2-4% water (DiatomiD®). The median particle size is 10 μm and particles range from 1 to 45 μm. Specific gravity is 0.22 g × cm^{-3} (Bilbao *et al*., 2007).

NSA exposure toxicity bioassays

The bioassay methodology was the same as that reported by Stadler *et al*. (2010a). Adult *S. oryzae* were exposed to NSA and DE respectively, at different concentrations and with continuous exposure of 7 days.

The wheat kernels used as bioassay substrate were acclimatized in an incubator at 27 ± 1 °C and 75% RH for one week. Once the grain reached the target moisture content, NSA or DE where added to achieve a concentration of 500 ppm. Then, 250 ppm, 125 ppm, and 62.5 ppm dilutions were prepared for each product by mixing with untreated wheat thoroughly to allow an even distribution of the powder through the entire grain mass. Twenty grams of each dilution were distributed in Petri dishes. A Petri dish containing 20 g of untreated wheat was used as control. This procedure was repeated ten times independently. Twenty 1-2 weeks old unsexed adult *S. oryzae* were placed in each Petri dish and these were then placed in an incubator at 27 ± 1 °C and 75% RH, in darkness. Adult mortality was assessed seven days after continuous exposure to the treated wheat.

Statistical analyses were performed using SAS (version 9.3, SAS Institute Inc.). Adult mortality was tested by analysis of variance (ANOVA) using a mixed-model design (PROC MIXED). Selection of covariance structure was based on the methods of Littell *et al*. (2006). Assumptions of normality were evaluated by visual examination of residual plots and homogeneity of variances by Levene's test (Levene, 1960). Data were transformed where necessary to meet these assumptions. When necessary, mortality data were corrected using Abbott's formula (Abbott, 1925). The LC_{50} and LC_{95} values (ppm) and 95% confidence limits (95% CLs) were calculated by probit analysis. The χ^2 value was used to measure the goodness of fit of the probit regression line. A significant χ^2 value indicated that the probit model failed to fit the observed dose-response data well.

Scanning electron microscopy (SEM) was used to analyse five dead insects from 500 ppm NSA and DE treated wheat exposure bioassays. Insects were dried in an oven at 60 °C and glued separately for SEM with conductive carbon paint on a 12 mm aluminium sample stub. Specimens were coated with an ultrathin graphite layer. SEM images and mapping of the relative proportion of *Al* and *Si* on insect body surface were performed with a JSM-6510 Series Scanning Electron Microscope with integrated Energy Dispersive Spectroscopy (EDS) at the MEByM, CONICET-Mendoza.

NSA intake toxicity bioassays

The insecticidal activity of NSA upon ingestion was assessed on *S. oryzae* adults using the flour disc bioassay adapted from Talukder and Howse (1994). Control disks were prepared with wheat flour and water and treated disks were prepared by mixing NSA with wheat flour and water (diet merging method, Talukder and Howse, 1994).

The flour disks were prepared as a suspension of NSA, wheat flour and water. This mixture was obtained by mixing white wheat flour, the insecticide and 150 mL of distilled water (mQ quality) in a 250 mL Griffin glass beaker, together with a Teflon-coated microbar. The mixture was stirred magnetically to homogeneity. Five different suspensions were prepared to achieve 500, 350, 250, 125, 75 and 36 ppm of NSA. Controls were prepared with wheat flour and water.

Table 1. Median lethal concentration values (ppm) and regression curve parameters for NSA and DE (DiatomiD®) in 7-day exposure laboratory bioassays using *S. oryzae* adults.

Product	LC_{50} (CLs)	LC_{95} (CLs)	Slope (SE)	Intercept (SE)	Goodness of fit χ^2 / P value
NSA	79.91 (102.70; 117.67)	213.14 (197.20; 233.85)	0.028 (0.01)	3.13 (0.22)	0.59 / 0.98
DE	365.76 (341.20; 393.82)	745.62 (686.97; 820.19)	0.008 (0.005)	−2.83 (0.16)	0.57 / 0.99

Aliquots of 100 to 200 µL were poured sequentially on a glass plate at 2 cm interval in rows. The preparation was air-dried overnight at room temperature, to produce the treated and control flour disks. Finally, the disks were placed in an incubation chamber at 27 ± 1 °C and 73 ± 5% RH for 24 h to stabilize the moisture content. The disks (range 80-170 mg) were transferred individually to 96 wells from Tissue Culture Test Plates (TPP) in order to feed the insects *ad libitum*.

Twenty unsexed *S. oryzae* adults, 1-2 weeks old, were transferred individually into the TPP wells. Then TPP were placed in the incubation chamber. The bioassay had duration of 39 days during which the insects were continuously exposed to the treated flour disks. Insect mortality was assessed nine times during that period.

The experimental design was totally randomized with ten replicates. The mean mortality and the standard error (SE) were calculated for each concentration and sampling time. The LC_{50} was calculated for the endpoint (39 days) using the Proc Probit procedure in SAS. Also, the LT_{50} was calculated for the maximum dose tested of 500 ppm using the Probit procedure in SAS (Littell *et al*., 2006).

The effect of NSA on insect water balance

The dehydrating effect of NSA on adult *S. oryzae* was analyzed indirectly by comparing the weight loss of insects exposed to four different treatments. Treatments consisted of insects killed by exposition to NSA in a 72 hours interval, killed with chloroform and killed with high temperature (60 °C) at the beginning of the test and one set of control insects. The total water loss of the body of each individual in all treatments was gravimetrically determined by the percentage weight difference, contrasting body weight at the beginning and the end of the experiment.

Unsexed *S. oryzae* adults (n = 132) 1-2 week old, were weighed with a scale (Chyo MOD JK 180; δ = 0.0001 g) and distributed individually in four TPP. Control insects were placed in a 12-well TPP (plate No. 1) with one flour disk as food supply (Talukder and Howse, 1994) placed in each well. Further 12 insects were placed in the second 12-well TPP (plate No. 2) and exposed immediately to chloroform vapours until dead. In the third plate (96-well TPP; plate No. 3), insects in were exposed to wheat kernels treated with 500 ppm NSA. Also, 12 insects were placed in a 12-well TPP (plate No. 4) and exposed immediately in a dry air oven at 60 °C until a constant weight was achieved. Higher temperatures were not used to avoid loss of low MW lipids and other compounds from the body. Finally, the four test plates were placed in an incubator at 27 ± 1 °C and 75% RH in darkness. After 72 hours ten individuals from each plate were weighed. From plates 3 and 4 only the weight of live individuals was analyzed and dead insects were discharged.

Results

NSA and DE were toxic to adult *S. oryzae* when these where exposed to treated wheat kernels and caused insect mortality even at the lower concentrations tested. However, NSA caused significantly greater mortality than DE (table 1). Prolonged exposure resulted in significant adult mortality for both products and at all concentrations tested. The LC_{50} as well as the LC_{95} were significantly lower for NSA (table 1). There was a product effect ($F = 3121.21$; $df = 3, 54$; $P < 0.0001$) on mortality as well as a concentration effect ($F = 105.45$; $df = 3, 54$; $P < 0.0001$). Overall mortality was greater in the NSA than in the DE treatments. At the higher doses, mortality of insects exposed to wheat treated with NSA at 500 ppm and 250 ppm was greater than the mortality attained by DE at 500 ppm (figure 1). At 500 ppm, mortality of adult *S. oryzae* after 7 days continuous exposure was 100% for NSA and 70.5 ± 1.5% for DE.

NSA as well as DE particles attach on insect body surface. As shown in figure 2, insects exposed to surfaces treated with NSA became massively and uniformly coated with NSA particles in contrast to unexposed insects (control) (figure 4). Insects exposed to DE showed a scant and diffuse distribution of particles on the insect surface (figure 3). On the insects exposed to NSA, spherical cuticle-wax blooms appear frequently (figure 5).

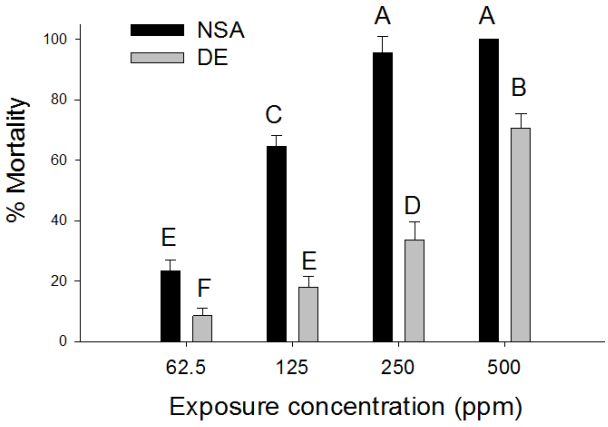

Figure 1. Mean mortality % (± SE) for *S. oryzae* after 7 days of continuous exposure to NSA and DE (DiatomiD®) treated wheat. Bars with different letters are statistically different ($P < 0.05$).

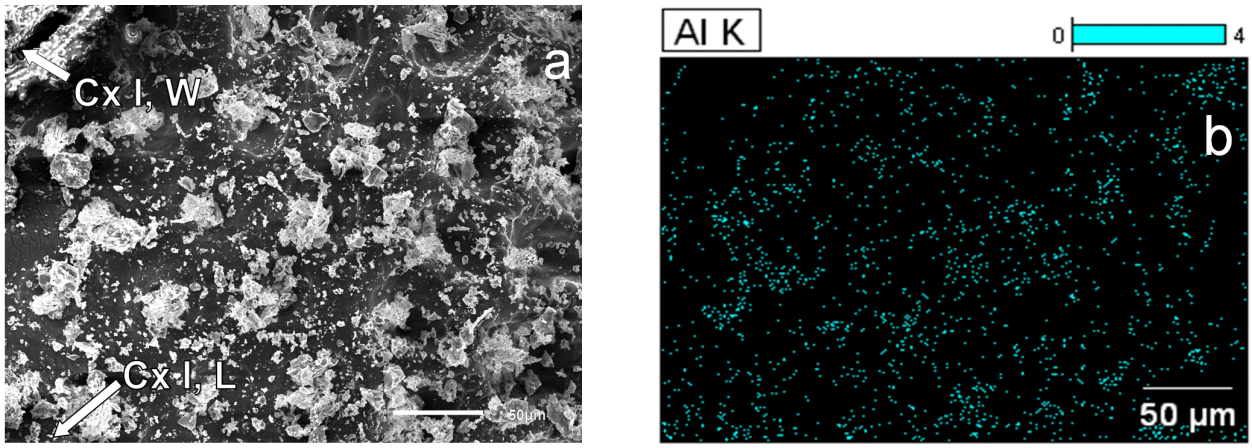

Figure 2. Prosternum of *S. oryzae* exposed to 500 ppm NSA. **a.** SEM image (400×); CxI, W: Coxa I, right; CxI, L: Coxa I, left. Format JEOL/EO, Version 1.0; Instrument JSM-6610; Acc. Volt 10; Mag 35; Spot Size 44; Vac Mode HV. **b.** Aluminium (*Al*) counts from Energy Dispersive Spectroscopy (EDS) - Filter Fit χ^2 value: 31.161; Errors: ± 1; Sigma Correction Method: Proza (Phi-Rho-Z); Acc. Voltage: 12.0 kV; Take Off Angle: 36.2 degree.

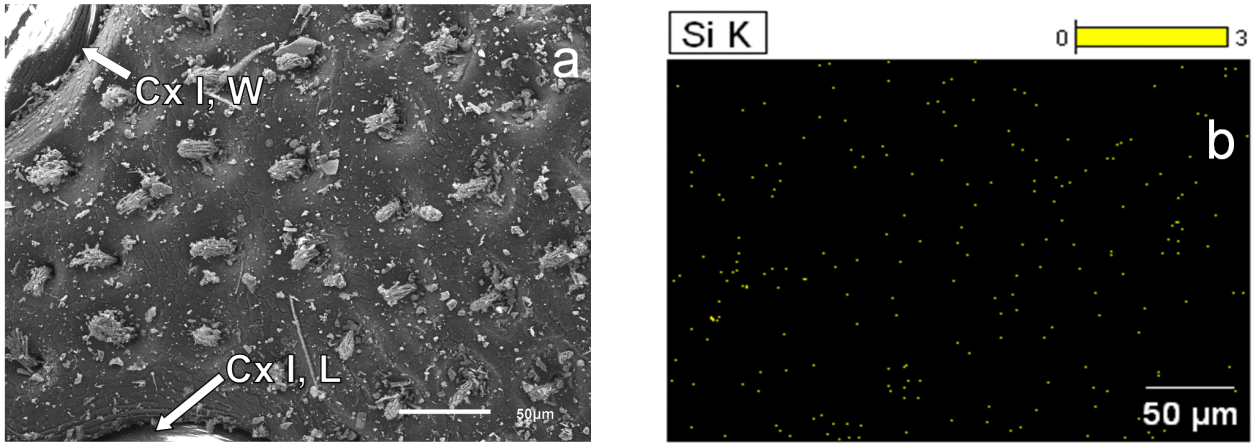

Figure 3. Prosternum of *S. oryzae* exposed to 500 ppm DE. **a.** SEM image (400×); CxI, W: Coxa I, right; CxI, L: Coxa I, left. Format JEOL/EO, Version 1.0; Instrument JSM-6610; Acc. Volt 10; Mag 35; Spot Size 44; Vac Mode HV. **b.** Silicium (*Si*) counts from Energy Dispersive Spectroscopy (EDS) - Filter Fit χ^2 value: 31.161; Errors: ± 1; Sigma Correction Method: Proza (Phi-Rho-Z); Acc. Voltage: 12.0 kV; Take Off Angle: 36.2 degree.

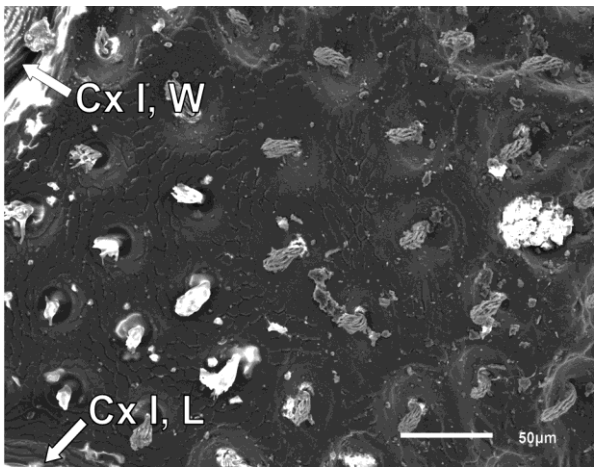

Figure 4. SEM image of *S. oryzae* prosternum (400×). Control insect (untreated). CxI, W: Coxa I, right; CxI, L: Coxa I, left. Format JEOL/EO, Version 1.0; Instrument JSM-6610; Accel Volt 10; Mag 35; Spot Size 44; Vac Mode HV.

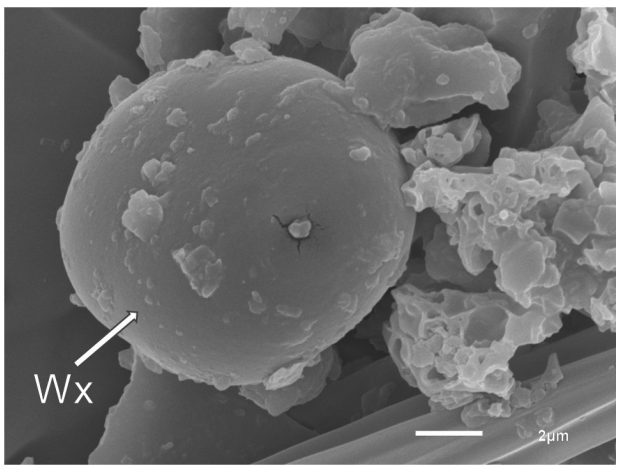

Figure 5. SEM image of a wax bloom (Wx) on the abdominal sterna of a *S. oryzae* individual exposed to 500 ppm NSA treated wheat. (Instrument JSM-6610, Acc. 12 V, Mag 7000×, Signal SEI, Vac Mode HV).

Results from NSA intake toxicity bioassays on *S. oryzae* show that adult mortality achieved after relatively short periods of exposure (7 days) to the higher concentrations of NSA-treated food was remarkable, indicating that toxicity due to ingestion is also a relevant mortality factor during exposure bioassays in *S. oryzae* adult.

There was a delayed response to NSA intake through ingestion after up to 39 days of continuous exposure to NSA treated flour disks at all tested concentrations. Mortality of adult *S. oryzae* was dose-dependent reaching up to 100% at concentrations of 50, 350 and 500 ppm, and up to 40% for concentrations below 125 ppm in wheat discs (figure 6).

The LC_{50} value calculated from intake bioassays on NSA treated flour disks was 180.97 ppm (CLs = 167.07; 195.91; slope = 0.01; intercept = −1.68) and the LT_{50} calculated for the maximum dose concentration tested of 500 ppm was 23.82 days (CLs = 22.05; 25.17; slope = 0.13; intercept = −3.04).

Results from exposure bioassays show the negative effect of NSA on adult *S. oryzae* water balance. At the beginning of the bioassay, no differences in body weight were observed between insects from different treatments (F = 0.04; df = 3, 36; P = 0.99). After 72 hours, there were significant differences among treatments (F = 138.51; df = 3, 36; P < 0.0001). Control insects had a significantly greater body weight than the insects in the rest of the treatments, illustrating the effects of dehydration at different environmental conditions (figure 7).

Changes in body weight of live individuals from the control treatment (TPP plate No. 1) fed with flour disks, decreased by 0.32% (± 2.95) after 72 hours incubation and where not significant. Dead individuals from the chloroform treatment (TPP plate No. 2) had a decrease in weight of 39.86% (± 3.95) on average (figure 7). The body weight of live individuals fed with NSA treated wheat kernels (TPP plate No. 3), presented a substantial reduction in body weight, of 51.6% (± 2.51) on average. The dry mass of individuals incubated at 60 °C (TPP plate No. 4) decreased an average of 62.5% (± 0.79) (figure 7). These results were expected, given that most insect species exhibit a water content of between 65% and 75% (Hadley, 1994) and the body water content in *S. oryzae* is almost 65% (Robinson, 1926). Furthermore, transpiration from the cuticle should proceed at a higher rate in dead than in living arthropods (Davies and Edney, 1952).

There was a significant effect of treatment in the weight loss of insects during the bioassay (F = 38.36; df = 3, 116; P < 0.0001). After 72 hours, control insects showed a very similar weight as that of the beginning of the study, while individuals exposed to NSA had a decrease in weight that was similar to that of insects killed by chloroform and exposed to a temperature of 60 °C. The difference in weight due to water loss is represented by the chloroform and high temperature treatments. Insects treated with NSA had a similar weight loss as those treated with chloroform and high temperature. Thus, these results suggest that water loss is a key factor in the insecticidal action of NSA.

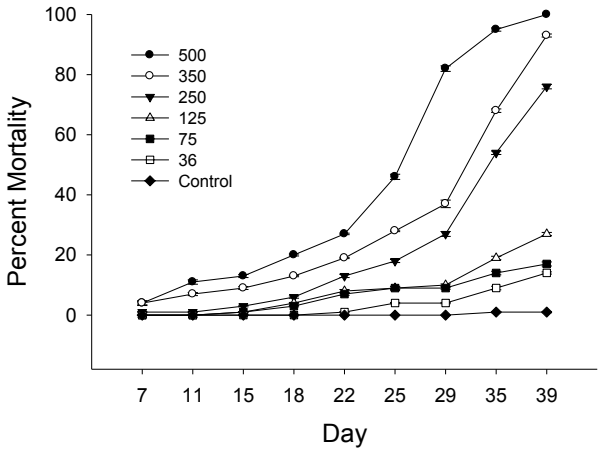

Figure 6. Mortality (%) (mean ± SE) of *S. oryzae* adults fed *ad libitum* with flour disks treated with 36, 75, 125, 250, 350 and 500 ppm of NSA after 39 days in the intake toxicity bioassay.

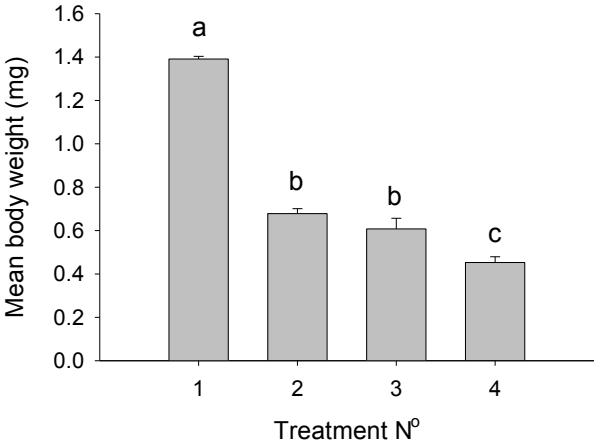

Figure 7. Mean individual body weight of *S. oryzae* adult exposed to different dehydration treatments after 72 hours at 27 ± 1 °C and 75% RH. 1: control group fed in untreated wheat *ad libitum*; 2: insects slay with chloroform and incubated; 3: live insects exposed to wheat treated with NSA 500 ppm; 4: dead insects incubated at 60 °C. Bars with different letters are statistically different (ANOVA: P < 0.0001).

Discussion

Our studies demonstrated that NSA is highly effective against *S. oryzae*, showing a higher insecticidal activity than the DE tested, DiatomiD®. Total mortality was achieved in insects exposed to wheat treated with 500 ppm of NSA after 7 days exposure.

Inert dusts act, in principle, on the base of the electrostatic charge of particles and triboelectrification phenomena in insects (Carlton, 1971) and through absorption of insect's cuticular waxes (Ebeling, 1971; Mewis and Reichmuth, 1998; Mimani and Patil, 2001). In nanomaterials synthesized by oxidation of metals, such as the NSA, the resulting particles are electrically charged, showing a dipole-dipole interaction that promotes aggregate formation with resistance to dissocia-

tion forces (Chacon, 2007). Thus, in those insects that exhibit electric charges generated by triboelectrification (McGonigle *et al*., 2002), NSA aggregates attach firmly to the body surface and to the insect cuticle wax layer through sorption because of its high specific surface area (14 $m^2 \times g^{-1}$; Mimani and Patil, 2001). Once the protective wax layer is disrupted, water loss becomes irreversible, leading to insect's dehydration and death (Cook, 2008).

Increased humidity leads to a decreasing mortality rate of adults for NSA according to Stadler *et al*. (2012) and Buteler *et al*. (2015) and for DE according to Korunic (1994) among others. The lower efficacy of insecticide dusts at higher relative humidity can be explained by a delayed drying process due to a slower rate of water loss through the surface of the insect cuticle, damaged by dust particles (Chiu, 1993a; 1993b; Alexander *et al*., 1944a; 1944b; Ebeling, 1971). The normal transpiration of an insect into the surrounding air is dependent on water vapour pressure. With increasing relative humidity the vapour pressure increases in the air and the water discharge from the insect body surface is reduced. These results are consistent with earlier findings which suggested that toxicity of insecticide dusts on arthropods is a consequence of the "cuticular water flux" (Edney, 1977).

Most insect species exhibit water content of between 65 and 75% (Hadley, 1994). There are big differences between the insect species in how they compensate for water loss when subjected to dehydrating conditions. Various authors report water losses were between 17 and 89% of the total water content observed (Hadley, 1994). According to Arlian (1979), the maximum body water loss for the rice weevil *S. oryzae* is 56%. The maximum body weight loss achieved in our study on *S. oryzae* was ≥ 66% when dead individuals were exposed to dry air at 60 °C during 72 hours. Nevertheless, differences in the water content values attained by different authors depend on the methodology used.

Weight reduction in surviving *S. oryzae* adults exposed to NSA treated wheat was ≥ 45% in 72 hours. A similar behaviour was documented by Alexander *et al*. (1994a) for the treatment of *Sitophilus granarius* (L.) with various dusts and by Trewin and Reichmuth (1997) after treatment of *Ephestia kuehniella* Zeller, *Oryzaephilus surinamensis* (L.), *Tenebrio molitor* L. and *Tribolium castaneum* (Herbst) with Aerosil® dispersions, a silica product.

Weight loss in dead *S. oryzae* after 72 hours at 27 ± 1 °C and 75% RH, was ≥ 50% in agreement with Davies and Edney (1952) who showed that transpiration from the cuticle should proceed at a higher rate in dead than in living arthropods. Since dead insects show a weight loss to ≥ 50% in a relatively short period of time, the cause can be attributed to the reduction of body mass by water loss rather than on the drop of fat reserves or proteins. Although the proportion of fatty substances in insects is considerable and can be up to 75 and 90% in adults and larvae respectively, fat can bind very little water (Vague and Fenasse, 1965).

NSA most likely increases the rate of water loss from insects. Absorption of body water by the NSA particles is unlikely, since only small amounts of water can be added to NSA and the water loss during the bioassays was 0.5 mg on average. It is more likely that there is an accelerated diffusion of water in air, across the cuticle that is disrupted by the particles.

As formerly assumed by Ebeling (1971), IDs disturb the external protective wax layer of the insect cuticle making it vulnerable to water loss and subsequent dehydration. Inert dusts may have abrasive, sorptive or combined effects (Wigglesworth, 1942; Beament, 1945; Ebeling and Wagner, 1959; Ebeling, 1964; 1971). According to studies by Ebeling (1961), abrasive dusts proved generally less effective than sorptive dusts. This is the case with NSA where a smaller exposure concentration was needed in comparison to the less sorptive DE dust in order to achieve a 100% mortality of *S. oryzae*.

NSA is an amorphous nanostructured material with sorptive properties (Stadler *et al*., 2010b) that adheres strongly to the cuticle and could only partially be washed off with water from insect body surface (personal observation). This is because the insect itself carries a net electric charge produced by contact with various surfaces or by friction (Edwards, 1962; McGonigle *et al*., 2002; Jackson and McGonigle, 2005). In insects the average negative charge is lower than the positive one (Collin *et al*., 1991). Measurements of surface potentials ranging between −14 mV and +8 mV on bees leaving the hive (Erickson, 1975). On the other hand, the majority of nanoalumina aggregates are charged either positively or negatively and some of the aggregates are dipoles (Karasev *et al*., 2004).

Insect mortality due to NSA depends mainly on contact phenomena. Our studies revealed that intake toxicity is a significant mortality factor that occurs simultaneously with contact toxicity during insect exposure to NSA. Intake concentrations lower than 75 ppm caused sub-lethal effects in *S. oryzae*. Results demonstrate that toxicity due to ingestion is a relevant long term mortality factor that should be taken into account when assessing the efficacy of NSA in the control of *S. oryzae*, and that would lead to a reduction in grain damage and generational survival (Smith, 1969).

A conceptual model of the mechanism of action of NSA to insects can be derived from the experimental results and the literature on inert dusts. After contact with the insect outermost layer by electrostatically induced binding, adsorption on the epicuticle and absorption of cuticular waxes (paraffins, polyphenols, esthers) occurs, resulting in a reduction of the superimposed outer epicuticular layer. Through these regions of the cuticle, in accordance with the Fick's law, there is higher diffusion of body water along the concentration gradient into the surrounding air (Wharton, 1985). On the other hand the water permeability of the integument is increased due to the increase in surface area by the particles. The result of the action of NSA is dehydration of the insect.

The toxicological aspect of insecticide dusts is still under discussion and needs to consider the chemical composition of the dust, the morphology of particles (crystalline and amorphous) and on also the potential

exposure manner (dermal, inhalatory, oral). Amorphous DE were classified in Category IV for oral toxicity by US-EPA (US-EPA, 1981). The only known harmful effect of DE is due to worker exposure (Omura, 1981) due to the risk of lung diseases and silicosis, as a result of continuous inhalation of dusts (Abrams, 1954; Saffioti, 1992). DE can also cause irritation of the eyes and the skin.

Given that nanomaterials possess novel properties as kinetics and bioactivity, their potential biological effects may differ from those of coarser bulk materials. Apart from the potential benefits, it is also necessary to anticipate and characterize potential health and environmental risk associated with this new technology. The information on NSA acute, chronic and environmental toxicity is still at an early stage of development. Hence, there remains a need for more research, especially with regard to hazard identification of this nanoinsecticide. Research efforts should also focus on the development of new variants of NSA by introducing modifications in the synthesis process aimed at higher insecticide performance as well as the search for potential new uses of NSA.

Acknowledgements

The authors would like to thank Lic. María Silvina Lassa for providing invaluable technical assistance in SEM and EDS analysis. We are also grateful to Ing. Mariano A. Bernardo, Nidera S.A., for providing standard seed material. This work was supported by the National Scientific and Technical Research Council, Argentina (Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET; grant number PIP N°11220130100382CO).

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Received April 13, 2016. Accepted October 14, 2016.