Particulate Nanoinsecticides: A New Concept in Insect Pest Management

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Abstract

Nanostructured alumina (NSA) has insecticidal properties and has been demonstrated to be effective against stored product insect pests in laboratory bioassays. NSA is a nano-engineered material synthesized by oxidation of metals, and resulting particles show fixed electric charges. On the other hand, insects exhibit their own electric charges generated by triboelectrification. We propose that the mechanism of action of NSA involves two steps occurring in sequential order. First, a strong electrical binding between negatively charged NSA particles and positively charged insect. Next, dehydration of the insect occurs due to the strong sorptive action of the NSA particles that remove the insect cuticular, leading to death by dehydration. As postulated for insecticidal inert powder in general, particles attach to the insect cuticle surface disrupting water balance, and effectiveness decreases as ambient humidity increases, given that electrostatic bond forces are reduced by electrostatic discharge. The high insecticidal efficacy of NSA is a result of its intrinsic electric charge, small particle size and high sorptive potential due to its large specific surface area. NSA could provide an alternative to conventional synthetic organic insecticides due to its strong insecticidal properties with the advantage that its mechanism of action involves physical and electrostatic phenomena.

Keywords: nanoinsecticides, mode of action, triboelectrification, *Sitophilus oryzae*, insecticide powders

1. Introduction

The advent of synthetic organic pesticides by mid-1950s made the control of insect pests highly effective and despite their drawbacks, most of these active principles are still used in modern agriculture. The use of synthetic insecticides has allowed an increase in yields and lowered the
the cost of farming. Synthetic organic pesticides also remain an important tool to control vectors of infectious diseases of both humans and domestic animals, leading to a great reduction in their incidence in many areas of the world. However, synthetic organic insecticides may impact negatively on human health and ecosystems, affecting populations of non-target organisms and biodiversity [1]. Moreover, the accumulation of active ingredients or their metabolites in the environment as well as in organisms, may lead to bioaccumulation, where these pollutants enter the food chain, posing a serious threat to both wildlife and humans [2, 3].

Caught in a vicious circle? Agriculture has waged a costly struggle fighting insects by constantly rotating obsolescent pesticides in a desperate strategy of chemical warfare. However, a comprehensive and successful strategy for minimizing acute and chronic risk from pesticide use should be based on research initiatives aimed at radical changes in pest management strategies and the replacement of the synthetic organic pesticides with effective but less hazardous substances [4]. Part of the research on new biorational pesticides focuses on natural products such as plant extracts, oils and inorganic products. These are frequently a source of new chemical classes of insecticides, as well as environmentally and toxicologically less hazardous active ingredients than many of the conventional products used for insect pest control. Furthermore, new active ingredients often have mechanisms of action or molecular target sites which still remain unexploited by conventional marketed pesticides [5]. Hence, substances with new properties are promising tools for crop protection and food production, opening new frontiers in pest management [6]. However, only 14% of the pesticides on the market are biorational products and only 1% consists of natural products like plant extracts, essential oils and insecticide powders [7].

1.1. Nanotechnology as a source of modern pesticides

Nanotechnology is a collective term for a wide range of technologies that deal with structures and processes at the nanometer scale. The transition to the nanometer scale ($10^{-9}$ m), leads to an increase in dominance of quantum-physical effects, optical, magnetic, electronic, mechanical and chemical properties [8]. Because of its potential for the fundamental transformation of entire technology fields, nanotechnology will not only influence technological development in the near future, but also have economic, ecological and social implications [9]. The size reduction to the nanometer range often leads to characteristic properties of materials which are useful for new applications and which do not occur in the case of macroscopic pieces of the same material. These include, for example, higher breaking strength at low temperatures as well as superplasticity at high temperatures, formation of additional electronic states, high chemical selectivity of the surface structures and a markedly increased surface energy [10].

Nanotechnology has advanced rapidly over the last 10 years and numerous nanomaterials, with a variety of potential applications, have been developed. For instance, improvements in medical science through nanotechnology offer the possibility to develop novel diagnostics and therapeutics [11], as well as new nano-engineered products with pesticide properties which have shown to be promising as tools for low impact or alternative organic agriculture and food production [12–15]. Engineered versions of conventional pesticides, growth regulators and seed treatment agents are among the first nano-chemicals that could be used in agriculture [13]. The use of nanoparticles could make pesticides more effective by reducing particle size to the nanoscale given the associated increase in surface area which introduces a fundamental
change in the physicochemical properties of nano-pesticides [16, 17]. Compared to larger particles of the same chemical substance, nanoparticles are more reactive, more biologically active and have a more catalytic action [18, 19]. Nanoparticles could help use pesticides and fertilizers more effectively [16], for example, by reducing agrochemical components to nanosize or to pack the active ingredients in nanocapsules, which release them selectively, would allow for lower amounts with the same effect, only under certain conditions of heat, sunlight or pH [20, 21].

The current levels of application of nanoparticles and the expected developments to come, suggest that nanotechnology will have a direct impact on the evolution of pest management practices in agriculture [16, 22–25]. Recently, the discovery of nanoinsecticides brings new alternatives to expand the spectrum of applications of inorganic powders [6, 24, 26, 27]. Nanoengineered aluminium oxide as nanostructured alumina (NSA), has been shown to have insecticidal properties, low non target toxicity, non-reactivity, low cost and reduced probabilities of generating resistance in insects [26].

In a previous work, Stadler et al. [6, 24, 28] assumed that “rice weevil” (S. oryzae) adults acquire electrostatic charge by triboelectrification when walking on a dielectric surface such as wheat kernels, and that these charges on the insect body lead NSA particles from the treated substrate to the insect body surface. In order to verify this phenomenon empirically, studies were undertaken to examine and model the tribo-charging of S. oryzae adults on a dielectric surface and to identify the type and magnitude of the electrostatic charge on NSA, diatomaceous earth (DE) samples and the net electrostatic charge density of wheat kernels.

1.2. Nanostructured alumina: a novel nano-engineered insecticidal powder

A reduction in particle size of a substance results in increased surface/volume ratio per unit weight, which generally correlates with increased toxicity [29]. This characteristic has been exploited by some researchers to control various microorganisms and insects by applying nanoparticles [26, 28]. For example, nano-engineered alumina (NSA) is the result of combustion synthesis, using a redox mixture, with glycine as fuel and aluminum nitrate as oxidizer, where the final product is a homogeneous powder of high purity with uniform characteristics and specific physicochemical properties [30]. During the combustion process, alumina nanoparticles (40–60 nm) aggregate in primary clusters of approximately equal diameter building electrically loaded amorphous micrometric agglomerates/aggregates with a specific surface area of 14 m²/g ranging in size from 0.1 to a few micrometers [31]. Due to its special characteristics as kinetics and bioactivity, it allows for varied and novel uses as a pesticide for human, livestock and agricultural use, stored product protection, treatments for wood preservation, carriers of pheromone or virus for insect pest control, etc. [32]. NSA has been shown to have strong insecticidal properties to several insect species through a mechanism of action different from conventional pesticides. Nanostructured alumina (NSA) has been shown to be an effective contact insecticide for several species of stored grain insect pests [6] as well as for leaf cutting ants [33].

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 dissociation forces [34]. Depending on the synthesis procedure, the greater part of the aggregates charged either positively or negatively and only some of these
are dipoles [31]. Thus, the combustion manufacturing process is the main factor responsible for the affinity of particles with the triboelectrically charged body surface of different insect species (Figure 1) and as a consequence, also responsible for insecticidal activity. However, the morphology of nanoalumina agglomerates can be influenced by different variables during the synthesis such as substrate concentration, additives and calcination temperature which play a decisive role in the final morphology and characteristics of nanoalumina [35].

The effect of NSA on insects has been investigated through contact as well as dietary intake toxicity bioassays. Also, the in vivo toxicity and the in vitro cytotoxicity of NSA particles were screened as reviewed below.

1.3. Insecticidal effect of NSA on stored product insect pests

1.3.1. The contact toxicity of NSA against stored product insect pests

The contact toxicity of NSA was first investigated using dry powder applications at three different relative ambient humidity levels [6]. Tests were conducted simultaneously with enhanced diatomaceous earth, Protect-It®, to compare the efficacy of NSA to that of commercial insecticide powders. Two major stored grain pests Rhizopertha dominica and Sitophilus oryzae were tested and significant delayed mortality was observed. Both species experienced significant mortality after 3 days of continuous exposure to treated wheat. Nine days after treatment, the median lethal doses (LD_{50}) observed ranged from 127 to 235 mg kg\(^{-1}\). Results showed that NSA was more effective in killing S. oryzae than Protect-It® and was equally toxic to R. dominica. R. dominica was, less susceptible to inert powders than S. oryzae [32]. According to Subramanyam and Roesli [36], S. oryzae is among the most susceptible species

![Figure 1: SEM images of individuals of three stored insect pest species (a, b) Sitophilus oryzae (Coleoptera: Curculionidae); (c, d) Ceratitis capitata (Diptera: Tephritidae); (e, f) Oryzaephilus surinamensis (Coleoptera: Silvanidae) showing the affinity of NSA particles to triboelectrically charged insect body surfaces. (a, c, e) after exposure to untreated wheat kernels; (b, d, f) after exposure to 125 ppm NSA-treated wheat kernels. Format JEOL/EO, version 1.0; instrument JSM-6610.](image-url)
to diatomaceous earth and *R. dominica* is among the least susceptible ones. Chemical makeup of epicuticular waxes varies across insect species [37], and this should translate into differences in susceptibility to nanoalumina and other inert powders due to differences in wetting. Treatment with NSA as well as Protect-It® also reduced progeny production although NSA powder was more effective in eliminating F1 adults than Protect-It®, for both species of insects tested. NSA reduced F1 progeny drastically at concentrations as low as 62.5 ppm for *S. oryzae* for high, medium and low humidity levels, and ranging from 250 to 500 ppm for *R. dominica* depending on the humidity level [32]. These results obtained with NSA are encouraging given that Protect-It® is one of the most effective DE-based products in the market [36, 38].

Comparison of these results with recommended rates for commercial insecticidal powders suggests that inorganic nanostructured alumina may prove a good alternative or complement to DE-based products, and encourage further testing with other insect pests and systems, plus experiments on delivery options to further enhance NSA products.

### 1.3.2. The intake toxicity of NSA in stored product insect pests

Although dehydration appears to be the main cause of mortality due NSA exposure, but it cannot be assumed as the only one, for it is found that at sub-lethal concentrations, insecticide powders in general exert further noxious effects on the insect [33, 36, 39]. Our studies revealed that intake toxicity is a significant mortality factor that occurs simultaneously with contact toxicity during insect exposure to NSA. Dietary intake of exposure to concentrations lower than 75 ppm caused sub-lethal effects in *S. oryzae* and adult mortality occurred after only 7 days exposure to NSA in food. These results indicate that toxicity due to ingestion is also a relevant mortality factor. There was a delayed response to NSA intake through ingestion which occurred up to 39 days of continuous exposure to NSA-treated flour discs. Mortality of adult *S. oryzae* was dose-dependent reaching up to 100% at concentrations of 250, 350 and 500 ppm, and up to 40% for concentrations below 125 ppm in wheat discs. The LC$_{50}$ value calculated from intake bioassays on NSA-treated flour discs was 180.97 ppm ([CI = 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 ([CI = 22.05; 25.17]; Slope = 0.13; Intercept = −3.04). The body weight of live individuals fed with NSA-treated wheat kernels (TPP plate No. 3), also presented a substantial reduction, of 51.6 (± 2.51)% on average [28]. These findings are similar to what Alexander et al. [40] observed after the treatment of *S. granarius* with various insecticide powders and by Trewin et al. [41] after treatment of *Ephestia kuehniella*, *Oryzaephilus surinamensis*, *Tenebrio molitor* and *Tribolium castaneum* with Aerosil® dispersions, a silica product. Results demonstrate that ingestion toxicity is a relevant long-term mortality factor that should be taken into account when assessing the efficacy of NSA and inert powders.

### 1.4. The *in vivo* toxicity and the *in vitro* cytotoxicity of NSA particles

An important characteristic of nanomaterials is their extremely large surface. For example, the same mass of material in the form of nanoparticles has a specific surface area which is many times larger than a coarse powder. This large surface can chemically react with materials that are otherwise non-reactive and non-toxic. However, it is not the size alone that contributes to the potential toxicity of nanomaterials. Rather, it has been shown that the toxicity of
nanomaterials is influenced by many parameters like size, shape, surface area, electric charge and texture. Moreover, it is possible to design a basic nanomaterial in its morphology and surface properties on the nanotechnology. This results in new properties of a new material but it is unclear to what extent significant changes in toxicological properties can result from changes in the morphology [42]. Soluble nanomaterials lose their nanostructure features after they have come into contact with biological fluids and a non-specific toxicity could possibly arise. However, insoluble nanomaterials that retain their nanostructures which are wrapped in a stable material and thus cannot come into contact with biological fluids or those that can be absorbed into organism are of lesser importance. Therefore, the free nanomaterials, which can be absorbed in different ways, are of particular toxicological significance. These nanomaterials are likely to lead to a higher exposure of humans and the environment [43].

Each nanostructured material has to be individually tested for its potential toxicity, since the knowledge about the complex relationships between physical and chemical parameters and a possible toxicity is missing. The toxicity of aluminum oxide nanoparticles has been discussed in many publications providing mixed results [44, 45]. On the other hand, toxicity of nanostructured aluminum oxide particles (NSA) [26] remains still an object of experimental work. Pochettino et al. [46] evaluated in vitro effect of the NSA on macrophages from the THP-1 cell line, exposed during two different time periods (6 and 24 hours) to different NSA concentrations (5, 25, 100 and 250 μg/mL). Cell cultures exposed to the lower concentrations of NSA during 6 hours show increased levels of the proinflammatory cytokine synthesized by macrophages IL-1β and a significant reduction of catalase (CAT) antioxidant enzyme activity. The two highest concentrations of NSA induced a decrease in cell viability (MTT assay) and an increase in lactate dehydrogenase activity (LDH: cytotoxicity indicator) and IL-1β release, in exposed cell cultures, and a decrease in CAT activity and thiol groups (−SH: thiols groups, antioxidants properties). These changes observed in CAT, LDH, −SH are indicators of oxidative stress. After NSA treatment, mitochondria lost their filamentous shape and displayed several morphological alterations. The effect of NSA on cell cultures after 24 hours of exposure was similar to that observed at 6 hours.

The exposure of THP-1 macrophage cell cultures to high NSA concentrations induces the release of IL-1β but also causes cell death, where NSA-mediated oxidative stress could play an important role. The generation of a controlled oxidative stress leads to the activation of intracellular mechanisms to compensate the production of reactive oxygen species (ROS), however a continuous overproduction of these species causes the onset of pathological states. Further studies should address the mechanisms involved in the oxidative stress caused by NSA in order to characterize and limit these. Also, further studies on the balance between pro- and anti-inflammatory molecules in vitro cell cultures exposed to NSA will be necessary looking for the mechanisms involved in acute effects of NSA exposure. On the other hand, low NSA concentrations raise the IL-1β levels without inducing changes in cell viability; so, this could be of relevance to enhance triggering immune responses. These results motivate further research on the mechanisms underlying the observed effects of NSA on THP-1 macrophage cells, as well as to analyze other mediators and immunological parameters in order to evaluate the potential of the NSA at low dose as a modulator of the immune response.

Deepening at the cellular level, Nadin [47] studied the genotoxicity effects of NSA at the cellular level. To determine whether NSA induces DNA damage, human peripheral blood
mononuclear cells (PBL) were isolated from a healthy donor venous blood. PBL were exposed for 24 hours to increasing concentrations of NSA (50, 100 and 200 μg/mL) and then collected. Concentrations used were the same as those tested by Pochettino [46]. DNA and chromosomal damage was assessed throughout the alkaline comet assay and micronuclei (MIN) test, respectively, and cell viability was tested with the resazurin assay. The comet assay allowed to quantify DNA damage and revealed no significant increase in DNA damage induced by NSA. No statistical significant differences were found in terms of cellular viability and NSA had no significant effect on MIN induction.

Regarding animal experiments (in vivo), the acute oral toxicity and the acute inhalation toxicity of engineered aluminum oxide nanostructured particles (avg. 100 nm) were assessed in Wistar albino rats [48]. Acute oral toxicity was assessed by a limit test at a test dose of 2000 mg/kg b.wt that was administrated in a single dose. No mortality was observed in treated animals and no significant differences in body weight were observed (p < 0.05) either. No morphological changes were observed through pathological examinations. After inhalation exposure (0.02 mg/L air), respectively, during 4 hours, no changes in body weight gain were noted. A decrease in body weight gain was observed after inhalation exposure with 0.07 mg/L. No morbidity or mortality was observed in inhalation NSA exposed rats. These studies provide information applicable to the early stage in the hazard identification process for this type of nanomaterials that could be useful in risk management in the context of production, handling and use of nanomaterials. These results show that acute oral and inhalation exposure to NSA did not result in morbidity or mortality in male rats.

The rapid proliferation of engineered nanomaterials and the limited toxicological data currently available on it presents a dilemma to regulators regarding risk assessment processes for these materials [49]. For recently developed nanomaterials, there are in many cases insufficient investigations into health effects. Therefore, no sufficiently reliable statement can be made about these nanomaterials. There is a need to determine the extent of absorption, systemic availability, accumulation and excretion of nanomaterials after inhalation and oral exposure. However, the necessary in vivo studies should be integrated into any toxicological studies to avoid unnecessary animal experiments. The influence of modifications in the NSA synthesis on the kinetic parameters [26] as well as on the toxicological properties of the nanomaterials should also be examined. Finally, oxidative stress and the formation of reactive oxygen species (ROS) are fundamental key mechanisms of cellular defense after particle capture.

In order to develop biorational pesticides through design of NSA synthesis, further research is necessary on the complex relationships between its physical and chemical parameters and its toxicity.

2. Identifying and understanding the mechanism of pesticide action of nanostructured alumina

2.1. Triboelectric charging in insects

Tribo-charging is the advent of electric charges based on the mechanisms of charge transfer which occurs when two different non-conductive bodies (materials) are brought into contact.
and separated or rubbed together acquiring positive or negative polarity [50]. Friction plays only a role in this respect, as the bodies are approached to molecular distances, thus permitting charge transfer (contact electricity). A triboelectric series can be established for the frictional electrification in which a material is positively charged when friction is applied to the following material, while friction is negative in the previous one. This series is based on Cohen’s rule according to which the substance with the higher dielectric constant is positively charged [51].

Insects also generate electrostatic charges by walking. This was first studied by Edwards [52, 53] who showed that rubbing dead insects against various substrates generated electrostatic charges. In a later study [54], this author monitored naturally acquired and retained electrostatic charges on living insects, showing that a net charge could be detected in flying insects. For example, a flying honeybee in a wind-tunnel reaches an average charge of $-23.1 \text{pC}$ [55] and this charge plays a key in the transfer of pollen grains from the flower to the insect [56]. Corbet et al. [57] showed that due to electrostatic charges, oilseed pollen grains pass from flower to freshly killed honeybee across an air gap of 0.5 mm. Electrostatic charges in insects may arise from frictional charging linked to contact with different types of surfaces through the migration of electrons from one surface to another, where equal but opposite charges arise on each surface [58–60]. However, insects may also acquire electrostatic charge by absorption via the insect cuticle through dermal pores [61], as well as through the adhesion of charged particles [55, 62, 63].

2.2. Electrostatic charge of insecticidal powder particles

Powders or more generically, solids in a high degree of subdivision, exist in an enormous variety of chemistries and morphologies. The discrete entities or “kinetic units” of interest typically range in linear dimension from a few micrometers to a few nanometers, at the colloid size range. Even in the nano-range, where powders take the form of quantum dots or nanowires, the objects are amenable to the descriptions afforded by macroscopic thermodynamics [64]. These particles tend to sediment from the air due to their greater density, depending on the environmental conditions and the shape and size of the particles. In dry atmospheres, the sedimentation or sink rate of the particles can be calculated as a function of their radius [65]. After landing, an adhesion process occurs immediately after the particle hits the surface and is a purely physical process. It is relatively weak, reversible and is based on unspecific capillary, van der Waals, electrostatic and hydrophobic forces between the particle and the surface [66]. These forces have a different strength and they also differ in their range. In order to get into the area of influence of molecular interactions, two surfaces have to approach below 10 nm. Capillary forces act in a range of 10–200 nm and electrostatic forces of 100nm–1 μm [67]. In some studies, it was found that there is an influence of surface hydrophobicity on adhesion [68, 69]. Thus, a stronger adhesion of particles to hydrophobic than to hydrophilic surfaces was detected. Furthermore, it has been shown that the surface roughness also has an influence on adhesion of the particles [67].

2.3. Electrostatic charge of wheat kernels

General characteristics of wheat seeds depend on a wide range of dielectric properties like conductance and bioelectric potentials related to ionic and structural heterogeneity of plant cells, tissues and organs. Biologically active substances as enzymes, contribute to bioelectric
polarity through powerful charge at the molecular level [70]. However, ionic activities inside the tissues dominate the low-frequency dielectric behavior of the tissues [71]. Additionally, the structure and shape of epidermal cells and epicuticular waxes of wheat seeds also contribute to their bioelectric activity [72]. Nonetheless, the bioelectric activity of a plant is an intrinsic structural feature of the organism and cannot be modified since it is genetically predetermined [70].

Bioelectric polarity is critical to adhesion or repellence of water or particles of different nature, shape and size from any surface [73, 74]. When particles, of whatever nature, reach a surface as, for example, the wheat seed epidermis (testa), interactions occur between particles and surface. If the particles are in the range of millimeters or above, gravitation and mass inertia are the decisive forces for these interactions where adhesion forces dominate [75]. These forces consists of different forces as capillary force, electrostatic force (Coulomb repulsion/attraction of different surplus charges, electrostatic double layer force) and molecular interactions (van der Waals forces, dipole-dipole interactions and hydrogen bonds) [76].

3. Assessment of tribo-charging in insects, electrostatic charge of insecticidal powder particles and wheat kernels

3.1. Materials and methods

3.1.1. Insects

*Sitophilus oryzae* (Linnaeus, 1763) (Insecta, Coleoptera: Curculionidae) were obtained from the Laboratory of Environmental Toxicology (IMBECU.CONICET, Argentina) culture, reared on wheat kernels (var. Baguette NIDERA) at 27 ± 2°C, 70 ± 5% RH in the dark. Adults used in all experiments were of unknown sex, mating status and age.

3.1.2. Insecticide powders

3.1.2.1. Nanostructured alumina (NSA)

Synthesized since Toniolo et al. [30] by glycine-nitrate combustion technique using a redox mixture, with glycine as fuel and aluminum nitrate nonahydrate as oxidizer. Nanostructured particles sized from approximately 0.1 μm up to a few micrometers.

3.1.2.2. Diatomaceous earth

Commercial diatomaceous earth (DiatomiD®) from fossilized sedimentary phytoplankton microalgae (diatoms) deposits from San Juan-Argentina, which contains over 85% amorphous SiO₂ and particles sizing from 1 to about 150 μm.

3.2. Experimental setup

Triboelectric charges on insects as well as charge densities on wheat kernels and insecticide powders were assessed under the same experimental and environmental conditions by means
of a Faraday cup connected to an ensemble of an electrometer based on a LMP7721 amplifier (NI, LMP7721 Multi-Function Evaluation Board amplifier in buffer mode) and a data acquisition system (NI USB 6009 (8 input, 14 bits, multifunction I/O, 10 bits DAQ system) controlled by NI Labview software (EFC). The detection limit of the EFC was 0.06pC. Electrometer calibrations were performed using ADA4530-1R-EBZ-BUF as the reference electrometer. Total electrometer input capacitance was assessed with Analog Devices AN-1373. The tribo-charging assessment method was validated since Greason [77] by using a stainless steel sphere (ø 2 mm) sliding along a slightly inward curved paperboard ramp (length 400 mm and 50 mm wide) coated with a smooth layer (1.5 ± 0.5 mm) of dried wheat paste (wheat flour and water). The ramp was tilted at 30°, so the stainless steel sphere slides into the Faraday cup at the end of the ramp.

Experiments were conducted within a grounded Faraday cage to avoid external sources of static electricity. In order to set the same baseline for each experiment, grounding was used to neutralize the initial charges carried by samples. Throughout data collection, the operator remained connected to the grounded Faraday cage. Temperature and humidity inside the Faraday cage were maintained at 25°C ± 2°C and 35 ± 5% RH and were constantly monitored during experiments.

Tribo-charging in S. oryzae was measured by using the paperboard ramp and EFC. Frictional charging experiments were developed by using live CO₂ anesthetized S. oryzae adults sliding smoothly from different distances on the ramp (1.25, 2.50, 5.00, 7.50, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0 and 40.0 cm). The insects slid at an almost constant speed under the action of gravity and fell into the Faraday cup down to the end of the ramp. The charge on the insect was detected by the EFC and the data were automatically stored in a computer. The process was repeated 12 times for each distance using different insects.

3.2.1. Assessment of electrostatic charge on insecticide powders

Charge density of nanostructured alumina (NSA) synthesized since Toniolo et al. [30] and diatomaceous earth (DE) [DiatomiD®] was measured by the static method [78]. Identical volumes of the inert powders were measured at 25°C, 35% RH, using a normalized copper cylinder \((h = 3.2 \text{ mm}; r = 8.75 \text{ mm, internal})\). By means of the earthed 0.769 mL cylinder, samples of 0.23 g of nanostructured alumina and on the other hand 0.74 g of diatomaceous earth were transferred into the Faraday cup. The process was repeated 20 times using always the same insecticide powder samples.

3.2.2. Assessment of electrostatic charge on wheat kernels

Electrostatic charge density of seed was measured by distributing 20 selected wheat kernels (55.2 mg/kernel (SD ±8.8 × 10⁻³) var. Baguette NIDERA (4 months after harvest) in a single layer on a grounded copper plate. Six randomly selected kernels were introduced one at a time, for 12 times each in the Faraday cup (EFC) under the experiments conditions described above.
4. Results

4.1. Tribo-charging in insects

Figure 2 shows tribo-charging of *S. oryzae* where the rate of charging at the start was proportional to the saturation charge and it decreased as the insects charge increased. The insect loses electrons as far as maximum charge is attained when the electron affinities reach equilibrium. The charge on the ramp surface has no influence on its particular electron affinity since the insect in motion rub sequentially different and uncharged sections of the ramp surface during sliding. The charge acquired by the insect with each additional distance covered on the ramp is equivalent to the difference between the insect maximum reachable charge and the charge of the ramp surface [58].

As shown in Figure 2, the magnitude of electric charge picked up by *S. oryzae* was approximately proportional to the distance it moved (d<sub>1.25cm</sub> = +0.766 (±0.254) pC/insect to d<sub>40.0cm</sub> = +2.560 (±0.221) pC/insect). In contrast to McGonigle et al. [58] and in some extent in concordance with Jackson and McGonigle [60], our results show a discrete evidence for a plateauing of charge and clearly demonstrate that saturation charge in *S. oryzae* was not reached (Figure 2).

4.2. Electrostatic charge in insecticide powders

The magnitude and sign of the net average electrostatic charge density measured was −93.91 (±2.62) pC/grain for NSA and −11.554 (±2.342) pC/grain for diatomaceous earth. Thus, both substances are negatively charged and consequently adhere on electropositive insects body surfaces.

![Figure 2](http://dx.doi.org/10.5772/intechopen.72448)

*Figure 2.* Mean charge (pC) generated by live anesthetized *S. oryzae* adults after sliding along different wheat flour ramp track sections (1.25–40 cm). Experimental were plotted (dots) alongside a modeled curve (entire line).
4.3. Electrostatic charge in wheat kernels

The electrostatic charge measured on wheat kernels var. Baguette NIDERA was weakly negative, averaging \(-0.191 \pm 7.15 \times 10^{-2}\) pC/grain.

5. Discussion

In principle, all life forms are immersed in an ionized environment. Ions bear an electric charge and thus an electric field may be influenced by another one. Thus, the electric fields from two bodies would interact in such a way that initially the ions would be driven or set into motion.

Our experiments showed that electrostatic charge in wheat seeds is weakly negative \((-0.191 \pm 7.15 \times 10^{-2}\) pC/grain), the electrostatic charge of diatomaceous earth is slightly negative \((-11.554 \pm 2.342\) pC/grain) and nanostructured alumina bears a strong negative electrostatic charge \((-93.91 \pm 2.62\) pC/grain). These data indicate despite the negative charge of wheat kernels, other characteristics such as rugosity and hairs on wheat kernels' surface are determinant for the surface attachment of DE and NSA particles (Figure 3a, b). Thus, even the repelling force between like charged particles and wheat kernels, the low net charge density of these will not be relevant enough for particle detachment from the kernels and therefore, the smaller the particles the denser the wheat grain surface coverage (Figure 3b).

As shown here and by different authors [55, 58, 60], insects possess bodily electric charges raised by walking or flying. In our experiments, the insects rubbing against flour on the 30° tilted ramp emulate their movement within a stored grain matrix where they charge themselves throughout friction (tribo-charging) and thereby enhance adherence of all particles bearing an opposite charge to their body.

As shown, the rate of insect tribo-charging at the start of the ramp was proportional to the saturation charge that decreases as the insects charge increases. This can be explained as follows: a sliding insect can be thought of as a conducting but electrically isolated object in motional contact with the ramp. The insect and ramp surface start with unequal electron affinities. The ramp surface has a high electron affinity so it takes electrons from the insect gaining negative charge and the insect gains a positive charge due to the loss of electrons [79].

The experimental results presented here (Figure 2) show that adults S. oryzae take up and retain a positive electrostatic charge on the cuticle, approximately proportional to the distance shifted on the experimental wheat flour ramp (d\(_{1.25\text{cm}}\) = +0.766 \((\pm0.254)\) pC/insect to d\(_{40.0\text{cm}}\) = +2.56 \((\pm0.221)\) pC/insect), which is consistent with the results obtained by Jackson and McGonigle [60] experiments. Thus, when S. oryzae was exposed to wheat kernels treated with NSA and/or DE dry powder, negatively charged particles became attracted to the positive tribo-charged insect body surface. However, bonding of DE particles on the insect body surface is 8.13 times weaker than NSA due to lower electric net charge \((-11.554 \pm 2.342\) pC/grain) of DE and its larger particle size (Figure 4b) and mass. Instead, bonding of NSA particles to the insect body surface is strong due the magnitude of its electric charge \((-93.91 \pm 2.62\) pC/grain) and because particles are smaller and lighter (Figure 4a).
These differences in attachment effectiveness are evidenced by the fact that insects exposed to surfaces treated with NSA became massively and uniformly coated with NSA particles (Figure 5a). In contrast, insects exposed to surfaces treated with DE showed a scant and diffuse distribution of particles on their body surface (Figure 5b) demonstrating that DE are not retained as NSA particles are (Figure 5c).

Insecticidal inert powders in general, attach to the insect cuticle surface (Figure 1) damaging the cuticle and producing a negative effect on insect water balance [36]; furthermore insecticidal efficacy decreases as ambient humidity increases and this may negatively impact the efficacy of inorganic powder insecticides [26, 32]. This decrease in efficacy in at higher relative ambient humidity of abrasive powders as DE can be explained by a delayed drying process [80] due to a slower rate of water loss through the damaged insect cuticle [40, 81–84]. The natural transpiration rate of an insect into the surrounding air is dependent on water vapor pressure. With increasing relative humidity the vapor pressure increases in the air and the water discharge from the insect body surface tends to decrease. These results are consistent with earlier findings for abrasive insecticide powders which suggested that toxicity of insecticide powders on arthropods is a consequence of the “cuticular water flux” [84]. On the other hand, the loss of insecticide efficacy in sorbitive insecticide powders such as NSA at higher relative humidity can be explained by analyzing the effect of moisture on the interaction of tribocharged insect body surface and the small but high electrically charged particles of NSA as follows: at constant temperature, a substance absorbs moisture from the air until the material and humidity are in equilibrium attaining the adsorption isotherm of the substance [85]. As shown, triboelectric charge is the main reason for insecticide powder adhesion to insect body surface. In general, the importance of the triboelectric effect increases with low humidities and with smaller particles. High relative humidity can influence the interparticle forces when certain quantity of water is condensed on particle surface reducing the electrostatic forces by electrostatic discharge [86]. Initially, at lower humidity (<65%) [87], the water adsorbs on the particle in the form of water vapor. So, the interparticle bond forces can be reduced as electrostatic forces are reduced. As the humidity increases exceeding the critical value, capillary condensation occurs at the contact points of the particles and liquid bridges form. Above
the critical value, the capillary forces are the predominant forces [87]. Due to the different contact angles, hydrophilic substances as DE [88, 89] are more exposed to the influence of moisture than hydrophobic materials (synthesized Al$_2$O$_3$; [90]). In addition, water adsorption also affects the surface energy of the particles [91]. Similar to liquids, solids have an imbalance in the surface forces. However, in solids the molecules are much more strongly bonded to one another and the surface energy is not evenly distributed on the particle surface.

Differences in insecticidal efficacy between DE an NSA arise from structural and physical differences between these two products. DE combines high abrasive and low sorptive properties due to sharp angular structure and large particle size (1 to about 150 μm [92]), (Figure 3b) and a relatively low specific surface area (ca. 4 m$^2$/g) [93]. In contrast, NSA particles (Figure 3a) are small aggregates (≈1.5 μm; [32]) assembled by coarse accumulations of nanoparticles (40–60 nm) which increase the overall specific surface area of the powder (ca.14 m$^2$/g [94]). Thus, DE insecticidal efficacy is lower than that of NSA due to its small electric affinity to the insect body surface (Figure 4b).
in addition to low sorptive properties. So, DE works, in general, stochastically by damaging the insect body surface mechanically when it moves within a stored grain matrix. On the other hand, NSA’s high insecticidal efficacy depends on its increased electrical affinity to the insect body surface (Figure 4c) in addition to having greater sorptive properties. The whole mechanism of action consists of two steps in sequential order. First, there is a strong electrical binding between negatively charged NSA particles and the positive tribo-charged insect. Next, dehydration of the insect occurs due to strong sorptive action of NSA particles removing the insect cuticular waxes responsible for protecting insects against water loss. Hence, the mechanism of action of NSA does target the water balance of the insect and dehydration is the leading cause of death.

6. Conclusions

Nanostructured alumina (NSA) is a nano-engineered material which has insecticide properties. The current study investigated its mode of action and demonstrated that tribo-charging is a key aspect in the interaction of NSA and the insects’ cuticle. In fact, triboelectric charge is the main reason for insecticide powder adhesion to the insect body surface, and could explain at least in part, the efficacy differences observed in previous studies between NSA and diatomaceous earth (DE). Insects exposed to surfaces treated with NSA became massively and uniformly coated with NSA particles while insects exposed to surfaces treated with DE showed a scant and diffuse distribution of particles on their body surface. This in turn, was accompanied by a difference in charge between both powders, where NSA has a greater intrinsic electric charge than DE. Moreover, NSA charges did not decay as a consequence of NSA low wettability. Thus, the current study supports previous studies showing that NSA has a greater affinity towards the insect cuticle and a greater insecticidal efficacy than other inert powders, and provides a reasonable explanation of its mechanism of action through triboelectric and sorptive phenomena. Further research is necessary to contribute to the knowledge of the complex relationships between physical and chemical parameters of insects and powders, responsible for insecticide activity. Future studies should focus on determining the insect chemical and physical characteristics that are involved in toxicity of inert powders such as NSA to insects. Measuring the triboelectric charges of different insect species could shed light on the basis of these differences in toxicity observed among different insect species to NSA, which may be related to their chemical composition as well as their physical structure, leading to electric charges of different sign and magnitude.

With regards to toxicity research studies should aim to determine the extent of absorption, systemic availability, accumulation and excretion of nanomaterials after inhalation and oral exposure, as well as genotoxicity. However, the necessary in vitro studies should be integrated into any toxicological studies to avoid unnecessary animal experiments. The influence of modifications in the NSA synthesis on the kinetic parameters as well as on the toxicological properties of the nanomaterials should also be examined. Finally, oxidative stress and the formation of reactive oxygen species (ROS) are fundamental key mechanisms of cellular defense after particle capture.

The current study, investigating the mode of action of NSA, supports previous studies demonstrating that NSA is more effective than other insecticide powders and has good potential...
as insecticide of stored grain insect pests since it possesses some of the characteristics of an ideal insecticide, given that it is not reactive, of low synthesis cost, with reduced probabilities of generating resistance in insects, and it is more effective than other commercially available insecticidal powders. It is likely that NSA may be used against other insect pests with similar and further research investigating this is warranted.

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References


[81] Chiu SF. Toxicity studies of so-called “inert” materials with the bean weevil, Acanthoscelidies obtectus (Say). Journal of Economic Entomology. 1939;32:240-248


