Insecticidal Effects of Different Application Techniques for Silica Dusts in Plant Protection on *Phaedon cochleariae* Fab. and *Pieris brassicae* L.

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**Abstract.** Since the 1900s, diatomaceous earth (DE) has been used as an alternative to chemical insecticides in stored product protection. New silica and DE formulations offer expanded possibilities for use in horticultural crops. However, many crop pests are found on the leaf underside and this is especially challenging when using silica because the substance must have direct contact with the insect to be effective. We tested three application techniques with three formulations of silica to evaluate their efficacy against different developmental stages of mustard leaf beetle (*Phaedon cochleariae* Fab.) and the cabbage worm of the large white butterfly (*Pieris brassicae* L.) on the host plant species pak choi (*Brassica rapa* ssp. *chinensis* L.). Formulations were applied manually with a powder blower, with an electrostatic spray gun, and in a closed chamber also working with electrostatic forces. The silica formulations used in the biotests were Fossil Shield 90.0s®, AE R974®, and a formulation developed at Humboldt University Berlin called AL-06-109. All formulations contained at least 60% silicon dioxide. Significant differences in efficacy were detected with different application methods and/or silica formulations. AL-06-109 electrostatic cabin-applied was the most effective combination. All formulations, if applied electrostatically, resulted in good coverage and in high plant protection against insect pests. Dusts applied manually were unevenly distributed and easily removed by wind from leaf surfaces. Electrostatic application with a spray gun resulted in even particle distribution on plants, but overspray was high. To accomplish even coverage without wasting so much active material, an enclosed mobile chamber with an electrostatic spray system and an attached exhaust system was developed.

The ubiquitous issues of pesticide residue and pest resistance to pesticides are becoming increasingly challenging for society to solve. Despite their hazards, many countries still produce, store, and use chemical pesticides on a regular basis. In the European Union and the United States, many chemical pesticides have been taken off the market because of stricter environmental and public health regulations. Therefore, there is an increased need for alternative plant protection materials. Naturally occurring silicas [e.g., diatomaceous earth (DE)] have already been verified for their insecticidal properties in the field of stored product protection by numerous authors (Athanassiou et al., 2003; Dowdy, 1999; Ebeling, 1971; Fields and Korunic, 2000; Mewis and Reichmuth, 1999; Mewis and Ulrichs, 1999; Prasanta, 2003; Stathers et al., 2004). In past years, DE and other silica-based products have proven to be suitable alternatives to chemical insecticide treatments (Erb-Brinkmann, 2000). Many silica compounds that are commercially available contain high amounts of silicon dioxide (SiO₂). Usually SiO₂ powder consists of mostly crystalline forms, which are proven to have a harmful effect on the lungs if inhaled. Fortunately, the silica used as insecticides in recent years has amorphous forms and only a very small portion of crystalline silicon oxide, so these products have no toxic side effects on mammals (Johnston et al., 2000; Korunic, 1998; Merget et al., 2002).

Early formulations of DE-based silica products were not effective under relative humidity conditions (Mewis and Ulrichs, 1999), but newly developed formulations have been shown to be effective above 70% relative humidity, which is common in greenhouse environments (Weishaupt et al., 2004). They also proved that the efficiency of silica formulations improves with the level of hydrophobicity. This is because the hydrophobic silica does not become saturated in humid environments compared with hydrophilic formulations. In addition to hydrophobicity, Ulrichs et al. (2006a) confirmed that formulations with smaller particle sizes have greater ability to absorb lipids from the insect’s epicuticle and thus increase insect mortality.

The application method of silica is central to its effectiveness because it is a contact insecticide and must reach pests that commonly live on the leaf underside. In earlier findings, Ulrichs et al. (2006b) demonstrated that FS90.0s® (Fossil Shield Company, Eiterfeld, Germany) can be successfully used for pest management under greenhouse conditions if applied electrostatically. The aim of this study was to examine different application methods concerning practicality and effectiveness. Additionally, through bioassays (“choice” and “force-feeding” experiments), the effectiveness of different applied silica products was investigated. Bioassays were conducted at different development stages of mustard leaf beetle (*Phaedon cochleariae* Fab.) and the cabbage worm of the large white butterfly (*Pieris brassicae* L.). These insects were chosen because they are serious pests of cruciferous crops in Europe (Kristensen, 1994; Schellhorn and Sork, 1997; Wang et al., 2009). All of the experiments were conducted on pak choi plants under greenhouse conditions.

**Materials and Methods**

**Application techniques**

*Manual application.* Silica formulations were manually applied to plant surfaces with a powder blower (“Bobby Duster”) (Fig. 1). Silica formulations were blown onto the plants with an air flow generated by a manual pumping process.

*Electrostatic application.* The current uses of electrostatic phenomenon are numerous (Hughes, 1997; Reddy, 1989; Schnick, 2009). For this project, we used an automatic electrostatic spray gun, which was originally developed for painting (Fig. 2). The advantages of using the powder painting method are even coverage on surfaces, including difficult-to-reach corners and crevices, and reduction of overspray and material waste. The electrostatic mechanism works according to several authors (Mandel, 2009; Moyle and Hughes, 1985; Pulli, 2006) as follows (Fig. 3): electrostatics involves static charges, their dispersion, and fields between charged solids. The forces between electrical charges are described by Coulomb’s law, and they have the ability to...
move particles in an electrical field. In the process of electrostatic powder application, the charged powder is transported by an electrical field onto the grounded plant and then the powder is deposited onto the plant subjected to experiment. The force between two electric charges (spherical–symmetrical) is proportional to the product of the quantity of the charges \( q_1 \) and \( q_2 \) and is reciprocally proportional to the squared distance \( r \) between both (\( \mathbf{C}15 = \text{matter constant} \)). If particles with the charge \( Q \) come into an electrical field \( E \), the force \( F \) is on these particles proportional to the charge \( Q \).

\[
F = \frac{q_1 \times q_2}{16 \pi \varepsilon_0 r^2} \quad \text{(Coulomb's law)}
\]

With these forces, particles move in an electrical field \( F = Q \times E \).

**Electrostatic spray gun application.** The spray gun application (Fig. 2) uses the “Corona-coating” method (Fig. 3). An electrical field is created between the spray gun and plant. Here the silica particles are loaded electrically and follow the electrical flux lines onto the grounded plants. Because the electrical flux lines are curved, they are able to reach the leaf underside.

**Electrostatic cabin application.** To improve the degree of efficiency and coating as well as the usability, a special mobile cabin was constructed (Fig. 4A). The cabin consisted of acrylic glass to minimize the weight. Two semicircular spray rims (of 180° each) were equipped with specially designed spray nozzles along with corona electrodes replacing electrostatic spray guns. The nozzles produce a “soft cloud,” which made it possible to surround the plants and cover them evenly with the silica powder (Fig. 4B). The intermittent exhaust system takes out the excess material left over during the application process in the chamber. The charging of the particles follows the “Corona-Principle” (Fig. 3).

**Insect trials: efficiency testing**

**Plant material.** The trials were carried out in a greenhouse at Humboldt-Universität zu Berlin (Germany). Pak choi (Brassica rapa ssp. chinensis L.) ‘Black Behi’ (East-West-Seed-Co., San Rafael, Bulacan, Philippines) plants were sown and, after 10 d, were single-planted into 10-cm diameter plastic pots in the greenhouse at 20 to 25 °C in standard potting soil (Gramoflor: 5.2 to 6.0 pH, 90 to \( \mathbf{1000} \)N–61 to \( \mathbf{100} \)P–158 to \( \mathbf{257} \)K and 0.7 to 1.2 L–\( \mathbf{1} \)g–\( \mathbf{1} \) salt). Light was provided with 10 klux intensity exposure for 12 h per day. The relative humidity (RH) ranged from 60% to 70%. During the experiments, temperatures and RH were monitored by a climate system in the greenhouse, and additionally, HOBO Pro Temp/R H data loggers were used to measure microclimate (Onset Comp., Pocasset, MA). Throughout the entire duration of testing, demand-driven irrigation was used.

**Insects.** \( \mathbf{P} \). cochleariae and \( \mathbf{P} \). brassicae were raised in an air-conditioned rearing room (22 °C, 60% RH) at Humboldt-Universität zu Berlin. Adults, eggs, and young larvae (L1) were reared on 4- to 5-week-old pak choi plants. Beginning with the second instar, the larvae of \( \mathbf{P} \). cochleariae were put on cellulose paper and savoy cabbage. From the fourth instar, it was necessary to avoid larval disappearance into the soil. Adult \( \mathbf{P} \). brassicae were fed on a honey/ascorbic acid in water mixture and laid eggs on cabbage turnip plants. After the first two development stages, they were transferred onto savoy cabbage on a bed of tissue paper. After pupation to simulate diapause, the pupae were cooled to 4 °C up to a maximum of 6 months.

**Silica formulations.** All silica materials used were obtained from the Fossil Shield Co. (Eiterfeld, Germany). The commercial circumferential equipment (fluidization instruments, powder and air supply, and the exhaust system) applies to standards for dust exposure regulations.
product “FS90.0s®” is a natural amorphous silica mixture based on DE with a particle size of 1 μm to 15 μm in diameter. These substances consist of 60% to 80% amorphous silicates, 12% to 16% aluminium oxide, and minor amounts of other oxides. The second group of silica were Aerosils® (Evonik Degussa GmbH, Frankfurt, Germany). They are synthetically produced in amorphous forms by flame hydrolysis processes or pyrogenesis in an oxyhydrogen flame starting from fluid chlorine silane. The average size of primary particles of the formulation AE R974® was 12 nm (Degussa/Evonik, 2009). Furthermore, our group developed a new product called “AL-06-109” based on naturally occurring silica. With an extensive pore system, it contains a high specific surface area of ≈800 m²·g⁻¹ with small hollow spherules. AL-06-109 is an amorphous material containing ≈86% silicon dioxide and has an average particle size of 6.70 μm. Through surface modification, “AL-06-109,” AE R974®, and FS90.0s® showed hydrophobic properties. As mentioned earlier, hydrophobic formulations are needed in high-humidity greenhouse surroundings.

Experimental design
Pak Choi plants were raised to the age of 5 weeks. Silica materials were either applied manually or electrostatically onto the plants. In one experimental setting, the silica dust remained on leaf surfaces, and in another setting, the dusts were rinsed off with water 48 h after treatment to examine residual effects of substances on the plants. These trials were conducted because particulate matter on leaf surfaces influences plants by reducing photosynthesis (Hirano et al., 1995; Pereira et al., 2009; Ulrichs et al., 2008b). All different bioassays were conducted three times with 10 replicates each for every formulation. In force-feeding tests, insects were placed into plastic cages with a gauze cover on single plants whereas in choice tests, they could choose between untreated and treated plants. Untreated plants in all trials served as controls. *P. cochleariae* was tested at different stages (eggs, larvae, adult) because all can cause plant damage. In the experiment with *P. brassicae*, second and third instar larvae were used because the larvae cause plant damage. In force-feeding tests and choice tests with *P. cochleariae*, 10 insects were applied to each plant in the trial. In the trials with *P. brassicae*, one larvae was placed onto each plant in the experiment. After 3 d (*P. cochleariae* beetles) and 5 d (*P. cochleariae* larvae, *P. brassicae* larvae), the mortalities of the insects were determined. Insects were counted as live if they showed movement. Insect leaf damage was measured using damage classes and scanning software (Sigma Scan Pro 5.0; STATCON, Witzenhausen, Germany) to quantify the amount of removed leaf area. In further observation, the reproductive capability of one pair of *P. cochleariae* per plant in the experiment was calculated after oviposition and hatching. Because the mode of action relies on the ability of silica-based substances to physically desiccate the insects (Mewis and Ulrichs, 2001a), the water content of the insects was calculated. For this, the insect body weight was evaluated before and after drying in an oven at 60 °C.

**Statistical analyses**
Data were subjected to analysis of variance (ANOVA) (one/two-way ANOVA) and Tukey’s honestly significant difference test to detect differences between treatments (*P* < 0.05). We used the statistical software package Statgraphics 15.2 (StatPoint Technologies, Inc., Warrenton, VA) and GLM Procedure in SAS 9 (SAS Institute Inc., Cary, NC).

**Results**
Comparison of application methods. It was impossible to achieve an even particle distribution on plant surfaces when using the “Bobby-Duster” system because the dust particles accumulated. Therefore, the formulations piled up on some leaf zones (Fig. 1). The average area of insect leaf damage (Table 1) in the manual application treatment after 3 d...
was above 53% with all used silica formulations and 84.5% in control plants (Fig. 5B). Similarly, the average insect mortality was below 40%, whereas on control plants, 2% of the insects died (Table 1; Fig. 5A). Nevertheless, the formulations showed a significant effect when compared with the control. In addition, a positive correlation between damage effect when compared with the control. In

Table 1. Comparison of application methods of silica substances on average mortality and leaf damage of Phaedon cochleariae beetles after 3 d treatment in a force-feeding test on Brassica chinensis plants (19 to 22 °C, 55% to 70% relative humidity).

| Substance | Application method | Mortality (%) | Leaf area damaged (%) |
|-----------|-------------------|---------------|----------------------|
| AL-06-109 | Manual            | 19.0 a        | 56.5 c               |
|           | Spray gun         | 16.0 a        | 15.7 b               |
|           | Cabin             | 66.0 b        | 2.5 a                |
| FS 90.0s® | Manual            | 37.0 a        | 53.2 b               |
|           | Spray gun         | 64.0 b        | 3.3 a                |
|           | Cabin             | 73.0 b        | 5.3 a                |
| AE R974®  | Manual            | 20.0 NS        | 63.0 b               |
|           | Spray gun         | 30.0 NS        | 2.5 a                |
|           | Cabin             | 17.0 NS        | 4.6 a                |
| Control   | Manual            | 2.0 NS         | 84.5 NS              |
|           | Spray gun         | 4.0 NS         | 70.7 NS              |
|           | Cabin             | 2.0 NS         | 77.8 NS              |

Different letters indicate significant differences between application methods within each substance (Tukey’s honestly significant difference at P < 0.05); NS = nonsignificant.

In the experiment with the electrostatic spray gun application (Table 1), leaf damage caused by insect feeding was generally lower than in the manual application trials. The leaf damage on AL-06-109-treated plants decreased from 56.5% to 15.7% (Table 1; Fig. 5B). Similar results were obtained for FS90.0s® and AE R974®. Average leaf damage in treated plants was in general significantly lower than in control plants. Similarly, the insect mortality was higher with electrostatic applications of silica than with manual applications. Mortality increased to 64% for the formulation FS90.0s®, whereas 16% occurred for AL-06-109, 30% for AE R974®, and 4% in the control (Table 1; Fig. 5A).

The electrostatic treatment in the cabin significantly increased the insect mortality compared with the spray gun treatment only for the substance AL-06-109 from 16% to 66% (Table 1). For FS90.0s® (73%), the average mortality was not increased when compared with the spray gun, whereas for AE R974® (17%), the mortality decreased. Likewise, the average leaf damage could be significantly reduced to 2.5% in the cabin trials with the formulation AL-06-109 (Table 1). In addition, the average leaf damage did not change for FS90.0s®, AE R974®, or the control. However, user safety and a clean working environment improved greatly after application as a result of decreased exposure to dust and reduced mess in general.

Beetle as well as larval mortality (T. Mucha-Pelzer, unpublished data) was not significantly influenced by leftover dust particulate matter of the different formulations (Table 2). Nevertheless, the material application and a following (48 h) rinse off still reduced the leaf damage of pak choi plants significantly below 44% for AL-06-109 and AE R974® compared with the control (70.7%), whereas FS90.0s® showed no difference (Table 2). Like with the manual application, a positive correlation between leaf damage and beetle mortality could be established ($R^2 = 0.8784$).

Comparison of silica products. The formulations were tested on different insect species and developmental stages because the efficacy of silica products depends not only on the application technique, but on the insect stages (Mewis and Ulrichs, 2001b) as well on the product formulation itself (Aldryhim, 1993; Aghanassiu et al., 2003). Also, the effectiveness of bioinsecticides depends on their mode of action. Therefore, to determine protective effects of formulations and application techniques, choice test bioassays were also conducted.

P. cochleariae larvae on dusted plants caused significantly lower leaf damage compared with the control (Fig. 6). However, average larval mortality did not increase significantly for FS90.0s® and AE R974®. Only AL-06-109 was able to reduce the larval population significantly with a mortality rate of 71% in contrast to the control (43%). In force-feeding experiments with P. brassicae...
latter (Fig. 7A), all silica formulations achieved a significant reduction of leaf damage in comparison with the control. The larval mortality on treated plants was significantly higher than the larval mortality on control plants. In addition, the treatments reduced larval weight significantly, whereas the control larvae even gained weight (Fig. 7B).

Application of silica formulations after oviposition had an ovicidal effect (Fig. 8). The present study verified that hydrophobic silica formulations can control insect pests on *B. chinensis* plants when they are applied electrostatically.

**Discussion**

Several studies have been conducted to determine the effectiveness of diatomaceous earth against insect pests. Many studies have reported a wide variation of susceptibility in stored product pests (Faulde et al., 2006; Fields and Konunic, 2000; Konunic, 1998), but very few studies have been conducted with horticultural pests (Ulrichs et al., 2008b). In contrast with horticultural pests, formulations with particle sizes from 1 μm to 50 μm used here. With the cabin construction, efficiency of silica formulations could be improved for transport and distribution, whereas the dosing unit and the injector bar guaranteed good fluidization. As a result of the construction of the peripheral equipment, smaller particles could be better fluidized and transported on all plant surfaces. An overall cover could be produced with a “soft cloud” of particles. As expected, the application in the mobile chamber was the cleanest way to cover the plants with the silica formulations. Nevertheless, the cabin design needs to be improved on further because currently it can only dust one plant at a time, and this is not feasible for a large greenhouse operation. Therefore, the cabin should be combined into other working steps in greenhouse procedures such as potting and separating plants.

**Comparison of silica formulations.** In earlier findings, Ulrichs et al. (2006b) demonstrated that FS 90.0® can be successfully used for pest management under greenhouse conditions if applied electrostatically. Our results also support the finding that silica formulations are effective protection agents in
greenhouse environments (Fig. 5A–B). However, the AL-06 formulation in the experiment conducted by Ulrichs et al. (2006b) achieved low insect mortality rates (10% to 20%) and more leaf damage, whereas the new formulation, AL-06-109, used here was more efficacious (Fig. 5A–B). The difference between AL-06 and AL-06-109 is that AL-06 had a 10-fold larger particle size than the newly developed AL-06-109 (average 6.70 μm). In addition to smaller particle size, AL-06-109 is also more hydrophobic than the original formulation. According to Weishaupt et al. (2004), the efficiency of silica formulations improves with the level of hydrophobicity. Hydrophobic silica also lasts longer in humid environments than more hydrophilic formulations. In addition, Ulrichs et al. (2006a) confirmed that formulations with smaller particle sizes have greater ability to absorb lipids from the insect’s epicuticle, therefore increasing mortality.

Insect populations in all conducted experiments were significantly reduced by the use of silica formulations. This reduction is consistent with many authors who have reported on the ability of silica to reduce various insect populations (Athanassiou and Palyvos, 2006; Mewis and Ulrichs, 2001a). In our trials, the average mortality did not exceed 82%, whereas other authors have reported higher mortality rates (Ceruti and Lazzari, 2005; Dowdy and Fields, 2002). This mortality reduction could be explained because most of their trials were conducted under lower RH and more consistent temperatures. Temperatures and humidities were less constant with values from 20 to 35 °C and 75% to 95% RH inside our cages. In these surroundings, silica tends to saturate with water leading to inactivation. According to Vayias and Athanassiou (2004), insect death is delayed in higher humidities. Mewis and Ulrichs (2001b) also established the idea that insects are able to extend their life through the uptake of food of which water might be metabolized. Nevertheless, although they were counted alive on body movement, they were near death as a result of the silica, and most insects were not able to cause any further damage. Because no oviposition in the bioassays was monitored when coverage was sufficient enough, this emphasizes the population-reducing effect, although the degrees of mortality were not substantial.

The electrostatically applied silica formulations showed an ovicidal effect (Fig. 8). Several authors reported the ability of silica to suppress progeny (Ferizli et al., 2005; Mewis and Ulrichs, 2001b; Stathers et al., 2004). In our trials, silica was generally more effective against beetles (Fig. 5; Tables 1 and 2) than larvae (Figs. 6 and 7), and this is consistent with research by Arthur (2006) and Mewis and Ulrichs (2001b). The explanation relies on the differences in morphological structure and physiology of the insects. In addition, the thickness of the cuticle during insect development can play a vital role because younger insects with thinner cuticles were killed more quickly in the trials. These findings were also supported by Mucha-Pelzer et al. (2008).

In the present study, the insect body water loss of P. brassicae larvae correlated with mortality (Fig. 7B). As a result of the mode of action of silica formulations, the insect cuticle becomes damaged, water is lost, and the insect is desiccated. The higher hydrophobic formulations resulted in a greater water loss.
and lower water content in insects (Mewis and Ulrichs, 2001a). The mode of action therefore relies on contact of silica with the insect surface. If plants are dusted sufficiently with silica, the modes of action of other materials can be weakened by fulfilling their mode of action. In choice tests, the preventive effect by use of silica was examined (Fig. 9). Force-feeding test plants in our experiment were protected from insect feeding by silica applications. There were no significant differences found in the effectiveness of different silica formulations. Nevertheless, silica causes a preventive layer and protective effect because there was no damage or eggs found on the plants.

**Impact on plant physiology.** Even after the removal of silica, protection against insect feeding could still be observed (Table 2). It is possible that particle residue had a repellent effect on the insects tested. However, many authors reported a negative influence of dust deposition on plant physiology because they found decreased photosynthesis and transpiration (Hirano et al., 1995; Pereira et al., 2008; Varadka et al., 1995). Because we observed decreases in plant growth and fading of leaf color, further research should be conducted to determine the effect of silica particles on plant appearance and physiology. Maybe these processes were originated by stress through coverage of the plants. Plants are possibly subjected to stress because of residual particles. These residual particles could be responsible for inducing changes in plant-secondary metabolite composition. This has to be taken into consideration, because secondary metabolites play an important role in plant defense mechanisms (Halkier and Gershenzon, 2006). It remains to be determined if the cuticle of plants gets damaged like the insect cuticle because the mode of action relies only on the physical activity and cannot distinguish between cuticle types. Because there is still the possibility that silica interact with the leaf surface, we currently recommend treatments only shortly before harvesting. A reduction in photosynthesis does not cause any substantial yield loss. This stage of plant development and synthetically chemicals cannot be applied any more because of possible residue problems. Further studies regarding the influence on plant physiology are ongoing. Nevertheless, our findings indicate that hydrophobic electronstatic interactions of silica formulations can assure effective plant protection under greenhouse conditions.

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