# Susceptibility of *Alabama argillacea* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) larvae to *Beauveria bassiana* associated with kaolin

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## Abstract

The mortality of the *Alabama argillacea* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) larvae caused by the kaolin inert powder and the entomopathogenic fungus *Beauveria bassiana* were determined under laboratory conditions. Using the caterpillar submersion method, the CG 138 *B. bassiana* isolate was more pathogenic to *A. argillacea* than the CG 70, GC 82, ESALQ 634, and ESALQ 645. All five tested isolates caused similar mortality of *C. includens*. The mortality of first-instar larvae of *A. argillacea* and *C. includens* by feeding on leaf-disc impregnated with *B. bassiana* (CG 138) and kaolin was also determined. Higher *A. argillacea* mortalities were observed in the *B. bassiana* (CG 138) treatments, regardless of the presence of kaolin. However, the activity of kaolin + *B. bassiana* (CG 138) against *C. includens* was higher than each ingredient alone, indicating an additive action against *C. includes* larvae. The mortality of *A. argillacea* and *C. includens* larvae treated with kaolin + *B. bassiana* (CG 138) was similar, and the *A. argillacea* mortality was higher than that of *C. includens* with kaolin and *B. bassiana* (GC 138) separated. The treatment kaolin + *B. bassiana* (CG 138) is promising for the simultaneous management of these two defoliator pests, mainly *A. includes*. In addition, the monophagous *A. argillacea* is more susceptible to both kaolin and *B. bassiana* (GC 138) than the polyphagous *C. includens*, suggesting that the nutritional ecology plays an important role in the susceptibility of these defoliator species to alternative insecticides.

*Keywords:* Associated use, cotton leafworm, *Gossypium hirsutum*, particle film, pathogenicity, soybean looper.

## Susceptibilidade de larvas de *Alabama argillacea* e *Chrysodeixis includens* (Lepidoptera: Noctuidae) a *Beauveria bassiana* associada com caulim

### Resumo

A mortalidade de larvas de *Alabama argillacea* e *Chrysodeixis includens* (Lepidoptera: Noctuidae), causada pelo pó inerte do caulim e pelo fungo entomopatogênico *Beauveria bassiana*, foi determinada em condições de laboratório. Usando o método de submersão da lagarta, o isolado CG 138 de *B. bassiana* foi mais patogênico para *A. argillacea* que os CG 70, GC 82, ESALQ 634 e ESALQ 645 desse fungo. Os cinco isolados testados causaram mortalidade semelhante de *C. includens*. A mortalidade de larvas de primeiro ínstar de *A. argillacea* e *C. includens* pelo método de alimentação em discos foliares impregnados com *B. bassiana* (CG 138) e caulim foi, também, determinada. A mortalidade de *A. argillacea* foi maior nos tratamentos com *B. bassiana* (CG 138), independentemente da presença do caulim. A atividade do caulim + *B. bassiana* (CG 138) contra *C. includens* foi maior que cada ingrediente isolado, indicando ação aditiva contra larvas desse Lepidoptera. A mortalidade de larvas de *A. argillacea* e *C. includens*, com caulim + *B. bassiana* (CG 138), foi semelhante e as de *A. argillacea* maiores que as de *C. includens* com caulim e *B. bassiana* (GC 138) isoladamente. O tratamento caulin + *B. bassiana* (CG 138) é promissor para o manejo simultâneo dessas duas pragas desfolhadoras, principalmente *A. includens*. Além disso, a monófaga, *A. argilacea*, é mais suscetível ao caulim e à *B. bassiana* (GC 138) que a polífaga, *C. includens*, sugerindo que a ecologia nutricional desempenha papel importante na suscetibilidade dessas espécies desfolhadoras a inseticidas alternativos.

*Palavras chave:* filme de partículas, *Gossypium hirsutum*, lagarta desfolhadora do algodão, lagarta desfolhadora da soja, patogenicidade, uso associado.

# 1. Introduction

The cotton leafworm, *Alabama argillacea* (Hübner, 1818) and the soybean looper, *Chrysodeixis includens* (Walker, 1857) (Lepidoptera: Noctuidae) are cotton defoliator pests (Viana et al., 2014). These Lepidoptera pests have different specialization levels (monophagy and polyphagy) on cotton plants, and are controlled with synthetic chemical insecticides (Oliveira et al., 2010). The chemical control can cause environmental problems, including ecological unbalance; the development of insect and mite resistance to agrochemicals (to date, more than 500 resistant pests have been identified); outbreak of secondary pests; pest resurgence; harmful effects on humans, natural enemies of pests, fish and other non-target animals, in addition to persistent chemical residues in food, water and soil (Nascimento et al., 2016).

The use of kaolin particle film technology can improve the efficiency of insecticides to controlling cotton pests (Silva and Ramalho, 2013), including aphids, boll weevils, cotton leafworms, pink larvae and white flies, as well as the cotton bollworm complex and those of the genus *Spodoptera* (Neves et al., 2014; Gonçalves et al., 2015). The film of kaolin particles, sprayed on the surface of the host plants, creates a physical or mechanical barrier that can alter plant tissue taste and digestibility, reducing the feeding and survival of chewing insects (Gonçalves et al., 2015; Guedes et al., 2020). Kaolin may affect the insect cuticle permeability to water, a limiting factor for these organisms (Cook et al., 2008).

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is produced and used in Brazil to manage insect pests of many orders (César Filho et al., 2002; Sree and Varma, 2015). The wide genetic variability of this entomopathogen increases its importance in microbial insect control. The combined use of kaolin with *B. bassiana* may increase the effect of this entomopathogen in the control of *A. argillacea* and *C. includens*.

Inert dusts, such as mineral clays and silica powders, remove the epicuticular lipid layers of arthropods, causing excessive water losses through the cuticle, and death (Storm et al., 2016). These dusts are used in the management of pests of both plants during cultivation and also in stored products (El-Aziz, 2011; Silva and Ramalho, 2013). The combination of entomopathogenic fungi with inert kaolin powder needs to be studied against pests of cultivated plants and grains. *Beauveria bassiana* formulated with kaolin has been shown to be more effective against *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (Storm et al., 2016), *Corcyra cephalonica*

Stainton (Lepidoptera: Pyralidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) adults than with tapioca flour or unformulated (Samodra and Ibrahim, 2006a, b).

The objective of this research was to determine the susceptibility of the cotton leafworm, *A. argillacea*, and *C. includes* larvae to the entomopathogenic fungus *B. bassiana* combined with kaolin.

# 2. Materials and Methods

## 2.1. Site of study

The work was conducted at the Embrapa Algodão Entomology Laboratory, Campina Grande, Paraíba state, Brazil. Two experiments were performed. The first assessed the pathogenicity of *B. bassiana* isolates and the second evaluated the additive activity of kaolin combined with the most effective one.

## 2.2. Insects and fungus isolates

*Alabama argillacea* and *C. includens* specimens were obtained from the rearing colonies of this laboratory and fed on a semi-natural diet (Bestete et al., 2017).

The strains of the fungus *B. bassiana*, CG 70, CG 82 and CG 138, were obtained from the mycology collection of the Biological Control Laboratory of *Embrapa Recursos Genéticos e Biotecnologia* in Brasília, Federal District, Brazil, and the Bb 634 and Bb 645 strains from the ESALQ-USP mycology collection, Piracicaba, São Paulo, Brazil. These strains have been tested because they are highly virulent to lepidopteran larvae (César Filho et al., 2002) and are not commercially available. These strains were stored at 7 °C in disposable Petri dishes with PDA (potato-dextrose-agar) and antibiotic (chloramphenicol) (PDA + A) and Nujol oil until the beginning of the bioassays (Table 1).

## 2.3. Isolate selection

The pathogenic action of the *B. bassiana* isolates was determined in a completely randomized experimental design with six treatments (five isolates and one control) and 50 replications per isolate. The replications were performed by neonate larvae of each of the tested lepidopteran species. The strains were multiplied in Petri dishes containing PDA culture medium and incubated for 15 days (Silva, 2001) at 25 °C under a 12 hours-photoperiod. The conidial suspensions of the *B. bassiana* strains were prepared with sterile solution of Tween 80® (Sigma-Aldrich, Saint Louis, MO, USA) at 0.1% as a surfactant. The control had only sterile water plus the surfactant. The first-instar larvae with one-day old of the *A. argillacea* or *C. includens* were submerged in the conidial suspension

**Table 1.** Identification of the strains, hosts and origin place of the entomopathogenic fungus *Beauveria bassiana*.

| Strains | Host | Origin Place |
|---------|------|--------------|
| CG070 | (Coleoptera: Curculionidae) | Brasília, DF |
| CG082 | *Diabrotica speciosa* (Coleoptera: Chysomelidae) | Tucumán, Argentina |
| CG138 | *Cosmopolites sordidus* (Coleoptera: Curculionidae) | Recife, PE |
| ESALQ 635 | Soil | Lavras, MG |
| ESALQ 645 | Soil | Alambari, SP |

at the concentration of $10^7$ conidia ml$^{-1}$ for five seconds. This concentration of *B. bassiana* conidia was used to facilitate the determination of the additive effect of kaolin. After drying on pre-sterilized filter paper, the larvae were transferred to plastic containers with cotton leaf disks, which were replaced daily until completion of the observations in a BOD-type climatic chamber at $25 \pm 1$ °C, $60 \pm 10\%$ relative humidity and photoperiod of 12 hours.

The percentage of insect mortality was evaluated during the first 10 days after pathogen application. The larvae that did not move after being touched with a brush bristle were considered dead. The dead larvae were immersed in 3% sodium hypochlorite solution for three seconds and then in distilled water and kept in a humid chamber for pathogen extrusion and confirmation of its pathogenicity.

### 2.4. Susceptibility of larvae to kaolin and *B. bassiana*

The larvae susceptibility to the *B. bassiana* combined with kaolin was evaluated in a completely randomized design, with a 4x2 factorial scheme, represented by the cotton leaf disks immersed in distilled water (control) and suspensions of kaolin (Caulisa®, Indústria de Caulim S.A., Campina Grande, Paraíba State, Brazil) at the concentration of 0.06 g.ml$^{-1}$; *B. bassiana* at the concentration of $10^7$ conidia ml$^{-1}$; and of the mixture of 0.06g kaolin + $10^7$ conidia ml$^{-1}$ of *B. bassiana*, each offered to first-instar *A. argillacea* and *C. includens* larvae with fifty replications. The *B. bassiana* conidia suspension was composed by the isolate selected in the experiment of this fungus against *A. argillacea* or *C. includens* first-instar larvae. The kaolin dose was chosen based on previous studies against the cotton lepidopteran pests *Spodoptera exigua* (Lepidoptera: Noctuidae) (Showler, 2003) and *A. argillacea* (Lepidoptera: Noctuidae) (Gonçalves et al., 2015) and the apple leafworm *Choristoneura rosacean* (Harris) (Lepidoptera: Tortricidae) (Sackett et al., 2005).

Cotton plants of the cultivar BRS 286 were cultivated in an experimental area at Embrapa Algodão in the first half of 2018 and used in this study. The cotton leaves were collected from 40 day-old plants when they reached the stage of 12-16 leaves per plant and provided as food to first-instar larvae with one-day old of the two Lepidoptera species.

The newly hatched first-instar *A. argillacea* and *C. includens* larvae were individually transferred to cotton leaf discs kept in a plastic container (3 cm high x 3 cm wide x 4 cm long) capped with a plastic plate. The cotton leaf discs were cut from the third expanded leaf counted from the apex of the plants. These were kept in a climatic chamber with the same conditions of the experiment of the "selection of isolates" until the end of the observations. Each replicate had one larvae per species of lepidopteran pest, fed with a cotton leaf disc and 50 replicates per treatment were used. The leaf discs were changed every two days until the end of the observations. The mortality of the larvae was evaluated daily for 10 days with the aid of a stereoscopic microscope.

The germination test of *B. bassiana* conidia was performed in both bioassays with the application of 50 mL of a suspension with $10^7$ conidia mL$^{-1}$ per isolate in Petri dishes with the PDA culture medium. Observations were made 24 hours after starting the bioassay in a total of 500 conidia.

### *Data analysis*

The $TL_{50}$ (time to death of 50% of the first-instar of the cotton leafworm and the soybean looper) was calculated by the Probit method (Finney, 1964) with the data of percentage of mortality of the larvae in the pathogenicity bioassays. The means of mortality percentages were corrected (Abbott, 1925) and compared by the Student Newman Keuls test at 5% probability. Data from the mortality (%) versus $TL_{50}$ variables were submitted to a correlation analysis. The percentage of larva mortality data in the susceptibility bioassay were corrected (Abbott, 1925) and submitted to variance analysis, and the means compared by the Student Newman Keuls test at 5% of probability. The statistical analyses were done with the System of Statistical and Genetic Analysis (Saeg) (Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil).

## 3. Results and Discussion

The viability of the conidia of *B. bassiana* strains tested was higher than 95% in both bioassays.

### 3.1. Selection of isolates

The mortality of *A. argillacea* first-instar larvae in the pathogenicity bioassay 10 days after inoculation ranged from 51 to 93% for all strains tested ($F_{4.25}$= 13.75; P< 0.001; Table 2). A negative correlation between the $TL_{50}$ values and the percentage of mortality of these larvae (r= -0.99; P <0.001) was found that is, the higher the percentage of *A. argillacea* neonate mortality, the shorter the time necessary to kill 50% of its population. The *B. bassiana* CG138 isolate was the most effective against *A. argillacea* first-instar larvae, followed by the ESALQ 634 and CG 82 and CG 70 and ESALQ 645 as the least effective (Table 2). Mortality of *A. argillacea* first-instar larvae with the GC 138 isolate was higher than that found (81, 74 and 40%) for third instar larvae of this species, with conidial suspension of *B. bassiana* strains UFRPE 645 ($10^9$ conidia mL$^{-1}$), UFRPE 604 ($10^8$ conidia mL$^{-1}$) and UFRPE 634 ($10^7$ conidia mL$^{-1}$), respectively (César Filho et al., 2002). These differences can be attributed to the inoculation and conidia concentration of this pathogen (Santoro et al., 2007), and to the larvae development stage (César Filho et al., 2002). Neonate larvae differ in scale, metabolism, digestive physiology, sensory modalities and intestinal microbiota from those of the late stages (Mason and Raffa, 2014; Despland, 2018). Furthermore, the neonate larvae are more selective to food and more sensitive to plant chemicals, possibly due to their more limited set of digestive enzymes (Hochuli, 2001). Therefore, they are often more vulnerable to plant compounds and pathogens that have a reduced effect on

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**Table 2.** Mortality (Mort.) and the $TL_{50}$ (lethal time of 50% of the insects) and confidence interval of *Beauveria bassiana* strains for *Alabama argillacea* and *Chrysodeixis includens* (Lepidoptera: Noctuidae).

| Pest insects | Strains | Mort (%) | $TL_{50}$ (Days)$^2$ | $\chi^2$ | $P$ | Conf. Int. |
|---|---|---|---|---|---|---|
| *Alabama argillacea* | CG 70 | 58 bc | 5.02 | 39.66 | < 0.01 | (4.16; 6.10) |
| | CG 82 | 68 b | 3.76 | 36.01 | < 0.01 | (3.09; 4.44) |
| | CG 138 | 93 a | 2.02 | 26.40 | < 0.01 | (1.68; 2.34) |
| | ESALQ 634 | 72 b | 3.72 | 34.53 | < 0.01 | (3.04; 4.40) |
| | ESALQ 645 | 51 c | 5.94 | 42.73 | < 0.01 | (4.92; 7.44) |
| *Chrysodeixis includes* | CG 70 | 19 a | 74.49 | 48.00 | < 0.01 | (25.36; 47.26) |
| | CG 82 | 23 a | 14.09 | 61.54 | < 0.01 | (10.92; 22.82) |
| | CG 138 | 26 a | 11.82 | 54.01 | < 0.01 | (10.25; 15.19) |
| | ESALQ 634 | 13 a | 26.25 | 53.09 | < 0.01 | (15.83; 99.06) |
| | ESALQ 645 | 18 a | 19.62 | 54.70 | < 0.01 | (13.60; 44.99) |

Mortality means followed by the same lower-case letter per insect-pest column do no differ by the Student Newman Keuls test at 5% probability. $^2$Calculated by the Probit method.

larvae of more advanced instars (Zalucki et al., 2002; Despland, 2018).

The mortality of first-instar *C. includens* larvae 10 days after inoculation ranged from 13 to 26% with all fungus strains ($F_{4.25}$= 1.52; P = 0.22; Table 2), with negative correlation between the values of $TL_{50}$ and the mortality percentage (r= -0.30; P= 0.031). This indicates that there is no conclusive evidence of the association between $TL_{50}$ values and the percentage mortality of *C. includens*. The low mortality of *C. includens* larvae is due to the low capacity of *B. bassiana* isolates to infect them. The insect cuticle is mainly composed of chitin with proteins, acting as a primary barrier against pathogens (Goettel et al., 2005; Xue et al., 2014). *Beauveria bassiana* produces chitinases and proteases to disintegrate the insect cuticle, but its efficacy depends on the spore size, conidia germination rate and metabolite production (Varea et al., 2012; Sree and Varma, 2015) and the composition of free fatty acids in the cuticle of the insects (Sree and Varma, 2015; Wronska et al., 2018). Long-chain fatty acids such as capric, undecanoic, stearic and oleic do not prevent *B. bassiana* conidia germination, but they may cause germ tube autolysis, preventing hyphae growth. Short-chain fatty acids such as butyric, valeric, caproic, heptanoic, caprylic, besides nanoparticulate can inhibit the germination tube of this fungus (Zhang et al., 2012).

*3.2. Susceptibility of larvae to kaolin and* B. bassiana

The mortality of *A. argillacea* and *C. includens* larvae with distilled water, kaolin, *B. bassiana* (CG 138) and the kaolin + conidia suspension of *B. bassiana* (CG 138) showed a significant interaction between treatments and larvae species ($F_{3.32}$= 24.08; P< 0.001; Table 3), indicating that the larvae mortality is dependent on the species of insects and treatments. The *B. bassiana* CG 138 isolate caused the highest mortality of *A. argillacea* first-instar larvae. Mortality was higher with kaolin + *B. bassiana* (CG 138) (Table 3), followed by the treatment with kaolin alone. The similar mortality of *A. argillacea* larvae with *B. bassiana* (CG 138) and kaolin + *B. bassiana* (CG 138) indicate no

additive or synergistic activity between this mineral and the fungus against this insect. The high mortality rates of kaolin-treated *A. argillacea* larvae corroborate results of lower survival and leaf consumption by first-instars of this pest on cotton plants treated with kaolin compared to non-treated ones (Gonçalves et al., 2015). The mortality of *A. argillacea* larvae was lower in the control.

The mortality of *C. includens* larvae was higher with kaolin + *B. bassiana* (CG 138), followed by those treated with *B. bassiana* (CG 138), and the lowest values were in the control and with kaolin alone ($F_{3.32}$= 24,08; P< 0.001; Table 3). The higher mortality of *C. includens* larvae with kaolin + *B. bassiana* (GC 138) indicates an additive effect of this mineral and the fungus against this insect, as observed for the rice pest, *C. cephalonica* with *B. bassiana* and kaolin (Samodra and Ibrahim, 2006a). This is probably due to the hygroscopic kaolin action, reducing the digestibility and the protective function of the cuticle that lines the digestive tract of these larvae, making feeding difficult. This fact may have facilitated the pathogen's action and increasing the infection process (Eigenbrode et al., 2006; Gonçalves et al., 2015).

The mortality of *C. includens* larvae fed with cotton leaf discs treated with *B. bassiana* conidial suspension was higher (Table 3) than those with the conidial suspension in the first bioassay (Table 2). Therefore, inoculation methods may influence the results of selecting entomopathogenic fungi strains (Santoro et al., 2007).

The mortality of *A. argillacea* larvae was higher than those of *C. includens* with kaolin or *B. bassiana* (CG 138) alone, and was similar between these species in the treatments with kaolin + *B. bassiana* (CG 138) or distilled water (Table 3). The higher mortality of *A. argillacea* larvae fed with cotton leaf discs treated with *B. bassiana* conidial suspension may be related to differences in the morphology and physiology of these fungus strains and their hosts, similar to the previous bioassay. The higher mortality of *A. argillacea* treated with kaolin suggests a greater abrasive action of this rock powder on the digestive tract of *A. argillacea* compared to *C. includens*. *Alabama*

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**Table 3.** Mortality (%) of *Alabama argillacea* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) larvae fed cotton leaf disks impregnated with distilled water (control), kaolin (0.06 g mL$^{-1}$), *Beauveria bassiana* (10$^7$ conidia mL$^{-1}$) and mixture of 0.06g kaolin + 10$^7$ conidia mL$^{-1}$ of *B. bassiana*.

| Treatments | *Alabama argillacea* | *Chrysodeixis includes* |
|---|---|---|
| Control (water) | 0.00 ± 0.00 c A | 0.00 ± 0.00 d A |
| Kaolin | 71.11 ± 4.44 b A | 33.33 ± 2.92 c B |
| *Beauveria bassiana* | 100.00 ± 0.00 a A | 83.33 ± 2.92 b B |
| Kaolin + *Beauveria bassiana* | 93.33 ± 2.72 a A | 92.86 ± 2.92 a A |

Means followed by the same lower case letter per column and capital letter per line do not differ by the Student Newman Keuls test at 5% probability.

*argillacea* is a monophagous species and *C. includens* is polyphagous and, therefore, the structure of the midgut epithelium of these species may differ with their food habits and phylogenetic position (Bellanda and Zucoloto, 2009). Moreover, the polyphagia requires more complex physiological mechanisms to confront the host plant chemicals (Sarate et al., 2012), which may explain the mortality differences between these two insect species. Thus, the response of lepidopteran species appears to depend on the host specialization level, with a stronger response among monophagous species to variations in host quality compared to polyphagous species (Bestete et al., 2017).

The combination of kaolin + *B. bassiana* may increase the mortality of *C. includens*, but not that of *A. argillacea*. Applications of kaolin particles and the entomopathogenic fungus *B. bassiana* are compatible and can increase their efficiency in controlling some lepidopteran pest species. Difficulties in formulations of entomopathogenic fungi to compete with chemical insecticides are related to their usually narrower host range, especially when crop damage results from a pest complex (Wraight et al., 2010). Strains of entomopathogenic fungi are rarely virulent for all species infesting the crop (Hajek, 1997) and, economically, it is not feasible for producers to target each pest with a different product and/or isolate (Wraight et al., 2010). This suggests the possibility of formulating stabilized propagules of *B. bassiana* mixed with kaolin as a promising biological insecticide against defoliating larvae.

## 4. Conclusions

The kaolin + *B. bassiana* (CG 138) treatment showed an additive effect against the polyphagous *C. includens* larvae, but not against the monophagous *A. argillacea* larvae, which is equally susceptible to treatment with only *B. bassiana* and also to kaolin. This suggests that ecological nutrition plays an important role in the susceptibility of these defoliating species to alternative insecticides.

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