Journal of Plant Protection Research eISSN 1899-007X

RAPID COMMUNICATION

Endophytic potential of *Beauveria bassiana* (Bals.-Criv.) Vuill against sap-sucking insect pests in kiwi trees and cotton plants

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Vol. 66, No. 1: 121–127, 2026

DOI: 10.24425/jppr.2026.158580

Received: August 24, 2025
Accepted: December 19, 2025
Online publication: March 17, 2026

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Responsible Editor:
Danuta Sosnowska

Abstract

Beauveria bassiana has proven to be an efficient crop colonizer due to its highly entomopathogenic activity against a wide range of sap-sucking pests. In this work, two different application methods of *B. bassiana* (Bals.-Criv.) strain PPRI 5339 were studied over two consecutive years: (A) seed coating on cotton and (B) trunk inoculation to kiwi trees. Untreated plants served as controls for both methods. The sucking insects, *Aphis gossypii* (Glover) on cotton plants, and *Halyomorpha halys* (Stål) on kiwi trees, were counted, and the total chlorophyll content and leaf area were measured as indicators of plant performance. The presence of *B. bassiana* slightly reduced the population of sucking pests but also enhanced the total chlorophyll content and the leaf area in both crops. These results demonstrate the dual role of strain PPRI 5339 as both a biocontrol agent and a growth promoter, highlighting its potential as an environmentally friendly alternative for managing sap-sucking pests that severely affect agricultural production in Greece

Keywords: *Aphis gossypii*, *Beauveria bassiana, Halyomorpha halys,* seed coating, trunk inoculation

Introduction

Kiwifruit, *Actinidia deliciosa* (A.Chev.) "Hayward" (Ericales: Actinidiaceae), cultivation is of major economic importance in Greece, which is one of the world's leading kiwifruit exporters. *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) has emerged as a serious exotic pest of kiwi in Greece, in recent years, causing significant damage to kiwifruit production through its piercing-sucking feeding behavior (Bariselli *et al.* 2016). Feeding by adults leads to the secretion of saliva rich in digestive enzymes, which damages fruit tissues and results in severe quality degradation and loss of market value (Peiffer and Felton 2014; Leskey and Nielsen 2018; Damos *et al.* 2020). Damaged tissues are also prone to secondary infections, further increasing economic losses (Andreadis *et al.* 2018). *Aphis gossypii* (Glover) (Hemiptera: Aphididae) is a major pest of cotton, and its sucking damage can cause nutrient reduction, particularly under drought conditions, leading to significant yield losses (Zhang *et al.* 2015; Liu *et al.* 2023). In addition, honeydew excretion by aphids interferes with photosynthesis by covering leaf surfaces and limiting light penetration, while also favoring the development of fungal pathogens (Srivastava and Shukla 2021; Li *et al.* 2022). As a result, infected cotton plants become more prone to fungal diseases, and cotton production is significantly decreased (Ramalho *et al.* 2012). Given that Greece is the largest cotton producer in Europe, the spread and persistence of *A. gossypii* pose a substantial threat to national cotton production (Tsaliki *et al.* 2024).

Chemical pesticides such as pyrethroids and organophosphates remain the most common control strategy against these pests; however, their intensive and often indiscriminate use has led to environmental concerns, pesticide resistance, and residue issues (Tozlu *et al.* 2019). In kiwi cultivation, chemical control is particularly problematic due to food safety considerations (Shan *et al.* 2021). Moreover, *A. gossypii* has developed resistance to many chemical compounds (Ahmad *et al.* 2003; Li *et al.* 2022), and available chemical options against *H. halys* are often effective only against adult stages (Parker *et al.* 2015; Leskey and Nielsen 2018; Shan *et al.* 2021).

The use of entomopathogenic fungi (EPF) represent an environmentally friendly alternative with high efficacy to reduce pest populations and can be included in integrated pest management (IPM) programs (Gurulingappa *et al.* 2011). Among them, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is well known for its capacity to infect a wide range of insect pests and to establish endophytic associations with plants (Brownbridge *et al.* 2012; Quesada-Moraga *et al.* 2014). Its endophytic action not only functions as an insecticide for many pests but may also promote plant growth (Rasool *et al.* 2021). These beneficial aspects could potentially enhance plant resilience to stress factors associated with climate change and entomological plant pests (Sánchez-Rodríguez *et al.* 2015; Liu *et al.* 2022). Various inoculation methods of *B. bassiana* have been successfully used to establish it as an endophyte in plants. These include foliar spraying (Wraight and Ramos 2002; Kasambala *et al.* 2018), seed treatment (Cherry *et al.* 2004; Canassa *et al.* 2019), and soil application (Ownley *et al.* 2008; Li *et al.* 2021).

The present study aimed to investigate whether *B. bassiana* strain PPRI 5339 can provide a dual benefit – pest suppression and growth promotion – in cotton and kiwi. Specifically, we evaluated the effectiveness of seed coating in cotton and trunk inoculation in kiwi trees against *A. gossypii* and *H. halys*, respectively.

Materials and Methods

Two independent experimental systems were established to evaluate the efficacy of *B. bassiana* strain PPRI 5339 (Velifer® OD 8 × 10^9 CFU · ml^{-1}, 92% Excipients) (BASF SE, Florham Park, NJ, USA). For the cotton experiment (treatment A), cotton seeds were placed in 9 l pots containing a peat-perlite substrate (1:1, *v/v*), and irrigated through an automated drip system (ARGOS Electronics, 2014). Irrigation frequency and volume were determined from climatic data collected from moisture and temperature sensors. In addition, frequent measurements of the substrate's relative moisture were taken by a soil moisture meter (ΔT-SM150 Kit, Delta T Devices, Cambridge, UK) to ensure rational irrigation. The kiwi experiment (treatment B) was conducted in a 7-year-old 'Hayward' commercial orchard A. deliciosa (cv) 'Hayward' trained on a T-bar trellis system, in the Kostakioi area, Arta, Greece. Kiwi trees were irrigated by a drip system connected to sensors providing evapotranspiration data. Untreated plants served as controls (C) in both experiments. Experiments followed a completely randomized design with three blocks per treatment, each block consisting of 12 plants. Trials were conducted from May to November in both 2022 and 2023.

Inoculation method of *Beauveria bassiana* on kiwi trunk

At 70 cm above the soil surface, a 4-cm-deep hole was drilled into the trunk at a 45° angle using sterile equipment. A slow-release tree injector (Chemjet, Stratagreen Co., Prestons WA, Australia) containing 10 ml of B. bassiana formulation was inserted, allowing gradual uptake through the vascular tissues. After complete release (72 h), the injector was removed and the wound sealed with grafting paste.

Cotton seed coating method of *Beauveria bassiana*

Seed coating was performed using 125 ml of formulated *B. bassiana* PPRI 5339 Velifer® per 100 kg of cotton seed. A precision balance (KERN PES 6200-2M) was used to separate the seed samples, and a Wintersteiger Hege 11 was used for the final coating application. The seeds were mixed thoroughly in a stainless-steel container, and the formulation was applied using precision pipettes. After coating, the seeds were air-dried and stored in open bags. All equipment was cleaned with double-distilled water between treatments to prevent cross-contamination.

Pest control and plant growth assessment

The presence of *H. halys* on kiwi trees and *A. gossypii* on cotton was recorded weekly by leaf inspection. To study the biostimulant effect of *B. bassiana* on the growth of kiwi trees (40 leaves per tree) and cotton plants (all leaves per plant), the leaf area (cm^2) was determined at the end of each experimental season for both 2022 and 2023 using Image J (v 1.54) following the protocol described by Bakr (2005). In addition, the total chlorophyll content (TCHL) (µg · cm^{-2}) of kiwi and cotton leaves was determined non-destructively using a SPAD-502 (Minolta Co., Ltd., Tokyo, Japan) instrument each week. SPAD readings were linearly correlated with actual chlorophyll values for kiwi (Razeto and Valdés 2006) and cotton

(Priya and Ghosh 2023) ($R^2 \geq 0.9$) (Fig. 1.1). For calibration, 0.04 g of homogenized leaf tissue was extracted in 10 ml of 100% acetone, crushed in a mortar with a pestle and placed in 10 ml glass tubes. Samples were vortexed and kept overnight at 4°C in the dark. The absorbance of the supernatant solution was determined at 644.8 and 661.6 nm using a spectrophotometer (Jasco-V630 UV-VIS, Jasco International Co., Ltd., Tokyo, Japan). Chlorophyll a and b were calculated by the equations of Lichtenthaler and Buschmann (2001):

$$\text{Ca } (\mu g \cdot ml^{-1}) = 11.24 \times A_{661.6} - 2.04 \times A_{644.8};$$

$$\text{Cb } (\mu g \cdot ml^{-1}) = 20.13\, A_{644.8} - 4.19\, A_{661.6}.$$

Data for leaf area, TCHL, and the presence of *H. halys* and *A. gossypii* were analyzed using two-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) to compare treatment means. Statistical analysis was made using SPSS v. 25 (IBM-SPSS Statistics, Armonk, NY, USA).

Results and Discussion

Application of *B. bassiana* significantly reduced pest populations in both cropping systems, in agreement with previous studies demonstrating its broad entomopathogenic activity (Dara 2019; Ramanujam *et al.* 2020; Jordan *et al.* 2021). The number of *H. halys* individuals was reduced in the treatment with *B. bassiana* (Fig. 1.3). Cotton plants grown from treated seeds (A) harbored significantly fewer *A. gossypii* individuals than control plants (Fig. 1.2) ($F = 11.88$, $df = 2$, $p < 0.001$). The seeds were coated with *B. bassiana* before each study year. This is likely attributed to this consistent effect. In addition, seed coating in crops such as cotton is a practice that may ensure higher EPF colonization (Posada-Vergara *et al.* 2022) as the EPF is preserved in the soil after seed planting (Garrido-Jurado *et al.* 2011) under favorable conditions for its growth, protected from UV radiation (Kaiser *et al.* 2019), and supported by moisture and the availability of nutrients, which increases its chance to establish itself as an endophyte. In the case of kiwi trees, the average number of *H. halys* in the control treatment (C) was significantly higher ($F = 19.88$, $df = 3$, $p < 0.001$) than in the B treatment. The markedly lower number of *H. halys* by *B. bassiana* has also been observed in other experiments (Özdemir *et al.* 2022), although by different inoculation methods. One of the aims of the kiwi experiment was to investigate the extreme scenario of a single application only in the first experimental year (2022) to explore the persistence limits of the strain. Annual applications of the strain at the beginning of each growing season may provide a greater decrease in *H. halys* numbers, but this should be further examined. This hypothesis aligns with findings from previous studies in which repeated field applications of *B. bassiana* and other EPF enhanced pest control efficacy (Shi and Feng 2006; Świergiel *et al.* 2016). Similar benefits of the trunk inoculation method with EPF have also been noted in other studies (Qin *et al.* 2021; Zhang *et al.* 2023). However, further research is needed to establish an annual initial application as the ideal strain utilization scenario for kiwi cultivation.

In addition to pest suppression, an increase in leaf area under *B. bassiana* treatments was also noticed, in agreement with other previous studies (Qin *et al.* 2021; Zhang *et al.* 2023), suggesting a potential growth-promoting role of the strain. In the cotton plants treated with *B. bassiana* (A), leaf area was significantly greater than in the control ($F = 11.55$, $df = 2$, $p < 0.001$) (Fig. 1.4). Similar effects of endophytic *B. bassiana* seed coatings have been detected for *Phaseolus vulgaris* L. (Fabales: Fabaceae) (Afandhi *et al.* 2019), and *Zea mays* L. (Poales: Poaceae) (Kuzhuppillymyal-Prabhakarankutty *et al.* 2021). Leaf area was also significantly higher in the case of kiwi tree trunk inoculation (B) than in the control ($F = 14.41$, $df = 3$, $p = 0.027$) (Fig. 1.5). It is important to mention that a substantial number of leaves from untreated cotton seeds (C) were damaged by *A. gossypii*, and this might also explain the smaller leaf area observed in the control. The same applies to the case of kiwi leaves, but to a lesser extent.

Regarding TCHL, higher chlorophyll levels in leaves are advantageous for the plant due to their key role in photosynthesis and metabolic processes (Martins *et al.* 2023). Pest presence in both cotton and kiwi reduced TCHL in the leaves' tissues of the control as a result of the sucking damage caused by *A. gossypii* and *H. halys,* respectively (Figures 1.6 and 1.7). The presence of *B. bassiana* in the plant tissues of both coated cotton seeds ($F = 13.22$, $df = 2$, $p < 0.001$) and trunk-inoculated kiwi ($F = 29.11$, $df = 3$, $p = 0.009$) also assisted the plants by limiting the pest number in these treatments. Thus, the leaf area remained intact, presenting less sucking damage in treatments A and B. The previous reasons may shape the environment for the greater TCHL in the plant tissues of the treatments with *B. bassiana*. These findings are in agreement with Geroh *et al.* (2014), who reported increased leaf TCHL in okra, *Abelmoschus esculentus* (L.) (Moench) (Malvales: Malvaceae), treated plots with *B. bassiana*, due to the EPF pathogenic action against *Tetranychus urticae* (C.L.Koch) (Trombidioformes: Tetranychidae), and its beneficial effect on plant metabolism. Moreover, the potential of *B. bassiana* regarding chlorophyll enhancement has been documented in *Oryza sativa* L.

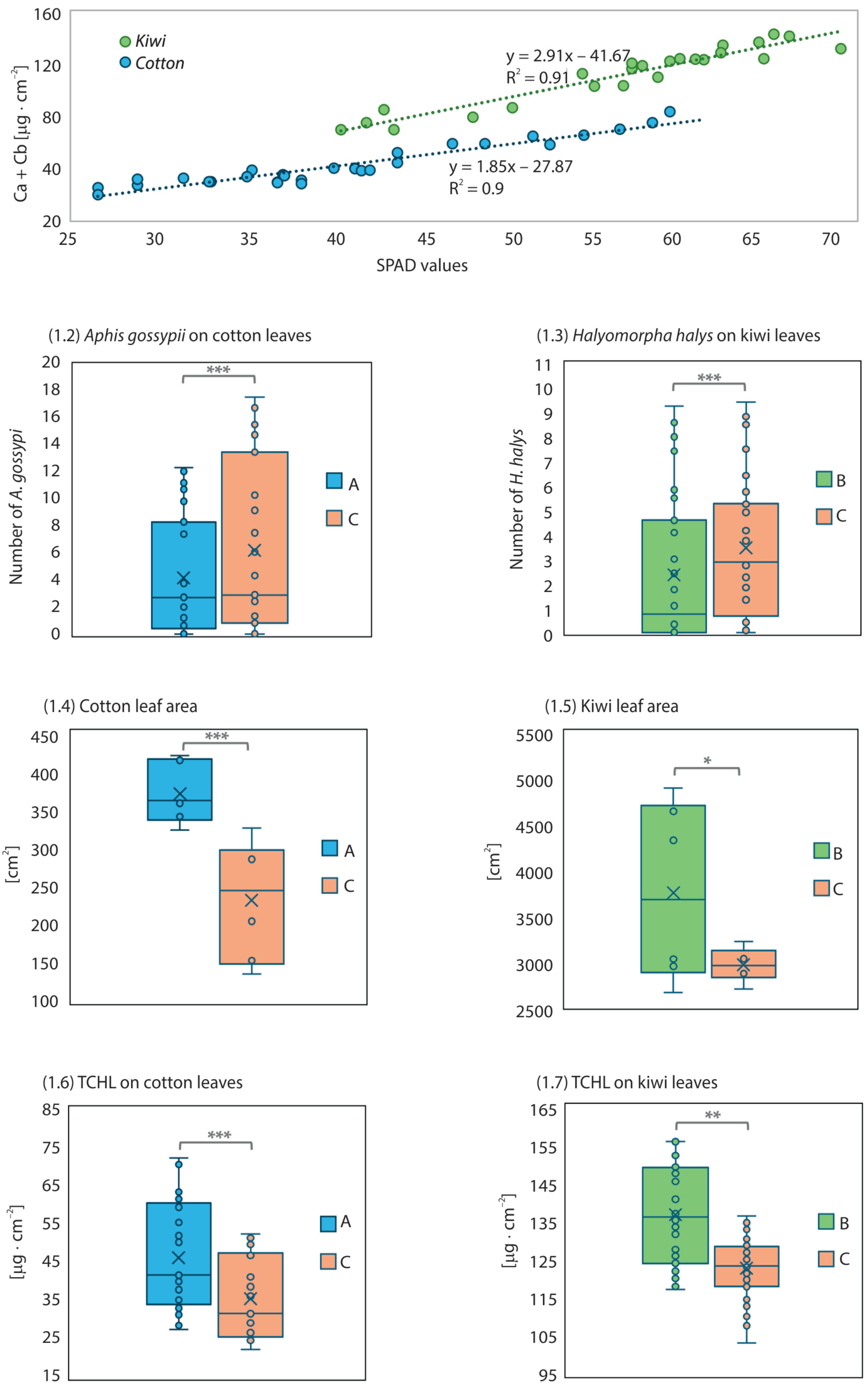

Fig. 1. Linear correlation of SPAD readings and spectrophotometrically determined TCHL on cotton leaves (blue) and kiwi leaves (green) (1.1). Variation in *A. gossypii* number on cotton leaves (1.2), and in *H. halys* number on kiwi *A. deliciosa* "Hayward" leaves (1.3). Variation in leaf area (cm^2) of cotton (1.4) and kiwi (1.5). Variation in TCHL (µg cm^2) on cotton leaves (1.6) and on kiwi leaves (1.7). Definition of treatments: (A) cottonseed coating of *B. bassiana*; (B) kiwi trunk-inoculation of *B. bassiana*; and (C) control. Asterisks *, **, ***, denote statistically significant differences between treatments at probability values of $p \leq 0.05$, ≤ 0.01, and ≤ 0.001, respectively, according to the Tukey test

(Poales: Poaceae) (Akter *et al.* 2023), *Brassica napus* (L.) (Brassicales: Brassicaceae) (Muola *et al.* 2023), *Lactuca sativa* (L.) (Asterales: Asteraceae) (Macuphe *et al.* 2021), and barley *Hordeum vulgare* (L.) (Poales: Poaceae) (Veloz-Badillo *et al.* 2019). Although studies on EPF seed coating and inoculation methods remain limited, available evidence indicates their beneficial contribution to plant metabolism (McKinnon *et al.* 2023), which is in accordance with the present results. Simultaneously, TCHL enhancement in plant tissues needs further studies to interpret how the strain is involved in crop physiology. Also, more comprehensive research is required to uncover the mechanisms underlying the strain's potential to stimulate plant metabolism, and the changes *B. bassiana* could cause to make the plant less attractive to the pest, or if feeding on the plant results in insect death.

Overall, the results demonstrate that *B. bassiana* strain PPRI 5339 can simultaneously enhance plant growth and suppress pest populations, supporting its potential inclusion in sustainable pest management strategies.

Several EPF application methods have proven efficiency across various crops, functioning both as bioinsecticides and biostimulants. In this study, these dual benefits were confirmed through the high values of plant growth and reduced pest populations in *B. bassiana* treatments. Increased chlorophyll content and leaf area in both kiwi and cotton were observed, while the number of *A. gossypii* and *H. halys* populations were significantly reduced. These findings support the development of environmentally friendly pest management protocols and highlight the potential of endophytic entomopathogenic fungi in sustainable agriculture.

Acknowledgements

The author, Vasileios Papantzikos, was granted a scholarship award from the Hellenic Entomological Society. The authors gratefully acknowledge the Hellenic Entomological Society for the doctoral grant and the Koliou Group Co., S.A., Arta, Greece, for providing an experimental kiwifruit orchard to conduct part of the experiment.

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