BIOLOGICAL CONTROL OF THE BOXWOOD MOTH (*CYDALIMA PERSPECTALIS*) BASED ON ENTOMOPATHOGENIC FUNGI AS ENDOPHYTES

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ANTECEDENTS

1. PROBLEM
Cydalima perspectalis invasion

Cydalima perspectalis **larvae** can cause total defoliation of natural and planted Boxwood trees (*Buxus*)

***Buxus* spp** are valuable ornamental plants to be preserved (Convention on Biological Biodiversity · art. 8) [1]

C. perspectalis populations has already developed resistance to **insecticides** [2]

2. POTENTIAL SOLUTION
an alternative for replacing insecticides

Biological control involves the introduction of natural enemies of *C. perspectalis*

Fungal pathogens, the most frequent mortality factor of insects in nature [3,4]

Beauveria bassiana (Ascomycota) is a remarkable entomopathogen used for insect pests control [5,6]

Its **efficiency** depends on the level of host-specificity [strains][3]

C. perspectalis infected by *B. bassiana* under natural conditions [9]

3. MECHANISMS OF ACTION
entomopathogenic endophyte

Beauveria bassiana is used for pest control after it is established as endophyte of plants [5,6]

As **endophyte**, it colonizes **host plants** without causing disease symptons [7]

As **hemibiotrophic entomopathogen**, it infects and kills **host insects** to use bodies to maximize the reproductive output (infective conidia) [8]

INITIAL HYPOTHESIS The artificial inoculation of efficient strains of *B. bassiana* in an attempt to establish them as endophytes of *Buxus* spp could result in the regulation of *Cydalima perspecatlis* populations

OBJECTIVE Screening strains of *B. bassiana* to describe their efficiency as biocontrol agents of *C. perspecatlis* through their establishment in *Buxus*

MATERIAL AND METHODS

1 Assess the ability of *B. bassiana* strains to colonize *Buxus* seedlings as endophytes

BIOASSAY I

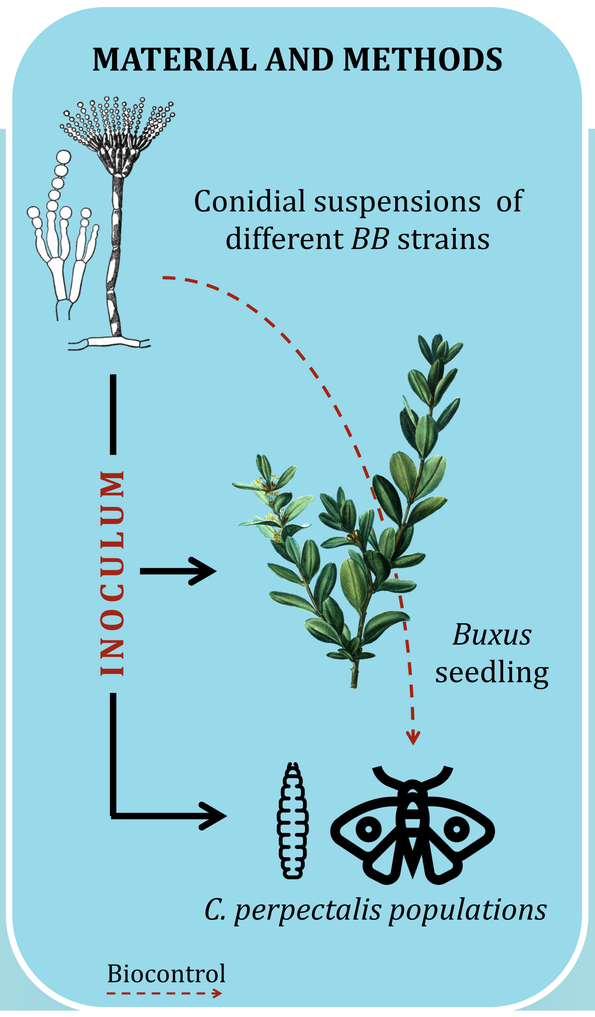
INOCULATION foliar application in *Buxus* seedlings

DETECTION the presence of *Bb* as endophyte of *Buxus*

I. Culture-dependent methods
II. Culture-independent methods (q-PCR)
III. SEM observations

INDICATOR measurement of *Bb* colonization
By the frequency of colonization

FACTORIAL ARRANGEMENT
Factors
- *Bb* strains
- Sampling times (post-inoculation weeks)
- Sampling tissues (leaves, stem, root)



2 Assess the pathogenicity of *B. bassiana* strains towards *C. perspectalis*

BIOASSAY II

INOCULATION physical exposure of *C. perspectalis*

DETECTION death of *C. perspectalis* caused by *Bb*

I. Progressive counting of dead bodies
II. Culture-dependent methods
III. Culture-independent methods (q-PCR)
IV. SEM observations

INDICATORS measurement of *Bb* pathogenicity
By the cumulative mortality rate, mean survival time, letal time

FACTORIAL ARRANGEMENT
Factors
- *Bb* strains
- Sampling populations (adults and larvae)

EXPECTED RESULTS

1. Bioassay I validates the presence of *B. bassiana* as endophyte of *Buxus*
Frequency of colonization significantly differ between strains

2. Bioassay II validates the pathogenicity of *B. bassiana* towards *C. perspectalis*
Indicators of pathogenicity significantly differ between strains

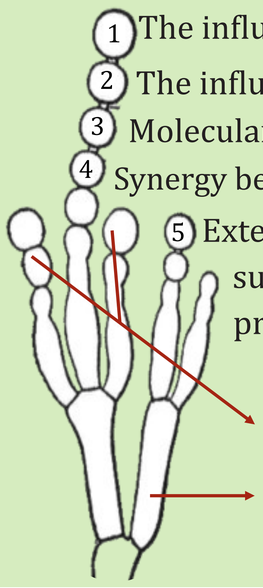
3. ANOVA allows to screen the most efficient strains

4. Positive correlations between both variables are expected for some strains

5. *B. bassiana* is observed/confirmed by SEM and qPCR

FURTHER CONSIDERATIONS

1. The influence of artificial inoculation methods
2. The influence of biotic and abiotic conditions
3. Molecular and genetic characterization ("omics"; NGS)
4. Synergy between *B. bassiana* and other entomopathogenic endophyte fungi
5. External validation to extend laboratory conclusions requires suitable methods to preserve virulent strains and for biomass production



DISSEMINATION PLAN

Lectures at Scientific Congresses for Innovative Agriculture Solutions

Submission to calls for proposal of projects offered by Environmental Institutions, Companies or Public Administrations

Through Master/PhD thesis and further projects

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