



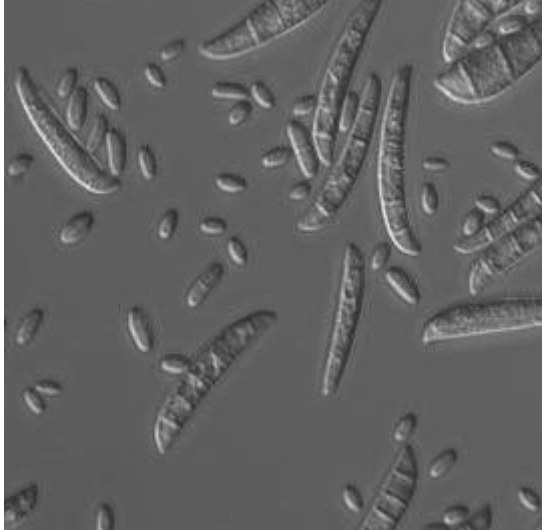
# Fungal symbiotic associations of the ambrosia beetle *Euwallacea nr. fornicatus*

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# Introduction



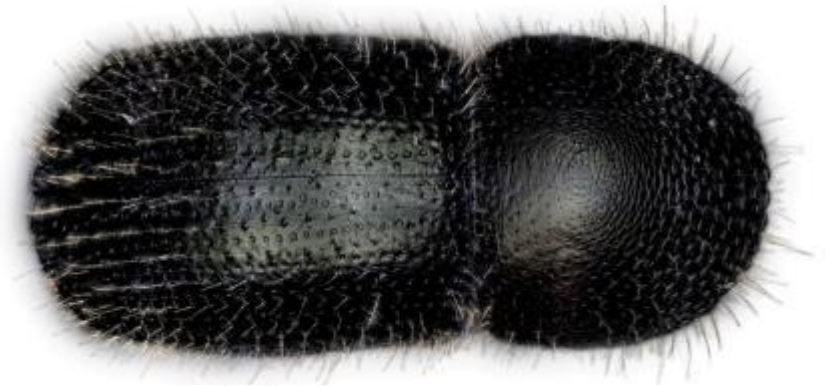
- *Euwallacea fornicatus* a complex of cryptic species attacking avocado.
- Fungal-farmers, presence of mycangia for fungal transport.
- *E. fornicatus* primary symbionts are members of the Ambrosia Fusarium Clade (AF-1 to AF- 12), *Graphium* spp. and *Acremonium* spp.
- Native to South Asia (Established in Israel, Australia and South Africa, Central and North America)

# Florida

## Tea Shot-hole Borer (TSHB)

*Euwallacea nr. fornicatus*

- 2002** Royal Poinciana
- 2012** First Avocado tree
- 2013-15** 10 avocado groves
- 2016** First outbreak  
(1500 trees in a grove)





- TSHB Attacks and kills medium and small branches
- Interior-shaded braches first, later outer branches
- Base of the branch, later all the branch







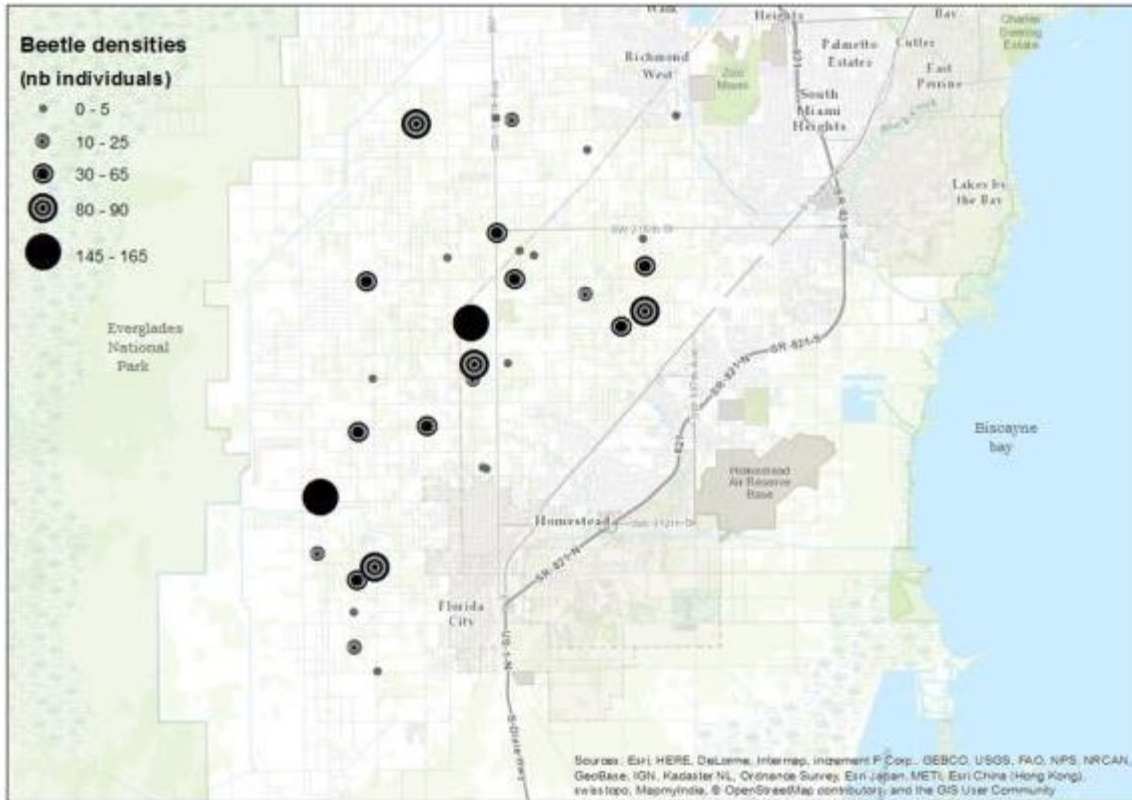
- Branch dieback
- May limit production



Article

# Distribution, Pest Status and Fungal Associates of *Euwallacea nr. fornicatus* in Florida Avocado Groves

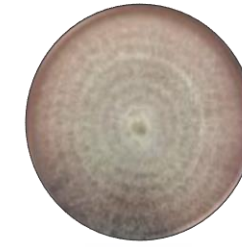
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*Fusarium sp.*



AF-8



AF-6



*A. morum*



*Acremonium sp.*



*Graphium sp.*

# Objective

To understand the symbiotic relationship of *E. fornicatus* and its fungal associates:

- I) Survival and development of larvae on individual cultures of the symbionts
- II) Rearing on avocado sawdust media inoculated with the individual symbionts
- III) Production of lines carrying a single *Fusarium* spp.
- IV) Infesting avocado trees with the monosymbiotic lines

## Beetles collection and rearing conditions and fungal strains



*Fusarium* sp.



AF-8



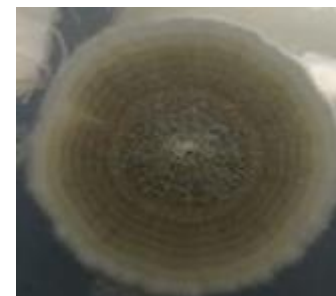
AF-6



*Acremonium* sp.



*A. morum*



*Graphium* sp.

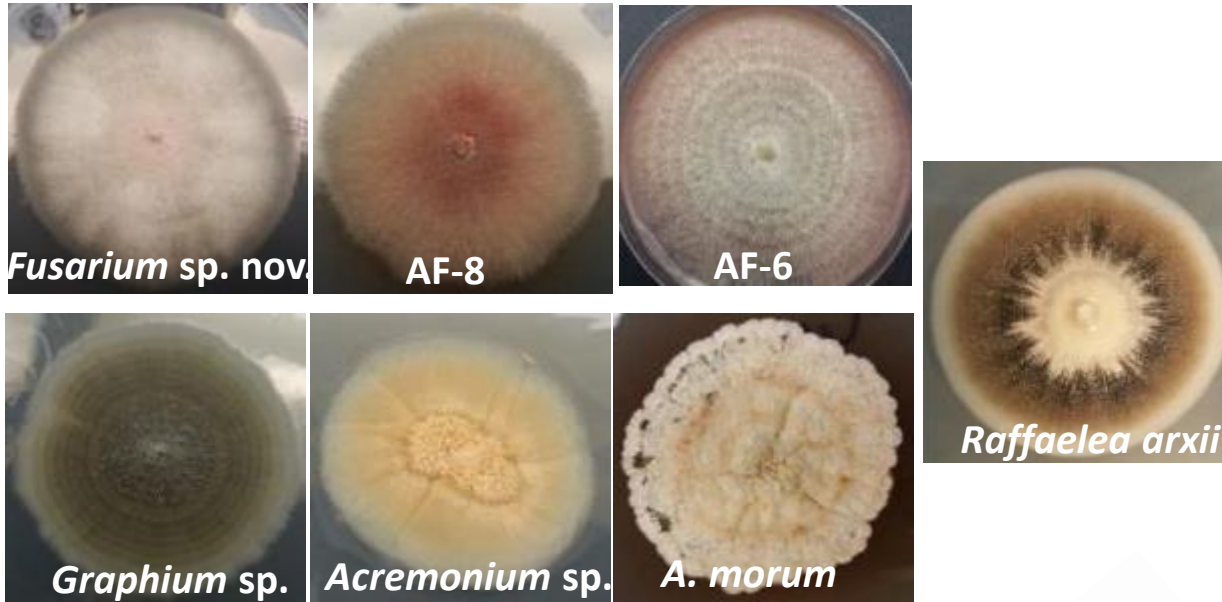


- *E. fornicatus* were excavated from infested avocado logs (Miami- Dade County Florida USA)
- Fungal isolates were obtained from sapwood tissue and beetles mycangia

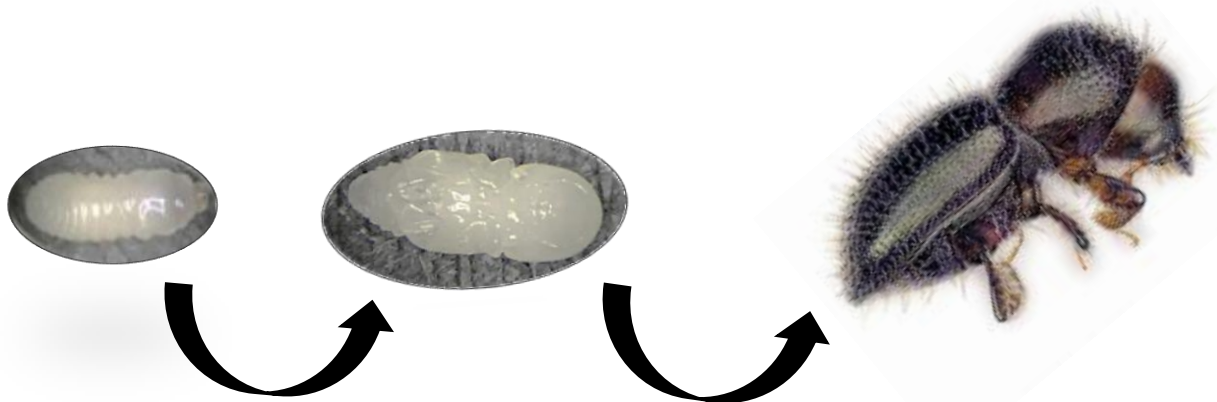




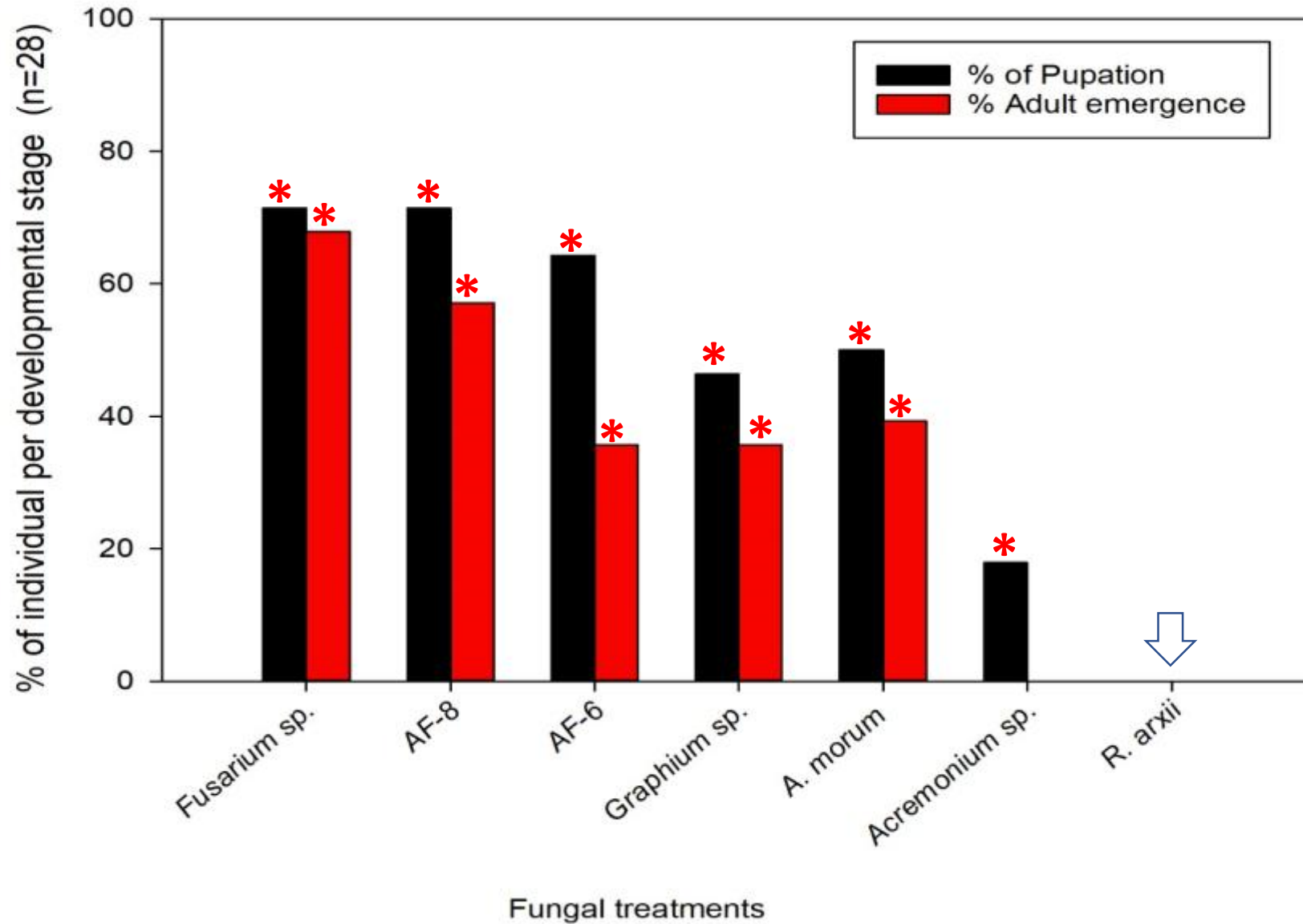
## I) Survival and development of *E. fornicatus* larvae on symbiont cultures



- Third instar larvae were collected and surface disinfected
- Symbiont cultures on PDA media were used as the larvae rearing substrate. *R. arxii* was used as a control
- Larva survival, pupation and emergence of adults were recorded



# I ) Development of *E. fornicatus* larvae on symbiont cultures

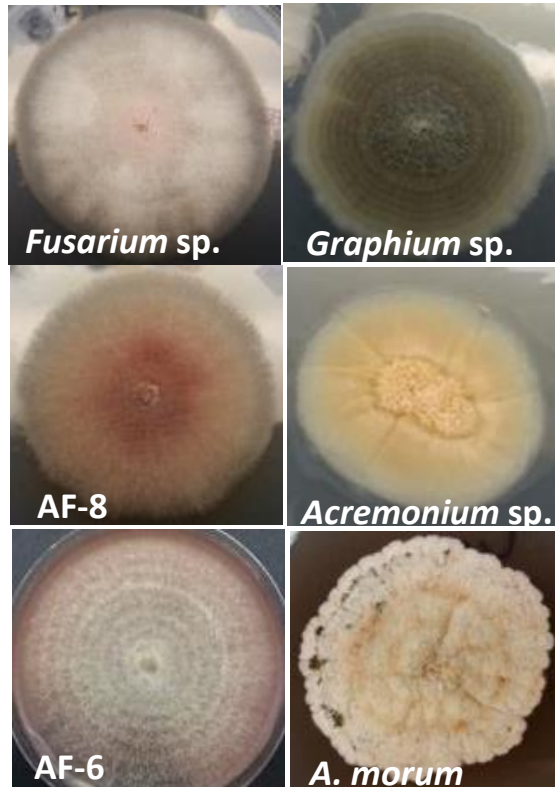




## I ) Development of *E. fornicatus* larvae on symbiont cultures

- Beetles can develop in a single symbiont but probably need more than one symbiont to develop strong colonies.
- *Fusarium* sp. and AF-8 important food for immature development
- AF-6, *Graphium* sp. and *Acremonium morum* important for adult stage.

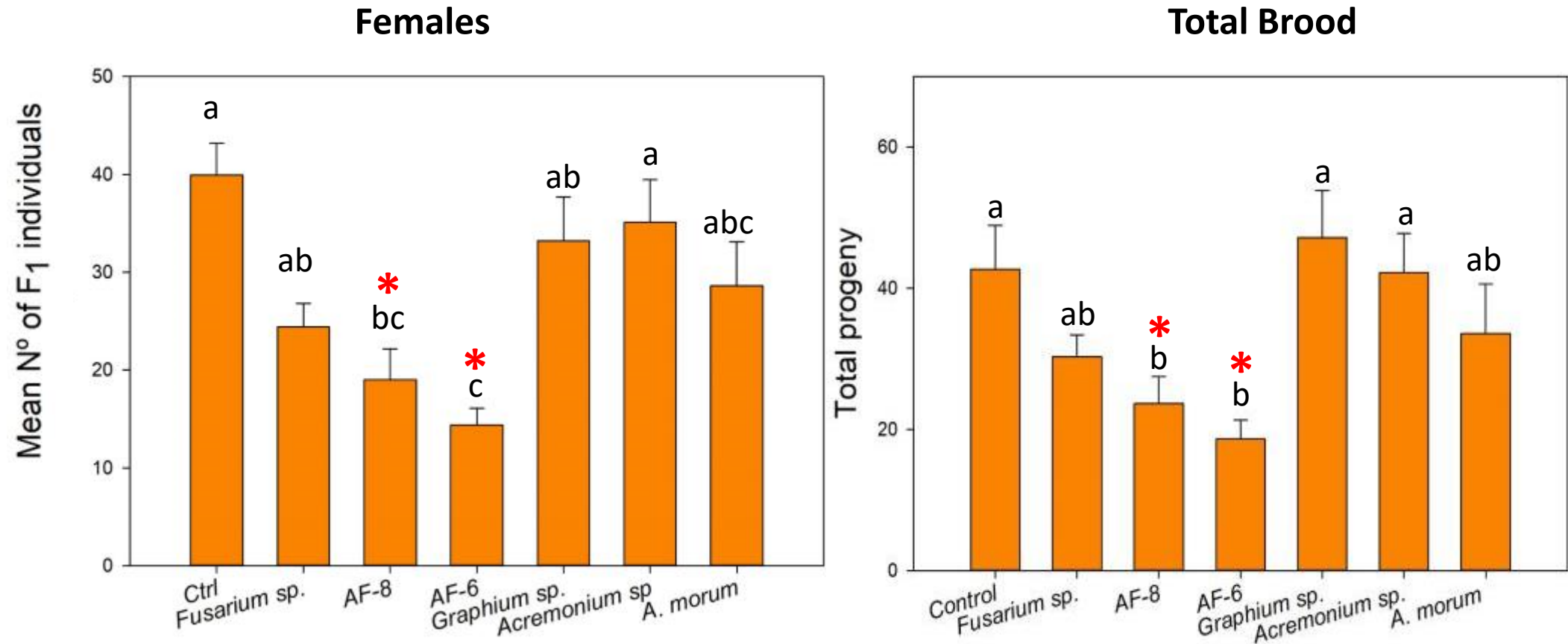
## II) Rearing of *E. fornicatus* on media inoculated with individual symbionts



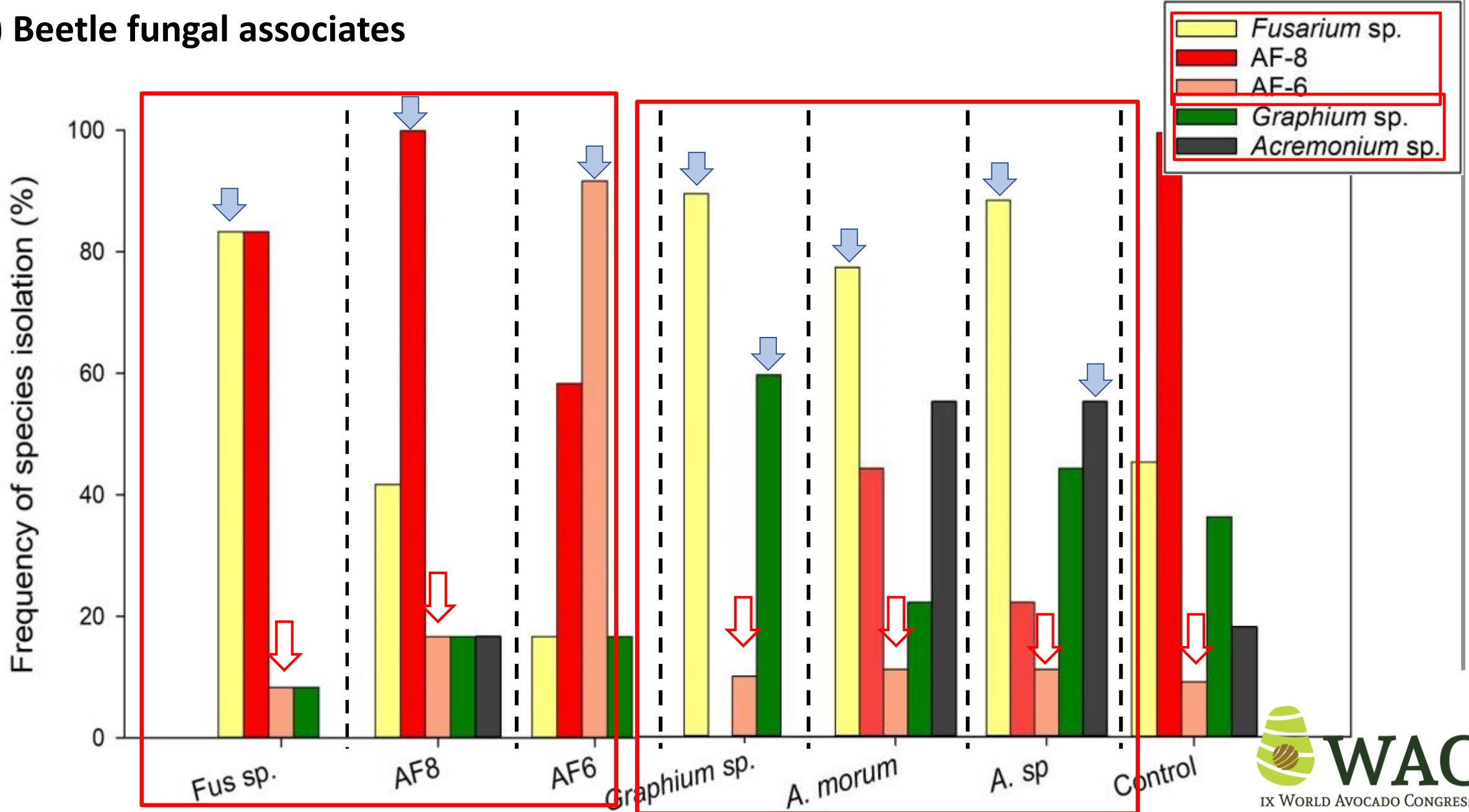
- Media inoculated with ( $1 \times 10^6$  CFU) a given symbiont
- Colony dissection 40 days
- The number of developmental stages was recorded
- Beetles symbionts were isolated and ID
- The experiment was carried out for two generations



## II) Effect of media inoculation on the reproduction of *E. fornicatus*



## II) Beetle fungal associates

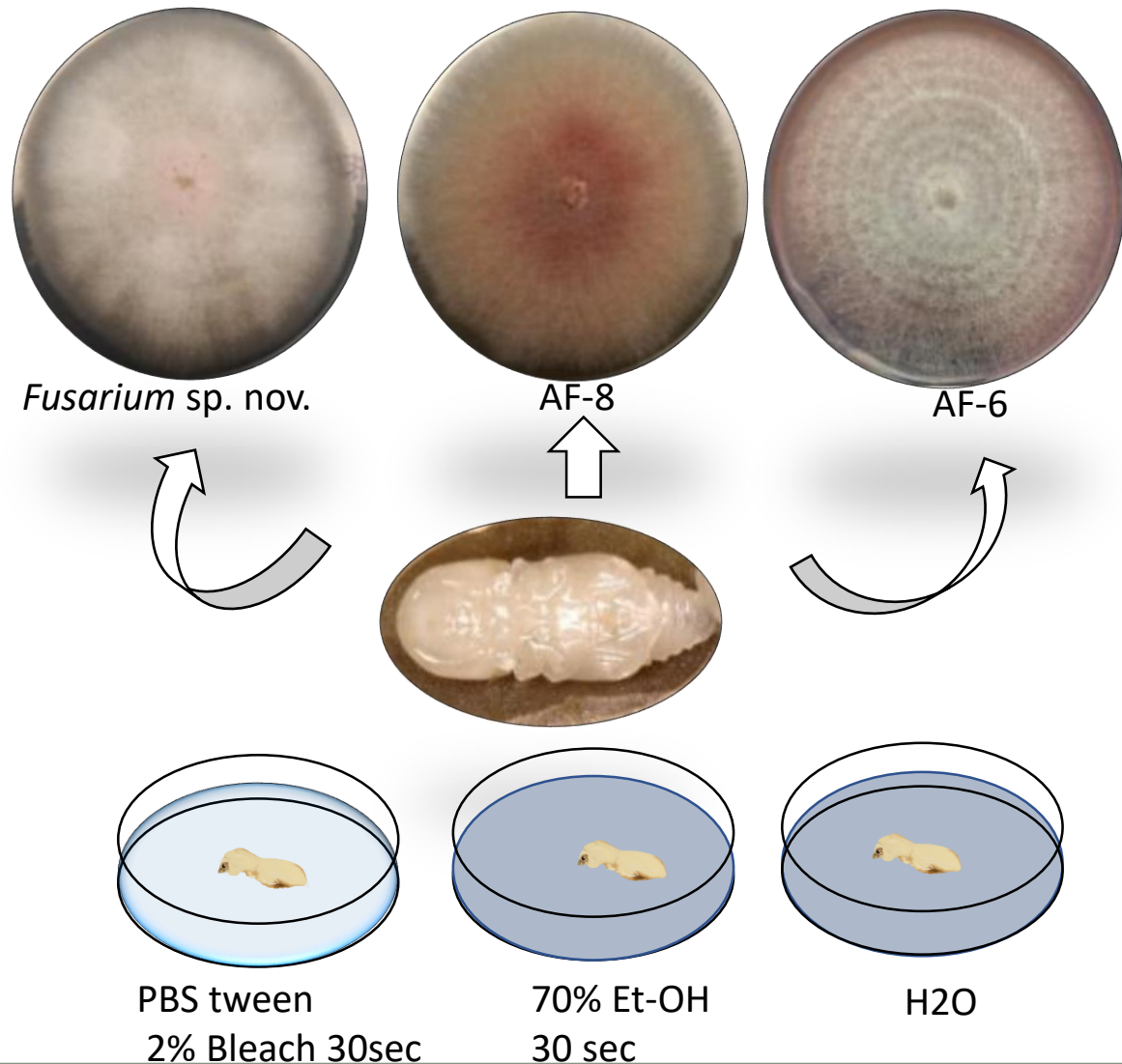




## II) Beetle fungal associates

- *Fusarium* spp. tend to colonize the mycangia of the beetles more than other symbionts.
- AF-6 was the symbiont with the lowest frequency in the mycangia.
- Reproduction was lower in the media inoculated with AF-8 and AF-6.

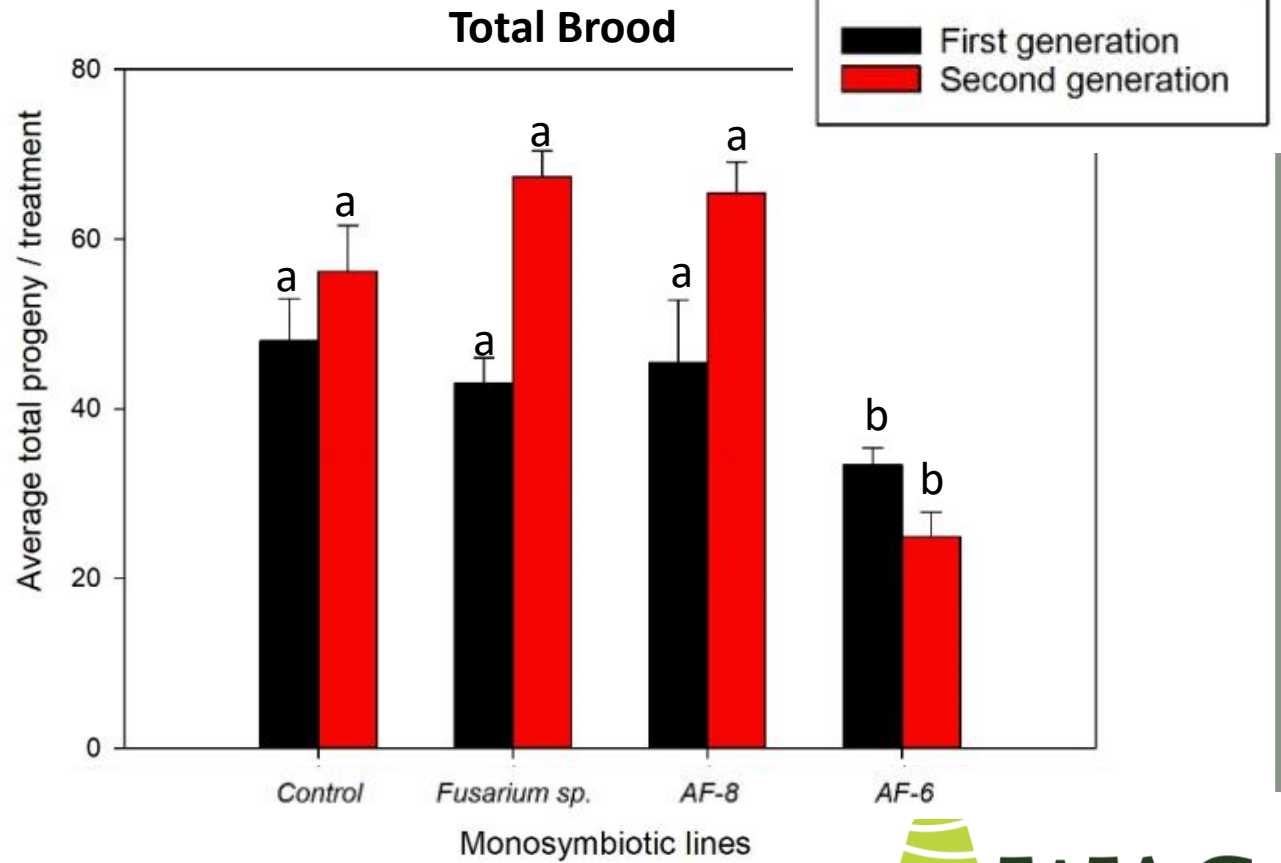
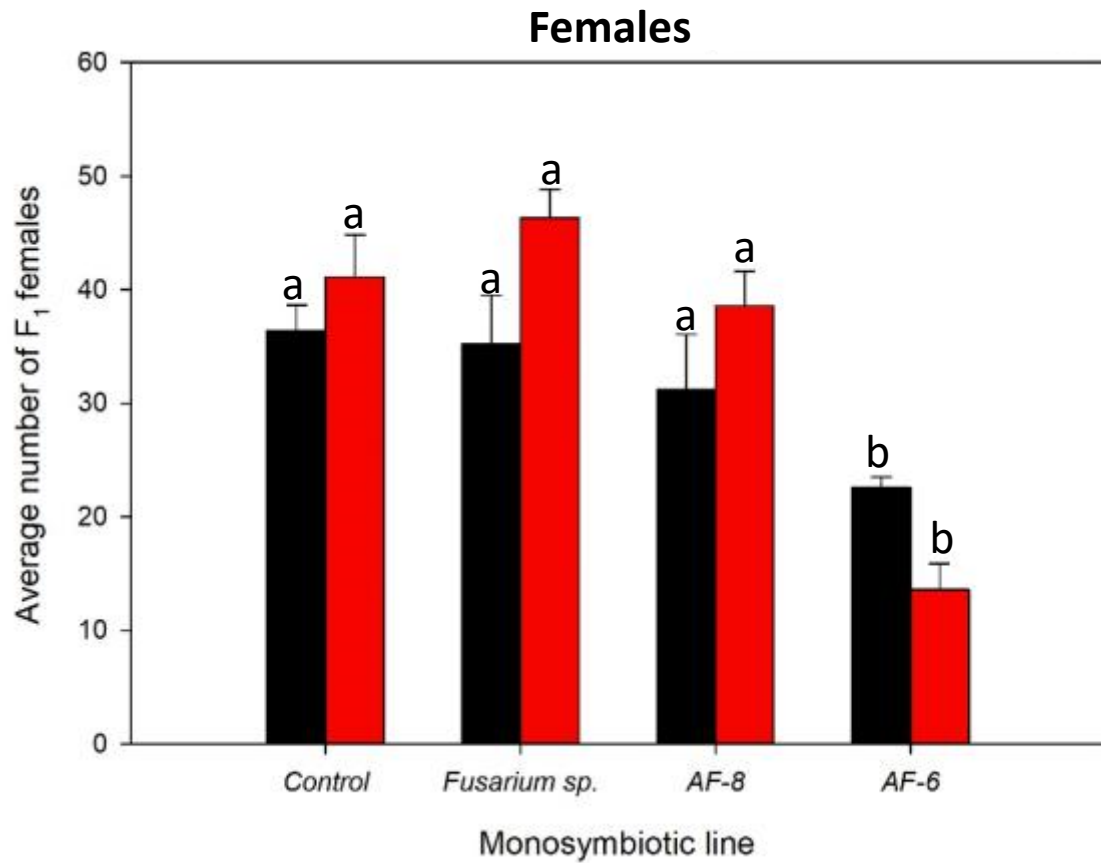
### III) Production of *E. fornicatus* lines monosymbiotic colonies



- Pupae were surface disinfected
- Reared ♂♀ on *Fusarium* spp. cultures
- Mature fertilized females were transferred to rearing tubes
- Offspring females were evaluated for the presence of a single *Fusarium* sp.



### III) *E. fornicatus* lines monosymbiotic lines reproduction



### III) *E. fornicatus* lines monosymbiotic lines reproduction

- *Fusarium* sp. and AF-8 were the best nutritional symbionts comparable to the control.

#### IV) Tree infestation with the *E. fornicatus* monosymbiotic lines



- Five beetles per monosymbiotic line and a lab colony
- Treatments were distributed in independent branches of the same tree
- Evaluation 50 days after infestation
  - \*Beetles activity (boring, reproduction)
  - \*Presence of the fungal pathogen



#### IV) Tree infestation with the *E. fornicatus* monosymbiotic colonies

Reproduction was not accomplished for any of the tree monosymbiotic lines nor the control laboratory colony

Monosymbiotic line	Nº infected trees	Xylem staining length (range)
<i>Fusarium</i> sp.	7/20	8.4 cm (2-17 cm)
AF-8	10/20	5.9cm (2-11 cm)
AF-6	3/20	4.5 cm (6-6 cm)
Control	7/20	8.5 cm (5-11 cm)



# Conclusions

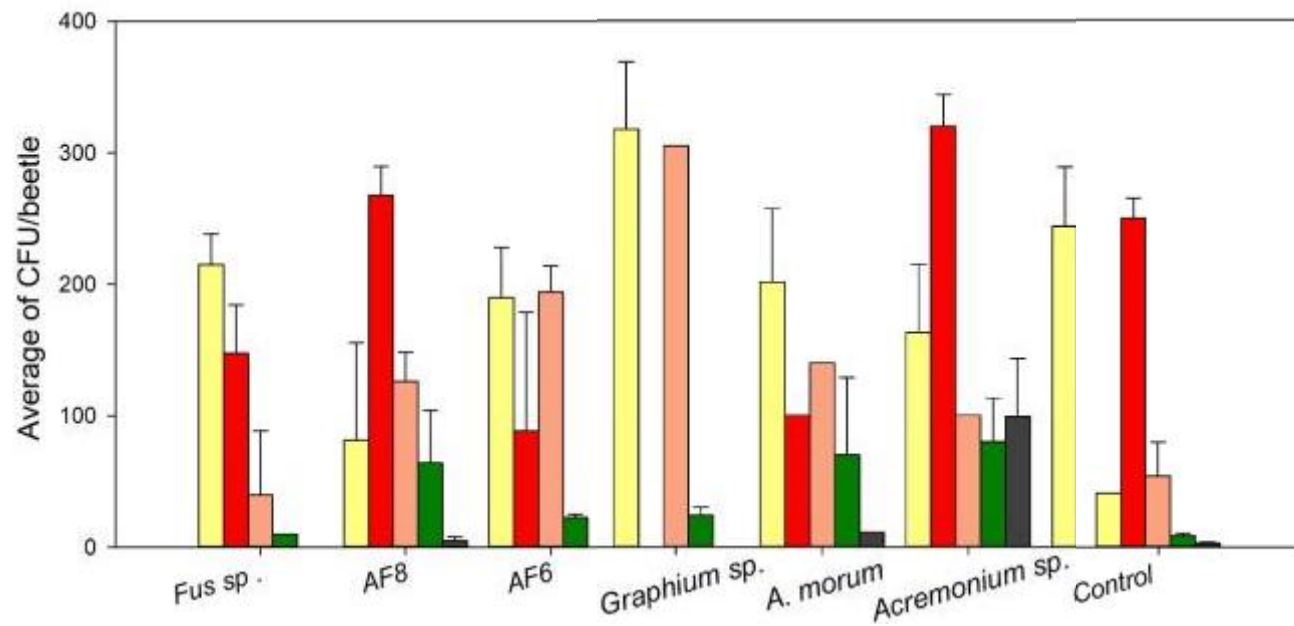
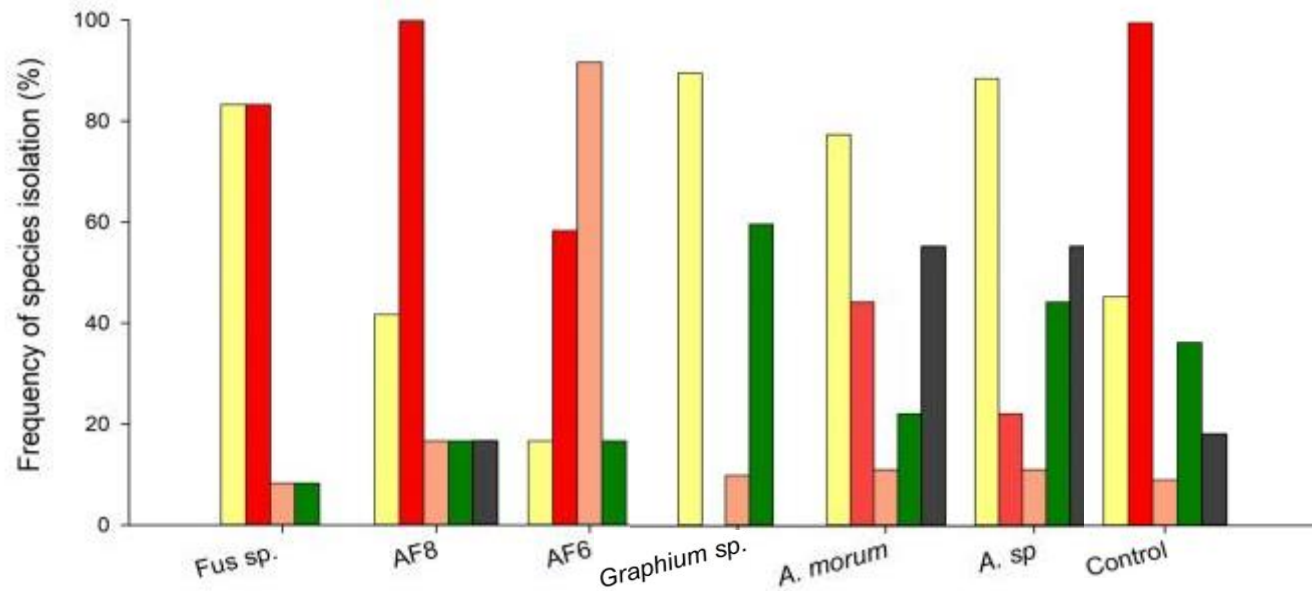
- *Fusarium* sp. and AF-8 appear to be the most important nutritional symbionts and the strongest pathogen.
- AF-6 seems to be a poor nutritional symbiont and a weak pathogen.

# Future research

- Pathogenicity of symbionts
- Influence of symbionts on the beetle's behavior.
- Can fungal volatiles improve current attractants

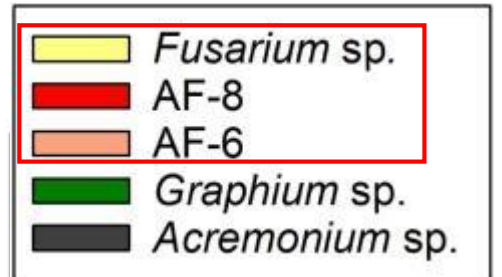
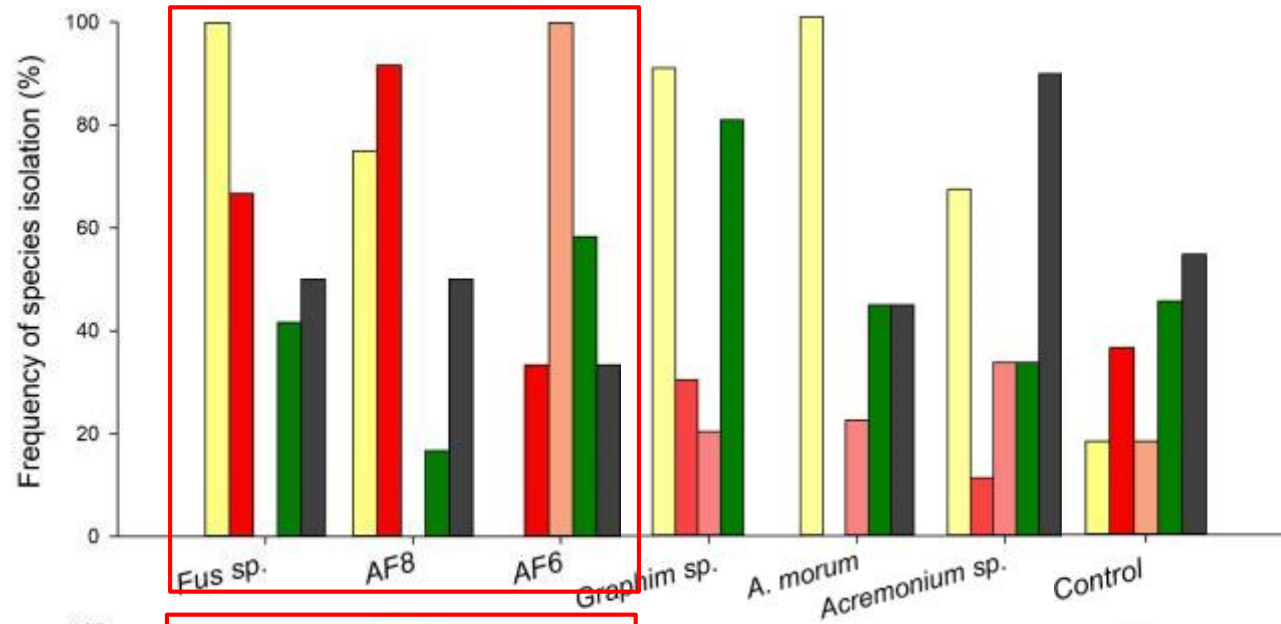
*¡Gracias!*



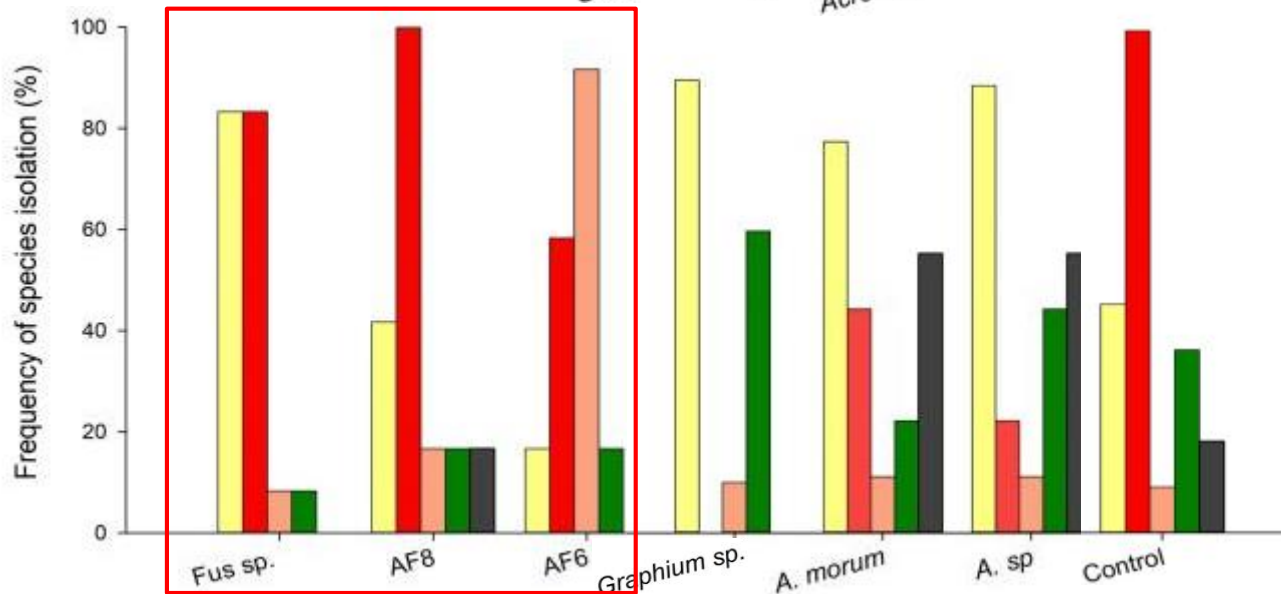


## II) Fungal associates

Galleries isolates



Beetles isolates



## II) Effect of media inoculation on the reproduction of *E. fornicatus*

