Invasive Ambrosia Beetle Conference
The Situation in California
August 12 – 14, 2012

Meeting sponsored by:

The Hofshi Foundation

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Session 4

Biology of the Fungal Symbiont

Update from Israel regarding the fungal symbiont *Fusarium* sp. nov., genetic diversity, interaction with its hosts as a plant pathogen, and as a feed for *Euwallacea fornicatus*

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Hosts of the fungus in Israel

To date, the fungus has been isolated from four botanical hosts:

1. California box elder, *Acer negunda*
2. Castor oil plant, *Ricinus communis*
3. Oak, *Quercus robur* sp. *brutia*
4. Avocado, *Persea americana*

Symptoms in avocado

Symptoms in mature avocado trees

Symptoms in mature avocado trees

Symptoms in mature avocado trees

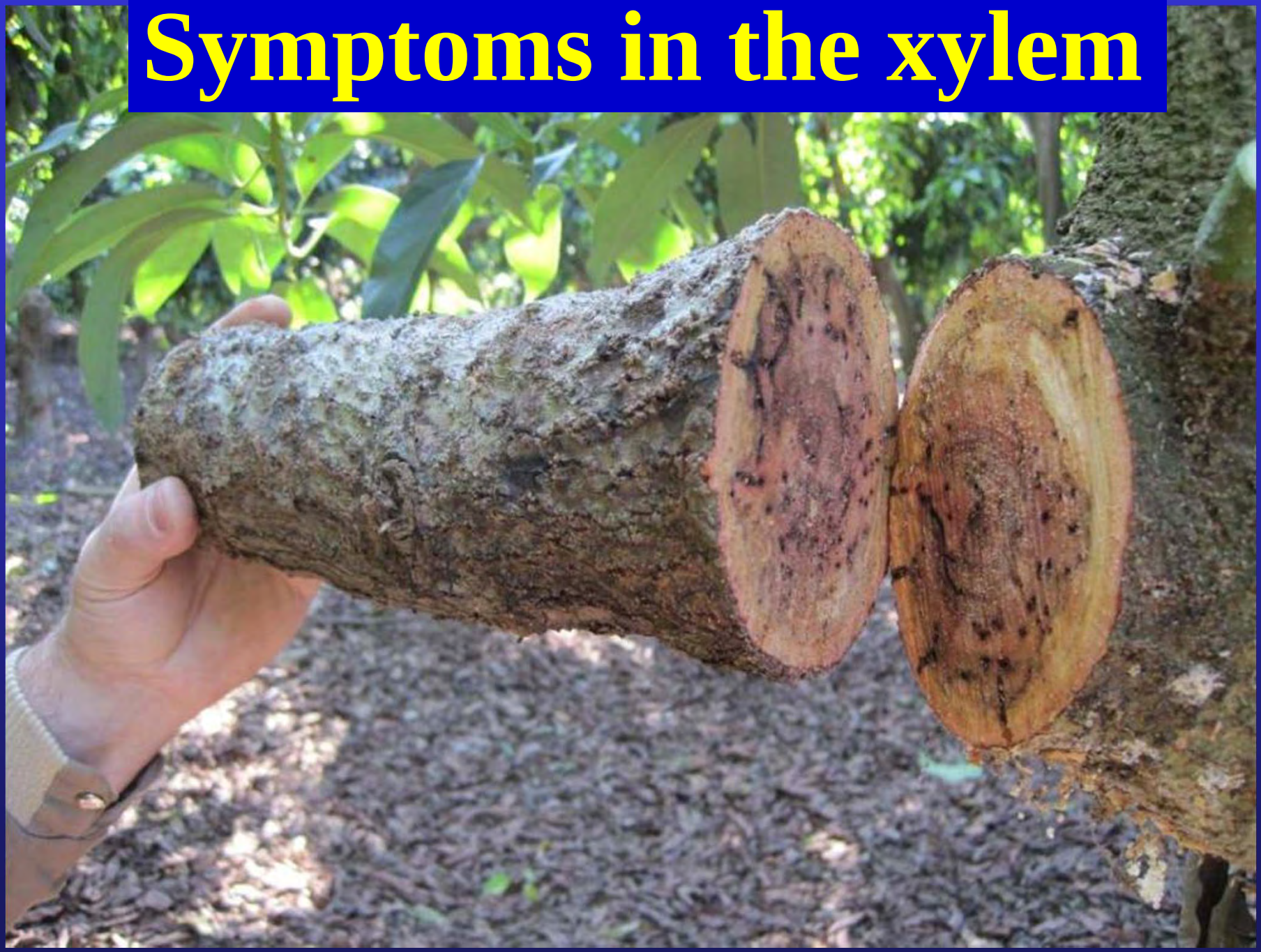
Symptoms in mature avocado trees

Symptoms in young avocado trees

Symptoms in young avocado trees

Symptoms in the xylem

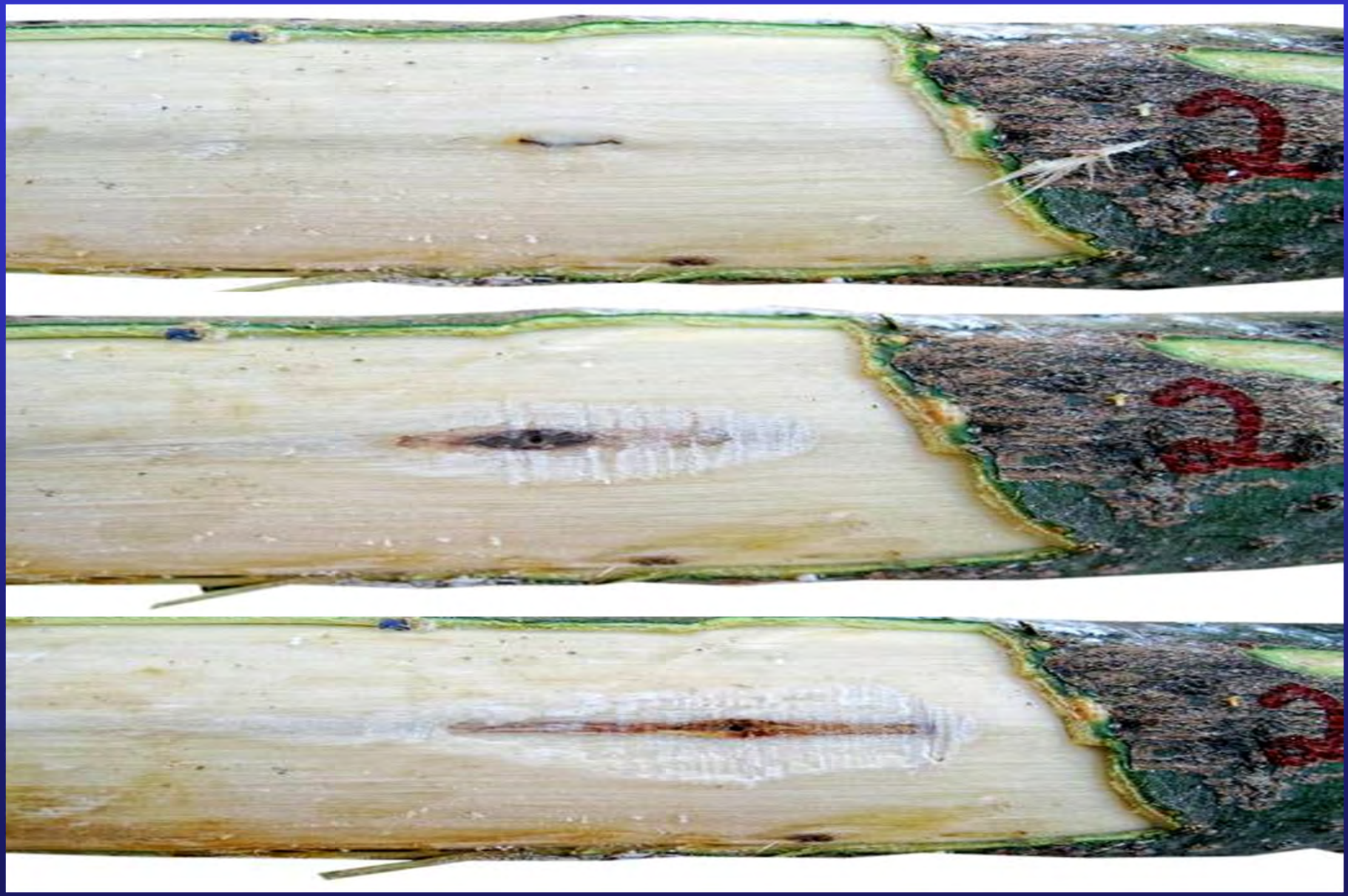
Symptoms in the xylem



Monitoring fungal presence in infected tissue

Peeling of bark at region of persitol

Exposure of bark to visualize beetle penetration



Fungus spread in xylem

Fungus spread in xylem

Fungus spread in xylem

Fungus spread in xylem

Fungus spread in xylem

Symptoms in maple

Symptoms in maple

Penetration of cortex

Fungal infection of xylem of maple

Infected

Healthy

Symptoms in castor bean

Symptoms in castor bean

Symptoms in castor bean

Fungal infection of xylem of castor bean

Infected

Healthy

Symptoms in oak

Symptoms in oak

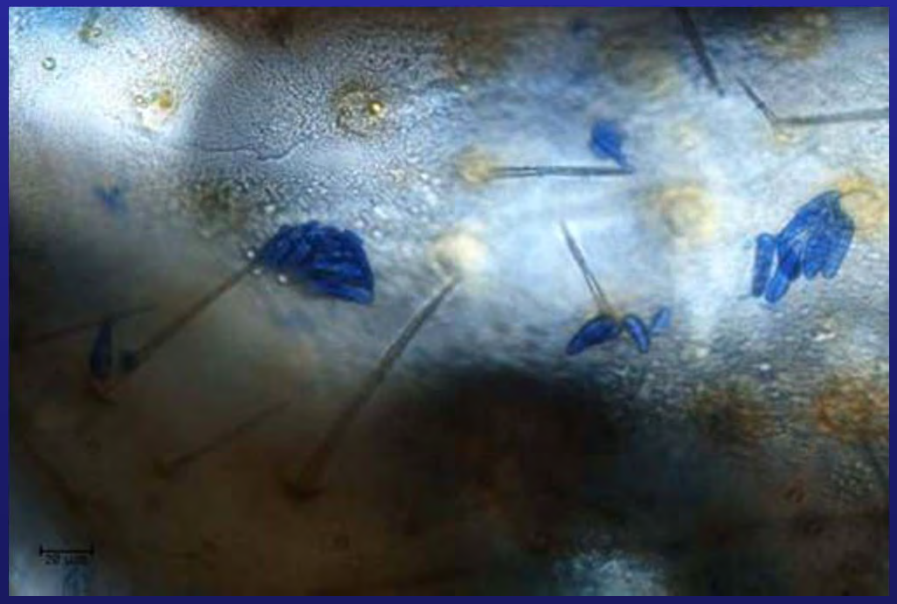
Symptoms in oak

Symptoms in oak

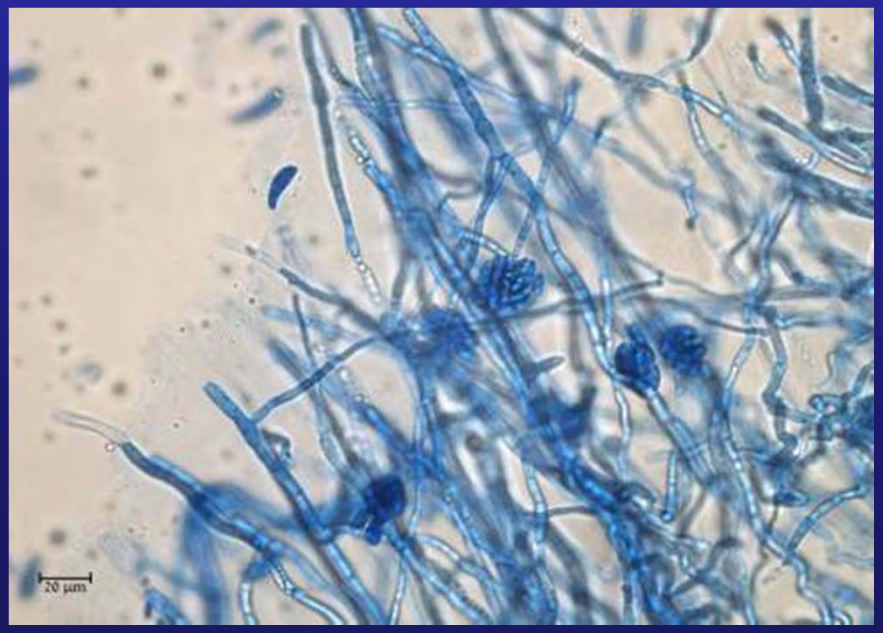
Symptoms in oak

Obligate symbiosis between the avocado beetle and its specific fungus

Fungus beetle interaction



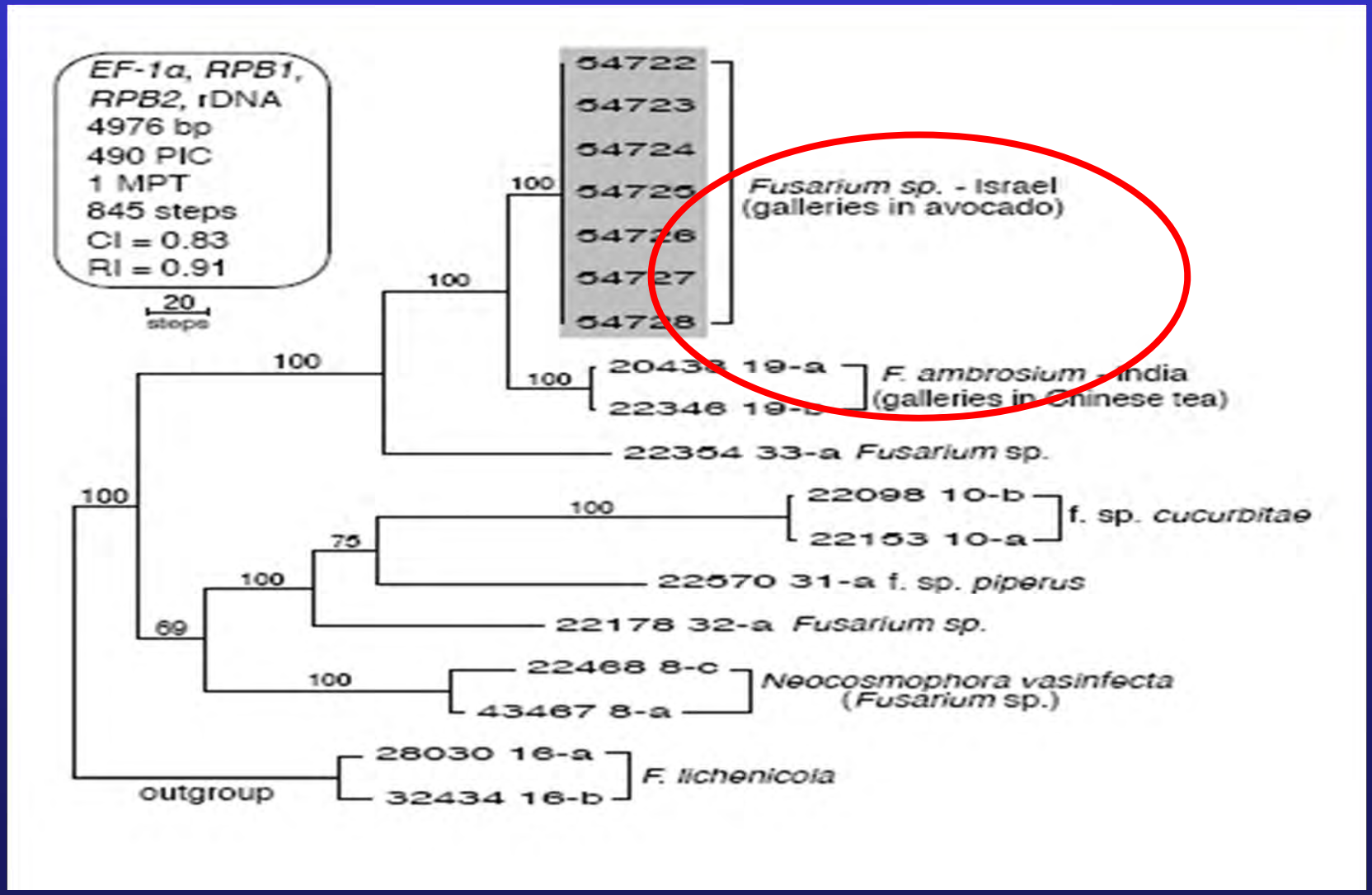
Fungal morphology – *Fusarium* sp.

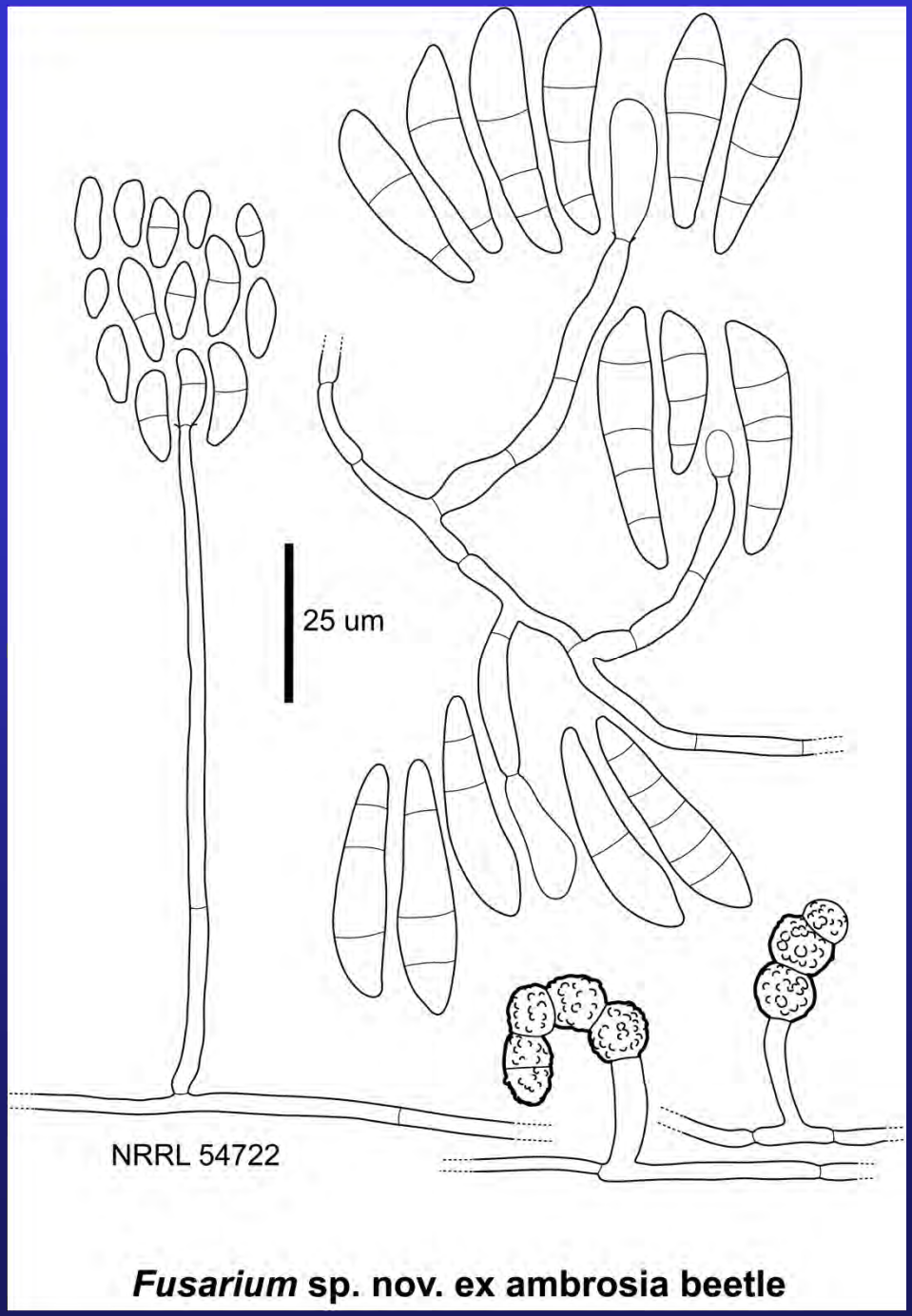


***Fusarium* spp. are NOT commonly associated with ambrosia beetles**

Phylogenetic tree

Fusarium from avocado belongs to the FSSC





Unique morphology currently being studied

Takayuki Aoki, unpubl.

Fusarium isolates (150) in collection

58 isolates from avocado (different locations and cultivars)

49 isolates from avocado beetles (dead and alive)

17 isolates from maple and beetles

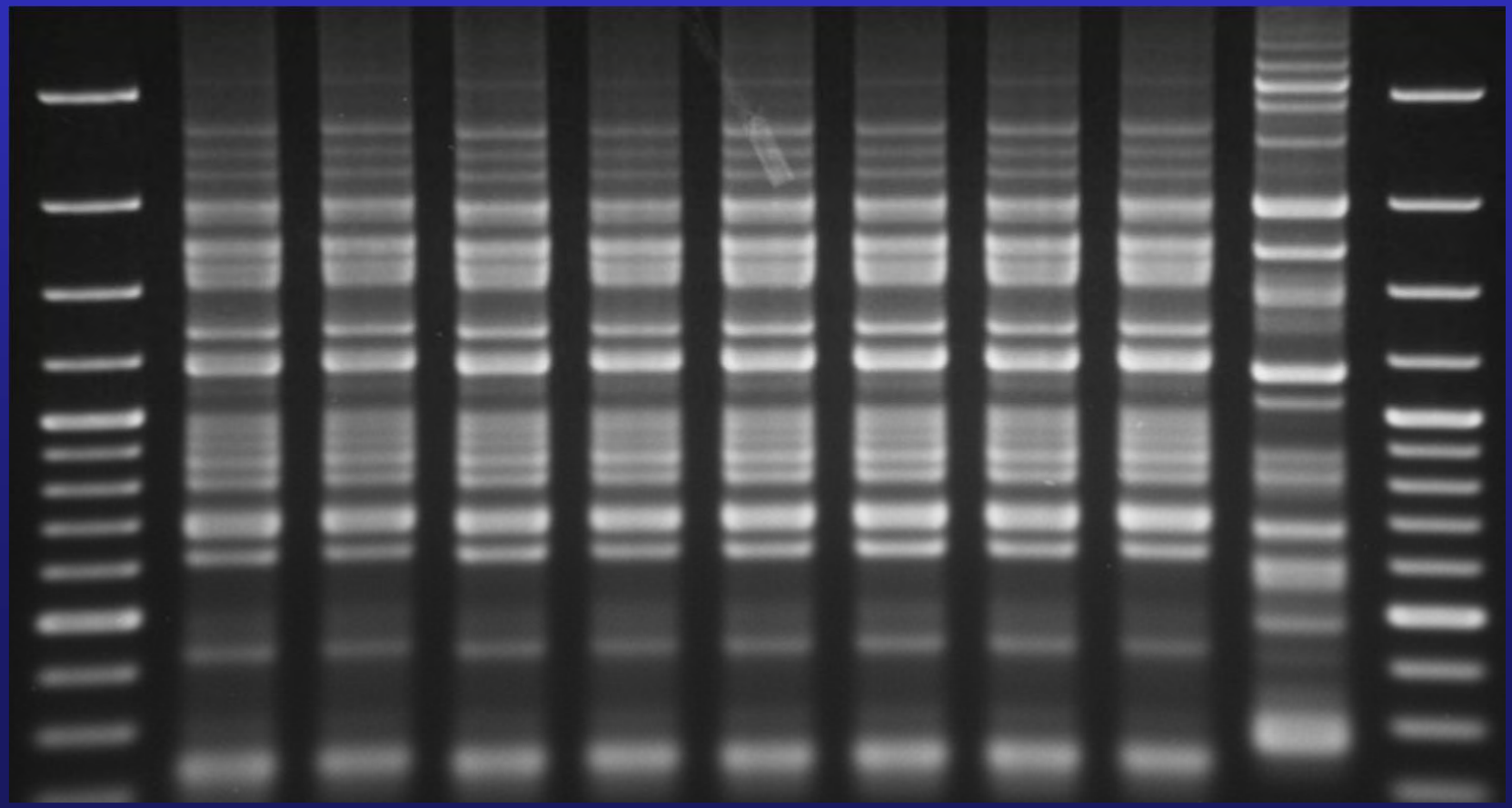
13 isolates from castor bean and beetles

9 isolates from oak and beetles

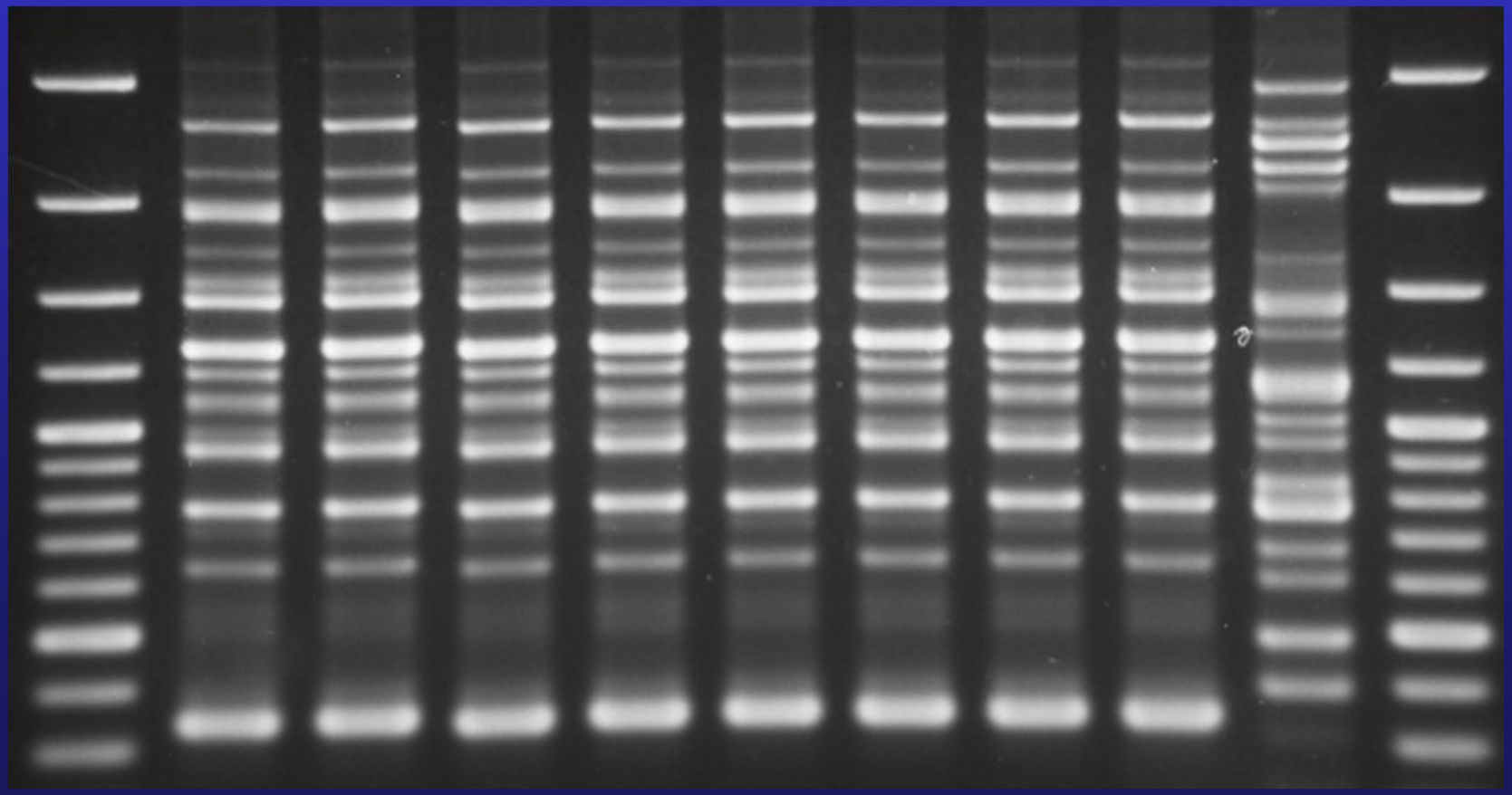
4 isolates from persimmon (resistant)

Genetic diversity of Israeli avocado *Fusarium* isolates

Ap-PCR $(CAG)_5$ primer

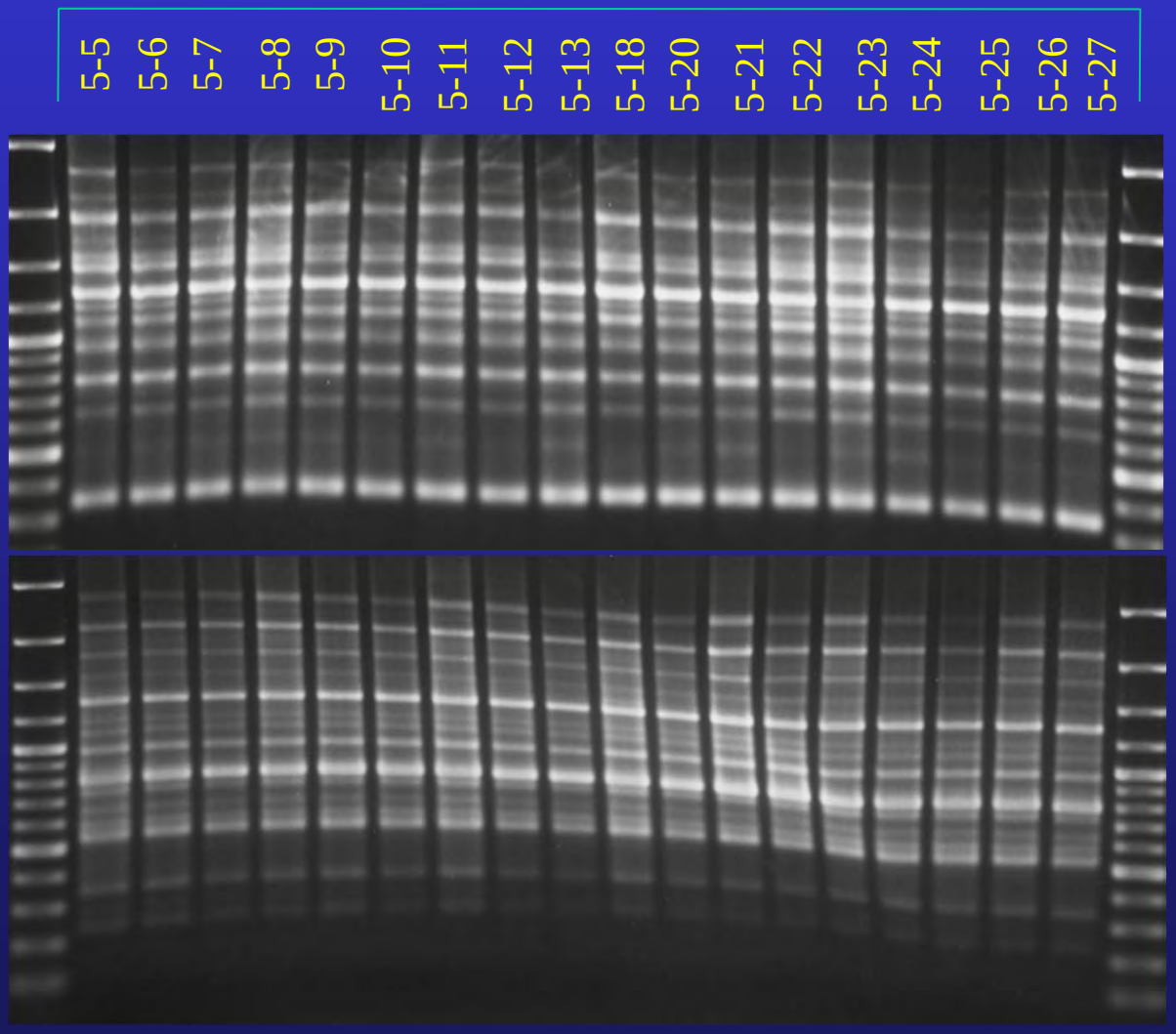


Ap-PCR $(GACA)_4$ primer



Ap-PCR of *Fusarium* sp. isolates

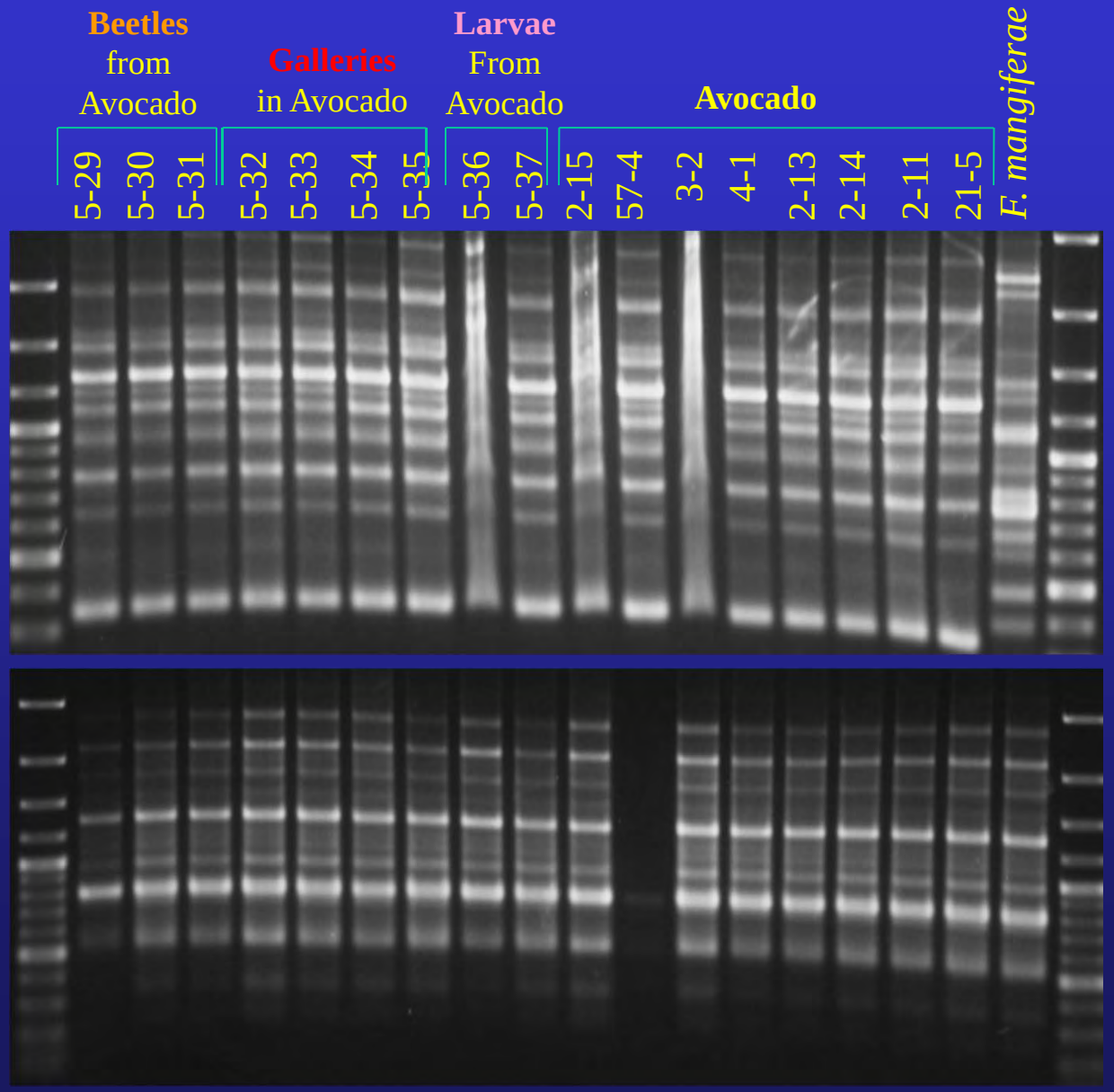
Beetles from Avocado



$(GACA)_4$

$(GACAC)_3$

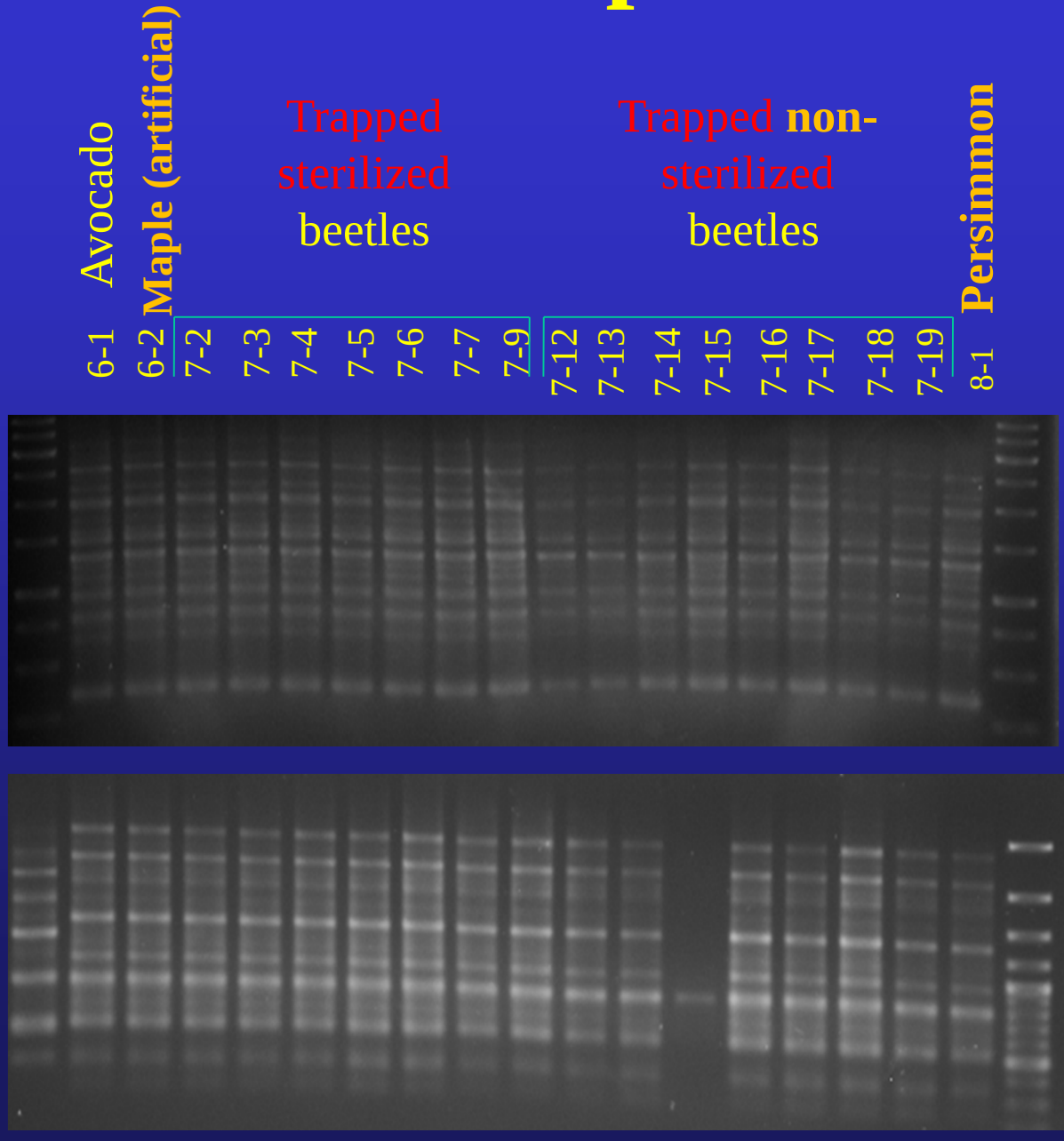
Ap-PCR of *Fusarium* sp. isolates



$(GACA)_4$

$(GACAC)_3$

Ap-PCR of *Fusarium* sp. isolates

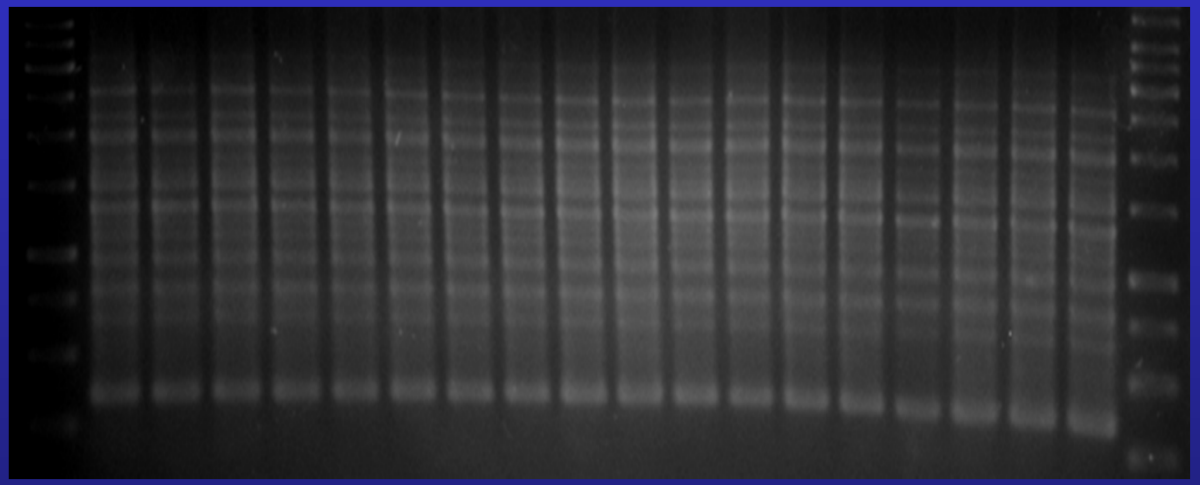


Ap-PCR of *Fusarium* sp. isolates

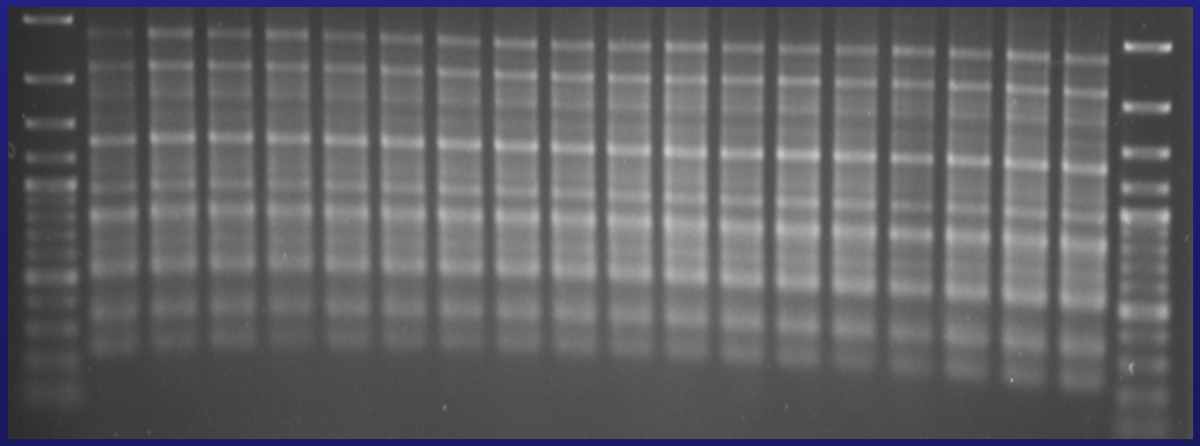
Persimmon **Avocado**

8-2 8-3 8-4 9-1 9-2 9-3 10-1 10-2 10-3 10-4 11-1 13-1 11-2 11-3 11-4 11-5 12-1 12-2

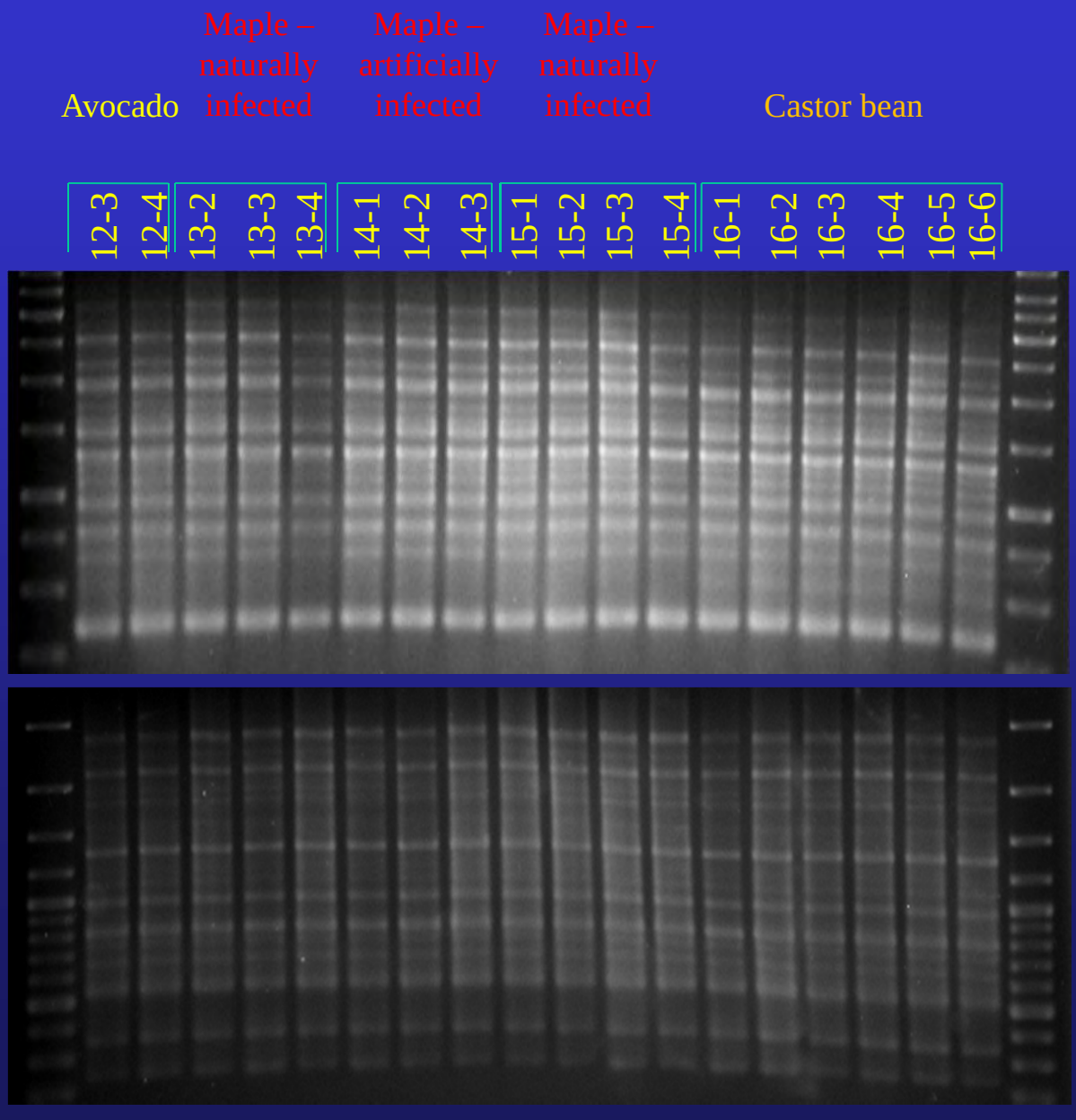
$(GACA)_4$



$(GACAC)_3$



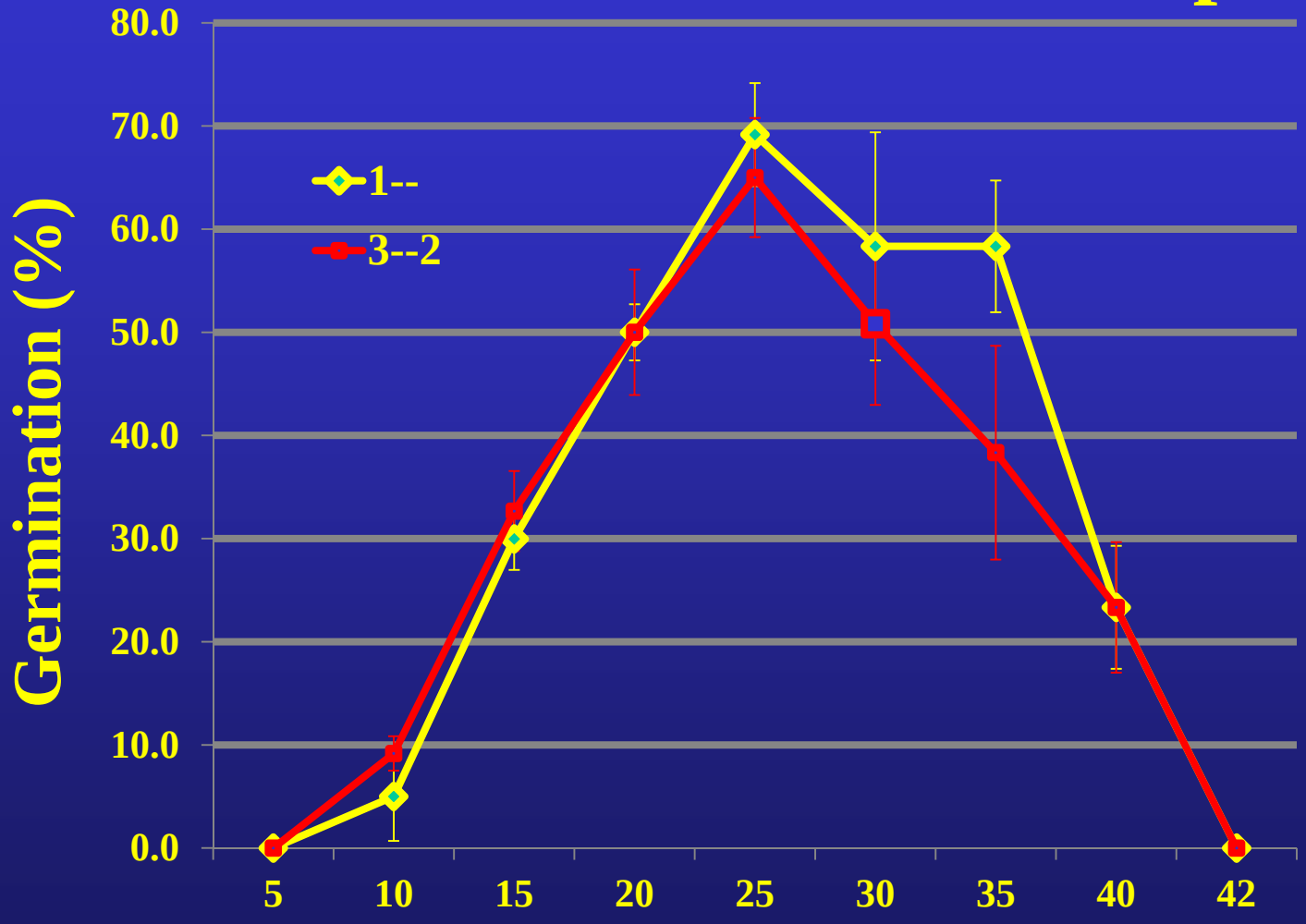
Ap-PCR of *Fusarium* sp. isolates



$(GACA)_4$

$(GACAC)_3$

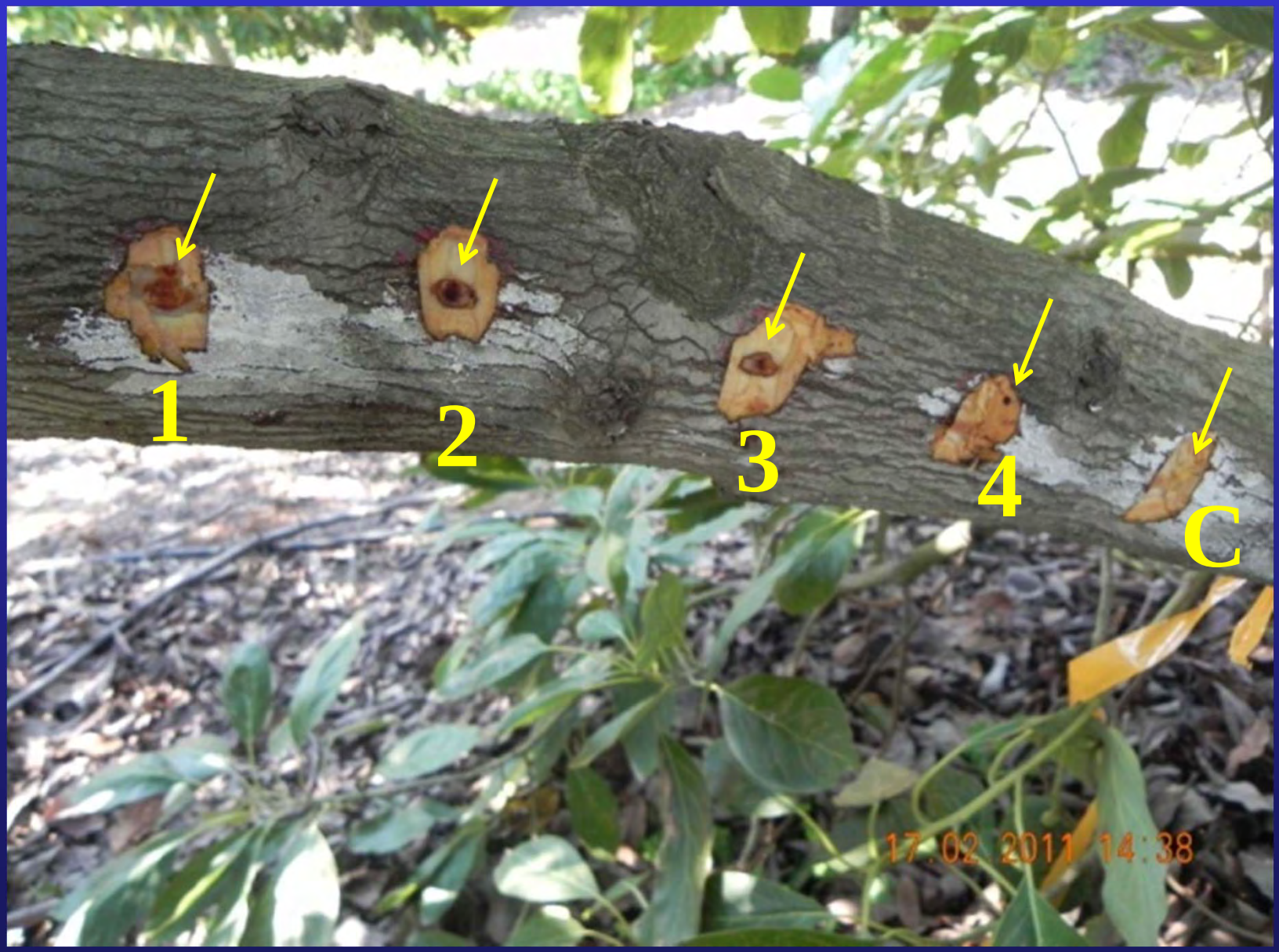
Temperature/germination conditions for the avocado *Fusarium* sp.



Koch’s postulates in mature avocado orchard

Inoculation of branches after 13 days with *Fusarium* sp.

Inoculation of branches after 6 weeks with *Fusarium* sp.



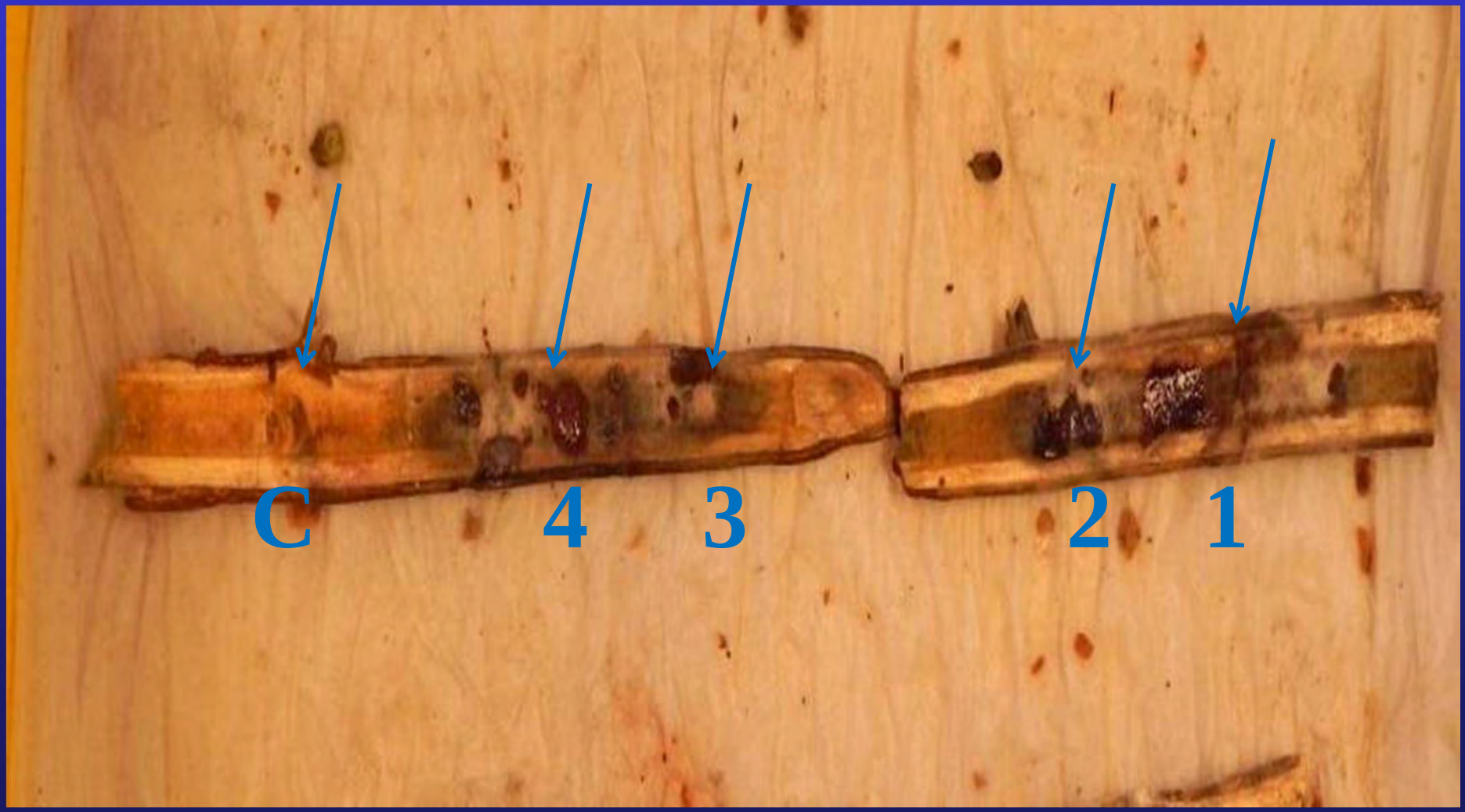
Inoculation of branches after 6 months with *Fusarium* sp.

Inoculation of branches after 11 months with *Fusarium* sp.

Inoculation of branches after 1 year with *Fusarium* sp.

Koch’s postulates in detached avocado branches

Koch postulates on detached branches of avocado, 22 days post inoculation



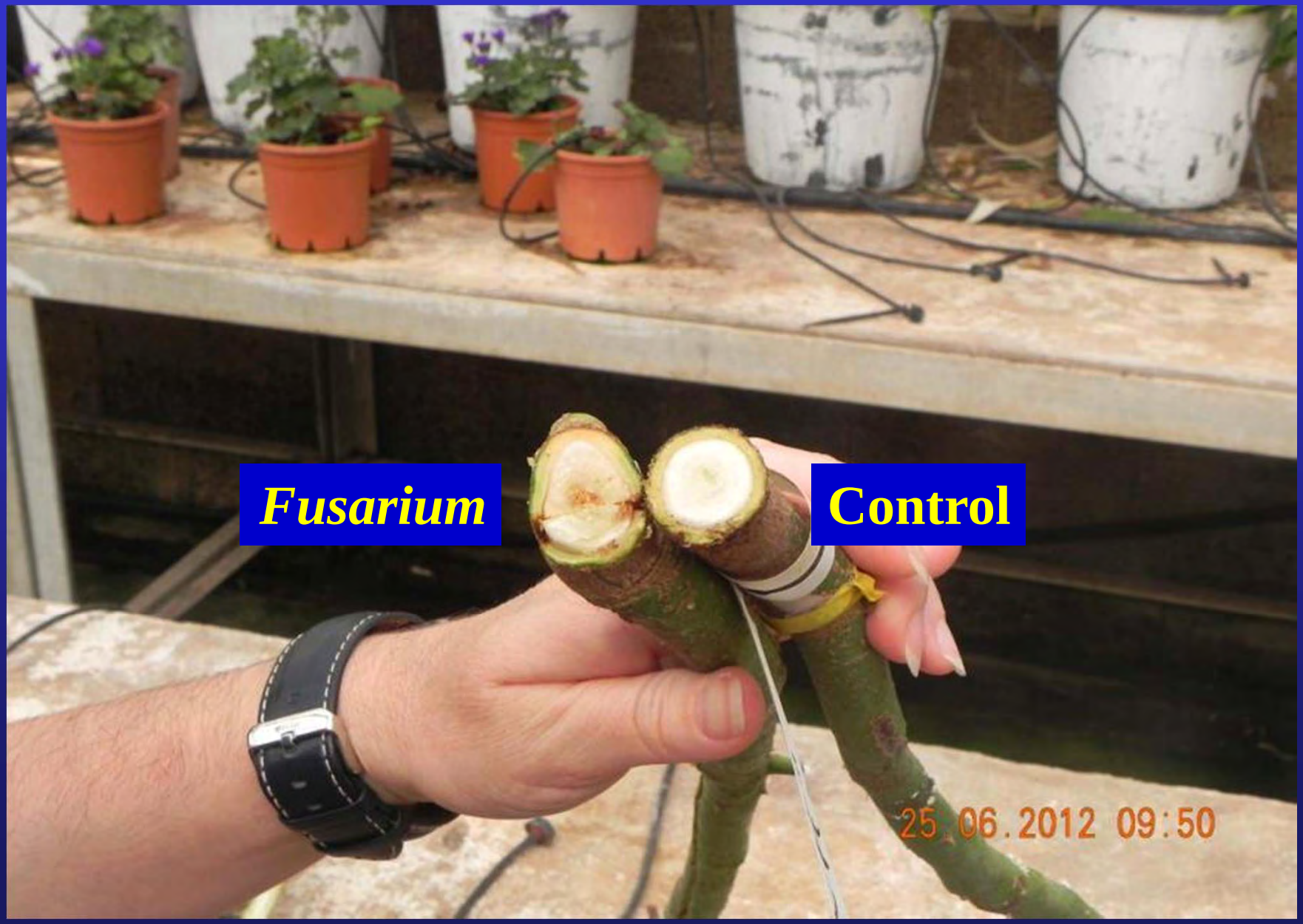
Koch's postulates in avocado plants in the greenhouse

Koch postulates in 1.5 yr-old plants in the greenhouse

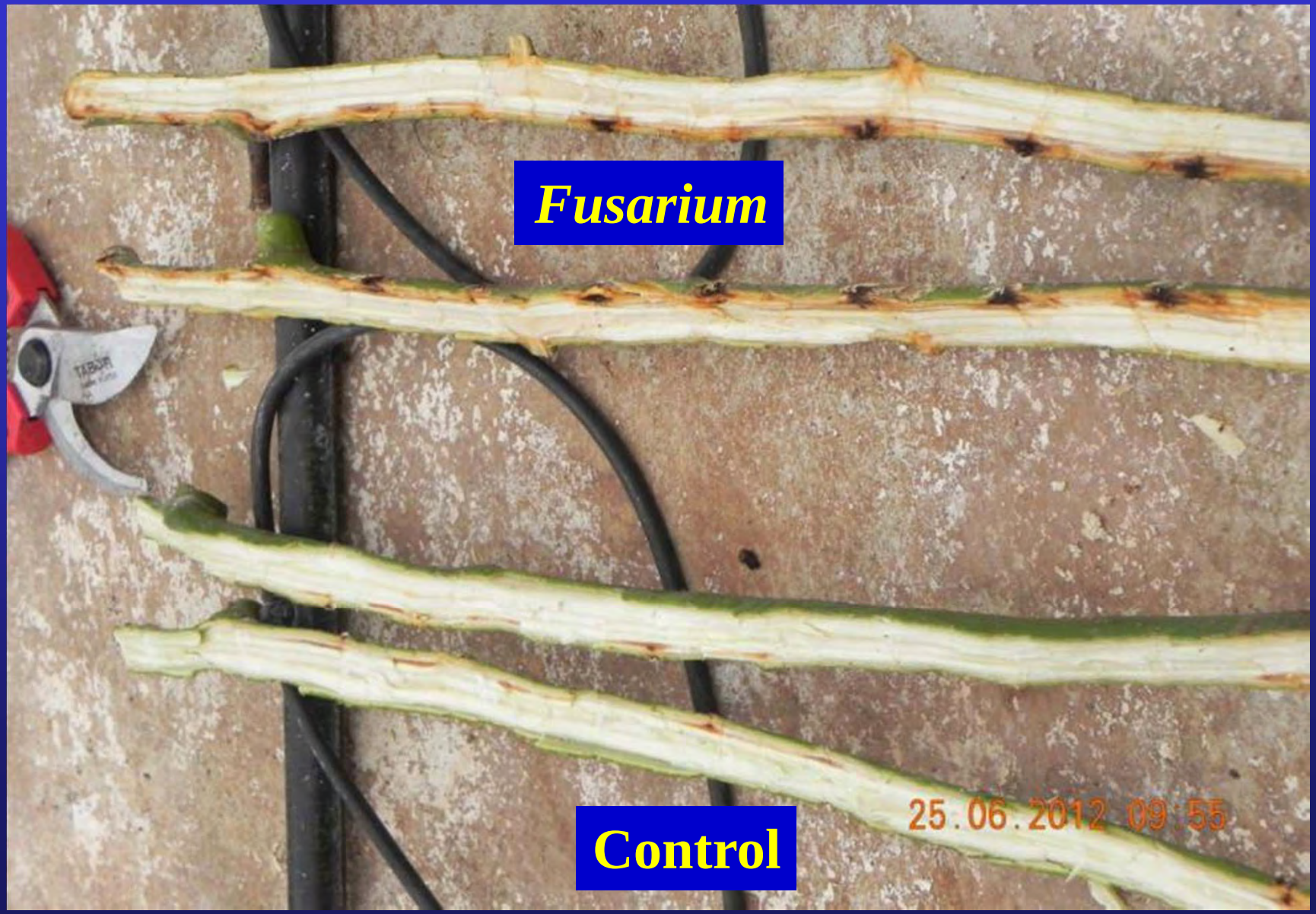
10 days post inoculation

60 days post inoculation

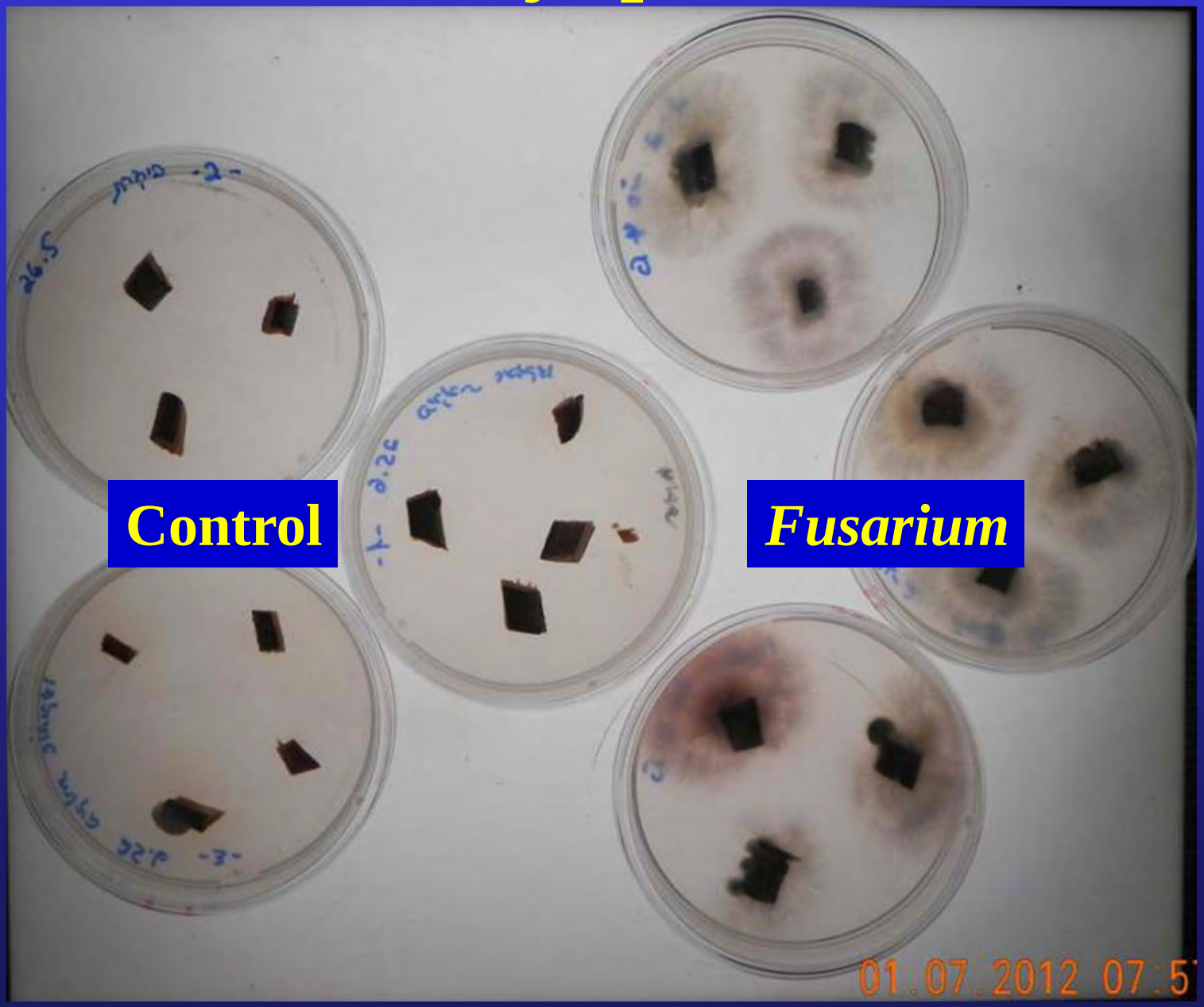
60 days post inoculation



60 days post inoculation



Isolations 60 days post inoculation



Koch's postulates in shoots and stems of young plants in the greenhouse

30 days post inoculation – Conidia injection

Control

Fusarium

30 days post inoculation – Conidia injection

Control

Fusarium

30 days post inoculation – Conidia injection

Control

Fusarium

30 days post inoculation – wound inoculation

Control

Fusarium

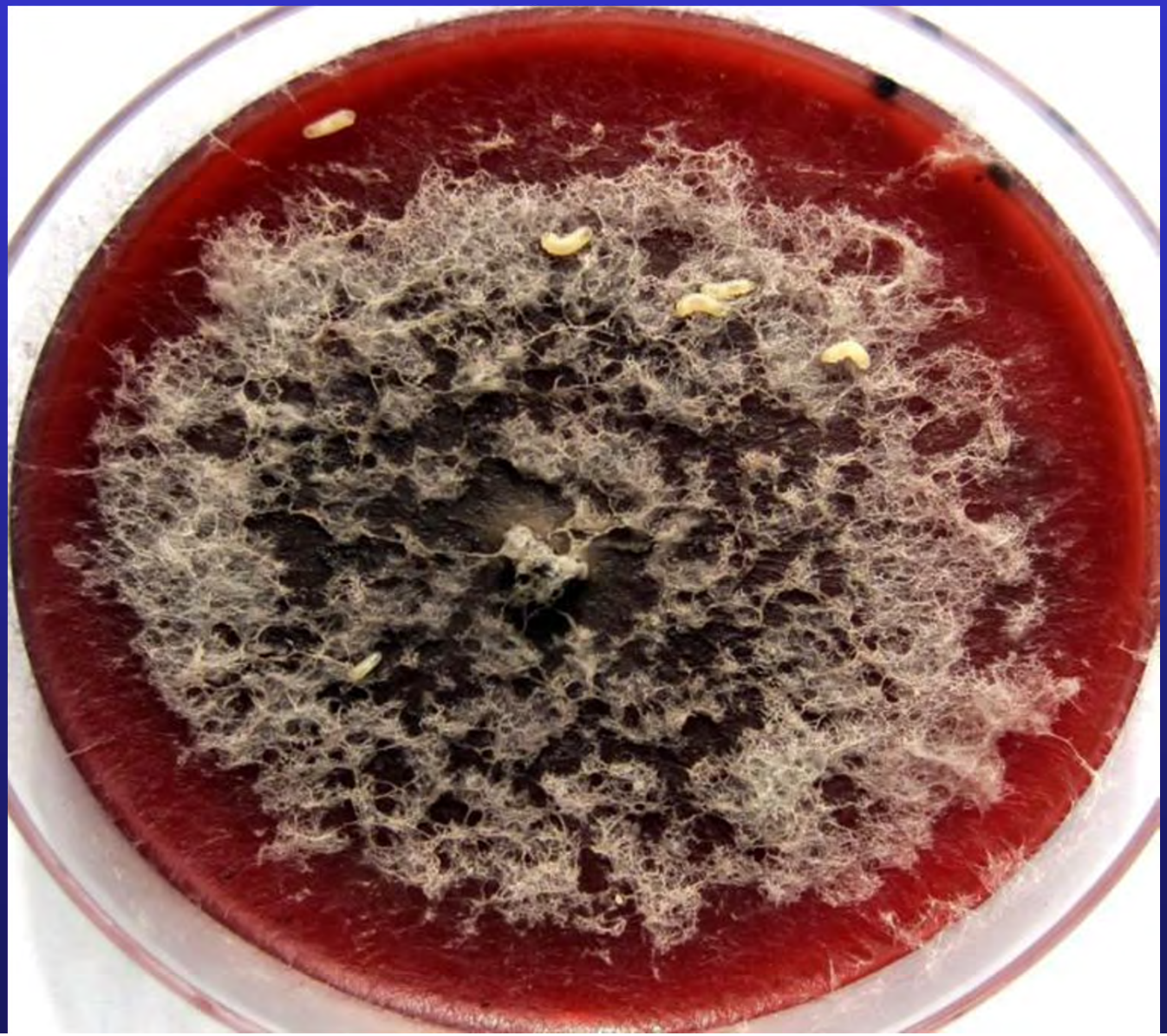
Fusarium sp. nov. is NOT a typical pathogen

Botryosphaeria

Raffaellea

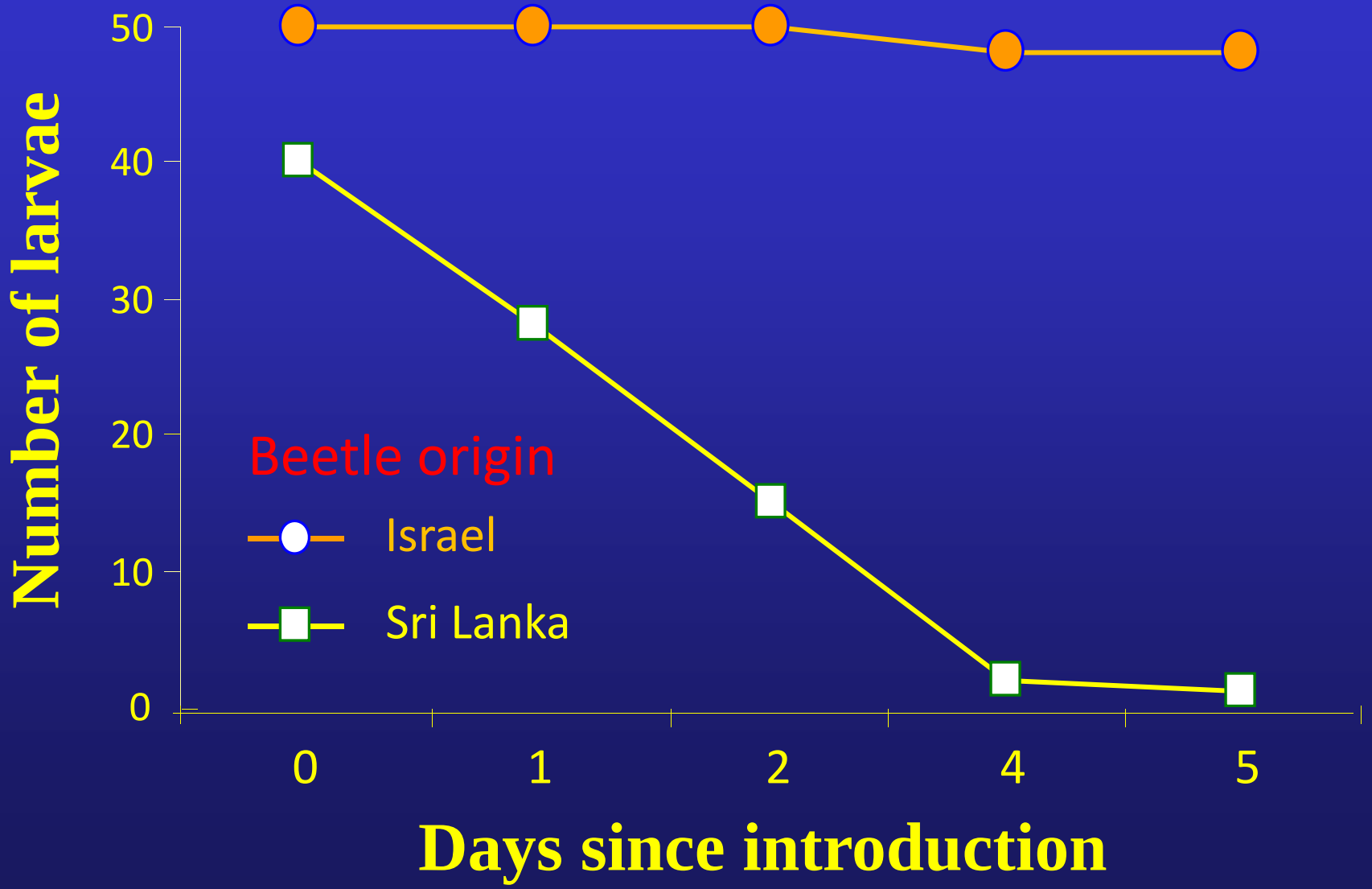
Beetle behavior from different sources fed with *Fusarium* species from avocado

Ambrosia larvae developing on fungal mycelium of *Fusarium* sp. in plates



Beetle galleries formed in agar plates

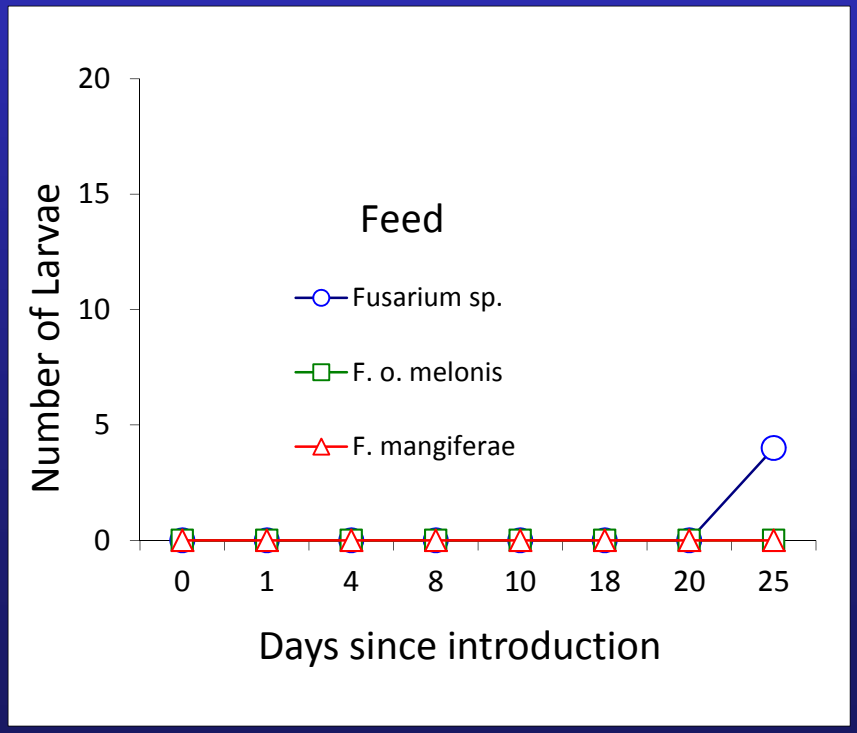
Survival of EF larvae (Israel and Sri Lanka) fed on *Fusarium* sp. from avocado in Israel



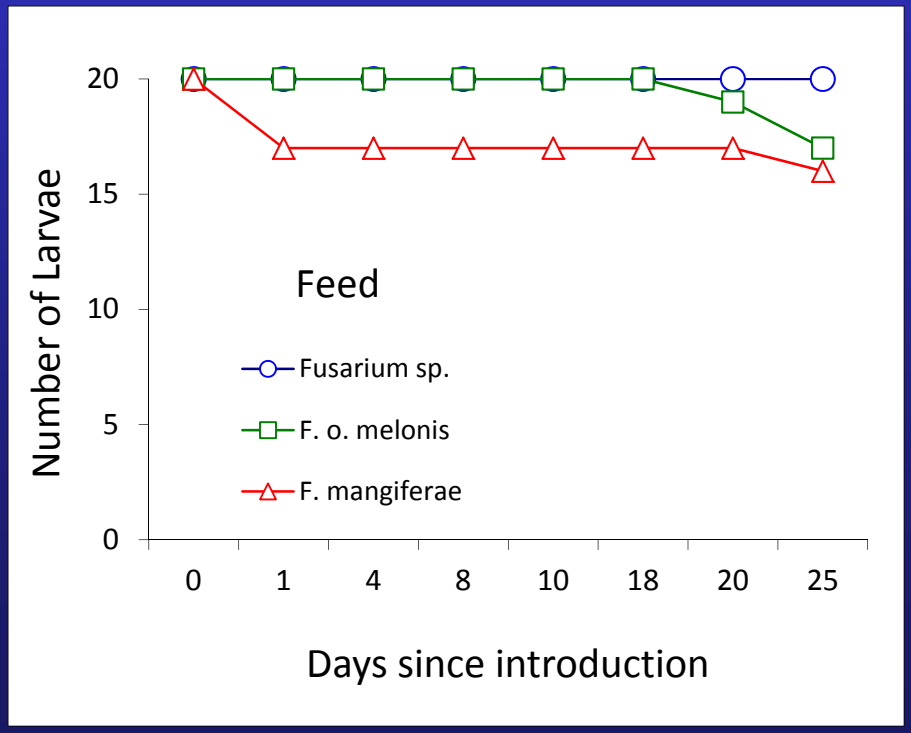
E. fornicatus behavior from avocado fed with *Fusarium* species from different sources

Pupation and survival of EF larvae from Israel fed on three *Fusarium* spp.

1st instar - pupation

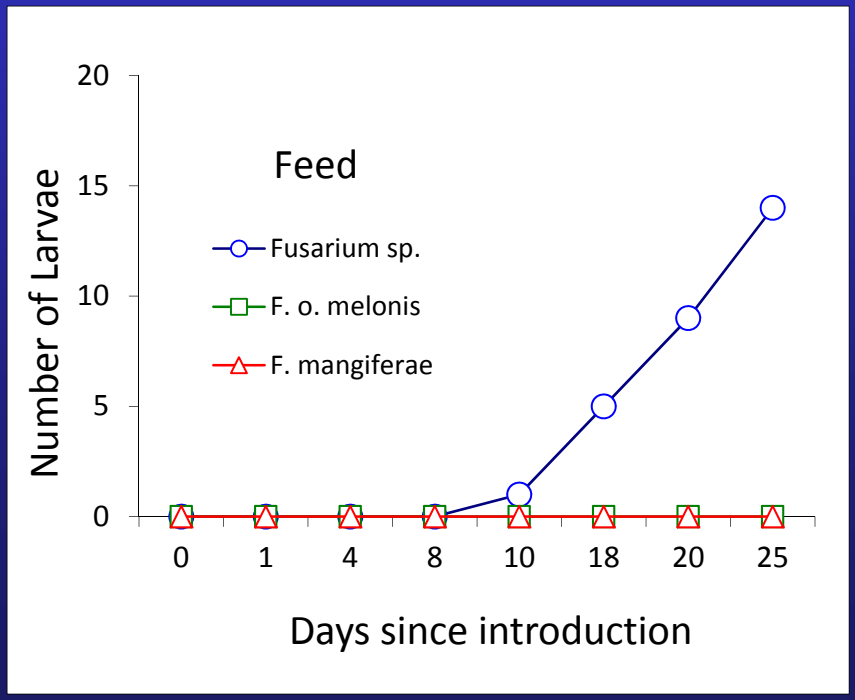


1st instar - survival

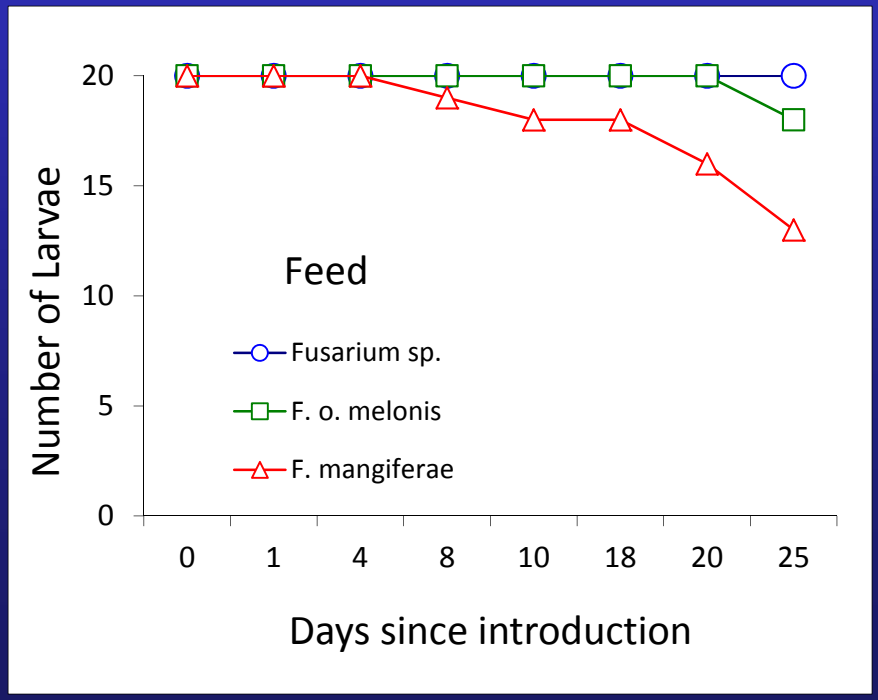


Pupation and survival of EF larvae fed on three *Fusarium* spp.

2nd instar - pupation

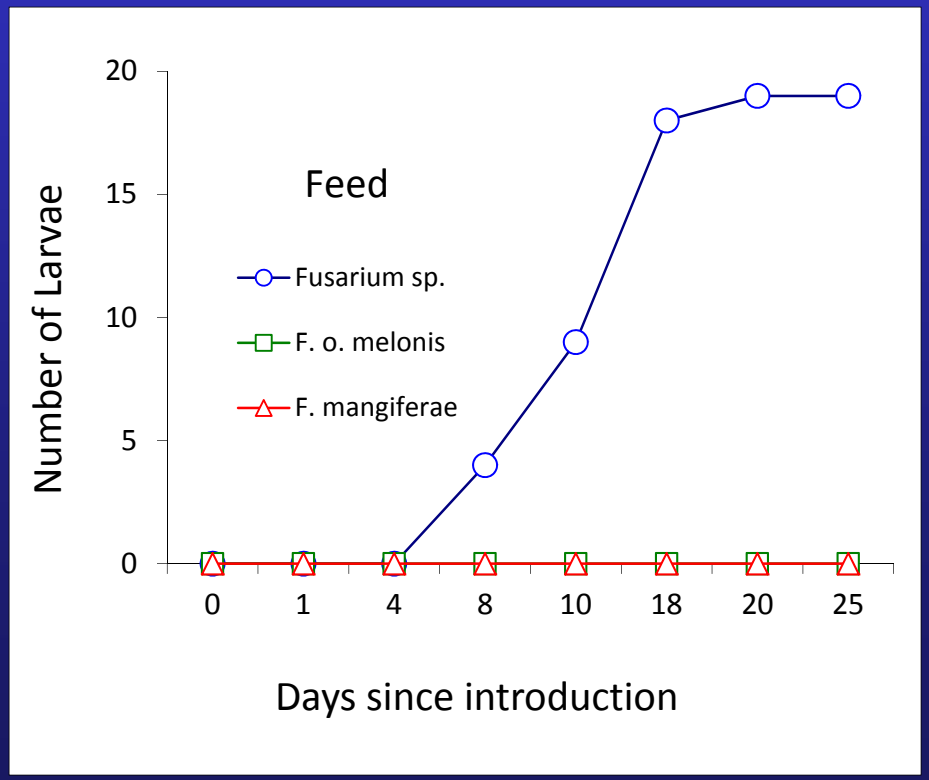


2nd instar - survival

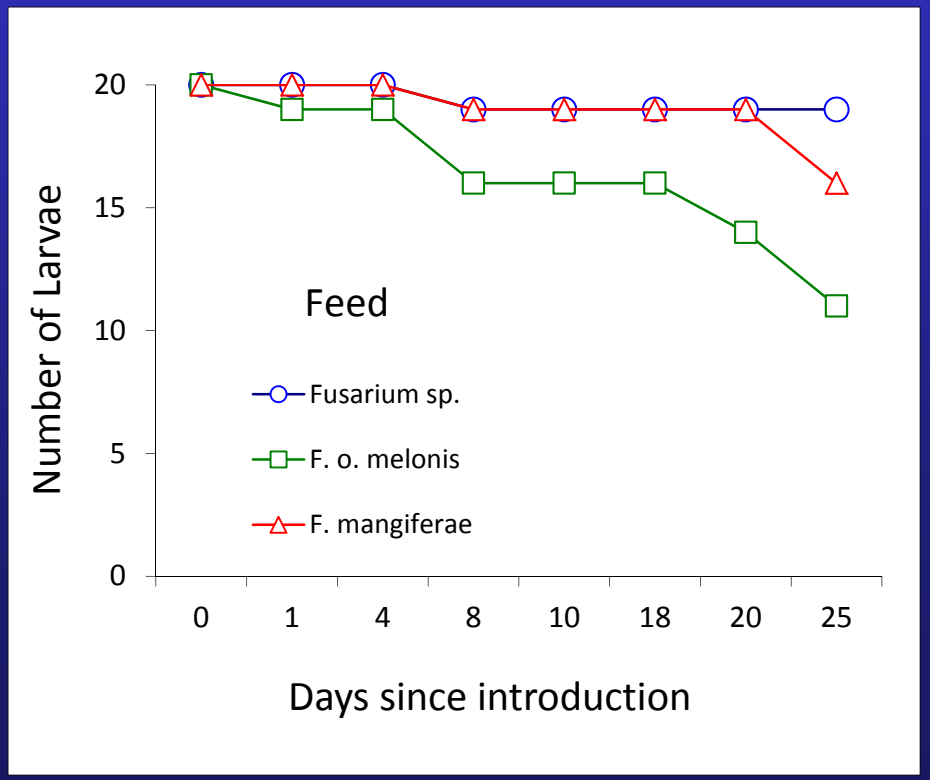


Pupation and survival of EF larvae fed on three *Fusarium* spp.

3rd instar - pupation



3rd instar - survival



Summary

1. *Fusarium* sp. nov from avocado is vectored in mycangium by the beetle

2. *Fusarium* sp. nov is not a typical pathogen such as *Rafaellea* and *Botryospaeria* as young plants are not killed

3. The beetle develops successfully on *Fusarium* but NOT on other associated *Fusaria* such as *F. mangiferae* and *F. oxysporum*

4. Only a single clone was isolated from infected trees colonized by the beetle

Summary (cont.)

5. The *Fusarium* sp. nov. has not developed as a pathogen on non-host trees attacked by the beetle, such as persimmon

6. *Fusarium* sp. nov is specific to avocado beetle and the tea beetle cannot develop on the *Fusarium* sp. nov

Questions ?

1. Monitor infection rate and levels of fungus in infected trees ?

2. If the beetle is eradicated or removed from the tree, can the infection rate of the fungus be monitored ?

3. Can infected trees survive colonization of the fungus after eradication of the beetle ?

4. How to treat infected trees ?

Future research

1. Interaction between beetles and fungi using GFP-marked strains

2. Determine specificity of beetle to botanical hosts

3. Determine specificity of fungal attraction to beetles

4. Control strategies

Thanks for your attention