

AGRAUXINE S.A.

**Research, Development, Registration
Production and Distribution of
Bio-Control products for plant protection, health and
Nutrition based on alive micro organisms and/or natural
substances**

Our aims and targets

**AGRAUXINE is developing different programs in the area
of plant bio-protection and nutrition**

AGRAUXINE is member of IBMA.

Esquive WP

A bio fungicide against pruning
wound diseases on vine and
plant root pathogens

Esquive WP *Trichoderma atroviride I-1237*

Many strains of Trichoderma have been selected in 90' by INRA for their capacity to fight most of the soil root pathogens, *Pythium*, *Sclerotinia*, *Phytophthora*, *Rhizoctonia*...

Among these strains we have selected one of the best for its ability to also stop development of fungi involved in the Eutypiose and ESCA diseases on vine.

Trichoderma atroviride - Strain T1
Collection : CNCM N° : I-1237
Patent belonging to Agrauxine

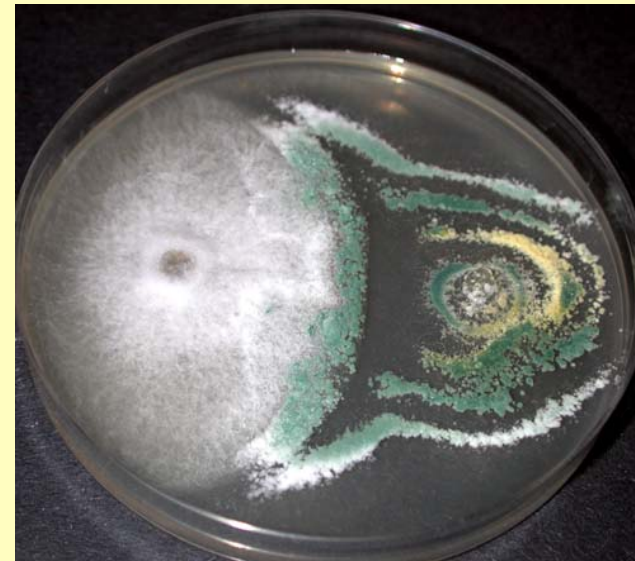
In vitro

- *Trichoderma strain T1* shown a strong antagonistic capacity to stop the development of fungi known to be involved in wound prunes diseases on vine (ESCA, Eutypiose).

Phaeomoniella chlamydospora



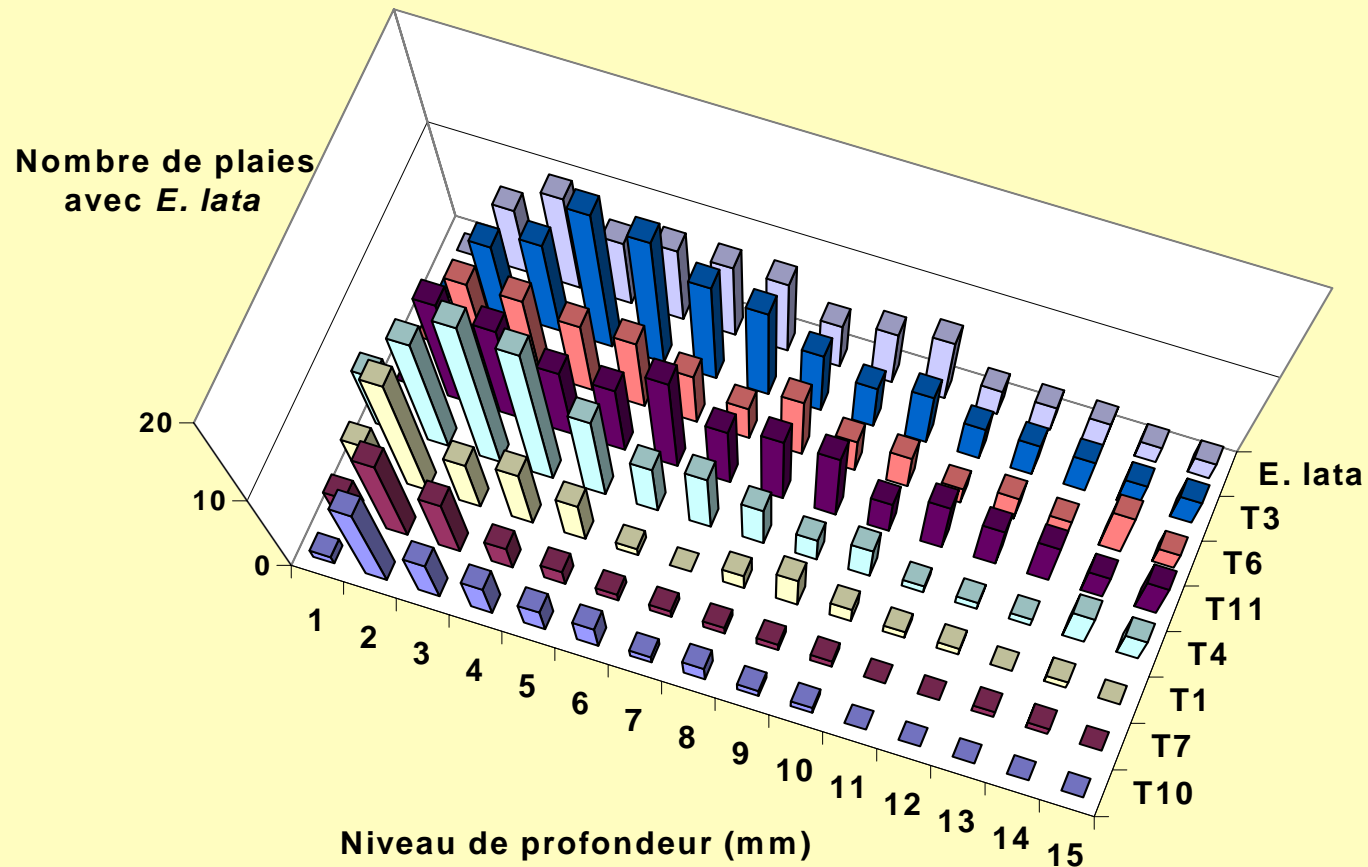
Eutypa lata



(collaboration with ITV Nîmes- France)

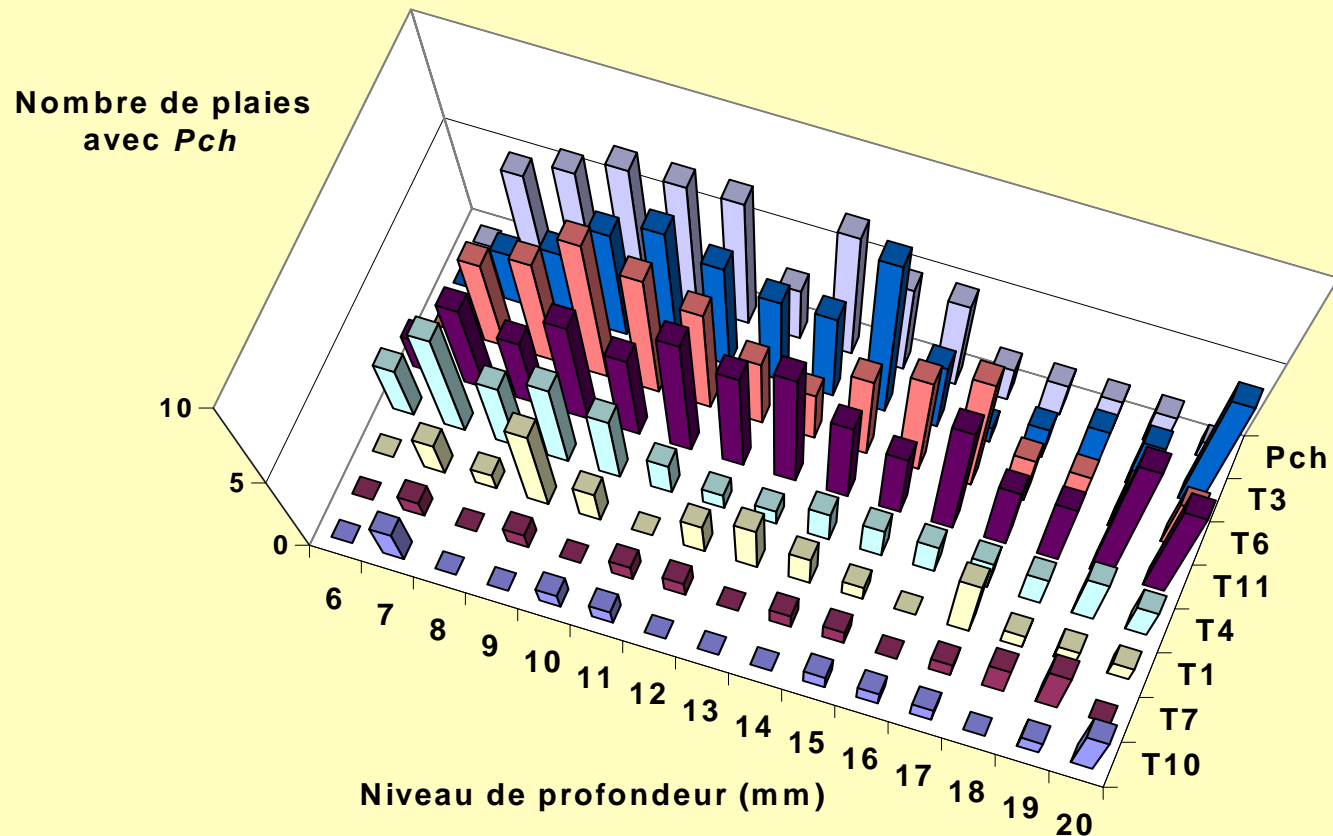
In situ Antagonistic activity of I-1237 against *Eutypa lata*

Le traitement avec la souche I-1237 (T1, T10, T4) après la taille permet de diminuer la présence d'*Eutypa lata* dans la profondeur du chicot (Etude ITV).



Antagonistic activity of I-1237 against fungi involved in Esca diseases

Le traitement avec la souche I-1237 (T1, T10, T4) après la taille permet de diminuer la présence de **Phaeomoniella chlamydospora** dans la profondeur du chicot (Etude ITV).



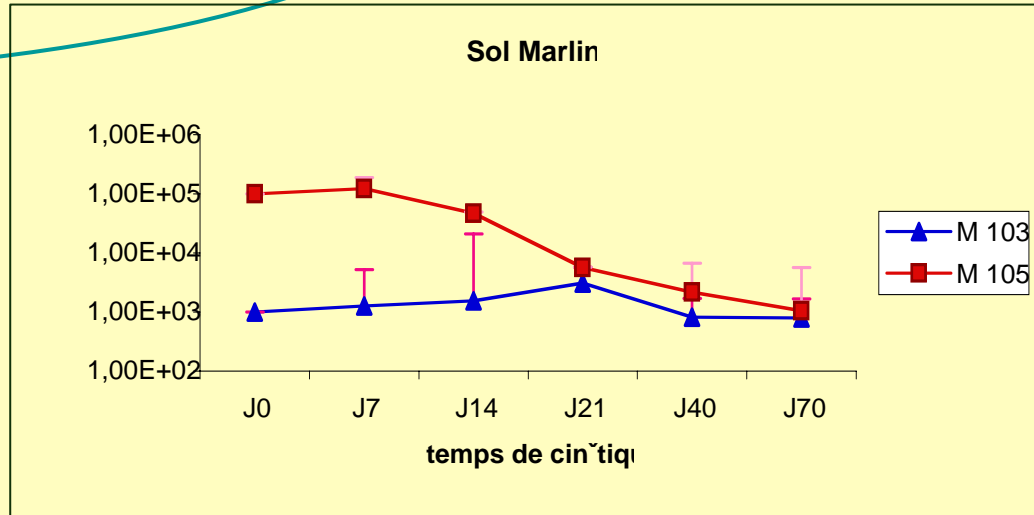
I- Ecology

- Dynamic of the microbe in soil, root or phyllosphere (Plant) etc..
- Identification / specific marker of the isolate / Detection in environment / physiology
- Effect on soil microbes communities
- Mechanism of action (competition, antibiosis, secondary metabolites Antibiotics,... ?

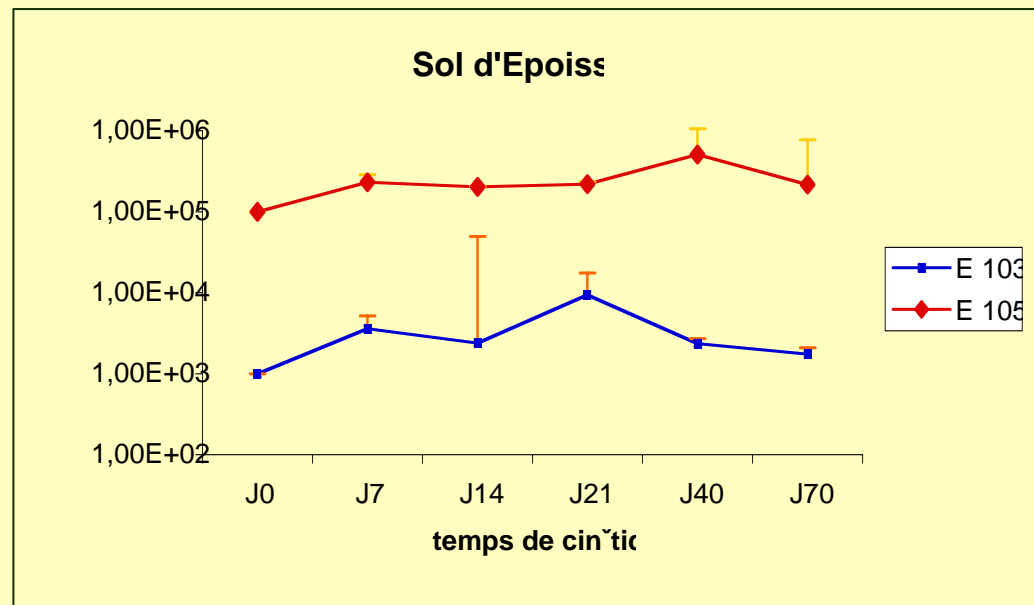
Ecology : Dynamic of the Trichoderma in 5 types of soil have been investigated to understand the development

	Sol d'Epouisses	Sol Marliins (Morvan)	Sol bordelais (Graves)	Tourbe	Sable
nature physico-chimical	Clay-loam	<u>Sandy loam</u>	sandy	peat	Pur sand
pH (H ₂ O)	8,2 (mesuré 6,8)	4,4 (mesuré 4,6)	5,8 (mesuré 6,4)	5,8 (mesuré 5,6)	
C/N	10,2	11	12,2	34,3 (après désinfection)	
MO	2,62 (élevé)	4,64 (très élevé)	1,09 (faible)	70% poids brut 82,2 (après désinfection)	

Natural soils



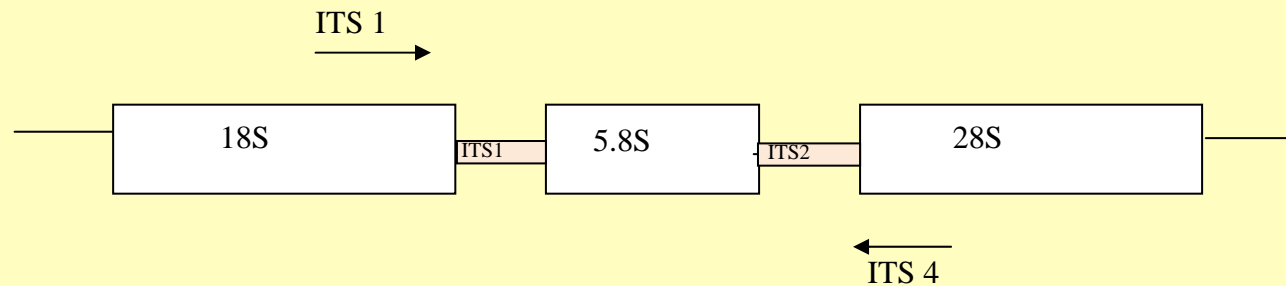
Soil Marlins : soil capacity to regulate population of Trichoderma T1 at 1×10^3 prop/gr sol whatever the starting rate, after 2 months: population is soil-dependant



Soil Epoisses. The population of Trichoderma in soil is still different in soil after 2 months. The population is rate-dependant and concentration of Trichoderma in the soil can be managed.

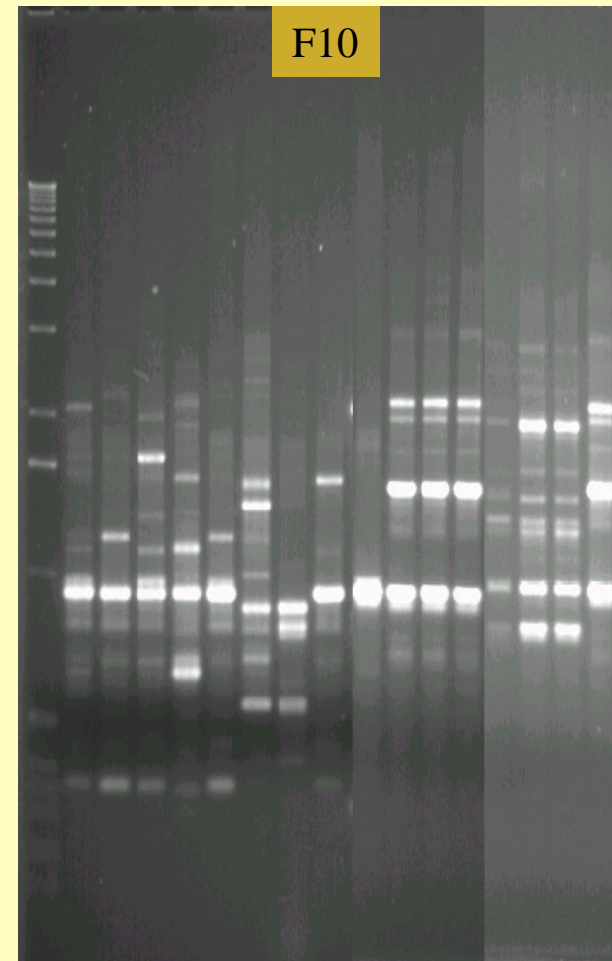
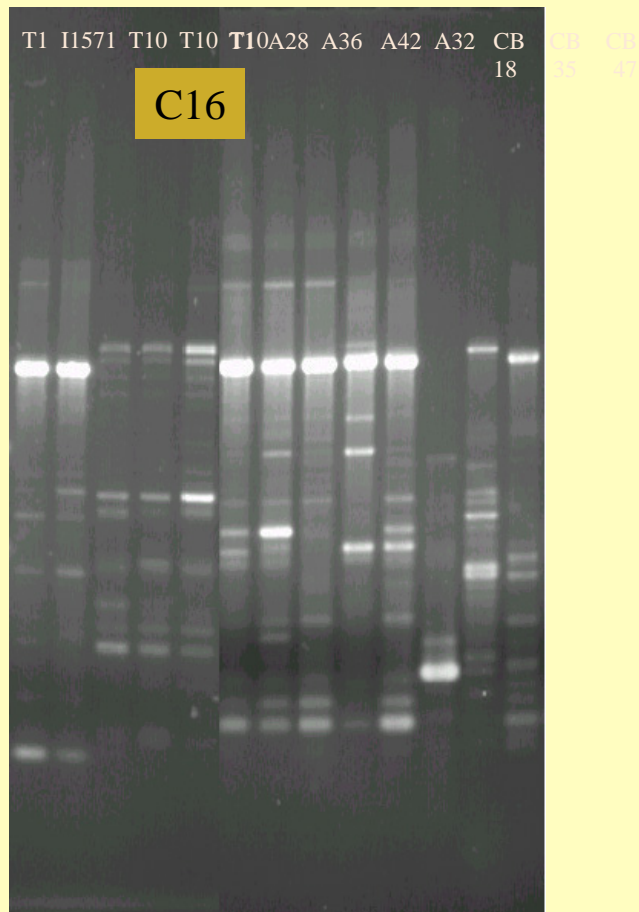
1 : Identification of the specie /development of a specific marker of the isolate I-1237

1- Specie identification ---- To confirm *atroviride*

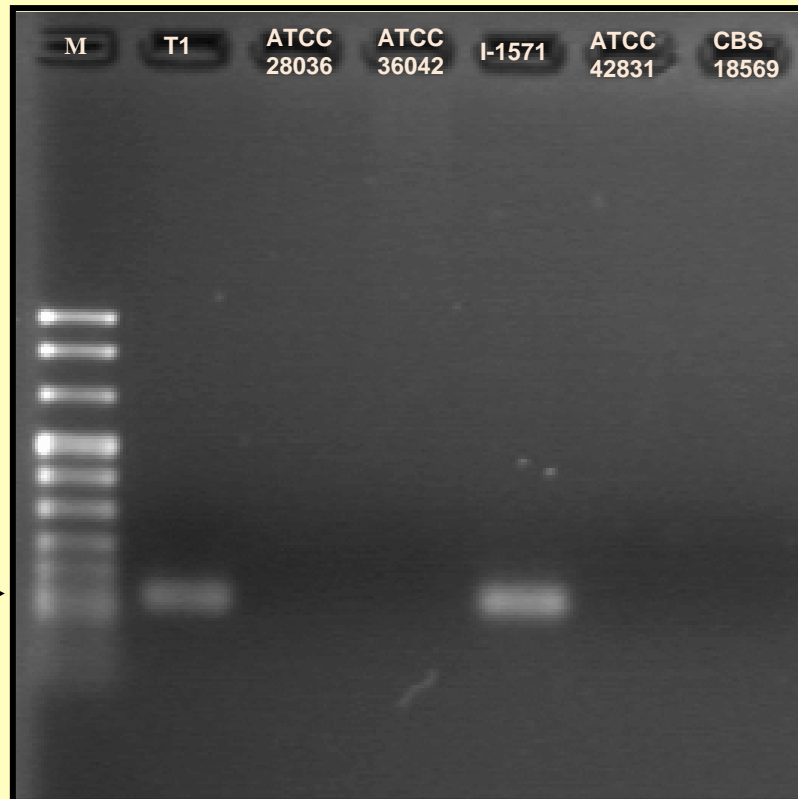


Scheme of the ITS 1 et ITS 2 have been targeted and amplified and sequenced using specific primers of these Ribosomal regions

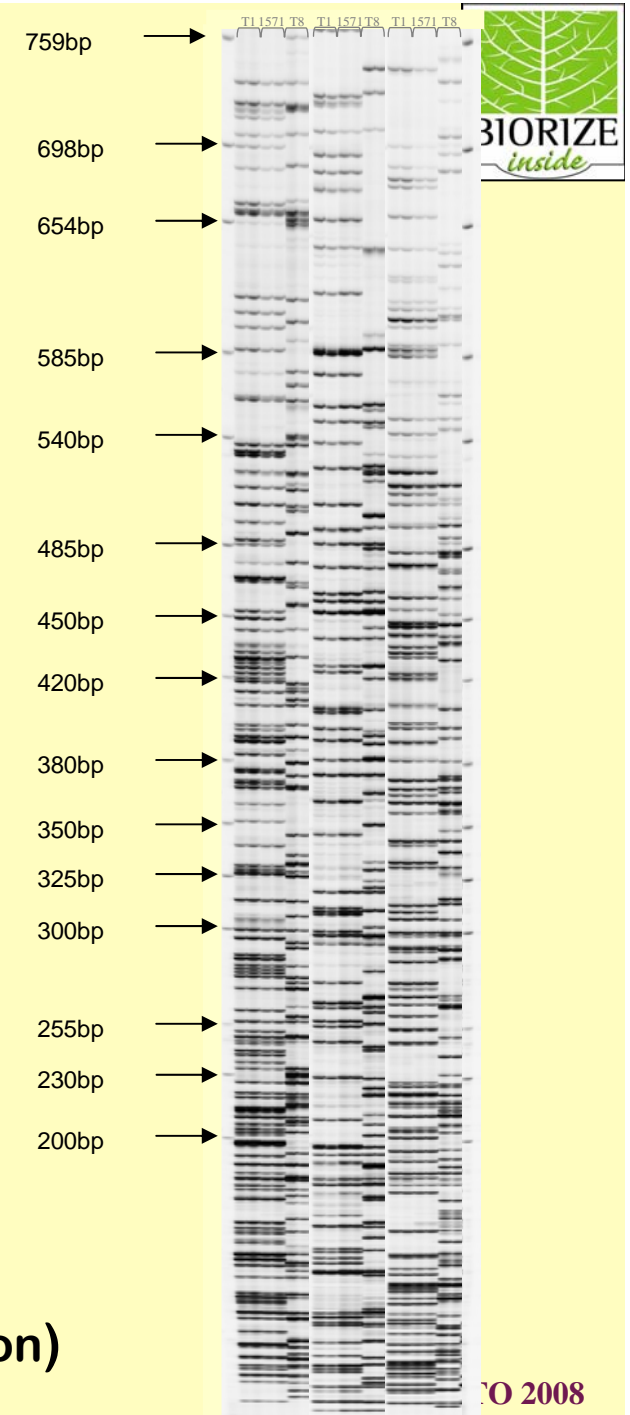
Different primers have been tested using the RAPD technique to reveal a polymorphism between more than 25 isolates



T. atroviride strains



Specific SCAR marker for T1 (I-1237)
(Sequence Characterized Amplified Region)



2 : Biotests on vine under practical conditions

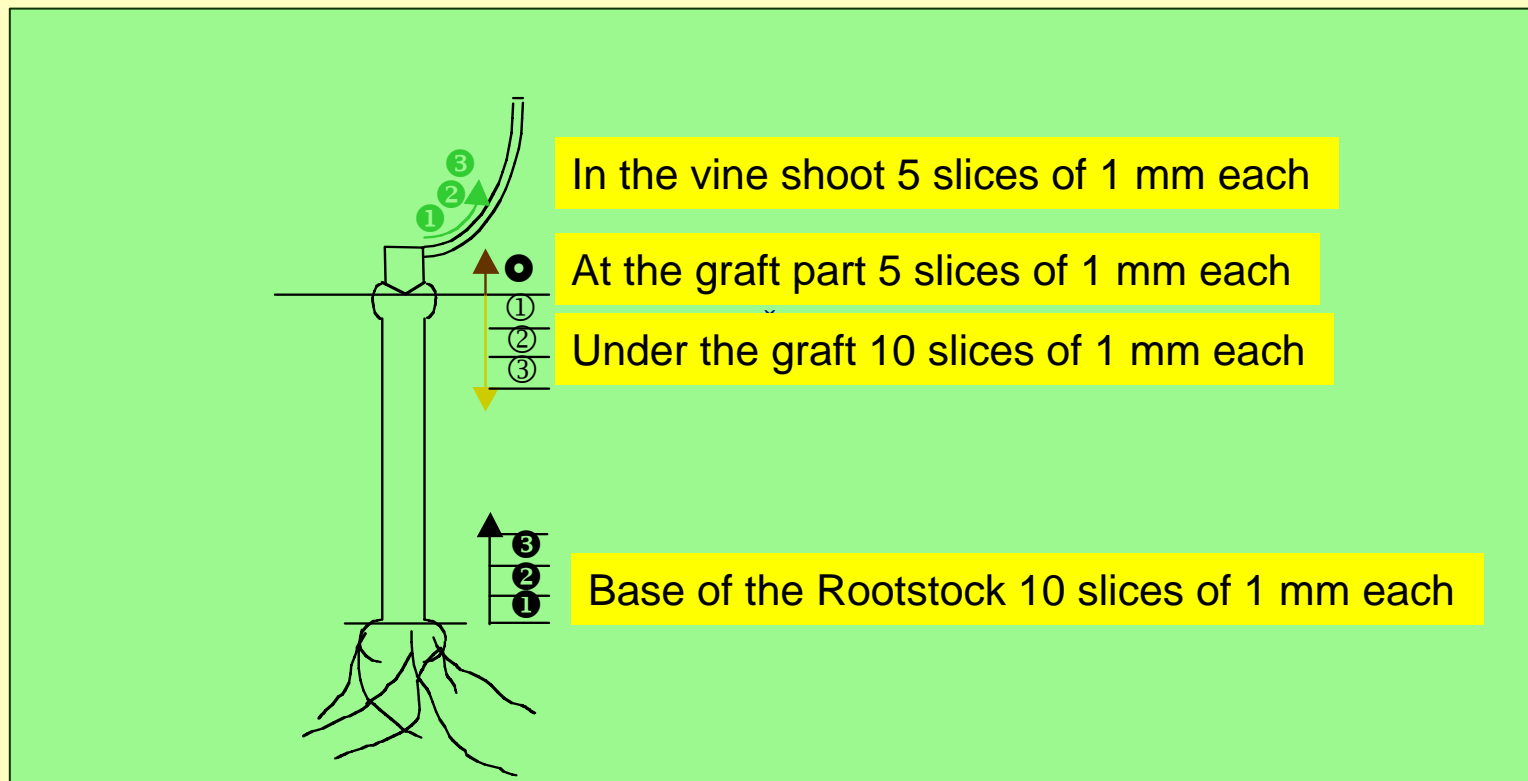
-tests during 4 years against :

- *Eutypa lata* (field trials) INRA Bordeaux

- Fungi involved in ESCA and BDA diseases on young plants in nursery. Mercier Novatech + INRA Dijon

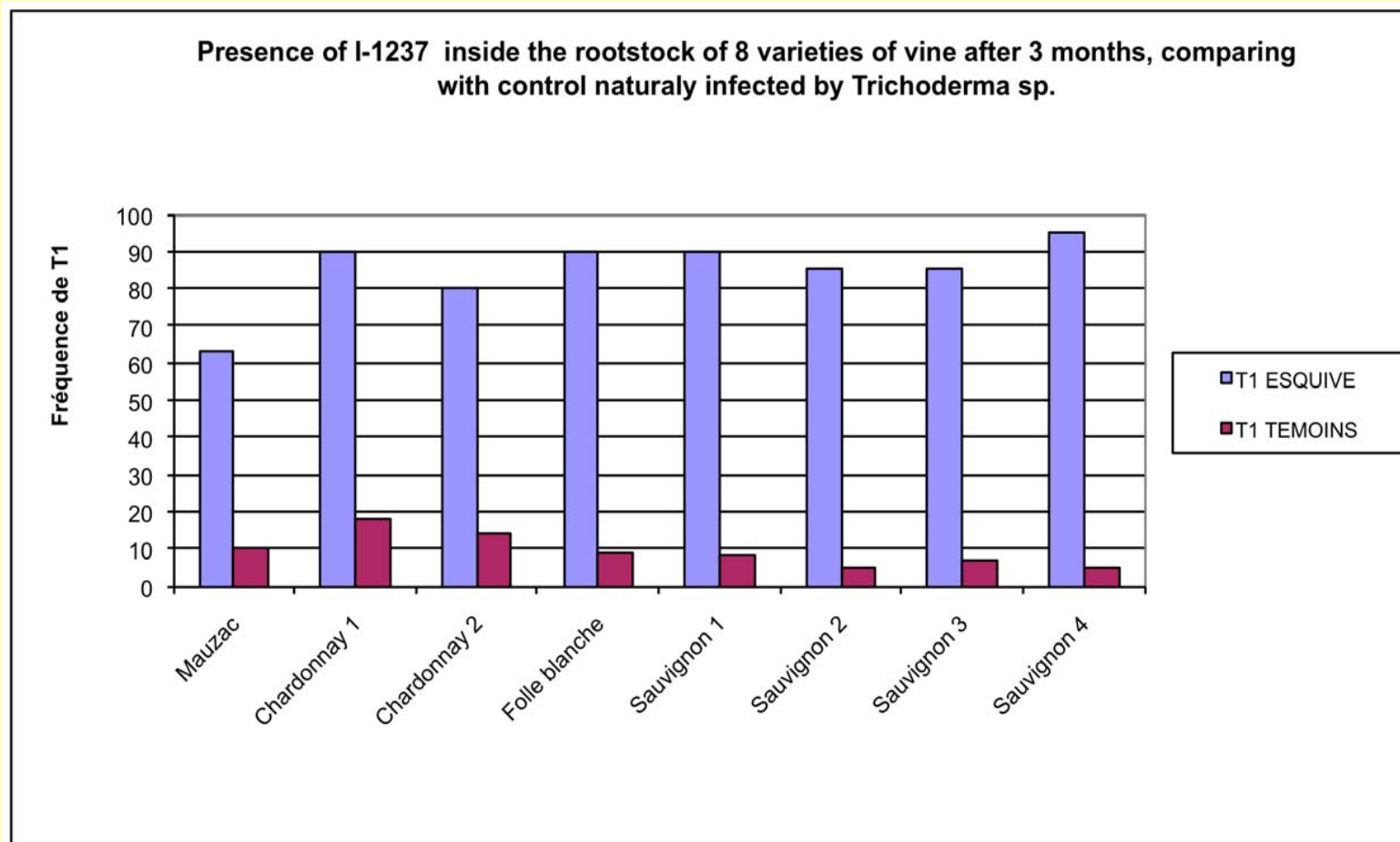
1- Capacity of I-1237 to colonize and persist in the vine wood following *ESQUIVE* application at nursery stage .

Objective : to follow and prove the development of I-1237 within the vessels of vine wood at all the levels of the plant development on 8 different varieties (collaboration MERCIER Novatech-France).



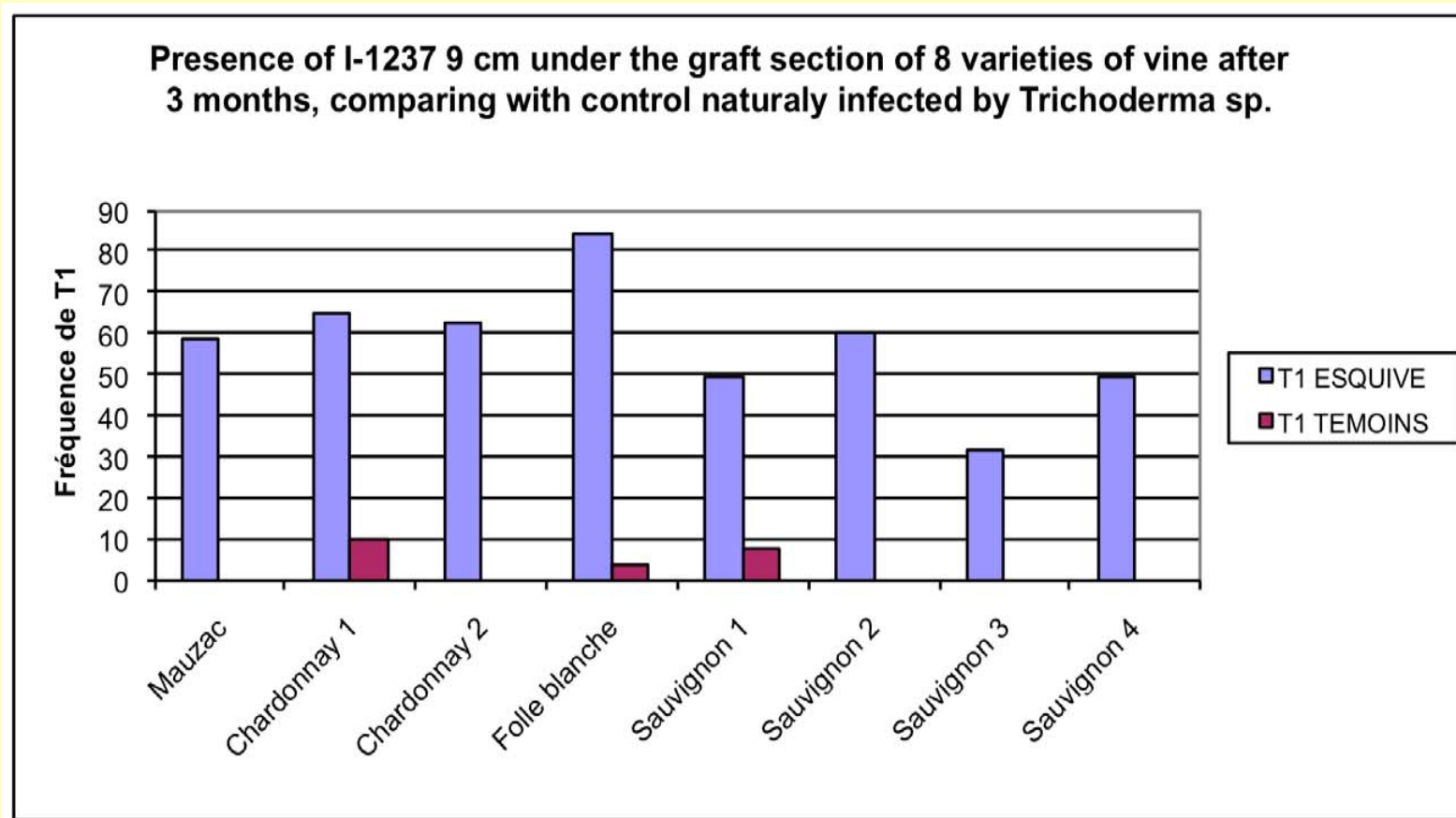
1- Capacity of I-1237 to colonize and persist in the vine wood following *ESQUIVE* application at nursery stage .

Presence of I-1237 within the base of rootstock of 8 different vine varieties



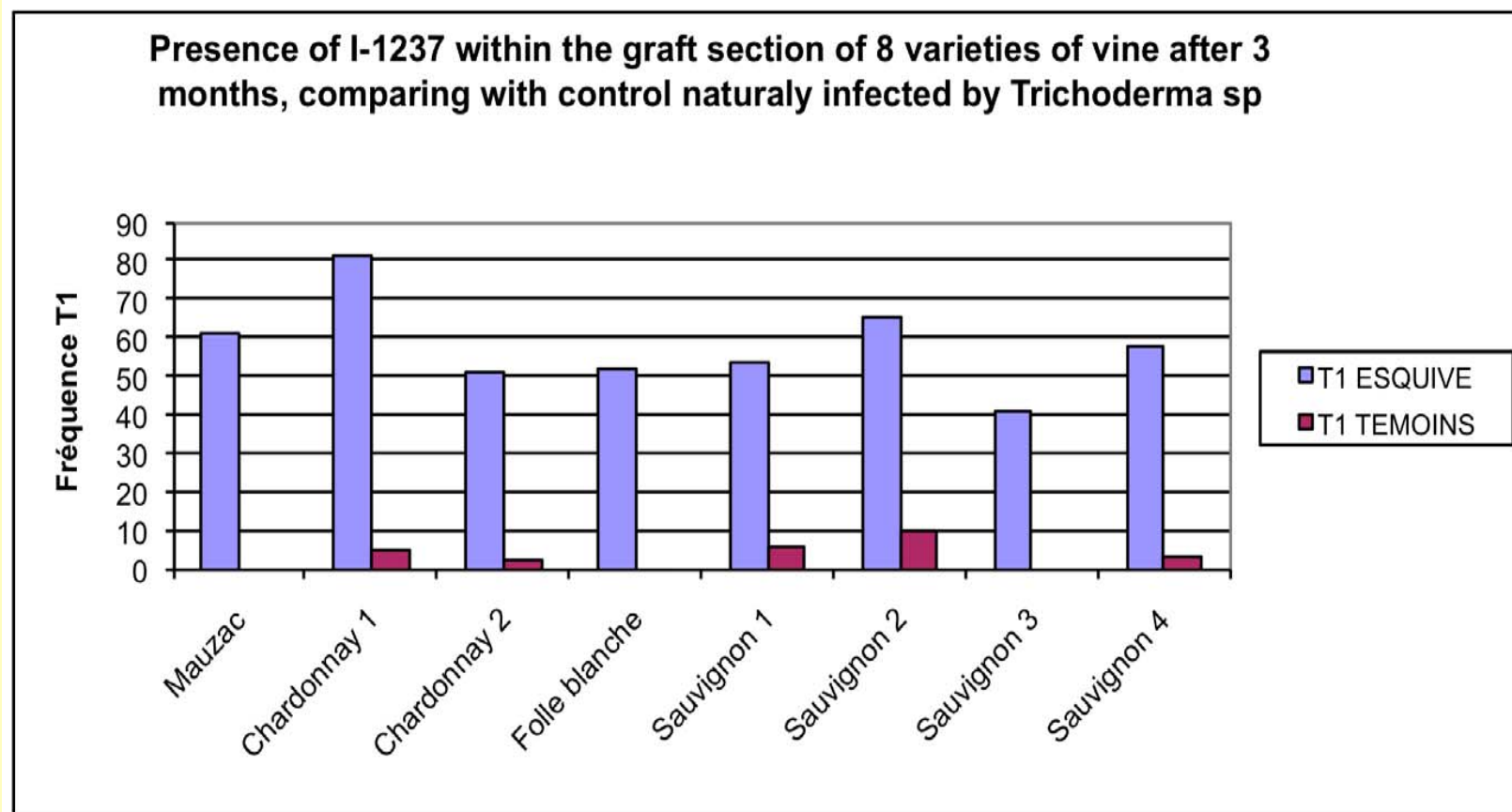
1- Capacity of I-1237 to colonize and persist in the vine wood following *ESQUIVE* application at nursery stage .

Presence of I-1237 within the mid of rootstock of 8 different vine varieties



1- Capacity of I-1237 to colonize and persist in the vine wood following *ESQUIRE* application at nursery stage .

Presence of I-1237 within graft section of 8 different vine varieties



2- Capacity of I-1237 to reduce ESCA pathogen development in the vine wood following *ESQUIVE* application at nursery stage .

Protocole:

- Esquive treatment of vine young plant after grafting
- Pathogens involved in Esca diseases : Botryosphaeria parva, B.obtusa, Phaeoacremonium aleophilum, Phaeomonelia chlamydospora
- Artificial inoculation of each Pathogen under the graft point
- Analysis of the presence of each pathogen and I-1237 after 6, 14 months.
- Analysis by INRA Dijon, in collaboration with Mercier Novatech.

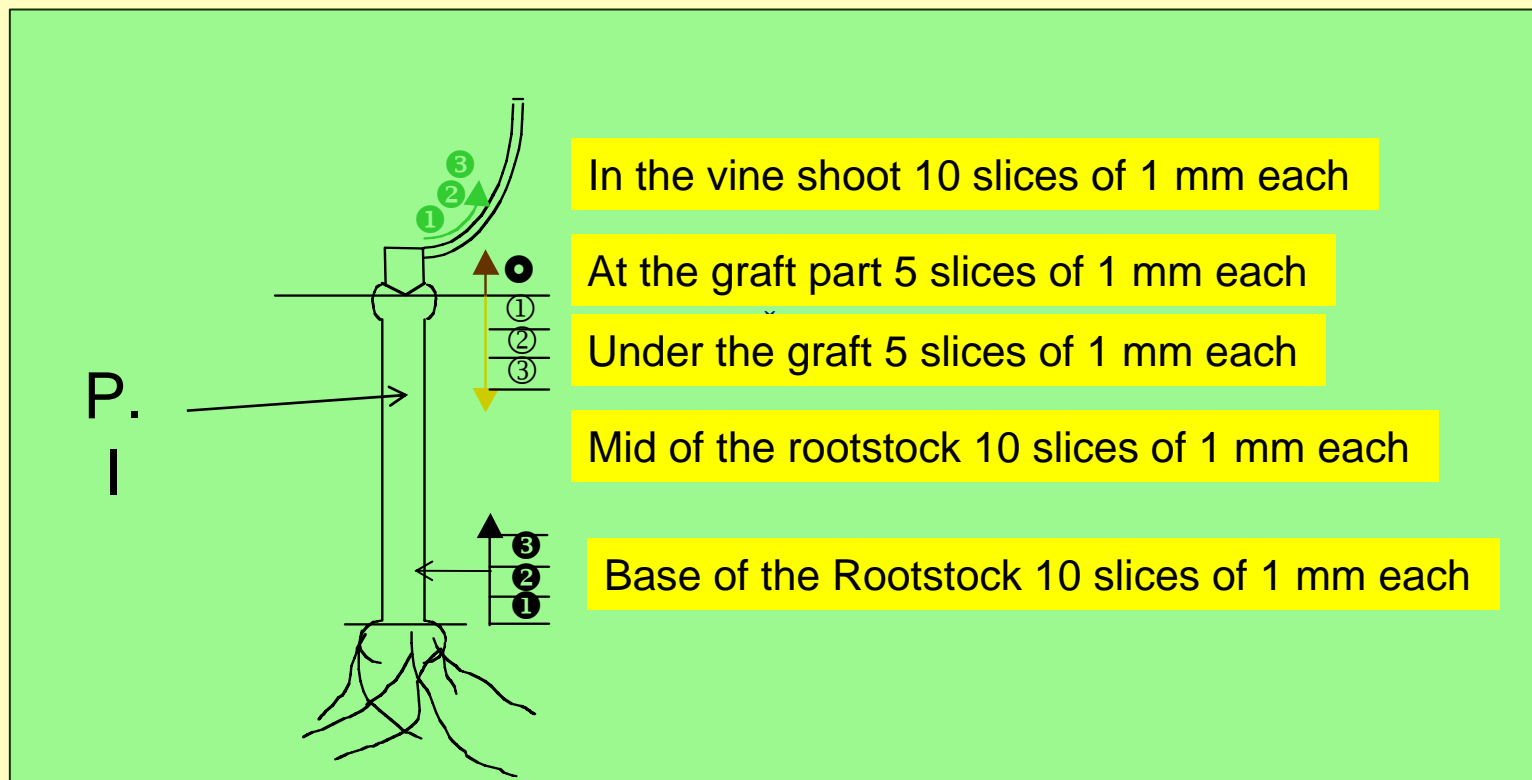
	+ PATHOGEN INOCULATION	+ PATHOGEN INOCULATION Esquive I-1237	Esquive I-1237
Phaeoacremonium aleophilum	YES	YES	YES
Phaemoniella chlamydospora	YES	YES	YES
Botryosphaeria parva	YES	YES	YES
Botryosphaeria obtusa	YES	YES	YES
CONTROL	NO		YES
CONTROL	NO		NO



Inoculation of the pathogen with a plug of PDA culture in a hole under the graft point

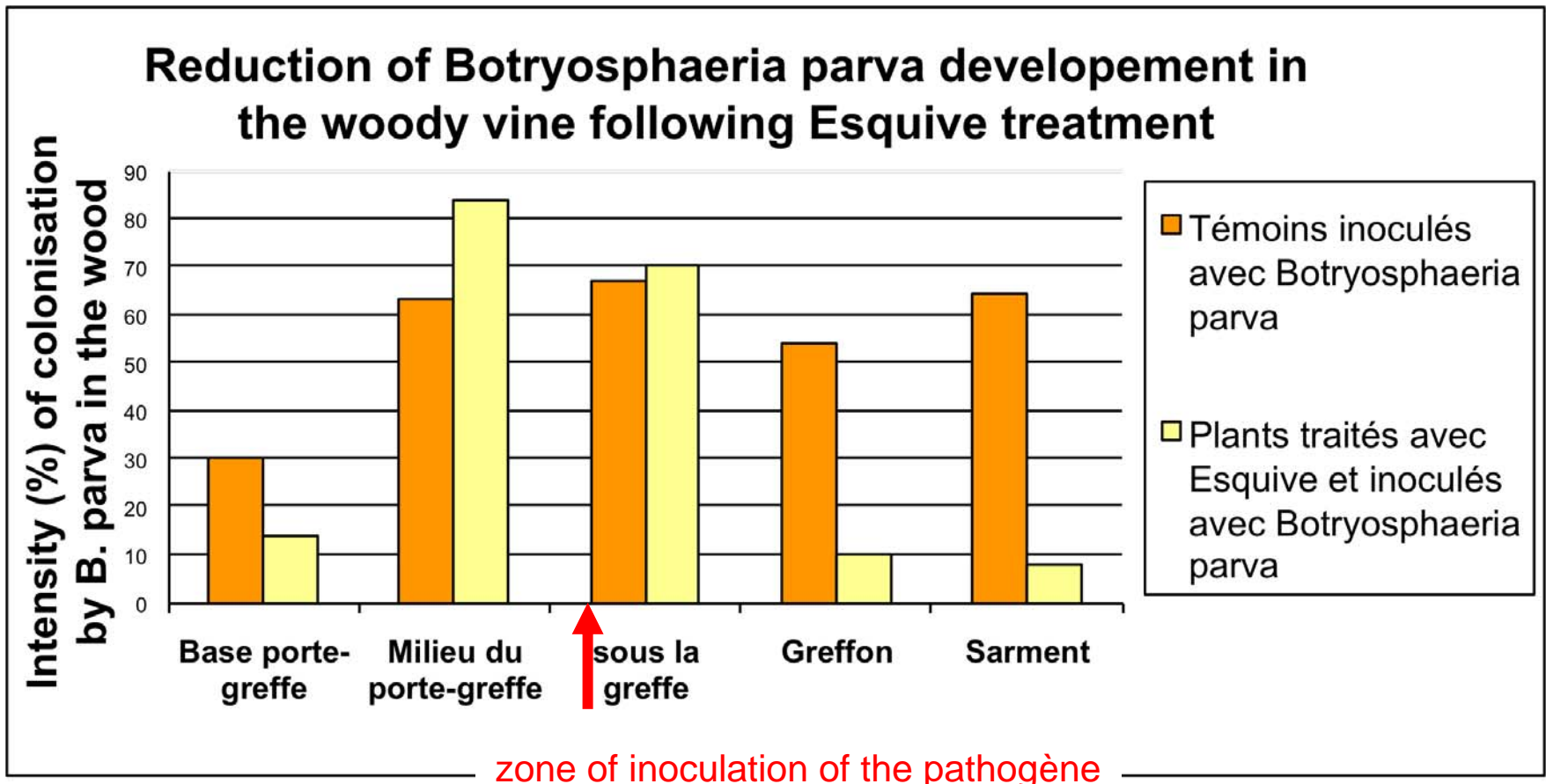


Protocole of the wood analysis

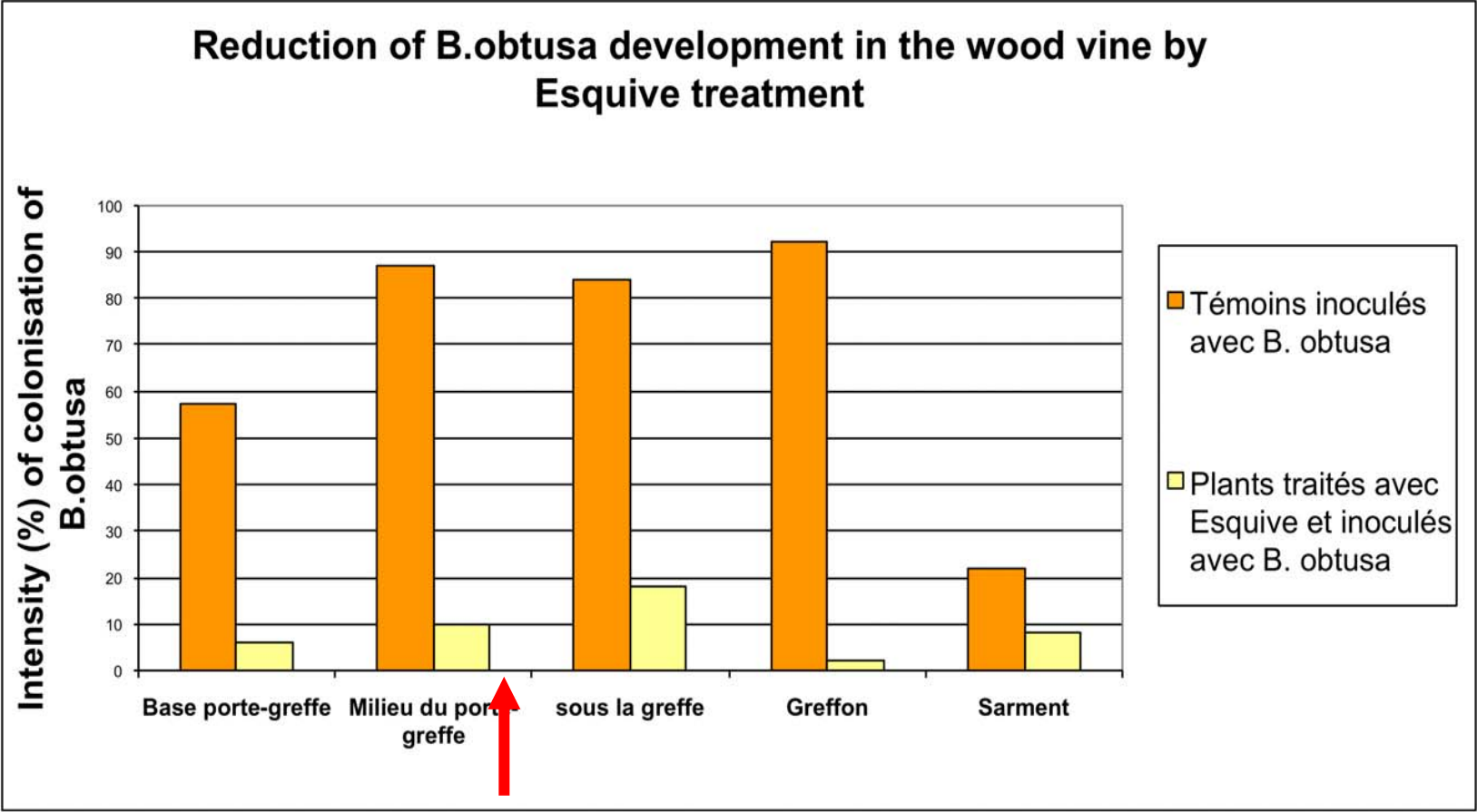


Result are expressed as intensity (%) of the pathogen development in each area of the vine

2- Capacity of *ESQUIVE* to reduce *B. parva* development in the wood of young vine plants.

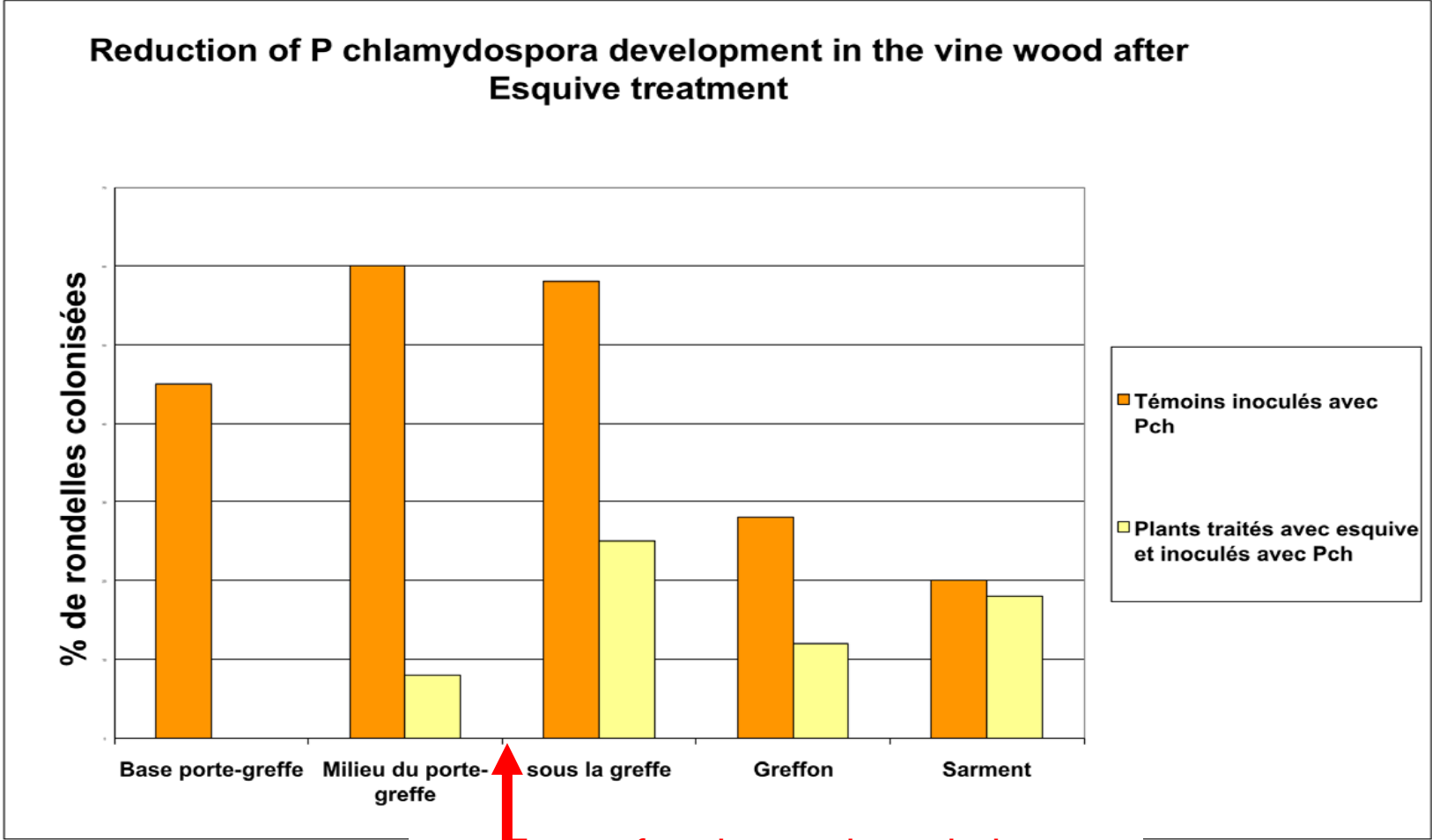


2- Capacity of *ESQUIVE* to reduce *B. obtusa* development in the wood of young vine plants.



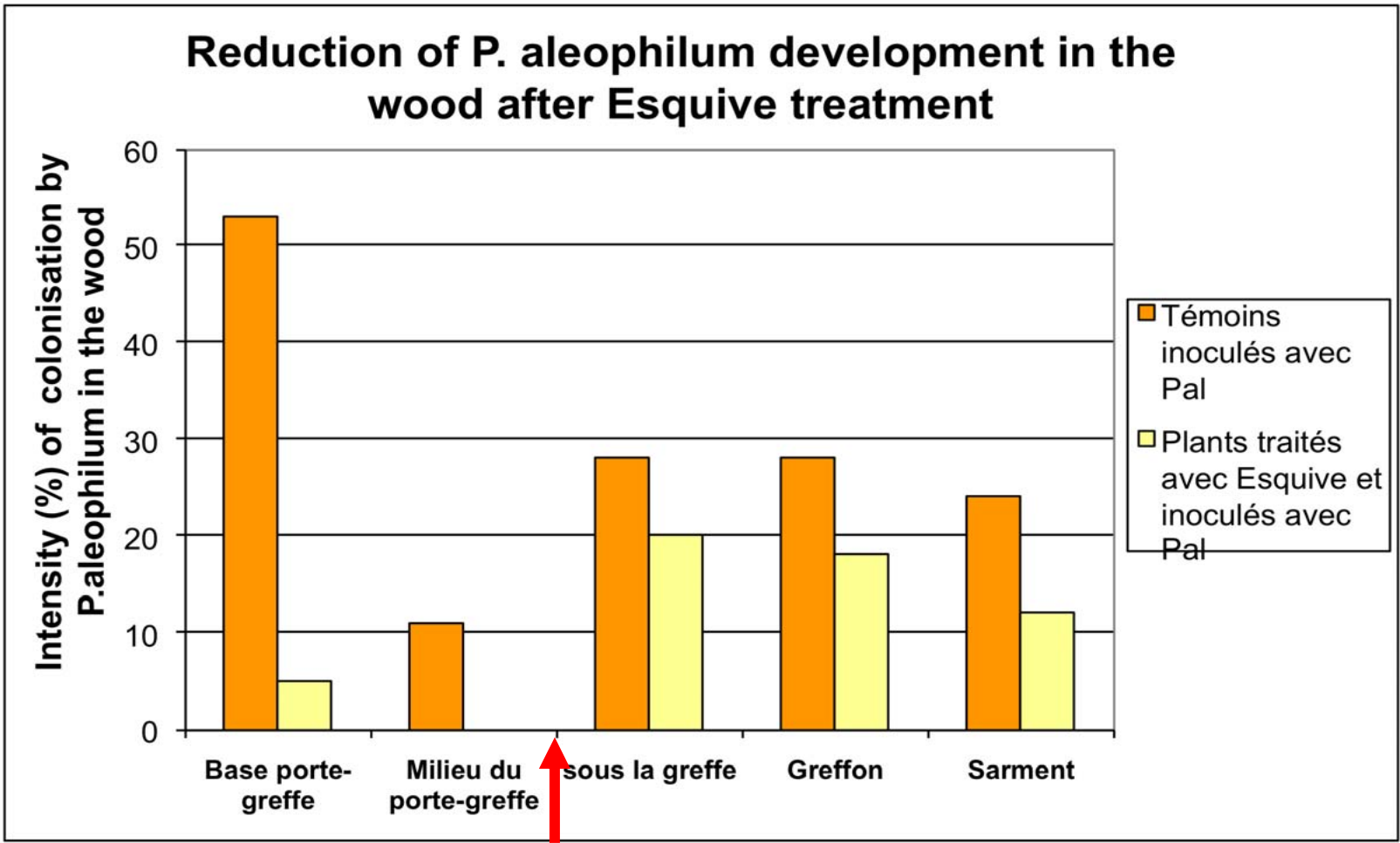
Zone of inoculation of the pathogen

2- Capacity of *ESQUIVE* to reduce *Phaeomoniella chlamydospora* development in the wood of young vine plants.



Zone of pathogen inoculation

2- Capacity of *ESQUIVE* to reduce *Phaeomoniella chlamydospora* development in the wood of young vine plants.



Zone of pathogen inoculation



3- Capacity of I-1237 to reduce *Eutypa lata* development in the vine wood by *ESQUIVE* application under field conditions.



- 3 years Field trials were conducted by INRA - Bordeaux (France) according the official method (CEB 155). 4
- The pathogen (spores) is inoculated one day after Esquive treatment and wood analysis are done 1 year later in the wound.
- Treatment of the prunes wound with *ESQUIVE* has shown its capacity to reduce *Eutypa lata* development in the wood

	On pruning wounds
Efficacy (%)	60% (Mean of 3 field trials during 3 years)

Conclusion

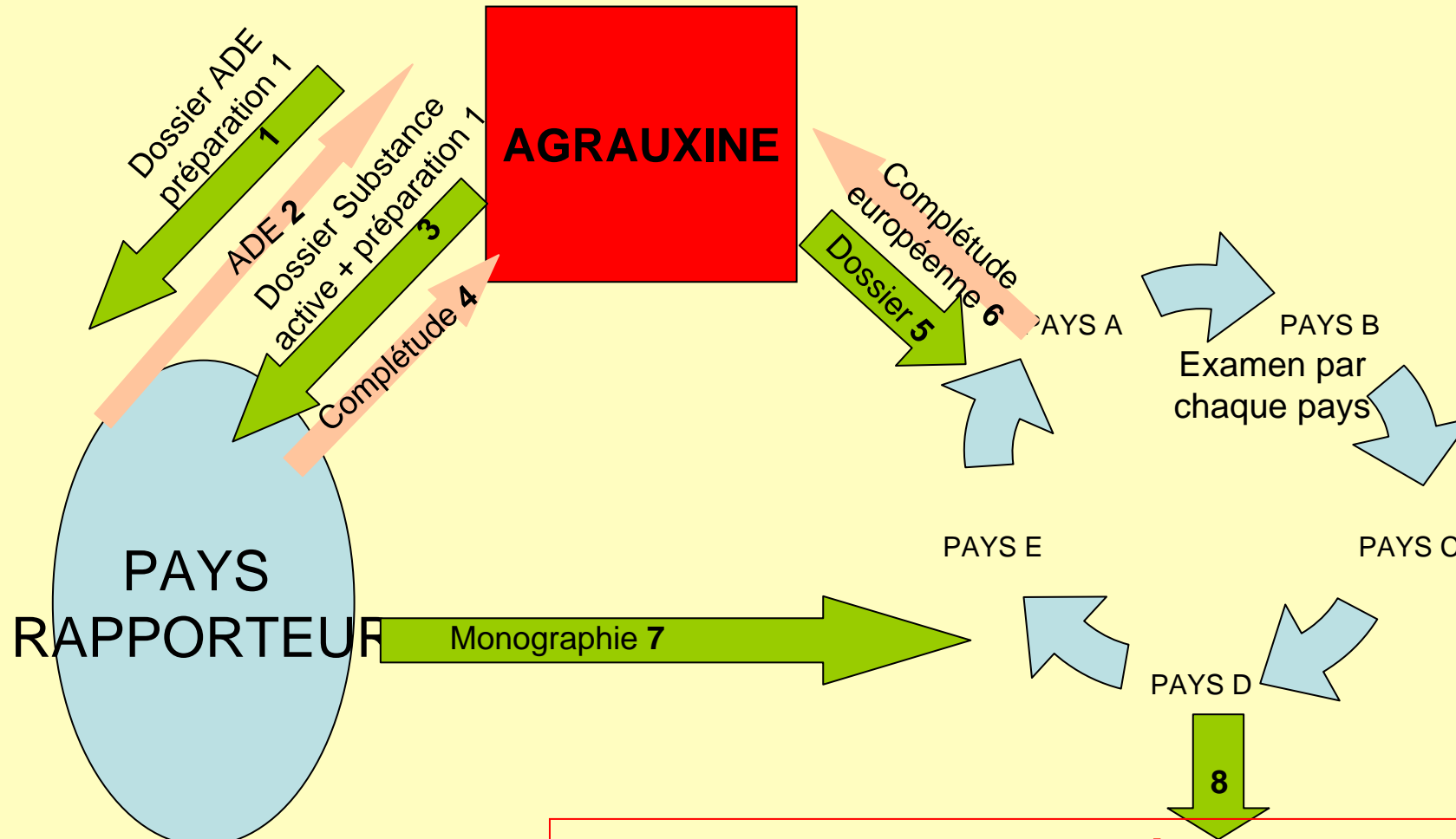
Trichoderma atroviride strain I-1237 :

- is able to colonize and persist within the vessels of vine wood and capable to develop as a natural barrier .
- has interesting antagonistic capacities against some of the Esca pathogens and *Eutypa lata*.
- Its utilization every year after pruning seems to be one of the good way to protect vine against ESCA and *Eutypa* diseases development.

Formulation as a WP

- *ESQUIVE WP* is a soluble powder containing alive spores of I-1237 with a high potential of germination and a proved capacity
- *ESQUIVE WP* = 1×10^{12} spores / kg
- *Storage* : 1 year at low temperature 4-10 °C
- *Application* : 20 gr/liter

Registration of the Active substance in the annexe 1



**Inscription de la matière active
à l'annexe 1 de la directive 91/414**

About registration !!!

- The European dossier has been submitted to AFSSA (France) to be supported for the registration of *Trichoderma atroviride* – strain I-1237 in the annex I of the EU Directive 91/414/CE.
- The completeness list ok for France and EU.
- We are waiting for response in October 2008.

Charte BIORIZE



To guaranty to users a high bio- product quality :

- Regulatory
- safe
- Proved Agronomical Efficiency
- Alive components or active ingredients