

FEBRUARY 2014 – Booklet #3

### OBJECTIVES

1. Assess the potential of selected combinations of defence- and priming-inducing agents to enhance the effectiveness of natural defence in tomato and wheat (Task 1)
2. Develop biocontrol strategies for use against plant pathogens (Task 2)
3. Develop biocontrol strategies for use against insect pests (Task 3)
4. Identify natural products that could function as biopesticides (Task 4)

### APPROACH

This activity identifies factors that can be used to control pest and disease infestations in order to develop innovative crop protection methods. Research is being conducted at a range of levels from molecular to whole organism to community or field level. Natural products with a direct effect against pests/microbes or their pathogenic behaviour as well as those providing indirect defence by attracting natural enemies of pests or priming plant defence are being investigated. Biocontrol agents are being evaluated to determine which show the most potential for use in crop protection. The knowledge of plant defence and interactions between pests and natural enemies gained in this activity will inform pest management practise and the development of bio-products or green products will supply the toolbox of Cropping systems activities.

### FIRST RESULTS

#### **1. Development of plant activator x genotype combinations to enhance tomato resistance against pests and diseases.**

The objective of task 1 is to optimise the benefits of different defence activators in tomato, and to extrapolate results from fundamental research with the model plant species *Arabidopsis* to tomato. So far, task 1. has revealed that the effects of chemical defence activators(s) on resistance and plant growth repression depend on the tomato cultivar used. This outcome creates opportunities for tomato breeding programmes to select for genetic traits that increase the resistance response to specific combinations of chemical defence activators, whilst minimizing the costs in terms of plant growth reduction. The most effective plant activators emerging from these trials are  $\beta$ -aminobutyric acid (BABA; *Figure 1*) and fructose. Fortunately, neither of these needs custom synthesis. BABA is available via Sigma and fructose is also readily obtainable and inexpensive. Protocols for their use are available from Jurriaan Ton (Sheffield University) and Nick Birch (JHI). Secondly, research on the model plant species *Arabidopsis* has revealed the identity of the receptor protein of BABA. This so-called IBI1 protein was

found to control disease resistance and plant growth suppression via separate signalling pathways. Hence, the costs of BABA-induced resistance can be separated genetically from the corresponding costs on plant growth. Subsequent experiments with tomato have revealed that tomato perceives BABA in a mechanistically similar manner as Arabidopsis. Currently, reverse genetics techniques are used to confirm the identity of the tomato *IBI1* gene homologue of in tomato. The discovery of the plant receptor of BABA allows for more targeted breeding programmes that specifically aim to increase crop responsiveness of BABA-induced resistance, whilst minimising the concurrent stress response to this chemical.

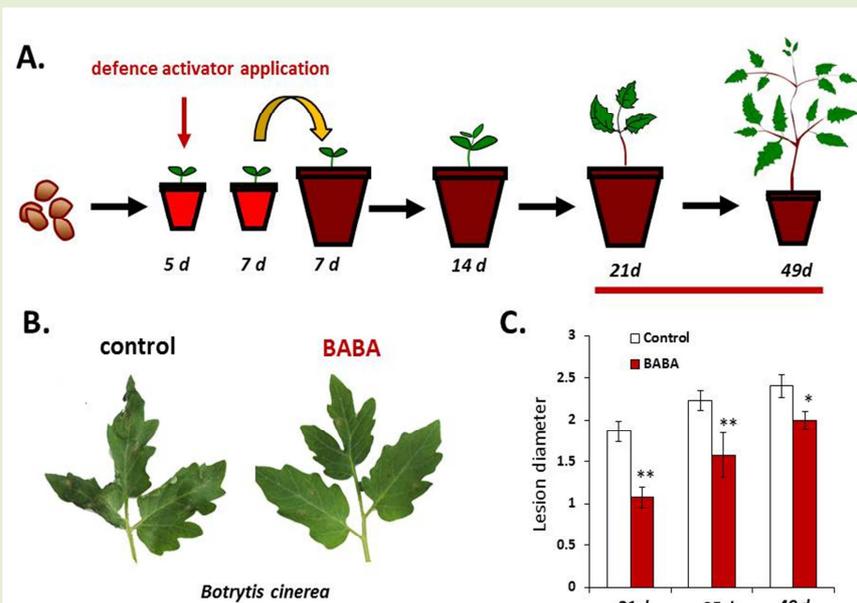


Figure 1. Long-lasting induced resistance in tomato against grey mould (*Botrytis cinerea*) upon seedling treatment with beta-amino butyric acid (BABA) **A:** Experimental procedure of seedling treatment **B:** representative differences in disease at 21 d after induction treatment and 5 d after inoculation with *B. cinerea*. **C:** Lesion size (cm) at 21d, 35d and 49d after induction treatment and 4 d after inoculation with *B. cinerea*.

## 2. Formulations to improve the longevity of fungal biocontrol agents and more efficacious strains have been developed.

A natural occurring hyperparasite, *Ampelomyces quisqualis* (Fig.2), is considered one of the best alternatives to chemicals against *Erysiphales*, the casual agents of powdery mildews that are among the most dangerous plant diseases worldwide.

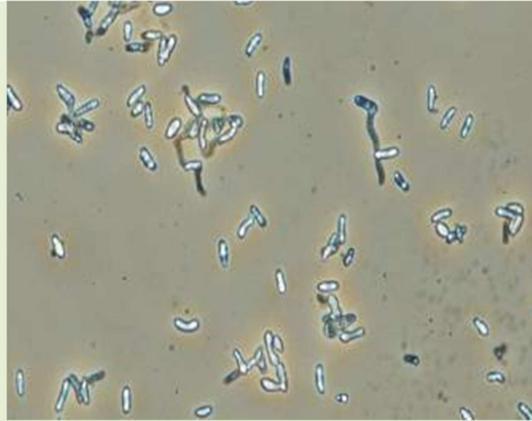


Figure 2: *Ampelomyces quisqualis*, fungal biocontrol agent viewed under the microscope

We evaluated the effect of five different substances (Powdery mildew extract, Shrimp Shells powder, Chitosan, Yeast cells and Mushroom powder) on the germination rates of three selected *A. quisqualis* strains (AQ10, ITA 3, ATCC 200245) (Fig.3). Conidial germination and tube elongation of strains were stimulated most strongly by shrimp shells. AQ10 exhibited significantly lower germination rates and less tube elongation in the presence of several substances stimulating conidia, as compared to the strain ITA3. *In vivo* biocontrol assays after stimulation of conidial spores of *A. quisqualis* were conducted with cucumber (*Podosphaera xanthii*), strawberry (*Podosphaera aphanis*) and grapevine (*Erysiphe necator*) powdery mildews. Best results were obtained with ITA 3 stimulated by chitoplant, shrimp shell and mushroom which reduced the infected leaf area on strawberry, grapevine and cucumber (62 to 91% less).

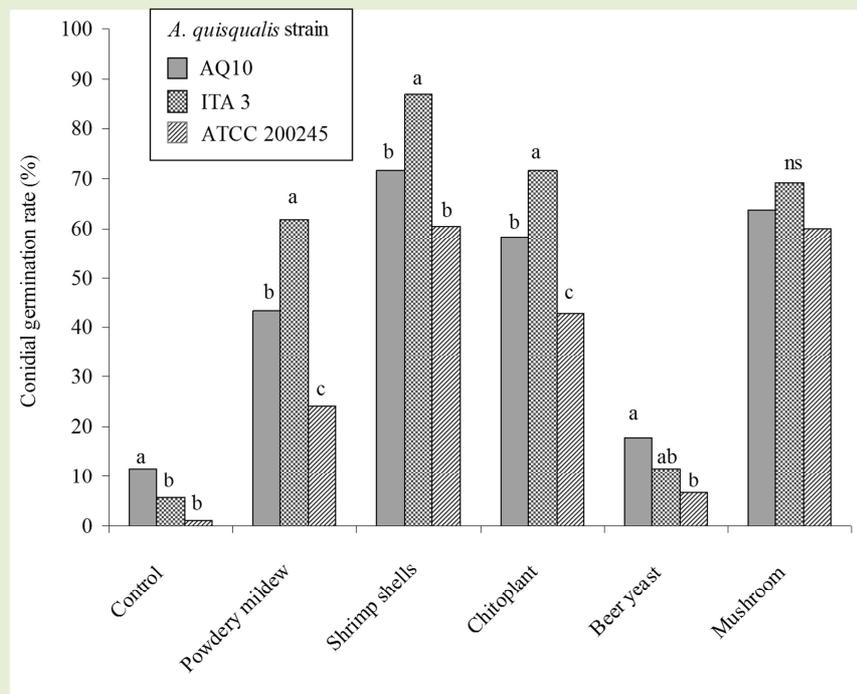


Figure 3: Effects of five different substances on the conidial germination (%) of three selected *A. quisqualis* strains

Several species of the fungal genus *Trichoderma* are good antagonists of soil borne diseases; they are competitors for space, nutrients and produce lytic enzymes. Trichodermas are common inhabitants of the soil; however their natural concentration is often too low to provide a sufficient antagonism against soilborne pathogens. We have developed an improved strain, *Trichoderma atroviride* SC1 and conifer bark formulations that improve its longevity.

### **3. Identification of biocontrol agents for the invasive insect pest *Tuta absoluta* (tomato leafminer).**



Figure 4. Egg parasitoid (*Trichogramma achaeae*) and the predator (*Necremnus artynes*) are being tested in glasshouse trials

We have been charactering and optimising biocontrol solutions for tomato leafminer (Fig. 4). Quality control is essential to achieve effective biocontrol because different species of insect parasitoids and predators look similar but have completely different performance in controlling the pest. For example, *Trichogramma brassicae* sold as *T. achaeae* by one company led to failures of biocontrol programmes. We have established morphological and molecular protocols for species characterisation. For *Trichogramma* species tested there was agreement of molecular and morphological data indicating that the strain sold by other companies such as Invivo Group (France) was a true *Trichogramma achaeae*.

In collaboration with “Protected crops” and “Emerging technologies” activities, a field test was conducted to assess the biological performances of *Necremnus artynes* against *Tuta absoluta* in a greenhouse in the South of Italy (Fig.4). Predation rate was 20-30% which was considered too low and future trials will focus on the egg parasitoid, *Trichogramma achaeae*.

### **4. Identification of natural products that could function as biopesticides**

Natural products with useful biological activity such as insect antifeedant activity or inhibition of fungal mycotoxin biosynthesis are being sought. 3-5 dicaffeoylquinic acid (DiCQ) has a significant activity on aphids by ingestion but not enough in field trials up to now. Similarly, Caffeic and Chlorogenic acids are the best plant derived

	<p>inhibitors of <i>Fusarium</i> mycotoxin <i>in vitro</i>, but their effect on treated plants is limited. For both series of compounds, improvement of their field effect will be tested through formulation using BayerCropScience expertise. <i>Ajuga chamaepitys</i> extract was tested at Rothamsted and found to have antifeedant activity against diamondbackmoth <i>Plutella xylostella</i> larvae.</p>
<p><b>INTERACTION WITH CROPPING SYSTEMS ACTIVITIES</b></p>	<p>Fungal biocontrol agents have been tested in the grapevine activity. Production of the <i>Trichoderma atroviride</i> SC1 strain was scaled up and is available for the field trials. It will be offered to the grapevine Cropping systems activities for trials in 2014.</p>
<p><b>NEXT STEPS</b></p>	<p>For the plant defence activator work, we will optimise application methods, evaluate effects on belowground interactions with plant beneficial microbes and develop tools for breeders.</p> <p>BCAs for plant diseases: Production of the ITA 3 <i>Ampelomyces quisqualis</i> strain and <i>Trichoderma atroviride</i> SC1 strains of biocontrol agents for use against plant diseases is being scaled up for a field application in summer 2014.</p> <p>BCAs for tomato leafminer control: Two different strains of <i>Trichogramma achaeae</i>, provided by IAS (strain A06 reared from eggs of <i>Ephestia kuehniella</i>, strain A02 reared from eggs of <i>Sitotroga cerealella</i>) at different of temperature and relative humidity regimes will be tested on <i>Tuta absoluta</i> eggs. These trials will allow us to understand how temperature before and after emergence affects the performances of this parasitoid in terms of parasitization rate coupled with longevity.</p> <p>Natural products: Tests of efficiency of curcumin to inhibit fungal growth and mycotoxin biosynthesis by <i>Fusarium</i>, phenolic acids metabolism by <i>F. graminearum</i> and field experimentations for the most active compounds.</p>
	<p><b>Acknowledgement:</b> The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/ 2007-2013) under the grant agreement n°265865-PURE</p>