Prospects for plant defence activators and biocontrol in IPM – concepts and lessons learnt so far

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ABSTRACT

There is an urgent need to develop new interventions to manage pests because evolution of pesticide resistance and changes in legislation are limiting conventional control options for farmers. We investigated β-aminobutyric acid (BABA), jasmonic acid (JA) and fructose as possible plant defence activators against grey mould disease, *Botrytis cinerea*, and root knot nematode, *Meloidogyne incognita.* We also tested *Trichogramma achaeae* parasitoid wasps and an antifeedant plant extract for biocontrol of the invasive tomato leafminer, *Tuta absoluta*. BABA and JA enhanced resistance of tomato plants to *B. cinerea* but neither treatment provided complete protection and the efficacy of treatment varied over time with BABA being more durable than JA. Efficacy was partly dependent on tomato cultivar, with some cultivars responding better to BABA treatment than others. Furthermore, treatment of tomato with BABA, JA and fructose led to partial suppression of *M. incognita* egg mass development. Biocontrol agent, *T. achaeae*, performance against *T. absoluta* could be enhanced by adjusting the rearing conditions. Both attack rate and longevity were improved by rearing the parasitoids on *T. achaeae* rather than on other insects. Finally, *Ajuga chamaepitys* extract was shown to have significant antifeedant activity against *T. absoluta*. Our findings suggest that there are potential new solutions for protection of crops but they are more complicated to deploy, more variable and require more biological knowledge than conventional pesticides. In isolation, they may not provide the same level of protection as pesticides but are likely to be more potent when deployed in combination in IPM strategies.

Keywords: induced resistance, plant defence activator, biocontrol, IPM, tomato

1. Introduction

Agricultural systems are vulnerable to attack and crop protection plays a key role in safeguarding crop productivity against losses caused by pests (Oerke and Dehne, 2004; Bruce, 2012). Here we use "pests" as a general term for attacking organisms, including weeds and diseases as well as animal pests, that reduce crop yield or quality. The availability of conventional pesticides for tackling crop pests is declining globally due to evolution of resistance and changes in legislation and there is an increasingly urgent need to find alternatives (Bruce, 2010; Bruce, 2012). Indeed, limiting the number of pesticides available increases the use of the ones which are permitted and intensifies selection pressure for resistance (Lamichhane et al. 2016). For sustainable crop protection it is better to have a range of options and not to rely too much on one tactic.

In the EU, the Sustainable Use Directive (2009/128/EC) requires member states to minimise pesticide use and risk while promoting the use of IPM and alternatives (Anon 2009; Hillocks, 2012). Such legislation is driven by concerns about potential effects of pesticides on human health and the environment. Lack of availability of pesticides has created a demand from farmers for alternative means to protect their crops and is a driver for innovation (Bruce, 2012; Stenberg et al., 2015), especially as pesticides are currently being restricted at a much faster rate than alternatives are being provided. A range of alternatives potentially exist such as resistant crops which can withstand pest or disease attack, biological control agents and changes to grower practice to reduce sources of infection or infestation. These need to be developed into practical tools which are usable in agriculture. Development of resistant crop cultivars was beyond the scope of our contribution to the PURE project and we focussed on three potential solutions for protecting tomato (*Solanum lycopersicum*) crops from attack: firstly, chemical priming of plant defence, secondly biocontrol by introduction of insect natural enemies and, finally, the use of insect antifeedants.

Plant defence activators (Walters et al., 2005) or priming agents (Conrath et al., 2006) are a new class of agrochemical that does not have a direct toxic effect on the target organism but instead

act by boosting plant defence. They have been proposed as potential tools for use within integrated pest management (IPM) strategies that aim to minimise the use of toxic products (Stout et al., 2002; Vallad and Goodman, 2004). A key advantage of plant activators, compared to broad spectrum toxicants, is that they are compatible with biocontrol agents and can even promote plant attractiveness to natural enemies of plant pests (Stout et al., 2002, Bruce 2010). Another advantage is that induced resistance via priming is based on an augmentation of basal defence resistance (Ahmad et al., 2010) and is controlled by a large number of defence related plant genes (also referred to as 'multigenic', 'quantitative', or 'horizontal' resistance). Consequently, induced resistance is a durable form of disease protection, since the augmentation of multigenic resistance is difficult to break by pathogens (Gardner et al., 1999; Ahmad et al., 2010). Moreover, unlike resistance that is controlled by single resistance (R) genes, induced resistance is non-specific and typically protects plants against a range of different pests. For plant defence activation studies, we focussed on grey mould (*Botrytis cinerea*) which, in addition to tomato, affects several hundred other host plants pre- and post-harvest. Losses due to this fungus are estimated at 10-100 billion euros per year (Weiberg et al., 2013). We also investigated elicitation of plant defence against the root knot nematode, *M. incognita,* which is also a globally important and polyphagous pest (Sasser, 1977).

Another promising alternative approach is the management of pathogens and insect pests with biocontrol agents. Research on biocontrol agents against plant diseases in the PURE project is described in the Mugnai et al. and Angeli et al. articles in this special issue. For insect pests, artificially introducing natural enemies of herbivorous insects provides a major opportunity for more sustainable management of crop pests and biocontrol strategies have been devised to protect the crops that rely on natural enemies to attack the pest species (Pilkington et al., 2000). These have been particularly successful in greenhouse environments, for example in the Almeria region of Spain where biocontrol has largely replaced conventional pesticides (Pilkington, 2010; Calvo et al., 2014). An increasing number of commercial greenhouse growers around the world employ beneficial

91 insects for crop protection and expenditure on biocontrol agents in greenhouses represents the
92 majority of sales of biological control agents globally. Greenhouses are ideal environments for
93 releasing biocontrol agents because they have contained conditions from which biocontrol agents
94 are less likely to escape. However, the invasive pest, tomato leafminer, *Tuta absoluta* (Lepidoptera:
95 Gelechiidae), threatens to undermine successful biocontrol programmes in greenhouses if toxic
96 pesticides have to be used to manage it and therefore we focussed on this species in exploring new
97 biocontrol options against it. Tomato leafminer can cause yield losses in tomato of 80-100%
98 (Desneux et al., 2010). In addition to investigating possible biocontrol agents for use against *T.*
99 *absoluta*, we explored the possibility of using antifeedants to reduce feeding damage by the pest.
100 We used an extract of *Ajuga chamaepitys* (Lamiaceae), the ground pine or yellow bugle, which
101 contains clerodane compounds (Camps et al, 1987) and has been shown previously to be active
102 against another lepidopteran pest, the diamondback moth, *Plutella xylostella* (Griffiths et al., 1991).

103 The current paper details our findings and discusses their implications for development of
104 new crop protection interventions. Some of the results are already published elsewhere and we
105 refer to these in the discussion section which is intentionally longer than usual to review potential
106 implications for research translation into new interventions for crop protection.

107

108 **2. Materials and methods**

109 *2.1 Chemical priming of plant defence*

110 Tomato cultivar 'MoneyMaker' was used for all experiments unless stated otherwise. Additional
111 tomato cultivars 'IL4', 'FCN93' and 'Motelle' were obtained from Wageningen University. BABA
112 (catalog number A4420-7) and JA (catalog number J2500) were obtained from Sigma-Aldrich. BABA
113 was prepared freshly in distilled water and diluted to appropriate concentrations. Stock solutions of
114 JA were prepared by dissolving 250 mg in 2 ml of ethanol, which was then diluted in distilled water

115 to a final stock concentration of 10 mM and kept at −20°C. Before usage, the 10 mM stock solution
116 was thawed and diluted in water to the indicated concentrations.

117 *Durability of BABA and JA*

118 Experiments were conducted as described in Luna et al., (2016) with some modifications. Briefly,
119 tomato cultivar 'MoneyMaker' plants were grown under greenhouse conditions at Rothamsted
120 Research with supplementary lighting to a total regime of 16h light, 150 µM m2 s^{-1} at 25^{o}C, and 8h
121 dark at 21^{o}C. Rothamsted standard substrate was use for cultivation of the tomatoes used in this
122 experiment. One-week old seedlings were treated with 0.5mM BABA or 0.05mM JA according to the
123 protocols used in Luna et al. (2016). One week after treatment, roots were washed to remove BABA
124 and JA and plants were placed in individual 2.2 L pots and grown until infection with *B. cinerea*.
125 Infection and disease assessment were performed as described before (Luna et al., 2016). Disease
126 levels were measured at 5 time points, starting at 2 weeks and finishing at 6 weeks after treatment.
127 At every time point, 10 plants per treatment were scored for *B. cinerea* lesion diameter size 3 days
128 after infection. Thus each experiment was replicated 10 times. The average lesion diameter per
129 plant was obtained from measurements of 12 independent lesions (6 per leaf; 2 young leaves per
130 plant). For statistical analysis of lesion diameters, normal distributions were confirmed by Shapiro-
131 Wilk tests, whereas equality of variances was determined by Levene's tests. If equality of variances
132 could be confirmed, differences between means were analyzed using independent-sample *t*-tests. If
133 the Levene's test revealed unequal variances between treatments, a Welch's t test was performed.

134 *Effect of cultivar*

135 Tomato cultivars 'MoneyMaker', 'Motelle' and 'FCN93' plants (*n* = 10 for each cultivar) were grown
136 in Levington M3 substrate for 4 weeks in a controlled environment chamber in Sheffield (UK) with a
137 light regime of 16h light; 150 µM m2 s^{-1} at 26^{o}C and 8h dark at 21^{o}C and ~ 65% relative humidity.
138 Four–week old plants were treated with 0.5mM BABA and after 5 days they were inoculated with *B.*

cinerea. Lesion diameter was recorded 4 days post inoculation. This experiment was performed according to the protocols used in Luna et al. (2016).

Effect on Root Knot Nematode

Tomato cultivar 'MoneyMaker', 'IL4', 'FCN93' and 'Motelle' seedlings were grown in a 2:1 sand:loam mix, in individual 10 cm diameter pots. They were treated with elicitors when they were 5 weeks old. There were 10 replicate plants of each cultivar for 6 treatments. The 100ml aqueous soil drench treatments consisted of 1) 0.5 mM BABA, 2) 10ppm fructose, 3) 0.05 mM JA, 4) 0.5 mM MJ, 5) 10ppm fructose + 0.05 mM JA, and 6) distilled water. Plants were inoculated with *M. javanica* (500 eggs or newly hatched second stage juveniles (J2s) per plant) 24h after treatment and egg masses were assessed after 6 weeks post inoculation using phloxine B (aqueous solution containing 15mg L^{-1}) to stain the egg masses (Daykin and Hussey, 1985). These were then counted under a stereoscopic microscope.

Egg masses were analysed using Analysis of Variance fitting Plant Genotype and Treatment. Although the residuals were approximately normal, there were several observations with large residuals. These were mainly caused by the presence of plants where no egg masses were present (mainly genotype FCN). The observations where no eggs were detected were dropped from further analyses. Unbalanced analysis of variance was used to test for differences between the Treatment and Plant Genotype means for egg masses. Pairwise multiple comparison tests for means were performed using Bonferroni's method in Genstat v17.1 with a comparison-wise error rate of 0.0003.

2.2 Optimising biocontrol

Two strains of *Trichogramma achaeae*, a biocontrol agent of the tomato leafminer *T. absoluta,* were obtained from IAS (InVivo AgroSolutions): A02, reared on eggs of *Ephestia kühniella* and A06 reared on eggs of *Sitotroga cerealella*. These were reared on their respective hosts and also on eggs of the natural host *T. absoluta*. The temperature was 25^{o}C and insects were fed with honey solution. Shape

and structure of male genitalia confirmed that specimens received for bioassays belonged to *T. achaeae* as did a PCR diagnostic (a fragment of ITS2 between 700 and 560 bps, not digested by EcoRI enzymes and sharing an identity of 100 % with sequence in NCBI JF415949). The performance of the different strains, in terms of longevity and attack rate against *T. absoluta*, was compared when reared on different hosts. Adult longevity was assessed for 45 females of each strain, composed of three different sets of 15 adults each to avoid any environmental bias. Adult parasitoids were isolated in aerated tubes (5.0 X 0.3 cm) and checked every 12 h. Longevity was recorded at 25° C, adults were fed with honey (water:honey solution 50:50, v:v) and keeping RH at 70 ± 5 % and 16:8 L:D photoperiod. Attack rate was assessed by releasing a single parasitoid female in a glass Petri dish (5 X 1 cm) containing 20 eggs of *T. absoluta* and a few honey drops at 25° C, 60 ± 5 % and 16:8 L:D photoperiod. After 48 h the parasitoid was removed and Petri dishes were kept at the same climatic conditions for two weeks. Attack rate was evaluated by counting the host eggs that had turned black excluding those were unparasitized and probed or host-fed. Overall, 15 females were tested for each strain. All normally distributed data in all experiments were analyzed using one-way ANOVA followed by Tukey post-hoc test to assign significant pairwise differences. Data that did not match a normal distribution were analyzed by Kruskal–Wallis ANOVA on ranks followed by Dunn's Method post-hoc tests.

2.3 Antifeedant Bioassay

An extract of *Ajuga chamaepitys* was obtained from Botanix Ltd, Hop Pocket Lane, Tonbridge TN12 6DQ (CO_2 extraction of field grown plants, batch no. SR 1870). A weighed amount of the extract was made up to 0.1% in absolute ethanol and absolute ethanol alone was used for the control treatment. The choice test method was based on that of Griffiths et. al., (1991). Individual leaflets were cut from approximately 3-4 week old tomato plants, cv 'Moneymaker', and the petioles placed in water in a tray to keep the leaflet fresh. The absolute ethanol control treatment was painted onto the upper and lower surfaces of the left-hand side of 10 replicate leaflets using a no. 5 sable paint brush.

188 The antifeedant extract (0.1% in absolute ethanol) was painted onto the upper and lower surfaces of
189 the right hand side of the leaflets using the midrib as the dividing line. The leaves were returned to
190 the water tray while the ethanol dried. Once dry, the leaflets were transferred to individual 15cm
191 diameter Petri dishes with two 12.5cm Whatman no. 1 filter papers and 3ml deionised water.
192 Batches of 5 small (2nd to 3rd instar) *T. absoluta* larvae were collected from culture and placed in the
193 centre of each of the treated leaflets. The larvae were left in the controlled environment room
194 (26°C, 16h light) for 25h after which the number of mines in the treated and control sides of the
195 leaflets were recorded. The data were analysed by Student's *t*-test (Genstat).

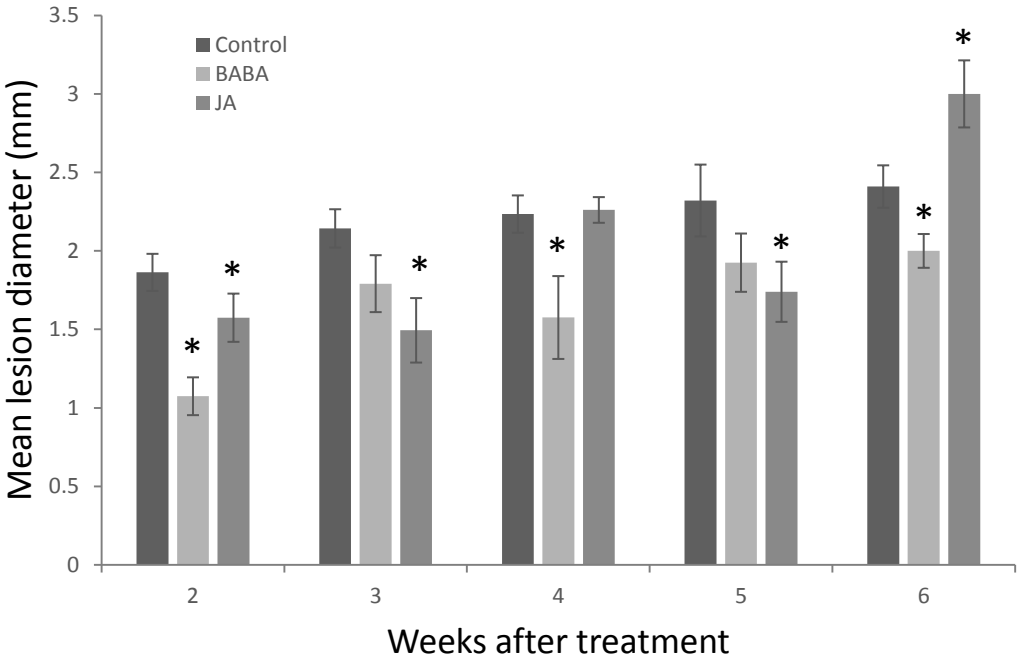
196

197 **3. Results**

198 *3.1 Chemical priming of plant defence*

199 *Durability of BABA and JA*

200 Disease levels of *B. cinerea* were significantly reduced by BABA treatment when mean lesion
201 diameter was measured 2, 4, 5 and 6 weeks after treatment in 'Moneymaker' tomato. JA treatment
202 was less reliable because it reduced the disease initially at 2 and 3 weeks after treatment, then lost
203 effectiveness at 4 weeks after treatment, but reduced disease at 5 weeks after treatment.
204 Surprisingly, JA increased susceptibility at 6 weeks after treatment. Because of the variability of the
205 result with JA we repeated the experiment and found no significant reduction in *B. cinerea* infection
206 levels when measured 4 and 5 weeks after treatment (Fig. S1). Although the protection by BABA was
207 more consistent and lasted up to 6 weeks after treatment, it only reduced lesion diameter by 17% at
208 6 weeks, indicating a partial effect.



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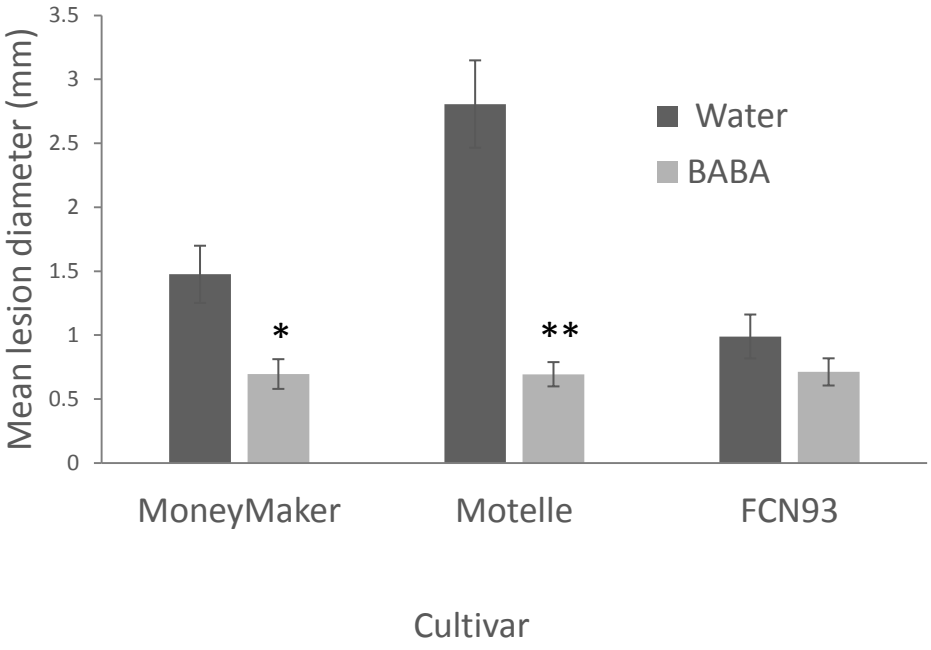
210 **Fig. 1** Mean *Botrytis cinerea* lesion diameter in tomato cultivar 'Moneymaker' 3 days after
211 inoculation at different times after treatment. Error bars denote SE. Asterisks indicate statistically
212 significant differences between control and treatment (*t*-test; $P < 0.05$; n=10)

213 *Effect of cultivar*

214 *B. cinerea* disease levels were reduced substantially more by BABA treatment in tomato cultivar
215 'Motelle' than in 'MoneyMaker', whereas there was no significant reduction in disease in 'FCN93'
216 (Fig. 2).



217

218 **Fig. 2** Mean *Botrytis cinerea* lesion diameter. Infection was performed 5 days after BABA treatment
219 and lesion diameter was measured 4 days after infection. Error bars denote SE. A single asterisk
220 indicates a significant difference, $P < 0.05$, and a double asterisk indicates a significant difference, $P <$
221 0.01, between water and BABA treatment.

222

Root Knot Nematode

Significant reductions in *M. incognita* egg mass development were obtained with BABA, fructose, JA and MJ but the effect depended on the cultivar used (Fig. 3). Egg masses were analysed using Analysis of Variance fitting Plant Genotype and Treatment. Although the residuals were approximately normal, there were several observations with large residuals. These were mainly caused by the presence of plants where no egg masses were present (mainly genotype FCN). The observations where no eggs were detected were dropped from further analyses. Unbalanced analysis of variance was used to test for differences between the Treatment and Plant Genotype means for egg masses. Both Treatment, ($p<0.01$) and Plant Genotype ($p<0.01$) were significant. There was no evidence of a Treatment by Genotype interaction.

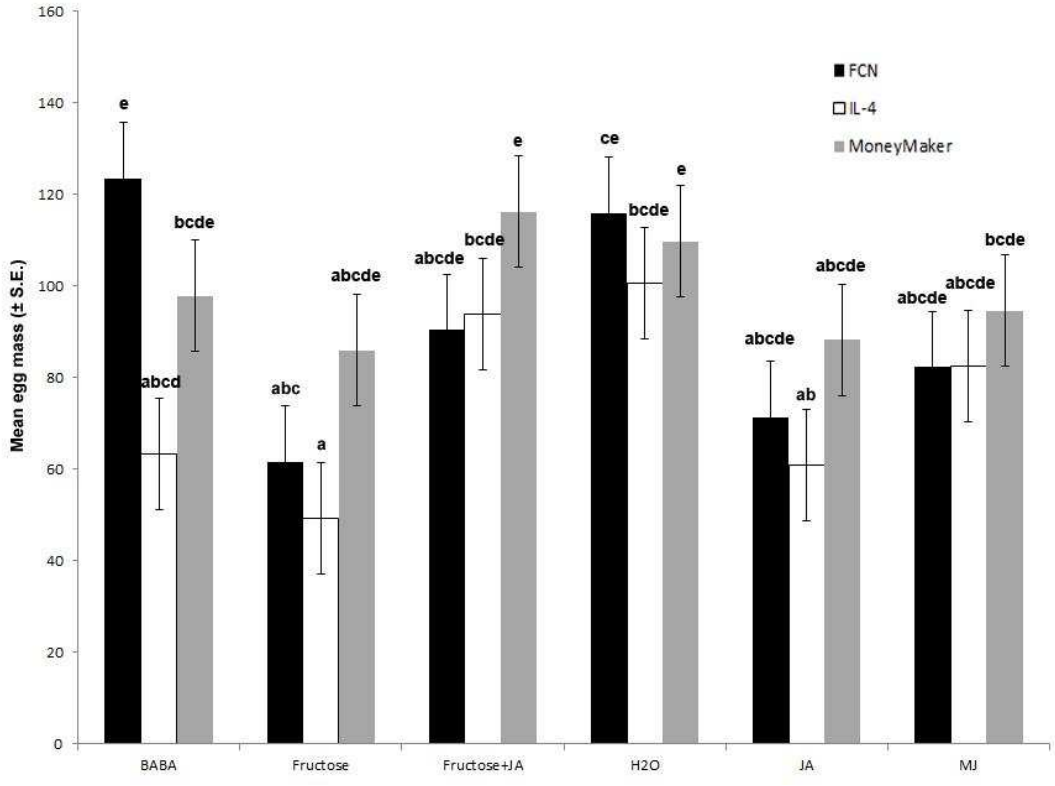


Fig. 3 Root knot nematode egg mass suppression by elicitors in three tomato genotypes. Letters above the bar denote a significant difference (Unbalanced ANOVA, Bonferroni; Experiment-wise error rate = $P < 0.05$, Comparison-wise error rate = 0.0003).

3.2 Optimising biocontrol

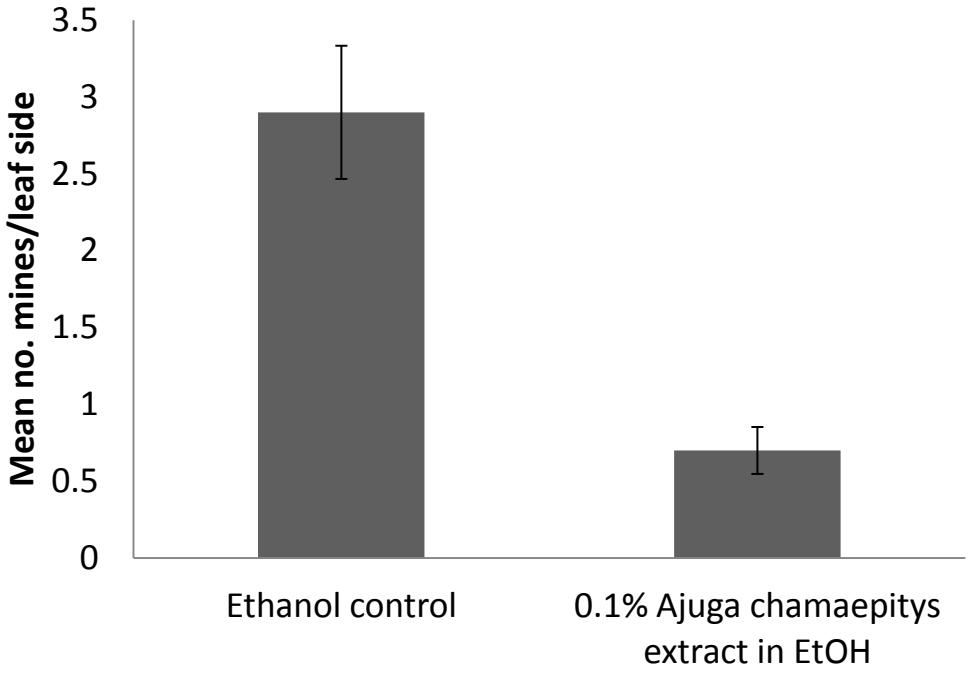
It was found that *T. achaeae* performance, as measured by attack rate and longevity, were significantly improved by rearing the parasitoids on *T. absoluta* on tomato plants rather than on other insects (ANOVA One Way, $P < 0.05$; Table 1). This was observed with both strains tested (A02 and A06).

Trichogramma acheae strain 	A02	A02	A06	A06
Host reared on	*Ephestia kühniella* 	*Tuta absoluta* 	*Sitotroga cerealella* 	*Tuta absoluta* 
Attack rate (number of black eggs / 20)	2.9 (± 0.7) c	13.4 (±1.4) a	6.5 (± 1.1) bc	8.0 (± 1.1) b
Longevity (days)	3.0 (± 0.1) b	5.9 (± 0.3) a	3.4 (± 0.1) b	4.9 (± 0.3) a

Table 1. Effect of rearing system on the performance and adult longevity of *Trichogramma achaeae*, a parasitoid of *Tuta absoluta.* Different letters indicate significant differences, assigned by One Way ANOVA followed by post-hoc tests ($P < 0.05$; Tukey test for adult longevity data; Dunn test for attack rate).

248 *3.3 Antifeedants*

249 A whole plant extract of *Ajuga chamaepitys* was tested for biological activity against larvae of *T.*
250 *absoluta*. In the choice test, the leaf side treated with *A. chamaepitys* extract had significantly fewer
251 leaf mines compared to the side treated with ethanol alone (P = 0.0011; Fig. 5).



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253 **Fig. 5** *Tuta absoluta* larval feeding in a choice test experiment

254

4. Discussion

Our experiments focused on tomato as a crop and revealed that plant defence activator treatments can partially suppress both a disease, *B. cinerea*, and attack by a herbivore, the root knot nematode, *M. incognita*. We also showed that biocontrol agents and an antifeedant plant extract can suppress tomato leafminer, *T. absoluta*. Our findings reported here and related studies from our research in the PURE project (e.g. Cascone et al., 2015; Luna et al., 2016) indicate that there are possibilities for developing novel approaches to crop protection. However, levels of control were not as high as would be expected with a pesticide, which may mean that such tactics have to be incorporated into a wider IPM strategy where they are used in combination with other approaches, such as basal host plant resistance or cultural control methods to reduce exposure to pests. Our experiments were relatively small scale and scaling up studies are required for translation of the research into field applications (e.g. Ruocco et al. in this special issue).

Obtaining crop protection with plant defence activators is more complicated than directly killing target organisms with a pesticide. The effect is plant-mediated and, therefore, dependent on plant genetics and physiology and can be altered by the environmental context (Bruce, 2014). Many chemical activators of induced defences against biotic attackers are known, and some of these, such as acibenzolar-S-methyl, an activator of SAR, have been commercialised for crop protection (Vallad and Goodman, 2004). However, sustained activation of defence throughout the plant is costly in terms of metabolic resources and energy requirements meaning that long-term activation of induced defences can result in yield penalties (Vallad and Goodman, 2004; van Hulten et al., 2006). A lower dose of activator only 'primes' defences which means they are potentiated for a faster and stronger response when the stress actually occurs and that this has a lower metabolic cost than immediately switching on defence pathways (van Hulten et al., 2006; Luna, 2016). The process of priming occurs when prior exposure to a biotic or an abiotic stimulus sensitises a plant to express a more efficient defence response to future stress exposure (Conrath et al., 2006; Bruce et al. 2007).

While JA and BABA can reduce tomato growth at higher concentrations, we have identified commercially feasible application methods of BABA and JA that induce durable disease resistance without negative impacts on plant growth or colonization by beneficial mycorrhizal fungi (Luna et al., 2016).

BABA was found to enhance resistance to *B. cinerea* in the tomato cultivar 'MoneyMaker' (Luna et al., 2016). Here we present new data on the longevity of the treatment, which can last for up to six weeks, and show that effect of treatments was partly dependent on which cultivar of tomato being grown with some cultivars responding better to the treatment than others. The tomato cultivar 'Motelle' responded better than 'MoneyMaker', whereas 'FCN93' performed worse. Similarly, there was an effect of tomato cultivar on the performance of plant defence activators against root knot nematode development. This outcome creates opportunities for tomato breeding programmes to select for genetic traits that increase the level of resistance response to specific combinations of chemical defence activators, whilst minimizing the costs in terms of plant growth reduction. Variability in plant defence activator performance between cultivars does mean that they are harder to use commercially, as data on efficacy in each cultivar would be required.

Fructose is a low cost, low toxicity natural product and can induce resistance to root knot nematode and *B. cinerea* at low dose application rates (Morkunas and Ratajczak, 2014; Moghaddam and Van den Ende, 2012). Although we investigated BABA as a plant defence activator, it is also a xenobiotic compound and is not rapidly metabolised meaning that it accumulates in the plant. This means it may not be the ideal compound for commercialization and further screening of plant defence activators is required to find more benign compounds. The regulatory process used for conventional pesticides is still required to ensure that potential plant defence activators do not have negative side effects on human health or the environment. Fructose, which we found had some activity against root knot nematode, may be a better prospect. Fructose is readily obtainable and inexpensive but has not yet been formulated as a commercial crop treatment. Registration of BABA

could be complicated because BABA is not rapidly metabolised in plants (Jakab et al., 2001) and it acts as a blocker of a ubiquitous enzyme in primary metabolism (Luna et al., 2014). However, analogues may be more promising in this respect. Our recent study with the model plant species *Arabidopsis thaliana* has shown that the resistance–inducing effects of BABA can be separated genetically from its growth repressing effects (Luna et al., 2014). This study revealed that the receptor for BABA controls defence priming and the accompanying stress response via separate signalling pathways. Apart from genetic strategies to optimise the cost-benefit balance of BABA-induced resistance, the elucidation of the molecular perception machinery of BABA allows designing structurally related compounds that retain resistance-inducing capacity, but that are less active in growth repression.

The use of biocontrol agents for reducing the populations of insect pests still remains a viable approach mostly because of its high sustainability. Nonetheless, modern techniques and recent investigations have highlighted at least four issues that need to be taken into account for successful application of biological control. The first, and possibly the most important one, is the correct identification of the species to be used. A modern integrative approach, that combines morphological, biological and molecular characterization should be followed not only by research institutions but also by the companies selling these precious allies. These considerations particularly apply to egg parasitoids within the family of Trichogrammatidae (Hymenoptera, Chalcidoidea) because some species that were considered for a long time to be “generalists” turned out to be in fact groups of separate species. It is now possible to identify these tiny wasps by combining morphological features of the male copulatory organ with the sequences of a gene (i.e. ITS2) that is able to separate close-related species attacking common hosts such as *T. absoluta* (Polaszek et al., 2012). Indeed, the specific parasitoid of the tomato leafminer, *T. achaeae*, is virtually undistinguishable from the closely related *T. evanescens* and this could lead to the failure of biocontrol of *T. absoluta* on tomato (Cascone et al., 2015).

330 The second issue is the rearing system for the biocontrol agent. We found that the
331 performance of the egg parasitoid *T. achaeae* as a biocontrol agent of *T. absoluta,* strongly depends
332 on the rearing system adopted, as composed of host plant and host egg. It was demonstrated that
333 the adult longevity of this parasitoid was higher if it was reared on its natural host *T. absoluta* on
334 tomato. Similarly, a better attack rate was recorded when the rearing and the target systems
335 coincided (Cascone et al., 2015). The consequences of these findings are clear because to reduce the
336 rearing costs, the companies use host eggs that are cheaper and often laid on artificial substrate in
337 total absence of host plant. In relation to the control of *T. absoluta*, this could explain why *T.*
338 *achaeae* has not attained the same performance in the field as those recorded in the laboratory.

339 The third issue is "conditioning": the negative effects of "artificial rearing systems" in place
340 of "natural" ones can be overcome by allowing the development of a single parasitoid generation on
341 its natural host (often the same as the target host). An example is again represented by *T. achaeae*
342 (Cascone et al., 2015) whose performance, in terms of attack rate, could also be enhanced by a
343 combination of temperature and time of exposure during preimaginal development (Cascone et al.,
344 2015). In the past, biological control has sometimes suffered from a lack of professionalism in
345 production systems but now is in a critical phase where big improvements are being made that will
346 allow development of more sustainable strategies for controlling pest populations (van Lenteren,
347 2012). We have now at hand all the tools and the techniques to optimise production. For biocontrol
348 agent *T. achaeae*, the best rearing temperature of the parasitoid is 25°C; food is important for
349 enhancing the survival of the parasitoid: food should be provided (particularly in protected tomato
350 where temperatures are higher than in the open field); a pre-oviposition treatment of 1h at 24°C or
351 28°C enhances the performance of the parasitoid in terms of attack rate and *T. achaeae* should be
352 reared for at least one generation on the natural host (*T. absoluta*) before its release in the field. The
353 fourth issue is registration of biocontrol agents to make them available to farmers and is reviewed
354 and discussed in detail by Lamichhane et al. (2016).

355 Antifeedants provide another possible direction. Here we found *A. chamaepitys* extract to
356 have activity against *T. absoluta*. For commercial production, the extraction process would have to
357 be scaled up and *A. chamaepitys* cultivated to obtain larger quantities for extraction. Here, the
358 extract suppressed feeding damage, but did not completely prevent it and a more detailed chemical
359 and biological investigation of the active antifeedant component/s may lead to improved efficacy.
360 The use of antifeedants requires very good coverage of the plant and the effect can be short-lived as
361 a result of non-persistence of the active compounds and/or the gradual habituation of the insect
362 after a period of starvation. The use of antifeedants in crop protection will require their integration
363 with other agents of control in specially devised management strategies (Griffiths et al, 1991) and
364 improvements in their formulation are required.

365 We recommend that policymakers consider implementation strategies involving further R&D
366 and practical training to make new approaches available to growers. We would like to highlight that
367 policymakers advocating the replacement of pesticides may have seriously underestimated the
368 challenge. Crop plants have been grown in a pesticide treated background for more than 60 years
369 and agricultural ecosystems are highly vulnerable to attack by adapted pests (Bruce, 2012). A
370 systems approach may be required to redesign the system with tactics integrated into appropriate
371 strategies to reduce the risk of crop damage. A conversation with farmers may help to co-design IPM
372 systems which are more user friendly. Schemes may be required to reduce risks for companies
373 considering commercialising the approaches we describe in this paper. There is a business
374 opportunity for innovative companies to develop new biological products to fill the gap left by
375 reduced availability of pesticides.

376 Although our experiments investigated plant defence activation and biocontrol agents
377 separately, these are very complementary approaches and could be used together in an IPM system.
378 It is likely that there would be an additive or even synergistic effect when combining these two
379 approaches. Unlike conventional toxic pesticides, plant defence activators would not have the
380 problem of causing collateral damage to populations of natural enemies through broad spectrum

toxic action. Furthermore, there are clear opportunities to use defence activators to make plants more attractive to natural enemies via activation of 'indirect defence' whereby the plant recruits natural enemies of herbivore pests by emitting attractive plant volatiles or providing supplementary nutrition (Heil, 2008). For example, it is known that natural elicitors contained in stemborer eggs can induce emission of volatiles that attract both larval and egg parasitoids (Tamiru et al 2011). Certain plant genotypes are more responsive to cues associated with attacking organisms and have a higher capacity for tritrophic interactions with their natural enemies which opens up the possibility of breeding crops that boost biocontrol (Tamiru et al 2015; Stenberg et al 2015). Induced resistance can complement constitutive resistance and diversifying crop protection tactics can reduce selection pressure for resistance.

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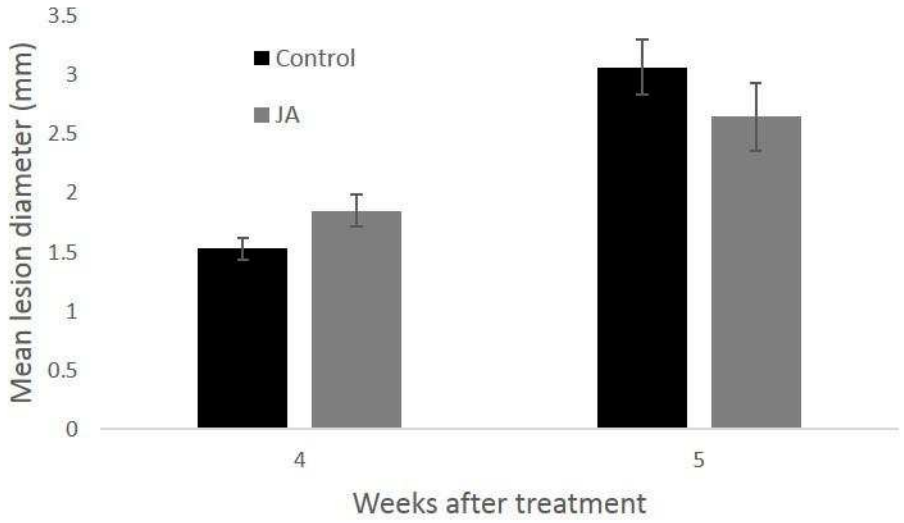

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493 **Fig. S1** Mean *Botrytis cinerea* lesion diameter in tomato cultivar 'Moneymaker' 3 days after
494 inoculation at different times after treatment. Error bars denote SE. No significant differences in
495 infection were observed 4 and 5 weeks after treatment.

Response to Reviewers

Line 1: We have referred to the Lamichhane et al. paper in the discussion as suggested

Line 23: “Parasitoid” is a particular Entomological term and is more precise than the general term “parasite”. It means that the female wasp injects her egg into the body of another insect in which the egg hatches and the parasitoid develops, eventually killing its host. We would prefer to continue using the term “parasitoid” because it describes more clearly the kind of biocontrol agent we are referring to.

Line 24: deleted “were found to” as suggested

Line 32: deleted “Taken together” as suggested

Line 49: deleted “as shown by” and added Lamichhane et al. citation details

Line 56: Added “pest or disease” to make the form of attack explicit

Line 77: induced resistance protects plants. Added “plants” to clarify

Line 98: Can cause yield losses of 80-100% in tomato in Europe. Added reference for this.

Line 114: the final concentration depended on the experiment and is provided on line 123 for the Durability of BABA and JA experiment and 139 for the Effect of cultivar experiment

Line 253/ Fig. 5: Capitalised first letter in axis title as suggested

Line 348: deleted repetition of optimise and corrected spelling