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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. absoluta* 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.

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, 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

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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., 2010). These have been particularly successful in greenhouse environments, for example in the Almeria region of Spain where biocontrol has largely replaced conventional pesticides (Pilkington et al., 2010; Calvo et al., 2014). An increasing number of commercial greenhouse growers around the world employ beneficial insects for crop protection and expenditure on biocontrol agents in greenhouses represents the majority of sales of biological control agents globally. Greenhouses are ideal environments for releasing biocontrol agents because they have contained conditions from which biocontrol agents are less likely to escape. However, the invasive pest, tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), threatens to undermine successful biocontrol programmes in greenhouses if toxic pesticides have to be used to manage it and therefore we focussed on this species in exploring new biocontrol options against it. Tomato leafminer can cause yield losses in tomato of 80–100% (Desneux et al., 2010). In addition to investigating possible biocontrol agents for use against *T. absoluta*, we explored the possibility of using antifeedants to reduce feeding damage by the pest. We used an extract of *Ajuga chamaepitys* (Lamiaceae), the ground pine or yellow bugle, which contains clerodane compounds (Camps et al., 1987) and has been shown previously to be active against another lepidopteran pest, the diamondback moth, *Plutella xylostella* (Griffiths et al., 1991).

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

2. Materials and methods

2.1. Chemical priming of plant defence

Tomato cultivar 'MoneyMaker' was used for all experiments unless stated otherwise. Additional tomato cultivars 'IL4', 'FCN93' and 'Motelle' were obtained from Wageningen University. BABA (catalog number A4420-7) and JA (catalog number J2500) were obtained from Sigma-Aldrich. BABA was prepared freshly in distilled water and diluted to appropriate concentrations. Stock solutions of JA were prepared by dissolving 250 mg in 2 ml of ethanol, which was then diluted in distilled water to a final stock concentration of 10 mM and kept at −20 °C. Before usage, the 10 mM stock solution was thawed and diluted in water to the indicated concentrations.

2.1.1. Durability of BABA and JA

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

2.1.2. Effect of cultivar

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

2.1.3. 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