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Crop Production in Southern Britain:
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EcoSyn: The potential for overcoming insecticide-resistance in arable crop pests using synergists

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Summary

Insect pests cause significant damage to agricultural crops and transmit several important diseases of humans and animals. Chemical insecticides have been used to control insect pests for many decades and remain essential to ensure a supply of affordable food and as part of disease vector control for the foreseeable future. Unfortunately, the world-wide use of insecticides over many years has led to increased resistance to insecticides and contributed to environmental contamination. One possible way to reduce insecticide use without compromising control is to use a synergist in combination with an insecticide. Synergists are themselves non-toxic at doses applied but increase efficacy of the co-applied insecticides by inhibiting the metabolic defence systems in insects that detoxify insecticides. The goal of this project is to develop ecofriendly synergists for use in formulations with insecticides, both in agriculture and in public health.

Key words: Synergists, insecticide resistance, piperonyl butoxide, aphids, oilseed rape

Introduction

The ever increasing incidence of resistance in target pest populations is causing control failures of many pests, whilst at the same time the number of insecticides available is declining as more and more become subject to bans or withdrawal. The recent ban on three neonicotinoid seed treatments by the European Commission is the latest example (EFSA, 2013), and is already causing concern among growers of oilseed rape and maize throughout Europe, as the alternatives are becoming compromised by the development of resistance. For example, in the target pests of neonicotinoid seed treatments in autumn sown oilseed rape, cabbage stem flea beetles, *Psylliodes chrysocephala,* and peach potato aphids, *Myzus persicae,* have developed widespread resistance to pyrethroids (Zimmer *et al.*, 2014; Foster *et al.*, 2013), and there are few or no alternatives for their control (Dewar, 2014).

New insecticidal actives are urgently required to fill the gap, but these take time to develop, and are subject to increasingly stringent regulatory procedures. An alternative or complementary strategy would be to use synergists such as piperonyl butoxide (PBO) in collaboration with existing insecticides. Synergists are "compounds that greatly enhance the toxicity of an insecticide, although they are usually practically non-toxic by themselves" (Matsumura, 1985). Insecticide synergists act by inhibiting metabolic enzymes that metabolise insecticides (Georghiou, 1983). Piperonyl butoxide is routinely used in household applications to increase efficacy of insecticides and has been shown to enhance the performance of insecticides in field situations (Young *et al.*, 2006; Bingham *et al.*, 2007).

This paper outlines proposals to investigate the potential of existing synergists, and the development of novel ones that might alleviate the looming crisis in pest control. On the basis of in-depth experimental analyses of the interactions of the known synergist piperonyl butoxide with metabolic enzymes in pest insects, new molecular structures will be designed, synthesized and evaluated on pest and beneficial species using laboratory bioassays and field trials. In addition, the synthesis process to manufacture these synergists will be evaluated with the aim of achieving an industrially and economically feasible process. Finally strategies will be developed that use the novel synergists to enhance the control of insect pests while preserving beneficial insects. As such this research has significant scientific, economic, and social impact as part of sustainable food production and disease control and will enhance the partners' competitiveness in this important industry by means of global patent and license agreements.

Concepts

Three main classes of enzymes have been shown to be involved in the detoxification of insecticides and metabolic resistance, these are esterases, cytochrome P450s (P450s) and glutathione-S-transferases (GSTs) (Oppenoorth, 1985). Esterases and P450s are involved in phase 1 detoxification as they can act on the intact insecticide, whereas GSTs are phase 2 enzymes, acting on degradation products of phase 1 enzyme activity. Synergists can increase the efficacy of insecticides by inhibiting one or more of these metabolic enzyme families. Because both resistant and susceptible insects express these enzymes, they can make susceptible populations hyper-sensitive to insecticides in addition to overcoming the resistance of insects that overexpress these enzymes.

Piperonyl butoxide was originally reported as a specific inhibitor of cytochrome P450s (Perry and Buckner, 1970) but is now known to also inhibit resistance-associated esterases [Gunning *et al.*, 1998]. As such it inhibits both Phase 1 metabolic enzyme systems allowing sensitisation of the target pest towards the insecticide. This not only abrogates resistance, but will also allow a reduced rate of insecticide to control susceptible pests,. with the concomitant environmental benefits. Sensitisation of beneficial insects such as pollinators is a concern of course, and there have been conflicting reports in the literature (Johnson *et al.*, 2006; Moores *et al.*, 2012). It is of particular importance to resolve this issue, clarify whether a reduction of insecticide can offset any synergist sensitisation or whether a 'bee-friendly' synergist can be designed.

Approaches

Initally, biochemical characterisation of the interactions between PBO/PBO analogues and esterases and P450s, which have been confirmed to confer insecticide resistance. To complement this, molecular modelling of the proteins/inhibitors will be made to give possible insights into the binding regimes. From such a structure activity relationship it is envisaged that novel synergists will be designed that give greater efficacy/ specificity.

Laboratory bioassays will then evaluate synergist/ insecticide mixtures with well-characterised insect populations, both resistant and susceptible to insecticides. Since a key aim of the project is the lack of adverse effects on beneficial insects, these laboratory bioassays will also be carried out in the presence of chosen beneficial insects such as honeybees (*Apis mellifera*).

Finally, field trials (including glasshouse trials) will be conducted to exemplify the performance of the formulations under environmental conditions encountered during control regimes. All trials will be conducted following published EPPO guidelines and will include both susceptible and resistant populations of insect species.

Since the effects of synergists on the rate of selection of resistance genes (both metabolic and target-site) has never been reported, this will also be carried out on selected populations with specific selection regimes.

Discussion

The proposed project, (EcoSyn), aims to develop eco-friendly synergists for insecticide formulations, and deployment strategies that enhance the effectiveness of several important classes of insecticide. It is envisaged that the novel synergists will be more potent than current synergists and enable a reduction in the amount of insecticidal active applied, thereby reducing the adverse effects of these insecticides on beneficial insects such as bees. As such, the novel synergists may be defined as eco-friendly.

The project will move in a systematic manner from the laboratory to the field, identifying powerful synergists and measuring their effects in the field against both pest and beneficial species and measuring changes in metabolic and target-site resistant genes.

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