Training natural enemies to enhance their biocontrol efficacy

The behaviour of natural enemies (NEs) is not merely genetically determined, but is also influenced for a large part by previous experience. Just like Pavlov's dog, natural enemies can internalize new information through associative learning. Through these associative learning mechanisms, NEs can be trained to respond more strongly and specifically to pest-induced volatiles of the crop, which can result in better pest control. This, in turn, can translate in an increased NE offspring production, thus providing longer-term effects. Considerable fundamental research has been performed on associative learning and memory formation in insects. However, until now most research on insect learning has been performed in the laboratory. The few existing semi-field studies - conducted with parasitic wasps - show strong effects of associative learning, with levels of parasitisation increasing up to 9-fold. More research is needed to identify how to best exploit the learning capability of NEs for the improvement of biocontrol.

The evolution of biocontrol

The efficacy of NEs is not always easy to predict and can vary considerably. Three main factors that determine natural enemy performance can be identified: 1) genetic traits of the NE, 2) the environment in which the NE is released, and 3) the experience the NE has obtained in this specific environment. A clear trend in research and development of biocontrol with respect to these three factors can be discerned. Initially, research focused predominantly on identifying NE species with the best genetic traits to control a certain pest species in a given environment. In other words; we were looking for *the best candidate to complete the task*. Since several years the focus has broadened: now we also investigate how the environment in the greenhouse can be adjusted in such a way that NEs can establish and function better. This can be done through supplementing additional food sources and/or functional biodiversity that provides extra shelter, food and/or egg-laying sites. In other words: we are now trying to *create the optimal working conditions for our staff*. Now, if we can prepare the NEs for the task they need to perform, namely locating and eliminating pest insects in the specific cropping environment, we may be able to further increase pest control efficacy. In other words: we will need to investigate *how to train our workers to do the job*.

Insect learning behaviour

The behaviour of insects is not merely genetically determined, but is also influenced for a large part by previous experience. Just like Pavlov's dog insects can internalize new information through associative learning.

Pavlov's dog was trained to associate the ringing of the bell with the food reward. Subsequently, the dog did not only start to salivate when food was presented, but also when the bell rang without any food being offered. Using a similar procedure, insects can be trained to associate odour, colour and/or form with a reward - such as a prey insect, a host insect used for egg deposition or an alternative food source (Dukas, 2009). Considerable fundamental research has been performed on insect associative learning and memory formation. From several parasitic wasps species, including commercially interesting species such as *Aphidius ervi* (Figure 1), it is known that they are excellent learners. Moreover, in recent years also several predatory species have been found to use learning to improve their searching efficacy for prey insects. However, until now most research on insect learning has been performed in the laboratory, and studies on the effects of learning behaviour under (semi-)field conditions remain scarce.

Figure 1. The aphid parasitoid *Aphidius ervi*.

Improving natural enemy searching efficacy through associative learning

But how can associative learning help NEs to find pest insects? This depends on the foraging behaviour of the NE. For many NEs, volatile information from the hosts' habitat and microhabitat - play a crucial role during foraging. By using such indirect cues, NEs can circumvent the problem of low detectability of pest-derived cues. An important source of indirect odour information used by NEs are the so-called herbivore-induced plant volatiles (HIPVs), which are emitted by the plant in response to feeding by pests. These HIPV blends differ from odour blends emitted by intact plants, thus providing a first indication of the presence of a pest insect. Even thus HIPVs are more easily detectable than the pest-derived odour, they are influenced by many different factors and are therefore less reliable. The solution to this so-called reliability-detectability problem is associative learning; every time the NE associates the presence of the pest with a specific HIPV blend, this HIPV blend becomes a more reliable indicator of pest presence.

Figure 2. Associative learning of herbivore-induced plant volatiles (HIPVs) as the solution to the reliability-detectability problem encountered by natural enemies.

Training natural enemies: potential benefits for biocontrol

Through these associative learning mechanisms NEs can be trained to respond more strongly and specifically to pest-induced volatiles of the crop, which can result in better pest control. This, in turn, can translate in an increased offspring production, thus providing longer-term effects. Even thus studies that investigated NE foraging success under (semi-) field conditions are scarce, the few existing semi-field studies - conducted with parasitic wasps - show strong effects of associative learning, with levels of parasitisation increasing up to 9-fold (Papaj & Vet, 1990; Lewis *et al.*, 1990; Kruidhof *et al.*, unpublished results). These results hold great promise for optimizing biocontrol.

Future research

By making is needed to identify how to best exploit the learning capability of NEs for the improvement of biocontrol.

More specifically we need:

- To investigate the learning ability of the most important NE species, and to assess how much the learning ability of these species can be improved through an artificial selection procedure.
- To assess the benefit of training NEs for biocontrol under different types of (semi-)field conditions (e.g. different pest species and -densities, different cropping systems).
- To develop easy-to-implement training procedures, both for training NEs during or directly following mass rearing and for training NEs in the cropping system where they are released. Because during associative learning, the pest insect serves both as the reward and the inducer of HIPVs, it will be important to identify alternative rewards as well as alternative inducers of HIPVs that can be used in such a training procedure.

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