Characterization of Foraging Behavior Of Yellowjacket Workers and Worker-Larva Trophallaxis Towards Feeding Baits Laced with Fipronil

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ABSTRACT

Foraging and trophallaxis behaviors towards a honeybee (HB) and a protein (P) based feeding baits were studied in *Vespula germanica* workers and larvae. Behavioral responses were described by observational methods and characterized by conventional statistics. Stereotypia indexes (SI) were estimated and contrasted within behaviors and between HB treatments. For foraging, the insecticide fipronil at two concentrations (LC_{20} and LC_{80} estimated for larvae) was contrasted against pure HB (control) but for trophallaxis, fipronil was compared at one concentration with the control. HB contaminated with a low concentration of fipronil did not significantly affect foraging and trophallaxis, allowing for larval exposure to this bait intended for workers. On the other hand, the P-based bait including fipronil at both concentrations was not foraged. The HB-bait loaded with the LC_{80} was foraged but trophallaxis did not occur. These results demonstrate the value of quantitative behavioral studies to develop pest management techniques.

Author Keywords

Ethogram, stereotypia index, chi-square test, Fisher test, *Vespula germanica*.

INTRODUCTION

The yellowjacket wasp, *Vespula germanica* (Hymenoptera: Vespidae), is a social insect species that has invaded several regions around the world, which is considered a serious pest in Chile. It forages in the vicinity of the nest to collect and provide food to larvae. During foraging the workers can bite and sting people and animals. They can also affect agriculture production (fruit bites), apiculture (destruction of hives), etc. Available techniques to manage this species include the use of non-specific traps to capture adults and nest destruction by either physical or chemical methods, but both are considered relatively inefficient. We have been working on developing a specific and efficient bait to attract *V. germanica* workers, and to allow them to forage, the collection of food at a bait station, and transport it back to the colony, which is followed by stages of trophallaxis, the exchange of food between members inside the colony, which distributes the bait to many individuals (larvae and adults), making it possible to deliver chemicals incorporated (e.g. insecticides) into the nest. To develop this technique, it was necessary to determine a baseline on feeding behaviors (both foraging and trophallaxis), and contrast behavioral responses on workers exposed to baits based on honeybee (HB) or a protein (P) matrix, laced with concentrations of fipronil, and eventually to distribute low amounts of the insecticide into the colony to decrease its vigor in a pest management strategy.

MATERIAL AND METHODS

Fipronil, a neurotoxic insecticide, was added into both liquid pure honeybee or a protein matrix (in the process of being patented), at concentrations equivalent to the LC_{20} and LC_{80}, which were estimated in previous studies with larvae, to test whether the use of a relatively low concentration of this toxicant affects workers and larvae differentially.

Nest comb pieces containing developed larvae, and workers marked with color tempera on the thorax dorsum were used in observation arenas (Flanders batteries), where both foraging and trophallaxis behaviors were videotaped. The tapes were revised to identify homogeneous, discrete, and observable steps in the sequences, and their respective frequencies were calculated. They were used to build ethograms for all treatments, which consisted on both concentrations of fipronil and the control without insecticide. To test if dependence occurred or not between the steps within behavioral sequences, the chi-square or Fisher tests were used. Also, a Stereotypia Index (SI) was calculated and used to test for eventual differences between

treatments in a particular behavior (during foraging or trophallaxis).

RESULTS

For both behaviors, foraging and trophallaxis, dependence between the steps included within sequences was found for HB with no insecticide, and also for all HB-fipronil-loaded with the LC_{20}, but not in the case of trophallaxis with the LC_{80}, since workers did forage but did not conduct trophallaxis, and died soon after exposure. With the P-based bait, dependence between the steps occurred only for foraging and trophallaxis both without fipronil. These behaviors were not observed when the P-bait was contaminated with the insecticide at either concentration.

When workers approach and recognize the protein bait without fipronil, they cut small pieces of food with their mandibles, which they hold between their front legs to carry them to the nest to conduct trophallaxis. On the other hand, pure liquid honey was swallowed and transported to the colony inside the crop of the worker. During trophallaxis with the P-bait, workers on the comb first manipulate the matrix with their front legs and mandibles, then approach and offer a flattened bread-like "tortilla" to larvae which take a piece with their mandibles. This process is repeated by workers until the whole food piece is consumed. Trophallaxis for honey occurs after regurgitation of fluid from the crop.

The SI values for foraging indicated a high degree of stereotypia for the pure HB-bait and HB with fipronil at the LC_{20}, but not for the LC_{80} treatment. With honey, trophallaxis at the control and the LC_{20} were also highly stereotyped, but this conduct was not observed at the LC_{80}. When trophallaxis did occur, it was observed between workers and larvae and between workers themselves. With the P-bait with no insecticide, the SI was also high for both foraging and trophallaxis. These behaviors were not observed when the P-bait was loaded with fipronil at any concentration.

By comparing SI between two behavioral responses (e.g. those occurring to the control vs. those occurring to the LC_{80}) using the chi-square test, we were able to discriminate between HB baits at two concentrations of fipronil to manage yellowjackets. In fact, we found that a low level of contamination with the insecticide (i.e. LC_{20}) did not alter significantly both foraging and trophallaxis. Although honeybee is not an appropriate matrix for preparation of feeding baits for this wasp (it also attracts bees and other insect pollinators), these data suggest that some sublethal concentrations of fipronil can be used to prepare feasible slow action feeding baits. Different specific feeding attractants to this yellowjacket species require to be studied.