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Cyantraniliprole can replace malathion in baits for *Ceratitis capitata* (Diptera: Tephritidae)

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ABSTRACT

Bait applications are frequently utilised to control the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), because they can be applied rapidly and have a limited impact on natural enemies. However, the preferred toxicant, malathion, sometimes results in unacceptable residues and resistance has been found to it and the most common alternative, spinosad. Cyantraniliprole 100 g/L SE was therefore evaluated as an alternative toxicant in protein hydrolysate bait in laboratory bioassays, field cages and commercial citrus orchards in South Africa. Field-cage studies showed that mean adult mortality with the registered dosage of malathion EC 875 ppm a. i. plus protein hydrolysate at 1% in water was not significantly different ($P > 0.05$) from mortality with cyantraniliprole at 50, 100, 150 or 200 ppm a. i. mixed with protein hydrolysate at 1%, or GF120 NF (containing spinosad) diluted to 3.3%. Five field trials conducted in citrus orchards with protein hydrolysate at 0.8% plus cyantraniliprole 100 ppm a. i. all showed equivalent efficacy to the registered protein hydrolysate plus malathion standard, and GF120 NF at 4 or 5%. Cyantraniliprole can therefore serve as an alternative toxicant to organophosphates such as malathion when used in combination with protein hydrolysate in baits for the control of *C. capitata*.

1. Introduction

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann), is a widespread and serious economic pest of many commercial crops in southern Africa (Hancock, 1989) and other parts of the world (White and Elson-Harris, 1992). Proteinaceous attractants combined with a suitable toxicant have been applied as bait sprays to control *C. capitata* for many years in South Africa (Georgala, 1959) and elsewhere (Steiner, 1957) and continue to be used around the world (Conway and Forrester, 2011; Mangan et al., 2006; Manrakhan and Kotze, 2011; Nigg et al., 2008). The organophosphates malathion and trichlorfon are still registered for use in fruit fly baits in South Africa, but pre-harvest intervals are increasing due to changes in acceptable maximum residue limits, so sometimes fruit must be protected with a bait containing spinosad just before harvest (Adan et al., 1996; Mangan et al., 2006). With spinosad being the only current registered alternative toxicant to the organophosphates (Manrakhan et al., 2013), the threat of resistance to both groups of toxicants is also a concern (Magaña et al., 2007; Couso-Ferrer et al., 2011).

This research was therefore conducted to evaluate Cyantraniliprole 100 g/L SE as a toxicant for *C. capitata* in proteinaceous baits. Cyantraniliprole is an anthranilic diamide that is effective against a wide range of insect pests (Selby et al., 2013) and was considered a better option for Diptera than the older, closely related product, chlorantraniliprole, which was primarily effective against Lepidoptera (Lahm et al., 2009) but had shown some mortality in adult fruit flies when used as a cover spray (Teixeira et al., 2009). Cyantraniliprole and other anthranilic diamides bind to the ryanodine receptor, leading to uncontrolled calcium release in muscles that impairs muscle contraction (Cordova et al., 2006; Lahm et al., 2009). The anthranilic diamides are more than 500 times more effective against insect cells than against mammalian receptors (Cordova et al., 2006), so are considered much safer than the organophosphates still in use as fruit fly bait toxicants. Research has been conducted with cyantraniliprole against the fruit fly *Bactrocera dorsalis* (Hendel) that involved selecting for resistance and the establishment of baseline susceptibility in the south of China (Zhang et al., 2014). Earlier unpublished bioassays by the authors using cyantraniliprole in sucrose solutions against *C. capitata* gave promising results and led to this work with protein hydrolysate combinations.

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2. Materials and methods

2.1. Laboratory bioassay and field cages

Ceratitis capitata flies used in laboratory and field cage experiments came from a culture at Citrus Research International in Nelspruit, South Africa, that had been maintained for more than 100 generations but with wild male flies added to the culture every two years to maintain genetic diversity.

2.1.1. Ingested toxicity using protein hydrolysate in boxes

The trial was conducted with 4-day-old *C. capitata* that had been deprived of protein. Approximately 15 female flies were used per plastic cake box (27 cm × 27 cm x 15 cm with gauze walls and glass lids), and three containers were used per treatment, although these were not treated as replicates for analysis purposes. Water and granulated sugar were provided at all times, and the experiment was run in the laboratory at approximately 23 °C, 60% relative humidity and 13/11 h day/night cycle. Treatments were compared by applying 10 × 10 μL droplets of each mixture on a 10 cm × 7 cm glass plate in each box. Green colourant (Moir's Double Concentrated green food colour containing CI 42090 and CI, 19140, Bromor Foods, Maitland, South Africa) was included in all treatment mixtures at 1% as an indicator for ingestion of the treatment solution by *C. capitata*. The following treatments were evaluated and included a sucrose control and cyantraniliprole treatment with sucrose in case it was found to be more palatable than the commercially used protein hydrolysate.

1. Control comprising 5% sucrose solution
2. Control comprising 1% Hym-Lure (425 g/L protein hydrolysate, Villa Crop, Johannesburg, South Africa)
3. 1% Hym-Lure + malathion (Mercaptothion 500 g/L EC, DowDuPont, Johannesburg, South Africa) at the registered rate of 875 ppm a. i.
4. 1% Hym-Lure + 200 ppm a. i. Cyantraniliprole 100 g/L SE (FMC Agricultural Solutions, Pretoria, South Africa)
5. 5% sucrose solution + 200 ppm a. i. Cyantraniliprole 100 g/L SE

The flies were placed in the boxes at 09h00, and 2 h later the glass plate with the bait droplets was placed on the floor of each box. After 4 h, observations were made of how many droplets had been completely consumed by the flies. After 24 h exposure, the glass plates were removed, and the first count of mortalities conducted as well as further observations on the further consumption of bait droplets. Mortality was determined without prodding after 48 and 72 h so that it could be done by observation through the glass lid. However, only flies with green colourant visible in their abdomens were included to exclude mortality due to causes other than feeding.

2.1.2. Field cage evaluations, part one

Two cages of 6.0 m × 8.0 m x 3.5 m covered with green shade net (50%) were used to run two trials simultaneously. Cages were situated in the grounds of Citrus Research International, Nelspruit, South Africa (25°28′47.75″S and 30°59′38.82″E). Twelve potted citrus plants were placed in each cage to provide a natural substrate for flies. In the experiments, 800, 7 to 9-day-old *C. capitata* with a 50:50 sex ratio were released into each field cage between 10h30 and 16h00 on a test day. These flies had only received granulated sugar and water since eclosing, so were protein-starved. A specially designed, two-tiered, multi-chamber trap was used to evaluate nine treatments simultaneously in each cage (Fig. 1) and these traps were suspended from the roof of the cage using string. The lower tier of this trap was a white bucket trap with four entry holes in the side (19 mm diam.) and transparent plastic inserts fitted in the holes that protruded into the chamber to prevent flies from escaping easily. The bottom of this trap (where the bait was placed) was removable and painted black to drive the flies to the upper tier, which comprised an inverted, clear plastic bowl (70 mm high and 113 mm diam.). The top, clear container had three evenly-spaced holes near the upper edge, each of which led via clear plastic tubes (19 mm diam.) to another clear plastic container (70 mm high and 113 mm diam.) with a gauze lid. The reason for using such a complex trap was that, because cyantraniliprole does not have knockdown action, we wanted to try to prevent flies escaping that remained active for a long period after feeding on bait in the lower tier.

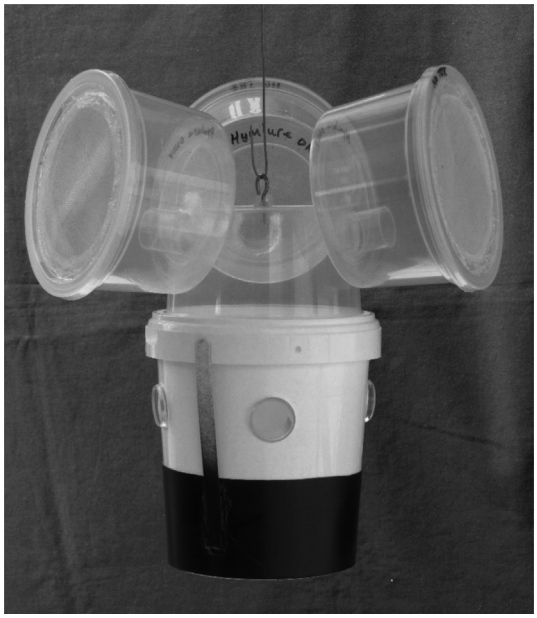

Fig. 1. A multi-chamber trap designed to prevent flies from escaping that remained active for a long time after feeding on bait without knockdown properties placed in the removable black base.

For each treatment, 5 ml were soaked into an approximately 8 mm thick cotton wool pad in a 34 mm-diameter Petri dish placed inside the bottom of the trap. Solbait, containing 15% invert sugar (Moreno and Mangan, 2002), replaced the protein hydrolysate in three treatments to see whether larger bait quantities would be consumed and thus cause more mortality than with the commercially-used Hym-Lure. At 17h00 on a test day, 9 traps containing different treatments were hung in two rows of 4 traps plus 1 trap in the centre of the cage. The traps were hung late in the day to give time for the flies to disperse throughout the cage after being released and so they could feed at dusk and from dawn the next morning. At 07h30 the next day the traps were all rotated (moved on) two positions, and this rotation was then repeated every 1.5 h until 16h00 to prevent certain traps from always being on either the shady or sunny side of the cage. At 17h00 the traps were closed (entrances plugged) and brought back to the laboratory and held at 23 °C, 60% relative humidity and with a 12:12 h day/night cycle. Immediately after removing the traps from the cages, the bait mixtures were removed from the traps to prevent further feeding, and the first count was conducted. Dead flies were placed in a small Petri dish in the bottom of each trap which could be removed without disturbing live flies in the upper chambers. Alive and dead flies in the upper chambers of the traps were also counted, and water and granulated sugar periodically provided to sustain the live flies in the upper chambers. Mortality of the surviving flies was determined after 1, 2, 3, 4 and 5 days.

The above technique was used in two cages simultaneously per week and repeated in a second and third week to provide a total of six replicates. The start dates (i.e. fly release dates) for these paired replicates were 20 September, 11 October and 18 October 2010. Maximum and minimum temperatures in the cages ranged between 37 °C and 13 °C during the evaluations and the relative humidity ranged from 20% to 89%. The day/night cycle was approximately 12/12 h.