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RESEARCH

Implementing a Spinosad-Based Local Bait Station to Control *Bactrocera cucurbitae* (Diptera: Tephritidae) in High Rainfall Areas of Reunion Island

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ABSTRACT. Three species of fruit flies cause serious damage to cucurbit crops on Reunion Island: *Bactrocera cucurbitae* (Diptera: Tephritidae) (Coquillett 1899), *Dacus ciliatus* (Loew 1901), and *Dacus demmerezi* (Bezzi 1917). To control them, a program of agroecological management of cucurbit flies has been implemented based on the application of Synéis-appât, especially spot sprays on corn borders. However, the high rainfall on Reunion Island limits the long-term efficiency of the bait; in addition, this method cannot be used for large chayote trellises, because corn borders cannot be planted around them. The aim of this study was to design a bait station adapted to prevailing conditions on Reunion Island. An 'umbrella trap' tested in Taiwan was used as a reference to compare its efficacy with our local bait station. Experiments were conducted in field cages on *B. cucurbitae* to test different characteristics of bait stations and to construct one using local materials. Results were validated in the field. The attractiveness of the bait station was related mainly to the color of the external surface, yellow being the most attractive color. The efficacy of the bait station with respect to fly mortality was found to be linked to the accessibility of the bait, and direct application of Synéis-appât on the bait station was found to be the most efficient. In the field, *B. cucurbitae* were more attracted to the local bait station than to the umbrella trap, while the two other fly species displayed equal attraction to both trap types. Our local bait station is a useful alternative to spot sprays of Synéis-appât and is now included in a local pest management program and is well accepted by farmers.

Key Words: Synéis-appât, Tephritidae, Integrated Pest Management, chayote, Indian Ocean

Fruit flies (Diptera: Tephritidae) are among the most destructive pests of horticultural systems in the world, and their impact is heightened in insular and tropical conditions (White and Elson-Harris 1992). On Reunion Island, three tephritid species, *Bactrocera cucurbitae* (Coquillett 1899), *Dacus ciliatus* (Loew 1901), and *Dacus demmerezi* (Bezzi 1917), attack cucurbit crops (cucumber, pumpkin, zucchini, chayote). The damage caused by the larvae feeding on the fruit can reach 90% of the crop yield (Ryckewaert et al. 2010). Up to now, the management of cucurbit fly species relied mainly on insecticides, but in the last decade chemical control has been shown to be inefficient and also have negative sanitary and environmental effects. Since 2009, a program of agroecological management of cucurbit flies has been successfully implemented on Reunion Island (Deguine et al. 2011). This program relies on different techniques, such as sanitation, spinosad-based baits, and male annihilation.

Adult *B. cucurbitae* are known to spend considerable time on roosting sites, such as corn (Nishida and Bess 1957; MacQuate and Vargas 2007). On Reunion Island, studies of the attractiveness of different candidate trap plants for cucurbit fly species recently showed corn to be the most attractive plant (Atiama-Nurbel et al. 2012). This result led us to combine corn borders planted around cucurbit crops with the application of spinosad-based bait on the corn plants (Deguine et al. 2012a).

This technique, using Synéis-appât (Dow AgroSciences, France) with a 1:5 dilution (1 unit volume of Synéis-appât for 4 units volumes of water), proved satisfactory in most farming situations and cropping systems (Deguine et al. 2012a). However, in high rainfall areas where cucurbits are grown, rain can wash away the spinosad-based bait spots applied on corn leaves, and applications have to be repeated (Prokopy et al. 2003). In addition, corn borders or corn patches cannot be planted under trellises of chayote, making application of Synéis-appât on a non-host plant impossible. This situation meant the flies continued to cause serious damage to chayote.

Bait stations can also be used in fruit fly suppression. In Taiwan, the umbrella trap (UT) is used as a rain-fast device for applications of methyl eugenol or cue lure (Kao et al. 2008). It was developed by Dr. Edward Y. Cheng at the Taiwan Agricultural Research Institute and consists of a yellow funnel with a hook. Recently, it was evaluated for application of protein baits (E. Y. Cheng, personal communication). In addition, in Hawaii, Piñero et al. (2009) designed a novel bait station that can be used against fruit flies. Sprayed with spinosad-based bait (GF-120, Dow AgroSciences), this bait station enhances the behavioral response of *Bactrocera dorsalis* (Hendel) adults due to the use of the visually attractive yellow color. It also provides shelter and protection against rainfall. In addition, the period of attractiveness of the bait station to *B. cucurbitae* was extended for one week after the bait is applied. This rain-fast bait station was then used in commercial papaya orchards and is considered to be an efficient method to control fruit flies (Piñero et al 2010).

When designing and implementing a local bait station, including spraying of Synéis-appât in high rainfall cucurbit cropping areas and under chayote trellises on Reunion Island, we benefited from the experience gained in Taiwan and Hawaii. In this study, we used the 'UT' from Taiwan as a control and compared this trap type with bait stations made from plastic bottles, as a model of a simple, low-cost bait traps constructed with cheap, locally available materials.

Initially, the response of *B. cucurbitae* adults to the UT is described and quantified in field cages in order to evaluate the importance of the different components affecting fly capture. Then, the efficacy of Synéis-appât bait applied in the UT and the local bait station is compared in the field.

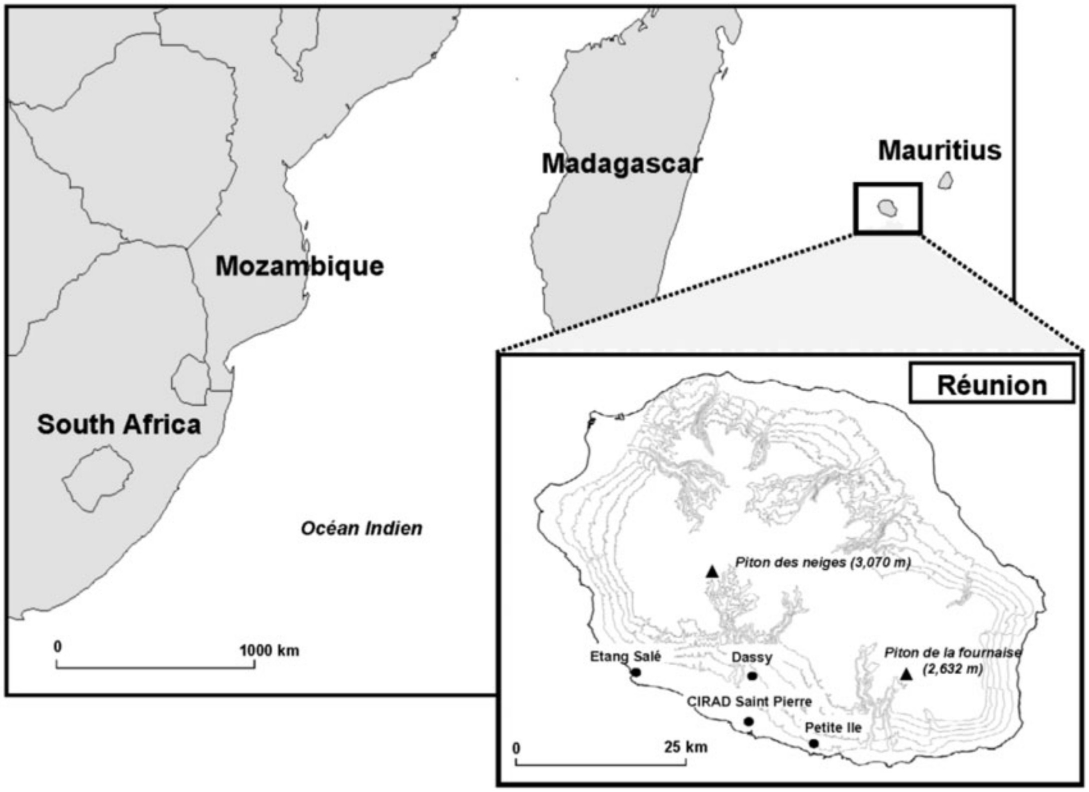

Fig. 1. Location of Reunion Island in the Indian Ocean and locations of the four experimental sites on the island with their altitude.

Materials and Methods

General Methodology. The study was conducted from 2009 to 2011 in the southern part of Reunion Island (Fig. 1). Field cage experiments were carried out in the French Agricultural Research Centre for International Development (CIRAD) experimental station at Saint-Pierre in cylindrical field cages (2.5 m in height by 3 m in diameter, amber High-density polyethylene (HDPE) screen cage, Synthetic Industries, Gainesville, GA) composed of PVC tubing and a mesh-screened canvas. During each day of the tests, ambient temperature and relative humidity (RH) around the cages were recorded every 30 min by a Hobo sensor (U12 Temp/RH/Light/External Data Logger, Onset Computer Corporation, Bourne, USA). Means per day were then calculated, and these climatic parameters were found to be near-constant throughout the experimental period. The field trials were conducted in three villages: Petite Ile, Etang-Salé and Dassy (Fig. 1). An experimental plot was chosen in each locality. The presence of fruit flies and vegetable crops (pumpkin, zucchini, and cucumber) were the main criteria for the choice of plots.

Field Cage Experiments

Biological Material. Strains of *B. cucurbitae* were collected from infested pumpkins, *Cucurbita maxima* cv. Duchesne in June 2000 at three localities on La Réunion (Petite Ile, Bassin Martin and Piton Saint Leu). Adult flies obtained from these samples were reared under controlled conditions for 35 generations [25 ± 1°C, 70 ± 10% RH and a photoperiod of 12:12 (L:D) h, following the method described by Rousse et al. (2005)]. Adult flies were fed with granulated sugar, enzymatic yeast hydrolysate (ICN Biomedicals, Aurora, OH) and water. Sexual maturity is attained at 7 d in the strain. Each day of the experiment, flies were released at 8 a.m. from a small cage (30 cm by 30 cm by 30 cm) placed inside the field cages. Unless specified, flies used in the experiments were 5 d old (± 2 d).

Bait Stations and Application of Synéis-appât. The reference bait station or 'UT' is produced by Agentec Technology CO. Ltd (Taichung City, Taiwan) and is included in an Integrated Pest Management(IPM) program in Taiwan, the 'Area-Wide Control of the Oriental fruit fly and Melon fly'. It is composed of a yellow plastic cone (12 cm in diameter, 12 cm in height, and 1-mm thick) equipped with a plastic black clip (10 cm long with 2.5 cm inside the plastic cone) making it possible to hang the trap and hook a rectangular wick (4 cm by 3 cm by 0.5 cm) inside the bait station (Fig. 2). The local bait station (Fig. 3) was constructed from a yellow plastic bottle cut in half length-wise (13.5 cm in height, 7 cm in diameter) (SPHB, St Pierre, Reunion Island) and attached with two strands of plasticized wire (1.1 mm in diameter) (Gamm Vert, Paris, France). In field cage experiments, the same device was also tested using other colors: transparent (Edena, La Possession, Reunion Island), red, and white (SPHB, St Pierre, Reunion Island).

Fig. 2. Reference bait station, or 'UT', with a cotton wick soaked in Synéis-appât.

Synéis-appât (Dow AgroSciences, N° AMM: 2060130) is a spinosad-based bait (spinosad concentration: 0.24 g/liters). The bait was

Fig. 3. Local bait station in chayote trellises, with Synéis-appât applied on the inside of the plastic bottle.

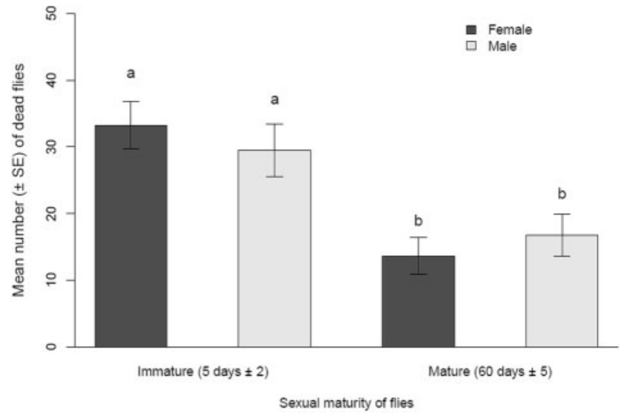

Fig. 4. Number of dead *B. cucurbitae* 7 h after application of Synéis-appât (UT) in field cages according to their sex and their age (immature and mature) (N = 8 replications, December 7 and 8, 2009). Bars with different letters are significantly different ($P < 0.05$).

diluted 1:5 as recommended (Dow AgroSciences 2001). Just after fly releases, Synéis-appât was applied at 8:30 a.m. on the bait stations in two different ways: 1) it was sprayed on the inside of the bait station (a total of 4.5 ml, equivalent to 5 sprays) or 2) when a cotton wick was used, the wick was soaked in the same volume of Synéis-appât until the liquid was completely absorbed. Immediately after Synéis-appât was applied, the bait stations were suspended at a height of 1.50 m inside the field cage.

Description of the Experiments. In the first experiment, after application of Synéis-appât in the UT, fly mortality was assessed according to sex and age. For each of the eight replicates, one hundred 2-month (± 1 wk) old mated flies (50% of each sex) were released in a field cage, and one hundred 5-d old virgin flies (50% of each sex) were released in another field cage. These age categories (representing mature and immature individuals, respectively) were selected from categories used in other field cage experiments (Deguine et al. 2012b). The number of dead flies (found on the floor of field cage) was recorded hourly for 7 h after the bait was applied. Living flies were also recorded 22 hours after the bait was applied. In addition, for each replicate, one field cage was used as a control, with an UT without spinosad (to verify that fly mortality was nil).

In the second experiment, fly mortality was assessed by comparing the two Synéis-appât application methods on the UT. Each method was tested in a cage with three replicates. One hundred *B. cucurbitae* adults (50% of each sex, 5 d old) were released in each cage. After Synéis-appât was applied, dead flies were counted every hour for 7 h. Living flies were recorded 29 h after the flies were released to estimate fly mortality over a longer period of time.

In the third experiment, the attractiveness of four different colored local bait stations (white, yellow, red, and transparent) was assessed in a choice experiment. Reflectance spectra of each bait station were measured with a spectrometer (LabSpec 5000/5100 Portable Vis/NIR Spectrometer, ASD Inc., Boulder, CO) focusing on the 400–680 nm spectral range. The four bait stations were suspended in a field cage in a line but in random order, with a 50-cm interval between each. Four replicates were conducted. The stations were coated with glue (Kollant S.P.A., Padova, Italy) to trap the attracted flies. In total, 200 immature flies (100 males and 100 females) were released per cage, and dead flies (caught by glue) were counted 6 h after application of Synéis-appât.

Field Trials. The efficiency of the local bait station was compared with that of the UT in the field. Each bait station was brushed with glue (Kollant S.P.A., Padova, Italy) on the inside in order to trap the attracted flies. Synéis-appât was then sprayed on top of the glued surface. In each of the three experimental plots, 10 bait stations of every kind were suspended alternately on 1 m poles at 10-m interval. They were removed 4 d later and taken to the laboratory to count the number of individuals of each cucurbit fly species (*B. cucurbitae*, *D. ciliatus*, *D. demmerezi*) trapped. The trial was repeated at seven different dates in February and March 2011.

Statistical Analysis. Generalized linear models were used to test for differences in fly mortality. In the case of field cage experiments, a binomial error distribution with logit link function was used, and in field trials a Poisson error distribution with a log link function was used. A likelihood ratio test, based on a chi-squared test, was performed to identify which factors were significant. When a factor with more than two levels was significant, a Tukey's HSD test was performed to test differences. All tests were performed with R software (version 2.14.2, R Development Core Team, 2011; Vienna, Austria). If a P-value (P) was < 0.05, the difference was considered to be significant.

Results

Fly Mortality According to Age and Sex. Over the initial 7 h after bait application, the maturity status of the flies had a significant effect on mortality ($P < 0.01$; Fig. 4). Mortality of immature flies was significantly higher than that of mature flies (62.8 ± 6.8 against 30.4 ± 5.8, respectively, for 100 released flies per sex). This significant difference persisted over time, and after 29 h numbers of dead flies recorded were 90.6 ± 2.2 and 85.1 ± 2.9 for immature and mature flies, respectively ($P < 0.01$). Seven hours after bait application, there was no effect of sex on mortality for either age ($P = 0.08$ and 0.10 for respectively mature and immature flies).

Fly Mortality According to the Bait Application Method. The method of bait application had a significant effect on mortality 7 h after application ($P < 0.01$; Fig. 5). Application on the inside of the UT attracted and killed significantly more flies (50.7 ± 6.7 for 100 released flies) than application on the wick (35.3 ± 3.8). Twenty-nine hours after application, there was no significant difference in mortality ($P = 0.051$) between the two methods (97.0 ± 0.6 and 93.7 ± 0.3 for umbrella and wick treatments, respectively).

Effect of the Color of the Bait Station Color on Its Attractiveness for the Flies. The color of the local bait station had a significant effect on the capture of *B. cucurbitae* ($P < 0.01$) (Fig. 6). Yellow was the most attractive color (50.8 ± 9.4) compared with white (30 ± 2.2, $P < 0.01$), red (12.5 ± 3.3, $P < 0.01$), and transparent (10.5 ± 3.9, $P < 0.01$). The white local bait station was significantly more attractive than the red ($P < 0.01$) and the transparent ($P < 0.01$) ones. Finally, the red and the transparent ones were equally attractive ($P = 0.81$).

This ranking of attractiveness was found to be the same as the ranking of the maximum values of reflectance (yellow LBS, 0.702; white LBS, 0.668; red LBS, 0.287; and transparent LBS, 0.034).

Efficacy of the Local Bait Station in the Field. In the fields, significant differences were observed between the UT and the local bait station (LBS; yellow color was selected based on the preceding experiment) at two locations (Etang Salé and Dassy, $P < 0.01$). In these locations, the local bait station was significantly more efficient than the UT (attracted three *Bactrocera* species combined per bait station—Dassy: 6.5 ± 1.9 for

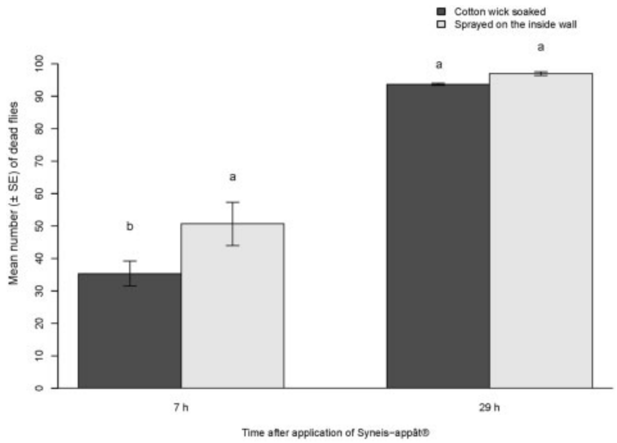

Fig. 5. Number of dead *B. cucurbitae* 7 and 29 h after application of Synéis-appât (UT) in field cages according to the bait application method ($N = 3$ replications, 30 November 2009 and 1 December 2009). For each time after application, bars with different letters are significantly different ($P < 0.05$).

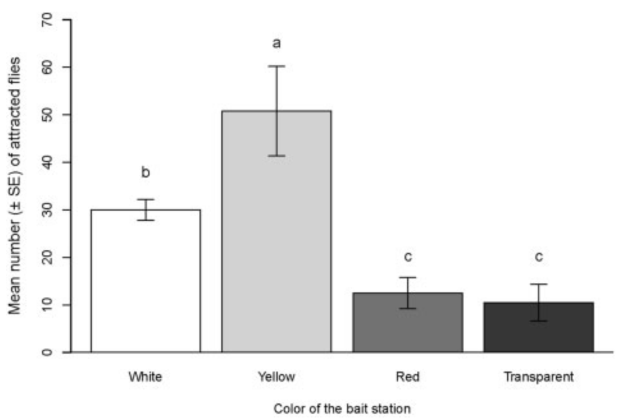

Fig. 6. Number of dead *B. cucurbitae* 6 h after application of Synéis-appât in field cages according to the color of the bait station ($N = 4$ replications, 13 January 2010). Bars with different letters are significantly different ($P < 0.05$).

LBS and 2.8 ± 1 for UT; Etang Salé: 3.7 ± 1 and 1.6 ± 0.7). However, in Petite Ile, no significant differences were observed between the two (7.3 + 1.3 and 6.0 + 1.3, respectively, $P = 0.05$).

Analysis of all the results of the whole trial revealed a significant difference in the numbers of flies attracted to the two bait stations (attracted three *Bactrocera* species combined per bait station: 5.5 ± 0.8 for LBS and 4.4 ± 0.7 UT, $P = 0.0044$). In addition, the effect of the bait stations differed with the species of fruit fly. No significant difference was observed in the efficiency of the two types of trap (LBS and UT) for *D. ciliatus* (attracted flies for 10 bait stations: LBS 6.3 ± 1.7, UT 6.4 ± 2, $P = 0.916$) or *D. demmerezi* (LBS 37 ± 14.2, UT 33.5 ± 11.6, $P = 0.2801$) species. *B. cucurbitae* flies were more attracted ($P < 0.01$) to the local bait station (flies attracted to 10 bait stations: 11.4 ± 4.9) than to the UT (4 ± 1.4).

Discussion

The efficacy of Synéis-appât had previously been demonstrated for different tephritid fruit flies species, including *B. cucurbitae*, *D. ciliatus*, and *D. demmerezi* (Deguine et al. 2012b; Prokopy et al. 2003). However, the technique of applying Synéis-appât on a border of corn plants, which is widely used in area wide pest management programs (Deguine et al. 2012a), is not suitable for the rainy eastern part of Reunion Island or for the configuration of chayote trellises. The bait rapidly loses its efficiency when exposed to rain. In Hawaii, Prokopy et al. (2003) and Revis et al. (2004) showed that the attractiveness of GF 120 NF Naturalyte Fruit Fly Bait for *B. cucurbitae* declined considerably within 24 h after exposure of the bait to 8 mm of rainfall. These results were confirmed by Vargas and Prokopy (2006) and Barry et al. (2006). This loss of efficiency led farmers to increase the frequency of applications, which resulted in costly fruit fly management.

Bait stations are a way to protect the bait from the rain and are a good alternative to spot sprays. Moreover, their use limits the environmental impact of the bait by reducing the release of toxic chemicals into the environment (Mangan and Moreno 2007). Over the years, different kinds of bait stations have been developed for different pests and crops (Espsky and Heath 1998). For example Piñero et al. (2009) designed the papaya leaf mimic (PLM), a novel bait station using GF-120 NF Naturalyte that represents a supernormal visual stimulus of papaya foliage. It was tested on *B. cucurbitae*, *Ceratitis capitata*, and *B. dorsalis* in Hawaiian papaya and coffee fields. In Taiwan, the UT greatly reduced *B. cucurbitae* populations under trellis grown crops (E. Y. Cheng, personal communication). This use corresponded to the purpose of our study, which was focused on developing a bait station adapted to the trellises used for chayote. For that reason, the UT was chosen in the field cage experiments as a reference for the development of the local bait station. Despite the diversity of the devices used, two characteristics are common to the design of all bait stations: they have to be attractive and have high lethality (Mangan and Moreno 2007).

The first part of this study was a preliminary phase to test flies response and to identify the important components of the bait station in order to optimize them.

The efficacy of the umbrella was confirmed under Reunion Island conditions for *B. cucurbitae* of both sexes and different ages. Results showed that 5-d old immature flies were more attracted by the UT than mature flies. To reach sexual maturity, female flies need proteins (Hendrichs et al. 1991). Proteins also play a role in males becoming sexually active. Kaspi et al. (2002) showed that protein-fed males of Mediterranean fruit fly become sexually active before protein-deprived males. In addition, Prabhu et al. (2008) showed that proteins (carbohydrate ratios) promote sexual activity and longevity of males of Queensland fruit fly *Bactrocera tryoni*. The importance of proteins for fruit flies to reach sexual maturity could explain the greater attraction of the protein bait for immature flies. We found no differences in mortality in the two sexes. Male and female flies were similarly attracted by Synéis-appât. This is expected in experiments with bait based on a food attractant (Fabre et al. 2003). The same result was previously obtained for the three species of fruit flies in cages by Deguine et al. (2012b). In our study, although laboratory flies may be good surrogates for wild flies, the latter have not been used in field cage experiments. The diet of the reared flies may affect their behavior and thus the results obtained in the experiments. Manrakhan and Lux (2008) showed that protein-deprived flies are more receptive to food odors than protein-fed flies reared on artificial diet. Indeed protein-deprived flies have less nutritional reserves than protein-fed flies reared on artificial diet (Kaspi et al. 2002) and appear to be more attracted by protein baits like Synéis-appât (Piñero et al. 2011). Taking these observations into account, the attractiveness of Synéis-appât associated with the bait station could be even greater in wild flies given their protein deprived diet.

Our results suggest that applying the bait on the entire inside wall of the bait station improves the efficiency of the device. This kind of application killed more flies than when the bait was in the wick. The external feature of the bait station is the first element in contact with the flies as they land on it. Contact with the bait is made easier when they walk toward the inside. The application surface is also larger than the wick, which increases the probability that the fly will come into contact with the bait. For the design of their PLM, Piñero et al. (2009), chose this method of application, which was shown to be efficient in the field.

Visual stimuli are one of the essential components of 'attract and kill' methods (Foster and Harris 1997; Vincent et al. 2003). In a limited space, visual stimuli take priority over olfactory stimuli (Rousse et al. 2005). This should be taken into account in the design of bait stations. Several authors have already demonstrated the strong attraction of yellow devices for *D. ciliatus*, *B. dorsalis* and *C. capitata*, and *B. cucurbitae* species (Vayssières 1999; Vargas et al. 2001; Piñero et al. 2006; Vayssières et al. 2008). The results of this study confirm this observation and show that yellow is more attractive to *B. cucurbitae* in field cages than white, red or transparent. We also found that colors with high reflectance are the most attractive to *B. cucurbitae* as Piñero et al. (2009) also reported in their study.

Adult fruit flies locate hosts using visual and olfactory stimuli (Prokopy 1986). Thus to be effective, a bait station needs to integrate visual and chemical cues (Espsky and Heath 1998). Ammonia is the main attractant of Synéis-appât, flies use this compound to locate sources of decaying protein (Mazor et al. 2003; Piñero et al. 2011). The visual stimuli of the bait station enhance the attractiveness of the Synéis-appât, especially when the compounds, such as ammonium acetate, volatilize. This phenomenon occurs some hours or days after the bait is sprayed (Bateman and Morton 1981; Prokopy et al. 2003; Yee 2007). Because spinosad based baits do not attract fruit flies from a long distance (Pelz-Stelinski et al. 2006), the use of a visual stimuli thus could give the bait station a longer range of action (even if visual cues do not act over long distances) and thereby could increase the efficacy of the device (Foster and Harris 1997). The feeding stimulant enhances the contact of the flies with the insecticide (Foster and Harris 1997). The combination of visual and chemical stimuli is frequently used in attract and kill methods and increases their efficacy compared with using one kind of stimulus alone (Foster and Harris 1997).

Taking into account our results in field cage experiments and those obtained in the study of Piñero et al. (2009), we chose to develop a local bait station adapted to our agroecosystem situation. Farmers' acceptance of new crop protection methods usually depends on cost but also on availability. It is important to design a device made of local low cost material that is easy to find, to build and to use. The aim of this study was to design a bait station adapted to these constraints. Our bait station was first developed to protect bait sprays against rainfall and then to enhance attractiveness of the bait when combined with yellow color.

In field trials, our local bait station appeared to be at least as effective as the «umbrella trap » from Taiwan for the three species of cucurbit fruit flies tested. It was found to be even more efficient against *B. cucurbitae*. These results are encouraging and show the ability of the local bait station to control the three species of fruit flies in cucurbit fields.

In Reunion Island, our Synéis-appât bait station is well suited for use in high rainfall zones and with chayote trellises and has been shown to be effective for the control of cucurbit flies. It is now well accepted by farmers (Deguine et al. 2011). Our local bait station is simple, effective, safe, and it can be used for a period of one year (Rousse, personal communication). This method is now an integral part of an area-wide program against cucurbit fruit flies, called GAMOUR, relying on the axioms of agroecological crop management: sanitation, habitat management and conservative biological control (Deguine et al. 2012a).

Biondi et al. (2012) showed that spinosad-based baits can have a lethal effect on different groups of arthropods, including beneficial insects. In the case of the local bait station, the Synéis-appât is only applied to the plastic part, and the environmental impact is thus less than that of spot sprays. However, the yellow visual stimulus is not specific to tephritid fruit flies and has been used on traps for monitoring certain beneficial insects, such as hover flies (Laubertie et al. 2006).

Beneficial insects may be attracted by the bait station and come into contact with the toxic substance of Synéis-appât. The impact of the local bait station on other arthropod populations thus needs to be further investigated. Future research should also be conducted to determine the optimal density of bait stations in open cucurbit fields and under chayote trellises. The duration of the bait station efficacy needs to be evaluated to know when to renew the application. It is important to determine the frequency of the Synéis-appât application and the shelf life of the plastic part of the bait station when exposed to climatic conditions. The local bait station should also be tested against other fruit fly species, mainly those that attack mango and citrus (*Bactrocera zonata*, *Ceratitis rosa*, and *C. capitata*) which are also important crops in Reunion Island.

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