# Adjustment of Field Cage Methodology for Testing Food Attractants for Fruit Flies (Diptera: Tephritidae)

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**ABSTRACT** To select efficient baits to attract fruit flies (Diptera: Tephritidae), the use of field cages represents a good compromise between laboratory and outdoor studies. Nevertheless, the methodological details of such experiments up to now have had little attention. To assess the influence of intrinsic and extrinsic factors on the efficiency of food attractants for *Bactrocera cucurbitae* (Coquillett), a series of methodological experiments were conducted in field cages with McPhail traps. Baited traps should initially be randomly placed and furthermore regularly rotated to lower the influence of climatic conditions. During an experiment lasting 8 h, the presence of already captured flies did not influence the attractiveness of the traps. The presence of potted host plants in the field cage allowed for better dispersion of the flies and enhanced the discrimination potential of the experiment. Moreover, yellow traps should be painted black to limit visual bias. Finally, in the conditions of these experiments, the sex of tested flies and the diet given to them during rearing, with or without protein, had no influence on the qualitative results of choice experiments. However, these factors greatly influenced the total amount of captured flies: protein-deprived females were more responsive than all others. Furthermore, whether the tested flies were sexually mature had a significant influence on their responsiveness to protein baits. These results are discussed to establish recommendations for further field cages experiments.

**KEY WORDS** field cages, methodology, Tephritidae, *Bactrocera cucurbitae*, protein baits

FRUIT FLIES (DIPTERA: TEPHRITIDAE) have been causing serious damage to fruit and vegetable crops for a long time in various parts of the world, and a large amount of research has been devoted to looking for food attractants for different species, particularly with the aim to use them in bait sprays (Roessler 1989) or monitoring (Matsumoto et al. 1985).

Screening of potential food attractants has been carried out in small cages in the laboratory (Robacker 1991, Robacker et al. 1991, Nigg et al. 1995) or in field assays with traps (Cunningham et al. 1978, Malavasi et al. 1990, Epsky et al. 1993, Heath et al. 1994). However, such experiments are more and more frequently conducted in field cages where seminatural conditions are created by introducing potted host plants. Field cage treatments represent a compromise between laboratory and field experiments. The food baits to be tested are presented in traps (Beroza and Green 1963, Bateman and Morton 1981, Fabre et al. 2003, Duyck et al. 2004), self-made plastic containers (Vickers 1997), or droplets on leaves (Prokopy et al. 1992, Vargas et al. 2002). Flies are released in the cages, and their relative recapture rate, in the different traps, for example, assesses the relative attractiveness of the tested products.

Nevertheless, the lack of standard protocol may lead to difficulty in interpreting or comparing results. The flies may be protein starved (Prokopy et al. 1992) or not (Fabre et al. 2003, Duyck et al. 2004). Some studies use yellow traps (Vickers 1997), whereas others use black-painted traps to avoid a possible visual bias (Fabre et al. 2003, Duyck et al. 2004). With respect to sexual maturity, the relative age of flies to be tested varies with the species studied and on the purpose of the experiment. For example, Prokopy et al. (1992) and Vargas et al. (2002) used 2–4-d or 10-d-old *Ceratitis capitata* (Wiedemann), respectively, Fabre et al. (2003) and Duyck et al. (2004) used 15–25-d-old *Bactrocera cucurbitae* (Coquillett), and Vickers (1997) used 7–11-d-old *Bactrocera tryoni* Frogatt.

This study aims to assess the influence of such factors. First, we examined whether the initial randomization and regular rotation of the traps within the cages were necessary. Then, the possible effect of the presence of flies in the traps was assessed. Also, we evaluated whether the presence of host plants interfered with fly distribution within the cages. Because tephritids respond to visual cues of wavelengths ≈500 nm (Agee et al. 1982), we tested whether the yellow color of the common plastic McPhail traps would interfere with the olfactory stimuli of the baits. Finally,

**Table 1. Range of temperature and relative humidity recorded during the experiments by the meteorological station of CIRAD, St Pierre**

| Date | Exp | Range (°C) | Avg temp (°C) | Relative humidity (%) |
|---|---|---|---|---|
| 03 July 2001 | 1/2 | 15.1–23.7 | 19.8 | 54–85 |
| 04 July 2001 | 1/2 | 16.3–24.0 | 20.5 | 63–90 |
| 10 Oct. 2001 | 3 | 17.1–26.6 | 21.2 | 46–87 |
| 11 Oct. 2001 | 3 | 17.2–27.7 | 21.2 | 55–87 |
| 17 Oct. 2001 | 3 | 18.1–27.2 | 21.6 | 45–98 |
| 18 Oct. 2001 | 3 | 16.9–26.4 | 21.5 | 67–100 |
| 16 July 2002 | 4 | 15.3–24.7 | 19.3 | 49–95 |
| 19 July 2002 | 4 | 14.6–24.5 | 19.0 | 47–89 |
| 23 July 2002 | 4 | 14.8–24.8 | 19.4 | 50–89 |
| 26 July 2002 | 4 | 16.4–26.4 | 20.3 | 58–93 |
| 09 July 2003 | 5 | 16.1–23.1 | 18.8 | 74–100 |
| 11 July 2003 | 5 | 17.7–23.0 | 19.9 | 74–98 |
| 01 Aug. 2003 | 5 | 13.8–21.2 | 16.3 | 63–96 |
| 22 Aug. 2003 | 5 | 13.5–25.5 | 18.9 | 51–87 |

because age and nutritional status of the flies have been proven to influence the response of tephritids to various stimuli (Chu and Lu 1987, Robacker 1991, Nigg et al. 1995), we looked at their possible influence on responses to food attractants. The experiments were conducted on the melon fly, *B. cucurbitae*, a species of great economic importance in many countries (Nishida and Bess 1957, Vijaysegaran 1985, Vayssières and Carel 1999, Sookar and Khayratee 2000).

## Materials and Methods

This study was conducted from 2001 to 2003 in the Agricultural Research Centre for International Development (CIRAD) experimental station of Saint-Pierre (Reunion Island, France). The range of temperature and relative humidity recorded during these experiments is reported in Table 1.

**Flies.** Wild pupae of *B. cucurbitae* were collected in June 2000 from infested pumpkin, *Cucurbita maxima* Duchesne, in three localities of Reunion Island (Petite Ile, Bassin Martin, and Piton Saint-Leu). Adult flies obtained from these samples were reared under controlled conditions of 25 ± 2°C, 70 ± 20% RH, and a photoperiod of 12:12 (L:D) h. They were given free access to granulated sugar, enzymatic yeast hydrolysate (ICN Biomedicals, Aurora, OH), and water. Three times a week, for 1 h, zucchini, *Cucurbita pepo* L., was used as an oviposition substrate. The larvae were subsequently fed on pumpkins and dehydrated potatoes. Pupae were then collected, and, from the beginning of emergence, adults were kept in 40 by 40 by 40-cm cages. With the exception of the last experiment, all flies were collectively provided sugar, protein, and water and were protein starved 24 h before each assay. When released into the field cages, adult flies were 15–25-d-old and were sexually mature (Vargas et al. 1984).

**General Procedure: Field Cages and Traps.** The experiments were conducted in cylindrical mesh-screened field cages (2.5 m in height by 3 m in diameter, Synthetic Industries, Gainesville, GA). Except when otherwise specified, 20 potted plants of *Cucurbita moschata* variety Martinica (Technisem, France) were placed in each field cage to provide the flies with seminatural conditions. These plants were replaced in 2003 by a dozen of more long-lived potted pumpkins (local variety). Except when otherwise specified, food baits were tested in bottom black painted plastic McPhail traps (Dome Trap, Agrisense, United Kingdom), with each trap containing 200 ml of solution. Traps were hung on an H-shaped base, 50 cm above the plant canopy, and arranged along a 1-m radius imaginary circle around the center of the field cage. Unless otherwise specified, four traps were randomly placed at the beginning of each experiment and then moved 90° clockwise along the circle every 2 h to minimize the influence of trap position. A fifth trap filled with water was placed centrally as control. Two hundred and fifty flies of a given sex were released per cage. Each experiment lasted 8 h and was repeated four times for each sex.

**Specific Methodology.** The points of the general procedure described above were modified to assess the influence of specific parameters. Depending on the experiment, the same solution was used in the four traps, or a series of treatments were presented as a choice experiment. In the latter case, the purpose was not to discriminate among the treatments, which has already been done by previous research (Fabre et al. 2003, Duyck et al. 2004) but to assess the impact of methodological modifications.

*Experiment 1, Three Treatments. Influence of Trap Position.* A 5% solution of liquid protein hydrolysate (Buminal, Bayer, France) was used in the four traps. In the first treatment, the initial respective positions of the four traps were identical in all cages, and the traps were kept in place during the whole experiment. In the second treatment, the traps were similarly initially set up but were rotated every 2 h. In the third treatment, the traps were differentiated by a number, and their initial position was randomized at the beginning of the experiment and then rotated every 2 h.

*Experiment 2, One Treatment. Influence of Number of Daily Recordings.* Buminal 5% was used in the four traps. Two of the traps were checked (by counting and removing the flies inside) every 2 h, whereas the two other traps were only checked at the end of the day.

*Experiment 3, Two Treatments. Influence of the Presence or Absence of Host Plants.* The same experiment (comparison of Buminal 5% at pH 3, 5, and 6, and water) was conducted with two treatments. In the first treatment, 20 potted plants of *C. moschata* were placed into the cage, whereas in the second treatment, the plants were removed from the field cages.

*Experiment 4, Two Treatments. Influence of Trap Color.* The same experiment (comparison of Buminal at 1, 5, 10, and 50%, and water) was conducted with two treatments. In the first treatment, yellow McPhail traps were used, whereas in the second treatment, the bottom of all traps was painted black to minimize a potential response of the flies to yellow.

*Experiment 5, Two Treatments. Influence of Age and Food Status of Flies.* The same experiment (comparison of a 5% Buminal solution and a papaïn-hydrolyzed solution of brewery waste) was conducted with two treatments. The brewery waste (Brewery "3 Brasseurs," Saint-Pierre, La Réunion, France) was boiled to reduce it to the most solid fraction possible (35–40% dry matter). Then, it was diluted in 6 volumes of water and hydrolyzed for 24 h at 65°C with 2% papaïn (Lloyd and Drew 1997).

In the first treatment, the effect of diet offered to 3–6-d-old flies was studied: four cages (two for males and two for females) received 250 normally fed flies, whereas four others received 250 protein-starved flies. The latter were given no protein hydrolyzate during their rearing process, and the cages were carefully washed with alcohol, detergent, and antifungal weekly to minimize the development of any commensal microorganisms that could be used as a protein source by flies (Drew and Lloyd 1989, 1991). Dissections were made to ensure that protein-starved females were not gravid. In the second treatment, the methodology was similar except that all flies were 15–20-d-old.

**Statistical Analysis.** For all experiments except experiment 2, the data analyzed for each cage were the total number of flies caught in one trap during 1 d divided by the total number of flies caught in all traps of the cage during the same day, i.e., the relative attractiveness of each trap. These percentages were transformed to stabilize the variance before analysis ($2\arcsin\sqrt{x}$). This analysis was necessary to minimize the variation of captured flies in relation with the varying climatic conditions during each experiment (Table 1).

Then, data were analyzed by a two-way analysis of variance (ANOVA) with first order interactions. The factors taken into consideration were sex (two levels) and trap content (three, four, or five levels according to the experiment). When trap content was identical (i.e., in experiments 1 and 2), the traps were differentiated by a number. When the $F$ value was significant ($P < 0.05$), Tukey's mean separation test was used. For all experiments, an ANOVA was made for each treatment. Then, the different ANOVA results of the same experiment were visually compared. For experiment 5, the factor diet (two levels) was added and a three-way ANOVA was performed for both tested ages. For the experiment 2, the data analyzed were the total number of flies caught in one trap during 1 d, which represent the absolute attractiveness of each trap.

## Results

Except in the last experiment, no sex effect was observed ($P > 0.05$ in all experiments), which meant that the relative or absolute (experiment 2) attractiveness of each trap/bait was similar for both sexes.

**Experiment 1: Influence of Trap Position.** Without any rotation nor initial randomization of the traps, a significant difference between the relative attractiveness of traps baited with the same attractants was

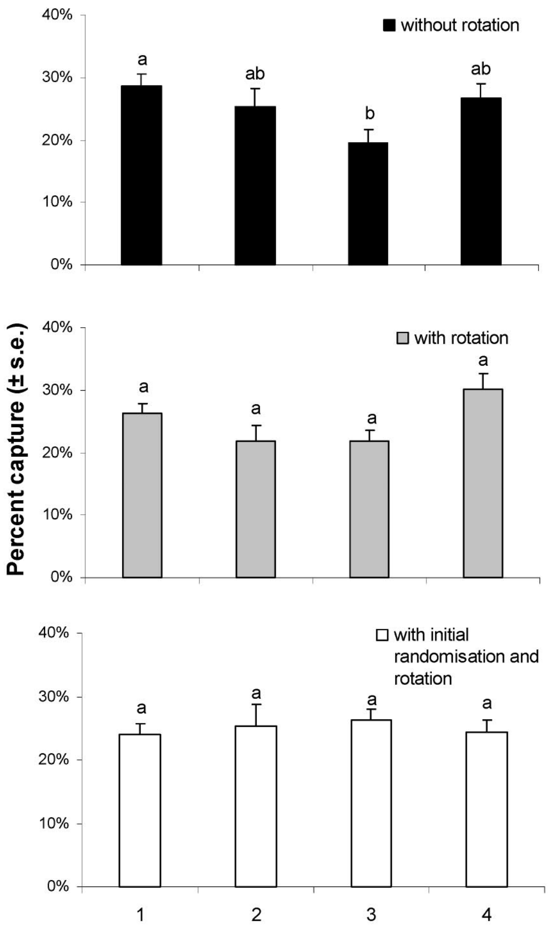


**Fig. 1.** Mean percentage of *B. cucurbitae* captured in four McPhail traps baited with Buminal 5% when using different trap placement methods. Data for males and females are combined. Bars with different letters are significantly different (1–4, four quarters of the field cage).

obtained ($F = 1653.72$; df = 3, 24; $P < 10^{-6}$). This difference disappeared when traps were rotated without initial randomization ($F = 3.30$; df = 3, 24; $P = 0.103$). However, the probability that trap position has no influence is higher when traps were randomly placed at the start ($F = 0.14$; df = 3, 24; $P = 0.932$) (Fig. 1).

**Experiment 2: Influence of Number of Daily Recordings.** There was no significant difference between the two recording methods ($F = 14.16$; df = 2, 34; $P < 10^{-4}$). Whether captured flies were counted and removed every 2 h (mean capture 26.3 ± 3.91) or counted once at the end of the experiment (mean capture 27.94 ± 2.65) did not influence the total number of flies caught.

**Experiment 3: Influence of Presence or Absence of Host Plants.** There was a significant effect of the pH on the relative attractiveness of Buminal with and without host plants ($F = 8.31$; df = 3, 24; $P < 10^{-3}$ and

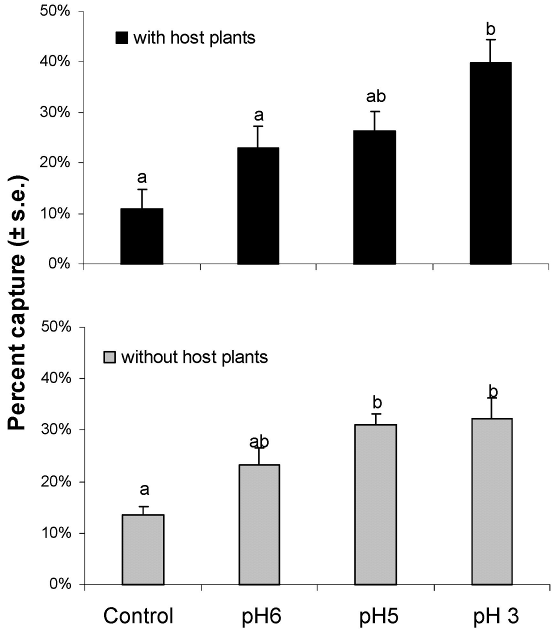


Fig. 2. Mean percentage of *B. cucurbitae* captured in McPhail traps baited with Buminal 5% at different pHs, with or without host plants. Data for males and females are combined. Bars with different letters are significantly different.

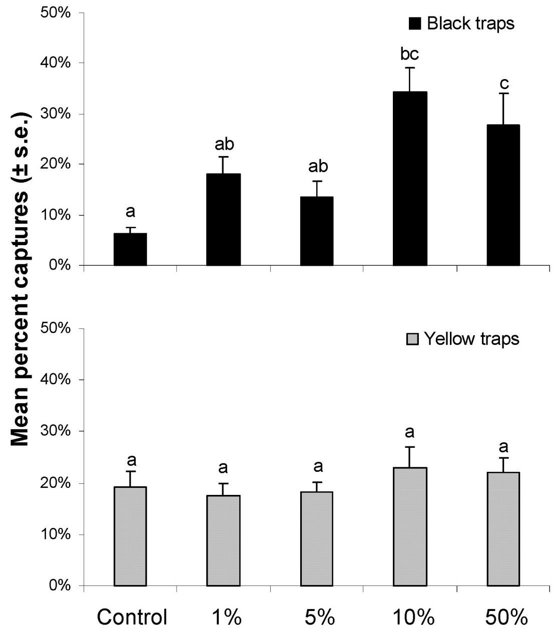


Fig. 3. Mean percentage of *B. cucurbitae* captured in five black or yellow McPhail traps containing Buminal at different concentrations. Data for males and females are combined. Bars with different letters are significantly different.

$F = 14.51$; df = 3, 24; $P < 10^{-3}$, respectively). However, the distribution of means differed between the treatments. The presence of host plants increased the differences between the means of captures in different traps and allowed detection of a significant difference in attractiveness between the Buminal solutions at pH 3 and 6 (Fig. 2).

**Experiment 4: Influence of Trap Color.** In the treatment using black-painted traps, there was a significant difference between the relative attractiveness of traps ($F = 5.66$; df = 4, 30; $P = 0.002$), whereas this difference was not significant when we used yellow traps ($F = 0.55$; df = 4, 30; $P = 0.699$) (Fig. 3). Regardless of the solution the trap contained, the proportions of flies captured in the yellow traps were not statistically different. By contrast, the use of black-painted traps enabled us to show significant differences in attractiveness between different protein bait concentrations.

**Experiment 5: Influence of Age and Food Status of Flies.** The bait contained in the traps had a significant effect on attractiveness for 3–6-d-old flies and 15–20-d-old flies ($F = 22.14$; df = 2, 14; $P < 10^{-4}$ and $F = 48.27$; df = 2, 14; $P < 10^{-5}$, respectively). Age had an effect on the response of flies to the traps: when flies were young, they were equally attracted to boiled brewery waste and Buminal. When they were older, they showed a preference for Buminal (Fig. 4).

Moreover, significant effects of the sex ($F = 11.97$; df = 1, 8; $P = 0.009$), diet ($F = 5.40$; df = 1, 8; $P = 0.049$) and diet × sex ($F = 5.62$; df = 1, 8; $P = 0.045$) factors were noticed. Females were captured significantly more than males, and protein-starved females came to the traps more than did protein-fed females. The diet had no significant effect on the number of males cap-

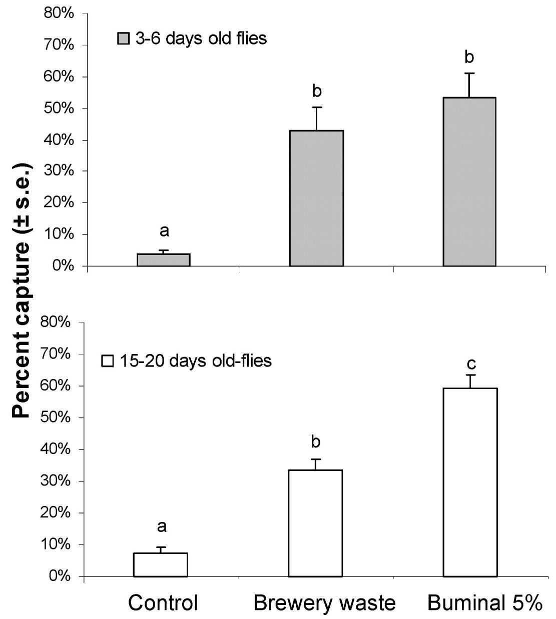


Fig. 4. Mean percentage of *B. cucurbitae* of two ages captured in three differently baited McPhail traps. Data for males and females are combined. Bars with different letters are significantly different.


**Fig. 5.** Mean total *B. cucurbitae* captured in experiment 5 according to their sex and nutritional status. Bars with different letters are significantly different.

tured, and age had no significant effect on the whole amount of captured flies (Fig. 5).

## Discussion

Usually, attractants for fruit flies are tested in the laboratory (in cages, wind tunnels, or olfactometers) or in the open field. Indeed, if laboratory tests allow a rapid screening of numerous products, their results need to be confirmed in field experiments. Also, numerous intrinsic and extrinsic parameters, which may modify the results, are sometimes ignored in laboratory experiments for greater convenience. For example, some studies stressed the influence of climatic conditions (Cunningham et al. 1978), physiological state of wild fly populations (Rull and Prokopy 2000), or temporal evolution of tested products (Epsky et al. 1993). Moreover, in some studies, the results of laboratory tests are barely confirmed in the field (Sharp 1987, Lloyd and Drew 1997, Vickers 1997).

However, field experiments carry costs as well: they are time-consuming (Gow 1954, Heath et al. 1994, Deshmukh and Patil 1996) and generally do not enable the testing of as many products as in laboratory tests. Moreover, the small number of flies caught in periods of low populations sometimes prevents a proper statistical analysis (Malavasi et al. 1990, Vickers 1997). Using a field cage method seems to be a good compromise to assess the relative attractiveness of several baits tested simultaneously (Beroza and Green 1963).

The current study enabled us to estimate the impact of several methodological parameters on the results of some field cage tests. The influence of trap rotation pinpoints the importance of climatic conditions. As for many insects, fruit fly behavior is largely influenced by light, wind, and heat. Thus, the position of the sun during assays may influence the distribution of flies within the field cages. The results show the importance of at least rotating the traps regularly to suppress the bias linked with abiotic factors.

Dead flies may alter the chemical composition of the bait and modify its attractiveness, whereas living flies might emit attractive or repulsive volatiles. Our results show that there are no such effects during an 8-h treatment on *B. cucurbitae.* Moreover, a single recording of captures at the end of the day is less time-consuming.

Host plants probably attract a portion of the flies present in the field cage, because there are fewer flies resting on the walls of the cage. This does not seem to modify the preferences of the flies for the tested baits. However, more flies are caught in the presence of host plants and the standard errors are reduced, allowing better accuracy of the ANOVA. Therefore, minor differences in attraction between baits may be pointed out.

Whatever the content of the yellow traps, the flies disperse equitably among them, which probably reflects the predominance of visual stimuli in the limited space within the field cage. These results tend to confirm that visual stimuli are probably predominant at a short distance, as they have been shown to be during host plant location processes (Prokopy 1983). It is probable that, like other phytophagous insects (Visser 1986) and pest flies (Notthingham 1988, Brévault 1999), *B. cucurbitae* is primarily responsive to long-range olfactory stimuli, whereas visual cues outdo the former in the vicinity of the target. Thus, it seems better to avoid the presence of attractive visual stimuli, such as a yellow color, when experiments on olfactory stimuli are to be conducted in a field cage.

Although most studies showed differences in attractiveness of food baits for males and females of tephritid species (Braga and Zucoloto 1981, Morton and Bateman 1981, Dodson 1982, Mason and Baranowski 1989, Prokopy et al. 1992, Vargas et al. 2002), Sharp (1987) mentions that female and male attraction to some hydrolysates may be equal. In terms of intensity of response, Robacker et al. (1991) found that male and female *Anastrepha ludens* (Loew) responded more or less equally to the odor of the bacterium *Staphylococcus aureus* Rosenbach. Moreover, from a qualitative point of view, Fabre et al. (2003) found no difference in the choice of males or females between a series of food attractants in their experiments on *B. cucurbitae.*

Our results showed that sex and diet had no effect on the distribution of flies among the traps. However, these factors influenced the number of captured flies, so that we caught numerically more females, especially when they were protein starved, than males. Thus, these factors modified the results quantitatively but not qualitatively. As mentioned previously, this may induce a greater accuracy of the statistical analysis and may enable us to observe minor differences between the relative attractiveness of baits. In *Rhagoletis pomonella* (Walsh), Rull and Prokopy (2000) found that the presence of food had little influence on responsiveness of either sex to host plant stimuli, whether they were protein fed or deprived and whether they were sexually mature or not. By contrast, bacteria, which contain a high proportion of protein (Johnson 1978), are especially attractive for previously yeast hydrolysate-deprived *A. ludens,* particularly during the ovarian maturation (Robacker 1991, Robacker and Garcia 1993). However, *A. ludens* adults not deprived of yeast hydrolysate also are very responsive to bacterial odor (Robacker and Garcia 1993).

Several studies have stressed the influence of age on responsiveness of tephritid flies to various attractants. Thus, the choice of the age of tested flies largely depends on the goal of the study. For example, Chu and Lu (1987) showed that the number of captured males of *B. cucurbitae* with cue-lure increases with the age of the cohort. Moreover, Robacker (1991) showed an increasing attractiveness of protein versus sugar to *A. ludens* females when they become sexually mature. Even when they were protein starved, immature males and females of *R. pomonella* showed little responsiveness to food or oviposition site stimuli (Rull and Prokopy 2000).

According to these results, some general recommendations can be made as to the methodology to be used for future field cage experiments. 1) Treatments should be placed in traps painted in an unattractive color, 2) initially randomized, and then 3) regularly rotated. 4) Potted plants should be placed within the field cages to provide a convenient seminatural environment, 5) the tested flies should be protein deprived from emergence onward, and 6) the age of flies should be selected according to the species tested (according to its sexual maturity) and the purpose of the experiment (food or sexual bait).

Like for laboratory tests, the results of field cage experiments should ideally be confirmed by field tests. However, a preliminary negative result obtained with field cage experiment avoids a labor-intensive and time-consuming field experiment. In any case, the field cage method allows a rapid and easy screening of numerous food attractants in outdoor conditions closer to a field situation.

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