

Effectiveness of foliar protein bait sprays in controlling *Bactrocera invadens* (Diptera: Tephritidae) on sweet oranges

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Abstract — Introduction. Damage caused by tephritid fruit flies has been identified as a major limiting factor to citrus production in Nigeria. Presently, no control measure is applied by the majority of the farmers. This study therefore seeks to evaluate the efficacy of locally-made protein bait sprays and the differences in the susceptibility of sweet orange varieties in reducing fruit fly damage. **Materials and methods.** In 2006 and 2007, one, two and three spot applications of locally-made protein bait mixed with chlorpyrifos insecticide were administered to Agege, Parson Brown and Valencia Late sweet oranges. Treatments were arranged in a randomized complete block design. Efficacy of the bait sprays in reducing fruit attack was assessed by randomly sampling 10 ripe or ripening fruits per stand as well as dropped fruits. **Results and discussion.** The bait sprays significantly ($p < 0.05$) reduced damage of sweet orange by lowering the infestation level of *Bactrocera invadens* Drew Tsurata & White, and consequently reducing the number of dropped fruits. Application of three spots of bait was most effective in reducing damage due to *B. invadens* on sweet orange varieties. The infestation level of *B. invadens* and the number of dropped fruits of the variety Agege were significantly ($p < 0.05$) higher than those of the varieties Parson Brown and Valencia Late. **Conclusion.** Our study shows that three spot applications were most efficacious in reducing fruit damage, and that the Valencia Late variety was the least damaged, followed by Parson Brown.

Nigeria / *Citrus sinensis* / fruits / Tephritidae / insect control / damage / fruit drop / chemical control / application methods / application rates

Efficacité de pulvérisations foliaires d'appâts protéinés pour le contrôle de *Bactrocera invadens* (Diptera: Tephritidae) sur orangers.

Résumé — Introduction. Au Nigéria, les dommages causés par les mouches des fruits ont été identifiés comme étant un facteur limitant majeur de la production d'agrumes. À l'heure actuelle, aucune mesure de lutte n'est appliquée par la majorité des agriculteurs. Cette étude vise donc à évaluer l'efficacité de pulvérisations d'appâts protéinés fabriqués localement et à mettre en évidence des différences de sensibilité de variétés d'orangers vis-à-vis de l'attaque des mouches des fruits. **Matériel et méthodes.** En 2006 et 2007, des traitements localisés, effectués avec un appât protéiné fabriqué localement et mélangé avec un insecticide, le chlorpyrifos, ont été pulvérisés à raison d'une, deux ou trois taches par arbre appartenant à chacune des trois variétés d'orangers : Agege, Parson Brown et Valencia Late. Les traitements ont été disposés en blocs aléatoires complets. L'efficacité des pulvérisations d'appât sur la réduction de fruits affectés a été évaluée sur un échantillonnage aléatoire de 10 fruits mûrs ou mûrissants par arbre étudié ainsi que par l'étude des fruits tombés sous les arbres traités. **Résultats et discussion.** Les pulvérisations d'appâts ont réduit de façon significative ($p < 0,05$) les dommages évalués sur les fruits en abaissant le niveau d'infestation par *Bactrocera invadens* Drew Tsurata & White et, par conséquent, en réduisant le nombre de fruits tombés. Le traitement localisé effectué par la pulvérisation d'appâts sur trois taches par arbre a été le plus efficace pour réduire les dommages dus à *B. invadens* sur les variétés d'oranges étudiées. Le niveau d'infestation de *B. invadens* et le nombre de fruits tombés de la variété Agege ont été significativement ($p < 0,05$) plus élevés que ceux des variétés Parson Brown et Valencia Late. **Conclusion.** Notre étude montre que des traitements localisés sur trois taches par arbre ont été les plus efficaces pour réduire les dommages des fruits, et que la variété Valencia Late a été la moins attaquée, suivie par la variété Parson Brown.

Nigéria / *Citrus sinensis* / fruits / Tephritidae / lutte anti-insecte / dégât / chute de fruit / lutte chimique / méthode d'application / dose d'application

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1. Introduction

Citrus is the most widely grown fruit crop in Nigeria. The recent awareness created in the country of the need to increase fruit intake and the presidential initiative on fruit tree production have contributed to the establishment of many more citrus orchards. However, pests and diseases have been identified as major limiting factors to citrus production [1, 2]. While some insect pests are noted for contributing to citrus decline [3], some play a major role in reducing fruit yields and rendering them unacceptable to consumers [4, 5].

Most often, damage of citrus fruits by fruit flies (Diptera: Tephritidae) results in economic losses. The situation is worsened by the recent introduction of an invasive species, *Bactrocera invadens* (Drew, Tsurata and White), a highly prolific and polyphagous species. It seems to have displaced the previously prevalent indigenous citrus fruit flies, *Ceratitidis* spp. [6, 7]. Observations have shown that more than 70% of fruit set can be lost due to fruit fly infestation in Nigeria [2].

The majority of citrus producers in Nigeria are small-holders who do not adopt standard citrus production practices, usually because of lack of funds. Insecticide application has been the readily available control method for insect pests of crops in Nigeria. However, for treated citrus fruits that are usually harvested by farmers without observing the necessary time interval between application and harvest, there is high risk of health hazard occurrences. Available environmentally-friendly fruit fly control methods include the application of the Male Annihilation Technique (MAT) by mass trapping with synthetic attractants fixed in traps [8–10]. Biological control agents have been tried but commercial application is still non-existent and their success in African farmers' situations still remains uncertain. Nevertheless, successful tests have proved more than 70% parasitism of *B. invadens* eggs by *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) [11–13]. In Nigeria, the majority of farmers have little or no knowledge of the presence of natural enemies and their conservation.

They are also not aware of any existing regulation on the types of pesticides to use, or the modes of usage that are acceptable without constituting danger to the environment, humans and natural enemies of fruit flies. Use of food bait (Bait Application Technique – “BAT”) either as commercially available protein hydrolysates or locally-made alternatives (protein autolysates) from brewery wastes shows good prospects [14]. The raw materials are cost-effective since they are usually discarded by breweries as spent yeast and are readily available due to the large number of breweries present in Nigeria [15].

Any fruit fly intervention to be developed for Nigeria must be readily available, affordable and acceptable to low-income farmers, who constitute the majority of citrus producers. Area-wide control programs are highly recommended since they will ensure reduction in the incidence of re-infestation from sites where control measures were not undertaken [16]. There is therefore the need to evaluate simple fruit fly control methods such as the use of locally-made bait sprays that can be adopted by small-scale farmers. The objective of our study was therefore to test the efficacy of foliar bait sprays made from brewery waste, and varietal differences in the reduction of fruit fly damage on sweet oranges.

2. Materials and methods

2.1. Preparation of crude protein bait

Bulked brewery yeast waste (yeast slurry) of Star™ and Gulder™ lager beer was obtained courtesy of the manufacturers; the Nigerian Brewery Ltd. Preparation of brewery waste was done at the National Horticultural Research Institute (NIHORT) using the method of Umeh and Garcia [15], which is a modification of Gopaul's method [17]. The brewery waste was poured into a 3-L aluminum pot immersed in a 5-L open vessel containing water. The vessel was heated with a hot plate and left to boil for 15 h. The process caused some degree of yeast cell autolysis and resulted in amber-colored

thick slurry with a strong yeast-like odor (about 7.5% protein). This was carried out repeatedly for the quantity of crude protein bait material needed. For each preparation, the pot was allowed to cool down to room temperature before use.

2.2. Field application of foliage bait spray

The orchard used for the trial was located at the National Horticultural Research Institute (NIHORT), Ibadan (3°5' E and 17°3' N). The trees were ≈ 2.8 m tall and ≈ 2.5 m in diameter along the outermost part of the canopy. Spot applications of the locally-made protein bait mixed with chlorpyrifos insecticide at $1 \text{ mL}\cdot\text{L}^{-1}$ of protein bait were carried out on three varieties of sweet orange (Agege, Valencia Late and Parson Brown) in two years (2006 and 2007). There were four bait treatments including the control with no bait application. The bait treatments, which included one, two and three spot applications, were separately applied to each sweet orange tree. Two trees out of the three arranged per variety were randomly picked for each treatment. The field layout was factorially arranged in a randomized complete block design with three replications. Thus, a total of 72 trees were used (2 trees \times 3 varieties \times 4 bait treatments \times 3 replicates). Each spot spray covered $0.5 \text{ m} \times 0.5 \text{ m}$ (0.25 m^2) of sweet orange foliage only. Trees earmarked for a particular number of spot treatments were maintained for the same treatment until the completion of the trial. The bait sprays were applied four times at intervals of two weeks, starting when the fruits were nearing maturity (5–6 cm in diameter). At this stage, fruit acidity was still high [18]. Spraying was done using a knapsack sprayer with a nozzle that dispenses long-distance spray jets. In all stands used for the trial, spraying the fruits was avoided as much as possible.

2.3. Field observation and sampling

Efficacy of the bait sprays in reducing fruit infestation was assessed by *in situ* observa-

tion of 10 ripe or ripening fruits per earmarked stand (with the aid of a hand lens) for fruit fly entry spots or damage. The above fruits that were observed for damage were sampled from five points each in the upper and lower halves of the circumference of the citrus canopy. All fruits were sampled at ≈ 30 cm from the open edge of the canopy. To confirm the species of fruit flies that contributed to sweet orange damage and the number of fruit flies harbored by the fruits in each treatment and variety, another set of 10 fruits was randomly harvested per sampled tree and added to the ones inspected (totaling 20 fruits). The latter were also used to assess the relative efficacy of the spot sprays in reducing fruit fly infestation by counting the number of adult fruit flies emerging from the fruits. To culture the larvae in the fruits, each set of 10 fruits was weighed, placed in a labeled wooden cage ($0.4 \text{ cm} \times 0.6 \text{ cm} \times 0.6 \text{ cm}$) at NIHORT insectaries and observed daily until adult fruit fly emergence. Each cage was covered at the sides by wire gauze, at the top by glass and at the base by sand-covered plywood. Fruit fly adults were captured after allowing an interval of three days post-emergence. They were counted and identified using available identification keys [4, 19]. The number of fruits that dropped due to fruit fly damage were examined and counted underneath each candidate tree. The dropped fruits were picked and buried away from the site after fruit observation and data taking. Furthermore, five out of the dropped fruits were randomly picked, weighed and set aside per treatment and per sweet orange variety for rearing of *B. invadens* and *C. capitata* larvae to adulthood where present.

2.4. Statistical analysis

Data collected for the number of fruit flies that emerged from fruit cultures (*i.e.*, fruits that were incubated in the laboratory) were transformed using square root transformation $(X + 0.5)^{0.5}$. The effect of insecticidal sprays and varieties on fruit damage and number of emerging *B. invadens* and *C. capitata* in fruit cultures were separately computed using PROC SORT [20]. All

parameters assessed were subjected to analysis of variance (ANOVA) procedures using SAS software [20]. Interactive effects between insecticidal treatments and varieties on assessed parameters were also computed in the ANOVA. Means of significantly different treatments were separated using the Student-Newman-Keuls test. Simple linear correlation analyses were conducted to determine the relationships between the numbers of fruit flies that emerged from the harvested fruits and their weights in 2006 and 2007; and the number of fruit flies that emerged from the harvested fruits and the number of dropped fruits. All tests were judged significant at $P \leq 0.05$.

3. Results and discussion

3.1. Effects of protein bait spray and variety on fruit fly damage

The most abundant fruit fly species associated with sweet orange in the study site was *Bactrocera invadens* (Drew, Tsurata and White). The presence of *Ceratitidis capitata*

(Wied) and *C. (Pardalaspis) ditissima* (Munro) was low ($\approx 3\%$), based on the number of adults that emerged from fruits incubated in the laboratory. This result corroborates other works reported on the displacement of indigenous fruit fly species by the invasive *B. invadens* [6, 7]. An earlier study conducted in the orchard in the mid- and late 90s showed that the predominant fruit flies belonged to the genus *Ceratitidis* [2], with no occurrence of *B. invadens*. In 2006 and 2007, the control (*i.e.*, sweet orange stands with no protein bait spray) had significantly ($P < 0.05$) higher numbers of attacked or dropped fruits than all the treated stands except those on trees given 1 or 2 spot applications or dropped fruits from trees given 2 spot applications in 2006 (*table I*).

The attractiveness of protein bait in the control of fruit flies other than *B. invadens* has been reported by various authors [8, 14, 15, 17]. The ultimate goal is to reduce fruit damage while minimizing insecticidal contact with fruits. The least numbers of infested fruits (0.9 and 1.3 fruits) were, respectively, observed in 2006 and 2007 in stands given 3 spot applications. However,

Table I.

Effects of different levels of protein bait spot applications on fruit fly damage on three varieties of sweet oranges (*Citrus sinensis*) in 2006 and 2007 (10 fruits sampled per tree, Nigeria).

Treatments	Mean number of fruits attacked on tree		Mean number of dropped fruits per tree		Mean total number of damaged fruits per tree	
	2006	2007	2006	2007	2006	2007
Cover spray						
1 spot	1.9 ab	2.2 b	2.7 b	13.0 b	5 b	15 b
2 spots	1.7 ab	2.0 b	5.2 ab	3.8 c	7 ab	6 c
3 spots	0.9 b	1.3 b	3.5 b	3.2 c	5 b	5 c
Control (no spray)	3.0 a	8.0 a	6.9 a	25.0 a	10 a	33 a
Variety						
Agege	4.3 a	4.9 a	7.8 a	6.6 a	11 a	11 a
Parson Brown	2.2 b	2.0 b	3.4 b	3.0 b	6 b	5b
Valencia	1.5 c	1.2 b	3.2 b	2.8 b	4 b	4 b
Interaction (Cover spray treatment \times Variety)	NS	NS	*	*	*	*

Means in the same column followed by the same letters are not significantly different by the Student-Newman-Keuls test ($P > 0.05$); NS: not significant; *: significant at $P = 0.01$.

the numbers of attacked fruits in the latter treatments were only significantly ($P < 0.05$) lower than that of the control. The result further indicates that the relative amount of bait applied per tree has a significant effect on fruit infestation levels, probably as a result of more flies responding to the bait. This was shown by the higher effectiveness of 3 spot applications, whereby less fruits were attacked compared with 1 spot application. Nevertheless, all the treatments performed better than the non-baited (control) stands in reducing fruit attack.

The number of fruits infested in the tree as well as those that dropped under the trees were also significantly influenced by the variety of sweet orange. Agege had a significantly ($P < 0.05$) higher number of fruits attacked on the tree as well as dropped fruits (minimum of 4.3 fruits and 6.6 fruits, respectively) than Parson Brown and Valencia (table I). Although Valencia had the least numbers of attacked and dropped fruits (1.2 fruits and 2.8 fruits, respectively), these were not significantly ($P > 0.05$) different from those of Parson Brown (2.2 fruits and 3.4 fruits, respectively) except in the lower number of attacked fruits on Valencia in

2006. The interactive effect of variety and cover spray only significantly ($P < 0.01$) affected the number of dropped fruits and the total number of damaged fruits (attacked + dropped) in 2006 and 2007.

3.2. Effect of protein bait spray and sweet orange variety on the emergence of fruit flies from harvested fruits

Significantly more adult *B. invadens* emerged from fruits sampled from untreated trees in 2006 and 2007 (mean of 12.8 and 21.8 *B. invadens* adults, respectively) than from treated ones (maximum mean of 5.3 and 7.0 *B. invadens* adults, respectively) (table II). Similarly, comparatively higher numbers of *Ceratitidis* spp. emerged from fruits sampled from untreated trees, although their incidence was low and did not exceed 3.2 fruit flies per fruit. Generally, the number of *B. invadens* and *Ceratitidis* spp. that emerged from sweet oranges decreased in the following order of application: 3 spots < 2 spots < 1 spot < control (no application), with the following respective numbers

Table II.

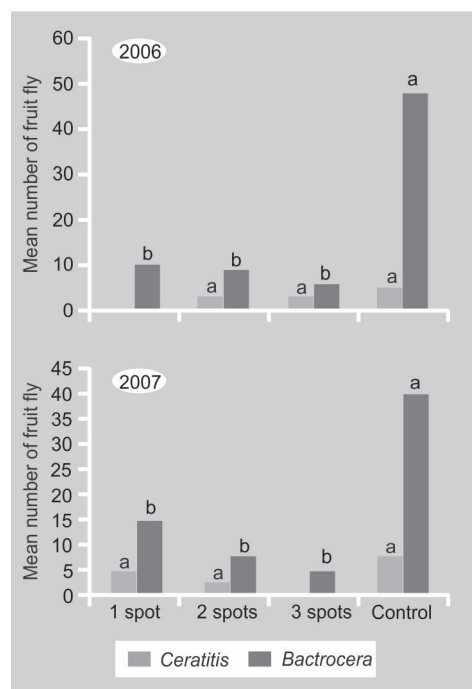
Numbers of emerged adult fruit flies from different varieties of sweet orange (*Citrus sinensis*) under different levels of protein bait applications in 2006 and 2007 (20 fruits per treatment, Nigeria).

Treatments	Mean weight of fruits (kg)		Mean number of <i>B. invadens</i> per fruit		Mean number of <i>Ceratitidis</i> spp. per fruit	
	2006	2007	2006	2007	2006	2007
Cover spray						
1 spot	8.0	8.5	5.2 b	7.0 b	1.2 b	1.5 b
2 spots	8.2	8.2	5.3 b	6.0 bc	1.0 b	1.2 b
3 spots	8.2	8.4	4.4 b	3.4 c	0.9 b	0.8 b
Control (no spray)	8.4	8.3	12.8 a	21.6 a	2.5 a	3.2 a
Variety						
Agege	8.4	8.0	8.2 a	10.0 a	3.8 a	3.6 a
Parson Brown	6.0	5.9	4.0 b	3.6 b	1.6 b	1.5 b
Valencia	8.4	8.2	3.7 b	4.6 b	1.4 b	1.6 b
Interaction (Cover spray treatment × Variety)	–	–	*	*	NS	NS

Means in the same column followed by the same letters are not significantly different by the Student-Newman-Keuls test ($P > 0.05$); NS: not significant; *: significant at $P = 0.05$.

Figure 1.

Number of adult fruit flies that emerged from dropped fruits of sweet oranges (*Citrus sinensis*) under different levels of protein bait sprays in 2006 and 2007. Bars with the same letters are not significantly ($P > 0.05$) different for each fruit fly genus.



in 2006 and 2007 for the predominant *B. invadens*: $4.4 < 5.3 < 5.2 < 2.8$ for 2006; and $3.4 < 6.0 < 7.0 < 21.6$ for 2007.

Our results indicate that locally-made brewery waste is effective, when mixed with an insecticide, in reducing fruit fly infestation in sweet orange. Protein bait made from brewery waste was comparable with commercial Torula™ yeast in attracting fruit flies in a citrus trial in which the bait was used in traps [5]. This is of advantage to fruit farmers in Nigeria. Commercial yeast baits are not readily available in the Nigerian markets. Even if they are imported, the cost of procurement may go beyond the reach of small-scale farmers. Therefore, the high cost of fruit fly control can be overcome by using locally-made protein baits.

The assessment of the varietal effect on fruit flies emerging from fruit cultures showed that only fruits of Agege had a significantly ($P < 0.05$) higher number of emerged *B. invadens* (≤ 14) and *Ceratitis* spp. (≤ 3.8) adults compared with the rest (maximum 4.6 and 1.6, respectively) (table II). There was no significant

($P > 0.05$) difference between the number of fruit flies that emerged from Valencia and Parson Brown in both years. Earlier studies have shown that some citrus varieties were less attacked by the indigenous *C. capitata* [2, 21], a finding that was also recorded for *Bactrocera invadens*.

The interactive effect of variety and cover spray only significantly affected the number of *B. invadens* that emerged from the harvested fruits, but did not affect that of *Ceratitis* spp.

3.3. Effect of protein bait spray and sweet orange varieties on the number of fruit flies that emerged from dropped fruits

Observations made on the number of fruit flies that emerged from fruit samples taken from beneath the sweet orange trees (dropped fruits) in the different treatments followed the same trend as those sampled from the trees. The numbers of adult *B. invadens* that emerged from dropped fruits were higher than those of *Ceratitis* spp. in 2006 and 2007 (figures 1, 2). The number of adult *B. invadens* that emerged from dropped fruits collected under the untreated trees (> 40) was significantly ($P < 0.01$) higher than that of treated trees (≤ 15) (figure 1). The number of *B. invadens* that emerged from dropped fruits picked under Agege sweet orange was significantly ($P < 0.05$) higher than those of the other varieties in 2006 and 2007 (figure 2).

Brewery waste is available all over Nigeria because of the high concentration of breweries in many parts of Nigeria. It is usually discarded as spent yeast by the brewers; and therefore can be harnessed and prepared at little or no cost by citrus farmers for use against fruit flies. Cover spray with locally-made protein bait can also be used for other species of fruits attacked by fruit flies. However, modification of the application method to take into cognizance the size of the crop species may be considered to achieve the desired results.

4. Conclusion

Our study shows that the use of protein bait spray can reduce fruit fly attack and consequent damage on sweet oranges. Meanwhile, research is still on-going to further improve the attractiveness of brewery waste. The latter can be used in Integrated Pest Management with other compatible methods to reduce the menace of fruit flies on citrus.

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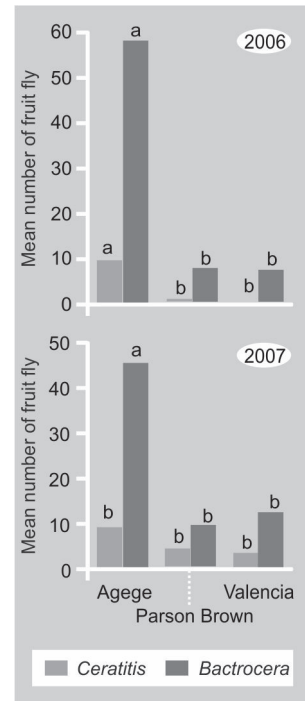


Figure 2. Number of adult fruit flies that emerged from dropped fruits collected from different sweet orange varieties (*Citrus sinensis*) in 2006 and 2007. Bars with the same letters are not significantly ($P > 0.05$) different for each fruit fly genus.

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Eficacia de las pulverizaciones foliares de cebos con proteínas para el control de *Bactrocera invadens* (Diptera: Tephritidae) en naranjos.

Resumen — Introducción. En Nigeria, los daños causados por las moscas de las frutas se identificaron como un importante factor que limitaba la producción de cítricos. En la actualidad, la mayoría de los agricultores no aplica ninguna medida de lucha. Por lo tanto, el presente trabajo pretende evaluar la eficacia de las pulverizaciones de cebos con proteínas fabricados localmente; así como poner de manifiesto las diferencias de sensibilidad de variedades de naranjos, frente al ataque de las moscas de las frutas. **Material y métodos.** En 2006 y 2007, se pulverizaron unos tratamientos localizados, producidos con un cebo con proteína fabricado localmente y mezclado con un insecticida, el clorpirifos, a razón de una, dos o tres manchas por árbol perteneciente a cada una de las tres variedades de naranjos: Agege, Parson Brown y Valencia Late. Los tratamientos se dispusieron en bloques aleatorios completos. Se evaluó la eficacia de las pulverizaciones de cebo en la reducción de frutos afectados, en un muestreo aleatorio de 10 frutos tanto maduros como en fase de maduración, por árbol estudiado, y mediante el estudio de los frutos caídos bajo los árboles tratados. **Resultados y discusión.** Las pulverizaciones de cebos redujeron de modo significativo ($p < 0,05$) los daños evaluados en los frutos, reduciendo el nivel de infestación por *Bactrocera invadens* Drew Tsurata & White y, en consecuencia, reduciendo el número de frutos caídos. El tratamiento localizado, efectuado a razón de pulverización de cebos en tres manchas por árbol, fue el más eficaz para reducir los daños causados por *B. invadens* en las variedades de naranjos estudiadas. El nivel de infestación de *B. invadens* y el número de frutos caídos de la variedad Agege fueron significativamente más altos ($p < 0,05$) que los de las variedades Parson Brown y Valencia Late. **Conclusión.** Nuestro estudio muestra que los tratamientos localizados en tres manchas por árbol fueron más eficaces para reducir los daños de los frutos, y que la variedad Valencia Late fue la menos atacada, seguida de la variedad Parson Brown.

Nigeria / *Citrus sinensis* / frutas / Tephritidae / control de insectos / daños / caída prematura de frutos / control químico / métodos de aplicación / dosis de aplicación

