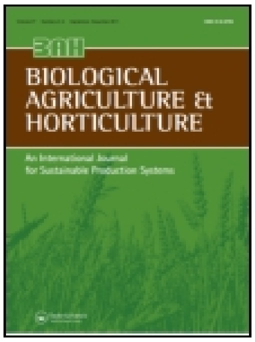

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High dilution of *Staphysagria* and fruit fly biotherapic preparations to manage South American fruit fly, *Anastrepha fraterculus,* in organic peach orchards

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The peach, *Prunus persica,* is an important economic crop in southern Brazil, where family farms are predominantly small. The South American fruit fly, *Anastrepha fraterculus* is a limiting factor to increased production in orchard crops. Increased organic production in Brazil has stimulated research to find ecological based pest management. This research was carried out in order to evaluate the efficacy of preparations at high dilution in managing *A. fraterculus in* organic peach orchards. Experiments were conducted under field conditions in randomized blocks during 2003/04 and 2004/05. Treatments consisted of *A. fraterculus* nosodes and *Staphysagria* homeopathic preparations, both at two high dilution levels, 3CH and 6CH (centesimal hahnemannian dilution method), applied at two spray intervals, 5 and 10 days, and a control. Fruit losses due to *A. fraterculus* infestation varied from 40 to 98.3%. Although there were significant differences among treatments in the first harvest of two of the three experiments there were no significant differences in the second harvest of any of the experiments. None of the homeopathic preparations reduced the incidence of infested fruit significantly below that of the water control in any of the three experiments. Further studies must combine other strategies such as bagging fruits and planting of early season cultivars. Variation on high dilution potency, dose, and frequency of application must also be considered.

Keywords: agro-homeopathy; *Anastrepha fraterculus*; high dilution preparations; plant protection; *Prunus persica*; *Staphysagria*

Introduction

The peach, *Prunus persica* (L.) Batsc, is an important economic crop for small family farms in south Brazil. Fruits are often damaged by *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae), which can cause a total loss of fruits (Botton et al. 2003). According to Salles (1995) and Kovaleski et al. (1999), the management of this pest is difficult due to its erratic fluctuation, depending on annual fruit set and climatic conditions, resulting in a broad use of insecticides, mainly organophosphorus compounds. Consumers are strongly concerned about the use of chemical pesticides and, whether or not this is justified, there is an incentive for farmers to adopt a more ecologically based system and for research to reduce losses in such a system (Boff et al. 2008). At present, bagging fruit

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is a common technique used to protect the fruits on organic peach orchards, but this technique is labour intensive and not suitable for medium or large orchards (João and Secchi 2002). A novel organic plant compound called Composto A is used as an alternative to chemical pesticides to control the fruit fly in organic peach orchards, but no toxicological studies or agronomic information about its hazardous effects and efficacy are available (Rupp et al. 2006).

Ecological-based technologies for agriculture must be developed to have a minimal effect on the environment and to be accessible to small farmers (Boff 2008). The use of homeopathy, with high dilution levels of compounds, in agricultural systems, is one such possibility (Betti et al. 2003). The science of homeopathy is based on the principle of simillimum, that a preparation can reproduce the symptoms of a disease in healthy organisms and that this has a curative capacity in sick organisms (Bellavite 2003). The application of homeopathy to plants by the principle of simillimum has received little attention and a derivation of that called isopathy seems to be more suitable to some situations (Bonato 2003). Isopathy consists of using preparations from the causal agent called nosodes or biotherapic preparations followed by dilution and succession to reach the desirable potential level that still has an effect on the host (Seco et al. 2001). In Brazil, homeopathy applies to plants, and its derivation of isopathy is supported by organic production legislation (Brazil 2003). Almeida (2003) demonstrated the efficacy of control of *Spodoptera frugiperda* on corn crops by applying homeopathic preparations from the *S. frugiperda* caterpillar at a high dilution level. Homeopathies from pharmacies, which are available for human and animal treatments, can also be used on plants by analogies described on homeopathic materia medica (Boff 2008; Casali 2003). The objective of this study was, therefore, to examine the efficacy of high dilution preparations in controlling South American fruit fly, *A. fraterculus,* in organic peach orchards.

Material and methods

Experimental set-up

The study was carried out during the 2003–2004 and 2004–2005 peach crop seasons. Three experiments were conducted using a randomized block design with six replicates in orchards containing 4-year-old peach cv. Cheripá in Experiment 1 and 13-year-old peach cv. Cheripá in Experiments 2 and 3. All of the experiments were conducted under an organic system and located in Antonio Prado, Rio Grande do Sul State, Brazil. Each experimental plot consisted of three peach plants in a row. Data were collected from the central plant.

High dilution preparations

The high dilution preparations were obtained from a mother extract of adult *Anastrepha fraterculus* (Weid.) fruit fly (biotherapic) and *Staphysagria* homeopathic remedy from the specialized pharmacy of Farmec®, Caxias do Sul, RS, Brazil. *Staphysagria* is a remedy obtained from seeds of the *Delphinum staphysagria,* a perennial plant of the family Ranunculaceae, used for suppression of deeply rooted emotions. *Staphysagria* is useful in treating behavior in patients who are excitable, easily disturbed, and easily aroused to anger (Boericke 2003).

Live adult female fruit flies were obtained from the laboratory of EPAGRI São Joaquim, Experimental Station of Agricultural Research and Extension Service of Santa Catarina State, Brazil and transferred to the drug store laboratory where they were processed. The mother extract of fruit fly was prepared by transferring the live adults into a glycerine, water and ethanol mixture (1:1:1), in the ratio of 1:20 (weight of fruit fly to volume of receptive solution). The macerate was kept for 20 days at 21°C and protected from direct light. The mixture was shaken for 1 min per day during the maceration time. After the maceration period, the preparation was filtered using qualitative paper JProlab™ (80 g) and successive dilutions at centesimal hahnemannian (CH) level were made with one hundred succussions immediately after each dilution, until the third and sixth CH potencies were reached according to *Farmacopeia Homeopatica Brasileira* [Brazilian Homeopathic Pharmacopeia] (Brazil 1997).

The high diluted preparations of *Staphysagria* were obtained directly from the specialized pharmacy at 3CH and 6CH dilution levels, ready for use in the sprayer. Both preparations, *Staphysagria* and biotherapic fruit fly, were prepared according to hahnemannian centesimal method, in which the proportion of 1 ml of mother extract or matrix was added to 99 ml of ethanol 70%, followed by 100 times succussion in angular-vertical movements (90°) assisted by a mechanical hand dynamizer.

Treatments

The first two experiments consisted of eight combination treatments arranged with biotherapic fruit fly or *Staphysagria* at 3CH or 6CH, sprayed at 5- or 10-day intervals. The control treatment was distilled water. The third experiment included an extra treatment, Composto A, a plant extract specifically used by organic farmers in south Brazil. The sprays were applied in the morning with a knapsack sprayer using a hollow-cone nozzle with an application rate of 400 l ha^{-1}. Using a double blind protocol, the treatments were identified by codes. Spray applications were done from 3 November 2003 until 7 January 2004 for the first and second experiments, and from 11 November 2004 until 29 December 2004 for the third experiment. Composto A was applied twice, on 25 November 2004 and 15 December 2004.

Fruit fly population monitored by traps

The adult populations of fruit fly were monitored using McPhail™ traps, with 5% hydrolysed protein BioAnastrepha™ as an attractant. Four traps per orchard were installed at 1.3 to 1.6 m from the soil surface hung from the principal plant branches. Trapped adults were counted every five days. After evaluation, the BioAnastrepha™ was replaced. Identity of fruit fly adults was confirmed by Dr. Flávio Roberto Mello Garcia, UNOCHAPECÓ University, Chapecó, SC, Brazil.

Fruit evaluations and data analysis

The fruit fly infestation was evaluated from 10 fruits sampled per plant per harvest. The fruits were picked at random from the four sides of the plant canopy. Fruits were harvest on 6 and 9 January 2004 for the first and second experiments and on 27 and 30 December 2004 for the third experiment. Harvested fruits were separated per plot into boxes and transported to a semi-climate room in the barn of Centro Ecologico Ipê, RS, Brazil, in which temperatures ranged from 20 to 25°C. The fruits were individually placed into 500 ml pots with 1 cm layer of vermiculite at the bottom. The pots were closed by voile net tied with elastic thread. After eight days, fruits were taken out and sliced to check for the presence of larvae and pupa. Larvae and pupa were also recovered from the vermiculite layer. Data were analysed using Assistat 7.2® software package for analysis of variances. The means were compared by Tukey's test at 5% significance, only if the *F* test showed significance. A *t* test was used to compare the fruit infestation of the first harvest with the second harvest.

Results and discussion

Fruit fly monitoring

Adults of flies sampled from all the experimental orchards were identified as *Anastrepha fraterculus* Wied. (Diptera: Tephritidae). This is supported by Salles (1995), who reported that more than 95% of fruit flies in Rio Grande do Sul are *A. fraterculus*. The total number of adult fruit flies trapped in the three experiments was 1,154. Fruit flies first appeared on 25 November 2003 for the 2003–2004 crop season and 14 November 2004 for the 2004–2005 crop season (Figure 1). The number of fruit flies trapped increased rapidly from the end of December in the areas of Experiments 1 and 2 but not in Experiment 3. On average, 13.2, 2.3, and 3.6 individuals per trap per day were found in Experiments 1, 2, and 3, respectively. Considering the economic threshold of 0.5 individuals per trap per day suggested by Hickel (1993) and Botton et al. (2003), the fruit fly population reached this level in both orchards at the end of December and control measures were needed from that period. The population fluctuation of fruit flies did not follow a similar pattern in the three experiments. This has already been pointed out by Souza Filho (2002), who found variations among seasons, places, and harvest time of particular cultivars. Salles (1995) reported that population fluctuation of fruit fly is influenced by the host itself, climatic conditions, and reproduction on alternative plant hosts. In Rio Grande do Sul state, where the experiments were carried out, several alternative plant hosts such as *Eugenia uniflora*, *Eryobotria japonica,* and *Campomanesia obscura*, which are native plants and facilitate reproduction of the fruit fly, have been identified (Salles 1995). The monitoring method was useful as a warning system but should be done in each season and orchard where it could be helpful to the implementation of control measures to keep the fruit fly population below the threshold level. More studies are needed to determine the effect of place and time of peach fruit harvest on the suitability of ecological sound pest control measures, such as homeopathic interventions.

High dilution preparation effects

Fruit infestation of peach by fruit fly was lower at the first harvest than in the second, in all the experiments (Tables 1, 2, and 3). These results indicate that the later the harvest of

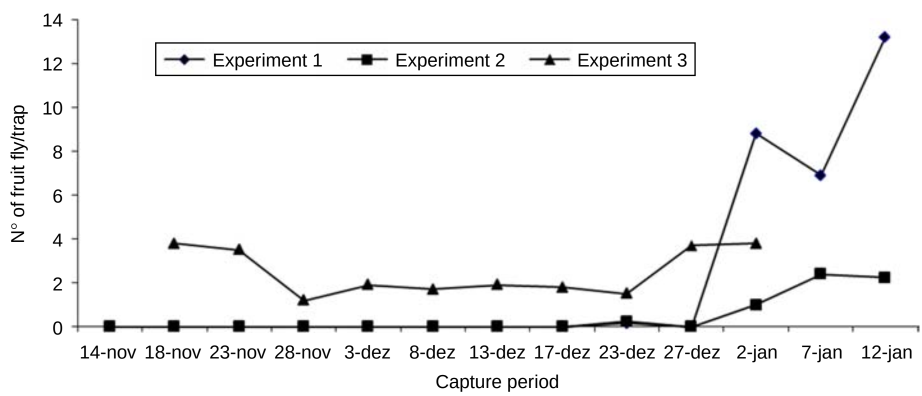

Figure 1. Population fluctuation of adults of *Anastrepha fraterculus* caught in Mc-Phail® traps in organic peach orchards in 2003–2004 (Experiments 1 and 2) and 2004–2005 (Experiment 3), Antônio Prado, RS, Brazil. Values are averages from four traps per orchard.

peach, the higher the probability of fruit fly infestation, and that more intensive control measures are needed for the later peach cultivars.

In the first experiment, in 2003–2004, the homeopathic preparation *Staphysagria* 6CH applied every 10 days showed lower fruit fly infestation in the first harvest than *Staphysagria* 3CH at 10-day intervals (Table 1). This tendency was also verified for the second experiment, but for five-day spray intervals for *Staphysagria* 3CH, with this treatment at five-day intervals actually giving a higher level of infestation than the water control (Table 2). In the third experiment, in 2004–2005, there were no significant differences among treatments in fruit fly infestation (Table 3). This may be due to the earlier harvest period than in the other two experiments, giving less exposure to fruit fly infestation and difficulties in discriminating between treatments. No statistically significant differences were observed among treatments for fruit fly infestation at the second harvest time in any of the three experiments. None of the homeopathic preparations reduced the incidence of infested fruit significantly below that of the water control in any of the three experiments.

The Composto A, evaluated only in the third experiment, did not differ significantly from the water control plot in fruit fly infestation. This result differs from Botton et al. (2003), who reported Composto A to be an effective product for the management of fruit fly, giving 80% reduction. Gonçalves et al. (2006a, 2006b) observed that Composto A (1%) associated with diatomaceous earth (0.5% and 1%) reduced the number of fruit damaged by *A. fraterculus* when sprayed on *Acca sellowiana* and *Prunus salicina* plants. Different doses and the frequency of sprays may alter the effectiveness of Composto A.

Overall, the homeopathic preparations tested were ineffective in reducing fruit fly infestations, and combination with other control strategies is needed. Moreover, it can be argued that the dynamization level of homeopathic preparations may also make a difference and other combinations of dilution, spray interval, and dose must be tested (Betti et al. 2003; Brizzi et al. 2000). Almeida (2003) demonstrated good control of corn caterpillar, *Spodoptera frugiperda,* with nosode preparations at 30CH or higher. However, Keske (2004) found inconsistent results with nosode of *A. fraterculus* applied at 30CH to control fruit fly on plum. This author started to spray only at the established threshold level

Table 1. Peach fruit infestation by fruit fly *Anastrepha fraterculus,* Experiment 1, 2003–2004, Antônio Prado, RS, Brazil.

Treatments (homeopathic preparation)	Dilution level[1]	Spray interval (days)	Infested fruits at harvest time (% ± *SE*) First harvest (6 January)	Second harvest (9 January)
Nosode Af	3CH	5	70.0 ± 10.3ab[2]	91.7 ± 3.1[ns]
Nosode Af	3CH	10	65.0 ± 12.3ab	85.0 ± 7.6
Nosode Af	6CH	5	61.1 ± 11.3ab	82.8 ± 8.3
Nosode Af	6CH	10	83.3 ± 5.6ab	96.7 ± 3.3
Staphysagria	3CH	5	86.7 ± 5.6ab	93.3 ± 2.1
Staphysagria	3CH	10	95.0 ± 3.4a	96.7 ± 2.1
Staphysagria	6CH	5	73.3 ± 8.8ab	92.9 ± 5.0
Staphysagria	6CH	10	57.4 ± 5.2b	91.7 ± 3.1
Water	–	5	83.3 ± 7.2ab	90.0 ± 4.5

Notes: [1]Third (3CH) and sixth (6CH) centesimal hahnemaniana dilution.
[2]Values within columns followed by the same letter are not significantly different (Tukey; $p < 0.05$).
Af = High dilution preparation from adults of *Anastrepha fraterculus;* ns = not significant by *F* test at 5%

Table 2. Peach fruit infestation by fruit fly *Anastrepha fraterculus,* Experiment 2, 2003–2004, Antônio Prado, RS, Brazil.

Treatments (homeopathic preparation)	Dilution level[1]	Spray interval (days)	Infested fruits at harvest (% ± *SE*) First harvest (6 January)	Second harvest (9 January)
Nosode Af	3CH	5	57.2 ± 9.2ab[2]	96.7 ± 2.1[ns]
Nosode Af	3CH	10	66.5 ± 11.9ab	90.0 ± 3.6
Nosode Af	6CH	5	40.0 ± 10.0bc	95.0 ± 2.2
Nosode Af	6CH	10	38.3 ± 7.9c	90.0 ± 6.3
Staphysagria	3CH	5	78.3 ± 7.0a	93.3 ± 3.3
Staphysagria	3CH	10	53.3 ± 6,7abc	100.00
Staphysagria	6CH	5	46.7 ± 8.8bc	88.2 ± 6.5
Staphysagria	6CH	10	67.8 ± 6.0ab	98.3 ± 1.7
Water	–	5	42.5 ± 12.3bc	93.2 ± 3.4

Notes: [1]Third (3CH) and sixth (6CH) centesimal hahnemaniana dilution.
[2]Values within columns followed by the same letter are not significantly different (Tukey; $p < 0.05$).
Af = High dilution preparation from adults of *Anastrepha fraterculus;* ns = not significant by *F* test at 5%.

Table 3. Peach fruit infestation by fruit fly *Anastrepha fraterculus,* Experiment 3, 2004–2005, Antônio Prado, RS, Brazil.

Treatments (homeopathic preparation)	Dilution level	Spray interval (days)	Infested fruits at harvest (% ± *SE*) First harvest (27 December)	Second harvest (30 December)
Nosode Af	3CH	5	53.3 ± 13.1[ns]	69.8 ± 12.0[ns]
Nosode Af	3CH	10	46.7 ± 11.2	60.0 ± 10.0
Nosode Af	6CH	5	48.3 ± 10.1	58.3 ± 11.9
Nosode Af	6CH	10	55.0 ± 14.8	60.6 ± 9.5
Staphysagria	3CH	5	45.0 ± 13.6	56.7 ± 13.1
Staphysagria	3CH	10	66.7 ± 10.5	68.3 ± 15.6
Staphysagria	6CH	5	46.7 ± 9.2	59.8 ± 11.4
Staphysagria	6CH	10	45.0 ± 12.0	60.0 ± 9.3
Composto A		–	28.3 ± 10.1	40.0 ± 13.9
Water	–	5	58.3 ± 10.8	69.5 ± 9.8

Notes: [1]Third (3CH) and sixth (6CH) centesimal hahnemaniana dilution.
Af = High dilution preparation from adults of *Anastrepha fraterculus;* ns = not significant by *F* test at 5%.

and made only one application, which means that homeopathic preparations may not work well as curatives at a single intervention.

A higher infestation of fruit fly observed at low dilution level of homeopathic preparation than at a higher level, as occurred with *Staphysagrica* in Experiments 1 and 2, may be due to pathogenesis. Casali (2003) attributed this anomalous behaviour to a hormesis phenomenon which may occur at low dynamization of the homeopathic preparation. It considered that as the homeopathic preparation is diluted and succussioned, its curative property is activated. Therefore, homeopathic preparations at high dilutions can have different effects than at low dilution, though this does not mean that there is a linear effect. Studies by Casa et al. (2007) showed a reverse effect in biomass production of willow by increasing in dilutions from 3CH to 12CH for several homeopathic preparations except the control with water. The non-linear effect must be considered in studies dealing with dynamic populations, even when chemical control is involved (Scoz et al. 2004). According to Bonato (2003), the response to rising dilutions can follow liner, oscillatory, decreasing/increasing, horizontal, or non-linear models.

In conclusion, *Anastrepha fraterculus* was the predominant species of fruit fly in organic peach orchards in the Serra Gaucha region of Brazil. The population level of fruit fly increased from December onward, precisely at the harvest time of the majority of peach cultivars growing in the south of Brazil. The use of homeopathic preparations from *A. fraterculus* nosodes or *Staphysagria* was ineffective in reducing peach fruit losses. However, high dilution preparations may be worth investigating in combination with early maturing cultivars and other strategies. Peach cultivars that are late harvested require more intensive control measures than early harvesting cultivars. Further studies are needed to test other homeopathic dilution levels and spray frequency of *A. fraterculus* nosodes and *Staphysagria* to evaluate possible reduction of fruit fly infestation in organic peach orchards.

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