

Laboratory Evaluation of Selective Pesticides Against the Storage Mite *Tyrophagus putrescentiae* (Acari: Acaridae)

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ABSTRACT The storage mite, *Tyrophagus putrescentiae* (Schränk), is an important source of airborne allergens, especially on farms. Three insect growth regulators (halofenozide, pyriproxyfen, hexaflumuron), one botanical insecticide (azadirachtin) and one naturalyte (spinosad) were tested to assess their efficacy against this mite. The organophosphate chlorpyrifos was used as a standard. Immature stages and adults were treated with different concentrations of the chemicals in diet-incorporation bioassays. All chemicals significantly increased the developmental time of immature stages at doses higher than 1,000 ppm. Pyriproxyfen and halofenozide yielded mortality rates of immature stages similar to that obtained with chlorpyrifos, with values near 100% at 10,000 ppm. At 1,000 ppm, only halofenozide significantly increased the mortality of immature stages. Similarly, adult survival and fecundity were significantly reduced with halofenozide at 1,000 ppm, whereas it required 10,000 ppm of chlorpyrifos to obtain a significant reduction in survival and fecundity, with no effect observed with pyriproxyfen. This data suggests that halofenozide could be a good alternative for the control of *T. putrescentiae* in farming environments.

KEY WORDS *Tyrophagus putrescentiae*, storage mite, insect growth regulator, naturalyte, neem

HOUSE DUST MITES are recognized worldwide to be one of the most important causes of allergic airway disease in many tropical and temperate climates. Approximately 93% of all mites belong to the order Astigmata, with the family Pyroglyphidae containing the most species associated with allergies (Hallas 1991). Nevertheless, exposure to nonpyroglyphid storage mites has been increasingly recognized as a cause of asthma and rhinitis (Musken et al. 2000). Storage mites have been identified as a part of the house dust acarofauna (Bernd et al. 1996, Sanchez Covisa et al. 1999), to the extent of being redefined as “domestic mites” (Platts-Mills et al. 1992).

One of the most prevalent storage mites associated with occupational allergy is the acarid mite *Tyrophagus putrescentiae* (Schränk) (Korsgaard et al. 1985, van Hage-Hamsten and Johansson, 1992), the allergenicity of which has been characterized (Eriksson et al. 1998, van Hage-Hamsten and Johansson, 1992). There is convincing evidence that *T. putrescentiae* is an important cause of asthma and rhinitis in farmers and others who work where grain is handled, stored, or processed (Cuthbert et al. 1979, Ingram et al. 1979, Terho et al. 1985, Iversen et al. 1992, Bernd et al. 1996). Other health problems reported are rhinoconjunctivitis and eczematous injuries as a result of the manipulation of salty ham (Armentia et al. 1994), or even systemic anaphylaxis after eating food contaminated by this

mite (Matsumoto et al. 1996). Moreover, the occurrence of *T. putrescentiae* in pulmonary cytology specimens has also been demonstrated (Farley et al. 1989).

A common strategy for indoor allergen avoidance is the control of storage mites by application of chemicals to infested commodities. Many synthetic pesticides have been reported to have acaricidal activity against *T. putrescentiae* and other storage mites (Zdarkova and Horak 1973, Wilkin and Hope 1973, Bowley and Bell 1981, Stables 1984, Tigar and Pinniger 1989). Organophosphate acaricides, particularly chlorpyrifos and pirimiphos-methyl, have been recommended for protection of empty stores (Zdarkova 1994). However, the use of conventional pesticides is often restricted in the food industry, as they must have low mammalian toxicity and minimum residual activity.

Insect growth regulators (IGRs) are synthetic chemicals that interfere with the normal growing and development of arthropods (Retnakaran et al. 1985). Commercial IGRs include chitin synthesis inhibitors (e.g., diflubenzuron and hexaflumuron), juvenile hormone mimics (fenoxycarb and pyriproxyfen), and molting hormone mimics (halofenozide and tebufenozide). These products have shown high efficacy and selectivity to target pests as well as low toxicity to nonarthropod organisms (Retnakaran et al. 1985, Dhadialla et al. 1998). The efficiency of IGRs for the control of stored product insects is well documented (Oberlander et al. 1997), although there is

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limited information on their potential application for the control of storage and house mites (Czaja-Topinska et al. 1979; Downing et al. 1990, 1993).

Products derived from leaves and kernels of neem (*Azadirachta indica*) are increasingly used to control stored pests (Rahim 1998, Lale and Abdulrahman 1999, Ulrichs and Mewis 2000) because they appear to be environmentally safe (Gahukar 2000). Moreover, essential oils constituents, such as monoterpenoids, have been tested to control mites (Perrucci 1995, Sánchez-Ramos and Castañera 2001). However, few studies have focused on the effect of azadirachtin on storage mites (Mansour and Ascher 1995, Mansour et al. 1997).

Spinosad is an insecticidal toxin of two macrocyclic lactones called spinosyns A and D, derived from metabolites of the bacterium *Saccharopolyspora spinosa*. Formulations of spinosad have proven to be effective against dipteran and lepidopteran pests (Adán et al. 1996, Sparks et al. 1998) and on phytophagous mites (Bret et al. 1997), and could be a good alternative to conventional chemicals because of its low human and environmental risk (Thompson et al. 1997).

The aim of this study is to provide reduced-risk chemicals for the control of mite populations in farming environments. We report here on the effect of different chemicals, incorporated onto brewers' yeast flakes, on survival and development of immature stages of *T. putrescentiae*, and on survival, fecundity, and egg hatching when applied to adult mites. The effectiveness of these chemicals was compared with that of the organophosphate pesticide chlorpyrifos, which is often used to control storage mites.

Materials and Methods

Mites. A stock culture of *T. putrescentiae* was established from infested samples of dry-cured ham obtained from drying store rooms in Almendralejo, Spain. The mites were maintained on brewer's yeast, and held in cylindrical plastic cages (9-cm diameter $\times$ 3-cm height). The cages were covered with round plastic plates having a 3-cm diameter hole in the center, sealed with filter paper disks for ventilation. The rearing cages were kept in an environmental chamber (Sanyo MLR-350H, Sanyo, Japan) at $25 \pm 0.5^\circ\text{C}$ and $90 \pm 5\%$ RH and dark conditions, within a watered-filled plastic tray to prevent mites escaping and to maintain a high humidity. Adults were sexed by observing their secondary sexual characters (Hughes 1976).

Chemicals. Four of the compounds were used in commercially available form: chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) purchased from Agrocros España as Dursban 48 (480 g [AI]/liter); hexaflumuron, (1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)-phenyl]-3-(2,6-difluorobenzoyl) urea) was supplied by Dow-Elanco España as Consult 10 EC (100 g [AI]/liter); pyriproxyfen (4-phenoxyphenyl (RS)-2-(2-pyridyl-

oxy)propyl ether) provided by Sumitomo Chemical Co. (Osaka, Japan; emulsified concentrate, 10%); spinosad (mixture of 50–95% of spinosyns A and D) provided by Dow-Elanco Ibérica Co. (Madrid, Spain) as Tracer SC (480 g [AI]/liter).

Technical halofenozide (N-*tert*-butyl-N'-(4-chlorobenzoyl) benzohydrazide), 99% purity, was obtained from Rohm and Haas Co. (Spring House, PA); technical azadirachtin (>95% purity) was supplied by Sigma (St Louis, MO).

Chemical Assays. Rearing cells were used to perform the assays. These cells consisted of cavity slides (15–18 mm cavity diameter and 0.5–0.8 mm depth of well) covered with a cover glass (22×22 mm) and sealed with a drop of water. Previously, the mites were introduced inside the cells with the help of a thin camel hair brush. Within each cell, mites were fed on a treated brewers' yeast flake of ≈ 1.3 mg.

To determine the efficacy of the selected chemicals on the survival and duration of mobile immature stages, larvae <16 h from hatching were used. Mite development was examined every 24 h until they became adults. The pesticides were added to brewers' yeast flakes at doses of 10, 100, 1,000, and 10,000 ppm of active ingredient (wt:wt). All compounds were added with a Gilson P2 microtiter pipette in 2 μl of distilled water except halofenozide and azadirachtin, which were added in 2 μl of methanol. Both solvents were used as controls. An additional starved control was used to elucidate antifeedant activity. Five individuals (within each cell) replicated ten times were used for each dose and for the controls.

Those products showing the highest activity on immature stages were selected to assess their effect on adult mite survival and fecundity (number of eggs per female). They were added to brewers' yeast flakes at doses of 1,000 and 10,000 ppm (wt:wt). Ten single pairs <24 h old were established for each compound and dose. The number of eggs laid was observed every 24 h for 25 d. Additionally, ten eggs per pair within the first 5 d of the oviposition period were transferred to new rearing cells to assess the hatching rate and the development of the progeny.

All assays were performed in an environmental chamber at $25 \pm 0.5^\circ\text{C}$ and $90 \pm 5\%$ RH and dark conditions.

Statistical Analysis. The effect of pesticides on the percentage of mortality of immature stages and on the percentage of egg hatching were analyzed by means of Kruskal-Wallis test. This was followed by a Dunn test (Dunn 1964) to determine significant differences from the controls. Development and fecundity data were analyzed using analysis of variance (ANOVA), followed by Newman-Keuls multiple range test for separation of significantly different means. The duration of the immature stages was analyzed by nesting the effects of slides within treatments. Chi-square tests were performed to establish differences in the survival of adults. The level of significance was $P < 0.05$ in all cases.

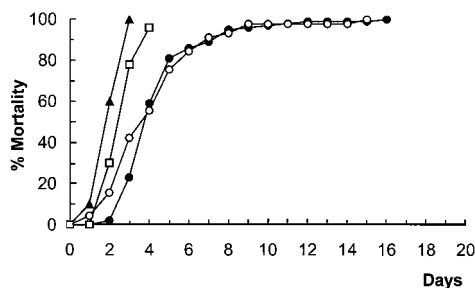
Table 1. Percentage of mortality (mean $\pm$ SE) of immature stages of *T. putrescentiae* fed with brewers' yeast flakes treated with six different pesticides

Doses (ppm)	Azadirachtin	Pyriproxyfen	Halofenozide	Hexaflumuron (Consult 10EC)	Spinosad (Tracer)	Chlorpyrifos (Dursban 48)
0 ^a	2 $\pm$ 2.0	0 $\pm$ 0.0	4 $\pm$ 4.0	0 $\pm$ 0.0	2 $\pm$ 2.0	4 $\pm$ 4.0
10	0 $\pm$ 0.0	6 $\pm$ 3.1	10 $\pm$ 5.4	0 $\pm$ 0.0	4 $\pm$ 2.7	2 $\pm$ 2.0
100	10 $\pm$ 3.3	2 $\pm$ 2.0	2 $\pm$ 2.0	4 $\pm$ 4.0	11.1 $\pm$ 6.8	4 $\pm$ 2.7
1,000	4.4 $\pm$ 2.9	4 $\pm$ 2.7	46 $\pm$ 11.9*	15.6 $\pm$ 10.4	2 $\pm$ 2.0	4 $\pm$ 2.7
10,000	2 $\pm$ 2.0	96 $\pm$ 2.7*	100 $\pm$ 0.0*	ND	8.9 $\pm$ 6.8	100 $\pm$ 0.0*

^a Controls: methanol for halofenozide and azadirachtin and distilled water for the rest.

ND, no data available.

* Indicates significant differences within columns ($P < 0.05$; Kruskal-Wallis test followed by Dunn test); $n = 50$ mites per chemical and dose.

**Fig. 1.** Mortality pattern of immature stages of *T. putrescentiae* fed with brewers' yeast flakes treated at a dose of 10,000 ppm with chlorpyrifos (▲), pyriproxyfen (□), halofenozide (○), and starved (●).

Results

In general, the incorporation of chemicals into the brewers' yeast flakes did not appear to have any overt negative effects on the behavior of the immature or adult stages. The mites readily started feeding on treated and untreated flakes. However, in those treated with halofenozide an antifeedant effect was noticed, because mites were away from the food at every sampling time.

Effects of Chemicals on Survival and Development of Immature Stages. The percentage mortality recorded for the immature stages of *T. putrescentiae* was very low and similar to the control for all chemicals except halofenozide at 1,000 and 10,000 ppm and pyriproxyfen and chlorpyrifos at 10,000 ppm (Table 1). No data were obtained for hexaflumuron applied at 10,000 ppm because of the sticky texture of the treated

flakes. At 1,000 ppm, halofenozide caused 46% mortality. None of the developing mites died at the larva stage, whereas 9% and 91% of the mortality occurred at the protonymph and tritonymph stages, respectively. At 10,000 ppm, pyriproxyfen, chlorpyrifos, and halofenozide produced highly significant mortality values ($\approx 100\%$), but they presented a different pattern (Fig. 1). Chlorpyrifos and pyriproxyfen produced a rapid increase of mortality from day 2, reaching values close to 100% at days 3 and 4, respectively. In contrast, the mortality was $\approx 60\%$ by day 4 with halofenozide and reached the maximum value at day 15 with a similar mortality pattern to that obtained for the starved control.

The developmental times of immature stages are shown in Table 2. No significant differences with the controls were recorded for any of the chemicals at doses of 10 and 100 ppm. However, significant increases on the developmental time were obtained for all pesticides at 1,000 ppm. The greatest effect was found for hexaflumuron and halofenozide, with mean increases in relation to the control of 37% and 22%, respectively. In those treatments where mites reached adulthood, the highest increase in developmental time was recorded for pyriproxyfen at 10,000 ppm, being approximately twofold that obtained for the control.

Accordingly, halofenozide, pyriproxyfen, and chlorpyrifos were selected to evaluate their effect on reproduction and survival of *T. putrescentiae* adults.

Effects of Chemicals on Adult Mortality, Reproduction, and Progeny Development. The percentage of adult mortality after being exposed for 25 d to halofenozide, pyriproxyfen, and chlorpyrifos is shown in Table 3. No effect was found for pyriproxyfen, and

Table 2. Developmental time (days $\pm$ SE) from larva to adult of *T. putrescentiae* immature stages fed with brewers' yeast flakes treated with six different pesticides

Doses (ppm)	Azadirachtin	Pyriproxyfen	Halofenozide	Hexaflumuron (Consult 10EC)	Spinosad (Tracer)	Chlorpyrifos (Dursban 48)
0 ^a	5.7 $\pm$ 0.1a (49)	5.8 $\pm$ 0.1a (50)	5.8 $\pm$ 0.1a (48)	5.7 $\pm$ 0.1a (50)	5.8 $\pm$ 0.1a (49)	5.8 $\pm$ 0.1a (48)
10	5.6 $\pm$ 0.1a (50)	5.8 $\pm$ 0.1a (47)	5.6 $\pm$ 0.1a (45)	5.8 $\pm$ 0.1a (50)	5.8 $\pm$ 0.1a (48)	5.7 $\pm$ 0.1a (49)
100	5.9 $\pm$ 0.1ab (45)	5.6 $\pm$ 0.1a (49)	5.6 $\pm$ 0.1a (49)	5.9 $\pm$ 0.1a (48)	5.8 $\pm$ 0.1a (40)	5.8 $\pm$ 0.1a (48)
1,000	6.0 $\pm$ 0.1b (43)	6.5 $\pm$ 0.1b (48)	7.1 $\pm$ 0.3b (27)	7.8 $\pm$ 0.1b (38)	6.1 $\pm$ 0.1b (49)	6.2 $\pm$ 0.1b (48)
10,000	6.5 $\pm$ 0.1c (49)	11.0 $\pm$ 1.0c (2)	–	ND	6.7 $\pm$ 0.1c (41)	–

Values followed by different letters within columns are significantly different ($P < 0.05$; nested ANOVA followed by Newman-Keuls test). The sample size is shown in parentheses under each value.

^a Control: methanol for halofenozide and azadirachtin and distilled water for the rest.

ND, no data available.

Table 3. Percentage of mortality of *T. putrescentiae* adults fed for 25 days with brewers' yeast flakes treated with three different pesticides

Doses (ppm)	Halofenozide	Pyriproxyfen	Chlorpyrifos
0	10a	20a	20a
1,000	70b	10a	5a
10,000	85b	30a	75b

Values followed by different letters within columns are significantly different ($P < 0.05$; chi-square tests); $n = 20$ mites per chemical and dose.

only the dose of 10,000 ppm for chlorpyrifos increased significantly the mortality of adults. In contrast, halofenozide yielded mortalities of 70% and 85% at 1,000 ppm and 10,000 ppm, respectively.

No significant differences on the fecundity of *T. putrescentiae* adults were observed for pyriproxyfen at any of the doses used. For chlorpyrifos, only the dose of 10,000 ppm yielded a significant reduction of fecundity with regard to the control. Halofenozide caused the greatest reduction on fecundity, as the total number of eggs laid was reduced >130 times at 10,000 ppm, and almost 5 times at 1,000 ppm (Table 4).

In all the chemicals tested, females were able to lay eggs during the 25 d of the assay, except for those treated with halofenozide, in which the oviposition period was reduced to 22 d at 1,000 ppm and to 8 d at 10,000 ppm (Fig. 2).

After adult exposure to diet-treated brewers yeast, the duration of egg stage, which ranged from 3.5 ± 0.1 to 3.8 ± 0.1 d, was similar to the values obtained for the controls, which varied from 3.7 ± 0.1 to 3.8 ± 0.1 d. Egg hatch was similar in all treatments, and always $>98\%$. No significant differences were found on the developmental time of the progeny from adults exposed to chemicals. Similarly, no differences were found in the total developmental time from egg to adult or in the total mortality corresponding to this period for the progeny of treated mites.

Discussion

It is well known that workers occupationally exposed to storage mites have a high prevalence of allergic airway diseases and that avoidance of mite allergen by chemical control of mites can effectively reduce allergic symptoms. We have shown that some reduced risk chemicals, such as the IGRs pyriproxyfen

Table 4. Fecundity (total number of eggs per female; mean $\pm$ SE) of *T. putrescentiae* females fed for 25 days with brewers' yeast flakes treated with three different pesticides

Doses (ppm)	Halofenozide	Pyriproxyfen	Chlorpyrifos
0	613.3 $\pm$ 71.1a	475.7 $\pm$ 78.9a	447.2 $\pm$ 60.9a
1,000	128.3 $\pm$ 23.9b	528.0 $\pm$ 45.2a	492.8 $\pm$ 51.4a
10,000	4.7 $\pm$ 1.7c	377.0 $\pm$ 51.8a	76.0 $\pm$ 38.4b

Values followed by different letters within columns are significantly different ($P < 0.05$; nested ANOVA followed by Newman-Keuls test); $n = 10$ pairs per chemical and dose.

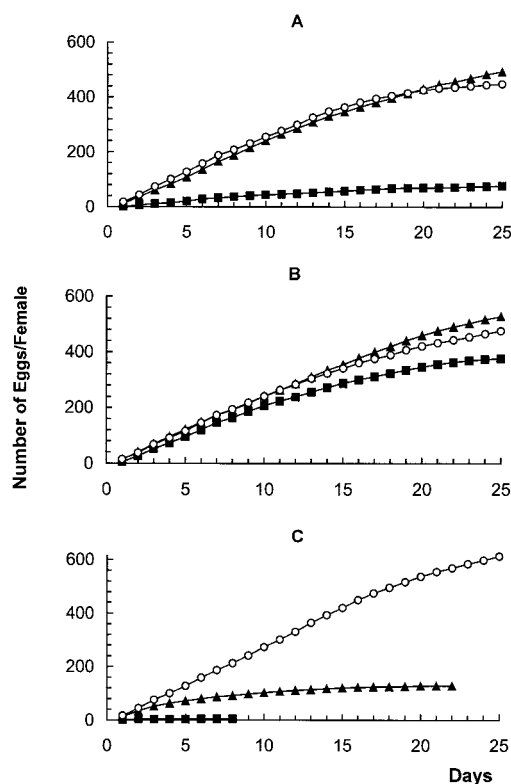


Fig. 2. Total number of eggs per female for *T. putrescentiae* females fed with brewers' yeast flakes treated with chlorpyrifos (A), pyriproxyfen (B) and halofenozide (C), at three different doses: 0 ppm (○), 1,000 ppm (▲), and 10,000 ppm (■).

and halofenozide, incorporated to brewers' yeast flakes, have an effect on survival and development of the acarid *T. putrescentiae* at similar or lower doses than chlorpyrifos.

The organophosphate pesticide chlorpyrifos was used as a standard because it is widely used for the control of astigmatid stored product mites. The dose needed to obtain 100% of mortality was 10,000 ppm. Doses of 0.2 mg of active ingredient per 100 g of grain has been reported in wheat sprayed with chlorpyrifos for the control of *Acarus siro* L., *T. putrescentiae*, and *Lepidoglyphus destructor* (Schrack) to obtain the same mortality rate (Wilkin and Hope 1973). Difference in the application procedure and in the strain of mite used may account for variations in the dose needed to control this mite.

We have found that technical azadirachtin incorporated to brewers' yeast flakes produced only a minor delay in the development of immature stages of *T. putrescentiae*, even at the highest dose tested (10,000 ppm). Other studies have also reported a small effect of neem products on mites (Sanguanpong and Schmutterer 1992). It may well be that higher doses could have greater effect on survival and development, but they would not be feasible. The miticidal

activity of spinosad against phytophagous mites is rather controversial. In fact, there is convincing evidence that its biological activity is rather low unless it is synergized by surfactants used in formulations (Cowles et al. 2000). Accordingly, we have detected a minor effect of spinosad on *T. putrescentiae*, as there were only small delays in developmental time at the highest dose tested (10,000 ppm). The use of surfactants was not considered as it was intended to elucidate the activity of spinosad alone.

Few studies have addressed the effect of chitin synthesis inhibitors on phytophagous or storage mites. We have recorded a low mortality ($\approx 16\%$) of the immature stages of *T. putrescentiae* even when they were exposed at the highest dose (1,000 ppm) of hexaflumuron tested. No further doses were assayed because at 10,000 ppm, the brewers' yeast flakes treated with this compound had a sticky and spongy texture that made impossible the development of the mite larvae. These results agree with those reported by Downing et al. (1990), who did not find any reduction in the number of mites in populations of *Dermatophagoides farinae* Hughes treated with the IGRs diflubenzuron and triflumuron at a dose as high as 50,000 ppm.

The juvenile hormone mimic pyriproxyfen and the molting hormone agonist halofenozide showed the highest efficacy, as these compounds negatively affected developmental time and survival of immature stages. Additionally, a differential effect on the reproduction was observed.

The exposure of *T. putrescentiae* to diet-incorporated with pyriproxyfen provoked a highly significant mortality of immature stages at a dose of 10,000 ppm and significant delays in the developmental time for doses $>1,000$ ppm. The later results are similar to that obtained by Czaja-Topinska et al. (1979) after treating topically immature mobile stages of this mite with several juvenile hormone analogues. The low activity of juvenile hormone analogues against astigmatid mites has also been reported by Downing et al. (1990, 1993). These authors evaluated the effect of methoprene and hydroxyphenol on *D. farinae* and pointed out that the population growth was suppressed at doses 25–67 times greater than what is generally used in an application for cat flea control at home (1,000–1,500 ppm).

Of all the pesticides tested, halofenozide showed the highest effectiveness. Interestingly, a strong and persistent antifeedant activity was noticed on those larvae of *T. putrescentiae* exposed to halofenozide in a no-choice situation. Moreover, they followed similar mortality pattern to that obtained for the starved population, and were unable to reach the protonymph stage. This result is consistent with the fact that when larvae were exposed to halofenozide or starved for three days, and then switched to nontreated brewers yeast, both presented similar mortality rates ($\approx 40\%$) and duration of the developmental time. When adults were exposed to a dose of 1,000 ppm of halofenozide, a significant reduction in adult survival and fecundity was obtained, whereas it required a dose of 10,000 ppm of chlorpyrifos to obtain similar results. This finding

cannot be clearly explained by an antifeedant activity of halofenozide on adults, because a high percentage of mites were able to survive and lay eggs at dose of 1000 ppm. It is likely that halofenozide can disrupt ecdysteroid-dependent physiological processes related with oocyte growth and embryo development, depending on its kinetics in the adult (Farinós et al. 1999). However, more information is warranted before a clear conclusion can be drawn on the mode of action of halofenozide on the mite reproduction system.

In summary, our results reveal that out of the six compounds tested halofenozide has the highest activity on immature and adult stages. The other chemicals yielded smaller effects than chlorpyrifos. The strong antifeedant effect of halofenozide on immature *T. putrescentiae*, together with the highly significant effect observed on female fecundity suggests that this chemical can be a good alternative for the control of *T. putrescentiae* in farming environments, because of its low toxicity for vertebrates (RohMid 1996). Nevertheless, more information is required at a large-scale situation to assess the practical application of this chemical.

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