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Contribución al desarrollo de estrategias de manejo integrado de plagas de la vid
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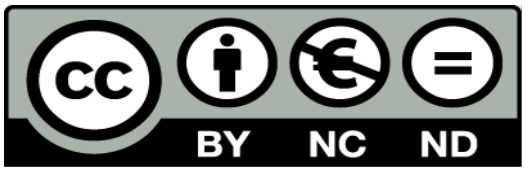

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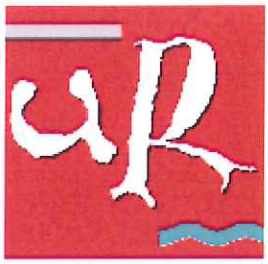

UNIVERSIDAD DE LA RIOJA

DEPARTAMENTO DE AGRICULTURA Y ALIMENTACIÓN

ÁREA DE PRODUCCIÓN VEGETAL

UNIDAD DE PROTECCIÓN DE CULTIVOS

Contribución al desarrollo de estrategias de manejo integrado de plagas de la vid.

TESIS DOCTORAL

Christina Elizabeth Pease

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DEPARTAMENT OF FOOD & AGRICULTURE

AGRICULTURAL PRODUCTION AREA

CROP PROTECTION DEVISION

Contribution to integrated pest management strategies for species commonly found in vineyards.

PhD THESIS

Christina Elizabeth Pease

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Memoria presentada por

Christina Elizabeth Pease

Para optar al grado de Doctora por la

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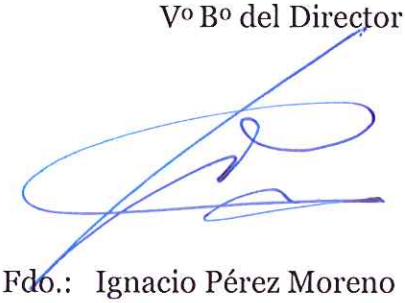

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Esta tesis esta dedicado a toda la gente
que me apoyaba,
que me daba su amistad,
y creía en mi durante los largos años de mi educación
y también, esta dedicada a la gente menos afortunada que yo...
espero que algún día con la experiencia adquirida
puedo ayudar en
la protección de vuestro cultivos.

This theses is dedicated to all the people
whom have supported me,
given me their friendship,
and believed in me during the long years of my education,
and also to those less fortunate than I...
hope that one day with the experience that I've gained
I can help in the protection of your crops.

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1 Global summary

To increase the available strategies for the control of populations of *Tetranychus urticae, Eotetranychus carpini* and *Lobesia botrana* bioassays were performed with kaolin, flufenoxuron and spirodiclofen. With the aim of protecting the natural enemy *Trichogramma cacoeciae* evaluation of possible secondary effects were also carried out.

Trials were carried out to evaluate if kaolin caused deterrent *T. urticae* patch use behavior. Effects of kaolin in mixtures with flufenoxuron or spirodiclofen and the interaction between the pesticides was evaluated in fecundity and fertility of directly treated females along with eggs and larvae.

Due to the impact on patch use behavior kaolin deterred feeding of *T. urticae* females on treated vegetation. Females didn't become accustomed to kaolin within the duration at which the bioassays were carried out. Kaolin did not detour the oviposition of treated females as flufenoxuron alone and in mixtures did. However, following the data, the percentage of eggs hatched can be reduced by treatments with kaolin in combination with flufenoxuron even at reduced rates.

Flufenoxuron and it's mixture with kaolin, when directly applied to eggs less than 24 hours old lowered the hatch percentage even at reduced concentrations and without antagonistic interactions between the two protection products. Due to greater larval survival with the mixture of kaolin and flufenoxuron an antagonistic interaction resulted when the χ^2 test was used. Thus treatments with the mixture could result in lower efficacy of the products combined on this life stage.

Due to the $\chi 2$ test resulting in an antagonistic interaction it appears that mixing spirodiclofen with kaolin would not result in higher efficacy for the control of *T. urticae* in the egg stage even though the suppression of this lipid synthesis inhibitor by kaolin was not complete.

Effects of spirodiclofen on eggs of *E. carpini* was evaluated. The probit analysis, with equation y = a + bx, resulted in the intercept, a = 4.788 and the slope of b = 2.662. Using the linear response curve to evaluate the data an increasing egg mortality of *E. carpini* with increasing concentration was obtained with a slope of 2.662. This was to be expected as a greater concentration of a toxin should give higher mortality. From the results herein presented spirodiclofen appears to be a very effective ovicide for this Tetranychidae.

When the effects of kaolin particle film were evaluated an inhibitory effect of the oviposition behavior of *Lobesia botrana* on both plastic oviposition chambers and on grape, in choice and no choice trials resulted. Treatments with kaolin in the product Surround WP® resulted in lower *Lobesia botrana* egg survival. This effect held true in all bioassay types and equally throughout the duration of the experiments.

The survival of neonate larvae was not demonstrated to be effected by treatments containing the kaolin product Surround WP®. Antifeedant effects on larval development and pupal weight were not effected by kaolin treatments with Surround WP®.

Trials were run to reveal if the kaolin treatment of *E. kuehniella* and *L. botrana* eggs had an effect on *T. cacoeciae* parasitism and parasitoid emergence. No effect in parasitism nor parasitoid emergence was detected when offered kaolin treated *E. kuehniella* host eggs. No secondary effect on *T. cacoeciae* parasitism was found from kaolin treatments of *L. botrana* eggs. There was, however, an inhibitory effect on parasitoid emergence in this host species. Due to these results the combination of the biological control agent, *T. cacoeciae,* and the kaolin particle film has been found to be compatible in these laboratory bioassays under the stated conditions.

1.2 Resumen global en español

El presente trabajo fue llevado a cabo con la finalidad global de generar conocimientos útiles para incrementar las herramientas de control incorporables al Manejo Integrado de importantes plagas de la vid tanto en España como en otras partes del mundo: *Lobesia botrana*, *Eotetranychus carpini* y *Tetranychus urticae*. En concreto, se desarrollaron bioensayos con caolín, flufenoxurón y spirodiclofén. Por otro lado, se llevaron a cabo investigaciones encaminadas a evaluar efectos secundarios del caolín sobre el parasitoide oófago *Trichogramma cacoeciae* con el fin de compatibilizar su utilización conjunta frente a *L. botrana*.

Se evaluó el efecto disuasorio de la alimentación del caolín sobre *T. urticae*, así como su efecto sobre la fecundidad y fertilidad de la plaga cuando era mezclado con flufenoxurón y spirodiclofén y se aplicaba a hembras adultas, a huevos y a larvas.

Aplicado directamente sobre la hembras de *T. urticae*, el caolín resultó ejercer un efecto disuasorio sobre su alimentación durante todo el tiempo de duración del bioensayo. Por otro lado, el caolín no afectó significativamente a la fecundidad del ácaro, mientras que sí lo hicieron, tanto el flufenoxurón como su mezcla con caolín. Sin embargo, el porcentaje de huevos eclosados se vio significativamente reducido por el tratamiento con los dos compuestos mezclados, incluso a concentraciones reducidas.

Aplicaciones de flufenoxurón y de su mezcla con caolín sobre huevos de menos de 24 h de edad de *T. urticae*, redujeron significativamente la eclosión de los mismos, incluso a bajas concentraciones, no observándose un efecto antagonista entre ambos compuestos. Por el contrario, cuando se trataron larvas del ácaro con la mezcla, se obtuvieron elevados porcentajes de supervivencia, mostrándose un efecto antagonista entre ambos.

El spirodiclofén y el caolín manifestaron una interacción antagónica cuando se aplicaron sobre huevos de menos de 24 h de edad de *T. urticae*. Así,

la mortalidad observada en el tratamiento con la mezcla no difirió significativamente de la observada en el testigo, a pesar de que el spirodiclofén solo afectaba significativamente a la eclosión de los huevos del ácaro.

Se caracterizó la actividad acaricida del spirodiclofén sobre huevos de menos de 24 h de edad de *E. carpini*. La recta de regresión ponderada probit obtenida tuvo una ordenada en el origen de 4,788 y una pendiente de 2,662. El valor de la LC_{90} resultó ser muy bajo, lo que pone de manifiesto un elevado efecto ovicida del spirodiclofén sobre el ácaro, resultando así prometedor para su incorporación al Manejo Integrado del mismo.

Cuando los efectos de las partículas de caolín fueron evaluados se observó un efecto inhibidor de la oviposición en *Lobesia botrana*, tanto sobre sustrato de plástico, como sobre uva, lo mismo en los ensayos de elección, como en los de no elección. Además, el tratamiento con caolín produjo una importante mortalidad de huevos en todos lo bioensayos.

El caolín no afectó a la supervivencia de las larvas neonatas de la polilla del racimo. Tampoco se encontró efecto antialimentario, por lo que no se vio afectado el desarrollo de las formas inmaduras.

Por último, se llevaron a cabo ensayos para averiguar si el tratamiento con caolín sobre los huevos de *Ephestia kuehniella* y de *Lobesia botrana* afecta de forma negativa al parasitismo por parte de *Trichogramma cacoeciae*, así como a la emergencia de los adultos del parasitoide. Sobre huevos de *E. kuehniella* no se detectó ningún efecto negativo. Tampoco se detectó efecto negativo sobre el parasitismo en el caso de huevos de *L. botrana*, aunque sí se encontró un efecto inhibidor de la emergencia de los adultos del parasitoide. Por tanto, los resultados indican que la combinación de la técnica de control biológico por inundación mediante *T. cacoeciae* y el uso del caolín puede ser compatible.

2 Global introduction

Various factors contribute to the difference in population densities of arthropod species in undisturbed settings and modern agroecosystems. Among the contributing factors which promote the appearance of the overpopulation of economically damaging arthropod populations in agroecosystems is the predictable super-abundance of available nutrients, the suppression of natural enemies by pesticides and the selection pressure promoting the occurrence of resistance (Huffaker *et al.*, 1969).

It has been repeated over and over again that we are in an arms race against the overpopulation of specific damaging arthropod species. This arms race concept comes about with the never ending appearance of resistance. Nearly all important greenhouse pests are documented to have some resistance to insecticides and acaricides (Georghiou & Mellon, 1983).

To combat resistance there are several emerging and re-emerging alternative crop protection techniques and combinations of those (Theiling & Croft, 1988; Zhang & Sanderson, 1990) such as, the promotion of natural enemies, dead end crops, non-toxic barrier products, breading of resistant plant species, and the sparing use of new generation, highly specific, rational pesticides. Pruning, meticulous pest monitoring, along with cultural or traditional farming practices are also being evaluated as possible options in the effort to combat resistance. Traditional farming practices include the cultivation of plants which naturally repel pests, and multiple species cropping systems.

The unification of appropriate available techniques, as those mentioned above, has become known as integrated pest management which has it's origins in integrated insect control (Frisbie & Adkisson, 1985; Horn, 1988). However, in it's early day integrated pest management focused on biological control agents (Horn, 1988; Luckman & Metcalif, 1994) and use of those agents to reduce the dependency on insecticides (Stern *et al.*, 1959). Nowadays the use of biological control agents can not be achieved unless the agent can survive the other strategies employed, i.e. toxic pesticide treatments.

Indubitably, the combination of chemical crop protection residues and non-specific, historically used pesticides constitute one of the noteworthy non-point sources of environmental contaminants (Weston *et al.,* 2009). The environmental contamination of these residues have been seen to negatively influence many species (Tarzwell, 1959). Contamination and negative impacts to non-target by-standard species appeared at the beginning of the industrial age. They were notably brought to light in popular literature in the 1960s with Rachel Carson's book, Silent Spring.

The accumulation of these non-selective pesticide residues is not only found in soil, air and water, but also in the cells of non-target species, such as fish (Cope, 1960), benthic invertebrates (Hintzen *et al.,* 2009), fairy shrimp (Brausch & Smith, 2009), songbirds (Mineau, 1988; U.S. EPA, 1989; Mineau, 1993; Stinson *et al.,* 1994), the Burrowing Owl, (Fox *et al.,* 1989), the Peregrine Falcon, (Newton, 1976), wildlife in general (DeWitt, 1956), aquatic invertebrates, bees (Kevan & LaBerge, 1979; Wayland, 1991), Sparrowhawks (Chen *et al.,* 2009) and unaccountably more species including humans. DDT for example, (long ago outlawed in many countries) was found in fat and breast milk of nursing human mothers (Laug, 1951).

The prevalence of cases of side effects to non-target species, such as beneficial arthropods and natural enemies has become extremely common. Guidelines for the risk assessment to natural enemies and beneficial arthropods have, of course, been written on the subject, (for example: Overmeer *et al.,* 1982; OEPP/EPPO, 1989; Samsøe-Petersen, 1990; Aldridge & Carter, 1992; Barrett, 1992; Bakker *et al.,* 1992; Carter *et al.,* 1992; Hassan, 1992a; Hassan, 1992b; Blümel *et al.,* 1993; Duso, 1994; OEPP/EPPO, 1994; Bakker & Jacas, 1995; Stark *et al.,* 2004).

These side effects to non-target species present worrisome situations due to lower regional biodiversity, lower crop yield due to the impact on pollination services (Kevan, 1975; Kevan & LaBerge, 1979) and in some cases, higher pest population density after treatment in consequence to disruption of the pest's natural enemies (Croft, 1990; Jansen *et al.,* 1994; Osborne *et al.,* 1999). The

influence of side effects to non-target species is often unforeseen and yet incredibly far reaching in our ecosystem.

There are, however, techniques that can protect non-target species and favour the use of natural enemies, such as timely applications, manipulation of pesticide formulations, method and special distribution of treatment (Croft, 1990) and use of pesticides which have little detrimental effect to natural enemies (Hoy & Cave, 1985; Hoy & Ouyang, 1986; Zhang & Sanderson, 1990; Kim & Paik, 1996a; Spollen & Isman, 1996).

With global warming and therefore the acceleration of the growth rates in arthropods we are beginning to find more and more cases damagingly high populations. These species, previously found at low densities, are now approaching the economic threshold levels of pest classification. This along with ever increasing human population the need for new integrated crop protection strategies are now more important than ever.

This project was funded for research on some of the most economically important and damaging arthropod species and one of their natural enemies present in Spanish vineyards. The natural enemies of these pests affect other pest species as well. Therefore, hopefully, this research will, not only be useful to Spanish agriculturalists in their vineyards, but also contribute to practical strategies for those agriculturalists without alternatives to harmful biocides.

2.2 Resumen del introducción global en español

Es una realidad que, en la agricultura actual, aparecen con mucha frecuencia elevadas poblaciones de especies (de insectos y ácaros principalmente) que, al superar los umbrales admisibles, se convierten en importantes plagas de los más variados cultivos en los más diversos agroecosistemas. Varios factores contribuyen a potenciar este hecho; entre ellos destaca la presencia de grandes cantidades de alimento fácilmente disponible, la drástica reducción de las poblaciones de sus enemigos naturales por la acción de productos fitosanitarios poco selectivos, o la aparición de resistencias por parte

de las plagas a estos productos fitosanitarios.

No es el anterior, el único problema generado por el empleo casi exclusivo de plaguicidas sintéticos neurotóxicos poco selectivos en el control de plagas. La adquisición del estatus de plaga por parte de especies que antes no lo tenían, la rápida resurgencia de las poblaciones plaga, o los problemas de contaminación ambiental por residuos plaguicidas son otros de los problemas más destacables.

Como consecuencia de lo anteriormente expuesto, la lucha contra las plagas ha evolucionado hasta cristalizar en la actualidad, en el denominado Manejo Integrado de Plagas que, entre otros principios, se basa en promover una integración racional de todos los métodos de control disponibles en cada caso.

La investigación presentada en este trabajo se ubica en este contexto, de modo que los diferentes objetivos planteados tienen como finalidad aportar información útil que permita incorporar e integrar interesantes medidas de control frente a algunas de las plagas más importantes que afectan a los viñedos españoles y de otras partes del mundo.

3 Crop protection products

The crop protection products, Surround® WP (95% kaolin), Cascade® (10% flufenoxuron), and Envidor® 240 SC (24% spirodiclofen) were employed in the thesis investigations.

3.1 Kaolin

Particle film technology was initially studied and used as a method for controlling arthropod pests of agricultural crops by Richardson and Glover, (1932) when they exposed the 12-spotted cucumber beetle, *Diabrotica duodecempunctata* (Fab.) (Coleoptera: Chrysomelidae), to calcium arsenate and kaolin. This technology has recently reemerged for the use of controlling agricultural diseases and wider variety of arthropod pests along with the reduction of sunburn and heat stress in the crop plants and fruit trees (Glenn *et al.*, 2001; Melgarejo *et al.*, 2004; Wand *et al.*, 2006).

Surround® WP, a kaolin product, was developed by D.M. Glenn and G.J. Puterka in cooperation with the Englhard Corporation and the Agricultural Research Station of the USDA in 1996. It was registered for use in 1998. This product is based on the white, purified, non toxic, fine-grained, nonabrasive aluminosilicate mineral ($Al_4Si_4O_{10}[OH]_8$), kaolin, which is inert over a wide pH range. Its small grain size makes it easily suspended in water.

Hydrophobic formulations of kaolin have been developed by the coating of the particles with organic zirconate, steric acid, chrome complexes, or other materials (Harben, 1995). The first prototype of the hydrophobic kaolin particle, M-96–018 (M96, Engelhard, Iselin, NJ) was applied to trees as a dust to make the plant surfaces water repellent (Glenn *et al.*, 1999). Hydrophobic kaolin particles have also been premixed with methanol for better dispersal in water (Sekutowski *et al.*, 1999). An even hydrophobic particle film resulted from the treatment with the mixture of kaolin and methanol which was found to prevent arthropod infestations and diseases (Puterka *et al.*, 2000a; Sekutowski *et al.*, 2000).

When the risks to human health due to kaolin were assessed by the US EPA, it was found that upon exposure to kaolin no evidence of toxicity to humans was detected. In addition, the FDA granted kaolin GRAS status (Generally Recognized as Safe) when used in human food. When the environmental risk assessment was completed the EPA found kaolin is not harmful to non-target organisms or to the environment. For example, studies with spiders and honeybees indicate that kaolin appears to have no adverse effects on beneficial insects/spiders. Aquatic organisms are not likely to be affected because kaolin does not dissolve in water. Due to the fact that kaolin has no mammalian toxicity, nor poses any danger to the environment and has been registered as safe in the Food Quality Protection Act, this strategy may also be valuable in organic farming (EPA, 1989; Showler, 2003; OMRI, 2007).

The suppression of arthropod pests, along with fungal and bacterial diseases by kaolin is due to a number of different nontoxic mechanisms. The hydrophobic particle film coating formulates a barrier causing the plant to be visually or tactilely unrecognizable to the arthropod pest. In addition, the attachment of particles to the bodies of the arthropods can disrupt insect movement, feeding, oviposition, and other activities. The particle film barrier also isolates the leaf surface from direct contact with water thus inhibiting some fungal and bacterial disease inoculums (Glenn *et al.*, 1999).

Since the development of these formulations a great number of studies have been carried out with the use of Surround® WP on many biological aspects of a wide range of crop pest. Kaolin has been shown to affect a variety of life stages of pest and, at times, their natural enemies. Egg viability and development, nymphal, larval and pupal development, adult emergence and population density have all been inspected in many arthropod pest species.

Not only has the affect of Surround® WP been investigated on arthropod species, but also on phytopathogenic bacteria and viral species as well. Studies from deterrence of feeding to oviposition to host selection with Surround® WP alone or in combinations with other crop protection products have also frequently appeared in the literature.

The results of previous studies found in the literature and the occasional disruption of natural enemies (Markó *et al.*, 2007) call for the expansion of investigations with Surround® WP, referred to as kaolin, against vineyard pests and prominent natural enemies.

3.2 Flufenoxuron

Cascade® (10% DC), commercialized by BASF, with the active ingredient, flufenoxuron, 1-{4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl}-3-(2,6-difluorobenzoyl)-urea, a benzoylurea, specifically a benzoilphenylurea, compound is a slow-acting growth regulator. This new generation pesticide is listed in group 15, the chitin synthesis inhibitors, type 0, IRAC, 2012.

Molting and metamorphosis are affected by insect growth regulators, IGRs, normally by interfering with cuticle formation, due to chitin synthesis inhibition, antagonizing juvenile hormone activity or mimicking juvenile hormone agonists or ecdysteroid agonists and ecdysone antagonist (Smet *et al.*, 1990; Oberlander *et al.*, 1991; Oberlander *et al.*, 1997). The synthesis and transport of specific proteins required for the assembly of N-acetyl-D-glucosamine (Glc-NAC) monomers into polymeric chitin is interrupted by chitin synthesis inhibitors (Oberlander & Silhacek, 1998). This mode of action has been illustrated in larval tissues (Salokhe *et al.*, 2006).

Flufenoxuron has been registered in more than 20 countries including South Korea, Japan, China, France, Italy, Spain, Greece, Latin American countries, Argentina, Chile, and Brazil and African countries for its application to fruits, vegetables, flowers, and beans (Japanese Food Safety Commission, 2009). It is registered for the control mites, namely, Tetranychid species, and insect defoliators, particularly, Lepidopteron species in crops such as citrus, and grapes.

Flufenoxuron is of low acute oral toxicity. It is not acutely toxic by skin contact or after inhalation. It is not irritant to skin or eyes. It is not a skin

sensitizer. No genotoxic potential was indicated. A low acute, short-term and long-term risk for insectivorous birds and a low acute risk for small herbivorous mammals was observed for it's representative use on grapevines. The exposure of bees should be completely avoided by the application of appropriate mitigation measures. A low risk was assessed to the earthworm, *Eisenia andrei* (Bouché), (Santos *et al.*, 2011) from its decomposition metabolite for the representative use on grapevines. No environmental exposure for soil organisms is expected following the representative uses in glasshouse. A low risk was identified for soil micro-organisms, terrestrial non-target plants and sewage treatment plants for the representative uses.

Flufenoxuron has been studied and used with success throughout the past two decades to control, for example, populations of *Agonoscena targionii* (Licht.) (Homoptera: Psylloidea), (Lababidi, 2002), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), (Salokhe *et al.*, 2006), neonates and 12 day old larvae of *Pandemis heparana* (Denis & Schiff.) (Lepidoptera: Tortricidae), (Ioriatti *et al.*, 2006), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), (Ibrahim & Ammar, 1988) and *T. urticae* (El Banhawy & Amer, 1992) just to name a few.

More recently sublethal effects of this chemical have been studied. One such investigation which focused on the effects of lowered concentrations of flufenoxuron was carried out (Salokhe, *et al.*, 2006). Their study confirmed a decrease in the protein amount in hemolymph in *Tribolium castaneum*. Lyra *et al.*, (1999) demonstrated effects on male copulating activity of *Spodoptera littoralis,* (Boisd.) (Lepidoptera: Noctuidae), when treated by ingestion.

Investigations with Cascade®, referred to as flufenoxuron, were programmed due to the efficacy found by previous authors with reduced dosages of flufenoxuron, and in combinations with other phytosanitary products, along with the low environmental and non-target species risk assessments.

3.3 Spirodiclofen

Spirodiclofen is classified within the tetronic acid derivative group which has acaricidal and insecticidal properties. Spirodiclofen is within the group 23, the lipid synthesis inhibitors (IRAC, 2012). The basic formation, Envidor® 240SC, was developed by Bayer Crop Science in 1992. It is selective, non-systematic and considered to be substantially active amongst the contact acaricides due to the fact that it remains on the waxy cuticle surface instead of penetrating deep into the plant tissue (Nauen, 2005). This pesticide is currently registered in many countries world wide.

Spirodiclofen has been found to be effective against various crop pests such as *Panonychus citri,* (McGregor) (Acari: Tetranychidae), in Chinese citrus orchards (Hu *et al.*, 2010), *Tetranychus evansi,* (Baker & Pritchard) (Acari: Tetranychidae), (Gotoh *et al.*, 2011), and on the reproductive capacity of *Tetranychus urticae,* (Kosh) (Acari: Tetranychidae), (Van Pottelberge *et al.*, 2009) in laboratory tests.

From the developer, spirodiclofen is said to have outstanding control of all important mites such as spider mites (e.g. *Tetranychus* spp., *Panonychus* spp.), eriophyd mites (e.g. *Phyllocoptutra* spp.), tarsenomid mites (e.g. *Hemitarsonemus* spp.), and false spider mites (e.g. *Brevipalpus* spp.).

Spirodiclofen acts to inhibit the production of acetyl Coenzyme A carboxylase, part of the first step in lipid synthesis. Lipids and their derived components play an important role in the formation of membranes and the integument therefore reducing the penetration of chemicals. Lipids are essential for the minimization of desiccation, and serve in chemical communication and numerous other biological processes (Blomquist *et al.*, 1987; Juárez, 1994).

An evaluation of the ecotoxicity of spirodiclofen was carried out in microcosm-based experiments, using a small-scale terrestrial ecosystem (STEM) containing, Mediterranean agricultural soil, earthworms, *Eisenia andrei* (Bouche) (Opisthopora: Lumbricidae), and turnip seeds, *Brassica rapa*

(L.) (Brassicales: Brassicaceae). In these bioassays spirodiclofen did not cause impairment of growth in *E. andrei,* at the recommended application rate. Spirodiclofen in combination with dimethoate, another commonly used pesticide which could easily come in contact with spirodiclofen in agricultural field conditions, was also evaluated. The binary mixtures did show an antagonistic effect on shoot length and fresh weight of *B. rapa* at all concentrations tested (Santos *et al.,* 2011).

Toxicity of spirodiclofen was also assessed on a beneficial pollinator insect, the bumblebee, *Bombus terrestris* (L.) (Hymenoptera: Apidae) in acute tests. When acute toxicity to worker bumblebees was tested by direct contact exposure, at the recommended concentration was safe, causing no acute toxicity, (<25%). Sublethal effects were also examined, causing a restriction in application times due to lower reproductive potential of females which experienced chronic contact (Besard *et al.,* 2010).

The following description of the toxicity and risk assessment of spirodiclofen is taken from the EPA's Pesticide Fact Sheet. Spirodiclofen is not an eye or dermal irritant and has low acute toxicity *via* oral, dermal, or inhalation routes, yet is a potential skin sensitizer. Spirodiclofen is practically nontoxic to terrestrial animals on an acute exposure basis. This compound does not appear to cause reproductive effects in avian species. Longer-term laboratory and field studies conducted using the formulated product show that populations of honey bees and the predaceous mite *Typhlodromus pyri* (Scheuten) (Acari: Phytoseiidae) are adversely affected (i.e., brood development, pupal and larval abundance, colony strength) at recommended application rates.

Since lipid stores are important for early life stage development in honey bees and other beneficial insects, chronic exposure to a compound that affects lipid biosynthesis could compromise the ability of organisms to successfully compete their lifecycle. Based on this information, beneficial insect populations appear to be at risk from chronic exposure to spirodiclofen at the proposed application rates. Label use restrictions are required for mitigating those risks

(EPA, 2005).

Bioassays to evaluate the effectiveness of Envidor® 240SC, referred to as spirodiclofen, were programmed due to the efficacy found by other investigators, and the low negative impacts on the environment and non-target species.

3.4 Resumen de capítulo en español

Recientemente, ha resurgido el interés por el empleo de tecnologías basadas en la producción de finas películas de partículas inertes para el control de plagas y enfermedades en agricultura, así como para reducir problemas de quemaduras de sol y de golpes de calor en frutales. El producto comercial Surround® WP, desarollado en 1996 a base de caolín, es uno de los más utilizados para estos fines. Este producto ha sido también el empleado en el presente trabajo para desarrollar investigaciones que faciliten su incorporación en el Manejo Integrado de importantes plagas de la vid.

El flufenoxurón es un compuesto inhibidor de la síntesis de quitina que, como consecuencia de ello, actúa como inhibidor del crecimiento de insectos y ácaros. Dado su modo de acción, es un plaguicida interesante para ser incorporado al Manejo Integrado de Plagas. Así, en el presente trabajo, el producto comercial Cascade®, cuyo ingrediente activo es flufenoxurón, fue utilizado para desarrollar investigaciones que orienten sobre su incorporación al Manejo Integrado de T. urticae en combinación con el caolín mencionado en el párrafo anterior.

Por su parte, el spirodiclofén es un derivado del ácido tetrónico con propiedades insecticidas y acaricidas. Dado que también se ha señalado como un plaguicida interesante para ser incorporado al Manejo Integrado de Plagas, en el presente trabajo se ha analizado la compatibilidad de su uso conjunto con caolín para el control de *T. urticae*. El producto comercial Envidor® 240 SC, cuyo ingrediente activo es spirodiclofén, fue el utilizado en los diferentes bioensayos.

Effects of kaolin alone and in mixtures with flufenoxuron & spirodiclofen on *Tetranychus urticae* Koch 1836

4.1 Introduction

Tetranychus urticae (Acari: Tetranychidae) often found in high densities causing great damage is recognized as a mite of important crop degradation and economic loss worldwide (Kim & Paik, 1996b; Cho, 2000).

It's rapid life cycle leads to a steep exponential growth curve under optimal nutrient and environmental conditions thus contributing to potential defoliation and or death of crop plants.

This tetranychid is polyphagous, and able to adapt to plant responses and pesticide treatments through a variety of detoxification activities involving various metabolic pathways. Therefore, it has an elevated host range and is prone to the formation of resistance.

Its arrhenotokous parthenogenesis reproduction also contributes to the ability of a surviving female to distribute her alleles rapidly. This can thus accelerate the formation of resistance when under pesticide selection pressure.

Its small size and efficient dispersion throughout individual plants or over expansive monoculture crops with its ballooning distribution strategy

along with other important biological characteristics all contribute to the global distribution of this species.

Taxonomic Position

T. urticae Koch is currently listed in the webpage: http://www.faunaeur.org, which corresponds to Fauna Europaea (2011) version 2.4. (accessed on October 28th, 2011) is as follows:

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Micrura
Infraclass	Acari
Superorder	Actinotrichida
Order	Prostigmata
Suborder	Eleutherengona
Superfamily	Tetranychoidea
Family	Tetranychidae
Subfamily	Tetranychinae
Tribe	Tetranychini
Genus	*Tetranychus*
Species	*urticae*

Morphology & Biology

The twospotted spider mite, *T. urticae,* is a phytophagous mite with great variability in adult appearance due to the coloration being extrinsic, or exogenous. In fact this species varies so much in its color that it is also known as the red spider mite and the common yellow mite in Spanish. Individuals can be found light yellow when having just come out of diapause (Evans, 1992a). Mature well nourished adult females can range in color from light green to quite reddish to almost black. Generally the large lateral spots of this species are

distinguishable, however, these spots blend into the body color when the individual is very dark. This is the case with the phenotype commonly found to infest Spanish vineyards which are generally very dark brown to black in color.

Females at their maximum size can measure 0.5 mm in length (Jeppson *et al.*, 1975). The species is markedly dimorphic, the males being substantially smaller than the females. The morphology of the male is particularly different than the female in shape. The anterior region of the male opisthosoma is markedly conical shaped, whereas the female opisthosoma is more squared off (Evans, 1992b). The legs of the male appear longer in relation to their idiosoma length (García-Marí *et al.*, 1994).

The eggs are spherical, and measure approximately 0.1 mm in diameter. They are transparent when freshly laid. They change from opaque to dark yellow through the course of their development. They are laid either on the leaf surface or suspended in the intricate webbing, normally on the underside of the leaf surface (Bueno Parra, 2004). This location depends upon the actual population density of the colony. For example, with high density and relatively great competition for space eggs are also found on the top side of the leaves.

Larvae are light in color easily distinguished from the protonymph and deutonymph stages by morphological characteristics. The most obvious of these is the number of legs on the posterior part of the opisthosoma; larvae are equipped with only one pare. Thus, in total the larvae only have three pares during this life stage (Evans, 1992a). Individuals in this stage are relatively venerable to predation and environmental extremes due to their fragility and their slow mobility.

Both the protonymph and deutonymph are equipped with four pares of legs making them octopod nymphal instars (Evans, 1992a). The mites actively feed during these stages and deutonymphs are slightly larger than protonymphs. They can normally only be told apart due to their age in cohorts. The life stages of *T. urticae* (figure 1).

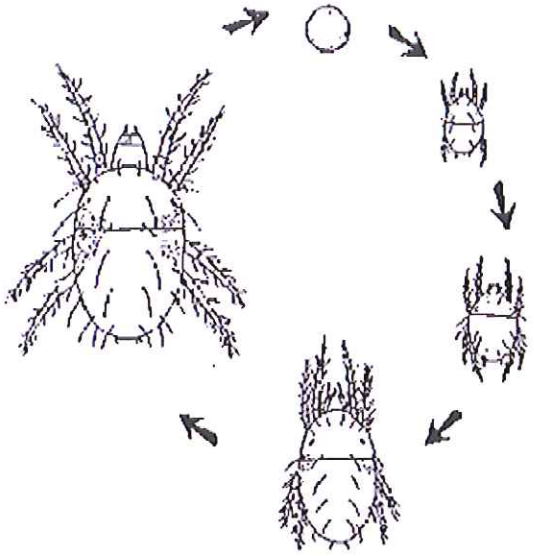


Figure 1. *T. urticae* life stages (UM Extension, 2009).

The protochrysalis, deutochrysalis, and teliochrysalis are all inactive stages of the individual (Evans, 1992a). During which the individual can be found gray in color due to the casting of the exuvia during apolysis (Jenkin & Hinton, 1966). Quiescent female deutonymphs can be distinguished from quiescent female protonymphs by the male guarding behavior. The quiescent female in the teliochrysalis stage produces a sex pheromone to which males are attracted. The males can be seen aggressively guarding the teliochrysalis females with whom they mate as soon emergence takes place (Penman & Cone, 1972). Due to the marked dimorphism in this species (figure 2) individuals can be easily separated by gender.

Figure 2. Sexual dimorphism of *T. urticae* (left: male, right: female).

Between January and March in the northern hemisphere, females become active, abandon their over wintering habitats and begin feeding. In vineyards this is before the shoots begin to grow and well before new leaves sprout. Due to the lack of green leaves and shoots the females are forced to use other sources of nutrients. Typically this is not a deterrence to population growth because they can use a great variety of plants, including many native species. In fact 150 economically important crop species are perfect hosts for this arthropod (Jeppson *et al.*, 1975) nowadays the number of plant species effected has grown to approximately 1,200 (Bolland *et al.*, 1998).

Reproduction of *T. urticae* is marked by arrhenotokous parthenogenesis. That is to say that unfertilized eggs result in male offspring. However a female when fertilized does not usually have internal fertilization of all eggs. This leads to the high observed variable in the sex ratio of offspring (Helle & Pijnacker, 1985). Even with this variability the ratio has been very often observed to be three females to every male offspring.

Within the life cycle of first generation females there are three periods, these being pre-oviposition, oviposition and post-oviposition. The fecundity of an individual female in optimal conditions ranges from forty to one hundred eggs over her two week oviposition period (Laing, 1969; Shih *et al.*, 1976; Helle & Pijnacker, 1985). This fecundity is influenced by many environmental and abiotic factors including temperature, relative humidity, sublethal doses of selected insecticides and host plant vigor (Wrensch & Young, 1985). Six to eight complete generations per year is typical in Mediterranean climates. However, the actual number of generations depends upon the temperature and humidity ranges experienced by each population (Brandenburg & Kennedy, 1987; Rao *et al.*, 1996).

Within the female lifespan web building and oviposition start soon after the end of diapause or overwintering. Due to the tendency to live in dense populations, webs play a crucial role in colony structure and reproductive behavior. On the leaf under surface trichomes support a buffering air layer within which a part of the plant transpiration humidity is trapped. The webbing

within this air layer microclimate acts as an incubator assuring a relative constant temperature and humidity. The eggs within this intricate barrier are also protected from some species of Phytoseiidae predators, and acaricidal treatments (Gerson, 1985; García-Marí *et al.*, 1994).

Eggs mature within 3 to 5 days after oviposition at normal seasonal temperature ranges in Mediterranean climates. After egg hatch the individual proceeds through the stages of larva, protochrysalis, protonymph, deutochrysalis, deutonymph, teliochrysalis, and adult. Males, on average, develop through the life cycle faster than females (Hussy & Huffaker, 1976; Helle & Sabelis, 1985; Zhang, 2003). The protochrysalis, deutochrysalis and teliochrysalis are inactive stages. During these molting stages a new cuticle is formed before the casting of the exuvia, and entrance into the proceeding active stage (Boudreaux, 1963). During the larval, protonymph and deutonymph stages the individual actively feeds and increases in weight.

The developmental time through each of these stages is highly correlated with temperature (Herbert, 1981). The preferred temperature for *T. urticae* is between 13 and 35 °C (Mori, 1961). However, between 30 to 32 °C the developmental time from egg to adult is said to have been less than a week (Carey & Bradley, 1982).

In the beginning of fall adult females stop feeding and overwinter in crevices, under the bark of vineyards and other woody species, in dirt clods and even leaf litter where they are protected from low temperatures and rain (Veerman, 1985). The timing for the entrance and exit of hibernation can be attributed to the change in photoperiod along with the average daily temperature (Brandenburg & Kennedy, 1987). However, if conditions are mild, populations can be reproductively active throughout the winter (Takafuji & Kambayashi, 1985).

Dispersal & Geographic distribution

The extension of the mite population throughout the plant is normally an active dispersion achieved by crawling (McEnroe & Dronka, 1971; Brandenburg & Kennedy, 1987). Mites commonly employ anemohoria, an inactive aerial drift (Fleschner *et al.,* 1956) for the relocation of individuals. This normally takes place when the population is motivated by a high level of competition for space, low relative humidity or insufficient level of nutrients (McEnroe & Dronka, 1971). This dispersion strategy is considered inactive due to the fact that the mite can not control its final destination. For this dispersion approach the mite adopts a posture wherein it orients itself toward the light source and lifts its hind appendages above its body (Suski & Naegele, 1963; Smitley & Kennedy, 1985). With this strategy mites have been transferred up to 100 meters on the wind (Brandenburg & Kennedy, 1982; Margolies & Kennedy, 1985; Miller *et al.*, 1985).

T. urticae is a temperate zone species, but has been found in the subtropical regions. It can be encountered on common ornamental plants including, but not limited to, arborvitae, azalea, camellia, citrus, evergreens, hollies, ligustrum, pittosporum, pyracantha, rose, and viburnum. It is found in berry crops including blackberry, blueberry and strawberry, and on a number of vegetable crops such as tomatoes, squash, eggplant, and cucumber. This mite is also a pest of ornamental trees and may damage maple, elm, redbud and has been reported on ash black locust and popular. It has been occasionally found on other trees (Johnson *et al.,* 1991). Tuttle and Baker, (1968) report this species to be a pest of deciduous fruit trees in northern regions of the U.S. and Europe. The species also has a array of weed plants on which it can complete it's life cycle. The global distribution of *T. urticae* includes Asia, Africa, the Americas, Australia, and New Zealand (García-Marí *et al.*, 1994). Following the revision of Pérez-Moreno in 1997, *T. urticae* is found to inhabit grape in the Check Republic, France, Germany, Italy, Spain and Switzerland (Galet, 1982; Schruft, 1985; Lozzia & Rota, 1988; Weber, 1991; Bueno-Parra, 2004).

Damage

T. urticae, like other tetranychid mites, normally feeds on the under side of the leaf by inserting its chelicerae into a plant cell and withdrawing cell contents (Baker & Connell, 1963). Thus rupturing the cell and causing necrotic discolorations of lighter spots wherein the level of chlorophyll is reduced (figure 3). Not only is this rupturing detrimental to the plant, but the saliva of *T. urticae* has been found to be phytotoxic in cotton agricultural systems (Sances *et al.*, 1982b). Defoliation, lower fruit, fiber, nut or seed production and death are common results of the uncontrolled feeding of these phytophagous mites (Huffaker *et al.*, 1969). The adaptability of *T. urticae* to new host plants and its world wide distribution lend to its status as one of the most important arthropod species of crop protection concern (Van De Vrie *et al.*, 1972; Zhang, 2003).

Figure 3. *T. urticae* leaf damage to *Phaseolus vulgaris,* var. Garrafal.

Treatment strategies

Historically and particularly since the end of world war two the most common strategy used to control *T. urticae* has been to use readily available non-selective pesticides and acaricides (Paik & Kim, 1996; Cho, 2000) or mixtures of them (Huffaker *et al.*, 1969) and at times with naive use.

In many cases the extensive use of pesticides has unwittingly exacerbated the incidence of *T. urticae* overpopulations due to the development of resistance (Kim & Paik, 1996b) and the deterioration of natural predator and parasite assemblages (Begljarov, 1957; Bartlett, 1963; Bartlett, 1964; Helle 1965a). There have arisen a number of other complications due to the sole use of chemical pesticides to control *T. urticae,* including threats to human health (Amer *et al.*, 1989).

Strategies which combine biological control, chemical usage, physical barriers and cultural practices (Lewis *et al.*, 1997) have also been explored. The application of alternative pesticides classes in a sequence, rotation or mosaic distribution of compounds acting on different target sites (Ahmad *et al.*, 2002) have all been widely used with the aim of managing resistance. Vegetable and essential oil mixtures to control *T. urticae* have been examined (Tsolakis & Ragusa, 2008). Essential oils or their vapors were shown to affect this pest (Kim & Yoo, 2002; Aslan *et al.*, 2004; Choi *et al.*, 2004). The effects of barrier films, new generation, and specialized pesticides which target specific pests continue to be investigated in laboratory and field trials. Investigations on this mite have also been carried out with the new generation pesticides, spirodiclofen (Rauch & Nauen, 2003; Marcic *et al.*, 2009) and flufenoxuron (El-Banhawy & Amer, 1992; Ahn *et al.*, 1993). Much research has been done with this species however investigations into alternatives continues due to the needs previously mentioned.

4.2 Objectives

With trials to evaluate if kaolin causes deterrent behavior on adult *T. urticae* females the first objective was carried out. The toxicity of kaolin and the interaction between the pesticides in mixtures with flufenoxuron on fecundity, and fertility of directly treated adult females was the second objective to be evaluated. The effect on *T. urticae* eggs due to direct treatments with kaolin and its mixtures with flufenoxuron was the next objective evaluated. The evaluation of effects of direct treatments to *T. urticae* larvae with kaolin and its mixture with flufenoxuron followed.

The effect on *T. urticae* eggs due to direct treatments of kaolin and its mixtures with spirodiclofen was the fifth objective evaluated. Effects of direct treatments to eggs along with pesticide interactions involving kaolin and spirodiclofen was the last objective evaluated with this species.

4.3 Materials & Methods

4.3.1 Mass Rearing of *T. urticae*

Mites used for bioassays were raised from a colony maintained in the Crop Protection Laboratory of the University of La Rioja originally collected from a natural Spanish population on ornamental crops in 2000 without pesticide selection pressure since collection. The mites were reared on green bean plants (*Phaseolus vulgaris*, var. Garrafal) in a growth chamber at the standard conditions of 24±1 °C, 65±5% relative humidity and photoperiod of 16:8 (L:D). The colony on the green bean plants was isolated in a translucent box (60 cm high, 45 cm long and 30 cm wide) covered with fine screen lid attached by Velcro (figure 4). Green bean plants were replaced when the conditions warranted the change.

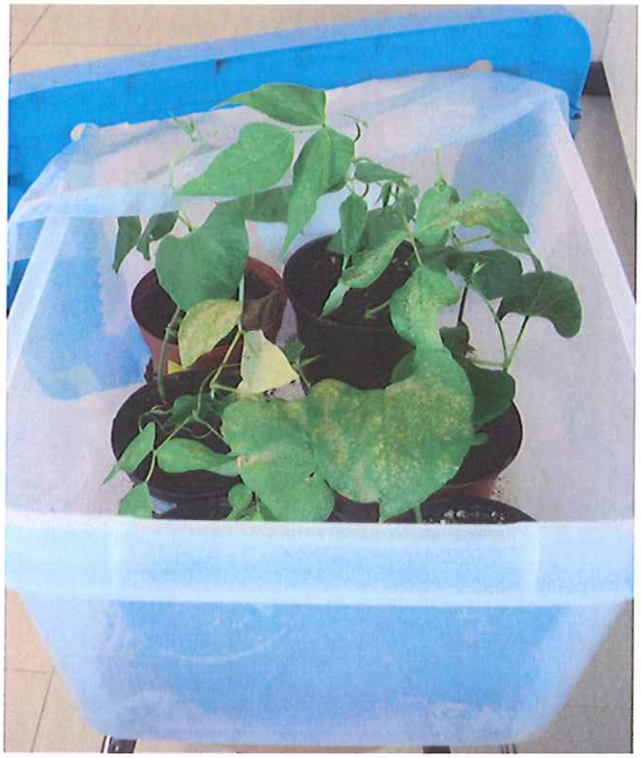


Figure 4. *T. urticae* colony.

4.3.2 Formation of cohorts

To synchronize the age of individuals for bioassays, cohorts were prepared. An individual excised green bean trifoliate leaflet (*Phaseolus vulgaris*, var. Garrafal) was placed up side down (stomatal side up) in a 9 cm diameter Petri dish previously lined with three disks of water soaked filter paper (figure 5). Gravid adult females were placed on the leaf to oviposit. Some of the cohorts used in experiments were started directly on the 2 cm leaf disks to be employed. Again the females were allowed to oviposit on the underside of the previously isolated leaf disks for 8 or 12 hours.

The Petri dishes containing the filter paper layers, the leaflets or leaf disks, and mites were placed into transparent cylindrical plastic boxes (5 cm high by 12 cm diameter) containing enough water to maintain the leaf surface isolated. A cloth wick and paper clip were attached to the side of the Petri dish to connect the filter paper layers with the water in the outer box. In this manner the humidity of the filter paper was maintained and thus the isolation of the

oviposition substrate (figure 6). The outer box was topped with a lid having two to four fine screen covered holes for air and humidity flow thus avoiding excessive condensation.

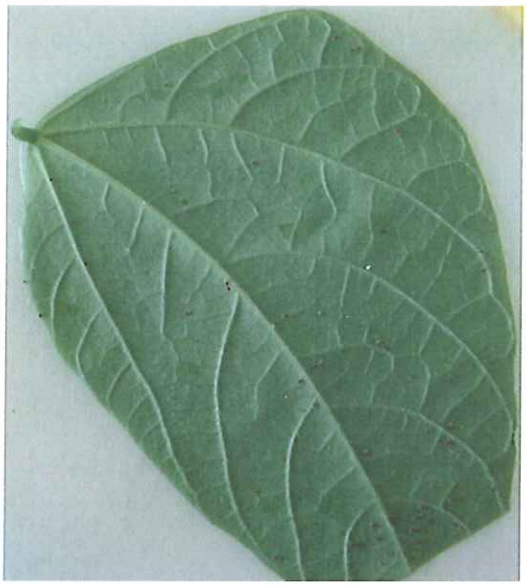


Figure 5. *T. urticae* cohort leaf.

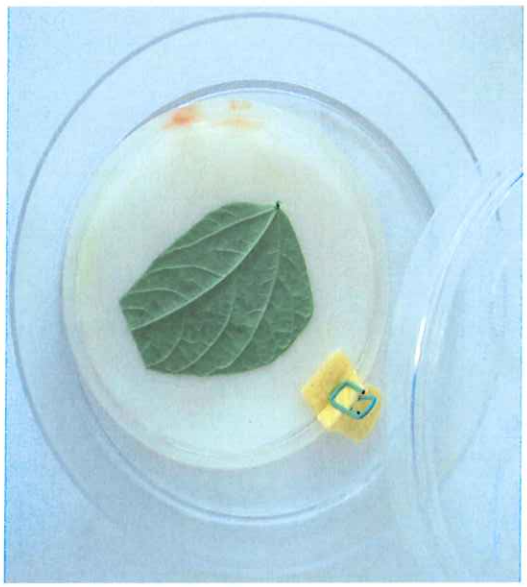


Figure 6. *T. urticae* cohort setup.

4.3.3 Deterrent effects of kaolin on *T. urticae*

All individuals used were taken from cohorts made using females originating in the laboratory colony. One, three and five 24 hour old *T. urticae* gravid adult females were placed on previously treated 2 cm diameter green bean leaf disks underside up.

Green bean plants used for leaf disk material were grown in the same conditions as those used for the laboratory colony. Treatments were carried out in the Potter tower at 0.5 bar with 5.5 ml of kaolin solution at a concentration of 57 g/L or distilled water alone for the control.

Leaf disks of each treatment were placed in a standard 9 cm Petri dish previously lined with two layers of moisten filter paper. The Petri dish was placed in a 12 cm diameter by 5 cm deep cylindrical box. Water was added to the outer cylindrical box. A piece of cloth used as a wick was connected the Petri dish using a paper clip. Thus conductivity of the water between the filter paper

of the Petri dish and the water reservoir of the outer box was maintained. The individual leaf disks were isolated from each other and the experimental set up in this manner. The arenas are illustrated below (figure 7). The outer box was topped with a lid containing four 2 cm diameter fine mesh covered holes. These arenas were used in both the choice and the no choice bioassays.

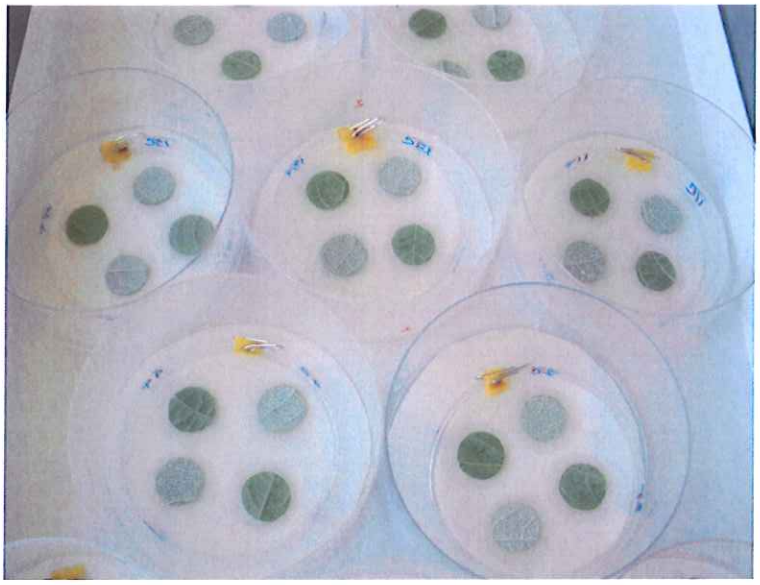


Figure 7. *T. urticae* bioassay arenas with kaolin, flufenoxuron and their mixture.

Choice deterrence trials

In these choice trials the green bean disks were previously half treated and dried to provide a choice to the young females. Treatments were preformed by covering then spraying the exposed half of each leaf disk in a Potter tower, twice, that is to say half of the disk was treated with kaolin while the other half with the water carrier. The number of females in each patch type was recorded for six consecutive 30 minute periods.

In these choice deterrence trials twenty nine replications were included in the statistical analysis for the assay with one female. In the three female assay thirty replications were included in the statistical analysis. Twenty-five replicates were statistically analyzed for the assay involving five females.

No choice deterrence trials

In these no choice trials the green bean leaf disks were either completely treated with kaolin at the above mentioned concentration or distilled water. The experimental set up differed in the location of the leaf disks upon 3 cm translucent plastic disks. The leaf disks were fixed to the plastic disks with a 20 µl drop of agar (figure 8).

The number of females upon the leaf disks or upon the plastic disk, surrounding the exterior border of the leaf disk, was recorded every 30 minutes during six consecutive periods. In these no choice trials, thirty replications of each treatment type were included in the statistical analysis for the trials involving one female.

Twenty six replicates each were included in the statistical analysis in the trial involving three females. For the trial with five females twenty replications were included in the statistical analysis.

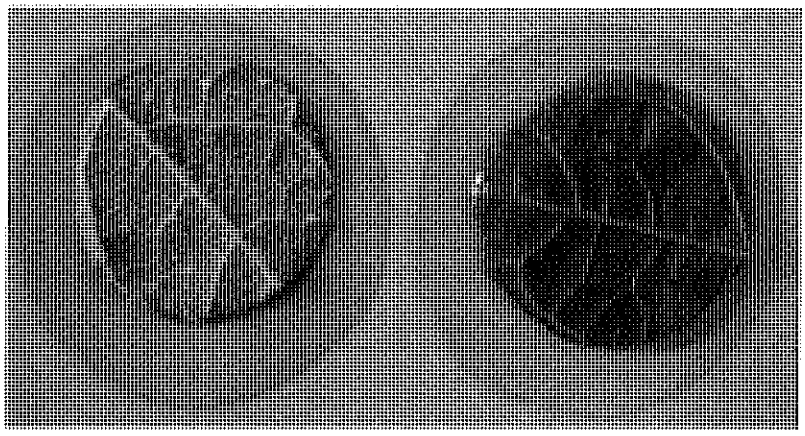


Figure 8. Leaf disks affixed to plastic disks.
(Left to right, kaoliu treated & water treated control).

4.3.4 Effect of kaolin and its mixture with flufenoxuron on *T. urticae* fecundity & fertility

The experimental arenas with all materials and methods were set up as those in the above *T. urticae* deterrence trail. However, females were treated directly on the leaf disks. The treatments were as follows: control (distilled water); kaolin at 57 g/L; flufenoxuron at 11 ppm; and the mixture of kaolin at 57 g/L and flufenoxuron at 11 ppm. In previous laboratory studies 11 ppm of flufenoxuron was found to be with in the limits of the LC_{40} for *T. urticae* females in their first day of adulthood (unpublished laboratory data).

One *T. urticae* 24 hour old gravid adult female was placed on the leaf disk and exposed to one of the four treatments in the Potter tower. The disks were allowed to dry then placed directly on the moistened filter paper of the arena. The complete set up was placed in the growth chamber at the same conditions as the cohorts were raised.

The number of eggs was recorded every 24 hours until the females were removed at the end of the fourth 24 hour period. At the end of the ninth 24 hour period the number of hatched and remaining eggs were recorded. Thirty replications of the four treatments were included in the statistical analysis.

4.3.5 Effects on *T. urticae* egg mortality from kaolin & its mixtures with flufenoxuron

Cohorts of approximately 25 eggs each replication were prepared directly on the 2 cm leaf disks employed as described in detail above. The experimental arenas used were equal to those described above in the deterrence trail.

The eggs were treated with an age of less than 24 hours. The treatments were as follows: control (treated with distilled water); kaolin alone; the mixture of kaolin with flufenoxuron; and flufenoxuron alone. Three sets of bioassays

were carried out differing only in the concentrations of flufenoxuron (11 ppm, 19 ppm and 33 ppm) alone and in mixture. The concentration of kaolin alone and in the three mixtures was 57 g/L and the distilled water used as the control was also employed as the carrier for all other treatments.

All bioassays were carried out in the standard trial conditions described previously. The number of hatched eggs was recorded at the end of the fifth 24 hour period in each set of bioassays. Eleven to twenty replications of each of the four treatments were included in the statistical analysis for each of the three sets of bioassays evaluating the effect of the mixtures of kaolin and flufenoxuron on egg mortality.

4.3.6 Mortality of *T. urticae* larvae due to direct treatments with kaolin and its mixture with flufenoxuron

Cohorts from 7 to 15 larvae each replication were prepared directly on leaf disks as described in detail above. The arenas and standard experimental conditions were equal to those described in the deterrence trail.

The larvae were treated within the first day after hatching. The treatments were as follows: control (treated with distilled water); kaolin 57 g/L; flufenoxuron at 11 ppm; and the mixture of kaolin with flufenoxuron at the same concentrations. The distilled water used as control was also employed as the carrier for all of the other treatments. Larval mortality was recorded five days after treatment. Ten to thirteen replications of each treatment were run to examine the mortality of *T. urticae* larvae due to direct treatments with kaolin and its mixture with flufenoxuron.

4.3.7 Mortality of *T. urticae* eggs due to direct treatments with kaolin and its mixtures with spirodiclofen

Cohorts from ten to thirty eggs each replication were prepared on leaf disks as described in detail in the section pertaining to cohorts. The

experimental arenas employed were equal to those described in detail in the deterrence trail. Trials with eggs were initially carried out to determine if the interaction of the mixture of spirodiclofen and kaolin was additive, synergistic or antagonistic.

The eggs were treated less than 24 hours after the beginning of the cohort oviposition period. The treatments were as follows: control (treated with distilled water), kaolin at 57 g/L, spirodiclofen, and a mixture of kaolin at 57 g/L with spirodiclofen. Three concentrations of spirodiclofen (0.5 ppm, 1.5 ppm and 2 ppm) alone and in mixture with kaolin were included in the bioassay. Distilled water used for the control as well as carrier for all other treatments.

All bioassays were held in the standard trial conditions described previously. The number of eggs hatched was recorded six days after treatment. Ten replications of each treatment were included in the statistical analysis for the bioassays involving spirodiclofen and kaolin.

4.4 Statistical Analysis

All statistical analyses were preformed with Statgraphics Plus for Windows 4.0. 1999 or Statgraphics Centurion XVI. In all comparisons the skewedness and kurtosis were both examined for their presence within the range for data sets with normal distributions. With the Levene test the equality of the standard deviations was also verified before other analysis were carried out.

The deterrence index equation was used in the statistical analysis for the six data sets in the deterrence experiments. This equation was modified from those in publications by Nawrot *et al.,* (1984), Sadek, (2003) and Zapata *et al.,* (2009).

$$\text{Deterrence index} = \left(\frac{F_c - F_k}{F_c + F_k} \right) \times 100$$

Fc represents the number of females in the control treatment.

Fk represents the nnmber of females in the kaolin treatment.

The χ^2 test equation and theory was used to evaluate interactions between two products in mixtures (Koppenhöfer & Fuzy, 2008; Morales-Rodriguez & Peck, 2009) and resulting impact on fertility, fecundity, egg and larval mortality of *T. urticae*. The χ^2 were calculated using the following formula.

$$\chi^2 = \frac{(M_{KF} - M_E)^2}{M_E}$$

The calculated theoretical expected mortality value, M_E for combined agents was obtained using the following formula also used after the authors mentioned above.

$$M_E = (M_K + M_F) \times \left(1 - \frac{M_K}{100}\right)$$

M_K - mean Abbott corrected mortality percentage observed in kaolin treated replications

M_F - mean Abbott corrected mortality percentage observed in the replications containing treatment with flufenoxuron or spirodiclofen

M_{KF} - mean Abbott corrected mortality percentage observed in the treatment containing the mixture of the two products (kaolin with flufenoxuron or spirodiclofen).

After the χ^2 values were calculated they were compared to the theoretical χ^2 table values for 1 degree of freedom at the confidence level of 95%. The null hypothesis, H_0, was formulated as "there is an additive effect of the two agents" if χ^2calculated < χ^2 theoretical value from the table. On the other hand, if χ^2 calculated > χ^2 theoretical value, and $M_{KF} - M_E$ was positive the interaction was considered to be synergistic. Yet, if $M_{KF} - M_E$ was negative, in the condition when χ^2 calculated > χ^2 theoretical value, the interaction was considered to be antagonistic.

4.5 Results & Discussion

4.5.1 Deterrent effects of kaolin on *T. urticae*

Significant differences between the two data sets for the treatments, control and kaolin, for both pared and independent data for all densities of females in the kaolin deterrent bioassay were evaluated using the deterrence

index equation. Within each of the six bioassays the differences of the indices between time blocks were compared using the t-Student test and the multiple range test with the Tukey $\alpha = 0.05$. The indices resulting from the data on patch use behavior showed a deterrent effect of kaolin on *T. urticae*. This deterrence was illustrated by the positive percentage values which represent greater patch use in the control replicates, for both the choice and no choice bioassays. This tendency was found in all time blocks in all densities of females except at the density involving five females per replicate. At the five female density two random, unrelated time blocks, one in the choice bioassay and one in the no choice bioassay displayed fewer individuals using the untreated foliage (table 1). When time blocks within treatments were compared, no tendencies were found.

Table 1. Deterrent effects of kaolin on *T. urticae* females.

	Deterrence indices (%)					
	Choice bioassays			No Choice bioassays		
Time (hours)	1 female	3 females	5 females	1 female	3 females	5 females
0.5	65.5 ± 14.3 a	51.7 ± 9.3 ab	7.2 ± 9.7 a	11.5 ± 9.0 a	15.2 ± 7.0 a	7.2 ± 3.1 a
1.0	65.5 ± 14.3 a	64.0 ± 7.2 a	8.3 ± 7.0 a	3.6 ± 6.0 a	4.9 ± 2.5 a	-1.8 ± 1.6 b
1.5	79.3 ± 11.5 a	40.0 ± 11.2 ab	-5.6 ± 11.3 a	0.0 ± 3.4 a	9.6 ± 3.6 a	0.9 ± 1.6 ab
2.0	72.4 ± 13.0 a	37.8 ± 10.6 ab	5.0 ± 7.9 a	3.4 ± 4.8 a	9.8 ± 3.6 a	1.5 ± 1.8 ab
2.5	65.5 ± 14.3 a	28.9 ± 12.3 b	4.0 ± 10.8 a	1.7 ± 4.9 a	13.9 ± 4.7 a	4.9 ± 2.8 ab
3.0	72.4 ± 13.0 a	26.7 ± 13.7 b	4.3 ± 8.2 a	1.7 ± 4.9 a	17.1 ± 6.0 a	5.5 ± 3.0 a

* Means with different letters within the same column have significant differences (Tukey test, $P \leq 0.05$).

There are various possible factors which attribute to the deterrence of patch use of *T. urticae*. The effects demonstrated may be a result of an inhibition to feeding due to the presence of the kaolin particles as suggested by Glenn *et al.*, 1999. Kaolin at the microscopic level may inhibit feeding owing to the irritation or possible damage of the chelicerae as the mite attempts to feed or a disruption of the digestive system (Richardson & Glover, 1932). This observed effect could also be a result of increased female search time for an

appropriate place to build webbing thus resulting in less time in each patch.

4.5.2 Effect of kaolin and its mixture with flufenoxuron on fecundity and fertility of treated females

A one-Way ANOVA was used in the statistical analysis of the effect on fecundity and fertility data sets from the bioassay with kaolin and its mixture with flufenoxuron. The F-test was employed to distinguish significant differences between the means. To determine which means were significantly different from which others the Newman-Keuls test within the multiple range test was used at a the 95% confidence level. The influence on fecundity and fertility of treated females by the interactions between the protection products was also evaluated using the χ^2 test.

No impact on fecundity and fertility of directly treated females resulted with the kaolin treatment (tables 2 & 3). This was in accordance with laboratory trials by Bostanian & Racette in 2008, using apple leaf disks. The cumulative mean fecundity (table 3) was found to be lowest in the treatment with both kaolin and flufenoxuron combined. Kim & Seo, (2001) reduced the reproduction rate of this mite with flufenoxuron treatments to 64–67% of that found in the control. Significant differences were found in the percentage of eggs hatched between treatments with and without flufenoxuron. The survival of eggs was highest in the control and kaolin treatments followed by the flufenoxuron and finally the mixture treatment.

When the interaction of kaolin and flufenoxuron was evaluated in egg viability the calculated $\chi 2$ value was less than the theoretical table value for one degree of freedom at the 95% confidence level. This result, according to the null hypothesis gave an additive effect between the two crop protection products. As with our trials, management strategies with mixtures of pesticides have been evaluated on various biological aspects of arthropods. Roush in 1993 theorized that mixtures, under certain conditions, can delay the development of resistance more effectively than sequences or rotations. Other combinations of pesticides

have been tested at varying sub-lethal concentrations and it appears our mixture was as effective with a relatively low concentration of flufenoxuron when mixed with the non-toxic kaolin.

Table 2. Effects on fecundity (± SE) per *T. urticae* female.

Mean fecundity (eggs/female)					
	Day 1	Day 2	Day 3	Day 4	Cumulative Total *
Control	10.0 ± 0.4	9.6 ± 0.6	9.6 ± 0.9	9.6 ± 1.0	41.4 ± 2.0 a
Kaolin	10.4 ± 0.2	11.5 ± 0.5	10.5 ± 0.8	10.5 ± 0.8	44.3± 1.6 a
Kaolin + Flufenoxuron	7.8 ± 0.4	7.9 ± 0.7	8.6 ± 1.0	8.6 ± 1.0	32.5 ± 2.7 b
Flufenoxuron	7.8 ± 0.4	6.8 ± 0.8	5.8 ± 1.0	6.1 ± 1.1	27.1 ± 2.9 b

* Cumulative means followed by different letters differ significantly ($\alpha < 0.05$).

Table 3. Effects on fertility % (± SE) of the oviposition periods in table 2.

Mean fertility (N° of eggs hatched, %)					
	Day 1	Day 2	Day 3	Day 4	Cumulative Total*
Control	45.0 ± 0.3	96.9 ± 0.6	93.8 ± 0.8	96.9 ± 1.5	88.6 ± 1.5 a
Kaolin	42.3 ± 0.4	92.2 ± 0.7	84.8 ± 0.6	80 ± 0.9	76.5 ± 1.2 a
Kaolin + Flufenoxuron	42.3 ± 0.4	51.9 ± 0.7	19.8 ± 0.6	17.4 ± 0.5	31.4 ± 1.5 c
Flufenoxuron	35.8 ± 0.3	79.4 ± 0.8	51.7 ± 0.7	68.9 ± 1.0	60.2 ± 2.2 b

* Cumulative means followed by different letters differ significantly ($\alpha < 0.05$).

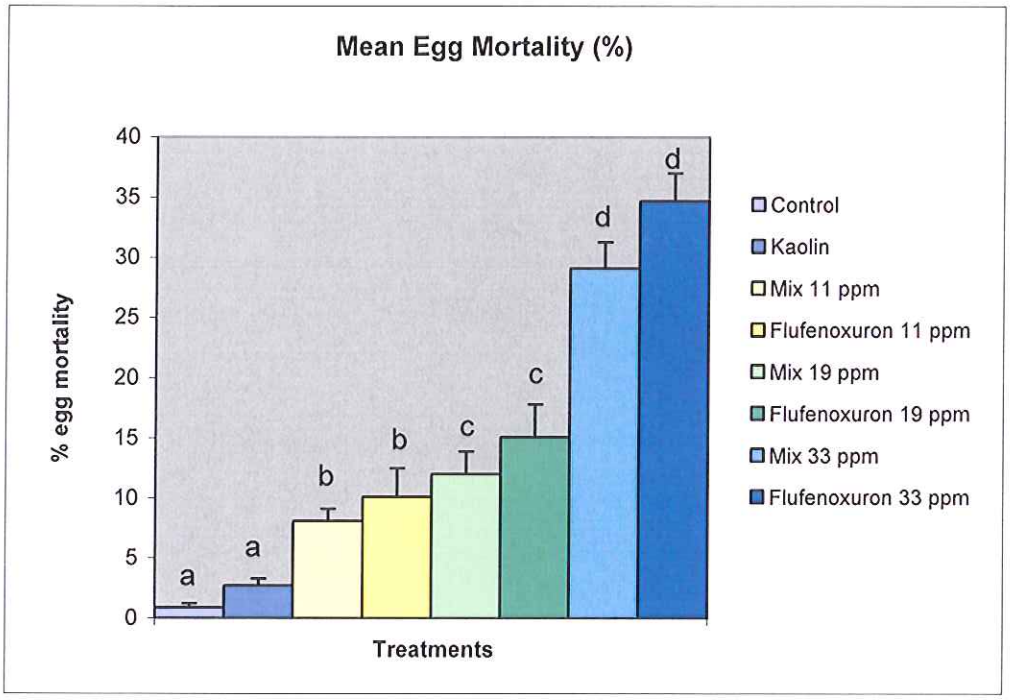
4.5.3 Effect on *T. urticae* eggs due to direct treatments with kaolin and its mixtures with flufenoxuron

The analysis of differences between treatments at all concentrations of flufenoxuron and the mixtures with kaolin were carried out using the multiple range test with LSD from Fisher and the Kruskal-Wallis test. The effects on mortality was also evaluated to determine if a synergic, additive or antagonistic

relationship between kaolin 57 g/L and flufenoxuron at 11 ppm, 19 ppm and 33 ppm existed and was illustrated by significant differences in the treatments. The analysis of interactions was carried out using the χ^2 test.

When eggs of *T. urticae* less than 24 hours old were directly exposed to kaolin there was no significant difference between this treatment and the control. All of the treatments containing flufenoxuron resulted in significantly greater mortality than those without flufenoxuron (figure 9 & table 4). This held true for all three bioassays with the concentrations of 11 ppm, 19 ppm and 33 ppm. No statistically significant differences were detected between mixture treatments and flufenoxuron treatments with the same concentration of flufenoxuron.

Figure 9. Average egg mortality with kaolin and flufenoxuron mixtures.



* Treatments followed by different letters differ significantly ($\alpha < 0.05$).

The observed results were in correlation with other studies on this spider mite by Kim and Seo, (2001). They found similar results, yet with a much higher concentration. They found flufenoxuron at 50 ppm to be effective against immature stages of *T. urticae* with 36% mortality in eggs and no survival to

adulthood when directly treated. Similar mortality is reported here yet with five times less product.

On the other hand, Ahn, *et al.*, 1993 found no effect at all on eggs with flufenoxuron treatments. The variation in results of these authors and our results could have been influenced by the differences in sensitivity of the distinct populations.

Table 4. Effects of kaolin & its mixtures with flufenoxuron at 11, 19 & 33 ppm on egg mortality .

Egg Mortality (%)			
Treatment	Mean Mortality (%)	Standard error	Abbot corrected Mean Mortality (%)
Control	0.87	0.30	-
Kaolin	2.71	0.60	1.84
Mixture 11 ppm	8.11	1.00	6.76
Flufenoxuron 11 ppm	10.13	2.35	8.82
Mixture 19 ppm	12.02	1.89	10.76
Flufenoxuron 19 ppm	15.09	2.70	13.88
Mixture 33 ppm	29.12	2.20	28.11
Flufenoxuron 33 ppm	34.70	2.30	33.77

When the χ^2 test was run with the Abbott corrected mean values (table 4). The calculated χ^2 value was found to be less than the theoretical χ^2 value from the table for 1 degree of freedom for all three bioassays at the 0.05 significance. The null hypothesis, H_o, was formulated as "there is an additive effect of the two agents" if χ^2 calculated< χ^2 theoretical value. This was the case in all of the trials involving directly treated eggs.

4.5.4 Effect of direct treatments to *T. urticae* larvae with kaolin and its mixture with flufenoxuron

An ANOVA was run to compare the data sets of the four treatments employing the F test. The Tukey test in the multiple range test was used to determine which means were different from each other. The χ^2 equations and

theory was also employed to analyze the interaction between the phytosanitary products kaolin at 57 g/L and flufenoxuron at 11 ppm and the effect of the interaction on larval mortality.

When larvae of *T. urticae* were directly exposed to treatments containing kaolin, flufenoxuron, and the mixture of the two there was a statistically significant higher rate of mortality in all treatment than in the control (table 5).

Table 5. Effects of kaolin, flufenoxuron & their mixture on larval mortality.

Larval mortality (%)				
Treatment	Mean (%)	Abbott corrected mean (%)	Standard error	Groups which differ significantly
Control	17.94	-	2.72	a
Kaolin	20.70	3.36	2.29	a
Kaolin + Flufenoxuron	30.76	15.62	3.26	b
Flufenoxuron	47.27	35.74	3.26	c

The calculated χ^2 value was greater than the theoretical χ^2 value from the table for 1 degree of freedom at both the 0.05 and 0.01 significance levels. Therefore, it was necessary to compare the M_E (calculated expected theoretical mortality percentage) with the M_{KF} (Abbott corrected mortality percentage observed in the mixture treatment). The M_E was greater than the M_{KF} thus resulting in a negative value of their difference. Therefore, the interaction is interpreted as antagonistic.

The impact of flufenoxuron directly treated larva was, again, in accordance with those found by Kim & Seo, (2001) wherein treatments with 50ppm flufenoxuron resulted in an average mortality of 64%. With the differences in the treatments with the mixture of kaolin and flufenoxuron and each alone in comparison to the control we see kaolin did not completely suppress the effect of flufenoxuron. The M_{KF} combined mortality being lower than the theoretical M_E gave a calculated χ^2 which was greater than the χ^2 theoretical value. Therefore in this case the interaction was found to be

antagonistic even with the incomplete suppression illustrated.

4.5.5 Effect on *T. urticae* eggs due to direct treatments of kaolin and its mixtures with spirodiclofen

The effect of the interactions between kaolin at 57 g/L and spirodiclofen at 0.5 ppm, 1.5 ppm and 2 ppm were analyzed with χ^2 test employing the egg mortality data sets.

When the eggs of *T. urticae* less than 24 hours old were directly exposed to treatments containing spirodiclofen at the concentrations of 0.50 ppm, 1.50 ppm and 2.0 ppm there was a statistically significant higher rate of mortality than in the control. The comparison of data sets illustrated the treatments containing only spirodiclofen also had higher mortality percentages than the treatments of mixtures with kaolin at each of the three concentrations when analyzed with the multiple range test employing LSD (lowest significant difference) from Fisher. The mortality percentage was also higher with greater concentration of spirodiclofen, as to be expected. Treatments with the mixtures did result in higher mortality percentages than the control and kaolin treatment alone. Thus it appears there was an incomplete suppression of spirodiclofen by kaolin (table 6 & figure 10).

In a 2009 publication Van Pottelberge and associates found a reduction in fertility of eggs placed by susceptible females due to spirodiclofen treatments. No eggs survived treatments of spirodiclofen when treated with 1000 ppm. In the bioassays herein presented 90% mortality of treated eggs was achieved with treatments of spirodiclofen at only 2 ppm. Nauen *et al.*, in 2000 found achieved an LC50 value of 0.33 ppm for larvae. It appears our population is relatively sensitive in comparison to the sensitive population of the Van Pottelberge group and similarly sensitive to that used in bioassays carried out by Nauen and associates.

When the χ^2 test was run for all three concentrations of spirodiclofen and

the mixtures using the Abbott corrected mortality percentages the calculated χ^2 value was greater than the theoretical χ^2 value from the table for 1 degree of freedom for all three bioassays. The M_E was greater than the M_{KF} in all three concentrations of spirodiclofen. The alternative hypothesis stated if the calculated χ^2 was greater than the theoretical χ^2 value from the table and the M_E was greater than the M_{KF}, the interaction was interpreted as antagonistic.

Figure 10. Average egg mortality due to kaolin and mixtures with spirodiclofen.

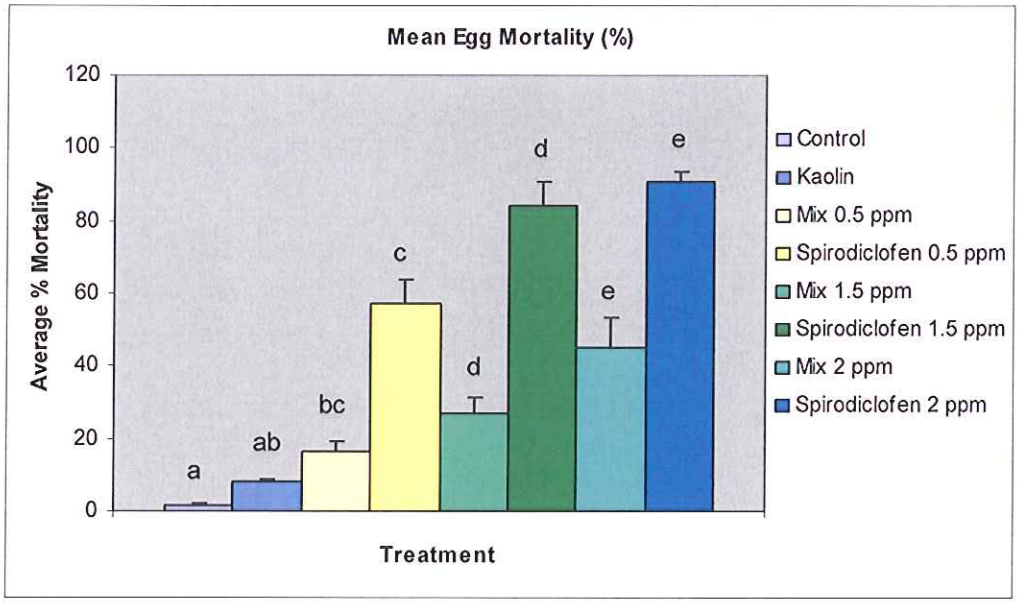


Table 6. Corresponding egg mortality values (%), standard errors & groups.

Egg Mortality				
Treatment	Mean(%)	Abbot Corrected Mean (%)	Standard Error	Groups which differ significantly
Control	1.41	-	0.95	a
Kaolin	8.04	6.72	0.86	ab
Mix 0.5 ppm	16.69	15.50	2.72	bc
Spirodiclofen 0.5 ppm	57.28	56.67	6.45	c
Mix 1.5 ppm	27.01	25.97	4.51	d
Spirodiclofen 1.5 ppm	84.13	83.90	6.51	d
Mix 2 ppm	45.34	44.56	7.94	e
Spirodiclofen 2 ppm	90.73	90.60	2.81	e

The results demonstrate the mixture of spirodiclofen and kaolin did not elevate the mortality rates in directly treated less than 24 hour old eggs. However, in the mixture treatments, spirodiclofen was not completely suppressed by kaolin as was demonstrated with a higher rate of mortality in the mixture treatment than in that of kaolin alone. This antagonistic interaction could be the result of a physical absorption of, or barrier to the spirodiclofen when applied on non-mobile life stages.

The impacts on biological aspects of target species due to the interactions of pesticides in mixtures appears to be highly variable. The interactions are strongly dependent on the treatment methods and the modes of action of each individual pesticide.

4.6 Chapter Conclusions

1. Due to the impact on patch use behavior kaolin deterred feeding of *T. urticae* females on treated vegetation. Females didn't become accustomed to kaolin within the duration at which the bioassays were carried out.

2. Kaolin did not detour the oviposition of treated females as flufenoxuron alone and in mixtures did. However, following the data, the percentage of eggs hatched can be reduced by treatments with kaolin in combination with flufenoxuron even at reduced rates.

3. Flufenoxuron and it's mixture with kaolin, when directly applied to eggs less than 24 hours old lowered the hatch percentage even at reduced concentrations and without antagonistic interactions between the two protection products.

4. Due to greater larval survival with the mixture of kaolin and flufenoxuron an antagonistic interaction resulted when the χ^2 test was used. Thus treatments with the mixture could result in lower efficacy of the products combined on this life stage.

5. Due to the $\chi 2$ test resulting in an antagonistic interaction it appears that mixing spirodiclofen with kaolin would not result in higher efficacy for the control of *T. urticae* in the egg stage even though the suppression of this lipid synthesis inhibitor by kaolin was not complete.

4.7 Resumen de capítulo en español

Tetranychus urticae es un ácaro fitófago que ha sido reconocido, a nivel mundial, como una de las plagas más importantes de los cultivos, ya que puede originar elevadas pérdidas económicas.

Se llevaron a cabo bioensayos de laboratorio para estudiar el efecto disuasorio del caolín sobre la oviposición de *T. urticae*. Además, se evaluó el efecto del caolín y de su mezcla con flufenoxurón sobre la mortalidad, fecundidad y fertilidad de hembras tratadas directamente, y sobre la mortalidad de huevos y larvas del ácaro. Por último, se estudió el efecto de la mezcla de caolín con spirodiclofén sobre la mortalidad de huevos.

El caolín mostró un efecto disuasorio de la oviposición sobre las hembras de *T. urticae*. No se encontraron diferencias significativas entre los índices de deterrencia al inicio y al final del ensayo, lo que indica que las hembras no se acostumbraron al caolín a lo largo del tiempo.

La fecundidad de las hembras tratadas con caolín y la fertilidad de sus huevos no se vio afectada por este producto. La mezcla de caolín con flufenoxurón sí afectó a la fecundidad de las hembras, sin embargo no se encontró un efecto sinergista entre ambos productos. Con respecto a la fertilidad, el efecto negativo fue superior cuando los dos compuestos se mezclaron que cuando se aplicaron por separado, obteniéndose un efecto aditivo entre ellos.

Cuando larvas de *T. urticae* fueron tratadas con caolín, la mortalidad fue significativamente mayor que en el control. La mezcla de caolín y flufenoxurón

mostró diferencias significativas en la mortalidad con respecto al tratamiento con flufenoxurón solo, y las pruebas estadísticas revelaron un efecto antagónico entre ambos productos.

La mortalidad de los huevos de *T. urticae* tratados con spirodiclofen fue muy superior a la del testigo. Sin embargo, la mortalidad se redujo de forma significativa cuando este producto se mezcló con caolín, encontrándose un efecto antagonista entre ambos compuestos.

Characterization of spirodiclofen toxicity on the eggs of *Eotetranychus carpini* (Oudemans 1905)

5.1 Introduction

Various subspecies of *Eotetranychus carpini* have been described. *Eotetranychus carpini vitis* (Oudemans), the yellow grapevine mite, is one of the subspecies differing only slightly in its biology and host range.

Historically, *E. carpini vitis*, has been found to cause damage in the majority of varieties of *Vitis vinifera* (L.) (Vitales: Vitaceae) throughout central and southern Europe and the Americas, typically in Mediterranean zones.

As *Eotetranychus carpini borealis*, this mite has adapted biological life stage parameters to lower temperatures. It has been seen to out compete *T. urticae* on grape in early spring when the temperatures are lower.

This subspecies is also oligophagous and has been commonly called the Hornbeam mite due to one of its primary hosts being the Hornbeam, *Carpinus betulus*. It completes the lifecycle at rates equal to those on grape.

Taxonomic position

E. carpini Oudemans is currently listed in the webpage: http://www.faunaeur.org which corresponds to Fauna Europaea (2011) version 2.4. (accessed on November 28th, 2011) is as follows:

Kingdom	Animalia
Subkingdom	Eumetazoa
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Micrura
Infraclass	Acari
Superorder	Actinotrichida
Order	Prostigmata
Suborder	Eleutherengona
Superfamily	Tetranychoidea
Family	Tetranychidae
Subfamily	Tetranychinae
Tribe	Tetranychini
Genus	*Eotetranychus*
Species	*carpini*

Morphology & Biology

Eotetranychus carpini is another phytophagous mite species which, at high densities, can cause economically important damage. In grapes adult females of the subspecies *E. carpini vitis* appear yellow with various black spots on each side of the body (Reyes Aybar, 2004) thus lending to the name yellow grapevine mite. In North America and the Pacific Northwest the species is described as flesh color to pale yellowish or even greenish.

Sex ratio of *E. carpini* expressed as percent of females, has been documented to range from 62 to 72% and even as high as 75% by some authors.

Males and females have similar developmental duration from fifteen to thirty degrees Celsius (Bounfour *et al.*, 2001). Temperature thresholds were published with the lower limit of 7 °C and the upper limit above 30 °C (probably 32 or 33 °C). The temperature at which this species displays their fastest growth rate is at 26 °C (Bonato *et al.*, 1993).

Females of the *E. carpini*, at their maximum size, can measure between 0.35 and 0.4 mm in length and 0.2 mm wide (Reyes Aybar, 2004). This species, as the majority of the Tetranychidae, is markedly dimorphic; the males are substantially smaller than the females, approximately one third the size. The anterior region of the opisthosoma is markedly conical shaped (Evans, 1992b). The dimorphic characteristic of this species is particularly useful in the selection of individuals for bioassays.

The eggs of *E. carpini* are smooth, spherical, and when recently laid, translucent in color, with a diameter of 0.1 mm (Reyes Aybar, 2004). *E. carpini* eggs contain a dorsal stipe (Jeppson *et al.*, 1975). The eggs darken to a light tan color with age and present the notable appearance of the eyes before hatching. There are varying data on the developmental times of *E. carpini* eggs. For example, in the subspecies *E. carpini borealis*, egg development was found to be between 3.5 and 4 days (Bounfour *et al.*, 2001), where as in *E. carpini vitis* egg development to hatch is said to average 7 days (Jeppson *et al.*, 1975). This species is haplodiploid, wherein unfertilized eggs result in male offspring with half the number of chromosomes as fertilized eggs which produce diploid females (Gutierrez *et al.*, 1979).

E. carpini develops through three active instars, between egg and adult the larva, protonymph and deutonymph. The larval stage differs from the other two immature stages in that it lacks a pair of rear legs (Evans, 1992b) and is rather pale in color and spherical shaped when recently hatched. Protonymphs and deutonymphs, with their four pairs of legs, are particularly difficult to differentiate in that the only difference in appearance is that of size.

The quiescent, non-feeding molting stages between the active instars are protochrysalis, which follows the larval instar, deutochrysalis which follows the protonymph stage and teliochrysalis from which the adult emerges (Krantz, 1986). In other publications these inactive stages have also been called larvochrysalis, protochrysalis and deutochrysalis (Bounfour *et al.*, 2001). To reduce confusion the first set of names is used which include the "teliochrysalis", or third chrysalis, to describe the last molt before adulthood.

Due to the fact that the last two chrysalis stages of the lifecycle, deutochrysalis and teliochrysalis, are particularly similar in appearance, the biology of this species is useful in distinguishing them. The immature female in the teliochrysalis stage liberates pheromones, thus attracting males which compete and guard them until fertilization (Zhang, 2003). Therefore, the teliochrysalis is discerned from the deutochrysalis not only by age but also by this male guarding behavior. On the other hand the protochrysalis is easily distinguishable from the deutochrysalis as they differ morphologically as the larva differs from the protonymph.

E. carpini survives the winter as females in hibernation form which are generally found under or in crevices of the bark of the grapevine and sometimes in the upper soil. Typically the gravid females congregate in small groups during the winter months. These females break hibernation and leave their winter refuges in early spring when temperatures permit. They establish themselves under the bud scale even before flowering takes place (Reyes Aybar, 2004). Heavy webbing is spun throughout the colony area under which all stages of the population reside. The females feed for approximately ten days after leaving hibernation before they begin laying eggs. They lay an average of one egg per day over a period of 12 to 20 days.

Eggs are characteristically placed on the underside of the leaf at the midrib or near to the veins where they are more sheltered from abiotic factors such as wind and rain. They are also slightly protected from predators between the leaf structures and the webbing. The fecundity of summer females, is usually

higher over their lifespan of 27 days, on average, and when temperatures are optimal with abundant resources (Jeppson *et al.*, 1975).

This species can cycle through six to eight generations per year depending upon the seasonal temperatures and available nutrition. In autumn when the day light hours begin to decrease, temperatures begin to fall and food is less abundant, fertilized hibernation form females prepare to overwinter. These females, even if temperatures are favorable, will not change to summer forms nor become active until they have passed a period of chilling.

Dispersal & Geographic distribution

As other species in the *Tetranychidae* family *E. carpini* invades all parts of the plant or neighboring plants by simply walking from one area to the next. This extension is an active dispersal process which is motivated by a number of factors such as population density, and low humidity resulting from depleted nutritional content of the occupied vegetation. Common means of dispersal described in the literature in species of *Eotetranychus* are on air currents or by other animals. The mite attaches a thread of silk to the plant and extends itself into the wind to be moved aloft. (McEnroe & Dronka, 1971; Brandenburg & Kennedy, 1987)

Like *T. urticae*, *E. carpini* is another temperate zone species, found in the subtropical regions. *E. carpini* is polyphagous thus has a variety of hosts such as Apple (Malus), Pear (Pyrus), Hazelnut (*Corylus*), Oak (*Quercus*), Willow (Salix), Maple (Acer), Alder (Alnus) and European Hornbeam (*Carpinus*) in Germany, England, Mexico, and New York (Jeppson *et al.*, 1975). Historically, however, in England it had only been found on unsprayed apple trees. In north America this species is a common resident of Raspberry (*Rubus*) and other cane fruits in northwestern Washington (Bounfour *et al.*, 1997).

The yellow mite is said to be present in the majority of vineyards in Southern Europe and Northern Africa, with initial outbreaks recognized over

fifty years ago (Rambier, 1958). Pérez-Moreno in his 1997 review listed substantial losses in vineyards sited in France (Galet, 1982; Weber, 1991), in Italy (Girolami, 1981; Lozzia & Rota, 1988) and in Switzerland (Baillod *et al.*, 1979). In Spain this mite is found throughout the peninsula in grape and other economically important crops such as cherry (Baltá, 1990). It has been documented along on the Mediterranean cost in Girona in the north and Valencia in the south. In northern vineyards in La Rioja and Navarra *E. carpini* has also become commonly known (Reyes Aybar, 2004).

Damage

E. carpini also normally feeds on the under surface of the leaf where in it ruptures the cell wall with its chelicerae to withdraw the cell contents (Kielkiewicz, 1985). This rupturing of the cell where in the chloroplast is damaged (Tanigoshi & Davis, 1978) results in crinkling, spotting and discolorations of leaf tissue (figure 1). In some grape varieties this damage results in a dusty appearance (Jeppson *et al.*, 1975).

At the beginning of the vegetative growth stages in spring, mite feeding can cause deformation of leaves, and reduce the size of the grape clusters. With high infestation levels the leaves can become almost completely red and darkened leaving only the veins green, thus giving an autumn senescence appearance. In extreme cases the entire grapevine can be defoliated promoting a second budding along with the disruption of hardening and winter acclimation of shoots. This stress not only causes obvious lower harvest weights but also fewer buds in the following year (Reyes Aybar, 2004).

The drying up of buds, flowers and or loss of pollen can also result from early spring infestations. In summer when mite density can be elevated, damage often appears as red sections in red wine grape leaves and yellow sections in white wine varieties all of which become brown when the leaf tissue dies. Damage by this species has been found to reduce sugar production (Reyes Aybar, 2004).

Figure 1. Advanced leaf damage by *E. carpini* in grape.

Treatment strategies

Generally, this species has been treated as other Tetranychid mites. Historically, lime-sulfur applications were said to be necessary in early spring to delay the development of summer populations (Rambier, 1958; Ambrosi & Lenarduzzi, 1959) as listed by Jeppson *et al.* (1975) in their chapter on injurious tetranychid mites previously sited.

Treatment with chemicals when the population is found in 60 to 70% of leaves in grape is currently recommended. Natural enemies are also within useful strategies for the control of tetranychid species, principally the Phytoseiidae family (Reyes Aybar, 2004). Predacious mites such as *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae) and other predacious insects have been found to feed on this species (Jeppson *et al.*, 1975).

Management strategies including the release of natural enemies, use of plant extracts and essential oils, treatments with new generation phytosanitary products, antagonistic bacteria and fungi and combinations of these have been and continue to be the focus of research.

Castagnoli *et al.* (2009) for example, have began studying releases and

establishment of various different predatory mites in vineyards for the control of *E. carpini* and other tetranychid pest mite species with much success.

Effectiveness of the antagonistic fungus *Beauveria bassiana* (Bals.-Criv.) (Hypocreales: Cordycipitaceae) on *E. carpini* and its side effects on a common predatory mite of Tetranychid species, *Kampimodromus aberrans*, (Nesbitt) (Acari: Phytoseiidae) were recently found (Simoni *et al.*, 2010).

Various new generation pesticides, such as chitin and lipid synthesis inhibitors, electron transport inhibitors, and proton gradient disruptors have recently been developed and their action in integrated management schemes evaluated. For example, investigations with bifenazate, acequinocyl, chlorfenapyr, flufenoxuron and fenbutatin oxide were found to have high efficacy along with compatibility with the commonly used predatory mite *P. persimilis* (Kim & Yoo, 2002). The resistance risk assessment of spirodiclofen in *T. urticae* has also been evaluated by Rauch & Nauen (2003). These authors found spirodiclofen to be active against all developmental stages and female adults of the two-spotted spider mite *T. urticae*. With these investigations in mind laboratory bioassays were carried out with the aim of offering more options in the management of *E. carpini*.

5.2 Objectives

The objective of this chapter was to characterize the acaricidal activity of spirodiclofen on *E. carpini* eggs thereby obtaining the probit regression line.

5.3 Materials & Methods

5.3.1 Mass rearing of *E. carpini*

The colony of *E. carpini* was raised on plants of the grapevine varieties Mazuelo and Tempranillo. The population was maintained in an isolated growth chamber at 27±1 °C and 60±10% relative humidity and a photoperiod of 16:8 (L:D). New plants were added to the colony when the grapevines were

deteriorated by mite damage.

The grapevines used to sustain the population and in all bioassays were propagated from the previous years field shoot cuttings. The shoots with approximately four buds each were kept in cold storage, 4±1 °C, until use. In order to end dormancy, the shoots were placed in a hot water bath at 30±1 °C for 48 hours following the protocol description by Martinez de Toda (1999). The top of the shoots were sealed with wax and the bases were treated with the Auxin growth hormone, Indole-3-butyric acid (1H-Indole-3-butanoic acid) (IBA) at a concentration of 200 µg/L to stimulate root growth. After treatment the shoots were planted in pots with approximately three buds above the soil surface. The grapevine plants were grown in chambers at 24±1 °C, 65±10% relative humidity and a photoperiod of 16:8 (L:D) without exposure to pesticides. When the grapevines had sufficient leaves they were added to the colony when needed.

5.3.2 Egg cohorts of *E. carpini*

Same age eggs were obtained by placing gravid adult females onto the underside of clean, disks of *Vitis vinifera* leaves, variety Tempranillo. After an oviposition period of 24 hours the females were removed, leaving the cohort isolated on leaf disks.

The leaf disks were placed up side down inside a 9 cm diameter Petri dish lined with wet cotton. The Petri dishes were placed into larger transparent cylindrical plastic boxes (5 cm high by 12 cm diameter) containing water.

A cloth wick and paper clip were attached to the side of the Petri dish to connect the cotton layer with the water in the outer box. This connection maintained the humidity of the cotton and thus the isolation of the individuals and turgor pressure of the leaf or disks. The complete chamber (figure 2) was topped with a lid containing four fine screen covered holes which allowed for air and humidity flow.

Figure 2. *E. carpini* egg cohorts chamber.

5.3.3 Toxicity of spirodiclofen on *E. carpini* eggs

The characterization of the toxicity of spirodiclofen on *E. carpini* eggs less than 24 hour old eggs of was carried out in the same conditions as the colony was raised. The bioassay arenas consisted of one 3 cm diameter grapevine leaf disk on top of a 10 ml layer of 3% agar. This was placed in a 3 cm diameter round box with lid containing a 1 cm diameter filter paper covered hole (figure 3).

Figure 3. Bioassay arenas for spirodiclofen treatments of *E. carpini* eggs.

The product Envidor® 240 SC with an original concentration of 24% spirodiclofen was used in all bioassays. Treatments were made in the Potter

tower at 0.5 bars of pressure. Ten to fifteen replicates of each treatment were completed. Five different ppm concentrations of the active ingredient were used in the analysis of the mortality to concentration relationship on eggs. These were as follows: 0.63 ppm, 1.25 ppm, 1.5 ppm, 5 ppm and 10 ppm. Water was employed as the pesticide carrier and control treatment. The mortality in each trial was considered 6 days after treatment by evaluation of larvae present and non-hatched eggs.

5.4 Statistical analysis

Estimates of LC50 and LC90 and their 95% fiducial limits were obtained using the PoloPlus Version 2.0 program (LeOra software), (Robertson & Preisler, 1992) based on Finney (1971) was used in all data analysis. Concentrations were converted to logarithms before analysis. Employing these logarithms of the concentrations the values for the regression equation, $y = a + bx$, were obtained from the PoloPlus analysis of the data.

5.5 Results & Discussion

The probit regression line with corresponding values for a and b is illustrated below (figure 4). Using the linear response function to evaluate the data an increasing mortality with increasing concentration was obtained with a slope of 2.662. This was to be expected as a greater concentration of any toxin should give higher mortality.

With these values the regression line was available to calculate any required lethal dose necessary. The lethal doses LC_{10}, LC_{50}, and LC_{90} with limits were extracted (table 1).

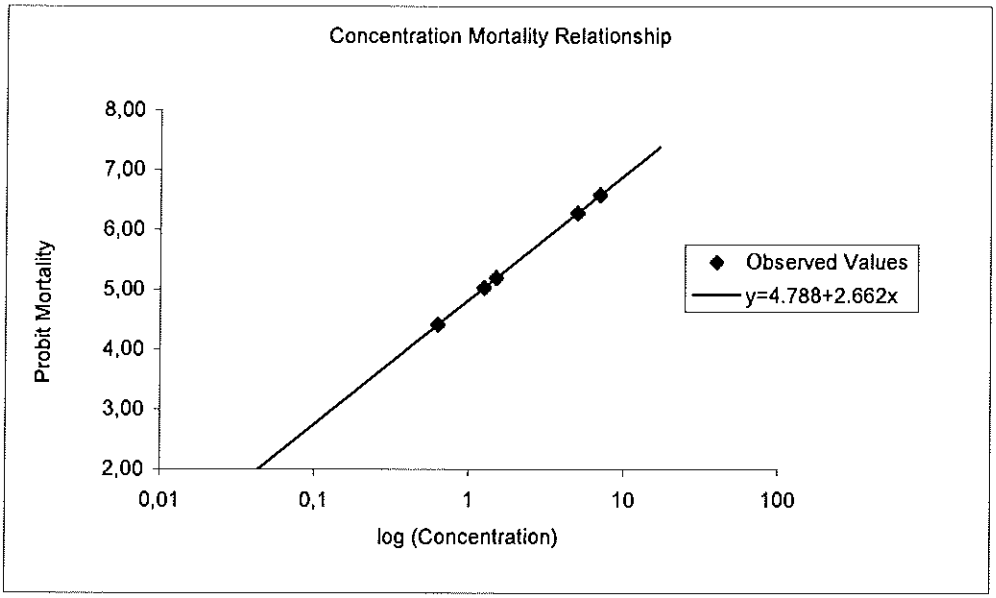


Figure 4. Probit regression line of spirodiclofen on *E. carpini* eggs.

Table 1. Predicted values from the probit analysis of spirodiclofen on *E. carpini* eggs.

Predicted concentration values for spirodiclofen on *E. carpini* eggs					
Lethal concentration	ppm	Limits	0.90	0.95	0.99
LC10	0.396	Lower	0.222	0.188	0.124
		Upper	0.553	0.581	0.634
LC50	1.201	Lower	0.972	0.924	0.822
		Upper	1.431	1.480	1.586
LC90	3.639	Lower	2.802	2.692	2.506
		Upper	5.622	6.358	8.654

From the results herein presented spirodiclofen appears to be a very effective ovicide for this Tetranychidae wherein the LC_{90} is at the maximum limit only 2.51 ppm. This was in comparison to results by Ullah *et al.* (2011) whom tested this pesticide and various others on the two Tetranychidae species *T. merganser* and *T. kanzawai*. The LC_{50} on eggs of their susceptible populations of both species at the maximum limit, reached 9.42 ppm.

Other studies with similar results as presented here on Tetranychidae species with pesticides of similar mode of action have also resulted in high

efficacy on eggs. The other tetronic acid, spiromesifen was illustrated to be an effective ovicide. The LC_{90} on 2-day old eggs of the Tetranychidae *T. urticae* was low with only 0.31 ppm (Nauen *et al.*, 2005) which was a response similar to that illustrated with *E. carpini*.

5.6 Chapter conclusions

1. Spirodiclofen gave a high level of efficacy on eggs of *E. carpini* even at very low concentrations.

5.7 Resumen de capítulo de en español

Se ha caracterizado la actividad acaricida del spirodiclofén sobre huevos de menos de un día de edad de *E. carpini* tratados mediante Torre para pulverizaciones de precisión en laboratorio, tipo Potter. Con este fin, se ha llevado a cabo el bioensayo necesario para la obtención de la recta de regresión ponderada Probit.

Al realizar el análisis estadístico, se obtuvo una ordenada en el origen de 4,788 y una pendiente de 2.662, con un valor de la LC_{50} de 3,6 ppm. Estos resultados indican que el spirodiclofén presenta un potente efecto ovicida frente al ácaro en las condiciones ensayadas. Este hecho hace interesante la realización de posteriores investigaciones para analizar la posibilidad de incluir el empleo de este compuesto en el Manejo Integrado de la plaga.

Effects of kaolin on *Lobesia botrana* (Denis & Schiffermüller, 1775)

6.1 Introduction

Lobesia botrana (Lepidoptera: Tortricidae) is a polyphagous species with a preference to grape, when available. However, it is able to complete its lifecycle on a wide variety of species including many non-crop plants.

This moth has been documented to cause damage to flowers and developing berries due to the larval feeding along with the entrance of fungal pathogens through the feeding wounds in the fruit.

In Mediterranean zones two to three generations are normal and when seasonal temperatures are very favorable, four generations have been reported.

The grape berry moth is among the most important species causing economic losses in vineyards world wide due to this feeding damage along with the associated secondary infections.

Taxonomic position

L. botrana (Denis & Schiffermüller 1775) is currently listed in the webpage: http://www.faunaeur.org which corresponds to Fauna Europaea (2011) version 2.4. (accessed on July 25th, 2011) is as follows:

Kingdom	Animalia
Subkingdom	Eumetazoa
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Order	Lepidoptera
Superfamily	Tortricoidea
Family	Tortricidae
Subfamily	Olethreutinae
Tribe	Lobesiini
Genus	*Lobesia*
Subgenus	*Lobesia*
Species	*botrana*

Morphology & Biology

The European grape berry moth, *L. botrana* is a relatively small Lepidoptera. The adult body length and wingspan has been described with a variation of observations. The complete range from the smallest average to the largest from the various authors is a length on average of 5 mm to 8 mm and a wingspan of 10 mm to 13 mm (Ruiz-Castro, 1943; Bovey, 1966; Galet, 1982; García-Marí, *et al.*, 1994; Torres-Vila *et al.*, 1997; Coscollá, 2004). This variation in the observations could be a result of various ecotype differences, or simply the impact of biotic and or abiotic environmental factors on the variation in gene expression or phenotype. For example Torres-Vila *et al.*, (1996a) published a melanic form of the moth and the genetic basis behind it. These same authors described phenotype differences in 1992.

Adults are cream and randomly speckled with black marks. The legs exhibit alternating white and brown bands. The forewings exhibit a mosaic pattern of black, brown, tan, gold and white ornamentation. The hind wings are brownish gray and hidden under the forewings when the individual is not in flight. The species is not markedly dimorphic in the adult stage (Ruiz-Castro, 1943) even though males are usually smaller and weigh less.

L. botrana eggs are flat and elliptical normally 0.8 mm by 0.6 mm, and give the impression of having a high surface to volume ratio because their height is very shallow. They appear to be stretched out upon the surface, not spherical as many other lepidopteran eggs. They are normally glued to grapes or inflorescences, one by one, dispersed throughout the available surface. Initially light yellow when laid the eggs grow to become transparent with three to four days age, depending upon temperature. The dark brown to black head capsule becomes evident at embryo maturity just before they hatch.

There are five larval instars. Laval sizes range from 1 mm to 15 mm and their colors progress through light tan to brown to green and finally to blue which is the last and pre-pupal stage. The color also depends upon the nutrition and instar. In the first larval stage, the neonate is on average 1 mm long and light tan with a black head capsule (Coscollá, 2004). The second instar is of greater length and usually darker in color, more brown than tan. The third larval instar is much like the second in the color and size. However, the fourth instar is a markedly bright green and the fifth a brilliant deep blue.

The elongated pupae is typical of the Tortricidae family with hooks on the posterior end. Their length ranges from 4 mm to 9 mm. They are initially light brown and later change to dark brown (González de Andrés, 1935). The pupa is encapsulated by a white silken cocoon during metamorphosis. The exuviate is shed outside the cocoon when the adult emerges. The pupal stage usually lasts 6.3 days at 24 ºC (Sáenz de Cabezón, 2003). However, diapause in the pupal stage is highly determined by photoperiod (Komarova, 1949; Roehrich, 1969). Individuals can be separated during this stage by gender (figure 1) using morphological characteristics such as antennal length (Belmonte & Peña, 1987).

Figure 1. *L. botrana* pupae (left male, and right female).

Adults of the first generation emerge from their cocoons over a period of two weeks to two months at the beginning of spring when conditions permit (Roehrich & Boller, 1991). Generally the first adults to dominate the primary population are males followed by a domination of females. Adult *L. botrana* are most active at dusk and normally stay hidden during the day (Ruiz-Castro, 1943; Bovey, 1966; Coscollá, 2004). The females typically mate once while males are capable of mating multiple times (Torres-Vila *et al.*, 1995). They produce from 50 to 80 eggs during approximately one week (García-Marí *et al.*, 1994).

Eggs are laid singly or in small groups on host reproductive organs and are usually found on dry, smooth, surfaces in the shade for protection against desiccation, which can kill them (Coscollá *et al.*, 1986). Eggs are also sensitive to excess humidity and sulfur residues which inhibit the oviposition (García-Marí *et al.*, 1994; Coscollá, 2004). Eggs hatch within three to five days after oviposition in favorable conditions, 10 to 11 days after oviposition in unfavorable conditions (Coscollá, 2004) and in particular at 24 °C, five days after oviposition (Sáenz de Cabezón, 2003). Larvae and eggs are capable of becoming dormant under extreme temperature conditions (Tzanakakis *et al.*, 1988).

The cumulative duration of the larval stages is highly temperature dependent as expected of poikilothermic species (Gabel, 1981; Briere & Pracros, 1998) and can take up to a month (Coscollá, 2004) but at 24 °C the larvae mature through the five instars in, on average, 20 days (Sáenz de Cabezón, 2003). For isolation and protection, the larva use silken threads to wrap themselves in clumps of buds thus hiding themselves from predators and insulating themselves from extremities of temperature. These silken threads are also used to construct the protective cocoon during the pupal stage.

Pupae, the inactive metamorphosis stage, are normally found on leaves and during the winter in the soil or in crevasse of branches and bark (García-Marí *et al.*, 1994). *L. botrana* individuals overwinter as pupae (Coscollá, 2004). In the fall when temperatures drop and the photoperiod shortens larva in the fifth instar prepare to convert itself into overwinter pupae. Temperature and photoperiod are said to be the most motivating factors to the entrance and exit of dormancy. However, not all pupae of late summer or early fall go into dormancy.

The number of annual generations is also especially influenced by humidity and temperature. Eghtedar (1996), found when under the moderate relative humidity of 40 to 45% and warm temperatures of 30 to 32 °C a generation could be completed within 30 to 32 days. With the eggs developing in 8 to 10 days, the larvae in 17 to 18 days, and the pupae in 7 to 8 days. However, low reproduction can occur even under optimal temperature and humidity conditions, illustrating how diapause and unknown factors can influence the population dynamics of this specie (Deseo *et al.*, 1981).

Dispersal & Geographic distribution

The dispersion of the European grape berry moth is most commonly achieved by an arched flight pattern (García-Marí *et al.*, 1994). Larvae are however capable of walking from one plant to another. Neonates have been seen to move throughout plants using their webbing (Torres-Vila *et al.*, 1997). Dispersal is effected by host plant, environmental conditions (Berger, 1989), and larval density (Torres-Vila *et al.*, 1997).

L. botrana has of alternate hosts to grape which include a variety of agricultural crops and fruit trees (Fowler & Lakin, 2002) along with many secondary non-cultivated hosts (Bradley *et al.*, 1979; Savopoulou-Soultani *et al.*, 1999). Climates in which this species thrives are generally characterized as temperate. Currently the global distribution of *L. botrana* is reported to be associated with plant species originating in Montane and Mediterranean scrub along with temperate broadleaf and mixed forests.

L. botrana has expanded its original palearctic range (CAB, 2003). It is now found in portions of the Afro-tropical, the Orient, Northern and Western Africa, throughout Europe, the Mediterranean region, Southern Russia, Japan, along with the Middle and Near East, (Avidov & Harpaz, 1969; CIE, 1974) and recently in Argentina, Chile, and California (Varela *et al.*, 2011; Lobos, 2011).

Damage

Also known as the European Vine Moth (Zhang, 1994), *L. botrana*, is one of the most important grapevine pests in Spain (Coscollá, 1997). In spring, neonates can cause damage to immature grapes when the larvae group the inflorescence together with silk forming a safe feeding zone.

The second and third generations are often the most harmful (Al-Zyoud & Elmosa, 2001). The direct damage is found primarily on the grapes by excessive punctures due to larval feeding. This damage alone results in lower crop yield and quality of harvested product and normally only noticeable with very high populations. On the other hand it is not the only dilemma linked with this species.

Fungal pathogens easily penetrate plant organs previously damaged by *L. botrana* feeding. These pathogens cause subsequent rotting and can also result in important economic losses (García-Marí *et al.*, 1994). Gray rot also known as Nobel rot is amongst the most characteristic fungal infections of grape which is caused by *Botrytis cinerea,* (De Bary) (Helotiales: Sclerotinicaeae), (Deseo *et al.*, 1981; Fermaud, 1998). In the case of wine grapes a disagreeable bouquet flavor from these secondary infections is a particularly undesirable outcome (Fowler & Lakin, 2002). The physical damage by larval feeding and fungal infections of *Botrytis cinerea* are illustrated below (figures 2 & 3).

Treatment strategies

Traditionally, *L. botrana* has been controlled by conventional pesticides (Ruiz-Castro, 1966; Coscollá, 2004). However, with increasing restrictions of available crop protection chemicals new strategies are being employed.

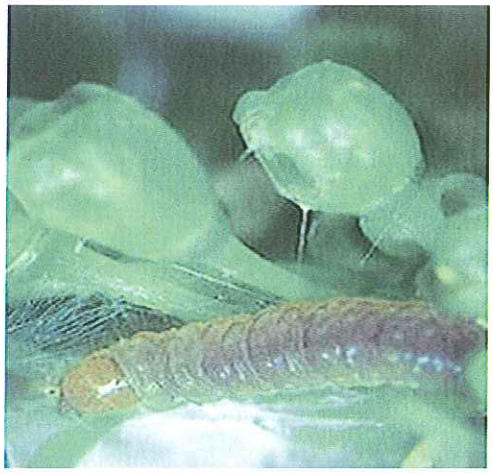


Figure 2. *L. botrana* larva on immature grape.

Figure 3. Gray rot due to *Botrytis cinerea*.

For example, mating disruption has been recently employed in Spanish vineyards with much success (García-Marí *et al.*, 1994; Varner *et al.*, 2001).

Amongst newly investigated management strategies are; microbiological pesticides employing *Bacillus thuringiensis* (Coscollá, 1997), biorational insecticides (Sáenz de Cabezón *et al.*, 2008) the application of plant extracts (Mondy *et al.*, 1997) and the use of biotechnology to manipulate plant physiology.

Biological control with predators and parasitoides (Coscollá, 1980; Pérez-Moreno *et al.*, 2000), namely the Trichogramma genus (Moreno-Grijalba, 2007; El-Wakeil *et al.*, 2008) have also entered the list of integrated strategies.

6.2 Objectives

The first goal with *L. botrana* was to evaluate effects on oviposition behavior when offered kaolin treated and non-treated surfaces on both plastic oviposition chambers and on grape. The second aim was to evaluate the survival of eggs from oviposition bioassays on kaolin treated surfaces in the four experimental types; choice, no choice on synthetic substrate and on grape.

Mortality of neonates due to kaolin inclusion in the semi-synthetic diet in treated experimental arenas was another target of these investigations also to be evaluated.

Finally, antifeedant effects due to kaolin inclusion in the semi-synthetic diet during larval development and on pupal reduction was the last objective to be evaluated.

6.3 Materials & Methods

6.3.1 Mass rearing of *L. botrana*

A colony of *L. botrana* was established from larvae collected in an ecological vineyard in La Rioja (Spain). It was maintained in the Crop Protection laboratory population of the University of La Rioja following techniques from Del Tío Moreno (1996) and Sáenz de Cabezón (2003). The original culture were augmented with new individuals once a year.

To obtain eggs, six to eight adult female moths along with six to eight adult male moths were isolated in oviposition chambers consisting in a 330 ml octagonal translucent plastic glass (10 cm high, 5 cm smaller diameter y 8.5 cm greater diameter) placed upside down in a 9 cm diameter Petri dish base (figure 4).

Adults were transferred to the oviposition chambers from the pupal boxes by anesthetized using a stream of CO_2 which was applied to the screen covered tops of the pupal boxes (figure 7) for 8-12 seconds. With this anesthetization the moths were unharmed nevertheless remain inactive for roughly 30 seconds.

To complete the chambers a 1 cm-high by 2.5 cm diameter cylindrical plastic container filled with cotton saturated with drinking water. The oviposition glasses were changed daily to provide a fresh surface on which the females could lay their eggs. This daily changing and isolation of the oviposition surface and eggs also provided cohorts when needed.

Figure 4. *L. botrana* oviposition chambers.

The plastic oviposition glasses with eggs attached were cut up and placed into cylindrical plastic colony boxes (5 cm high by 12 cm diameter). After four to five days the newly hatched neonates were transferred from the egg incubation boxes to the larval growth boxes. These larval boxes consisted of cylindrical translucent plastic containers of the same dimensions as the egg incubation boxes, yet with three layers of filter paper and more or less 10 pieces of semi-synthetic diet 1 cm by 3cm by 4mm (figure 5).

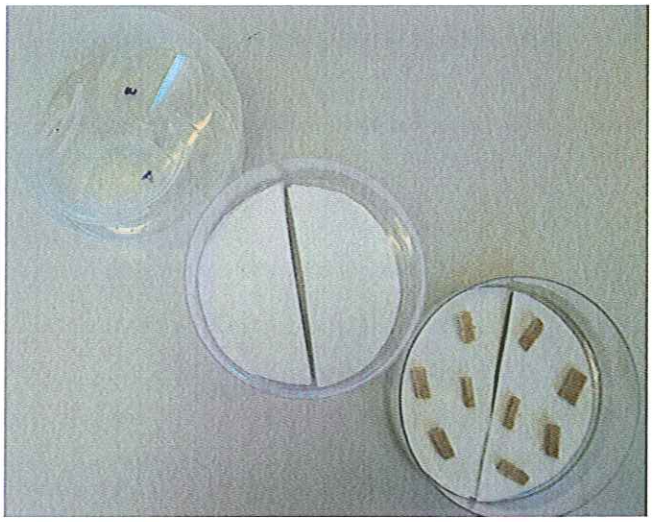


Figure 5. *L. botrana* mass rearing chambers. (from left to right, first box: *L. botrana* eggs on oviposition chambers; second box: paper layers; third box: neonates & diet)

The semi-synthetic larval diet, found in Torres-Vila (1994) consisted of the following ingredients and amounts listed (table 1).

Table 1. *L. botrana* larval diet.

Ingredients	Quantity
Agar	20 g
Ascorbic acid (L+)	6.4 g
Benzoic acid	2 g
Brewer's yeast	65 g
Chlortetracycline (3)	0.75 g
Corn meal	65 g
Fumagillin (4)	0.03 g
Iprodione (2)	0.75 g
Methylparahidroxybenzoate (1)	2 g
Vegetable oil	2 ml
Water (distilled)	1,110 ml
Wheat germ	78 g

(1): Nipagin®; (2): Rovral® ; (3): Aureomicina® ; (4): Fumidil B®.

Methylparahidroxybenzoate is a preservative and antiseptic, Iprodione is a wide spectrum fungicide, Chlortetracycline an antibiotic whereas Fumagillin is an inhibitor of intestinal protozoan parasites.

The individuals passed through all of the developmental stages in these chambers until they were removed as pupae. These boxes were topped with a lid containing one to three screen covered holes for air flow. The diet was replaced three times a week, roughly every 48 hours.

After pupation had taken place, the pupae were carefully withdrawn from their silken cocoons, separated by gender, and isolated in cylindrical plastic boxes which are 2.7 cm high and 10 cm in diameter (figure 6). These pupal containers were topped with a lid containing one 5 cm diameter screen covered hole. Upon emergence the adult moths were removed to the oviposition chambers previously described.

Figure 6. *L. botrana* pupae separated by gender with emerged adults.

6.3.2 Effects of kaolin on oviposition behavior & egg viability on synthetic substrate

All individuals used in these trials were taken from the laboratory colony maintained at previously mentioned conditions. The trial was run in a growth chamber at 24±1 ºC, relative humidity of 60±5% and photoperiod of 16:8 (L:D).

To synchronize the age of adult *L. botrana* used in the oviposition preference trials, pupae were isolated in the population every day by removing the emerged adults from the pupal incubation chambers. When sufficient unmated adults emerged in the same 24 hour period they were isolated by groups of three for 24 hours in preoviposition chambers. These groups consisted of two males and one female. Two males were used to ensure insemination of the female in each experimental replication.

After the preoviposition period of 24 hours these groups were placed in the experimental arenas. These arenas were nearly equal to the oviposition chambers employed in the massive rearing of the colony. The only difference was the water dish which was a 3 mm high by 2 cm diameter lid filled with water soaked cotton.

All treated surfaces were sprayed with 5.5 ml of the kaolin solution at a concentration of 57 g/L using a Potter Tower. All arenas for all of the periods were treated and set to dry for roughly two hours before the beginning of the trial.

The duration of the assay consisted of three consecutive 48 hour periods. The adults were moved to new arenas (oviposition chambers) at the end of each period. This was done by chilling the entire arena in a refrigerator at 3±1 °C with a relative humidity of 50±5% in complete darkness for 3 to 5 minutes before moving the three adults.

The number of eggs on both the glasses and bases of each period's oviposition chamber was counted under a stereomicroscope and recorded accordingly. The locations of the eggs on the glasses and bases were marked with a fine tip red or blue marking pen. The number of hatched eggs was also recorded after the emergence of the neonates. All replicates were preformed in blocks of five to ten.

Choice trial on synthetic substrate

Both the standard Petri dish base and the plastic glass top of all the 48 hour periods were half treated with kaolin and set to dry before the beginning of the trial (figure 7). This was done by covering sections of each glass top and Petri dish base during the treatment with the Potter Tower. Forty-eight replicates were compared in this choice trial on synthetic substrate.

Figure 7. *L. botrana* assay oviposition chamber. (Left: half treated top & Right: half treated base with water recipient).

No choice trial on synthetic substrate

In this no choice trial, both the standard Petri dish base and the plastic glass of the treated replicates were completely treated with kaolin at a concentration of 57 g/L. All arenas for all of the 48 hour periods were sprayed and set to dry before the beginning of the trial. Thirty-one of each control and kaolin treated replicates were compared.

6.3.3 Effects of kaolin on oviposition behavior & egg viability on grape

The number of adult moths used in each replicate and their origin was the same as described above. These individuals were added to the complete experimental arenas by use of a stream of CO_2 which anesthetized them.

The experimental arenas consisted of one translucent plastic cylinder 9 cm diameter by 21 cm tall placed inside in the base of a 9 cm diameter Petri dish. One hydration container 3 mm high by 2 cm diameter lid filled with water soaked cotton was added to the inside of the arena base. The arena was topped

with another 9 cm diameter Petri dish which contained two 1 cm diameter respiration holes. All plastic surfaces were covered with filter paper to deter oviposition on the arena itself and the escape of adults through the respiration holes. Illustrated below are the experimental arenas and treated grape clusters (figure 8).

Figure 8. Complete experimental arena and grape clusters for oviposition and egg viability experiments.

In all trials, Tempranillo grape clusters were offered for oviposition. The grape clusters were collected and kept in cold storage until treatment and placement in the bioassay. Clusters were selected with three to four berries and an accumulative weight of 6±1g. The grape clusters were treated by immersion in a 47.5 g/L solution of kaolin or the water carrier during continual mixing for 5 seconds. This immersion was done to simulate a treatment to runoff.

All clusters were hung to dry completely before their transfer to the experimental arenas. Hanging the clusters allowed all excess product to drip off the berries yet left the same quantity as would a field treatment to run-off. The coverage of kaolin on grape is illustrated below (figure 9).

Figure 9. Coverage of kaolin particle film on grape.

All bioassays in this set of experiments took place over a duration of eleven days in a growth chamber at 24±1 °C, with a relative humidity of 60±5% and a photoperiod of 16:8 (L:D).

Oviposition was allowed for each of three consecutive 48 hour periods after which the grape clusters were changed. The individuals in each replicate were subjected to a stream of CO_2 to anesthetize them while the previous periods grape clusters were replaced in the oviposition chambers.

The grape clusters removed from the oviposition chambers were isolated and kept in the same environmental conditions as the trial. After the incubation period of five days the number of eggs laid and the number of hatched eggs were recorded.

Choice trial on grape

In the choice trial, two clusters, one kaolin treated and one control treated were added to each experimental arena, as illustrated in figure 10. Twenty-five replicates were evaluated in the choice trials.

Figure 10. Choice grape trial inside experimental arena.

No choice trial on grape

In the no choice trials one kaolin treated or one control grape cluster was placed inside each chamber for each of the consecutive 48 hour periods. All grape clusters were treated and set to dry before the beginning of the trial. Twenty and thirteen replicates; control and kaolin treated, respectively, were evaluated in the no choice trials.

6.3.4 Effects of kaolin on neonate survival

Five *L. botrana* neonate larvae, of the synchronized age of less than 24 hours old were used in each replicate. They were moved from the colony with a fine camel hair brush and placed in the center of each complete experimental arena.

The arenas consisted of two layers of filter paper in the base of a 9 cm diameter by 3 cm tall cylindrical plastic container with one 2 cm diameter filter paper covered hole in the top (figure 11). All experimental arenas, with the filter paper layers, were treated in the Potter Tower and set to dry one hour before the start of the trial.

Five cylinders of semi-synthetic diet, 1 cm diameter by 3.5 mm thick (0.30 - 0.39 g) were treated by submersion for 5 seconds in a solution of kaolin at a concentration of 47.5 g/L or in the water carrier. After the diet dried it was added, upside down, to the experimental arenas (figure 11).

The number of live neonate larvae was recorded at 72 hours. Fifteen replicates of each, treatment and control were compared with t-student. The conditions of the growth chamber in which the trial took place were: 24±1 ºC, 60±5% relative humidity and 16:8 (L:D) photoperiod.

Figure 11. Experimental arena for neonate assay, water treated control.

6.3.5 Antifeedant effects of kaolin on larval development & pupal weight

Sixteen neonate larvae, less than 24 hours old, taken from the laboratory colony, were used in each replicate. The larvae were moved to the center of the experimental arenas with a fine camel hair brush after each arena was completed with treatment or control diet.

The experimental arenas were equal to those used for the massive rearing in the larval stages, as seen in the figure above. They consisted of three layers of filter paper cut in half, in the base of a cylindrical box with a diameter of 12 cm

and a height of 5 cm. The lid of which contained two screen covered respiration holes. Eight disks of diet, 2 cm diameter by 4 mm thick, four on each side of the arena, were placed equal distance from the center of the arena. The synthetic diet was replaced three times a week.

The number of live larvae was recorded three times a week during the addition of diet. The pupae were removed and the weights of both male and female were also recorded when the synthetic diet was changed. The trial was run in a growth chamber at 24±1 °C, 60±5% relative humidity with a photoperiod of 16:8 (L:D).

For the treatment, kaolin was added to the semi-synthetic diet at a concentration of 57 g/L in relation to the quantity of water necessary for the diet preparation.

Differences between twelve replicates of each, treatment and control, were statistically analyzed with Student's t test. All replicates in this set of experiments were evaluated until all individuals died or pupated.

6.4 Indices of activity equations

Various authors, for example Bentley *et al.*, 1984 and Raffa & Frazier, 1988 have developed and used indices for both choice and no choice trials to represent comparison of data sets. Indices lend clarity and ease of data analysis, including the unification of two treatments into one value for each set of data points. Inhibition of oviposition and egg viability is demonstrated by positive values in the accumulative totals.

Index of activity equations for both the oviposition and egg hatch evaluation experiments were modified from those used by Raffa & Frazier (1988). The resulting equation used for the index of oviposition inhibition (IOI) was as follows:

$$IOI = \left(\frac{Ec - Ek}{Ec} \right) \times 100$$

Ec represents the number of eggs laid in the control.
Ek represents the number of eggs laid on the kaolin treated surfaces.

Likewise the equation used for the evaluation of the index of egg hatch inhibition (IEI) data was:

$$IEI = \left(\frac{PEc - PEk}{PEc} \right) \times 100$$

PEc represents the percent of hatched eggs in control.
PEk represents the percent of hatched eggs in the kaolin treatments.

6.5 Statistical analysis

All statistical analyses for this set of bioassays were performed with Statgraphics Centurion XVI (2010). The Bartlett or Levene test was employed to evaluate if the variances within each treatment were similar to those in the other treatments therefore being homogenous. The ANOVA simple or the t student test followed by the Tukey test or LSD.

If the variances were not found to be homogeneous, the Kruskal-Wallis test was employed. Differences between time periods within treatments of indices of activity in percent were compared using the Tukey test a = 0.05.

6.6 Results & Discussion

6.6.1 Effects of kaolin on oviposition

Inhibition of oviposition and reduction of *L. botrana* egg hatch by kaolin were found in all bioassays both choice and no choice on plastic oviposition chambers and on grape. Differences and between time blocks within treatments of indices of activity in percent were compared using the Tukey test a=0.05. Females were more attracted to untreated surfaces markedly illustrated in the

trials with grape. The inhibition level is represented by the extent of the positive values (table 2).

No significant difference between time periods was observed in the statistical analysis in both the choice and no choice trials on synthetic substrate. However, a trend of increased inhibition of oviposition with time was found in the trials on plastic chambers. Curiously, the level of inhibition on grape was much greater than those on plastic, with mean index values of 87.39 and 94.36 percent in the choice and no choice experiments on grape, respectively.

Table 2. Indices of inhibition of oviposition on synthetic substrate & grape.

Time (hours)	Index of Inhibition of Oviposition (Mean ± SE)			
	Synthetic Substrate		Grape	
	Choice	No Choice	Choice	No Choice
0 – 48	16.5 ± 7.0 a	31.5 ± 10.4 a	86.79 ± 3.97 a	98.07 ± 1.04 a
48 – 96	37.0 ± 6.8 a	37.2 ± 9.0 a	91.61 ± 3.86 a	93.63 ± 3.14 a
96 – 144	42.7 ± 9.8 a	55.8 ± 5.4 a	83.77 ± 6.45 a	91.93 ± 2.93 a
Totals (0 – 144)	31.7 ± 4.6 **	40.4 ± 5.2 **	87.39 ± 4.76**	94.36 ± 2.37**

Within each column, means followed by the same letter are not significantly different ($p>0.05$); within each bioassay the ** indicate a statistically significance difference between the kaolin and control treatments.

The reduced attraction to treated grapes could be a result of the masking of the natural volatiles of the fruit when covered with the kaolin solution. This type of effect on oviposition was also found in the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in apple and pear (Unruh *et al.*, 2000).

There have been various suggestions of how this mineral affects arthropods. Particles can stick to the insect body causing difficulty during oviposition activities (Lapointe, 2000) thus leading to excessive time spent grooming instead of oviposition (Glenn *et al.*, 1999). In addition, the attachment of particles to the insect body can disrupt movement, and feeding (Glenn *et al.*,

1999). Cottrell *et al.*, (2002), observed this type of difficulty by the black pecan aphid, *Melanocallis caryaefoliae,* (Davis) (Hemiptera: Aphididae) which lead to a lower population density. The lower level of oviposition in the kaolin replications on synthetic substrate in no choice trials may have been due to this irritation of the females by the kaolin particles.

6.6.2 Effects of kaolin on egg viability

The kaolin treatment had an inhibitory effect on egg hatch in all trials as represented by the positive index values (table 3). A significant difference was found between the replicates compared in the choice trial along with no choice assay on the plastic chambers. These are illustrated below by the letters following the index values.

Table 3. Indices of egg hatch inhibition on synthetic substrate & grape.

Time (hours)	Index of Egg Hatch Inhibition (Mean ± SE)			
	Synthetic Substrate		Grape	
	Choice	No Choice	Choice	No Choice
0 – 48	14.7 ± 2.8 a	13.7 ± 3.7 a	88.61 ± 4.05 a	79.17 ± 13.99 a
48 – 96	13.8 ± 8.5 a	28.1 ± 6.9 ab	94.48 ± 3.16 a	91.67 ± 8.33 a
96 – 144	27.1 ± 9.6 a	42.1 ± 7.0 b	86.68 ± 6.61 a	85.42 ± 9.95 a
Totals (0 – 144)	17.9 ± 4.1 **	27.8 ± 3.8 **	89.92 ± 4.60 **	85.42 ± 10.76 **

Within each column, means followed by the same letter are not significantly different ($p>0.05$); within each bioassay the ** indicate a statistically significance difference between the kaolin and control treatments.

The inhibition of egg hatch on grape was found to be substantially higher than that obtained in the trials on the plastic oviposition chambers. Average values were nearly four times higher in the choice and three times greater in the no choice trial with mean inhibition values of ~18% on plastic compared to ~90% on grape in the choice trial and ~28% on plastic compared to ~85% on grape, in the no choice trial. This effect could have been a result of the difference in the application on plastic and on grape thus leading to a greater deposit of

kaolin on the grapes along with a greatly restricted surface area in the grape trial.

Knight *et al.*, (2000), found high efficacy of kaolin on oviposition with the obliquebanded leafroller, *Choristoneura rosaceana*, (Harris) (Lepidoptera: Tortricidae) in semi-field trials with apple seedlings, however they found no significant difference in larval hatch between the kaolin and control treatments. In contrast, the surface area to volume ratio appears great in the isolated eggs of *L. botrana*, as apposed to the egg clusters of similar species. There should be, therefore, a high percentage of the chorion in contact with the kaolin treated surface. Kaolin is known to be particularly absorbent, thus influences moisture levels on treated surfaces. It has also been suggested that kaolin effects cuticle lipids resulting in eventual dehydration (Korunic, 1998). The absorbent quality and the high percent of chorion contact with the kaolin treated surface could have contributed to the significant difference in our treatments.

6.6.3 Effects of kaolin on neonate survival

There was a significant difference, with $p<0.01$, between the means of the survival rates of the treatment and the control. The average percentage of neonate mortality in the kaolin treatment was 78.7%, compared to 37.1% in the control. This difference was supported by the Abbot corrected mortality in the treatment of 66.1%.

The results presented here are similar to those found by other researches. For example, Larentzaki *et al.* (2008) encountered delayed larval development and increased mortality of *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) in kaolin treatments which they attribute to difficulty in penetrating the particle film and dissuasion of feeding. Cottrell *et al.*, (2002), suggested mortality by starvation in progeny of the black pecan aphid, *M. caryaefoliae*, (Davis) (Hemiptera: Aphididae) raised on kaolin treated foliage due to feeding obstruction by the particles. Showler (2003), also found larvae of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), to be effected when feeding on kaolin treated leaf surfaces. The effects on the neonate

larvae demonstrated in our trial could have be attributed to dehydration which is in concurrence with the proposed effect of arthropod irritation (Puterka *et al.*, 2000b) and the previous mentioned authors.

6.6.4 Antifeedant effects of kaolin on larval development & pupal weight

No Antifeedant effects of kaolin were evident with the experimental conditions tested. There was no significant difference, with p=0.069, of survival rates nor pupal weights between the means of the treatment and control when kaolin was added to the synthetic diet was evaluated. It is possible that the mixing of kaolin into the diet may have lent to a buffering effect due to the incorporation of the mineral within the synthetic diet. The ingestion of kaolin was found by other authors to have effects when the surface of foliage was treated with kaolin. The dehydration effect found with *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) larvae by Díaz *et al.,* (2002), when leaf disks were treated, the it can be conjectured that in this trial, was negated by the high moisture quantity in the synthetic diet fed to the larvae.

The kaolin particle film has also been found to cause host plants to be visually or tactilely unrecognizable to the arthropods contributing confusion and an interference with tactile perception (Puterka *et al.*, 2000a). The boll weevil, *Anthonomous grandis* (Boheman) (Coleoptera: Curculionidae) had difficulty with visual recognition of host plants in trials by Showler (2003).

6.7 Chapter conclusions

1. An inhibitory effect of the oviposition behavior of *L. botrana* on both plastic oviposition chambers, more on grape, in choice and no choice trials resulted from treatments with kaolin treatments.

2. Treatments with kaolin resulted in a reduction of survival of *L. botrana* eggs laid on treated surfaces. This effect held true in all bioassay types and equally throughout the duration of the experiments and more so on grape.

3. The survival of neonate larvae was not demonstrated to be affected by treatments containing kaolin.

4. Antifeedant effects during larval development and reduction of pupal weight were not observed due to kaolin treatments.

5. The results observed indicate that kaolin could be another useful reduced-risk alternative in the management of *L. botrana* when the individuals come into contact with treated surfaces.

6.8 Resumen de capítulo en español

Lobesia botrana es un lepidóptero que constituye una de las plagas más importantes de la vid a nivel mundial. Ello es consecuencia de los daños directos que produce al alimentarse sobre las bayas y, sobre todo, de potenciar la proliferación de podredumbres, fundamentalmente fúngicas, favorecidas por las heridas que provoca en dichas bayas.

En este contexto, la presente investigación se plantea para evaluar la eficacia del caolín (Surround® WP) en el control de la plaga.

Los experimentos para conseguir dicho objetivo, se llevaron a cabo mediante aplicaciones con la Torre para Pulverizaciones de Precisión en Laboratorio tipo Potter o tratando la dieta semisintética de las larvas por inmersión o por adición del compuesto en su elaboración. En todos los bioensayos se emplearon condiciones ambientales controladas.

Se evaluó el efecto sobre la fecundidad, fertilidad y supervivencia de larvas neonatas. Los análisis estadísticos necesarios aplicados a los resultados se llevaron a cabo mediante el programa estadístico Statgraphics Centurion XVI. Para obtener los índices indicativos del efecto sobre la fecundidad y fertilidad se utilizaron fórmulas obtenidas mediante modificaciones de las propuestas en

1988 por Raffa y Frazier.

El caolín tuvo un efecto inhibitorio de la oviposición tanto empleando plástico como bayas de uva, como sustrato de puesta. Curiosamente, el nivel de inhibición fue significativamente mayor sobre bayas que sobre plástico.

El tratamiento con caolín tuvo un efecto inhibitorio de la eclosión de los huevos. De nuevo, el nivel de inhibición fue mucho mayor cuando se utilizaron bayas de uva como substrato de puesta, respecto a cuando se empleó plástico para tal fin.

La supervivencia de larvas neonatas se vio significativamente reducida cuando la dieta semisintética con que se alimentaban era tratada por inmersión con caolín.

No se observaron efectos del caolín referidos a provocar disuasión de la alimentación cuando se aplicó mezclado con la dieta larvaria semisintética. Este hecho quedó demostrado al no observarse diferencias significativas ni en los porcentajes de supervivencia ni en el peso de las pupas, entre los individuos tratados y los empleados como testigo.

En conclusion, los resultados obtenidos indican que el caolín podría ser incorporado como una útil herramienta de control en el Manejo Integrado de *L. botrana*. Debido al hecho de que el compuesto es natural, no tóxico para mamíferos, ni presenta otros riesgos ambientales, tal como han certificado diferentes agencias oficiales en diferentes partes del mundo, puede ser incorporado en agricultura ecológica.

Evaluation of side effects of kaolin on *Trichogramma cacoeciae* Marchal 1927

7.1 Introduction

The use of natural enemies in crop protection rose to awareness for entomologists and agriculturists alike more than a century ago. Yet, with the appearance of chemical pesticides with their low price and ease of use, the number of investigation involving natural enemies dropped off. However, with the quick fix chemical eradication paradigm problems arose.

Starting in the late 1940´s with those pesticide use problems, such as pest resistance and secondary pest infestations, resulting economic losses, the interest in biological control agents increased. Later, ecosystem contamination in soil, air and water, along with harm to bystander species, such as birds (Carson, 1962) and beneficial species such as pollinators, the interest in the search for alternative pest management strategies and therefore the Trichogrammatidae family was maintained (De Bach, 1973).

This renewed interest in the use of natural enemies to reduce pest populations to tolerable levels has brought about the definition of biological control agents (De Bach & Rosen, 1991). With initial concerns previously mentioned along with the new concerns which include occurrence and detection of pesticide toxicity in humans, and the need for satisfactory pest control in

organic, sustainable farming (Board of Agriculture, US National Research Council, 1996), along with the benefits obtained from the use of natural enemies interest in biological control continues to grow.

Currently, integrated pest management (IPM) programs that investigate and incorporate agents such as those species in the *Trichogramma* genus is being employed in a variety of crops due to the numerous benefits of their use (Boettner *et al.,* 2000; Neuenschwander, 2001; Louda & Stiling 2004; Roltsch *et al.*, 2006; Beggs *et al.,* 2008). Amongst those benefits are, the absence of resistance pressure to the target species, and absence of toxicity on human or environmental health.

Therefore, with the benefits of its use in mind researchers are investigating strategies with *Trichogramma* which parasitizes economically important lepidopteran species such as, rose chafers, and leafrollers, just to mention a few. Recently, a native population of *T. cacoeciae* (Moreno-Grijalba *et al.,* 2001) has been described to occur in or need Spanish vineyards. Due to this, the genus has become the focus trials for its use in Spanish vineyards. However, there are still questions pertaining to the practical application to achieve the highest effectiveness of *T. cacoeciae* when in combination with other crop protection strategies.

This species is easily reared and maintained in lab colonies and is a natural enemy of *L. botrana* one of the principle lepidopteran pests of current concern. With these aspects in mind along with the success with this family found by other investigators and farmers worldwide, bioassays were planned and carried out to examine compatibility of *T. cacoeciae* with other crop protection strategies and to fully take advantage of the benefits of the use of this species.

Taxonomic Position

T. cacoeciae Marchal 1927 is currently listed in the webpage: http://www.faunaeur.org which corresponds to Fauna Europaea (2011) version 2.4. (accessed on May 24th, 2011) is as follows:

Kingdom	Animalia
Subkingdom	Eumetazoa
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Order	Hymenoptera
Suborder	Apocrita
Superfamily	Chalcidoidea
Family	Trichogrammatidae
Genus	*Trichogramma*
Species	*cacaeciae*

Since the beginning of the use of the Trichogrammatidae as biological control agents the list of know parasitoid species in this family has become great. This is partially due to the 1970's discovery and use of the male genitalia in systematic classifications (Nagarkatti & Nagaraja, 1968; Nagarkatti & Nagaraja, 1971).

However, authors differ in their count of species. Currently it is said that there are from 145 to 225 species which comprise the *Trichogramma* genus, within which there are three subgenera (García-González *et al.*, 2005). The classification system of these species has been troublesome due to the use of morphological characteristics and inaccurate usage of scientific names (Olkowski & Zhang, 1990).

The study of molecular characteristics has become a new tool in the classification and systematics of the *Trichogramma* genus (Pinto & Stouthamer, 1994; Stouthamer *et al.*, 1999; Stouthamer *et al.*, 2000).

Trichogrammatidae Morphology, Biology & Life cycle

These hymenopterous wasps are true parasitoids by definition. That is to say they require a host egg to complete their life cycle making them an excellent biological control agent. They consume the host egg contents by feeding on it from within. The dependency upon and destruction of the host egg is a distinct characteristic of true parasitoids by the definition of De Bach in 1973.

Chalcidoid wasps parasitize a large number of lepidopteran species, in a variety of habitats. However, the eggs of beetles (Coleoptera), flies (Diptera), true bugs (Heteroptera), other wasps (Hymenoptera), lacewings and their relatives (Neuroptera) have also been parasitized by Trichogrammatidae species (Pinto & Stouthamer, 1994). Due to their great host range and ease of rearing *Trichogramma* has been widely employed in the control of persistent crop pests, such as codling moths, cotton bollworms, spruce budworms, and many others.

Two types of parthenogenesis are displayed in the *Trichogramma* genus. The first type displayed, is haplodiploid arrhenotoky wherein fertilized eggs result in a diploid females and unfertilized eggs result in haploid males. The second type of parthenogenesis in the *Trichogramma* genus is thelytoky, wherein unfertilized eggs result in females (Stouthamer *et al.*, 1990; Lundgren & Heimpel, 2003) which is the case with *T. cacoeciae* (Volkoff *et al.*, 1995).

Curiously though, populations of genetically haplo-diploid arrhenotokous individuals can be found displaying thelytoky due to the presence of an endosymbiotic bacterial genera *Wolbachia* (Johanowicz & Hoy 1998; Pintureau *et al.*, 1999; Grenier *et al.*, 2002; Vavre *et al.*, 2004). In these micro-hymenopterous species, males typically emerge first and remain at the host patch to increase their prospect of mating with emerging females. In haplo-diploid populations, males are the result of unfertilized eggs. Therefore, the placement of male off-spring generally increases the fitness of the female (Hamilton, 1967).

Females are typically found to mate locally at their site of emergence due to their low active dispersal capacity, short longevity (Martel & Boivin, 2004) and minuscule inbreeding depression (Brückner, 1978; Antolin, 1993; Werren, 1993). Even though the probability of encountering a mate outside the patch is lower (Waage & Ming, 1984; Nunney & Luck, 1988) virgin females have been found at off-patch sites in nature (Godfray & Hardy, 1993).

In spring, when conditions become favorable with increased temperature and hours of light, adults begin to search for hosts. In the search for a suitable host the female is cued to possible populations by plant symptoms such as plant volatiles and the radiation by infested or stressed plants (Nordlund, 1994; Bjorksten & Hoffmann, 1998).

The parasitoid female uses various characteristics to select host eggs including state of the egg case, and age, particularly before the larva host head capsule is visible. The size, shape and color of the eggs are said to be the main visual cues used by the female wasp (Ruberson, & Kring, 1993; Schmidt, 1994). Kairomones or host sex pheromones, inadvertently left in scales by host females at the oviposition site, are important volatile chemical clues recognized by the female parasitoid in her search for host eggs (Nordlund *et al.*, 1981; Ruberson & Kring, 1993).

After the female parasitoid encounters a host egg, she inspects it evaluating if it is appropriate for oviposition (Schmidt, 1994). With a particular posture the female punctures a tiny hole through the chorion with her ovipositor and evaluates the quality which determines how many eggs will be deposited (Schmidt, 1994). At the time of the puncturing of the chorion the female deposits metabolic regulation chemicals which are believed to cause pre-digestion of the host egg content (Schmidt, 1994).

Upon the insertion of parasitoid eggs, the internal pressure of the host egg is increased. In a number of host species this increase of pressure forces a small percentage of the egg contents out of the oviposition hole. The longevity of

various species with in this genus has been found to be increased due to the feeding on this displaced yolk (Knutson, 2005). *Trichogramma* females have been documented to parasitize from one to twenty eggs per day, under laboratory conditions from ten to 190 during their life span.

These tiny, quasi-gregarious endoparasites range in length up to 1.5 mm, when adults (Mills & Kuhlmann, 2000). Even though a range of adult size has been established, large females have been found to have a higher fitness, parasitizing more eggs than smaller ones. The longevity of females was seen to be increased when provided with honey (Moreno-Grijalba *et al.*, 2010; Ruberson & Kring, 1993). One study found an average adult life span to be 24 days (Suh, 1998). However, this is dependent on the species, the temperature, and availability of nutrients.

Girault described *Trichogramma* eggs in 1912 to be 0.04 mm wide, 0.14 mm long, and oblique in shape instead of spherical. They hatch at optimal temperatures inside the host egg after a rapid gestation period of approximately 24 hours after oviposition (Volkoff *et al.*, 1995; Jarjees & Merritt, 2002).

The literature regarding the number of larval instars in the *Trichogramma* genus is contradictory. It has been said there is one instar (Silvestri, 1908), four (Pak & Oatman, 1982), or even five instars (Hagen, 1964). However, most authors list three larval stages, depending on the species. The larva has been described to develop through these stages over a period of three to four days, during which time dark melanin granules accumulate on the inner surface of the host egg (Jarjees & Merritt, 2002).

The final larval, or pre-pupal stage, lasts approximately 2 days after which the parasitic larva pupates inside the host egg (Volkoff *et al.*, 1995). When temperatures and hours of light begin to drop in fall it is in this last larval stage that the majority of the Trichogrammatidae overwinter within the host egg (Boivin, 1994).

The pupae stage lasts up to 4 days (Jarjees & Merritt, 2002). The time of emergence varies among the species and again, is temperature dependant. Researchers have found developmental times to be 8 days for *T. australicum* at 29 °C (Jarjees & Merritt, 2002), and 10 days for *T. cacoeciae* at 25 °C (Volkoff *et al.*, 1995).

When the individual is completely developed it chews a hole through the host egg chorion and emerges from the egg as a fully developed sexually mature adult. The black color of the host egg and the exit hole are the typical characteristics of parasitism and resulting parasitoid emergence by *Trichogramma* (Jarjees & Merritt, 2002).

A great plasticity of behaviors within the Trichogrammatidae family for survival during unfavorable conditions has been recognized. Some *Trichogramma* overwinter as immature forms, such as mature larva inside host eggs. In many species these adverse conditions are tolerated by a state of diapause which is initiated by changes in photoperiod and temperature (Boivin, 1994). Some species are able to pass adverse conditions by simply slowing their rate of development instead of going into diapause (Lopez & Morrison, 1980; Keller, 1986). Still other species in this genus have evolved a combination of both strategies, employing a lower metabolic rate for one period and diapause during another (Boivin, 1994).

Figure 1, (Knutson, 2005), demonstrates the generalized development of species in the *Trichogramma* genus. Phase 1, the female parasitizes the egg which is generally before the host egg displays the black head stage, normally undesirable to the parasitoid. Phase 2, parasitoid hatches and begins to feed within the host egg. Phase 3, the dark melanin granules begin to accumulate on the inner surface of the host egg and pupation begins. Phase 4, pupation finishes and the adult emerges.

Figure 1. Generalized life cycle of a *Trichogramma* wasp (Knutson, 2005).

Dispersal & Geographic distribution

The dispersion of these tiny species is accomplished passively by employing wind currents. They can theoretically be carried on the wind for various meters to kilometers due to their minuet size (Nordlund, 1994; Fournier & Boivin, 2000).

Trichogrammatidae ecotypes or native species have been document in all terrestrial habitats (Pinto & Stouthamer, 1994). Therefore it is not surprising that the *Trichogramma* genus is released in 30 countries on approximately 80 million acres of agricultural crops and forests (Olkowski & Zhang, 1990; Li, 1994). The Trichogrammatidae family has become one of the natural enemies used most in the world due to these aspects (Knutson & Gilstrap, 1989). They have been documented to be in use for the control of pests on apple, cabbage, chestnut, corn, cotton, plum, pomegranate, sugarcane, sugar beet, sweet pepper, tomato, in pasture, and paddy fields (Hassan *et al.*, 1998). Research continues and several species are now commercially available in many parts of the world.

T. cacoeciae is also a cosmopolitan species of the Trichogrammatidae family, native, but not limit to, the Nearcrtic and Neotropical regions. It has been cited in the majority of the European union countries (Pintureau, 1997; Fursov & Pintureau, 1999) and is native to European forests and apple orchards, and French vineyards. It is amongst the most commonly found *Trichogramma* species in the Mediterranean region (Herz *et al.*, 2007). *T. cacoecae* recently

documented as native to Spain (Moreno-Grijalba *et al.*, 2010) is currently commercially available in Germany to control *L. botrana* and *Eupoecilia ambiguella* (Hübner) (Lepidoptera: Tortricidae) in grape and *Cydia pomonella* (Hübner) (Lepidoptera: Tortricidae) and *Adoxophyes orana* (Fischer v. Roslerstamm) (Lepidoptera: Tortricidae) in fruit trees (Zimmermann, 2004).

7.2 Objectives

The first objective of these studies was to evaluate the side effects of kaolin on *T. cacoeciae* parasitism and parasitoid progeny emergence in eggs of the factitious host, *E. kuehniella*.

To complete the study pertaining to side effects on parasitism and parasitoid progeny emergence of kaolin on *T. cacoeciae,* the second objective was carried out in laboratory experiments in the target vineyard pest *L. botrana*.

7.3 Materials & Methods

7.3.1 Mass rearing of *T. cacoeciae*

Wasps used for all bioassays were taken from a colony reared in the Crop Protection Laboratory of the University of La Rioja on the factitious host, *E. kuehniella* (Zeller) (Lepidoptera: Pyralidae) at 24±1 °C, 60±5% RH and a photoperiod of 16:8 (L:D). This laboratory colony was created using individuals originally captured in 2003, in Hormilleja, (La Rioja). The identification, traps used and the exact methodology is described in Moreno-Grijalba (2007).

Adults were placed in cylindrical plastic boxes (5 cm high by 12 cm diameter) with one to two 2 cm diameter filter paper covered holes in the lids (figure 2). Two drops of honey were placed on the inner surface of the lid as food for adult wasps as fitness has been shown to be greater in fed females.

Approximately ten square 1 cm by 1 cm yellow cards containing the *E. kuehniella* eggs were introduced into each box. The *E. kuehniella* eggs used were UV sterilized for 90 minutes and stored at 3±1 °C, 70±5% RH in complete darkness no more than a week before being glued onto the yellow cards (figure 3). Tragacanth gum adhesive, synthesis grade by Scharlau Chemis S.A., product G00030 at a rate of 3 g/L was shown to be innocuous to *Trichogramma* species (Hassan, 1992a; Schöller & Hassan, 2001).

Every three to four days new host eggs were provided to the adults by replacing the yellow cards which contained the newly parasitized host eggs. This was accomplished using a stream of CO_2 gas applied to the tops of the cylindrical boxes for 10 to 20 seconds, thereby anesthetizing the adult *T. cacoeciae* individuals. During the changing of host eggs the honey was also refreshed. Dead *T. cacoeciae* adults were withdrawn every ten days and boxes replaced when needed. These rearing boxes and all materials contained within are illustrated in figure 2. The pre-parasitized and parasitized eggs glued to cards are illustrated below (figure 3).

Figure 2. Multiple trays of boxes containing *T. cacoeciae* colony.

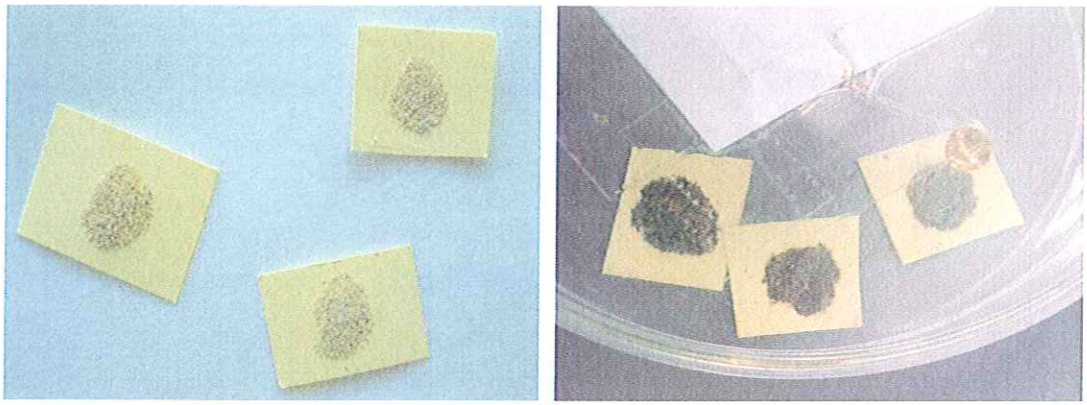


Figure 3. Cards containing UV sterilized *E. kuehniella* eggs (Left: pre-parasitism, and right: parasitized.)

7.3.2 Mass rearing of *E. kuehniella*

Ephestia kuehniella has been repeatedly proven as an adequate factitious host to rear various *Trichogramma* species. In fact its control of *E. kuehniella* in food processing facilities has been accomplished with another species of parasitic wasp, *Trichogramma pretiosum* (Steidle *et al.*, 2001). This Pyralidae moth is not highly vulnerable to infections, bacterial nor fungal. It is easily established and maintained in lab colonies producing an abundant quantity of eggs using minimal resources. Due to its ease of production and handling, cost effectiveness, and versatility of use in laboratory trials, along with the fact that *T. cacoeciae* completes its lifecycle perfectly via the parasitism of this factitious host, *E. kuehniella* was employed.

The stock culture of this host was established in the Crop Protection Laboratory of the University of La Rioja in 2002. This colony was created from individuals reared in the Agricultural Entomology Dept. of the University Politécnica of Madrid. The original colony was started in 1988 and maintained with addition of individuals. The rearing of this species described below was carried out following Marco 1994.

Eggs used in the bioassays were taken from the laboratory colony. To obtain large quantities, approximately 100 adults are placed inside prismatic inverted boxes (22.7 x 16.8 x 3.5 cm). A plastic screen was set between the lid

and the top of the box. The eggs were thus separated from the adults in the bottom of the box. The bottom of the box was lined with black patent paper to ease egg collection.

The adult moths do not feed, therefore, it was not necessary to add nutrients to the cages. Adults were transferred to the oviposition boxes by a stream of CO_2 which acts as anesthesia in short durations of approximately 15 seconds. The eggs were removed from the oviposition boxes daily. The oviposition materials and boxes are illustrated in figures 4 and 5.

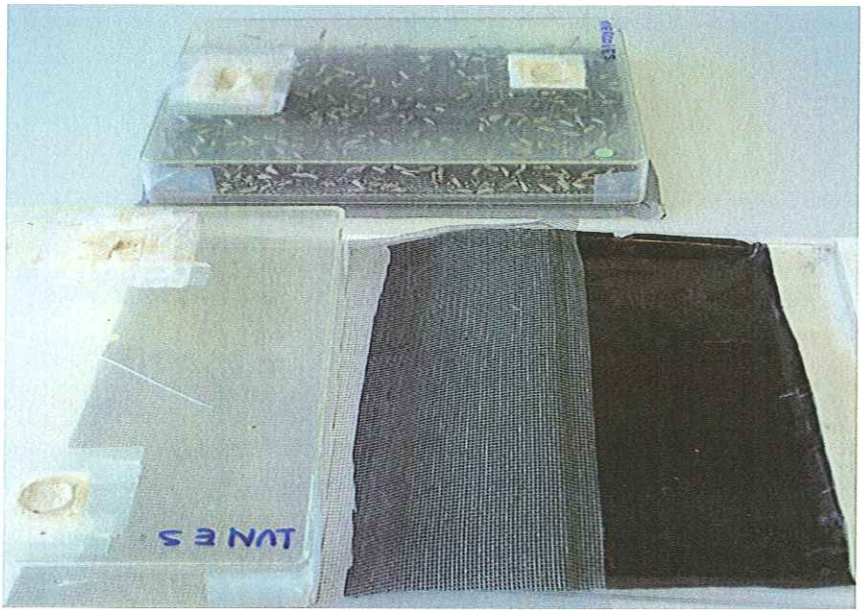


Figure 4. Cage and materials containing *E. kuehniella* adults for oviposition.

Figure 5. Cages containing *E. kuehniella* eggs for collection.

The production of colony individuals was carried out by placing 0.05 grams of eggs into prismatic boxes (22.7 x 16.8 x 3.5 cm) containing 200 grams of larval diet. These eggs and therefore the resulting larva and pupae were maintained in the same boxes to develop until adult emergence (figure 6).

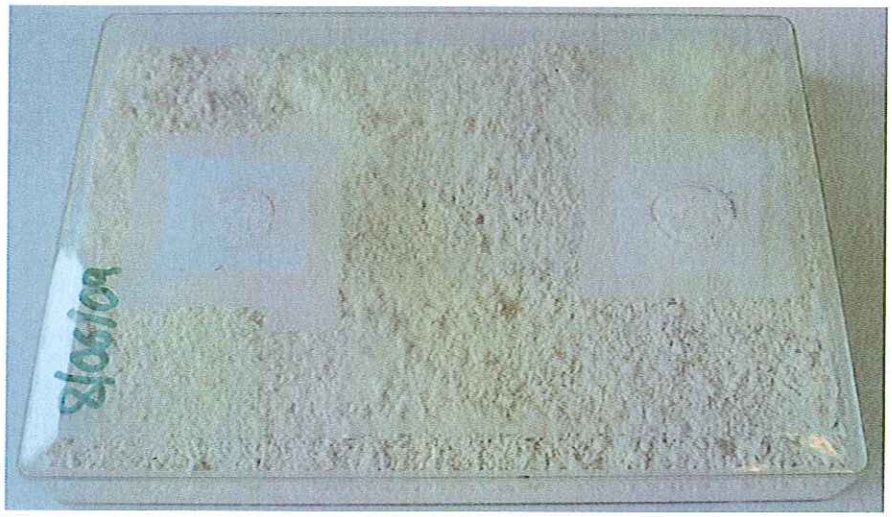


Figure 6. Newly filled *E. kuehniella* colony production cage containing diet and eggs.

The lids contain two 3 cm diameter screen covered holes which provide ventilation and permit the application of CO_2. The larval diet consists of 95% wheat flour and 5% brewers yeast. The diet is well mixed to assure its complete homogenization.

The eggs are distributed evenly in the diet in relatively low densities (400 eggs per box) due to the fact that *E. kuehniella* larvae are territorial. Once added, the eggs hatch and neonate larvae feed on the diet and develop within it. Fully developed larvae build a cocoon and pupate inside the diet or on the walls or lid (figure 7). When the adults emerge they are transferred from the larval boxes to the oviposition chambers as stated above.

Figure 7. Colony production cage containing pupae and emerged *E. kuehniella* adults.

7.3.3 Effects of kaolin on *T. cacoeciae* parasitism of *E. kuehniella* eggs and the emergence of parasitoid offspring

In the following assays involving *E. kuehniella*, all eggs used were produced in the laboratory colonies. All bioassays employed adult *T. cacoeciae* females less than 48 hours old.

Groups of 20 *E. kuehniella* eggs less than 24 hours old were sterilized with UV light for 1.5 hours in order to arrest embryonic development which has been demonstrated not to affect the development of *T. cacoeciae* (Moreno-Grijalba *et al.*, 2010). The egg groups were glued to 1 by 1 cm yellow cards using tragacanth gum adhesive.

The cards containing *E. kuehniella* eggs were treated with kaolin at a rate of 47.5 g/L, which represents one of the commercially recommended concentrations of Surround® WP at 50 g/L. Water was used for the control and the carrier in the treatment. Five and a half ml of the treatment solution was sprayed with the Potter tower at 0.5 bars of pressure. Treatments were carried out and set to dry before the start of the trial.

The experimental arenas consisted of one 6.5 cm long, 4.5 cm wide, 2.5 cm tall rectangular, translucent plastic box. The lids contained one filter paper covered, 2 cm diameter ventilation hole. On the inner side of the lid two 5 µl drops of honey were placed at opposite edges of this ventilation hole. The small size of the drops enabled the female to feed without great risk of becoming trapped in the honey. In each arena one *T. cacoeciae* female was introduced via a fine camel hair brush.

Cards were replaced every 24 hours for four consecutive periods. These egg groups were then isolated in the same condition as the trial until parasitoid emergence. At the end of the ten day developmental period the number of parasitized eggs was recorded along with the number of parasitized eggs which contained a parasitoid exit hole.

Choice trial in *E. kuehniella* host

One kaolin treated card along side one control card containing the host eggs were placed in all of the experiment arenas. The presence of both conditions gave the adult female parasitoid the choice of parasitism between kaolin treated and control egg groups. Thus, allowing the evaluation of the presence a preference. In this trial on average 27 replications of each treatment were included in the statistical analysis of the effect on parasitism, whereas 20 replications were included in the statistical analysis of the effect on parasitoid progeny emergence.

No choice trial in *E. kuehniella* host

One kaolin or control treated card was placed in each experimental arena with one adult female parasitoid. In this trial 40 replications of each treatment were included in the statistical analysis of parasitism. However, only 20 replications were available to be included in the statistical analysis for offspring emergence totaled approximately.

7.3.4 Effects of kaolin on *T. cacoeciae* parasitism of *L. botrana* eggs and parasitoid emergence

In the following two bioassays with *L. botrana* eggs and *T. cacoeciae* adult females, all individuals were taken from the laboratory colonies. *T. cacoeciae* females less than 48 hours old were employed. One female parasitoid was added to each arena using a fine camel hair brush at the beginning of each trial. These arenas were the same as those used in previous trials with *T. cacoeciae* and illustrated in figure 8 which contained two drops of honey one on either side of the ventilation hole.

Groups of approximately 20 *L. botrana* eggs used were less than 24 hours old having been laid on plastic oviposition chamber cups were previously sterilized with UV for 90 minutes. The egg groups were then treated with kaolin at a concentration of 57 g/L in the Potter tower with water as carrier. The elevated concentration was selected for these bioassays due to the fact that this pest, is a naturally occurring host for the Trichogrammatidae. This elevated concentration also more closely represents possible conditions which could be found in vineyards. Every 24 hours, for four consecutive periods, the *L. botrana* eggs were replaced and those of the previous days isolated.

Choice trial in *L. botrana* host

Two pieces of oviposition chamber containing groups of *L. botrana* eggs, one treated with kaolin along side the control, were placed in each of the experimental arenas along with the female parasitoid. The number of parasitized and un-parasitized eggs for both treatments was recorded for all of the four consecutive 24 hour periods. After the developmental phase of 10 days, the number of parasitized host eggs containing emergence holes was also recorded for each of the 24 hour periods. This data was collected after the ten day emergence period had taken place. Approximately 20 replications, each with both conditions of parasitism and emergence were included in the statistical analysis in this choice trial.

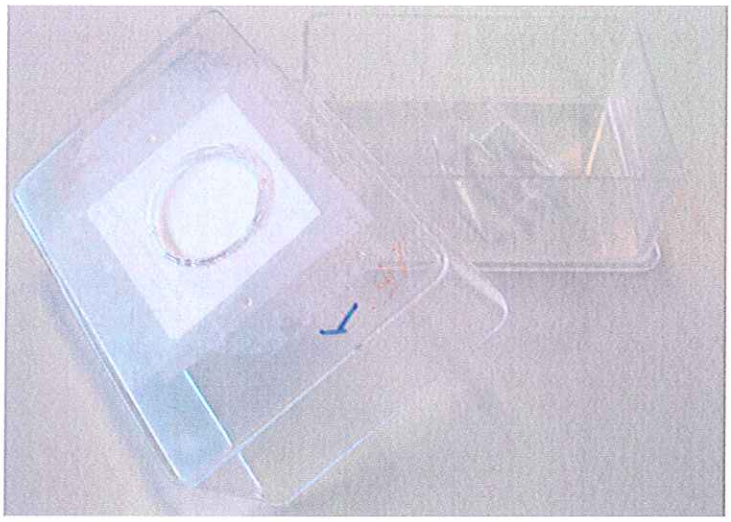


Figure 8. Experimental arena for parasitism and emergence of *T. cacoeciae*.

No choice trial in *L. botrana* host

One group of *L. botrana* eggs, kaolin treated or control, was placed in each experimental arena along with the *T. cacoeciae* female. The number of parasitized host eggs for both treatments were recorded along with the number of parasitized eggs which contain an exit hole. These values were taken for each of the four 24 hour periods, as in the choice trial. In the no choice trial, on average, 20 replications of each condition were included in the statistical analysis of parasitism and emergence.

7.4 Statistical analysis

All statistical analyses were performed with Statgraphics Centurion XVI (2010) at a confidence level of 95%. The Bartlett or Levene test was employed for the comparison of the standard deviations. When the variance between the treatments was found to be equal and the skewness and kurtosis were within appropriate ranges, a standard analysis of variance was carried out with t-student.

7.5 Results & Discussion

7.5.1 Effects of kaolin on *T. cacoeciae* parasitism of *E. kuehniella* eggs and offspring emergence

The parasitism and emergence data of the choice trial involving *E. kuehniella* eggs was evaluated by comparison of the means using t-student, wherein no significant difference was found (table 1).

No significant difference between treatments was found when the parasitism and emergence data of the no choice bioassay involving *E. kuehniella* eggs was evaluated by comparison of means (table 1).

Table 1. Effects of kaolin on *T. cacoeciae* parasitism and emergence in *E. kuehniella* eggs.

Means (%) of parasitism & emergence (± SE) in *E. kuehniella* eggs.								
Assay type	Choice Bioassays				No Choice Bioassays			
Trait	Parasitism*		Parasitoid Emergence*		Parasitism*		Parasitoid Emergence*	
Means	control	kaolin	control	kaolin	control	kaolin	control	kaolin
	17.8±2.4	22.1±2.8	97.7±1.8	97.6±1.8	49.3±2.7	45.7±2.9	97.7±0.7	99.0±0.4
p value	0.25		1.0		0.30		0.16	

* t-student test employed

The impact of kaolin on auxiliary fauna including natural enemies in both lab and field conditions is highly variable. There is of course, an array of diverse trophic relationships and interacting biological and ecological aspects which contribute to the varying results of the secondary effect, or lack thereof, with kaolin use. In an assortment of crop settings, no unfavorable repercussion to many parasitoid species were established. However, altered species composition of generalist predator assemblages along with a reduced relative abundance in kaolin treated apple orchard plots was encountered (Sackett *et al.*, 2007). Yet, in those same kaolin treated plots the proportion of parasitized Obliquebanded Leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), by

various different parasitoid species was not demonstrated to be perturbed (Sackett *et al.*, 2007). Thus the alteration in assemblage could have been a result of low pest species or availability.

On the other hand, some authors have purposed an interruption of the ability of the parasitoid to recognize host species due to kaolin treatments. The parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae) for example, did not parasitize kaolin treated larvae in laboratory studies (Adán *et al.*, 2007). This response has also been recognized in citrus field studies with two other Hymenopteran parasitoids, *Aphytis melinus* (DeBach) (Hymenoptera: Aphelinidae), and *Comperiella bifasciata* (Howard) (Hymenoptera: Encyrtidae) (Rill *et al.*, 2008). In light of previous studies which encountered detrimental effects on auxiliary fauna with the use of kaolin, the lack of inhibitory effects on *T. cacoeciae* in the host *E. kuehniella* was an encouraging result.

7.5.2 Effects of kaolin on *T. cacoeciae* parasitism and parasitoid emergence in *L. botrana* host eggs

When the accumulative data sets of parasitism and emergence for both choice and no choice experiments were compared no adverse effect from kaolin on the parasitism by *T. cacoeciae* was found in the host *L. botrana* (table 2). However, an effect on parasitoid emergence was illustrated by a lower percentage of parasitized host eggs containing the parasitoid exit hole in the kaolin treated replicates of the no choice bioassay (table 2).

This trend of lesser parasitoid emergence in the kaolin treated replication, even though not statistically illustrated in the control bioassay, could have been influenced by the shape of the egg. The shape of the *L. botrana* host eggs are flat, not spherical eggs unlike those of *E. kuehniella*. Therefore greater surface area should have been exposed to and in contact with the kaolin particle film. It has been suggested that kaolin affects cuticle lipids resulting in eventual dehydration (Korunic, 1998). Thus a heightened contact with the particle film treatment could lead to greater dehydration. A higher rate of

desiccation in the *L. botrana* eggs could in fact contribute to the difference in parasitoid emergence found between the two hosts.

Table 2. Effects of kaolin on *T. cacoeciae* parasitism and emergence in *L. botrana* host eggs.

Means (%) of parasitism & emergence (± SE) in *L. botrana* eggs.								
Assay type	Choice Bioassays				No Choice Bioassays			
Biological trait	Parasitism*		Parasitoid Emergence*		Parasitism*		Parasitoid Emergence*	
Means	control	kaolin	control	kaolin	control	kaolin	control	kaolin
	15.9±2.1	18.6±2.1	79.7±3.5	73.6±3.4	33.2±2.3	34.3±2.7	69.6±3.0	57.9±3.5
p values	0.36		0.21		0.93		0.00	

* t-student test used

This generalist parasitizes various lepidopteran crop pests with similar biological characteristics as the factitious host employed thus it's conservation is an important issue to be taken into consideration. If the use of kaolin in combination with the release or augmentation of native populations of *Trichogramma* is to be considered timing of is paramount. These tiny wasps are relatively delicate therefore direct treatment of them with a clay kaolin layer, even though non-toxic, could be problematic.

The ability of the parasitoid to survive and or thrive in combination with kaolin treatments could lead to a greater range of possibilities for crop protection.

7.6 Chapter conclusions

1. When offered kaolin treated *E. kuehniella* eggs no impact on parasitism nor adult emergence was observed on *T. cacoeciae*.

2. There was no significant difference between parasitism of *L. botrana* host eggs by *T. cacoeciae* when the kaolin and control treatments were compared. However, the emergence of *T. cacoeciae* progeny was affected when *L. botrana*

eggs were treated with kaolin.

7.7 Resumen de capítulo en español

Numerosas especies del género *Trichogramma* han sido utilizadas en el control biológico de importantes plagas de los cultivos (en decenas de países y en millones de hectáreas), debido a su amplio rango de huéspedes (sobre todo del orden Lepidoptera) y a la facilidad de su cría masiva.

En el presente trabajo, se llevaron a cabo bioensayos de elección y no elección para determinar si el tratamiento con caolín de huevos del huésped de sustitución, *E. kuehniella*, afectaba a la parasitación y a la emergencia de *T. cacaeciae*. Los resultados obtenidos indicaron que el compuesto no tuvo efecto significativo sobre ninguno de estos dos parámetros.

Cuando se desarrollaron bioensayos paralelos con el mismo fin, pero utilizando como huésped *L. botrana*, no se observó tampoco efecto sobre la parasitación. Sin embargo, sí se produjo un efecto inhibitorio de la emergencia cuando los huevos huésped eran tratados con el compuesto.

A la vista de estos resultados, la combinación de los dos métodos de control (aplicación de caolín y liberación de *T. cacoeciae*) en el manejo integrado de *L. botrana*, podría ser compatible. No obstante, más ensayos de semicampo y campo, así como sobre otros parámetros biológicos deberían ser llevados a cabo antes de confirmar con rotundidad dicha compatibilidad.

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