

1 Juan-Blasco *et al.*: Micro-encapsulated formulation to exclude citrus ants

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3 **Efficacy of a micro-encapsulated formulation to exclude ants from citrus canopies.**

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23 **Abstract**

24 Inesfly IGR FITO[®] is an insecticidal paint based on chlorpyrifos and pyriproxyfen, in a micro-
25 encapsulated formulation that confers the advantage of releasing active ingredients slowly. In
26 this study, a 15 cm band of Inesfly IGR FITO[®] was painted around citrus trunks. The efficacy of
27 this paint to exclude ants from foraging in citrus trees was evaluated in two citrus orchards
28 along the season in two different ant communities, one dominated by *Lasius grandis* and the
29 another by *Linepithema humile*. Field results proved that a single application of Inesfly IGR
30 FITO[®] at the beginning of the season resulted highly effective excluding ants from canopies
31 throughout the season. Inesfly IGR FITO[®] represents an efficient and more economical
32 alternative than current ant exclusion strategies used in many perennial crops. Further studies
33 should be performed to determine the effects of this strategy on other pests and on citrus
34 beneficials.

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36 **Key words:** *Lasius grandis*, *Linepithema humile*, Inesfly IGR FITO[®], ant barriers

37

38 **Introduction**

39 Ants have long been associated with outbreaks of citrus pest (DeBach 1951; Buckley 1987;
40 James et al. 1999). Current strategies to control ants generally include the use of insecticides
41 and physical barriers to prevent their access to canopies. Physical barriers, based on sticky
42 materials banding the trunk, exclude rapidly and effectively ants from foraging in the canopies.
43 This method is based on monthly replacements of bands, which makes the method too
44 laborious and economically unaffordable (Vincent et al. 2001; Vega and Rust 2001; Pekas et al.
45 2010a). Insecticide treatments include surface sprays, toxic baits and contact insecticides that
46 act as barriers. The first option mostly kills the aboveground foragers but the colony itself may
47 not be eliminated (Davis and Van Schagen 1993) and, moreover, it is not a selective strategy to
48 natural enemies (Smith et al. 1996). Broadcast application of toxic baits is generally considered
49 the most efficient method to control multiple colonies over a large area in different crops
50 (Stanley 2004; Greenberg et al. 2006; Daane et al. 2008). Toxic baits usually kill brood and
51 sterilize or kill the colony queen(s), which eliminates the entire colony (Williams 1993).
52 However, their action is not immediate and does not last all the season-long (Rust et al. 2004;
53 Daane et al. 2007, 2008). Moreover, important technical measures as bait station deployment
54 rate, seasonal periods of deployment and bait insecticide formulations need to be properly
55 designed for Mediterranean citrus ants. Finally, chemical barriers, as physical barriers, exclude
56 ants from foraging in trees instantaneously and, moreover, kill foraging workers that come into
57 contact with the insecticide. The effectiveness of chemical barriers is limited by high
58 temperature, irrigation and ground cover (Rust et al. 2000) and they usually degrade within 2-3
59 months, increasing the need for repeated applications (Tollerup et al. 2006).

60 Inesfly IGR FITO[®] (Industrias Químicas Inesba S.L. o INESFLY Ingeniería de Salud S.L., Paiporta,
61 Spain) is an insecticidal paint (active ingredients 3.0% chlorpyrifos and 0.063% pyriproxyfen) in
62 a micro-encapsulated formulation. This micro-encapsulated formulation is a polymer
63 composed of two parts: 1) Vinyl Acetate that gives the polymer its consistence and its capacity
64 of carrying active ingredients, and 2) Versaic Acid Ester VeoVa that provides wet scrub and
65 photolytic resistance. Inesfly IGR FITO[®] confers the advantage of releasing active ingredients
66 slowly, so paint does not have to be applied frequently and its effect can last for a long time
67 (López et al. 1999; Mosqueira et al. 2005; Dias and Jemmio 2008; Amelotti 2009; Llácer et al.
68 2010). To our knowledge, no formulation of this polymer has been previously tested to exclude
69 ants from tree canopies.

70 The objective of this study was to evaluate the efficacy and persistence of the paint Inesfly IGR
71 FITO[®] as chemical barrier to exclude two different ant communities found in Mediterranean
72 citrus orchards from foraging in canopies.

73 **Material and methods**

74 *Study sites*

75 The study was conducted in two clemetine (*Citrus sinensis* var. *clemenules*) orchards located at
76 La Pobla de Vallbona (UTM X713419 Y4390371; 145 m altitude) and Bétera (UTM X722481
77 Y4385219; 35 m altitude) (Valencia, Spain) in 2009. Both orchards were drip irrigated and a
78 vegetal cover crop was maintained. The orchard of La Pobla de Vallbona (8.24 ha) was sown
79 with a monoespecific sod of *Festuca arundinacea* Schreber (Poacea) that was mowed in middle
80 spring. The ant community of this orchard was dominated by *Lasius grandis* Forel and *Pheidole*
81 *pallidula* (Nylander) (Hymenoptera: Formicidae) (Vanaclocha et al. 2005), the native ant
82 species commonly found in Spanish citrus agroecosystems (Alvis 2003; Vanaclocha et al. 2005;
83 Cerdá et al. 2009). The orchard of Bétera (3.49 ha) preserved a spontaneous cover crop that
84 was mowed twice during spring and once at the beginning of fall. Ant community was
85 dominated by the exotic ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae)
86 (Vanaclocha et al. 2005).

87 *Ant-exclusion and ant activity*

88 In each orchard, 12 blocks were randomly selected. Each block consisted of four adjoining
89 trees in a square distribution. Four of these blocks were treated with Inesfly IGR FITO[®], four
90 with the physical barrier Tangle-Trap[®] (Bioestimulantes Agrícolas S.L., Massalfassar, Spain) and
91 the other four were not treated. The chemical barrier, Inesfly IGR FITO[®], was applied by
92 painting a 15 cm width band of the trunks with 170-200 g/m² paint deposit. The physical
93 barrier, Tangle-Trap[®], was monthly sprayed over 15 cm width adhesive bands placed in the
94 trunk of the trees. Each barrier was placed 20 cm high from the soil. In order to prevent ants
95 from using alternative routes into the canopies, trees were pruned before barrier applications.
96 Ant activity was evaluated monthly from April to December 2009 by counting the number of
97 ants ascending and descending through a 15 cm width imaginary band during 2 minutes (Pekas
98 et al. 2010a). The imaginary band was defined 5 cm above barriers. Ant activity on selected
99 trees was assessed previously to barrier applications, in order to confirm the homogeneity
100 between the 12 selected blocks.

101 *Statistical analysis*

102 All statistical comparisons were performed using one-way ANOVA's and means were
103 compared using a Tukey test ($P < 0.05$). Ant activity was log transformed ($\log(x+1)$) to correct
104 heterogeneity of variance. Trees were used as independent samples because no significant
105 differences were found between ant activity in the 12 blocks of each orchard in the sample
106 previous to the application of the barriers.

107 **Results**

108 *Ant abundance.*

109 *Lasius grandis* was the most active ant in the orchard of La Pobla de Vallbona comprising
110 75.5% of the individuals counted in citrus trunks along the year, followed by *P. pallidula* and
111 *Formica rufibarbis* Fabricius with 16.2% and 7.5%, respectively. *Linepithema humile* was the
112 unique species present in the orchard of Bétera (Table 1). At the beginning of the season,
113 before the barriers were applied, there were no significant differences on ant activity in the
114 blocks of each orchard (La Pobla de Vallbona: $F = 0.83$; $df = 11, 36$; $P = 0.62$; Bétera: $F = 1.29$; df
115 $= 11, 30$; $P = 0.28$).

116 *Seasonal activity and efficacy of barriers.*

117 In La Pobla de Vallbona, ant complex remained active until November (Fig. 1). Monthly
118 applications of Tangle-Trap[®] and a single application of Inesfly IGR FITO[®] in April significantly
119 reduced ant activity until September. In this month, ant activity in trees treated with Inesfly IGR
120 FITO[®] increased and became similar to control trees. The most active ant during this month
121 was *P. pallidula* (129 ants) followed by *F. rufibarbis* (29 ants) whereas *L. grandis* activity was
122 almost null (3 ants). The barriers of Tangle-Trap[®] totally excluded foraging activity of ants in
123 citrus canopies until the end of the season. In Bétera, *L. humile* remained active until
124 December, with higher activity from July to September (Fig. 2). Tangle-Trap[®] and Inesfly IGR
125 FITO[®] barriers avoided ant transit to citrus canopies. Tangle-Trap[®] barriers showed a non-
126 continuous efficacy along the experiment.

127 **Discussion**

128 The present work demonstrates that Inesfly IGR FITO[®] can effectively exclude ants from citrus
129 canopies. Field results obtained in two citrus orchards with different ant complex prove that
130 this micro-encapsulated formulation results effective against both *L. grandis*, the most
131 abundant ant in Mediterranean citrus (Alvis 2003; Vanaclocha et al. 2005; Cerdá et al. 2009),
132 and the invasive Argentine ant *L. humile*, the most damaging ant in other citrus areas such as
133 Californian citrus (DeBach 1951; Vega and Rust 2001).

134 A single application of Inesfly IGR FITO[®] at the beginning of the season, was enough to exclude
135 ants from canopies along the season. The seasonal activity of *L. grandis* described herein
136 coincides with the published by Pekas et al. (2010b) and Alvis (2003). The increase of ant
137 activity in trees treated with Inesfly IGR FITO[®] at the end of the summer was due to the
138 presence of *P. pallidula* and *F. rufibarbis* which may benefit from the exclusion of *L. grandis*.
139 This species and *P. pallidula* avoid nesting and foraging in the same trees (Pekas et al. 2010b).
140 The number of Argentine ant specimens ascending and descending the trunk was much higher
141 than that of the Mediterranean native ants from April to December. *Linepithema humile* was
142 even able to elude Tangle-Trap[®] barriers. Despite this activity pattern, Inesfly IGR FITO[®]
143 maintained the citrus canopies free of *L. humile*. The exclusion of ants started in April, when
144 high densities of *L. humile* were already present in canopies. Considering this high activity at
145 the beginning of the season and the long persistence and high efficacy showed by this
146 insecticidal paint, we suggest to apply this barrier at least one month earlier, in late February
147 to early March as recommended by Markin (1970). Finally, as physical barriers, Inesfly IGR
148 FITO[®] showed an immediate effect and avoid ant transit immediately after its application. This
149 represents an advantage in respect of the use of baits, which action mode is slower. In
150 addition, non phytotoxic effects were observed in the trunk and canopy of the assayed trees.
151 Further researches should be focused on the side effects of this methodology and on its
152 possibilities of implementation in large citrus areas.

153

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Tables

Table 1. Ant species and total number of ants counted in ant-allowed (control) and ant-excluded (Tangle-Trap[®] and Inesfly IGR FITO[®]) trees in two citrus orchards sampled from April to December 2009.

Ant species	La Pobla de Vallbona			Bétera		
	Control	Tangle-Trap [®]	Inesfly IGR FITO [®]	Control	Tangle-Trap [®]	Inesfly IGR FITO [®]
<i>Linepithema humile</i> (Mayr)	0	0	0	9871	1,323	17
<i>Lasius grandis</i> Forel	2,492	0	38	0	0	0
<i>Pheidole pallidula</i> (Nylander)	399	0	144	0	0	0
<i>Formica rufibarbis</i> Fabricius	103	0	150	0	0	0
<i>Camponotus sylvaticus</i> (Olivier)	12	0	6	0	0	0
<i>Plagiolepis schmitzii</i> Forel	7	0	1	0	0	0
Total	3,013	0	339	9,871	1,323	17

Figures

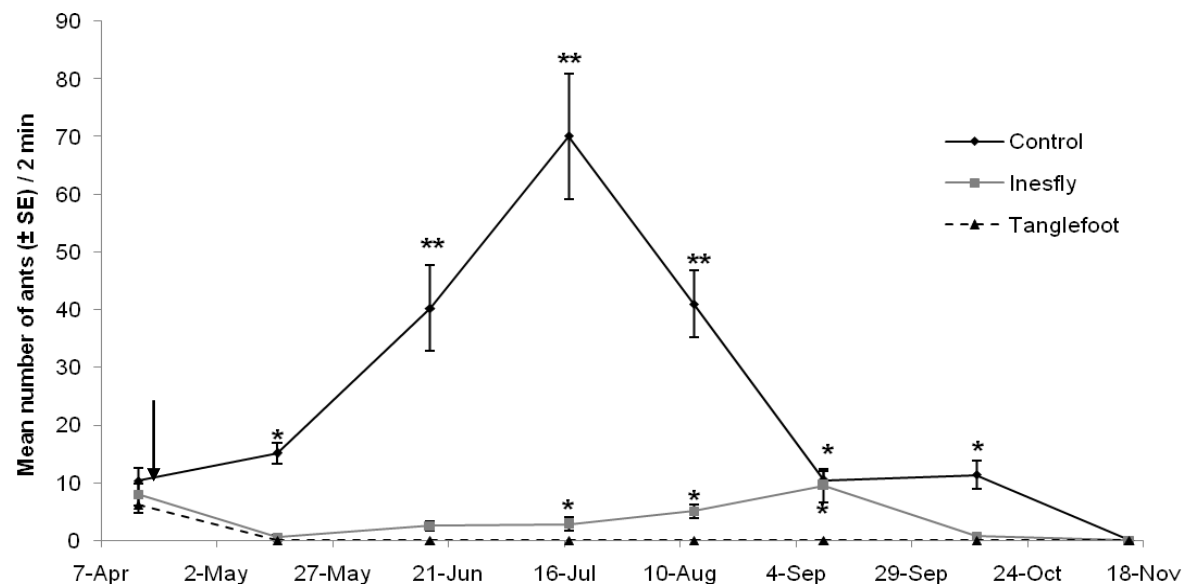


Figure 1. Seasonal activity of a Mediterranean citrus ant community on canopies of trees where Inesfly IGR FITO[®] and Tangle-Trap[®] were used as barriers and without barriers (control) along 2009 in La Pobla de Vallbona citrus orchard. Activity is presented as the mean (\pm SE) of the ants ascending and descending the tree trunk during two minutes. The arrow shows the day that barriers were applied (April 20). For each sample date, a different number of asterisks following the means indicates significant differences between treatments (Tukey test. $P < 0.05$).

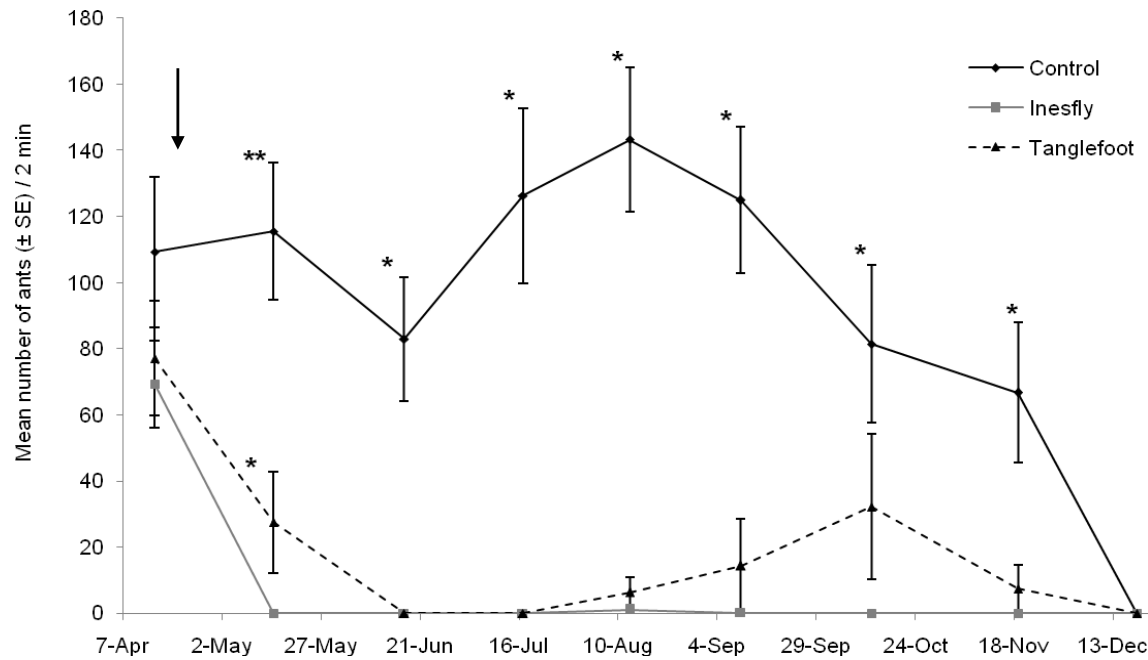


Figure 2. Seasonal activity of *L. humile* on canopies of trees where Inesfly IGR FITO[®] and Tangle-Trap[®] were used as barriers and without barriers (control) along 2009 in Bétera citrus orchard. Activity is presented as the mean (\pm SE) of the ants ascending and descending the tree trunk during two minutes. The arrow shows the day that barriers were applied (April 11). For each sample date, a different number of asterisks following the means indicates significant differences between treatments (Tukey test. $P < 0.05$).

