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A new mechanised cultural practice to reduce *Ceratitis capitata* Wied. populations in area-wide IPM

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Abstract

The Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), affects most of the fruit species grown in temperate and tropical climate regions, causing significant economic damage. One of the classical cultural strategies against this pest is to gather and bury the remaining fruit after harvest, but this is economically unfeasible today. Wood shredders already available in current Spanish groves can be used to grind or crush fruits laying on the soil as an alternative to this practice and to the use of pesticides in area-wide integrated pest management (IPM). With the purpose of evaluating this alternative, the initial step of this study was to perform laboratory tests to assess the efficacy of crushing and grinding as a method for controlling medflies. The results showed that grinding was 78% effective against larval stages, while crushing resulted in a 17% efficacy, leading us to choose the first alternative. As a second step, the operational parameters (type of cutting tool, shaft rotation speed and tractor speed) of the wood shredders were adjusted to efficiently carry out this practice under field conditions. Finally, the effect of the mechanised grinding of fallen fruit on *C. capitata* populations was evaluated for two consecutive years in commercial citrus orchards. The results showed a significant 27-46% reduction in *C. capitata* populations the following spring, thus demonstrating that the newly proposed mechanised alternative can be included in the current area-wide IPM of the pest in Spain.

Additional key words: Mediterranean fruit fly; *Citrus sinensis; Citrus unshiu;* wood grinders; shredders; non-chemical.

Introduction

Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most devastating fruit pests worldwide. Medfly affects most of the fruit species that are grown in temperate and tropical climate regions. The female oviposits under the skin of the fruits so the eggs and subsequent larvae are invisible to the naked eye. This activity produces both direct and indirect economic losses. Direct losses are due to the internal deterioration of the fruit as a result of the larval feeding in the pulp, often accompanied by different types of rottenness. Indirect losses are due to the quarantine periods imposed by fruit-importing countries to avoid presence of the insect inside of the fruit (USDA, 2002; Alonso *et al.*, 2005; Jacas *et al.*, 2008).

In Spain, Mediterranean fruit fly has traditionally been managed by pesticide applications against adult flies (Urbaneja *et al.*, 2009) because this stage of development is the only stage that can be easily exposed to chemicals. Chemical control is still widely used in citrus against *C. capitata* (Urbaneja *et al.*, 2011), although its use poses serious problems not only because it can negatively affect natural enemies (Ehler & Endicott, 1984; Urbaneja *et al.*, 2004) or generate resistance problems (Magaña *et al.*, 2007), but also because the presence of pesticide residues on the fruits can make them unmarketable (Fernández *et al.*, 2001; Ortelli *et al.*, 2005). Therefore, important efforts have recently been made to move medfly chemical-based management towards environmentally safer measures. In 2003 an ongoing area-wide sterile insect technique (SIT) program was

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Abbreviations used: IPM (Integrated Pest Management); IVIA (Instituto Valenciano de Investigaciones Agrarias); PTO (Tractor power take-off); SIT (Sterile Insect Technique).

initiated, covering an area of 150,000 ha (Argilés & Tejedo, 2007). Mass trapping, biological control, selective pesticide applications, chemosterilisation, area-wide SIT and cultural practices are assessed to be used in combination as a part of integrated pest management program (IPM) in Spanish citrus areas (Castañera, 2003; Castañera & Urbaneja, 2007; Juan-Blasco *et al*., 2012). The quantitative effects of cultural practices on *C. capitata* mortality have been generally less studied than other control methods. Indeed, there is a lack of scientific literature on this topic, although many classical citations describe different cultural approaches that contribute to control medflies and could be included in the area-wide IPM program.

Spanish pest control regulators recommend gathering and burying all citrus fruits that either have not been harvested or have fallen to the ground (Generalitat Valenciana, 2005; Urbaneja *et al.,* 2011). This operation may reduce the number of oviposition substrates in which females could continue laying eggs and, at the same time, may kill larvae that would be protected inside the fruit during winter and which are the origin of spring populations (Papadopoulos *et al*., 1996, 1998; Aleixandre & García- Marí, 2007). Gómez-Clemente (1932) reported that when third larval instar individuals are buried a few centimetres into the soil, teneral adults have difficulty emerging from the soil. However, Bodenheimer (1951) noted that larvae bury themselves between 5 and 15 cm deep to become pupae, and adults can emerge from depths of 45 cm in loose soil. Del Pino (2000) also observed that adult flies are capable of reaching the soil surface without being injured after emerging from pupae that were buried at depths of 40 cm in loose soil. All of these findings are in favour of deeply burying fruit, but burying operations have traditionally been performed manually. This practice is costly, what makes it unfeasible for the majority of growers.

The Instituto Valenciano de Investigaciones Agrarias (IVIA) launched a project to efficiently reduce the amount of fruit on the ground that is susceptible to becoming medfly reservoirs. Mechanical removal of fruit from the orchard using sweepers and pick-up machines was rejected due to their high costs. Moreover, these machines are not commonly used in Spanish citrus groves. Burying the fruit in the orchard by means of rotocultivators was also considered, but the depth of burial depends on the moisture and texture of the soil and these machines do not provide deep burial. Furthermore, the integrated production protocols for citrus recommend maintaining the vegetation cover to prevent soil erosion (BOE, 2002), making this alternative unfeasible.

However, wood shredders or grinders are frequently used by Spanish farmers to manage vegetal residues after pruning (Ortí, 2001). The most common types of such machines in Spanish citrus groves are horizontal shaft shredders. These implements have a rotating horizontal shaft where the cutting tools are attached and are often equipped with a roller at the rear that crushes the grinded wood. It is important to note that these machines do not affect the soil and are thus compatible with integrated production practices.

There is no published information on the use of grinding or crushing methods to destroy the fruit remaining on the ground as a means of reducing *C. capitata* populations. For this reason, the present work aims to determine whether the use of shredders can be a non-chemical alternative to burying the fruit compatible with area-wide IPM, considering two possibilities: (1) grinding the fruits meanwhile the rotation of the shaft, (2) crushing the fruits by passing over just with the roller (this practice is cheaper). First, in the laboratory, we evaluated the mortality caused by crushing and grinding the fruit infested by *C. capitata* larvae to determine whether crushing, which demands less energy, could be sufficient to reduce medfly populations. The second part was performed under field conditions and was aimed at adjusting the operational parameters of wood shredders to perform this practice efficiently. Finally, we studied the effect of apply this practice in autumn or winter under field conditions on the population of *C. capitata* in the subsequent spring season.

Material and methods

Effect of crushing and grinding fruit on the mortality of *C. capitata* larvae under laboratory conditions

Citrus sinensis (L.) Osb. var. Lanelate oranges from orchards in the Moncada (Valencia, Spain) area were hand harvested at commercial maturity and transferred to the IVIA Agroingeniería facilities. Fruits without external damage were selected and dipped in a fungicide solution of imazalil {1-[2-(2,4 dichlorophenyl)-2 (2-propenyloxy) ethyl]-1H imidazole} (Deccozil S-7.5, Decco Ibérica Post Cosecha S.A.U., Paterna, Valencia, Spain) at 0.2% for 1 min and left undisturbed to air dry for 24 hours. The oranges were artificially infested with *C. capitata* larvae following a similar methodology described by Alonso *et al*. (2005). A hole was done in each fruit by puncturing and removing a plug 10 mm in diameter and 20 mm deep with a cork borer. Then, 15 larvae of second and third instar were introduced into each fruit hole and finally the hole was covered with cotton.

Three treatments were tested: (T1) fruit crush treatment, infested fruits were individually introduced in a black plastic bag (54 × 60 cm), which was hand crushed with a wooden roller (6 cm diameter and 35 cm long); when larvae left the fruit due to the pressure of the roller, they were introduced again into the fruit; (T2) fruit grind treatment, the infested fruits were individually cut into halves, perpendicular to the imaginary axis of the infested holes, and were then ground for 8 s at 455 rpm using a household electric blender (MR 555 CA Minipimer, Braun Spain SA, Barcelona); and (T0) control treatment, no treatment was applied to the infested fruit. One fruit was considered a replicate and 150 replicates were used per treatment.

After treatment, each fruit was individually placed in a plastic container (12 cm diameter and 14 cm high) with 0.3 mm diameter holes at its base to allow fruit juice to flow but keep larvae inside of the container. The fruit was placed on wire netting (mesh of 1 × 1 cm) located in the middle of the plastic container in such a way that the larvae could jump to the base of the container for pupation. The containers were covered with muslin cloth to prevent the larvae from escaping by jumping. The fruits were placed in a climate-controlled chamber under optimal conditions for larval development and pupation (25 ± 3°C, 75 ± 10% RH and a dark photoperiod) (Putruele, 1998).

The fruits were observed daily and the number of pupae found per fruit was recorded. The observations ended when the number of pupae per fruit did not increase within a 10 day period.

Survival rates expressed as percentage were calculated as the ratio of the number of pupae found per fruit to the total number of larvae introduced per fruit (15). The mortality rates were calculated subtracting the survival rate from 100.

Dunnett's test (Dunnett, 1985) was used to compare the mortality rates of each management treatment with the control and of each treatment. When significant differences were found, the efficacies of each treatment compared to control were calculated using the Schneider-Orelli formula (Püntener, 1981). These efficacies between treatments were compared by means of a one-factorial ANOVA. The ANOVA assumption of the normal distribution of residues was assessed by plotting the residuals on normal probability paper. The assumption of homoscedasticity was assessed by Levene's test (Levene, 1960). An LSD test was employed to study the differences between treatments. All tests were considered at the 95% confidence level.

Setting up the operational parameters of a wood shredder

The main settings that can be changed when operating wood shredders are the following: the type of cutting tools, their rotation speed and the tractor speed. To determine the effect of these factors on the quality of grinding and the required power, a 3-factorial design was employed as follows: (i) type of cutting tool, with two variants (knives and hammers), (ii) shaft rotation speed, with two levels (1,840 and 2,500 rpm), and (iii) tractor speed, with two levels (2 and 4.4 km h^{-1}).

Because the characteristics of the fruit (*e.g.,* size, weight and firmness) may have an influence on the quality of grinding, the experiments were conducted on both orange and mandarin species. Both citrus species are susceptible to attack by *C. capitata*. The tests with mandarins were conducted in an orchard planted with *Citrus unshiu* (Mak.) Marc var. Clausellina located in Moncada (Valencia, Spain) (UTM X723582 Y4384822). The tests with oranges were conducted in an orchard planted with orange *Citrus sinensis* (L.) Osb. var. Navelina located in Moncada (Valencia, Spain) (UTM X723627 Y4384731). Each experiment was repeated three times, resulting in a total of 24 tests per fruit species. The tests were conducted in random order to avoid systematic errors.

Two identical horizontal shaft-suspended shredders (Enguix, TRR-150 model, Enguix, SL, Valencia, Spain) were used. One shredder was equipped with 32 knives and the other with 16 hammers.

For each trial, 300 mandarins and 200 oranges were placed in the middle of an orchard lane, forming a 100 m long row. The fruits were harvested on the day before grinding in the same orchard. In all of the experiments, the distance between the base of the shredder and the ground was 1 cm.

To define the quality of grinding, the remains of the fruits were classified into five levels: whole fruits without apparent damage (intact fruits), whole fruits with cuts and/or shocks (damaged fruits), pieces with 40%-60% of the initial fruit (half fruits), pieces of 15%-35% of the initial fruit (quarter fruits), and completely ground fruits. For the first two levels, the percentage of fruit was calculated by dividing the number of classified fruits by the total number of fruits that were used in the experiment. A similar approach was followed for the half and quarter fruits, but the number of pieces were divided by two and four, respectively. The percentage of fruit that was completely grinded was calculated by subtracting the total amount of percentages calculated at each level from 100.

The power required by the fruit shredder was calculated by measuring the speed and torque at the tractor power take-off (PTO) (Eq. [1]). In each test, both parameters were averaged every 0.5 sec during the effective operation of the machine.

$$P = (M \cdot n) / 9550 \qquad [1]$$

where P = average power required by the PTO (kW); M = average torque on the PTO (Nm); n = average rotation speed of the PTO (rpm).

A torque sensor of slip rings (Lebow model 1248, Honeywell Sensing & Control, Freeport, ME, USA) with a signal conditioner (model Daytronic 3270, Daytronic Corporation, OH, USA) was coupled to the PTO to measure this parameter.

The rotation speed of the PTO was measured with a pulsed magnetic sensor (Lebow model 550, Honeywell Sensing & Control, Freeport, ME, USA). Data acquisition was performed with a commercial data logger with software for data visualisation (Yokohama model DC 100, Technol Seven, Yokohama, Japan).

To analyse the effect of the three factors (type of cutting tools, shaft rotation speed and tractor speed) on the two dependent variables (percentage of fruit ground and average power required by the tractor), a multi-factorial ANOVA for each response variable and citrus species was performed. The normality of the data was assessed by plotting the residuals on normal probability paper. An LSD test was employed to study the separation between variants. The confidence level for all of the analyses was set to 95%.

Effect of grinding on *C. capitata* population under field conditions

In 2004 and 2005, a field experiment was conducted to determine the effect of grinding of the fruit that remained in the field after harvest on the subsequent year's first generation of the *C. capitata* population. In addition, there was a control treatment with no treatment applied to the remaining fruit. The experiment was conducted in two adjacent orchards (plot A and plot B) in Sagunto (Valencia, Spain) (UTM X726240 Y4391074) with the same management characteristics. Both plots had an area of 1 ha and were planted in 1997 with *Citrus clementina* Hort. ex Tan. var. Marisol, one of the most sensitive varieties to *C. capitata* (Paniseo-Tafalla *et al.*, 2009). In 2004, plot A was used to perform fruit grinding and plot B was the control. In 2005, grinding was performed in plot B and plot A was the control. Both grinding treatments were performed after harvest, on 10 November 2004 and on 23 December 2005. The grinding treatment was set up according to the results obtained in the previously described experiment: the wood shredder was equipped with 16 hammers (the machine used was the same described in the previous experiment), the tractor speed was 4.4 km h^{-1} and the rotation speed of the shaft was 1,840 rpm.

Treatment efficacy was assessed by comparing the total number of medfly adult captured on both plots during the following spring. We assumed that the captures in spring came from flies that emerged from fruits or soil in the experimental fields. Therefore, we considered that the first spring generation development started in March, when the captures were very low or zero, and that development was complete when there was a significant increase in captures corresponding to the medfly emergences that originated from later generations (Aleixandre & Garcia-Marí, 2007). The evaluation period occurred from 16 March to 17 June in 2005 and from 1 March to 31 May in 2006. Six Tephritraps® (Sorygar, S.L., Madrid) were randomly placed in each plot for this purpose. The traps used Tri-pack® (5 g a.i. ammonium acetate, 50 mg a.i. putrescine and 2.50 g a.i. trimethylamine) (Kenogard S.A., Barcelona) as bait. The traps also included a tablet of diclorvos (0.5 g a.i. dimethyl 2.2-dichloroethenyl phosphate) (Biagro S.L., Valencia, Spain) as a pesticide, which was replaced every 6 weeks. The trapped flies were counted weekly.

The effect of the grinding treatment was assessed each year by comparing total number of medfly adult captured of the two plots using a chi-squared test at a 95% confidence level. If the control plot had more captures than treated plot the percentage of reduction of medflies per year was calculated by Eq. [2].

$$\% \text{ Reduction} = [(C - T) / C] \cdot 100 \qquad [2]$$

where C = total of medfly captured in the control plot; T = total of medfly captured in the treated plot.

Results

Effect of crushing and grinding of fruit on the mortality of *C. capitata* larvae under laboratory conditions

The percentages of larval mortality (Table 1) resulting from both treatments (crushing and grinding) were significantly different from the control (Dunnett's test, $p < 0.05$). The efficacy of the grinding treatment was 78%, which was significantly higher than the efficacy observed in the crushing treatment at 17% ($F_{1,299} = 696.14$; $p < 0.001$) (Table 1).

Table 1. Percentage of larval mortality (mean ± SE) and efficacy (mean ± SE) for each treatment

Treatment	Percentage of mortality (%)	Efficacy (%)[1]
T0, Control	15.10 ± 1.18	—
T1, Crushing	29.20 ± 1.59*	16.64 ± 1.88[b]
T2, Grinding	81.33 ± 1.16*	78.02 ± 1.37[a]

[1] Mean values followed by a different letter are significantly different ($p < 0.05$, LSD test). * Mean values are significantly different to the control ($p < 0.05$, Dunnet's test).

Setting up the operational parameters of fruit gridding with a wood shredder under field conditions

The percentage of intact fruits was close to zero in all cases, indicating that all the fruits were affected by the shredder. The percentage of damaged fruits, half fruits and quarter fruits were low in all cases. This resulted in very high percentage of completely ground fruits, with values between 78.50% and 97.25%. In both the mandarin and orange experiments (Table 2), the type of tool was the only statistically significant factor for the percentage of ground fruit. In both cases, this percentage was significantly higher when hammers were used. Neither the speed of the tractor nor the rotation speed of the shaft produced statistically significant differences for the percentage of ground fruit.

In mandarin experiments, the required power was significantly higher when the cutting tools were hammers. As expected, the increase of the rotation speed of the shaft from 1,840 rpm to 2,500 rpm required significantly more power for both knives and hammers. However, this increase was greater for hammers (Fig. 1), and thus, the interaction between the type of tools and the rotation speed of the shaft was significant ($F_{1,23} = 36.46$; $p < 0.0001$). In the experiments with oranges, the interaction between the type of tools and the rotation speed of the shaft was also significant for the required power ($F_{1,23} = 26.42$; $p < 0.0001$). The trends were

Table 2. Percentage of ground fruit (mean ± SE) depending on the type of tool, tractor speed and rotation speed of the shaft for mandarin and orange experiment

Type of fruit	Factor		Completely ground fruit (%)*
Mandarin	Type of tool ($F_{1,21} = 25.79$; $p < 0.001$)	Knives	91.95 ± 1.17[b]
		Hammers	97.60 ± 0.36[a]
	Tractor speed ($F_{1,21} = 0.01$; $p = 0.92$)	2 km h^{-1}	95.17 ± 1.04[a]
		4 km h^{-1}	94.89 ± 1.33[a]
	Rotation speed of the shaft ($F_{1,21} = 2.64$; $p = 0.12$)	1,840 rpm	94.12 ± 1.37[a]
		2,500 rpm	95.93 ± 0.90[a]
Orange	Type of tool ($F_{1,23} = 63.02$; $p < 0.001$	Knives	89.24 ± 0.84[b]
		Hammers	97,33 ± 0.55[a]
	Tractor speed ($F_{1,23} = 0.38$; $p = 0.55$)	2 km h^{-1}	92.97 ± 1.60[a]
		4 km h^{-1}	93.60 ± 1.19[a]
	Rotation speed of shaft ($F_{1,23} = 1.04$; $p = 0.32$)	1,840 rpm	93.81 ± 1.58[a]
		2,500 rpm	92.76 ± 1.20[a]

* For each factor, mean values followed by a different letter are significantly different ($p < 0.05$, LSD test).

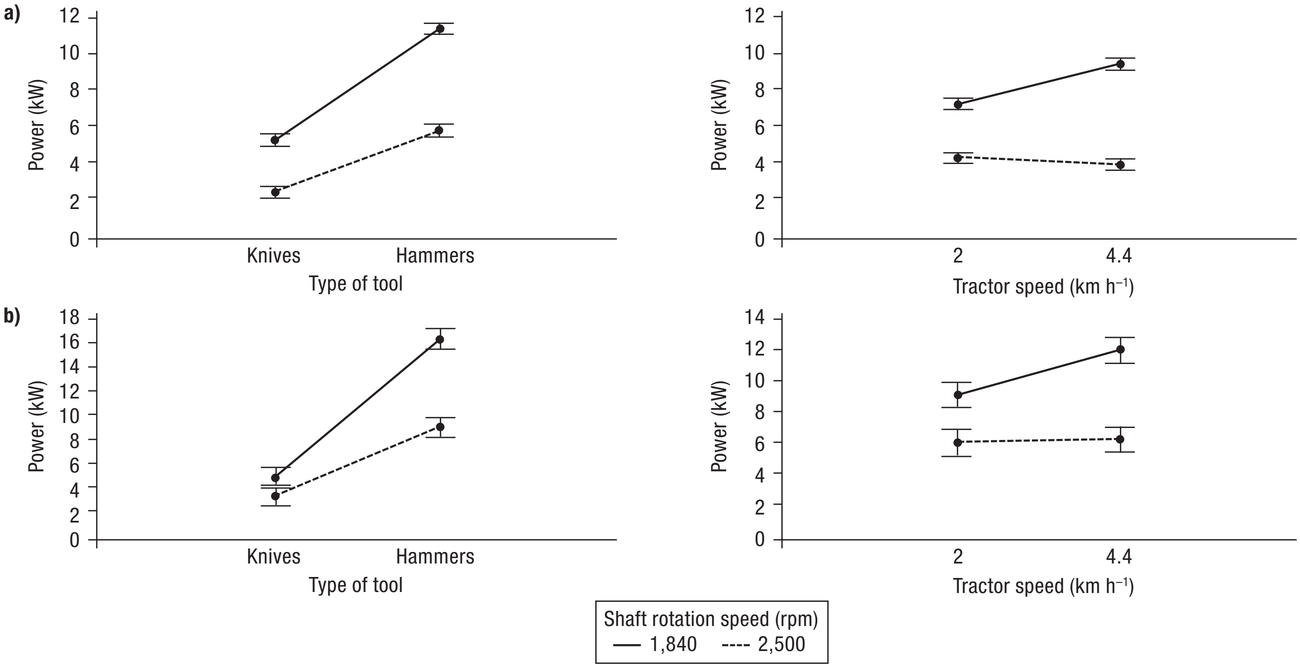

Figure 1. Interaction between the type of tools and rotation speed of the shaft for the required power (left) and interaction between the speed of the tractor and the rotation speed of the shaft for the required power (right) in the mandarin tests (a) and the orange tests (b). LSD intervals with a confidence level of 95%.

similar to those in the mandarin experiments, but the required power values were higher (Fig. 1).

No significant differences in the required power were found between 2 km h^{-1} and 4.4 km h^{-1} when the rotation speed of the shaft was 1840 rpm in the experiments with both mandarins and oranges. However, when the rotation speed of the shaft was 2,500 rpm, the required power was significantly lower at 2 km h^{-1} (Fig. 1), and thus, the interaction between both factors was significant for the experiments with mandarins ($F_{1,23} = 35.13$; $p < 0.0001$) and for the experiments with oranges ($F_{1,23} = 5.5$; $p < 0.0315$).

Effect of grinding on the *C. capitata* population under field conditions

The curves of cumulative *C. capitata* captures per trap and day during the spring of 2005 and the spring of 2006 in the control plot and in the plot in which the clementine fruits were ground are shown in Figs. 2a and 2b, respectively. In the spring of both years, the control plot always had more captures than the plot in which the fruits were ground, although in the spring of 2005, the quantity of captures was lower than in spring 2006. In fact, during the spring of 2005, the total medfly captures in the control plot was 67, which was significantly higher than in the treated plot and had a value of 36 (Chi-squared test, $p < 0.05$). In 2005, the reduction in captures in the treated plot was 46% compared to control. Likewise, during the spring of 2006, the total of medfly captures in the control plot was 215, which was significantly higher than in the treated plot and had a value of 156 (Chi-squared test, $p < 0.05$). In 2006, the reduction in medfly captures in the treated plot was 27% compared to control.

Discussion

Both the grinding and crushing treatments were effective at reducing the survival rate of *C. capitata* under laboratory conditions. The observed efficacy of grinding was 78% and the efficacy of crushing was 17%. This difference was so large that we decided to rotate the horizontal shaft of the shredder to break the fruit in the field, and we discarded the shredder in favour of using the roller alone.

The expected efficacy of these treatments would increase under field conditions because we reintroduced the larvae to the fruit in the laboratory experiments, and the environmental conditions (humidity, tempera-

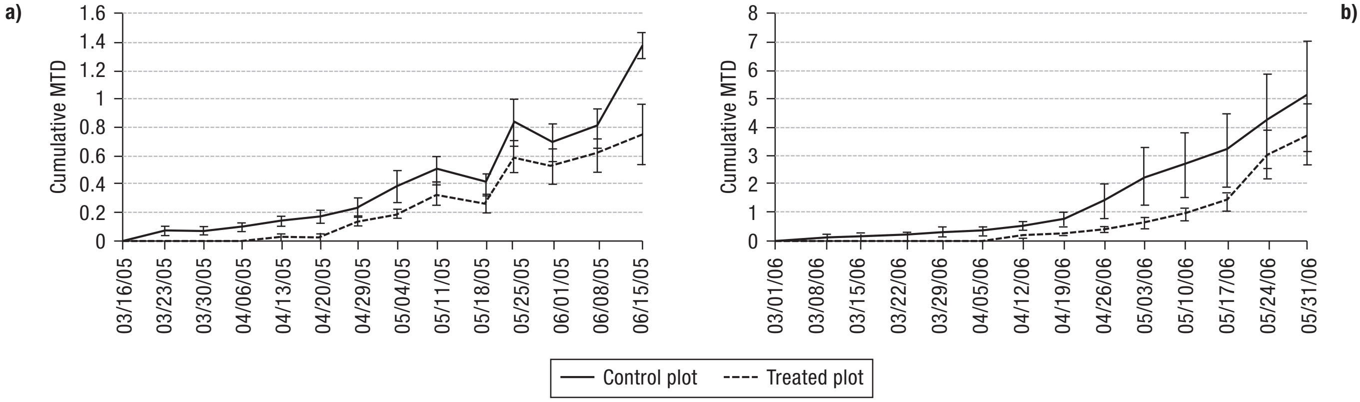

Figure 2. Cumulative medfly captures per trap and day (MTD) during the spring of 2005 (a) and the spring of 2006 (b) in the control plot (T0) and in the plot in which the fruits were ground (T2).

ture and substrate) were optimal for larval development, although these conditions would not occur outdoors. In addition, laboratory fruit were treated with fungicide, however, damaged fruits in the field are easily infested by fungi under field conditions in Spain, mostly by *Penicillium* spp. (Brown & Miller, 1999), and we observed in previous experiments that larval survival is reduced in rotten fruits (unpublished data).

Our work was focused only on second and third instar larvae, although all medfly stages could be detected on fallen fruit. However, eggs and first instar larvae are not capable to overwinter on fallen fruit (Aleixandre & Garcia-Marí, 2006) and, additionally eggs and first instar larvae are very sensitive to desiccation conditions (Putruelle, 1998).

The wood shredder with hammers was found to be the best option to maximise the percentage of ground fruit (~97%). The shredder with knives produced a statistically significant lower percentage of ground fruit, but the value was still high (~90%). However, in practice, both types of tools could be recommended to growers. They would not be required to change the tools of their wood shredders to significantly improve the quality of grinding. Because the speed of the tractor and the rotation speed of the shaft did not affect the percentage of ground fruit, it would be advisable to use the combinations that require less power. The shaft rotation speed of 1,840 rpm required significantly less power, and therefore, it would be recommended to conserve energy and consequently reduce costs. At this shaft rotation speed, there were no significant differences in the required power at a tractor speed of 2 and 4.4 km h^{-1}, so it would be advisable to use the fastest speed to reduce operational time.

The field results indicate that grinding the fruit that remains after harvesting with wood shredders produces a clear reduction in adult captures in the subsequent first generation of *C. capitata*, demonstrating that this technique can be a new effective practice in area-wide IPM. Moreover, the use of wood shredders is compatible with a multi-tactical IPM program that would combine it with complementary non-chemical techniques, such as the release of sterile males or mass trapping, which have been demonstrated to be capable of controlling low levels of *C. capitata* populations.

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