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Biological and mechanical control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice

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Abstract

The combination of mechanical and biological methods was evaluated in the laboratory to assess their impact on the rice weevil *Sitophilus oryzae* in rice. Mechanical methods comprising the conventional polishing process applied either before or after infestation, resulting in reduced nutritional quality plus the added effect of mechanical impact in the presence of the pest, biological methods including parasitism by the pteromalids *Anisopteromalus calandrae* and *Lariophagus distinguendus*, and combined methods (combinations of mechanical and subsequent biological treatments), were tested in the laboratory. All treatments significantly reduced the number of weevil progeny and individual weevil weight. Of the two parasitoids, *L. distinguendus* had the greater effect, reducing weevil populations by 98% in unpolished rice. In the combined treatments, parasitism increased the effect of mechanical methods. However, mechanical methods had a detrimental impact on parasitoid survival, especially in *A. calandrae*. Furthermore, in the presence of mechanical treatments, the sex ratio of *A. calandrae* was unbalanced in favour of males indicating the high vulnerability of females. The impact of both parasitoid species on weevils was attributed to successful parasitism as well as to aborted parasitism and host-feeding. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Polishing process; Rice quality; Parasitism; *Anisopteromalus calandrae*; *Lariophagus distinguendus*

1. Introduction

Despite the progressive reduction of availability of methyl bromide for use in fumigation to control stored product pests (White and Leesch, 1996), many control options remain available but

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few have been evaluated thoroughly. The options include physical (Watters, 1991; Fields and Muir, 1996), chemical (Harein, 1991; White and Leesch, 1996) and biological control methods (Brower et al., 1996).

Both white and brown rice are susceptible to the rice weevil, *Sitophilus oryzae* (L.), as the adults feed on rice and the larva develops inside the rice kernel (Arbogast, 1991; Beckett et al., 1994). During the process of polishing rice grain, kernels are rubbed, lose their pericarp layer and become white. The polishing process has a negative impact on rice weevils, firstly because of the mechanical action of the process (Riudavets and Lucas, 2000; Lucas and Riudavets, 2000) and secondly because of the subsequent low nutritional quality of white rice (McGaughey, 1974; Cho et al., 1988; Singh, 1981; Lucas and Riudavets, 2000; Ryoo and Cho, 1992; Haryadi and Fleurat-Lessard, 1994).

The female of the rice weevil chews a hole in the kernel, lays the egg inside, and seals the hole with a gelatinous secretion which protects the egg (Arbogast, 1991). As the polishing process rubs the rice kernels, it could damage this protection, and consequently could increase the vulnerability of larvae to natural enemies. Moreover, as the polishing process removes some nutritional factors and slows weevil development, it should extend the period of larval susceptibility to parasitism.

Several species of hymenopteran parasitoids including *Anisopteromalus calandrae* (Howard), *Lariophagus distinguendus* (Förster), *Pteromalus* (*Habrocytus*) *cerealellae* (Ashmead) and *Theocolax* (*Choetospila*) *elegans* (Westwood) have been used to attack the rice weevil (Gordh and Hartman, 1991; Brower et al., 1996). These species have a highly developed ability to detect prey and can attack numerous host species. Both *A. calandrae* and *L. distinguendus* attack only older weevil larvae or pupae (Yoo and Ryoo, 1989). The potential of *L. distinguendus* (Cho, 1989; Ryoo et al., 1991) and *A. calandrae* (Press et al., 1984; Cline et al., 1985; Arbogast and Mullen, 1990; Wen et al., 1994) as biological control agents has been shown.

The simultaneous use of compatible methods may lead to a higher level of control. Thus, this study aims to evaluate the compatibility of the conventional mechanical (polishing) process carried out in Europe and subsequent biological control methods based on parasitism, and the efficacy of their combined use in reducing rice weevil populations in rice.

2. Materials and methods

2.1. Insects and rearing conditions

Insects were originally collected from storage areas near Barcelona (Spain). *S. oryzae* was reared on polished rice from 1997 at $25\pm1°C$, $70\pm10\%$ r.h., 16L : 8D. *A. calandrae* and *L. distinguendus* were reared on *S. oryzae* in white rice under the same conditions. The experiments were conducted in a climatic chamber in similar conditions. The rice (cv. Senia and cv. Bahia) came from the Ebro delta area (Catalonia, Spain).

2.2. Experimental technique

The experimental arenas, 0.5 l-glass jars containing 83 g of rice (approx. 3260 kernels), were ventilated. Adult *S. oryzae* were added (410 adults/kg) to each jar at the beginning of the test and removed after 7 days with a 2.0 mm sieve.

Table 1
List of treatments carried out, with their biological and mechanical components

Treatment	Polishing process (PP)	Parasitoid	Rice
Control			
Control	No PP	No parasitoid	Brown
Mechanical			
Rice quality	PP before weevil infestation	No parasitoid	White
Polishing	PP after weevil infestation	No parasitoid	White
Biological			
Lario	No PP	*L. distinguendus*	Brown
Aniso	No PP	*A. calandrae*	Brown
Combined			
Lario + Rice quality	PP before weevil infestation	*L. distinguendus*	White
Aniso + Rice quality	PP before weevil infestation	*A. calandrae*	White
Lario + Polishing	PP after weevil infestation	*L. distinguendus*	White
Aniso + Polishing	PP after weevil infestation	*A. calandrae*	White

Nine treatments were carried out (Table 1). (1) a "Control" without any mechanical or biological treatment of the rice (brown rice); (2) two mechanical treatments: "Polishing" where the rice was polished after weevil infestation (white rice), and "Rice quality" where commercial rice was polished before weevil infestation (white rice). Thus, weevils in the "Polishing" suffered both the mechanical effect and the post-polishing effect on rice quality, while those in the "Rice quality" suffered only the impact due to reduced rice quality. The rice was polished using a laboratory polisher (A. Guid'etti, Universal Brevetto 65378, vertical type) (with a weight loss of 11%); (3) two biological treatments: the "Lario" with an infestation by *L. distinguendus* and the "Aniso" with an infestation by *A. calandrae* (brown rice). Parasitoid infestation with 10 individuals (7 females, 3 males) for each 0.5-l glass jars (83 g of rice) was initiated 14 days after weevil infestation. This interval was selected because both parasitoids demonstrated a significant preference for the older larval instars (Smith, 1993; Ryoo et al., 1996); (4) four combined treatments corresponding to the combination of the mechanical and biological treatments ("Lario + Rice quality", "Aniso + Rice quality", "Lario + Polishing", "Aniso + Polishing") (white rice). In all cases, the biological treatment was applied after the mechanical treatment.

Parasitoids were removed after 7 days. From four weeks after weevil infestation, emerging weevil and parasitoid progeny were counted, removed and frozen weekly for a 6-week period. Weevils were weighed and parasitoids were sexed. Twelve replicates were carried out for each treatment.

2.3. Statistics

Weevil progeny densities (number of weevils in the 2nd generation) and weevil weights were compared using one-way analysis of variance (ANOVA) and subsequent Tukey tests (SAS

Institute, 1996). The number of parasitoids and the mean proportion of males (sex ratio) emerging from the biological and combined treatments were also compared using an ANOVA. A Bartlett test was applied before ANOVA, to test variance homogeneity, and data were log-transformed when necessary (Sokal and Rohlf, 1981). The number of weevils dying for reasons other than successful parasitism (DW) (host-feeding or aborted parasitism), was evaluated by comparing the total number of weevils alive in the control (TWC) (=expected number of emerging weevils in the absence of parasitoids) with the number of emerged weevils (AW) and the corresponding number of emerged parasitoids (PW). For evaluation of the biological treatments, the TWC value was obtained from the "Control" treatment. For evaluation of the combined treatments, the TWC was obtained from the corresponding mechanical treatment (for example, the TWC for "Lario + Polishing" came from the "Polishing").

$$DW = TWC - (AW + PW)$$

The proportions of live, parasitized with success (=adult parasitoids) or dead weevils (mortality due to aborted parasitism or to host-feeding by the parasitoid) were compared in the biological and combined treatments, using a likelihood ratio *G*-test.

3. Results

3.1. Weevil progeny

Compared to the control, the number of weevil progeny was significantly reduced in all treatments ($F = 64.64$; df = 8/94; $P < 0.0001$) (Fig. 1). More than 43% of the weevils were killed in the "Rice quality" treatment and more than 80% in the "Polishing process" treatment. The biological treatments led, respectively, for *A. calandrae* ("Aniso") and *L. distinguendus* ("Lario") to a decrease of 79% and 98%. *Lariophagus distinguendus* was more efficient than *A. calandrae* in all combined and biological treatments. The optimal control was achieved with *L. distinguendus* only. In the combined treatments with *L. distinguendus*, the polishing process hampered the effectiveness of the parasitoids. However, in the combined treatments with *A. calandrae*, the polishing process improved the control of the weevils by the parasitoids but the effect on rice quality had no significant influence ("Aniso + Rice quality").

3.2. Weevil weight

Emerging adult weevils were heavier (2.06 mg) in the "Control" than in any other treatments (1.27–1.66 mg) ($F = 7.74$; df = 8/33; $P < 0.0001$) (Fig. 2). Weevils of the "Aniso + Polishing" treatments were lighter than those of the "Aniso" treatments which constituted the only significant difference among biological, mechanical and combined treatments.

3.3. Parasitoid progeny density

Parasitoid progeny were most abundant in the biological treatments with an average of 54 *A. calandrae* ("Aniso") and 41 *L. distinguendus* ("Lario") per replicate ($F = 49.08$; df = 5/61;

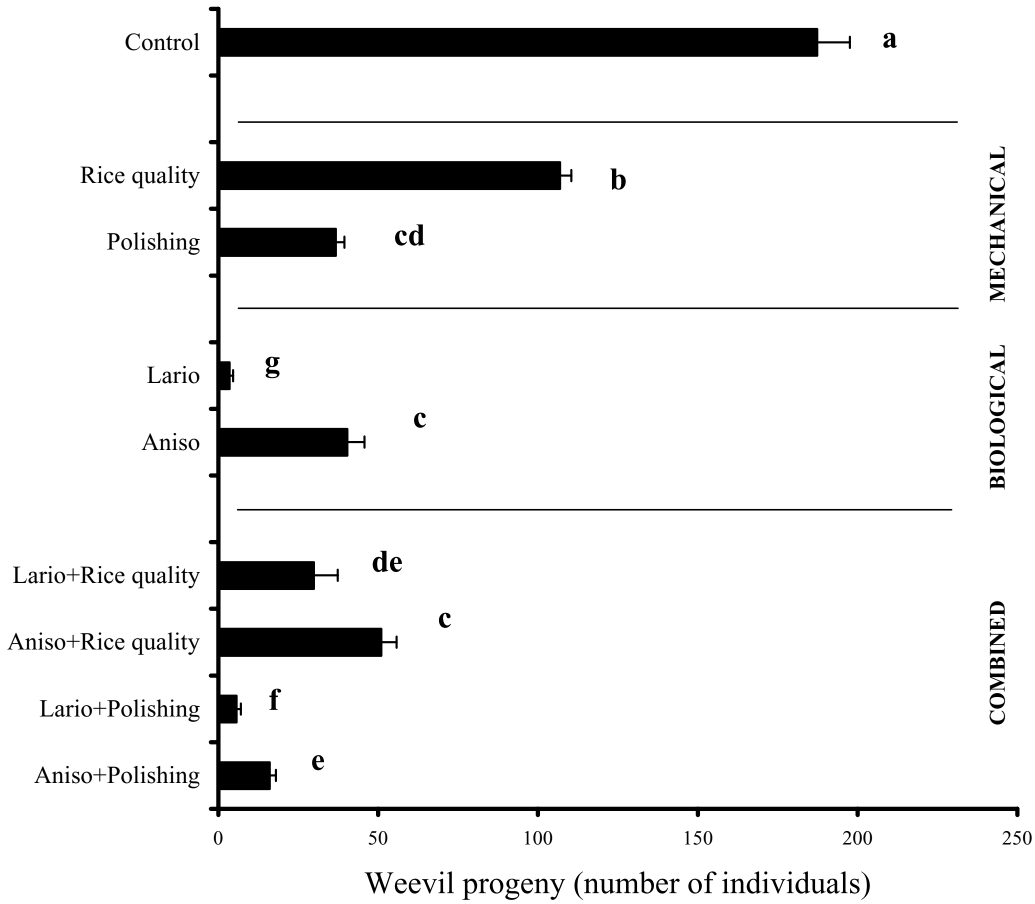

Fig. 1. Total number of *S. oryzae* progeny collected during 9 weeks from control, mechanical, biological and combined treatments. Different letters indicate a significant difference between treatments (Tukey, $P<0.05$). For explanations of treatments see Table 1.

$P<0.0001$) (Fig. 3). All the combined treatments (mechanical and subsequent biological) resulted in significantly less parasitoid progeny. *Lariophagus* progeny in the "Lario + Rice quality" and in the "Lario + Polishing" treatments were, respectively, reduced by 54% and 92%. *Anisopteromalus* progeny were more affected with a diminution of 94% in the "Aniso + Rice quality" and a diminution of 99% in the "Aniso + Polishing".

3.4. Parasitoid sex ratio

The proportion of male *A. calandrae* was lower in the biological treatment ("Aniso") than in the combined ones ("Aniso + Rice quality" and "Aniso + Polishing") ($F = 4.14$; df = 5/47; $P<0.0034$) (Fig. 4). By contrast, the sex ratio (proportion of males) of the parasitoid progeny was similar among all treatments using *L. distinguendus*. The total number of emerging parasitoids used to calculate the sex ratio was much higher in biological treatments than in

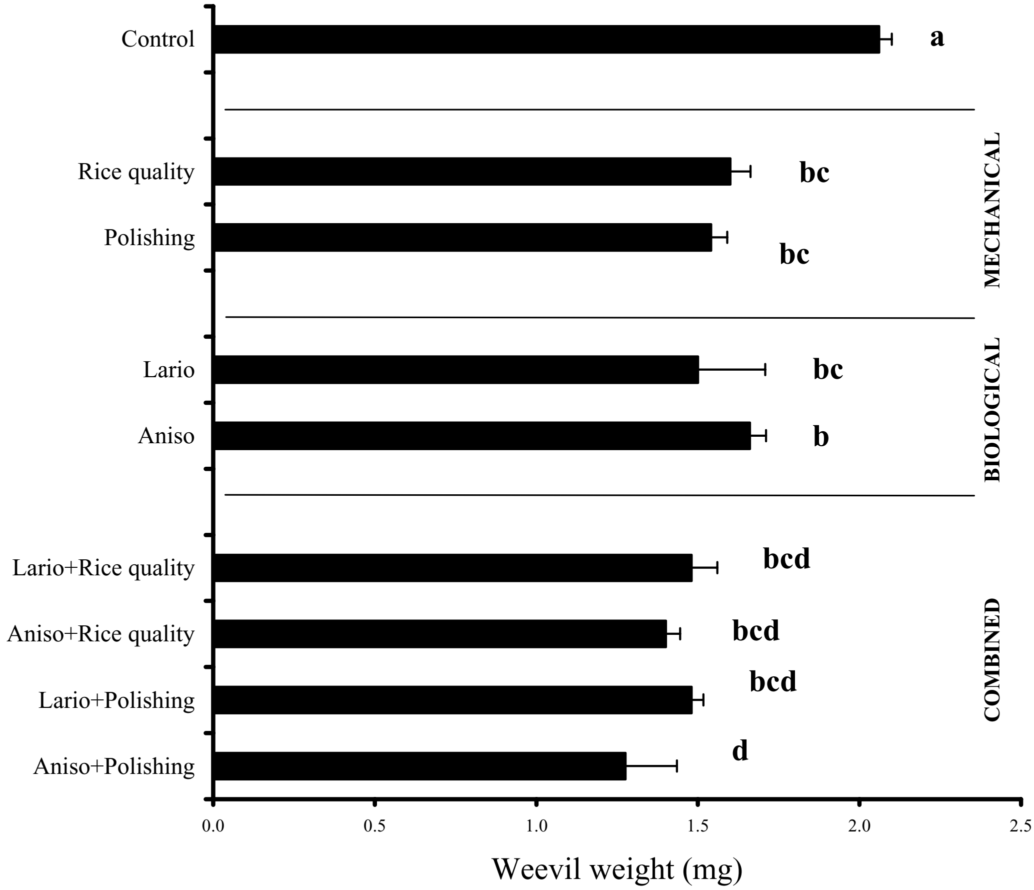

Fig. 2. Individual weight of *S. oryzae* progeny collected during 9 weeks from control, mechanical, biological and combined treatments. Different letters indicate a significant difference between treatments (Tukey, $P<0.05$). For explanations of treatments see Table 1.

combined treatments (see Fig. 3). Finally, both parasitoid species had similar sex ratios in the absence of polishing.

3.5. Proportion of alive, parasitized and dead hosts

The proportions of weevils alive, parasitized or killed by host-feeding or aborted parasitism differed greatly among treatments ($G_{10} = 1774.1$; $P<0.0001$) (Fig. 5). In the biological treatments, *L. distinguendus* successfully parasitized 22% and caused an additional 76% mortality of the weevils, while *A. calandrae* parasitized 29% and caused an additional 50% mortality. The combined use of mechanical and biological treatments always reduced the proportion of successful parasitism and increased the proportion of surviving weevils from the levels obtained by biological control alone. However, this diminution of parasitism was much greater with

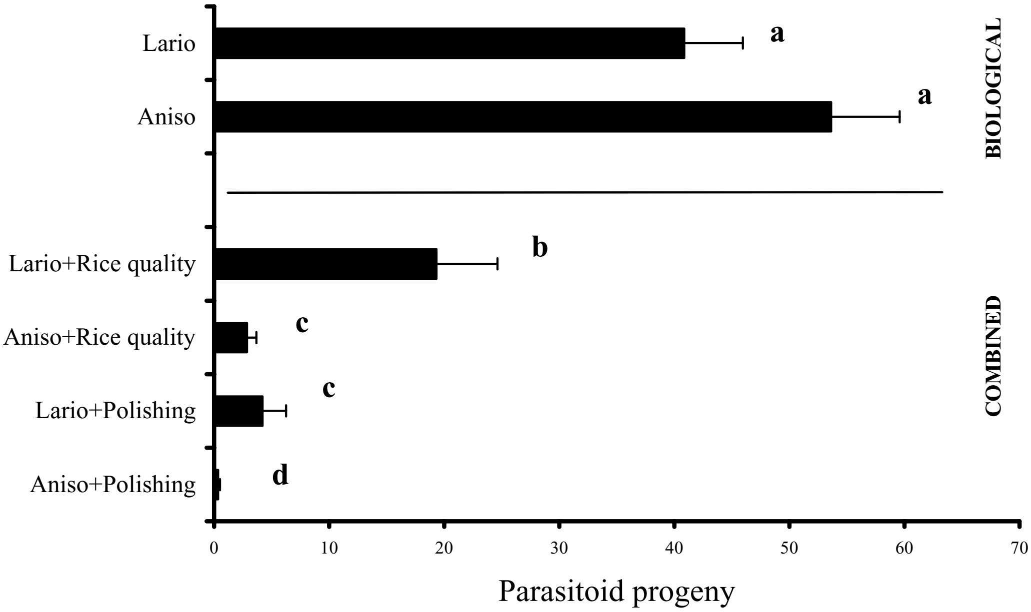

Fig. 3. Total number of parasitoid progeny collected during 9 weeks from biological and combined treatments. Different letters indicate a significant difference between treatments (Tukey, $P<0.05$). For explanations of treatments see Table 1.

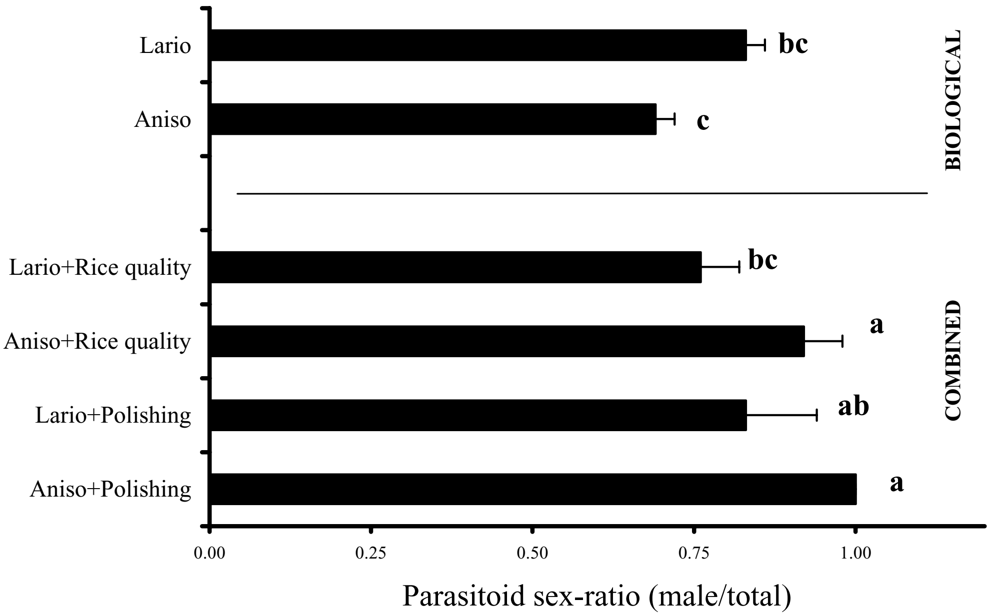

Fig. 4. Sex ratio (males/total) of parasitoid progeny collected during 9 weeks from biological and combined treatments. Different letters indicate a significant difference between treatments (Tukey, $P<0.05$). For explanations of treatments see Table 1.

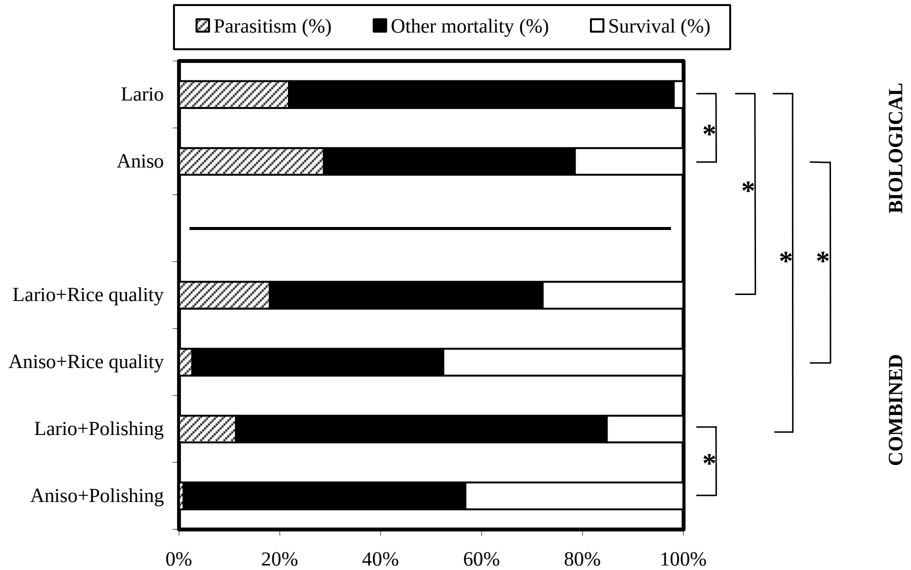

Fig. 5. Proportion of surviving, successfully parasitized and dead (but not parasitized) *S. oryzae,* collected during 9 weeks from biological and combined treatments. An asterisk indicates a significant difference between the two treatments (*G*-test, $P<0.05$). For explanations of treatments see Table 1.

A. calandrae than with *L. distinguendus*. In the combined treatments, as in the biological ones, the proportion of weevils killed was higher with *L. distinguendus* than with *A. calandrae*.

4. Discussion

In our study, all the methods tested for control of the rice weevil had a significant impact on the number of weevil progeny and on weevil adult weight. In the mechanical treatments, both the polishing process itself, and the effect of reduced rice quality, had a significant impact on weevil density, confirming previous results (McGaughey, 1974; Yamanouchi and Takano, 1980; Ryoo and Cho, 1992; Haryadi and Fleurat-Lessard, 1994; Lucas and Riudavets, 2000). It may be possible to alter the conditions of the polishing process (polisher type, polishing intensity, etc.), in order to further reduce pest populations. However, biological control had a greater effect on the weevils (except comparing "Polishing" and "Aniso" treatments) than mechanical control or a combination of the two. Both parasitoid species were efficient but the greater control was achieved by *L. distinguendus*, which killed 98% of the weevils. The superiority of this species over *A. calandrae* was confirmed in the combined treatments. The joint use of parasitoids and the mechanical process was effective in reducing the pest population. Therefore, as polishing is required in the commercial production of white rice, the use of pteromalid parasitoids, especially *L. distinguendus,* before the polishing could improve weevil control.

All treatments resulted in reduced weevil weight compared to the control. This could be due, in the mechanical treatments, to the poor quality of the white rice for development of the weevil

(McGaughey, 1974; Ryoo and Cho, 1992; Haryadi and Fleurat-Lessard, 1994; Riudavets and Lucas, 2000; Lucas and Riudavets, 2000), and, in the biological treatments, to a selection by the parasitoids of larger weevil larvae, or infested rice kernels of larger size, which may produce larger larvae and pupae (Steffan, 1963). This reduction of weight should increase any impact on the weevil population, as lower weight is normally correlated with reduced fecundity (Smith et al., 1995; Honek, 1993). It means that, for example, the parasitism of *L. distinguendus* generated 98% weevil mortality and 2% of lighter weevils with a presumably reduced fecundity.

Both parasitoid species were affected by polishing the rice before and after infestation. This could have been a direct effect, polished rice disrupting the capacity of parasitoids to detect, reach or parasitize their prey. For example, Brower et al. (1996) demonstrated that *A. calandrae* had a lower attack rate on hosts in rough rice than in brown rice. This loss of efficacy could also result from the indirect effect of the polishing process reducing weevil density and consequently, the level of parasitism or by delaying weevil development (Riudavets and Lucas, 2000). It was shown with *A. calandrae* that grain type affected host size and host suitability for parasitism (Smith et al., 1995). Changes to the weevil host caused by the processing of the rice adversely affected *A. calandrae* more than *L. distinguendus*. This second species appears therefore to be more useful against the rice weevil in white rice.

The sex ratio was constant in *L. distinguendus* but the proportion of males increased in the combined treatments with *A. calandrae*. The females of both species are known to lay more female eggs on large larvae and more male eggs on small larvae (van den Assem, 1971; van den Assem et al., 1984; Yoo and Ryoo, 1989; Smith et al., 1995). The weight of the adult weevils was decreased significantly in combined treatments with *A. calandrae*. Moreover, if the parasitism had occurred during the first days of contact between parasitoids and weevils, the weevil larvae would have been relatively young (third stage). Yoo and Ryoo (1989) demonstrated that *L. distinguendus* lays a majority of male eggs on younger larvae which may explain the high proportion of males in all treatments. Furthermore, the combined treatments were performed with white rice which is known to delay the development of the rice weevil (Lucas and Riudavets, 2000). The presence of younger (and smaller) hosts in the combined treatments may have influenced the sex ratio, as shown in parasitism of the bruchid *Zabrotes subfasciatus* (Boheman) (Kistler, 1985).

Finally, with *A. calandrae* the percentage of parasitism was greater in the biological treatments than with *L. distinguendus* despite a greater effect of the latter species. It means that in an inundative biological control program *L. distinguendus* should be more efficient, but in an inoculative program *A. calandrae* should have a higher impact. With both species, the greater effect on the weevils was achieved by non-parasitism-mortality which could occur by host-feeding of adult parasitoids or from aborted parasitism. Generally, both species lay only one egg per rice kernel, but more than one could be laid at low host densities (Gonen and Kugler, 1970; van den Assem, 1971). The non-parasitism-mortality was relatively constant among treatments for each parasitoid species. Parasitoid action was recorded in all treatments but mechanical treatments reduced successful parasitization of weevils. The weevil density (number of available hosts) could in part explain this phenomenon, as the percentage of parasitism decreases with host availability (Control > Rice quality > Polishing).

In commercial rice storage, the use of both mechanical and biological control methods may be limited by many factors. Firstly, increasing the intensity of the polishing process would enhance the impact on pest populations but would also increase costs (Lucas and Riudavets, 2000).

Secondly, rearing and releasing high populations of parasitoids in vertical silos may be very difficult and costly, and the efficacy of the parasitoids in such a different situation may be reduced. Thirdly, there are many pest species associated with rice and not all may be susceptible to attack by the parasitoids. Furthermore, the presence of the parasitoids in parts of the mill may not be acceptable in the retail trade. However, since the present control procedure of methyl bromide fumigation has to be replaced in developed countries by 2005, both mechanical and biological control techniques may have some potential.

In conclusion, biological control by the parasitoids was very efficient in brown rice, confirming the potential of both *A. calandrae* (Smith, 1993; Brower et al., 1996 and ref. therein) and *L. distinguendus* (Cho, 1989; Hong and Ryoo, 1991; Ryoo et al., 1991). By contrast, in white rice, the survival of both parasitoids was severely reduced. Since in Europe brown rice is the product stored and the polishing process occurs only before commercial packaging, pest control during storage may be improved by *L. distinguendus* and/or *A. calandrae*. Furthermore, both species attack an array of internal-feeding grain pests and thus may contribute to the control of the weevils *S. granarius* (L.) and *S. zeamais* Motschulsky, the lesser grain borer *Rhyzopertha dominica* (F.) and the Angoumois grain moth *Sitotroga cerealella* Olivier (Brower et al., 1996).

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