J. Appl. Entomol.

ORIGINAL CONTRIBUTION

Japonica cultivars' susceptibility to the rice water weevil *Lissorhoptrus oryzophilus* (Coleoptera: Curculionoidea: Brachyceridae)

D. Lupi[1], M. L. Giudici[2], C. Cenghialta[2], A. Rocco[1], C. Jucker[1] & M. Colombo[1]

1 Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milano, Italy
2 National Rice Institute (ENR), Rice Research Centre, Castello d'Agogna (PV), Italy

Keywords
alien species, damage, feeding preference, hosts, laboratory assay, *Oryza sativa*

Correspondence
Daniela Lupi (corresponding author), Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Celoria 2. 20133 Milano, Italy.
E-mail: daniela.lupi@unimi.it

Received: March 23, 2012; accepted: June 26, 2012.

doi: 10.1111/j.1439-0418.2012.01750.x

Abstract

Italy is the largest rice-producing country in the European Union. In Italy, only japonica cultivars are listed in the Italian National Register. Almost all of the rice production ~~in Italy~~ is concentrated in the Po Valley, where the rice water weevil *Lissorhoptrus oryzophilus* Kuschel was first detected and settled. This study investigated the performance of this pest in terms of feeding, reproduction and plant injury on 10 rice cultivars chosen among the most widely grown in Italy. No-choice experiments were conducted to evaluate the plant susceptibility to larval attack and to find out how cultivars can influence the adult leaf area consumption. The results gave evidence of different types of attack depending on the density of the insect (0.6 adults/plant vs. 0.9 adults/plant), the cultivar type and climatic conditions. Different cultivars with the same level of infestation gave different results in terms of productivity. Production was significantly affected by the larval presence in ~~four~~ of the 10 cultivars tested. A different population structure reflected a different damage severity. Statistically different values for total adult leaf area consumption were found according to adult female age and to the cultivar.

Introduction

The rice water weevil (RWW) *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionoidea: Brachyceridae) (*sensu* Bouchard et al. 2011) was detected for the first time in Europe, specifically in Italy, in 2004 (Caldara et al. 2004). Since then, the insect has rapidly spread in northern Italy, where rice cultivation is most widespread and the connected rice fields form corridors that facilitate its expansion (Lupi et al. 2010; Wang et al. 2011). Native to the United States (Webb 1914; Tindall and Stout 2003), the RWW is considered as one of the most destructive pests of this crop in all the countries in which it is present.

The RWW is a polyphagous species. Its host preferences include many monocotyledonous and some dicotyledonous species (Lupi et al. 2009a). Poaceae and Cyperaceae are the preferred hosts, and the insect can develop on both rice and many wild grasses (Tindall and Stout 2003; Chen et al. 2005).

Rice water weevil larvae cause significant damage to the rice crop. They initially feed within the rice sheath, and then they migrate downward to feed on and within the roots (Zou et al. 2004a). This causes shearing of the roots, which leads to stunted plant growth, delayed maturation and a loss in productivity. Furthermore, the plant's anchoring to the soil is weakened to the extent that wind and water turbulence may uproot it and leave it floating in the paddy field (Wu and Wilson 1997). The adults cause minimal economic damage; they feed on the leaves, scraping the epidermis and leaving longitudinal scars parallel to leaf veins, but do not typically reduce the yield of the plant. However, they are a good indicator of subsequent larval infestation and damage (Way and Wallace 1992).

UNCORRECTED PROOF

© 2012 Blackwell Verlag, GmbH

UNCORRECTED PROOF

The RWW has separate sexes only in its area of origin in North America; in California, Asia, and Europe, it is a parthenogenetic species (Saito et al. 2005; Lupi et al. 2007a, 2010). The insect's success in many different countries is attributed to its ability to undergo an adult reproductive diapause in cold winters (Jiang et al. 2004). Thus, it overwinters as an adult in or near rice fields, and after emergence in the spring begins feeding first on wild plants and then on rice. After a small period necessary for the insect to regenerate its flight muscles and develop ovaries, it oviposits in submerged rice leaves. Multiyear field experimental data have established that in Italy, the RWW completes only one generation per year. A combination of factors, such as photoperiod and temperature, is not adequate to allow the development of a second generation in northern Italy (Lupi et al. 2007b). Overwintered adults can be observed on vegetation from April until the end of June. Oviposition generally begins in May, and larvae are found from the end of May until July. Adults generally emerge in late June–July and overwinter in the litter or in the first few centimetres of the soil (Lupi et al. 2007a, 2009b, 2010).

Water is the prerequisite for oviposition. If the rice is not submerged, the plant is not suitable. In fact, according to many authors, delayed flooding negatively influences and postpones the feeding and oviposition of the insect (Rice et al. 1999; Lupi et al. 2007a). Other factors contributing to the tolerance to insect feeding are the rice cultivar and the plant age (Smith and Robinson 1982; Stout et al. 2002). Various studies have been done on *indica* cultivars in North America to identify rice lines exhibiting tolerance or resistance to the RWW (Smith and Robinson 1982, 1984; N'Guessan and Quisenberry 1994; N'Guessan et al. 1994a,b,c; Stout et al. 2001; Zou et al. 2004b). Despite these considerable efforts, however, no rice lines possessing high levels of resistance to the RWW have been identified, and very little progress has been made in integrating plant resistance into management programs for this insect (Way 1990; Stout et al. 2001).

In Italy, only *japonica* cultivars are used as the climatic conditions are generally inadequate for authentic *indica* ones. The cultivars include the traditional Italian grain type (generally long and broad, with a soft cooking grain) and the so-called *indica* type (long and slender grain), which is actually another *japonica* cultivar (Angelini et al. 2008). The former covers nearly 70% of the rice cultivation area, whereas the latter covers 30%. Studies conducted in Italy have demonstrated that the rice cultivar cycle (long and short periods) can influence the RWW attack. Long-period cultivars with their early sowing were more susceptible to attack by the insect because of the synchronization of the plant and the insect cycle (Lupi et al. 2008, 2009b).

As the susceptibilities of the various *japonica* cultivars have not been fully defined yet, the aim of this study was to investigate the performance of the RWW in terms of feeding and reproduction on some Italian rice cultivars. Italy is the largest rice-producing country in the European Union, and almost all of its rice production is concentrated in the Po Valley, where the RWW has settled. Nearly 200 cultivars are listed in the Italian National Register, but the most cultivated varieties number about 30. The notable differences among Italian cultivars are grain type and productivity, which are very important to rice marketability. They have different quality characteristics that include parameters such as the crude amount of starch, proteins, fibres and lipids; the ratio between the two starch components (amylose/amylopectin); size, shape and uniformity; and other components (Cirillo et al. 2009). Because the susceptibility of plants to insects is the result of many other factors, such as plant palatability, the growth of the plant and root system as a function of genetic factors or agronomic aspects, and the possible presence of antifeedants (Zou et al. 2004b; Stout et al. 2009; Hamm et al. 2010; Cosme et al. 2011), a first screening on some of the most widespread cultivars used in Italy is necessary to evaluate susceptibility to feeding and damage from RWW infestation.

Materials and Methods

Two different no-choice experiments were conducted in 2008 and 2009. Experiment 1 was conducted inside tanks positioned in open air at the Rice Research Centre at Castello d'Agogna in Pavia province (45° 14.88N; 8°41.97E) to evaluate the plant susceptibility to larval attack. Experiment 2 was conducted inside a rearing chamber in a laboratory to find out how cultivars can influence the adult leaf area consumption. In this experiment, leaves from plants reared in open air at the Faculty of Agriculture of Milan (45°28.53N; 9° 13.60E) were used.

Rice water weevil collection

To maximize the subject's uniformity, parthenogenetic adults were manually collected from rice plants in Bereguardo, Pavia province (45°15.26N; 9°01.27E), on the same day. The weevils were maintained until

© 2012 Blackwell Verlag, GmbH

use in petri dishes with a thin layer of water and rice leaves. The time of collection depended on the experiment and the year.

Experiment 1

Some days before the experiment, some females were dissected to find the presence of chorionated eggs stored in egg calyxes. After egg detection, the necessary number of specimens was collected in the field.

Experiment 2

In 2008, after the emergence of the RWWs from overwintering, observations were carried out on females captured in the field just after the detection of chorionated eggs stored in egg calyxes and on new emerging adults. In 2009, observations were carried out on specimens at the beginning of their oviposition period and on females at the end of their reproductive cycle captured one month later.

After collection, the weevils were brought to the laboratory of DeFENS (Faculty of Agriculture, Milan) and preconditioned on rice leaves (*Oryza sativa* L. var. *sylvatica*) in a climatic chamber with a 14L : 10D photoperiod at 28°C.

No-choice tests

Experiment 1

Trials were carried out in 2008 and 2009. The test tanks were made of plastic and had a base of 60 × 100 cm and a height of 52 cm. The tanks were filled with medium-textured soil.

In the first and second years, 7 and 10 cultivars, respectively, were tested (table 1). All seeds were placed in petri dishes on moist filter paper and maintained in a clean room oven at 27°C for 3 days to favour germination. After germination when the radical length was about 1–2 cm, 100 seeds/cultivar were transferred into the tanks. The seedlings were placed at each corner of an 8 × 5 cm grid and covered with sterile sand. When the seedlings reached the 2–3 leaf stage, the tanks were flooded. Water management and fertilization were conducted similarly to water-seeded rice fields.

Waterproof data loggers (Hobo® ~~U22 Pro~~ Water Temp) were used to record the temperature fluctuations inside the flooded soil once every hour. The daily maximum and minimum air temperatures, humidity and precipitation were detected in both years by a permanent weather station of the regional meteorological system (ARPA) located at the Rice Research Centre.

Table 1 Cultivars tested in 2008 and 2009

Cultivar	Days from sowing to maturity	Experiment 1		Experiment 2	
		2008	2009	2008	2009
Baldo	150		*		*
Balilla	160	*	*	*	*
Centauro	140	*	*	*	*
Creso	145	*	*	*	*
Gladio	135	*	*	*	*
Libero	160	*	*	*	*
Loto	130		*		*
Nembo	135	*	*	*	*
S. Andrea	150		*		*
Volano	150	*	*	*	*

In 2008, eight tanks per cultivar were sown: four were used as non-treated controls and the other four to evaluate the effect of the RWW activity. In these, 60 adults/tank (=0.6 RWW/rice plant) were added at the plant tillering stage. Tanks were arranged in a randomized block design with four replications.

In 2009, 12 tanks per cultivar were sown: four were used as controls, four to evaluate the effect of the attack of 90 adults/tank (=0.9 RWW/rice plant) introduced on the plants at the three-leaf stage (1st adult introduction) and four to evaluate the effect of 90 adults/tank (=0.9 RWW/rice plant) introduced 2 weeks later at the plant tillering stage (2nd adult introduction). After their introduction, the adults were left in the tank until their death. Tanks were arranged in a randomized block design with four replications.

To avoid RWW escape, undesired infestation, light shading and, consequently, spindly plants, each tank was covered with a 80-cm-high box-shaped cage with a wood frame and draped with a fine mesh (17 g/m^2). After 40 days, all the box-shaped cages were removed to allow plant growth, flowering and grain ripening.

To determine the larval density, samples from both years were taken at 25 and 32 days after adult introduction. Each time, four cores (1 plant/core) were collected in each tank with a metal sampler (10 cm in diameter and 10 cm deep). The samples were individually placed in a tub and soaked in water to force the larvae to float to the surface. The larvae were then counted, collected and preserved in 70% alcohol. To detect the structure of the larval population in different treatments, the larval head capsule was measured in the laboratory according to the scale suggested by Cave and Smith (1983). To relate the RWW development to the year temperatures, the averaging method was applied to calculate the number of degree days to

UNCORRECTED PROOF

© 2012 Blackwell Verlag, GmbH

UNCORRECTED PROOF

the RWW adults and larvae according to the following formula (Herms 2004):

$$\mathrm{DD} = \sum_{1}^{n}\left[\frac{(T_{\max} + T_{\min})}{2} - t_{\mathrm{r}}\right]$$

where DD, degree days; n, number of days of observation; $T_{\max}$, maximum daily temperature; $T_{\min}$, minimum daily temperature; and t_{r}, minimum temperature threshold for insect development. The temperature thresholds chosen were 18 and 10°C, respectively, for the *L. oryzophilus* adult and larval development, according to Zou et al. 2004c. As the temperature prior to adult introduction in tanks can influence their development and oviposition, DD were calculated before (30 days) and after (25 days) adult introduction in tanks. For pre-imaginal development, DD sum was calculated from the day after adult introduction in tanks to larval sampling (25 and 32 days).

The damage was evaluated on the following productive parameters estimated after the harvest: production (grain yield calculated for the whole plot and expressed at 14% RH), milling yield, culm length (measured from the soil surface to the neck node), panicle length, number of culms/m^2 (calculated for the whole plot), dry matter (calculated for the whole plot considering all the plants, excluding their roots), the weight of 1.000 seeds and number of spikelets per panicle.

Experiment 2

In 2008 and 2009, trials were carried out on the same cultivars used in experiment 1. No-choice tests were executed in climatic chambers; as according to the previous tests, these conditions are favourable for RWW trophic activity (Lupi et al. 2009a).

In both years, petri dishes were prepared with a sheet of paper towel at the bottom. Adults were individually placed in each petri dish. A piece of rice leaf of about 5 cm in length was added to each petri plate at the beginning of the trial. Water was added to create a film, necessary for both insect survival and leaf preservation. Leaves were removed after 24 h. Trials were continued until 40 observations/cultivar were obtained. The leaf pieces removed from the petri dishes were prepared to measure the total daily area consumption per adult according to the procedure of Lupi and Jucker (2004). Daily food consumption was calculated as the total area per day as the leaf thickness was assumed to be the same for the different leaves.

Leaves were obtained from the test tanks prepared and positioned in open air at the Faculty of Agriculture of Milan. The tanks were made of plastic, with a base of 45 × 100 cm and a height of 49 cm. Each tank was filled with medium-textured soil, cultivated with one cultivar and covered with a 80-cm-high box-shaped cage whose base fit the tank base to preclude plant infestation by insects. Rice was seeded and cultivated according to the procedure in experiment 1. Leaves were cut at the plant tillering stage. To obtain leaves from plant at the same stage, the tanks were seeded at different periods.

In 2008, trials were repeated on females that had emerged from winter recovery, found on rice and captured in the field at the end of May (young ovipositing females) and on just-emerged females captured in the field at the beginning of July (newly emerged females). In 2009, trials were performed on young ovipositing females, as in 2008, and on females at the end of their reproductive career (old females). Trophic activity was evaluated in terms of the percentage of specimens that fed in the period considered and of the total daily leaf area consumption.

Statistical analysis

All statistical analyses were performed using the PASW Statistics 18.0® software for Windows. Differences among trials in the 2 years and among treatments in the same year were analysed using the independent samples *t* test with replications. Differences among cultivars were analysed by general linear model univariate analysis of variance with replications and means separated by the Duncan multiple rate test ($P < 0.05$). The correlation between loss of production and adult leaf feeding was analysed with the Spearman rank order correlation coefficient r_s ($P < 0.05$).

Results

Experiment 1

The mean density of larvae per core across all the cultivars in the tests was significantly different in the 2 years (2008 vs. 2009, $t = 26.07$, significantly different at $P < 0.001$). The mean density was 0.30 ± 0.04 larvae/core (n = 224 cores) in 2008 and 4.09 ± 0.13 larvae/core (n = 640 cores) in 2009. The analysis of the RWW population age structure allowed the detection of differences in the 2 years: 25 days after adult introduction in 2008, 66% of the samples were equally divided between the first and second ages, and only 10% was in the 4th instar, whereas in 2009 (1st introduction), 73% was already in the 4th instar

© 2012 Blackwell Verlag, GmbH

(fig. 1). A comparison of the climatic data showed that 2008 was significantly more rainy than 2009 (fig. 2), with mean air temperatures significantly lower in the period before adult introduction into the tanks (fig. 3). The water temperature detected from the introduction of the ovipositing females until the emergence of the new adults had a mean value of 24.04 ± 3.11°C and a range from 16.82 to 31.56°C in 2008, compared with a mean value of 26.44 ± 1.33°C and a range from 23.64 to 28.96°C in 2009. In addition, the adult degree-day accumulation differed in the 2 years, with an accumulation of 30.6 DD in 2008 and 92 DD in 2009 prior to adult introduction. In the period after adult introduction, the temperature fluctuation also differed in the 2 years. The adult degree-day accumulation was 115.5 DD in 2008 and 91.6 DD in 2009. The larval degree-day accumulation in the first year was 384.4 DD and 483 DD after 25 and 32 days from adult introduction, respectively. The larval degree-day accumulation in the second year was equal in the two treatments, with an accumulation of 388.2 DD and 500.3 DD after 25 and 32 days from the 1st adult introduction, respectively, and of 420.5 DD and 534.4 after 25 and 32 days in the larvae from the 2nd adult introduction, respectively.

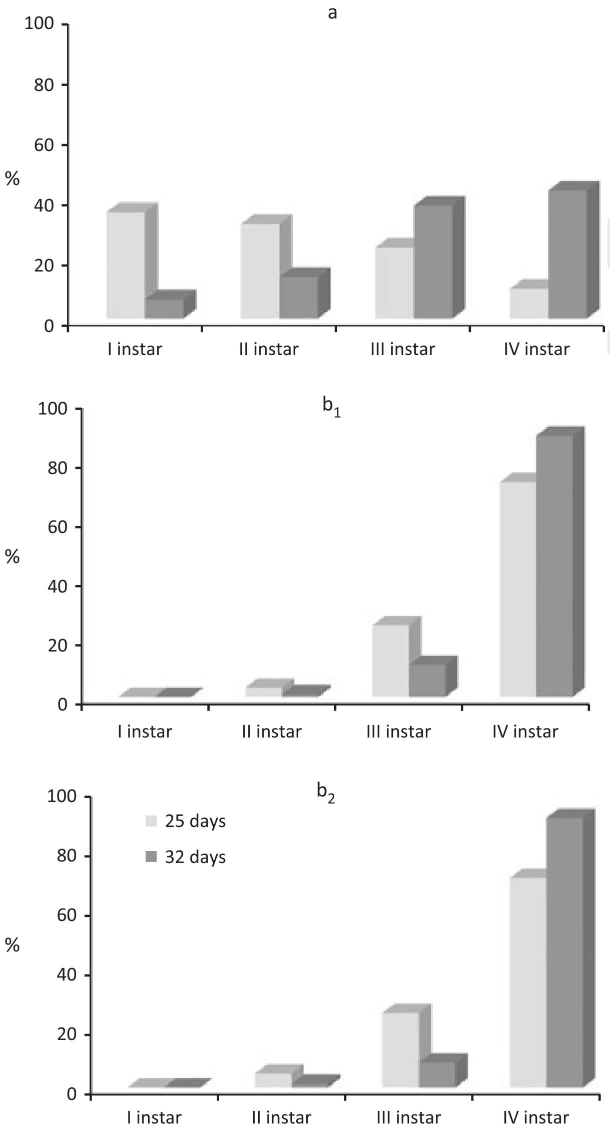


Fig. 1 Rice water weevil (RWW) larval population age structure in the 2 years (a = 2008 and b = 2009) and in the different treatments in 2009 (b_1 = 1st adult introduction and b_2 = 2nd adult introduction).

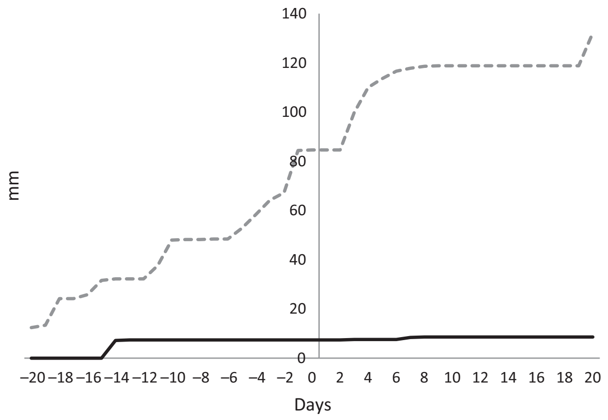


Fig. 2 Cumulative pluviometric data (mm of rain) for 40 days before and after adult introduction in 2008 (dotted line) and 2009 (unbroken line).

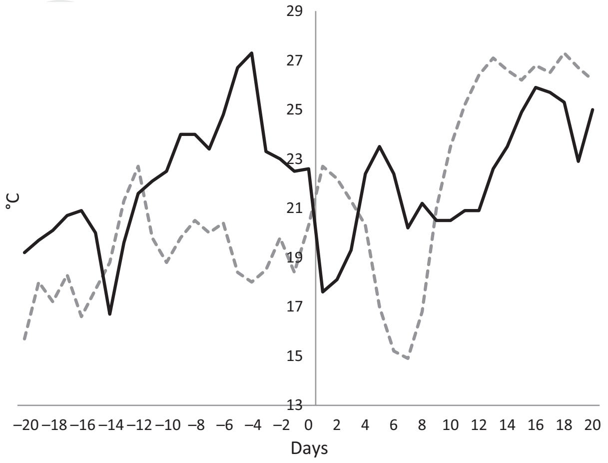


Fig. 3 Daily mean air temperature in °C for 40 days before and after adult introduction 2008 (dotted line) and 2009 (unbroken line).

The trials in 2008 showed no statistical differences in the larval presence among cultivars (I sampling: $F = 1.015$, d.f. = 6, P = 0.417; II sampling: $F = 0.739$, d.f. = 9, P = 3.619). No statistical differences were found between non-infested and the infested plants in terms of productive parameters. No cultivar

© 2012 Blackwell Verlag, GmbH

UNCORRECTED PROOF

showed evidence of alteration of productive parameters caused by RWW larval load (table 2).

In 2009, the mean density was significantly different between the treatments (1st vs. 2nd adult introduction, $t = 8.36$, significantly different at $P < 0.001$). In the 1st adult introduction, the mean density was 5.16 ± 0.19 larvae/core ($n = 320$), while in the 2nd adult introduction, it was 3.02 ± 0.17 larvae/core ($n = 320$) (table 3). The distribution of instars was similar in the two treatments.

Over all cultivars, production was significantly affected by the larval presence in 2009 (1st adult introduction: $F = 6.597$, d.f. = 9, $P < 0.001$; 2nd adult introduction: $F = 3.402$, d.f. = 9, $P < 0.01$) (table 2) in four of the 10 cultivars tested (fig. 4). Major larval load was associated with all these four cultivar in 1st adult introduction and with only three in 2nd adult introduction (table 3). Other parameters, such as grain size and weight, and plant structure (number of culms per plant, etc.), were not influenced by larval presence.

Experiment 2

Because of some problems in the germination of the cultivars Balilla and Creso in the first trial in 2008, it was not possible to synchronize the presence of females and tillering plants for all the cultivars. This trial was therefore performed only on the other five cultivars. A second trial had all seven cultivars.

The trials in 2008 and 2009 allowed detection of differences among cultivars and treatments for the percentage of adults that fed and the leaf area consumed. In 2008, a lower percentage of young ovipositing females compared with newly emerged ones fed on leaves (fig. 5). In the same year, the analysis of variance of the total area consumption for young ovipositing females did not show significant differences ($F = 1.953$, d.f. = 4, $P = 0.106$). The same analysis on the newly emerging females yielded significant differences ($F = 3.351$, d.f. = 6, $P < 0.01$). Table 4 shows the differences among the cultivars according to the treatment. Statistically different values for total leaf area consumption ($F = 3.676$, d.f. = 9, $P < 0.001$) were found between young ovipositing females and newly emerged ones. Newly emerged females consumed more leaf area than young ovipositing females.

In 2009, a higher percentage of younger females fed than in the older females (fig. 6), except for the cultivars Balilla and Volano. Also, total area consumption by young was significantly different among cultivars ($F = 10.713$, d.f. = 9, $P < 0.001$), but was not for old females ($F = 1.087$, d.f. = 9, $P = 0.386$) (table 4). Statistically different values for total leaf area consumption ($F = 6.751$, d.f. = 18, $P < 0.001$) were found between young and old females. Young females consumed more leaf area than old females.

The Spearman rank order correlation coefficient did not indicate a statistically significant linear relationship between loss of production and adult leaf feeding [r_s (20) = 0.645, $P > 0.05$].

Discussion

The results of experiment 1 indicate that it is not only the adult density that is important in determining damage but also the population structure and its synchronization with plant. Developmental stage is a critical determinant of the ability of most arthropods to cause damage. A different population structure can be reflected in a different damage severity. In experiment 1, conducted in the first year, the lower air temperature before adult introduction probably delayed oviposition; in fact, more than half of the

Table 2 Mean ± SE of rice cultivar production (expressed in t/ha) in different treatments in 2008 and 2009 and ANOVA results

	Production 2008 (t/ha)		Production 2009 (t/ha)		
	1st adult introduction	Not infested	1st adult introduction	2nd adult introduction	Not infested
Baldo	–	–	9.09 ± 0.54b	9.74 ± 0.56ab	10.99 ± 1.01a
Balilla	9.52 ± 0.55a	9.86 ± 0.68a	10.05 ± 0.59a	9.69 ± 0.88a	11.6 ± 0.57a
Centauro	10.27 ± 1.25a	9.88 ± 1.03a	10.08 ± 0.77a	9.68 ± 0.63a	10.65 ± 0.62a
Creso	7.74 ± 0.88a	7.63 ± 0.56a	7.65 ± 0.85a	8.22 ± 0.68a	10.03 ± 0.69a
Gladio	6.81 ± 0.24a	6.70 ± 0.67a	6.16 ± 0.67b	7.54 ± 0.73a	7.99 ± 0.65a
Libero	8.32 ± 0.78a	8.05 ± 1.46a	8.54 ± 0.72a	7.95 ± 0.81a	8.98 ± 0.76a
Loto	–	–	7.32 ± 0.64a	7.58 ± 0.76a	9.55 ± 0.65a
Nembo	7.36 ± 0.44a	6.80 ± 0.61a	7.14 ± 0.59ab	6.55 ± 0.77b	8.53 ± 0.65a
S. Andrea	–	–	12.46 ± 0.87a	11.74 ± 0.78a	13.16 ± 0.74a
Volano	9.71 ± 0.77a	9.00 ± 1.09a	8.82 ± 0.97b	9.08 ± 0.67b	11.63 ± 0.89a

Means followed by different letters in the same column are significantly different at $P < 0.05$ by one-way ANOVA and Duncan multiple rate test.

© 2012 Blackwell Verlag, GmbH

UNCORRECTED

Table 3 Mean ± SE of larval density in 2009 (expressed as larvae/core) and ANOVA results

	Sampling	Baldo	Balilla	Centauro	Creso	Gladio	Libero	Loto	Nembo	S. Andrea	Volano
Adult I introduction	25 days	7.63 ± 1.13c	3.81 ± 0.67a	4.75 ± 0.74ab	5.13 ± 0.70abc	5.00 ± 0.91abc	4.75 ± 0.69ab	5.75 ± 0.71abc	5.19 ± 0.63abc	7.19 ± 1.45bc	6.56 ± 0.81bc
	32 days	5.81 ± 0.56b	5.44 ± 0.86ab	3.13 ± 0.72a	4.13 ± 0.81ab	5.81 ± 0.90b	4.75 ± 0.97ab	4.63 ± 0.81ab	5.06 ± 0.74ab	5.13 ± 0.79ab	3.63 ± 0.73ab
Adult II introduction	25 days	4.00 ± 0.65ab	1.75 ± 0.33a	1.81 ± 0.41a	3.56 ± 0.86ab	3.13 ± 0.78ab	4.31 ± 0.87b	3.75 ± 0.83ab	4.00 ± 0.49ab	2.63 ± 0.73ab	3.88 ± 1.25ab
	32 days	1.38 ± 0.22a	2.44 ± 0.53a	3.44 ± 0.58a	1.94 ± 0.5 a	2.75 ± 0.31a	1.81 ± 0.48a	2.44 ± 0.47a	6.00 ± 1.31b	3.19 ± 0.87a	2.13 ± 0.50a

Means followed by different letters in the same row are significantly different at $P < 0.05$ by one-way ANOVA and Duncan multiple rate test.

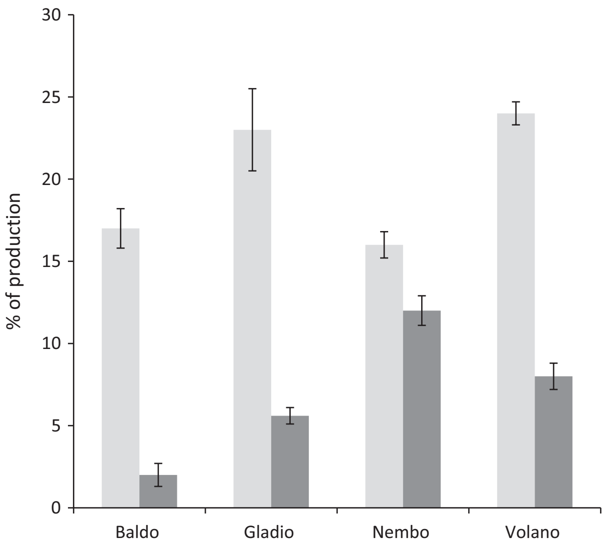


Fig. 4 Percentage reductions in production of cultivars infested with rice water weevil (RWW) when compared to non-infested plants in 2009 (light grey = 1st adult introduction and dark grey = 2nd adult introduction).

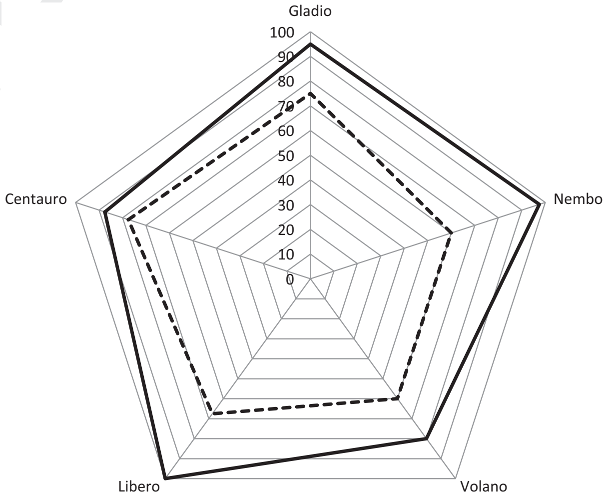


Fig. 5 Percentage of specimens with trophic activity in 2008 (dotted line – young ovipositing females; unbroken line – new emerging females).

larva specimens were still first and second instars 25 days after adult introduction, suggesting that oviposition was delayed. In the second year, the mean density was augmented to 0.9 adults/plant, and higher mean temperatures probably accelerated oviposition, with nearly all of the larvae occurring in the 3rd and 4th instars after 25 days. This means that the females oviposited just after introduction into the tanks. A second explanation can be furnished considering the relation between RWW and the root system.

© 2012 Blackwell Verlag, GmbH

Table 4 Mean ± SE of daily adult leaf consumption (expressed as mm^2 of leaf) and ANOVA results

	2008		2009	
	Young ovipositing females	New emerging females	Young ovipositing females	Old females
Baldo	–	–	9.43 ± 1.01ab	11.32 ± 2.47a
Balilla	–	24.47 ± 1.81c	10.65 ± 1.83abc	9.24 ± 3.16a
Centauro	19.23 ± 3.03b	18.68 ± 1.51ab	12.22 ± 1.29bc	6.67 ± 0.10a
Creso	–	16.84 ± 1.56a	11.52 ± 1.13abc	6.62 ± 0.01a
Gladio	18.66 ± 2.12b	23.00 ± 1.46bc	6.89 ± 1.19a	14.01 ± 3.68a
Libero	16.80 ± 1.90ab	25.52 ± 1.87c	22.18 ± 1.38e	9.80 ± 1.47a
Loto	–	–	17.65 ± 1.20d	12.65 ± 2.10a
Nembo	15.27 ± 1.72ab	18.82 ± 1.99ab	9.30 ± 1.43ab	10.65 ± 1.40a
S. Andrea	–	–	14.72 ± 2.10cd	7.31 ± 1.13a
Volano	11.08 ± 1.95a	21.03 ± 2.30abc	14.16 ± 1.98bcd	8.53 ± 1.23a

Means followed by different letters in the same column are significantly different at $P < 0.05$ by one-way ANOVA and Duncan multiple rate test.

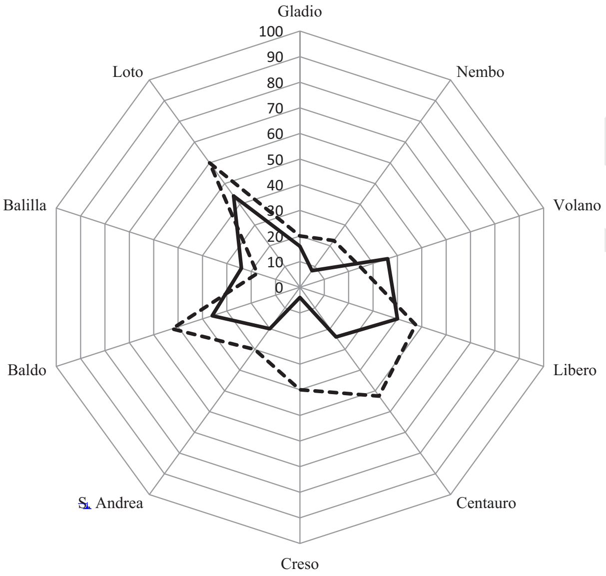


Fig. 6 Percentage of specimens with trophic activity in 2009 (dotted line – young ovipositing females; unbroken line – old females).

In 2008, the primary cool period before the introduction of the adults into field plots and the high precipitation during this time with cloudy conditions could have reduced root growth (Varade et al. 1971; Kato-Noguchi 2007). The lack of early-season root growth could have caused the survival of the RWW that hatched out of the eggs to be poor as many larvae could not find a source of food. This would have been most evident with the eggs that hatch first since at that time the roots would have been smallest. By the time the larvae occurred from the later hatching eggs, root tissue could develop and survival would have been higher. That could account for the shift in the population to 1st and 2nd instars. Root system development just after adult introduction was not investigated; 25 days later, cores did not show evident root reduction probably because the higher temperature in the following period can have stimulate root system growing. In 2009, 4.09 ± 0.13 larvae/core were present on plants infested at the three-leaf stage and resulted in considerable damage in some cultivars. In contrast, 0.30 ± 0.04 larvae/core were present on plants infested at the tillering stage and very little if any damage occurred. This resulted in major damage in some cultivars. There is a strict relationship between insect attack, weather, cultivar, plant and insect phenology. All of them play an important role and hence need to be evaluated to estimate damage. In addition, we must add that the root systems develop differently depending on the cultivar and soil moisture (Yoshida and Hasegawa 1982). Little information is available on the root system of rice Italian cultivars; therefore, the relationship between the insect and the root system needs to be investigated. Our trials were performed on flooded rice, which develops greater aerenchyma formation in comparison with dry-seeded rice. The roots of water-seeded rice are more suitable for RWW larval development because they have a bigger diameter in comparison with dry-seeded rice flooded after 30–40 days, which develops longer and finer structures with more adventitious roots (Gowda et al. 2011). This consideration is very important because in Italy, rice is water-seeded in about 70% of the rice-growing areas, with short dry periods that only allow cultural operations such as herbicide application.

Experiment 2 allowed evaluating the plant susceptibility to adult feeding. It was possible to estimate that the leaf area consumed by one RWW alone can vary as a function of the adult's age. This is noteworthy

UNCORRECTED PROOF

UNCORRECTED PROOF

because insects can consume different amounts of food at different periods of their life cycle. A specimen that has just emerged needs to prepare for overwintering. This reflects a major insect activity and has a major influence on the leaf area consumed. Similarly behaves a female that has emerged from overwintering sites and is at the beginning of its ovipositing period. A female at the end of its ovipositing career needs less food than a younger one. In addition, we found that some unknown factors influenced feeding by adults on cultivars and patterns susceptibility. Nembo, for example, is always less favoured for RWW feeding, while Creso and Libero are in the medium–low and medium–high consumption groups, respectively. Inconsistent results were detected in association with Gladio and Volano because they are sometimes among the most-appreciated cultivars and sometimes among the less-appreciated ones. Studies are required to verify whether the palatability in the laboratory is related to susceptibility in the field.

Both experiments show that the japonica rice cultivars commonly grown in Italy differ substantially in their susceptibility to infestation by RWW in terms of adult leaf area consumption and tolerance to larval infestation and feeding, expressed in terms of loss of production. This study only indicates a difference in tolerance; we maintain that none of the cultivars possess a real resistance to the weevil. Painter (1951) defined tolerance as the mechanism whereby the host plant can grow and reproduce normally or compensate for injury while supporting an insect pest population that severely damages a susceptible host. Therefore, host plant tolerance is a valuable component in the management of the RWW. However, as these experiments were executed in trials in tanks and in climatic chambers in laboratory, further study is required to verify whether the RWW behaviour is the same in the field.

Acknowledgement

The authors give a special thank to Bruno Villa, ENR technician now retired, for his help in field and to all students and collaborators who helped to realize this work. ~~I am~~ also very grateful to anonymous reviewers for helpful comments. This research was financially supported by the Lombardy Region as part of the research project 'Entomological pests in rice fields: control and biology of *Lissorhoptrus oryzophilus* and other newly introduced pests', by the University of Milan in the project 'UNIMI 5 PER MILLE: Evaluation of arthropod role in rice areas to maintain ecosystem equilibrium'.

References

Angelini R, Ferrero A, Ponti I (eds.), 2008. Il riso. Bayer Crop Science Edscript, Bologna, Italy, 1–680.

Bouchard P, Bousquet Y, Davies AE, Alonso-Zarazaga MA, Lawrence JF, Lyal CHC, Newton AF, Reid CAM, Schmitt M, Ślipiński SA, Smith ABT, 2011. Family-group names in Coleoptera (Insecta). ZooKeys 88, 1–972.

Caldara R, Diotti L, Regalin R, 2004. First record for Europe of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera, Curculionoidea, Erirhinidae). Boll. Zool. Agr. Bachic. ser. II. 36, 165–171.

Cave GL, Smith CM, 1983. Number of instars of the rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 76, 293–294.

Chen H, Chen Z, Zhou Y, 2005. Rice Water Weevil (Coleoptera: Curculionidae) in mainland China: invasion, spread and control. Crop Prot. 24, 695–702.

Cirillo A, Del Gaudio S, Di Bernardo G, Galderisi U, Cascino A, Cipollaro M, 2009. Molecular characterization of Italian rice cultivars. Eur. Food Res. Technol. 228, 875–881.

Cosme M, Stout MJ, Wurst S, 2011. Effect of arbuscolar mycorryzal fungi (*Glomus intraradices)* on the oviposition of the rice water weevil (*Lissorhoptrus oryzophilus*). Mycorrhiza 21, 651–658.

Gowda VRP, Henry A, Yamauchi A, Shashidhar HE, Serraj R, 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crop. Res. 122, 1–13.

Hamm JC, Stout MJ, Riggio RM, 2010. Herbivore- and elicitor-induced resistance in rice to the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) in the laboratory and field. J. Chem. Ecol. 36, 192–199.

Herms DA, 2004. Using degree-days and plant phenology to predict pest activity. In: IPM (Integrated Pest Management) of Midwest landscapes. Ed. by Krischik V, Davidson J, Minnesota Agricultural Experiment Station ~~Publication SB-07645, ?????~~, 49–59. 3

Jiang MX, Zhang W, Cheng J, 2004. Termination of reproductive diapause in the rice water weevil with particular reference to the effects of temperature. Appl. Entomol. Zool. 39, 683–689.

Kato-Noguchi H, 2007. Subemergence acclimation to low-temperature stress in rice roots. Plant. Prod. Sci. 10, 297–302.

Lupi D, Jucker C, 2004. Method to quantify *Cameraria ohridella* leaf damages on *Aesculus hippocastanum* using image analysis. In: Cameraria ohridella and other invasive leaf-miners in Europe, 1st International Cameraria Symposium, Prague, Czech Republic 24–27 March, 2004, 29.

Lupi D, Colombo M, Giudici ML, Villa B, Sparacino AC, Ranghino F, 2007a. Present status of knowledge on *Lissorhoptrus oryzophilus* Kuschel (Rice Water Weevil) in Italy. In: Proceedings of the fourth temperate rice

© 2012 Blackwell Verlag, GmbH

UNCORRECTED PROOF

conference, Ed. by Bocchi S, Ferrero A, Porro A, Novara, Italy, June 2007, 138–139.

Lupi D, Baumgärtner J, Cola G, Mariani L, 2007b. Studi preliminari per la rappresentazione della fenologia della specie invasiva *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Erirhinidae). Proceedings XXI Congresso Nazionale Italiano di Entomologia, Campobasso, Italy 11–16 June 2007, 200.

Lupi D, Cenghialta C, Giudici ML, Villa B, Tabacchi M, 2008. Prime acquisizioni sulla biologia e sul contenimento di *Lissorhoptrus oryzophilus* (punteruolo acquatico del riso) in Lombardia. Atti Giornate Fitopatologiche 1, 245–246.

Lupi D, Cenghialta C, Colombo M, 2009a. Adult feeding by the rice water weevil *Lissorhoptrus oryzophilus* on different host plants. Bull. Insectology 62, 229–236.

Lupi D, Cenghialta C, Giudici ML, Villa B, Colombo M, 2009b. Suscettibilità di cultivar di riso all'attacco di *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Erirhinidae). Proceedings XXII Congresso Nazionale Italiano di Entomologia, Ancona, Italy 15–18 June 2009, 213.

Lupi D, Giudici ML, Cenghialta C, Villa B, Passoni D, Colombo M, 2010. On the spatial spread of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Erirhinidae), in Italy. J. Ent. Acar. Res. ~~Ser II,~~ 42, 81–90. 4

N'Guessan FK, Quisenberry SS, 1994. Screening selected lines for resistance to the rice water weevil (Coleoptera: Curculionidae). Environ. Entomol. 23, 665–675.

N'Guessan FK, Quisenberry SS, Croughan TP, 1994a. Evaluation of rice anther culture lines for tolerance to the rice water weevil (Coleoptera: Curculionidae). Environ. Entomol. 23, 331–336.

N'Guessan FK, Quisenberry SS, Croughan TP, 1994b. Evaluation of rice tissue culture lines for resistance to the rice water weevil (Coleoptera: Curculionidae). J. Econ. Entomol. 87, 504–513.

N'Guessan FK, Quisenberry SS, Thompson RA, Linscombe SD, 1994c. Assessment of Louisiana rice breeding lines for tolerance to the rice water weevil (Coleoptera: Curculionidae). J. Econ. Entomol. 87, 476–481.

Painter RH, 1951. Insect resistance in crop plants. The Macmillan Co, New York, 520.

Rice WC, Croughan TP, Ring DR, Muegge MA, Stout MJ, 1999. Delayed flood for management of Rice Water Weevil (Coleoptera: Curculionidae). Environ. Entomol. 28, 1130–1135.

Saito T, Hirai K, Way MO, 2005. The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae). Appl. Entomol. Zool. 40, 31–39.

Smith CM, Robinson JF, 1982. Evaluation of rice cultivars grown in North America for resistance to the rice water weevil. Environ. Entomol. 11, 334–336.

Smith CM, Robinson JF, 1984. Progress in identification of rice genotypes with rice water weevil resistance. Proc. Rice Technol. Work. Group 25, 73.

Stout MJ, Rice WC, Linscombe SD, Bollich PA, 2001. Identification of rice cultivars resistant to *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae), and their use in an integrated management program. J. Econ. Entomol. 94, 963–970.

Stout MJ, Rice WC, Ring DR, 2002. The influence of plant age on tolerance of rice to injury by the rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae). Bull. Entomol. Res. 92, 177–184.

Stout MJ, Riggio MR, Yang Y, 2009. Direct induced resistance in *Oryza sativa* to *Spodoptera frugiperda*. Environ. Entomol. 38, 1174–1181.

Tindall KV, Stout MJ, 2003. Use of common weeds of rice as hosts for the rice water weevil (Coleoptera: Curculionidae). Environ. Entomol. 32, 1227–1233.

Varade BS, Letey J, Stolzy LH, 1971. Growth responses and root porosity of rice in relation to temperature, light intensity and aeration. Plant Soil 34, 415–420.

Wang Z, Wu J, Shang H, Cheng J, 2011. Landscape connectivity shapes the spread pattern of the rice water weevil: a case study from Zhejiang, China. Environ. Manage. 41, 254–262.

Way MO, 1990. Insect pest management in rice in the United States. In: Pest management in rice. Ed. by Grayson BT, Green MB, Copping LG, Elsevier, New York, 181–189.

Way WO, Wallace RG, 1992. Rice water weevil pest management in the United States with emphasis on the south. In: Proc of workshop: "Establishment, spread, and management of the rice water weevil and migratory rice pests in East Asia" Suwon, Korea September 20–24, 1992, 58–83.

Webb JL, 1914. Notes on the rice water weevil. J. Econ. Entomol. 7, 432–438.

Wu GW, Wilson LT, 1997. Growth and yield response of rice to rice water weevil injury. Environ. Entomol. 26, 1191–1201.

Yoshida S, Hasegawa S, 1982. The rice root system: its development and function. In: Drought resistance in crops with emphasis on rice. ~~Ed. by~~ ~~???? ?~~ [illegible] ~~International Rice Research Institute,~~ Manila, Philippines, 97–115. 5

Zou L, Stout MJ, Dunand RT, 2004a. The effects of feeding by the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, on the growth and yield components of rice, *Oryza sativa*. Agric. For. Entomol. 6, 47–53.

Zou L, Stout MJ, Ring DR, 2004b. Density–yield relationships for rice water weevil on rice for different varieties and under different water management regimes. Crop Prot. 23, 543–550.

Zou L, Stout MJ, Ring DR, 2004c. Degree-day models for emergence and development of the Rice Water Weevil (Coleoptera: Curculionidae) in Southwestern Louisiana. Environ. Entomol. 33, 1541–1548.

© 2012 Blackwell Verlag, GmbH

Author Query Form

Journal: JEN

Article: 1750

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

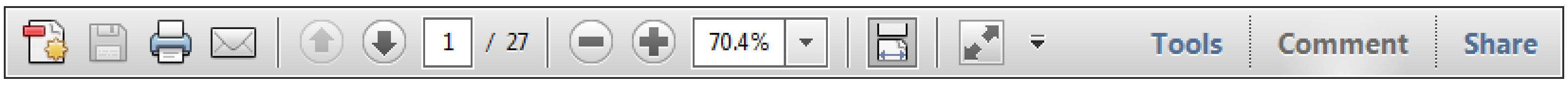
Many thanks for your assistance.

Query reference	Query	Remarks
1	**AUTHOR: Please give manufacturer information for Hobo® U22 Pro Water Temp: company name, town, state (if USA), and country.**	
2	**AUTHOR: Please give manufacturer information for PASW Statistics 18.0®: company name, town, state (if USA), and country.**	
3	**AUTHOR: Please provide the volume, edition, city location of publisher, group name for reference Herms (2004).**	
4	**AUTHOR: Please check the journal name J. Ent. Acar. Res. Ser II. for correctness.**	
5	**AUTHOR: Please provide the editor name(s) for reference Yoshida and Hasegawa (1982).**	

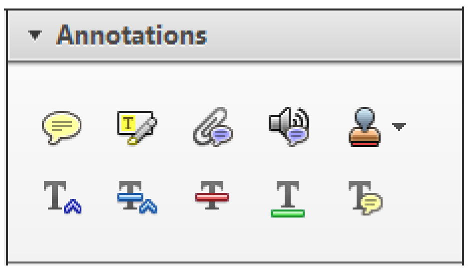
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/uk/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

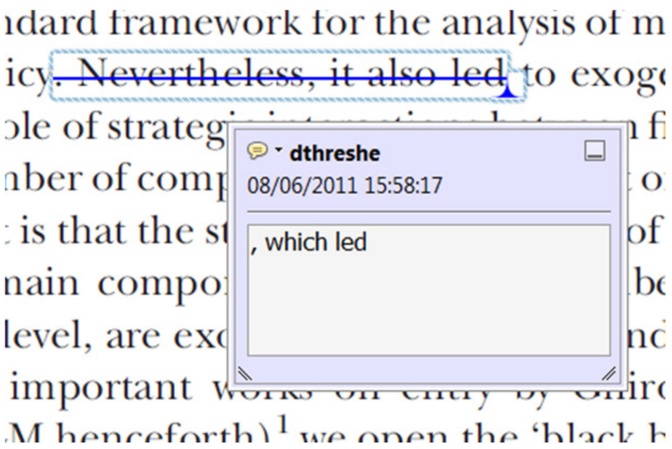


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.



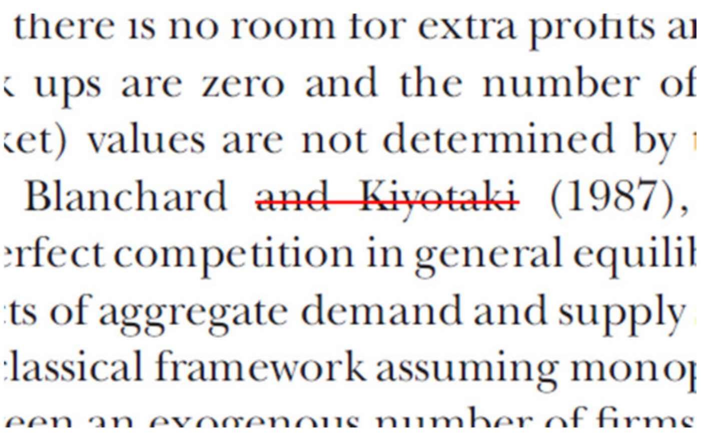
dthreshe
08/06/2011 15:58:17
, which led

2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

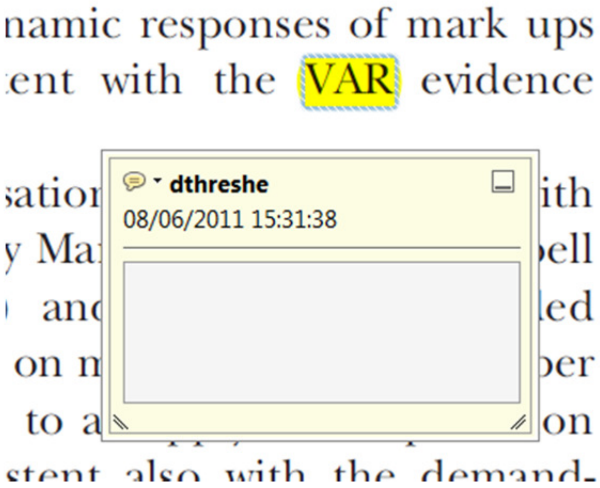


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.



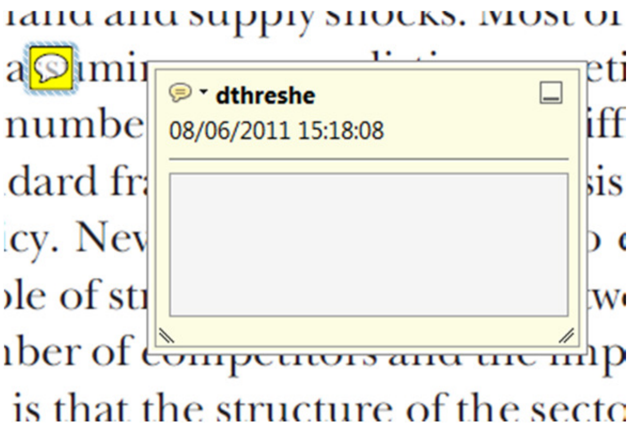
dthreshe
08/06/2011 15:31:38

4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



dthreshe
08/06/2011 15:18:08

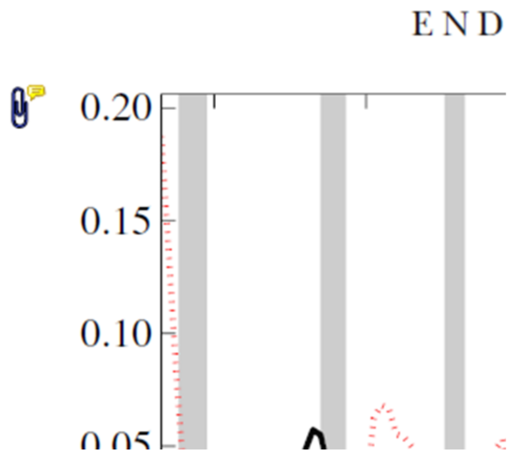
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

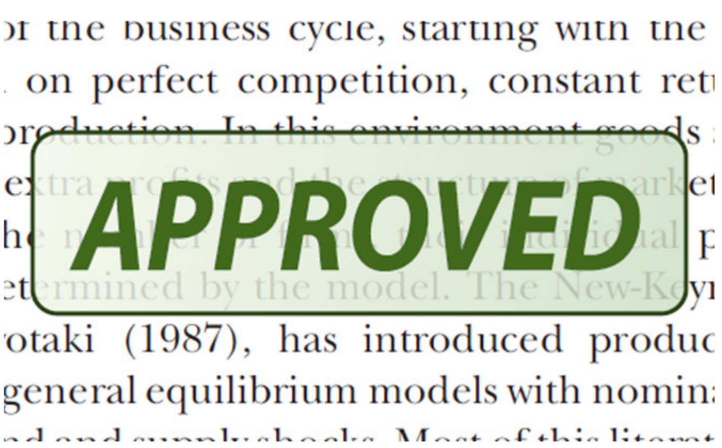


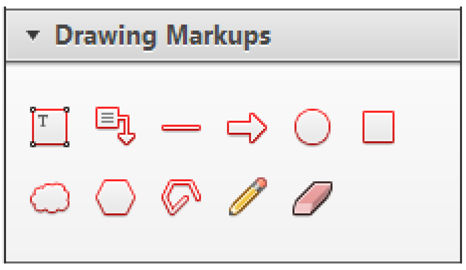
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

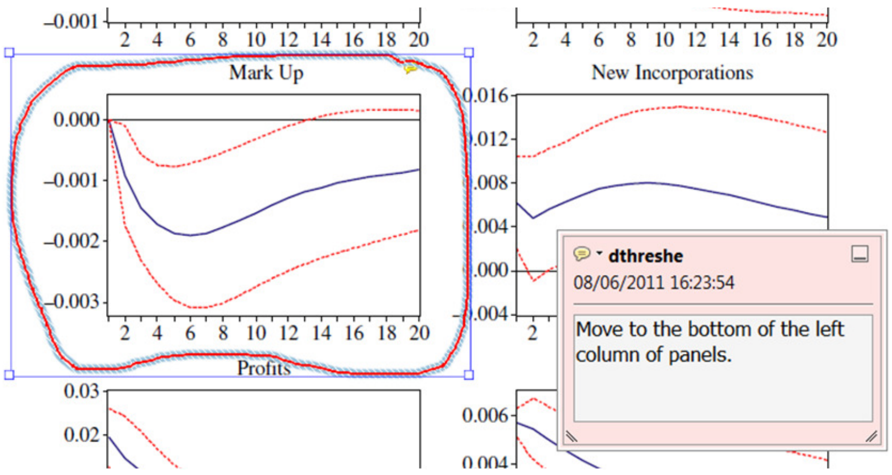
- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).





7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..



dthreshe
08/06/2011 16:23:54
Move to the bottom of the left column of panels.

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: