**Short running page heading**

Insects affect cardoon achene yields

**Title**

Novel crop, novel pests: assessment of insect damage to achenes of cardoon grown in a Mediterranean environment

**Authors:**

L. Sulas[1], L. Loru[2], G.A. Re[1], G. Campesi[1], R. Mannu[2, 3] and R.A. Pantaleoni[2, 3]

[1]Consiglio Nazionale delle Ricerche, Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo (CNR-ISPAAM), Sassari, Italy

[2]Consiglio Nazionale delle Ricerche, Istituto per lo Studio degli Ecosistemi (CNR-ISE), Sassari, Italy

[3]Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39, Sassari, Italy

**Summary**

Cardoon (*Cynara cardunculus* var. *altilis*) has have gained interest as a novel crop for bioenergy, multipurpose uses, and industrial bio-based productions, also based on the achene yield. However, achenes can be affected by various insects, which are considered as minor pests in traditional *Cynara* crops. However very little information is available on these pests. The aim of this research was to identify the frequency of different insect species with particular regard to weevils, and to quantify the achene yields and losses caused by these spermophagous insects on cardoon. Field measurements were performed across three consecutive years in Sardinia (Italy). Mature cardoon heads of different insertion orders (primary, secondary, tertiary and quaternary heads, respectively) were manually harvested. For each head, the achenes were counted and weighed, and bare receptacles were scored in terms of damaged areas by weevil larvae (i.e. the percentage of total area). The results highlighted a higher frequency of *Larinus scolymi* (90%) compared to *L. cynarae*. The total number of achenes per head and the one thousand achene weight were markedly affected by head order. Actual achene yield ranged from 86.8 to 107.9 g per plant. The estimates of achene yield losses per plant due to *Larinus* weevils ranged from 36.3 in 2014 to 113.0 g per plant in 2015, corresponding to about 0.4 to 1.1 t per hectare, respectively. Our results showed that achene losses were caused mainly by *L. scolymi*, indicating that cardoon crop profitability could be jeopardised in the absence of insect controls. Based on experimental data, appropriate strategies aimed at controlling weevil infestations are needed to preserve cardoon achene yields.

**Keywords:** *Larinus*, Curculionidae, achenes, *Cynara cardunculus* var *altilis*, industrial crop

**Introduction**

The high-yielding cardoon (*Cynara cardunculus* var. *altilis* DC) has gained increasing interest in the last few decades for its use as a bioenergy and multipurpose crop. Biological characteristics such as the perenniality, potential biomass yield and drought tolerance make this plant particularly suited to semi-arid Mediterranean environments (Fernández *et al.,* 2006; Christodoulou *et al.,* 2014; Pedroso *et al.,* 2014; Vasilakoglou & Dhima, 2014; Gominho *et al.*, 2018). The vigorous and deep root system, the annual growth cycle with vegetative stasis in summer (drought-escape strategy), as well as the considerable competitive ability against spontaneous species, are important traits that enable cardoon to be grown under dry regimes and particularly in environments characterized by reduced rainfall and infertile soils (Ledda *et al.,* 2013). The production of solid (wood pellets) and liquid (biogas and biodiesel) biofuels, pulp for the paper industry (Fernández *et al.,* 2006; Gominho *et al.*, 2009; Oliveira *et al.,* 2012) and inulin from the vigorous tap roots (Raccuia & Melilli, 2004, Raccuia & Melilli, 2010) have already been explored. In addition, cardoon biomass is a valuable source of bioactive compounds with antimicrobial, pharmaceutical and antioxidant properties (Kukić *et al.,* 2008; Pandino *et al.,* 2011: Genovese *et al.,* 2016). The cardoon crop has also been increasingly exploited in biorefinery for the production of novel industrial products such as bio-intermediates, bio-lubricants, bio-plastic and additives (Centi & Perathoner, 2012; Cravero *et al.,* 2012; Ramos *et al.,* 2013).

The basic type of cardoon inflorescence is the capitulum (head), in which the florets (flowers) are located on a flattened surface (receptacle), surrounded by bracts (Archontoulis *et al.,* 2010a). Cardoon growth stages are characterized by the emergence, development and maturation of main stem heads and heads on the side branches with different insertion orders or positions. The final number of heads may range from 1–4 to 8–12 capitula per plant, in poor and fertile soils, respectively (Archontoulis *et al.,* 2010a); in addition, it can be affected by plant density and seeding time (Puglia *et al.,* 2016)

The achenes (i.e. fruits, constituted by an embryo and pericarp, also called the kernel and hull, respectively), often referred to as seeds, can be used for the production of edible oil and biodiesel through chemical processes (Fernandez *et al.,* 2007; Raccuia *et al.*, 2011). The fatty acid composition of cardoon oil, which is similar to sunflower, enables it to be used for producing bioplastics, whereas the protein-rich press cake is an important biorefinery by-product that can be used for animal feeding (Genovese *et al.,* 2016).

The first studies on cardoon cultivation for bioenergy began in the 1980s (Fernández *et al.,* 2006). Research conducted in southern Europe has shown that the production of dry matter ranges from 9 to 26 t $ha^{-1}$ per year depending on the yearly amount of rainfall (Gramelis *et al.*, 2008; Gominho *et* *al.,* 2011). The annual growing period of cardoon begins after late summer rains and lasts until the following summer. In Italy, where our research was carried out, it is usually harvested from August to mid-September (Pari *et al.,* 2016a).

Cardoon achene yields may be variable and markedly affected by both the genotype and management practices adopted, including plant density, fertilization and irrigation ( Gominho *et al.*, 2011; Raccuia *et al.*, 2012; Vasilakoglou & Dhima, 2014). However, recent experiments carried out in central Italy found that cardoon achene yields ranged from 0.6 to about 3 t ha$^{-1}$ (Francaviglia *et al.*, 2016; Neri *et al.*, 2017; Ottaiano *et al.,* 2017).

Cardoon cultivation is now widespread in the Mediterranean (Angelini *et al.*, 2009; Cocco *et al.*, 2014; Deligios *et al.*, 2017), but little information is available on its pests. Although the majority, if not all, of the insect pests attacking the conspecific artichoke (*Cynara cardunculus* var. *scolymus* L.) are also expected to attack cardoon crops, their relative importance might be very different depending on the specific cropping systems and different genotypes. The most widespread artichoke autumn-spring cultivars produce flower heads in different periods and the achene production is unusual because the open pollinated cultivars reproduced by the seeds are very scarce.

According to Martelli (1948, 1952), Prota (1958) and Loru et al. (2016), some spermophagous pests on *Cynara* have been recorded in Italy: three beetles, including two weevils, *Larinus (Larinus) cynarae* (Fabricius, 1787) and *Larinus (Larinomesius) scolymi* (Olivier, 1808) and one anobiid, *Lasioderma baudii* Schilsky, 1899, and the tephritid fly *Terellia fuscicornis* (Loew, 1844). Generic information on cardoon insect pests indicates few problems in Spain (Fernández *et al.,* 2006) and there seems to be no problem in Sicily (Mauromicale *et al.,* 2014). In southern Italy, Gherbin *et al.* (2001) reported attacks due to achene feeding insects, referring to the fruit fly genus *Terellia* and *Larinus* weevils, whereas Fagnano *et al.* (2015) ascribed attacks to the species *T. fuscicornis* and *L. scolymi*. In all cases, achene damage has not been quantified and/or reported. In Sardinia, Rosnati *et al.* (2015) considered *L. cynarae* to be one of the main causes for the reduction or failure of achene production in the absence of pest control management. Finally, a preliminary study (Loru *et al.,* 2016) showed that weevil damage was not negligible and could seriously affect cardoon achene yields.

In order to assess the insect damage to cardoon achene yield, a multidisciplinary three-year study was carried out. This work aimed to: (i) identify the relative frequency and ascertain the damage of spermophages with particular regard to *L. scolymi* and *L. cynarae*, and (ii) quantify the achene yield, its components and losses from different head orders of cardoon plants.

**Materials and methods**

*Field site*~~*Stand description*~~

Field measurements and observations were carried out at the National Research Council experimental station in Leccari, Sassari (40°45'17"N 08°25'02"E; 27 m a.s.l.) across three consecutive years, from 2014 to 2016. The climate is attenuated thermo-Mediterranean with a four-month drought period in the summer. The hottest and coldest months are July and February, respectively. Long-term average crop year (September to August) precipitation is about 550 mm. The soil is classified as Eutric, Calcaric and Mollic Fluvisol (Soil Survey Staff, 2014), and is sandy-clay-loam, alkaline with a low average nitrogen content (0.96 g $kg^{-1}$) and adequate phosphorous (20.33 ppm), organic matter (1.46%) and organic carbon (0.85%) contents. Daily maximum and minimum air temperatures and rainfall events were monitored in the experimental site by a weather station. In the first productive cardoon growing season, total rainfall reached 643 mm from September 2013 to August 2014, exceeding climatic means by 17%. In contrast, total rainfall was 444 mm (minus 20%), in the subsequent growing season, and 415 mm (minus 25%) from September 2015 to August 2016. Mean temperatures were 17.8, 15.6, and 16.1 °C in the year 2013-2014, 2014-2015, and 2015-2016, respectively.

Sampling and measurements

Observations were carried out on three sampling areas~~ in plots replicated three times~~, each one 50 $m^2$ in size, planted in 2013 at a maximum density of 4 plants $m^{-2}$ and ~~,All plots were~~ thinned at the four-leaf stage (BBCH code 14) (Archontoulis *et al.,* 2010a) to adjust to the standard density of 1.25 plants $m^{-2}$. The sowing of a Spanish genotype of cardoon, kindly supplied by the Department of Plant Production, Polytechnic University of Madrid, was carried out in April, after ploughing, followed by disk harrowing. In all the plots, 100 kg $ha^{-1}$ of phosphorus fertilizer ($P_2O_5$) were applied before sowing. No other chemical inputs were applied throughout the experiment lifespan, and plants were grown without supplemental irrigation across the subsequent growing seasons.

In the three consecutive productive years, from middle to late July, mature heads were harvested manually from eight plants per plot randomly selected. With the exception of primary heads, heads from branches with a different insertion order on the main stem (i.e.~~,~~ secondary, tertiary and quaternary heads, respectively) were subsampled in the same plant or shoot. Overall, about 100 heads were processed each year.

In the laboratory, all harvested heads were weighed and manipulated in order to clean the receptacle. The bracts were then opened manually and all the pappi and achenes were removed. For each head the following measures were performed: i) number of undamaged achenes; ii) weight of achenes (thereafter referred to as "actual achene weight per head"); iii) percentage of the damaged area of the total surface of the receptacle. The achenes were considered "undamaged" when they were normally ripened without any detectable damage. Since damaged achenes were either totally destroyed, or reduced to head remains (i.e.~~,~~ a mix of achene fragments and insect excrement~~s~~), their contribution to the "actual achene weight per head" was not considered. The percentage of damaged area on total receptacle surface (rounded to 5%) was assessed for all bare receptacles by quantifying the untouched surface and the area damaged by insect pests with the aid of a 360° protractor (Fig. 1).

The following values were also calculated: i) the number of damaged achenes; ii) total number of achenes per head; iii) average achene weight; iv) lost achene weight; v) potential achene weight; vi) expected achene weight.

The number of damaged achenes was estimated by the proportion:

$$\frac{\text{No. damaged achenes}}{\text{\% damaged area}} = \frac{\text{No. undamaged achenes}}{100 - \text{\% damaged area}}$$

The total number of achenes per head was obtained by summing the damaged and undamaged achenes. The average achene weight was obtained by dividing the actual achene weight by the number of undamaged achenes and it was referred to one thousand achene weight. The lost achene weight was obtained by multiplying the number of damaged achenes by the average achene weight. The potential achene weight was obtained as the sum of actual and lost achene weights. The expected achene weight per head was obtained using the function reported by Archontoulis *et al.* (2010b), based on head weight:

$$\text{expected achene weight} = 0.429*(\text{head weight}) - 2.9$$

Finally, the total number of heads was counted on an additional four plants per plot in each year, which were randomly selected. The achene yield per plant was obtained by multiplying the actual achene weight of each head per the number of heads having the same order (data in Table 3) and then by summing the values of the four orders.

*Insect counting*

The presence-absence of the four main spermophagous insects feeding on cardoon was recorded by identifying the pest by the damage they caused. Based on the different feeding behaviours, it was possible to identify which species attacked the heads manipulated during our investigations. In fact, the larval feeding behaviours of the two *Larinus* species are different and correspond to the two biologically and morphologically different types in which the *Larinus* species can be grouped (Zwölfer & Brandl, 1989). *L. scolymi* attacks achenes, which are located above the surface of the receptacle, whereas *L. cynarae* attacks below. The *L. scolymi* activity produces "buboes", one for every larva, which are formed by pappi, the remains of achenes, and faeces, with a central cell where pupation will take place. The damage by *L. cynarae* barely affect the achenes and the attack is less evident on the exterior (Loru *et al.*, 2016). The presence of *Terellia fuscicornis* can be seen from the typical damage caused to the achenes, the presence of puparia or the characteristic cocoons, which are not always built, as described in Martelli (1952). The presence of *Lasioderma baudii* can be detected from the damage of the peripheral achenes, near the bracts (Prota, 1958).

The presence/absence of spermophagous insects was recorded for each sampled head. The amount of damage by *Larinus* weevils was directly correlated to the damaged area of the receptacle (as above reported). As regards *Terellia* and *Lasioderma*, empirical scores on the abundance (low, medium, high) for every head were recorded.

Primary and secondary head subsamples were carefully examined in order to record the number and species of *Larinus* weevil larvae. On subsamples, the identification of the larvae was confirmed by morphological examination of the specimen. The discrimination between the larvae of the two *Larinus* species was based on the comparative key provided by Martelli (1948). In order to ensure that examination was made on mature larvae, samplings were carried out exclusively on mature cardoon heads. The Martelli's identification keys have been already evaluated in ~~a~~ previous work (Loru et al., 2016) in which identification was confirmed observing adults emerged from reared larvae.

*Statistical analysis*

All statistical analyses were performed with R, version 3.2.2 (R Core Team, 2016).

Fisher's exact test was used to compare the frequency of the two *Larinus* species in the primary and secondary subsampled heads. A Mann-Whitney-Wilcoxon test was used to test for differences between the mean number of *L. cynarae* and *L. scolymi* specimens observed in the same subsampling.

The effects of head order, year and their interactions were explored on the following variables: (1) percentage of the damaged area of the receptacle, (2) number of undamaged achenes per head, (3) number of damaged achenes per head, (4) total number of achenes per head, (5) one thousand achene weight, (6) actual achene weight per head, (7) lost achene weight per head, (8) potential achene weight per head. Linear Mixed Models (LMMs) were thus fitted separately for each variable using the lmer function from "lme4" package in R (Bates *et al.,* 2015). Before the analysis, all data were explored for normal distribution by visually evaluating the kurtosis and skewness. When necessary, data were transformed as either log(x+1) or arcsin($x^{1/2}$) to satisfy the normality assumption. LMMs are strongly recommended when unbalanced samples occur (Pinheiro & Bates, 2000).

Since samples were taken from the same plots throughout the entire sampling period, the effect of each plot was considered as a crossed random effect. Analysis of variance (ANOVA) considering Type III Wald F tests with Kenward-Roger correction of degrees of freedom was used to test the significance of each factor, reducing both the standard error and F-statistic bias due to large sample size (Luke, 2017). The least significant difference (LSD) test was used for means separation at $p<0.05$.

The mean of actual achene weight per head was compared with the mean of expected achene weight per head [based on head weight, according to the Archontoulis *et al.* (2010b) function] performing a Monte-Carlo permutation test (9999 permutations).

One-way ANOVA followed by the LSD test at the 5% significant level were considered to evaluate differences among years in the average number of second, third, and fourth-order heads, for the actual, lost and potential achene yield per plant, respectively.



**Results**

*Frequency of Larinus species and observations on other spermophagous insects*

In our samplings, both *Larinus* weevil species attacking cardoon in Sardinia were recorded. Among the total examined heads, 82% and 3% were infested exclusively by *L. scolymi* and *L. cynarae*, respectively, 7% by both species, whereas 8% were not damaged. These results concur with those detected in the subsamples (Table 1), where the frequency of *L. scolymi* larvae was significantly higher than that of *L. cynarae* (Fisher exact test, $p<0.001$). In addition, the number of *L. scolymi* specimens counted in the subsamples was significantly higher than that of *L. cynarae* (Mann-Whitney-Wilcoxon test, $W = 1134$, $p < 0.001$). *L. scolymi*, which is slightly smaller, was able to colonize up to near twenty larvae in larger heads. In contrast, *L. cynarae* colonized heads with one or two specimens only (Table 1).

The constant presence of *T. fuscicornis* was also recorded in the sampled heads (frequency near 100%). The abundance was always registered as low. The presence of *L. baudii* was also recorded, the abundance was constantly low leading to insignificant damage.

*Receptacle damaged area, undamaged, damaged, and total achene number per head*

The values of these variables for each head order and year are reported in Table 2.

The total number of achenes was strongly influenced by head order and ranged from 439 to 215 in the primary and quaternary heads, respectively (Fig. 2). The average number of damaged achenes of the quaternary heads was approximately one third of the other three orders. Consequently,

observed in 2016 than in the previous years (Table 4). On a three-year average, about 12 heads were counted for each cardoon plant (Fig. 5). Achene yield per plant, which ranged from 86.8 to 107.9 g, was not significantly affected by year (Fig. 5). The cumulative achene yields from secondary and tertiary heads, which contributed almost equally, represented about 75% of the total plant yield. The estimates of cumulative achene yield losses per plant due to the *Larinus* species ranged from 36.3 in 2014 to 113.0 g per plant in 2015, representing in the same years 25.2 and 51.4% of the total potential achene yield per plant. Based on an average plant density of approximately 1 plant per $m^2$, the expected achene yield losses per hectare ranged from about 0.4 to 1.1 t.

**Discussion**

*Cynara cardunculus* L. is a perennial species native to the Mediterranean basin, which includes artichoke, cultivated cardoon, and their common ancestor, wild cardoon (var. *sylvestris* Lam.) (Portis *et al.*, 2014). As a consequence of conspecificity among the different *Cynara* botanical varieties, the phytophagous insects attacking the wild cardoon are expected to also feed on cultivated varieties. Currently, there is no evidence that states the contrary, although the wide differences in growth and flowering season, edible plant portion, and cropping management might strongly affect the relative importance of the phytophagous pests on both artichoke and cardoon. Spermophagous insects were very rarely considered as pests in the past. Indeed, their economic importance has been limited to date to the damage on the seed production in the small niche market of both traditional vegetable cardoon cultivars and rare open-pollinated artichoke cultivars. The expansion of cardoon, which today represents a novel cropping system supplying large amounts of biomass and seeds, not previously available, is facing new pest issues (Loru *et al.*, 2016; Gominho *et al.*, 2018).

Four main spermophagous pests feed on achenes of the *Cynara* botanical varieties in the Italian peninsula and islands: the weevils *Larinus cynarae* and *L. scolymi* (Martelli, 1948; Loru *et al.*, 2016), the tephritid fly *Terellia fuscicornis* (Martelli, 1952), and the anobiid beetle *Lasioderma baudii* (Prota, 1958). Our results showed that the two *Larinus* species were the most frequent and harmful pests in these experimental conditions. Undoubtedly, they represent the most important achene pests, whereas the tephritid fly and the anobiid beetle were found to be minor pests. Previous records of both *L. cynarae* and *L. scolymi* as pests of *Cynara* spp. have always been qualitative, and no information regarding the single and/or simultaneous presence of the two species has been provided to date (Gherbin *et al.*, 2001; Fernández *et al.*, 2006; Mauromicale *et al.*, 2014; Fagnano *et al.*, 2015; Rosnati *et al.*, 2015). In addition, the impact of each species has never been identified nor have the infestation levels been quantified (Loru *et al.*, 2016). Our survey of both the whole set of heads (for presence/absence data) and the subsamples (for larvae count) registered an over 90% infestation due to *Larinus*, of which 82-83% was caused by *L. scolymi* alone (Table 1). Moreover, the number of *L. scolymi* larvae recorded in one head reached about twenty, versus only one or two in *L. cynarae* (Table 1). These findings show for the first time that *L. scolymi* might be the most common and destructive spermophagous pests on cardoon under Mediterranean conditions. Our three-year study has provided a detailed evaluation of achene yields and losses, differentiated in terms of primary, secondary, tertiary and quaternary heads. There was a significant effect of head order on the total number of achenes, one-thousand achene weight and potential achene weight, which have to date not been available for cardoon. The one thousand achene weight gradually decreased from the first head order to the fourth head order, partially contributing to the decreasing potential weight of achenes per head (Fig. 2). Our findings are in agreement with previous results obtained in artichoke, indicating that the seed set was greater in primary heads than in heads of remaining orders (Basnizki & Zohary, 1994).

The damage caused by *Larinus* weevils seemed similar for the first three head orders, while lower values were recorded for the fourth one. Considering that each larva of *L. scolymi* feeds on a fixed number of achenes (approximately 10 achenes) (Martelli, 1948), the same number of larvae would damage a higher percentage of achenes in small heads than in big ones. In contrast, the percentage of damaged achenes was lower in quaternary heads (31% vs. 40/47%). Consequently, it is very probable that quaternary heads were more likely to be less attacked by weevils as a consequence of the smaller dimension and late maturing period, making them less attractive for *Larinus* adults.

Our study also indicated variations due to growing year. The number and the weight of achenes per head, as well as the number of damaged achenes, the achene losses per head and the damaged area were significantly lower in the first growing year than in the following two years. Conversely, in the second year all the variables directly linked to the damage value were significantly higher, together with high values for both total achenes and potential achene weight per head. Finally, the third year was characterized by a relative low insect damage and a good "per-head" productivity.

The consumption of achenes by *Larinus* larvae produced a weight loss in the heads. Even if the head weight was used to estimate the achene weight per head (Archontoulis *et al.,* 2010b), the weight of heavily damaged heads was an unreliable predictor of achene weight per head.

In order to understand the annual variation in crop yield and the effective losses due to *Larinus* pests, it is necessary to take into account the whole achene production per plant. In fact, the results per plant differed from those per head, because in 2016 there was a lower number of heads per plant (i.e. achenes), probably as a consequence of a second consecutive season of drought (Table 4; Fig. 5). Instead, 2015 was the most productive year but, at the same time, was the most damaged by insects. The peak of potential achene yield recorded in 2015 could be explained by the typical production trend of cardoon, as described by Angelini *et al.* (2009), and by the meteorological trend over the three years. However, as the seasonal trend might simultaneously affect the cardoon crop and insect growth, the weather conditions could have operated on the weevil population both directly and indirectly, via plant growth, in ways that are not clearly discernible. In any case, the actual yields did not differ significantly among years. Concurrently, a higher pest attack was also recorded in the most productive year, thus the differences in actual yields were mitigated.

Compared with other studies carried out in Greece and Italy (Raccuia *et al.*, 2007b; Archontoulis *et al.*, 2010b; Fagnano *et al.*, 2015; Francaviglia *et al.*, 2016), the actual achene yields estimated in our study could potentially reach a peak value of about 3.0 t ha$^{-1}$ only in the absence of achene damage by *Larinus* weevils. However, considering the biology of both *Larinus* species, the infestation levels and relative abundance of the two species may well differ greatly among different localities. In fact, *Larinus* weevils overwinter in shelters away from the cardoon crops and then in the spring the adults begin to search for host plants (Martelli, 1948). Apart from the size of the overwintering weevil population and the meteorological trend, colonization of the cardoon crop may therefore be strongly influenced by the landscape pattern (position of shelters in relation to the presence of wild host plants, new crops, artichoke crops, etc.). Genetic differentiation among weevil populations could also condition their harmfulness, influencing, for example, plant host specificity (Olivieri *et al.*, 2008). Finally, the interactions among the different spermophagous insects need to be fully~~deeply~~ investigated in future studies (Skuhrovec *et al.*, 2008; Abela-Hofbauerova *et al.*, 2011).

In conclusion, the achene losses caused by spermophagous insects exceeded half of the potential yield in the second growing year, suggesting a significant impact on the cardoon under our environmental and cropping conditions. Our research highlighted the preeminent role of *L. scolymi* compared to *L. cynarae*, and overall, the high incidence of damage from weevils compared to the other insects investigated.

Our study also highlighted new information related to cardoon plant architecture:

- head order affected achene yields and losses and its components, such as (undamaged, damaged and total) number of achenes per head and the one-thousand achene weight;
- significant reductions in one thousand achene weight and potential achene weight per head occurred from primary to quaternary heads;
- at the plant level, achene yields from secondary and tertiary heads, contributed almost equally, representing 75% of the total yield;
- quaternary heads were less attacked by *Larinus* weevils, presumably due to their smaller size and later maturing period.

Although the high productive potential of cardoon was confirmed, crop profitability might be jeopardised in the absence of appropriate insect control. Management and control strategies must be developed in future studies, starting from the recorded quantitative damage and weevil behaviour and by considering the possible insect relationships with the entire cropping system.

**Acknowledgements**

This work was partially funded by the FP7 OPTIMA project "Optimization of Perennial Grasses for Biomass production (Grant Agreement 289642)". Authors wish to thank Ms. Maria Maddalena Sassu, Mr. Daniele Nieddu, Mr. Alessandro Ortu at CNR ISPAAM, Ms. Gavina Serra at CNR ISPA, and Dr. Simone Canu at CNR ICB for their valuable technical assistance in the field and laboratory.

**References**

Abela-Hofbauerova I., Münzbergová Z., Skuhrovec J. (2011) The effect of different natural enemies on the performance of *Cirsium arvense* in its native range. *Weed Research*, **51**, 4, 394-403.

Angelini L.G., Ceccarini L., Nassi o Di Nasso N., Bonari E. (2009) Long-term evaluation of biomass production and quality of two cardoon (*Cynara cardunculus* L.) cultivars for energy use. *Biomass and Bioenergy,* **33**, 810-816.

Archontoulis S.V., Struik P.C., Vos J., Danalatos N.G. (2010a) Phenological growth stages of *Cynara cardunculus*: codification and description according to the BBCH scale. *Annals of Applied Biology*, **156**, 253–270.

Archontoulis S.V., Struik P.C., Yin X., Bastiaans L., Vos J., Danalatos N.G. (2010b) Inflorescence characteristics, seed composition, and allometric relationships predicting seed yields in the biomass crop *Cynara cardunculus*. *Gcb Bioenergy*, **2**, 113-129.

Basnizki J., Zohary D. (1994) Breeding of seed planted artichoke. *Plant Breeding Reviews*, **12**, 253-269.

Bates D., Maechler M., Bolker B., Walker S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1-48.

Centi G., Perathoner S. (2012) Is there a strategy for sustainable (green) chemistry in Italy? *La chimica & l'Industria*, **10**, 86–90.

Christodoulou C., Tsekos C., Tsalidis G., Fantini M., Panopoulos K.D., De Jong W., Kakaras E. (2014) Attempts on cardoon gasification in two different circulating fluidized beds. *Case Studies in Thermal Engineering*, **4**, 42-52.

Cocco D., Deligios P.A., Ledda L., Sulas L., Virdis A., Carboni G. (2014) LCA study of oleaginous bioenergy chains in a Mediterranean environment. *Energies,* **7**, 6258-6281.

Cravero V., Martin E., Crippa I., Anido F.L., García S.M., Cointry E. (2012) Fresh biomass production and partitioning of aboveground growth in the three botanical varieties of *Cynara cardunculus* L. *Industrial Crops and Products,* **37**, 253-258.

Deligios P.A., Sulas L., Spissu E., Re G.A., Farci R., Ledda L. (2017) Effect of input management on yield and energy balance of cardoon crop systems in Mediterranean environment. *European Journal of Agronomy*, **82**, 173-181.

Fagnano M., Mori M., Di Mola I., Ottaiano L., Laudonia S., Sinno M. (2015) Cresce l'interesse per il cardo. *Terra e Vita*, **8**, 56-58.

Fernández J., Curt M.D., Aguado P.L. (2006) Industrial applications of *Cynara cardunculus* L. for energy and other uses. *Industrial Crops and Products*, **24**, 222-229.

Francaviglia R., Bruno A., Falcucci M., Farina R., Renzi G., Russo D.E., Neri U. (2016) Yields and quality of *Cynara cardunculus* L. wild and cultivated cardoon genotypes. A case study from a marginal land in Central Italy. *European Journal of Agronomy*, **72**, 10-19.

Genovese C., Platania C., Venticinque M., Calderaro P., Argento S., Scandurra S., Raccuia S.A. (2015) Evaluation of cardoon seeds presscake for animal feeding. *Proceedings IX International Symposium on Artichoke, Cardoon and Their Wild Relatives*, 323-328.

Genovese C, Brundo M.V., Toscano V, Tibullo D. Puglisi F., Raccuia S.A. (2016) Effect of *Cynara* extracts on multiple myeloma cell Lines. *Acta Horticulturae*, *1147*, 113-117.

Gherbin P., Monteleone M., Tarantino E. (2001) Five year evaluation on cardoon (*Cynara cardunculus* L. var. *altilis*) biomass production in a Mediterranean environment. *Italian Journal of Agronomy*, **5**, 11-19.

Gominho J., Lourenco A., Curt M., Fernandez J., Pereira H. (2009) Characterization of hairs and pappi from *Cynara cardunculus* capitula and their suitability for paper production. *Industrial Crops and Products*, **29**, 116–125.

Gominho J., Lourenço A., Palma P., Lourenço M.E., Curt M.D., Fernández J., Pereira H. (2011) Large scale cultivation of *Cynara cardunculus* L. for biomass production – a case study. *Industrial Crops and Products*, **33**, 1-6.

Gominho J., Curt M.D., Lourenço A., Fernández J., Pereira H. (2018) *Cynara cardunculus* L. as a biomass and multi-purpose crop: A review of 30 years of research. *Biomass and Bioenergy*, **109**, 257-275.

Grammelis P, Malliopoulou A., Basinas P., Danalatos N.G. (2008) Cultivation and characterization of *Cynara cardunculus* for solid biofuels production in the Mediterranean region. *International Journal of Molecular Science*, **9**, 1241-1258.

Kukić J., Popović V., Petrović S., Mucaji P., Ćirić A., Stojković D., Soković M. (2008) Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. *Food chemistry*, **107**, 861-868.

Ledda L., Deligios P., Farci R., Sulas L. (2013) Biomass supply for energetic purposes from some *Carduae* species grown in a Mediterranean rainfed low input cropping system. *Industrial Crops and Products*, **47**, 218-226.

Loru L., Pantaleoni R.A., Re G.A., Sulas L. (2016) Danni da insetti ai capolini del cardo gentile in Sardegna con note sulla biologia di *Larinus cynarae* e *L. scolymi*. *Atti Giornate Fitopatologiche,* 2016, **1**, 375-384.

Luke S.G. (2017) Evaluating significance in linear mixed-effects models in R. *Behavior Research Methods*, **49**, 1494-1502.

Martelli M. (1948) Osservazioni su due specie del genere *Larinus* Germ Coleoptera Curculionidae. *Redia*, **33**, 221-286.

Martelli M. (1952) Reperti sulla *Terellia fuscicornis* Leow. (Diptera, Trypetidae), *Redia*, **37**, 405-442.

Mauromicale G., Sortino O., Pesce G.R., Agnello M., Mauro R.P. (2014) Suitability of cultivated and wild cardoon as a sustainable bioenergy crop for low input cultivation in low quality Mediterranean soils. *Industrial Crops and Products,* **57**, 82-89.

Neri U., Pennelli B., Simonetti G., Francaviglia R. (2017) Biomass partition and productive aptitude of wild and cultivated cardoon genotypes (*Cynara cardunculus* L.) in a marginal land of Central Italy. *Industrial Crops and Products*, **95**, 191-201.

Oliveira I., Gominho J., Diberardino S., Duarte E. (2012) Characterization of *Cynara cardunculus* L. stalks and their suitability for biogas production. *Industrial Crops and Products*, **40**, 318-323.

Olivieri I., Singer M.C., Magalhães S., Courtiol A., Dubois Y., Carbonell D., Justy F., Beldade P., Parmesan C., Michalakis Y. (2008) Genetic, ecological, behavioral and geographic differentiation of populations in a thistle weevil: implications for speciation and biocontrol. *Evolutionary Applications*, **1**, 112-128.

Ottaiano L., Di Mola I., Impagliazzo A., Cozzolino E., Masucci F., Mori M., Fagnano M. (2017) Yields and quality of biomasses and grain in *Cynara cardunculus* L. grown in southern Italy, as affected by genotype and environmental conditions. *Italian Journal of Agronomy*, **12**, 375-382.

Pandino G., Lombardo S., Mauromicale G., Williamson G. (2011) Phenolic acids and flavonoids in leaf and floral stem of cultivated and wild *Cynara cardunculus* L. genotypes. *Food Chemistry*, **126**, 417-422.

Pari L., Alfano V., Acampora A., Del Giudice A., Scarfone A., Sanzone E. (2016a) Harvesting and Separation of Different Plant Fractions in *Cynara cardunculus* L. In *Perennial Biomass Crops for a Resource-Constrained World,* pp. 261-271. Eds S Barth, D. Murphy-Bokern, O. Kalinina, G. Taylor, M. Jones. Switzerland Springer International Publishing.

Pedroso G.M., Hutmacher R.B., Putnam D., Six J., van Kessel C., Linquist B.A. (2014) Biomass yield and nitrogen use of potential C4 and C3 dedicated energy crops in a Mediterranean climate. *Field Crops Research,* **161**, 149-157.

Pinheiro J.C., Bates D.M. (2000) Mixed-effects models in S and S-Plus. New York, Springer.

Portis E., Mauro R.P., Barchi L., Acquadro A., Mauromicale G., Lanteri S. (2014) Mapping yield-associated QTL in globe artichoke. *Molecular breeding*, **34**, 615-630.

Prota R. (1958) Ricerche sull'entomofauna del Carciofo (*Cynara cardunculus* v. *scolymus* L.). II *Lasioderma baudii* Schils. (Coleoptera Anobiidae). *Studi Sassaresi, Sez. III*, **6,** 210-255.

Puglia G., Raccuia S.A. Pappalardo, H., Genovese, C., Argento, S., Melilli, M.G. (2016) Characterization of a MADS flowering locus C - Like (MFL) in *Cynara cardunculus* var. *Altilis* under different sowing and planting density, *Acta Horticulturae*, **1147**, 301-308.

R Core Team, (2016) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://R-project.org/.

Raccuia S.A., Melilli M.G. (2004) Cynara cardunculus L., a potential source of inulin in the Mediterranean environment: Screening of genetic variability. *Australian Journal of Agricultural Research*, **55**, 693-698.

Raccuia S.A., Melilli M.G. (2010) Seasonal dynamics of biomass, inulin, and water-soluble sugars in roots of Cynara cardunculus L. *Field Crops Research*, **116**, 147-153.

Raccuia S.A., Piscioneri I., Sharma N., Melilli M.G. (2011) Genetic variability in Cynara cardunculus L. domestic and wild types for grain oil production and fatty acids composition. *Biomass and Bioenergy*, **35**, 3167-3173.

Raccuia S.A., Gallo G., Melilli M.G. (2012) Effect of plant density on biomass and grain yields in *Cynara cardunculus* var. *altilis* cultivated in Sicily. *Acta Horticulturae*, **942**, 303-308.

Ramos P.A.B., Guerra A.R., Guerreiro O., Freire C.S.R., Silva A.M.S., Duarte M.F., Silvestre A.J.D. (2013) Lipophilic extracts of *Cynara cardunculus* L. var *altilis* (DC): a source of valuable bioactive terpenic compounds. *Journal of Agriculture and Food Chemistry,* **61**, 8420–8429.

Rosnati C., Mangone M., Cossu Q.A. (2015) SEA and biomass energy industries planning. *Proceedings 35th Annual Conference of the International Association for Impact Assessment, Florence, Italy,* 1-6. www.iaia.org

Skuhrovec J., Koprdová S., Mikulka J. (2008) How can seed feeders regulate dispersion of thistles, *Cirsium arvense* and *C. heterophyllum*? *Journal of Plant Diseases and Protection*, Special Issue **21**, 281-284.

Soil Survey Staff (2014) *Keys to Soil Taxonomy*. 12th edn. Washington, DC: USDA-Natural Resources Conservation Service.

Vasilakoglou I., Dhima K. (2014) Potential of two cardoon varieties to produce biomass and oil under reduced irrigation and weed control inputs. *Biomass and Bioenergy,* **63**, 177-186.

Zwölfer H., Brandl R. (1989) Niches and size relationships in Coleoptera associated with Cardueae host plants: adaptations to resource gradients. *Oecologia*, **78**, 60-68.

Table 1. Presence and average number of specimens per head of the *Larinus* species attacking cardoon. The heads examined were primary or secondary.

| **Year** | **Heads** | **Presence** | | | **Individuals per head** (mean ± SE) | |
|---|---|---|---|---|---|---|
| | | *L. scolymi* | *L. cynarae* | *Both* | *L. scolymi* | *L. cynarae* |
| **2014** | 11 | 9 | 1 | 1 | 6.36 ± 1.55 | 0.36 ± 0.24 |
| **2015** | 12 | 11 | 0 | 1 | 4.33 ± 0.58 | 0.08 ± 0.08 |
| **2016** | 12 | 9 | 0 | 1 | 3.08 ± 0.96 | 0.08 ± 0.08 |
| **TOTAL** | 35 | 29 | 1 | 3 | 4.54 ± 0.64 | 0.17 ± 0.09 |

**Table 2.** Estimate of damaged area in the head receptacles, undamaged and damaged achenes number and total achene number per head of cardoon across 2014 – 2016 and different head orders (means ± SE).

| Year | Head order | Samples (no.) | Damaged area (%) | Undamaged achenes (no. head$^{-1}$) | Damaged achenes (no. head$^{-1}$) | Total achenes (no. head$^{-1}$) |
|---|---|---|---|---|---|---|
| **2014** | | | | | | |
| | **Primary** | 23 | 36.5 ± 1.8 | 217.1 ± 17.2 | 128.5 ± 18.3 | 345.6 ± 18.6 |
| | **Secondary** | 23 | 34.1 ± 2.7 | 189.5 ± 15.0 | 93.2 ± 7.4 | 282.7 ± 16.1 |
| | **Tertiary** | 21 | 18.1 ± 2.8 | 156.4 ± 18.8 | 30.1 ± 5.2 | 186.5 ± 19.5 |
| | **Quaternary** | 14 | 22.5 ± 3.9 | 105.7 ± 16.5 | 27.2 ± 5.6 | 132.9 ± 18.8 |
| **2015** | | | | | | |
| | **Primary** | 12 | 58.3 ± 7.9 | 209.6 ± 38.6 | 305.4 ± 51.9 | 515.0 ± 41.3 |
| | **Secondary** | 54 | 48.8 ± 3.4 | 220.7 ± 14.9 | 221.1 ± 19.8 | 441.8 ± 20.2 |
| | **Tertiary** | 69 | 51.3 ± 3.0 | 159.3 ± 10.1 | 201.3 ± 16.7 | 360.6 ± 17.9 |
| | **Quaternary** | 15 | 37.3 ± 5.0 | 169.4 ± 18.1 | 121.1 ± 18.9 | 290.5 ± 27.5 |
| **2016** | | | | | | |
| | **Primary** | 24 | 29.4 ± 4.2 | 309.9 ± 33.5 | 145.0 ± 30.4 | 454.9 ± 40.0 |
| | **Secondary** | 24 | 40.2 ± 4.7 | 296.6 ± 26.8 | 182.7 ± 19.7 | 479.3 ± 26.0 |
| | **Tertiary** | 22 | 41.4 ± 5.7 | 242.9 ± 27.4 | 149.2 ± 24.4 | 392.1 ± 31.0 |
| | **Quaternary** | 11 | 19.5 ± 7.0 | 164.1 ± 17.5 | 44.0 ± 16.7 | 208.1 ± 14.3 |
| **LMM ANOVA** | | | | | | |
| | **Head order** | | 3.38 | 9.61 ** | 8.09 * | 18.36 ** |
| | **Year** | | 11.68 * | 11.39 * | 29.01 ** | 32.95 ** |
| | **Head order × Year** | | 2.23 * | 0.68 | 1.63 | 1.34 |

*$P < 0.5$, ** $P < 0.01$, *** $P < 0.001$.

**Table 3.** One thousand achene weight, actual, lost and potential achene weight across 2014 – 2016 and different head orders (means ± SE).

| Year | Head order | Samples (no.) | One thousand achene weight (g) | Actual achene weight (g head$^{-1}$) | Lost achene weight (g head$^{-1}$) | Potential achene weight (g head$^{-1}$) |
|---|---|---|---|---|---|---|
| **2014** | | | | | | |
| | **Primary** | 23 | 53.6 ± 1.5 | 11.8 ± 1.0 | 6.9 ± 1.0 | 18.7 ± 1.2 |
| | **Secondary** | 23 | 48.4 ± 2.2 | 9.3 ± 0.9 | 4.5 ± 0.4 | 13.8 ± 1.0 |
| | **Tertiary** | 21 | 43.4 ± 2.3 | 7.1 ± 0.9 | 1.3 ± 0.2 | 8.4 ± 1.0 |
| | **Quaternary** | 14 | 37.2 ± 2.0 | 4.1 ± 0.7 | 1.0 ± 0.2 | 5.1 ± 0. 8 |
| **2015** | | | | | | |
| | **Primary** | 12 | 56.4 ± 2.6 | 11. 9 ± 2.2 | 17.6 ± 3.6 | 29.5 ± 3.0 |
| | **Secondary** | 54 | 50.4 ± 1.5 | 11.6 ± 0.9 | 11.2 ± 1.0 | 22.8 ± 1.4 |
| | **Tertiary** | 69 | 44.5 ± 1.3 | 7.2 ± 0.5 | 9.4 ± 1.0 | 16.6 ± 1.1 |
| | **Quaternary** | 15 | 35.0 ± 2.4 | 5.7 ± 0.7 | 3.9 ± 0.7 | 9.6 ± 1.0 |
| **2016** | | | | | | |
| | **Primary** | 24 | 50.2 ± 1.9 | 15.0 ± 1.5 | 7.3 ± 1.7 | 22.3 ± 2.0 |
| | **Secondary** | 24 | 42.0 ± 1.9 | 12.7 ± 1.2 | 7.5 ± 0.9 | 20.2 ± 1.4 |
| | **Tertiary** | 22 | 36.4 ± 1.7 | 8.9 ± 1.2 | 4.9 ± 0.7 | 13.8 ± 1.1 |
| | **Quaternary** | 11 | 29.8 ± 2.6 | 4.9 ± 0.7 | 1.1 ± 0.3 | 6.0 ± 0.5 |
| **LMM ANOVA** | | | | | | |
| | **Head order** | | 28.83 *** | 24.38 *** | 14.43 ** | 44.65 *** |
| | **Year** | | 2.44 | 1.83 | 20.35 ** | 6.48 * |
| | **Head order × Year** | | 1.06 | 0.45 | 1.59 | 1.16 |

*$P < 0.5$, ** $P < 0.01$, *** $P < 0.001$.

**Table 4.** Head number of different order per cardoon plant across 2014 – 2016 (means ± SE).

| **Year** | **Number of head per plant** | | | |
|---|---|---|---|---|
| | **Primary** | **Secondary** | **Tertiary** | **Quaternary** |
| **2014** | 1.0 ± 0.0 | 3.9 ± 0.2 a | 6.2 ± 0.7 a | 3.7 ± 0.4 a |
| **2015** | 1.0 ± 0.0 | 3.6 ± 0.1 a | 5.1 ± 0.4 a | 2.5 ± 0.6 a |
| **2016** | 1.0 ± 0.0 | 2.9 ± 0.1 b | 3.4 ± 0.5 b | 1.0 ± 0.1 b |
| **ANOVA** | | | | |
| **F** | - | 8.88 *** | 7.00 ** | 10.09 *** |
| **df** | | 33 | 33 | 33 |
| **SED** | | 0.47 | 1.52 | 1.19 |

** $P < 0.01$, *** $P < 0.001$.

Figure 1. Cardoon bare receptacle scored for *Larinus* damage: damaged area was valued with the aid of a 360° protractor (after the removal of bracts, pappi, and achenes).

Figure 2. Effect of cardoon head order on receptacle damaged area (%), undamaged, damaged and total achene number per head (no.), one thousand achene weight (g), actual, lost and potential achene weight per head (g). For each variable mean ± standard error are reported. Different letters above the bars indicate significant differences at $p<0.05$ following ANOVA and LSD test for multiple comparison.

Figure 3. Effect of year on cardoon receptacle damaged area (%), undamaged, damaged and total achene number per head (no.), one thousand achene weight (g), actual, lost and potential achene weight per head (g). For each variable mean ± standard error are reported. Different letters above the bars indicate significant differences at $p<0.05$ following ANOVA and LSD test for multiple comparison.

Figure 4. Relationships between the undamaged achene weight and the total weight of the sampled heads (diamonds). The dashed line represents the linear regression model (equation and the $R^2$ reported). The black circles in line represent the expected achene weights calculated following Archontoulis *et al.* (2010b).

Figure 5. Three-year average of head number per plant of different order and actual, lost and potential achene yield per plant (g) across the three years.

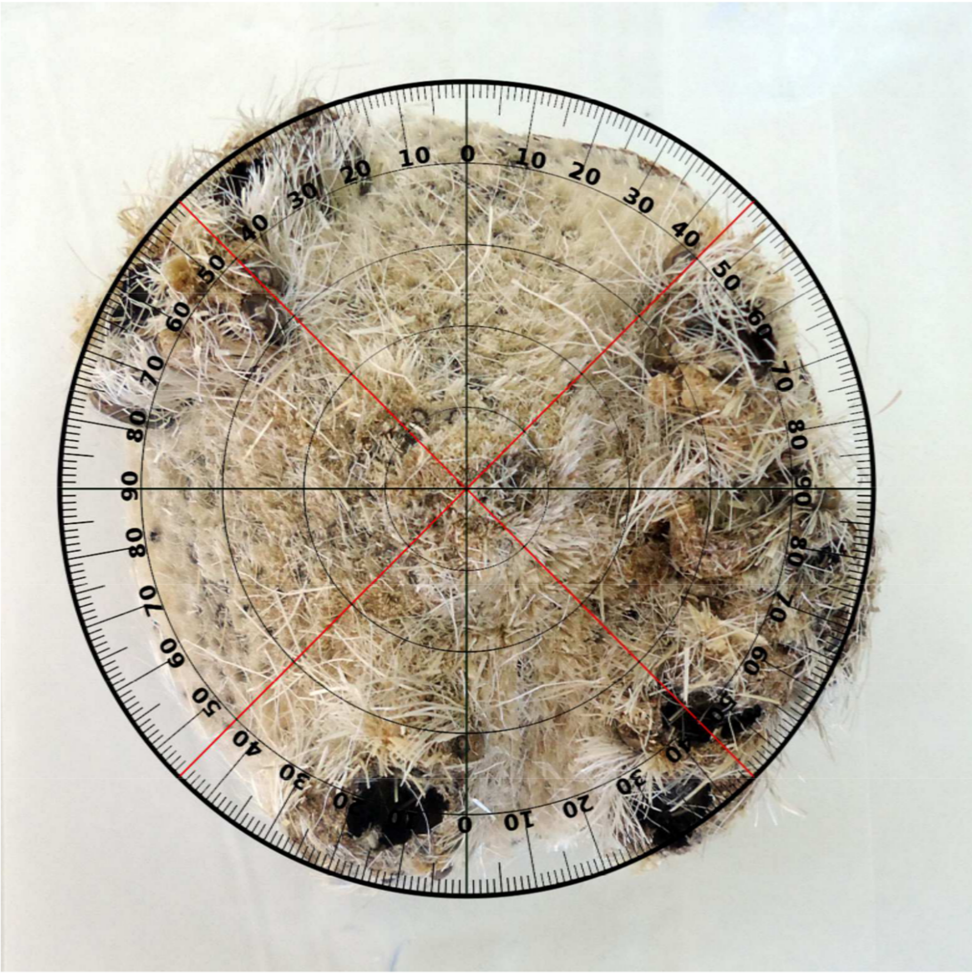
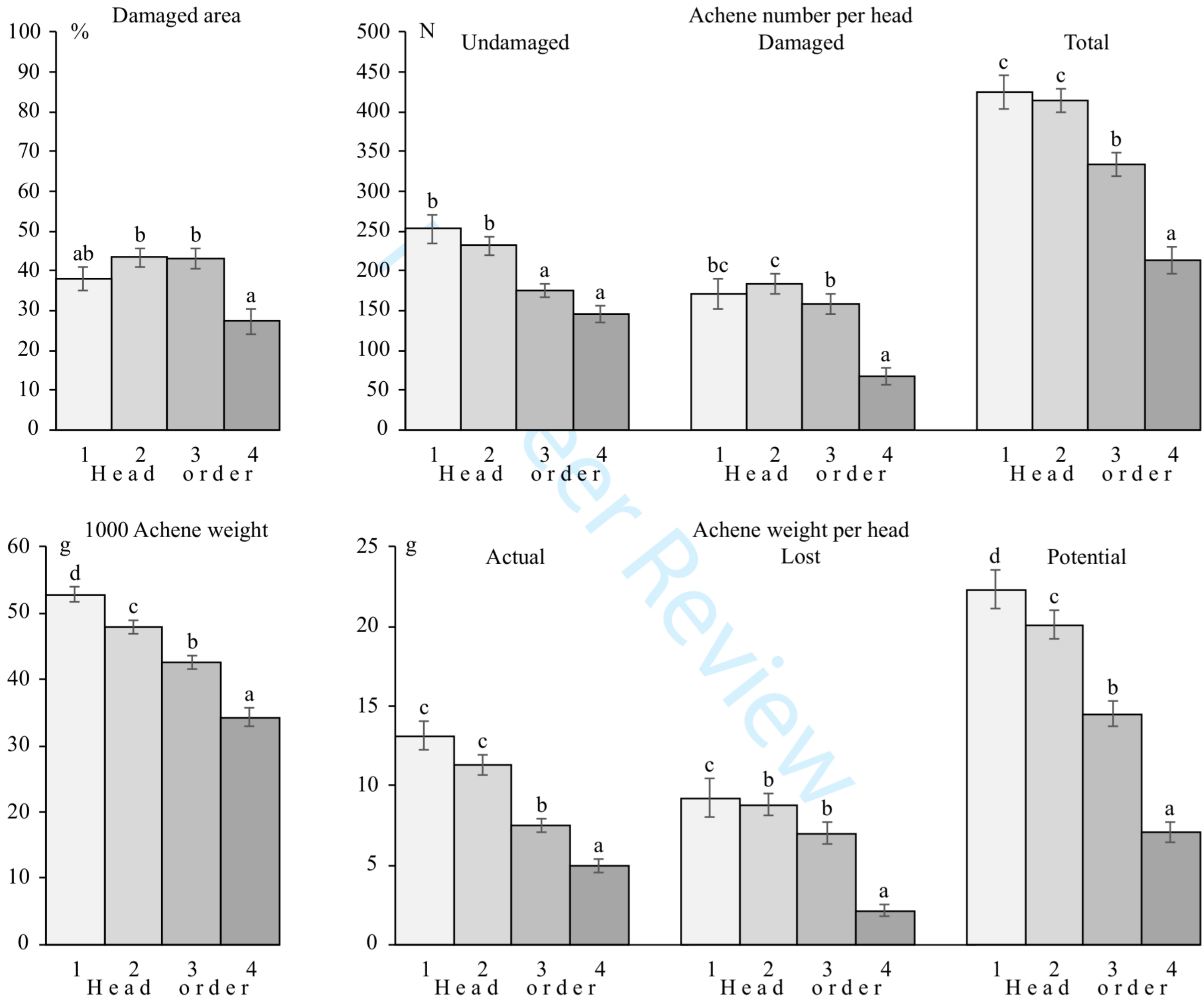
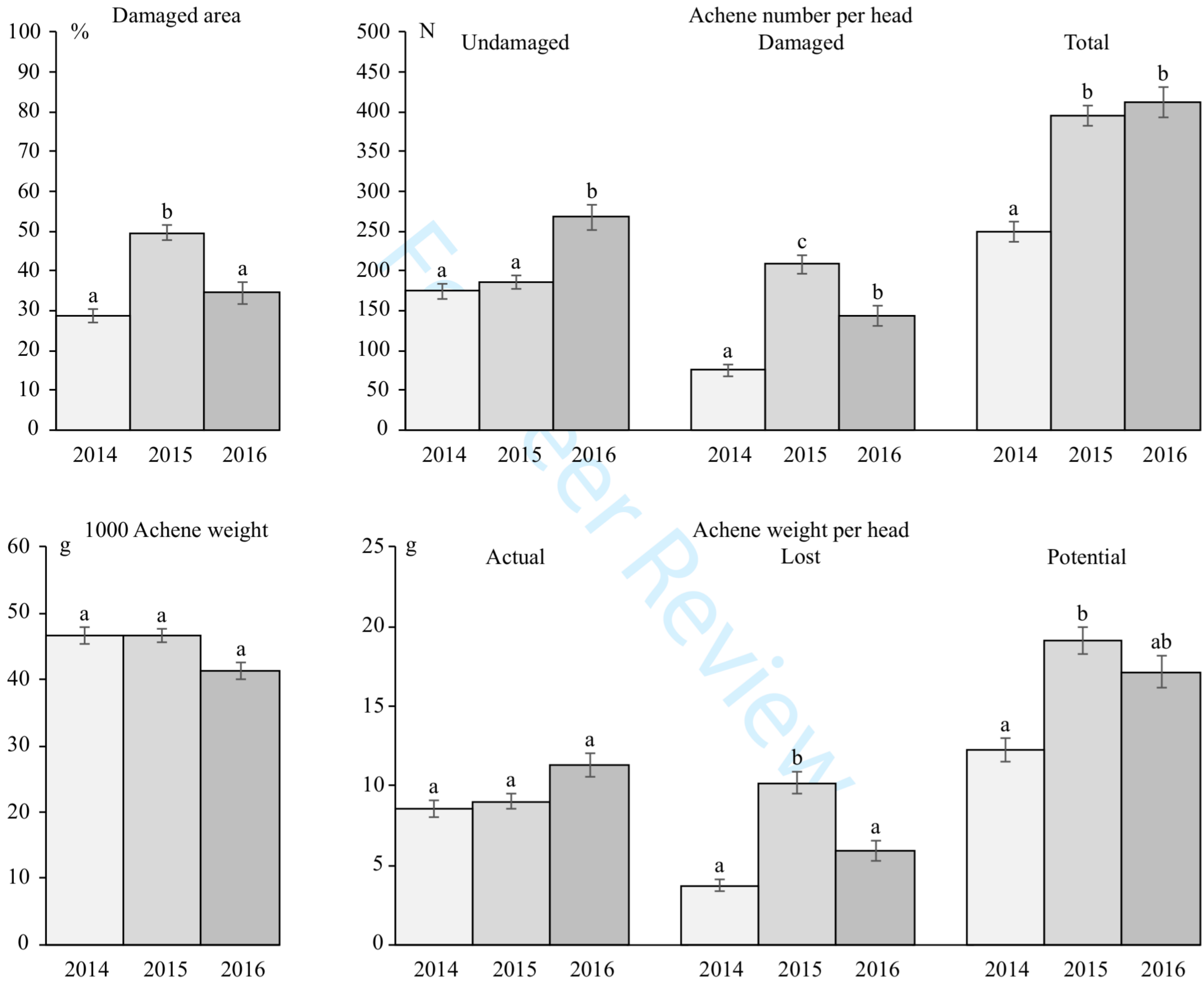


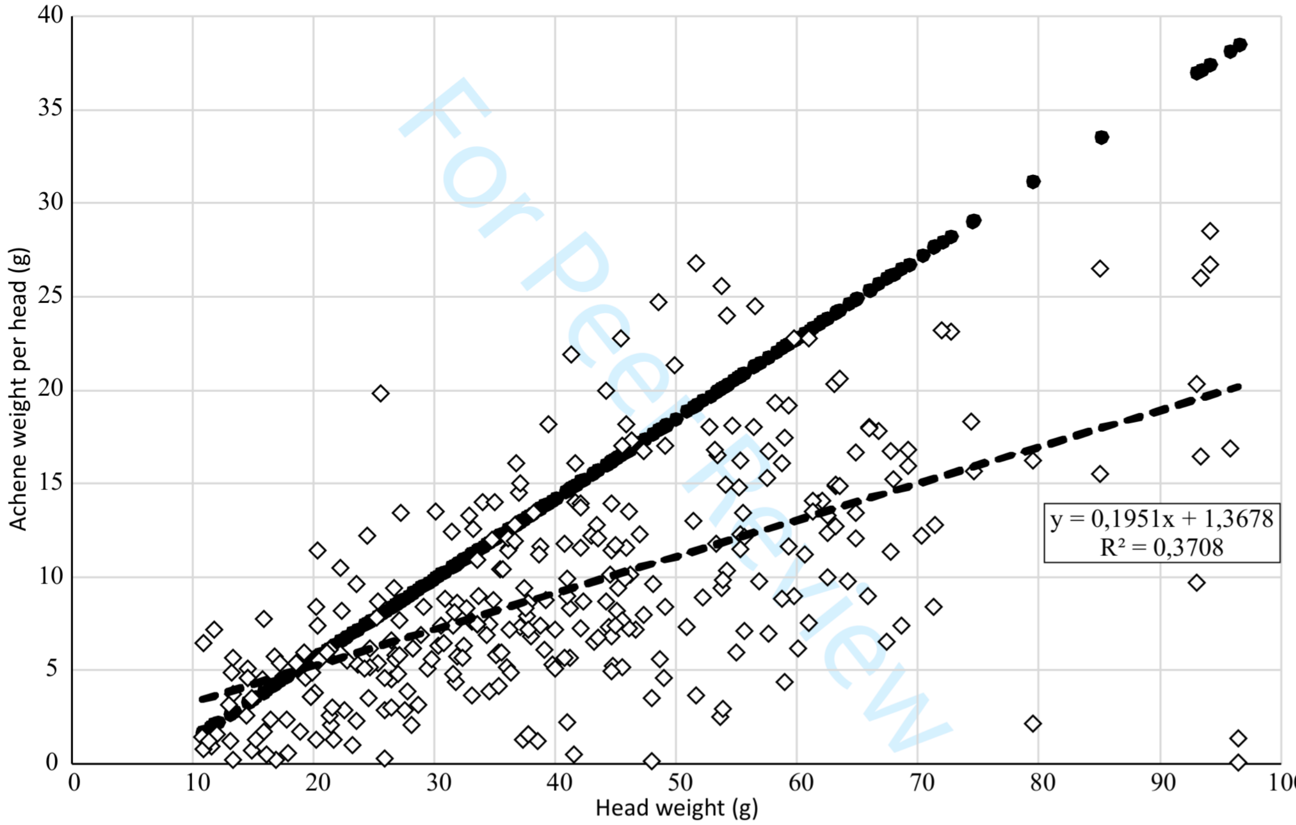

Figure 1

447x444mm (72 x 72 DPI)


Damaged area
%
Achene number per head
N
Undamaged
Damaged
Total
Head order
1000 Achene weight
g
Achene weight per head
Actual
Lost
Potential



Damaged area
%
100
90
80
70
60
50
40
30
20
10
0
2014
2015
2016
Achene number per head
N
Undamaged
Damaged
Total
500
450
400
350
300
250
200
150
100
50
1000 Achene weight
g
60
Achene weight per head
Actual
Lost
Potential
25
15
5
a
b
c
ab



Achene weight per head (g)
Head weight (g)
y = 0,1951x + 1,3678
R² = 0,3708
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
70
80
90
100

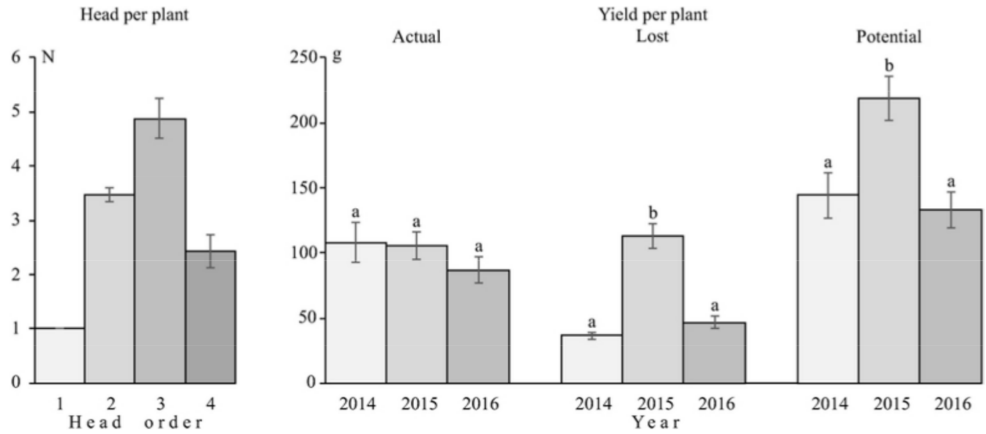



Figure 5

81x35mm (300 x 300 DPI)