SHORT COMMUNICATION

THE CASE OF THE 2014 CROP SEASON IN TUSCANY: A SURVEY OF THE EFFECT OF THE OLIVE FRUIT FLY ATTACK

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ABSTRACT

In this work we compare the chemical composition of olive fruits (*cv Moraiolo*) and the sensory and chemical quality of olive oils extracted in two crop seasons: the 2013 crop season, characterized by a very minor attack by the *Bactrocera oleae*, and the 2014 crop season, characterized by one of the strongest attacks of *Bactrocera oleae* in the last decades.
Results show that during the 2014 crop season the pulp/stone ratio, moisture, phenolic content, oil content, and sugar content were lower than in the 2013 crop season. Moreover, the olive oils from the 2014 crop season were characterized both by higher free acidity values, peroxide value and K_{232}, and by lower values of antioxidants (phenolic compounds and tocopherols). The olive oils from the 2013 crop season did not present any defects, while among those from the 2014 crop season, 41% were defective and did not fall into the extra virgin olive oil category.

Keywords: extra virgin olive oil, *Moraiolo* cultivar, olive degradation, olive fly, olive oil quality

1. INTRODUCTION

In recent decades, extra virgin olive oil (EVOO) has been attracting the interest of the scientific community, also due to its well-recognized nutraceutical and sensory properties (CECCHI *et al*., 2015). These properties go to define the quality of EVOO and are affected by various factors, one of which is the olive fruits' health status (MIGLIORINI *et al*., 2012).
One of the main risk factors for the health of olive fruits is related to the attack of pathogens and phytophagy, the most dangerous aggressor being the olive fly, *Bactrocera oleae* (PEROVIC *et al*., 2007; WANG *et al*., 2009; WANG *et al*., 2013).
In most crop seasons in the inland areas of Tuscany, attacks by the olive fly are scarce, so the effect on EVOO quality is low. Instead, in some crop seasons, olive fly attacks can affect up to 100% of the olive fruits in some areas, with very heavy consequences on the sensory and nutritional properties of EVOO.
Various parameters can be used to make a quantitative assessment of olive fly attacks. Among these parameters, active infestation only accounts for the stages of development of the larvae up to the second age and is used to establish the threshold beyond which a chemical treatment is justified (this threshold is 10% of active infestation). The total infestation takes into account all the types of infestation caused by olive fly and, therefore, can be better correlated to the variations in the chemical parameters of the olive fruits (CALECA *et al*., 2007). In fact, the main risk factors for the fruits' state of health are those that cause the rupture of the skin. The main consequences of such a rupture are: (i) exposure to oxygen of the olive fruits' chemical constituents; evaporation of water from the fruit no longer protected by the outer waxy layer; (ii) alteration of the metabolic processes of the fruit; (iii) attacks from exogenous and endogenous enzymes. Each of these phenomena affects the olive fruits' chemical composition, which can compromise the quality parameters of the EVOOs (SERVILI *et al*., 2007), as also stated in studies conducted on oils obtained by milling different mixtures of healthy and damaged olives on a laboratory scale (KOPRIVNJAK *et al*., 2010; PEREIRA *et al*., 2004; MRAICHA *et al*., 2010) or on oils from partially damaged olives in different industrial mills (GOMEZ-CARAVACA *et al*., 2008). The decrease in the oil quality mainly depends on: i) the kind of infestation, ii) the percentage of damaged fruits, iii) the cultivar, and iv) the fruits' stage of development (GUCCI *et al*., 2012).
The 2014 crop season was characterized by a very intense attack by the olive fly and, in Tuscany, almost all the olive plantations were totally damaged. One of the main reasons for this attack was the many climatic anomalies during the Fall 2013 - Fall 2014 period (LaMMA, 2013; LaMMA, 2014a; LaMMA, 2014b; LaMMA, 2015).
The main goal of this study was to represent the chemical composition of the olive fruits and the extracted EVOOs in light of the olive fruit fly attack in the 2014 crop season in Tuscany.

2. MATERIALS AND METHODS

2.1. Data relating to olive fly infestation

Data relating to active infestation and total infestation were collected from the Azienda Agricola Buonamici organic farm (Fiesole, Florence, Italy) by AgroAmbiente (2014), a Tuscany regional government website which collects data on olive infestation.

2.2 Olive fruit samples

During the 2013 and 2014 crop seasons 10 olive trees of both the *Frantoio* and *Moraiolo* cvs were selected from the Azienda Agricola Buonamici organic farm (Fiesole, Florence, Italy) and Fattoria Altomena (Pelago, Florence, Italy). Olive fruit samples, of approximately 500 g, were collected from these plants on a weekly basis: from September 8 to November 24 in 2013, and from September 1 to October 27 in 2014. All the samples were analyzed as soon as they were delivered to the laboratory.

2.3 Oil samples

The oil samples consisted of olive oils produced in Tuscany, mainly in inland areas, which were analyzed by the Laboratorio Chimico Merceologico (Promofirenze, Special Agency of the Florence Chamber of Commerce, Florence, Italy) in the 2013 and 2014 seasons. The oils were from the same farms in the two crop seasons, with the exception of the farms which did not harvest the olives in 2014 because they had been completely ruined by the olive fly.

2.4 Chemical analyses on the olive fruits

The total phenolic content, water content and oil content in the olive fruit samples were evaluated as previously reported (CECCHI *et al.*, 2013).

One hundred olive fruits were weighed and pitted to calculate the pulp/stone ratio. The stones were weighed, and the pulp weight was calculated as the difference between the weight of the whole olive fruit and the weight of the stones. The pulp/stone ratio was calculated by dividing the pulp weight by the stone weight.

To measure the sugar content of the olive fruits, eight grams of olive paste were cold extracted (6 ± 2°C) with distilled water in a 200 ml flask for 2 hours. The content of the flask was filtered through paper and 10 ml of the solution obtained were diluted with water in a 20 ml flask. The sugar content was analyzed with an enzymatic method using the ChemWell automatic analyzer (Awareness Technology, ChemWell 9210, Palm City, FL). Three enzymatic kits were used to measure respectively (i) the sum of two monosaccharide contents, namely glucose and fructose; (ii) the disaccharide sucrose content; and (iii) the polyol mannitol content. All the kits were purchased from R-Biopharm (Darmstadt, Germany). Measurements were performed by means of external calibration standards: fructose and glucose (purity > 99%, Sigma Aldrich SrL, Milan, Italy), and mannitol (purity > 98%, Sigma Aldrich Srl, Milan, Italy). The results provided by the instrument were expressed in g/l; they were also converted into sugar content on a dry matter basis (g/kg dm) as the average of two readings carried out for each sample. The sucrose contents were determined by multiplying the difference between the sum of glucose, fructose, and sucrose contents, and the sum of glucose and fructose contents, by 0.95.

2.5 Chemical and sensory analyses on the olive oils

The free acidity, peroxide number, and UV spectrophotometric indices (K_{232}, K_{270}, ΔK) of the oil samples were determined according to the analytical methods (EEC Reg. 2568/1991).

The tocopherols of the olive oil samples were determined according to the ISO 9936:2006/Corr. 1:2008 analytical method (ISO 9936/2008), while the total phenolic compounds were quantified according to the COI/T.20/Doc. no. 29 analytical method (COI/T.20/Doc.29, 2009).

Sensory evaluation of the olive oil samples was performed according to EEC 2568/91 regulations and the following amendments (EEC Reg. 2568/1991). The form used for the sensory evaluation was developed according to the EEC 2568/91 regulation (EEC Reg. 2568/1991) and the COI/T.20/Doc. No 15/Rev. 8 February 2015 document (COI/T.20/Doc. 15/ Rev.7., 2015).

2.6 Statistical analyses

The F-Test was used for each parameter to test if the variances in the data from the two crop seasons were unequal. Statistical significance was determined using Microsoft Excel statistical software (t-test: two-sample assuming unequal variances and using two-tailed distribution). Results of $P < 0.05$ were deemed as differences.

3. RESULTS AND DISCUSSIONS

Table 1 compares levels of active and total olive fly infestation in the 2013 and 2014 crop seasons in Tuscany. As shown, in the 2013 crop season, the active infestation never exceeded three attacks per 100 olives, remaining below the threshold for chemical treatment (10 attacks per 100 olive fruits). Instead, in the 2014 crop season, at the end of July, the active infestation had already passed this threshold. Regarding the total infestation, it remained below six attacks per 100 olives throughout the 2013 crop season, while during the 2014 crop season it exceeded 100 attacks per 100 olive fruits.
To understand the effect of the attack on the olive fruits' state of health and on the quality of the extracted EVOO, it is enough to observe that at the peak of attack in the 2014 crop season, there was an active infestation of approximately 40% while the total infestation even exceeded 100%; this suggests that at least 60% of the fruits had an infestation due to the presence of exit holes or larvae older than the second age, with an obvious detrimental effect on the quality of the fruit itself.

Table 1. Levels of active and total olive fly infestation in the 2013 and 2014 crop seasons. Data are shown as number of attacks per 100 olive fruits.

	2013		2014	
	Active infestation	Total infestation	Active infestation	Total infestation
Jul 17	-	-	4	4
Jul 23	0	0	6	6
Jul 31	0	0	12	12
Aug 07	0	0	15	16
Aug 14	0	0	5	18
Aug 20	0	0	9	22
Aug 27	1	2	13	30
Sep 04	1	2	20	47
Sep 11	1	2	26	72
Sep 18	3	5	32	79
Sep 25	3	3	36	88
Oct 02	3	6	40	95
Oct 09	2	3	42	111

Many factors seem to have contributed to the intense olive fly attack in Tuscany in the 2014 crop season. First, the 2013 crop season was a year of vegetative charge, as a consequence, in some cases the harvest was not completed; while the 2014 crop season was a year of vegetative discharge. Second, Tuscany was affected by major climatic anomalies during the period of Fall 2013 - Fall 2014. The mild winter temperatures meant that the flies could overwinter. Therefore, they were able to lay their eggs on the olive fruits remaining on the ground, especially in the olive groves where the harvest in 2013 had not been completed; in addition, the June "heat wave" limited the olives' productivity, due to the concomitant start of the fruit set. Finally, the mild temperatures combined with the high summer rainfall created the perfect precondition for a wide and rapid spread of the fly from generation to generation (LaMMA, 2014b).

Figures 1 and 2 show the chemical content trends during ripening of the cv *Moraiolo* olive fruits from Azienda Agricola Buonamici in the two crop seasons. The data regarding the cv *Frantoio* olives and the olives from Fattoria Altomena gave the same conclusions, so they are not shown in this short communication.

In the 2014 crop season the pulp/stone ratio did not increase during the ripening period, in contrast to what was observed in 2013 (Fig. 1). This may be due to several effects of the olive fly attack, such as the modification or impairment of the growth processes of the fruit and decrease in pulp owing to the trophic effect caused by the parasite. The natural consequence of the lower contribution of the pulp to the total weight of the olive fruit was a lower moisture content in the olives (Fig. 1). The moisture contents were comparable until September 22, when the pulp/stone ratio in the two olive oil seasons was not very different. After that date, the moisture in the 2014 crop season plummeted dramatically to values of up to 30% less than in 2013.

The sugar content of the olives was also affected by the olive fly attack (Fig. 2). In the 2013 crop season we can observe a sugar content of 40-45 g/kg of dry matter at the optimal ripening time (MIGLIORINI *et al.*, 2011; CECCHI *et al.*, 2013). Instead, in the 2014 crop season (October 22) the sugar content was already less than 10-15 g/kg of the dry matter. This drastic reduction may be due to both to the parasite compromising the biochemical maturation process and the evident loss of pulp.

A similar effect was seen for the oil content of the olive fruits (Fig. 2) and, consequently, the theoretical yields in the mill. In October we observed a break in the biochemical phenomenon of oil accumulation, together with a removal of pulp due to the fly's trophic activity.

Finally, the phenolic content of the 2014 crop season was always lower than that of the 2013 crop season, reaching a difference of 35% at the end of October (Fig. 2). This was probably due to the combined effect of the fly attack and the high water availability (PANNELLI *et al.*, 1994) in the 2014 crop season.

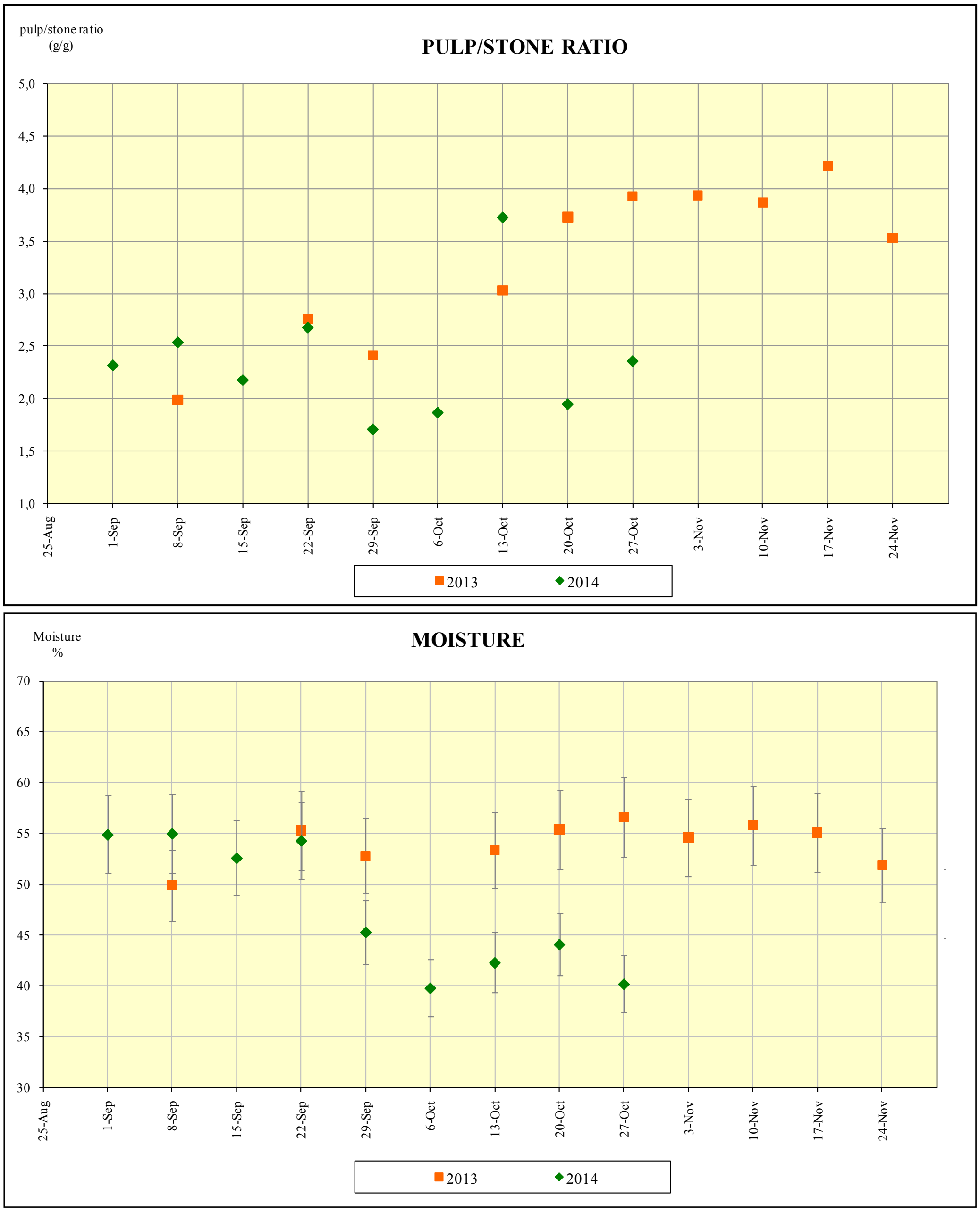

Figure 1: Pulp/stone ratio and moisture content trends in cv *Moraiolo* olive fruits during ripening in the 2013 and 2014 crop seasons.

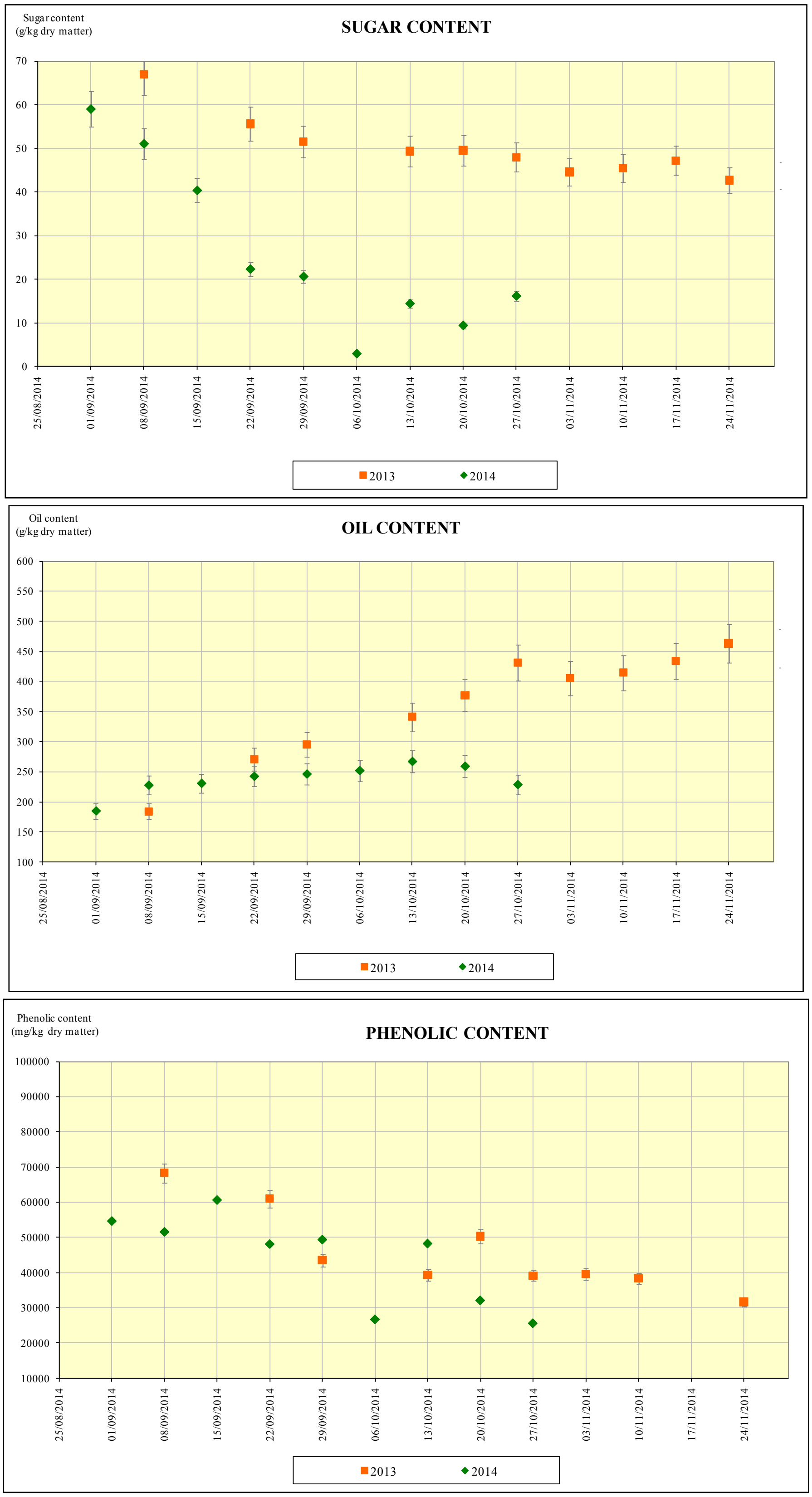

Figure 2. The chemical content trends in cv *Moraiolo* olive fruits during ripening in the 2013 and 2014 crop seasons.

The olive fly attack had a strong negative impact on the oil quality. Table 2 shows the results of the parameters used to describe oil quality. In the 2014 crop season, the olive fly attack, causing the oil to come into contact with the water in the olive fruits and then with the exogenous and endogenous lipases, may have induced the hydrolytic degradation of the triglycerides, with a clear increase in free acidity. Consequently, in the 2014 crop season, as much as 16% of the analyzed oils was found to be outside the legal limits which oil must respect to be classified as extra virgin (EEC Reg. 2568/1991), while in the 2013 crop season only one oil sample was found to be outside the legal limit of 0.80% free acidity.

The attack of the olive fly may have caused the fatty acids' exposure to strong oxidative conditions; hence, a sharp increase in both the peroxide number and the value of K_{232} occurred.

Table 2. Results of the parameters used to describe oil quality in the 2013 and 2014 crop seasons. For the mean value of each parameter, columns with letters indicate significant differences at $P < 0.05$.

	Free Acidity (%)	Peroxide number (meqO_2/kg)	Tocopherol content (mg/kg)	Phenolic content (mg_{tyr}/kg)	K232	K270	ΔK
2013 Crop Season							
Number of samples	356	354	202	212	235	235	235
Mean value	0.21 a	5.2 a	277 a	372 a	1.74 a	0.13 a	0.000 a
Maximum value	0.91	19.8	616	817	2.30	0.20	0.007
Minimum value	0.11	1.4	173	122	1.24	0.08	0.001
Standard deviation	0.08	2.08	67	128	0.15	0.02	0.001
2014 Crop Season							
Number of samples	221	212	35	52	76	76	76
Mean value	0.51 b	7.8 b	202 b	330 a	1.90 b	0.16 b	0.004 b
Maximum value	4.21	29.2	352	637	2.62	0.53	0.053
Minimum value	0.11	2.6	120	53	1.56	0.08	0.000
Standard deviation	0.39	3.5	49	140	0.22	0.07	0.008

Regarding the antioxidant components of the oil, the tocopherols were also compromised by the infestation, while the decrease in phenolic content was not statistically significant, confirming the unclear correlation between the % of fly attack and the phenolic content reported previously (GOMEZ-CARAVACA *et al*. 2008).

Regarding the oil sensory analysis, during the 2013 crop season all of the oil samples were free from defects, so they fell into the extra virgin olive oil category. In the 2014 crop season, instead, due to the major olive fly attack, 41% of the analyzed oils were defective, so they did not fall into the extra virgin olive oil category. Among these, 29% could be categorized as virgin olive oils, while the remaining 12% fell into the "*lampante*" olive oil category.

It also has to be taken into account that several farms did not harvest the completely ruined olives in 2014. This probably caused an underestimation of the negative effects of the olive fly attack on the oils from this crop season in Tuscany.

In conclusion, this research confirmed that the olive fruit fly is a strong risk factor for the quality of olive oil. A strong infestation by the olive fly causes a significant degradation in the chemical properties of the olive fruits and, as a result, poor oil quality.

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