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Olive fly management with allowed formulations in organic agriculture

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Imadeddine Rouini (Morocco)

Olive fly management with allowed formulations in organic agriculture

Abstract

The olive fly is a serious pest in the Mediterranean. Copper is used to manage it in organic groves, but its use is restricted. Therefore alternative products should be utilized.
This work carried out in 2007-2008 in three Apulian olive groves on Coratina and Termite di Bitetto cultivars aimed at testing: i) copper oxichloride, kaolin and propolis effectiveness, ii) their influence on oil quality, and iii) their residues of trace elements in olive fruits and oil.
Infestation level and quality criteria such as acidity, peroxide index, K_{232}, K_{270}, ΔK, and panel test were evaluated. Trace elements were determined by Inductively Coupled Plasma Mass Spectrometry.
Results showed that tested products were able to limit infestation and to produce high quality olives and oils. Aluminum, copper and silicon contents monitored in the fruits and oil did not show any significant risk for consumers.

Keywords: olive fly, organic farming, copper, kaolin, propolis, quality, trace elements.

La gestion de la mouche de l'olive avec les formulations admises en agriculture biologique

Résumé

La mouche de l'olive est un insecte important en région méditerranéenne. Le cuivre est employé pour la lutte dans les oliveraies biologiques, mais son application est soumise à des limitations.
Les travaux effectués en 2007-2008 dans trois oliveraies des Pouilles sur les cultivars Coratina et Termite di Bitteto visaient à tester : i) l'efficacité de l'oxychlorure de cuivre, de la kaolinite et de la propolis, ii) leur influence sur la qualité d'huile, et iii) leurs résidus en éléments-traces dans les olives et les huiles.
Le niveau de l'infestation et les critères de qualité tels que l'acidité, l'indice de peroxyde, K_{232}, K_{270}, ΔK, et le panel test ont été évalués. Les éléments-traces ont été analysés par ICP- MS.
Les résultats ont montré que les produits testés ont été en mesure de limiter l'infestation et de produire des olives et les huiles de haute qualité. L'analyse des résidus d'aluminium, de cuivre et de silicium dans les olives et les huiles n'a pas révélé de risque significatif pour les consommateurs

Mots-clés: mouche *de l'olive, agriculture biologique, cuivre, kaolinite, propolis, qualité, éléments-traces.*

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Dedication

I would like to thank my family, for their invaluable support, inspiration and love.

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Sisters
And brothers

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And of course to all

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List of abbreviations

%	Percentage
µl	Micro litter
°C	Degree celsius
µg	Microgram
A	Adult
AAS	Atomic Absorption Spectrometry
AI	Active Infestation
Al	Aluminum
B	Bactrocera
BRC	Bureau Communautaire de Réference
cm	Centimeter
conc	Concentration
CRA	Istituto sperimentale per l'olivicultura
CRM	Certificate Reference Materials
Cu	Copper
cv.	Cultivar
E1%1 cm	The extinction of 1% solution of the fat in the specified solvent, in a thickness of 1 cm
ECN42	Equivalent Carbon Number 42
CEE	Commission of the European communities
Fig.	Figure
g	Gram
Ha	Hectare
HNO_3	Nitric Acid
HPLC	High-performance liquid chromatography
IAMB	Mediterranean Agronomic Institute of Bari MAIB
ICP	Inductively Coupled Plasma
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
INRA	Institut National de la Recherche Agronomique
IOOC	International olive oil council
ISMEA	Istituto di Servizi per il Mercato Agricolo Alimentare
Kg	kilogram
L	larvae
LOD	Limits of detection
Md	Median of defects
mEq O_2/kg	Milliequivalents of active oxygen per kilogram

Mf	Median of fruity
min	Minute
mL	Milliliter
mm	Millimeter
MRL	Maximum Residues Limit
MS	Mass Spectrometry
MT	Million tonnes
MΩ	Milliohm
N A	Not Available
ng	Nanogram
nm	Nanometer
NR	Non detectable
OPSAPM	Osservatorio permanente sul sistema agroalimentare dei paesi del mediterraneo
EC	European commission
PC	Personal computer
PDO	Protected Designations of Origin
Si	Silicon
T	Tonne
TI	Total infestation
Trt	Treatment
USA	United States of America
v/v	volume to volume
V	Volume

1. Introduction

1.1. Statement of the problem

The olive fruit fly (*Bactrocera oleae* (Rossi), 1790), formerly *Dacus oleae*, is a serious pest of olives in most of the countries around the Mediterranean sea. In fact, about 30% of olive lost has been evaluated owing its attack, especially in Greece and Italy where large commercial production occurs (Weems and Nation, 2003).Damages are due to the quantitative (premature fall of the fruit and destruction of the pulp by grub) and qualitative (increases the acidity of olive and causes off-flavours, increase the peroxide index value, decreases total phenols content, while oviposition stings destroy the value of table fruit) damages caused by the different stages of the insect development.
Organic farming aims at reaching a sustainable development by lowering the chemical input. Copper is becoming more and more restricted in organic farming from the 1st January 2006 according to the annex II of the EU regulation N° 2092/91. Therefore, alternatively, organic pesticides or other tools are required to substitute copper or to minimize its use.

Kaolin is usable in organic farming as a biostimulant and its use against insects and parasites as dust of rock has been tested and proved. The powdery film formed by Kaolin on plants may prevent insects from identifying a host crop and consequently insects do not land, feed or lay eggs on the host crop. The coating may also cause insects to deem the fruit or leaves unsuitable.
The use of cupric products as antibacterial was recently introduced with a good effectiveness against *Bactrocera oleae* also in organic farming. This action arise from the symbiosis interruption between the insect and some of the bacteria present on the olives phylloplane, which are vital for the survival of the larva (Iannotta *et al*. ,2007b; Belcari *et al*., 2008).
Recently, studies have also been performed on the use of propolis, a resinous substance also credited to have antibacterial property. These active substances have already demonstrated their effectiveness against active fly infestation, inhibiting the development of the pre-immaginal stages of olive fruit fly (Iannotta et al., 2007a; Iannotta *et al*., 2007b).

The environmental impact of this kind of products remains an important issue in organic olive growing as well as for the consumer's health. Therefore, the use of copper oxichloride should not result in the occurrence of copper in edible oils and table olives above a critical level. For this reason, their content should be monitored. Concerning kaolin-based products, the active ingredient kaolin is a common soil inorganic constituent and it is not uptaken by plants. Therefore, methods for residue analysis of plants and plant products are not required (Pest Management Regulatory Agency, 2003; European community, 2008).

Consequently, in our study, it was necessary to monitoring trace elements resulting from kaolin in order to evaluate mainly: i) how and how much olive oil or table olives could be affected and ii) if there is an eventual risk or relationship with olive oil quality alteration.

Organic oil is by definition a virgin olive oil produced by pressing fresh olives cultivated with organic farming procedures. It is a high value-added product and highly supervised by European regulations. The classification of virgin olive oils takes into account the physical and chemical criteria but also the organoleptic characteristics of oils, in order to ensure to consumers a good quality product, particularly through sensory analysis. For the assessment of these characteristics and criteria the analyses have been carried out in CRA Research Centre for Olive Growing and Olive Industry in Rende (Cosenza) Italy, where the organic olive oil quality was evaluated according to the International Standards.

Accordingly, this study will present a work done in southern Italy, in three olive groves of Apulia region situated in the communes of Bitetto, Barletta and Conversano in Bari province. The farms involved in this research produce two cultivars of olive, Coratina oil cv. (in Barletta and Conversano) and Termite di Bitetto table olive cv. (in Bitetto). Both cultivars were managed under organic farming. The climatic conditions are different and can have an effect on the dynamics of *Bactrocera oleae* population but the challenge for those Apulian farms remains to control the olive fruit fly.

1.2. Objectives

The main objectives of the present study are:

- Evaluating the effectiveness of two kaolin commercial formulations and propolis with respect to copper oxichloride for the control of *Bactrocera oleae* (Rossi);
- Evaluation of the influence of those formulations on the physical and chemical criteria of two olive varieties (Coratina and Termite di Bitetto) but also on the organoleptic characteristics of their oils;
- Monitoring of the trace elements (aluminium, silicon and copper) resulting from the formulations use in olive drupes and oils.

2. Literature Review

2.1. Historical overview and distribution of olive tree "*Olea europaea* L."

Today the olive plant (O*lea europaea*, var. sativa) is distributed throughout the countries of the Mediterranean basin, particularly in the central and southern areas of Spain and Italy, and Greece, Turkey, Tunisia, and Morocco. The present situation is a result of slow but continuous development of the various civilizations in the coastal territories of the Mediterranean and more inland areas of the Middle East (Harwood and Aparcio, 2000).
The most ancient civilizations in the history of man have left clear evidence that cultivation and oil production were adopted. This longevous tree characterizes many landscapes, even since the Greek and later in Roman Age. For the residents of the Mediterranean, olive oil constituted the main source of nutritional fats, their most valuable export product, and was identified with their culture (Harwood and Aparicio, 2000).

2.2. Importance and challenges of olive growing

Today, olive cultivation in the Mediterranean is an additional income source and supports the population in rural areas during the winter period, which profit from summer and sea tourism activity. Although an agro-ecosystem, the olive grove resembles the natural Mediterranean ecosystem and abandonment transforms them into natural Mediterranean type forests. Their change of use from olive cultivation to pasture degrades the ecosystem and decreases the natural resources, because of over-grazing. At this time, two major factors threaten the traditional olive cultivation (i) the competition of the intensive olive groves in plain and irrigated areas and (ii) the cheaper seed-oils, which intensify the abandonment of traditional olive groves and change them into pasture, resulting in the deterioration of the ecosystem. Olive cultivation has left its mark on life in the Mediterranean and has contributed to the sustainability of natural resources (Loumou and Giourga, 2003).
Nevertheless, it succumbs under the pressure of current socioeconomic situations. Today, the conservation of olives in production constitutes a necessity for the fragile Mediterranean ecosystems and a challenge for everybody involved (Loumou and Giourga, 2003) showing a large diversity of biological conditions. In many cases olive groves are among the arboreal agro-ecosystems that host the highest numbers of herbivorous and carnivorous arthropods (hundreds of species; more than 300 species of parasitoids)(Conti, 2007).

Thus, several authors agree in considering olive groves as important reservoirs for biodiversity. Indeed, groves may be conducted traditionally or with modern and intensive techniques, but still remain among the cultures that produce lowest environmental impact.

2.3. Olive production

2.3.1. World olive production

International olive production is currently 7,439,300 tons: Italy contributes to this amount with little less than 8% while Spain is by far the most relevant country both from its surface-related profile and olive growing areas amounting to about 1.7 million tons per year.
With its 580,000 t, Italy is also approached by Greece, whose production exceeds 455,000 t of good quality olive (Deidda *et al*., 2006).
Outside the European area, Turkey, Tunisia and Syria have crops that, depending on harvests, can reach the entire Italian production as well as that of North Africa. Morocco, which benefits from a policy that fosters olive growing by means of its new plantation, is also becoming a "competitive" producer. Outside the Mediterranean, olive production is considered not relevant knowing the fact that its value does not exceed 25,000t (Deidda *et al*., 2006).
The olive tree area trends to be spread in the mild-hot areas, up to 30° latitude (north and south), in areas that are characterised by hot, dry summers. This "new" growing area will affect oil composition and organoleptic features future.
Olive planted areas are developing at international level, with present estimated increases up to 100,000 -120,000 ha/years. Due to this growth, olive growing areas are expected to increase up to 1-2 million ha in the next twenty years, as a direct consequence of the enlarged area (ISMEA, 2004).
The consumption trend is also developing at international level, but its growth probably will not be proportionate to the increased availability rate in the near future (Deidda *et al*., 2006).

2.3.2 Italian olive production

In Italy the olive cultivation is particularly massed in 4 southern regions and actually Apulia, Calabria, Sicily and Campania contribute to the national statistics with the 67.6% of the acreage, 79.9% of olive yield and 86.8% of olive oil. Apulia is the leading region in terms of olive acreage (31.9%) (Table1), olive fruit (36.9%) and olive oil production (39.7%). Olive groves cover about 370.000 hectares (i.e the 25% of the regional arable land). The number of olive trees is around 45 million and 99.4% of the total yield is utilized for oil making (Godini A., 2006).
As concerns the altimetry, 56.3% of the regional number of olive acreage is lowland, 42.7% hilly and only 1.0% mountainous. The regional number of olive farms is about 250,000 and the average acreage of single olive farm is 1.4 ha. In particular, farms smaller than 5ha are 84% whereas those ones larger than 20ha are 3.0% (Godini and Contò, 2004).
Along the 450km from the north-west to the south–east of Apulia, soil and climatic characteristics notably change as well as the varietal assortments, the training systems and the management techniques. It is not impossible to meet up trees aged about 10 centuries and more. With regards to the training systems, the cylindrical vase (open centre), high-headed is the common denominator of the local traditional olive growing (Morettini, 1972). Depending on the environmental conditions and varieties, there are several

variations in the regions and these variations end up by depicting the most intriguing aspect of the regional olive growing (Godini, 2002).

Table 1: Olive acreage and yield of the first four Italian regions, average 1999/2005 (Godini, 2006).

Region	Acreage		Olive production		Oil production		Yield/ha	
	Ha	%	(1000 MT)	%	(1000 MT)	%	Olive (t)	Oil(t)
Apulia	371,199	31.9	1,364,643	36.9	243,587	39.7	3.7	0.7
Calabria	185,922	16	1,064,424	28.7	197,724	32.3	5.7	1.1
Sicily	158,585	13.6	303,629	8.2	50,513	8.2	1.9	0.3
Campania	71,902	6.2	223,705	6	40,308	6.6	3.1	0.6
Others	376,672	32.4	745,004	20.1	80,908	13.2	2	0.2
Total	1,164,279	100	3,699,405	100	613,040	100	_	

The Italian olive production scenario is dominated by 148 cultivars (Muzzalupo *et al.*, 2006). This is due to the long-standing tradition of millennia, particular ecopedological conditions and the tormented history of Italy, divided into many small states up until the second half of the nineteenth century. As a matter of fact, the Italian situation strongly differs from those ones in other olive growing countries, where a handful of cultivars (even one in Morocco, the “Picholine Marocaine”) meet the requirements of most olive production (Pannelli, 2005).
Each single variety requires specific canopy, pruning and harvesting management criteria, just as each specimen produces oil which is different and characteristic both due to the genotype and its interaction with the target location. A list of 255 cultivars reveals a lack of knowledge as well as incorrect orientations (Deidda *et al*, 2006). In the case of this study Coratina (olive oil cv.) and Termite di Bitetto (table olive cv.) were the treated cultivars. Some informations about these two cultivars are reported hereafter.

a. Coratina cultivar: Cultivar grown in the region of Apulia, with 60,000 ha in the province of Bari (Corato, Andria, Canosa, Molfetta, etc.) and 10,000 ha in the province of Foggia (Cerignola, Foggia, Manfredonia, Ortanova and Trinitapoli). We can find it also in the other regions of Italy with a significant presence (Lombardo, 2004).
Coratina is mainly destined to the extraction of oil. It is characterized by early production and high and constant productivity. It adapts easily to different climates and is characterized by a good rooting capacity. Its drupes are late maturing and have different sizes. Some years, the fruit is also used for green olives confectionery in brine. Its oil yield is high, with a strong presence of phenols (Perri *et al.*, 2002; Perri and Cavallo, 2007). It should be emphasized the particular tolerance of this variety for cold (IOOC, 2006).

b. Termite di Bitetto cultivar: The Termite di Bitetto cultivar seems homonym of the original town in the province of Bari where it is also known as Bitetto Apples. For over a decade the cultivar is spreading in the area of Bari then in other provinces of Apulia for the exclusive use for direct consumption (Ferrara *et al*., 1980).

2.4. Organic olive production

2.4.1. World organic olive

In Europe, permanent crops account for seven percent of organic agricultural land. More than half of this land is used for olives, followed by fruits, nuts, and grapes (Willer and Yussefi, 2006).
Growing olive oil and table olive under organic farming, including the biodynamic method, is found in all countries around the Mediterranean, including Jordan. The reasons for such situation are obvious: the olive tree is spread almost everywhere characterizes environments often difficult, rocky and drought, and olive oil is an essential component for the Mediterranean diet.
According to the latest data released by IFOAM (Willer and Yussefi, 2007) and other concerned by this study, the organic olive would extend for about 36,2000 ha, including the area under conversion. This represents 11% of the total organic area and almost 5% of the entire olive area of concerned countries (OPSAPM, 2008).
With regard to organic, countries where the organic oliviculture is particularly important (Table 2) are Jordan (100%), Tunisia (56%), Malta and Palestine (50%), Algeria (47%) and Cyprus (34%). With regard to the national olive area, Italy has the highest rate (9.2%), followed by Portugal (7.8%) and Spain (7.6%). The lack of some data, such as the organic olive grove area in France, or in Palestine, as well as the total absence of information on Libya, makes impossible an exact estimation. Overall, approximately 30% of the area cultivated with organic olive is in Italy, followed by Spain (25%), Tunisia (22%) and Greece (11%) (OPSAPM, 2008).

Table 2: Organic olive area by country (OPSAPM, 2008). **NA**: not available.

Country	A) Organic area (ha)	B) Organic olive groves (ha)	Total olive groves (ha)	B/A %	B/C %
Albania	1,170	70	28500	6.0	0.25
Algeria	887	416	239,350	46.9	0.17
Croatia	3,184	27	18000	0.8	0.15
Cyprus	1,698	576	14,830	33.9	3.88
Egypt	24,548	23	49,000	0.1	0.05
France	560,838	NA	18,340		
Greece	288,255	39636	784500	13.8	5.05
Israel	6,685	340	22,000	5.1	1.55
Italy	1,067,102	106,938	1,167,980	10.0	9.16
Jordan	10	10	64,520	100.0	0.02
Lebanon	2,465	475	58,000	19.3	0.82
Libya	NA	NA	200,000		
Malta	14	7	150	50.0	4.67
Morocco	20,040	100	504,700	0.5	0.02
Palestine	1,000	500	NA	50.0	
Portugal	233,458	28,152	360,000	12.1	7.82
Slovenia	23,499	7	780	0	0.90
Spain	807,569	91,485	1,199,090	11.3	7.63
Syria	205,500	5,000	500,000	24.4	1.00
Tunisia	143,099	80,016	1,500,000	55.9	5.33
Turkey	93,133	7,732	649,350	8.3	1.19
Total	3,299,154	361,510	7,379,090	11.0	4.90

2.4.2. Organic olive growing in Apulia

Apulia with over 47,638 ha (Fig. 1) of organic olive invested in 2006 is the first producing region in Italy, followed by Calabria and Sicily. Apulia, with yields more than double compared to the national average, provides approximately half the production of organic olives.The good performance of Apulian organic oliviculture is emphasized by the positive trend in the evolution of its surfaces, which since 2001 still characterizes the region. Traditional olive vocation of the region has enabled the development of organic farming, thanks to the conversion of existing olive orchards and high quality product allows to increase prices making the crop remunerative (Guario *et al*., 2007).

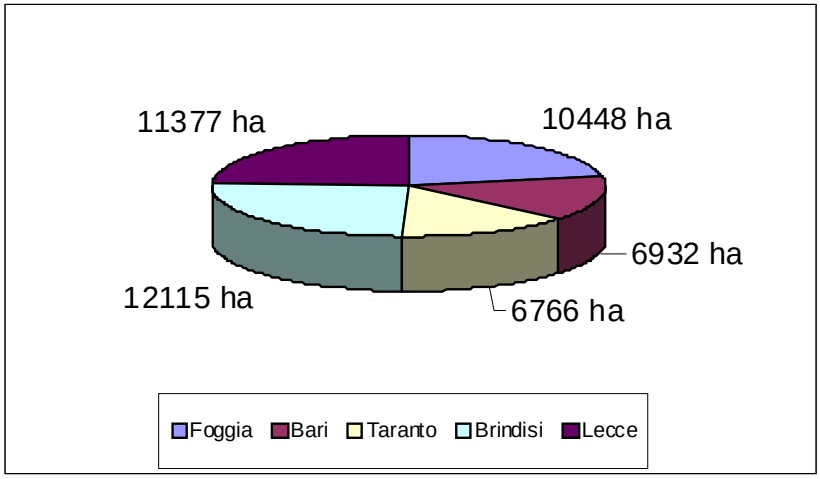

Figure 1: Organic farmland (ha) per province in Apulia (Guario *et al*., 2007).

2.5. Pests of olive tree in the Mediterranean region

The most important species in olive groves attack fruits (carpophagous) and/or flowers (antophagous), thus causing direct damages. However, several other species that attack other organs are able to cause indirect damages. Among them, depending on the cultivation area and climatic conditions, some sap-sucking species, leaf chewers and xylophages sometimes become economically relevant pests (Conti, 2007). The most common are listed in (Table 3), but many other secondary species are known.

Table 3: Primary pests and some of the most common secondary pests of olive groves in the Mediterranean region, related to the type of damage (Conti, 2007).

Pest status	Genus and species	Order: Family	Type of feeding	Organs attacked	Damage
Primary (key)	*Bactrocera oleae*	Diptera: Tephritidae	lacerating (larvae)	fruits	galleries
Primary	*Prays oleae*	Lepidoptera: Yponomeutidae	chewing (larvae)	flowers fruits leaves	erosions galleries mines
	Saissetia oleae	Homoptera: Coccidae	sap sucking (nymphs and adults)	leaves, twigs and fruits	sap loss, saliva inject. and honeydew
Secondary	*Liothrips oleae*	Thysanoptera: Phloeothripidae	cell sucking (nymphs and adults)	Buds, leaves, flowers and fruits	deformed leaves and fruits
	Euphyllura olivine	Homoptera: Aphalaridae	sap sucking (nymphs and adults)	shoots, leaves and flowers	sap loss, shoot wilt, honeydew and wax
	Parlatoria oleae	Homoptera: Diaspididae	sap sucking (nymphs and adults)	shoots, leaves, fruits, and twigs	sap loss, saliva inject. and fruit spotting
	Palpita unionalis	Lepidoptera: Pyraustidae	chewing (larvae)	Shoots and fruits	destroys shoots (grafting, nursery)
	Zeuzera pyrina	Lepidoptera: Cossidae	chewing (larvae)	Branches and shoots	borer
	Otiorrhynchus cribricollis	Coleoptera: Curculionidae	chewing (adults)	leaves, shoots	new plantations, grafts
	Phloeotribus scarabeoides	Coleoptera: Scolytidae	chewing (adults and larvae)	branches, twigs and buds	borer

2.6. Olive fruit fly (Diptera: Brachycera: Tephritidae)

2.6.1. Introduction

The olive fruit fly *Bactrocera oleae* (Rossi) is a serious pest of olives in most of the countries around the Mediterranean sea. The larvae are monophagous, and feed exclusively on olive fruits. Adults feed on nectar, honey dew, and other opportunistic sources of liquid or semi-liquid food. The damage caused by tunnelling of larvae in the fruit results in about 20-30 percent loss of the olive crop in Mediterranean countries, and especially in Greece and Italy where large commercial production occurs (Weems and Nation, 2003).

2.6.2. Identification

Adult: The adult olive fly is normally 4-5 mm long with large reddish eyes and small antennae (Fig. 2). The thorax is dark brown with 2-4 gray or black longitudinal stripes. The scutellum is yellow to white; there are also several yellow-white patches on each side of the thorax. The abdomen is brown with darker areas on the sides of each segment (this character is quite variable). The wings of olive fly are clear except for a small distinct black spot at the tips; wing veins may also be slightly dark. Olive fly does not have colored wing bands or patterns typical of many other species of fruit flies such as the Mediterranean fruit fly, *Ceratitis capitata* (Rice, 2000).

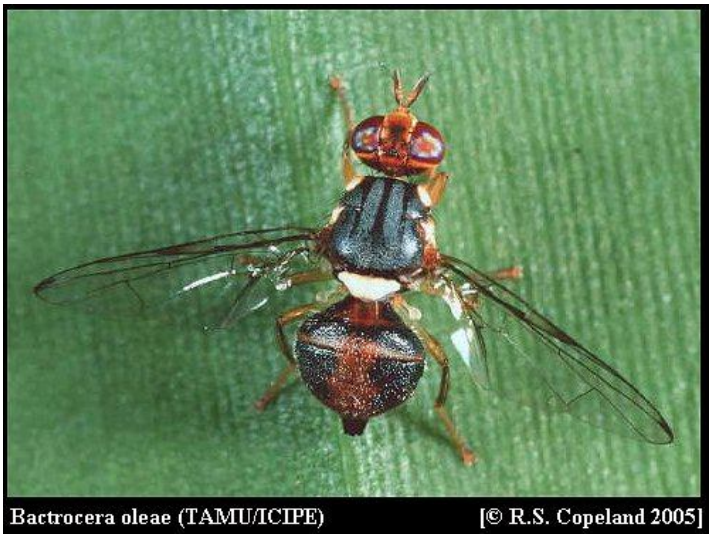

Figure 2: *Bactrocera oleae* adult (De Meyer *et al*., 2008).

Egg: The egg is elongated, smooth, very small, hardly visible with the naked eye, pearly white. Its dimensions are about 0.7 mm long and 0.2 wide (Cappello *et al*, 2008).

Larva: the larvae are white-yellow legless maggots (Fig. 3) with a point on one end (head) (Vossen *et al*., 2004).

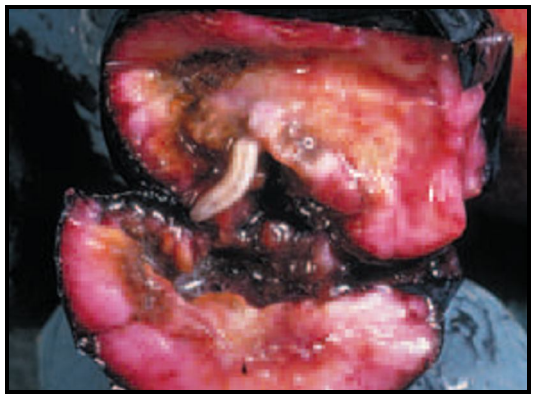

Figure 3: *B*. *oleae* larvae feeding on olive pulp (Vossen *et al*. 2004).

Pupa: The pupa is concealed inside the last larval exuvia, called puparium, which therefore is the only visible part. This is 3.5-4.5 mm long and shows an ellipsoidal shape (INRA, 2003), whereas its colour is light yellow initially; it slowly turns to brown with the pupa aging (Cappello *et al*, 2008).

2.6.3. Economic importance and damage

The adult female can lay 50-400 eggs in her lifetime, usually one in each fruit. These ones hatch into tiny larvae (maggots) that are very difficult to see until they feed for a while and get larger. While feeding, they tunnel throughout the fruit, destroying the pulp and allowing secondary infestations of bacteria and fungi that rot the fruit (Vossen *et al.*, 2006).
The damage caused by tunneling of larvae in the fruit results in about 30 % loss of the olive crop in Mediterranean countries, especially in Greece and Italy where large commercial production occurs (Economopoulos *et al.,* 1982 ; Athar, 2005). The damage is not only quantitative but also qualitative; it greatly increases the free fatty acid level (acidity), the number of peroxides and determines sterolic and phenolic fraction alteration of the olive oil causing off flavors. Feeding damage may cause premature fruit drop. Oviposition stings, caused by the female laying eggs inside the olive, destroy the value of table fruit (Vossen *et al*., 2006). The number of peroxides increasing and polyphenols decreasing considerably reduce conservation time. However, quality strongly depends also on harvest time and stocking duration. Heavily infested olives collected early and immediately processed may give an extra-virgin olive oil. Instead, especially when olives are stocked for several days, galleries in the fruit are quickly infected with fungi. Between the mould and galleries, oxidative damage also occurs, causing lower oil quality (Conti, 2007).

2.6.4. Distribution

This species is associated to plants of the genus *Olea* and, in particular, to olive. It is present throughout the Mediterranean basin in South Africa and in the countries where olive farming recently spread as United States (Table 4).

Table 4: World distribution of the olive fly, *B. Oleae* (Rice, 2000).

Albania	Italy	South Africa
Algeria	Jordan	Spain
Canary Islands	Lebanon	Syria
Corsica	Libya	Tunisia
Egypt	Mexico	Turkey
Eritrea	Morocco	United States
France	Pakistan	Yugoslavia
Greece	Portugal	
Israel	Sardinia	

2.6.5. Life history and population habit

In the Mediterranean region, two to five generations of flies occur yearly. The winter is spent in the pupal stage several cm below the soil and leaf litter, and adult flies emerge from March to May, depending upon the latitude and temperature. Under summer conditions, a preoviposition period of six to ten days elapses before mating, with longer time required earlier when temperatures are not as high. Adults feed on nectar, honey dew, and other opportunistic sources of liquid or semi-liquid food (Weems and Nation, 2003). Because this diet mostly has a low protein content, which is important for egg production and development, they are particularly attracted by materials with volatile nitrogen compounds (Conti, 2007). “Female olive fruit flies are oligogamous and mate 1-3 times during their life (Tzanakakis *et al*., 1968; Cavalloro and Delrio, 1971). Male olive fruit flies on the other hand are polygamous and they can mate daily if receptive females are available (Zervas, 1982). During the mating period sexually active males stridulate by fanning their wings over a pair of combs which forms bristles on the third abdominal tergite, the high frequency sound that is produced has been interpreted by Feron and Andriew (1962) as a mating stimulus. Haniotakis (1974 and 1977) demonstrated that sexually mature females attracted males during the mating period and he suggested that virgin females release an airborne sex pheromone which attracts males. The major female pheromone component was confirmed by Mazomenos *et al*, 1981 and Gariboldi *et al*. 1983, which is the 1.7-dioxaspiro[5.5]undecane. Male response to virgin females occurs in the late afternoon or at dusk” (Mazomenos, 1989).

Females can be distinguished from males by the ovipositor, a pointed structure at the end of the female’s abdomen (Collier and van Steenwyk, 2003). During the preoviposition period the female is maturing the ovary and a first set of eggs. Beginning in June, females actively seek and oviposit in early maturing olive fruits (Collier and Van Steenwyk, 2003). The female punctures the fruit with the ovipositor and deposits an egg beneath the skin.

From 10 to 12 eggs may be laid daily, usually one per olive fruit, and about 200 to 250 are laid in a lifetime (Weems and Nation, 2003).
The new-born, legless larva (maggot) feeds upon the fruit (mesocarp) tissue; it initially bores a superficial gallery which becomes deeper with larval development, reaching the stone (endocarp), which anyway is not injured as the larva does not enter into it. Such galleries may cause early fruit drop. In summer mature larvae pupate in fruit when these are green. Therefore, when close to the 3rd moulting, the III-instar larva move close to the fruit surface saving only the thin epidermis film, well visible from the exterior, and then pupates. The emerging adult breaks the film and flies away leaving an emerging hole. During late autumn and winter, instead, the fly behaviour changes, as the mature larva leaves the ripen fruit and pupates in the soil under the tree, where it spends the winter. Although the olive fruit fly does not have a true diapause, development is sufficiently slowed during the winter, so pupae produced in late fall do not emerge until the following spring (March to May). Depending on the environmental conditions, the olive fruit flies also overwinter as larvae in fruit and to a lesser extent as adults (Conti, 2007).
The life cycle of the olive fruit fly is closely linked to the seasonal development of its main host (Table 5), the cultivated olive (*Olea europaea*), and to the local climate (Zalom *et al*., 2003). The climate influence the cycle especially with temperature and to lower extent humidity. The duration of individual biological phases is summarized in the following table:

Table 5: Duration of individual biological phases of olive fruit fly (Cappello *et al.*, 2008).

Stage	Summer	Autumn-Winter
Egg	2-3 days	10 days (autumn)
Larva	10-13 days	20 days or more
Pupa	10 days	to 4 months (pupae wintering)
Adult	Even several months	

The size of the population varies during the year, but generally two peaks are found: the first one in full spring at the emergence adult wintering generation, the second one, more intense, early autumn when the olives present the highest level of receptivity, slightly lower temperatures and the climate becomes rainier. In Sardinia these peaks population usually occur in the months of April to May and September to October. The population dynamic is characterized by two peaks, a smaller peak in spring (April-May in Sardinia) with the adults emerging from the overwintering generation, and a higher peak at early autumn (September-October) when the olives show the highest level of receptivity, temperatures slightly decreases and the weather becomes rainier (Delrio, 1992).

2.6.6. Biotic and abiotic factors affecting populations

The severity of the fly attacks is tied to several factors, both intrinsic and extrinsic. The main natural factor is climate (temperature and humidity, but other genetic or agronomic factors are not to be neglected.

a. Limiting factors

Both high temperature regime and low humidity could have an impact on:

- The inhibition of ovarian maturation;
- Flight distance and duration of the flies;
- Abiotic mortality of *B. oleae*.

A series of experiment on the olives flies by (Fletcher *et al.*, 1978) in constant temperature cabinets indicated that high temperatures (i.e. 26–29°C) in conjunction with a low humidity (45 ± 50%) inhibited ovarian maturation, Examination of the ovaries of female olive flies from wild populations on Corfu (Greece) during the summer months of 1975 indicated that all were non-gravid for a period of several weeks during June and July and the terminal follicles were resorbed.

Regardless of the food and temperature conditions, both flight distance and duration of the flies decreased with increasing exposure time to high temperature regime. The high temperature (heat stress) could dramatically influence the fly's survival. It is thus critical for the fly to seek out food resource (water and carbohydrates), and refuge during summer.

Abiotic mortality of *B. oleae* is growing at values higher than 25 °C and is especially high above 37 °C up to become total in just a few minutes over the temperature of 43 °C. For adults it has been observed that, for temperatures between 38-43 °C, the availability of water increases the chances of survival, while according to Tremblay (1990) already at temperatures around 35 °C adults remain immobile in contact with moist surfaces. For example, at 40 °C adults who otherwise will die within two hours, can survive up to three days if they have water available. Values of very low relative humidity (20% in August) concomitantly with high summer temperatures lead to sudden spikes mortality of eggs and larvae (Delrio and Prota 1976; Pucci *et al*., 1985). The percentage of larvae mortality increases with the temperature and the decrease of relative humidity, so that at 38 °C and 22% of humidity, you have 90% of larval mortality.

With condition of low air humidity and high temperatures, adults block reproduction and expect more favourable conditions like late summer rains that do regain turgidity, make drupes more thick and the temperature fall below the thermic threshold of sexual organs maturation.

In irrigated oil and table olive groves, olive drupes are more susceptible than those ones conducted under non irrigated system, because more microclimate is humid more it reduces the harmful effects of high temperatures and also the mortality of eggs and larvae (Cappello *et al.*, 2008).

b. Genetic or agronomic factors

- Table cultivar or dual attitude: early and larger table olives are more susceptible than oil olives. Larval development is influenced by fruit size and pulp consistency, as well as the water content. Large fruits offer better protection against high summer temperatures, and high water content reduces risk of desiccation, therefore increasing larval survival. These characteristics describe most table olive varieties. Thus, the olive fruit fly has definite variety preferences (e.g., in Italy the variety Frantoio is less susceptible than Leccino; both are less susceptible than table olive

varieties). This could be important for oil producers who have multiple varieties to choose for planting;
- Another factor apparently unique is the relationship between the alternation of production. Infestations are often more serious during years of low production compared to those ones of high production (Conti, 2007);
- In irrigated oil and table olive groves, olive drupes are more susceptible than those conducted under non irrigated system, because more microclimate is humid more it reduces the harmful effects of high temperatures and also the mortality of eggs and larvae (Cappello *et al*., 2008).

c. Natural enemies:

Natural antagonists of olive fruit fly are several, but in most cases they fail alone to maintain attacks within the economically sustainable limits. Usually, these antagonists can break attacks when the fly population is low, while they are ineffective in the case of heavy infestations. The enemies of olive fruit fly are mostly predators and parasitoids (Cappello *et al.*, 2008).

The most important parasitoids are:

- *Eupelmus urozonus* Dalm. (Hymenoptera: Chalcidoidea: Eupelmidae): This is an ectophagous larval parasitoid of different insect species; it can be reared on the Mediterranean fruit fly, *Ceratitis capitata*.
- *Pnigalio agraules* (Walk.) (Hymenoptera: Chalcidoidea: Eulophidae): This is one of the most effective external larval parasitoid of the olive fruit fly.
- *Cyrtoptyx latipes* Rond. (Hymenoptera: Chalcidoidea: Pteromalidae): Also an external larval parasitoid, this species is less common.
- *Eurytoma martellii* Dom. (Hymenoptera: Chalcidoidea: Eurytomidae): Also an external larval parasitoid of the olive fruit fly.
- *Psyttalia* (=*Opius*) *concolor* Sz. (Hymenoptera: Ichneumonoidea: Braconidae): This is an endoparasitoid of the 3rd instar larva of the olive fruit fly, native of the subtropical area. This species can be reared on *Ceratitis capitata* and can be used in inundative biological control programmes.

A well known predator *of B. oleae* is *Prolasioptera berlesiana* Paoli (Diptera: Cecidomyidae). This midge oviposits in the lesion made by the olive fly, and the emerged larva preys on the olive fly eggs, being quite effective. However, this species has a curious status, as it is a beneficial because of its predation habits, but it is also a pest as the female during oviposition transmits the symbiotic fungus *Camarosporium dalmaticum* (Thüm) Zachos & Tzav.-Klon. Which develops in the fruit tissue. Probably the midge is more beneficial on the first summer generations, whereas should be considered as a pest in autumn.

Other important predators living in soil, such as predaceous beetles and ants, may also cause high mortality of larvae and pupae (Conti, 2007).

2.6.7. Damage thresholds

In Europe, the damage threshold for commercial table fruit orchards is 1% and the most commonly cited European damage threshold level for olive oil production is 10-15% (Iannotta *et al.*, 1995). However, research has shown that even with 100% of the fruit sustaining olive fly damage, extra virgin olive oil can be produced as long as the fruit shows no signs of rot. The real problem occurs when larval feeding introduces fruit rotting organisms that create off flavours. Since this is more likely to happen toward the end of the larval feeding cycle when the maggots get quite large; earlier harvest may be one of the options for dealing with this pest. It is also important to note that when olives are damaged by olive fruit fly, the fruit is more sensitive to oxidative and microbial breakdown, therefore the time from harvest to milling should be kept as short as possible (Vossen *et al.*, 2006).

2.6.8. Monitoring of olive fly

In order to maintain threshold levels at less than 1% damage for table fruit and at 10-15% damage for oil production, certain monitoring thresholds have been established in Europe, but these ones are based on the use of conventional insecticides usually applied with bait sprays. The yellow sticky trap is currently the monitoring standard in California, but the McPhail trap catches may actually be higher giving an indicative of early season population numbers. The monitoring is usually performed by using traps, fruit sampling, panel sticky traps (yellow or other color) (Vossen *et al*., 2004).

a. Panel sticky traps

Yellow sticky traps baited with a sex lure (spiroketal pheromone) and a food attractant (ammonium carbonate or bicarbonate) are used to capture both male and female adult flies. The bait packet and pheromone lure hang on the top edge of the trap. Hang the trap in the shade on the north side of a tree with fruit, inside the canopy with 8-10 inches of clearance from foliage. Traps can last from 1 to 8 weeks depending on how dirty they get (and thus how sticky they remain). Yellow sticky traps can also be used for mass trapping (Vossen *et al*, 2006).

b. McPhail traps (glass or plastic)

Plastic McPhail traps have been used in large numbers to reduce damage levels in organic olive oil orchards in Europe. They are difficult to manage and keep filled with the ammonium bait attractant (Varela and Vossen, 2003) because they dry out quickly in hot weather. The McPhail trap is used extensively for monitoring and for mass trapping. They have a reservoir for liquid bait and use torula yeast as the bait attractant. Flies enter from below and drown in the solution. McPhail-type traps tend to have the highest catches of all the common traps. They also require the most maintenance because of their tendency to dry out in hot weather. So, they require to be hung in the shade checking the water level frequently and the use of three torula yeast bait tablets per trap adding water to the fill line. Besides, they require the change of the solution at least once a month (Vossen *et al.*, 2006).

2.6.9. Control of *Bactrocera oleae* in organic farming

Control is based on a combination of tactics including agronomic, biological, biotechnological strategies and the use of natural pesticides (Table 7).

Table 6: Olive fruit fly management in organic farming (Iannotta, 2003).
A: adult, L: larvae.

Type of Control		Action on	Efficacy	Environmental impact	Cost	Observations
Agronomic	Choose of cultivar	-	Discret	-	-	It is required a better genotypes knowledge.
	Early harvest	-	Good	-	-	Increased efficiency with the delay of infestation.
	Increase of plant diversity	-	Discret	-	Low	Improvement of conservative biological control.
Biologic	Conservative control	L/A	Medium	-	-	
	Beneficial insects (*Opius concolor*)	L	Low	Low	Very high	Requires Biofactories for breeding. Applicable only in areas with defined perimeter.
	Bacillus thuringiensis	L/A	Low	Low	Medium /high	
Biotecnology	Mass trapping	A	Medium	Low	Medium	Effectiveness inversely proportional to population density.
	Miting desruption	A	Low	Low	Medium	It requires early repeated interventions in extensive territories
	Autocide	A	Low	-	High	Difficult application in practice.
Natural Pesticide	Protein baits with plant biocides (azadiractin pyrethrins, and rotenone)	A	Good	Medi-um	Medium	Requires early and repeated interventions; Effectiveness increases with the increase of treated area.

Other products are recently discovered to have an effect on the olive fly management in organic farming such as repellent products and those ones having antibacterial activities.
The use of repellent and ovipositional products for *B. oleae* (Rossi) control turns out to be a great topical interest in organic farming, because of the lack

of effective products able to kill the olive fly preimmaginal stages(Caleca and Rizzo, 2007).
From 1937 to 1953 Russo and some other entomologist (Russo, 1937; Russo and Fenili, 1950; Russo, 1954) tested the effectiveness of clay and Bordeaux mixture against the olive fly, obtaining a similar protection, suggesting their use for early ripening olives to harvest before autumnal rainfall.
Visual and chemical stimuli lead the female olive fly to oviposit into fruits (Katsoyannos and Kouloussis, 2001; Rotundo *et al.,* 2001; Solinas *et al.,* 2001); so the clay, especially white clays as Kaolin, disrupts ovipositing females, while copper salts through their antibacterial action make fruits less attractive to ovipositing females because of lack of some bacterial compounds on the surface of fruits (Tsanakakis, 1985; Belcari *et al.,* 2003). Furthermore the presence of the particles of those products on fruit surface could be another obstacle for the fruit recognition of female olive fly (Caleca and Rizzo, 2007).
"More recently some authors tested copper products (Prophetou-Athanaisiadou *et al.,* 1991; Belcari and Bobbio, 1999; Petacchi and Minnocci, 2002; Tsolakis and Ragusa, 2002) and Kaolin (Saour and Makee 2004; Iannotta *et al.*, 2006a; Pennino *et al.*, 2006; Perri *et al.*, 2006 Iannotta *et al*., 2007a; Iannotta *et al*., 2007b) against *B. oleae* obtaining interesting results" (Caleca and Rizzo, 2007). The aim of this research is to test the effectiveness of Kaolin against the olive fly, comparing it with copper hydroxide, in different conditions of infestation level.

a. Repellent and ovipositional (Surround®WP and Kaolin®)

Both Kaolin® (commercial name) and Surround®WP are kaolin-based products composed of 95 % of kaolin but differ on the 5% adjuvant components that remain.

Surround®WP: The particle film of surround is a highly refined kaolinic mineral [$Al_2Si_2O_5(OH)_4$], white in appearance and hydrophilic. Although it is not directly toxic to insect pests, its insecticidal properties are thought to be a result of its repellent nature, anti-ovipositional qualities and/or due to its highly reflective white coating. In practice, when Surround is sprayed onto tree foliage as a liquid suspension, water evaporates leaving kaolin as a white porous protective powdery film on the surface of the leaves and fruits (Saour and Makee, 2004).
Surround WP's highly reflective white coating is reported to achieve a crop protection effect by reducing egg laying activity and feeding damage from insects. This protection may be accomplished by several different ways. The powdery film formed on the plants may prevent insects from identifying a host crop and consequently the insects do not land, feed or lay eggs on the host crop. The coating may also cause insects to deem the fruit or leaves unsuitable. If insects recognize the crop and land on it, clay particles from the coating may stick to the insects causing them to become agitated and stimulated to move to other more attractive plants rather than remaining to feed or lay eggs.

Kaolin®: Kaolin with micronized powder is a natural mineral containing 950g/kg of kaolin miscible in water. It is used as rock dust in Organic Farming, as D.P.R 290/91 art. 38s, the same as Surround WP (Pennino *et al.*, 2006; Perri, 2008).

b. Antibacterial products (Cupric products and Propolis)

Bacteria are present on the leaf surface of host plants and in many food sources of fruit flies, such as fruits and fermented feces of birds. They are known as symbionts in food channels of different fruit flies. Fruit flies are attracted to bacteria and they can form four kinds of association types;

- "Mutual mandatory symbiosis (Petri, 1906; Capuzzo *et al.*, 2005);
- Mutual optional symbiosis (Lauzon *et al.*, 2000);
- Symbionts temporary rapport (Huston, 1972);
- Source of food (Drew *et al*., 1983)" (Belcari *et al.,* 2006).

Petri presented inconclusive experimental micro-biological data to support his interpretation of symbiosis. In studying the alimentary tract bacteria of *B. oleae* Petri described *Ascobacterium luteum* bacterium, as occurring in association with *Pseudomonas savastanoi*. In this work it was stated that bacteria are smeared onto the egg from rectal glands which open near the oviduct, the bacteria then enter the egg through the micropyle and are thus incorporated into the newly hatched larva (Fig. 4). In the larvae, the bacteria develop colonies in four blind sacs at the anterior end of the mid-gut and from here are transferred into the adult when some bacteria are incorporated into the oesophageal bulb which develops in 5 day old pupae (Mazomenos, 1989). Many years after it was shown how the bacteria are transferred from the female larva through the egg by Mazzini and Vita in 1981 (Belcari *et al.,* 2006).In view of those studies, it was suggested the use of antibacterial products, in combating *B. oleae*, as a cause of the symbiosis interruption between the insect and some of the bacteria present on the olives phylloplane, which are vital for the survival of the larva (Iannotta *et al*. ,2007b; Belcari *et al*., 2008) (Fig. 5).

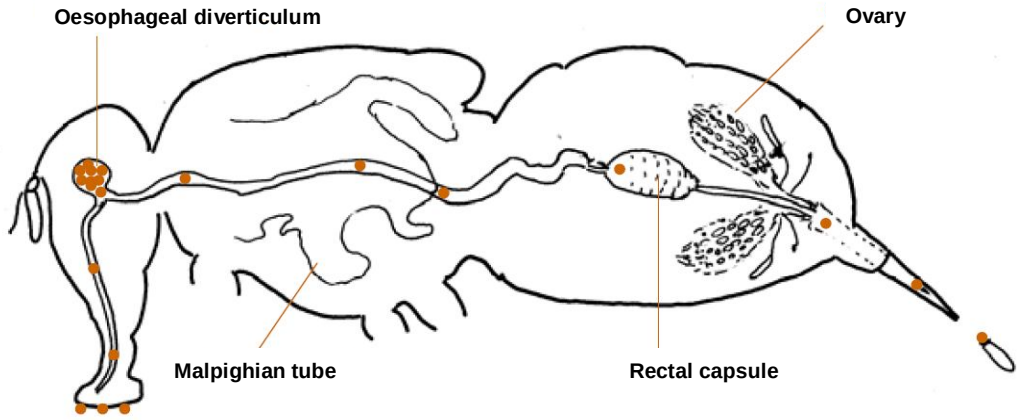

Figure 4: Bacteria associated to olive fly symbiosis and transmission mechanisms (Belcari *et al*., 2006).

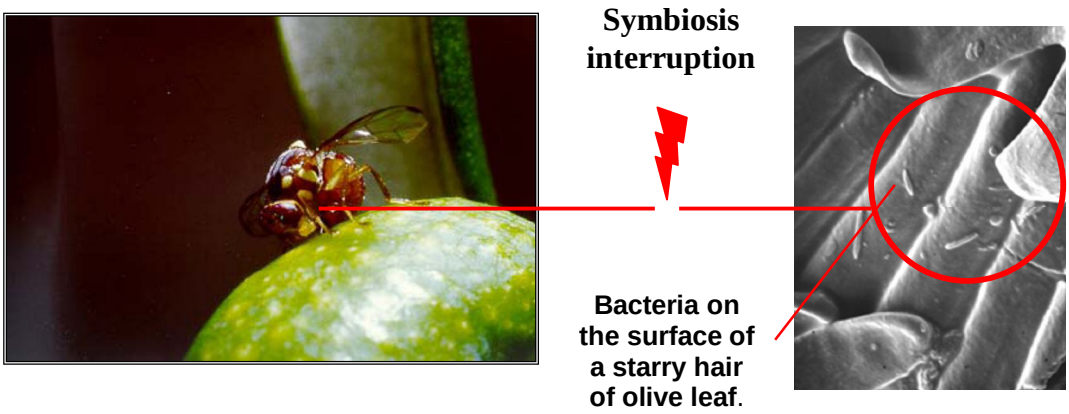

Figure 5: Inhibition of symbiotic bacteria as a means of olive fly control (Belcari *et al*., 2006).

Cupric products: The use of cupric products acting as antibacterial, also used in organic farming, was recently introduced with good effectiveness in combating *B. oleae*, as a cause of the symbiosis interruption between the insect and some of the bacteria present on the olives phylloplane, which are vital for the survival of the larva (Iannotta *et al.*, 2007b; Belcari *et al*., 2008). Copper salts through their antibacterial action make fruits less attractive to ovipositing females because of lack of some bacterial compounds on the surface of fruits (Tsanakakis, 1985; Belcari *et al.*, 2003), furthermore the presence of the particles of those products on fruit surface could be another obstacle for the fruit recognition of the female olive fly (Caleca and Rizzo, 2007). In organic agriculture the utilization of copper oxycloride as fungicide is allowed, with the recognition of the inspection body or inspection authority, from 1 January 2006 up to 6 kg copper per ha per year, for perennial crops (European Community, 1991b).

Propolis : Propolis is a resinous substance collected by honeybees from leaf buds and cracks in the bark of various plants, and it is composed of 50% resin (composed of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds (Pietta *et al.*, 2002).
Propolis has been used extensively in folk medicine for many years, and there is a substantial evidence indicating that propolis has antiseptic, antifungal, antibacterical, antiviral, anti-inflammatory and antioxidant properties (Ghisalberti, 1979).
Recently, studies have been performed concerning the use of propolis, resinous substance also credited to have antibacterial property, for olive fly control. Iannotta certifies that these active substances have already demonstrated their effectiveness against active fly infestation, inhibiting the development of the pre-immaginal stages (Iannotta *et al.*, 2006a; Iannotta *et al.,* 2006c; Iannotta *et al*., 2007b).
In Italy, the Ministerial Circulars No 9890634, May 6, 1998 and No 90678 of April 4, 2000, allow the use of certain substances including propolis, which are not listed in Annex II B of EEC 2092/91 Regulations, used as phytostimulants and as protective (Forconi, 2005).

2.7. The International Olive Oil Council and olive oil standardization

Olive oil is a high value-added product, highly supervised by European regulations. The classification of virgin olive oils takes into account the physical and chemical criteria but also the organoleptic characteristics of oils, in order to ensure to consumers a good quality product, particularly through sensory plan.
The International Olive Oil Council (IOOC) is the intergovernmental organization responsible for administering the International Agreement on Olive Oil and Table Olives, which has been negotiated at United Nations commodity conferences. Olive oil is the only commodity in the fats and oils sector to have its own international accord (Harwood and Aparicio, 2000).
The International Standards under resolution (IOOC, 2007e) lists ten grades of olive oil under two primary categories; i) Olive Oil, and ii) Olive Pomace Oil. The standard is in compliance with Codex Alimentarius standards regarding olive oil. The IOOC standard oils must meet certain criteria for inclusion into specific categories. The olive oils must comply with the concept of authenticity not be adulterated with any other type of oil, must pass a sensory analysis by a certified panel of tasters, and meet the analytical criteria. The standard indicates all the tests used to determine genuineness and purity plus the legal requirements for the label. Olive oil is defined as oil obtained solely from fruit of the olive tree (*Olea europaea sativa*). Virgin oils further are obtained solely by mechanical means that do not lead to alterations in the oil.

2.7.1. IOOC authenticity and quality standards

The concept of authenticity means that the oil is obtained only from olives with absolute exclusion of the presence of other oils or other fruit oil.
The product classification of olive oils is defined in the EEC 2568/91 regulations (European community, 2003b) as amended several times to take account of scientific progress, which sets qualitative standards which must be followed by the different commercial categories, so the consumers can feel protected by the legislature. The main physico-chemical analysis (Table 8) measuring acidity, peroxide value and UV spectrophotometric values (K_{232}, K_{270} and Δk), which indicate the level of hydrolytic alteration and primary and secondary oxidative alteration of the oil, respectively. Other analyses, also, ensure authenticity and purity of the product, considering an indices series regarding both glyceridic fraction, and some components of unsaponifiable fraction that are the sterols, aliphatic and triterpenic alcohols, waxes, etc. (Pannelli *et al*., 2003).

Table 7: Characteristics of olive oil types (European Community, 2002).

Category	***Extra virgin olive oil***	***Virgin olive oil***	***Lampante olive oil***	***Refined olive oil***
Acidity (%)	≤ 0,8	≤ 2,0	> 2,0	≤ 0,3
Peroxide value mEq O2/kg	≤ 20	≤ 20	—	≤ 5
Waxes mg/kg	≤ 250	≤ 250	≤ 300	≤ 350
Saturated acids in 2- position of the triglyceride	≤ 1,5	≤ 1,5	≤ 1,5	≤ 1,8
Stigmastadienes mg/ kg	≤ 0,15	≤ 0,15	≤ 0,50	—
Difference between HPLC ECN42 and theoretical ECN42	≤ 0,2	≤ 0,2	≤ 0,3	≤ 0,3
K_{232}	≤ 2,50	≤ 2,60	—	—
K_{270}	≤ 0,22	≤ 0,25	—	≤ 1,10
ΔK	≤ 0,01	≤ 0,01	—	≤ 0,16
Organoleptic assessment Median of defects (Md)	Md = 0	Md ≤ 3,5	Md > 3,5	—
Organoleptic assessment Median of fruity (Mf)	Mf > 0	Mf > 0	—	—

2.7.2. Sensory characteristics

One of the most important aspects of olive oil classification and value determination is sensory analysis. Human sensory evaluation is much more accurate (100 times) for olive oil than laboratory equipment for certain characteristics. Aroma and taste are very complex and cannot be determined in the laboratory. The tongue can also detect texture differences difficult to measure analytically. The first and primary objective in sensory evaluation for olive oil is to determine if oils contain one or more of the defects that commonly occur in oils from improper fruit storage, handling, pest infestation, oil storage, or processing problems (Vossen, 2007).
The numerical sensory values for each of the first three grades (extra virgin, virgin, and lampante olive oils) come from a rating of the oil by a qualified taste panel that has been officially recognized by the International Olive Oil Council (IOOC). The previous cited grades are defined for the European Community according to the regulation (EEC) 1989/2003, concerning the relevant methods of analysis they are reported into the Regulation (EEC) 796/2002, recently amended by Commission Regulation (EEC) 640/2008.

The majority of the tasters, usually 5 of 8, must agree statistically on the rating of the oil indicating the same defect, if any is present and similar intensity for fruitiness, bitterness, and pungency (Vossen, 2007).
In the specific vocabulary of the IOOC comprise, as positive attributes, the following sensations:

- **Fruity:** Set of olfactory sensations characteristic of the oil which depends on the variety and comes from sound, fresh olives, either ripe or unripe. It is perceived directly and/or through the back of the nose;

- **Bitter:** Characteristic primary taste of oil obtained from green olives or olives turning colour. It is perceived in the circumvallate papillae on the "V" region of the tongue;
- **Pungent:** Biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are still unripe. It can be perceived throughout the whole of the mouth cavity, particularly in the throat (IOOC, 2007e).

It also show negative attributes which are: fusty/muddy sediment, musty-humid, winey-vinegary/acid-sour, metallic, rancid, heated or burnt, hay-wood, rough, greasy, vegetable water, brine, esparto, earthy, grubby, cucumber, wet wood.

2.8. Trace elements residues determination in organic olive and extra virgin olive oil

The determination of trace elements in olive oil is important because of both the metabolic role of metals and the detection of possible defects in the final product and the oil characterization (Zeiner *et al.*, 2005; Benincasa *et al.*, 2007b).
Beside the technological risk, metal contamination in oils may derive from the environmental exposure to a large variety of elements. They reach olive plants via deposition as well as bioaccumulation from the soil due to natural metal sources and/or environmental pollution. In addition, the agricultural habits of the olive growers play a role in the metal contents of their products, such as the application of fertilizers or metal containing plant protection agents (Zeiner *et al.*, 2005; Benincasa *et al*., 2007b).

The presence of metals in oil, copper in particular, accelerates the phenomena of autoxidation inducing the increased risk of quality deterioration, but also of hygiene lack owing to free radicals formation (D'Alessandro, 2000). In addition, the presence of copper in olive oil is limited to 0.1 mg/Kg (Codex alimentarius, 2001). For these reasons copper monitoring in olive oils is extremely required.
Aluminum is one of the most abundant elements in the earth crust and biological system probably evolved in the presence of appreciable concentrations. Silicon, as well, is the most abundant electropositive element in the Earth's crust; its environmental effects are negligible and the safe upper level of this element is about 700 mg/day for 60 kg adult (Expert Group on Vitamins and Minerals, 2003).
It is necessary to emphasize that, in our study, Al and Si forms in the case of kaolin-based treatment are present as aluminum silicates. The kaolin is a mineral found in soil and is not uptaken by plants. Therefore, methods for residue analysis of plants and plant products were not required (Pest Management Regulatory Agency, 2003; European community, 2008) in terms of consumer's safety.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Zeiner *et al*, 2005) and Atomic Absorption Spectrometry (AAS) are the most commonly used techniques for the determination of metals in different

samples (Maurillo *et al.*,1999; Prohaska *et al.*, 2000; Zeiner *et al.,*2005). Fats and oils are particularly difficult to analyze for their trace metal contents since some of them are present at very low concentration levels. For this reason Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Castillo *et al.,* 1999; Becker and Dietze, 2003; Jimenez *et al.,* 2003; Beauchemin, 2006) could be an interesting tool, not only because of its well-known high sensitivity, but also because it allows an easy multielement analysis for each single sampling of a complex matrix such as olive oil (Benincasa *et al.,* 2007a).

3. Materials and methods

3.1. Experimental design

The experimental design answered to the following conditions:

- The olive grove should be at least two hectares (16 trees x 5 treatments x 3 = 240 replicates trees) conducted according to the method of organic farming with uniform production (excluding those ones with poor production);
- Each treatment (control, copper oxychloride, two commercial formulations of kaolin and propolis) was repeated on 3 plots (three repetitions) us shown in Fig. 6;
- Each plot was composed of 16 trees (4 x 4) and only 4 trees situated in the centre of the plot will be monitored and treated;
- The experimental design was randomized complete block design, with the exception of Tateo farm where it was not possible to verify this condition. In fact, In Tateo farm a completely randomized design was used

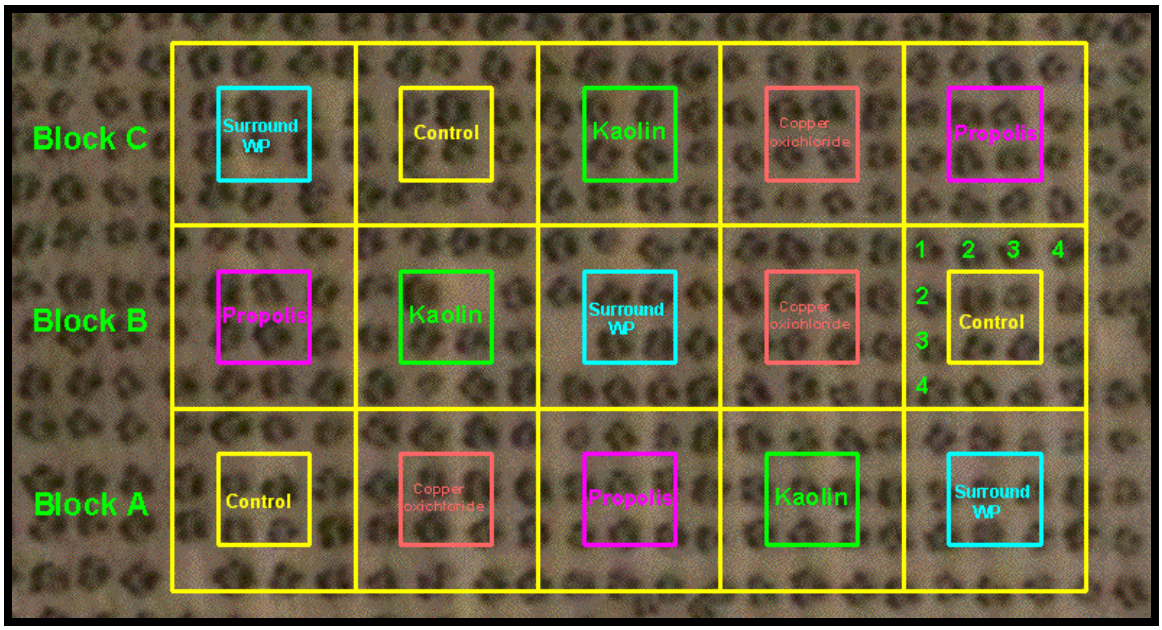

Figure 6: Experimental design projection on Rasciatano experimental olive grove.

3.2. Treatments and experimental sites

The trials have been performed in 2007 in three different olive areas. The first experimental field called the Tenuta Rasciatano is situated half way between the seaside and the buttresses of Murgia hills in that particular area of Barletta commune where the olive oil tradition has roots going back several centuries. The mean age of olive trees is 200 years which extend on 170 ha

and the main cultivar is Coratina with spacing of 8 x 8 m. The field was divided in different plots corresponding to the experimental design (Fig. 6) in which substances allowed in organic farming were tested. The first treatment (Figs. 7 and 8) of this field was performed on 21st September, the second one was on the 6th November (Table 9) and consisted on: kaolin (5 kg/hl) (Kaolin® progetto Geovita S.r.l, Turin, Italy), Kaolin (5 Kg/hl) (SURROUND®WP Crop Protectant, Engelhard Corporation, Iselin, NJ, USA), propolis (150 ml/hl) (PROPOLI®+SERBIOS srl, Badia Polesine, Rovigo, Italy) and copper oxycloride (300 gr/hl) (Ossiclor 20®, Manica S.p.A, Trento, Italy) (Table 10).

Table 8: Dates of the first and the second treatment carried out in the three farms.

Farm	Rasciatano	Damone	Tateo
First treatment	21/09/2007	10/09/2007	10/10/2007
Second treatment	6/11/2007	08/11/2007	14/11/2007

Table 9: Formulations concentration used in the different farms under experimentation.

Products	Active ingredients	Concentration per 100l
OSSICLOR 20®	Copper oxycloride	300 gr
KAOLIN®	Kaolin	5kg
PROPOLI®	Propolis	150ml
SURROUND®WP	Kaolin	5Kg

The second field, “Damone”, is a small farm (no more than 2 ha) in the commune of Bitetto that produce organic table olive of the region which is called “Termite di Bitetto”. This orchard contains irrigated 15 years old trees under a “Vase” training system which is common for the three farms. The plant spacing is 6 x 6m.The third farm “Tateo“ is located in the commune of Sammichele di Bari at about 300m above the sea level. Spacing of olive trees is 6x7m associated with cherry trees. For the last two farms the same experimental protocol was adopted as in Rasciatano one.

Figure 7: Worker treating the four central olive trees of each plot.

Figure 8: Kaolin protective powdery film on olive fruits surface.

3.4. Monitoring

In the tree investigated fields, the flight trend of *B. oleae* was followed by decadal reading of pheromonic traps (Fig. 9 Dacotrap ®) since July placed in number of 3 per hectare and situated in the control plots. Those traps were accompanied by three other chromotropic traps (Fig. 10 Glutor ®) (Raspi and Malfatti 1985) in order to determine the biocoenoses of the field. The insect recognition was carried out in the research centre of olivicolture of Rende.
Active infestation (presence of eggs, larvae and pupae), the number of sterile bits and total infestation (Active infestation, pre-imago stages, emergency holes and feeding tunnels) were determined in the insectarium of MAIB by microscopy analysis of drupes samples (100/replicate) collected from 4 central trees of each replication every ten days. Climatic conditions, concerning temperature and humidity, were also monitored by the mean of a Datalogger.

Figure 9: Reading of pheromonic traps (Dacotrap ®).

Figure 10: Reading of chromotropic traps (Glutor ®).

3.5. Olives sampling

Residues determination, in particular aluminum, silicon and copper, was carried out in March. Samples were taken from the contour of four central treated olive trees at different periods (Table 11) from the second treatment in order to monitor the concentration trend of the studied elements in olive drupes. These samples weighing ½ kg have been sent immediately to the Research centre of olive growing and olive oil industry in Rende, where they have been dried, weighed, and conserved in order to make residues analysis (Fig. 11).

Table 10: Olive sampling periods.

Sampling period	Quantity	Objective of sampling	Damone	Rasciatano	Tateao
First	½ Kg	Analysis of Al, Si and Cu residues in olive drupes	09/11 (1 day)	10/11 (4 days)	28/11 (14 days)
Second	½ Kg		19/11 (11 days)	17/11 (11 days)	–
Third	½ Kg		–	27/11 (21 days)	–

(x): number of days after the second treatment (last treatment).

Figure 11: Olive samples dried and conserved for residues analysis.

3.6. Harvest and olive milling

At the time of the olive harvest (Figs 12 and 13), a total of 15 samples of olive drupes from each olive grove were picked (15 replications per farm), to produce the corresponding olive oil samples for: (i) sensory analysis (Panel test), (ii) the main physico-chemical analysis: measuring acidity, peroxide value and UV spectrophotometric values; and (iii) residues determination in particular aluminum, silicon and copper in olive oil.
The quantity required for this kind of tests is 20 Kg of olive (Table 12), picked from the four central treated trees of each repetition plus control plots. The olive drupes were not washed before oil extraction. It is important to underline that harvest date largely goes beyond 20 days from last treatment date.
Timing of harvesting was delayed for two reasons, the first is to expose olives to *B. oleae* attacks and the second is the respect pre-harvest interval of 20 days (Mainly for copper oxichloride).

Table 11: Information about the olive harvest regarding date, quantity and objective of harvest.

Objective of sampling	**Quantity**	**Harvesting date**		
		Damone	*Rasciatano*	*Tateao*
Oil extraction for sensorial, physico-chemical and residues analysis	20 Kg per plot	19/11	20/12	02/12

Figure 12: Olive harvesting with olive shakers.

Figure 13: Trunk vibrator provoke olives fall on the net covering ground.

3.7. Oil extraction

For the oil extraction, the Spremoliva® (Fig. 16) discontinuous (pressing) system was used. The machine processes 8.5 kg of olive per cycle and it is able to carry out milling (crushing), mixing and extraction of extra virgin olive oil and waste discharge through a single cylinder/decanter. The whole working cycle lasts about two hours. Cleaning of the machine was indispensable after every mill and takes 20 minutes. All parts in contact with the product are made of AISI 304 stainless steel (Olive center web site, 2008).

Figure 14: Spremoliva® for extra virgin oil extraction

3.8. Trace elements residues determination in organic olive and extra virgin olive oil

3.8.1. Introduction

The goal of this study was the monitoring of aluminum, silicon and copper in olives and olive oils. This was carried out on the oils and drupes whose plants have been treated with copper oxichloride, propolis and two commercial formulation of kaolin. To perform this study, multi-element analysis of organic virgin olive oils was carried out by ICP-MS.

3.8.2. Materials and equipments

The reagents used were all of analytical-reagent grade certified for the impurities: nitric acid (Normaton ultrapure, VWP Prolabo); single element standard (Certipur, Merck, Darmstadt, Germany). Ultrapure water (18 MΩ cm) was prepared using a Milli-Q system (Millipore). All glassware was decontaminated with nitric acid (2% v/v) over night, rinsed with ultrapure water

and dried. The experimental work was carried out using the following system for the microwave digestion: Milestone MLS-1200 MEGA oven (Fig. 15) with programmable power control; segmented rotor MRP 600/10M; construction materials TFM-HTC for vessel and polypropylene for the segmented rotor.

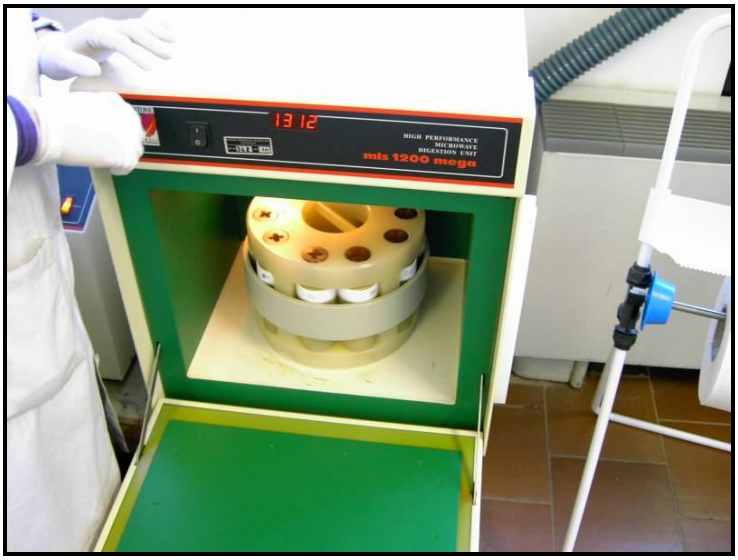

Figure 15: Microwave digestion; Milestone MLS-1200 MEGA oven with programmable power control.

The determination of elements of interest in the solutions obtained is carried out utilizing an Agilent 5700a ICP-MS instrument (Agilent Technologies, USA). The digested olive/oil samples are introduced by means of quartz nebulizer (Fig. 16); a propylene spray chamber of Scott type is used. The ICP torch is a shield Torch System. The performance of the ICP-MS instrument strongly depends on the operating conditions. A tuning solution is used to optimize the instrument in terms of sensitivity, resolution and mass calibration (Benincasa *et al.*, 2006).

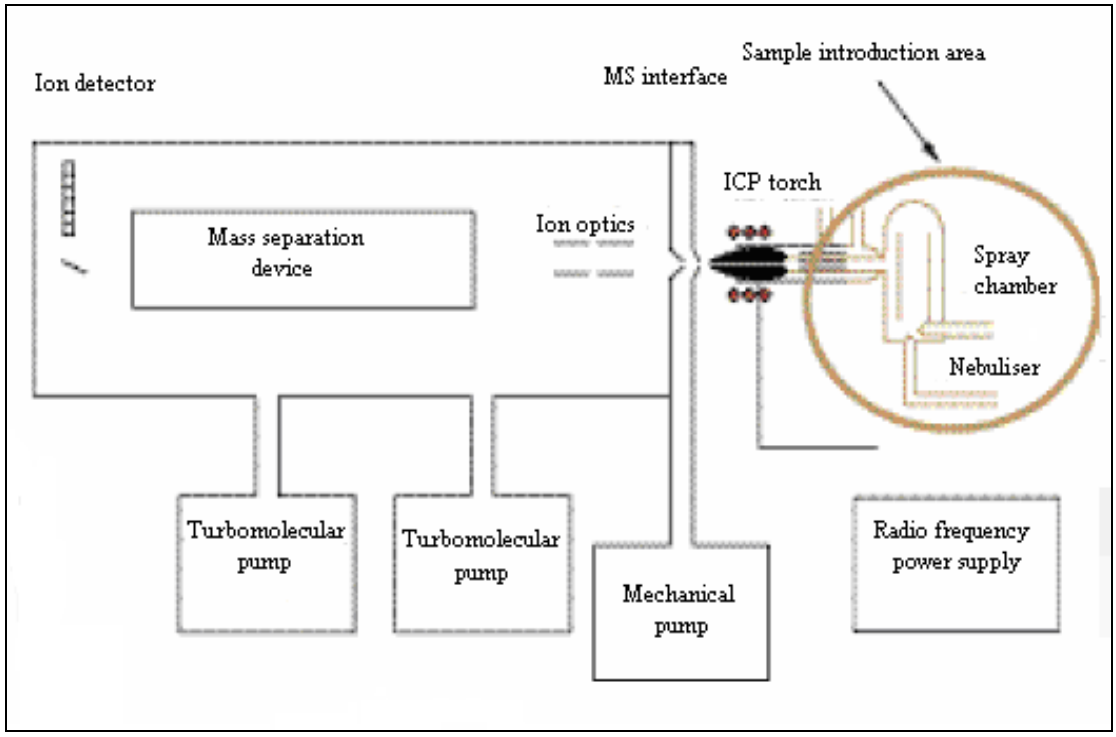

Figure 16: ICP-MS system diagram showing the location of the sample introduction area relative to the rest of the ICP mass spectrometer.

3.8.3. Calibration procedure

For the quantitative analysis, a calibration curve has been built on five different concentrations. Standard solutions have been prepared by diluting a solution of Al, Si and Cu (10µg/ml). The concentration ranges should be between 0.5 to 50 ng/ml (Benincasa *et al.*, 2007b).

3.8.4. Analytical procedure

The analysis concerns two kinds of preparation: one is for the olive oil and the second is for the olive fruit. Dried olive fruits are crashed by the mean of mortar and pestled to small pieces (Fig.17), while olive oil is directly used. Prior to analysis by ICP-MS, each sample is thoroughly shaken. An aliquot (0.5 g of oil/ olive paste) of sample is weighed directly into the digestion vessel. The digestion is performed by adding HNO_3 conc. (5 mL) to each sample. After cooling at room temperature, all the digestion liquors are quantitatively transferred into volumetric flask and diluted to volume (25 mL) with 18.2 MΩ cm ultrapure water and then injected in the ICP-MS via an auto injector (1 ml/min).

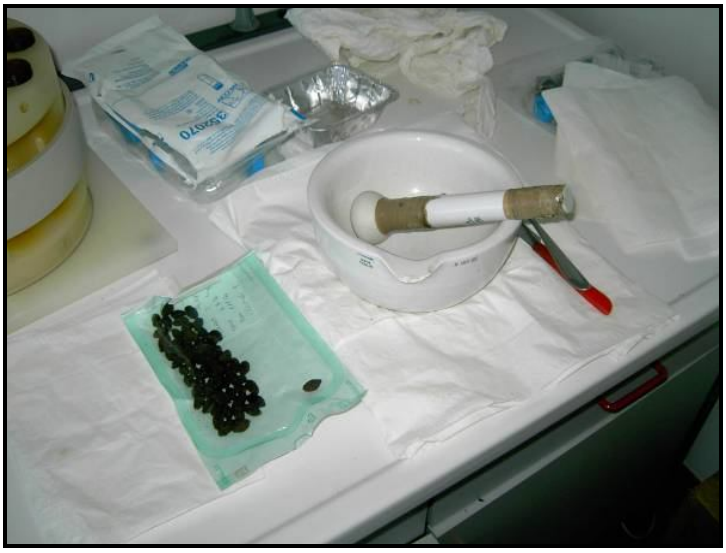

Figure 17: Dried olive ready for crashing by the mean of mortar and pestle.

An analytical batch was prepared with a minimum of 3 procedural blanks; one procedural blank spiked with a standard solution containing Al, Si and Cu and two Certificate Reference Materials (CRM) from Bureau Communautaire de Réference (BRC): BRC 100 (beech leaves) and BRC 62 (olive leaves) for quality assurance material performance data. A mid-range calibration standards is measured at the end of each analytical run, for quality control purposes, i.e., to assess instrumental drift throughout the run. Limits of detection (LODs) should be defined as 3 times the standard deviation of the signal from reagent blanks, after correction for sample weight and dilution (Benincasa *et al.*, 2006).

3.9. Olive oil quality criteria determination

Olive oil classification as defined in the EEC 1989/2003 regulations has been performed in the laboratory of CRA (Research Centre of Olive Growing and Olive Oil Industry). The quality criteria and their determination procedure were carried out according to the following scheme (Figs. 18 and 19; Annexes 3, 4 and 5) defined by the indicated regulation.

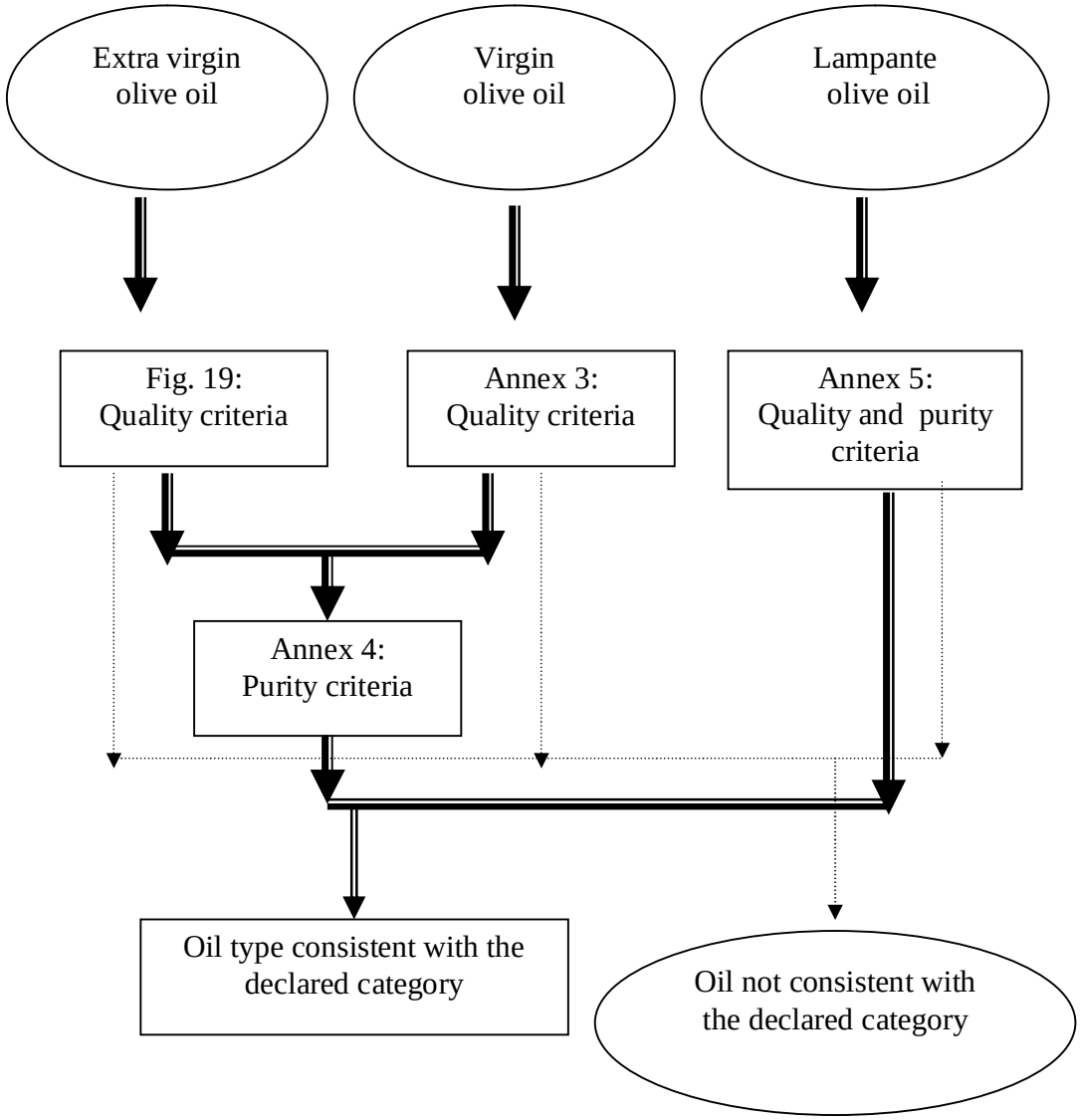

Figure 18: Olive oil grades and determination procedure (European Community, 2003a).

NB: The double line (═) indicates the route to be followed in case of compliance (positive answer) with the criteria specified in the preceding box. The dotted line (.....) indicates the alternative route to be followed in case of non-compliance.

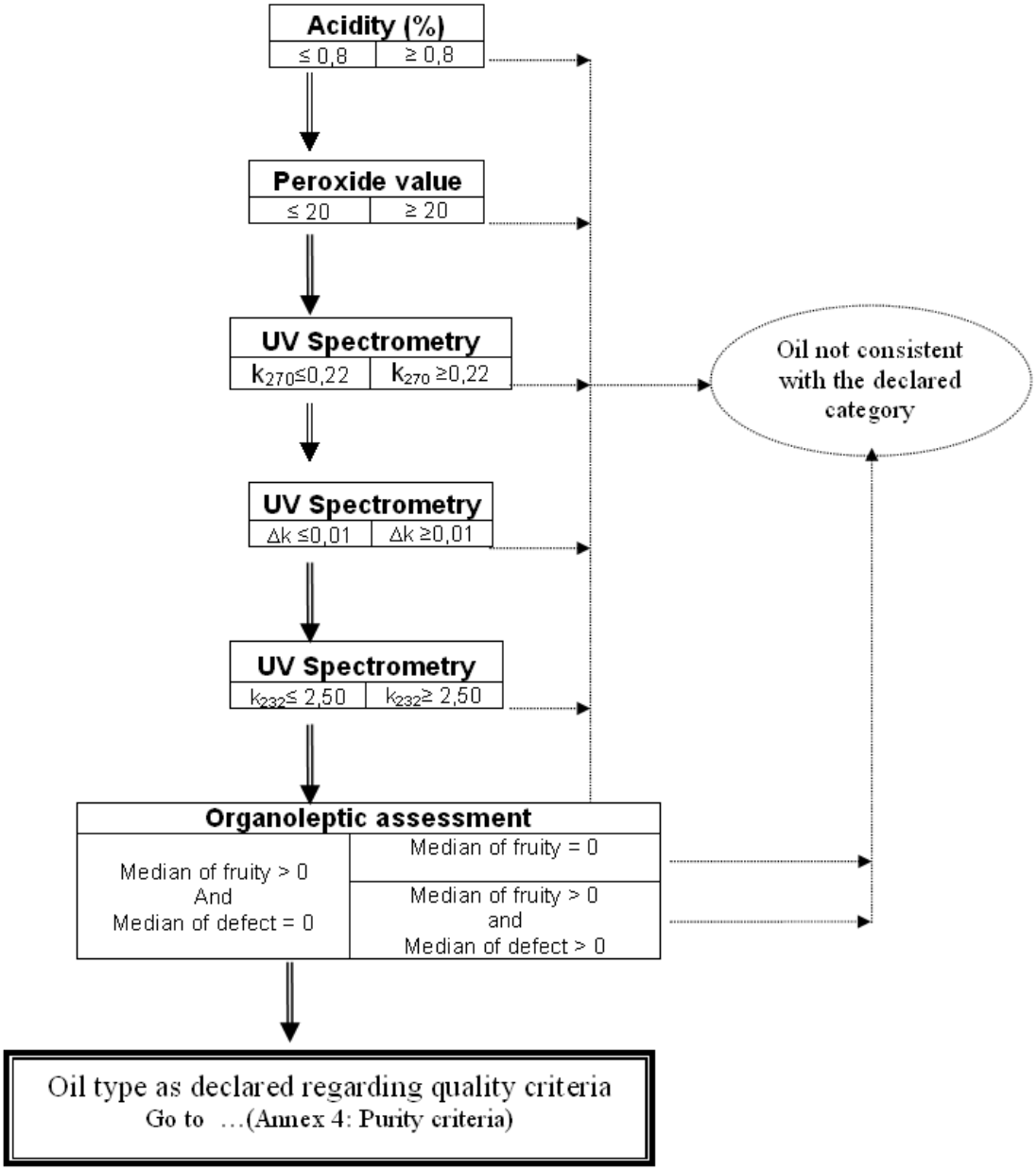

Figure 19: Extra virgin olive oil Quality criteria (European Community, 2003a).

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3.9.1. Acidity determination

The test consists on the determination of free fatty acids in olive oils (Fig. 23). The content of free fatty acids is expressed as acidity calculated conventionally. A sample is dissolved in a mixture of solvents and the free fatty acids present

titrated using an ethanolic solution of potassium hydroxide (European Community, 1991a).

- Apparatus:

The apparatus used was; analytical balance, 250 ml conical flask, 10 ml burette graduated in 0.05 ml.

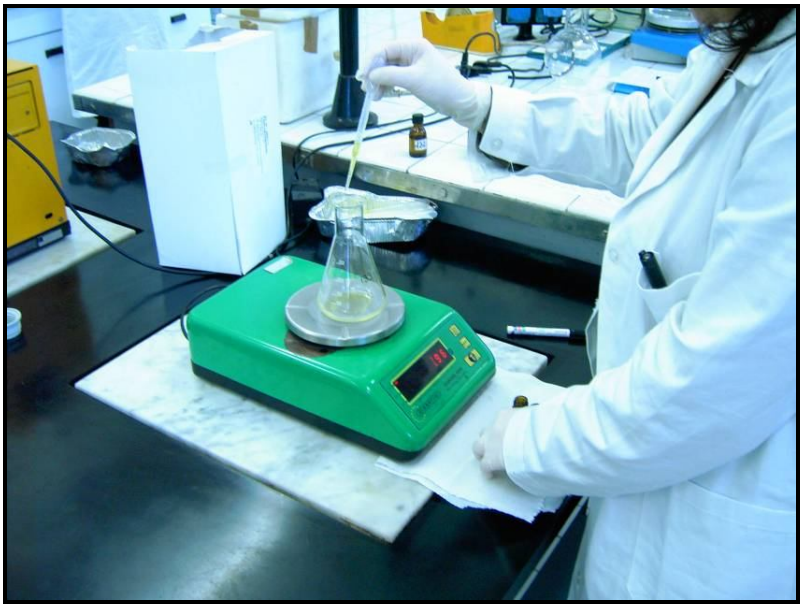

Figure 20: Weighting of 2g of oil in 250 ml conical flask by the mean of an analytical balance.

- Procedure:

We dissolved a 2g sample of oil already weighted in 100 ml of a neutralized mixture of diethyl oxide and ethanol. We titrated while stirring with 0.1 mol/l potassium hydroxide solution until the indicator colour (0.3 ml phenolphthalein) changes into bright red.

- Determination:

The volume of titrated potassium hydroxide solution used, in milliliters, is multiplied by 1.41 to give the acidity value of the sample expressed as percentage of oleic acid.

3.9.2. Determination of peroxide value

The peroxide value is the quantity of those substances in the sample, expressed in terms of milliequivalents of active oxygen per kilogram (mEq O_2/kg), which oxidize potassium iodide under the operating conditions of the analysis.

- Apparatus:

The apparatus used was: 3 ml glass scoop; flasks, with ground necks and stoppers, of about 250 ml capacity, dried beforehand; 50ml burette, graduated in 0.1 ml.

- Procedure

The test shall be carried out in diffuse daylight or in artificial light. A quantity of 5 g of olive oil was weighted in a flask. We added 25 ml of solution containing acetic acid and chloroform (3:1), then 50µl of potassium iodide solution. We inserted the stopper quickly, shacked for one minute, and left for exactly five minutes away from the light at a temperature from 15 to 25 °C. 75 ml of distilled water were added, after that, we titrated the liberated iodine with the sodium thiosulphate solution and shacked vigorously, using starch solution as indicator (Fig. 21).

Figure 21: Titration step for the determination of peroxide value.

- Determination:

The peroxide value, expressed in milliequivalents of active oxygen per kilogram (mEq O_2/kg), is given by the multiplication of V (Volume in ml of the standardized sodium thiosulphate solution used for the test) by 2.

3.9.3. Spectrophotometric investigation in the ultraviolet

Spectrophotometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about in it by technological processes. The absorption at the wavelengths specified in the

method is due to the presence of conjugated diene and triene systems. These absorptions are expressed as specific extinctions $E^{1\%}1\ cm$ (the extinction of 1% solution of the fat in the specified solvent, in a thickness of 1 cm) conventionally indicated by K (also referred to as 'extinction coefficient`)(European Community, 1991a).
The oil in question is dissolved in the required solvent and the extinction of the solution is then determined at the specified wavelengths with reference to pure solvent. Specific extinctions are calculated from the spectrophotometer readings.

- Apparatus:

Equipment used was: a spectrophotometer for measuring extinction in the ultraviolet; rectangular quartz cuvettes; 25 ml graduated flasks; and high precision balance.

- Procedure

We weighed 0,25 g, to the nearest 0,01 g, of the olive sample into a 25 ml graduated flask, made up to the mark with the Spectrophotometrically pure iso-octane (2,2,4-trimethylpentane) and we homogenized. The resulting solution must be perfectly clear. We filled a cuvette with the solution obtained and measured the extinctions at an appropriate wavelength between 232 and 276 nm, using the solvent used as a reference.

- Determination:

ADL Shell Program® is used to define the constants values k_{232}, k_{270} and Δk (Fig. 22).

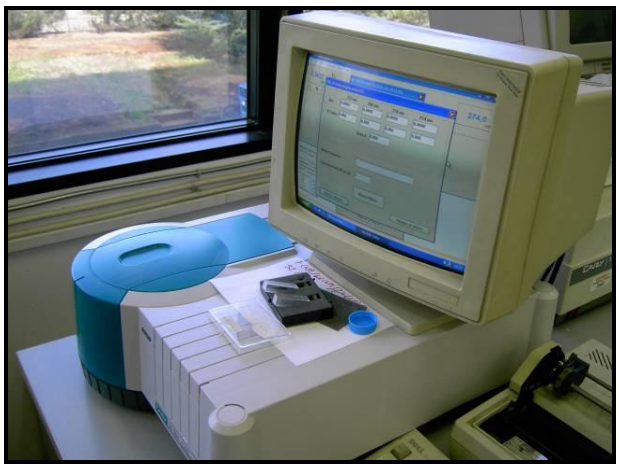

Figure 22: PC used to measure the extinction coefficients.

3.9.4. Sensory virgin olive oils assessment method

a. Olive oil categories

The IOOC has developed a method for assessing sensory virgin olive oils adopted by European Communities on July 11, 1991. The EEC Regulation 2568/91 has been amended several times (European Community, 1991a). At present the CE Regulation 1989/2003 states that olive oil destined for international trade can be classified in three categories:

- Extra virgin olive oil;
- Virgin olive oil;
- Lampante olive oil.

b. Skills and specialized facilities required for olive oil tasting

One of the most important aspects of olive oil classification and value determination is sensory analysis. Human sensory evaluation is much more accurate (100 times) for olive oil than laboratory equipment for certain characteristics (Vossen, 2007). Tasting olive oils require specific skills and specialized facilities:

- A head of the jury and a panel of tasters selected and trained consisting of eight to twelve experts (IOOC, 2007a);
- Installations, sensory analysis laboratory with individual cabins, and the equipment needed for the tasting:
    - Glass shall contain 14–16 ml of oil, or between 12.8 and 14.6 g if the samples are to be weighed, and shall be covered with a watch-glass. Each glass shall be marked with a code made up of digits or a combination of letters and digits chosen at random. The code will be marked by means of an odour free system (IOOC, 2007c).
    - Heating device in each cabin to maintain oil temperature at 28 ± 2 °C for the entire duration of a meeting. This temperature has been chosen because it makes it easier to observe organoleptic differences than at ambient temperature and because at lower temperatures the aromatic compounds peculiar to these oils volatilise poorly while higher temperatures lead to the formation of volatile compounds peculiar to heated oils (IOOC, 2007c).
- The test room (Fig. 23) must be at a temperature between 20 and 25 °C (IOOC, 2007d) and the best time for tasting oils is the morning. It has been proved that there are optimum perception periods as regards taste and smell during the day. Meals are preceded by a period in which olfactory–gustatory sensitivity increases, whereas afterwards this perception decreases. However, this criterion should not be taken to the extreme where hunger may distract the tasters, thus decreasing their discriminatory capacity; therefore, it is recommended to hold the tasting sessions between 10.00 in the morning and 12 noon (IOOC, 2007e).

- The use of vocabulary specific to the sensory analysis of olive (IOOC, 2007b).

Figure 23: Panel test room.

c. European community procedure for organoleptic assessment and grading in compliance with IOOC

❖ **Use of profile sheet by taster**

Tasting sheet has been simplified and begins with the evaluation of the defects; it consists of a profile (Annex 1) of flavors and a table rating the overall quality. The list of descriptors includes two categories of words representative of oil quality the first is called positive attributes (fruity, bitter and pungent) and the second are negative attributes (Winey-vinegary-Acid-sour, Metallic, Rancid, heated or burnt, hay-wood, rough, greasy, vegetable water, brine, esparto, earthy, grubby, cucumber and wet wood). They are measured by using a structured scale from zero to five. After the profile, each taster assigns an overall score between 1 and 9, depending on the intensity of major defects and the presence or absence of the attribute "fruity olive" (European Community, 2002).

❖ **Processing of data by panel head**

The panel head collects the profile sheets completed by the tasters and scrutinises the intensities assigned. He may feed each tester's data into a computer program for calculating the median. If a negative attribute is mentioned under “Other” by 50 % of the panel the head must calculate the median for this attribute and grade accordingly.

❖ **Grading of oils**

The oil is graded as follows in line with the median of the defects and the median for “fruity”. By this is understood the median of the negative attribute perceived with greatest intensity. The value of the robust variation coefficient for this negative attribute must be no greater than 20 %.

(i) Extra virgin olive oil: the median of the defects is 0 and the median for “fruity” is above 0;

(ii) Virgin olive oil: the median of the defects is above 0 but not above 3.5 and the median for “fruity” is above 0;

(iii) Lampante olive oil: the median of the defects is above 3.5; or the median of the defects is not above 3.5 and the median for “fruity” is 0.

d. Organoleptic profile

Olive oil obtains its distinctive taste from the climate, geography and vegetation of growing area. Olive oil organoleptic profiles differ according to the previous cited parameters but also according to the variety. In addition to the profile sheet destinated to olive grading, CRA-OLI has elaborated a new kind of sheet for extra virgin oil to describe as much as possible organoleptic traits of olive oils by adopting the suggestions of IOOC for the PDO characterization (Annex 2).

4. Results and discussion

4.1. Treatments and climate effect on *Bactrocera oleae* infestation level

4.1.1. Effect of climatic conditions on *Bactrocera oleae* development

Olive growing season in Apulia region was exceptional in 2007 in term of olive infestation caused by *Bactrocera oleae* (Rossi). The probable explanation for this phenomenon is the hard climatic conditions interfering with olive fruit fly development. Both high temperature regime and low humidity could have an impact on:

- The inhibition of ovarian maturation;
- Flight distance and duration of the flies;
- Abiotic mortality of *B. oleae*.

4.1.1.1. Temperature and humidity in 2007 summer

From half of June 2007, temperature starts to reach critical values for *B. oleae* (Table 12). On June 19th and 21st hourly temperature overcomes 35°C, on the 25th of June temperature reached values between 40 and 43 °C for more than 8 hours. This increase of temperature remained for several days and recover to moderate summer temperatures, until the second decade of July when temperature displays more than 38°C for at least 6 hours on July 22nd.
On the 24th July, temperature reached the highest value (44°C). On this date temperature varied between 40 and 44°C during at least 10 hours. After this period temperature was less than 35°C till the third decade of August where hourly temperature registered more than 38°C for 4 hours on the 30th of August. This year was exceptional in Apulia region in terms of temperature and humidity especially in summer. In fact, the temperature reached high values combined with low humidity levels. This is due probably to the global warming characterizing the last 93 years according to the World Meteorological Organization and the United Nations Environment Programme (Green and Armstrong, 2007).

Table 12: Temperature and humidity impact on *B. oleae* biology (Cappello *et al*., 2008).

Resumption of the adult	*>6-7 °C*
Mating	*>14-15°C at sunset*
Full activities (eggs maturation and oviposition)	*20-30°C*
Blocking eggs production	*>30°C with low humidity*
Death of eggs and larvae I and II instars	*>32°C with low humidity*
Death of all stages	*<-9°C and >42°C*

4.1.1.2. *B. oleae* flight at Damone

The flight trend was monitored from July 21. No adult was found on the pheromonic traps during the first two months. This is may be due to the high temperature and low humidity that characterized especially the ends of June and July. The first adults appeared on September 17th and the number of adults captured per week remained low (one to 4 adults) until the olives harvest that was carried out on November 19th (Fig. 24).

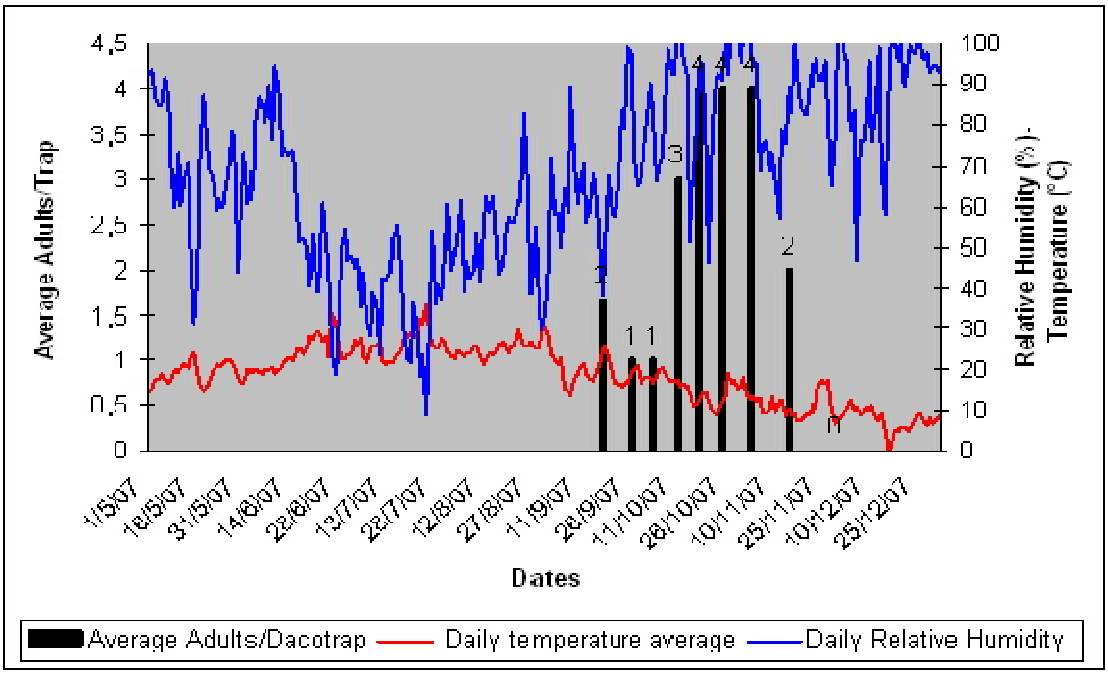

Figure 24: Adults' population trend and average temperature and humidity at Damone farm. Climatic data are from IAM-B meteorological station.

4.1.1.3. *B. oleae* flight at Tateo

Again, high temperature and low humidity delayed olive fly adults' apparition to the end of summer. As matter of fact, adults appeared at the end of September and captured adults number increased continuously until the end of November. Cold weather led population to decrease starting from the end of November ending in the harvest date, on December 2nd, when monitoring activities were stopped (Fig. 25). This generation peak noticed in the mid-November, can be considered late comparing to the usual second olive fly population peak generally encountered at early autumn (Delrio, 1992).

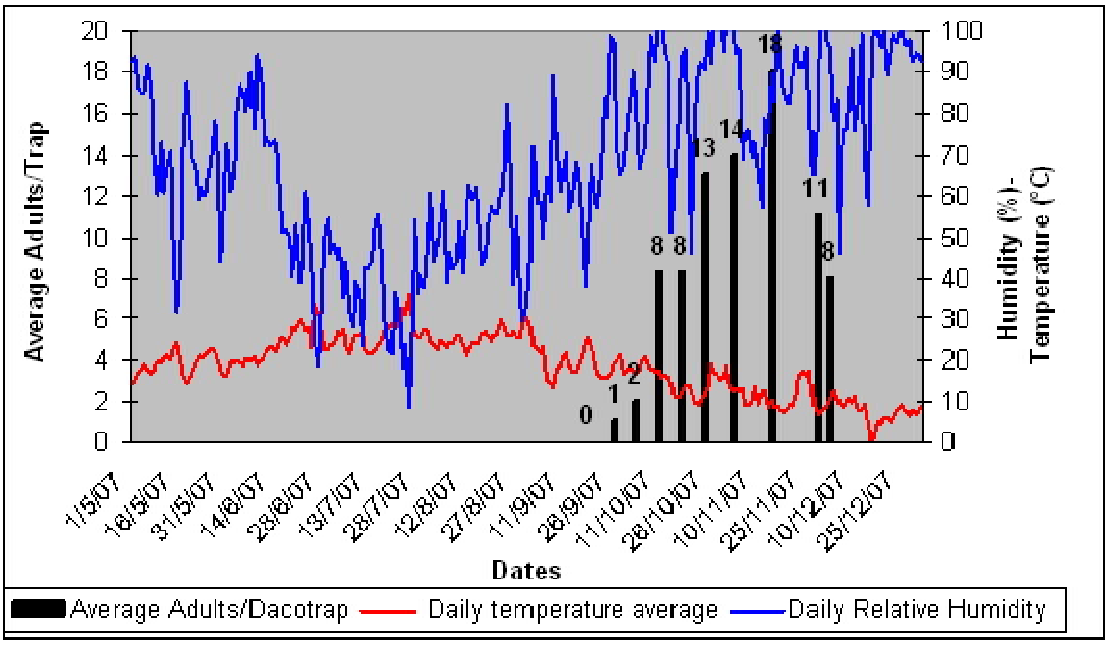

Figure 25: Population trend and average temperature and humidity at Tateo farm. Climatic data are from IAM-B meteorological station.

4.1.1.4. *B. oleae* flight at Rasciatano

At Rasciatano farm, the beginning of *B. oleae* adults' appearance was on September 26th roughly two months from the start of monitoring in July. From the beginning of September, favourable temperature and humidity favoured *B. oleae* adults' population increase. There were two adults capture peaks (16 adults per trap and per week) on October 24th and November 14th. Adults' population started to decrease in December; this is due probably to low temperatures (Fig. 26).

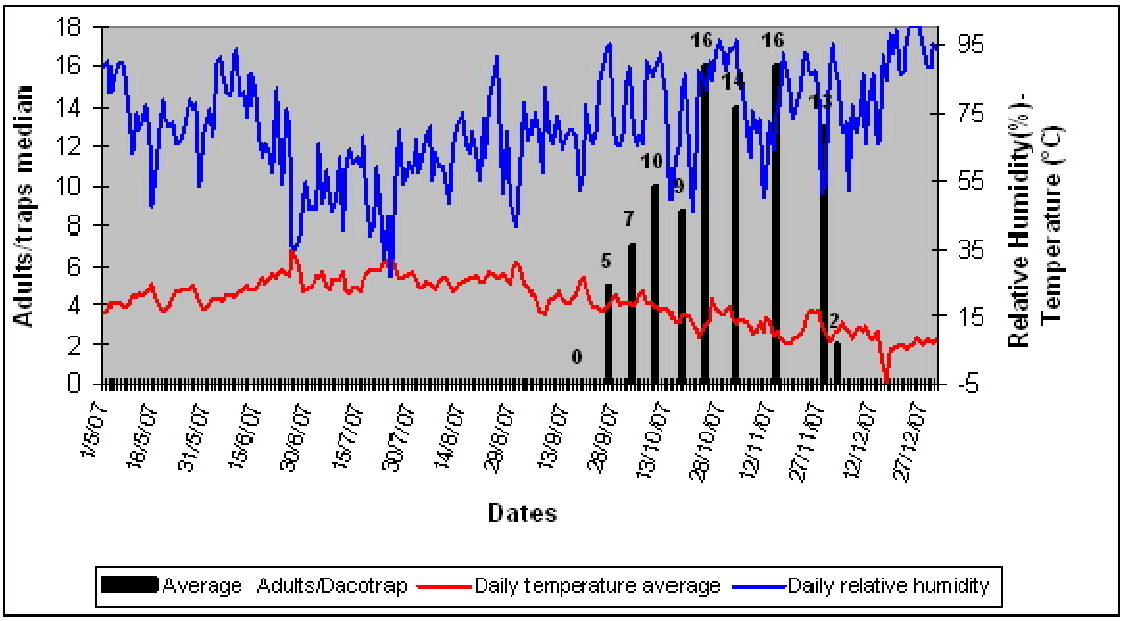

Figure 26: Population trend and average temperature and humidity at Rasciatano farm. Climatic data are from Molfeta meteorological station.

4.1.2. Treatment effect on *B. oleae* olives infestation

4.1.2.1. Damone

Considering the earlier harvesting (November 19th) of table olives, low susceptibility of cv. Termite di Bitteto to *B. oleae* attacks (Iannotta *et al.*, 2006b) and particularly the low pressure of olive fruit fly, Damone grove was intact from infestation.

4.1.2.2. Rasciatano

Active and total infestations values in Rasciatano farm were, generally speaking, null or very low during the whole monitoring period starting from the beginning of September. Olive fly damage did not exceed in the worst case 2.7 % total infestation in control plots registered on December 3rd.

Even if infested olive drupes were found two times, on November 27th and December 3rd respectively, no significant difference was noticed between treatments; this is may be due to the very low infestation level registered (Fig. 27).

The only observation that can be emphasized is that Surround®WP treated plots have not been attacked during all the summer period. The treatments were applied two times on September 21st and November 6th. It was noticed during field observation that even after several precipitations (12th, 16th and 23rd November and 16th, 17th and 19th December) Surround®WP remains longer on the surface of the fruits and leaves in comparison to the other product which

means that the barrier effect still has an effect until the harvest period. Kaolin® comes after in terms of fixation on the fruits and leaves surface but it was clearly affected by precipitations.

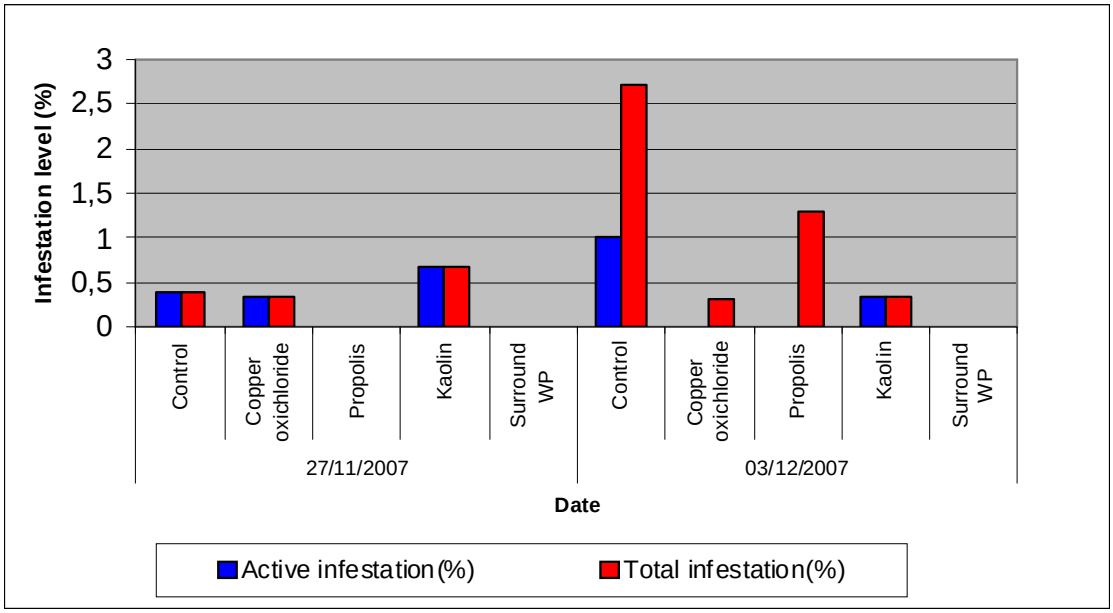

Figure 27: Olive fly active and total infestations at Rasciatano farm.

4.1.2.3. Tateo

Tateo was the only farm where a statistically significant difference between treatments was highlighted. However, *B. oleae* damages was found only once on November 28th with low levels. Main conclusions were that; on one side the highest value of olive fly infestation (4% of total infestation, TI) was recorded in untreated plots (control). On the other side, copper oxichloride has been the most effective treatment (0% as TI) followed by Propolis treatment (1.233 % as TI).

Infestation coincides with generation peak of *B. oleae* (Fig. 28) and comes 20 days after the second treatment, during this period it rained two times on 16th and 29th of November (treatment wash-off); may be for these reasons the attack of *B.oleae* happened. We can suppose that under those conditions, copper oxichloride had more efficacy than kaolin-based treatments.

According to results, we can deduce that the treated plots were less susceptible to *B. oleae* attack than the untreated ones.

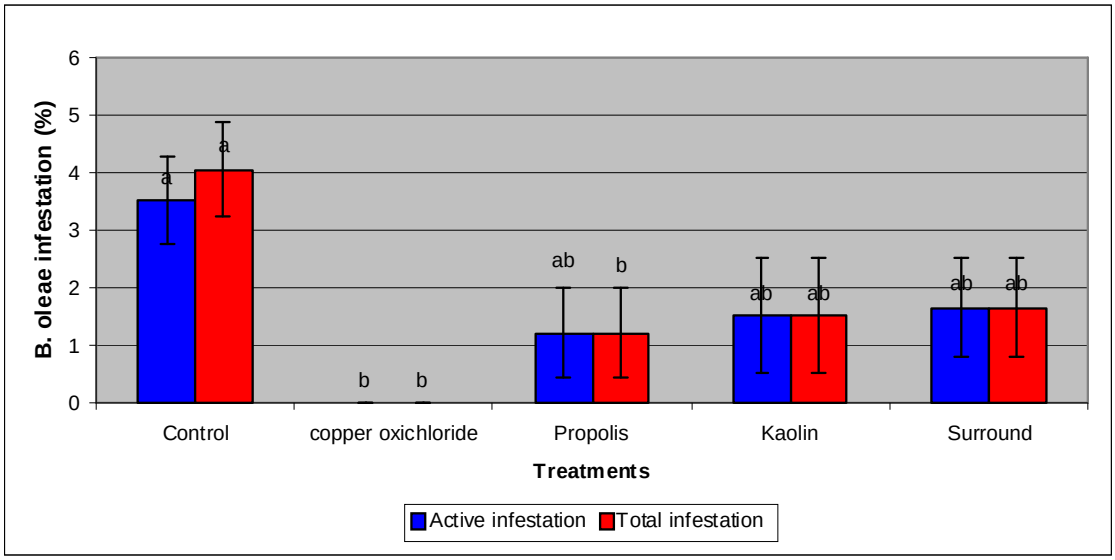

Figure 28: Olive fly active and total infestations at Tateo farm. Error bars represent standard errors of the means. Histograms with different letters are statistically significant using ANOVA followed by Duncan's Multiple-Range test; $p<0.05$.

4.1.3. *B. oleae* infestation and climatic condition comparison of 2002 and 2007

In order to show the high incidence of climatic conditions on *B. oleae* pressure, olive fruit fly infestation has been compared in 2002 and 2007. As it has been mentioned, 2007 summer was exceptional in terms of low olive fruit fly pressure in Apulia region. Five years ago, in 2002, the infestation scenario was the opposite and presented a high olive fruit fly damages.
Infestation data of 2002 were assessed in N. Motolose and Rubino Michele and compared respectively to those ones of Tateo and Damone.
The observation of temperature and humidity differences leads to conclude the severe life conditions of olive fruit fly during the summer of 2007 compared to the 2002 summer. Data, provided from the meteorological station of MAIB, of these two years show that the daily temperature average outruns rarely 28°C in 2002, while in 2007 summer temperatures were high, exceeding 30°C in several occasions. And as the hourly temperature is crucial for the survival of *B. oleae*, the graph (Fig. 29) shows that in 2007, hourly temperature in summer;
i) was always higher than in 2002 with a large differences sometimes (especially in the ends of June, July and September) and ii) overcomes 40°C during many hours a day.

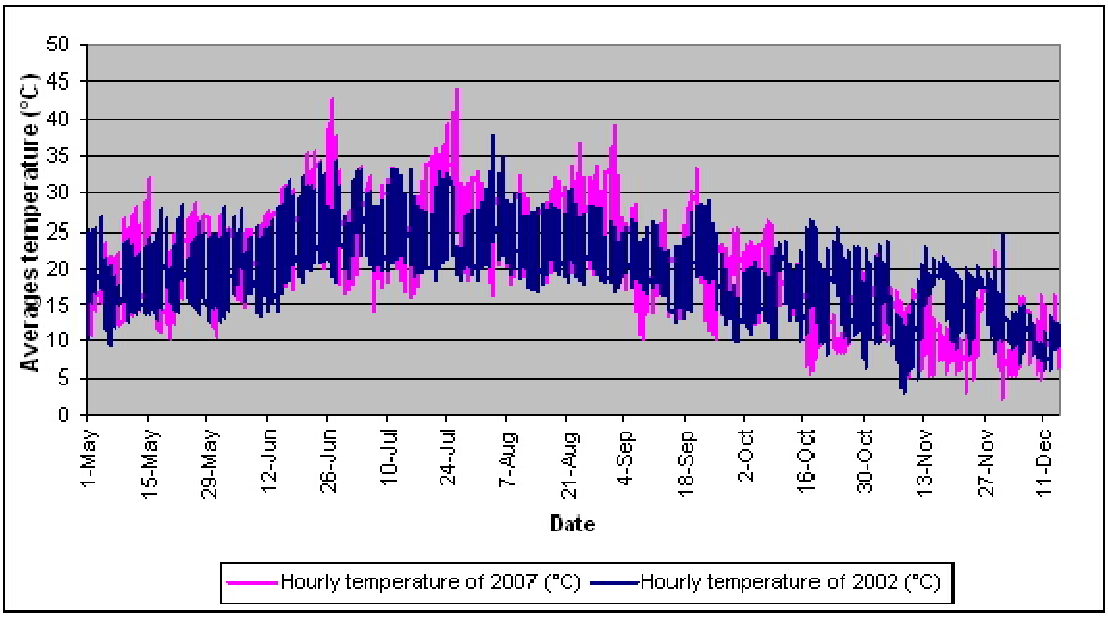

Figure 29: Hourly temperature average comparison, 2002 versus 2007.

Relative humidity from the end of June to the end of September in 2002 was superior of these ones registered in the same period of 2007. This means that *B. oleae* did not suffer from the low humidity level in the summer of 2002 as it was in 2007 (Fig. 30).

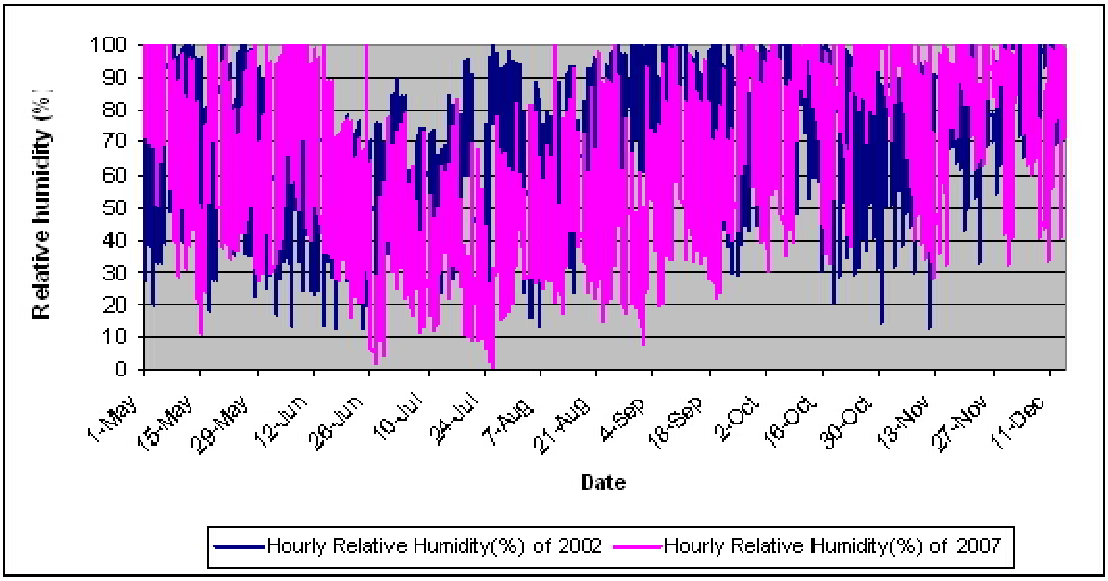

Figure 30: Daily relative humidity average comparison 2002 versus 2007.

2002 was characterized by a high rainfall and mild temperatures conducive to the development of olive fly, so infestations were early, since July, and continuing till October. Instead of 2007 when high regime temperature and low humidity covered a big part of summer and resumed on low or almost null infestation level.
In the following graph (Fig. 31) it is obvious that the infestation pressure in 2002 was higher than it is in 2007. N. Motolose is a farm located not far from Tateo while A. Rubino Michele is situated approximately 5 Km from Damone. Infestation at N. Motolose started in the third decade of August and reached the 100% in the end of September 2002, while in Tateo it was almost null in 2007. The infestation in Rubino was early in the end of June 2002 with considerable increase reaching 80% of total infestation in the end of October. Five years after in Tateo the infestation was null during all 2007 summer.

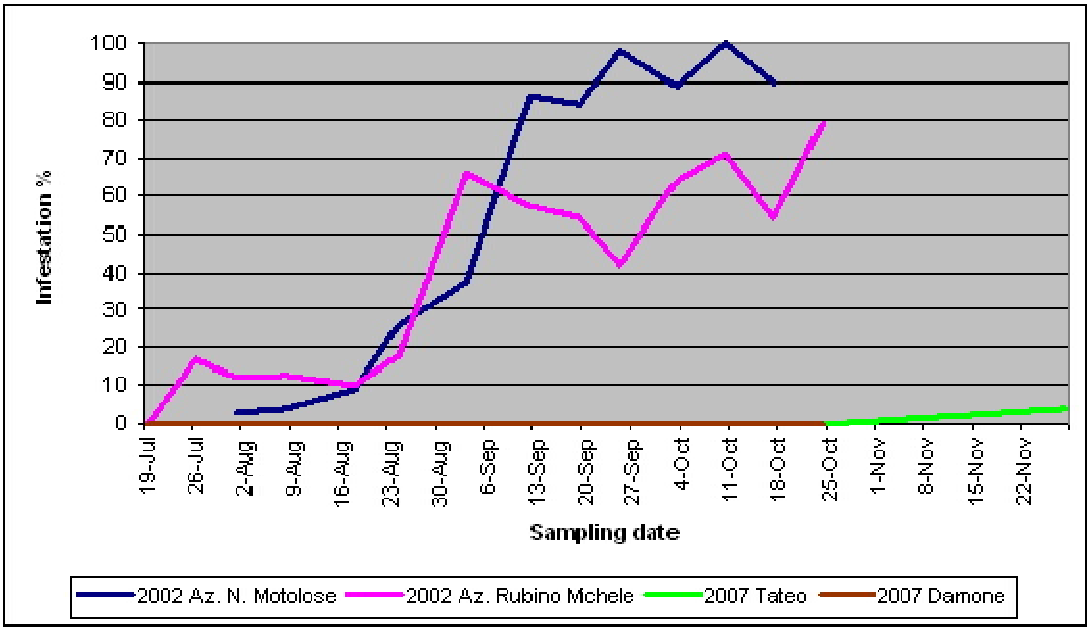

Figure 31: *B. oleae* infestation comparison of 2002 versus 2007

4.2. Aluminium, silicon and copper monitoring on olive drupes and oils

Aluminum, silicon and copper monitoring has been made in the olive oils whose plants have been treated with copper oxichloride, propolis, Kaolin® and Surround®WP in order to evaluate: i) how and how much olive oil or table olives could be affected and ii) if there is an eventual risk or relationship with olive oil quality alteration. In addition, samples of olive from each experimental farm have been carried out to determine the concentrations of those elements at different periods from the last treatments application.

4.2.1. Damone

The second treatment in Damone has been carried out on November 8th. Olive sampling was done in two occasions; on November 9th (one day after the second treatment) and November 19th (11 days after the second treatment) (Figs. 35 and 36).

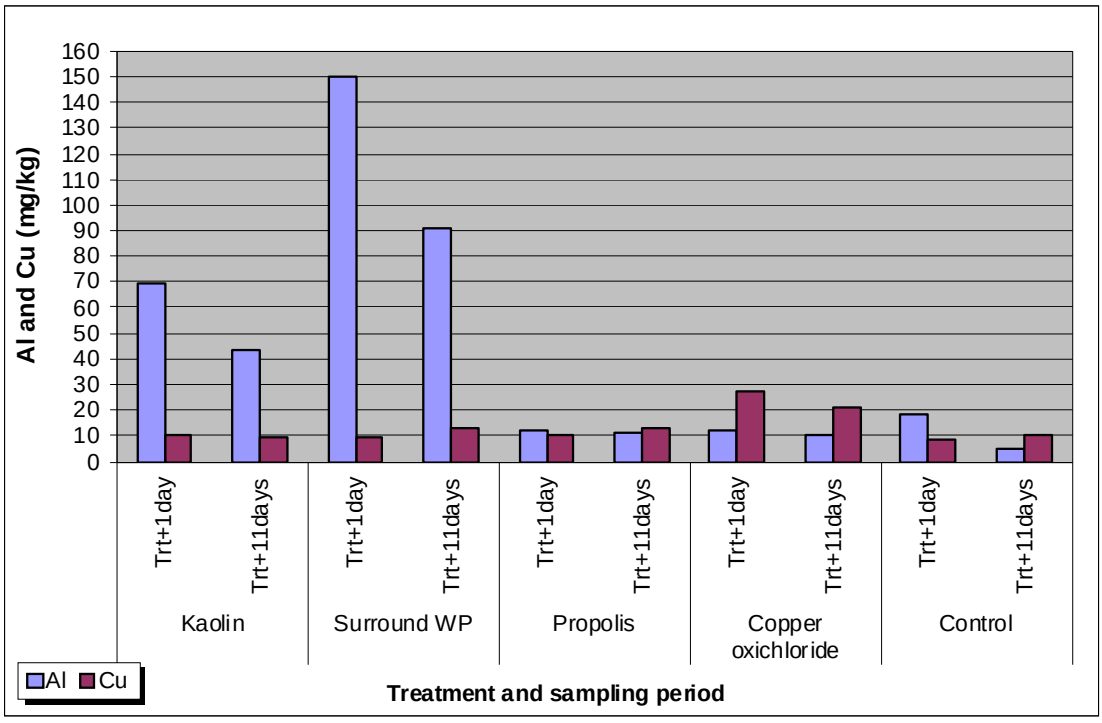

Figure 32: Aluminium and copper concentrations in Damone olive drupes at two different periods from the second treatment (Trt+1 day and Trt+11 days).

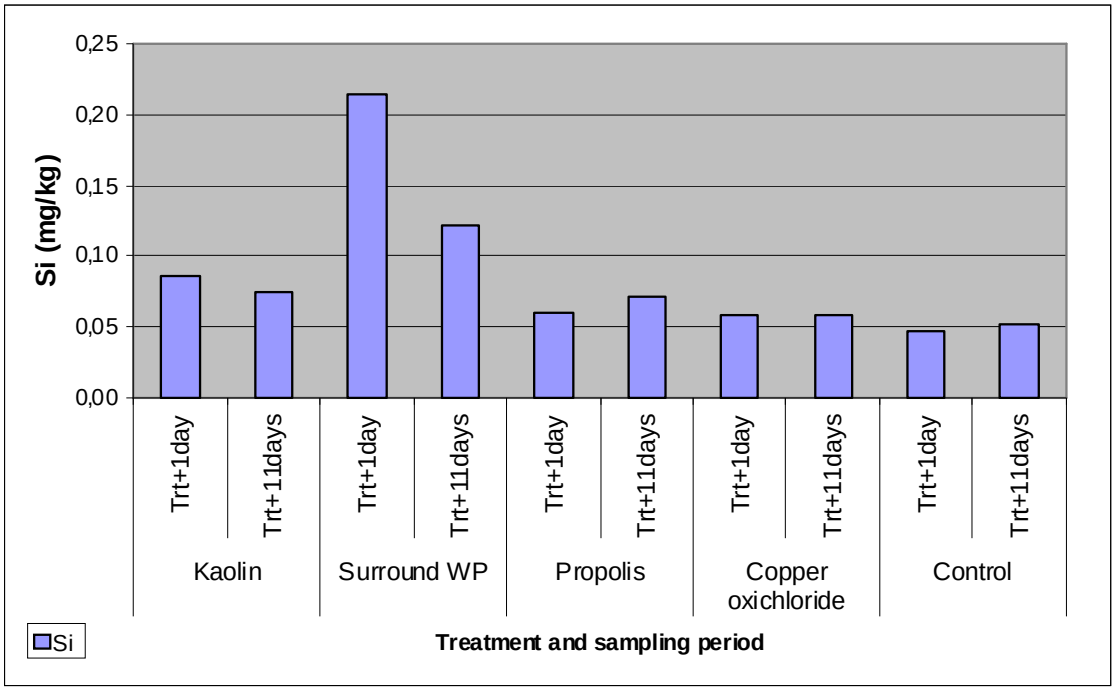

Figure 33: Silicon concentration in Damone olive drupes at two different periods from the second treatment (Trt+1 day and Trt+11 days).

Results obtained for the concentration of the extracted aluminium, silicon and copper (soluble fraction in concentrated HNO_3) in olive drupes, show that propolis does not contribute to any changes in the concentration of those trace elements. In fact there was no significant difference between propolis and control plots. Unlike Kaolin® and Surround®WP where the amount of aluminum and silicon were high in both treatments but diminishing over time.
Even Kaolin based treatments (Kaolin® and Surround®WP) have almost similar component, which is kaolinite, the deposition of this element on the olive fruits differs according to the commercial product used. It is remarkable that aluminium and silicon concentrations are higher in olives treated by Surround®WP; actually during our visits to the different farms we noticed that Surround compounds remain more on the surface of leaves and fruits. Even after rain, the powdery film persists longer than in Kaolin ® plots.
Concerning copper, the highest amount was registered in copper oxichloride treatment. This amount remains until the day 11 after treatment over the MRL of 20 mg/kg, whereas the safety interval is 20 days (Ordinary supplement of the Italian Official Journal n. 292 14/12/2004 and n.232, 6/10/2003).
In our case, the trend of copper residues concentration, coming from the copper-based treatment on olives, tends to decrease. In fact, copper concentration value respectively one day (27.4 mg/kg) and 11 days (21 mg/Kg) after the

second treatment, account for 23% of copper concentration reduction in 10 days.
Those results confirm the necessity of the safety interval period of at least 20 days after the last application of copper oxichloride (Ossiclor 20®) to guarantee the concentration reduction of this heavy metal below the MRL.
The treatment effect does not affect the amount of silicon and copper extracted in Damone olive oil (Table 13). For copper, the highest concentration (0.011 mg/kg) at the harvest moment (November 19th) was registered in copper oxichloride treatment. This amount is lower than 0.1 mg/Kg which is the copper residues level fixed by Codex standards for olive oil (Codex alimentarius, 2001).

Aluminum, silicon and copper residues in the olive oil were very low, especially when compared with olives. In the case of copper, such reduction can be explained by the discharge of this element in the mill waste waters by centrifugation for both: i) natural highly soluble copper contained in olive fruits (Simeone *et al.,* 2007) and ii) non soluble copper fixed on the surface of the fruit in copper oxhichloride treated plots. Regarding Al and Si in kaolin treated plots, the larger portion could be released during the mill water phase as kaolinite because of the high stability of this active ingredient.
This result clearly shows that the higher element fraction present in olives is discarded with the olive mill water phase during oil extraction. Therefore, these olive waste waters can contain high concentrations of Cu and their spreading on the soil without any preventive treatment can be detrimental to the soil biological activity (Simeone *et al.,* 2007).

Table 13: Aluminim, silicon and copper residues in Damone olive oil.

Treatment	Al (mg/Kg)	Si (mg/Kg)	Cu (mg/Kg)
Kaolin®	ND	0.021	ND
Surround®WP	ND	0.031	ND
Propoli®	ND	0.046	0.003
Ossiclor 20®	ND	0.021	0.011
Control	ND	0.065	0.005

ND: Non detectable element, considering the following Limits Of Detection (LODs) for: (Al) 51.972 10^{-3} mg/kg, (Si) 14.089 10^{-3} mg/kg and (Cu) 2.808 10^{-3} mg/kg.

4.2.2. Tateo

Regarding the amount of aluminum and silicon found in olives (Figs. 34 and 35), Kaolin® and Surround®WP treatments revealed significant difference compared to control plots at 14 days after the second treatment. At this time, copper concentration in copper oxichloride plots (14.99 mg/Kg) was under the MRL reported in the Italian official journal. Concerning Surround®WP and Kaolin® deposition on the surface of olives and leaves, the difference was not statistically convincingly but field observations show that Surround®WP powder remains more fixed.

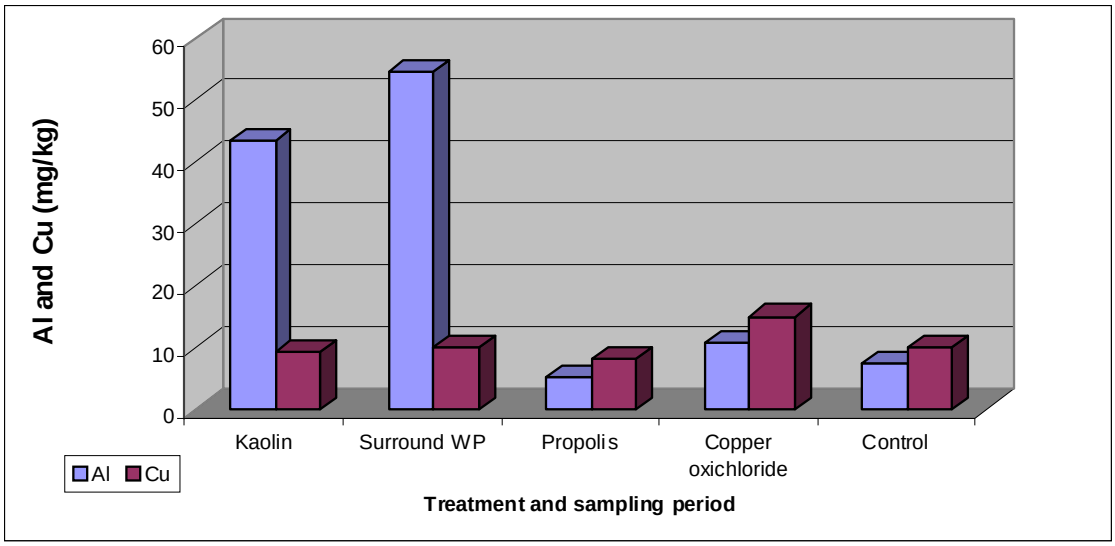

Figure 34: Aluminium and copper concentrations in Tateo olive drupes 14 days after the second treatment.

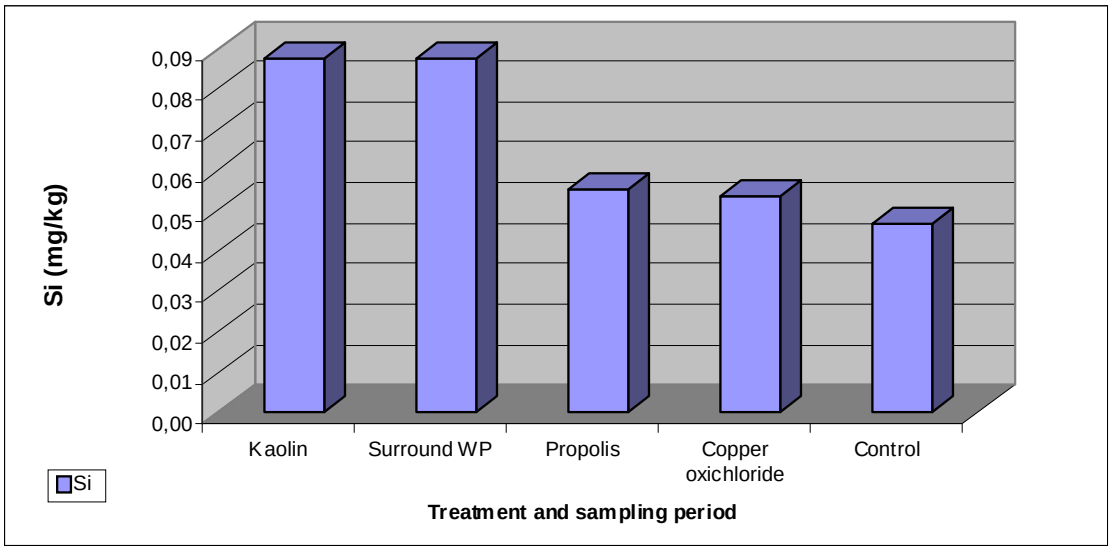

Figure 35: Silicon concentration in Tateo olive drupes 14 days after the second treatment.

Olive oil can be considered safe at 18 days from the second treatment regarding the levels of trace element deriving from the different treatments. Copper is

under the copper residues level 0.1 mg/Kg fixed by Codex standard for olive oil (Table 14).

Table 14: Aluminim, silicon and copper residues in Tateo olive oil.

Treatment	Al (mg/Kg)	Si (mg/Kg)	Cu (mg/Kg)
Kaolin®	ND	0,023a	ND
Surround®WP	ND	0,022a	0,003
Propoli®	ND	0,035a	ND
Ossiclor 20®	ND	0,039a	0,008
Control	ND	0,022a	ND

ND: Non detectable element, considering the following Limits Of Detection (LODs) for: (Al) 51.972 10^{-3} mg/kg, (Si) 14.089 10^{-3} mg/kg and (Cu) 2.808 10^{-3} mg/kg.

4.2.3. Rasciatano

After the second treatment, on November 6th, olive sampling was carried out 3 times for analysis, respectively 4, 11 and 21 days following the indicated date (Figs. 36 and 37).

Even if no significant difference was found between the 3 periods of sampling in each treatment, the trend of aluminum and silicon residues concentrations in olives were decreasing over time in Kaolin® and Surround®WP plots. Highest amounts were noticed in the 4th day after the second treatment and less concentration in the 21st day. The same observation was obtained in copper oxichloride plots; copper was slightly above the MRL of 20 mg/kg but a remarkable reduction at acceptable value in terms of human safety (17.8 mg/kg) has been shown.

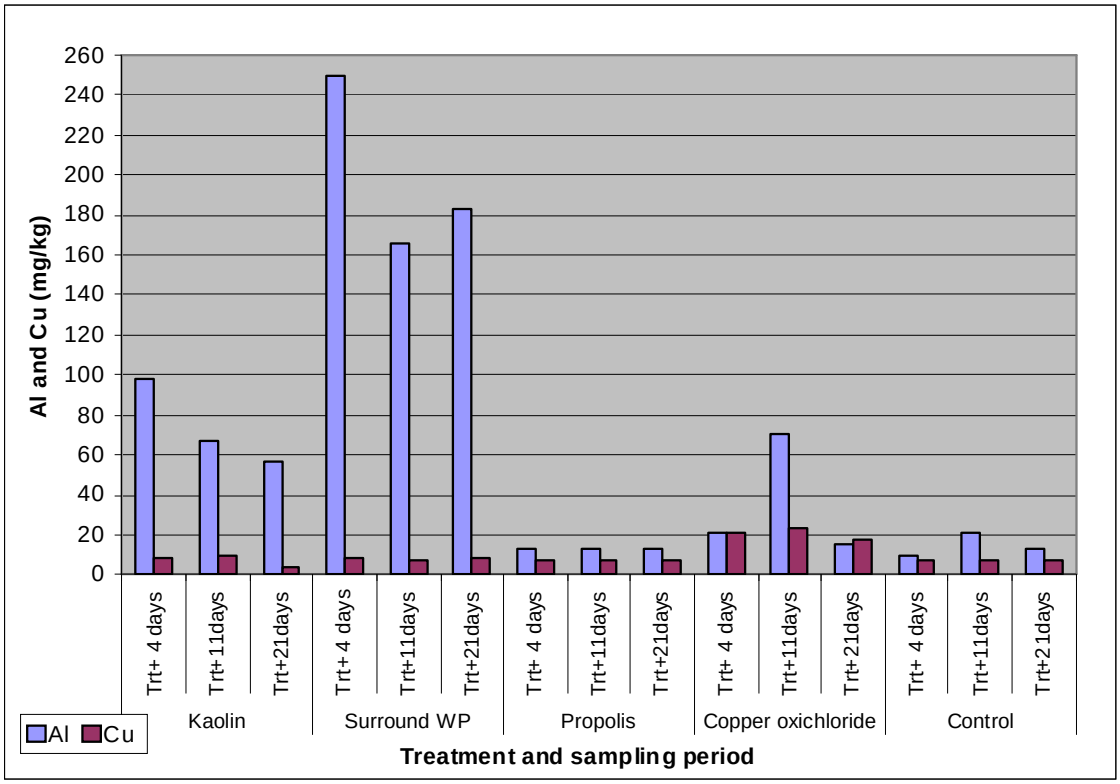

Figure 36: Aluminium and copper concentrations in Rasciatano olive drupes at three different periods from the second treatment.

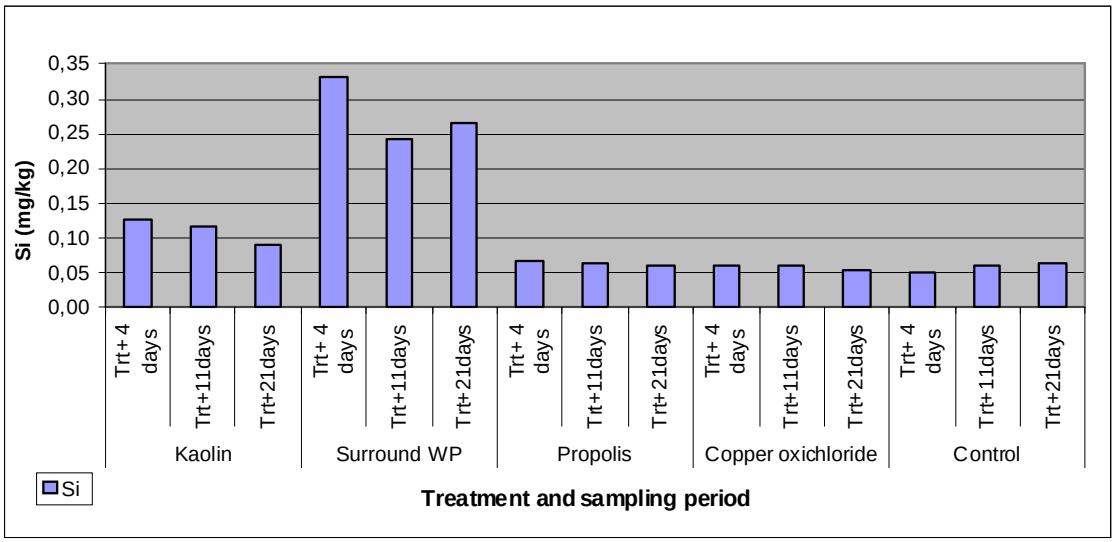

Figure 37: Silicon concentration in Rasciatano olive drupes at three different periods from the second treatment.

As reported for the two previous farms, Surround®WP shows a good adhesive capacity to the surface of the fruits and resists more to rain comparing to Kaolin®. The concentrations of aluminum and silicon, presenting the main elements of the kaolin based treatments, are higher in Surround®WP at the three sampling dates following the second treatment (Figs. 38 and 39).
High concentration of aluminum in olives treated by Surround WP® were found even at 21 days after the last treatment. Actually, the drupes have not been washed before analyses which explain these high values of aluminum concentration in olives.
Al and Si resulting from treatments based on kaolin, whose formula is $Al_2Si_2O_5(OH)_4$, have approximately a close atomic weights. The atomic weights for Al and Si are respectively 26.981 g/mol and 28.085 g/mol. In addition their stoichiometric ratio is equal. Consequently, their final concentrations on olives drupes should not show a big difference, but it is not the case. This let us suppose that the nitric acid HNO_3 used in digestion procedure was not efficient for Si compounds.

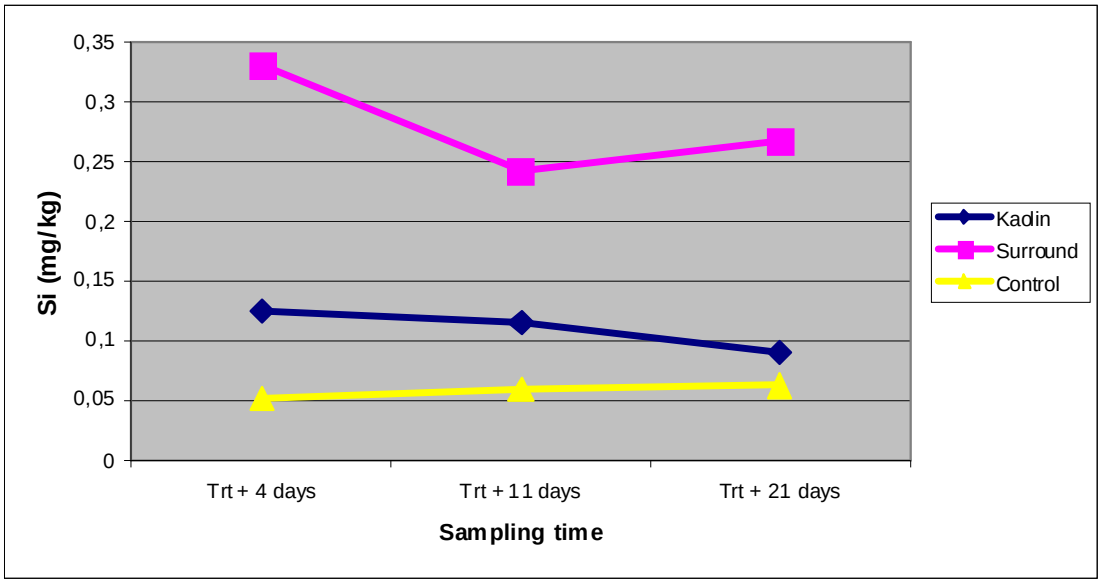

Figure 38: Silicon monitoring within time in olive drupes at Rasciatano Control, Surround®WP and Kaolin® plots.

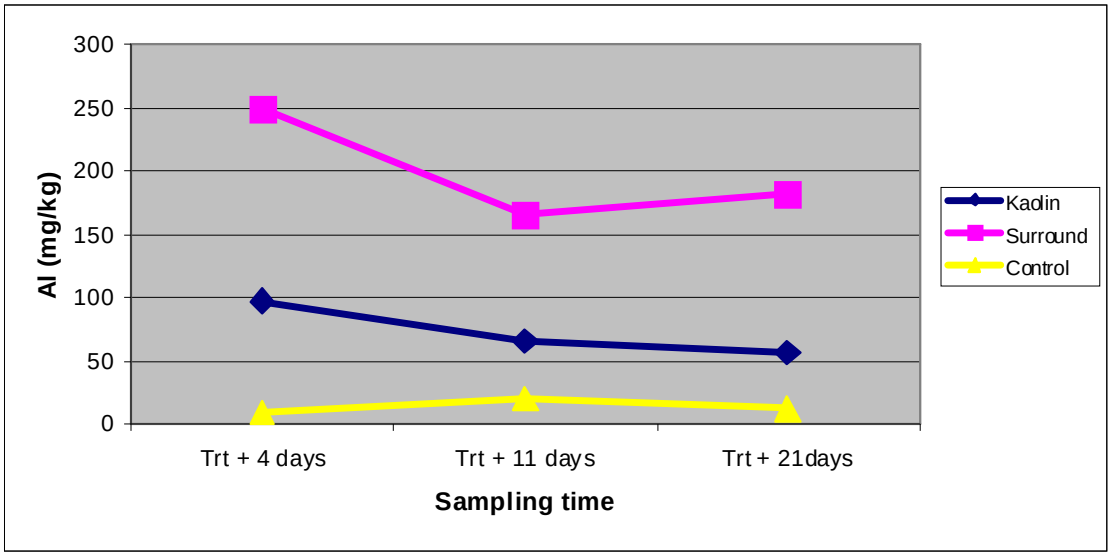

Figure 39: Aluminum monitoring within time in olive drupes at Rasciatano Control, Surround®WP and Kaolin® plots.

The treatment does not affect the amount of silicon and copper residues in Damone olive oil (Table 15). For copper the highest residues concentration (0.007 mg/kg), in December 20th, was registered in copper oxichloride treatment. This amount is under 0.1 mg/Kg (Codex alimentarius, 2001).

Table 15: Aluminum, Silicon and cupper residues in Rasciatano olive oil.

Treatment	Al (mg/Kg)	Si (mg/Kg)	Cu (mg/Kg)
Kaolin®	ND	0,026a	ND
Surround®WP	ND	0,023a	ND
Propoli®	ND	0,028a	ND
Ossiclor 20®	ND	0,037a	0,007
Control	ND	0,033a	0,005

ND: Non detectable element, considering the following Limits Of Detection (LODs) for: (Al) 51.972 10^{-3} mg/kg, (Si) 14.089 10^{-3} mg/kg and (Cu) 2.808 10^{-3} mg/kg.

Here also, aluminum, silicon and copper residues in the olive oil were very low, especially if compared with olives. As previously explained, such reduction is explained by the discharge of these elements in the mill waste waters.

Since aluminum, silicon and copper concentrations are very low in oils extracted from the treated plants, and did not show significant difference with oils emanating from untreated plants. We can suppose that any eventual alterations in olive oils, treated either by kaolin or copper based treatments, are not evident to be due to the presence of kaolin and copper residues.

4.3. Olive oil quality criteria determination

4.3.1. Damone

4.3.1.1. Acidity

Total acidity of Termite di Bitetto is generally between 0.38-0.36 percent (Ferrara et *al*, 1980). The acidity analysis results of Damone olive oil did not go over the rate of 0.28% in all treatments. Thus, olive oil responds to the extra virgin olive oil criterion regarding acidity that fixes 0.8% as maximum total acidity for an extra virgin olive oil (European Community, 2003a). Also the difference between treatments is not significant; which means that there is no treatment effect on the Coratina olive oil acidity (Fig. 40).

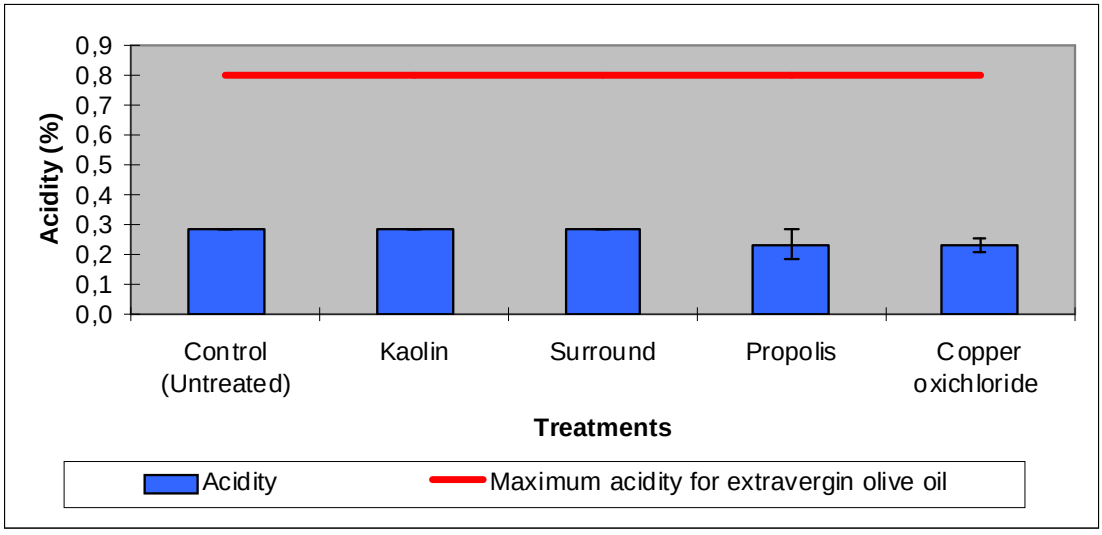

Figure 40: Damone olive oil free acidity under different treatments of Coratina cultivar.

4.3.1.2. Peroxide value

The peroxide index is caused by hydroperoxides (primary stage of oxidation). The maximum limit fixed for extra virgin olive oil is 20 (European Community, 2003a). All the treatments in the experiment have registered lower values. While it was registered a significant difference between untreated and treated plots; where control seems to be more concerned by oxidation (Fig. 41). In addition, Kaolin® has shown the best performance.

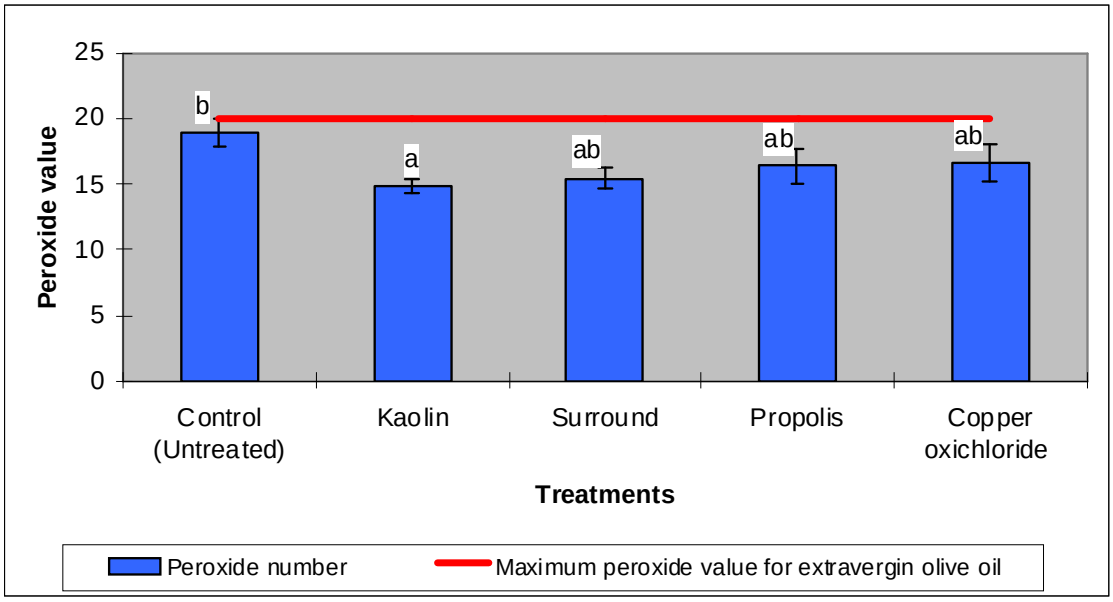

Figure 41: Damone olive oil peroxide value under different treatment of Coratina cultivar. Error bars represent standard errors of the means. Histograms with different letters are statistically significant using ANOVA followed by Duncan's Multiple-Range test; $p<0.05$.

4.3.1.3. Spectrophotometric investigation in the ultraviolet

According to the EU Regulations, spectrophotometric examination in the ultraviolet is the absorption at the wavelengths 232nm and 270nm. The absorbency at 232nm is caused both by hydroperoxides (primary stage of oxidation) and conjugated dienes (intermediate stage of oxidation).
While the absorbency at 270nm is caused by either carbonylic compounds (secondary stage of oxidation) and conjugated trienes (technological treatments) (Christopoulou, 2005).
UV spectrophotometric values K_{232} and K_{270} registered in the treatments during the analysis of Damone olive oil were all under the maximum values fixed for extra virgin olive oils. We have to underline the highest K_{232} value (Fig. 42) distinguished in copper oxichloride plot. The presence of metals in oil, copper in particular, can accelerate the phenomena of autoxidation inducing the increased risk of quality deterioration (D'Alessandro, 2000). For K_{270} (Fig. 43), all treatments responded approximately the same way and all are under the upper value fixed for exravirgin olive oil grade (0.22).
The index Delta-K is a criterion of discrimination between a bad quality virgin olive oil and a virgin olive oil adulterated with refined olive oil. For extra virgin olive oil Delta-K should be less or equal to 0.01 (Table 16). No significant difference was found between treatments.

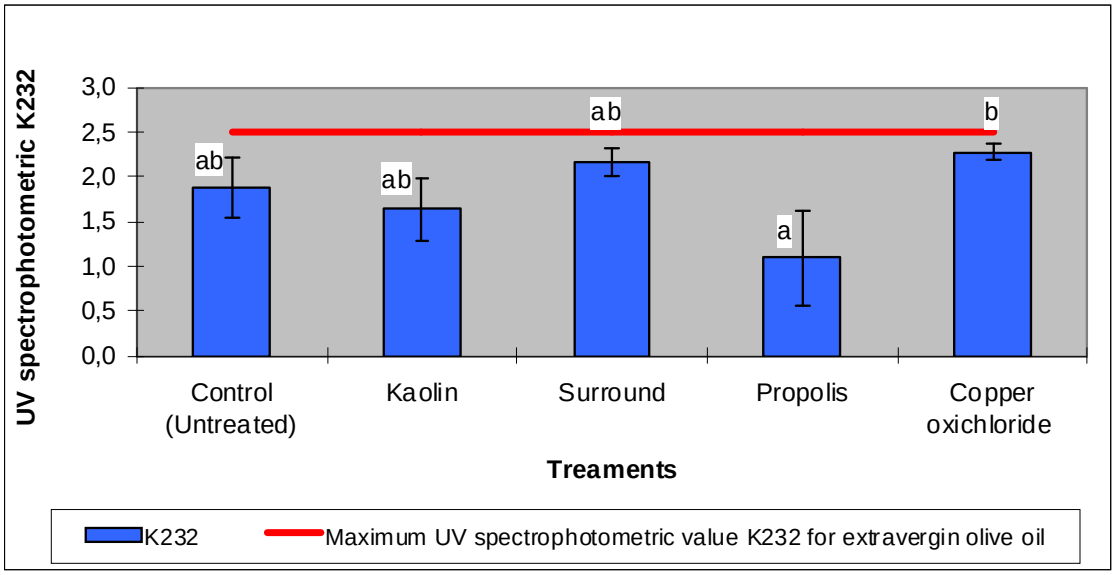

Figure 42: UV spectrophotometric value K_{232} of Termite di Bitetto cultivar at Damone farm. Error bars represent standard errors of the means. Histograms with different letters are statistically significant using ANOVA followed by Duncan's Multiple-Range test; $p<0.05$.

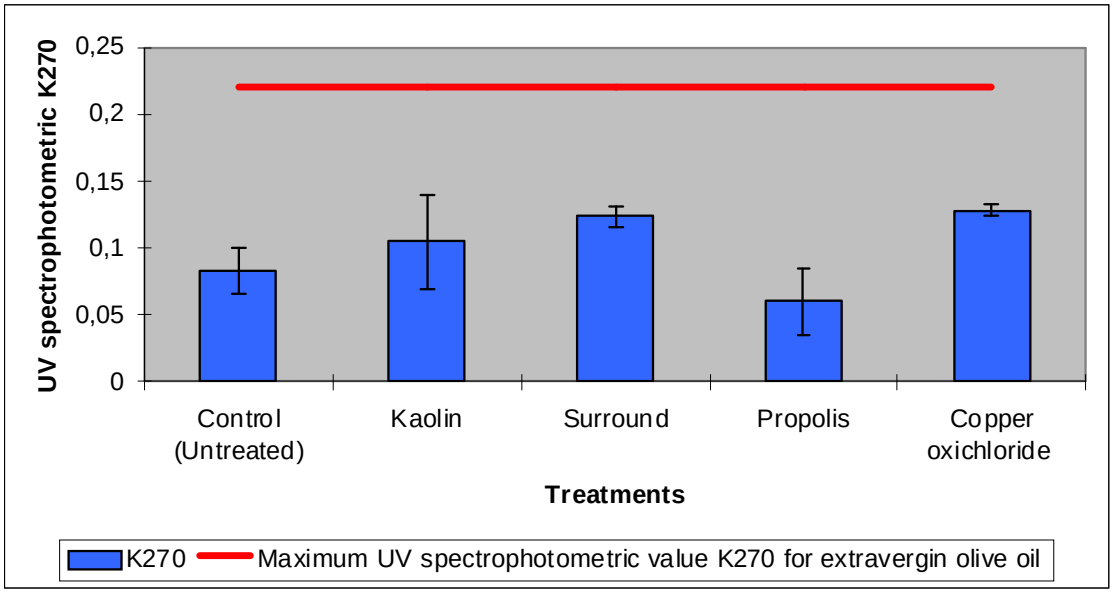

Figure 43: UV spectrophotometric value K_{270} of Termite di Bitetto cultivar at Damone farm.

Table 16: Delta-k value of Termite di Bitetto cultuvar at Damone farm.

Treatment	Control (Untreated)	Kaolin®	Surround®WP	Propolis	Copper oxichloride
ΔK	0.000577	0.000333	0.000000	0.000000	0.000000

4.3.1.4. Organoleptic assessment

The determination of chemical parameters alone is not sufficient to guarantee the goodness of oil from an organoleptic point of view. It may happen that oils with good chemical parameters have organoleptic defects (taste, aroma, flavors) that can declassify a product. Sensory analysis through tasting is a good way to highlight the organoleptic characteristics of virgin oils (Cappello and Dugo, 2005) In our case, the treatment effect did not show any significant difference (Table 17) and there is no correlation that can be mentioned between the level of infestation and oil sensorial analysis. Consequently, *B. oleae* infestation did not affect the organoleptic traits at low infestation level.

Table 17: Sensory analysis classification of Damone olive oil from different experimental plots.

Treatment	Sensory analysis category
Kaolin®	Extra virgin
Surround®WP	Extra virgin
Propoli®	Extra virgin
Ossiclor 20®	Extra virgin
Control	Extra virgin

4.3.1.5. Organoleptic profile

Olive oil obtains its distinctive taste from the climate, geography and vegetation of growing area. Olive oil organoleptic profiles of Damone in the different treatment present coloration more or less intense varying from green to yellow. Olfactory sensation is typical of oils obtained from olives that have been harvested when fully ripe, sweet and pungent sensation. Almond and grass are the direct aromatic olfactory sensations predominant in the oils analysis (Fig. 44).

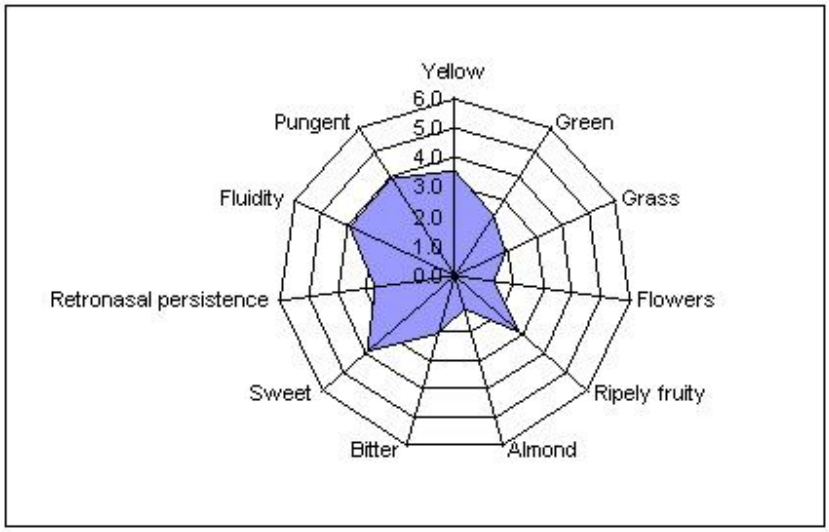

Figure 44: Organoleptic profile of olive oil from Damone control.

4.3.2. Rasciatano

4.3.2.1. Acidity

The acidity analysis of olive oil in Rasciatano complied with olive oil criterion regarding acidity that fixes the rate of 0.8% as maximum acidity for an extra virgin olive oil grade (Fig. 45). The difference between treatments is not significant; which means that there is no treatment significant influence on the acidity of Coratina cv. oil.

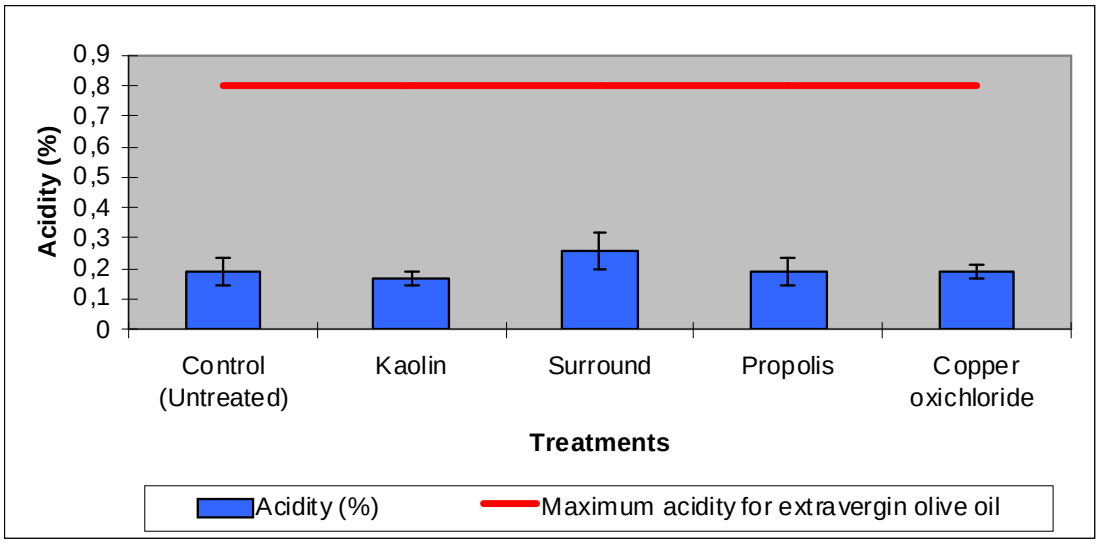

Figure 45: Rasciatano olive oil free acidity in different treatments of Coratina cultivar.

4.3.2.2. Peroxide value

Hydroperoxides (primary stage of oxidation) synthesis was not at the level of affecting the extra virgin quality of Coratina olive oil in Rasciatano. As it is shown in the graph (Fig. 46), the maximum value of peroxide for extra virgin olive oil was not reached.

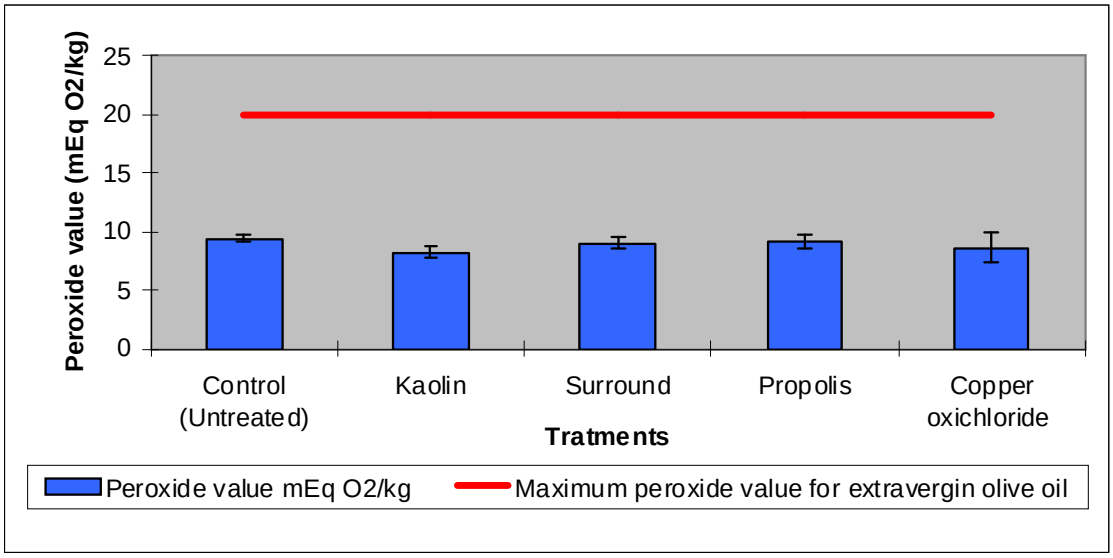

Figure 46: Olive oil peroxide value of Coratina cultivar at Rasciatano.

4.3.2.3. Spectrophotometric investigation in the ultraviolet

UV spectrophotometric values K_{232} , K_{270} (Figs. 47 and 48) and Delta-K registered for the treatments during the analysis of Rasciatano olive oil were all under the maximum values fixed for extra virgin olive oil grade. No significant difference was noticed for the spectrophotometric analysis between treatments.

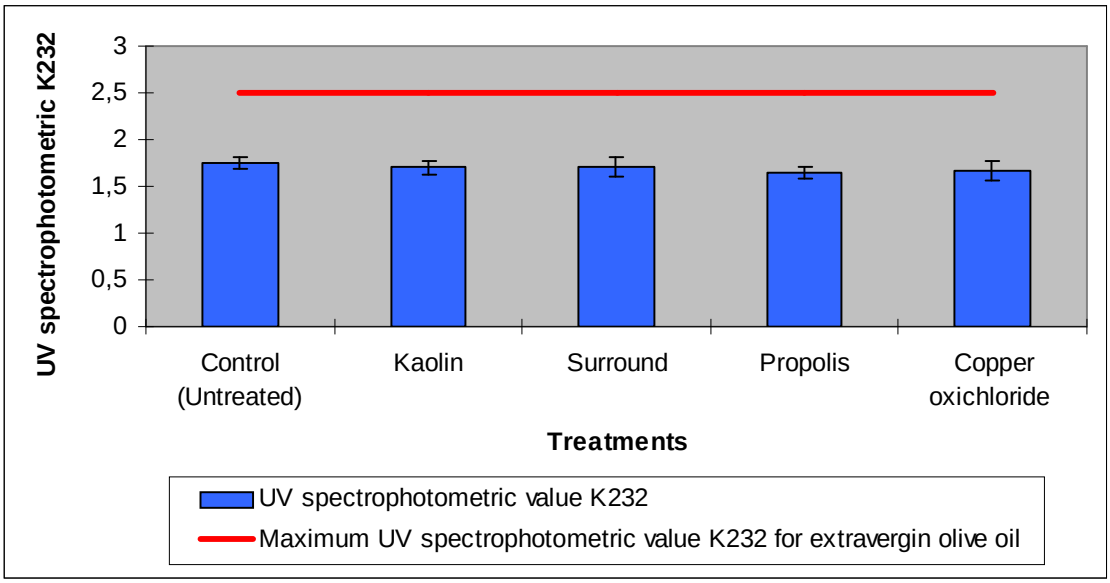

Figure 47: K_{232} UV spectrophotometric value of Rasciatano Coratina cultuvar.

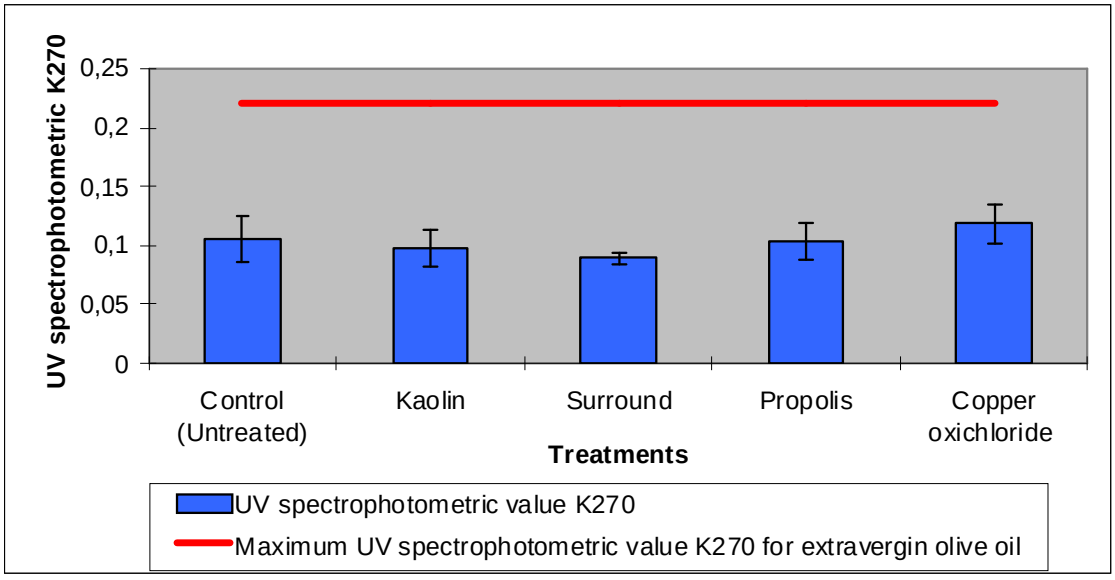

Figure 48: UV spectrophotometric value K_{270} of Rasciatano Coratina cultuvar.

4.3.2.4. Organoleptic assessment

Organoleptic assessment of olive oil in Rasciatano shows that nor infestation neither treatment type (Table 18) affect the sensorial characteristics of olive oil.

Table 18: Sensory analysis classification of Rasciatano olive oil from different experimental plots.

Treatment	Sensory analysis category
Kaolin®	Extra virgin
Surround®WP	Extra virgin
Propoli®	Extra virgin
Ossiclor 20®	Extra virgin
Control	Extra virgin

4.3.2.5. Organoleptic profile

Olive oil organoleptic profiles of Rasciatano in the different treatments present coloration varying from yellow to green. Olfactory sensation in control, Surround® and Propolis is typical of oils obtained from olives that have been harvested during coulor change (greenly fruity) but in Kaolin® and copper oxichloride treatments the sensorial analysis shows ripely fruity sensation. Almond and grass are the direct aromatic olfactory sensations predominant in the oils analysis. Bitter and pungent are more or less intense in the Coratina oil of Rasciatano which is one of the most important criteria of this variety in Apulia (Lombardo, 2004).

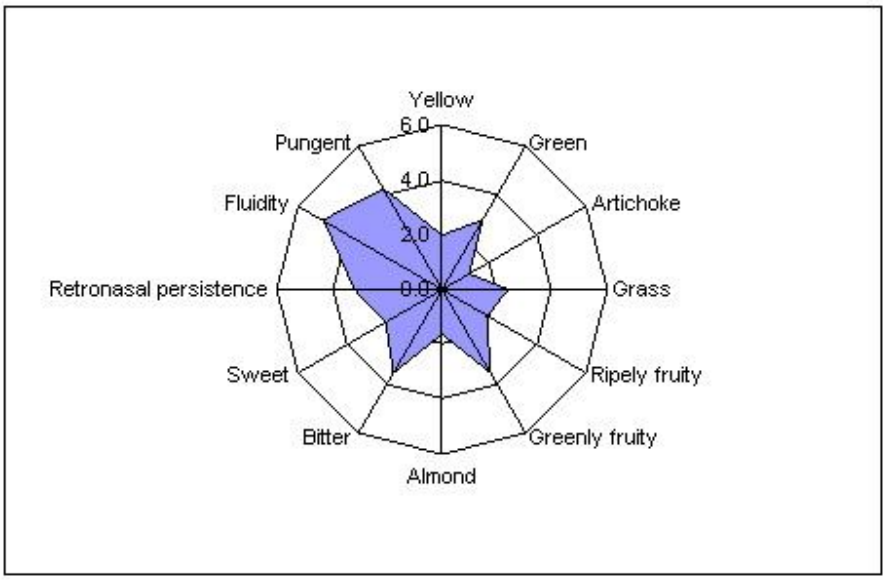

Figure 49: Organoleptic profile of olive oil from Rasciatano control.

4.3.3. Tateo

4.3.3.1. Acidity

Olive oil acidity in the different plots was low; between 0.14% in control and propolis treatments and 0.21% at Kaolin® and Surround® (Fig. 50). This difference is significant in statistic terms but did not affect considerably the acidity of the oil.

Vossen *et al.*, (2006) confirm that olive fruit fly infestation greatly increases the free fatty acid level of olive oil. *Bactrocera oleae* infestation in Tateo did not show any correlation with acidity, this is may be due to the low infestation values. In fact control plot, that has the maximum infestation level (4%), has the lower acidity through all treatments (0.141%).

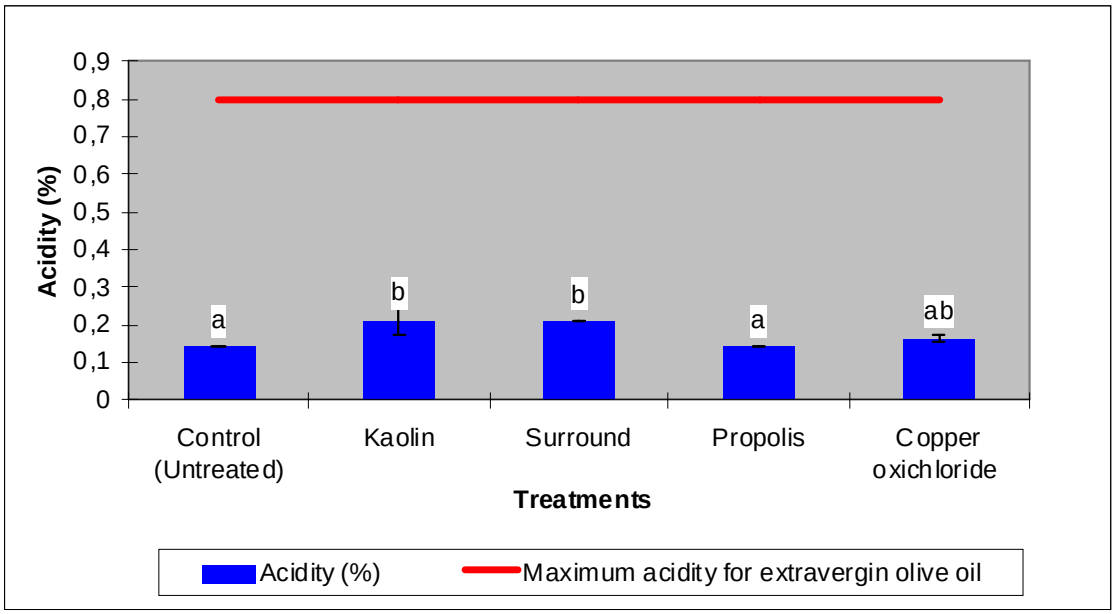

Figure 50: Olive oil free acidity of Coratina cultivar in Tateo farm. Error bars represent standard errors of the means. Histograms with different letters are statistically significant using ANOVA followed by Duncan's Multiple-Range test; $p<0.05$.

4.3.3.2. Peroxide value

Peroxide index did not show differences through the different treatments and fill the value required for extra virgin oil grade (Fig. 51).

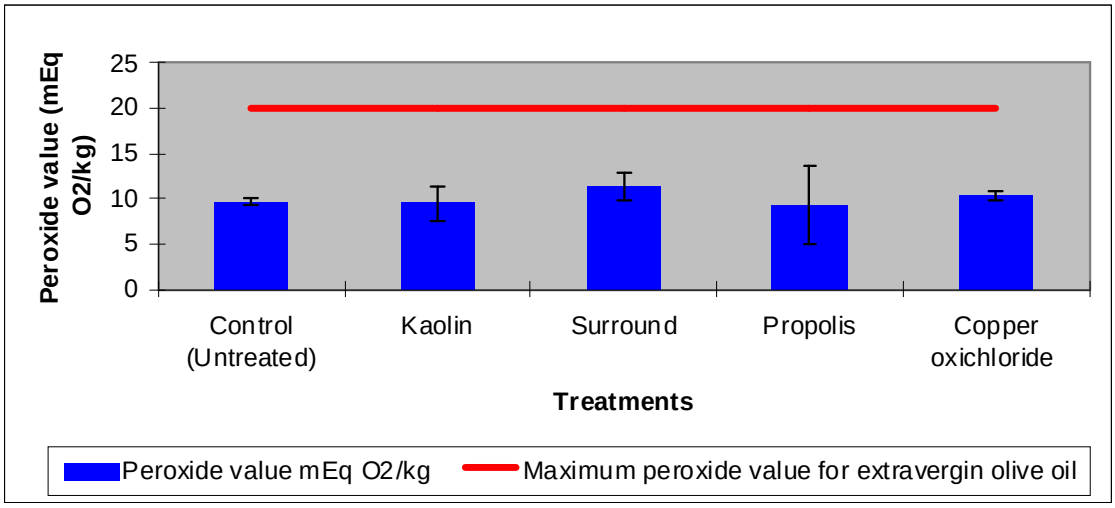

Figure 51: Olive oil peroxide value of Coratina cultivar in Tateo farm.

4.3.3.3. Spectrophotometric investigation in the ultraviolet

UV spectrophotometric values K_{232}, K_{270} and Delta-K registered for the treatments during the analysis of Tateo olive oil were all under the maximum values fixed for extra virgin olive oil grade. No significant differences were noticed for the spectrophotometeric analysis between treatments (Figs. 52 and 53).

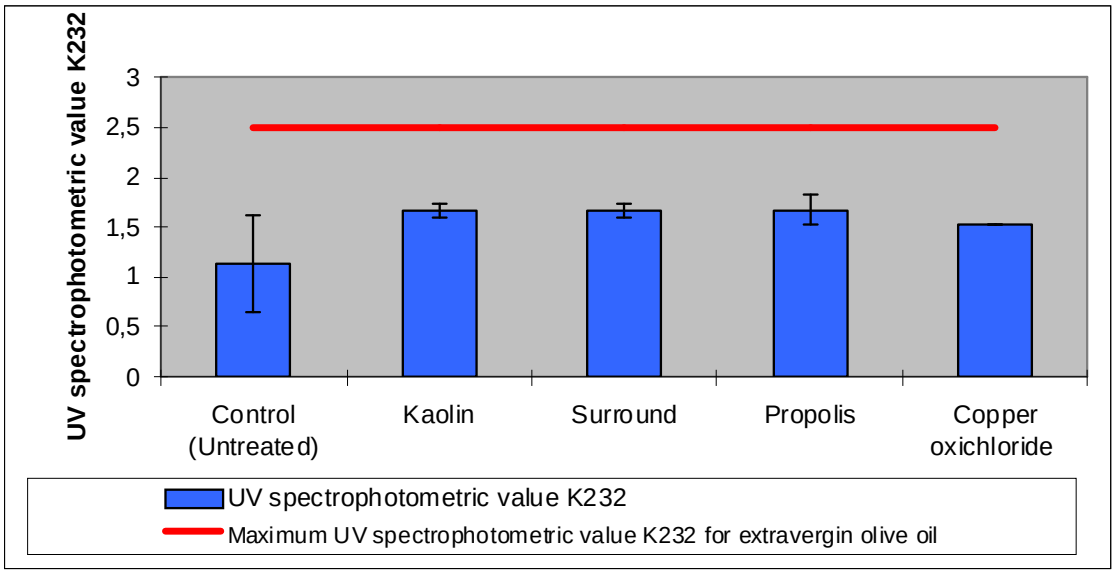

Figure 52: K_{232} UV spectrophotometric value of Tateo Coratina cultivar.

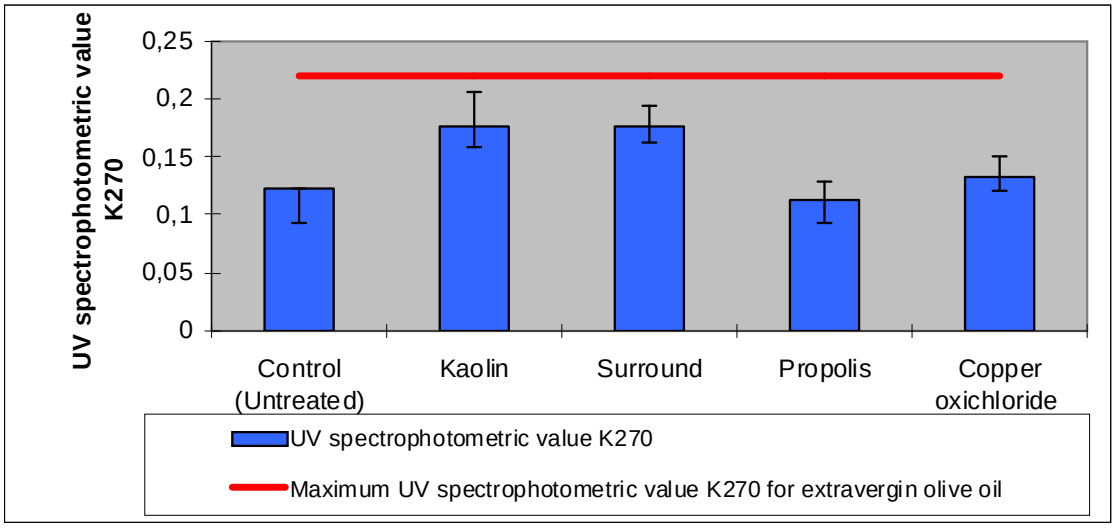

Figure 53: K_{270} UV spectrophotometric value of Tateo Coratina cultivar.

4.3.3.4. Organoleptic assessment

The relationship between the infestation level and sensory analysis (Table 19) does not seem to be clear, probably because the low *B. oleae* infestation level does not affect significantly the olive oil organoleptic traits, as well as the different kind of treatment. Therefore, only technical problems during olive milling can be taking into account to explain the classification into the "virgin" category for Propolis and Control oil samples.

Table 19: Sensory analysis classification of Tateo olive oil from different experimental plots.

Treatment	Sensory analysis category
Kaolin®	Extra virgin
Surround®WP	Extra virgin
Propoli®	Virgin
Ossiclor 20®	Extra virgin
Control	Virgin

4.3.3.5. Organoleptic profile

Olive oil in Tateo is bitter and pungent with olfactory sensation of oils obtained from olives that have been fully ripe with hints of almond and grass (Fig. 54).

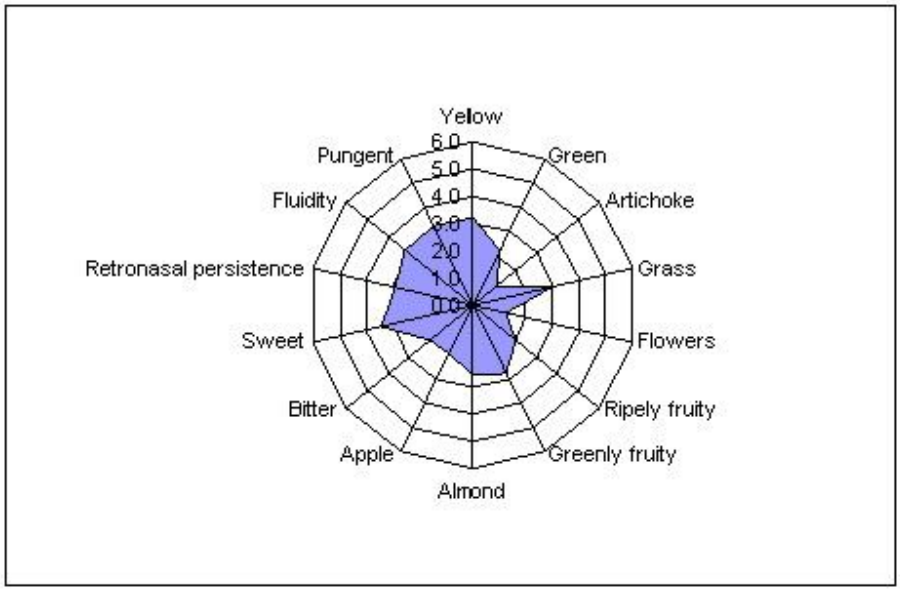

Figure 54: Organoleptic profile of olive oil from Tateo control.

5. Conclusions and recommendations

Effectiveness evaluation of kaolin-based treatments and propolis compared to copper oxichloride for the control of *B. oleae* (Rossi) tested in Damone, Rasciatano and Tateo farms was very hard in 2007 summer. In fact, high temperatures accompanied with low humidity conditioned olive fruit fly population dynamic and can lead to: (i) ovarian maturation inhibition; (ii) flies disturbing; and (iii) abiotic mortality of *B. oleae*. In consequence, the fly pressure in Apulia reached an unusual level of low infestation that hampered our study. However, results proved that tested products; containing kaolin, copper oxichloride and propolis; are able to limit *B. oleae* infestation for the production of high grade olive oil and high quality table olives. Generally speaking, treatments response to *B. oleae* infestation did not show significant differences. This probably means that copper-based treatment can be replaced by other products such as kaolin and propolis. Moreover, considering the earlier harvesting of table olive in Damone farm and the low susceptibility of Termite di Bitteto cv, the olive fly attack in this last case could be neglected.

The monitoring of aluminum, silicon and copper in olives and olive oils revealed important conclusions.

The copper arising from copper oxichloride treatment in olive drupes is reduced to an acceptable content value only if the safety pre-harvest period of at least 20 days is respected. For that reason the necessity of the safety interval of at least 20 days after the last treatment of copper oxichloride is confirmed to guarantee the concentration reduction of copper below the MRL in olive fruits (20 mg/kg). On the contrary, aluminum concentration in kaoilin treated olive fruits, even with a noticeable decrease, remains high (183 mg/kg in Rasciatano at 21 days after the second treatment application). Actually, the drupes have not been washed before milling which explain these high values of aluminum concentration in olives.

Aluminum, silicon and copper residues in the olive oil were very low, especially if compared with olives. In the case of copper, such reduction is explained by the discharge of this element in the mill waste waters by centrifugation for both; i) natural highly soluble copper contained in olive fruits and ii) non soluble copper deposited on the surface of the fruit in copper oxhichloride treated plots.

Regarding Al and Si in kaolin treated plots, the major part could be evacuated during the mill water phase as kaolinite because of the high stability of this active ingredient. Therefore, these olive waste waters can contain high concentrations of Cu and their spreading on the soil without any preventive treatment can be detrimental to the soil biological activity.

Since aluminum, silicon and copper concentrations are very low in oils extracted from the treated plants, and did not show significant difference with oils emanating from untreated plants. We can suppose that any eventual alterations in olive oils, treated either by kaolin or copper based treatments, are not evident to be due to the presence of kaolin and copper residues.

In fact, the determination of chemical parameters in oils obtained from olives treated by kaolin based products, copper oxychloride and propolis revealed that, in the trial conditions, there is no relation between the product used and quality criteria (acidity, peroxide number, K_{232}, K_{270} and ΔK), probably owing to the low infestation levels. Furthermore, the organoleptic traits correspond to a high quality grade olive oil. Oils are all extra virgin, except those ones treated with propolis in Damone trial that resulted virgin because of some technical troubles during olive milling. Table olive "Termite di Bitetto" could be more exposed to oxidation because of the highest value of peroxide index and k_{232} constant with respect to olive oil from Coratina cv.

To suggest a substituent to copper oxichloride is not easy, especially with the low olive fruit fly pressure of this year. In terms of efficacy Surround®WP has shown a good response to *B. oleae* attacks, the product is more adhesive to the surface of the fruits and leaves which mean that its treatment number can be reduced compared to the others. In addition results showed that kaolin-based treatments do not affect the olive oil quality criteria. The presence of aluminum and silicon in olive dupes residues resulting from kaolin application do not present risk for consumer's health. The kaolinite, existing on the treated fruits surface, is an inert substance that can only affect the olives natural coloration appearance in the case of olive table cultivars. Therefore, a fruit washing is enough to market this product without doubt.

Future studies about kaolin-based treatments and copper oxichloride should concern:

- The influence of kaolin on the agronomic parameters of olive plants;
- The mill waste water resulting from olives treated with copper oxichloride in order to assess the related environmental risk;
- The cost of the above mentioned treatments compared with eventual products existing on the market and being authorized in organic agriculture.

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Annex 1: CRA organoleptic profile sheet.

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Esame visivo	**INTENSITÀ**
Giallo	⟶
Verde	⟶
Sensazioni aromatiche olfattive dirette o retronasali	
Acerbo	⟶
Agrumi	⟶
Camomilla	⟶
Carciofo	⟶
Erba	⟶
Erbe aromatiche	⟶
Floreale	⟶
Foglia di olivo	⟶
Frutta esotica	⟶
Fruttato di oliva maturo	⟶
Fruttato di oliva verde	⟶
Frutti rossi	⟶
Mandorla	⟶
Mela	⟶
Noce	⟶
Pepe verde	⟶
Peperone	⟶
Pera	⟶
Pinolo	⟶
Pomodoro	⟶
Vaniglia	⟶
Sensazioni Gustative	
Amaro	⟶
Dolce	⟶
Sensazione retrolfattiva qualitativa	
Persistenza retrolfattiva	⟶
Sensazioni tattili o cinestetiche	
Fluidità	⟶
Piccante	⟶

VOTO: _____ **DATA:** _____ **Codice campione:** _____

FIRMA

Assaggiatore: _____ _____

Annex 2: Profile sheet for virgin olive oil (IOOC, 2007e).

PROFILE SHEET FOR VIRGIN OLIVE OIL

INTENSITY OF PERCEPTION OF DEFECTS:

Fusty/ muddy sediment ——————➡

Musty-humid-earthy ——————➡

Winey-vinegary - acid-sour ——————➡

Metallic ——————➡

Rancid ——————➡

Others (specify) ——————➡

INTENSITY OF PERCEPTION OF POSITIVE ATTRIBUTES:

Fruity ——————➡

greenly ☐ ripely ☐

Bitter ——————➡

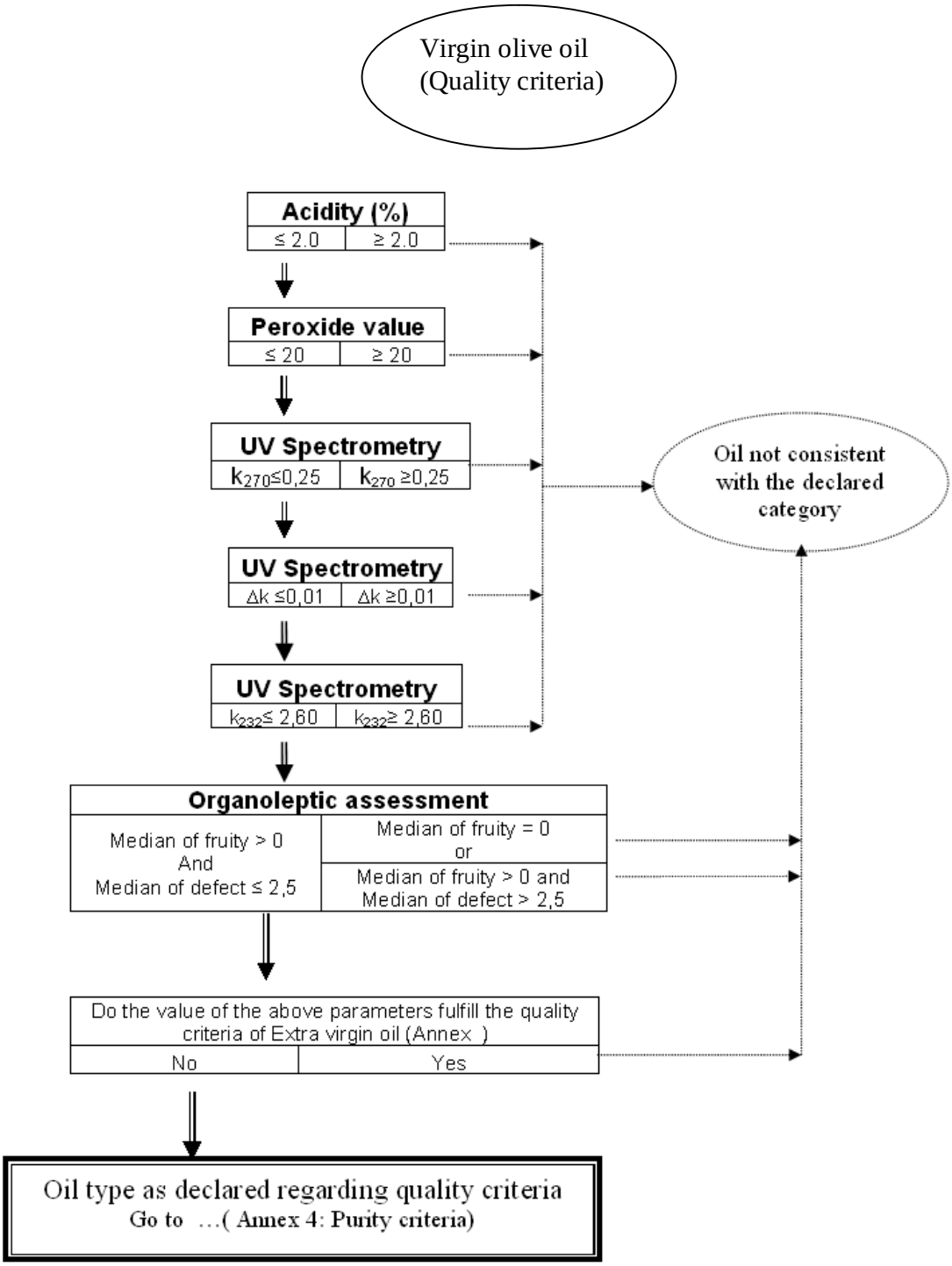
Pungent ——————➡

Name of taster:

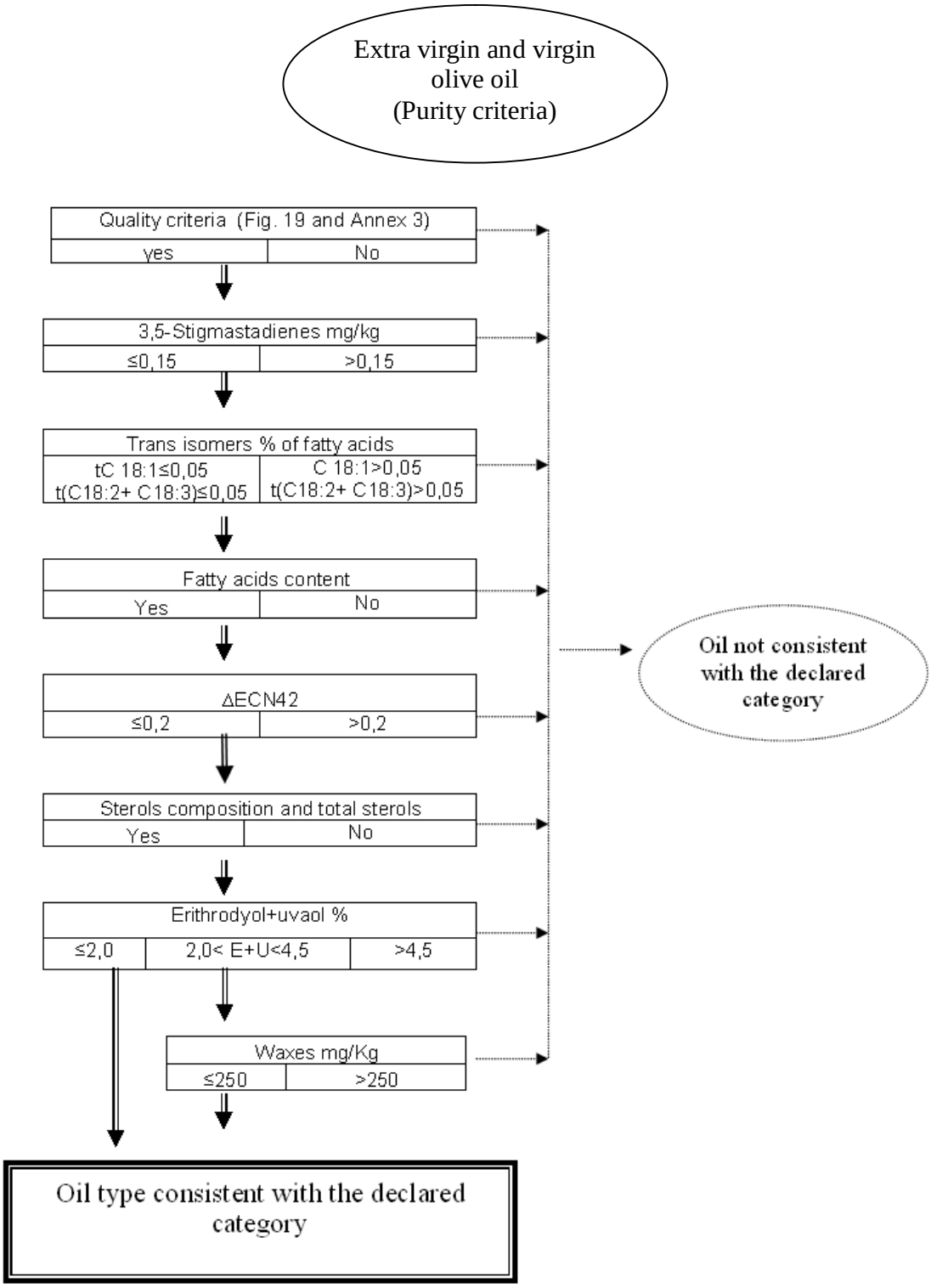
Sample code: **Date:**

Comments:

Annex 3: Virgin olive oil (Quality criteria) (European Community, 2003a).



Annex 4: Extra virgin and virgin olive oil (purity criteria) (European Community, 2003a).



Annex 5: Lampante olive oil (Quality and purity criteria) (European Community, 2003a).