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Effects of crop method and harvest seasons on yield and quality of green asparagus under tunnel in southern Italy

G. Caruso*[1], G. Villari, C. Borrelli*, G. Russo*****

* *Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università degli Studi di Napoli Federico II, Via Università, 100, 80055 Portici (NA), Italy.*

** *Stazione Sperimentale per l'Industria delle Conserve Alimentari, Via Nazionale, 121, 84012 Angri (SA), Italy.*

*** *Ph.D., Università degli Studi di Napoli Federico II, Via Università, 100, 80055 Portici (NA), Italy.*

Key words: annual double harvest, amino acids, *Asparagus officinalis* L., fiber, organic management, sugars, vitamin C.

Abstract: A three-year study (2007-2009) was carried out on green asparagus under tunnel in Campania (southern Italy) with the purpose of verifying both the possibility to practise organic management and annual double harvest in order to extend the eco-compatible production period and to avoid expensive imports. Comparisons among eight experimental treatments were made. Treatments were obtained by factorial combination of two crop methods (conventional and organic) and four spring and summer harvest periods of 90-day total duration (75 days in spring plus 15 in summer; 60 days in spring plus 30 in summer; 45 days in spring plus 45 in summer; as a control, 90 days in spring), arranging a split plot design with three replicates. The conventional management led to the highest yield, as a consequence of the higher spear number per plant, while the organic management resulted in both spear calibre and mean weight increase. Organic spears showed a higher level of residues and sugars but a lower content of nitrate and fibre. The treatment with 75-day harvest in spring and 15 in summer proved the best double harvest combination, leading to the highest comprehensive yield (11.5 t·ha^{-1}), not different from the control harvested only in spring for 90 days. Summer spears showed higher values of optical residue, glucose, fructose, vitamin C and some mineral nutrients; instead, spring spears attained lower nitrate and average fibre content. Asparagus annual double harvest revealed economically interesting results, but the profits are strictly related to the prices of summer spears, which were evidently higher in summer than in spring in the three years of research.

1. Introduction

Asparagus (*Asparagus officinalis* L.) is widely cultivated in Italy and the total surface area devoted to this crop is as much as 6347 ha, mainly located in Veneto (1610), Campania (1347) and Apulia (1070); notably, Campania is the only Italian region where the species is significantly grown in greenhouse (1052 ha) (ISTAT, 2012).

In the temperate area of the northern hemisphere, asparagus harvest begins after winter rhizome dormancy, when soil temperature is favourable to hypogeous bud resumption (McCormick and Geddes, 1996; Heiβner *et al*., 2006). Therefore, the latter is earlier at lower latitudes and gradually delayed towards the north: in fact, it starts in January in California desert valleys, in February in Italian southern regions and in south Carolina, in May in Holland, end of June in Michigan and in Washington districts (Dufault, 1994 a). The spring harvest duration in greenhouse in southern Italy is usually extended to ninety days, as it occurs in other areas with similar climatic conditions (Shou *et al*., 2007). Indeed, in such cases the favourable season length allows the plants to completely recover rhizome reservoirs, insuring crop vigour and longevity (Takatori *et al*., 1970). However, as in Italy the production period is exclusively concentrated in spring, in the other seasons of the year the product demand is satisfied by imports from warmer areas (South Africa, Chile, Mexico, southern California). As an alternative, asparagus production can be achieved by summer forcing, through total aerial biomass cutting and subsequent irrigation a few days before the scheduled harvest. The possibility of annual double harvest has also been proposed in South Carolina (Dufault, 1991, 1995 and 1996) and its convenience seems to be mainly dependent on the favourable season duration. Particularly, the crop forcing achieved at the end of spring or summer could reveal, respectively, too early or late and therefore damage the rhizome reservoir and consequently plant longevity. Instead, a second harvest

(1) Corresponding author: gcaruso@unina.it

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period managed in July-August could allow the plants to reconstruct the aerial biomass both after the spring production and before the winter quiescence. In comparing the traditional spring three-month yield and the comprehensive production derived from the spring and summer harvest, it should be taken into account that the double harvest period can satisfy the consumer requirement in both seasons. Particularly, in summer higher economic profits could be achieved due to both a lack of asparagus spear offer at Italian markets and the potential product demand by tourists. Also, harvest season influences the quality of asparagus spears, which are considered a good source of essential minerals, vitamins, amino acids and dietary fibers (Lopez *et al*., 1996). In fact, spear nutritional features are highly affected by environmental factors, especially temperature and light (Makus, 1994, 1995; Papadopoulou *et al*., 2003). In addition, Bhowmik and coworkers (2001) proved that carbohydrate and organic acid contents in asparagus spears were season-dependent. Also fibrousness, though being a varietal peculiarity (Poll and van Kuistrum, 1990; Simón, 1990; Billau *et al*., 1990; Gast *et al*., 1991; Sanchez, 1996), is influenced by crop environment and seasonal climate. In particular, it was inversely correlated with rainfall (Keulder and Riedel, 1990) and temperature (Sosa Coronel *et al*., 1976; Keulder and Riedel, 1990; Poll, 1996), which regulate the spear growth rate. Fibrousness is the consequence of wall lignification of the pericycle cells and vascular bundles (Baxter *et al*., 1987). According to Haard *et al*. (1974), spear removal causes endogenous ethylene development at an amount stimulating the peroxidase activity involved in lignin synthesis. Also spear diameter influences fibrousness (Clore *et al*., 1976; Billau *et al*., 1990; Poll, 1996) because the fibrous bundle number does not change and thus the fibre has a lower relative incidence in higher calibre spears (Herner, 1973).

With the purpose of studying the different aspects mentioned above, research was carried out in Salerno province (southern Italy) on green asparagus under tunnel with the aim of evaluating the effects of crop method and annual double harvest on spear yield and quality.

2. Materials and Methods

Research was carried out in the period 2007-2009 in Fisciano (Salerno province) under tunnel (40°46' N, 14° 48' E, 150 m a.s.l.). The asparagus crop was planted in 2004 on clay-loam soil (Table 1), using cultivar Desto crowns spaced 1.20 m between rows and 0.40 m between the plants along rows. Temperature time course of the three research years are shown in Figure 1.

Eight experimental treatments were compared. Treatments were obtained by factorial combination of two crop methods (conventional and organic) and four spring and summer harvest periods of 90-day total duration (75 days in spring plus 15 in summer, labelled as 75+15; 60 days in spring plus 30 in summer, labelled as 60+30; 45 days in spring plus 45 in summer, labelled as 45+45; as a control, 90 days in spring). A split plot design was used, with three replicates, assigning the crop methods to plots and the harvest periods to sub-plots; the latter had a 17.3 m^2 surface area.

Table 1 - Soil characteristics

Constituents		
Coarse sand	%	24.2
Fine sand	%	29.8
Silt	%	27.2
Clay	%	18.9
Composition		
Organic matter (Walkley and Black method)	%	3.47
Total nitrogen (N) - Kjeldahl method	%	0.21
Available phosphate (P_2O_5) - Olsen method	ppm	12.5
Available potassium (K_2O) - ammonium acetate method	ppm	146.9
Reaction	pH	6.57

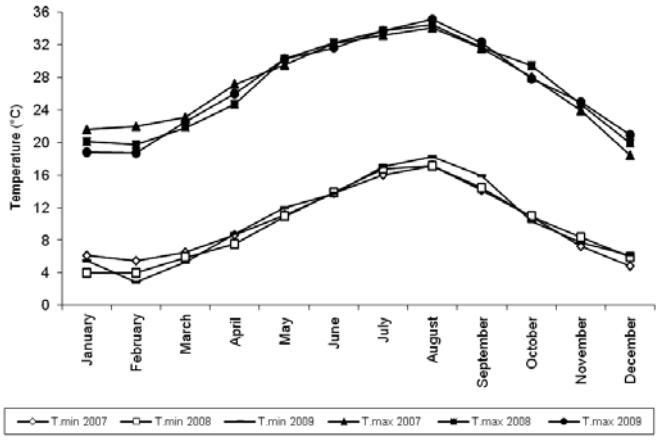

Fig. 1 - Trend of monthly temperatures in greenhouse.

Polytunnels, covered by thermal polyethylene, were made of three structural units, each of them 20 m long, 8 m wide, 2 m high at wall and 3.5 m at roof.

The organic system was managed according to EC Regulation 834/2007, using only substances permitted under the law. For the crop, three fertilizations per year were achieved, before and after spring harvest and after summer harvest, each with 60 kg·ha^{-1} of N, 40 of P_2O_5 and 70 of K_2O, using mineral fertilizer for the conventional method and manure for the "organic". Moreover, weed control was undertaken using linuron chemical treatment (0.75 l·ha^{-1} of a. p.), at spear pre-emergence, in conventional plots and by hand in organic. Drip irrigation was activated at 80% soil available water consumption.

Harvest was carried out twice a year, in spring and in summer, except for the control harvested only in spring. Spear emission in summer was urged by cutting the crop aerial biomass and, at the same time, activating irrigation. In particular, these interventions were made at the beginning of July for the 45+45 treatment, at mid-July for 60+30 and at the end of July for 75+15.

Harvest was practised by hand when the spears emerged 150 mm out of soil and it began on 28 February, 3 March and 2 March, respectively in 2007, 2008 and 2009. At each picking, the plot product was separated in marketable and waste fractions. The latter included the folded, damaged or less than 10 mm thick spears. After harvesting, marketable spears were cut at 30 mm from the base and their calibre was measured on 30-unit random samples.

Moreover, with the aim of estimating product prices during spring harvests, data published by Infomercati (www.infomercati.com) were used. Informercati is a consortium of the main Italian fruit and vegetable wholesale markets, which records and processes the daily prices of each product. In July and August the prices were detected by local sellers to whom the research spears were given, as in summer no asparagus transactions were recorded.

In order to evaluate spear qualitative characteristics, random 30-unit samples were collected in each plot (for three replicates) both in conventional and organic treatments. In the control, this procedure was achieved 45 days after beginning of harvest; in the 75+15, 60+30 and 45+45 treatments sample collection was made 37, 30 or 22 days, respectively after spring harvest and 7, 15 or 22 days after summer harvest. Spears were immediately transferred to the Experimental Station for the Food Preserving Industry, Angri (Salerno) Branch, where the following determinations were made:

- dry residue: in an oven at 70°C under vacuum until steady weight;
- optical residue (expressed in °Brix): on spear sap after squeezing, at a temperature of 20°C, by means of a digital refractometer, model R.F.M81, from BS (Bellingham+Stanley);
- reducing sugars (glucose and fructose) and sucrose: by HPLC, using the 600E Waters chromatographic system and a column Sugar-pak Waters at 85°C, EDTA-Ca in water solution as eluent (50 $mg \cdot l^{-1}$);
- titratable acidity: expressed as grams of monohydrate citric acid per 100 g of product in agreement with the official analysis methods for vegetable preserves of the Italian Ministry of Agriculture and Forestry (MiPAF, 1973);
- proteins: with the Kjeldahl method, utilising a Foss Tecator digestor with a Kjeltec 2300 distiller;
- lipids: measured in accordance with the official analysis methods for vegetable preserves of the Italian Ministry of Agriculture and Forestry (MiPAF, 1973);
- vitamin C: by HPLC using the model 600E Waters chromatographic system, equipped with a 486 Waters UV detector set to 410 nm λ and a column Biorad mod. HPX87H at 35°C;
- fibre: the SIGMA Chemical Company Enzymatic kit was used. The samples were weighed, dried (105°C), gelatinized in the presence of heat-resistant α–amylase and digested enzymatically by proteases and amydoglucosydase, to remove proteins and starch, whereas soluble fibre was precipitated by ethanol. The residue, filtered, washed with ethanol and acetone, dried and weighed, was split into two fractions used to determine, respectively, proteins and ash. Fibre content was obtained by the difference between the weights of the residue and the proteins and ash;
- free amino acids: by means of HPLC with a Waters 600E chromatographic system, connected to a personal computer using Millenium32 software, version 3·05·01, and equipped with a Waters 717 autosampler and a fluorescence detector set at a λ of 205 nm-395 nm. The measurements were carried out utilising: a Waters AccQTag column (spherical C-18, 4 µm 150 x 3.9 mm, at a temperature of 35°C; in condition of gradient with eluent A, or a 140 mM sodium acetate trihydrate buffer, TEA, EDTA-Na2, sodium azide, eluent B, consisting of acetonitrile, and eluent C, which consists of water and with an injection volume equal to 5 µL;
- mineral anions (chlorides, nitrates, phosphates): by HPLC with 600E Waters system, a mod. 717 autosampler and a Dionex column (mod. AS11, 4 x 250 mm);
- mineral cations: (calcium, magnesium, potassium, sodium, iron, copper, zinc): by atomic adsorption spectrophotometry, after sulpho-nitric mineralization, with a model 1100 Perkin-Elmer spectrophotometer.

Data were processed by analysis of variance and Duncan multiple range test was used for mean separation at 0.05 and 0.01 probability levels (n = 3).

3. Results and Discussion

From the data reported in Table 2 it can be stated that there are no significant differences among the research

Table 2 - Asparagus yield results as influenced by crop method and harvest seasons

	Marketable spears			
Treatment	Yield $t \cdot ha^{-1}$	no. per plant	Mean weight g	Calibre mm
Year				
2006	10.3	14.3	35.8	15.6
2007	10.1	14.6	34.4	15.5
2008	10.2	15.1	33.9	15.3
	NS	NS	NS	NS
Crop method				
Conventional	10.7	15.6	34.0	15.1
Organic	9.7	13.7	35.3	15.8
	*	*	*	*
Harvest seasons				
Spring 90 days	11.7 a	16.4 a	35.4 a	15.8 a
Spring 75 days + Summer 15 days	11.5 a	16.5 a	35.1 a	15.6 a
Spring 60 days + Summer 30 days	9.5 b	13.7 b	34.7 a	15.5 a
Spring 45 days + Summer 45 days	8.1 c	12.1 c	33.4 b	14.9 b

NS= not significant; * significant at p≤ 0.05.
Means followed by different letters are significantly different according to the Duncan test at p≤0.05 (n=3).

years in terms of yield. This means that spring harvest did not condition the subsequent summer plant productive behaviour and summer harvest did not condition the crop performances in following year's spring. Moreover, no plant density reductions were recorded.

Conventional asparagus management caused the highest yield, as a consequence of the higher spear number per plant (Table 2). This is consistent with Poll and van Kruistum (1987) referring to a correlation between spear number and yield. Organic management, instead, resulted in spear calibre and mean weight increase. These differences between the two crop methods are the consequence of the yield differences recorded both in spring and summer (Table 3), though the conventional method resulted in higher production than the organic one by 14.2% in spring but only by 6% in summer. Contrary to our results, in previous research (Warman, 1991) no significant difference was detected between the two crop methods.

As for the comparison among the annual double production periods (Table 2), the 75+15 treatment (75-day spring harvest plus 15 days in summer) caused the highest comprehensive yield (11.5 t·ha^{-1}), owing to the highest spear number, calibre and mean weight. However, it was not different from the control harvested only in spring for 90 days. The lowest production was attained by the combination of 45-day spring harvest plus 45 days in summer (8.1 t·ha^{-1}). Notably, the comprehensive yield obtained by the double harvest crops was influenced to a greater extent by spring results than by summer ones, as in the first season more remarkable differences among the treatments were recorded (Table 3). In fact, in spring the production increases caused by the 75+15 treatment were, respectively, by 54% and 170% in comparison with 60+30 and 45+45, though correspondingly the harvest period was 25% and 67% longer. In summer, however, an opposite yield trend was shown by the annual double harvest treatments, but unable to balance the spring results. In fact, in summer the 45+45 treatment had a longer harvest period than 60+30 and 75+15, respectively, by 100% and 200%, to which the same percentage yield increases corresponded. The productive results were, therefore, affected by the lower temperatures recorded in March which, in agreement with previous reports (Heiβner *et al*., 2006), conditioned the shooting rate. The latter was, instead, favoured by the gradual temperature increase, which allowed in the 75+15 treatment to get more than 60% of the whole spring production in the last 30 days of harvest. In fact, quick spear emission is promoted by an air temperature of about 24°C (Bouwcamp and McCully, 1975) or soil temperature of about 20°C and it is correlated with the total yield (Poll and van Kruistrum, 1987; Keulder and Riedel, 1996). Therefore, summer temperature was always very favourable to spear immediate and continuous emission, in agreement with the reports of Dufault (1994 b), thus differences among the harvest treatments were not so remarkable as those recorded in spring. But in summer, yield results were conditioned by spear calibre, with values showing an inverse relation with temperature, consistent with previous findings (Wagenvoort *et al*., 1980; Pill *et al*., 1993). Notably, both in spring and summer spear calibre and mean weight decreased from the shortest to the longest harvest period, confirming the effects of harvest pressure on these variables (McGrady and Tilt, 1990).

Table 3 - Asparagus spring and summer yield results as influenced by crop method and harvest seasons

	Marketable spears							
	Yield (t ha^{-1})		No. per plant		Mean weight (g)		Calibre (mm)	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Year								
2006	8.0	3.0	10.6	4.9	38.2	31.0	16.5	14.1
2007	7.9	3.0	11.0	4.9	36.4	30.6	16.3	13.9
2008	8.0	2.9	11.5	4.8	35.7	30.4	16.2	13.8
	NS	NS	NS	NS	NS	NS	NS	NS
Crop method								
Conventional	8.4	3.1	11.8	5.1	36.0	30.1	15.9	13.7
Organic	7.6	2.9	10.2	4.6	37.6	31.2	16.8	14.1
	*	*	*	*	*	*	*	*
Harvest seasons								
Spring 90 days	11.7 a		16.4 a		35.4 c		15.8 c	
Spring 75 days + Summer 15 days	10.0 b	1.5 c	14.1 b	2.4 c	35.7 c	31.4 a	15.7 c	14.6 a
Spring 60 days + Summer 30 days	6.5 c	3.0 b	8.8 c	4.9 b	37.1 b	30.6 b	16.5 b	13.8 b
Spring 45 days + Summer 45 days	3.7 d	4.4 a	4.8 d	7.4 a	38.9 a	30.0 c	17.2 a	13.4 c

NS = not significant; * significant at p≤ 0.05.
Means followed by different letters are significantly different according to the Duncan test at p≤0.05 (n=3).

Table 4 - Asparagus spear quality as influenced by crop method and harvest seasons

Treatment	Dry residue (g·100 g^{-1} FW)	Optical residue (°Brix)	Glucose (g·100 g^{-1} DW)	Fructose (g·100 g^{-1} DW)	Sucrose (g·100 g^{-1} DW)	Acidity (g·100 g^{-1} DW)	Protein (g·100 g^{-1} DW)	Lipids (g·100 g^{-1} DW)	Vitamin C (mg·100 g^{-1} DW)
Crop method									
Conventional	8.38	6.72	4.54	7.54	1.55	4.93	31.4	6.20	659.9
Organic	9.11	7.26	5.40	8.41	1.77	5.02	32.4	6.26	673.0
	*	*	*	*	*	NS	NS	NS	NS
Harvest seasons									
Spring	8.64	6.81	4.83	7.78	1.63	4.87	31.7	6.13	627.0
Summer	8.85	7.18	5.11	8.17	1.69	5.08	32.1	6.33	705.9
	NS	*	*	*	NS	NS	NS	NS	*

NS= not significant; * significant at p≤ 0.05 (n=3).

Our research showed that the goal of annual double harvest is pursuable in terms of yield in irrigation regime, if the traditional spring harvest is reduced from 90 to 75 days and integrated with 15 days of summer harvest. Alternative double harvest combinations, represented by 60-day spring harvest plus 30-day summer harvest or by 45-day harvest both in spring and summer, caused significantly lower yields than the control. Nevertheless, their economic profits are strictly related to the prices of summer spears. In this regard, the mean prices of green asparagus spears recorded in the three years of research were evidently higher in summer (5.5 €·kg^{-1} in July and 6.0 in August) than in spring (3.45 €·kg^{-1} in March, 2.68 in April and 2.36 in May).

From the statistical processing of spear quality data no significant differences were recorded both between the examined years (2008 and 2009) and among the different harvest durations within the same season. Therefore, only the results concerning the comparison between conventional and organic methods and between spring and summer harvest are reported (Tables 4, 5, 6 and 7).

With regard to quality indicators (Table 4), organic management resulted in higher values of dry and optical residues, glucose, fructose and sucrose than conventional cultivation, whereas acidity, proteins and lipids were not affected by crop method.

Also the harvest season had significant influence on quality, as summer spears showed higher values of optical residue, glucose, fructose and vitamin C than the spring ones; instead, no significant change was detected for dry residue, sucrose, titratable acidity, lipids and proteins. The latter result was similar to that of Shou *et al*. (2007) but, differently from our findings, these authors reported a decreasing trend for dry residue and reducing sugars and an increasing trend for acidity. Among the sugars, fructose attained higher incidence than glucose and sucrose, as also it resulted in our research. With respect to ascorbic acid, both Esteve *et al*. (1995) and Shou *et al*. (2007) found a content decrease in summer spears compared with spring ones.

Fibre content was affected by crop method (Table 5), as organic spears attained lower values than conventional ones. Also significant differences were recorded between spring and summer spears, as the latter showed higher average fibrousness, consistent with previous reports (Shou *et al*., 2007). Presumably, in summer the spear fibre increase caused by calibre reduction prevailed the opposite effect of temperature; in fact, the latter is inversely correlated with fibre content during spear growth, as reported by Sanchez (1996).

With respect to the fibre content of each 5 cm fraction obtained by spear split, the organic method led to higher values than the conventional in all the comparisons (Table 5). Regarding harvest season, it was found that summer spears showed higher fibre content than the spring ones only from the basal to the middle fraction. As for fibre content along the spear, the highest value was detected in the white basal fraction and it was as much as 2.6 times higher than the one recorded in the green tip (respectively, 28.1 and 11.0%). Haard *et al*. (1974) stated that fibre reduction towards the spear tip is the consequence of isoperoxidase changes, which start from the cut surface and gradually extend to the tip.

Table 5 - Asparagus spear fibrousness as influenced by crop method and harvest seasons

Treatment	Spear fibre content	Fibre content per spear section from tip to base (cm)				
		0-5 (g·100 g^{-1} DW)	5-10 (g·100 g^{-1} DW)	10-15 (g·100 g^{-1} DW)	15-20 (g·100 g^{-1} DW)	20-25 (g·100 g^{-1} DW)
Crop method						
Conventional	18.8	11.7	12.8	14.1	24.8	30.7
Organic	15.9	10.3	11.1	11.7	20.8	25.5
	*	*	*	*	*	*
Harvest seasons						
Spring	16.6	11.0	11.6	12.1	21.6	26.5
Summer	18.1	10.9	12.3	13.7	24.0	29.7
	*	NS	NS	*	*	*

NS= not significant; * significant at p≤ 0.05 (n=3).

Concerning the amino acids, we have reported only the mean composition and the statistical differences among the individual compounds (Table 6), as both crop method and harvest season did not cause any significant effect on this variable. With regard to composition, glutamine and asparagine showed the highest content in the asparagus spears, whereas cystine, methionine, tyrosine and histidine displayed the lowest. These results are consistent with those reported by other authors (Shou *et al*., 2007; Slupski *et al*., 2010).

As for spear chemical composition (Table 7), sodium and copper showed significant higher contents in organic crops, whereas the conventional method led to higher nitrate accumulation; the other ions examined were not affected by crop management. In previous research (Warman, 1991) no significant differences were recorded between conventional and organic methods with regard to spear mineral nutrients. With respect to harvest season, summer spears showed higher sodium, iron and zinc than the spring ones. In the latter season, contrary to the findings of other authors (Shou *et al*., 2007), a significantly higher nitrate content was detected, which was however lower than the one recorded in other research (Shalaby *et al*., 2004).

Table 6 - Asparagus spear amino acid composition (average of crop methods and harvest seasons)

Amino acids	Content (g·100 g^{-1} DW)
Alanine	1.18 bd
Arginine	1.24 bc
Asparagine	3.60 a
Cystine	0.16 h
Glutamine	3.97 a
Glycine	0.76 cf
Histidine	0.40 fh
Isoleucine	1.03 ce
Leucine	1.11 bd
Lysine	1.23 bd
Methionine	0.29 gh
Phenylalanine	0.62 eg
Proline	1.56 b
Serine	1.05 cd
Threonine	0.74 df
Tyrosine	0.37 fh
Valine	0.97 ce

Means followed by different letters are significantly different according to the Duncan test at p≤0.05 (n=3).

4. Conclusions

A three-year investigation (2007-2009) was carried out on green asparagus under tunnel in Salerno province (Campania, Italy) in order to investigate the effects of crop method and annual double harvest on spear yield and quality. Organic crops did not give as high yields as the conventional, but produced better spear quality in terms of higher level of residues and sugars and lower content of nitrate and fibre. Asparagus summer forcing, achieved through the plant aerial biomass cut and irrigation, did not limit the crop productivity but revealed interesting economically. Compared with the traditional 90-day harvest period, the annual double production practised for 75 day in spring and 15 in summer did not significantly differ in terms of yield. However, production decreased with the other spring-summer combinations, made up of 60-day harvest in spring plus 30 in summer or of 45-day harvest in both seasons. Nevertheless, the annual double harvest effectiveness also depends on asparagus market receptivity, which in summer is potentially interesting owing to tourist demand. This perspective is supported by the investigation conducted during our research: higher spear prices in summer than in spring (on average, respectively 5.75 €·kg^{-1} vs 2.83) due to the lack of Italian product offer. From a qualitative point of view, summer spears showed higher

Table 7 - Asparagus spear chemical composition as influenced by crop method and harvest seasons

Treatment	Calcium (mg·100 g^{-1} DW)	Magnesium (mg·100 g^{-1} DW)	Potassium (mg·100 g^{-1} DW)	Sodium (mg·100 g^{-1} DW)	Iron (mg·100 g^{-1} DW)	Copper (mg·100 g^{-1} DW)	Zinc (mg·100 g^{-1} DW)	Chlorides (mg·100 g^{-1} DW)	Phosphates (mg·100 g^{-1} DW)	Nitrates (mg·100 g^{-1} DW)
Crop method										
Conventional	25.0	22.2	249.6	3.5	1.19	0.12	0.95	57.1	62.4	45.9
Organic	23.5	21.7	263.9	4.2	1.16	0.15	0.93	57.7	66.7	14.2
	NS	NS	NS	*	NS	*	NS	NS	NS	*
Harvest seasons										
Spring	24.0	21.7	254.0	3.7	1.11	0.13	0.89	55.2	63.9	43.2
Summer	24.5	22.2	259.5	4.0	1.24	0.14	0.99	59.6	65.3	16.9
	NS	NS	NS	*	*	NS	*	NS	NS	*

NS= not significant; * significant at p≤ 0.05 (n=3).

values of optical residue, glucose, fructose, vitamin C and some mineral nutrients, compared with the spring product. Nevertheless, the latter attained lower fibre content, though only from the middle to the basal fraction.

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Pear resistance to Psilla (*Cacopsylla pyri* L.). A review

S. Nin*, A. Ferri*, P. Sacchetti, E. Giordani***

* *Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Università degli Studi di Firenze, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.*

** *Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze, Via Maragliano, 75-77, 50144 Firenze, Italy.*

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Abstract: Pear psylla, *Cacopsylla pyri* L., is one of the most important insect pests in European pear production areas. Control measures are directed specifically at controlling pear psylla and require accurate and timely information about insect densities in the orchard. Thus, there is a widespread interest in the search for suitable biological control agents and in breeding for resistance to pear psylla. Modes of host plant resistance to pear psylla damage have been studied extensively by several authors and the susceptibility of many European pear genotypes have been investigated in order to detect cultivars resistant or highly tolerant to this pest useful in breeding programs. This review presents an update of published results and knowledge on psylla life, host finding for feeding and oviposition, type of damages, monitoring and control strategies with renewed and improved efficacies, resistance characterization and breeding, with particular regard to the identified sources of resistance and the screening methods.

1. Introduction

Pear psylla is one of the most important pests affecting production of pears of *Pyrus communis* parentage. A sucking insect that causes severe wilting and defoliation, which reduces yields and weakens the trees. At least seven species of pear (*Pyrus*)-feeding psyllids in the genus *Cacopsylla* (formerly *Psylla*) are recognized, but there are three major species which occur primarily west of China: *C. pyricola* Foerster is the only species found in North America; *C. pyri* L. and *C. pyrisuga* Foerster are also endemic to Europe. *C. pyricola* probably originated in Western Eurasia in contact with wild *Pyrus* (Bell *et al.*, 1996).

All of the major cultivars of the European pear are susceptible to this Homopteran insect, varying only slightly in the degree of infestation and tolerance to feeding. Major cultivars of *P. pyrifolia* parentage are slightly less susceptible, while those of *P.* x *P. bretschneideri* or *P. ussuriensis* origin (e.g., Ya Li and Tzu Li) appear to be moderately resistant (Beutel, 1985).

2. Morfology and life of *Psylla*

C. pyri is characterized by a seasonal dimorphism, which is strong enough that the two morphotypes were at one time considered to be distinct species (Slingerland, 1892). The winter form is a large dark red overwintering adult with wide blackish longitudinal and traverses scratches, that is quite larger (2.6-2.9 mm) than the smaller and light-colored summerform adult (2.1-2.7 mm).

Pear psylla spends much of the winter in reproductive diapause, characterized by immature ovaries and a lack of mating. Dispersal of winterforms from the orchard in autumn begins in early September (Civolani and Pasqualini, 2003), and peaks during late-October and early-November, coinciding with leaf fall in pear. This winterform adults overwinter both alone or in small groups in bark crevices, branch intersections and at the base of shoots on the pear host plant (Priore, 1991) and away from the host plant, in the earth, or under rocks and clods which are exposed to sun irradiance (Nguyen, 1962). So, some individuals spend the entire winter on pears, others recolonize pear early in spring before bud break, long before any sign of green foliage, since they would feed on a plant other than pear but not complete develop (Fye, 1983). Cool, wet autumns result in a reduction in dispersal out of the orchard. As soon as the weather conditions become favourable, winterforms reach the apical twigs and pierce the plant by inflicting their stylets at the bud base. In Sicily overwintering nymphs from which adults flutter in February have been noticed as well (Nucifora, 1969; Tremblay, 1995).

The egg maturation is very slow in overwintering females and seems to be accelerated when psylla adults ceases to disperse among orchards (Rieux *et al.*, 1992; Lyoussoufi *et al.*, 1994). Generally diapause terminate in mid December, but in the more precocious females this event may occur starting from the end of November (Rieux *et al.*, 1990). At the end of January all females are mature

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and inseminated, but for the beginning of egg-laying by overwintered winterforms a temperature over 10°C for 2 consecutive days (thermic quiescence) is required (Nguyen, 1975). In Italy egg-laying begins in late February in Campania (Priore, 1991), early March in Emilia-Romagna (Giunchi, 1959) or early April in Veneto (Terza and Pavan, 1988). Because of the absence of foliage at this time, the first eggs are deposited directly on wood, generally at the base of unopened buds (spurs), in a number of 300-400 per female. As foliage becomes available in mid- to late-March, oviposition shifts to occur primarily on expanding leaves and flowers: eggs are deposited along mid-veins and petioles of developing leaves and on stems and sepals of blossoms. First nymphal instars escape from wintereggs in concurrence with bud opening and leaves sprouting and infest the new vegetation. The first generation of summerform adults appears in April and fecundity of females appears to be quite higher compared to winterforms due to their major longevity (on average about 600 eggs per female) (Stratopoulou and Kapatos, 1995), although high temperatures can cause a substantial reduction in fecundity. Spring- and summer-deposited eggs require ca. 6-10 days to hatch, depending upon temperature (McMullen and Jong, 1977). There are 5 nymphal instars. Nymphs require 3-4 weeks to complete development at moderate (21-27°C) temperatures (Georgala, 1956; McMullen and Jong, 1977). Male and female progeny are produced in equivalent numbers (Burts and Fischer, 1967). Afterwards (May) feeding nymphs of second generation develop on growing shoots with an aggregate distribution on leaves and internodes (Deronzier and Atger, 1980; Pasqualini *et al.*, 1997) immersed in pools of honeydew, which they produce in extremely large amounts. Further generations overlap with all the ontogenetic stages and phases till autumn. In the warmer periods aestivation phenomenons may occur (Stratopoulou and Kapatos, 1995). *C. pyri* develops 5-7 generation per year.

3. Host finding, probing and feeding behaviour

Host finding for feeding and oviposition contemplates a sequence of phases of hierarchic nature. The first, out of the three principal phases, consist of a host selection that the insect makes from distance using visual and olfactive impulses. The second phase occurs when the insect takes contact with the plant surface getting information about its physical structure. These impulses may strongly influence female egg-laying. The third phase regards the discrimination between host and non host plant through gustative impulses perceived during the survey of the internal tissues, the so called 'probing behaviour'.

Although most of the Homopteran insects have been reported to make little use of volatile compounds for the long and mid distance host finding and acceptance, the chemical characteristics are thought to be much more plant specific than the quality of the visual spectrum (Prokopy and Owens, 1983; Dethier, 1982). The role of olfactive and gustative sensilli existing in the antennas and tarsi in not yet known, whereas that of gustative sensilli in the mouth apparatus is evident.

Psylla rarely initiates oviposition activities immediately upon leaf contact. Rather, oviposition activities tend to be preceded by settling-probing activity, as evidence that plant cues received on initial contact are insufficient to release oviposition activity but that plant cues received during settling-probing activity release oviposition activities. Thus plant cues received during oviposition activities ultimately affect whether the egg is depositated (Horton and Krysan, 1991). Deprivation or habituation may result in higher number of eggs oviposited on less suitable genotypes than in free-choice tests.

Indeed, *C. pyricola* adult has been found to be more selective in oviposition activities than in its settling-probing activities, i.e. probing is not likely to be an indicator of a variety's acceptability, since *C. pyricola* is able to colonize and feed nonhosts like *Pyrus calleriana* and *Malus* spp., but without laying eggs (Horton and Krysan, 1990). As for plant cues that mediate host acceptance it has been reported that pear psylla readily settled on nonhosts to an extent that initially unacceptable species eventually receive eggs, thus suggesting either that plant cues that release settling attivity differ from those that release abdomen bend activity and oviposition, or that thersholds for these activities differ. Yet, there is evidence that settling is partially mediated by leaf surface characteristics for winterform psylla. First, settling-probing activity differs between upper and lower leaf surfaces, suggesting that cues received at the leaf surface affect activity. Second, at leaf contact, pear psylla scrape the leaf surface with their tarsi (Ullman and McLean, 1988; Horton and Krysan, 1990). Finally, despite the tendency to settle readily on apple, the amount of time between initial leaf contact and onset of settling-probing was smaller for psylla encountering Bartlett pear than for those encountering apple, suggesting that leaf surface cues affected behaviour.

Psylla adults ingest more frequently xylematic tissue, while nymphs prefer phloematic tissue or at least that of vascular fasces. A good knowledge on the different *C. pyri* feeding phases by means of EPG may allow to discriminate between susceptible and resistant selections and to locate the mechanisms of resistance within plant tissues (Civolani *et al.*, 2010). Up to know only little differences have been found in the feeding behavior of psylla adults on the susceptible William and the resistant NY10353 pears. However, lasting of the first and second non-probing is longer on William compared to NY10353, and *C. pyri* needs less time to reach the phloematic fasces in the susceptible plant, in accordance wih the assumption of Horton and Krysan (1990, 1991).

4. Types of damage

Pear psylla causes three primary types of damage: fruit russet, psylla shock, and pear decline (Burts, 1970; Westi-

gard *et al.*, 1979; Beers *et al.*, 1993). Fruit russet is caused by the feeding activities of nymphs and is of most concern to growers, and control programs are generally directed at preventing this injury, since it can be caused by relatively low population densities (Burts, 1988).

As in other Homoptera, pear psylla ingests excessive quantities of plant juices and other plant products that must be eliminated (as honeydew) during the digestive process. In fact, lymph in the phloematic tissue is rich of carbohydrates and poor of nitrogenous substances, due to this deficiency the insect has to absorb a great quantity of lymph that it afterwards excretes through his digestive apparatus producing honeydew. Adult psylla excrete these waste products as small, waxy pellets that cause no harm to the plant or fruit. Conversely, the immature form of the insect secretes copious quantities of honeydew, a sugary, sticky substance. If nymphal-produced honeydew is in contact with fruit for a significant period of time it causes dark blotches or streaks on the surface of the fruit (russetting), which in turn results in downgrading of the fruit at harvest (Burts, 1970). Honeydew allows a black, sooty mold fungus (*Antennaria, Aureobasidium, Capnodium, Ceratocarpia, Cladosporum, Torula, Ulocladium*) to grow on both fruit and leaves, not only reducing the quality of the fruit, but also blocking sunlight from the leaves and decreasing photosynthesis.

A second type of injury, also caused by the sucking nymphs at high densities, is of a more indirect nature than that previously mentioned. Infected leaves turn brown and often fall and the fruits drop prematurely or are small and of poor quality, thus suppressing root growth and reducing tree vigor and yield. (Westigard and Zwick, 1972). These symptoms have collectively been termed psylla shock, and are caused by a toxin in the saliva of feeding nymphs (Beers *et al.*, 1993). Symptoms of the injury can be similar in appearance to those associated with pear decline disease. Psylla shock can be particularly damaging because the effects are not always restricted to the year of infestation, but symptoms may carry-over into a second year even if densities are not high the second year (Beers *et al.*, 1993). Cultivars that are less preferred by psylla, such as some red pears or pears of Asian origin, tend less likely to experience this type of damage.

Finally, adult pear psylla vector the mycoplasma-like organism (Hibino and Schneider, 1970) that is the causal agent of pear decline disease especially during vegetative growth (Carraro *et al.*, 1998; Davies *et al.*, 1998; Guerrini *et al.*, 2000). The feed and phytoplasma are assumed together from a diseased plant and transmitted to a healthy plant during the salivation of phloematic feedings (Carraro *et al.*, 1998). The way of transmission is persistent-dispersive, as pear decline phytoplasma propagate in the insect body. Disease acquisition and inoculation require at least 1-2 hours of phloematic feeding; thereafter the vector undergoes to a period of latency (about 1-2 weeks) during which the phytoplasma circulates and propagate within its body till he reaches the slave glands. Both winterform and summerform pear psylla can be important in the transmission of pear decline (Blomquist and Kirkpatrick, 2002).

This pathogen causes sieve-tube necrosis at or below the graft union (Batjer and Schneider, 1960; Westigard *et al.*, 1979), preventing tree-synthesized nutrients from reaching the roots and resulting in starvation of the roots (Wilde and McIntosh, 1964). Symptoms of the disease include a slow to abrupt decline or collapse in growth and vigor, causing a reduction in yield and (often) death of the tree. Certain affected pear trees may recover if psylla densities are kept low or during winter quiescence thanks to the degeneration of epigeous phloematic tubes (Giunchedi and Refatti, 1997; Davies *et al.*, 1998). Severity of the disease depends upon psylla density and type of rootstock (Beers *et al.*, 1993). Cultivars that have been grafted onto *P. communis* rootstock are less susceptible than those grafted onto *P. pyrifolia* or *P. ussuriensis* rootstock. Quince (*Cydonia oblongata*) rootstocks posses a limited aptitude to allow phytoplasma survival between one vegetative cycle and another. Resistant rootstock has largely remedied this problem in various pear growing regions.

5. Monitoring and control tactics

The psyllid *C. pyri*, along with its natural enemies, needs to be carefully monitored for correct integrate pest management and biological pest control decision making. Moreover, timing of spray application against *C. pyri* is crucial because recommended insecticides are only efficient at certain stages. Monitoring should provide starting from spring density of eggs, nymphs, adults and presence of the principal antagonist. A simple method for estimating densities of pear psylla is desirable. Monitoring pear psylla is made difficult by the uneven distribution of insects (eggs and nymphs) on the trees, a distribution that may in fact change seasonally. Densities of psylla may also vary with height in the tree canopy (and sex) both for *C. pyricola* (Brunner, 1984; Horton, 1994) and *C. pyri* (Stratopoulou and Kapatos, 1995).

Currently, sampling of the adult population is necessary to determine the onset of reentry in late winter or the popolation density, and sampling of fruit spurs for eggs is often the easiest way to determine the beginning of egg-laying.

Counts have been obtained in USA using frappage, sticky traps, beat trays and open-ended organdy bags, the last one providing direct estimates of psylla numbers per leaf but being extremely time consuming (Horton, 1994; Horton and Lewis, 1997). Effectiveness of yellow sticky-board traps have been examined by several authors and seasonality of the catch and flight activity of pear psylla (*C. pyricola*) according to weather conditions have been reported (Krysan and Horton, 1991; Horton, 1994; Civolani and Pasqualini, 2003; Erler, 2004), as well as diurnal difference (Horton, 1993) and intraorchard changes in distribution associated with leaf fall (Horton *et al.*, 1993). Laboratory study have shown that males of both the sum-

merform and winterform morphotypes in *C. pyricola* are attracted to volatiles given off by females, whereas in the field male has shown a clear preference for sticky traps that have been baited with live females compared with traps baited with live males or left unbaited (Brown *et al.*, 2009). Limb beating or limb jarring to collect arthropod specimens from trees has been known for a long time and in several variations: frappage or beating tray (two-dimensional) and beating umbrella (three-dimensional). The first of this procedure has been applied by several authors and has been reported by Jenser *et al.* (2010) to depend considerably on weather condition, while the second one to be much less temperature and wind-sensitive, due to its vertical extension, and much more suitable for collecting fast moving or flying beneficial organisms than the two-dimensional method. In contrast with the hypothesis that any data collected for the adults using a beating umbrella would be influenced more by weather conditions than those gathered using funnels, Sanchez and Ortìn-Angulo (2011) have found a higher efficacy of the net in relation to the funnel. The same authors also have stated a low efficacy of the beating techniques for sampling nymphs that may be due to the fact that they hold tight to the substrate and are not easily removed by the act of beating.

Both the application of the sticky board traps and beating tray provide accurate information about the changes of pear psylla population density (Jenser *et al.*, 2010). In particular the capture of adults using either the funnel or the net may be used to estimate the absolute number of *C. pyri* nymphs on trees, thanks to the high correlation found by Sanchez and Ortìn-Angulo (2011) between nymphs counted on shoots and the capture of adults using either of the beating techniques. Several authors have reported the same relationship for other psyllid species (Horton, 1994; Jenser *et al.*, 2010).

The beating techniques also have the advantage of being less time-consuming than the sampling of leaves and, for beating over a net or tray, samples may be processed directly in the field, although the amount of collected insects using beating umbrella some times makes necessary the laboratory process.

In Europe the dinamic of *C. pyri* populations have been studied using frappage by various authors (Deronzier, 1984; Rieux *at al*., 1992). According to Civolani and Pasqualini (2005) frappage is the sampling method which best represents the dinamics of populations of psylla and its predators (Antocoridis, Coccinellids, lacewings). Predators overwintering in bark cervices may be estimated by using corrugated cardboard traps (Bogya *et al.,* 1999; Horton *et al.*, 2002; Civolani and Pasqualini, 2003; Jenser *et al.*, 2010).

Alternatively the psylla eggs have been counted on the shoots and leaves using a binocular dissecting microscope by several authors (Jenser *et al.*, 2010) and a few of the authors investigated and counted both the eggs and larvae. This method provides real data, but it's time consuming; the sample must be taken into the laboratory and the counting completed within a short time. Berlese funnel is a widespread technique for extracting arthropods mainly from soil and litter samples (Stäubli *et al.*, 1992). Moreover the mite brushing machine or leaf brushing machine developed by Henderson and McBurney (1943) is a technology that can reduce the time required to obtain either absolute counts or estimates of arthropods on leaves from samples. Recently developed, the wash-down method described by Jenser *et al.* (2010) offers the advantages of the independence of the weather conditions (temperature, wind, rain) and the daily rhythm of the examined psylla stages. Since practically every larvae developing on the flowers and shoots are extracted, it provides suitable data about the pear psylla population density and its changes, as well as about the effectiveness of the insecticides. This method has been suggested to provide also significant data to judge the susceptibility or tolerance of the pear cultivars to pear psylla species.

Since observing the population development of pear psylla is time-consuming and prone to error, phenological models could assist growers in the timing of monitoring and control measures, as they simulate and predict, by means of driving variables (usually temperature), the timing of natural events. There have been modest attempts to develop degree-day models that predict onset of egg-laying and appearance of first generation nymphs (Westigard and Zwick, 1972; Brunner, 1984; Beránková and Kocourek, 1994) and timing of reentry (Horton *et al.*, 1992), with aims toward improving timing of the dormant spray. Morgan and Solomon (1993) have provided a phenological model for *C. pyricola* which have been integrated into a multipest forecasting system. Further on, a phenological model for *C. pyri* based on biological mechanisms, in particular the emergence of juvenile instars of the second generation, has been developed by Schaub *et al.* (2005).

In Italy the defence against *C. pyri* is mainly based on integrated pest management (IPM), supported by natural control aimed to equilibrate the complex biological relationships of the field community (Civolani, 2012). Among the basic strategies there are the 'good agricultural practice' (GAP) techniques that reduce tree suitability for growth and reproduction of pear psylla by avoiding overuse of fertilizers, incorrect or over pruning, and reducing excessive plant vigor (Beers *et al.*, 1993; Civolani, 2012). Suckers or water sprouts should be removed from scaffold limbs (Beers *et al.* 1993), because these are a source of rapidly growing and highly nutritious foliage. Also the strategies to control other pest species, such as the technique of mating disruption and the use of granulosis virus (CpGV) employed to control codling moth (*Cydia pomonella*), may influence psylla and assist in chemical control.

However, in the last decade commercial pear growers have relied primarily on the use of synthetic products to control pear psylla, and the advantages and disadvantages of the main strategies performed in the last 20 years in integrated and conventional farms have been described by Civolani (2012).

Unfortunately, these methods are not always entirely effective, as their efficiency depends both on the active in-

gredients employed and the weather conditions at the time of treatment and moreover pear psylla has developed resistance to several classes of commonly used insecticides (Riedl *et al.*, 1981; Follett *et al.*, 1985; Burts *et al.*, 1989; Croft *et al.*, 1989).

Current control recommendations emphasize destruction of the overwintered generation, or offspring of the overwintered generation with insecticides. A typical control program for overwintered adults is performed at leaf fall, commonly in France on *C. pyri* and in North America on *C. pyricola*, with the application of pesticides belonging to the pyrethroid family (with or without mineral oil added), repeated as necessary in late winter (at bud swelling stage or bud break) to break down the population of females emerging from winter shelters and about to lay eggs.

These pyrethtoids are completely non-selective but broad spectrum and therefore dangerous for the beneficial insects. For this reason the treatment must be performed only at complete leaf fall (late November or early December), when *A. nemoralis* populations have already found shelter in bark crevices while *C. pyri* adult winter forms are still active on plants. Efficiency of treatments may vary considerable upon seasonal conditions. For example most psylla adults take shelter early and survive to the late autumn treatments when an early frost occurs at the beginning of autumn. Similarly the efficiency of chemicals is reduced after frost waves at the end of winter which interrupt and delay the emergence of adults, while activity of pesticides is best after a mild winter when almost all adults leave their shelters at the time of treatment (Civolani, 2000; Civolani and Pasqualini, 2003).

In Italy the dormant sprays are discouraged since the pest population, after an initial sharp decline, soon recovers in spring because the natural control by its predator *A. nemoralis* is limited, then increases again in May, reaching the economic threshold for spring-summer treatments (Civolani, 2012).

The main side effect of the use of pyrethroids in late winter is that they sharply reduces the psylla first generation and therefore could starve the anthocorids, interfering with their settlement during early plant growth in spring. Various alternative biorational solutions to synthetic pesticides have been tested against the overwintering generations, and among them kaolin and some oily compounds. Kaolin, a white, non-abrasive, fine-grained allumosilicate mineral that is purified and sized so that it can be easily dispersed in water, creates a mineral barrier on plants that prevents oviposition and insect feeding (Puterka *et al.*, 2000). Treatment with kaolin has been reported to hinder egg anchorage on the leaf surface and inhibiting host-plant acceptance. Moreover, some insects have been found to be less mobile and unable to reach the laying site (host location) on plants, as their body and wings have became soiled (Pasqualini *et al.*, 2003; Daniel and Wyss, 2006).

Further on, Puterka *et al.* (2005) investigated the effects of particle film type (hydrophobic versus hydrophilic) and formulation determining that there are a number of biological effects particle films have on pear psylla beyond the deterrence of adult settling and oviposition.

Alternatively, mineral oils and oily compounds could also be used to interfere with egg deposition by psylla adults. A good reduction of the number of eggs laid has been obtained in Northern Italy with pure mineral oil alone ('dormant oil') (Pasqualini *et al.*, 2003) and in Turkey with fish-liver oil and summer oil (Erler, 2004).

Some growth regulators have proved to possess a good activity against eggs and nymphs of first and second generation by interfering on the cuticle transport and deposition during larvae development (Erler and Cetin, 2005).

At the beginning of the second generation growers can assess the risk to their orchard and still target specific stages. Therefore, treatments target mostly eggs and/or young larvae of the second generation. The treatments against summer generations can be performed towards eggs or nymphs.

Chitin inhibitors, usually employed against *C. pomonella*, have shown a secondary effect on second generation eggs, usually laid in the first decade of May, especially when they are applied on newly laid eggs (white eggs) or on eggs laid in a short time after the treatment.

However, control strategies against juvenile stages are of most relevance, and were performed in the past with generic organophosphorates, whereas are based in present times on specific synthetic active ingredients, often acaricides. Among these, abamectin (produced by the soil bacterium *Streptomyces avermitilis*) is the basic chemical employed today against young nymphs of second generation (usually in May) and included in the Italian Disciplinary of Integrated Management. The best results are obtained when yellow eggs are mostly present and when the hatching peak has not yet achieved (Pasqualini and Civolani, 2006). Abamectin is allowed only once in a year or twice in case of young orchards; since it's not systemic, the addition of mineral oil may improve its penetration within 24 hour time. A new broad spectrum acaricides, namely spirodiclofen (BAJ2740, trade name: Envidor®), belonging to the new chemical class of tetronic acid derivatives, has been discovered by Bayer CropScience during the 1990s and is commercially available since 2007. Spirodiclofen has a new original mode of action (interference with lipid biosynthesis) and shows no cross-resistance to any resistant mite or whitefly field population, representing an invaluable new tool to manage insecticide resistance in rotation with abamectin. It's efficiency is best on yellow eggs some days before the hatching of first instar nymphs and is improved by addition of mineral oil, although often lower than that of abamectin (Pasqualini and Civolani, 2007; Boselli and Cristiani, 2008; Marčić *et al.*, 2009).

Besides summer mineral oils, whose main action is that of dissolving honeydew, sodium dioctyl sulphosuccinate or other vegetal free fatty acids may be used for washing the trees (Briolini *et al.*, 1989). Recently some other novel compounds have been used, similar to liquid glue and capable of controlling almost all juvenile instars of *C. pyri.* These products are synthetic sugar esters (sucrose oc-

tanoate) and represent a relatively new class of insecticidal compounds that are produced by the reaction of sugars with fatty acids, valuable in crop integrated pest management programs (Puterka *et al.*, 2003).

It's important to keep in mind that summer psylla infestation depend on the antagonists development in spring, first of all the most important one, *A. nemoralis*, which has to be protected. For first instar nymphs control threshold is given by the ratio between number of infested shoots and number of shoots with the antagonist Antocoride, which have to be ≥ 5 (Marani and Reggidori, 2007).

Also the relevant effect of weather conditions on pest populations should not be underestimated. In fact, the development of psylla is strongly reduced by high summer temperatures that cause the death of eggs and the slowdown of juvenile growth. On the other hand, cold and rainy periods during blossoming and petal fall encourage nymph spreading on plants, often clustering in flower calyxes, sometimes causing russet blotches or young fruit drop (Civolani, 2012). Climate conditions, such as wind, have been demonstrated to have an impact on the clustering of psyllids, wheras spatial factors, such as distance from a mixed hedge have been found to be related to beneficial arthropod community (Debras *et al.*, 2008).

Localized resistance cases to organophosphorates insecticides, pyrethroids and carbamates pesticide families and chitin inhibitors family have been developed and have been largely documented, especially for *C. pyricola* in North America since 1960 (Harries and Burts, 1965). Resistance rates among the active ingredients has been reported to be very variable in laboratory tests and probably there are different mechanisms involved in the resistance to different pesticide families, as reported by Civolani (2012).

In Italy, cases of loss of efficiency of abamectin have been noticed in some orchards in Emilia-Romagna region, indicating that there is a high risk of selection for resistance to abamectin, especially if the number of treatments per year is high. Up to now, the tests data indicate that no apparent resistance to abamectin has been developed in *C. pyri* populations of that region, but may rather be related to incorrect pest defence management (Civolani *et al.*, 2007).

Control strategies should be based on a limited use of pesticides, possibly selective ones, in order to foster the development of *A. nemoralis* populations, which become a relevant factor to control the pest, preying on both eggs and nymphs of psylla. In Emilia-Romagna *A. nemoralis* generally shows three generations and may feed also on other insects, for example aphids and the pear sawfly *Hoplocampa brevis*. Laboratory tests have shown an average predation of about 300 psylla nymphs during the entire life of an adult, which lasts about 60 days (Civolani, 2012).

One problem is that the populations of this anthocorid grow rapidly in spring only if there is psylla of first generation in the orchard for feeding, therefore in May-June some amounts of the pest have to be tolerated. A further weakening of the wild *A. nemoralis* populations may be caused by the large amount of active ingredients used against other pests, having significant toxic effects on *A. nemoralis*. Among these, thiacloprid, the most frequently pesticide used against the codling moth, *C. pomonella*, as well as the neonicotinoids, not employed in Italy and Europe as specific psyllicides, but against aphids and the pear sawfly *H. brevis*.

The artificial introduction of the antagonist Antocoride at the end of March - beginning of April is a very useful mean for controlling eggs and young nymphs of the first generation. The flow is made with about thousand individuals per hectar fractioned in 3 times at weekly intervals. Resettlement is much more feasible much wider the cultivated area is (minimum 1 hectar).

Some authors however retain that 500 individuals would be sufficient for each introduction (Beninato and Morella, 2000). Good natural equilibrium have been obtained in Veneto with the introduction of 500-600 psylla adults per ha in a sole time in May (Mori and Sancassani, 1984). In France the introduction of *A. nemoralis* has been performed by the distribution of *Pelargonium* stems containing 2.940 eggs of the psylla antagonist (Fauvel *et al.*, 1994; Rieux *et al.*, 1994).

Some authors indicated that the efficacy of this predator is not strongly mediated by plant quality, at least at tree scale, thus, for systems where pest population growth is strongly tied to plant vigor or quality, the reduction of fertilizers to the minimum level required for proper fruit set is likely to improve the success of pest biocontrol (Daugherty *et al.*, 2007).

6. New pesticides and strategies for Integrated Pest Management

In the last years new pesticides have been developed with generally low toxicity towards beneficial insects. AkseBio2 is a mixture of various aromatic plant essential oils, edible plant extracts and a bacterium TR 2000 which decreases oviposition and immature stages of the pest (Erler *et al.*, 2007).

Spirotetramat (Movento®) is a new, fully systemic and ambimobile active ingredient particularly effective against a broad range of sucking pests, similar to the tetronic acid derivate spirodiclofen. Its singularity depends upon its unique translocation property, which allows the protection of new shoots or leaves appearing after foliar application, in fact after foliar uptake the insecticidal activity is translocated within the entire vascular system (Nauen *et al.*, 2008). Due to the lack of any cross-resistance to existing chemical classes of insecticides, spirotetramat is a very interesting alternative to be used in rotation schedules.

Natural plant compounds, fungal pathogens and different orchard ground cover all seem promising controls. Among nontoxic plant compounds, sugar-ester extracted from wild tobacco has proved to be most successful in psylla control (USA), killing most nymphs within 2 hours. Even nymphs that hatched 3 to 5 days after spraying die as soon as they walk on leaves (Stanley, 1993). Rapeseed

oil and petroleum oil as well have showed a total efficacy against eggs laid by winterforms females of *C. pyri* (Marčić *et al.*, 2008, 2009). Several naturally occurring fungal pathogens (spores of *Beauveria*, *Verticillium*, and *Paecilomyces* mixed with either oil or water) have given 100-percent control as well within 5 days. The advantage is that fungi can last indefinitely compared to the sugar-ester that may persist on the plant for about a week. They are host-specific, completing their life cycle on infected insects on the plant, and therefore nontoxic to humans, animals and beneficial insects. After killing their host, the fungi release hundreds of spores, each capable of infecting another pear psylla. Since pear psylla also have several predators (Table 1), planting ground covers with perennial crops between tree rows to attract them could provide a measure of control (Stanley, 1993).

New strategies for integrated pest management of psylla may be offered in the future by the optimization of the recently identified sex attractant pheromone, the 13-methylheptacosane, for *C. pyricola* winterforms males (Guédot *et al.*, 2009).

7. Resistance to pear psylla

All of the main cultivars of the European pear grown commercially (Abate Fétel, William, Conference, Doyenne de Comice, Kaiser, etc.) (Bellini and Nin, 2002) are susceptible to this arthropod pest and biological controls are becoming of limited effectiveness since resistance to insecticides has developed rapidly. Host plant resistance would therefore be a valuable control strategy.

Resistance to the pear psylla has been demonstrated in the East Asian pear species, *P. betulifolia* Bunge, *P. calleryana* Decne., *P. fauriei* Schneid., *P. ussuriensis* Maxim., and *P.* x *bretschneideri* Redh. (Westigard *et al.*, 1970; Quamme, 1984; Moore and Ballington, 1991). Hybrids of *P. ussuriensis* x *P. communis* have been found to be resistant to *C. pyricola* (Harris, 1973; Harris and Lamb, 1973; Quamme, 1984) as well as to *C. pyri* (Robert *et al.*, 2004). Different interspecific hybrids between *P. communis* and *P. longipes* or *P. pyrifolia* have shown high levels of resistance to *C. pyri*, too (Robert *et al.*, 2004). Resistance has been reported also for a few genotypes of *P. nivalis* Jacq. and *Sorbopyrus* (Westigard *et al.*, 1970; Bell, 1992). Small fruit size of the pure species and gritty or coarse texture of both the pure species and interspecific hybrids may limit the utility of some of this germplasm for rapid transfer of resistance into cultivars with *P. communis* type fruit. Within *P. communis,* moderate resistance has been demonstrated in the old Italian cultivar Spina Carpi (Quarta and Puggioni, 1985), and in eleven 'primitive' cultivars from Yugoslavia and Hungary (Bell and Stuart, 1990; Bell, 1992). All of these genotypes have relatively poor fruit quality but are important sources of resistance within the primary gene pool available for improvement of *P. communis* cultivars.

In many countries *ex situ* pear collections have been established in some important pear growing areas with a great diversity of national, local and foreign cultivars, mainly for evaluation of resistance to major disease and insects, to be used as potential parents in breeding (Quarta and Puggioni, 1985; Braniste *et al.*, 1994; Braniste and Militaru, 2008; Benedek *et al.*, 2010). More than 200 pear cultivars of Tuscan, national and international origin, in-

Table 1 - Natural enemies associated with pear psylla in Europe

Natural enemy	Taxonomic group	Species
Predators	Arachnida, Araneae	Unidentified spiders
	Dermaptera	*Forficula auricularia* Linnaeus
	Heteroptera, Anthocoridae	*Anthocoris nemoralis* (Fabricius)
		Orius spp.
	Heteroptera, Nabidae	*Nabis* spp.
	Heteroptera, Miridae	Several species
	Heteroptera, Lygaeidae	Several species
	Neuroptera, Chrysopidae	*Chrysoperla carnea* (Stephens) *Chrysopa* formosa Brauer *Chrysopa septempunctata* Wesmael *Anisochrysa prasina* (Burmeister)
	Coleoptera, Coccinellidae	Several species belonging to different genus
	Diptera, Syrphidae	*Episyrphus balteatus* (De Geer) *Epistrophe* spp.
Parasitoids	Hymenoptera, Encyrtidae	*Trechnites psyllae* (Ruschka) *Prionomitus mitratus* (Dalman)
	Hymenoptera, Pteromalidae	*Syrphophagus mamitus* (Walker)
Entomopathogenic fungi		*Entomophthora sphaerosperma*

From: Armand *et al*., 1991; Tremblay, 1995; Civolani and Pasqualini, 2003; Erler, 2004.

cluding also 25 Afghan accessions, are presently being evaluated in *ex situ* and *in situ* collections for psylla resistance at the Department of Plant, Soil and Environmental Science of the Florence University (DiPSA-UNIFI) within the AGER project 'INNOVAPERO: Management and crop innovations for high-quality pear production'. Moreover, evaluation of insect preference in tunnel is being in progress on 26 local cultivars showing good pomological traits.

Taking earlier and present results into account almost 60 European pear cultivars being resistant or highly tolerant to pear psylla infestation and damage can actually be listed (Table 2). Some of these ancient or local cultivars may be exploited both in organic farming or in breeding, but further investigations are needed to estimate their yield capacity and fruit quality (Benedek *et al.*, 2010; Szabó *et al*., 2010). Moreover, some varieties considered resistant in field have shown to be susceptible, if isolated and articially infested by adults (Westigard *et al.*, 1970; Harris, 1975).

Methods of evaluating host resistance are sufficiently developed and rapid nymphal feeding bioassays have been developed to screen pear germplasm for antibiosis-based resistance by Harris (1973, 1975) and Butt *et al.* (1989) for the evaluation of pear germplasm introduced in North America from Eastern Europe, and then modified by different Authors (Table 3). The results of tests can vary, depending on the type of assay and host phenological stage, which affects ovipositional preference (Bell and Puterka, 2003).

Genetic psylla resistance do not follow a general rule and is supposed to be often polygenically inherited (Harris and Lamb, 1973). Lespinasse *et al.* (2008) found that psylla resistance was not well transmitted from the *P. ussuriensis* x *P. communis* hybrid NY10355 to its progenies, assuming that genetic resistance in NY10355 may result

Table 2 - Pear cultivars showing some degree of resistance to psylla as reported by different authors

Cultivar	Tolerant	Resistant	Moderately resistant	Low susceptible	Country	Reference
20th Century			x		Serbia	Stamenkovic *et al*., 1993
Bartjarka		x			USA	Bell, 1992
Bókoló Körte		x			Hungary	Benedek *et al.*, 2010; Szabó *et al*., 2010
Bötermö Kálmán		x			Hungary	Benedek *et al.*, 2010; Szabó *et al*., 2010
Bulgaresti		x			Romania	Braniste *et al*., 1994
Cantalupesti		x			Romania	Braniste *et al*., 1994
Cantari				x	Romania	Sestras *et al*., 2009
Cj16-9-13		x			Romania	Straulea *et al*., 1992
Craiesc		x			Romania	Braniste *et al*., 1994
Cure				x	Romania	Straulea *et al*., 1992
Cure-6	x				Hungary	Benedek *et al.*, 2010
D'Aout Lamer		x			France	Robert and Raimbault, 2005
Daoyenné de Poitiers		x			France	Robert and Raimbault, 2005
Erabasma		x			USA	Bell and Stuart, 1990
Ewerd		x			Romania	Braniste *et al*., 1994
Füge Alakú		x			Hungary	Benedek *et al.*, 2010
General Osmanwill		x			Romania	Braniste *et al*., 1994
Haydeea		x			Romania	Sestras *et al*., 2009
Honeysweet		x			USA	Quamme, 1984
Imperiale		x			Romania	Braniste *et al*., 1994
Imperiale				x	Romania	Sestras *et al*., 2009
Jerisbasma		x			USA	Bell, 2003
Kajzerka		x			USA	Bell, 1992
Karamanka		x			USA	Bell, 2003
Karamanka			x		Serbia	Stamenkovic *et al*., 1993
Karamanlika		x			USA	Bell and Stuart, 1990
Katman		x			USA	Bell and Stuart, 1990
Katman		x			France	Robert and Raimbault, 2005
Kései Kálmán	x				Hungary	Benedek *et al.*, 2010
Kieffer seedling		x			Romania	Braniste *et al*., 1994

Cultivar	Tolerant	Resistant	Moderately resistant	Low susceptible	Country	Reference
Kieffer	x				Hungary	Benedek *et al.*, 2010
Kieffer Éd	x				Hungary	Benedek *et al.*, 2010
Krupen Burnusus		x			USA	Bell and Stuart, 1990
Krupen Burnusus		x			USA	Puterka, 1997
Lorencz Kovacs				x	Romania	Sestras *et al*., 2009
Lorenz		x			Romania	Braniste *et al*., 1994
Lucele		x			USA	Bell, 1992
Magness		x			Romania	Braniste *et al*., 1994
Magness			x		Serbia	Stamenkovic *et al*., 1993
Mednik		x			USA	Bell and Stuart, 1990
Mednik		x			USA	Puterka, 1997
Monglow				x	Italy	Quarta and Puggioni, 1985
Nagyasszony Körte		x			Hungary	Benedek *et al.*, 2010
Nyári Kálmán		x			Hungary	Benedek *et al.*, 2010
Obican Vodenac		x			USA	Bell and Stuart, 1990
Obican Vodenac		x			USA	Puterka, 1997
Pinguoli			x		Serbia	Stamenkovic *et al*., 1993
Rocha Portugheza		x			Romania	Braniste *et al*., 1994
Rozs Nyári Körte		x			Hungary	Benedek *et al.*, 2010
Severinka				x	Romania	Sestras *et al*., 2009
Sierra		x			USA	Quamme, 1984
Sirrine	x				USA	Quamme, 1984
Sirrine				x	Italy	Quarta and Puggioni, 1985
Smokvarka		x			USA	Bell and Stuart, 1990
Spadona		x			Romania	Braniste *et al*., 1994
Spina Carpi				x	Italy	Quarta and Puggioni, 1985
Spina Carpi				x	France	Robert and Raimbault, 2005
Steiner	x				Hungary	Benedek *et al.*, 2010
Téli Kálmán	x				Hungary	Benedek *et al.*, 2010
Tomnatice		x			Romania	Braniste *et al*., 1994
Topka		x			USA	Bell and Stuart, 1990
Triomphe de Joidogne		x			Romania	Braniste *et al*., 1994
Triomphe de Joidogne				x	Romania	Sestras *et al*., 2009
Vidovaca			x		Serbia	Stamenkovic *et al*., 1993
Viki Körte		x			Hungary	Benedek *et al.*, 2010; Szabó *et al*., 2010
William Precoce Morettini		x			Romania	Braniste *et al*., 1994
Zelinka		x			USA	Bell and Stuart, 1990
Zelinka		x			USA	Puterka, 1997

either from the combination of several small-effect resistance genes, according to Pasqualini *et al.* (2006), or from a combination of dominance or epistatic effects or from both. A genetic mapping approach should help researchers to understand the genetic mechanism of psylla resistance. The molecular interaction between pear tree and the piercing/sucking psylla has been investigated through the construction and characterization of cDNA subtracted libraries. Genes expressed upon insect infestation were identified in a susceptible and a resistant pear genotype. The two expression profiles were found to be different: in the resistant plant more genes involved in the response to biotic and abiotic stress were activated than in the susceptible one. The further characterization of the identified genes could lead to the development of molecular markers associated with tolerance/resistance to psylla (Salvianti *et al.*, 2006). The quantitative resistance to pear psylla has been analyzed recently in a progeny of the European pear Angelys crossed with the resistant genotype NY10355, and by screening parents/seedlings with microsatellite markers a QTL (Quantitative Trait Loci) that explained 15% of the phenotypic variability has been determined and mapped on the linkage group 17 (Bouvier *et al*., 2011).

Table 3 - Pear resistance to psylla: assay methods adopted in controlled conditions

Reference	Number of insects used for artificial infestation	Site of infestation	Replications per cultivar/selection	Observations (hours or days after infestation)
Harris, 1975	100 adults	plant	1	4 days: removal of adults, growth and development of the resultant progeny
Butt *et al.*, 1988	1 nymph	lower midrib of 10 fully expanded detached-leaves	3-10	2 h, 4 h, 6 h, 24 h: position of nymph and presence of honeydew
Butt *et al.*, 1988	1 nymph	lower midrib of 10 fully expanded leaves of potted trees	2	2 h, 4 h, 6 h, 24 h: position of nymph and presence of honeydew
Butt *et al*., 1988	10 first instars	lower midrib of the 2 youngest fully expanded leaves of potted trees	2	24 h: position of nymph and presence of honeydew
Butt *et al*., 1989	25 first-instar nymphs	2 youngest and fully expanded leaves of a shoot	4	each day: feeding determined by excretion of honeydew
Puterka *et al.*, 1993	2-6 females	excised twig collected at different stages of bud development	8	24 h after infestation at stages of dormant bud, green tip, fully expanded leaf: adults per twig 48 h: adults per twig 72 h: eggs per twig
Berrada *et al*., 1995	15 pairs of sexually mature adults	10-16 leaves (≈300 cm^2)	4	24 h: removal of adults and egg count each day: survival of eggs and larvae until they developed into adults
Baldassari *et al*., 1996	6-10 third-fourth instar nymphs	2 younger and more expanded leaves of a shoot	2-3	5 days: vitality of nymphs, amounts of produced honeydew, possible development of sooty moulds 15 days: number of deaths
Baldassari *et al*., 1996	10 first instar nymphs	2 younger and more expanded leaves of a shoot	3	Every day: number and age of dead nymphs, days needed for possible development of adults
Puterka *et al*., 1997	5 nymphs	4 fully expanded terminal leaves	5	4 days: nymphal survival and development alternating 3rd and 4th day up to day 29: nymphal survival and development
Robert *et. al.*, 1999	1 female	plant	1	2-7 days: female removal after 50 eggs on average per plant had been laid Each week: larval mortality and count of different instars
Bell, 2003	10 second or third instar nymphs	underside of the top 2 youngest fully expanded leaves of	5	48 h: number of surviving and actively feeding nymphs
Robert and Raimbault, 2005	4 females and 1-2 males in two times at 8 day-interval	plant	7-8	15 days: number of eggs on the 8 upper leaves of shoots 36, 63 and 98 days: number of nymphs 134 days: shoot and leaf state
Pasqualini *et al*., 2006	300-400 males and females	plant	5-16	10 -25-50 days: number of adults per plant 10-25 days: number of eggs per plant 25-50 days: number of nymphs producing honeydew per plant
Pasqualini *et al*., 2006	1 female	upper surface of a leaf in a clip-cage	3-11	48-72 h: number of laid eggs per female
Bouvier *et al*., 2011	8 insects	plant	7	presence of honeydew on the first, second and last third of the plant. The quantity of larvae present on the whole plant

8. Resistance characterization

Resistance is characterized by both ovipositional non-preference (antixenosis = settling and oviposition) and feeding inhibition, delayed development and increased nymphal mortality (antibiosis) (Bell and Stuart, 1990). While antixenosis influences the size of the initial nymphal population, antibiosis probably exerts the greatest effect on population levels over a season. So, feeding rejection is a major component of resistance and leads directly to a precocious nymphal mortality; the mechanism for feeding acceptance or rejection is probably internal to the leaf as reported by Butt *et al.* (1988).

Volatile substances emitted by the leaves of different varieties are not substantially dissimilar and therefore do not probably play a basilar role in the affinity an repulsion of psylla adults (Miller *et al.*, 1989), but bioassays on this topic are still lacking. Resistance of genotypes is not directly proportional to leaf cuticle thickness, the resistant genotype NY10355 for instance has a lower content of cutin compared to the susceptible William variety (Gérard *et al*., 1993). Pubescence is not a major factor in feeding deterrence according to Bell and Stuart (1990). However, antixenosis is influenced by both the physiological status (Bigre and Lefeuvre, 1982) and bud phenological phase (Stuart *et al.*, 1989; Puterka *et al.*, 1993) of pear tree. Thus differences in leaf morphology may influence psylla oviposition, bearing in mind that the insect prefers to lay the eggs on prominent structures such as leaf vein or crevices at the base of fruiting spurs.

Resistant genotypes express antibiosis with the production of a limited amount of honeydew and a strong nymphal mortality (Butt *et al.*, 1988, 1989). However, the quantity of produced honeydew has not been denotive of the infection intensity on selections obtained by induced mutagenesis (Baldassari *et al*., 1996). Ingestion of substances belonging to the group of polyphenols (for example tannins) has been suggested to be the cause of this mortality (Bell, 1984). Challice and Williams (1968) underlined the presence of the group of active components flavone glycosides in the Asiatic *Pyrus ussuriensis*, which is lacking in *Pyrus communis*. Braniste *et al.* (1994) evidenced a lower total isoperoxidase activity in resistant pear cultivar compared to susceptible ones. Also sugar content in leaves differed between resistant and susceptible genotypes, an increase of sugar content due to a reduced level of starch synthesis and also its rapid degradation was noticed in susceptible cultivars.

Fiori and Lamb (1982) found the presence of secretory cells to be much more extensive in the phloem of leaf midveins of pear genotypes with resistance against *P. pyricola* and suggested that average percentage of the phloem area occupied by secretory cells in May-June may provide a valid method for determining the resistance of pear trees to *P. pyricola.*

Antibiosis towards preimmaginal stages is accompanied by a reduction of feeding frequency, which may be linked to the presence of nutritional inhibitors (Butt *et al.*, 1989) or to an insufficient plant alimental appetizer (Chang and Philogène, 1975). Later on, the *ex novo* induction of a phenolic compound (3-O-trans-p-cumaroyltormentic acid) has been demonstrated after 12 hours from the phytophaga attack with a pick after 30 days from infection (Scutareanu *et al.*, 1999). This induction has been recently shown to be local (Conference) or systemic (William and NY10355), but there are no evidence on whether this induction can modify *C. pyri* behavior or not (Scutareanu *et al.*, 1999). A different effect has been attributed to other volatile substances still originating during the wounding process of the mouth apparatus. Some of these essences released from infected pear leaves, i.e. the monoterpene (E,E)-α-farnesene and the phenolic compound methyl salicylate, are primarily responsible of the attraction of the main psylla predators, namely *Anthocoris nemoralis* and *Anthocoris nemorum* (Scutareanu *et al.*, 1997, 1999, 2001). The capacity of some plant species to emit mixture of volatile compounds, dominated by terpenes, to attract carnivorous arthropods that prey on or parasitise herbivorous insects or mites, has been well documented as plant defence strategy (Degenhardt *et al.*, 2003).

Finally, antixenosis and antibiosis are often associated in resistant genotypes, but are supposed to be indipendent from a genetic point of view, since only one of this two mechanisms of resistance exist in some genotypes (Puterka *et al.*, 1993).

9. Breeding

Fortunately, pear species vary considerably in their resistance to pear psylla and breeding for resistance is possible. For breeding, the use of the larger fruited species (*P. ussuriensis* and *P*. x *bretschneideri*) should prove to be more efficient for combining resistance with European-type fruit quality.

In Italy, the Experimental Institute for Fruit Crops, Rome, Forlì section (ISF-FO) has been studying genetic improvement of pear for about 35 years, looking with particular regard for fire blight and pear psylla resistant cultivars. The breeding activity for the transfer of pear psylla resistance lists 22 crossing combinations, about 8,200 seedlings and 13 advanced selections, 3 of which are rather tolerant to pear psylla (Baldassarri *et al.*, 1996). Praiseworthy is the selection ISF.68-14-44-11, which is rather tolerant to pear psylla, although the fruit does not have sufficient eating quality (Rivalta and Dradi, 1998). Transfer of resistance traits have been reported more recently in crosses with different NY selections (ISF 94-1/174-267, ISF 94-4/103-267, ISF 94-5/-51-268) and selections of *P. pyrifolia* (ISF 98-5-70-150, ISF 90-12/110-149) (Pasqualini *et al.*, 2006).

The Department for Tree Crops, Bologna University (DCA-UBO), has been implementing a programme of both intervarietal and interspecific cross breeding, which began about 30 years ago, in order to develop diversified pears for quality and ripening calendar, without disregarding the evaluation of fire blight and psylla resistance (Sansavini and Rosati, 1986; Sansavini, 1999). NY10353 and NY10355 have been used as male parents, while Max Red Bartlett and Doyenne de Comice as female parents, and eight seedlings have been selected and are under evaluation. Among these, DCA 92052105-119 (NY10353 x Doyenne de Comice) has shown a great degree of psylla resistance in controlled growth chamber and is actually under evaluation in open field (Musacchi *et al.*, 2005; Pasqualini *et al.*, 2006). Moreover, a number of 90 AFLP primer combination has allowed to indentify, through a Bulk Segregant Analysis, a first step of molecular markers linked to psylla (Sansavini, pers. com.).

In France, a close collaboration between INRA and the National Institute of Horticulture (INH), Angers, has been recently started for the definition of precocious tests for evaluation of pear psylla resistance as well as potential parents to be used in the breeding project. Now, some 10,000 resistant hybrids and 60 selections are under study using artificial inoculation tests (Le Lezéc, 1991; Le Lezéc, pers. com.).

Of great importance is also the breeding programme which is undergoing at the Fruit Research Stations of Pitesti-Maracineni, Cluj-Napoca and Voinesti, Romania, whose goals have been focused since many years on resistance improvement to fire blight, pear psylla and scab by means of intra and interspecific hybridization followed by backcross. The initial sources concerning psylla resistance were represented by biotypes derived from *P. serotina* and subsequent F_1 and F_2 interspecific selections. Some foreign and native *P. communis* cultivars were used as parents (Napoca, Butirra Precoce Morettini, Butirra Hardy, Butirra Six, Doyenné d'Hiver, etc.) and the psylla resistant or tolerant cultivars Haydeea, Euras, Getica and Ina Estival have been promoted and named (Andreis, pers. com.; Braniste, pers. com.; Sestras *et al.*, 2009).

In North America the breeding programmes initiated in the 1920's and 1930's developed in the 1960's into two impressive programmes for disease and insect resistance at Harrow in Canada and at Kearneysville (USDA) in the United States, based on hybridisation with cultivars and selections from *P. ussuriensis* and *P. pyrifolia* (characterised by a higher resistance, probably of monogenic type), with fruit characteristics of *P. communis* being recovered by backcrossing to selected *P. communis* cultivars (Bellini and Nin, 1997). Resistance to pear psylla represent an additional breeding objective of the pear programme at Harrow (Brunner, 1997; Hunter, 1994; Hunter, pers. com.), while has been added as specific primary objective of the United States Department of Agriculture (USDA) breeding programme. Selection methods have been developed from detailed studies of the modes of resistance (a 24-hour nimphal feeding bioassay plus choice and non choice oviposition assays for further resistance characterization), almost 4,400 seedlings have been evaluated and RAPD markers associated with resistance to nymphal feeding antixenosis are in progress. Among the most recent cultivars coming from United States are Elliot, Gourmet, Potomac and Blacke's Pride (Bell and van der Zwet, 1992; Bell *et al.*, 1996).

Often, parallel studies are carried on in order to support and speed up the attainment of the pursued goals. Generally, the future direction of such programs will include a biotechnology component, with the objective of identifying and transferring genes for resistance to fire blight and pear psylla (Bellini and Nin, 1997; Bellini *et al.*, 2000).

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The influence of a non-pathogenic *Pseudomonas putida* strain BTP1 on reproduction and development of grape phylloxera

A. Adam[(1)], H. Makee, I. Idris
Department of Biotechnology, Atomic Energy Commission of Syria. P.O. Box 6091, Damascus, Syria.

Key words: developmental time, fecundity, grape phylloxera, oviposition period, PGPR, *Pseudomonas putida* BTP1.

Abstract: Some non-pathogenic rhizobacteria called Plant Growth Promoting Rhizobacteria (PGPR) possess the capacity to induce defense mechanisms effective in plant against pathogens. The effect of *Pseudomonas putida* BTP1 on reproduction and development of phylloxera, which infested the roots of our local grape variety "Balady", was evaluated. Our results showed that the life table of grape phylloxera was different between treated and control plants. The percentage of matured females, developmental time, fecundity and oviposition period were reduced when plants were treated with bacteria. The results showed that the phylloxera resistance was influenced by root soaking duration in *P. putida* BTP1 suspension. The present study provides good information on the possibility of using *Pseudomonas putida* BTP1 to increase the resistance of grape to phylloxera.

1. Introduction

Grape phylloxera (*Daktulosphaira vitifoliae*), aphid-like gall-forming parasite, is an economically important homopteran pest of grape vines *Vitis vinifera* L. world wide. In Syria, there are more than 70,000 ha planted with grapevine and the annual production is about 540,000 t. Grape phylloxera annually causes millions of dollars in losses in grape production.

Grape phylloxera causes direct damage to grapevine by forming damaging root galls. The fleshy galls formed on immature roots, by swelling of the root cortex, are called "nodosities", while on mature roots they are called "tuberosities"; these latter are considered more damaging to the vine. These galls are metabolically active organs suited to match the nutritional requirements of phylloxera and can support populations with high reproductive rates, making this pest capable of destroying the root system of *V. vinifera* vines (Granett *et al.*, 2001). In most cases the swelling stops rootlet growth, and the affected portion dies. Root injuries reduce the vines' ability to absorb nutrients and water, causing decline in vigor and productivity. Weakened plants probably become more susceptible to secondary infections from fungal diseases and other insects, and to environmental stresses.

Since there is not an effective control method for grape phylloxera, sanitation and quarantine can be considered required procedures to prevent the spread of this insect pest. Insecticides and hot water dips are used as quarantine treatments (Granett *et al*., 2001). Makee *et al.* (2010) proposed that gamma irradiation could be economically very useful in quarantine treatments against phylloxera. However, once phylloxera is in a vineyard, the use of resistant rootstocks is the most common and effective means of managing phylloxera. It should be mentioned that some rootstocks were more resistant than others to grape phylloxera (Makee *et al*., 2003). Moreover, for unknown reasons, the resistance of some rootstocks may break down and farmers must replant vineyards (Granett *et al*., 1983; Song and Granett, 1990; De Benedictis and Granett, 1993). Because replanting is costly in money, time, and labor, additional ways to control this pest should be considered. All plants have active defense mechanisms against pathogen attacks. Some plant growth-promoting rhizobacteria (PGPR) are able to reduce disease through the stimulation of inducible plant defense mechanisms that render the host plant more resistant to further pathogen ingress. This induced systemic resistance (ISR) (Pieterse *et al*., 2002) can be the basis of integrated plant disease management strategies (Ramamoorthy *et al*., 2001; Zehnder *et al*., 2001; Saravanakumar *et al*., 2007).

Many studies in plants on PGPR against pathogens have been performed. However, only a few of them determined the protective effect of PGPR against insects (Zehnder *et al*., 1997 a, b; Zehnder *et al*., 2001; Kloepper *et al*., 2004; Vijayasamundeeswari *et al*., 2009; Valenzuela-Soto *et al*., 2010). A non-pathogenic *Pseudomonas putida* strain (BTP1) was shown to enhance the level

[(1)] Corresponding author: ascientific@aec.org.sy
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of resistance in cucumber, bean and tomato against the fungal pathogens *Pythium aphanidermatum* and *Botrytis cinerea,* respectively (Ongena *et al*., 1999; Adam *et al*., 2008). These studies revealed that the disease-protective effect was associated with stimulation of defense mechanisms in host plant (Ongena *et al*., 2000, 2004; Adam *et al*., 2008).

The main objective of the present study was to evaluate the ability of strain *P. putida* BTP1 to protect grape roots against grape phylloxera. Thus, the effect of concentrations and root soaking duration in *P. putida* BTP1 suspension on percentage of matured females, developmental time, fecundity and oviposition period of local phylloxera strain were determined.

2. Materials and Methods

Establishment of the phylloxera colony

Grape phylloxera was originally collected from field-infested roots of the local grape varieties in southern parts of Syria. The phylloxera colony was established following similar procedures to those mentioned by Makee *et al*. (2003). Fresh and healthy pieces of roots (4-7 mm in diameter and 5-7 cm long) of local grape cultivar Helwani (*V. vinifera*) were taken and washed with tap water. Each piece was wrapped with moist cotton wool around one end, and then 10 to 15 phylloxera eggs were placed on each piece. The infested root pieces were then placed on a wet filter paper disk inside a plastic Petri dish (12 cm diameter). Each dish had three to four root pieces. For ventilation purposes the Petri dish lid was modified with a 1-1.5 cm cloth-screened hole. The edges of the dishes were sealed with parafilm and they were kept in plastic boxes with tightly fitting lids and incubated at 25±1°C, 70±5% RH and 24 hr darkness. The root pieces were replaced when they desiccated, rotted or the phylloxera became crowded.

Microbial strains and inoculum preparation

P. putida strain BTP1, isolated from barley roots, was originally selected for its specific features regarding pyoverdine-mediated iron transport (Jacques *et al*., 1995; Ongena *et al*., 2002); it was maintained and prepared for use in the ISR assays as previously described by Ongena *et al*. (2002). For bioassays, two different concentrations (10^8 and $2x10^8$ CFU/ml) of bacterial suspension were prepared.

Effect of bacteria-treated roots on phylloxera

Fresh root pieces were soaked for 3 hr in solutions with various *P. putida* concentrations: 0 (roots were soaked in distilled water as a control), 10^8, and $2x10^8$ CFU/ml. All root pieces were then left to air-dry. For each concentration five root pieces were taken. Each root piece was infested with 50 newly-laid phylloxera eggs (<24 hr old) and then all root pieces were kept at 25±1°C, 70±5% RH and 24 hr darkness.

Evaluation procedure

A daily microscope inspection of all phylloxera stages on all root pieces was carried out. The number of eggs hatched, feeding nymphs and adults were detected to calculate the percentage of emerged mature females on each root piece at each concentration. Also, the mean developmental time (egg to egg) was determined. Fecundity (total number of eggs) of phylloxera was evaluated by randomly choosing five individuals of root-feeding phylloxera females on each root piece at each concentration. Thus, at each tested concentration 20 females were examined. All eggs laid by each female were observed and counted till the female's death. Additionally**,** the oviposition period (the time from the first laid egg to the natural death of individual ovipositing females) was recorded for the females chosen for the fecundity measurement.

Effect of root soaking duration in bacterial suspension on phylloxera

Fresh root pieces were soaked in solutions with various *P. putida* concentrations: 0, 10^8, and $2x10^8$ CFU/ml. At each tested concentration the root pieces were soaked for various periods: 0, 3, 5 and 15 hr. All root pieces were then left to air-dry. At each concentration and soaking duration, five root pieces were taken. Each root piece, at each concentration and soaking duration, was infested with 50 newly-laid phylloxera eggs (<24 hr old) and then all root pieces were kept at 25±1°C, 70±5% RH and 24 hr darkness.

The same evaluation procedure as described above was followed to determine the percentage of emerged mature females, mean developmental time, fecundity and the oviposition period of phylloxera.

Statistical analysis

All statistical analyses were performed using STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% level (P = 0.05). Data were subjected to analysis of variance (ANOVA) for the determination of differences in means between tested plants at each dose. Differences between means were tested for significance using Tukey HSD test.

3. Results

Effect of bacterial treated roots on phylloxera

Table 1 shows that when root pieces were treated with *P. putida*, the percentage of emerged matured females was significantly affected compared to the control ($F=84.69$; $df=15, 64$; $P<0.05$). However, the percentage emerged of matured females was not significantly increased by increasing *P. putida* concentrations.

The result illustrates that the developmental time of phylloxera was significantly decreased by the application of *P. putida* ($F= 15$; $df = 2, 42$; $P < 0.05$). There was a significant reduction in developmental time as the concentration of *P. putida* was increased (Table 1).

There were significant differences in the mean number of laid eggs between all tested root pieces, regardless of concen-

Table 1 - Mean percentage of matured females, developmental time, fecundity and oviposition when phylloxera was reared on *P.putida*-treated grape root pieces

Oviposition period/d (Mean±SE)	Fecundity (Mean±SE)	Developmental time/d (Mean±SE)	% matured female (Mean±SE)	Bacterial Concentration CFU/ml
14.2±0.54 a	44.73±0.85 a	28.27±0.57 a	78.16±1.48 a	0
12.07±0.27 b	40.07±1.3 b	26.53±0.27 b	36.33±0.88 b	108
9.53±.53 c	34.2±1.3 c	24.53±0.54 c	32.03±1.49 b	2x108

Means, in a column, followed by the same letter are not significantly different at the $P < 0.05$ (Tukey HSD test).

tration (F = 19.9; df = 2, 42; P< 0.05). On untreated root pieces, the mean number of eggs was significantly higher than that on treated ones (Table 1). When phylloxera females were reared on *P. Putida*-treated root pieces, the mean number of eggs was markedly reduced by increasing *P. putida* concentration.

Table 1 shows that the oviposition period of phylloxera on untreated root pieces was significantly longer than that on treated ones, irrespective of the concentration of *P. putida* (F = 25.5; df = 2, 42; P < 0.05). There was a significant difference in the oviposition period between *P. Putida*-treated root pieces. The oviposition period on 10^8 CFU/ml-treated root pieces was significantly longer than that on $2x10^8$ CFU/ml-treated ones.

Effect of root soaking duration in bacterial suspension on phylloxera

There was a significant effect of the soaking duration in *P. putida* suspension on the percentage emerged of matured females, regardless of the concentration (F= 620.87; df= 11, 24; P<0.05) (Fig. 1). At each concentration, the percentage of emerged matured females on un-soaked root pieces was significantly higher than that on soaked ones, regardless of soaking duration. When the root pieces were soaked, the percentage of emerged matured females with 3 hr soaking duration significantly differed from that on 5 and 15 hr, whatever the *P. putida* concentration. At each *P. putida* concentration, there was no significant difference in the percentage of emerged matured females between 5 and 15 hr soaking duration (Fig. 1).

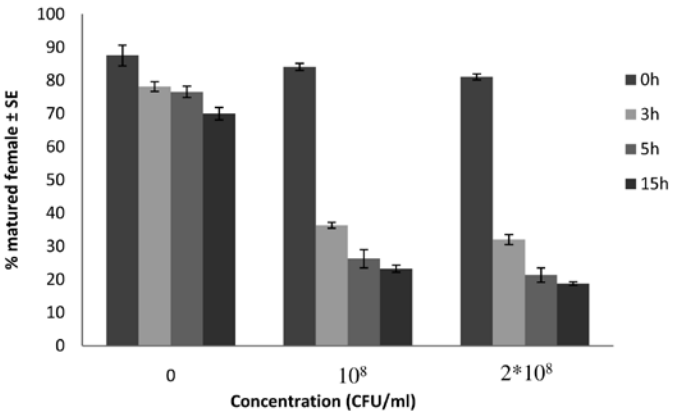

Fig. 1 - Effect of root soaking duration in bacterial suspension of percentage emerged matured females of phylloxera.

There was a significant effect of soaking in *P. putida* suspension on the mean developmental time, regardless of the soaking duration and concentration (F= 16; df= 11, 168; P< 0.05) (Fig. 2). At each concentration, the mean developmental time on un-soaked root pieces was significantly higher than that on soaked ones, regardless of soaking duration. The result illustrates that at $2x10^8$ CFU/ml the observed differences in mean development between soaking durations were not significant.

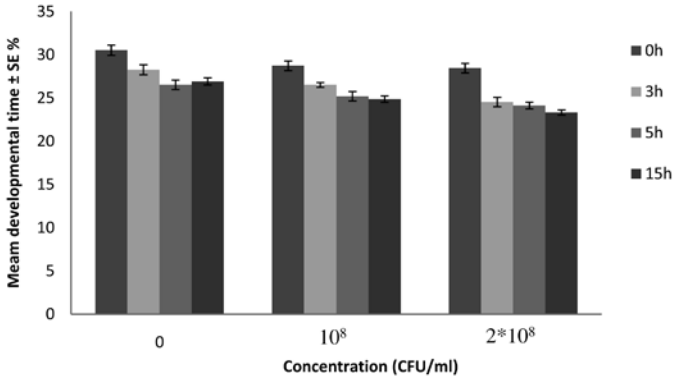

Fig. 2 - Effect of root soaking duration in bacterial suspension on mean developmental time of phylloxera.

There was a significant effect of the soaking duration in *P. putida* suspension on mean number of eggs, regardless of the concentration (F= 112; df = 11, 168; P<0.05) (Fig. 3). At each concentration, mean number of eggs on soaked root pieces was significantly higher than that on un-soaked ones, regardless of soaking duration. At concentration 0 (root pieces soaked with distilled water), there was no significant difference in the mean number of eggs between 3 and 5 hr soaking duration. However, the mean number of eggs was significantly reduced with 15 hr soaking duration. At 10^8 and $2x10^8$ CFU/ml concentrations, the mean number of eggs on 3 hr soaking duration was significantly higher than that on 5 and 15 hr (Fig. 3).

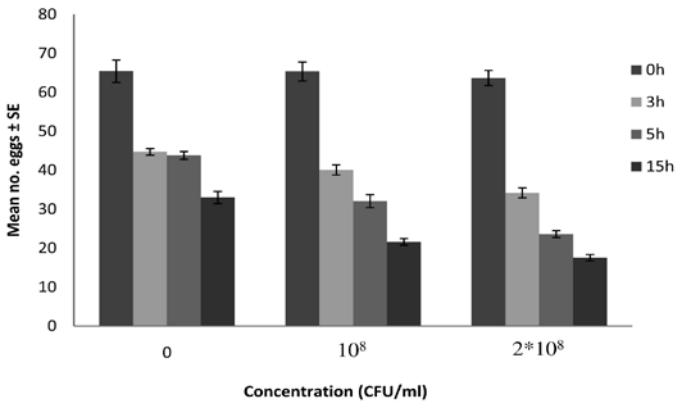

Fig. 3 - Effect of root soaking duration in bacterial suspension on mean number of eggs of phylloxera.

A significant effect of the soaking duration in *P. putida* suspension on mean oviposition period was found, regardless of the concentration (F= 46; df = 11, 168; P<0.05) (Fig. 4). At each concentration, the mean oviposition period on un-soaked root pieces was significantly higher than that on soaked ones, regardless of soaking duration. At both 0 and 10^8 CFU/ml, the mean oviposition period with 3 hr soaking duration was significantly higher than that with 15 hr. At 0 concentration, there was no significant difference in the mean oviposition period between on 3 and 5 hr soaking duration, while at 10^8 CFU/ml there was. At $2x10^8$ CFU/ml, there was no significant difference in the mean oviposition period between 3, 5 and 15 hr soaking duration.

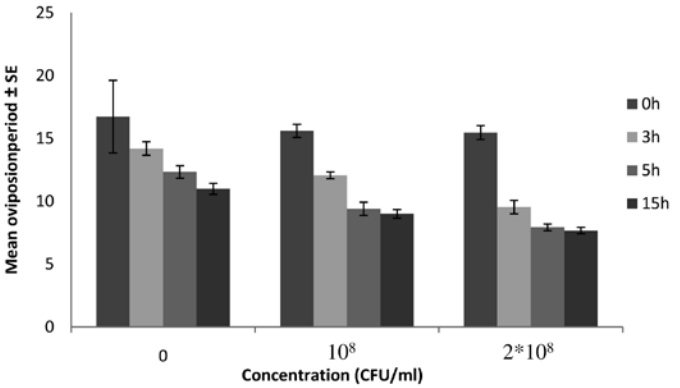

Fig. 4 - Effect of root soaking duration in bacterial suspension on mean oviposition period of phylloxera.

4. Discussion and Conclusions

Several studies reported the use of rhizobacteria as a biological control of pests (Racke and Sikora, 1992; Zehnder *et al*., 1997 a, b). To our knowledge, the application of rhizobacteria against phylloxera has not been investigated. Our study indicates that when *P. putida* BTP1-treated roots were infested by phylloxera eggs, the percentage of matured females was negatively influenced (Table 1). The result illustrates that the percentage of matured females was significantly decreased when the concentration and the soaking duration were increased (Fig. 1). The reduction of the number of matured females on the treated roots could be attributed to the inability of phylloxera to feed. Thus, nymphs fed on *P. putida* BTP1-treated roots showed antifeeding behaviour. When *Heliothis zeae* (Boddie) diet was contaminated with the bacterium *Pseudomonas maltophila,* a 60% reduction in adult emergence and high pupal and adult malformations were observed (Bong and Sikorowski, 1991). Moreover, when chestnut was treated with *Pseudomonas fluorescens*, 20% chestnut weevil mortality was recorded (Yaman *et al*., 1999). Therefore, the antifeeding behaviour of phylloxera nymphs on the PGPR treatments could be related to the reduction in the feeding stimulant.

Consequently, the developmental time was altered leading to early emergence of matured females (Table 1). A comparison between *P. putida* BTP1-treated and untreated roots showed that the phylloxera development on treated roots was faster than that on untreated ones. Similar results were reported when cucumber beetles and American bollworm were fed on PGPR-treated cucumber plants and cotton bolls, respectively (Zehnder *et al*., 1997 a, b; Vijayasamundeeswari, 2009). Our current study indicates that the developmental time was significantly shorter on treated root pieces soaked for 15 hr (Fig. 2). Qingwen *et al*. (1988) detected a reduction of relative growth rate, consumption rate and digestibility of feed when *Heliothis armigera* fed on *P. gladioli*-treated cotton plants.

It is known that fecundity could be considered an essential factor in assessing the effect of *P. putida* BTP1on phylloxera. Phylloxera fecundity on *P. putida* BTP1-treated root pieces was distinctly lower than that on untreated ones. When phylloxera females were reared on treated roots they were unable to produce a normal number of eggs compared to the control, especially at high concentration (Table 1). Nevertheless, when soaking duration in *P. putida* BTP1 suspension was prolonged, a defective reproductive capacity was obtained (Fig. 3). The average number of eggs was 65.3, 40, 32 and 21.6 eggs on 10^8 CFU/ml-treated roots pieces soaked for 0, 3, 5 and 15 hr, respectively. While on $2x10^8$ CFU/ml-treated roots pieces soaked for 0, 3, 5 and 15 hr, the average number of eggs was 63.7, 34.2, 23.6 and 17.5 eggs, respectively. Inadequate nutrition and inability to establish good feeding sites could directly affect the number of eggs laid. Therefore, phylloxera resistance could be reflected in a strong relationship between poor feeding and the reduction of insect reproduction (Granett *et al*., 1983). Thus, phylloxera produced more eggs on untreated roots compared with treated ones.

Correspondingly, the oviposition period of phylloxera on treated roots was markedly shorter than that on untreated ones (Table 1). When the concentration of *P. putida* BTP1 was increased a great reduction in the oviposition period was obtained. Such reduction was noticeably increased by prolonging the root soaking duration in *P. putida* BTP1 (Fig. 4).

It is known that the resistance mechanisms of phylloxera could be related to several factors including: 1) reduction of phylloxera fitness (antibiosis); 2) decrease in plant attractiveness to phylloxera (antixenosis) (Granett *et al*., 2001). Zehnder *et al*. (2001) mentioned that the PGPR treatment led to alteration in the plant metabolic pathway which elicited the induction of plant defense compounds. Qingwen *et al.* (1998) reported that polyphenol and terpenoid content were increased with cotton treated with *P. gladioli.* The synthesis and formation of such materials in *P. putida* BTP1-treated roots would have a negative influence on phylloxera feeding and development.

The results illustrate that the soaking of grape roots with *P. putida* BTP1 suspension for 5 hr and 15 hr, regardless of concentration, led to a great reduction in matured females, developmental time, fecundity and oviposition

period. Such reduction might be attributed to increased bacterial cell concentration by prolonging the soaking duration. Therefore, roots pieces soaked with $2x10^8$ CFU/ml for 15 hr showed more resistance to phylloxera than lower concentrations and a shorter soaking duration.

These results provide essential information about the relationship between *P. putida* BTP1 and phylloxera resistance of *grape plants*. Phylloxera encounters serious difficulties in surviving, feeding and reproducing on *P. putida* BTP1-treated roots. *P. putida* BTP1 reduced the susceptibility of roots to phylloxera. Nevertheless, in future it is essential to determine for how long such effects could persist. Therefore, we acknowledge that these laboratory studies cannot simulate all conditions that exist in nature. Therefore, care must be taken when laboratory-based results are applied in nature.

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Effect of type of fertilization and maturity on quality of fresh-cut red and yellow peppers (*Capsicum annuum* L.)

F. Piazzolla*, M.L. Amodio*, R. Rinaldi*, F. Raimo, G. Colelli***

* *Dipartimento di Scienze delle Produzioni e dell'Innovazione dei Sistemi Agroalimentari Mediterranei, Università degli Studi di Foggia, Via Napoli, 25, 71121 Foggia, Italy.*

** *Consiglio per la Ricerca e Sperimentazione in Agricoltura, Unità di Ricerca per le Colture Alternative al Tabacco, CRA-CAT, Via P. Vitiello, 108, 84018 Scafati (SA), Italy.*

Key words: appearance, organic fertilization, phenolics, Vitamin C.

Abstract: The aim of this work was to evaluate the effect of the type of fertilization (mineral and combined fertilization with compost in pre-transplant plus mineral addition during cultivation) and stage of maturity at harvest (mature-green and full-colored) on post-cutting quality of red and yellow 'Cazzone' peppers. Peppers were cut into strips, and air-stored for 8 days at 5°C. During storage, color, appearance score, firmness, respiration rate, soluble solids, acidity, pH, vitamin C, total phenols, and antioxidant activity were measured. The maturity stage influenced color parameters and soluble solids, acidity and pH for both yellow and red types. Full-colored peppers showed a lower respiration rate, and higher SSC than mature-green peppers; for the yellow type, a lower firmness value was observed for full-colored fruits compared to the mature-green ones. A lower antioxidant activity was also observed in the yellow type fertilized with the combined treatment, while phenol content in full-colored peppers was higher than in mature-green ones. Fresh-cut yellow peppers showed higher susceptibility to decay compared to red types: after 8 days of storage, the appearance score in mineral fertilized full-colored yellow peppers dramatically decreased below the limit of marketability. The results of this experiment show that the type of fertilization and maturity stage can have varying impact on the quality of yellow and red peppers.

1. Introduction

Due to consumer demand for high-convenience foods, fresh-cut peppers may represent an interesting product to add to the existing fresh-cut products. The quality of a fresh-cut product is generally affected by pre-harvest and post-harvest factors, including processing. Genotype, growing conditions, cultural practices, and maturity stage at harvest, from a pre-harvest point of view, may greatly influence initial quality of the product to be processed; on the other hand, postharvest handling and storage of raw materials, and processing conditions, including finished product fate throughout the distribution chain, markedly determine its final quality. Maturity stage is an important factor conditioning final quality and processability of fresh-cut products, particularly fruits. Less mature fruits, in fact, are more suitable for processing due to their greater firmness, compared to more mature fruits, but this can result in lower sensorial quality as observed in melons (Watada and Qi, 1999) and mangoes (Bender *et al.*, 2000). In peppers, the maturity stage was found to have an impact on flavonoids, carotenoids, and ascorbic acid concentration (Marin *et al.*, 2004; Fox *et al*., 2005), with carotenoids and ascorbic acid increasing, and flavonoids decreasing, as maturity proceeds, while Deepa *et al.* (2007) observed an increase in the main antioxidant compounds, including phenols ascorbic acid, capsicin, and carotenoids for 10 different genotypes. Moreover, in 'Domino' bell peppers, an increase in firmness was observed with the increasing of fruit size, occurring with ripening, most probably due to the increase of pericarp thickness (Tadesse *et al.*, 2002). The same authors reported an increase in soluble solids, and a decrease in respiration rate and ethylene production and suggested as maturity index at harvest for this cultivar firmness values of 35 N and a minimum of 6°Brix. Molinari *et al.* (1999) reported greater titrable acidity on full-ripe peppers ripened on the plant compared to those harvested at the color-break stage and ripened in storage.

In addition, agricultural practices, soil, and climate conditions may differently affect quality attributes of fresh produce, including external attributes (Kays, 1999), firmness (Sams, 1999), and nutritional composition (Lee and Kader, 2000). In particular, nitrogen fertilization seems to decrease the concentration of vitamin C in many fruits and vegetables (Lisiewska and Kmiecik, 1996). All these factors can consequently affect quality of fresh-cut produce

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and their storability, but very few works have directly investigated the impact of pre-harvest factors on the quality of fresh-cut products, and none of them regard bell peppers. More works are available on the impact of processing, and among them Artés-Hernández *et al.* (2010) studied the effect of the cut type and of the modified atmosphere packaging on quality of bell peppers (cv. Requena) observing that in general peppers cut in a 'ring' suffer greater weight loss than peppers cut in strips or dice, although respiration was not affected by the cutting mode and was not significantly different from the whole product.

The objective of the present work was to evaluate the effect of the type of fertilization (mineral and combined fertilization with compost in pre-transplant plus mineral addition during cultivation) and stage of maturity at harvest (mature-green and full-colored) on post-cutting quality of red and yellow *'*Cazzone*'* peppers.

2. Materials and Methods

Plant material

The experiment was carried out in Scafati (SA, Italy, coordinates 40° 44'N, 14° 30'E, 10 m a.s.l.), on soil of sandy loam texture, basic pH and with organic and mineral content as reported in Table 1.

Table 1 - Soil parameters

Soil parameters	
pH in water (1:2.5)	8.4
EC 25°C (1:2) (dS/m)	0.6
C (g/kg)	15.4
Organic matter (g/kg)	26.6
N (g/kg)	1.3
C/N	11.6
Assimilated P_2O_5 (mg/kg)	85.0
Exchangeable K (ppm)	917.1
Exchangeable Na (ppm)	251.8
Exchangeable Ca (ppm)	2090.9
Exchangeable Mg (ppm)	497

Ecotypes of red and yellow 'Cazzone' pepper (*Capsicum annuum* L.) (density of 3.3 plants m^{-2}) were subjected to two different tecniques of fertilization: mineral and combined fertilization with compost in pre-transplant plus mineral addition during cultivation. Mineral fertilization was in compliance with the Campania Region guidelines: 100 kg ha^{-1} of nitrogen were applied for 1/3 in pre-transplant and 2/3 during plant growth. For the combined fertilization, 20 t ha^{-1} of dry organic compost obtained from urban organic waste (characteristics reported in Table 2) were applied in pre-transplant, and integrated with 50 kg ha^{-1} of mineral nitrogen during plant growth. Transplanting was carried out on 25 May. Pepper fruits were harvested on 15 September at two stages of maturity (mature-green and full-colored), and transported to the Laboratory of Postharvest Technology at the University of Foggia (Italy).

Table 2 - Compost parameters

Compost parameters	
Humidity (%)	31
pH	6-8
C (% DM)	28
Humic and fulvic carbon	8
N (% DM)	2
C/N	14
Cu (mg/kg DM)	110
Zn (mg/kg DM)	250
Salinity (meq/100 g)	21

Experimental design and protocol

For each ecotype/fertilization/stage of maturity combination, six lots (two replicates x three storage sampling) of 15 strips were individually placed in plastic trays closed in PET macroperforated bags, together with wet paper (to maintain high level of RH), and stored at 5°C for eight days. Initially, and after four and eight days of storage at 5°C quality attributes (including color, appearance score, firmness, respiration rate, weight loss, soluble solids, titrable acidity, pH, vitamin C, phenols content, and antioxidant activity) were monitored.

Respiration rate and weight loss

Respiration rate (ml CO_2 kg^{-1} hr^{-1}) was measured using the static system, measuring the amount of CO_2 accumulated in the headspace of sealed PVC containers (5 l). CO_2 concentration, determined by a Shimadzu gas chromatograph (model 17A) equipped with a TCD detector, was then referred to the weight of the sample, to the volume of the headspace, and to the elapsed time. Samples were individually weighed and the weight loss was calculated as % of the initial fresh weight.

Physical analysis

The colour of the strips was measured in two different points of the mesocarp, randomly selected, using a spectrophotometer (CM 2600d Konica Minolta, Osaka, Japan) in the reflectance mode using the CIE L*a*b* colour scale. Hue angle and saturation were then calculated.

Appearance score evaluation was subjectively assessed using a scale of 5 to 1 where 5= excellent, no defects; 4= very good, few defects; 3= good, moderate defects, limit of marketability; 2= poor, many defects; and 1= inedible. A score of 3 was considered as the limit of marketability and a score of 2 as the limit of edibility (Amodio *et al.*, 2007).

Firmness was measured on two points of the mesocarp, as resistance of the strips to a 2-mm penetration by a probe

of 6-mm diameter, using a digital penetometer (Tierre S.r.l., Torino).

Chemical analysis

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata and Dufour (1992), with some modifications. Samples of 20 µL were analysed with an Agilent 1200 Series HPLC (Waldbronn, Germany) equipped with a binary pump, an autosampler, and a photodiode array detector. Separations of DHAA and AA were achieved on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm; 5 µm of particle size; Agilent Technologies, Santa Clara, CA, USA). AA and DHA contents were expressed as milligrams of ascorbic or dehydroascorbic acid per kilogram of fresh weight (mg kg^{-1}).

Total phenols were determined according to the method of Singleton and Rossi (1965). The absorbance was read at 725 nm against a blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). The content of total phenols was calculated on the basis of the calibration curve of gallic acid, and was expressed as grams of gallic acid per kilogram of fresh weight (g GA kg^{-1}). Antioxidant assay was performed following the procedure described by Brand-Williams *et al.* (1995), with minor modifications. Trolox was used as a standard and the antioxidant activity was reported in milligrams of Trolox equivalents per kilogram of fresh weight (g TE kg^{-1}).

Four grams of fresh juice were used to determined total soluble solids (TSS) (measured with a digital refractometer, ATAGO PR32), pH and titratable acidity (TA). Titratable acidity was determined with an automatic titrator (TitroMatic CRISON 1S), using the juice samples and titrating with 0.1 N NaOH up to pH 8.1, and the value was expressed as percentage of citric acid.

Data analysis

For mean data at harvest of peppers of both ecotypes, a two-way ANOVA for stage of maturity and fertilization effects was run, while on the whole data set, a three-way ANOVA was performed with stage of maturity, fertilization treatment and time of storage as factors. When interactions among factors were not significant, main effects were analyzed. Mean separation among treatments at each time of storage was performed with the Tukey test. ($P< 0.05$, N=10).

3. Results and Discussion

Most of the quality attributes were not affected by the type of fertilization, except respiration rate, a* value and pH, whereas, as expected, most of them were affected by the stage of maturity. The interaction between the type of fertilization and the stage of maturity was statistically significant for respiration rate and pH (Table 3).

Table 3 - Effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored), and their interaction on quality attributes of fresh-cut yellow 'Cazzone' peppers at harvest

Quality attributes	Fertilization (F)	Stage of maturity (S)	FxS
Firmness (N)	NS	NS	NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	*	***	***
L*	NS	****	NS
a*	*	****	NS
b*	NS	****	NS
Hue angle (*)	NS	****	NS
Chroma	NS	****	NS
Soluble solids (°Brix)	NS	***	NS
Titrable acidity (% citric acid)	NS	NS	NS
pH	**	****	***
Vitamin C (mg/100 g FW)	NS	NS	NS
Ascorbic acid (mg/100 g FW)	NS	NS	NS
L-dehydroascorbic acid (mg/100 g)	NS	NS	NS
Antioxidant activity (mg Trolox/100 g FW)	NS	NS	NS
Total phenol content (mg gallic acid/100 g FW)	NS	NS	NS

* when P≤ 0.05; ** when P≤ 0.01; *** when P≤ 0.001; **** when P≤ 0.0001; NS when not significant; N= 10.

Pepper fruits treated with mineral fertilization showed a higher respiration rate (9.6 ml CO_2 kg^{-1} hr^{-1}) than fruits treated with the combined fertilization (8.8 ml CO_2 kg^{-1} hr^{-1}), a higher a* value (0.6 vs. -1.1) and pH (5.44 vs. 5.35) although the absolute pH difference was very little (Table 4). Table 4 illustrates the effect of stage of maturity on quality attributes of fresh-cut yellow peppers at harvest. In particular, the stage of maturity affected the respiration rate, L*, a*, and b* values, hue angle, chroma, soluble solids, and pH. Full-colored peppers showed lower respiration rate than mature-green peppers (7.6 and 10.7 ml CO_2 kg^{-1} hr^{-1} respectively), higher soluble solids (6.1 vs. 4.7°Brix) and lower pH (5.75 vs. 5.04). These findings confirmed that reported by Tadesse *et al.* (2002) for 'Domino' bell peppers, and mainly that soluble solids, respiration rate and firmness (other than color) evolve during fruit ripening, and may be used as maturity index at harvest. The increase of titratable acidity and decrease of pH during bell pepper growth and ripening has also been observed in several studies (Fox *et al*., 2005), during plant ripening (Molinari *et al.*, 1999; Serrano *et al.*, 2010) and, in particular Serrano *et al.* (2010) observed an increase of citric acid during ripening.

For red 'Cazzone' peppers, no differences were observed for the measured attributes according to the type of fertilization and interaction between the type of fertilization and the stage of maturity, whereas the stage of matu-

Table 4 - Main effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut yellow 'Cazzone' peppers at harvest

Quality attributes	Type of Fertilization		Stage of maturity	
	Mineral	Combined	Mature green	Full-colored
Firmness (N)	19.0 NS	18.3 NS	19.2 NS	18.1 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	9.6 a	8.8 b	10.7 a	7.6 b
L*	45.2 NS	46.1 NS	39.3 b	52.1 a
a*	0.6 a	-1.1 b	-13.2 b	12.7 a
b*	37.7 NS	38.3 NS	26.4 b	49.6 a
Hue angle (*)	95.0 NS	97.3 NS	116.6 a	75.6 a
Chroma	40.3 NS	40.5 NS	29.5 b	51.3 a
Soluble solids (°Brix)	5.4 NS	5.5 NS	4.7 b	61.0 a
Titrable acidity (% citric acid)	0.16 NS	0.14 NS	0.14 NS	0.15 NS
pH	5.44 a	5.36 b	5.75 a	5.04 b
Vitamin C (mg/100 g FW)	59.4 NS	58.8 NS	61.4 NS	56.8 NS
Ascorbic acid (mg/100 g FW)	58.6 NS	58.0 NS	60.4 NS	56.2 NS
L-dehydroascorbic acid (mg/100 g)	0.8 NS	0.8 NS	1.0 NS	0.7 NS
Antioxidant activity (mg Trolox/100 g FW)	169.8 NS	155.0 NS	153.7 NS	171.1 NS
Total phenol content (mg gallic acid/100 g FW)	114.1 NS	119.5 NS	108.0 NS	125.5 NS

For each row, mean values followed by a different letter are significantly different (N=10 and P≤0.05).

rity affected several quality attributes (Table 5). The respiration rate of full-colored peppers was about half that of peppers at a mature green stage; soluble solids increased by about 2°Brix with ripening. In addition, an increase of acidity from 0.1% to 0.15% and a decrease of pH were also observed (5.93 and 4.93, respectively) (Table 6).

Most of the differences observed at harvest were maintained during storage for yellow peppers. Some differences among chemical constituents were found for yellow

Table 5 - Effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored), and their interaction on quality attributes of fresh-cut red 'Cazzone' peppers at harvest

Quality attributes	Fertilization (F)	Stage of maturity (S)	FxS
Firmness (N)	NS	NS	NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	NS	**	NS
L*	NS	****	NS
a*	NS	****	NS
b*	NS	***	NS
Hue angle (*)	NS	****	NS
Chroma	NS	**	NS
Soluble solids (°Brix)	NS	***	NS
Titrable acidity (% citric acid)	NS	***	NS
pH	NS	***	NS
Vitamin C (mg/100 g FW)	NS	NS	NS
Ascorbic acid (mg/100 g FW)	NS	NS	NS
L-dehydroascorbic acid (mg/100 g)	NS	*	NS
Antioxidant activity (mg Trolox/100 g FW)	NS	NS	NS
Total phenol content (mg gallic acid/100 g FW)	NS	NS	NS

* when P≤ 0.05; ** when P≤ 0.01; *** when P≤ 0.001; **** when P≤ 0.0001; NS when not significant; N= 10.

Table 6 - Main effect of stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut red 'Cazzone' peppers at harvest

Quality attributes	Mature green	Full-colored
Firmness (N)	18.9 NS	18.9 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	9.8 a	4.9 b
L*	40.2 b	31.8 b
a*	-13.1 b	32.4 a
b*	27.0 b	16.6 b
Hue angle (*)	115.9 a	27.2 b
Chroma	30.0 b	36.4 a
Soluble solids (°Brix)	4.1 b	6.3 a
Titrable acidity (% citric acid)	0.10 b	0.16 a
pH	5.93 a	4.93 b
Vitamin C (mg/100 g FW)	55.0 NS	52.0 NS
Ascorbic acid (mg/100 g FW)	53.6 NS	51.4 NS
L-dehydroascorbic acid (mg/100 g)	1.4 a	0.6 b
Antioxidant activity (mg Trolox/100 g FW)	149.1 NS	163.9 NS
Total phenol content (mg gallic acid/100 g FW)	107.2 NS	123.2 NS

For each row mean values followed by a different letter are significantly different (N=10; P≤0.05).

'Cazzone' peppers, depending on the fertilization treatment. For this last ecotype, Table 7, the main effect of the fertilization treatment over storage on quality attributes is shown. In particular, a lower antioxidant activity was observed for peppers fertilized with the combined treatment (139 mg Trolox/100g FW) compared to mineral fertilization (157 mg Trolox/100g FW), whereas phenol content was positively affected by the stage of maturity, showing a value 10% higher for full-colored peppers compared to mature green (Table 7). The higher phenol content of full-coloured peppers compared to mature green ones confirms that phenols and antioxidant compounds increase during ripening, as found by Marin *et al.* (2004) and Deepa *et al.* (2007). In addition, a higher susceptibility to decay of fresh-cut yellow peppers compared to red type was observed. As shown in figure 1, after eight days of storage the appearance score in mineral-fertilized full-colored yellow peppers dramatically decreased below the limit of marketability (score 3) due to the presence of decay and most likely to the high respiration rate, even if it was found not significant among treatments (Fig. 1).

For red peppers, no differences related to the fertilization treatment were observed during storage; on the other hand, the stage of maturity had effects on many attributes of fresh-cut red peppers after storage (Table 8). In particular, the mature-green peppers showed a higher respiration rate (8.6 ml CO_2/kg/h) than full-colored peppers (5.9 ml CO_2/kg/h), probably causing the greater weight loss (0.9% and 0.5%, respectively). Full-colored red peppers accumulated 2.4°Brix with respect to the mature green fruits, also showing an increase of titrable acidity and lower pH (0.20% citric acid vs. 0.11%) which resulted in a lower pH (4.72 vs. 5.80).

Table 8 - Main effect of type stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut yellow 'Cazzone' peppers during storage

Quality attributes	Mature green	Full-colored
Firmness (N)	16.5 NS	17.2 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	8.6 a	5.9 b
L*	39.7 a	30.9 a
a*	-12.6 b	31.4 a
b*	26.6 a	16.3 b
Hue angle (*)	115.3 a	27.4 b
Chroma	29.5 b	35.4 a
Appearance score	4.2 NS	4.2 NS
Soluble solids (°Brix)	4.4 b	6.8 a
Titrable acidity (% citric acid)	0.11 b	0.20 a
pH	5.80 a	4.72 b
Weight loss (%)	0.9 a	0.5 b
Vitamin C (mg/100 g FW)	54.5 NS	58.8 NS
Ascorbic acid (mg/100 g FW)	51.8 NS	56.3 NS
L-dehydroascorbic acid (mg/100 g)	2.7 NS	2.5 NS
Antioxidant activity (mg Trolox/100 g FW)	136.6 NS	147.9 NS
Total phenol content (mg gallic acid/100 g FW)	96.1 NS	107.2 NS

For each row mean values followed by a different letter are significantly different (N=10; P ≤0.05).

Table 7 - Main effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut yellow 'Cazzone' peppers during storage

Quality attributes	Type of Fertilization		Stage of maturity	
	Mineral	Combined	Mature - green	Full-colored
Firmness (N)	16.5 NS	17.0 NS	15.9 NS	17.5 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	9.7 NS	7.8 NS	9.2 NS	8.3 NS
L*	44.6 NS	45.0 NS	39.0 b	50.6 a
a*	0.4 a	-0.8 b	-12.6 b	12.2 a
b*	37.5 NS	37.7 NS	26.4 b	48.8 a
Hue angle (*)	95.0 NS	96.6 a	115.6 a	76.0 b
Chroma	39.8 NS	39.8 NS	29.3 b	50.3 a
Appearence score	4.0 b	4.2 a	4.1 NS	4.0 NS
Soluble solids (°Brix)	5.9 NS	5.8 NS	4.8 b	6.9 a
Titrable acidity (% citric acid)	0.15 NS	0.14 NS	0.11 b	0.18 a
pH	5.26 NS	5.26 NS	5.66 a	4.86 b
Weight loss (%)	0.8 NS	0.7 NS	0.9 NS	0.6 NS
Vitamin C (mg/100 g FW)	60.9 NS	61.8 NS	58.5 NS	64.2 NS
Ascorbic acid (mg/100 g FW)	58.6 NS	59.3 NS	55.9 NS	62.1 NS
L-dehydroascorbic acid (mg/100 g)	2.3 NS	2.5 NS	2.6 NS	2.2 NS
Antioxidant activity (mg Trolox/100 g FW)	157.0 a	139.0 b	141.7 NS	154.3 NS
Total phenol content (mg gallic acid/100 g FW)	111.4 NS	106.8 NS	103.6 b	114.6 a

For each row mean values followed by a different letter are significantly different (N=10; P ≤0.05).

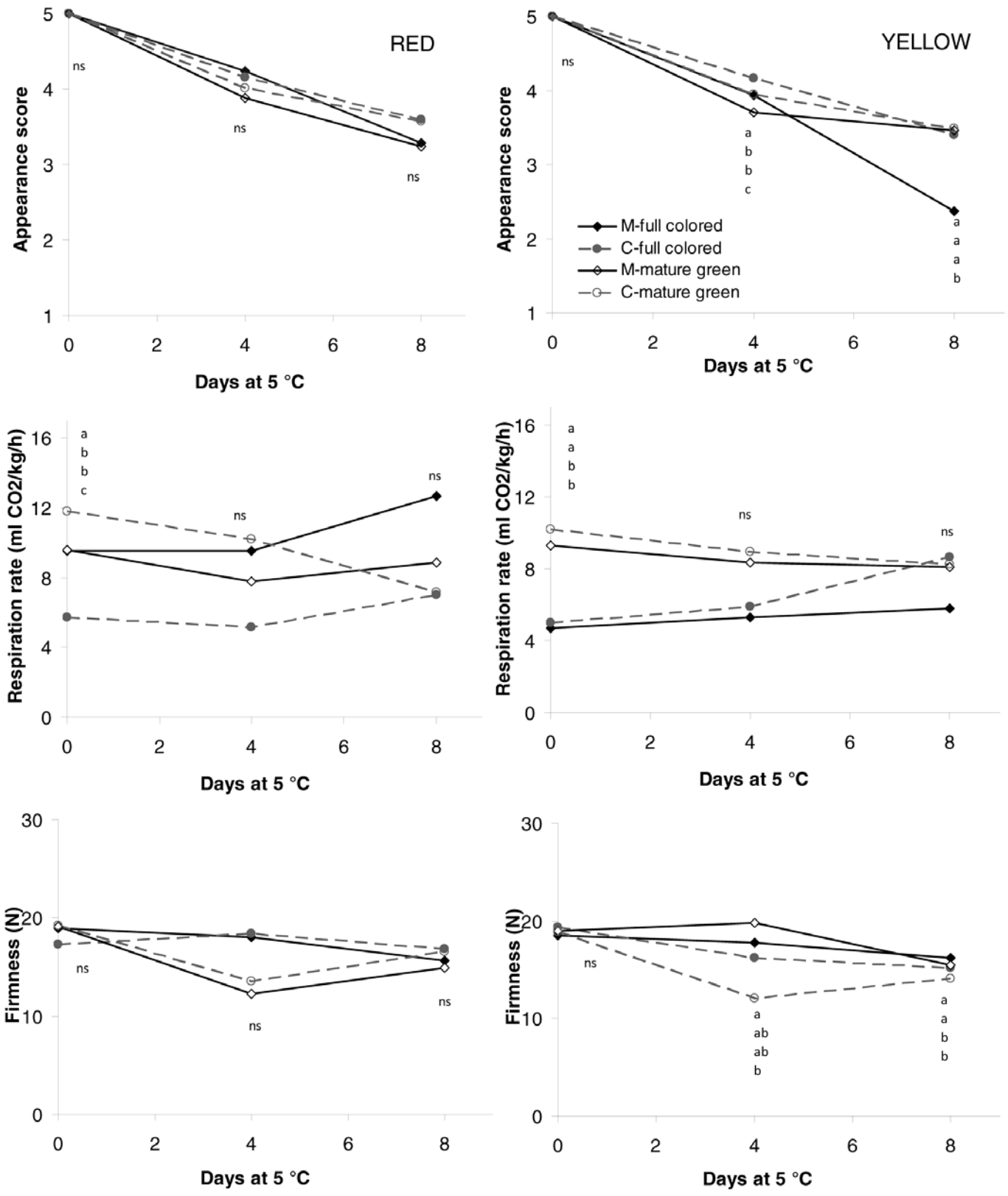

Fig. 1 - Effect of storage at 5°C on appearance score, respiration rate and firmness of fresh-cut yellow and red *'Cazzone'* peppers (M=mineral fertilization; C=combined fertilization). At each storage evaluation different letters indicate significant differences among treatments (N=10; P≤0.05).

All red peppers received a score higher than 3 at the end of storage, without differences among treatments, whereas a lower firmness was observed for peppers fertilized with the combined treatment (Fig. 1). Particularly, after four days of storage, the mature-green peppers fertilized with the mineral system showed greater firmness (19.80 N) than mature-green peppers fertilized with the combined system (12.09 N), while intermediate results were observed for full-colored peppers. At eight days of storage, full-colored and mature-green peppers treated with mineral fertilization showed greater firmness than peppers fertilized with the combined treatment. For yellow peppers, no significant differences in firmness were observed.

In conclusion, the results of this experiment show that the type of fertilization and the maturity stage had a different impact on quality of yellow and red peppers. In particular, for yellow full-colored peppers, the combined fertilization treatment allowed a longer shelf-life than the mineral treatment, and this should be considered when processing fresh-cut peppers. These results may also encourage further study of the feasibility of using 'environmentally friendly' fertilization techniques on bell peppers and eventually to extend these trials to other species.

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Growth, yield and fruit quality of strawberry under protected cultivation in South Kashmir

A. Kumar, I. Ahad
Krishi Vigyan Kendra, Extension Training Centre, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, Malangpora (Pulwama) Jammu & Kashmir, India.

Key words: fruit quality, growth, protected cultivation, strawberry, yield.

Abstract: Field experiments were conducted at Krishi Vigyan Kendra, Pulwama, Jammu and Kashmir at an altitude of 1601 m amsl to identify the suitable strawberry cultivars for higher production of good quality fruits. Eight strawberry cultivars were evaluated for two consecutive years (2008-09 and 2009-10) under polyhouse conditions. Maximum plant spread (27.43 cm) along with maximum number of runners (8.54) was produced by 'Chandler'. 'Tioga' produced first flower in 97 days after planting, while runners of 'Chandler' (56.79) flowered for maximum number of days. 'Chandler' produced maximum number of flowers per plant (27.23) and set maximum berries (86.01%), however final yield of berries was more in 'Tioga' which recorded maximum yield per plot (2.26 kg), closely followed by 'Chandler' (2.19 kg). Berry weight (12.24 g) and berry size (5.10 cm length and 4.73 cm) was maximum in 'Tioga'. 'Catskill' registered maximum for all the biochemical characters, closely followed by 'Tioga'. Overall, 'Chandler', 'Catskill' and 'Tioga' performed well under polyhouse conditions in Kashmir Valley.

1. Introduction

Strawberry (*Fragaria x annanasa* Dutch) has been grown commercially in various parts of the world for many years but in India it was only introduced in the early 1960's (Sharma and Sharma, 2004) and it has now acclimatized well in different parts of India. This is essentially a temperate fruit crop hence its expansion in the Kashmir valley has been gaining popularity in the last decade. It is not only consumed as fresh fruit but is also used in processed foods such as jam, ice cream, biscuits and so on. The demand for strawberry fruits for domestic as well as export markets has been increasing steadily. The standard planting time in the valley ranges from late October to the first fortnight of November, and harvesting from 15 March to late April in controlled conditions and from the second fortnight of April to late May in open conditions. Climatic conditions under various production methods during the growing season may affect fruit quality. Soluble solids concentration, acidity and colour of strawberry fruit have all been reported to be affected by environmental factors (Sacks and Shaw, 1994; Vlachonasios *et al.*, 1995), as well as harvest date (Shaw, 1988). Temperature is an important factor for floral initiation under short day conditions. The optimum temperature for short day floral initiation is 15-18°C, while below 10°C and above 25°C short day induction is rather ineffective (Manakasem and Goodwin, 2001; Sonsteby and Heide, 2006; Verheul *et al.*, 2007). Although both diurnal and nocturnal temperatures are important, strawberry requires an optimum daytime temperature of 22°C and nighttime temperature of 13°C for maximum growth and yield (Shoemaker, 1977). Growing strawberries under polyhouse decreases the dependence of fruit quality on climate and soil conditions. Such cultivation system also enables better water, light and temperature control to a certain extent. In Kashmir valley, cultivation is generally carried out under open field conditions and takes advantage of the local climatic conditions, but due to the long duration of winters the availability of fruit is very late. There is no information regarding the protected cultivation and management practices on the performance of commercialized cultivars of strawberry in the Kashmir valley. Hence, the present study was conducted to study the feasibility of growing strawberry under polyhouse to obtain an early crop and to evaluate the cultivars which may be suitable for commercial exploitation.

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2. Materials and Methods

Experimental site and material

The experiment was laid out under polyhouse during 2008-09 and 2009-10 at Krishi Vigyan Kendra, Pulwama, Jammu and Kashmir. The KVK is located at 33° North and 74° East at an altitude of 1601 m amsl. The mean annual rainfall ranges from 500 to 850 mm. The minimum and maximum temperatures of the station during summers range between 10 and 30°C and between -4 and 10°C dur-

ing winter under open conditions. A polyhouse with steel pipe framework clad with twin layer UV stabilized 200 µm plastic sheet of was used to create a modified environment. The polyhouse was additionally fitted with a high pressure fan on each west side. Under the polyhouse the temperature was maintained up to 25°C. The soil of the location is silty clay, loam neutral in reaction (pH 7.07) having organic C 10.02 g/kg, available N 248.6 kg/ha, available P 14.7 kg/ha and available K 250.3 kg/ha. The experimental materials were comprised of eight commercial cultivars ('Catskill', 'Chandler', 'Confutura', 'Gorella', 'Pajaro', 'Selva', 'Tioga' and 'Fern') collected from SKUAST-K and the Department of Horticulture, Ramban, Jammu and Kashmir. The experiment was laid out in a completely randomized block design (CRBD) with three replications. The spacing between the runners was 30 x 30 cm on 1 x 1 m^2 raised beds of 15 cm height with 55 cm spacing between the beds. Uniform runners were planted in the first week of November 2008 in three rows on each bed accommodating nine runners. For the second year crop, the emerged runners were removed in the last week of October 2009 in order to maintain the proper spacing for the next year's crop. Usual irrigations, manures and fertilizers, weeding and hoeing were applied equally to the experimental plots during the study years.

Observations recorded

Data were recorded for different growth, flowering and fruiting characters for three years. Plant spread (cm) and length of the runners (cm) was measured with the help of a measuring tape. Number of runners per plant, number of flowers per plant and number of berries per plant were counted from five randomly selected plants. Days to first flower was recorded from the date of planting of runners to initiation of first flower. Flower duration was counted by subtracting the date of initiation of first flower from the date of last flowering. Percentage of berry set was calculated by dividing the number of berries by the number of flowers. Yield per plant (g) was calculated by weighing whole fruits from a single plant. Ten fruits were randomly selected for all the physio-chemical characters. Berry weight was determined with the help of a weighing scale; berry length and width were determined using a Vernier Calliper. TSS, acidity and TSS/acid ratio were estimated using standard procedures. Total sugar and reducing sugar were determined by Shaffer Somogy, micro method (Ranganna, 1991). Data on temperature and humidity under polyhouse were recorded with a portable thermohygrometer.

Data analysis

The pooled data of two years were statistically analyzed following Panse and Sukhatme (1985). The mean of attributes was compared by paired 't' test and the least significant difference was calculated at 5% level.

3. Results and Discussion

The average monthly data on minimum and maximum temperature and relative humidity inside the polyhouse from transplanting to harvesting are presented in figure 1.

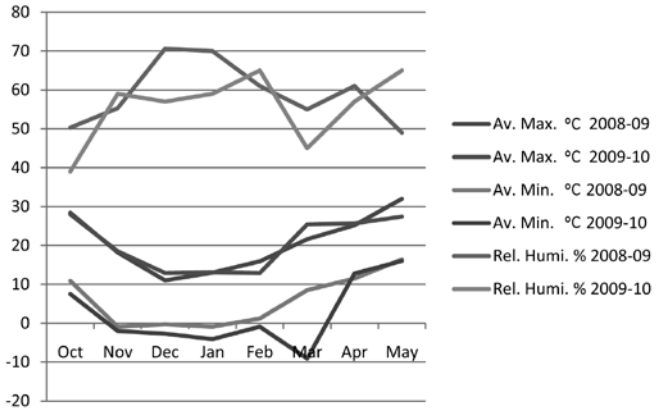

Fig. 1 - Average maximum, minimum temperature (°C) and relative humidity (%) month wise from transplanting to harvesting time.

The pooled data of two consecutive years shown in Table 1 reveal that 'Chandler' had maximum plant spread (27.43 cm) which was statistically at par with 'Catskill' (25.49

Table 1 - Growth and flowering characters of strawberry cultivars under polyhouse

Cultivar	Plant spread (cm)	No. of runners/ plant	Runners length (cm)	Days taken first flower to produce	Duration of flowering	Number of flower/ plant
Catskill	25.49 ef	7.09 ef	81.94 f	106 def	50.86 ab	26.92 e
Chandler	27.43 f	8.54 h	82.59 fg	101 bc	56.79 d	27.23 f
Confutura	24.18 cde	6.11 d	89.78 h	99 ab	53.92 bcd	20.93 ab
Gorella	25.44 def	5.67 bc	75.40 de	103 cd	51.12 abc	21.90 bc
Pajaro	21.75 ab	4.89 a	67.58 b	105 de	49.39 a	20.48 a
Selva	23.08 bcd	5.44 b	73.95 cd	116 h	47.82 a	21.84 bc
Tioga	20.19 a	7.35 fg	70.42 bc	97 a	55.49 cd	25.64 d
Fern	22.35 abc	6.93 e	60.92 a	110 g	48.91 a	21.45 bc
Mean	23.74	6.50	75.32	104.6	51.79	23.31
$CD_{0.05}$	2.40	0.29	3.70	3.84	4.43	0.99

cm) and 'Gorella' (25.44 cm), while minimum plant spread was recorded for 'Tioga' (20.19 cm). Maximum number of runners per plant in pooled data was found in 'Chandler' (8.54) which differed significantly from all other cultivars, whereas minimum number of runners per plant was recorded in 'Pajaro' (4.89). The pooled data of two years shows that 'Confutura' (89.78 cm) produced maximum runner length which differed significantly from the other cultivars; minimum length of runners was observed in 'Selva' (67.58 cm). Two years of data relative to runner length shows that in the first year runner length was greater than in the second year: the material may have degenerated with the passage of time (Childers, 1975). 'Tioga' (97) produced flowers earlier among the considered cultivars and was closely followed by 'Confutura' (99) and 'Chandler' (101); 'Selva' ranked last and took 116 days to produce first flower (Table 1). Runners of 'Chandler' (56.79) flowered for the maximum number of days followed by 'Tioga' (55.49) and 'Confutura' (53.92) which was statistically at par with both the cultivars, whereas 'Selva' (47.82) produced flowers for the fewest number of days. Kaska *et al*. (1997) cultivate nine cultivars of strawberry under high tunnels in Adana (Turkey) observed that 'Selva' took maximum days to produce first flower and flowered for least number of days while 'Chandler' flowered for maximum number of days.

Maximum number of flowers per plant (27.73) (Table 1) and maximum number of berries per plant (23.42) (Table 2) were both produced by 'Chandler' which was statistically at par with 'Catskill' (26.92) with respect to the number of flowers per plant, while for the number of berries per plant the former was significantly higher than all the cultivars. This indicates that the number of flowers per plant certainly has a bearing on the number of fruits per plant to be harvested, i.e. greater the number of flowers/plant, greater the number of fruits to be harvested but the total yield per plant may vary due to berry weight. 'Pajaro' (20.48) produced the fewest flowers per plant, which was statistically at par with 'Fern' (21.45), however the lowest number of berries per plant was produced by the latter (14.49) which was statistically at par with 'Pajaro' (14.74). Paraskevopoulou-Paroussi *et al*. (1990) also recorded a minimum number of flowers per plant and number of berries per plant in 'Pajaro' and 'Fern' while growing these cultivars under greenhouse in northern Greece.

The data in Table 2 reveal that 'Chandler' (86.01%) gave the maximum berry set, which was statistically at par with 'Tioga' (83.48 %); the minimum berry set (71.84%) was recorded for 'Pajaro'. Maximum yield per plot of berries was recorded in 'Tioga' (2.26 kg) which was statistically at par with 'Chandler' (2.19 kg) and 'Catskill' (2.17 kg), and minimum yield per plot was recorded in 'Selva' (1.08 kg). Paraskevopoulou-Paroussi *et al*. (1990) also noted that 'Pajaro' (24%) and 'Fern' (38%) produced marketable yield under greenhouse conditions in northern Greece, however, Kaska *et al.* (1997) reported that 'Chandler' and 'Selva' produced the highest and lowest yield, respectively under high tunnel conditions in Adana (Turkey). Observations from two years of data on yield per plant shows that yield was greater in the second year than the first which might be due to substantial annual variation in fruit set in strawberry (Smolarz *et al*., 1968; Misic *et al*., 1976). 'Tioga' scored as having maximum berry weight (12.24 g) along with maximum berry length (5.10 cm) and berry breadth (4.73 cm), closely followed by 'Chandler'. 'Selva' had minimum berry weight (8.71 g), however minimum size [i.e. berry length (3.70 cm) and berry breadth (3.62 cm)] was recorded for 'Pajaro'. Pathak *et al.* (2006) observed similar results with respect to weight, length and breadth of berries while growing strawberry cultivars under cover.

Maximum TSS was scored by 'Catskill' (9.85%), followed by 'Tioga' (9.32%), however both these cultivars significantly differed from each other (Table 3); 'Selva' had minimum TSS (6.72%). Minimum acidity was observed in 'Catskill' (0.88%), closely followed by 'Tioga' (0.89%), yet the pooled data of two years showed non significant results. 'Catskill' (10.51) showed maximum TSS/acid ratio which was statistically at par with Tioga (10.47) while minimum TSS/acid ratio was recorded for 'Selva' (6.25). Maximum reducing sugar (6.27 %) and total sugar (8.09%) were observed in 'Catskill' which was the highest

Table 2 - Yield and fruiting characters of strawberry cultivars under polyhouse

Cultivar	Number of berries/ plant	Berry set (%)	Yield/plot (kg)	Berry weight (g)	Berry length (cm)	Berry breadth (cm)
Catskill	22.32 e	82.90 e	2.17 d	11.11 e	4.98 de	4.55 de
Chandler	23.42 f	86.01 f	2.19 d	12.11 f	4.79 d	4.31 c
Confutura	16.93 c	80.86 d	1.44 d	10.54 d	4.78 d	4.48 cd
Gorella	16.62 c	75.81 c	1.28 c	9.76 c	4.25 bc	3.76 a
Pajaro	14.74 a	71.84 b	1.18 b	9.03 b	3.70 a	3.62 a
Selva	15.94 b	72.73 b	1.08 a	8.71 a	4.39 c	3.72 a
Tioga	21.32 d	83.48 ef	2.26 d	12.24 f	5.10 e	4.73 e
Fern	14.49 a	66.95 a	1.13 ab	9.22 b	4.03 b	4.01 b
Mean	18.22	77.57	1.59	10.34	4.50	4.14
$CD_{0.05}$	0.64	2.73	0.09	0.27	0.22	0.23

Table 3 - Biochemical characters of strawberry cultivars under polyhouse

Cultivar	TSS (%)	Acidity (%)	TSS/acid ratio	Reducing sugar (%)	Total sugar (%)
Catskill	9.85 h	0.88	10.51 g	6.27 f	8.09 e
Chandler	9.06 ef	0.94	9.64 d	5.45 d	7.18 d
Confutura	9.24 fg	0.97	10.11 e	5.85 e	7.14 d
Gorella	8.03 d	1.02	7.85 c	4.63 c	6.65 c
Pajaro	7.81 b	1.00	7.78 c	3.65 a	5.55 a
Selva	6.72 a	1.04	6.25 a	4.04 b	5.86 b
Tioga	9.32 g	0.89	10.47 fg	5.88 e	7.19 d
Fern	7.83 cd	1.02	7.64 bc	3.76 a	5.70 a
Mean	8.48	0.97	8.78	4.94	6.67
$CD_{0.05}$	0.20	NS	0.35	0.19	0.22

among all the studied cultivars, and 'Pajaro' scored minimum reducing sugar (3.71%) and total sugar (5.55%). Our investigation showed much variation in the various cultivars for all the characters and this could be attributed to the genetic make up of the cultivars (Dhaliwal and Singh, 1983; Chandel and Badiyala, 1996). Factors which may significantly influence strawberry composition include mineral and organic fertilization but weather conditions and variety are also important.

It is concluded from the present study that 'Chandler' for growth and yield characteristics, 'Catskill' for biochemical characters and 'Tioga' for yield and physical characters of strawberry fruits are profitable for cultivation under polyhouse conditions in the Kashmir valley.

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‘Verdello’, ‘Verdicchio’ and ‘Verduschia’: an example of integrated multidisciplinary study to clarify grapevine cultivar identity

M. Crespan*, A. Armanni, G. Da Rold*, B. De Nardi*, M. Gardiman*, D. Migliaro*, S. Soligo***, P. Storchi****

* *Consiglio per la ricerca e la sperimentazione in agricoltura, Centro di ricerca per la viticoltura, CRA-VIT, Viale XXVIII Aprile, 26, 31015 Conegliano (TV), Italy.*

** *Consiglio per la ricerca e la sperimentazione in agricoltura, Unità di ricerca per la viticoltura, CRA-VIC, Via Romea, 53, 52100 Arezzo, Italy.*

*** *Centro Regionale per la Viticoltura, l’Enologia e la Grappa, CeRVEG, Veneto Agricoltura, Via Zamboni, 31015 Conegliano (TV), Italy.*

Key words: ampelography, ‘Duropersico’, SSR markers, ‘Trebbiano di Soave’, ‘Turbiana’, ‘Uva Angiola’.

Abstract: ‘Verdello’, ‘Verdicchio’ and ‘Verduschia’ are registered in the official Italian Catalogue as three distinct grapevine varieties. Twenty-five accessions of these cultivars, encompassing known or presumed synonyms, coming from CRA repositories and from vineyards where they are traditionally cultivated, have been genotyped with eleven SSR markers. For morphological comparison, one accession for each variety has been described with 57 characters of OIV 2009 list; phenological and yield traits have also been recorded. In addition, the phenotypic comparison has been extended to the literature descriptions. The same DNA profile has been obtained for all 25 accessions; moreover, present and historical ampelographic data showed a very high similarity. All this information leads to the conclusion that these three varieties are, in fact, the same cultivar.

1. Introduction

The spread of grapevine varieties through different countries over time has produced many of cases of synonymy that now need clarification. Correct identification is very important in order to gain more precise knowledge of the varietal assortment and to better manage grapevine catalogues and germplasm repositories.

The Italian Catalogue of Grapevine Varieties is one of the most copious in the world. Nonetheless, it contains various duplicates or triplicates, such as ‘Alicante’/‘Cannonao’/‘Tocai rosso’, ‘Vermentino’/‘Pigato’/‘Favorita’, ‘Biancame’/‘Trebbiano toscano’, ‘Albarola’/‘Bianchetta genovese’.

Also ‘Verdicchio’, one of the most prized white grape varieties of the Marche region (central Italy) and registered in the Italian Catalogue with code no. 254, has an officially recognized duplicate, ‘Trebbiano di Soave’ (code no. 239), the name used in several provinces of the Veneto region (north-eastern Italy). Other synonyms in the same region are ‘Trebbiano di Lugana’ and ‘Turbiana’. In the Lazio region (central Italy) it is known as ‘Trebbiano verde’ (Bruni, 1962). To our knowledge, the first citation of ‘Verdicchio’ was by the botanist Costanzo Felici as far back as 1569 (in Arbizzoni, 1986).

‘Verdello’ is considered a minor grapevine variety of the Umbria region (central Italy). It is registered in the Italian Catalogue with code no. 253 and is used for the DOC wines of Orvieto, Colli Amerini, Colli del Trasimeno and Torgiano. Scalabrelli and Grasselli (1988) report that ‘Verdello’ is also cultivated in the south of Tuscany under the synonym ‘Duropersico’, mainly in the territory of Pitigliano DOC, where it has been present for at least two centuries and contributes to the wine of the same name. The two authors refer that in the same territory a variety known as ‘Uva Angiola’ is also present; they generically link it to the Trebbiano group, but this variety more specifically resembles to those analyzed in the present study.

Furthermore, in the most northern part of Tuscany, a variety morphologically similar to ‘Verdicchio’ has been grown for a long time. It is called ‘Verduschia’ (Soderini, 1600; Acerbi, 1825), ‘Verdella’ or ‘Verdarella’, depending on the cultivation area. The diffusion of this variety is limited to the small Lunigiana territory (provinces of La Spezia and Massa Carrara) and to a few residual specimens in old vineyards (Scalabrelli and Dodi, 1998). ‘Verduschia’ falls into the category of minor grapevine varieties on the

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verge of extinction; although registered in the Italian Catalogue since 1971 with code no. 297, it does not seem to have been propagated.

DNA genotyping allowed us to hypothesise an unrecognized synonymy among 'Verdicchio', 'Verdello' and 'Verduschia'. In fact, based on previous results obtained with molecular analysis of some accessions of 'Verdello', interesting for the clonal selection of materials from Lazio, we found that they had the same profile of the 'Verdicchio' accession held in the Centro di ricerca per la viticoltura (CRA-VIT) repository. Given the interest aroused by this preliminary information, we extended the molecular comparison to numerous accessions of 'Verdicchio', 'Verdello' and their synonyms, as well as to 'Verduschia', the Tuscan variety morphologically similar to 'Verdicchio'. The samples for genotypic comparison were singled out both in the CRA-VIT repositories and in the cultivation areas of each variety, for a total of 25 accessions, including a commercial clone of 'Verdello' (clone VCR1) and another of 'Verdicchio bianco' (clone R2). This study was integrated with morphological comparison: one accession for each variety was characterized with 57 descriptors of the OIV 2009 list; phenological and yield traits were also recorded. In addition, ampelographic comparison was extended to the literature descriptions of these three varieties: most of the traits reported in literature were retrieved and harmonized according to the OIV 2009 descriptors list used for examining the actual materials.

Table 1 - List of the analysed accessions

ID	Accession name	Provenance
1	Verdicchio	CRA-VIT repository
2	Verdello	CRA-VIT repository
3	Verdello clone VCR1	Vivai Cooperativi of Rauscedo (Pordenone) - Italy
4	Verdello covio 6	Orvieto (Terni) - Italy
5	Verdello sugano	Orvieto (Terni) - Italy
6	Verdello fausto 1	Porano (Terni) - Italy
7	Verdello 553	Ercolani farm, Capodimonte (Viterbo) - Italy
8	Verdello 550	Ercolani farm, Capodimonte (Viterbo) - Italy
9	Verdello 549	Ercolani farm, Capodimonte (Viterbo) - Italy
10	Verdello 514	Catercia farm, Capodimonte (Viterbo) - Italy
11	Verdello 405	Bianchi farm, Bagnoreggio (Viterbo) - Italy
12	Verdello 146 ar	Pitigliano (Grosseto) - Italy
13	Verdello	Lazio
14	Verdello	CRA-VIT repository
15	Verduschia	Aulla (Massa Carrara) - Italy
16	Verdella	Aulla (Massa Carrara) - Italy
17	Verdicchio carr 10	Terranuova Bracciolini (Arezzo) - Italy
18	Turbiana	Vicenza - Italy
19	Trebbiano verde	Viterbo - Italy
20	Trebbiano di Soave	CRA-VIT repository
21	Trebbiano di Soave	CRA-VIT repository
22	Trebbiano di Lugana	Veneto
23	Duropersico 165 ar	Pitigliano (Grosseto) - Italy
24	Uva angiola	CRA-VIT repository
25	Verdicchio clone R2	Marche

2. Materials and Methods

Plant material

Twenty-five accessions were sampled, coming from CRA-VIT repositories in Spresiano (TV) and Susegana (TV), from Unità di ricerca per la viticoltura (CRA-VIC) repositories in Arezzo, from the Veneto Agricoltura collection and from vineyards in Veneto, Tuscany, Umbria, Marche and Lazio regions (Table 1).

Ampelographic, phenological and yield data comparison

Three accessions were described: one 'Verdello' of Umbrian origin (accession no. 4 in Table 1) and one 'Verduschia' (no. 15 in Table 1) coming from Lunigiana (province of Massa Carrara), both held in the CRA-VIC germplasm collection in Arezzo, and one 'Verdicchio' coming from Marche (no. 25 in Table 1), held at the Azienda Poggio Gagliardo in Montescudaio (Pisa, Italy). Morphological descriptions of the three varieties were performed according to 57 descriptors (OIV, 2009); phyllometric analyses were also carried out on samples of 20 leaves per cultivar using SuperAmpelo software (Soldavini *et al*., 2009).

The comparison was widened to the 'Verdello', 'Verdicchio' and 'Verduschia' descriptions given in the literature: in particular, the Umbrian 'Verdello' was described by Cartechini and Moretti (1989), the 'Verdicchio' from Marche by Bruni (1962) and the Tuscan 'Verduschia' by Breviglieri and Casini (1965). To facilitate the comparison, all these descriptions were standardized according to the OIV 2009 descriptor list.

DNA extraction

Total genomic DNA was extracted from young leaves of the 25 accessions using NucleoSpin® 8 Plant kit (MACHEREY-NAGEL GmbH, Düren, Germany) automated on the Microlab® STAR liquid handling robot according to the MACHEREY-NAGEL NucleoSpin® 8 Plant protocol. DNA concentration and quality were assessed with a spectrophotometer and by 1% agarose gel electrophoresis. DNA samples were then diluted to 10 ng/µl prior to amplification.

SSR analysis

SSR analysis was performed in order to verify the varietal identity of the studied accessions. Eleven microsatellite loci were analyzed: the six core loci selected within Genres 081 European Project (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79) (This *et al*., 2004); VVMD28 (Bowers *et al*., 1999); ISV2 (VMC6e1), ISV3 (VMC6f1) and ISV4 (VMC6g1) (Crespan, 2003); VMCNG4b9 (Welter *et al*., 2007).

A multiplex PCR mixture was prepared, amplifying simultaneously all 11 SSR loci. The reaction mixture consisted of: 1 X PCR buffer (Promega; Pharmacia Biotech), 200 µM of each dNTPs, 1 U of Taq DNA polymerase (Promega; Pharmacia Biotech), 2.0 mM $MgCl_2$ and the primer concentrations ranged between 0.09 µM and 0.40 µM, according to signal intensity; the forward primers were labelled with 6-FAM, VIC, PET or NED fluorescent

dyes; the final volume was 12.5 µl. PCR was carried out in GeneAMP 9700 (Applied Biosystems) with the following thermal profile: 2 min at 94°C, followed by 30 cycles at 94°C for 45 sec, 55°C for 1 min and 30 sec, 72°C for 1 min and a final step at 72°C for 30 min.

PCR products (1 µl) were added to 0.1 µl LIZ 500 size standard and 8.9 µl Hi-Di formamide (Applied Biosystems) and separated by capillary electrophoresis using an ABI Prism 3110xl DNA analyzer (Applied Biosystems) and POP-7 polymer (Applied Biosystems). After data collection, genotyping analysis was performed with ABI Prism® GeneMapperTM software version 3.0.

3. Results

The results of the ampelographic comparison between 'Verdello', 'Verdicchio' and 'Verduschia' are reported in Table 2. The descriptions from the present study show that the three grapevine varieties share 47 out of 57 traits. Ten diverge (in bold in Table 2): in particular, 'Verdello' differs from 'Verdicchio' and 'Verduschia' by having a slight bronze tinge on the young leaf and the mature leaf with less pronounced lower lobes and shorter petiole compared to the length of the middle vein. 'Verdicchio' showed a larger average size of the mature leaf, a longer middle vein N1 and a greater density of prostrate hairs on the lower side of the blade (descriptor no. 84), as well as longer internodes (no. 353). Finally, three morphological traits differ in 'Verduschia' with respect to 'Verdello' and 'Verdicchio': the dorsal side of the shoot is green (OIV codes 007 and 009) and berry size is smaller. Therefore, comparison of our descriptions shows a substantial analogy among these varieties, which share 82% of the expression levels, with very minor discrepancies. Their similarity is also confirmed by the elaboration of the mature leaf measurements with SuperAmpelo software: the cluster analysis performed by the program shows that the degree of similarity is even higher and above 93% (Fig. 1).

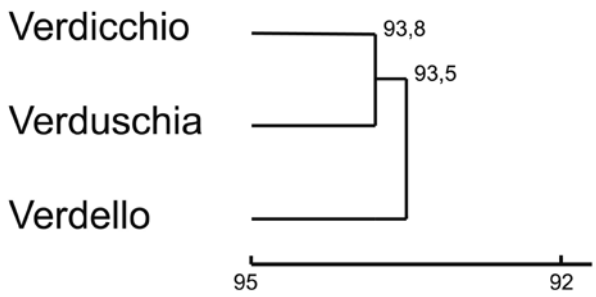

Fig. 1 - Cluster analysis of 'Verdello', 'Verdicchio' and 'Verduschia' phyllometric data elaborated with SuperAmpelo software. Percentage of similarity values are indicated.

Table 2 - List of ampelographic traits of the varieties 'Verdello', 'Verdicchio' and 'Verduschia'. There are two types of comparisons: i) among current descriptions: discordant characters observed in the present study are written in bold; ii) among descriptions from other authors: slightly discordant traits inferred from the literature are underlined; clearly discordant characters are underlined in italics. An asterisk indicates the characters defined by the software SuperAmpelo. nr: not recorded character.

Organ	OIV Code 2009	Description	Verdello		Verdicchio		Verduschia	
			present study	Cartechini and Moretti, 1989	present study	Bruni, 1962	present study	Breviglieri and Casini, 1965
shoot	002	young shoot: distribution of anthocyanin coloration on prostrate hairs of the shoot tip	absent	absent	absent	absent	absent	piping
	003	young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip	none or very low	none or very low	none or very low	none or very low	none or very low	low
	004	young shoot: density of prostrate hairs on the shoot tip	medium	very high	medium	very high	medium	medium or very high
	006	attitude	semi-erect	semi-erect	semi-erect	semi-erect	semi-erect	semi-erect
	007	colour of the dorsal side of internodes	**green and red**	green and red	**green and red**	green and red	**green**	green
	008	colour of the ventral side of internodes	green and red	green and red	green and red	green and red	green and red	green and red
	009	colour of the dorsal side of nodes	**green and red**	green and red	**green and red**	green and red	**green**	green
	010	colour of the ventral side of nodes	green and red or red	green	green and red or red	green and red	green and red	green and red
	013	density of prostrate hairs on nodes	low	none or very low	low	none or very low	low	low
	015_2	intensity of anthocyanin coloration on the bud scales	weak	weak	weak	nr	weak	nr
	016	number of consecutive tendrils	2 or less	2 or less	2 or less	2 or less	2 or less	2 or less

Organ	OIV Code 2009	Description	Verdello		Verdicchio		Verduschia	
			present study	Cartechini and Moretti, 1989	present study	Bruni, 1962	present study	Breviglieri and Casini, 1965
young leaf	051	colour of upper side of blade (4th leaf)	**green / bronze / yellow**	green	**green / yellow**	green / bronze	**green / yellow**	green
	053	density of prostrate hairs between main veins on lower side of blade (4th leaf)	high	very high	high	very high	high	very high
	055	density of prostrate hairs on main veins on lower side of blade (4th leaf)	low	low	low	nr	low	nr
mature leaf	065	size of blade	**medium-small***	medium	**medium***	medium	**medium-small***	medium-small
	067	shape of blade	wedge-shaped or pentagonal*	circular	wedge-shaped or pentagonal*	circular or pentagonal	wedge-shaped or pentagonal*	pentagonal
	068	number of lobes	**five or three**	five	**five**	five or three	**five**	five or three
	069	colour of the upper side of the blade	between medium green and dark green	medium green	between medium green and dark green	between medium green and dark green	between medium green and dark green	dark green
	070	area of anthocyanin coloration of main veins on upper side of blade	absent	absent	absent	absent	absent	absent
	071	area of anthocyanin coloration of main veins on lower side of blade	absent	absent	absent	absent	absent	absent
	073	undulation of blade between main or lateral veins	present	present	present	present	present	present
	074	profile of blade in cross section	involute, V-shaped or twisted	*V-shaped*	involute, V-shaped or twisted	*flat or twisted*	involute, V-shaped or twisted	*flat or twisted*
	075	blistering of upper side of blade	medium	weak	medium	medium	medium	nr
	076	shape of teeth	mixture between straight and convex sides	*one side concave, one side convex*	mixture between straight and convex sides	*mixture between straight and convex sides*	mixture between straight and convex sides	*both sides convex*
	078	length of teeth compared with their width	short	short	short	short	short	short
	079	degree of opening/overlapping petiole sinus	overlapped	strongly overlapped	overlapped	closed or overlapped	overlapped	overlapped
	084	density of prostrate hairs between main veins on lower side of blade	**medium**	*medium*	**high**	*very high*	**medium**	*very high*
	086	density of prostrate hairs on main veins on lower side of blade	low	*low*	low	*high*	low	nr
	090	density of prostrate hairs on petiole	low	none or very low	low	none or very low	low	none or very low
	093	length of petiole compared to length of middle vein (N1)	**shorter than N1**	longer than N1	**equal to N1**	nr	**equal to N1**	nr
	601	length of vein N1	**short***	nr	**medium***	nr	**short***	nr
	602	length of vein N2	medium*	nr	medium*	nr	medium*	nr
	603	length of vein N3	medium*	nr	medium*	nr	medium*	nr
	604	length of vein N4	very long*	nr	very long*	nr	very long*	nr
woody shoot	101	cross section	circular or elliptic	circular	circular or elliptic	circular or elliptic	circular or elliptic	circular or elliptic
	103	main colour	brownish	brownish	brownish	between brownish and grey	brownish	brownish
inflorescence	151	flower: sexual organs	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite
	152	insertion of 1st inflorescence	3rd and 4th node	3rd and 4th node	3rd and 4th node	3rd and 4th node	3rd and 4th node	3rd and 4th node
	153	number of inflorescences per shoot	1.1 to 2	1.1 to 2	1.1 to 2	1.1 to 2	1.1 to 2	1.1 to 2

Organ	OIV Code 2009	Description	Verdello		Verdicchio		Verduschia	
			present study	Cartechini and Moretti, 1989	present study	Bruni, 1962	present study	Breviglieri and Casini, 1965
bunch	202	length	medium-long	medium	medium-long	medium-long	medium-long	long
	204	density	dense	*very dense*	dense	*dense or medium*	dense	*medium*
	206	length of peduncle	short	*very short*	short	*medium*	short	*short*
	208	shape	conical / funnel shaped / cylindrical	conical	conical / funnel shaped / cylindrical	conical or cylindrical-conical	conical / funnel shaped / cylindrical	conical
	209	number of wings of the primary bunch	1-2/3-4	with wings	1-2/3-4 wings	with wings	1-2/3-4	with wings
berry	220	length	**medium**	medium	**medium**	medium	**between medium and short**	short
	222	uniformity of size	uniform	uniform	uniform	uniform	uniform	uniform
	223	shape	globose	globose	globose	globose	globose	globose
	225	colour of skin	yellow	yellow	yellow	yellow	yellow	yellow
	227	bloom	medium	medium	medium	medium	medium	medium
	228	thickness of skin	thin	*thick*	thin	*thin*	thin	*thick*
	229	hilum	visible	visible	visible	visible	visible	visible
	241	formation of seeds	complete	complete	complete	complete	complete	complete
vegetation	306	autumn coloration of leaves	yellow	yellow	yellow	yellow	yellow	yellow
	351	vigour of shoot growth	medium-strong	medium	medium-strong	medium-strong	medium-strong	medium-strong
	352	growth of lateral shoots	weak	weak	weak	medium	weak	weak
	353	length of internodes	**medium**	long	**long**	medium-long	**medium**	medium
	354	diameter of internodes	medium	small	medium	medium	medium	medium

With regard to the descriptions from the literature, most of the 57 OIV descriptors we used were retrieved. Only the measurements from code 601 to 604 relative to the mature leaf, three traits of 'Verdicchio' and five of 'Verduschia' were excluded (indicated as not recorded characters in Table 2) and so a total of 50 descriptors for 'Verdello' and 'Verdicchio' and 48 for 'Verduschia' were taken into consideration. As expected, the literature data comparison highlighted greater differences, given that the three descriptions were made by different authors. Twenty-eight descriptors, underlined in Table 2, showed slightly discordant expression levels, as they are limited to a single interval or with different coexisting or intermediate expression levels. These are mainly characters whose expression may be influenced by environmental factors like the exposure to the light or by cropping factors and vegetative vigour, in addition to the subjectivity of the observer. Other descriptors (7 out of 52), underlined in italics in Table 2, showed markedly different expression levels. Four of them regard the mature leaf and in particular the density of prostrate hairs on the lower side of the blade (no. 84 and 86) and the profile and shape of the teeth (no. 74 and 76); the other three regard the bunch and the berry (no. 204, bunch density; no. 206, peduncle length; no. 228, thickness of skin). Comparing this last group of seven morphological features with the set of results from our field measurements, it appears that our descriptions match better with those of 'Verdicchio' described by Bruni (1962); instead, fewer similarities are noted with the descriptions in the literature for 'Verduschia' and even fewer for 'Verdello'.

Lastly, the comparison of the phenological and yield data collected in the two environments [Arezzo and Montescudaio (Pisa)] are reported in Table 3. The data obtained from the same references for ampelographic descriptions are also shown for comparison and 'Trebbiano toscano' is proposed as common reference variety to enhance data significance. 'Verduschia' differs by the longer vegetative cycle and the lower berry weight. Instead, there are no relevant differences between the other two varieties.

Molecular analysis of the 25 accessions listed in Table 1 produced the same DNA profile, reported in Table 4 together with 'Sangiovese', 'Pinot noir' and 'Muscat blanc à petits grains' profiles, proposed as reference varieties for easier data comparison. To our knowledge this is the first time that the synonymy with 'Trebbiano verde' from Lazio, 'Duropersico', 'Verduschia' or 'Verdella' and 'Uva Angiola' from Tuscany and 'Turbiana' from Veneto, is confirmed by molecular data.

Table 3 - Phenology and yield data for 'Verdello', 'Verdicchio' and 'Verduschia': average of 2007-2010 for the vineyard in Arezzo, average of 2004-2008 for the vineyard in Montescudaio (Pisa). The data from some reference ampelographic descriptions are also shown for comparison. 'Trebbiano toscano' is proposed as common reference variety to enhance data significance

	'Verdello'		'Verdicchio'		'Verduschia'		'Trebbiano toscano'	
Place of data collection	Arezzo[1]	Orvieto (Terni)[2]	Montescudaio (Pisa)[3]	Iesi (Ancona)[4]	Arezzo[1]	Firenze[5]	Arezzo[1]	Montescudaio (Pisa)[3]
Bud burst	13 April (medium)	medium	7 April (medium)	medium-late	12 April (medium)	10 - 20 April (medium)	16 April (medium)	7 April (medium)
Flowering	3 June (medium - early)	medium	2 June (medium -early)	early	7 June (medium)	1 - 10 June (medium)	8 June (medium)	3 June (medium)
Veraison	11 August (medium)	medium	5 August (medium)	medium	13 August (medium late)	21 - 31 August (late)	17 August (medium late)	6 August (medium)
Ripening	25 September (medium)	late	20 September (medium)	medium-late	27 September (late)	1 - 10 October (late)	4 October (late)	28 September (late)
Average weight of the bunch (g ± SD)	320±95	269	455±43	280	352±66	245	357±51	402±60
Average weight of the berry (g ± SD)	1.87±0.18	1.80	1.94	2.05	1.60±0.64	0.80	1.71±0.21	1.82±0.19
° Brix (± SD)	22.0±1.2	22.3	21.4±0.6	20.0	20.6±0.7	21.0	18.9±1.2	21.1±0.8
Titratable acidity (g/l ± SD)	6.71±1.36	8.90	6.80±0.40	8.25	6.42± 0.75	6.97	7.32±0.48	5.29±0.62

[1] CRA-VIC repository, Lon: 11°49'29'' E, Lat: 43°28'30'' N.
[2] Data from Cartechini and Moretti (1989). Lon: 12° 12' E, Lat: 42° 42' N.
[3] Poggio Gagliardo Farm, Lon: 10°32'53'' E, Lat: 43°18'52'' N.
[4] Data from Bruni (1962), Lon: 13° 11' E, Lat: 43° 38' N.
[5] Data from Breviglieri and Casini (1965), Lon: 11°19'21'' E, Lat: 43°45'11'' N.

Table 4 - SSR profiles of 'Verdicchio'/'Verdello'/'Verduschia' and three cultivars proposed as reference to favour comparison with other databases

SSR loci	Verdicchio, Verdello and Verduschia	Sangiovese	Pinot noir	Muscat blanc à petits grains
VVS2	133	133	137	133
	155	133	151	133
VVMD5	228	226	228	228
	240	236	238	236
VVMD7	239	239	239	233
	247	263	243	249
VVMD27	179	179	185	179
	185	185	189	194
VVMD28	239	237	221	249
	261	247	239	271
VrZAG62	195	193	187	185
	195	195	193	195
VrZAG79	248	242	238	250
	256	258	244	254
ISV2 (VMC6e1)	165	143	151	141
	165	165	165	143
ISV3 (VMC6f1)	135	139	133	133
	139	139	145	139
ISV4 (VMC6g1)	169	177	169	169
	197	197	177	187
VMCNG4b9	164	158	158	158
	166	168	162	166

4. Discussion and Conclusions

The large number of accessions analyzed, most of which come from traditional cultivation areas, together with the results of the ampelographic comparisons, allow us to affirm that 'Verdicchio', 'Verdello' and 'Verduschia' represent a new group of synonyms. This discovery is important because the three varieties are registered as distinct in the Italian Catalogue. This is further supported by the results of the molecular analysis on the commercial clone of 'Verdello' (clone VCR1), which showed to be identical to the other 'Verdello' accessions analyzed in this study.

It emerges from present work that the extent of the cultivation area in Italy and the interest in 'Verdicchio'/'Verdello'/'Verduschia' are greater than previously supposed from the information available on the already known synonyms. According to the Italian census of Agriculture Data (ISTAT, 2004) the total area under 'Verdicchio', 'Verdello', 'Verduschia' and 'Trebbiano di Soave' cultivation in Italy is about 6000 ha, mainly in Marche (53%) and Veneto (30%) and, to a much lesser extent, in Umbria (8.3%) and Lazio (1.6%). Cultivation is also authorized in Emilia Romagna, Tuscany, Abruzzo, Molise and even in Sardinia. The success with which this variety has been cultivated for centuries in central and north-eastern Italy is obvious; the broad and historic diversification of the denominations, even in very close areas, supports the old age of the variety and also the interest in the locally produced wines, with recognised quality.

The synonymy 'Verdicchio'/'Verdello'/'Verduschia' is added to a growing number of redundancies found in the Italian Catalogue and highlights the value of molecular analysis to facilitate rapid comparison among varieties. This tool has been very useful in revealing other, never previously suspected cases of synonymy, such as 'Greco di Tufo' and 'Asprinio' (Costantini *et al*., 2005), 'Malvasia delle Lipari' and 'Malvasia di Sardegna' (Crespan *et al*., 2006), or to definitively clarify long disputed synonymies, such as 'Malvasia nera di Brindisi' and 'Malvasia nera di Lecce' (Crespan *et al*., 2008; Gasparro *et al*., 2008).

It is extremely difficult to hypothesize the centre of diffusion of 'Verdicchio'/'Verdello'/'Verduschia', also because the pedigree of this variety is unknown. One not scientifically supported hypothesis infers that this cultivar arrived in Marche from northern Italy in the second half of the 15th century, with colonies of farmers from Veneto and Lombardy, and from there it moved to Lazio, to finally turn up in Tuscany (Pollini, 2006).

The discovery of this new group of synonyms has been greatly facilitated by the use of SSR markers, which suggest comparisons among varieties independently from preliminary ampelographic indications. Given the wealth of varieties registered in the Italian Catalogue, molecular analysis, associated with the building of a reliable and complete molecular database of reference, has been shown to be very useful to highlight redundancies. Ampelographic comparison is indispensable to confirm the preliminary data acquired via molecular fingerprinting. Indeed, the definition of a cultivated variety is tied to the DUS criteria (Distinctness, Uniformity and Stability), since the somatic mutants for agronomically important characters are legally registered as separate cultivars, as are mutants for berry colour or earliness of ripening. Lastly, genotyping represents a strategic tool in order to avoid the recording of duplicates in the future.

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Production of strawberries (*Fragaria x ananassa* Duch.) in mountain areas: a comparative evaluation of berries from two June-bearing cultivars

E. Giordani, W.A. Petrucci, D. Morelli, A. Ferri
Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Università degli Studi di Firenze, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.

Key words: elevation cultivation, 'Elsanta', garden strawberry, 'Marmolada®Onebor'.

Abstract: Two uniferous strawberry cultivars, 'Marmolada®Onebor' and 'Elsanta', were tested in three experimental fields located at 1,200 m asl; phenological phases and fruit quality of first and second season crops were compared. Harvesting time in the first year crop occurred 40 days later than in the second season crop. Cultivar significantly affected fruit skin brightness and chroma index, flesh firmness, titratable acidity and yield in the first season crop; the experimental field location exerted a significant effect also on fruit weight and diameter, but not on total acidity. Fruit weight was higher in the second season crop, and 'Marmolada®Onebor' fruits (22.7 g) resulted heavier than those of 'Elsanta' (19.7 g). Total solid soluble content ranged from 7.4 to 8°Brix in 'Elsanta', compared to 6.2 to 7.4°Brix in 'Marmolada®Onebor'. Neither diseases nor arthropod attacks were noticed on plants and fruits.

1. Introduction

Open-field elevation cultivation of strawberries (*Fragaria x ananassa* Duch.) is being developed for fruit production in tropical regions (López *et al*., 2002; Pirlak *et al*., 2002; Riyaphan *et al*., 2005; Pádua *et al*., 2009) and also in temperate areas (Faedi, 2010; Gambardella, 2010; Rowley *et al*., 2010). Nevertheless, little information is available on the quality of strawberries obtained from plants grown in mountain areas. A strong variability of horticultural characteristics and antioxidant profiles were observed among 12 cultivars grown at 730 m asl in the Trentino Region (Italian Alps), the most relevant attributes limiting quality being represented by poor taste and low flesh firmness (Giongo *et al*., 2006). In the same area, a study by Faedi *et al*. (2009) reported that 'Elsanta' showed the best qualitative results in the first season crop among the tested cultivars.

Two commercial, ordinary June-bearing strawberry cultivars, 'Elsanta' and 'Marmolada®Onebor', were selected for an elevation cultivation trial in the area of Abetone Mountain (Apennine) in Italy.

The present paper reports the results observed on the quality of berries of these cultivars grown in open field mountain area. Two cultivation strategies are also comparatively discussed - one based on "first season crop" (FSC), carried out in 2008, and the subsequent year (2009) "second season crop" (SSC) - as well as some aspects related to the ecological environment.

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2. Materials and Methods

Location and environmental characteristics

Three experimental fields (EF1, EF2 and EF3) were established in three different locations around the Abetone pass (Apennine Mountains) in Tuscany (Italy); details of the sites are reported in Table 1. The three experimental fields had good exposure to light; all of them laid on Humic Umbrisols soils (LAMMA, 2012) with similar chemico-physical characteristics. The yearly average rainfall for the area is 2,000 mm; snow is present from late November until late spring (May). Historical average of air temperature and humidity data and rainfall for the period 2008-2009 are reported in figure 1. From an ecological point of view, the selected sites for the experiment are represented by scarcely anthropized natural grasslands, highly biodiverse in terms of flora being constituted mainly by *Festuca pucinellii*, *Trifolium thalii*, *Plantago alpina*, *Poa alpina*, *Brachypodium genuense* and *Nardus stricta,* and surrounded by forests of European beech (*Fagus silvatica*), European silver fir (*Abies alba* Mill.) and elder (*Sambucus racemosa* L.) often consociated with blueberry (*Vaccinium myrtillus* L.) (Dondini and Vergari, 2009).

Table 1 - Description of experimental fields (EF): altitude, coordinates, soil texture, N concentration (‰), C concentration (%), pH, field capacity (F.C.) and wilting point (W.P.)

Experimental fields	Altitude (m a.s.l.)	Coordinates (N/W)	Soil texture	N‰	C%	pH	F.C. (-0,33 bar)	W.P. (-15 bar)
EF 1	1,213	N 44°08.650' W 10°41.412'	Sandy-loam	0.3	3.5	5.2	20.1	11.6
EF 2	1,250	N 44°08.517' W 10°42.043'	Loam	0.1	3.6	6.2	18.4	11.1
EF 3	1,250	N 44°07.810' W 10°45.336'	Sandy-loam	0.1	3.7	5.9	23.6	16.5

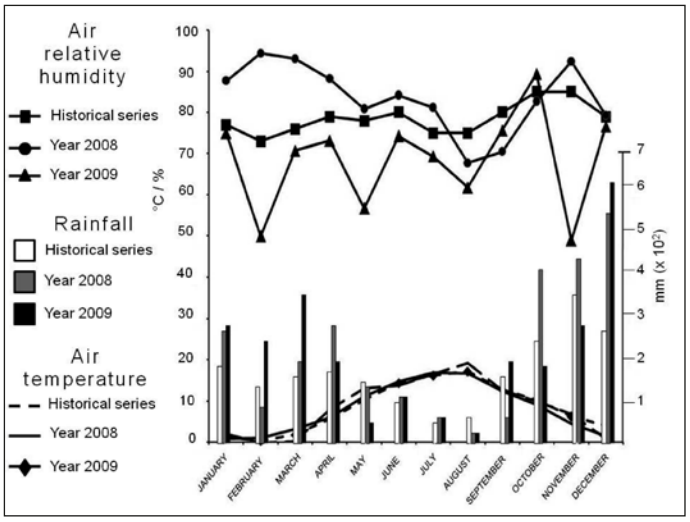

Fig. 1 - Mean air temperature (°C), relative humidity (%) and rainfall (mm) of the trial location of years 2008, 2009 and historical series (data elaborated from LAMMA).

Soil management and plant spacing

In May 2008 the soil area destined for the three experimental fields (500 m^2 each) was ploughed 30 cm deep and mature manure was distributed uniformly at the rate of 80 t/ha; soil was not fumigated. The strawberries were planted in parallel hill rows 110 cm apart with a distance of 30 cm between plants within the row, with a density of 4 plants/m^2; the plants of each cultivar were distributed in an alternate four-row scheme for a total of eight rows for each experimental field. A black polyethylene film was used along the rows for mulching. Each plot was surrounded by guard rows.

Plant material, planting and management

One-crown homogeneous-size certified refrigerated plants of 'Elsanta' and 'Marmolada®Onebor' were supplied by a qualified commercial nursery. In early May 2008 strawberries were planted in the experimental fields, from which the FSC was obtained; the same open field unprotected, overwintered and not de-crowned strawberry plants generated the SSC in 2009. Plants were drip irrigated with 1,000 l/d average per experimental field, from flowering time up to the end of the productive season; no treatment was adopted for pest and disease control. Weeds were manually removed.

Data collection

During the two years of study (2008 and 2009), observations regarded phenological, morphological and chemical characteristics. Flowering and ripening time were recorded on one sample every five plants and reported as days from the beginning of the year. Morphological data were measured on four replicated sets of 25 ripened fruits each for every experimental unit (cultivar/location) during the period of maximum productive peak. Fruits were harvested and promptly characterized for weight and maximum diameter, skin colour, flesh firmness (two sides), fruit shape and presence of internal cavity. Weight (g) was measured with a precision balance (Sartorius TE 150/2s - SARTORIUS) and diameter (mm) with hand calliper; colour (L, a, b coordinates) was determined with a Minolta Chromameter CR200 - KONICA MINOLTA electronic colorimeter and the a and b coordinates were transformed in the chroma index $(a^2 + b^2)^{1/2}$; flesh firmness (g) was measured with a 6 mm diameter plunger hand penetrometer TR 53200. Fruit shape and presence of an internal cavity were visually assessed following the UPOV descriptor for strawberry (UPOV, 2008). Chemical parameters such as pH, titratable acidity and total solid soluble (TSS) content were assessed on samples (four replicates per experimental unit) of juice extracted from 10 fruits. Titratable acidity (meq malic acid/100 g fresh weight) was determined at pH 8 with a 0.1 N solution of NaOH, adopting a Basic 20 - CRISON pH meter; total soluble solid content (°Brix) was quantified with a hand refractometer (Atago N1 - ATAGO CO., LTD). Fruits were tasted by five experts to assess sensorial quality and flavour quality was rated on a five-score scale (1 - very weak; 2 - medium weak; 3 - medium; 4 - good; 5 - very good), while for persistence of taste 3 min after ingestion and scent intensity, a three-score scale (1 - weak; 2 - medium; 3 - strong) was adopted.

Experimental design and statistical analysis

Each of the three experimental fields was split into 16 rows (eight alternate rows per cultivar); every row held 125 plants. A two-way analysis of variance was employed to test the significance of the effects due to cultivar ('El-

santa' and 'Marmolada®Onebor') and experimental field location (EFL1, EFL2 and EFL3), both considered as independent factors, and their interaction. Duncan's test was applied for mean separation; the averages were reported with standard errors. Two-tail Chi^2 test was applied to qualitative and scale-scored parameters exposed as frequencies. SPSS Statistics 17.0 software was used for analyses; differences at $P < 0.05$ and $P < 0.01$ were considered significant and very significant, respectively.

3. Results

The ANOVA statistical significance of the different factors on the studied parameters are reported in Table 2; each of them will be discussed in the following sections.

First season crop (FSC)

Both Elsanta and Marmolada®Onebor cultivars showed the same pattern of phenological stages in the three locations; in detail the full blooming started on 8 July 2008 (day 190) and portrayed until day 203 for a total of 13 days. The first fruits of both cultivars ripened 24 days later (day 214), and harvest time closed on day 234; the period of maximum peak of ripened strawberries fell in the period 4-11 August 2008 (days 217-224) (Fig. 2).

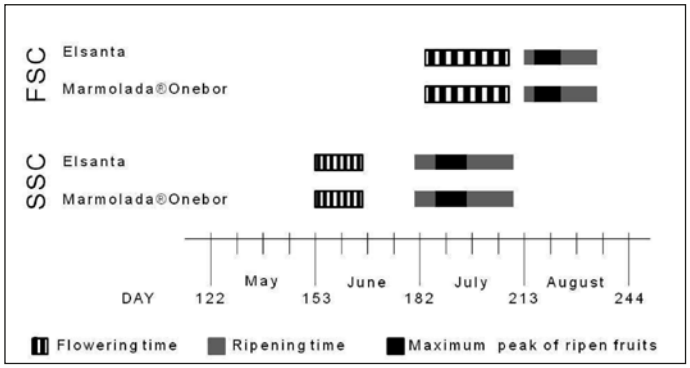

Fig. 2 - Blooming and fruit ripening time in years 2008 (FSC) and 2009 (SSC).

Average fruit weight resulted identical for both cultivars (15.0±0.4 g)(ANOVA; P = 0.69) (Table 2). Field location and its interaction with cultivar exerted a statistically significant effect on this parameter (ANOVA; $P < 0.01$), with the highest and the lowest weights of 19.1±0.7 g and 11.8±0.7 for 'Marmolada®Onebor' obtained in EF1 and EF3, respectively; for 'Elsanta' the maximum and minimum values were 15.9±0.7 g in EF1 and 13.8±0.7 g in EF3 (Fig. 3A). As expected, the fruit maximum diameter (average 32.9±6.1 mm in 'Elsanta' and 32.04±6.2 mm in 'Marmolada®Onebor') followed a behaviour similar to that observed for fruit weight, and it was affected significantly by the experimental field and its interaction with cultivar (Fig. 3A).

Table 2 - Significance of cultivar, experimental field location and their interactions on the quantitative parameters analyzed on strawberry fruits resulted from analysis of variance (ANOVA)

Attribute	Crop	Cultivar CV	Experimental field - EF	CV*EF	Replicates
Fruit weight	FSC	NS	**	**	NS
	SSC	**	**	**	NS
Fruit diameter	FSC	NS	**	*	NS
	SSC	NS	**	NS	NS
Fruit brightness (L)	FSC	**	**	*	NS
	SSC	NS	**	**	NS
Chroma index	FSC	**	**	**	NS
	SSC	NS	**	**	NS
Flesh firmness	FSC	**	*	**	NS
	SSC	**	**	**	NS
Fruit pH	FSC	NS	NS	NS	NS
	SSC	NS	NS	NS	NS
Titratable acidity	FSC	*	NS	NS	NS
	SSC	**	**	NS	NS
TSS content	FSC	NS	NS	NS	NS
	SSC	*	NS	NS	NS

NS= non significant; * = 0.05<p<0.01; **= p<0.01.

Skin colour was analysed taking into account brightness (L) and chroma index. L values ranged from 30.9 to 47.6 for 'Elsanta' and 24.7 to 47.8 for 'Marmolada®Onebor', with averages of 38±0.3 and 35.2±0.3, respectively (ANOVA; P <0.01). Fruit skin brightness showed differences of 2 units (≈ 35 against ≈ 37) due to the effect of the experimental field location (ANOVA; $P < 0.01$). Analogous results were observed for chroma index, with average values of 40.3±0.4 and 37.1±0.4 for 'Elsanta' and 'Marmolada®Onebor' (ANOVA; $P < 0.01$); similarly to skin brightness, EF exerted a very significant influence on chroma index, with a variation of ≈ 2 units.

Flesh firmness resulted statistically different (ANOVA; P<0.01) between the cultivars; the highest average value was observed in 'Marmolada®Onebor' (473.1±9 g), which is about 33% higher than the firmness of 'Elsanta' fruits (355.6±7.9 g). The experimental field location exerted a significant effect on fruit firmness (P<0.05); a 5% difference between the maximum and the minimum average values was observed.

No relevant differences were found in pH of juice, showing a value of 3.6 for both cultivars, and no effect was attributable to the location of the experimental fields. Different results were obtained for titratable acidity, which was higher in 'Elsanta' fruits (12.6±0.8 meq/100 g FW) than in 'Marmolada®Onebor' (9.6±0.4 meq/100 g FW) (ANOVA; P<0.05); location did not affect this parameter. Taking into account the total solid soluble content of strawberry juice, the cultivar Elsanta showed an average value of 7.4±0.3 °Brix, against 6.2±0.3 °Brix of

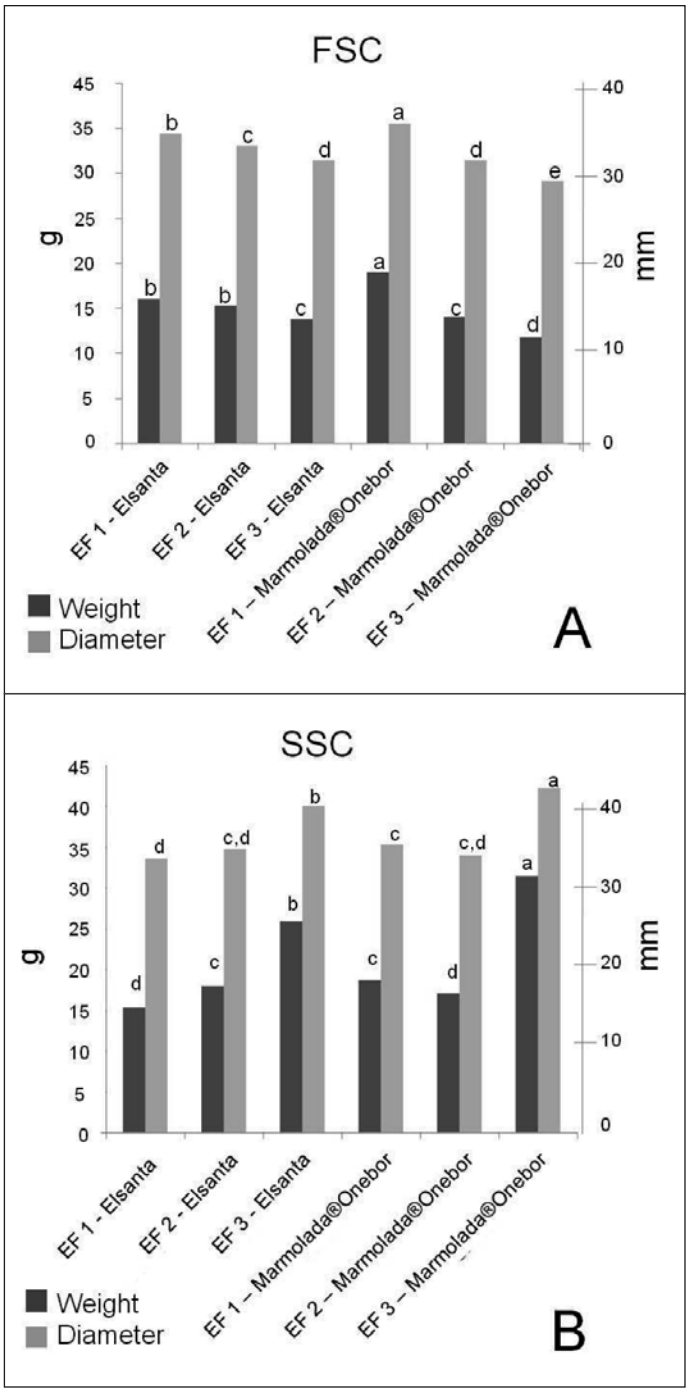

Fig. 3 - Fruit weight and diameter for 'Elsanta' and 'Marmolada®Onebor' in the first (A) and second (B) season crop observed in each experimental field (EF). Bars with different letters are statistically different (Duncan's test; P<0.05).

	Strawberry shape	LONG CONIC	CONICAL	GLOBOSE - CONIC	GLOBOSE	OBLATE
FSC	Elsanta	4	48	40	6	2
FSC	Marmolada® Onebor	6	55	27	7	5
FSC	Significance	NS	NS	*	NS	NS
SSC	Elsanta	1	26	68	4	1
SSC	Marmolada® Onebor	1	58	36	3	2
SSC	Significance	NS	*	*	NS	NS

Fig. 4 - Fruit shape distribution (%) of 'Elsanta' and 'Marmolda® Onebor' for years 2008 (FSC) and 2009 (SSC). CHI^2 statistical differences within columns: NS= not significant; * significant (P<0.05).

'Marmolada®Onebor'; such differences were not statistically significant (ANOVA; P>0.05).

The distribution of fruit shape resulted similar among cultivars; no statistical differences were observed between cultivars for conical and globose-conic classes, the most frequent fruit shapes. Significant differences (CHI^2; P< 0.01) were found in the percentages (40% and 27%) of globose-conic fruits of 'Elsanta' and 'Marmolada®Onebor', respectively (Fig. 4).

The internal cavity was present in approximately 81% of fruits of both cultivars; a significant statistical difference (CHI^2; P<0.01) was observed taking into account the experimental field (EF factor), since almost 100% of fruits from EF2 showed internal cavity, against about 75% of those obtained in the other two sites.

The distribution of fruits for each cultivar taking into account the quality of taste, resulted as an average value of five taste assessors, is reported in figure 5A. 'Elsanta' showed a percentage (86%) of fruits of acceptable quality (namely the sum of the "medium", "good" and "very good" classes) which was higher than the one observed in 'Marmolada®Onebor' (63%); such difference resulted non significant (CHI^2; P>0.05). On the other hand, no relevant effect was exerted by the experimental field location. The fruits of 'Elsanta' showed a higher percentage of fruits with medium (69%) and strong (21%) persistence of taste 3 min after ingestion, against 61% and 3% observed on 'Marmolada®Onebor' fruits. The effect of the location of the experimental field was not statistically significant for this parameter, but the best results were obtained in EF2. A similar situation was observed for scent intensity, which resulted superior in 'Elsanta' fruits compared to those of 'Marmolada®Onebor', with a higher percentage of strongly scented fruits (26%) against (8%)(CHI^2; P <0.01)(Fig. 6A); the location producing more fruits with strong scent was EF2.

Second season crop (SSC)

Full blooming of 'Elsanta' and 'Marmolada®Onebor' was observed in the three plots on 29 May (day 150) and it finished 14 days later (day 164). The first fruits ripened at day 177 (26 June 2009) and the last ripened on day 209 (28 July 2009), with the maximum peak of ripened fruits between days 186 and 192 (Fig. 2).

The highest fruit weight was observed for 'Marmolada®Onebor' (average 22.4±0.5 g) while a lower mean value was found in 'Elsanta' fruits (19.7±0.5 g)

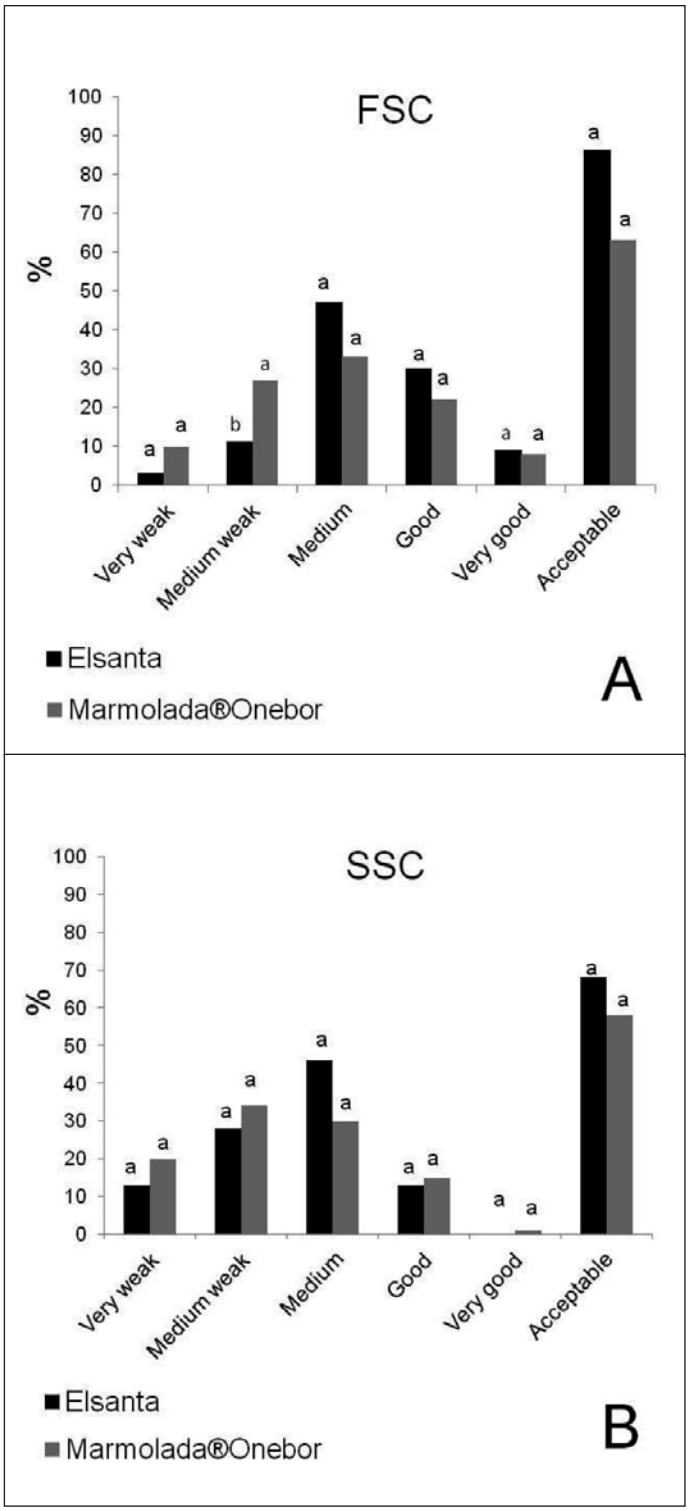

Fig. 5 - Fruit taste: distribution (%) of the first (A) and second (B) season crop strawberries for different quality classes of 'Elsanta' and 'Marmolada®Onebor'. Acceptable fruit is the sum of medium, good and very good classes. Bars with different letters are statistically different (CHI^2; $P<0.05$).

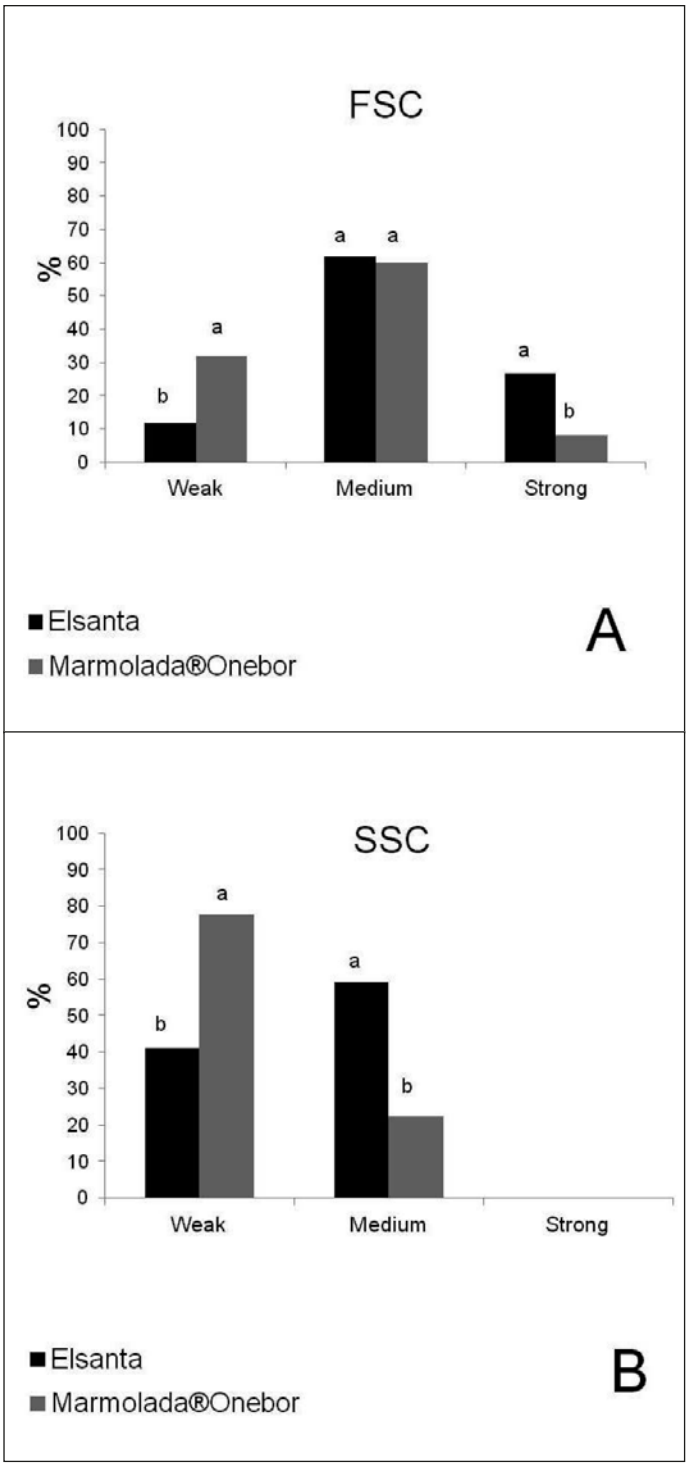

Fig. 6 - Fruit scent intensity: distribution (%) of strawberries of Elsanta and Marmolada®Onebor cultivars for the first season crop (A) and the second season crop (B). Bars with different letters are statistically different (CHI^2; $P<0.05$).

(ANOVA; $P< 0.01$). Also the experimental field location affected fruit weight very significantly, with the highest mean values observed in EF3 (28.7±0.7 g); a reduction of about 40% of this parameter was observed in EF1 and EF2. The effect of the interaction cultivar by location on fruit weight was also very significant (ANOVA; $P<0.01$) with the highest mean values found in fruits of 'Marmolada®Onebor' grown in EF3 (31.5±0.9 g) and the lowest for those of 'Elsanta' grown in EF1 (15.3±1.0 g).

Fruit diameter resulted practically identical between the cultivars (37.2±0.4 mm and 36.1±0.4 mm for 'Marmolada®Onebor' and 'Elsanta', respectively); in ac-

cordance with fruit weight, the greatest diameters were observed in EF3 for fruits of 'Marmolada®Onebor' (Fig. 3B).

Skin brightness (L coordinate) was similar between the cultivars, ranging from 29.3 to 47.8 for 'Elsanta' and 29.2 to 47.1 for 'Marmolada®Onebor', with averages of 37.7±0.3 and 37.3±0.3 respectively. Only field location exerted a strong effect on this parameter (ANOVA; P<0.01); skin brightness reached the highest value (39.7±0.4) in EF2, followed by EF3 (36.8±0.4) and EF1 (35.9±0.3). Similarly, chroma index of skin colour was influenced by the location of experimental field (ANOVA; P<0.01) with the highest values in fruits from EF2, decreasing then in those obtained in EF3 and EF1. The chroma index average value for fruits of 'Elsanta' was 34.3±0.5 while for 'Marmolada®Onebor' it was 33.8±0.5. The interaction of cultivar and location affected very significantly these two colour parameters (Table 2).

Taking into account fruit flesh firmness, 'Elsanta' showed the highest average (490±0.5 g) compared to that of 'Marmolada®Onebor' fruits (460.2±4.7 g)(P<0.01). The experimental field location and its interaction with cultivar exerted a very significant effect on fruit firmness (P<0.01), with differences of around 13% between the maximum and the minimum average value found in EF3 and EF1, respectively.

The juice of 'Elsanta' and 'Marmolada®Onebor' fruits showed the same pH value (3.6), while titratable acidity was affected by the cultivar and location of cultivation. 'Elsanta' showed a higher titratable acidity (average 12.8±0.6 meq/100 g FW) than that observed in 'Marmolada®Onebor' fruits (10.7±0.5 meq/100 g FW) (ANOVA; P< 0.01). Analogously, the location of cultivation exerted a very significant effect on this parameter; the highest mean value (13.5 meq/100 g FW) was found in berries obtained in EF2, against 10.9 meq/100 g FW of those harvested in EF1.

The cultivar Elsanta showed an average value of 8.1±0.7 °Brix for total solid soluble content of juice, against 7.4±0.8 °Brix of 'Marmolada®Onebor' (ANOVA; P <0.05). No significant differences were observed due to the location of the experimental field; in any case, the highest value was observed in 'Elsanta' cultivated in EF2 (8.6±0.3 °Brix); 'Marmolada®Onebor' showed the maximum value of total solid soluble content in EF1 (8±0.3°Brix).

The most frequent fruit shapes were conical and globose-conic (Fig. 4). The differences between the cultivars resulted statistically very significant (P<0.01) for both shapes; 'Elsanta' presented 68% and 26% of fruits belonging to the globose-conic and conical shapes, while in 'Marmolada®Onebor' the frequencies were of 36% and 58%, respectively (Fig. 4).

The difference between cultivars for the presence of internal cavity was non significant, with values of 35% and 45% of cases in 'Elsanta' and 'Marmolada®Onebor' respectively. The location of cultivation exerted a significant effect (Chi^2; P<0.05) on this characteristic; the lower percentage of fruits showing internal cavity was observed in EF1.

The distribution of cases for fruit taste is illustrated for both cultivars in figure 5B. Taking into account the percentage of acceptable fruits in terms of quality of taste, 59% and 46% for 'Elsanta' and 'Marmolada®Onebor' respectively, the cultivar effect resulted statistically not significant. On the contrary, the effect of the experimental field location on fruit taste was very significant: EF1 showed 72% of fruits with acceptable taste, while these values were of 44% and 18% for EF2 and EF3, respectively (CHI^2; P<0.01). The persistence of taste after ingestion resulted identical for both cultivars and the location did not exert a relevant effect on this parameter. None of the cultivars produced fruits with strong scent; 'Elsanta' showed higher percentages of fruits belonging to the medium class (59%) against 22% of 'Marmolada®Onebor' (CHI^2; P<0.01) (Fig. 6B). Taking into account the location of the experimental field, EF3 showed the worst result, with the highest percentage of fruits with weak scent (80.7%), against ≈ 55% observed in the other two fields.

4. Discussion and Conclusions

No differences were observed for flowering and fruit ripening time between cultivars and experimental fields. In 2008, the interval between planting date and flowering time corresponded to 24 days for both cultivars, which is almost half the time indicated by Perez de Camacaro *et al*. (2004) for 'Elsanta' plants grown in the UK; conversely the interval flowering-ripening time (36 days) coincided with that found by Perez de Camacaro *et al*. (2004). In the second season crop, this interval was eight days shorter for the strawberries grown in Tuscany (28 days), and the same (36 days) as in the trial reported by Perez de Camacaro *et al*. (2004). The FSC ripening time for 'Elsanta' and 'Marmolada®Onebor' cultivars showed a delay of 40 days in relation to the second year crop. This finding confirms the possibility of scheduling the strawberry harvesting time offered by elevation cultivation, as indicated by Faedi *et al*. (2009).

The average weight of FSC fruits was about 15 g for both cultivars, but experimental field and its interaction with the cultivar exerted a strong effect on this parameter. The heaviest fruits (average 19.1 g) were obtained from the plants of 'Marmolada®Onebor' grown in EF1; they weighed about 7 g more than the fruits collected in EF3. A lower magnitude difference was observed in 'Elsanta' fruits. In the second season, crop fruit weight was on average 25% higher than the value for 2008; furthermore the cultivar factor was more relevant, since 'Marmolada®Onebor' fruits (average 22.7 g) were about 3 g heavier than those of 'Elsanta' (19.7 g). A similar situation was observed by Coman *et al*. (2002) for the same cultivars grown on the north-eastern coast of the USA (19 and 14 g/fruit for 'Marmolada®Onebor' and 'Elsanta', respectively). The values obtained for fruit weight of 'Elsan-

ta' in the second season crop are similar to those indicated by Fitogest (2011) and much higher than those observed by Giongo *et al*. (2006) in similar growing conditions and by Palha *et al*. (2009) in soilless culture. On the other hand, the fruit weight of 'Marmolada®Onebor' fruits was very close to the value indicated by Fitogest (2011). Slightly lower average weights (17.2 g) were found in a set of cultivars grown in Brazil at 1,370 m asl by Pádua *et al*. (2009), while a higher value (26.3 g/fruit) was observed for 'Marmolada®Onebor' in Cesena (Italy) on one-year-old planted strawberries (Tagliavini *et al*., 2005). The observations confirm that average fruit weight is lower in the first season crop than in the second, which is in accordance with the results of previous studies on 'Elsanta' and 'Bolero' (Perez de Camacaro *et al*., 2004). Also the experimental field influenced fruit weight with heavier fruits obtained in EF3. The opposite influence of EF3 on fruit weight in the second year crop can be attributed, more than to the intrinsic characteristics of the location (e.g. soil chemico-physical properties), to a predominant effect of the cultivar due to cumulate effects associated to the changes in vegetative growth of the second year. In strawberry, the predominant sink is represented by fruits (Olsen *et al*., 1985); fruiting affects dry-matter partitioning, hence the changes in vegetative development may significantly interfere with fruit production as suggested by Perez de Camacaro *et al*. (2004). FSC strawberries were underweight by a few grams when taking into account the optimal market values indicated by Lovati (2010). Conversely this parameter was acceptable for the second year crop. Fruit diameter resulted highly correlated with fruit weight (R^2= 0.87; P<0.01), nevertheless it was less influenced than fruit weight by cultivar effect. Again, the observed values of 'Elsanta' fruit diameter are in accordance to those indicated by Giongo *et al*. (2006).

Taking into account fruit colour, brightness resulted close or superior to the acceptable threshold of 37, as indicated by Lovati (2010) for both cultivars and for the two crops; on the other hand, chroma index was lower than the limit value (40). Colour parameters were less affected by cultivar in the second season crop; conversely experimental field was found to be a more relevant factor. During 2008 the fruits obtained in F1 showed the highest values of brightness and chroma index, while in 2009 the most attractive fruits in terms of brightness and chroma index were grown in EF2. Brightness values in both years were almost the same: chroma index was 4 points higher in 2008 than in 2009.

Flesh firmness of fruits was significantly affected by the studied factors and by their interactions. The optimal range of flesh firmness for harvesting and handling strawberries is indicated as 300-400 g (Lovati, 2010), about 20% lower than that observed for first and second season crops for both cultivars under study. The average firmness found for 'Elsanta', regardless of year and experimental field, was 423 g, approximately 25% higher than that found by Giongo *et al*. (2006) in plants grown at 730 m asl, demonstrating a good texture of the fruits at ripening time, and hence better suitability to handling and transport.

Fruit juice pH was not affected by any studied factor, resulting substantially stable with a value of 3.6 in both FSC and SSC. Titratable acidity was significantly influenced by the cultivar in both FSC and SSC, with average values of about 12.7 and 10.2 meq/100 g FW for 'Elsanta' and 'Marmolada®Onebor' respectively. Such values are within the range of 10-15 meq/100 g FW indicated as optimal for strawberries by Roudellac and Trajkovsky (2003) and Lovati (2010). Giongo *et al*. (2006), observed a higher value (13.6 meq/100 g FW) in 'Elsanta' fruits obtained in similar growing conditions; in a trial conducted in Slovenia, 'Elsanta' showed a total acid content higher than the one found for 'Marmolada®Onebor' (Sturm *et al*., 2003), which is in accordance with the results of this research. Titratable acidity was lower (7 meq/100 g FW) in the juice of 'Marmolada®Onebor' fruits collected in Cesena (Italy) from one-year-old plants (Tagliavini *et al*., 2005); location seems to have a strong effect on this parameter, as confirmed in our experiment for the second season crop where location exerted a significant influence with a maximum variation of about 30% of the amount of titratable acidity.

Taking into account the total solid soluble content of strawberry juice, the cultivar 'Elsanta' showed an average value of 7.4 and 8°Brix, against 6.2 and 7.4°Brix of 'Marmolada®Onebor', in 2008 and 2009 respectively. These results, even if higher in value, show a similar trend to those found for 'Elsanta' (about 5.8°Brix) and 'Marmolada®Onebor' (about 5.2°Brix) grown under identical conditions in Slovenia by Sturm *et al*. (2003). Similar results were obtained by Radajewska and Dejwor-Borowiak (2002) on both cultivars grown in Poland. Nevertheless, different values of total solid soluble content of 'Elsanta' fruits have been found by different authors and for plants growing in diverse areas and cultivation systems. Kovačević *et al*. (2008) noted a value of 7.2°Brix in fruits grown under organic and conventional cultivation. Palha *et al*. (2009) found values ranging from around 7.7 up to 10 °Brix on strawberries in soil less cultivation, depending on planting date, tray and bare-rooted plant type, and similar values were observed by Voća *et al*. (2007) in Croatia for the same cultivar. Giongo *et al*. (2006) recorded an average value of 8.4°Brix in fruits obtained from cultivation in buckets at 730 m asl. Lower mean values of solid soluble content (7.2°Brix) were observed by Pádua *et al*. (2009) on a different set of cultivars grown in Brazil at 1,370 m asl. The values observed for 'Marmolada®Onebor' were higher than those shown by the same cultivar grown in flat areas (4.7°Brix) (Tagliavini *et al*., 2005), thus confirming the positive influence of environmental conditions of mountain areas on fruit quality. No statistical differences were observed taking into account the experimental fields and their interaction with the cultivar, nevertheless it is worth noting that 'Elsanta' fruits obtained in EF1 reached 8.1°Brix, which is in accordance with the values found by Giongo *et al*. (2006). However the results obtained in the present study are higher than the threshold of 7°Brix,

adopted as a standard quality parameter for strawberries (Roudeillac and Trajkosky, 2003). The results here obtained for 'Marmolada®Onebor' are higher than those reported by Kovačević *et al*. (2008) and Plantgest (2012) of 5.7-6.0 and 5.7 °Brix respectively.

In this study total solid soluble content was associated with taste quality, even if this sensorial parameter is influenced also by many other factors such as acidity, flavours, flesh texture, etc. 'Elsanta' fruits resulted better in taste quality than those of 'Marmolada®Onebor' for the two years of observation (Fig. 5) and in taste persistence in the FSC. Similarly 'Elsanta' fruits showed a higher percentage of fruits in the superior class of scent intensity (Fig. 6). These results are in accordance with those found by Coman *et al*. (2002) in fruits of 'Marmolada®Onebor' and 'Elsanta' grown on the north-eastern coast of the USA and with those of Radajewska and Dejwor-Borowiak (2002) in a trial conducted in Poland. Fruits of the FSC resulted better than those obtained as SSC for all the analysed sensorial attributes (taste, taste persistence and scent intensity); this result may be associated to the about 15% higher total soluble content and slightly lower titratable acidity observed in the juice of strawberries derived from the FSC, with respect to those of the SSC.

Most of consumers prefer conical, or slightly round-conical strawberries (Tirelli, 2010). The predominant fruit shapes found in the present study were conical and globose-conic; a cultivar effect was noted, but location did not seem to exert any influence on this parameter. The most frequent class of fruit shape in the FSC was conical while conversely globose-conic was predominant for the second season crop. The variation from one class to the other was more evident in 'Elsanta' fruits (Fig. 4).

The internal cavity is a morphological attribute that does not have a relevant effect on quality but may modify the shape and volume of fruits. This parameter was not cultivar-dependent but location had a significant influence on the percentage of fruits with an evident internal cavity. Furthermore, the growing system (FSC or SSC) seemed to exert a similar effect, since during the FSC around 80% of fruits showed this attribute compared to 40% in the second year crop.

'Elsanta' is considered highly susceptible to soil-born diseases and powdery mildew, while 'Marmolada®Onebor' is deemed to be less susceptible to fungal diseases (Baruzzi *et al*., 2009). Nevertheless, no disease or arthropod attacks were observed in this trial on either of the cultivars in both years of experimentation. Conversely Łabanowska *et al*. (2004) observed heavy symptoms caused by powdery mildew and strong attacks of mites (*Tetranychus urticae* and *Phytonemus pallidus* ssp. *fragariae*) on 'Elsanta' plants, while 'Marmolada®Onebor' was strongly infected by leaf spot (*Mycosphaerella fragariae*) and attacked by strawberry blossom weevil (*Anthonomus rubi*). Analogously, Hietaranta *et al*. (2004) evaluated 'Elsanta' as insufficient for resistance to pest and diseases in Nordic European countries. This means that the environmental characteristics of the area chosen for the trial exert a positive effect on plant health. This is not a secondary aspect in strawberry production, since the practices adopted to reach high yields in flat area cultivation (including a massive use of pesticides, soil fumigants and fertilizers) are in contrast with the market demand oriented towards healthy, organic and high quality strawberries (Gengotti *et al*., 2008; Mennone *et al*., 2008). Furthermore, the demand by consumers for healthy fresh fruits, rich in antioxidants, produced locally and in unpolluted environments is noticeably increasing (Tulipani *et al*., 2008). Strawberry high-altitude cultivation opens a new scenario since the ecological conditions (climate, soil, and spontaneous flora and fauna, associated with a generally low anthropized environment), seem to positively influence the nutritional properties of berries, for example increasing their polyphenol content as reported by Szajdek and Borowska (2008), and above all exert a strong influence on the amount and spectrum of harmful pests and diseases and hence on the cultural practices needed for production. Guerena and Born (2007) found that spontaneous flora alongside strawberry fields represented a shelter and a source of pollen and nectar to predators and parasites of insect pests, thus reducing the amount of damaged plants and strawberries. Furthermore, in almost natural conditions and sustainable farming systems the soils are rich in arbuscular mycorrhizal fungi which strongly contribute to counter-balance the appraisal and diffusion of soil-born diseases, such as fungi like *Fusarium, Phytophthora, Pythium, Rhizoctonia* and *Verticillium*, and nematodes (Branzanti *et al*., 2002; Harrier and Watson, 2004). Additionally, naturally fertile soils, namely in terms of organic matter content, are typical of many mountain areas. In this regard, Gonzalez and Acuña (2009) found that in strawberry cultivation a soil rich in organic matter may supply fertilization at least for the first year of establishment, hence reducing the chemical inputs which are a source of pollution to adjacent ecosystems (Tagliavini *et al*., 2005).

Strawberry high-elevation cultivation is feasible also in temperate areas as shown by this study. The main factors affecting fruit quality and productivity are related to cultivar and to location, this latter being a relevant aspect even under highly homogeneous environmental conditions. The first season crop, showing higher fruit quality than the second season crop, is particularly adequate for scheduling ripening time, hence filling the gaps in product offer by flat-area cultivation during specific periods. Furthermore, mountain cultivation in marginal and low anthropized areas allows production of high quality marketable strawberries. Further comparative tests are needed to study in detail the effect of climate, soil, and especially of the ecological background of mountain areas on strawberry cultivation.

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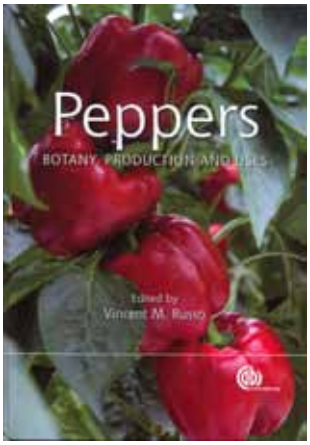
BOOK REVIEWS

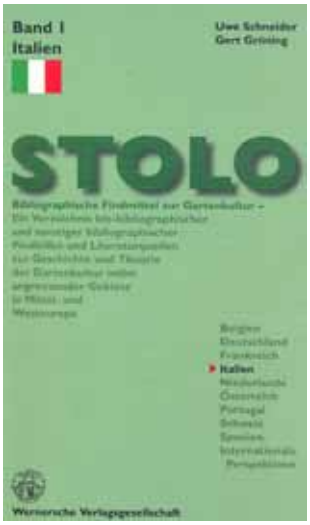


PEPPERS. BOTANY, PRODUCTION AND USES. *Russo Vincent M.* (ed.). CAB International, Wallingford, Oxfordshire, UK, 2012, pp. 280. ISBN 978-1-84593-767-6. € 110.00.

The purpose of the book (which contains 19 chapters) is to provide a general description of the world of peppers. Even though difficulties exist with pepper taxonomy, the book presents a good introduction where all disputes are clearly elucidated and discussed. Botany and cultivation techniques are widely described and throughout the book several unanswered questions relating to the lack of information about peppers emerge, keeping up the reader's interest. Some information can result a bit redundant in the chapters, which are written by different authors, but this offers the reader the opportunity to easily jump from one part to another. Moreover, all the chapters are clearly written and present a thorough bibliography, making the book suitable for both scientists and common devotees of peppers. The book can be used as a manual for cultivation, starting from a single plant to a massive production of peppers, with both biotic and abiotic stresses properly described. Particular attention is given to cultivation problems such as pests and weed control and for descriptions and reports, causes and effects of various types of diseases are also listed. Other common problems relating to peppers and analyzed in the book include irrigation, fertilization and soil composition, and economic and logistic problems regarding field and greenhouse production of plants. From the molecular side, the book is a good anthology of the majority of molecules involved in peppers and underlying their functions and physiology. While a large part of the work is fully dedicated to field production, a brief chapter is dedicated to tissue culture of peppers despite their recalcitrant nature. Finally, germination and transplant techniques are illustrated, making this book complete and useful for a wide audience.

Diego Comparini

STOLO. BIBLIOGRAPHISCHE FINDMITELL ZUR GARTENKULTUR. BAND I. ITALIEN. STRUMENTO BIBLIOGRAFICO SULLA CULTURA DEI GIARDINI. VOLUME 1. ITALIA. *(Bibliographic tools for the culture of gardens. Volume 1. Italy) Schneider U.*, and *G. Gröning.* Wernersche Verlgasgesellschaft mbH, Worms, Germany, 2009. pp. 576. ISBN 978-3-88462-248-3



This volume, dedicated to Italy, is the first of nine in the series STOLO on the history and cultural theory of gardens and related topics in the center-west of Europe. Each volume treats a single country and can be consulted individually, as the material presented pertains to the subject country. At the same time, the ample literature reported opens up international connections, presenting to the reader the geographic impact and extension of the treated topic.

The work required to compile the bibliographic material was carried out between 2004 and 2007, and required numerous trips to public and private libraries.

In its complex, this work by Stolo is a notable tool to access the history of gardens in various European countries, and it is presented in a way that is easy-to-use and in-depth.

This particular work on Italy is divided into eight chapters: *Sull'importanza sistematica delle conclusioni letterarie nella cultura dei giardini*. (The systematic importance of literary conclusions in terms of garden culture) 2. *Strumenti bibliografici generali e documentazione letteraria.* (General bibliographic tools and literary documentation) 3. *Bibliografie specifiche e strumenti bibliografici sulla cultura dei giardini e materie affini*. (Specific bibliographies and bibliographic tools regarding the culture of gardens and related topics) 4. *Manuali e opere generali sulla cultura dei giardini come contributi e temi speciali.* (Manuals and general works on the culture of gardens as contributions and special themes) 5. *Materiale primario*. (Primary material) 6. *Periodici e collane* (Periodicals and series) 7. *Ricerca in internet.* (Internet research) 8. *Organizzazioni, federazioni, associazioni, biblioteche nella cultura dei giardini.* (Organizations, federations, associations and libraries for the culture of gardens).

Enrico Rinaldelli