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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · FEBRUARY 2004

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Nutrients and Antioxidant Molecules in Yellow Plums (*Prunus domestica* L.) from Conventional and Organic Productions: A Comparative Study

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Yellow plums (*Prunus domestica* L.) conventionally and organically grown in the same farm were selected to study the influence of different agronomic practices on antioxidant vitamins (ascorbic acid, vitamin E, β -carotene) and phenolics (total polyphenols, phenolic acids, flavonols) concentration. Conventional plums were grown on tilled soil. Three organic cultivations were performed: tilled soil, soil covered with trifolium, and soil covered with natural meadow. Differences in macronutrients were marginal, whereas antioxidant vitamins and phenolic compounds concentration markedly differed among cultivations. Ascorbic acid, α -, γ -tocopherols, and β -carotene were higher in organic plums grown on soil covered with natural meadow. The highest phenolic acids content was detected in plums grown on soil covered with trifolium. Total polyphenols content was higher in conventional plums. Quercetin was higher in conventional plums, but myricetin and kaempferol were higher in organic plums. Under the same cultivar and climate conditions, the type of soil management turned out of primary importance in influencing the concentration of health-promoting compounds.

KEYWORDS: Organic plum; antioxidant vitamins; phenolic acids; flavonols

INTRODUCTION

The ever growing concerns of the consumers toward the health and safety attributes of foods emphasized the role of the agronomic practices as one of the main determinant of the food quality. It is beyond doubt that the healthfulness of the agronomic practices has a direct impact on human health; therefore, today the concept of food quality require an approach that takes into account also the type of soil management (organic/conventional) as quality and safety determinant. Notwithstanding the organic productions have now a wide diffusion, very few comparative studies on nutrient and bioactive compounds content in foods conventionally and organically grown are available and the number of foods examined is small. Studies on organically produced fruits are available mainly on apples (1, 2), strawberries (3), and pineapple (4). Further studies are thus necessary to gather more data on this topic. Bioactive compounds (vitamins, phenolics) in fruits play a role in the plant defense mechanisms as well as in the antioxidant expression of the plant. The concentration and composition of phenolic compounds in plants is influenced by a number of factors, among these the resistance to fungal activity (5, 6) and the use of pesticides in agriculture (7) are of great importance. Some herbicides reduce the carbon fixation by plants, decreasing the proportion of carbon available for the synthesis of secondary metabolites; other herbicides block the shikimate pathway reducing the synthesis of aromatic amino acids at the starting

point of the synthesis of phenolic compounds (7). The consequence may be a side effect on human health, because phenolic compounds are considered to exert an inhibitory action on the development of some degenerative diseases (8, 9). Therefore, the composition and the level of phenolic compounds in plums provides a spin-off on the total quality of the food product. Studies measuring the total antioxidant capacity of a number of fruits found that plums showed one of the highest antioxidant activity and the highest among mediterranean fruits (10–12). Among the molecules exerting antioxidant activity in plums, the contribution of phenolic compounds was found much greater than that of vitamin C and carotenoids (13).

The aim of this study was to evaluate some aspects of the nutritional quality of conventionally or organically grown plums (*Prunus domestica* L., var. *Shiro*). Other than the evaluation of the nutritional attributes of the plums, the attention was centered on the influence of different agronomic practices on the concentration of antioxidant molecules. In particular, the concentration in plums of antioxidant vitamins (ascorbic acid, vitamin E, β -carotene), and of those compounds (total polyphenols, phenolic acids, flavonols) more susceptible to modifications because they are linked up to specific plant functions (eg., molecules involved in plant defense mechanisms), was studied.

MATERIALS AND METHODS

Plums (*Prunus domestica* L.) var. *Shiro* (yellow pulp and skin) either conventionally or organically grown in the same farm (Fruit Farming

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Institute, Rome, Italy) were studied. Organic fields were placed at 600 m from the conventional fields and encircled by a thick hedge. Conventional fields were fertilized with synthetic fertilizers. Organic fields were manured with organic fertilizers (guano, dried fowl dung). The organic cultivation was performed utilizing three different types of soil management: (i) tilled soil, the same utilized for the conventional cultivation (milling each 30–40 days), (ii) soil covered with *trifolium subterraneum* (sowed in october, mowed in spring leaving the biomass in situ) (trifolium), and (iii) soil covered with natural meadow (mowed in spring leaving the biomass in situ) (meadow).

In all the types of cultivation, the vegetation did not touch the trunks.

		Cultivations:
Conventional	tilled soil	
	tilled soil	
Organic	soil covered with trifolium (trifolium)	
	soil covered with natural meadow (meadow)	

The study was carried out for three growing seasons (years): 1999–2000–2001. Plums were picked at commercial ripeness and sampled on receipt in the laboratory. Equal amounts of fruits for each type of cultivation were pooled and held in a -30°C refrigerator. Injured fruits were not selected. The pooled plums were homogenized (with skin), frozen to avoid oxidative phenomena, aliquots were then taken for subsequent analysis and some aliquots were freeze-dried. Other than the macronutrients composition of the plums, citric, and malic acids, ascorbic acid, vitamin E, β -carotene, vitamin K1, total polyphenols, phenolic acids (protocatechuic, caffeic, ferulic, p-cumaric, chlorogenic, iso-chlorogenic), and flavonols (myricetin, quercetin, kaempferol) contents were determined. For each type of cultivation, triplicate analysis for each year were done.

Proximate Analysis. Moisture, protein (nitrogen \times 6.25), lipid, and ash were determined by AOAC methods (14).

Minerals and Trace Elements. Minerals and trace elements were determined by ICP–Plasma (Optima 3200–Perkin-Elmer) following liquid ashing of the samples (4 mL HNO_3 + 1 mL H_2O_2) in a microwave digestion system (Milestone, 1200 Mega). Standard Reference Materials: Mixed diet (NBS 8431, National Bureau of Standards, Gaithersburg, MD 20899) and Wholemeal flour (BCR 189, Community Bureau of Reference, Brussels) were analyzed as a check on the accuracy of the analysis.

Sugar Analysis. Free sugars were determined by RP-HPLC (Waters 996), equipped with a RI detector, on a Alltima-NH2 column (Alltech) (250 mm \times 4.6 mm), mobile phase acetonitrile/water (75:25, v/v).

Dietary Fiber. Total, soluble, and insoluble fiber content was detected following the method of Prosky et al. (15).

Organic Acids. Ascorbic acid, citric acid, and malic acid were determined by HPLC (16). Samples were extracted in 5% metaphosphoric acid and separated on Hypersil ODS C18 column (0.46 \times 25 cm, Sigma Aldrich).

Vitamin E, K1. Both vitamins were identified and quantified by RP-HPLC (Waters 996, PAD detector), comparing peaks with respective standards. Vitamin E was determined following the method of Halbalá-Hurtado (17).

β -Carotene. β -carotene was extracted and quantified as described by Tonucci et al. (18). Stock solution of trans β -carotene was prepared and kept at -30°C . β -carotene was injected at three different level of concentration at 4°C with autosampler (Water, model 717 plus). Standards were injected both before and after samples injections (bracket method).

Total Polyphenols. were determined following the method of Singleton et al. (19).

Phenolic Acids. Chlorogenic and neo-chlorogenic acids were extracted following the method of Donovan et al. (20). Protocatechuic, caffeic, ferulic, and trans-*p*-coumaric acids were extracted following the method of Hanna et al. (21): two hydrolyses, 10 min for protocatechuic and caffeic and 24 h for ferulic and trans-*p*-coumaric acids, were performed. Phenolic acids were separated by RP-HPLC

Table 1. Chemical Composition of Conventionally and Organically Grown Plums (Fresh Weight)^a

	conventional tilled soil	organic tilled soil	trifolium	meadow
edible portion (%)	95.5 \pm 0.6	96.8 \pm 0.7	96.6 \pm 0.7	96.9 \pm 0.7
water (g/100 g)	88.7 \pm 0.1	88.7 \pm 0.1	88.1 \pm 0.2	88.1 \pm 0.5
ash (g/100 g)	0.33 \pm 0.1	0.37 \pm 0.1	0.37 \pm 0.2	0.37 \pm 0.1
protein (g/100 g)	0.51 \pm 0.1	0.51 \pm 0.1	0.53 \pm 0.1	0.51 \pm 0.1
lipid (g/100 g)	0.13 \pm 0.1	0.13 \pm 0.1	0.13 \pm 0.1	0.13 \pm 0.1
minerals				
P (mg/100 g)	11.9 \pm 4.5	14.6 \pm 2.8	15.6 \pm 2.2	15.1 \pm 3.6
Na (mg/100 g)	1.46 \pm 0.4	1.0 \pm 0.4	1.2 \pm 0.3	1.3 \pm 0.6
K (mg/100 g)	174 \pm 16	201 \pm 8	218 \pm 2.2	207 \pm 3.9
Mg (mg/100 g)	4.90 \pm 0.8	5.80 \pm 0.3	5.70 \pm 0.3	5.40 \pm 0.8
Ca (mg/100 g)	4.16 \pm 0.7	4.85 \pm 1.2	4.49 \pm 0.8	4.26 \pm 0.7
Fe (mg/100 g)	0.29 \pm 0.1	0.27 \pm 0.1	0.26 \pm 0.1	0.27 \pm 0.1
Zn (μg /100 g)	60 \pm 0.01	70 \pm 0.08	40 \pm 0.02	90 \pm 0.06
Cu (μg /100 g)	60 \pm 0.01	50 \pm 0.01	60 \pm 0.01	60 \pm 0.01
Mn (mg/100 g)	50 \pm 0.01	50 \pm 0.01	50 \pm 0.01	40 \pm 0.01
dietary fiber				
total fiber (g/100 g)	1.38 \pm 0.1	1.36 \pm 0.1	1.50 \pm 0.1	1.41 \pm 0.1
soluble fr. (g/100 g)	0.56 \pm 0.1	0.55 \pm 0.1	0.61 \pm 0.1	0.56 \pm 0.1
insoluble fr. (g/100 g)	0.82 \pm 0.1	0.82 \pm 0.1	0.89 \pm 0.1	0.80 \pm 0.1
S/t	0.41	0.40	0.41	0.36
carbohydrates				
fructose (g/100 g)	1.08 \pm 0.2	1.38 \pm 0.3	1.21 \pm 0.3	1.35 \pm 0.1
glucose (g/100 g)	1.97 \pm 0.8	2.11 \pm 0.6	2.00 \pm 0.7	2.00 \pm 0.9
sucrose (g/100 g)	2.23 \pm 0.1	1.82 \pm 0.5	2.75 \pm 2.0	2.50 \pm 0.9
sorbitol (g/100 g)	1.16 \pm 0.7	0.96 \pm 0.2	1.18 \pm 0.1	1.20 \pm 0.2
total sugar (g/100 g)	6.44 \pm 1.8	6.27 \pm 0.4	7.14 \pm 0.9	7.05 \pm 0.5
organic acids				
citric acid (mg/100 g)	27.0 \pm 2.1	25.7 \pm 1.0	25.8 \pm 2.1	25.2 \pm 2.7
malic acid (g/100 g)	1.98 \pm 0.3	1.98 \pm 0.2	2.02 \pm 0.3	2.02 \pm 0.2
vitamins				
ascorbic ac. (mg/100 g)	2.00 \pm 1.4	1.60 \pm 0.9	2.10 \pm 1.0	2.20 \pm 0.2
α -tocopherol (μg /100 g)	446 \pm 15	415 \pm 10	411 \pm 11	585 \pm 16
γ -tocopherol (μg /100 g)	7.2 \pm 0.1	9.7 \pm 0.1	9.7 \pm 0.1	11.0 \pm 0.7
β -carotene (μg /100 g)	107 \pm 5.0	68 \pm 8.0	77 \pm 8.0	117 \pm 8.0
K1 (mg/100 g)	12.4 \pm 0.6	10.9 \pm 0.4	12.6 \pm 0.6	9.7 \pm 0.7

^a Values are the $M \pm$ SD of triplicates for each harvest year.

(Waters 996) equipped with a detector PAD, on an Alltima C18 column (4.6 mm \times 250 mm, Alltech Associates).

Flavonols. Flavonols were determined by RP-HPLC (Waters 996, PAD detector) as aglycones after acid hydrolysis as described by Hertog et al. (22).

Statistical analysis were performed utilizing the student *t*-test for comparing conventional versus organic samples grown on tilled soil and the analysis of variance (ANOVA) for comparing the three organic cultivations.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of plums deriving from both conventional and organic cultivations. The comparisons were performed either between conventionally and organically grown plums (both grown on tilled soil) or among the three types of organic cultivations, each characterized by a different type of soil management (tilled soil, trifolium, meadow). Conventionally grown plums generally weighed 52 ± 0.7 g, organic plums weighed between 61.3 ± 0.7 g (tilled soil), 56 ± 0.7 g (trifolium) and 63.2 ± 0.7 g (meadow). No differences in moisture, protein, and lipid content neither between the fruits deriving from conventional and organic cultivations (tilled soil), nor among the three type of the organic productions were observed (**Table 1**). All the organic products showed an ash content slightly higher than that found in the conventional product; this depended on the highest mineral concentration showed by the organic plums. Indeed, the organically grown plums (tilled soil) were richest ($p < 0.001$)

in K, Mg, and Zn compared to the conventional cultivation; Na and Cu were higher ($p < 0.05$) in the conventional cultivation (**Table 1**). The main factor influencing mineral concentration in vegetable products is the type of soil. In this study, however, this type of influence can be excluded, being both the cultivations grown in the same experimental fields. Therefore, the differences in mineral content could be ascribed to the different types of soil management (see Materials and Methods). Total dietary fiber content was similar in both conventionally and organically grown plums cultivated on tilled soil. On the other hand, significant differences ($p < 0.05$) were evidenced among the other two organically grown plums (**Table 1**). In all the plums analyzed, however, the insoluble fraction constituted about 59% of the total fiber content and the soluble fraction about 40% of it. In succession, in **Table 1**, the total sugar content of the fruits and the composition in the individual sugar was reported. The total sugar content was similar to that reported by Holland et al. (23) for yellow plum. Even in this study, the total sugar content of the plums was not significantly different between the conventional and the organic fruits grown on tilled soil. On the other hand, significant differences ($p < 0.05$) were observed among the three organic cultivations with the highest value observed in the plums grown on soil covered with trifolium. The sugar pattern showed the characteristic distribution reported by Forni et al. (24) for plums and by Stacewicz-Sapuntzakis et al. (25) for prune-making plums. Plums contained more glucose than fructose with a G/F ratio of 1.8 for the conventionally grown fruits and of about 1.6 for the organically grown fruits; this ratio was in the range typical for this fruit, and it was suggested to be considered a taxonomic feature for plums (24, 26). On average, in the conventionally grown plums, glucose constituted 31% of the total sugar content, fructose 17%, sucrose 35%, and sorbitol 18%, values not significantly different from those detected in the correspondent organic cultivation (tilled soil). In the organically grown plums (tilled soil, trifolium, meadow) glucose constituted from 28 to 34% of the total sugar content, fructose from 17 to 22%, sucrose from 29 to 39%, and sorbitol from 15 to 17%. Sorbitol, a sugar characteristic of the *Rosaceae* family, was in the range already reported in the literature for this sugar (21), a concentration which tended to increase with the total sugar content. The individual sugar content in the plums varied among the three years of production, therefore the differences observed were probably not referable to the different type of soil management but rather to the degree of ripeness of the fruits. As far as organic acids were concerned (**Table 1**), the conventionally grown fruits showed slightly higher content of citric acid compared to the organic products. Among the organically grown plums no differences in both citric and malic acid content was observed. In **Table 1**, the concentration of some vitamins in plums was also reported. The presence of ascorbic acid, vitamin E, and β -carotene was of particular interest either for their activity as vitamin or for the antioxidant properties exhibited by these compounds. Ascorbic acid (**Table 1**) detected in all the plums analyzed was lower than that reported in the literature. Holland et al. (23) reported about 5 mg/100 g of ascorbic acid in yellow plum, higher amounts were generally referred to the prune-making varieties (25). Samples analyzed in this study did not differ significantly in ascorbic acid content, which varied from 1.6 to 2.2 mg/100 g. Conventionally grown plums showed significantly higher amounts ($p < 0.01$) of α - but not of γ -tocopherol compared with the organic plums grown on the same type of soil (**Table 1**). However, the organic plums grown on soil covered with natural meadow showed the highest

Table 2. Total Polyphenols, Phenolic Acids, and Flavonols in Conventionally and Organically Grown Plums (Tilled Soil) (f.w.).

	conventional plum	organic plum
total polyphenols mg tann.ac/100 g	121 \pm 21a	88 \pm 4b
phenolic acids mg/Kg		
protocatechuic	0.8 \pm 0.03a	0.6 \pm 0.061b
caffeic	20.6 \pm 1.23c	22.6 \pm 1.05a
trans- <i>p</i> -cumarico	8.5 \pm 0.34c	8.9 \pm 0.32a
ferulic	8.0 \pm 0.63b	9.3 \pm 0.42a
chlorogenic	25.2 \pm 1.25b	37.5 \pm 2.94a
neo-chlorogenic	52.0 \pm 2.76a	46.0 \pm 6.95c
flavonols mg/Kg		
myricetin	0.9 \pm 0.2c	1.1 \pm 0.1a
quercetin	30.2 \pm 0.8a	19.6 \pm 1.2b
kaempferol	0.6 \pm 0.2b	1.7 \pm 0.3a

^a Values are the $M \pm$ SD of three harvest years. Values, within rows, with different letters are significantly different (a vs b, $p < 0.001$; a vs c, $p < 0.05$).

Table 3. Total Polyphenols, Phenolic Acids, and Flavonols in Organic Plums Grown under Different Management Systems (f.w.).

	organic plums		
	tilled soil	trifolium	meadow
total polyphenols mg tan.ac/100 g	88 \pm 4a	94.7 \pm 6b	103.2 \pm 4c
phenolic acids mg/Kg			
protocatechuic	0.6 \pm 0.06b	0.6 \pm 0.03b	0.7 \pm 0.03a
caffeic	22.6 \pm 1.05b	24.3 \pm 1.31a	13.8 \pm 0.96c
trans- <i>p</i> -cumarico	8.9 \pm 0.32c	9.7 \pm 0.32a	9.3 \pm 0.29b
ferulic	9.3 \pm 0.42b	10.4 \pm 0.27b	9.4 \pm 0.13b
chlorogenic	37.5 \pm 2.94b	41.5 \pm 4.41a	22.3 \pm 2.98c
neo-chlorogenic	46.0 \pm 6.95b	60.7 \pm 1.84a	26.8 \pm 3.20c
flavonols mg/Kg			
myricetin	1.1 \pm 0.1a	1.1 \pm 0.3a	0.7 \pm 0.1b
quercetin	19.6 \pm 1.2b	28.0 \pm 2.4a	28.3 \pm 2.0a
kaempferol	1.7 \pm 0.3a	0.6 \pm 0.2c	0.9 \pm 0.1b

^a Values are the $M \pm$ SD of three harvest years. Values, within rows, with different letters are significantly different ($p < 0.001$).

amounts of both α - and γ -tocopherol ($p < 0.01$) compared with all the other samples analyzed. β -carotene concentration in organically grown plums (tilled soil) was higher than that found in conventionally grown plums, the organic plums grown on natural meadow showing the highest amount. The amount of β -carotene detected in this study was in the range of values reported by Holland et al. (23) and by Gil et al. (13) for yellow varieties of plum. Vitamin K content was higher in the plums grown on conventional fields compared to the respective organically grown plums, small differences were detected among the three organic cultivations (**Table 1**).

Plums contain different types of molecules exerting antioxidant activity; therefore, other than the above-described compounds showing vitamin activity, the total polyphenols content and the composition in phenolic acids and flavonols of the plums was also studied (**Tables 2 and 3**). In **Table 2**, the concentration in phenolic compounds in conventional and organic plums, both grown on tilled soil, was reported. Total polyphenols content in conventionally grown plums was significantly higher ($p < 0.001$) compared to that found in the organic cultivation. Among the phenolic acids detected, neo-chlorogenic acid was predominant in both the cultivations accounting for over 45% of total phenolics in conventional plums and for over 37% in organic plums. Higher concentrations in caffeic ($p < 0.05$), *p*-cumaric ($p < 0.05$), ferulic ($p < 0.001$) and chlorogenic ($p < 0.001$) acids were steadily detected in the organically grown plums. On the other hand, protocatechuic ($p < 0.001$) and neo-

chlorogenic ($p < 0.05$) acids were higher in conventionally grown plums (**Table 2**). Among flavonols, quercetin, which was the flavonol predominant in both conventional and organic plums, was significantly higher in conventionally grown plums ($p < 0.001$). By contrast, both myricetin ($p < 0.05$) and kaempferol ($p < 0.001$) were significantly higher in the organically grown plums (**Table 2**). In **Table 3**, the comparison in phenolic compounds content among the three types of organic cultivations was reported. The three type of organically grown plums differed significantly in total polyphenols concentration ($p < 0.001$), with plums grown on natural meadow showing the highest. The differences in phenolic acids content among the three organic cultivations were always significant; apart from protocatechuic acid, the organic plums grown on soil covered with trifolium showed the highest concentrations ($p < 0.001$) of all the other phenolic acids determined. As already found by Herrmann (27), in this study among the hydroxycinnamoylquinic acids, neo-chlorogenic was predominant on chlorogenic acid (**Table 3**). As far as flavonols were concerned, it is noteworthy that quercetin concentration in plums grown on tilled soil was significantly lower ($p < 0.001$) than not only that found in the conventionally grown plums (**Table 2**) but also of that detected in the other two organically grown plums (**Table 2** and **3**). *Prunus domestica* L. was characterized by the occurrence of kaempferol 3-rutinoside, 3-glucoside, 3-galactoside, and 3-arabinoside-7-rhamnoside, in addition to the quercetin analogues plus quercetin 3-xyloside and 3-rhamnoside (13). However, under the conditions utilized in this study (22), only the aglycone forms of flavonoids were detected.

Our findings indicated that differences in macronutrients content between conventional and organic plums were marginal, whereas the concentration of some antioxidant vitamins and of secondary metabolites (phenolics) markedly differed among the cultivations examined. In this study, both conventional and organic plums were grown in the same farm, this allowed the variability due to the location of the farm, length of insolation, climate, choice of cultivar, factors which strongly influence the content and the composition of these compounds in fruits (28, 29, 30, 31, 32), to be overcome. Our results showed that the differences in phenolics concentration among cultivations became appreciable when the organic cultivations were performed with different soil managements. Among all the cultivations analyzed, the organic plums grown on soil covered with trifolium or with natural meadow steadily showed the highest amounts of the antioxidant compounds: Plums grown on soil covered with trifolium had the highest phenolic acids content and a flavonol content similar to that of the conventional cultivation. Plums grown on soil covered with natural meadow had the highest antioxidant vitamins content. Therefore, under the same cultivar and climate conditions, the type of soil management turned out to be of primary importance in influencing the concentration in these health-promoting compounds.

A number of studies showed that the increase in phenolics concentration in fruits (chlorogenic acid is one of the most active) together with PPO activation was often a consequence of damages induced by microorganisms (33, 34), a type of attack to which organic productions are more susceptible. It is known that plant tissue injury influences the metabolism of preformed antifungal agents and increases the activity of some enzymatic systems. (6, 35). This so-called "hypersensitive reaction" of the host tissues (5) represents a barrier to the spreading of infectious process. In the framework of defense mechanisms, special attention is deserved also for the ferulic and *p*-coumaric acids

ability to form esterified bonds with the cell wall's polysaccharides (36). An increase in their content could be related to an augmented fruit firmness, and consequently, to an augmented mechanical resistance. The hypothesis that ferulic and *p*-coumaric acids are covalently bound to polysaccharides may be confirmed by the highest hydrolysis time (24 h), in that it was necessary for their detection.

In conclusion, our findings did not provide evidence of major differences in macronutrients content between conventionally and organically grown plums. On the other hand, differences were evidenced in some antioxidant vitamins (α - γ -tocopherols, β -carotene) and phenolic compounds (total polyphenols, phenolic acids, flavonols), which showed higher concentrations in the organically grown plums. Therefore, and also in the light of their possible utilization as markers of different agronomic practices, further studies should be addressed to the understanding of to what extent the cultural systems utilized can influence the synthetic pathways of the antioxidant compounds.

ACKNOWLEDGMENT

We thank Dr. M. Antonelli (Istituto Nazionale di Frutticoltura, Rome, Italy) for supplying plums.

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Received for review May 7, 2003. Revised manuscript received November 6, 2003. Accepted November 11, 2003.

JF0344690