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Quantitative Seasonal Changes in the Leaf Phenolic Content Related to the Alternate-Bearing Patterns of Olive (*Olea europaea* L. cv. Gemlik)

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ABSTRACT

Using HPLC, the seasonal changes in the phenolic compound content of the leaves of the Gemlik olive cultivar (*Olea europaea* L) has been investigated with respect to the effects on the alternate bearing of the cultivar. For this purpose, the leaf concentrations of oleuropein, chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid, scopolin and p-coumaric acid were analyzed at 10 day intervals around the years. The quantity and distribution of these phenolics in the leaves showed significant differences in 2008 (off year) and 2009 (on year). In the "on" year, the levels of chlorogenic and p-coumaric acids were high, whereas the abundance of other phenolic compounds was low. In contrast, during the "off" year, the chlorogenic and p-coumaric acid levels were at low levels, whereas the levels of the other phenolics were high. We found a negative relationship between the chlorogenic acid and caffeic acid concentrations in the "on" and "off" years: the amount of caffeic acid in the leaves was high, and the chlorogenic acid level was low. The contents of chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid and p-coumaric acid were significantly different in the "on" and "off" years. These findings indicated that these compounds and the other phenolics examined in this study were related to alternate bearing. Interestingly, the levels of all of the phenolic compounds examined in this study were at their highest during the dormant season.

Keywords: Caffeic acid, Chlorogenic acid, Oleuropein, Olive leaves, P-coumaric acid, Scopolin, 3-hydroxycinnamic acid.

INTRODUCTION

Olive is an important fruit crop due to its higher value in human nutrition for its processed fruit and its oil which are necessary for a healthy life. It is naturally grown in the subtropical climates and the Mediterranean countries. Consequently, many countries have grown the olive tree for its economic value. However, this valuable fruit tree has commonly shown alternate bearing habit. Due to this unfavorable attribute, the crop production decreases significantly, resulting in important economic loss in some years. Therefore, this condition needs to be improved and the first step to overcome the problem is to enlighten the physiological basis of this phenomenon. In this respect, the phenolic compounds must be investigated.

Although the nature of phenolic metabolism in higher plants is complex and not well understood, the phenolic compounds in plant leaves are known to be involved in several physiological mechanisms. Phenolics act as UV-protecting agents in plant tissues (Takeda *et al*., 1994) and are often involved in plant–pathogen interactions, both constitutively and as newly induced compounds (Clerivet *et al*., 1996). The composition of these phenolics in plant tissues is markedly influenced by

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such environmental conditions as UV light (Markham *et al*., 1998), temperature (Rivero *et al*., 2001) and nutrition (Ruehmann *et al*., 2002). Therefore, some phenolic compounds in plant tissues are significantly affected by environmental conditions.

It has been reported that the phenolic compounds in olive leaves are highly beneficial for human health (Benavente-Garcia *et al*., 2000); accordingly, the phenolics in olive leaves, particularly oleuropein, have attracted the attention of researchers. Many procedures and extraction methods have been employed to obtain oleuropein and the other polyphenols in olive leaves (Soler-Rivas *et al*., 2000; Malik and Bradford, 2008; Papoti and Tsimidou, 2009), and several studies have been conducted to determine the phenolic compounds in the olive fruit, oil, and seed (Amiot *et al*., 1986; Ryan *et al*., 1999; Servili *et al*., 1999; Allalout *et al*., 2009). In addition, some research has been conducted on the annual fluctuations of certain phenolic compounds in olive leaves (Heimler *et al*., 1996; Ryan *et al*., 2003; Ercan and Özkaya, 2008).

There is an extensive body of literature devoted to the phenomenon of alternate bearing in *O. europaea*, and it is believed that the initial signal for alternate bearing may be received by the leaves (Lavee, 1989; Akillioglu, 1995; Ryan *et al*., 2002; Ryan *et al*., 2003). The first chemical signal of alternate bearing, which is produced by the developing embryo, is intercepted by the leaves to induce certain chemical changes. The leaves then produce an inhibitor at a rate determined by the intensity of the signal and the environmental conditions, determining the degree of flower bud differentiation. The degree of the response of the leaves to this signal is affected by the environmental conditions. It has been stated in the literature that these chemical changes in the leaves include phenolic and flavanolic compounds, which arrest flower bud formation during the physiological initiation period. Recent studies have shown that one of these phenolic compounds, i.e. chlorogenic acid, is an important physiological signal for periodicity and is correlated with noteworthy changes in relation to the crop load (Lavee *et al.*, 1994; Lavee, 1996). Lavee *et al*. (1986) have reported that cinnamic acid, one of the phenolic compounds present in olive leaves, can play a role in the crop load. In contrast, there is insufficient information about oleuropein, caffeic acid, 3-hydroxycinnamic acid, scopolin and p-coumaric acid with regard to their annual variations in olive leaves and their effects on alternate bearing.

Therefore, the objective of this study was to determine the accumulation and variation of oleuropein, chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid and p-coumaric acid in olive leaves in "off" (2008) and "on" (2009) years and to establish the relationships between alternate bearing and the abundance of these phenolic compounds.

MATERIALS AND METHODS

In this study, the following phenolic compounds in the leaves of olive cultivar Gemlik have been determined using HPLC analysis during 2008 (off year) and 2009 (on year): oleuropein, chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid, scopolin, and p-coumaric acid. For this purpose, three trees from the same orchard row were selected based upon the similarity of tree size, number of branches, and yield. The leaf samples were collected from the middle of the previous year's shoots at 10 day intervals; approximately 50 g of leaf samples was collected from each tree. The collection of the leaf samples began at beginning of January 2008 and continued until the end of December 2009. All of the samples were immediately frozen and stored at -20^{o}C until used for the analysis.

Oleuropein was purchased from Extrasynthese (Genay-France); chlorogenic acid, 3-hydroxycinnamic acid, and p-coumaric acid were from Sigma Chemical Company (St. Louis, MO). Caffeic acid was from Fluka Sigma-Aldrich Chemie

(Steinheim-Germany) and Scopolin from AApin Chemical (United Kingdom).

Extraction Method

The extraction was performed using methanol: acetonitrile: acetic acid, (85: 13.5: 1.5). The frozen plant material (2 g in 30 ml of solvent) was incubated in an ultrasonic bath at room temperature for 30 minutes. The samples were then centrifuged (1,000 rpm, 15 minutes) and filtered through a 0.45 µm general purpose filter prior to HPLC injection.

Quantitative Analysis

The phenolic compounds in the hydrolysed extracts were determined using a Schimadzu 10 Series HPLC equipped with a UV detector. The detector was operated at a wavelength of 280 nm. An aliquot of the extract was diluted with an equal amount of water, and the injection volume was 20 µL. The phenolic compounds were separated using a C18 column (5 µm; 250×4.6 mm) at 40°C. The mobile phase for oleuropein was a mixture of 0.01% Trifluoroacetic acid (solution A) and acetonitrile (solution B) at a flow rate of 1 ml min^{-1}. The gradient elution for oleuropein was as (Table1):

Table1. The gradient elution for oleuropein.

Time (Min)	A (%)	B (%)
0.01	95	5
10.00	90	10
34.00	70	30
40.00	60	40
40.01	95	5
50.00	95	5

The mobile phase for the other phenolics consisted of a mixture of 0.01% 50 mM H_3PO_4 at pH 2.5 (solution A) and acetonitrile (solution B) at a flow rate of 1 ml min^{-1}. The gradient elution for the other phenolics was as (Table2):

Table2. The gradient elution for other phenolics.

Time (Min)	A (%)	B (%)
0.01	95	5
5.00	95	5
55.00	50	50
55.01	95	5
70.00	95	5

The phenolic compounds in the extract were identified by comparing their retention times and UV spectra of the peaks in the chromatogram with the peaks of known standard compounds.

Statistical Analysis

The data were tested using a one-way analysis of variance with the Minitab 14.0 software, and the means were compared using a Duncan's multiple range test ($P< 0.05$).

RESULTS AND DISCUSSION

The annual changes ("off" year, 2008, and "on" year, 2009) in the oleuropein, chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid, scopolin, and p-coumaric acid levels in olive leaves are presented in Tables 3and 4 and Figures (1-a to 1-f). The concentrations and variation in the accumulation of these phenolics in the leaves showed different fluctuations in both study years. The following values were determined for the variation in the phenolic compounds during 2008 (off) and 2009 (on): chlorogenic acid (5-caffeoylquinic acid), 6.94-41.37 mg g^{-1} in 2008 and 9.60-70.71 mg g^{-1} in 2009; caffeic acid, 9.62-22.19 mg g^{-1} in 2008 and 1.35-19.51 mg g^{-1} in 2009; 3-hydroxycinnamic acid, 5.38-32.69 mg g^{-1} in 2008 and 5.04-31.66 mg g^{-1} in 2009; p-coumaric acid, 0.26-14.73 mg g^{-1} in 2008 and 4.13-19.07 mg g^{-1} in 2009; scopolin, 0.35-11.19 mg g^{-1} in 2008 and 0.32-4.68 mg g^{-1} in 2009; and oleuropein, 0.73-7.05 mg g^{-1}

Table 3. The annual variation in the contents of phenolic compounds in the leaves of olive cultivar, 'Gemlik' in 2008.

Day	Example on day	Chlorogenic acid [Mean±SE (mg g^{-1})]	Caffeic acid [Mean±SE (mg g^{-1})]	3-hydroxycinnamic acid [Mean±SE (mg g^{-1})]	p-coumaric acid [Mean±SE (mg g^{-1})]	Oleuropein [Mean±SE (mg g^{-1})]	Scopolin [Mean±SE (mg g^{-1})]
3	03.01.08	9.91±0.88 o[a]	18.56±0.69 de	24.67±1.82 e	6.01±0.17 h	3.07±0.04 gh	1.33±0.21 m
10	10.01.08	19.66±0.63 jkl	16.90±0.10 gh	25.69±0.42 cde	10.18±0.03 d	4.57±0.05 c	1.43±0.07 lm
21	21.01.08	20.77±1.28 jkl	21.45±0.21 ab	26.61±0.10 c	7.21±0.18 g	2.88±0.06 u	2.79±0.03 k
31	31.01.08	14.67±0.12 n	18.40±0.25 def	27.41±0.53 c	14.73±0.20 a	3.42±0.00 f	0.35±0.01 n
42	11.02.08	21.13±0.78jk	20.49±0.27 c	32.69±1.98 a	10.77±0.03 c	3.88±0.00 e	2.67±0.05 k
51	20.02.08	19.36±0.08 kl	17.08±0.03 gh	24.97±0.10 de	12.99±0.13 b	2.73±0.36 ıj	1.30±0.00 n
63	03.03.08	17.54±0.01 m	18.01±0.37 efg	23.04±0.25 f	5.09±0.24 k	2.38±0.05 l	2.36±0.08 kl
77	17.03.08	19.07±0.00 lm	16.94±0.41 h	24.72±1.42 e	4.26±0.01 mn	2.12±0.12 m	2.52±0.08 k
91	31.03.08	14.29±1.61 n	20.63±0.35 c	30.11±0.84 b	5.64±0.24 j	2.16±0.14 m	1.88±0.04 klm
101	10.04.08	10.28±0.10 o	21.06±0.13 bc	26.93±0.15 c	5.67±0.06 ıj	2.01±0.00 mn	2.05±0.04 klm
112	21.04.08	6.94±0.35 p	22.19±0.15 a	24.66±0.13 e	5.86±0.13 hı	1.85±0.01 nop	1.07±0.02 mn
122	01.05.08	10.67±0.30 o	16.85±1.03 gh	22.04±0.35 f	4.96±0.05 k	4.39±0.05 c	2.03±0.41 klm
134	13.05.08	14.34±0.18 n	18.03±0.25 ef	29.89±0.71 b	8.21±0.06 f	7.05±0.04 a	4.03±0.10 j
147	26.05.08	21.74±0.96 j	13.26±0.03 kl	15.63±0.69 ı	3.32±0.03 pq	2.88±0.01 ıj	8.41±0.25 bc
155	03.06.08	27.30±0.06 gh	9.62±0.22 n	13.65±0.05 jk	3.47±0.00 p	3.74±0.01 e	6.60±0.01 d
162	10.06.08	27.66±0.52 fg	11.04±0.18 m	12.54±0.25 jk	2.97±0.06 qr	3.14±0.02 g	8.97±0.00 b
175	23.06.08	24.87±1.14 ı	10.62±0.32 m	5.38±0.26 m	0.26±0.05 u	1.56±0.03 rs	4.30±0.10 ıj
184	02.07.08	36.95±0.20 d	11.96±0.01 m	8.67±0.00 l	2.11±0.05 t	0.87±0.04 u	8.54±0.00 c
192	10.07.08	40.97±0.90 b	15.83±0.35 ı	13.13±0.12 jk	4.18±0.05 no	1.66±0.07 qr	11.19±0.01 a
217	04.08.08	37.10±0.10 c	12.97±0.12 l	12.24±0.10 k	2.46±0.03 s	0.77±0.06 u	5.20±2.42 fghı
224	11.08.08	30.13±0.56 e	13.18±0.05 kl	13.78±0.46 j	2.28±0.29 st	0.73±0.07 u	9.28±0.22 b
233	20.08.08	41.37±0.03 a	15.61±0.13 ı	17.47±0.38 h	4.52±0.05 lm	1.17±0.03 t	7.91±0.00 c
245	01.09.08	36.78±1.57 c	17.04±0.13 gh	19.91±0.20 g	3.84±0.10 o	2.10±0.02 m	11.17±0.02 a
254	10.09.08	30.83±0.87 e	13.11±1.55 kl	13.83±0.99 j	2.96±0.22 r	1.41±0.05 s	8.66±0.40 bc
266	22.09.08	29.82±1.64 e	16.49±0.37 h	21.89±0.47 f	4.26±0.08 mn	1.78±0.07 opq	5.10±0.11 fghı
280	06.10.08	30.17±0.03 e	15.55±0.01 ı	19.46±0.01 g	3.90±0.08 o	2.03±0.02 mn	5.17±0.06 fghı
289	15.10.08	32.86±1.10 e	16.09±0.41 ıj	18.64±0.63 h	3.50±0.03 pqr	2.11±0.04 mno	6.37±0.01 defg
297	23.10.08	25.39±0.87 ı	13.64±0.41 l	19.05±0.09 h	2.24±0.05 t	2.07±0.13 mno	6.61±0.10 de
304	30.10.08	26.22±0.29 ı	16.21±0.12 ı	20.54±0.72 g	4.58±0.18 mn	3.09±0.00 hı	6.37±0.06 def
316	11.11.08	26.40±1.31 hı	17.94±0.57 fgh	22.26±0.35 f	5.08±0.33 k	2.62±0.03 kl	5.52±0.01 efgh
325	20.11.08	29.19±1.75 ef	17.49±0.15 fgh	22.77±0.69 f	5.82±0.15 hıj	4.09±0.03 d	6.01±0.06 def
336	01.12.08	28.11± 0.74 fg	14.16±0.55 jk	15.42±0.06 ı	3.16±0.15 qr	1.72±0.18 pqr	4.59±0.23 hıj
351	16.12.08	24.19±0.10 ı	17.12±0.05 h	26.63±0.48 cd	4.69±0.01 l	2.74±0.07 jk	5.64±0.07 efg
365	30.12.08	32.98±0.01 d	19.00±0.23 d	28.68±0.11 b	8.78±0.24 e	5.81±0.06 b	4.88±0.09 ghıj

[a] Mean values followed by different lower-case letters differ significantly by Duncan's multiple range test at $P< 0.05$.

Table 4. The annual variation in the contents of phenolic compounds in the leaves of olive cultivar, 'Gemlik' in 2009.

Day	Example on day	Chlorogenic acid [Mean±SE (mg g^{-1})]	Caffeic acid [Mean±SE (mg g^{-1})]	3-hydroxycinnamic acid [Mean±SE (mg g^{-1})]	p-coumaric acid [Mean±SE (mg g^{-1})]	Oleuropein [Mean±SE (mg g^{-1})]	Scopolin [Mean±SE (mg g^{-1})]
14	14.01.09	13.42±0.92 p[a]	12.01±0.15 f	14.82±0.34 ıj	11.05±0.34 ghıj	3.08±0.08 c	1.05±0.01 s
22	22.01.09	20.74±1.85 o	14.75±0.21 c	19.83±1.71 g	10.52±0.17 ghıj	1.68±0.03 n	0.82±0.04 t
34	03.02.09	25.07±0.24 n	17.33±0.03 b	24.93±2.02 d	11.51±0.59 fghıj	2.04±0.05 ı	0.78±0.03 t
41	10.02.09	20.96±0.49 o	14.87±0.03 c	17.09±0.16 h	12.19±0.16 fgh	1.82±0.06 m	1.36±0.06 q
50	19.02.09	38.30±0.78 jk	12.98±0.71 e	22.70±0.92 e	15.07±0.21 a	2.46±0.02 g	0.32±0.02 w
70	10.03.09	25.01±0.86 n	19.46±0.22 a	15.33±0.08 ı	13.78±1.28 def	1.01±0.01 r	0.67±0.03 u
83	23.03.09	30.73±0.34 m	13.55±0.18 de	15.62±0.59 ı	14.89±0.07 cde	1.14±0.01 q	1.26±0.05 r
95	02.04.09	14.46±0.46 p	14.12±0.30 cd	22.12±0.02 ef	15.20±0.86 bcde	1.66±0.01 n	1.41±0.06 q
101	10.04.09	16.10±0.42 p	19.37±0.55 a	19.87±0.00 g	15.14±0.47 bcde	0.76±0.00 s	0.57±0.01 u
111	20.04.09	9.60±1.66 q	19.51±1.06 a	14.56±0.68 ıj	19.07±0.85 a	1.26±0.00 p	1.52±0.03 p
125	04.05.09	15.67±1.43 p	11.69±0.64 fg	14.98±0.30 ıj	17.41±0.81 abc	1.27±0.02 p	2.39±0.03 mn
131	10.05.09	15.83±1.65 p	11.78±0.47 fg	12.50±0.14 l	17.43±1.39 abc	0.53±0.01 t	2.83±0.04 ı
141	20.05.09	43.43±2.37 ı	10.97±0.63 g	26.73±1.12 c	17.65±0.70 ab	1.01±0.01 r	2.68±0.02 j
153	01.06.09	54.86±0.88 f	4.10±0.08 kl	29.57±0.28 b	9.08±0.03 jklm	1.03±0.01 r	4.03±0.05 d
162	10.06.09	70.22±0.02 a	1.35±0.28 m	31.66±0.35 a	6.66±0.23 mnop	1.20±0.00 q	4.51±0.08 b
172	20.06.09	54.44±0.91 f	5.69±0.34 j	12.45±0.90 l	5.80±0.25 op	1.67±0.06 n	3.68±0.03 e
183	01.07.09	70.71±1.82 a	2.05±0.17 m	21.04±1.12 fg	7.22±0.50 lmno	1.91±0.00 k	3.57±0.01 f
192	10.07.09	65.38±1.33 b	3.19±0.05 l	17.89±0.28 h	4.13±0.03 p	2.44±0.06 g	4.68±0.05 a
202	20.07.09	48.33±0.91 g	2.27±0.08 m	14.41±0.16 ıjk	5.24±0.00 op	1.06±0.00 r	4.31±0.04 c
214	01.08.09	59.58±0.14 d	2.22±0.00 m	13.01±0.06 kl	10.16±0.40 ghıjk	1.44±0.01 o	4.50±0.02 b
223	10.08.09	44.20±0.06 ı	1.74±0.04 m	5.04±0.12 q	6.43±0.10 nop	1.82±0.01 m	3.46±0.07 g
234	21.08.09	44.32±0.42 ı	1.54±0.05 m	5.14±0.03 q	4.63±0.10 p	3.04±0.01 c	4.61±0.05 a
245	01.09.09	47.33±1.29 gh	2.00±0.00 m	10.04±0.01 mn	18.25±1.33 a	2.12±0.03 h	3.27±0.08 h
254	10.09.09	48.80±0.33 g	1.61±0.07 m	5.07±0.24 q	12.74±0.04 efg	2.17±0.02 h	4.05±0.06 d
263	19.09.09	62.73±1.10 c	6.12±0.03 ıj	10.76±0.28 m	13.87±0.05 def	1.99±0.02 ıj	3.52±0.04 fg
275	01.10.09	45.65±0.40 hı	4.40±0.11 k	10.97±1.00 m	10.23±0.94 ghıjk	1.99±0.01 ıj	2.46±0.03 lm
280	06.10.09	53.30±0.18 f	3.40±0.29 kl	8.70±0.14 no	15.53±0.09 bcd	1.96±0.01 jk	2.67±0.01 j
293	19.10.09	57.66±1.03 de	6.82±0.13 ı	8.31±0.01 op	18.54±0.05 a	2.02±0.02 ıj	2.55±0.01 kl
306	01.11.09	58.29±0.72 de	3.93±0.36 kl	7.27±0.30 op	11.80±0.06 fghı	1.85±0.02 lm	2.62±0.01 jk
316	10.11.09	65.46±0.37 b	8.69±0.61 h	7.14±0.47 p	11.34±0.18 fghıj	1.90±0.00 kl	2.40±0.04 mn
325	20.11.09	39.77±2.96 j	8.20±0.83 h	7.66±0.44 op	11.63±0.67 fghıj	2.92±0.03 d	2.20±0.03 o
336	01.12.09	55.84±1.93 ef	6.83±0.52 ı	7.19±0.85 p	9.20±0.64 ıjklm	2.64±0.01 f	2.33±0.05 n
345	10.12.09	36.22±1.16 kl	11.00±0.90 g	13.50±0.15 jkl	9.56±0.14 hıjkl	3.54±0.01 b	2.51±0.03 l
355	20.12.09	35.42±1.44 l	8.49±0.31 h	14.65±0.05 ıj	9.46±0.10 ıjkl	2.85±0.01 e	2.33±0.02 n
366	31.12.09	39.76±1.31 j	6.50±1.09 ıj	14.65±0.76 ıj	8.35±0.41 klmn	3.95±0.02 a	2.80±0.06 ı

[a] Mean values followed by different lower-case letters differ significantly by Duncan's multiple range test at $P< 0.05$.

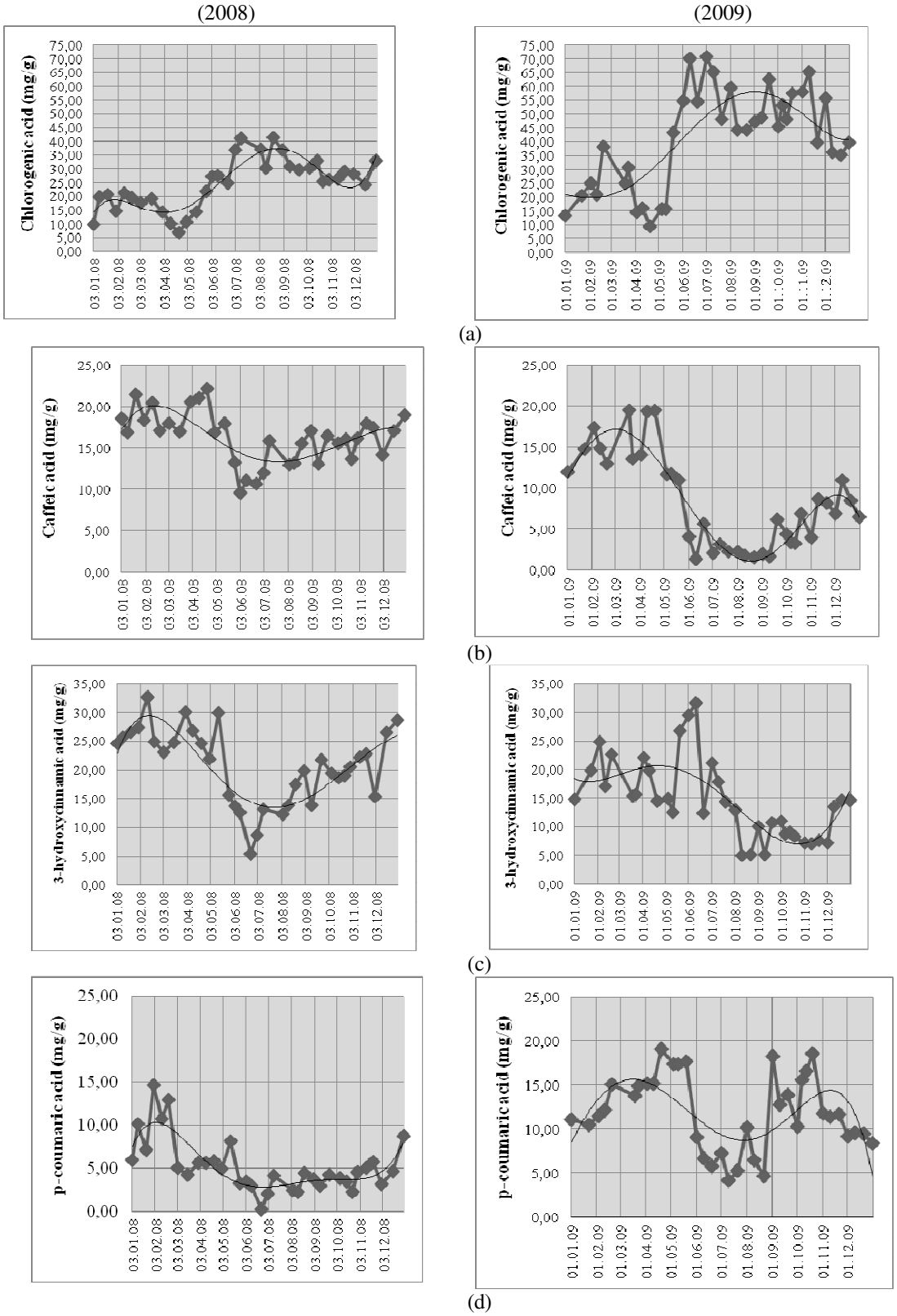

Figure 1. The change in level of (a) chlorogenic acid, (b) caffeic acid, (c) 3-hydroxycinnamic acid, (d) p-coumaric acid , (e) scopolin (f) oleuropein, in the leaves of cultivar, 'Gemlik' in 2008 and 2009.

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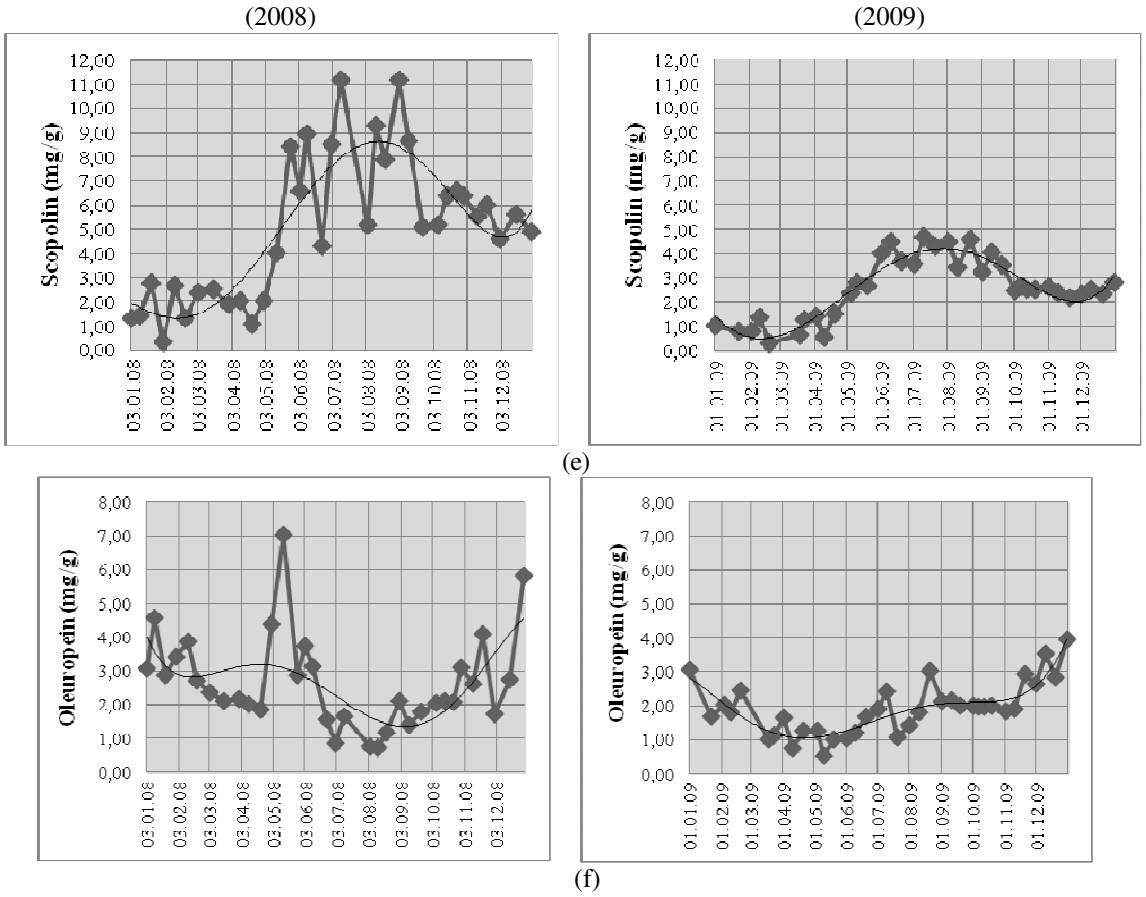

Figure 1. Continued.

in 2008 and 0.53-3.95 mg g^{-1} in 2009 (Tables 3 and 4).

Chlorogenic Acid (5-caffeoylquinic)

The change in the level of chlorogenic acid in the leaves showed similar distributions in both years (Figure 1-a). In 2008, the chlorogenic acid level increased markedly in January (9.91-20.77 mg g^{-1}), ranged between 17.54 and 21.13 mg g^{-1} in February and March, and decreased to a minimum level in April (6.94 mg g^{-1}) (Table 3, Figure 1-a). Thereafter, the chlorogenic acid level gradually increased in May through June and reached a maximum level in July-August (30.13-41.37 mg g^{-1}). Although the level of accumulation of chlorogenic acid in the leaves was low in September, it increased again during the period between September and December (Table 3, Figure 1-a). Higher levels of chlorogenic acid were noted in 2009 (on year) compared with the levels in 2008 (off year) (Figure 1-a). In 2009, it gradually increased from January to March (13.42-38.30 mg g^{-1}) and then decreased sharply in April (14.46 mg g^{-1}), reaching a minimum level at the end of April (9.60 mg g^{-1}). The level of chlorogenic acid began to increase in the middle of May and reached a maximum level in June (70.22 mg g^{-1}) and July (70.71). Although the abundance decreased slightly, it generally reached the highest level from August to December (Table 4, Figure 1-a). As reported by some researchers, chlorogenic acid appears to play an important role in flower bud induction (Lavee *et al.*, 1986; Lavee, 1989; Lavee *et*

al., 1994; Ryan *et al*., 2003; Ercan and Ozkaya, 2008). The time of flower bud induction was reported by some researchers to be July (Fernandez-Escobar *et al.*, 1992), whereas several studies have indicated that the main period of flower bud differentiation occurs in October, November, January and March (Pinney and Polito, 1990; Ferguson *et al*., 1994; Barut and Erturk, 2002). Heimler *et al*. (1996) stated that the level of chlorogenic acid in olive leaves reaches its maximum in early winter (December). In another study, chlorogenic acid was found to begin accumulating in the leaves of fruiting olive trees after fruit set and continued increasing until pit hardening. The level was reported to remain high until the next fruit set period in the following year (Lavee *et al*., 1986). Lavee (1989) has proposed a metabolic relationship between olive fruits and leaves, whereby alternate bearing is initiated by a signal, probably hormones, which diffuse from the developing fruits to the leaves. In our study, chlorogenic acid was found to increase during embryo development and reached its highest amount during the pit-hardening period in July and August in both study years. In addition, several reports have identified the central role of chlorogenic acid in alternate bearing (Lavee *et al*., 1986; Lavee, 1989; Lavee *et al*., 1994; Ryan *et al*., 2003): concentrations of chlorogenic acid have been found to be 3-4 times higher in the mature full-size leaves from the previous season's fruit-bearing trees, as compared to the leaves of non-fruiting trees (Lavee, 1989). The changes in the levels of chlorogenic acid that occur during the flowering and fruit-set period have also been examined (Lavee *et al*., 1986). These results are in agreement with the trend observed in the present study.

Caffeic Acid

The alterations in the caffeic acid level in the leaves showed similar distributions in 2008 and 2009 (Figure 1-b). The level was high from January to April and reached its highest value in April; it started to decrease at the beginning of May and reached the minimum value in June-August. The amount of caffeic acid gradually increased again during the September-December period (Tables 3 and 4, Figure 1-b). However, lower accumulation levels of caffeic acid in the leaves were observed in 2009 (on year) compared with the levels in 2008 (off year) (Figure 1-b). In 2009, the amount of caffeic acid was notably lower, and this result may be related to alternate bearing. A probable relationship between the caffeic acid and chlorogenic acid levels in the leaves was found: whereas the level of one shows an increasing tendency, the other compound starts to decrease in both years (Figures 1 a and 1-b). Ryan *et al.* (2003) reported caffeic acid as a probable metabolic precursor of chlorogenic acid and that, when the caffeic acid level was high, the chlorogenic acid content was low in olive cultivar 'Hardy's Mammoth'. Our results agree with the results of that study.

Hydroxycinnamic Acid

The 3-hydroxycinnamic acid levels in the leaves were generally high in both years. In 2008, the 3-hydroxycinnamic acid level was high during the January-May period and increased to maximum levels in the middle of February (32.69 mg g^{-1}) and at the end of March (30.11 mg g^{-1}). Thereafter, the level began to decrease at the end of May and reached a minimum at the end of June (5.38 mg g^{-1}). This phenolic compound was found in lower levels in the leaves from June to August and then increased gradually during the September-December period (13.78-28.68 mg g^{-1}) (Table 3, Figure 1-c). As is the case with caffeic acid, lower levels of 3-hydroxycinnamic acid were found in 2009, as compared with the levels in 2008 (Figure 1-c). In 2009, the 3-hydroxycinnamic acid levels in the leaves were lower during the August-November period and higher in the other months; it reached a maximum in June and began to decrease in the middle of July and reached a minimum in the August-November period (Table 4, Figure 1-c). This compound was present in the leaves in different quantities in each year and tended to decrease when

chlorogenic acid was in abundance. Due to this tendency, this phenolic is probably related to the alternate bearing of olive trees.

p-Coumaric Acid

In 2008, it was found that the accumulation of p-coumaric acid reached the maximum level during the January-February period (10.77; 14.73 mg g^{-1}). The level of this compound sharply decreased in March (5.09 mg g^{-1}) and reached a minimum in June (0.26-2.97 mg g^{-1}) (Table 3, Figure 1-d), and the accumulation of p-coumaric acid was lower in the leaves during the June-October period. A higher accumulation of p-coumaric acid in the leaves was determined in 2009, and the level of accumulation of this phenolic compound was lower from June to August than in the other months (Table 3, Figure 1-d). In 2009, the accumulation of p-coumaric acid gradually increased from January to May and reached a maximum level at the end of April (19.07 mg g^{-1}) through May (17.65 mg g^{-1}). The amount of this compound sharply decreased from June to July, and the lowest level was observed in July (Table 4, Figure 1-d). The same fluctuation pattern in the production of caffeic acid was found for p-coumaric acid in 2009, but the p-coumaric acid level was higher in this year than in 2008 (Figures 1-b and 1-c). Thus, p-coumaric acid may play an important role in determining alternate bearing.

Scopolin

The scopolin level in the leaves fluctuated similarly in both years (Figure 1-e): this product was at a low level from January to April and started to increase at the beginning of May, reaching a maximum level from June to September (Tables 3 and 4, Figure 1-e). Although the variations in the levels of scopolin were generally in parallel with the variation in the chlorogenic acid levels in both years (Figures 1-a and 1-e), it was determined that the year in which the accumulation of chlorogenic acid increased, the scopolin accumulation decreased. Thus, it was suggested that scopolin may be related to alternate bearing.

Oleuropein

For 2008, the annual variation in the quantity of oleuropein in the leaves demonstrated the following pattern: during the January-April period, the level changed between 1.85-4.57 mg g^{-1} and reached the maximum level in the middle of May (7.05 mg g^{-1}); in June, the level sharply decreased (2.88 mg g^{-1}), and then a gradual decrease continued in July and August (0.73-1.56 mg g^{-1}), reaching a minimum level in this period; the leaf level showed a gradual increase during the September-December period (Table 3, Figure 1-f). In 2009, lower oleuropein quantities were determined in the leaves compared to the quantities in 2008 (Tables 3 and 4). In 2009, higher levels were found in December, January and February, with lower levels in the March-June period (Table 4, Figure 1-f). Malik and Bradford (2006) studied the changes in the levels of oleuropein during the early stages of flower formation and during fruit development and maturation periods. These authors stated that, after floral bud differentiation, the oleuropein levels progressively increased from the flowering to fruiting stages and then declined as the fruit began to mature, reaching a negligible level in the fully mature black fruit stage. In another study, the polyphenol and oleuropein levels were determined in the leaves of six olive cultivars for three different periods (May, July, and December), with the oleuropein content of the leaves reaching a maximum level in May for cultivar 'Maurino' (4.8 mg g^{-1}), in July for cultivar 'Leccio del corno' (7.43 mg g^{-1}), and in December for the other 4 cultivars (Fabbri *et al.*, 2008). In our study, the oleuropein level was found to be at the lowest level during embryo development and the pit-hardening stage, and the highest level was found during the dormant period.

CONCLUSIONS

In conclusion, the annual variation in the amount of chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid, p-coumaric acid, scopolin and oleuropein in the leaves of the 'Gemlik' olive cultivar showed different quantities and distributions in "on" and "off" years. In the "on" year, the chlorogenic acid and p-coumaric acid levels were high, whereas the caffeic acid, 3-hydroxcinnamic acid and scopolin levels were significantly lower than in the "off" year. In contrast, the oleuropein level did not fluctuate notably in either year. Based on our results, we conclude that these phenolics, particularly chlorogenic acid, are involved in the alternate-bearing pattern of the olive cultivar 'Gemlik'.

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تغییرات فصلی و کمی مواد فنولی برگ در ارتباط با باردهی متناوب(سال آوری) درختان زیتون(*Olea europaea* L. cv. Gemlik)

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چکیده

در این پژوهش، با استفاده از دستگاه اچ پی ال سی(HPLC) اثر تغییرات فصلی و کمی مواد فنولی برگ در ارتباط با باردهی متناوب(سال آوری) درختان زیتون (*Olea europaea* L) رقم Gemlik مطالعه شد. به این منظور، غلظت موادی شامل oleuropein وchlorogenic acid ، caffeic acid، 3-hydroxycinnamic acid، Scopolin وp-coumaric acid در برگ زیتون در دوره های ده روزه در طی سال اندازه گیری شد. مقدار این مواد فنولی و توزیع آنها در طی سالهای ۲۰۰۸ (سال کم بار) و ۲۰۰۹(سال پر بار) تفاوتهای عمده ای نشان داد. در سال پر بار، غلظت

اسید های chlorogenic وp-coumaric زیاد بود در حالیکه مقدار دیگر مواد فنولی کم بود. بر خلاف این وضع، در طی سال کم بار دهی، اسیدهای chlorogenic و p-coumaric در غلظت کم و دیگر مواد فنولی در مقادیر بالا بودند. نتایج به دست آمده رابطه ای منفی بین غلظت دو اسید chlorogenic و caffeic در سالهای کم بار و پر بار نشان می داد به این معنا که مقدار اسید caffeic در برگه زیاد بود ولی مقدار اسید chlorogenic کم بود. مقدار موجود اسیدهای chlorogenic، caffeic، 3-hydroxycinnamic، وp-coumaric به طور معنی داری در سالهای کم بار وپربار با هم تفاوت داشت. این نتایج اشاره به آن دارد که مواد مزبور و دیگر مواد فنولی مطالعه شده در این پژوهش با پدیده باردهی متناوب ارتباط دارند. جالب اینکه مقدار همه مواد فنولی مطالعه شده در این بررسی در فصل خفتگی در حد بیشینه بود.