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Effect of Natural Lipid on Flowering, Pollination Traits and Fruit Set on Loquat (*Eriobotrya japonica* Lindl.)

T.H. DEMIRKESER*, A.A. POLAT and O. CALISKAN
Department of Horticulture, Faculty of Agriculture
University of Mustafa Kemal, Antakya, Hatay 31034, Turkey
E-mail: demirkeser@gmail.com

In this study, the possible effects of lysophosphatidylethanolamine when applied onto eight-year-old *Eriobotrya japonica* Lindl. loquat cultivar is investigated. Treatments included both single and double applications of lysophosphatidylethanolamine sprayed before bloom and an untreated control. The treated buds were sampled from trees for laboratory pollen experiment and the trees were observed for several flowering, fruit set and harvest traits. In laboratory experiment, the pollen grains were sowed on media having various sucrose concentrations (5, 10, 15 and 20 %) and incubated on 10, 15 and 20 °C. It was observed that pollen germination and tube growth increased on 15 and 20 °C incubation temperatures and 10 and 20 % concentrations. The experiment also indicated that single lysophosphatidylethanolamine application increased the pollen viability rate (%). Pollen germination rate (%) and pollen tube growth were negatively affected by lysophosphatidylethanolamine treatments when evaluated after 3, 6 and 9 h incubation. The field observations indicated that lysophosphatidylethanolamine conditioned earliness for flowering and harvest date. The double application also had significantly higher fruit set comparing the control. Taken together, the results indicate that lysophosphatidylethanolamine applications may be beneficial for fruit set and earliness in loquat production.

Key Words: Lysophosphatidylethanolamine, *Eriobotrya japonica*, Pollination, Fruit set, Mediterranean, Pollen viability.

INTRODUCTION

An evergreen species loquat (*Eriobotrya japonica* Lindl.) is a member of Rosaceae family and exhibit unusual flowering habit. The loquat flowers are borne in fall or early winter in panicles at the ends of the branches. Under costal Mediterranean conditions of Turkey for several loquat cultivar, beginning of flowering varied between middle of November to middle of January while full flowering was in January; flowering in all cultivars tested ended in February[1]. Regions with Mediterranean climates are suitable for loquat production. However, because the loquat flowers during winter the occasional low temperatures might be harmful for flowering and fruit set. Frost or cold temperatures damage or kill flowers or flowers' organs. Indeed,

temperatures below -2 ºC damage open flowers and small fruits, while set fruit is damaged[2,3] at a temperature of -1 ºC. Viability and germination of pollen can also be affected by low temperatures[4,5].

In an earlier report, it was shown that lysophosphatidylethanolamine application prior to bloom may be advantageous for loquat cultivars grown on Mediterranean ecological conditions as lysophosphatidylethanolamine increased pollen germination rate (%) and enhanced pollen tube growth for 'Sayda' cultivar[6]. Indeed, other effect of lysophosphatidylethanolamine in being plant growth regulators for accelerating ripening, retarding ethylene-promoted leaf senescence and softening of some of agrochemicals have been reported recently[7-11].

Eriobotrya japonica is an important, early loquat cultivar. Polat *et al.*[12] compared the flowering and harvest period for three loquat cultivar on open-field and protected fruit culture under greenhouse conditions. Appearance of flower buds are first observed in *Eriobotrya japonica* on both of the growing treatments. Moreover, the earliest fruits were harvested from *Eriobotrya japonica* on both open field and greenhouse. Therefore, *Eriobotrya japonica* is an ideal cultivar to investigate possible effects of lysophosphatidylethanolamine application, as these effects may be more profound for early cultivars.

The objective of the present study was to determine the effect of lysophosphatidylethanolamine applications on pollen viability, pollen germination rate and pollen tube growth. It also aimed at determining the flowering and harvesting traits under growing conditions.

EXPERIMENTAL

The experiments were conducted on the Mediterranean costal region in Hatay, Turkey. The orchard was located on 36°51' N, 36°09' E, 8 m. Three randomly-chosen *Eriobotrya japonica* trees, eighth-year old and grafted on seedlings were used for the study. Lysophosphatidylethanolamine is a naturally occurring phospholipid derived by phospholipase A2 hydrolysis of phosphatidylethanolamine and was commercialized for use as a bioregulator due to its ability to stimulate fruit ripening but delay senescence[4]. The treatments included applications of lysophosphatidylethanolamine: (1) control treatments were left untreated; (2) single application of lysophosphatidylethanolamine sprayed on selected branches on 26 October 2005 and (3) a second application on the same branches on 7 December 2005. Two weeks after the second application buds were sampled for further study. The experiments included laboratory and field studies.

Experiment-1: In laboratory experiments the pollen viability test was conducted on the pollen collected from these treatments by standard methods[13]. Shortly, the pollen grains were sowed at 1 % triphenyl tetrazolium

chloride solution and examined after 2 h under light microscope. In each treatment six randomly chosen areas were investigated. The pollen grains with dark red colour were considered as viable while light red-coloured ones were counted as semi-viable. The dead grains were not coloured. Only the percentage viability was subjected to the analysis of variance (Anova) using SAS[14]. The experimental design was a completely randomized design (CRD). The data were $\sqrt{\arcsin}$ transformed to improve normality, but original data were used to present means.

Experiment-2: For the pollen germination rate and pollen tube growth determinations, randomly sampled buds were kept on a room temperature. The pollen were germinated on various temperature (incubated at 10, 15 and 20 ºC) and sucrose concentration (5, 10, 15 and 20 %) treatments. At the end of these treatments the petri dishes were placed on -20 ºC. Germination rates and pollen tube growths were examined under a light microscope after 3, 6, or 9 h incubation. In each petri six different regions with equal size were evaluated. In each region, germinated and total numbers of pollen were counted and the ratios were calculated. The lengths of the five randomly chosen germinated pollen tubes were measured. The average pollen tube growth was calculated from the means of five values. Statistical analyses were conducted using GLM procedure of SAS[14]. A factorial design using a split plot model was employed. In this model, lysophosphatidylethanolamine treatment was whole-plot, incubation temperature was sub-plot and sucrose concentration was sub-sub-plot. Means of main effects were separated by Tukey and using appropriate error terms at 5 %. Although pollen germination data was $\sqrt{\arcsin}$ transformed to improve normality, original data were used to present means.

Experiment-3: On the field experiment beginning of lowering, full flowering, end of flowering, fruit set and harvest dates were recorded. The beginning of flowering, full flowering, end of flowering was determined when 5, 50 and 95 % of the flowers opened, respectively. These percentages were estimated considering all of the experimental units; that is to say, these values were not recorded as each replication. Therefore, no statistical tests were carried out for this part of data set.

In each replication of lysophosphatidylethanolamine treatments number of buds was counted on 11 November 2005. The flower, initial fruit and harvested fruits were counted on 14 December 2005, 24 February 2006 and 6 May 2006. These numbers were converted into the flowering rate (%), initial fruit set (%) and final fruit set (%) by dividing them by bud number. Similar to Experiment-1, the experimental design was a CRD. Because they were in percentage, the data were $\sqrt{\arcsin}$ transformed to improve normality, but original data were used to present means.

RESULTS AND DISCUSSION

Experiment-1: The effect of lysophosphatidylethanolamine treatments on pollen viability variables is presented in Table-1. The pollen viability was highest on single application (91.5 %) of lysophosphatidylethanolamine followed by double application (81.4 %) and control (75.5 %).

TABLE-1
MEANS AND STANDARD DEVIATION OF LYSOPHOSPHATIDYL-ETHANOLAMINE TREATMENTS FOR POLLEN VIABILTY VARIABLES ON *Eriobotrya japonica* CULTIVAR

Treatment[1]	Viable	Semi-viable	Dead	Viability (%)
Control	20.9 ± 5.3	12.2 ± 8.6	10.3 ± 3.3	$75.5^{b,2}$ ± 9.0
Single application	20.8 ± 5.0	13.8 ± 5.4	3.2 ± 1.3	91.5^{a} ± 3.5
Double application	14.8 ± 2.5	19.2 ± 3.9	7.8 ± 3.1	81.4^{ab} ± 6.4
Mean	19.0 ± 5.2	14.9 ± 6.8	6.9 ± 4.0	83.1 ± 9.4

[1]Control treatments were left untreated while 100 ppm lysophosphatidylethanolamine was sprayed before bloom for single application and an additional 100 ppm lysophosphatidylethanolamine sprayed after 6 weeks from the first one for the double application treatment.
[2]Significance test was carried out using LSD at 5 %.

Experiment-2: The lysophosphatidylethanolamine treatments significantly affected the pollen germination rate for 3, 6 and 9 h incubation (Table-2). The germination rate increased as incubation duration expanded (Table-3). The averages for 3, 6 and 9 h lysophosphatidylethanolamine treatments were 84.1, 86.3 and 88.8 %, respectively. Regardless the incubation duration, the highest pollen germination rates were recovered for control while the differences between lysophosphatidylethanolamine treatments were not statistically significant (Table-3). The incubation temperature effects were also statistically significant for all incubation duration treatments (Table-2). As the temperature increased, the pollen germination rate also increase for all duration treatments. For example, at 3 h incubation at 10, 15 and 20 °C, treatments had the averages of 72.8, 88.5 and 91.0 %, respectively (Table-3). The main effect of the sucrose concentration was also significant for all incubations (Table-2). However, there were no obvious trends to explain the effect. The two- and three-way interactions were also significant at 3, 6 and 9 h which were difficult to explain. Indeed, the sucrose effect was not consistent as different treatments had the highest values for different incubation durations. In general, however, 10 and 20 % sucrose concentrations gave higher pollen germination rates (Table-3).

The pollen tube growth results had similar trends to those of pollen germination rate. For example, all main effects were statistically significant for all incubation treatments (Table-2). Overall, the pollen tube length

TABLE-2
RESULTS OF THE SIGNIFICANCE TEST OF LYSOPHOSPHATIDYL-ETHANOLAMINE TREATMENTS FOR POLLEN GERMINATION RATE AND POLLEN TUBE GROWTH ON *Eriobotrya japonica* CULTIVAR

Source	df	Pollen germination rate (%)			Pollen tube growth (μ)		
		3 h	6 h	9 h	3 h	6 h	9 h
Treatment (T)[1]	2	949.5‡	432.7‡	1086.4‡	1433.0‡	436.8‡	1002.5‡
Whole-plot error	15	22.7	18.5	21.1	4.9	9.9	26.6†
Temperature (C)[2]	2	4597.3‡	3462.8‡	2393.9‡	120.6‡	775.1‡	1842.3‡
T × C	4	35.4	49	67.0‡	36.6	29.8	39.1‡
Sub-plot error	30	23.2	18.8	14.6	14.3	14.1	9.2
Media (M)[3]	3	1145.4‡	1282.1‡	704.2‡	2625.8‡	2966.0‡	5696.3‡
M × T	6	392.9‡	391.9‡	294.8‡	265.8‡	349.8‡	270.2‡
M × C	6	117.9‡	168.6‡	113.7‡	115.3‡	98.8‡	692.3‡
M × T × C	12	71.8‡	75.0‡	84.5‡	62.8‡	39.0‡	142.9‡
Error	135	29.7	23.9	25.8	12.5	12.3	14.5

[1]Control treatments were left untreated while 100 ppm lysophosphatidylethanolamine was sprayed before bloom for single application and an additional 100 ppm lysophosphatidylethanolamine sprayed after six weeks from the first one for the double application treatment.
[2]The pollen was incubated at 10, 15 or 20 °C.
[3]The growing media had 5, 10, 15 or 20 % sucrose concentration.

also increased as incubation temperature increased (Table-3). In addition, the two- and three-way interactions of media were significant for all incubations. However, the pollen tube growth averages were not progressively increased based on incubation durations (31.1, 29.0 and 35.5 for 3, 6 and 9 h).

Experiment-3: The effect of the lysophosphatidylethanolamine treatments on flowering, fruit set and harvest date are presented in Table-4. Because the data were collected considering all replications for each treatment, it was not possible to conduct a significance test. Also, the experimental plots were started to be investigated on 9 November 2006 and beginning of flowering for double application which preceded that date was missed. However, full flowering and end of flowering dates were similar for both single and double applications which were 5 and 7 d earlier than untreated control. Single and double applications also had the same days for fruit set and harvest dates. The fruit set was 5 d earlier in lysophosphatidylethanolamine treatments when compared to the control while they were harvested 8 days earlier than the control.

Flowering rate, initial fruit set and final fruit set are presented in Fig. 1. The lysophosphatidylethanolamine treatment did not have a significant effect on flowering rate and initial fruit set. However, the final fruit set was higher in double application when compared to the control.

TABLE-3
MEANS AND STANDARD DEVIATIONS OF RESULTS OF LYSOPHOSPHATIDYLETHANOLAMINE TREATMENTS FOR POLLEN GERMINATION RATE AND POLLEN TUBE GROWTH ON *Eriobotrya japonica* CULTIVAR

Source	Pollen germination rate (%) Incubation duration			Pollen tube growth (μ) Incubation duration		
	3 h	6 h	9 h	3 h	6 h	9 h
Treatment[1]						
Control	88.8^{a}±9.4	90.0^{a}±6.5	93.1^{a}±5.2	36.1^{a}±7.3	31.7^{a}±6.4	39.7^{a}±10.9
Single application	82.2^{b}±15.3	85.2^{b}±14.0	86.1^{b}±11.6	29.4^{b}±8.4	28.1^{b}±9.7	32.6^{b}±13.1
Double application	81.3^{b}±11.9	83.8^{c}±13.2	87.2^{b}±8.2	27.7^{c}±8.1	27.0^{b}±9.5	34.1^{b}±12.0
Incubation temperature (°C)						
10	72.8^{c}±12.9	77.2^{c}±14.3	83.2^{c}±8.3	30.3^{b}±7.8	26.9^{b}±8.6	31.1^{c}±9.2
15	88.5^{b}±9.5	89.0^{b}±8.2	89.0^{b}±10.3	30.3^{b}±10.0	27.2^{b}±7.4	34.4^{b}±9.9
20	91.0^{a}±6.5	92.8^{a}±5.3	94.2^{a}±4.8	32.6^{a}±8.2	32.8^{a}±9.3	41.0^{a}±15.2
Sucrose concentration (%)						
5	83.0^{b}±11.6	86.8^{b}±7.7	88.7^{b}±5.9	25.6^{c}±4.5	25.5^{c}±4.4	28.3^{c}±5.8
10	88.2^{a}±8.0	91.0^{a}±4.8	91.6^{a}±5.7	31.8^{b}±6.1	30.2^{b}±4.6	36.7^{b}±5.2
15	76.1^{c}±16.5	77.1^{c}±17.7	82.6^{c}±13.7	26.2^{c}±9.0	21.5^{d}±8.8	27.4^{c}±9.7
20	89.0^{a}±9.5	90.3^{a}±7.8	92.3^{a}±5.8	40.7^{a}±4.8	38.7^{a}±5.6	49.6^{a}±12.1
Mean	84.1±12.8	86.3±12.0	88.8±9.2	31.1±8.7	29.0±8.8	35.5±12.4

[1]Control treatments were left untreated while 100 ppm lysophosphatidylethanolamine was sprayed before bloom for single application and an additional 100 ppm lysophosphatidylethanolamine sprayed after six weeks from the first one for the double application treatment.

TABLE-4
EFFECTS OF LYSOPHOSPHATIDYLETHANOLAMINE TREATMENTS FOR FLOWERING, FRUIT SET AND HARVEST DATE *Eriobotrya japonica* CULTIVAR

Treatment[1]	Beginning of flowering (5 %)	Before full flowering (50 %)	End of flowering (95 %)	Fruit set	Harvest date
Control	9 Nov.	12 Dec.	4 Jan.	23 Jan.	11 May
Single application	9 Nov.	7 Dec.	28 Dec.	18 Jan.	3 May
Double application	–	7 Dec.	28 Dec.	18 Jan.	3 May

[1]Control treatments were left untreated while 100 ppm lysophosphatidylethanolamine was sprayed before bloom for single application and an additional 100 ppm lysophosphatidylethanolamine sprayed after six weeks from the first one for the double application treatment.

Single application of lysophosphatidylethanolamine increased the pollen viability. However, it is not known why the mean double application was on the same mean group by both single application and the control. It is possible that the date of the double application was too late to be beneficial for the pollen. In earlier report, lysophosphatidylethanolamine application on pollen viability was not statistically significant for 'Sayda' cultivar[6].

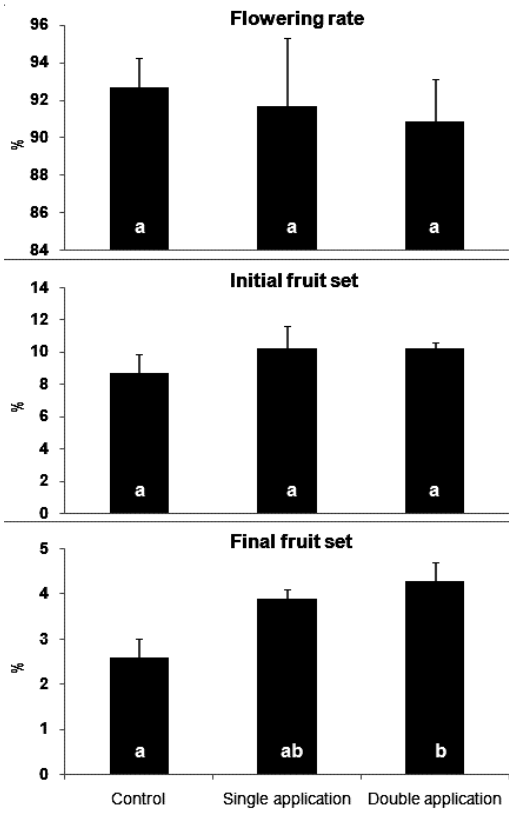

Fig. 1. Effect of lysophosphatidylethanolamine treatments[1] for flowering characteristics on *Eriobotrya japonica* cultivar

[1]Control treatments were left untreated while 100 ppm lysophosphatidylethanolamine was sprayed before bloom for single application and an additional 100 ppm lysophosphatidyl-ethanolamine sprayed after six weeks from the first one for the double application treatment.

Lysophosphatidylethanolamine applications significantly reduced the pollen germination rates as well as pollen tube growth in the laboratory experiments for *Eriobotrya japonica*. This is contradictory to the earlier report where both treatments gave higher mean values for both of these variables[6]. It is not clear why two studies gave different results. A possible explanation of this discrepancy is the cultivar effect. In any case, although the pollen germination ratio was reduced up to 9 % and pollen tube growth was inhibited up to 18 % when compared to the control it is arguable that these statistically significant differences were indeed biologically significant. Even our minimum values for pollen germination rate and pollen tube growth are satisfactory for loquat pollination[15].

The incubation temperature and sucrose concentration affected both the traits evaluated in the experiments. Overall, an increasing trend was present by incubation temperature for pollen germination rate and tube growth.

These finding are similar to those of Demirkeser *et al.*[6]. It was found that the most preferable sucrose concentrations for pollen germination were 10 or 20 % treatments. Bolat and Pirlak[12] found that 15 % sucrose concentration gave the highest pollen germination for several apricot, sweet and sour cherry cultivar. The differences in two studies may be caused by the pollen sources (*i.e.*, cultivar differences) as 'Sayda' had its highest rate in 20 % sucrose concentration as well[6].

The effects of lysophosphatidylethanolamine treatments would be critical if they are supported by experiment conducted on growing conditions. The field experiment might serve as an indicator for this purpose. The field studies indicated that lysophosphatidylethanolamine application conditioned earliness for all flowering traits. The beneficial effect was also present for fruit set and harvest date. The single and double applications were not found to be different for these traits. lysophosphatidylethanolamine did not affect flowering rate and initial fruit set. However, final fruit set was improved by double application of lysophosphatidylethanolamine when compared to control.

It was found that lysophosphatidylethanolamine had significant effect on pollen viability, pollen germination rate and pollen tube growth under laboratory conditions for *Eriobotrya japonica* cultivar grown under Mediterranean conditions. The positive effects were detectable on flowering traits, fruit set and harvest date. In conclusion, the results of the present study indicate that lysophosphatidylethanolamine applications may be beneficial for fruit set and earliness in loquat production.

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