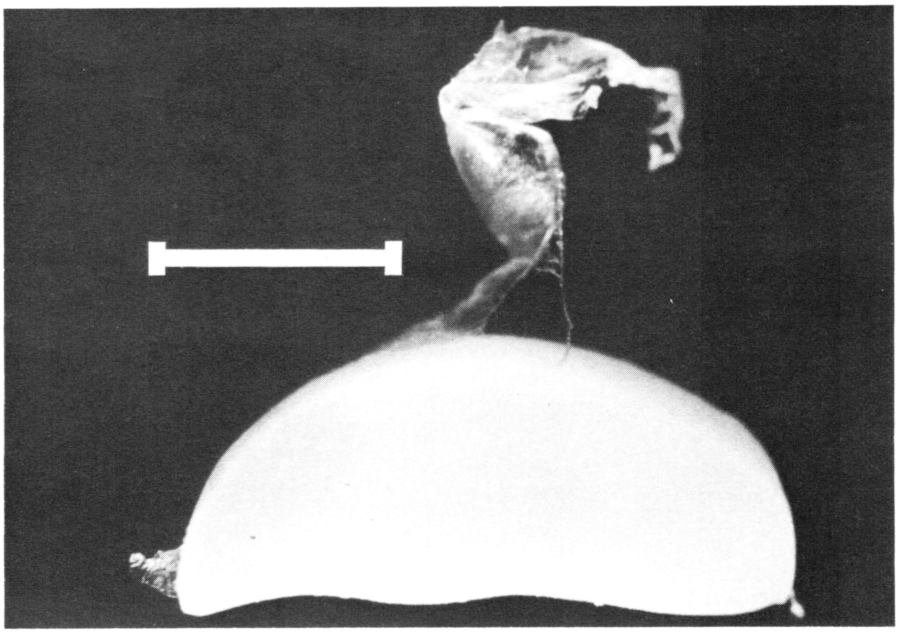

Fig. 4. A single cotyledon of a 'Temple' tangor coated with Polyox, partially removed to show its film forming ability (bar = 0.5 cm).

lyox WSR-N 750 was chosen for further consideration as a synthetic seed coat for in vitro-produced asexual embryos.

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Improving 'Manzanillo' Olive Seed Germination

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Additional index words. *Olea europaea*, stratification, chemical scarification

Abstract. **'Manzanillo' olive (*Olea europaea* L.) seeds were subjected to chemical scarification with NaOH and H_2SO_4 for various periods of time to determine the most appropriate treatment for improving the germination of the seeds. A critical balance of concentration and time was necessary to achieve high germination percentages without loss of viability of the seeds. H_2SO_4 was more effective than NaOH in increasing germination percentages. Germination percentages as high as 98% were obtained on stratified seed using H_2SO_4, compared to 0% without chemical scarification.**

Although several cultivars are used in olive production, none have the less pendulous form suitable for mechanical harvesting, resistance to *Verticillium*, or dwarf stature. These qualitites in a scion or rootstock would increase productivity and reduce production costs significantly. Yet, in spite of these needs, little breeding for cultivar and rootstock improvement is being conducted on olives. One reason for the lack of effort is the low germination percentage of olive seeds (1, 2, 6, 8, 10), often as low as 5% to 10%, especially in the highly desirable, large-fruited varieties grown in California (7).

Olive seeds can reach high germination percentages if the endocarp is removed and internal dormancy is eliminated (4, 5, 9). Crisosto (3) determined that the endocarp prevented olive seed germination by mechanical resistance of embryo expansion. Lagarda (7) reported that 'Manzanillo' embryos require 800–1000 hr of stratification at 15°C to obtain high germination percentages, but he worked only on seeds which had the endocarp removed.

Practical considerations preclude excising the embryo when many seeds are to be planted, as in a breeding program. Thus, it is imperative that a means for overcoming the restrictive role of the endocarp be found which would result in improving germination of 'Manzanillo' seeds. This paper describes the development of methods to improve the germination of 'Manzanillo' olive seeds planted intact with endocarp.

'Manzanillo' olive fruit were collected from a block of 5 trees in the Univ. of California, Davis, Pomology Experimental Orchard in 1981 and 1982, and the exocarp was removed with a seed cleaner. The heavy, well-developed seeds were separated from light, empty seeds by using an aqueous solution of common salt (30%, w:v). Seeds that floated in the solution had no embryo and were discarded.

Concentrated sulfuric acid (H_2SO_4) and 3% sodium hydroxide (NaOH) were used for scarification. Four samples of 60 seeds each were scarified with either H_2SO_4 or NaOH for various time periods. After scarification, the seeds were rinsed in cold running water for 15 min.

After scarification, seeds were mixed with moist vermiculite and placed in polyethylene bags for stratification at 15°C for 30 days. The seeds then were germinated for 30 days at 20° to 25°C under a 16-hr photoperiod. Seeds were evaluated for germination immediately after removal from stratification and after the 30-day germination period. Germination was determined as the total number of seeds with a radicle protruding, and of seeds that were split and showing a swollen, slightly elongated radicle.

Table 1. Effect of scarification with NaOH prior to stratification at 15°C on germination of 'Manzanillo' olive seeds harvested in 1981.

Scarification treatment (hr)	Germination 30 days after stratification[z] (%)
0	0
1	21
3	18
6	24
12	26
24	79
36	73
48	89
72	57
96	15
LSD	12.8

[z]Data analyzed after arc sine transformation.

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Table 2. Effect of scarification with H_2SO_4 prior to stratification at 15°C on germination of 'Manzanillo' olive seeds harvested in 1981.

Scarification treatment (hr)	Germination (%)[z]	
	During stratification	1 month after stratification
0	0	0
6	2	79
12	35	83
18	94	98
24	85	94
30	94	97
36	88	67
42	22	75
48	0	0
LSD	9.6	11.6

[z]Data analyzed after arc sine transformation.

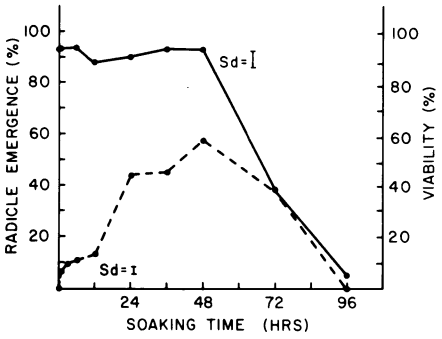

Fig. 1. Effect of soaking time in 3% NaOH on germination and viability of stratified 'Manzanillo' olive seeds harvested in 1981. Dotted line refers to germination (radicle emergence), and strought line refers to viability.

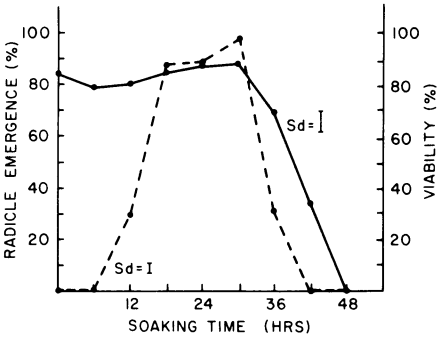

Fig. 2. Effect of soaking time in concentrated H_2SO_4 on germination and viability of stratified 'Manzanillo' olive seeds harvested in 1981. Dotted line refers to germination (radicle emergence), and straight line refers to viability.

Tests for seed viability were performed using a direct germination test on samples from all treatments. The endocarp was removed from the whole seed (embryo, endosperm, and seedcoat) with a rotary scarificator. The seeds were surface-sterilized with 20% laundry bleach (1.05% sodium hypochlorite) for 20 min and were rinsed twice with sterile water. Sixty embryos from each treatment were placed in 100 × 15 mm plastic petri dishes (15 per dish) on 2 layers of Whatman No. 1 filter paper moistened with 75% Thiram fungicide in a 2% aqueous slurry. Since the embryos were not excised, the seeds were stratified at 15°C for 30 days and then were incubated at 25° under 16-hr photoperiod until maximum germination percentage was reached. The embryo was considered to be viable if the radicle had elongated 2 mm at the time of recording.

Analysis of variance was conducted on data after arc sine transformation. The least significant difference (LSD) was calculated for pairwise multiple comparisons among treatment means in order to determine those means significantly different from controls.

The use of NaOH significantly increased germination over controls (Table 1). Soaking times from 24 to 48 hr produced the highest percentage of germination compared to other treatments. Soaking seeds in NaOH for 72 hr and longer resulted in significantly lower percentages of germination than for a 48-hr treatment period. Viability, which was high for most of the treatments, dropped significantly when seeds were soaked for 72 and 96 hr in NaOH (Fig 1).

The use of H_2SO_4 for scarification significantly increased germination of olive seeds (1981 harvest) over controls (Table 2). Treatments from 18–36 hr produced high levels of germination during the stratification period itself. During the additional month in which the seeds were incubated at 20° to 25°C for germination, very high rates of germination were achieved in all treatments except the control and the 48-hr soak, neither of which had any germination. Soaking times greater than 30 hr resulted in a marked decrease in viability and a significant decrease in germination (Fig. 2).

The effect of H_2SO_4 scarification was similar on 1981 and 1982 seeds. Since a decrease in viability had been observed with 36 hr of acid treatment using 1981 seeds, treatment periods only up to 30 hr were applied to 1982 seeds. The highest germination percentages were obtained using 20 and 30 hr of treatment (Table 3), and results were consistent with those from seeds harvested in 1981.

Sulfuric acid was more effective than NaOH for scarification, producing a higher germination percentage than NaOH at the same durations of treatment. The use of H_2SO_4 on seeds harvested in 1981 resulted in 98% germination, compared with an optimum of 89% when NaOH was used, and was more efficient than NaOH in decreasing the mechanical resistance of the endocarp without damaging the embryo.

Table 3. Effect of scarification with H_2SO_4 prior to stratification at 15°C on germination of 'Manzanillo' olive seeds harvested in 1982.

Scarification treatment (hr)	Germination (%)[z]					
	During stratification			30 days after stratification		
	Total[y]	Radicle[x]	Split[w]	Total	Radicle	Split
0	0	0	0	0	0	0
10	68	22	45	82	47	35
20	83	31	52	100	87	13
30	85	37	47	100	95	5
LSD	12.3	13.9	6.8	9.3	6.6	7.0

[z]Data analyzed after arc sin transformation.
[y]Includes split seeds plus seeds that show radicle emergence 1.0 cm.
[x]Radicle emergence greater than 1.0 cm.
[w]Open seeds with radicles less than 1.0 cm.

Results with 1982 seeds indicated that H_2SO_4 treatment of less than 18 hr may give higher germination percentages than those obtained in this study if prolonged germination periods are used after stratification. After 30 days of incubation, the percentages of split seeds decreased with a concomitant increase in the percentages of seeds with radicle emergence (Table 3). It is possible that most seeds that split during stratification germinate, although the time to germinate might be longer than the 30 days used in these experiments. If so, short treatment times of 20 hr, which produced the same number of split seeds during stratification as 30 hr, could be used. Longer periods to germination would be required.

The possibility of using mechanical scarification with a rotary scarificator instead of acid or alkali was tested. Althouh the use of the rotary scarificator was rapid, taking only 0.5 min to scarify 100 seeds, only 31% of the seeds had an intact embryo after treatment:

Using H_2SO_4 is more practical than excising embryos, especially when the inner seedcoat must be removed. The use of a rotary scarificator, although rapid and simple, resulted in many damaged embryos. The loss of a great number of seeds with potentially valuable germplasm would be unacceptable in a breeding program. Thus, the increased percentages of germination obtained when olive seeds are scarified with H_2SO_4 would improve the feasibility of developing breeding programs for olives and would enable nurserymen to grow olives from seed without serious loss.

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Increased Yield in the Olive with Putrescine Treatment

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Additional index words. *Olea europaea*, fruit-set

Abstract. **Aqueous solutions of putrescine and putrescine dichloride were sprayed on flowers and fruit of self-incompatible olive (*Olea europaea* L.) 'Leccino' and 'Pendolino'. Both diamine formulas increased fruit-set and yield when applied on flowers before anthesis ('Pendolino') and at full bloom ('Leccino'), but they slightly decreased fresh fruit weight. Putrescine dichloride was more effective than putrescine base, stimulating fruit-set with 5×10^{-2} M solution compared to 5×10^{-1} M to 1 M of putrescine base.**

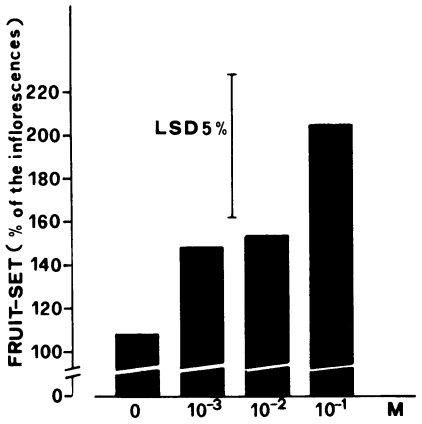

Fig. 1. Effects of putrescine base concentrations applied before anthesis on 'Pendolino' fruit-set, 1982.

Low productivity in the olive results from very low fruit-set and subsequent fruit abscission. Various attempts have been made to increase fruit-set and decrease fruit abscission with auxins (6), nitrogenous products (9) and GA_3 or BA (13) but with rather disappointing results. Field use of growth regulators has resulted in variable response because of environmental variation and lack of knowledge about penetrating capacity and the physiological mechanisms regulating abscission. It is known, for example, that abscission is related to the emission of ethylene by the tissues (1) and that ethylene has the same precursor as the polyamines, S-Adenosylmethionine (2). Whereas the former promotes senescence (11), the polyamine retards this process (7). Furthermore, polyamine treatment inhibits ethylene biosynthesis in apple fruit slices and protoplasts (4). It is uncertain whether the polyamine response is direct or indirect. In fact, polyamines inhibit the development of RNAase and protease activity (3, 12), increase RNA synthesis and cell division in *Helianthus tuberosus* L. (5) and induce DNA synthesis (10).

On the basis of these considerations, we tested one of the polyamines, putrescine, in 2 different formulations in an attempt to increase fruit-set and decrease fruit abscission. In 1982, treatments were applied on the flowers of 2 self-incompatible cultivars (Pendolino and Leccino) and on the fruit of the 'Leccino'. An aqueous solution of putrescine (1,4 diaminobutane) at pH 12 and putrescine dichloride (1,4 diaminobutane dichloride) (Merck) at pH 7 plus 0.01% Tween 80, as wetting agent, was sprayed until run-off at concentrations of 5×10^{-4} to 1 M on flowers and on fruit.

Tests were carried out on uniform 12-year-old trees, vase-trained and grown in a non-irrigated orchard. Ten one-year-old fruit bearing shoots, uniform in vigour and average number of inflorescences (about 60, with about 14 flowers each or with an average of 25 fruit when treatments were carried out after full bloom), were labelled for each concentration of each putrescine formula and control. The 10 shoots were distributed on 5 trees (2 per tree). A total number of 20 trees was selected for the experiments: a) five trees of 'Pendolino' were used to test putrescine base applied on flowers before anthesis (0.5 to 1% of open flowers); b) five trees of 'Leccino' to test both putrescine base and dichloride, applying them on flowers at full bloom; c) five trees of 'Leccino' to test both putrescine formulas applied on fruit at 4 weeks after full bloom in an attempt to decrease fruitlet-drop; and d) five trees of 'Leccino' to test both putrescine formulas on fruit at 20 weeks after full bloom, when the epicarp was completely black, in an attempt to decrease preharvest drop. Control shoots, just as in the other treatments, were distributed on the same trees and sprayed with H_2O and wetting agent.

In 1983, on the basis of the results of the previous year, treatments were repeated only on 'Leccino' at full bloom with putrescine base (see Fig. 2B for concentrations), using the same experimental procedure as the previous year. Furthermore, since, in the previous year, putrescine base was found to cause immediate necrosis of the stigma and styles, NaOH (3×10^{-1} M) solution also was applied to cause the same damage. For this experiment 10 shoots, distributed among the 5 trees used for putrescine base and control, were used. Data on fruit-set percentage (percentage of the fruit set, referred to the number of inflorescences) were collected 3 weeks after treatments. Then, in order to determine relative fruit abscission in experiments carried out on flowers and on the fruit at 4 weeks after full bloom, the fruit still remaining at 10, 14, and 18 weeks were counted. Olives were harvested at 18 weeks after full bloom, when the epicarp was almost completely black, except for those from the last treat-

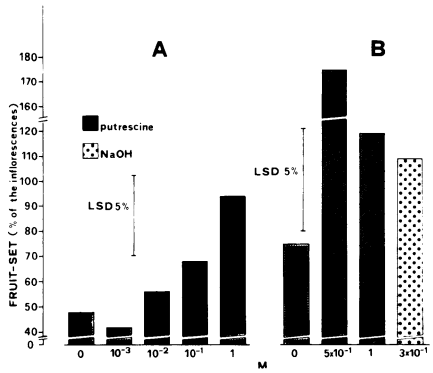

Fig. 2. Effect of putrescine base and NaOH concentrations applied at full bloom on 'Leccino' fruit-set; **A** = 1982, **B** = 1983.

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