Reprinted from HortScience, Vol. 113(5), September 1988
A publication of the American Society for Horticultural Science, Alexandria, Virginia

J. Amer. Soc. Hort. Sci. 113(5):708–712. 1988.

Putrescine Influences Ovule Senescence, Fertilization Time, and Fruit Set in 'Comice' Pear

C.H. Crisosto[1]
Departamento de Fruticultura, Universidad Católica de Chile, Santiago, Chile

P.B. Lombard[2] **and David Sugar**[3]
Department of Horticulture, Oregon State University, Corvallis, OR 97331

V.S. Polito[2]
Department of Pomology, University of California, Davis, CA 95616

Additional index words. *Pyrus communis*, polyamines, yield, nitrogen, effective pollination period, aniline blue

Abstract. **Putrescine at 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M applied at anthesis increased fruit set of 'Comice' pear (*Pyrus communis* L.) in 1985 and 1986. Ovule longevity and the effective pollination period were extended 2 days in 1985 and 5 days in 1986 by putrescine at 10^{-3} M. Pollen tubes reached the micropyle 2 days earlier in treated than in untreated flowers. High levels of evolved ethylene in flowers were related to pollination and fertilization and were unaffected by putrescine application. Increased ovule longevity and fruit set in treated flowers were associated with increased foliar and flower N and B levels after fertilization. Chemical name used: 1,4-diaminobutane dihydrochloride (putrescine).**

'Comice' pear trees produce sufficient numbers of flowers, but, due to heavy post-bloom and preharvest drop, seldom produce an economic crop (5, 17, 19). In Oregon, Stephen (25) reported a low level of self-fruitfulness in 'Comice', with little parthenocarpic fruit set. Poor fruit set in 'Comice' may be due to a short effective pollination period (EPP) (29) or to the lack of cross-pollination (6). EPP is the remaining period of ovule viability beyond the time required for the first pollen tube to reach and fertilize the ovule. Lombard et al. (19) found that the EPP of 'Comice' flowers was 1 to 2 days at 9° to 10°C. In 1985, Crisosto et al. (10) reported an EPP period of 5 days for 'Comice' pear in Corvallis, Ore., and suggested that extension of the EPP could improve fruit set.

Polyamines have been described as growth promoters in higher plants (1–3, 12). Polyamines and ethylene are derived from the same precursor, *S*-adenosyl-methionine, and substrate competition has been demonstrated in ripening fruits and leaves (1, 2). Since polyamines may inhibit ethylene production (1–3, 13) and appear to delay the onset of senescence (1, 2), EPP might be prolonged by polyamine treatments. Fruit set and yield increases of apple and pear have been obtained with [*S*-(*E*)]-2-amino-4-(2-aminoethoxy)-3-butyric acid (AVG) (13, 17) and polyamines (8). Postbloom polyamine sprays in apple (8) and olive (22) resulted in increased fruit size during the early period of fruit growth. Because of the success in achieving increased fruit set with polyamines and ethylene inhibitors on apples, we believed that it was appropriate to look at their effects on 'Comice' pear and their possible mode of action in increasing fruit set.

Received for publication 6 July 1987. Oregon Agr. Expt. Sta. Tech. Paper no. 8371. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Assistant Professor.
[2]Professor.
[3]Instructor.

Materials and Methods

Putrescine at 0 and 10^{-3} M was applied in 1985 two days before anthesis on six replicate trees of 8-year-old 'Comice' pear, planted 1 × 5 m at the Lewis Brown Horticulture Research Farm, Oregon State Univ., Corvallis. The following year, putrescine at 0, 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M was applied to each of ten 12-year-old 'Comice' pear trees at the Southern Oregon Experiment Station, Medford. Ten single-tree replicates were used for each concentration. Control (0 putrescene) trees were sprayed until runoff with 0.01% Tween-80 (v/v); treated trees received a mixture of Tween-80 and putrescine in a pH 6.7 aqueous solution.

Fruit set and yield. Fruit set was measured on four branches with a total of about 300 flower-cluster buds for each of the six replicate trees in 1985 and the 10 replicate trees in 1986. Fruit set was based on the number of fruit retained until harvest per 100 flower clusters. Yield was expressed as yield efficiency (yield per unit of trunk cross-sectional area). In both seasons, fruit weight, fruit diameter, flesh firmness, and seed number were determined at each harvest. Return bloom was determined in all trees the year following treatment and measured as the number of flower clusters relative to the flower clusters of the previous year.

Ovule longevity and pollen tube growth. To determine ovule longevity, additional limbs were bagged and flowers not at anthesis were removed. Bagged and emasculated 'Comice' flowers were hand-pollinated with 'Bartlett' pollen at anthesis (1986). Samples of 40 emasculated flowers from the bagged branches were collected and fixed in formaldehyde : propionic acid : 95%

ethanol in a proportion of 5:5:90 (FPA) every 2 days for 12 days following anthesis in 1985 and 1986. Flowers were removed from FPA, washed in distilled water for 30 min, soaked in 1% sodium carbonate for 1 hr, and rinsed three times in distilled water. The pistil and ovary samples were softened by autoclaving in 1% sodium sulfite for 2 min. The ovaries were rinsed in water, split longitudinally, and the ovules were removed with a fine forceps. Ovules and styles were then mounted on slides, squashed directly in 0.5% aniline blue in 0.15 M K_3PO_4, and observed in a Zeiss fluorescence microscope equipped for epi-illumination using near-UV excitation (9, 21).

Ovule longevity was based on the differential intensity of ovule fluorescence after staining with aniline blue (9), with ovules showing very intense fluorescence considered non-viable or senescent (Fig. 1 A and B). Time from pollination to fertilization was determined when the pollen tubes could be seen entering the micropyle (Fig. 1 C and D). Maximum fertilization was defined as occurring after the highest number of ovules were penetrated by 'Bartlett' pollen tubes.

Ethylene determination. Evolved ethylene was determined in 1985 on treated and untreated non-pollinated and hand-pollinated flowers. 'Comice' flower samples of ≈5 g per replicate were held for 20 min to release wound ethylene before being placed in 25-ml vials and capped with a rubber stopper. After 5 hr, a 1-ml gas sample was withdrawn and assayed for ethylene by gas chromatography (Carle model 311, Hach Co., Loveland, Colo.) equipped with a flame ionization detector and activated alumina column at 55°C.

Tissue mineral analysis. Leaves from fruiting spurs and ovary samples from untreated and 10^{-3} M putrescine-treated trees were collected for nutritional analysis 12 days after anthesis (1986). Five replicate samples of 40 leaves each from two trees were randomly sampled from fruiting spurs on all trees 12 days after anthesis. Ovary samples were taken every 2 days during bloom for N and B determinations. The spray solutions of surfactant plus putrescine and surfactant alone were analyzed for mineral content. The modified Kjeldahl method (23) was used for total N analysis and ICP spectrometry (14) for the following nutrients: P, K, Ca, Mg, Mn, Cu, Fe, Zn, and B.

Analyses of variance and of regression were carried out using the Number Cruncher Statistical System package (NCSS, c/o T. Hintze, 865 East 400 North, Kaysville, Utah).

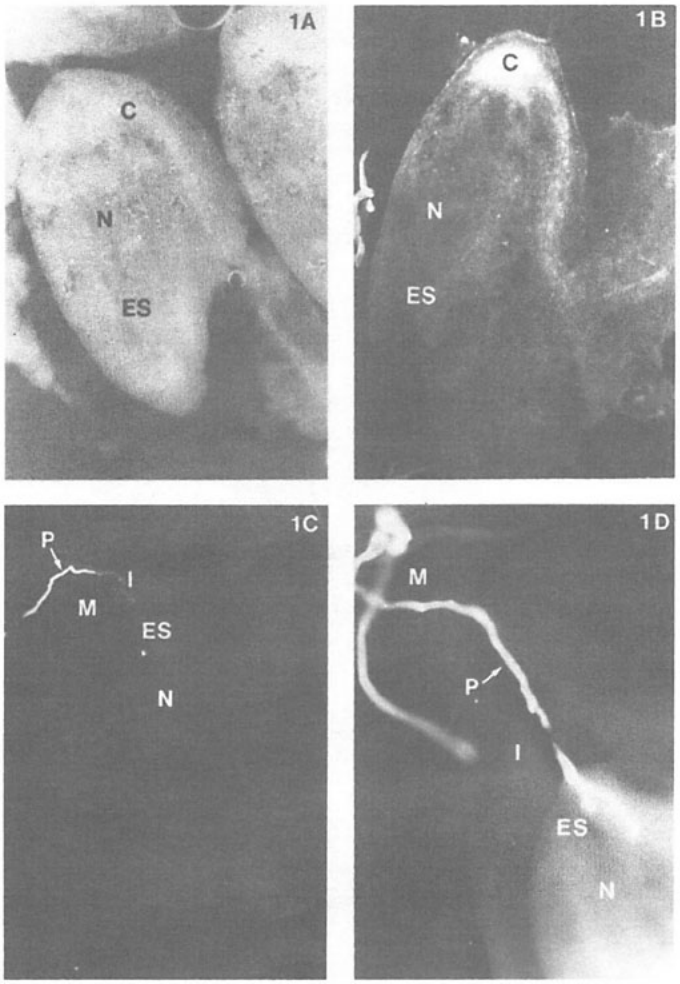

Fig. 1. Fluorescent photomicrographs of 'Comice' pear ovules and pollen tube penetration using aniline-blue stain. C = chalazal end, I = integuments, M = micropylar end, N = nucellus, ES = embryo sac, P = pollen tube. (**A**) Viable ovules, ×39. (**B**) Senescent ovules; ×39 (fluorescence in the chalazal end indicates senescent tissue). (**C**) Fluorescent photomicrograph of 'Bartlett' pollen tube at the micropyle end of the 'Comice' pear ovule; ×39. (**D**) 'Bartlett' pollen tube at the micropyle of 'Comice' ovule; ×96.

Results and Discussion

Fruit set and yield. Fruit set and yield efficiency were enhanced by the application of 10^{-3} and 10^{-2} M putrescine at

Table 1. Effect of putrescine applications at anthesis on 'Comice' yield components, Medford, 1986.

Putrescine (M)	Fruit set (%)	Crop density[z] (no. fruit/cm²)	Yield efficiency[y] (kg·cm⁻²)	Fruit wt (g)
0	32	3.9	0.17	180
10^{-5}	47	5.0	0.20	200
10^{-4}	49	5.5	0.21	200
10^{-3}	50	5.7	0.25	210
10^{-2}	50	6.1	0.24	185
Logarithmic[x]	*	*	*	NS
Model r^2	0.30	0.27	0.38	---

[z]No. fruit/trunk cross-sectional area.
[y]Fruit weight/trunk cross-sectional area.
[x] Regression model significance: *, $P = 0.05$; NS = nonsignificant.

Table 2. Influence of putrescine application at anthesis on 'Comice' ovule senescence in 1985 and 1986.

	Fluorescent ovules[zy]					
	Days after anthesis					
Treatment	0	2	4	6	8	10
	1985					
Control	0 a	0 a	0 a	72 a	98 a	100 a
Putrescine (10^{-3} M)	0 a	0 a	0 a	5 b	76 b	100 a

	1986							
	Days after anthesis							
	2	4	6	8	10[x]	12	14	16
Control	0 a	0 a	5 a	30 a	45 a	90 a	100 a	100 a
Putrescine (10^{-3} M)	0 a	0 a	0 a	0 a	5 b	28 b	75 b	100 a

[z]A high degree of fluorescence indicates senescence of ovules; aniline blue stain method; 200 ovules examined at each period.
[y]Mean separation between treatments in same year (same column) by analysis of variance ($P = 0.01$).
[x]Maximum fertilization time.

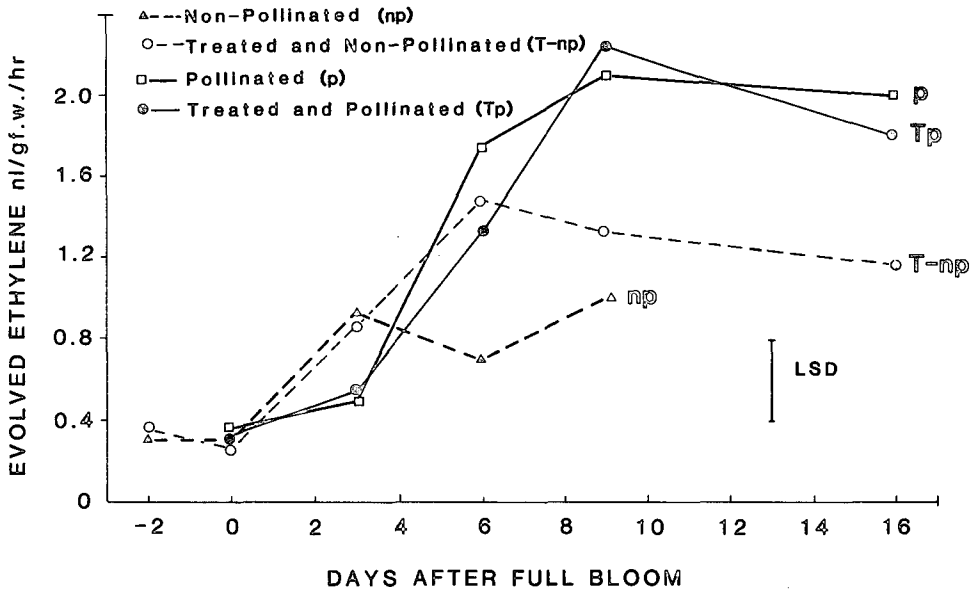

Fig. 2. Evolved ethylene from non-pollinated and cross-pollinated 'Comice' flowers with or without an application of 10^{-3} M putrescine at anthesis (Corvallis, 1985). Bar indicates LSD at the 5% level.

bloom in 1986 (Table 1) and in 1985 (data not shown). Fruit set, crop density, and yield efficiency were increased 44%, 46%, and 47%, respectively, by putrescine at 10^{-3} M. In spite of the greater fruit set and crop density, putrescine at 10^{-3} M did not decrease fruit weight and fruit diameter (data not shown) as compared to the control. Seed content was increased by the various putrescine treatments (data not shown). A relationship between high number of seed per fruit and high fruit size was established by Callan and Lombard (6). Thus, higher numbers of seed might explain the maintenance of fruit size with increased fruit load resulting from putrescine application. However, the variation in seed number accounted for 61% of the fruit size (diameter) variation [$Y = 6.16 + 1.27 \log$ (seed no.), $r^2 = 0.61$], indicating only a partial effect from seed content.

Fruit firmness and percent of fruit with russet measured 2 months after storage were not influenced by putrescine treatments during the two seasons of this study (data not shown). Putrescine-treated trees developed more flower clusters than the control trees the year after treatment as compared to the previous year (data not shown). The same situation was reported by Costa and Bagni (8) for apples after polyamine application to the 'Delicious' strain 'Ruby Spur'.

Ovule longevity. Senescence of 'Comice' ovules, as determined by the aniline blue method, was delayed by putrescine sprays (Table 2). At anthesis all ovules were viable and senescence commenced between 5 to 8 days after anthesis. Under a mean ambient temperature of 8.5°C at Corvallis, Ore. in 1985, untreated flowers had 28% viable ovules 6 days after anthesis, while those treated with putrescine had 95% viable ovules. The mean temperature during bloom was 11.7° in Medford, Ore. during 1986. Under these conditions, only 10% of the ovules in untreated flowers remained viable at day 12 and none were viable at day 14. In the putrescine-treated flowers, 72% of ovules were viable at day 12, while none were viable 4 days later. These observations are in agreement with those of Bini and Bellini (4), who failed to find evidence of early embryo-sac degeneration in 'Comice' flowers.

A similar degree of 'Comice' ovule senescence was reported by Jaumien (16). She observed that 95% of embryos examined had degenerated 17 days after anthesis. In our studies, the untreated ovules lost most of their viability by 12 days and all of it by 14 days in 1986 (Table 2). We observed 'Comice' ovule breakdown in the nucellus at the chalazal end several days after anthesis. These observations are similar to those on 'Italian' prune (27) and cherry (26). Both groups concluded that ovule degeneration at the chalazal end of the nucellus rather than in the embryo sac resulted in low fruit set.

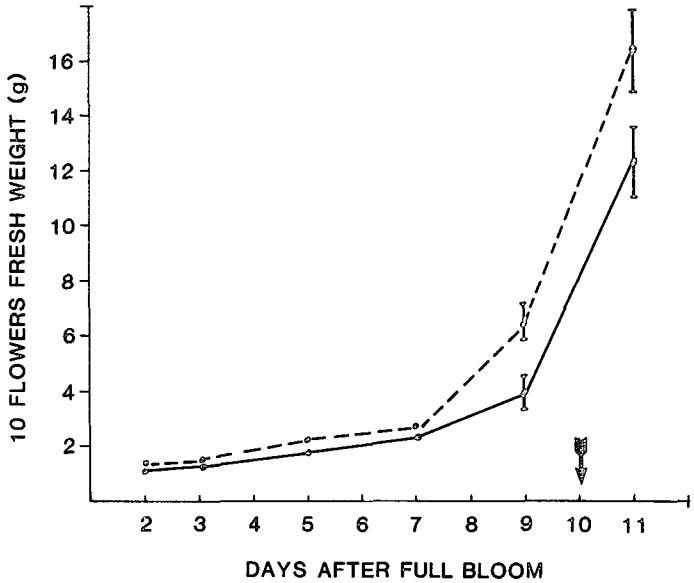

Fig. 3. Effect of putrescine application at full bloom on fresh weight of 'Comice' flowers, 1986. Bars represent SD of the four means per treatment. Arrow indicates the time of maximum fertilization (——— control; ------- putrescine at 10^{-3} M).

More ethylene was evolved from pollinated flowers than from non-pollinated flowers (Fig. 2). This observation suggests a possible role of ethylene in fertilization. Nichols et al. (20) found an increase in 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor, in hand-pollinated styles of carnation flowers.

Table 3. Effect of putrescine application on mineral content of 'Comice' pear flowers and leaves 12 days after anthesis, 1986.

Treatment	Macroelements (% dry wt) N	P	K	S	CA	Mg		Microelements (% dry wt) Mn	Fe	Cu	B	Zn	Al
							Leaves						
Control	2.56	0.33	1.94	0.13	1.00	0.37		26	114	13	40	28	42
Putrescine	2.57	0.33	1.65	0.14	0.81	0.37		31	104	13	56	24	41
Significance	NS	NS	NS	NS	NS	NS		NS	NS	NS	*	NS	NS
							Flowers						
Control	2.72	0.79	2.1	0.39	1.00	0.56		20	85	30	73	51	6
Putrescine	3.29	0.70	1.9	0.38	0.88	0.48		22	67	25	114	43	5
Significance	*	NS	NS	NS	NS		NS	NS	NS	NS	*	NS	NS

NS,*Nonsignificant or significant by analysis of variance ($P = 0.05$), respectively.

Putrescine applied at anthesis did not reduce the levels of ethylene evolved from flowers during the bloom period, although it extended ovule longevity and increased fruit set and yield. These data suggest that the increase in fruit set and yield by putrescine is not due to ethylene inhibition during the bloom period. However, because the measured ethylene may be evolving from various floral tissues that differ physiologically, it is difficult to determine the physiological role ethylene has in fertilization. Therefore, a more accurate method of ethylene determination needs to be developed to clarify the possible relationships between polyamine application and ethylene in flower tissues.

Pollen tube growth. 'Bartlett' pollen tube growth in the 'Comice' styles was difficult to assess (9), especially in the lower two-thirds of the style. Lombard et al. (18) reported that rate of growth was less for pollen tubes growing from the base of the 'Comice' style into the micropyle (0.37 mm·day^{-1} at 8°C) than from the stigma to the style (0.95 mm·day^{-1}). Similar observations have been described for almond (21), sour cherry (26), avocado (24), and, in pear, for 'Conference' pollen tubes in 'Bartlett' styles (18). Because of this difference, the effect of putrescine on pollen-tube growth was determined as the time when pollen tubes reached the micropyle. Pollen tubes in the micropyle fluoresce brightly and are easily identified (Fig. 1 C and D), providing a more accurate determination of the pollen tube growth than when pollen tubes reach the base of the style in pear. At a mean ambient temperature of 11.7°, 'Bartlett' pollen tubes reached some of the 'Comice' micropyles in 8 days for untreated and 6 days for treated flowers, and, in both, the most pollen tubes reached the micropyles 10 days after hand-pollination (maximum fertilization). At this time, nearly all the putrescine-treated ovules were viable, while only 55% of the control ovules were alive (Table 2). Although the effect of putrescine on maximum fertilization time was not statistically significant, treated flowers had a higher percentage of pollen tubes reaching the micropyles earlier and greater fresh weight after fertilization than the control treatment (Fig. 3). The fresh weight differences may be due to a higher number of fertilized ovules and/or from a putrescine effect on cell division in the developing ovary and external tissues.

EPP was at least 2 days longer for putrescine-treated flowers than for the controls in 1985 and 5 days longer in 1986. According to Williams (29), EPP is affected by prevailing temperatures. Vasilakakis and Porlingis (28) reported an EPP of 13 days at 8°C, 5 days at 15°, and 1 day at 20° for 'Tsakoniki' pear. In our study, the difference in EPP and ovule longevity in the two orchards cannot be explained by temperature alone, but other factors are also important in determining EPP. In pear (29) and in grape (11), N fertilization had a positive effect on extending ovule viability and increasing fruit set. Putrescine treatment resulted in significant increases in N and B concentrations in flower tissue 12 days after anthesis (Table 3), although spray solutions did not differ in N and B content (data not shown). Boron was also higher on treated than untreated leaves. Nitrogen and B measured during bloom and expressed as milligrams per flower were also greater in hand-pollinated and putrescine-treated flowers than in hand-pollinated and untreated flowers; these differences became more evident after fertilization (data not shown). Galston et al. (12) reported that aliphatic polyamines are metabolically related to basic amino acids, such as arginine and ornithine, and that arginine decarboxylase activity and N levels can increase after putrescine application (3). Also, polyamine accumulation in tissues may cause alteration in the levels of inorganic ions as a result of adjustment of the cellular ionic balance (7). Thus, B and N, which are known to increase fruit set in several fruit trees (15, 29), could increase after putrescine application and be associated with the increase in fruit set from the extension of ovule longevity.

Application of putrescine at anthesis resulted in increased fruit set and yield of 'Comice' pear with no adverse effects to fruit size and quality. The results of this study indicate that putrescine extends ovule longevity and EPP, increases N and B, and may increase the rate of pollen tube growth in the styles of pears. The effect of putrescine on increasing floral cluster numbers was also evident the year after treatment. In practice, these studies indicate that putrescine may be useful in increasing yields of 'Comice' pear.

Literature Cited

1. Altman, A. and U. Bachrach. 1981. Involvement of polyamines in plant growth, p. 365–375. In: C.M. Caldarera, V. Zappia, and U. Bachrach (eds.). Advances in polyamine research, Vol. 3. Raven, New York.
2. Apelbaum, A., A.C. Burgoon, J.O. Anderson, M. Lieberman, R. Ben-Arie, and A.K. Matoo. 1981. Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts through polyamine mediated inhibition of senescence. Plant Physiol. 60:570–574.
3. Bagni, G. and E. Bellini. 1982. Polyamines and cellular growth processes in higher plants, p. 473–482. In: P.F. Wareing (ed.). Plant growth substances. Academic, New York.
4. Bini, G. and E. Bellini. 1971. Macrosporogenesi nella cultivar di pero 'Decana di Comizio' e ricerca del periodo piú opportuno

per la sua impollinazione (in Italian). Riv. Ortoflorofrutt. Ital. 4:322–337.
5. Callan, N.W. 1977. Effect of boron sprays on fruit set, fruit quality, boron distribution, and floral morphology of 'Italian' prune (*Prunus domestica* L.). PhD Diss., Oregon State Univ. Corvallis. (Diss. Abstr. 77–15366.)
6. Callan, N.W. and P.B. Lombard. 1978. Pollination effects on fruit and seed development in 'Comice' pear. J. Amer. Soc. Hort. Sci. 103:469–500.
7. Cho, S.C. 1983. Effect of cytokinin and several inorganic cations on the polyamine content of lettuce cotyledons. Plant and Cell Physiol. 24:27–32.
8. Costa, G. and N. Bagni. 1983. Effects of polyamines on fruit set of apple. HortScience 18:59–61.
9. Crisosto, C.H. 1987. The effect of ethylene manipulation on flower bud development of *Prunus domestica* and *persica* and on 'Comice' pear ovule senescence. PhD Diss., Oregon State Univ. Corvallis.
10. Crisosto, C.H., M. Vasilakakis, P.B. Lombard, D.G. Richardson, and R. Tetley. 1986. Effect of ethylene inhibitors on fruit set, ovule longevity, and polyamine levels in 'Comice' pear. Acta Hort. 179:229–236.
11. Ewart, A. and W.M. Kliewer. 1977. Effects of controlled day and night temperatures and nitrogen on fruit set, ovule fertility, and fruit composition of several wine grape cultivars. Amer. J. Enol. Viticult. 28:88–95.
12. Galston, A.W. and R. Kaur-Sawhney. 1980. Polyamines in plants and plant cells. What's New in Plant Physiol. 11:5–8.
13. Greene, D.W. 1980. Effect of silver nitrate, aminoethoxyvinyl glycine, and gibberellins A_{4+7} plus 6-benzylamino purine on fruit set and development of 'Delicious' apples. J. Amer. Soc. Hort. Sci. 105:717–720.
14. Isaac, R.A. and W.C. Johnson. 1985. Elemental analysis of plant tissue by plasma emission spectroscopy: Collaborative study. J. Assn. Anal. Chem. 68:449–505.
15. Hanson, E.J. and P.J. Breen. 1985. Effects of fall boron sprays and environmental factors on fruit set and boron accumulation in 'Italian' prune flowers. J. Amer. Soc. Hort. Sci. 110:389–392.
16. Jaumien, F. 1968. The causes of poor bearing of pear trees of the variety 'Doyenne du Comice'. Acta Agrobot. 21:75–106.
17. Lombard, P.B. and D.G. Richardson. 1982. Increased fruit set and cropping of 'Comice' pear trees with an ethylene inhibitor, aminoethoxyvinylglycine. Acta Hort. 124:165–169.
18. Lombard, P.B., and R.R. Williams, K.C. Scott, and C.J. Jeffries. 1973. Temperature effects on pollen tube growth in styles of 'Williams' pear with a note on pollination deficiencies of 'Comice' pear. Proc. Symp. Pear Growing 64:43–46.
19. Lombard, P.B., R.R. Williams, and M.M. Thompson. 1971. Effective pollination—the facts of life behind uniform pear cropping. Proc. Ore. Hort. Soc. 62:31–36.
20. Nichols, R., G. Bufler, Y. Mor, D.W. Fujino, and M.S. Reid. 1983. Changes in ethylene production and 1-aminocyclopropane-1-carboxylic acid content of pollinated carnation flowers. J. Plant Growth Regulat. 2:1–8.
21. Pimienta, A.E., V.S. Polito, and D.E. Kester. 1983. Pollen tube growth in cross-and self-pollinated 'Nonpareil' almond. J. Amer. Soc. Hort. Sci. 108:643–647.
22. Rugini, E. and M. Mencuccini. 1985. Increased yield in the olive with putrescine treatment. HortScience 20:102–103.
23. Schuman, G.E., A.M. Stanley, and D. Knudson. 1973. Automated total nitrogen analysis of soil and plant samples. Soil Sci. Soc. Amer. Proc. 37:480–481.
24. Sedgley, M. 1979. Light microscope study of pollen tube growth, fertilization, and early embryo and endosperm development in the avocado varieties Fuerte and Hass. Ann. Bot. 44:353–359.
25. Stephen, W.P. 1958. Pear pollination studies in Oregon. Ore. State Univ. Agr. Exp. Sta. Tech. Bul. 43.
26. Stosser, R. and S.F. Anvari. 1983. Pollen tube growth and fruit set as influenced by senescence of stigma, style, and ovules. Acta Hort. 139:13–22.
27. Thompson, M.M. and L.J. Liu. 1973. Temperature, fruit set, and embryo sac development in 'Italian' prune. J. Amer. Soc. Hort. Sci. 98:193–197.
28. Vasilakakis, M. and I.C. Porlingis. 1984. Effect of temperature on pollen germination, pollen tube growth, stigma receptivity, and fruit and set of 'Tsakoniki' or 'Spada' pear. HortScience. 19(3):528. (Abstr.)
29. Williams, R.R. 1966. II. The effective pollination period for some apple and pear varieties. Annu. Rpt. Long Ashton Agr. and Hort. Res. Sta. p. 136–138.