12 F_2 families and found TuMV-susceptible. The evidence in the crosses indicates the same single recessive allele for susceptibility in *L. serriola* lines and *L. sativa* cvs. Calmar, Imperial 410, and E-4.

Discussion

Zink and Duffus (10) reported linkage of the mildew reaction gene, *Dm dm* with the TuMV reaction gene, *Tu tu,* with a cross-over value of 12.5% ± 1.6 in *L. sativa.* In the present study we found a similar genetic relationship in the 3 *L. serriola* lines that were TuMV-susceptible and mildew-resistant. The cross-over value for the *L. serriola* crosses was 12.0% ± 2.9. These 2 linkage values are probably estimates of the same value. Also the same allele for mildew-resistance and the same allele for TuMV-susceptibility was demonstrated in cvs. Calmar, Imperial 410, and E-4, and in the 3 *L. serriola* lines. These genetic relationships are additional strong circumstantial evidence that TuMV susceptibility in *L. sativa* is associated with mildew-resistant progenies derived from *L. serriola* (P.I. 91532).

'Imperial 410' was derived from original crosses of 'Imperial D' and P.I. 91532, and 'Imperial D' crossed to a mildew-resistant strain of 'Chinese Stem' lettuce (1). *Lactuca serriola* (P.I. 91532) is the source of mildew resistance in 'Calmar' (7, 8), and crosses between 'Calmar' and 'Imperial 410' indicated the same allele for mildew-resistance and the same allele for TuMV-susceptibility. It is probable that resistance in 'Imperial 410' stems from P.I. 91532.

Cultivar E-4 was released in 1943 by the late Dr. LeRoy E. Weaver, Growers Ice and Development Company, Salinas, California. The source of resistance to mildew and susceptibility to TuMV is not known, as no pedigree record is available. Crosses between 'Calmar' and 'E-4', or 'E-4' and 'Imperial 410' indicated the same allele for mildew-resistance and the same allele for TuMV-susceptibility. It appears likely, based on the history of 'E-4', and the genetic relationships reported herein, that 'E-4' is a selection from one of the late Dr. I. C. Jagger's advanced breeding lines derived from a cross with P.I. 91532.

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Fruit-set and Development in the Pear: Diffusible Growth Substances from Seeded and Seedless Fruits[1]

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Abstract. Seasonal changes in natural growth substances were studied by collecting the diffusate, via the pedicel, from intact seeded 'Winter Nelis', seeded 'Bartlett', and parthenocarpic 'Bartlett' pear fruits. The diffusate of 'Bartlett' fruits collected 10 to 25 days after full bloom (AFB) contained more auxin-like promoter than did that of 'Winter Nelis' fruits. With the exception of the 45-day sample, the diffusate from parthenocarpic 'Bartlett' fruits had more promoter from 25 to 70 days (AFB) than did either seeded pear. In contrast, more gibberellin (GA)-like materials diffused from 'Winter Nelis' fruits than from seeded or parthenocarpic 'Bartlett' fruits. With all types of pears the concentration of abscisic acid-like materials in the diffusate was similar until harvest when the concentration was greater for 'Winter Nelis' than for 'Bartlett'. The combined effect of relatively low amounts of auxin-like and greater amounts of GA-like materials may explain why the presence of seeded pears during the postbloom period has a greater inhibitory effect on flower bud formation in 'Winter Nelis' than in 'Bartlett'.

In most studies concerning concn of endogenous growth regulators in fruits, the substances have been extracted from intact fruits, using organic solvents. The extractable hormones in pear fruits have been investigated by Rudnicki et al. (15) and by Gil et al. (6). There was no evidence, however, that these substances were involved in processes outside the fruits. The seasonal variations of diffusible growth promoter(s) collected from intact 'Bartlett' pear fruits were reported recently (7). In that investigation most of the growth promoter diffused from seedless, rather than seeded, fruits and this was considered related to the ability of spurs carrying seedless fruits to differentiate flower buds.

The purpose of the present work was to investigate the seasonal changes of natural growth-promoting and growth-inhibiting compounds diffusing from intact seeded and seedless pear fruits.

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Materials and Methods

The diffusible auxin-like, gibberellin-like (GA-like), and abscisic acid-like (ABA-like) substances of developing 'Winter Nelis' and seeded and seedless 'Bartlett' fruits were studied in the seasons of 1970 and 1971. The fruits were obtained from the Leary and Fay-Sturtz Orchards, Sacramento River district, California.

The technique of Griggs et al. (7) was used to collect diffusible growth substances from entire intact fruits in 1970. It takes 'Bartlett' approximately 100 days and 'Winter Nelis' approximately 170 days to mature. On the basis of previous (7) and concurrent (6) growth regulator estimations, samples of each cultivar were often taken on different days. Five-ml vials each containing 1 ml of 10^{-2}M phosphate buffer, pH 5.0, were taken to the field, where 1 or 2 flowers or fruits were placed in each of 10 vials, with the pedicel immersed in the buffer. After a diffusion period of 6 hr, the flowers or fruits were removed and the diffusate was partitioned with organic solvents as previously described (6). The resulting acidic ether and butanol phases were saved for assay. Some modifications were introduced the second year in order to obtain larger quantities of GA-like and ABA-like substances. Agar at 1.5% was used instead of buffer, and the diffusion time was lengthened to 24 hr. At the end of the diffusion period, the agar was freeze-dried and extracted several times with 80% methanol. The combined methanol extracts were concd to an aqueous phase under reduced pressure at 30°C. After acidification (pH 2.5), growth substances in the aqueous phase were shaken once with ½ and twice with ¼ its volume of ethyl acetate, and twice with ¼ its volume of diethyl ether. The combined organic phase was examined for growth substances and the aqueous phase was discarded.

The methods used to purify and test the growth substances were those described previously (6). To determine the presence of auxin-like growth promoters, portions equivalent to the diffusate from 200 mg dry wt of tissue were streaked on Whatmann 3 MM paper strips (2.5 x 30 cm), and the chromatograms developed with isopropanol:ammonia:water (IAW, 8:1:1, v/v/v) to a distance of 20 cm. After being dried, each chromatogram was cut into 10 equal horizontal strips. These were individually bioassayed by the wheat coleoptile test (14). To study GA-like substances, extracts equivalent to the diffusate from 2.0 g dry wt of tissue was chromatographed, using Gelman Instant Thin-Layer plates (TLC) with IAW (8:1:1, v/v/v), and estimated by a dwarf rice bioassay (13). The ABA-like inhibitors diffused from an equivalent of 3.0 g dry wt of tissue, were separated by TLC with double development, using benzene:acetic acid:water (BAW, 8:3:5, v/v/v, upper phase). The TLC zone between Rf 0.05 and 0.25 was examined by means of the wheat coleoptile test (14).

Results and Discussion

Auxin-like promoters. The diffusate from the 1970 samples contained an acidic growth promoter which migrated to Rf 0.3-0.5 with IAW, as did standards of indoleacetic acid (IAA). No quantitative difference in auxin-like activity was found among the 3 groups of pears ('Winter Nelis', seeded and seedless 'Bartlett') at bloom time (Fig. 1). Activity in the diffusate of 'Bartlett' increased slightly until 25 days AFB. Thereafter, the promoter activity from seeded fruits decreased continually, whereas that of seedless fruits continued to increase until 70 days AFB, after which it decreased sharply. The auxin-like activity in the diffusate of the seeded 'Winter Nelis' pears fluctuated, but the values were below those for either seeded or seedless 'Bartletts' at 10, 35, and 60 days AFB.

No auxin-like substances were found in the diffusate of the 1971 samples. The procedure was altered in that year, as samples were dried under a stream of air rather than by vacuum. In 1971, the absence of auxin-like materials was also reported for extracts of intact fruits which were similarly handled (6).

In 1970 the seasonal changes observed in these auxin-like substances corresponded, in many respects, with those of an extractable growth promoter we reported previously (6). Since the diffusate was collected from intact fruits via the pedicel in the present work, the promoter was most likely translocated from the fruits. Grochowska (8) demonstrated that ^{14}C-IAA, when injected into apple seeds, was translocated to the spur.

One role that can be ascribed to auxin-like compounds in the pedicel is inhibition of abscission. Luckwill (11) found a correlation between auxins produced by the seeds of apple fruits and the cessation of fruit drop. Addicott (1), after reviewing the physiology of abscission, concluded that auxin reaching the abscission zone of the pedicel from the distal subtending organ is the major hormonal factor tending to inhibit abscission. Gil et al. (5) found that exogenous application of 2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop) inhibited abscission of pear fruits at the time of "June drop."

Griggs et al. (7) found that parthenocarpic 'Bartlett' pear fruits diffused more auxin-like promoter than did seeded fruits of the same cultivar during the period 21 to 60 days AFB. They related the presence of that promoter in the diffusate with the greater tendency to initiate flower buds by those spurs bearing seedless fruits. Grochowska (9) found that naphthalenaecetic acid (NAA) stimulated flower bud formation in apple spurs when applied to the core of fruits. In our study, the fruits of 'Bartlett', which has a greater tendency to differentiate flower buds than does 'Winter Nelis', diffused more auxin-like substances than did the latter cultivar, and, in the case of parthenocarpic fruits, did so for a longer period of time.

Gibberellin-like growth promoters. Diffusate from the equivalent of 1 g extracted dry wt of pear fruit tissue educed only a slight growth response in the dwarf rice assay in 1970. Greater activity was found the following year, when diffusion time was increased from 6 to 24 hr, and agar was substituted for buffer as receiver. Diffusate data for 1971 were taken on the basis of 2 g dry wt equivalents. The peaks of GA-like concn on the chromatograms were usually between Rf 0.6 and 0.7. A striking feature was the greater GA-like activity in the diffusates from flowers and fruits of 'Winter Nelis' than in those from 'Bartlett'. This was particularly marked in diffusates from flowers and from 10-day-old fruitlets (Fig. 2). No GA-like activity was detected in the diffusates of fruits beyond 26 days AFB.

Endogenous gibberellins produced in fruits seem to inhibit

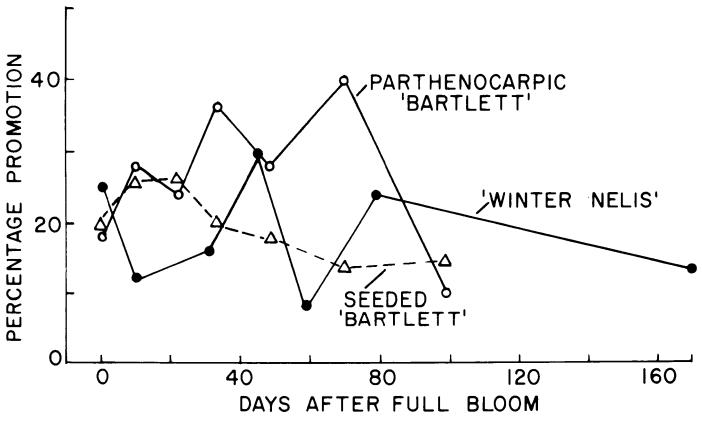

Fig. 1. Seasonal changes in diffusible auxin-like promoter from pear flowers and fruits as estimated by the wheat coleoptile straight growth test. Each point represents the promotion given by the auxin-like material in 200 mg dry material which migrated to Rf 0.4-0.5 on Whatmann 3 MM paper with isopropanol:ammonia:water (8:1:1, v/v/v).

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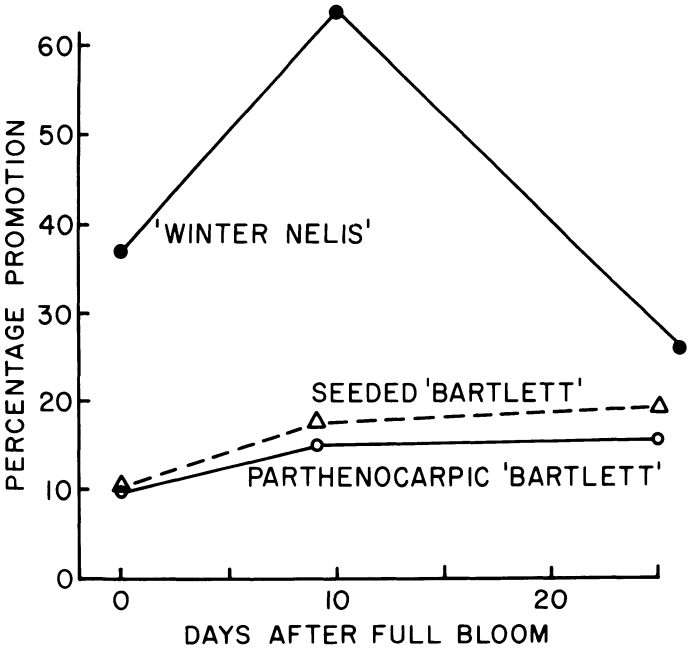

Fig. 2. Diffusible GA-like promoter from pear flowers and fruits as estimated by the dwarf rice test. Results correspond to GA-like promoters in 2 g dry matter of flowers or fruits which migrated to Rf 0.5-0.8 on thin-layer chromatogram with isopropanol:ammonia:water (8:1:1, v/v/v).

flower bud differentiation. Chan and Cain (2) demonstrated that the seeds of apple inhibited formation of flower buds in the adjacent spurs. The studies of Grochowska (9) and Luckwill (12) indicate that gibberellin(s) may be the inhibitory substances. Griggs et al. (7) reported that the presence of seeded fruits inhibited flower bud formation in 'Winter Nelis' and, to a lesser extent, in 'Bartlett'. Our results provide a possible explanation. The diffusate from 'Winter Nelis' pears contained a higher concn of GA-like substances than that from seeded or seedless 'Bartlett' pears during the postbloom period when the presence of seeded fruits was shown by Griggs et al. (7) to inhibit flower bud formation.

It is difficult to understand why similar concn of extractable GA-like substances were found in the intact fruit extracts from 'Bartlett' and 'Winter Nelis' pears (6). Yet our present results indicate that the diffusate from 'Winter Nelis' contains a greater concn of GA-like substances. Further investigation will be necessary to explain this contrast. It is possible that the diffusing gibberellins are of seed origin, and not from the remainder of the fruit. If this is the explanation, then seeds of 'Winter Nelis' should have a higher GA-like concn than seeds of 'Bartlett'. In apple, Luckwill (12) found a correlation between high production of GA-like substances in seeds and high content of GA-like substances in spurs.

A mechanism that could explain the difference in GA-like activity between the diffusates of the 2 cultivars involves the transport form of GA. Evidence for this is the work of Jones (10), who found GA_1 and GA_5 in extracts of pea tissue, but only GA_1 in the diffusate. Crozier and Reid (3) reported that the roots of *Phaseolus coccineus* converted GA_{19} into GA_1, which was then transported to the shoot.

Abscisic acid-like inhibitors. No inhibitory activity was found in the diffusate collected in 1970 with dry matter equivalents up to 2 g. In 1971, however, when the diffusate was collected in agar for a longer time, a clear inhibitory activity was detected with an equivalent of 3 g dry matter. Only the diffusates of the last 3 or 4 samples were bioassayed, as the earlier ones were

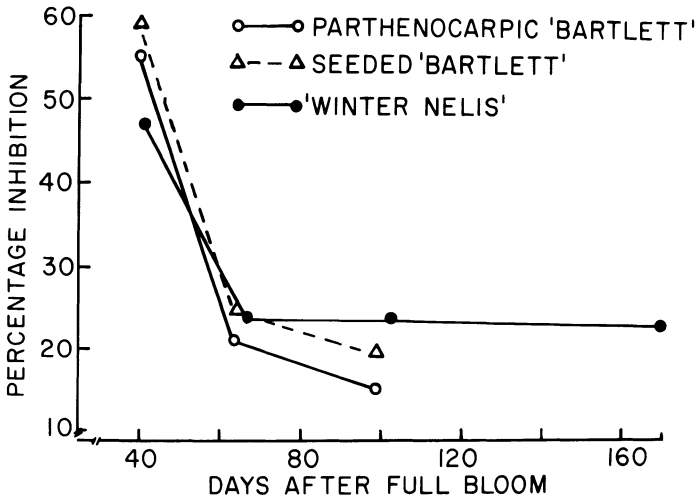

Fig. 3. Seasonal changes in diffusible ABA-like inhibitor from developing pear fruits assayed by the wheat coleoptile straight growth test. Each point was obtained with an extract equivalent to 3 g dry wt of fruits which migrated to Rf 0.05-0.25 on thin-layer chromatogram with benzene:acetic acid:water (8:3:5, v/v/v).

consumed while assaying for GA-like activity.

Forty days AFB, the diffusate equivalent to 3 g of dried fruits caused inhibition of wheat coleoptile growth that ranged from 47% for 'Winter Nelis' to 58% for seeded 'Bartlett' (Fig. 3). The concn decreased until harvest in all pears. At harvest, when the fruits were nearly equal in size and maturity, approximately the same amount of inhibitor was found in the diffusates of both cultivars. Although the concn of diffusing ABA-like compound was low at harvest if calculated on a per fruit basis, the total ABA-like compound was at its greatest level for the year. On this basis, the ABA-like inhibitor diffusing through the fruit pedicel may influence the abscission layer and promote fruit drop at harvest. This situation is similar to that in cotton (4), where 2 to 4 times as much ABA was found in abscising fruits.

We previously reported that the inhibitor extracted from entire pear fruits was at a high concn (6). In the present work in which only the diffusate was examined, a low concn of inhibitor was found. During the 1970 season no inhibitory activity was found in the diffusate. These variations seem to indicate that only a small part of the total inhibitor moves from pear fruit tissue. Most of the inhibitor could exist functionally inactive, with only a portion moving to active sites. This would account for the high concn of extractable inhibitor existing at times of active growth.

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Morphological Studies of Flower Bud Initiation and Development in Bulbous Iris Stored at Various Temperatures[1,3]

Joseph Uhring[2]

Abstract. Flower buds are absent in dormant iris bulbs, but floral initiation occurs after subjecting them to a high temp, heat curing treatment, followed by a holding period at a moderate temp, and then a low temp pre-cooling period. The effects of cultivars, digging dates, ethylene gas treatment, and different storage temp on earliness and uniformity of flowering were studied in microscopic sections of bulb growing points of samples collected at intervals from the different treatment lots. The results indicated that variations in field growing conditions produced bulbs with varying degrees of maturity, of which some would respond properly to curing treatments and others would not. Properly matured bulbs grown in the Pacific Northwest can be heat cured by exposing them to a temp of 32.2°C for 10 days. Holding temp lower than 15.5°C delayed subsequent flower bud initiation. Ethylene gas treatment prior to heat curing appeared to stimulate floral initiation.

The variations in flowering time noted from year to year in flowering trials of bulbous iris suggest that several factors must affect their time of floral initiation and development sequence leading to flowering as influenced by storage and ethylene treatments.

Blaauw (1934) showed that a specific storage temp was required for floral initiation in bulbous iris (5). Hartsema et al. (4) reported that bulbs could be held in the vegetative state (retarded condition) for 12 months at a temp of 25°C. Pereira (5) reported that floral initiation could begin at temp between 20-25°C, but abortion occurred if these temp continued. Kamerbeek (4) pointed out that the bulb was more retarded at 30°C than at 25°C as determined by measurement of the length of the first foliar leaf after 1 year of storage. He concluded this measurement to be a good indicator of shoot growth in dry bulbs without roots. Storage of dry bulbs at temp above 40°C resulted in dehydration and death (4). Stuart et al. (8) recommended a min heat curing of 10 days at 32°C. Ethylene treated bulbs bloomed earlier and within a shorter period of time (Stuart et al. 6). In their experiment, 99% of the treated bulbs bloomed while nearly one third of the untreated plants failed to flower.

Materials and Methods

Bulbs of the hybrid group of Dutch Iris, 'Wedgwood' and 'Ideal', which are derivatives from crosses of *Iris xiphium* L. var. *Praecox* x *I. tingitana* Boiss and Reut. and x *I. lusitanica* Ker-Gawl. were grown at Sumner, Washington. Bulbs dug in July and August were heat cured at the Western Washington Research and Extension Center, Puyallup, before shipment to Beltsville. These bulbs were then cold stored for various periods of time at various temp and forced in the greenhouses during 3 different years. Specific treatments are described in the results section. The 9 to 10-cm diam graded bulbs were grown in flats and were discarded at the end of each forcing season. Small samples were removed from the various bulb treatments at periodic intervals for microscopic determination of flower bud initiation. The growing point was removed with a min of extraneous tissue to facilitate penetration of the fixing solutions and then processed through infiltration and embedding in paraffin. Longitudinal median sections were cut at a thickness of 10 to 12 microns. The sections were stained with either iron-hematoxylin or satranin and fast green (3). The ethylene gas treatment consisted of exposing the bulbs in 1-gal glass containers at 21°C. Bulbs were forced in 35.6 X 50.8 cm wood flats of steamed soil at a night temp of 12.7°C.

Results and Discussion

The vegetative growing point is situated very close to the base of the bulb (Fig. 1 and 2) up to the time of pre-cooling. At this dormant stage, it is not readily distinguishable by macroscopic observation because of its small size and lack of color contrast. Early stages of floral initiation can be identified by low power microscopic observation (x 80), without staining the paraffin sections.

According to Pereira (5), floral initiation can occur after the bulbs have been pre-cooled at 13°C for approx 4 weeks. The first indication of transition from the vegetative to the reproductive state is an increase in the rate of cell division in the rib meristem about 10 cell layers below the tunica. This increased mitotic activity gradually occurs over the entire apical dome region resulting in a change of the vegetative zonation pattern. According to Blaauw cited by Hartsema (1), the development of the flower occurs as follows: Three primordia

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