Measurable biochemical variations in leaves, shoots and flower buds exist among peach cultivars of varying degrees of cold hardiness. These differences involving sugars, protein, total free amino acids and certain individual amino acids appear significant only at certain times throughout the year, mostly during the winter and early spring.

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Embryo Sac Development in Relation to Virus Infection of Four Red Raspberry Cultivars[1,2]

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Abstract. In a factorial experiment each of 4 red raspberry cultivars was treated with each of 4 viruses. Embryo sacs were examined in treated and control flowers collected at anthesis and 4 days later. A numerical index was devised to quantify stages of embryo sac development, and thus facilitate statistical analysis. Effects on embryo sac development of cultivar differences, viruses, and year difference were observed. The virus effects on embryo sac development as such were not considered severe enough to account for failure of fruit-set or yield reduction. Embryo sacs of the 'Sumner' cultivar were retarded in development compared to the other cultivars.

Developmental studies of the embryo sac have explained the unreliable cropping behavior in sweet cherry (6) and led to practical recommendations for commercial sweet cherry orchards (9). Embryo sac studies have also been used to correlate temperature with poor fruit set in bean (13) and to serve as a basis for tracing the possible cause of poor fruit set in apricot (8).

Studies correlating pollen abortion and retarded embryo sac development in radiation-induced mutants of apple (1, 2) and sweet cherry (7) indicated that retarded embryo sac development might be expected in red raspberries. A correlation probably exists between pollen abortion and retarded embryo sac development in virus free 'Sumner', possibly explaining crumbliness (failure of fruit-set) in one naturally occurring somatic mutant of this cultivar (5). Increased pollen abortion induced by virus has already been established in red raspberry (10).

Virus interference with cell division necessary for fruit set in tomato has been found (3), and a similar interference with drupelet set in red raspberry has been suggested (4, 12, 15).

This study was undertaken to examine the effects of known viruses on red raspberry embryo sac development.

Materials and Methods

The material used in this study was from a replicated field trial involving 4 red raspberry cultivars deliberately infected with known viruses, established by Dr. J. A. Freeman at Abbotsford, British Columbia.

Flowers were collected from a factorial experiment involving 4 red raspberry cultivars: 'Willamette', 'Fairview', 'Newburgh', and 'Sumner'; and 4 viruses: black raspberry necrosis (BRNV), raspberry mosaic (RMV) (a combination of BRNV and *Rubus* yellow net viruses), tomato ringspot (TomRSV), and raspberry vein chlorosis (RVCV); and a virus free control (VF). There are

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20 possible treatment combinations of the 4 cultivars and 5 viruses.

Samples of 3 to 5 flowers each were taken from each treatment in 2 replications in both 1968 and 1969. Tomato ring spot virus infected plants showing advanced decline symptoms were not sampled because they had few flowers. Cytological examinations were made of 'Fairview' and 'Willamette' embryo sacs in flowers collected at anthesis (defined here as that stage of flower development occurring when the stigmas are first exposed by the expansion of the sepals) and of flowers of all 4 cultivars collected 4 days after anthesis. The flowers were fixed in Belling's modified Navashin fluid, embedded in paraplast, sectioned serially at 10 microns thickness and stained with Heidenhain's iron haematoxylin for microscopic examinations. Five or more pistils in each flower were surveyed to determine the stage of embryo sac development. Longitudinal sections of 3907 embryo sacs were examined in all.

In order to carry out statistical analysis of the results, each stage of embryo sac development, from megaspore mother cell to a 16-celled or larger embryo, was given a numerical index between 0 and 100 based upon the ordinates of a cumulative stanine distribution. Differentiated eggs (enlarged, vacuolate and with one nucleolus) were assigned a value of 40. Zygotes (with 2 nucleoli) were given a value of 60. Two-nucleate, 4-nucleate, and 8-nucleate sacs were valued at 4, 11, and 22 respectively, and 2-, 4-, and 8-celled embryos were valued at 77, 89, and 96 respectively. Abnormal and degenerate embryo sacs were given an index of 29, the same as that of an undifferentiated micropylar triplet. Analysis of variance and Duncan's New Multiple Range tests were then carried out to determine the comparative stage of embryo sac development of the different cultivars and the treatments. The 5 percent level of significance was used in evaluating the results.

Results

Embryo sac development in the red raspberry is generally of the familiar monosporic 8-nucleate normal or Polygonum type, and is similar to that described for salmonberry, *Rubus spectabilis*, (16) and other syngamic *Rubus* species (11, 14).

In some ovules 2 or more sacs were observed. Such sacs might both be functional, but this is thought to be unlikely. Generally no egg cell had reached maturity in multiple sacs. In some sacs the antipodal cells, instead of disintegrating, proliferated, especially in 'Fairview'. The fate of these cells is not known. They lacked organization, especially the polarity, of embryos.

Embryo sac development at anthesis ranged from a 2-nucleate stage to a fully differentiated egg. In collections 4 days after anthesis, embryo sac growth stages ranged from an unfertilized egg to a 20-celled embryo.

The endosperm was coenocytic. The primary endosperm nucleus apparently divided much more rapidly than the embryo cells. An embryo sac containing a 2-celled embryo contained 10 to 20 endosperm nuclei. The division rate of the endosperm nuclei appeared to decrease as the embryo grew and one 14-celled embryo had an endosperm of 50 to 60 nuclei.

The results (Tables 1, 2) demonstrated differences in embryo sac development due to virus treatments, cultivars, and years, although none of these effects was consistent throughout the experiment. The standard deviation of indices of ovules within flowers at anthesis was 4.9 (2362 degrees of freedom) and 4 days after anthesis was 11.9 (1168 degrees of freedom).

Embryo sacs of only 2 cultivars, 'Willamette' and 'Fairview', were examined at anthesis. In 'Willamette', both BRNV and RVCV retarded embryo sac development when compared to VF. The RVCV effect was more severe than that of BRNV. The effects of TomRSV and RMV were not significantly different from those of VF. 'Fairview' plants infected with TomRSV had advanced embryo sac development compared to VF. Raspberry mosaic virus, although not significantly different in effect from VF and BRNV, retarded development compared to RVCV and TomRSV.

In 1968 the virus treatments had no effect on mean embryo sac index at anthesis. In 1969, RVCV resulted in retarded embryo sac development at anthesis compared to VF and the

Table 1. The effect of virus on raspberry embryo sac index[a]

		Treatment				
		BRNV	RMV	TomRSV	RVC	VF (control)
Willamette, at anthesis (2 years)	mean	33.5b	34.0ab	33.9ab	31.4c	35.5a
	no. of observations	383	299	193	234	107
Fairview, at anthesis (2 years)	mean	31.7bc	30.9c	33.2[a]	33.0ab	31.8bc
	no. of observations	214	239	235	254	289
1968, at anthesis (2 cultivars)	mean	32.6a	32.2a	33.5a	33.0a	32.2a
	no. of observations	381	398	209	165	221
1969, at anthesis (2 cultivars)	mean	33.3a	33.8a	33.5a	31.8b	33.5a
	no. of observations	216	140	219	323	175
1968, 4 days after anthesis (4 cultivars)	mean	65.1a	60.8ab	52.5c	56.7bc	57.3bc
	no. of observations	155	155	140	155	155
1969, 4 days after anthesis (4 cultivars)	mean	82.9a	84.0a	85.2a	86.1a	85.1a
	no. of observations	140	135	150	145	130

[a]Means in the same row not sharing the same letter were significantly different at the 5% level.

Table 2. The effect of cultivar on raspberry embryo sac index[a]

		Cultivar			
		Willamette	Fairview	Newburgh	Sumner
1968, 4 days after anthesis (5 treatments)	mean	59.6b	50.8c	76.2a	47.9c
	no. of observations	175	200	195	190
1969, 4 days after anthesis (5 treatments)	mean	88.1ab	89.2a	83.7b	77.9c
	no. of observations	175	195	130	200

[a]Means in the same row not sharing the same letter were significantly different at the 5% level.

other virus treatments.

Embryo sac development 4 days after anthesis was most advanced in 'Newburgh' in 1968. 'Willamette' embryo sacs were not as advanced in development as 'Newburgh' but were at a more advanced stage of development than those of 'Fairview' and 'Sumner'. The last 2 were at a similar stage of embryo sac development. In 1969, the average index of embryo sac development in 'Fairview' though not significantly different from that of 'Willamette' was higher than that of either 'Newburgh' or 'Sumner'. 'Willamette' and 'Newburgh' sacs were not significantly different in development, but sacs of both cultivars were more advanced in development than those of 'Sumner'.

Some virus effects were apparent in the combined cultivars 4 days after anthesis in 1968. Plants infected with BRNV had a higher mean embryo sac index than VF plants, although it was not significantly higher than that of RMV infected plants. Tomato ring spot virus caused retardation of embryo sac development, compared to BRNV and RMV, but was not significantly different in effect from RVCV and VF. No differences in the effects of the 5 treatments could be discerned in the 4-day flowers in 1969.

Discussion

The index system used here to quantify the stages of embryo sac development does not seem to have been used previously. It may have some limitations, particularly in the evaluation of abnormally developed embryo sacs. The results, however, agreed well with various preliminary qualitative tabulations of the same data. There are definite advantages in quantifying the stages of embryo sac development since statistical analyses are greatly facilitated.

In a previous study (5) it was demonstrated that one crumbly fruited somatic mutant of 'Sumner' had increased pollen abortion and retarded embryo sac development when compared to normal 'Sumner'. The results of this study and the results of pollen abortion studies (10) make it apparent that even normal 'Sumner' has a high percentage of pollen abortion and increased retardation of embryo sac development when compared to the other raspberry cultivars.

Since the effects attributed to virus treatment and cultivars were very inconsistent in this study, it is difficult to draw definite conclusions about the susceptibility of certain cultivars to specific virus-induced effects on embryo sac development. In some cases, particularly with BRNV treated plants 4 days after anthesis in 1968, virus infected plants had a more advanced stage of embryo sac development than virus free plants. It may be that viruses stimulated embryo sac development under some circumstances, but retarded it in others. It is doubtful whether the differences noted in embryo sac indices are large enough, even where statistically significant, to account for differences in fruit-set or yield of virus infected raspberry plants. It is not known whether differences in fruit set of virus-infected plants are due to pollen abortion (10) or to break-down in later stages of embryo development.

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