

Breeding and genetics of fruit-rot resistance and polyphenolics in the American cranberry

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Cranberry fruit rot poses a serious threat to cranberry growers and is increasing in severity and scope.

- In New Jersey, crop losses of 25% are common even with multiple fungicide applications.
- Wisconsin, where fruit rot has not been a concern, now has numerous reports of severe fruit rot.
- This disease complex is caused by 10-15 fungal species, primarily *Phyllosticta vaccinii*, *Physalospora vaccinii*, *Colletotrichum gloeosporioides*, and *Coleophoma empetri*.
- Severe disease pressure in NJ makes our program optimal for fruit rot resistance breeding.
- We have identified multiple sources of broad-based field fruit rot resistance (FFRR).
- From initial crosses, we have established that FFRR is transmitted to progeny, and appears to be heritable.

Thus our ultimate objective is to develop high yielding cranberry varieties with increased levels of FFRR, and enhanced polyphenolic profiles. These varieties will improve the economic and environmental sustainability of cranberry production.

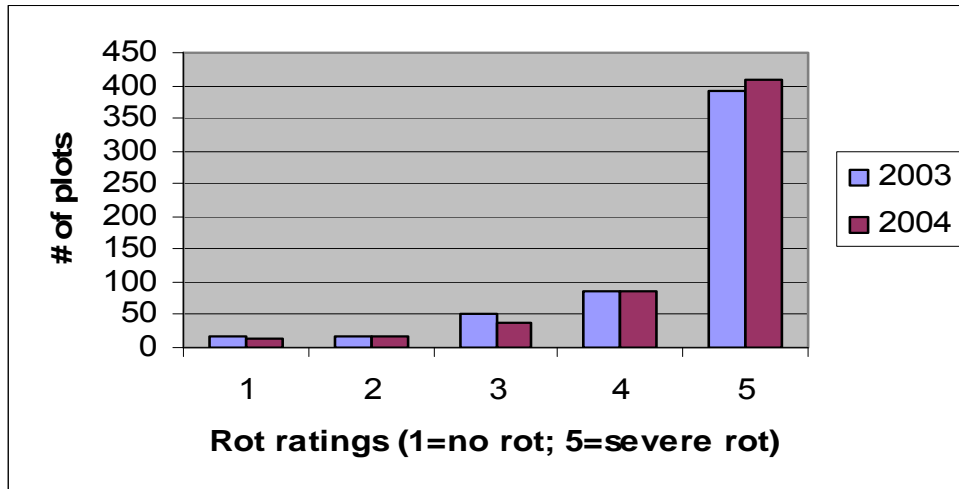
Aims of the Project:

- Estimate field fruit rot resistance (FFRR) heritability and introgress FFRR into high yielding diverse genetic backgrounds with superior polyphenolic content.
- Determine the relationship of polyphenolics with FFRR during the flowering and fruit development phases.
- Identify molecular markers (QTLs) linked to FFRR.
- Determine the correlation between horticultural traits (yield, establishment, berry size) and polyphenolic profiles.

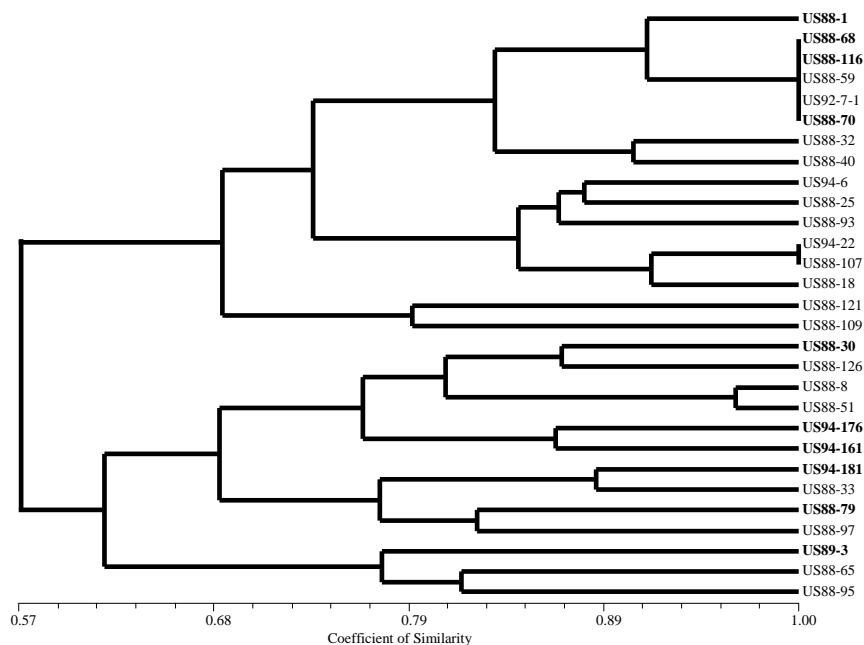


Germplasm Evaluation

- Of 562 germplasm accessions evaluated, most were highly susceptible to fruit rot (rating of 5), but a few showed consistent resistance.
- Fruit rot counts ranged from 5% to 100% rotten fruit; counts and ratings were highly correlated ($r=0.9$).
- Limited sampling in 2004 found several fruit rot fungal pathogens in berries of both resistant and susceptible accessions, suggesting broad-based resistance.



DNA fingerprinting (SCARS) of fruit-rot resistant accessions (bold font) identified several genetically distinct types.

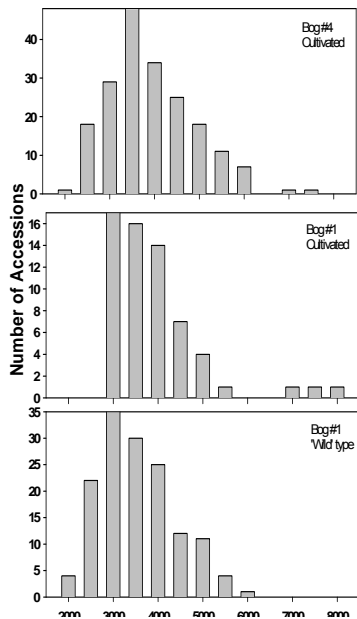


**Fruit rot ratings of accessions in a germplasm evaluation trial,
Chatsworth, NJ (1-5 scale, where 1=no rot, 5=100% rot)**

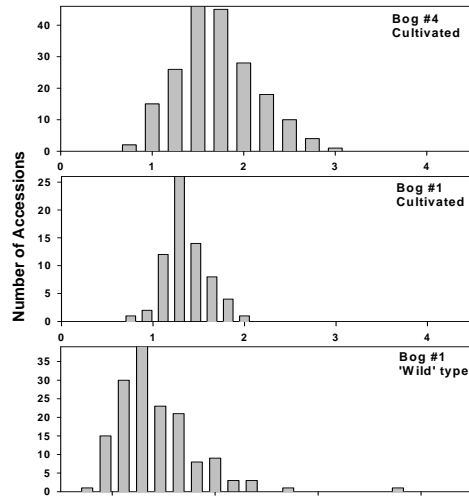
Cultivar/Accession	Code	Fruit rot rating	
		22-Sep-03	7-Sep-04
DREVER	US88-1	1.0	1.0
HAINES BLUES-1	US94-176	1.0	1.0
HAINES BLUES-2	US94-181	1.0	1.0
BUDD'S BLUES	US88-30	1.0	1.0
BUDD'S BLUES-TYPE	US93-34	1.0	1.0
CHAMPION	US88-116	2.0	1.0
CUMBERLAND	US88-79	2.0	1.0
HOLLISTON-TYPE	US88-68	2.0	1.0
PARADISE MEADOW-1	US88-97	1.0	3.0
US88-121	US88-121	2.0	2.0
US89-3	US89-3	2.0	2.0
PARADISE MEADOW-2	US88-85	3.0	2.0
GRYGLESKI HYBRID #3	US94-6	3.0	2.0
WALES HENRY	US88-67	2.0	3.0
AR2	US88-43	2.0	3.0
CUTTS BOG TETPLD B	US94-57	3.0	2.0
US94-93	US94-93	3.0	2.0
GEBHARDT'S BEAUTY	US88-115	2.0	3.0
US94-12	US94-12	2.0	3.0
HOLLISTON-TYPE	US88-59	3.0	3.0
GRYGLESKI HYBRID #2	US94-5	3.0	3.0
PILGRIM LAKE, MASS	NJ91-13-7	3.0	3.0
HOLLISTER RED	US88-70	3.0	4.0
LEMUNYON		3.8	3.9
FRANKLIN		4.0	4.0
WILCOX		4.0	4.5
#35		4.5	4.5
PILGRIM		4.0	5.0
EARLY BLACK		4.5	4.6
POTTER		4.6	4.6
STEVENS		4.8	4.5
CROPPER		4.6	5.0
HOWES		4.8	4.9
MCFARLIN		5.0	4.8
BEN LEAR		-	5.0
BLACK VEIL		5.0	5.0
EARLY RICHARD		5.0	5.0
Mean of 562 accessions		4.5	4.5

*Code is the designation given to each accession when collected in 1988-1994. Accessions without codes are the means of multiple plots of that cultivar.

Levels of phenolic compounds vary widely among cranberry germplasm. Cranberry fruit contains high concentrations of phenolic compounds which have human health-related benefits, and may be associated with fruit rot resistance.

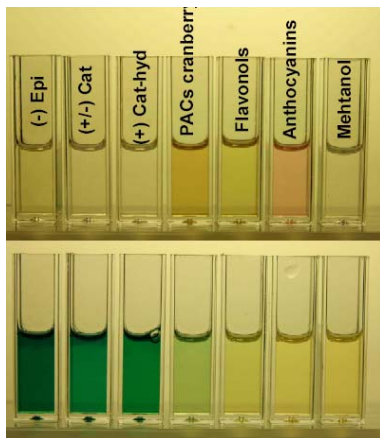


Frequency distribution for **total phenolic content** in cranberry germplasm collection (from cultivated beds and wild populations), using HPLC-PDA at 280nm (Vorsa et al. 2002).

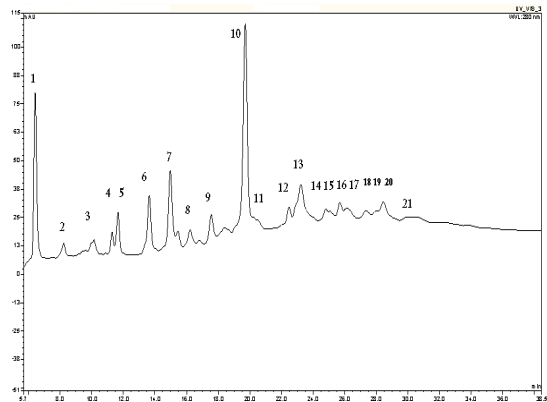


Frequency distribution for **total proanthocyanidin content** in cranberry germplasm collected from cultivated beds and from wild populations using vanillin-sulphuric acid assay (Vorsa et al. 2002).

New protocols allow for concise and efficient evaluations of **proanthocyanidins**, compounds associated with disease resistance in other plants.

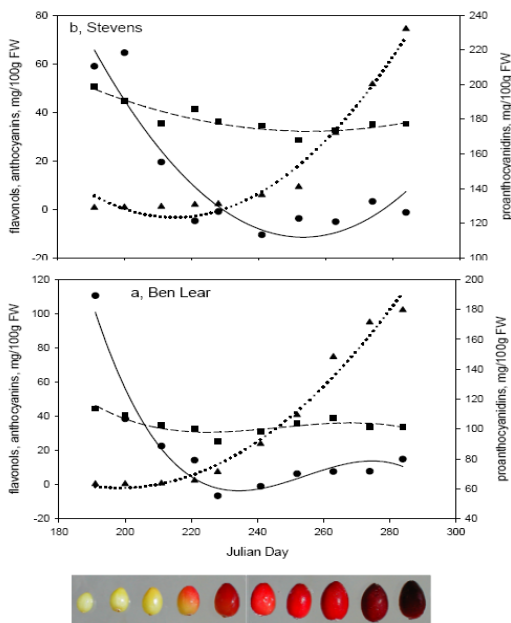


Proanthocyanidins (PAC) can be efficiently measured with DAC reagent. Because absorption is measured at 640nm, anthocyanins do not interfere, eliminating an extra sample preparation step. In the lower vials, DAC has reacted with the PAC monomers epicatchin (Epi), catechin (Cat), and catechin-hyd, and cranberry PAC's.

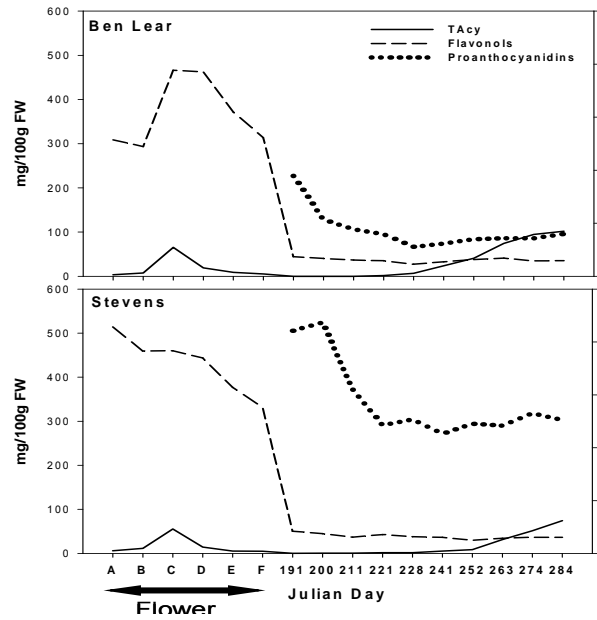


HPLC chromatogram demonstrating **separation of purified proanthocyanidins** of cranberry (using a Sephadex column): Peaks 1-3 correspond to dimers (DP-2), peaks 4-7 to trimers (DP-3), peaks 8-10 to tetramers (DP-4), peak 11 to pentamers (DP-5), peak 12 to hexamers (DP-6), peak 13 to heptamers (DP-7) peak 14 to octamers (DP-8), peak 15 to nonamers (DP-9), peak 16 to decamers (DP-10), peak 17 to undecamers (DP-11), peak 18 to dodecamers (DP-12), peak 19 to tridecamers (DP-13), peak 20 to tetradecamers (DP-14), and peak 21 to pentadecamers (DP-15).

Phenolic Profile of Resistant Varieties: To identify a possible mechanism of resistance, we are evaluating the polyphenolic profiles of fruit rot-resistant vs. susceptible selections, starting at bloom.

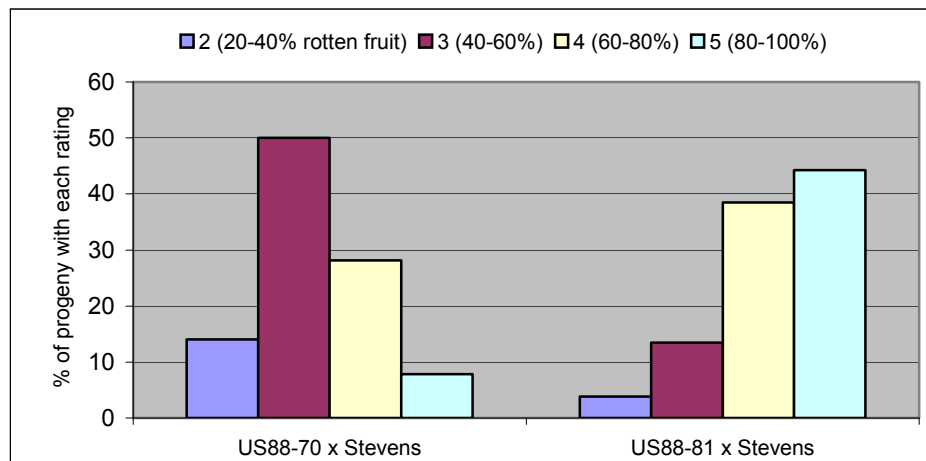


Levels of flavonoid classes during the fruit development period for cranberry varieties 'Ben Lear' and 'Stevens', and fit to curves as a function of Julian Day. Flavonoid classes are anthocyanins (●▲●), flavonols (■), and proanthocyanidins (●). **Proanthocyanidins** are highest at fruit set (Vvededskaya & Vorsa, 2004).



Flavonol and anthocyanin levels in flowers: A – unopened, B – anthesis, C – style elongation, D – anther diarticulation, E – petal drop, and F – ovary enlargement. **Flavonol levels** were exceptional high in unopened flowers and dropped rapidly at petal drop (Vvededskaya & Vorsa, 2004).

Progeny Evaluation: Over 1600 progeny from 30 families were evaluated over three years. Families with a resistant parent had higher frequencies of resistant progeny; indicating potential for improving fruit rot resistance. For example, moderately resistant US88-70 x 'Stevens', as compared to susceptible US88-81 x 'Stevens'



Infection Process and Mechanism of Resistance: The current hypothesis is that fruit rot fungi infect during flowering in cranberry, and then remain latent until fruit ripening.

- In 2008, we evaluated flowering characteristics and found no relationship between FFRR and bud development rate, % bloom, or flowering period.
- In 2009, flowers from resistant and susceptible selections were collected weekly, surface sterilized and plated on PDA.
- A total of 1276 cranberry ovaries were plated and fungi were isolated from 53% of them, including: *Alternaria* spp., *Cladosporium* spp, *Colletotrichum gloeosporioides*, *Penicillium* spp., *Pestalotia vaccinii*, *Phyllosticta elongata*, *Phyllosticta vaccinii*, *Physalospora vaccinii*, *Rhizoctonia* spp., mycelia sterilia and yeasts.
- We will continue sampling fruit over the season and compare the fungal profile of resistant and susceptible selections to determine when fruit are infected, and with which pathogens.



Molecular Markers for Resistance: In order to discover molecular markers for genetic mapping of FFRR, we are:

- preparing cranberry DNA (from inbred lines) for sequencing by a state-of-the-art high-throughput method
- evaluating microsatellite primers from blueberry in our cranberry progenies segregating for FFRR

DNA from Stevens and US88-70 (parents of a cross segregating for resistance) were amplified using four microsatellite primer sets; differences in banding were found with two sets (noted with arrows).

These markers, along with others, will be evaluated in the progeny to identify molecular genetic markers linked to FFRR.

