A DNA fingerprinting study of Washington State and Newfoundland 'Stevens': is 'Stevens' becoming more contaminated with off-types?

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Washington State beds of Stevens have variable yields, with many having yields of less than 100 bbl/acre. A DNA fingerprinting study of 17 Stevens beds from Washington State was carried out in July 2008 using a sequence characterized amplified regions (SCAR) marker methodology. The 17 Stevens' beds ranged in productivity from **31** to **396 bbl**/acre (2 year average) in productivity. For each bed, five areas across the bed were sampled by taking 5 uprights/runners in a radius about a given point (Fig. 1). Leaves from each subsample were bulked into one sample, to give one DNA fingerprint. For example, Fig. 2 shows one leaf from separate uprights bulked into one tube to give one fingerprint in lane 2. Although bulking does lead to loss of information, this is the most economical way to search for off-types in a bed. In this study 450 subsamples of uprights/runners were run as 90 samples. From Bed 1, lanes 2 through 4 and 6 match our Stevens control in lane 1, and suggest that these samples (all 5 subsamples in each sample) are likely Stevens. Lane 5 has extra bands indicating there was something other than Stevens in this sample. From Bed 2, the fingerprints in lanes 7 through 12 suggest that off-types are present in all samples.

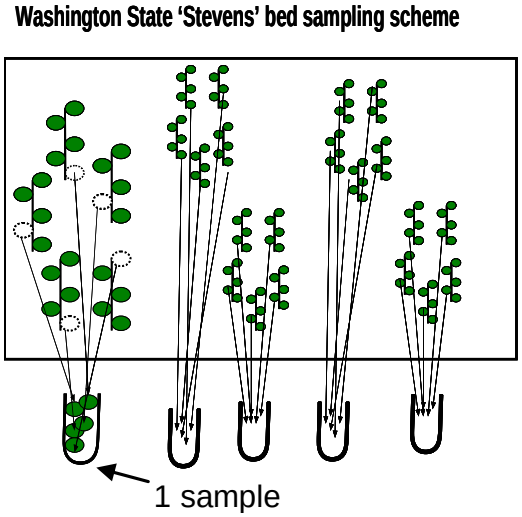

Fig. 1. Each cranberry bed had total of 25 subsamples taken. Five evenly distributed areas through out the bed were sampled, with 5 subsamples (uprights or runners) selected to be bulked into one sample (to keep costs down).

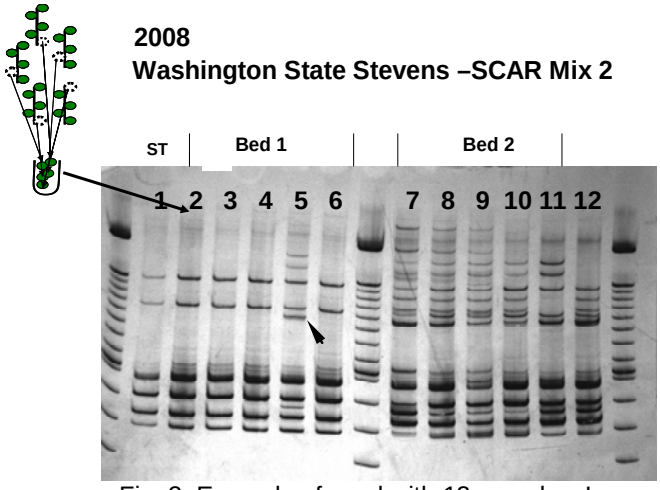

Fig. 2. Example of a gel with 12 samples. Lane 1 is our Stevens control. Lanes 2 – 6 represent 5 samples from 1 Stevens bed, and lanes 7-12 represent samples from a 2nd bed. Note: lanes 2 – 4 and 6 match our Stevens control (lane 1), whereas lane 5 does not. It has some additional bands indicating there at least one subsample was not Stevens. Bed 2 (Lanes 7 – 12) has no match to Stevens, indicting considerable contamination. Arrow indicates extra non-Stevens bands in lane 5.

When we looked at the relationship between the number off-type fingerprints and yield, we found that beds which had a greater number of 'non-Stevens' bands present in the fingerprint,

generally were lower yielding (Fig. 3). **Therefore, productivity of Washington State Stevens beds decreases when a greater number of off-types are present.**

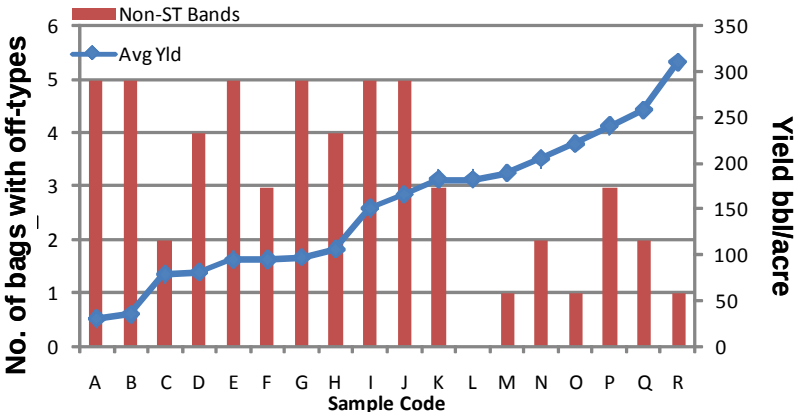

Fig 3. The number of non-Stevens bands in the 5 fingerprint samples and the production bbl/acre (2 year average) of the bed.

Establishing new beds with 'prunings' may result in poorly producing beds - For one bed, uprights and runners were sampled separately. We found uprights to have both Stevens and non Stevens types, whereas, for runners all 5 samples (all 25 runner subsamples) lacked Stevens bands. Thus, if one were to prune runners for propagation from this bed, one would establish a new bed with little or no Stevens vines in the new bed. One could mow the bed. However, even this practice does not guarantee that the newly established bed would be the same varietal composition as the original bed. Stevens may have a greater tendency to begin fruiting, at the expense of runners, more readily than the off-type varieties.

Fingerprinting (with SCARs) Newfoundland cranberry

Newfoundland initiated cranberry propagation in the late 1990's, and obtained rooted cutting from commercial nurseries to initiate Newfoundland's cranberry industry. The principal varieties were Stevens, Pilgrim and Ben Lear. Earlier this year we obtained samples from various farms from Newfoundland. Below, are DNA fingerprints obtained for beds of Ben Lear (Fig. 4) and Stevens (Fig. 5) from two different farms for each of the varieties. The bed of Ben Lear of Farm 1 appears to be very consistent with

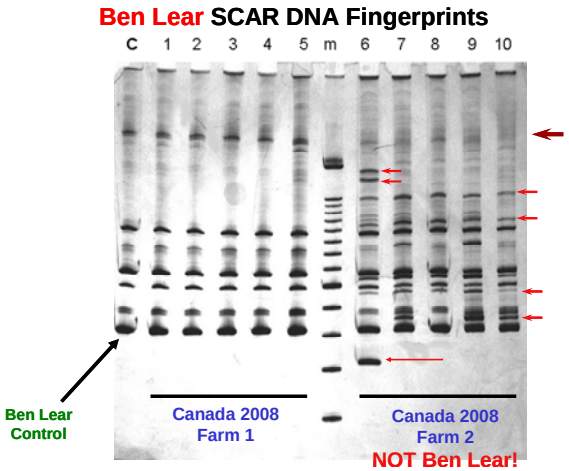

Fig. 4. DNA SCAR fingerprints of samples from Newfoundland Ben Lear beds from 2 different farms. Farm 1 DNA fingerprints (lanes 1-5) match pretty well our Ben Lear control (lane c) whereas, DNA fingerprints from Farm 2 (lanes 6 – 10) do not match. Arrows indicate non Ben Lear bands.

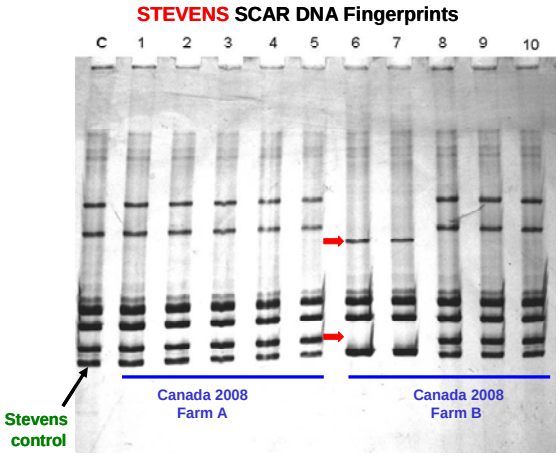

Fig. 5. DNA SCAR fingerprints of samples from Newfoundland Stevens beds from 2 different farms. Farm A DNA fingerprints (lanes 1-5) match pretty well our Stevens control (lane c) whereas, DNA fingerprints from Farm 2 (lanes 6 – 10) do not match. Arrows indicate non Stevens bands.

our Ben Lear fingerprint control (lane c), whereas none of fingerprints from Farm 2 are consistent with the control. Similarly, Farm A has fingerprints consistent with Stevens control in Fig. 5 (lane c). Farm B, however, has only three samples matching (lanes 8 – 10), and lanes 6 and 7 which lack Stevens bands, plus non-Stevens bands. Thus, Newfoundland beds have uncertain varietal purity.

Conclusion: As more samples are sent to us from various growing areas, we are finding numerous beds considered to be Stevens, which are moderately or severely contaminated with off-type varieties. It would also appear that the off-types typically are less productive than Stevens, apparently partitioning their resources to ‘runnering’ as opposed to fruit production. **Therefore, prunings, unless absolutely certain of their varietal integrity should be avoided in establishment of new plantings.**