The New Cranberry Wisconsin Research Station: Renovation Priorities of a ‘Stevens’ Cranberry Marsh Based on Visual Mapping, Genetic Testing, and Yield Data

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ABSTRACT
Cultivar contamination is a common issue in commercial cranberry production. Unknown or unwanted cranberry genotypes are found in commercial cranberry beds that are intended to be a single uniform genotype. Identification of contamination and the impact of contamination remain crucial issues for the cranberry industry to maintain long-term high productivity. To address this issue, tissue samples were taken from the former commercial beds of the new Wisconsin Cranberry Research Station (WCRS) for genetic fingerprinting analysis. The goal of this collection was to analyze the 10 beds for genetic uniformity to determine if any should be maintained or replaced, and to assess the accuracy of visual perception of genetic contamination in the field. A total of 288 DNA samples were collected in the 10 cranberry beds, and the ‘Stevens’ cultivar represented 180 samples or 69% of the 261 samples expected to be ‘Stevens.’ Therefore, the genotype contamination in the ‘Stevens’ beds was 31% overall. Overall, visual differentiation was accurate in distinguishing between genotypes and detecting large areas of contamination. A yield analysis was conducted along with the genotypic uniformity assessments, and a significant correlation was found between the 2017 yield of the beds and their level of genetic contamination. This study demonstrates the usefulness of genetic uniformity testing and mapping for cranberry bed management and renovation decision-making.

KEYWORDS
SSRs; microsatellites; DNA fingerprinting; genetic uniformity; clonal purity; barren berry; cranberry bed management; Wisconsin Cranberry Research Station

Introduction
The American cranberry (Vaccinium macrocarpon Ait.) is native to the bogs and wetlands of central Wisconsin and Eastern North America (Vander Kloet, 1983, 1988). The species was a staple in the diet of Native Americans long before it became a commercially cultivated crop (Durand, 1942; Eck, 1990; Peltier, 1970). The first attempt to cultivate cranberries occurred in Massachusetts in 1810, and it expanded to Wisconsin in 1853 (Chandler and DeMoranville, 1958; Dana, 1983; Eck, 1990; Voris and Zalapa, 2019). The Wisconsin cranberry industry grew rapidly in the late-19th century, and by the 1940s, Wisconsin had become one of the top producers of cranberries in the nation, second only to Massachusetts (Durand, 1942; Eck, 1990; Peltier, 1970). In 2019, cranberry yield in the US totaled over 8.9 million barrels or 445,000 tons, and of this, Wisconsin led the nation with nearly 60% of all production (USDA NASS, 2020).
Cranberries are often found colonizing large areas of natural bogs as single genotypes by way of their strong prostrate growth habit (Rodriguez-Bonilla et al., 2019; Rodriguez-Bonilla et al., 2020; Zalapa et al., 2015). This ability of cranberries to colonize their environment is used in commercial production to grow a single genotype in large production areas (Eck, 1990). Thus, cranberry is a clonal crop in which a single genotype or cultivar is propagated vegetatively until enough material can be used to plant a cranberry bed, the unit area of production, which in Wisconsin is variable in size, but typically corresponds to a square or rectangular sunken bed surrounded by dikes (Fajardo et al., 2013). However, under commercial growing practices, particularly under sand cultivation, cranberries are poor competitors with most other plants, which leads to the spread of many weed species that cause significant losses to cranberry growers (Dana, 1987; Else et al., 1995; Mason et al., 2006). In addition to weed species, cultivated cranberry genotypes also have to compete with other unwanted cranberry genotypes, some of which are more vegetative in nature, and can easily outcompete fruiting/reproductive cranberry genotypes (Fajardo et al., 2013; Novy and Vorsa, 1995; R.g. et al., 1996; Roper et al., 1995). Thus, commercial cranberry plantings can be contaminated with multiple unintended genotypes when the starting propagation material is not pure or overtime due to chance seedlings and/or other propagules invading the pure original planting (Fajardo et al., 2013).

Genetic markers such as simple sequence repeats (SSRs) or microsatellite markers can be implemented to genetically fingerprint, identify, and classify wild and cultivated cranberry genotypes (Fajardo et al., 2013; Rodriguez-Bonilla et al., 2019; Rodriguez-Bonilla et al., 2020; Schlautman et al., 2017, 2018, 2015; Zalapa et al., 2015). Several cranberry SSR studies have shown that it is possible to identify clonality within commercial plantings and wild stands (Fajardo et al., 2013; Rodriguez-Bonilla et al., 2019; Rodriguez-Bonilla et al., 2020; Zalapa et al., 2015), as well as to detect contamination due to selfing, outcrossing, and clonal misidentification in commercial plantings and collections (Fajardo et al., 2013; Schlautman et al., 2018). Detecting and estimating genetic contamination is a critical aspect of maintaining cranberry production efficiency since contaminants may be associated with lower productivity and decreased fruit quality (Fajardo et al., 2013; Novy and Vorsa, 1995; Polashock and Vorsa, 2002; Schlautman et al., 2018). For example, “barren berry” cranberry variants, which produce little to no fruit have been identified in Wisconsin using random amplified polymorphic DNA (RAPD) (Roper et al., 1995).

The first Wisconsin Cranberry Research Station (WCRS) existed from 1903 to 1917, although researchers began using the Wisconsin plots in 1893 (Eck, 1990; Peltier, 1970). Notably, a number of regional native selections, including Ben Lear and Prolific, were selected at the original cranberry station. In 1917, the station was closed due to lack of funding, and the research plots became commercial cranberry beds (Peltier, 1970). In 2017, exactly 100 years later, a 155-acre commercial cranberry marsh was purchased to serve as a second WCRS. Research at the station began in 2018 using the existing commercial cranberry beds, which provided an opportunity to conduct genotype fingerprinting analysis, visual perception of uniformity, and its correlation with historical whole-bed yield. The station was composed of 10 existing beds, nine of which were planted to the ‘Stevens’ cultivar, which is the most popular hybrid release in the US and around the world. ‘Stevens’ was released in the 1950s, and many new and renovated beds are still being planted with this cultivar in Wisconsin and other states (Dana, 1983; Eck, 1990; Vorsa and Zalapa, 2019).

The purpose of this study using the 10 production beds at the new WCRS was to 1) conduct a visual mapping of cultivar contamination, 2) sample and analyze the genotypes present in each bed in the context of a visual map to assess accuracy of visual mapping, 3) analyze the historical yield of each bed in the context of genotypic data to determine if there is a correlation between yield and genetic uniformity, and 4) use the genetic and yield data to determine which beds should be maintained or renovated. We aim to provide a model for the cranberry industry of how genotype testing combined with visual inspection and yield data can help growers make management decisions, such as replanting, versus changing cultural practices, such as fertilizing, mowing, and sanding.
Materials and Methods

Visual Mapping of Contamination

The main reasoning behind monoculture in cranberry is that different cultivars fruit and ripen at different points in the season, and therefore separating cultivars allows for an entire bed to be harvested at once (Eck, 1990). Moreover, each genotype may have a slightly different developmental timing from breaking dormancy in the spring, to flowering in mid-June, to fruit setting, sizing, and coloring in late summer, and finally going back to dormancy in the fall. Additionally, cranberry plants also have different proportions of vegetative versus reproductive growth, which produce varied vine heights and colors (due light exposure) in different genotypes throughout the growing year.

When cranberry beds are contaminated with multiple genotypes, these phenotypic differences are clearly observable in cranberry beds (Figure 1). Growers often observe and report pockets of phenotypically odd-looking and unproductive vines in otherwise homogenous cranberry beds (Figure 1) (Novy and Vorsa, 1995; R.g. et al., 1996; Roper et al., 1995). These differences in fruiting and other characteristics can be easily mapped (Figure 2), similarly to the visual mapping of weeds, which is a known component of integrated pest management in cranberry production (Else et al., 1995).

The 10 existing cranberry beds at the Wisconsin Cranberry Research Station (WCRS), Robinson Creek Cranberry Marsh, in Black River Falls, Wisconsin (Long. 44.18586, Lat. −90.74192) were visually assessed for contamination. Each of the 10 beds was visually assessed based on qualitative differences in vine color (green versus red), height (tall versus short), and fruiting habits (fruiting versus non-fruiting) (Figure 1). The visual mapping was intended to identify major areas that might

Figure 1. Visual mapping of differences in the cranberry beds at the Wisconsin Cranberry Research Station. A) Example of color (red) and vine height differences, B) mapping of perceived differences (the blue lines indicate areas with perceived differences), C) close-up of color and height differences, and D) example of lack fruit development in the differentially mapped areas.
Figure 2. Aerial view of the 10 cranberry beds at the Wisconsin Cranberry Research Station. Visual mapping (Figure 1) was used to divide each bed (blue lines) in different areas of apparent phenotypic differences. Each point represents the GPS locations of each sample (n = 285) collected and genotyped using nine microsatellite markers. The color of the points represents each of the genotypes detected in the genetic analysis. The yellow arrow indicates the location of the cranberry bed in Figure 1 which is given as an example of the mapped differences. Nine of the beds were originally planted with the 'Stevens' cultivar and the single 'BG' bed is identified in the figure. Each bed was numbered from 1 to 10, with the first bed being furthest south and bed 10 being the furthest north in the satellite image.

correspond to distinct cultivars or genotypes in the map. A map was developed based on the perceived visual differences in each of the 10 cranberry beds, nine of which were originally planted with the 'Stevens' cultivar and one was planted with 'BG' (Figure 2).

Sampling for Genetic Analysis

A total of 288 samples (three 96 well plates) were collected throughout the 10 beds of the WCRS, with sampling occurring to cover the different areas identified in the visual inspection map (Figs. 1 and 2). The coordinates of each sample were recorded using a global positioning system (GPS). Each sample was an individual upright (i.e., vertical stem) or runner (i.e., stem that grows parallel to the soil surface) of 10–15 cm long. The plant material collected corresponded to the current year’s growth and contained at least 20 leaves. Each sample was packaged separately in an envelope, kept cool in ice and frozen until further processing for DNA extraction. Each sample was labeled with the site name, date, bed #, cultivar, phenotype, and GPS coordinates.

DNA Extraction

Genomic DNA extractions for microsatellite analysis of 288 samples collected at the WCRS were performed using leaf tissue per CTAB method (Doyle and Doyle, 1987) modified in our laboratory to isolate high quality, clean DNA. Briefly, the leaf tissue was lyophilized using a BenchTop lyophilizer (Virtis Inc., Gardiner, N.Y.), and a total of 10–20 mg of tissue were placed in 2.0 mL tubes. Glass and metal beads were added to the tubes, and the samples were placed in a tissue grinder for 60 seconds, or until all samples ground into powder. 700 μL of 2% CTAB extraction buffer [20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB] was added to the tube and then mixed. The solution was then incubated at 65°C for 45 min. After incubation, 400 μL of a chloroform-isoamyl alcohol (24:1) solution was added to the tubes and gently mixed by inversion. Samples were then centrifuged for 5 min at 14,000 rpm; 500 μL of the supernatant was then transferred to a fresh 1.5 mL tube containing 50 μL of 10% CTAB buffer and then mixed. 750 μL of cold isopropanol (100%) was added, and samples were
then incubated from 2 to 48 hours at −20°C. Samples were then centrifuged at 14,000 rpm for 20 min. After centrifugation, the supernatant was discarded, and the resulting pellet air-dried for 5 minutes. The pellet was then washed with 700 µL of cold 70% ethanol, vortexed, and centrifuged at 14,000 rpm for 4 min. The ethanol was discarded, and the pellet air-dried for approximately 24 hours. The DNA was then re-suspended in 100 µL TE 10:1 buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) plus 5 µL of ribonuclease (RNAse 10 mg mL−1) in each tube, and was incubated at 37°C for 2 hours prior to storage at −20°C.

**Polymerase Chain Reactions (PCRs)**

PCRs were performed according to Schlautman et al. (2018) using carboxyfluorescein (FAM) and hexachlorofluorescein (HEX) M13 tags (5’-CACGTTGTAAAACGAC-3´). Briefly, reactions were performed in 8 µL total volume using 3.5 µL 1× JumpStart REDTaq ReadyMix (Sigma, St. Louis, MO, USA), 1.0 µL of 15 ng/µL DNA, 2.0 µL of ddH₂O, 0.5 µL of 5 µM forward primer, 0.5 µL of 50 µM reverse primer, and 0.5 µL of 0.5 µM M13-FAM, M13-HEX, or M13-NED primer. Thermocycling conditions included a 3 min melting step of 94°C, followed by 33 cycles of 94°C for 15 s, 55°C for 90 s, and 72°C for 2 min, and a final extension step of 72°C for 30 min. One microliter each of FAM and HEX labeled PCR product was mixed with 10 µL formamide and a carboxy-X-rhodamin (ROX; GeneFlo-625 ROX; CHIMERx, Milwaukee, WI, USA) ladder, and the pool-plexed mix was sent to the University of Wisconsin Biotechnology Center DNA Sequencing Facility for fragment analysis using an ABI 3730 fluorescent sequencer (Pop-6 and a 50 cm capillary array; Applied Biosystems, Foster City, CA, USA). Allele genotyping was performed using the GeneMarker software v 1.91 (SoftGenetics LLC, State College, PA, USA). To avoid technical and human error during genotyping, PCR was repeated as needed (up to three times) and multiple scorers were used to ensure repeatability of the results.

**Genetic Data Analysis**

Nine microsatellite loci were used to genotype the 288 samples collected at the WCRS according to Fajardo et al. (2013) and Zhu et al. (2012). The primers used were vm04084, vm26877, cvm28527, vm31701, vm38401, vm39030, vm51985, vm52682, and vm78806. Genotype diversity was examined using GenAlEx 6.4 (Peakall and Smouse, 2012). Unique clones were initially detected by calculating a simple genetic distance matrix between pairs of individuals based on the nine microsatellite loci as estimated by GenAlEx 6.4. Then, the alleles of each genotype were individually examined to determine clones. When the two genotypes had identical allelic constitutions in all nine microsatellite markers used, they were considered clones. Missing data points are imputed based on the clonal information using a data matrix consisting of 288 rows and 18 columns, when necessary. The final pairwise genetic distance between plants was estimated and used to construct a principal coordinate analysis (PCoA) using a standardized covariance matrix of the genotypes identified in the WCRS (PCoA; Peakall and Smouse, 2012). For fingerprint identification, the genotypes identified at the WCRS were compared to a database of cranberry cultivar variants present at the National Clonal Germplasm Repository (NCGR; Schlautman et al., 2018).

**Results**

**Visual Mapping of Contamination**

Ten existing cranberry beds at the Wisconsin Cranberry Research Station (WCRS) were visually assessed for differences in vine color, height, and fruiting habits (Figure 1). The mapping of visual differences was done to divide the beds into major areas that might correspond to distinct genotypes or clones (Figure 1). The full map with the integrated visually identified differences for each of the 10
cranberry beds, nine ‘Stevens’ and one ‘BG’ bed, is presented in Figure 2. Leaf samples for genetic analysis with corresponding GPS locations at WCRS collected to cover each of the phenotypically distinct areas identified in visual analysis are also presented in Figure 2.

**Genetic Analysis**

A total of 288 samples were collected at the WCRS and used for genotyping and analysis. In total, 217 samples had no missing data. However, one marker was imputed for 32 samples that had one marker missing, and for 33 samples, 2–3 markers were imputed based on the clonal information in the matrix. Three samples were discarded due to excessive missing data. Out of the 285 samples, only 16 genotypes were detected (Table 1). For fingerprint identification, the 16 genotypes identified were compared to a database of cranberry cultivar variants present at the NCGR (Schlautman et al., 2018). As expected, the nine ‘Stevens’ beds were composed mostly of the ‘Stevens’ genotype and the ‘BG’ bed was composed predominantly of the ‘BG’ genotype. However, 88 samples, or ~30% of the 285 samples collected, did not match the expected genotype. Overall, the ‘Stevens’ cultivar represented 180 samples, or 69% of the 261 samples expected to be ‘Stevens.’ Therefore, the genotype contamination in the nine ‘Stevens’ beds was 31% overall. Similarly, 17 of the 24 samples, or 71% of the samples, collected in the ‘BG’ bed matched the expected genetic fingerprint, which translates into 29% contamination in the ‘BG’ bed overall. The 16 genotypes identified in the WCRS are presented in Table 1, and the locations of each genotype are presented in the genotype map in Figure 2. The major genotype contaminant at the WCRS was matched to genotype 13 (‘Perry Red’) of the NCGR collection (Schlautman et al., 2018). Overall, six out of the nine ‘Stevens’ beds contained the ‘Perry Red’ contaminant; however, this genotype was not detected in the ‘BG’ bed. Overall, a total of 57 samples, or 22%, of the contamination detected in the ‘Stevens’ beds samples were matched to ‘Perry Red.’ Furthermore, the ‘Perry Red’ genotype represented 70% of the detected contamination throughout the nine ‘Stevens’ beds. Other named cultivar contaminants detected in the ‘Stevens’ beds at the WCRS were ‘Howes’ (n = 10), ‘Potter’s Favorite’ (n = 1), and ‘Bugle Mashpee Type’ (n = 1). The ‘BG’ bed contamination mostly consists of a known ‘BG’-closely related variant called BG48 (Fajardo et al., 2013). Additionally, nine unknown genotype variants were identified at the WCRS, which did not match any accession at the NCGR collection (Table 1). In order to investigate the genetic identity of unknown genotypes found at the WCRS, a PCoA was constructed, which is presented in Figure 3. The PCoA analysis included the known cultivars found at the station plus a set of commonly used cultivars in the cranberry industry (Figure 3). Overall, the PCoA showed that the unknown genotypes found at WCRS have a unique

**Table 1.** The 16 cranberry genotypes were detected using nine microsatellite markers and 285 (n) samples at the Wisconsin Cranberry Research Station in 2017 by genotyping 285 samples collected over 10 beds.

| Genotype     | (n) | ct04084 | ct26877 | ct28527 | ct31701 | ct38401 | ct39030 | ct51985 | ct52682 | ct78806 |
|--------------|-----|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| ‘Stevens’   | 180 | 151     | 151     | 268     | 268     | 216     | 218     | 257     | 268     | 185     | 201     | 196     | 202     | 122     | 182     | 269     | 279     | 223     | 223     |
| ‘Perry Red’ | 47  | 173     | 179     | 271     | 271     | 216     | 252     | 257     | 257     | 187     | 195     | 214     | 214     | 171     | 178     | 291     | 304     | 227     | 227     |
| ‘BG’        | 17  | 151     | 153     | 268     | 217     | 232     | 242     | 257     | 257     | 190     | 185     | 196     | 200     | 171     | 171     | 269     | 269     | 227     | 227     |
| ‘BG48’      | 6   | 151     | 151     | 268     | 217     | 232     | 242     | 257     | 257     | 189     | 201     | 200     | 200     | 171     | 171     | 269     | 283     | 223     | 223     |
| ‘Howes’     | 10  | 153     | 159     | 246     | 268     | 252     | 254     | 257     | 303     | 173     | 177     | 202     | 212     | 171     | 178     | 269     | 291     | 231     | 231     |
| ‘Potter’s Fav.’ | 1  | 151     | 199     | 268     | 271     | 216     | 220     | 257     | 268     | 185     | 193     | 196     | 200     | 171     | 182     | 269     | 269     | 223     | 227     |
| ‘Bugl. Mash.’ | 1 | 161    | 183     | 271     | 280     | 216     | 224     | 257     | 268     | 173     | 201     | 212     | 214     | 180     | 180     | 279     | 291     | 227     | 227     |
| Unknown1    | 3   | 155     | 185     | 268     | 271     | 242     | 250     | 282     | 288     | 183     | 208     | 202     | 202     | 171     | 171     | 269     | 269     | 233     | 233     |
| Unknown2    | 2   | 159     | 199     | 246     | 280     | 224     | 254     | 257     | 257     | 173     | 173     | 212     | 214     | 171     | 180     | 291     | 291     | 231     | 231     |
| Unknown3    | 2   | 151     | 153     | -       | 216     | 256     | 257     | 282     | 289     | 195     | -       | -       | -       | -       | -       | 269     | 269     | 231     | 231     |
| Unknown4    | 1   | 151     | 153     | 268     | 292     | 242     | 257     | 257     | 268     | 185     | 199     | 196     | 200     | 171     | 171     | 269     | 269     | 223     | 223     |
| Unknown5    | 1   | 151     | 153     | 268     | 268     | 232     | 242     | 257     | 257     | 268     | 185     | 197     | 196     | 202     | 171     | 182     | 269     | 269     | -       | -       |
| Unknown6    | 1   | 151     | 153     | 265     | 268     | 216     | 232     | 257     | 301     | 185     | 191     | 206     | 206     | 174     | 178     | 271     | 308     | 227     | 237     |
| Unknown7    | 1   | 133     | 199     | 271     | 271     | 216     | 257     | 257     | 189     | 201     | 196     | 214     | 180     | 180     | 279     | 291     | -       | -       | -       |
| Unknown8    | 1   | 151     | 199     | 259     | 280     | 216     | 236     | 286     | 286     | 173     | 189     | 212     | 212     | 171     | 180     | 291     | 291     | 227     | 227     |
| Unknown9    | 1   | 151     | 151     | 246     | 268     | 216     | 216     | 268     | 268     | 201     | 201     | 202     | 202     | 122     | 122     | 269     | 269     | 223     | 223     |

a = allelic constitution of each genotype based on nine microsatellite markers, two alleles per marker.
genetic profile and are diverse compared with the rest of the cultivars in the plot. Based on the full allelic constitutions of these genotypes in Table 1 and genotype NCGR data (Schlautman et al., 2018), Unk4 is very closely related to 'BG' and 'BG48', Unk9 is likely a self of 'Stevens,' Unk5 shares most of its alleles with 'Potter’s Favorite,' Unk2 is a closely related variant of ‘Hoes,’ and Unk6 shares a large proportion of alleles with ‘BenLear’ and ‘Demorranville’ (‘BenLear’ × ‘Franklin’), 11 and 10 alleles, respectively. Unk1, Unk3, Unk7, Unk8 did not closely relate to our samples in the database of the NCGR collection.

**Historical Yield Analysis and Bed Contamination Levels**

Yield data were available for 2013, 2014, and 2017. The 'BG' bed was planted in 2014, so it is only represented by the 2017 yield data. The goal of this analysis was to compare levels of genetic contamination and yield by bed to evaluate the correlation between historical yield and genetic analysis. The historical yields of each bed along with the percentage of genetic contamination for each bed measured in 2017 are presented in Table 2. Each bed was numbered from 1 to 10, with the first bed being furthest south and bed 10 being the furthest north in the satellite image (Figure 2). Genetic contamination ranged from 0% to 69%, and the least contaminated bed was bed 5, while the most contaminated was bed 9, both of which were ‘Stevens’ beds. The ‘BG’ bed had 29% contamination overall. Some of the beds with the highest levels of contamination, such as bed 9 and bed 10, had comparatively low average yield. However, bed 3 and bed 8 also had high levels of contamination, and they were some of the highest average yielding beds. The one bed with no measured genetic

![Figure 3. Principal coordinates analyses (PCoA) of the cranberry genotypes identified using nine microsatellite markers at the Wisconsin Cranberry Research Station. The green triangles are known cultivars commonly used by the cranberry industry. The red points are the nine unknown genotypes identified at the station.](image)

| Bed | (%) | 2013 Yield | 2014 Yield | 2017 Yield | Average Yield |
|-----|-----|------------|------------|------------|---------------|
| 1   | 8%  | 216.84     | 145.26     | 241.05     | 201.05        |
| 2   | 21% | 231        | 115.50     | 215        | 187.17        |
| 3   | 48% | 301.32     | 180.13     | 223.84     | 235.1         |
| 4   | 30% | 143.45     | 210.12     | 213.1      | 188.89        |
| 5   | 0%  | 197.96     | 110.71     | 236.22     | 181.63        |
| 6   | 29% | -          | -          | 202.42     | 202.42        |
| 7   | 19% | 242.82     | 153.76     | 264.24     | 220.27        |
| 8   | 36% | 153.86     | 207.39     | 287.36     | 216.20        |
| 9   | 69% | 110.39     | 175.99     | 164.16     | 150.18        |
| 10  | 28% | 186.43     | 159.29     | 181.72     | 175.81        |
contamination, bed 5, was fairly low average yielding. Contamination data for each bed was plotted with the yield of that bed in 2017 (Figure 4). This is the most direct comparison between genetic contamination and yield possible, as the genetic contamination data is only available for 2017. A trendline and $R^2$ value (0.48) were found, showing a significant negative correlation ($P < .039$) between yield and genetic contamination in 2017. Contamination data for each bed was also plotted with the average yield of that bed across 2013, 2014, and 2017. A trendline and $R^2$ value (0.03) were found, showing no correlation between yield and contamination. WCRS renovation (phase 1) of the previous production beds (Figure 2) was done based on the genetic analysis of the production beds conducted in 2017 and historical yield data from the same beds, 2013–2017.

**Discussion**

In this study, we combined visual contamination mapping, genotypic testing, and yield data to help growers at the Wisconsin Cranberry Research Station (WCRS) make critical renovation decisions, which can cost $25,000 to $30,000 per acre, in addition to the loss of yield for 5 years or longer (Roper, 2008). Visual mapping of cranberry vines in the field (Figure 1) coupled with genetic testing of a large sample set ($n = 285$) was conducted in the 10 existing commercial production beds at the WCRS (Figure 2). The 285 samples collected and analyzed using nine microsatellite markers from Fajardo et al. (2013) indicated relatively large amounts of genetic contamination in eight of the beds, low contamination in one bed, and no contamination in one bed (Figure 2; Table 2).

‘Perry Red’ was the main contaminant at the WCRS with 70% of the detected contamination throughout the nine ‘Stevens’ beds (Figure 2; Table 1), and in almost all cases, visual mapping was able to discern ‘Stevens’ from ‘Perry Red,’ ‘Perry Red’ is a wild cultivar selected in Massachusetts in 1888 (Dana, 1983; Eck, 1990). The ‘Perry Red’ genotype (shown in yellow in Figure 2) is a common contaminant detected in samples sent in by growers for genetic testing to the USDA, UW-Madison, Cranberry Genetics and Genomics Laboratory (CGGL). Based on the visual mapping patterns at the WCRS, it appears ‘Perry Red’ may have been unintentionally planted along with ‘Stevens’ in an alternating fashion, as is indicated by the alternating color patterns of red and yellow (Figure 2). This pattern may be due to the practice of mowing and baling cranberry vines (Eck, 1980; Roper, 2008) and combining multiple bales from different genetically untested sources during replanting.
Some areas of the ‘Stevens’ beds visually appeared as different genotypes, but these apparent differences turned out to be the same genotype (example bed 5, Figure 2). These perceived differences are likely due to the use of machinery running up and down the beds making patterns that appear as different phenotypes in the visual analysis (Figure 2). Also, many of the smaller areas of contamination also went undetected in the visual analysis, and in the ‘BG’ beds selves could not be differentiated visually. Because relatively few other genotypes were present at the station (Table 1), these genotypes were likely more difficult to distinguish in the cranberry beds compared to the big patches of ‘Stevens’ and ‘Perry Red.’ Different visual techniques or sensing technology, including the use of drones, may be able to increase the accuracy of visually discriminating genotypes in cranberry beds (Zhao et al., 2019). The unknown genotypes detected were mostly related to other genotypes already growing in the cranberry beds at WCRS, which indicates some level of volunteerism or natural diversity creation in the beds (Fajardo et al., 2013; Vorsa and Zalapa, 2019), but the contribution of such genotypes to contamination was minimal. Thus, the main source of contamination at the WCRS was like during a renovation event through the introduction of an unintended genotype, ‘Perry Red,’ in mowing material used for replanting. Cranberry cultivar misclassification and cultivar variants are a common problem in the cranberry industry that can easily be avoided by using genetic testing of propagation materials and sources (Fajardo et al., 2013; Novy and Vorsa, 1995; Novy et al., 1994; Polashock and Vorsa, 2002; R.g. et al., 1996; Schlautman et al., 2018).

The correlation analysis using the 2017 yield data indicated that there was a negative correlation between the amount of genetic contamination and cranberry bed productivity (Figure 4). However, the average yield data from 2013, 2014, and 2017 was not correlated with genetic contamination. The correlation was perhaps not detectable in previous years as patches of contamination grew over time and became more prominent in 2017, when visual mapping and genetic uniformity testing were performed. Thus, genetic testing of contamination should be validated by conducting multiple years of genetic testing and using many more years of average yield data to perform the correlation analysis. Alternatively, yield could be collected using the square foot area method at the exact locations of DNA sampling for genetic uniformity testing. This would ensure that the correlation between yield and genetic uniformity would be more direct and not affected by the sample size of the genetic test and the acreage of the bed.

An alternative explanation for the low or lack of correlation between genetic uniformity and historical yield is that cultivar mixes may confer a yield advantage (Gallandt et al., 2001). While the predominant preference is for genetically pure cranberry beds, it is possible that beds in which multiple cultivars are grown together or even beds with “contamination” (i.e., distinct genotypes from the intended cultivar of the bed) may actually have increased yield. Cultivar mixtures, in which several cultivars are planted together, which offer intraspecific genetic diversity (Reiss and Drinkwater, 2018), have been utilized in crops such as rice (Zhu et al., 2000) and wheat (Mille et al., 2006). Cultivar mixtures can potentially decrease the severity of disease (Didelot et al., 2007; Finckh, 1992) and insect pest damage (Shoffner and Tooker, 2013), and increase overall yield compared to monocrops (Gallandt et al., 2001). Beyond these advantages, cranberry also prefers to outcross, and thus a cultivar mixture may allow for increased yield based on the hybrid seed increased fitness (Bruederle et al., 1996). In other crops, the cultivars chosen for a specific cultivar mixture are intentional, whereas the widespread contamination of a cranberry bed is incidental (Fajardo et al., 2013; Novy and Vorsa, 1995; Novy et al., 1994; Polashock and Vorsa, 2002; R.g. et al., 1996; Schlautman et al., 2018). For example, the distinct genotypes present in the cranberry beds analyzed here were not chosen to complement each other in order to serve any purpose. Common contamination genotypes are often unwanted due to their lower level of productivity (Novy and Vorsa, 1995; R.g. et al., 1996; Roper et al., 1995). Additionally, as seen in Figure 2, contamination is often clustered. Therefore, mixing any cultivars together in a bed will not necessarily increase yield, as cultivars would have to be more thoroughly mixed together. However, in bed 3, two cultivars appear to be more evenly mixed, with two distinct rows of each in the bed (Figure 2). Bed 3 was also the highest yielding bed on average (Table 2). This may be an indication that cultivar mixtures in cranberry may convey some
benefit, whether it be related to disease or pest resistance, the preference for outcrossing in cranberry, or another aspect of cranberry productivity. A more thorough and intentional research of cultivar mixtures should be conducted in order to determine the efficacy of this technique in cranberry. The special mixing of cultivars in each bed would also have to be studied for impact management practices such fertilizing, mowing, sanding, and harvesting.

**Conclusion**

While visual analysis was not perfectly accurate, it was a very useful indicator of cranberry bed genetic contamination. As previously stated, the visual analysis was able to distinguish between the two main genotypes, ‘Stevens’ and ‘Perry Red’ and identify large pockets of contamination of these genotypes at the WCRS. However, small pockets of contamination were not easily identifiable using visual mapping. However, the goal of analyzing each bed for genetic uniformity and integrating the visual and yield data was accomplished to determine beds to maintain or renovate for the long-term productivity of the Wisconsin Cranberry Research Station (WCRS). Thus, renovation decisions of the whole marsh were made based on these results and logistics of cranberry management and remodeling determined by Wisconsin growers. Phase 1 of the renovation at WCRS concluded in 2020 with a variety of new cultivars (all genetically tested) planted in five newly renovated beds. This study provides cranberry growers with a model for using genotype testing, visual inspection, and yield data to make management and renovation decisions. Future studies should focus on studying statewide low yielding and vegetative ‘barren berry’ genotypes to aid growers’ decisions on when to replant. To increase the accuracy of data collection, future studies should include square foot yield and fruit quality data, as well as soil, water, and plant nutrient analyses that will allow for a comprehensive understanding of genetic and trait data in the context of environmental effects.

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