

The Barren Berry Phenotype: Untangling Cranberry (*Vaccinium macrocarpon* Ait.) Genetic Contamination in Central Wisconsin and Beyond

F. Lazar

University of Wisconsin

H. Lopez-Moreno

University of Wisconsin

E. Wiesman

United States Department of Agriculture

F. La Torre

University of Wisconsin

P. Verhulst

Lady Bug IPM

J. Sojka

Lady Bug IPM

I. Maureira

Universidad Austral de Chile Valdivia

David Millar

United States Department of Agriculture

C. Kennedy

United States Department of Agriculture

J. Mura

United States Department of Agriculture

J. Zalapa

juan.zalapa@usda.gov

United States Department of Agriculture

Research Article

Keywords: SSRs, microsatellites, DNA fingerprinting. genetic uniformity, clonal purity, barren berry, cranberry bed management

Posted Date: May 15th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4385161/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Wisconsin is the world's leading producer of cranberries (*Vaccinium macrocarpon* Ait.; $2n = 2x = 24$). The state produces over twenty-thousand acres that contribute to more than 50% of the global total production, with more than one billion dollars in value. Cranberry growers in the “central sands” of Wisconsin have been experiencing yield decline due to vegetative unproductive genotypes, popularly known as “Barren Berry”, which consistently remain vegetative and produce no fruit. The purpose of this study was to compare visual inspection in the field to DNA fingerprinting for the early detection of unproductive/barren genotypes. Additionally, the study served as a survey of unproductive/barren genotypes in central Wisconsin, the largest growing region in the world. Growers submitted 839 cranberry leaf samples for DNA testing of two visually determined phenotypes: unproductive or barren ($n = 646$; those that produced little to no fruit), versus the normal productive or fruiting ($n = 193$). We conducted genetic fingerprinting on the leaf tissue using nine microsatellite markers previously shown to differentiate cranberry genotypes. This study identified a barren berry genotype unique to the samples submitted by growers across central Wisconsin, which we denoted “Barren Berry 1”. This genotype accounted for 44% of samples submitted as the barren berry phenotype. Genetic fingerprinting revealed sixty-five different genotypes in beds which were thought to be monocultures of just four cultivated genotypes. Early detection of genetic contamination through visual inspection, genetic testing, and responsible propagation can drastically aid in the management and longevity of cranberry beds, and in turn save time and money to the growers.

Introduction

The American cranberry (*Vaccinium macrocarpon* Ait.; $2n = 2x = 24$) is a fruit-bearing woody perennial plant, native to North America (Durand 1942). Cranberries were first cultivated in 1810 on the peatlands on Cape Cod, Massachusetts, which were later surrounded by ditches and dikes (raised earthen dams) to enhance production and ease management. The state of Wisconsin began cultivating cranberries in 1853 (Matusinec et al. 2022; Schlautman et al. 2018), and rapidly became a global producer by the 20th century, producing 14.7 million barrels or 735,000 tons of fruit and contributing 50% of the global cranberry ecommerce in 2021 (Vorsa and Zalapa 2019).

Cranberries are grown as a monoculture, and most new plantings or renovations of cranberry beds are carried out using cuttings often mowed from existing beds to allow for vegetative clonal reproduction of the desired cultivar (Schlautman et al. 2018). Therefore, genotype purity of the mowed beds is often a key factor contributing to variation in crop yields (Matusinec et al. 2022), and plantings of new uncontaminated hybrid cultivars produce ~ 2-4x more fruit than mixed genotype native varieties (Hoekstra et al. 2020; Vorsa and Zalapa 2019). Additionally, cranberry genotypes exhibit marked phenotypic differences including dormancy, plant architecture, flowering, and fruiting, thus maintaining the genetic purity of beds is crucial to optimize agronomic management, harvest, and post-harvest storage (Eck 1990; Diaz-Garcia et al. 2019; Lopez-Moreno et al. 2023). This cultivar-specific phenotypic variation makes it feasible for growers to identify genetic contamination in cranberry beds by visually inspecting plant characteristics in the field (e.g., leaf color, flowering, canopy thickness, yield, etc.) (Matusinec et al. 2022; Roper and Vorsa 2010).

Visual observation has shown promise as an accurate and cost-effective tool for identifying areas of genetic contamination compared with more rigorous detection methods such as analysis of molecular markers, as demonstrated by Matusinec et al. (2022). For instance, results from the pilot study of Matusinec et al. (2022) were a key factor contributing to the decision to renovate most of the beds of the Wisconsin Cranberry Research Station (WCRS) and demonstrated the usefulness of molecular fingerprinting of genetic contamination to inform decisions on cranberry farm renovations (Matusinec et al. 2022).

In this study, we conducted a large-scale assessment of genetic contamination in commercial cranberry beds in central Wisconsin, popularly known among growers as the “Wisconsin Cranberry Country”, by combining visual inspection and molecular fingerprinting testing for identification. Our research objective was to quantify the extent of unproductive or “barren” genotypes among Wisconsin growers. Unproductive genotypes are thought to be a problem in central Wisconsin (Roper et al. 1995; Matusinec et al. 2022), but they may also be affecting cranberry production in northern Wisconsin, southeastern Massachusetts, New Jersey, and states in the Pacific Northwest. If present, unproductive genotypes may be a cause of decreased cranberry yields with an unknown impact, yet a rigorous analysis of visual inspection as a tool for identifying barren genotypes is lacking. To fill this gap, we compared visual inspection and molecular testing for identifying genetic contamination across 14 cranberry growers in central Wisconsin, which represent one of the most productive cranberry production areas worldwide. We report a breakdown of predominant genotypes associated with the unproductive/barren phenotype of Central Wisconsin and beyond, including the discovery of a new genotype, denominated Barren Berry 1 (BB1), associated with the barren phenotype.

Materials and methods

Tissue Collection and Phenotype Identification

To assess contamination by unproductive cranberry genotypes in central Wisconsin, 839 plant tissue samples were collected from 14 growers based on visual evaluation of cranberry beds in the field during the month of September 2021 shortly before the cranberry harvest so that a clear fruit presence or absence could be identified. (Table 1; Fig. 1A). The cranberry samples were collected from beds planted to one of four cultivars: Stevens (n = 552), Ben Lear (n = 146), Searles (n = 113), or HyRed (n = 28). The 14 growers were divided into three general geographic regions or sections based on the assumption that growers in proximity to one another are more likely to share vegetative propagules to plant newly constructed beds or to renovate existing ones (Fig. 1A). To this end, we grouped the 14 growers into three sections based on a radial distance of 15 km to three towns, Tomah, City Point, and Wisconsin Rapids (i.e., growers within 15 km to each town were grouped together; the exact location of each grower was hidden due to privacy concerns; Fig. 1A). Regardless of the cultivar, each grower visually assessed their plantings based on qualitative differences of fruiting (productive) versus non-fruiting (unproductive) phenotypes. (Fig. 1B). From the 14 growers, a total of 193 samples were collected as productive or fruiting (Table 1; Fig. 1B, right), while 646 samples were collected as unproductive (i.e., barren phenotype which lacked fruit; Table 1; Fig. 1B, left). The unproductive phenotype usually also had a hairy or bushy (vegetative) growth habit, which could be easily distinguished from the productive areas of the cranberry bed. Lady Bug IPM, LLC, a cranberry crop consultant located in Pittsville, WI, was responsible for collecting samples along with each participating grower, and problematic areas were determined in conjunction with the grower. Samples with or without fruit (i.e. productive and unproductive, respectively) were collected to include both uprights (i.e., reproductive vertical stems) or runners (i.e., stem that grows parallel to the soil surface) of 4–5 cm long. The plant material collected corresponded to the current year’s growth and contained at least 10 leaves. Samples were labeled with site name, date, bed identification, cultivar, and phenotype (unproductive or barren and productive or fruiting), kept cool on ice in the field, and then frozen in laboratory until further processing for DNA extraction.

Table 1

Genetic contamination identified using nine microsatellite markers among 839 cranberry samples collected at 14 grower sites in central Wisconsin. Three grower sections or regions were designated based on geographical proximity of growers sampled (Fig. 1A). N = number of samples submitted in two categories productive and unproductive (i.e., barren). Samples were genetically analyzed and divided into cultivars, cultivars, selfs/crosses, Barren Berry 1 (BB1), Perry Red (PR), and unknown (UNK) genotypes.

Grower #	Section	N	Productive	Unproductive	Cultivar	Self/Cross	BB1	PR	UNK
1	3	16	4	12	3	2	0	0	11
2	2	96	24	72	35	0	56	0	5
3	2	32	8	24	20	0	0	11	1
4	1	33	8	25	3	4	25	0	1
5	1	82	20	62	21	39	0	2	20
6	3	64	16	48	23	1	10	14	16
7	2	32	8	24	1	3	24	0	4
8	1	119	35	84	37	5	53	11	13
9	1	16	4	12	4	3	0	8	1
10	3	16	4	12	15	0	0	0	1
11	2	152	20	132	27	6	103	14	2
12	2	64	16	48	6	23	12	10	13
13	3	80	20	60	46	0	0	29	5
14	3	37	6	31	9	0	0	21	7
Totals		839	193	646	250	86	283	120	100

DNA Extraction and Polymerase Chain Reactions (PCRs)

Genomic DNA extractions for microsatellite analysis of the 839 samples (~ nine 96 well plates), collected from the 14 growers (Fig. 1A), were performed using leaf tissue per a CTAB method (Doyle 1987) modified to isolate high quality, clean DNA (Matusinec et al. 2022). 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-325 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 were repeated as needed (up three times) and multiple scorers were used to ensure the repeatability of the results.

Data Analysis

Nine microsatellite loci, previously used by Fajardo et al. (2013) and Zhu et al. (2012) to differentiate cultivars, were used to genotype the samples collected from the 14 participating growers. The primers used were vm04084, vm26877, vm28527, vm31701, vm38401, vm39030, vm51985, vm52682, and vm78806. Genotype diversity was examined using GenAEx 6.5. 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 GenAEx 6.5. Then, the alleles of each genotype were individually examined to determine clones. When two genotypes had identical allelic constitutions at all nine microsatellite markers used, they were considered clones. When necessary, missing data points were imputed based on the clonal information using the data matrix consisting of the 839 samples. 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 at the Wisconsin Cranberry Research Station (WCRS) (Matusinec et al. 2022). 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 (Schlautman et al. 2018). Based on this database and the genetic test results of this study, samples were divided into two categories 1) productive or likely productive and 2) unproductive or likely unproductive. We group known cultivar genotypes and their derivative self/cross genotypes as productive and likely productive. The unproductive or likely unproductive genotypes are grouped as a new genotype associated with the barren phenotype, denominate Barren Berry 1 (BB1), which was identified in the current study, and Perry Red and unknown genotypes, which were previously associated with the unproductive phenotype (Matusinec et al. 2022).

Binomial Exact Test

To assess the growers' ability to visually differentiate pure cultivars from contaminated unproductive genotypes in the field, we performed a Binomial exact test, which is a statistical method used to determine whether the observed success rate in a binomial experiment (trials with two possible outcomes, e.g. productive vs. unproductive) significantly deviates from a specified theoretical expectation derived from the null hypothesis (i.e., the growers can differentiate between productive and unproductive genotypes in the field based on phenotypic evaluation). In an exact binomial test, a test statistic (TS) is calculated:

TS = Probability Mass Function (PMF) for $x = 1$ - PMF (PMF($X = x$) = $C(n, x) * p^x * (1 - p)^{(n - x)}$, where $C(n, x) = n! / (x!(n - x)!)$),

n represents the total number of trials, x represents successful outcomes, and p represents the probability of success on any single trial. This test statistic is then compared to the chosen critical value of significance ($p < 0.05$). If the computed test statistic is less than the critical value, then the null hypothesis is accepted.

Results

Overall Genetic Contamination Analysis

Our genetic analysis results, representing 839 samples from 14 growers, yielded a total of 60% unproductive or likely unproductive genotypes (i.e., Barren Berry 1, Perry Red, and unknown genotypes) (Table 1; Fig. 2A). Of these unproductive samples, 34% were Barren Berry 1 ($n = 283$, first time identified in this study), followed by 14% Perry Red ($n = 120$), which has been identified as an unproductive cultivar in central Wisconsin (Matusinec et al. 2022). Unknown genotypes, which were not genotypically matched to any named cultivar genotype in our database, represented 12% ($n = 100$) of the total (839) samples. Additionally, cultivar genotypes constituted 30% ($n = 250$) of all samples while self/crosses of cultivar represented 10% ($n = 86$) (Table 1; Fig. 2A).

To further explore these results, we partitioned the samples into two categories: 1) those samples perceived by growers as (productive) pure cultivars (Table 1; Fig. 2B), and 2) those samples perceived by growers as (unproductive) genetically contaminated (Table 1; Fig. 2C). Of the 839 samples, 193 samples were submitted as perceived pure cultivar by phenotypic inspection in the field (Table 1; Fig. 1B). In comparison, DNA fingerprinting revealed that 138 (72%) of those samples were pure cultivars (Fig. 2B). To assess if growers can visually differentiate pure cultivars from unproductive genotypes in the field, we performed an exact binomial test of the 138 confirmed cultivar samples versus the 193 samples submitted as pure. The result was significant at $p < 0.001$, indicating that growers can visually identify pure cultivars from contaminated genotypes through visual inspection (Fig. 1B).

Overall, 646 of the 839 submitted samples were identified as unproductive (Table 1). Our DNA fingerprinting results confirmed that 281 samples (44%) belonged to Barren Berry 1 (Fig. 2C), Perry Red accounted for 119 unproductive samples (18%), while unknown genotypes accounted for 77 samples (12%) (Fig. 2C). Thus, unproductive genotypes accounted for 74% of unproductive samples. To assess if growers can visually differentiate unproductive genotypes from pure cultivars in the field, we performed a second exact binomial test of the 477 confirmed unproductive samples versus the 646 samples submitted as unproductive. The result was significant at $p < 0.001$, confirming that growers can visually distinguish unproductive genotypes from cultivars through visual inspection in the field (Fig. 1B).

Geographical Genetic Contamination Analysis

To analyze the spatial distribution of contaminating genotypes in central Wisconsin, we divided the 14 growers into three regions or groupings based on distance proximity (Table 1; Fig. 1A; Fig. 3). Every grower in all three sections showed substantial contamination by unproductive genotypes (i.e., Barren Berry 1, Perry Red, and unknowns) (Fig. 3A), except for grower 10. Seven of the growers in all three sections showed contamination by Barren Berry 1 with most of the contamination concentrating in section 2 (Fig. 3B). While nine of the growers, three growers per section, showed genetic contamination by Perry Red (Fig. 3C), contamination by unknown genotypes appeared in all growers and all sections (Fig. 3D).

We further explored a historic national and international data set available through a free fingerprinting service to growers by the USDA-ARS Cranberry Genetics and Genomics Lab (Supplementary Table 1). The data set was composed of 2,912 samples submitted for DNA fingerprinting by 58 different grower contacts between the years 2011–2022. The grower contacts represent mostly Wisconsin and its growing areas, but samples were also submitted by growers in Minnesota, New Jersey, Oregon, Washington, and Europe. Barren Berry 1 and Perry Red represented 130 and 467 of genetically contaminated samples while unknowns accounted for 517, altogether representing ~ 38% percent of the total submitted samples. Based on this data set, Barren Berry 1 was first identified in 2016 in Wood County (section 2 in Table 1 and Fig. 2) Wisconsin, and no cases were detected in other growing regions outside Wisconsin. Thus, the Barren Berry 1 genotype was detected in eight growers mostly in central Wisconsin (Jackson, Monroe, Juneau and Wood counties), but also in the northern county of Vilas and in the western county of Crawford. The Perry Red genotype was first identified in 2011 in Monroe County. The genotype was more widely spread-out among 27 grower contacts with geographical representation in Central Wisconsin (all three sections in Table 1 and Fig. 3) and outside in La Crosse County, and in the state of Washington. Finally, unknown genotypes were a problem for 44 grower contacts in all growing regions represented in the sample, except for New Jersey (Supplementary Table 1).

Cultivar Contamination Analysis

Additionally, we looked at genetic contamination by the submitted samples by expected cultivar. Table 2 shows the expected cultivar names and the breakdown of productive and unproductive submitted samples. The fingerprinting

analysis revealed a parallel between the number of samples submitted as a productive phenotype and the number of samples that genetically matched the expected cultivar name, except for Searles (Table 2). We further broke down the contamination by cultivar, showing that most of the samples in this study were submitted from Stevens beds (n = 552) in two categories: productive (n = 123) and unproductive (n = 429). Stevens beds were mostly contaminated with Barren Berry 1 (n = 222), followed by Perry Red (n = 78), and unknowns (n = 50). A considerable number of Stevens bed samples did not match the correct genotype, but many of them (n = 65) matched other commercially grown cultivars. Ben Lear bed samples (n = 146; productive n = 36; unproductive n = 110) were contaminated with the unproductive Perry Red (n = 21) and unknowns (n = 33), but a considerable number of samples were determined to be self/cross (n = 50). For samples submitted from Sealers beds, there were none matching the correct Searles genotype in our database; however, a considerable number of samples appeared to be self/crosses of the cultivar Searles. While most of the unproductive genetic contamination in Searles beds was due to Barren Berry 1, the unproductive genetic contamination in HyRed beds was mostly Perry Red.

Table 2

Cultivar identity and genetic contamination identified using nine microsatellite markers among 839 cranberry samples collected at 14 grower sites in central Wisconsin. Submitted cultivar bed, N = number of samples submitted in two categories productive and unproductive (i.e., barren). Samples were genetically analyzed and divided into cultivars (match or other), cultivars selfs/crosses, Barren Berry 1 (BB1), Perry Red (PR), and unknown (UNK) genotypes.

Cultivar (Bed)	N	Productive	Unproductive	Genotype Match	Other Cultivar	Cultivar Self/Cross	BB1	Perry Red	Unknown
Stevens	552	123	429	131	65	6	222	78	50
BenLear	146	36	110	39	3	50	0	21	33
Searles	113	28	85	0	5	30	61	0	17
HyRed	28	6	22	7	0	0	0	21	0
Totals	839	193	646	177	73	86	283	120	100

Principal Coordinate Analysis

To understand the genetic relationships among Barren Berry 1, Perry Red, and the 839 samples in this study, we conducted a PCoA analysis. Figure 4 displays the relationships of all the samples to Barren Berry 1 and Perry Red and the correct genotypes of the Stevens, Ben Lear, Searles, and HyRed (cultivars represented in this study). The PCoA of the 839 samples resulted in 47.73 percent of variance (PCoA 1 = 26.88% + PCoA 2 = 20.85%) explained by the first two axes (Fig. 4). The Barren Berry 1 position represents 283 samples, the Perry Red position represents 120 samples, the Stevens position represents 131 samples, the Ben Lear position represents 39 samples, HyRed represents 7 samples, and the Searles genotype was not found among the samples of this study, but it is shown for comparison (Table 2). The Barren Berry 1 genotype was part of a differentiated cluster of samples, and the closest cultivar to that cluster was Ben Lear. Looking at the genotypes of Barren Berry 1 and Ben Lear (Supplementary Table 2), they share six alleles at six markers. The Perry Red genotype was slightly differentiated from the main genetic cluster but remained connected and far away from the positions of the Stevens, Ben Lear, Searles, and HyRed genotypes. Finally, to better assess the genetic relationships, we conducted two additional PCoA with all the redundant samples removed from the data set (Supplementary Fig. 1) and another one with additional genotypes of representative and diverse commercially grown cultivars (Supplementary Fig. 2). The resulting PCoAs included the 65 unique genotypes discovered in the 839 samples through the fingerprinting analysis, and an added Searles genotype for reference. Besides Stevens, Ben Lear, Searles, and HyRed, other cultivars detected in the samples submitted by

growers, included 73 samples (Table 2), Howes = 61, Potter's Favorite = 7, Stankavich = 3, and Harju = 2. The total variation explained for the two PCoAs were 26.07% and 24.24%, respectively. In these two PCoAs, there were no differentiated clusters, and the genotypes of Barren Berry 1 or Perry Red appeared as part of the main cluster (Supplementary Figs. 1 and 2). However, as expected, the Barren Berry 1 genotype grouped closer to Ben Lear and its derivatives varieties while Perry Red clustered remained close to eastern native varieties.

Discussion

The American cranberry is one of Wisconsin's largest staple crops and has utmost economic importance, contributing over one billion dollars in revenue annually (USDA 2022; Vorsa and Zalapa 2019). Genetic purity is very important in relation to the overall production and health of a cranberry bed as it is managed as a monoculture. Maintaining the genetic purity of cranberry beds is crucial to ensure productivity and facilitate agronomic management, harvest, and post-harvest storage (Eck 1990; Diaz-Garcia et al. 2019; Lopez-Moreno et al. 2023). Unproductive yield patterns have been reported by growers in cranberry beds in all growing regions in the U.S. and have also been reported in Southern Chile and Canada. In this study, we analyzed 839 samples using nine microsatellite markers submitted by growers as productive and unproductive/barren. We compared visual inspection and molecular testing for identifying genetic contamination across 14 cranberry growers in central Wisconsin. We report the genotypes of the main unproductive contaminants of cranberry beds in Central Wisconsin and beyond.

Visual inspection versus genetic testing

Based on visual inspection growers were successful in identifying 138 out of 193 samples (72%) as pure cultivars (Table 1; Fig. 2B). An exact binomial test significant at $p < 0.001$ confirmed that growers can visually identify pure cultivars from contaminated genotypes through visual inspection in the field (Fig. 1B). Additionally, cultivar self/crosses represented 15% of the samples submitted as perceived pure cultivars. Considering that cultivar self/crosses may have similar productivity characteristics as their parental cultivars, genetically confirmed pure cultivars plus self/crosses added up to 87%, which provides further evidence that visual inspection is a valid tool for distinguishing cultivars within cranberry beds. Additionally, unproductive, or likely unproductive genotypes detected in the samples submitted as pure cultivars accounted for less than 13%, unknown genotypes ($n = 23$; 12%), Perry Red ($n = 1$; <1%), and Barren Berry 1 ($n = 2$; <1%), which further provided evidence that growers can successfully identify cultivars in the field based on phenotype alone. Similarly, of the 646 samples submitted as unproductive or barren phenotype observed in the field (Table 1), 477 samples (74%) belonged to Barren Berry 1, Perry Red, and unknown genotypes (Fig. 2C). An exact binomial test significant at $p < 0.001$ confirmed that growers can visually distinguish contaminated genotypes from cultivars through visual inspection in the field based on phenotype alone (Fig. 1B). Finally, pure cultivars and cultivar self/crosses accounted for only 26%.

Barren Berry 1

We discovered a new genotype associated with a barren phenotype of central Wisconsin, which we denominated Barren Berry 1 (Supplementary Table 2). Of the 646 samples submitted as unproductive or barren samples, 44% were Barren Berry 1 (Table 1; Fig. 2). This newly identified genotype creates a new concern for growers in terms of productivity. To date, Barren Berry 1 is the first cranberry genotype known to be almost completely vegetative with no fruit production. To our knowledge, this is the only invasive barren genotype affecting fruit crops recorded in literature. According to grower descriptions, the barren phenotype is "extremely aggressive and forms large clumps of hairy or bushy vine" (vegetative growth habit), which could be easily distinguished from the productive part of the cranberry bed (Fig. 1B).

Barren Berry 1 appears to produce an abundance of vegetative growth and prioritizes its clonal reproduction over fruit production. Cranberries originate in natural bogs, which are typically very low nutrient environments (Eck 1990; Vander Kloet 1988); thus, the wild cranberry vines seldom produce fruit or are thought to be mostly 'biennial-bearers' (Vorsa 1994; Vorsa and Zalapa 2019). Cranberry vegetative reproduction and biennial-bearing are thought to be evolutionary adaptations allowing cranberries to survive in poor nutrient environments (DeVetter et al. 2015). Moreover, cranberries are often found in the wild in large clonal pockets throughout Wisconsin and across their natural range (Rodriguez-Bonilla et al. 2020). However, some wild populations have been found to harbor clonal diversity (Rodriguez-Bonilla et al. 2020). Through human selection, cranberries have been domesticated and bred to produce abundant fruit and "rebud" each year (i.e., avoid biennial-bearing) (Vorsa and Johnson-Cicalese 2012). Focusing on vegetative growth allows Barren Berry 1 to be extremely vigorous and aggressive compared to cultivars in fruit production in terms of perennial development (such as bud development, fruit setting, ripening, etc.) and resource use efficiencies (such as carbohydrates) due to seasonal constraints (DeVetter et al. 2015; Obeso 2002).

The first report of barren berry in the cranberry literature is Roper et al. (1995), who reported a genetic dissimilarity based on dominant markers among barren and productive Searles vines. Based on codominant marker fingerprints, we now speculate that the Barren Berry 1 genotype originated from the wild, likely in Wisconsin since the genotype appeared more closely related to Ben Lear and its derivative cultivars in the PCoAs (Fig. 4; Supplementary Figs. 1 and 2). Ben Lear is one of Wisconsin's first wild selection cultivars first discovered in 1901 (Eck 1990). In the geographical analysis, Barren Berry 1 appears to be more prevalent in section 2, and it seems to be expanding into section 1 and 3 (Fig. 3B). Additionally, in a national and international historical fingerprinting database, Barren Berry 1 was detected for the first time in 2016 also in section 2, with additional presence in the northern and western cranberry growing regions of Wisconsin, but it was not detected in any other grower regions outside of Wisconsin (Supplementary Table 1). Thus, the Barren Berry 1 genotype likely entered grower's marshes in Wisconsin during management practices, such as through flooding from its natural source (Eck 1990; Matusinec et al. 2022). Alternatively, Barren Berry 1 could have originated through a process of de-domestication where a wild genotype of cranberry crossed with that of a cultivated variety (e.g., Ben Lear), and the resulting genotype expressed a more wild-like phenotype (Dongya et al. 2021). The expansion of Barren Berry 1 is likely happening due to the practice of mowing and sharing vine among growers for renovation of new cranberry beds (Matusinec et al. 2022). It is important to note that Barren Berry 1 appears to be completely barren or non-reproductive under cultivated conditions, but that may not be the case in the wild. This genotype is working its way into commonly cultivated cranberry plantings, and its response to high nitrogen input appears to turn it to a vegetative state rather than reproductive (DeVetter et al. 2015). In weed cropping systems it is well known that weeds take input and compete with agronomic crops for nutrients and environment (Korres 2018). Cranberries must be fertilized at the correct levels and timing for optimal production, as naturally, cranberries seldom produce fruit, or are known to be biennial bearers (Strik et al. 1991; Eck 1976; Davenport & Vorsa 1999). Mullica Queen for example, a new variety that is being readily accepted for its superior fruit qualities and yields can accept heavy fertilizer inputs and turn that into production for growers (Jeranyama et al. 2023). Stevens, on the other hand, receiving a similar regime of fertilizers would turn vegetative and avoid flowering altogether. Barren Berry 1's reproductive abilities will need to be further tested under controlled conditions in a greenhouse or field experiment.

Other genetic contaminants

Perry Red

In this study, we identified several additional non-cultivated genotype contaminants in grower's beds in Central Wisconsin. One such genetic contaminant was an unproductive genotype, denominated Perry Red, which accounted

for 18% of the contamination among unproductive grower-submitted samples. Perry Red was previously identified and associated with low productivity in Wisconsin during the renovation of the Wisconsin Cranberry Research Station (Matusinec et al. 2022). Based on the PCoA Perry Red is more closely related to eastern wild selection cultivars such as Howes, Early Black, and Stankavich (Fig. 4; Supplementary Figs. 1 and 2). In the geographical analysis (Fig. 3C) and national and international fingerprinting data set (Supplementary Table 1), Perry Red appears more widespread in all growing regions and was observed in Washington state. The origin of Perry Red is unknown, but the name was adopted from a genotype match with an accession at the USDA-ARS national cranberry collection in Corvallis, OR. From historical information, Perry Red was first selected in 1888 from wild stands in Massachusetts (Eck 1990 and Dana 1983). All historical accounts suggest that the cultivar was once productive and commercially grown, but it is likely that the correct genotype was lost to the current unproductive genotype in the east coast of the US. Previous work suggests that Perry Red was introduced in Stevens beds through contaminated bales of vegetative propagule and planted in an alternating fashion (Matusinec et al. 2022). More recently, Perry Red was identified in Massachusetts (unpublished data), which supports the idea that the genotype originated in the east and was transferred to Wisconsin through the practice of sharing of vegetative propagules among growers.

Unknown genotypes

Contamination by unknown genotypes unrelated to cultivars appeared in all growers and all growing regions (Fig. 3D) and in other states and internationally (Supplementary Table 1). In this study, we detected a total of 65 unique genotypes (Supplementary Figs. 1 and 2), and of those 32 were unknown genotypes (which are genotypes that do not match any cultivar in a cranberry genotype database utilizing the previously observed 9 microsatellite markers). Although not all unknown genotypes may be unproductive or barren, unknown genotypes are likely less productive than known cultivars based on their overrepresentation in the samples submitted as unproductive (Table 1). Unknown genotypes likely originate from a wild gene bank, typically located nearby, and are naturally flowing through the waterways as seed or vegetative propagule. Unselected wild genotypes tend to be more vegetative and biennial-bearing, traits that promote survival in the wild and would confer advantage in a cultivated system (Matusinec et al. 2022). One example of volunteerism reducing yields in a cultivated system is “weedy” rice, which invades the standard rice cropping systems from nearby wild stands (Kanapeckas et al. 2016; Qui et al. 2017).

Contamination with other cultivars and self/crosses

Other unexpected cultivars detected in the samples submitted by growers, included 73 total samples: Howes = 61, Potter’s Favorite = 7, Stankavich = 3, and Harju = 2 (Table 2). All of these cultivars are wild selections known to have at least 2–3 times lower yield performance than Stevens (Eck 1990; Vorsa and Zalapa 2019). Thus, contamination with these cultivars could also result in lower yield and perception as unproductive/barren by the growers. Previous studies have found that some genotypes can derive from cultivar selfing and/or crossing with cultivars (Sarracino and Vorsa 1991; Huevel and DeMoranville 2009; Matusinec et al. 2022). In the current study, self/crosses were designated as likely productive based on their close relationship with commercial cultivars. However, it is possible that some of these self/crosses maybe be unproductive; for example, in the case of grower 5 in our study, the number of submitted unproductive samples can only be explained by adding up some the self/crosses to the list of unproductive genotypes (Table 1).

Other causes of low productivity

When analyzing geographical analysis, it was noted that a few growers (growers 5 and 10) had submitted a large portion of their unproductive phenotype samples as a productive genotype. This is likely due to inappropriate management practices affecting such as nutrient or carbohydrate availability at critical times, which may have hindered their production for this particular year of sampling (Jeranyama et al. 2023). In the future, it will be

important to assess growers' management practices to determine what issues may be causing low yield when genetic contamination is not an issue.

Conclusions and grower recommendations

As previously reported, our study of submitted unproductive/barren samples from growers showed that visual inspection in the field is a useful tool for growers to assess genetic contamination of their planting. Together, visual inspection and genetic fingerprinting of grower fields can explain discrepancies in the performances of cranberry beds and enable growers to make informed decisions for renovations and new plantings. Generally, the further the bed deviates from monocultures of cranberry cultivars with known performances, the less predictable and lower the yield will be. Cranberries genetic contamination can arise from vegetative propagation, selfing, and outcrossing. Based on our data, genetic contamination is occurring by all three means in growers' fields, and contamination is likely coming from the wild gene bank nearby. Even genotypes closely related to cultivated germplasm can affect productivity negatively. Only cranberry true-to-type cultivars specifically chosen by the grower for their known productivity and phenotypic attributes can deliver the desired results, and no amount of management will make an unproductive genotype productive. Barren Berry 1, Perry Red, unknowns, and other off types are all likely a detriment to cranberry production. If left unaddressed, genetic contamination will spread and could grow very costly to growers due to its negative effect on production and renovation costs. The information derived from this study, including the identification of Barren Berry 1, will help growers better manage their plantings by genetically testing vines to prevent genetic contamination at the time of renovation. One of the main reasons that cranberry contaminants are so common is that there are currently no regulations on the propagation of unpatented cranberry germplasm such as Stevens. We recommend that growers responsibly buy, share, or sell only tested propagule. Furthermore, if a bed contains small areas of contamination, it can be mediated by the grower through vine destruction and overseeding vegetative propagule of the affected surrounding area. Visual inspection, genetic testing, and responsible propagation can substantially aid in the management and longevity of cranberry beds, and in turn save time and money to growers.

Declarations

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any

medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The

images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to

obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

Funding

This project was supported by USDA-ARS (project no. 5090-21220-007-00-D provided to J.Z.); Ocean Spray Cranberries, Inc.; Wisconsin Cranberry Growers Association; and Cranberry Institute. The funders had no role in the study design, data collection, and analysis, interpretation of the data, writing and preparation of this article, or decision to publish.

Author Contribution

Conceptualization, JZ, JM, CK, DM, IM, PV, JS; Methodology, FL, HL-M, EW, FDLT, PV, JS; Software, FL, HL-M, FDLT; Validation, FL, HL-M, FDLT; Formal Analysis, FL, HL-M, EW, FDLT; Investigation, FL, HL-M, EW, FDLT; Resources, JZ, JM, CK, DM, IM; Data Curation, FL, HL-M, EW, FDLT; Writing—Original Draft Preparation, FL, JZ; Writing— Review & Editing, JZ, JM, CK, DM, IM, FL, HL-M, FDLT, PV, JS; Visualization, FL, HL-M, FDLT; Supervision, JZ, JM; Project Administration, JZ, JM; Funding Acquisition, JZ, JM. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors also thank the reviewers who helped enhance the quality of this paper. JZ would like to express his gratitude through PS 136:1.

Data Availability

The data is available with the corresponding author upon request.

References

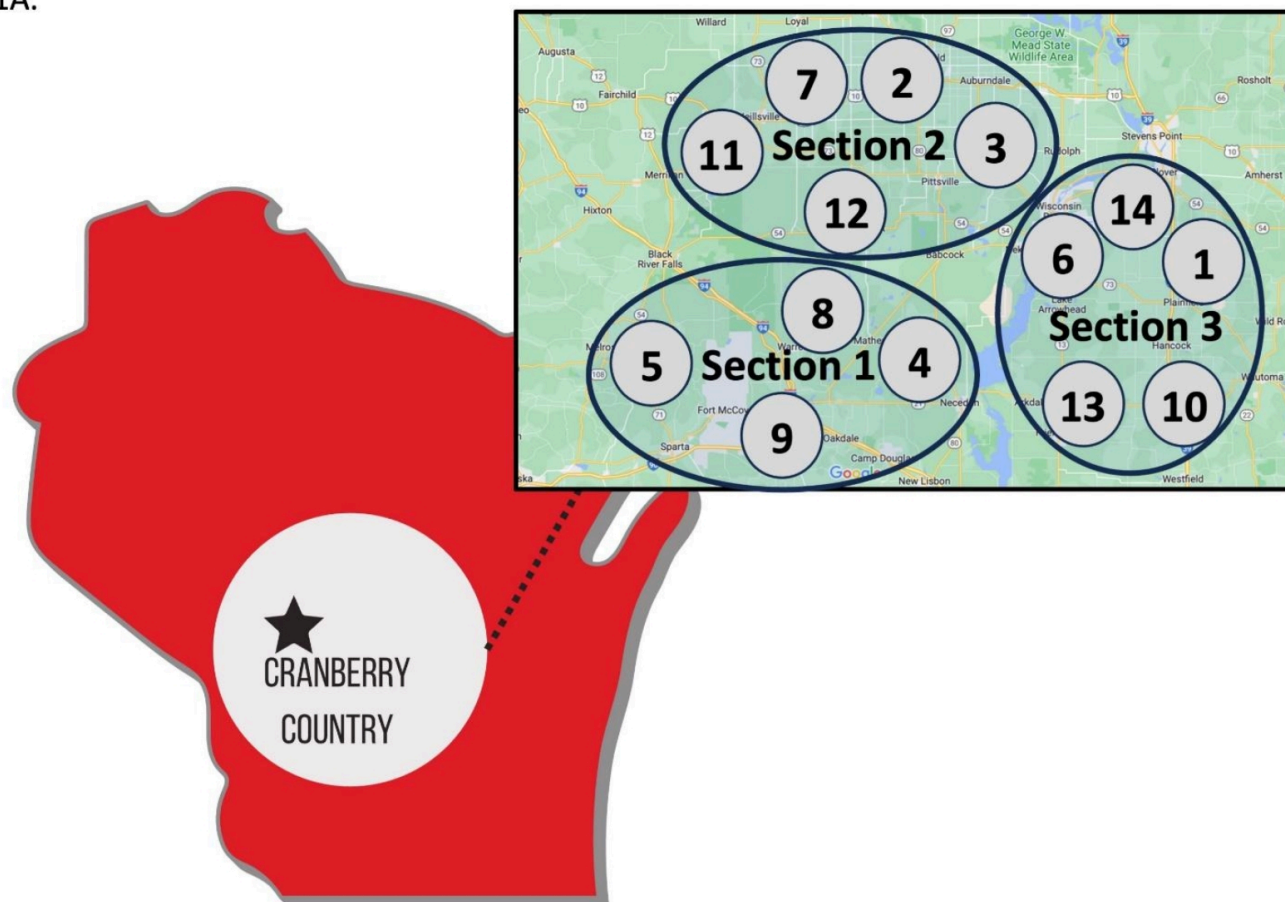
1. Dana MN (1983) Cranberry cultivar list. *Fruit Var. J.* 37:88–95.
2. DeVetter L, Colquhoun J, Zalapa J, and Harbut R (2015) Analysis of genetic, physiological, and environmental yield components within commercial cranberry production systems. *Scientia Horticulturae*. 190:83–93.
3. Diaz-Garcia L, Rodriguez-Bonilla L, Phillips M, Lopez-Hernandez A, Grygleski E, Atucha A, Zalapa J (2019) Comprehensive analysis of the internal structure and firmness in American cranberry (*Vaccinium macrocarpon* Ait.) fruit. *PLoS ONE* 14(9): e0222451. <https://doi.org/10.1371/journal.pone.0222451>
4. Dongya W, Sangting L, Longjiang F (2021) De-Domestication: An Extension of Crop Evolution, *Trends in Plant Science*, Volume 26, Issue 6, 2021, Pages 560–574, ISSN 1360–1385. <https://doi.org/10.1016/j.tplants.2021.02.003>.
5. Doyle J (1991) DNA Protocols for Plants. In: Hewitt, G.M., Johnston, A.W.B., Young, J.P.W. (eds) *Molecular Techniques in Taxonomy*. NATO ASI Series, vol 57. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-83962-7_18
6. Durand L (1942) Wisconsin Cranberry Industry. *Economic Geography*, 18(2), 159–172. <https://doi.org/10.2307/141796>.

7. Eck P (1976). Relationship of Nitrogen Nutrition of 'Early Black' Cranberry to Vegetative Growth, Fruit Yield and Quality^{1,2}, *Journal of the American Society for Horticultural Science*, 101(4), 375–377. Retrieved Apr 12, 2023, from <https://doi.org/10.21273/JASHS.101.4.375>
8. Eck P (1990) *The American cranberry*. New Brunswick: Rutgers University Press.
9. Fajardo D, Morales J, Zhu H, Steffan S, Harbut R, Bassil N, Hummer K, Polashock J, Vorsa N, Zalapa J (2013) Discrimination of American Cranberry Cultivars and Assessment of Clonal Heterogeneity Using Microsatellite Markers. *Plant Mol Biol Rep* 31, 264–271 (2013). <https://doi.org/10.1007/s11105-012-0497-4>
10. Hoekstra BR, Neill C, Kennedy CD (2020) Trends in the Massachusetts cranberry industry create opportunities for the restoration of cultivated riparian wetlands. *Restor Ecol*, 28: 185–195. <https://doi.org/10.1111/rec.13037>
11. Jeranyama P, Mupambi G, Kennedy C (2023) Optimal nitrogen fertilizer application rates for second-generation cranberry hybrids. *Acta Hortic.* 1357, 9–12.
12. DOI: 10.17660/ActaHortic.2023.1357.2 <https://doi.org/10.17660/ActaHortic.2023.1357.2>
13. Kanapeckas KL, Vigueira CC, Ortiz A, Gettler KA, Burgos NR, et al (2016) Escape to Fertility: The Endoferal Origin of Weedy Rice from Crop Rice through De-Domestication. *PLOS ONE* 11(9): e0162676. <https://doi.org/10.1371/journal.pone.0162676>
14. Korres N (2018) Chap. 6 - Agronomic Weed Control: A Trustworthy Approach for Sustainable Weed Management, *Non-Chemical Weed Control*, Academic Press, 2018, Pages 97–114, ISBN 9780128098813. <https://doi.org/10.1016/B978-0-12-809881-3.00006-1>
15. Lopez-Moreno H, Phillips M, Diaz-Garcia L, Torres-Meraz MA, de La Torre F, Berro I, Loarca J, Mura J, Ikeda S, Atucha A, Giongo L, Iorizzo M, Zalapa J, (2023) A Survey of Key Methods, Traits, Parameters, and Conditions for Measuring Texture in Cranberry (*Vaccinium macrocarpon* Ait.). *Horticulturae* 2023, 9, 479. <https://doi.org/10.3390/horticulturae9040479>
16. Matusinec D., Maule A., Wiesman E., Atucha A., Mura JD, Zalapa J (2022) The New Cranberry Wisconsin Research Station: Renovation Priorities of a 'Stevens' Cranberry Marsh Based on Visual Mapping, Genetic Testing, and Yield Data, *International Journal of Fruit Science*, 22:1, 121–132. DOI: 10.1080/15538362.2021.2014016
17. Obeso JR (2002) The costs of reproduction in plants. *New Phytologist*, 155: 321–348. <https://doi.org/10.1046/j.1469-8137.2002.00477.x>
18. Qiu J, Zhou Y, Mao L et al. (2017) Genomic variation associated with local adaptation of weedy rice during domestication. *Nat Commun* 8, 15323 (2017). <https://doi.org/10.1038/ncomms15323>
19. Rodriguez-Bonilla L, Williams K.A, Rodríguez Bonilla F, Matusinec, D, Maule A, Coe K, Wiesman E, Diaz-Garcia L, Zalapa, J (2020) The Genetic Diversity of Cranberry Crop Wild Relatives, *Vaccinium macrocarpon* Aiton and *V. oxycoccos* L., in the US, with Special Emphasis on National Forests. *Plants* 2020, 9, 1446. <https://doi.org/10.3390/plants9111446>
20. Roper T, Vorsa N (2010) Cranberry: Botany and Horticulture. 10.1002/9780470650660.ch7.
21. Roper TR, Skroch P, Nienhuis J (1995). Barren berry and Searles vines are genetically dissimilar. *Cranberries* 59(3): 14–15.
22. Sarracino J.M, Vorsa N (1991) Self and cross fertility in cranberry. *Euphytica* 58, 129–136 (1991). <https://doi.org/10.1007/BF00022813>
23. Schlautman B, Covarrubias-Pazaran G, Rodriguez-Bonilla L, Hummer K, Bassil N, Smith T, Zalapa J (2018) Genetic diversity and cultivar variants in the National Clonal Germplasm Repository cranberry (*Vaccinium macrocarpon* Aiton) collection. *Journal of Genetics* 97(5):1339–1351.

24. Strik BC, Roper TR, DeMoranville CJ, Davenport JR, Poole AP (1991) Cultivar and Growing Region Influence Return Bloom in Cranberry Uprights, *HortScience HortSci*, 26(11), 1366–1367. Retrieved Apr 12, 2023, from <https://doi.org/10.21273/HORTSCI.26.11.1366>
25. United States Department of Agriculture (2022) USDA, 22 May 2022, https://www.nass.usda.gov/Statistics_by_State/Wisconsin/Publications/Crops/2022/WI-Cranberries-Annual-05-22.pdf.
26. Vorsa N, Zalapa J (2019) Domestication, Genetics, and Genomics of the American Cranberry. In *Plant Breeding Reviews*, I. Goldman (Ed.). <https://doi.org/10.1002/9781119616801.ch8>
27. Zhu H, Senalik D, McCown BH, Zeldin EL, Speers J, Hyman J, Bassil N, Hummer K, Simon P, Zalapa J (2012) Mining and validation of pyrosequenced simple sequence repeats (SSRs) from American cranberry (*Vaccinium macrocarpon* Ait.). *Theor Appl Genet* 124:87–96
28. Vanden Heuvel JE, DeMoranville CJ (2009) Competition Between Vegetative and Reproductive Growth in Cranberry. *HortScience horts*, 44(2), 322–327. Retrieved Jul 17, 2023, from <https://doi.org/10.21273/HORTSCI.44.2.322>
29. Vander Kloet SP (1988). The genus *Vaccinium* in North America. Agriculture Canada.
30. Vorsa, N (1994) Breeding the American cranberry. Wisconsin State Cranberry Growers Association. <http://researchguides.library.wisc.edu/cranberry> (1994): 1–4.
31. Vorsa N, Johnson-Cicalese J (2012) American Cranberry. In: Badenes M, Byrne D. (eds) *Fruit Breeding. Handbook of Plant Breeding*, vol 8. Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-0763-9_6

Figures

1A.



1B.



Figure 1

(A) Central Wisconsin cranberry growing area denoted in the gray circle. The Wisconsin cranberry country is the world's most prominent producer of cranberries. Three grower sections or regions were designated based on geographical proximity of growers sampled. (B) The phenotype of 'Barren Berry 1' (left, unproductive) versus that of the industry standard 'Stevens' (right, productive). The exact location of the growers is not shown for privacy.

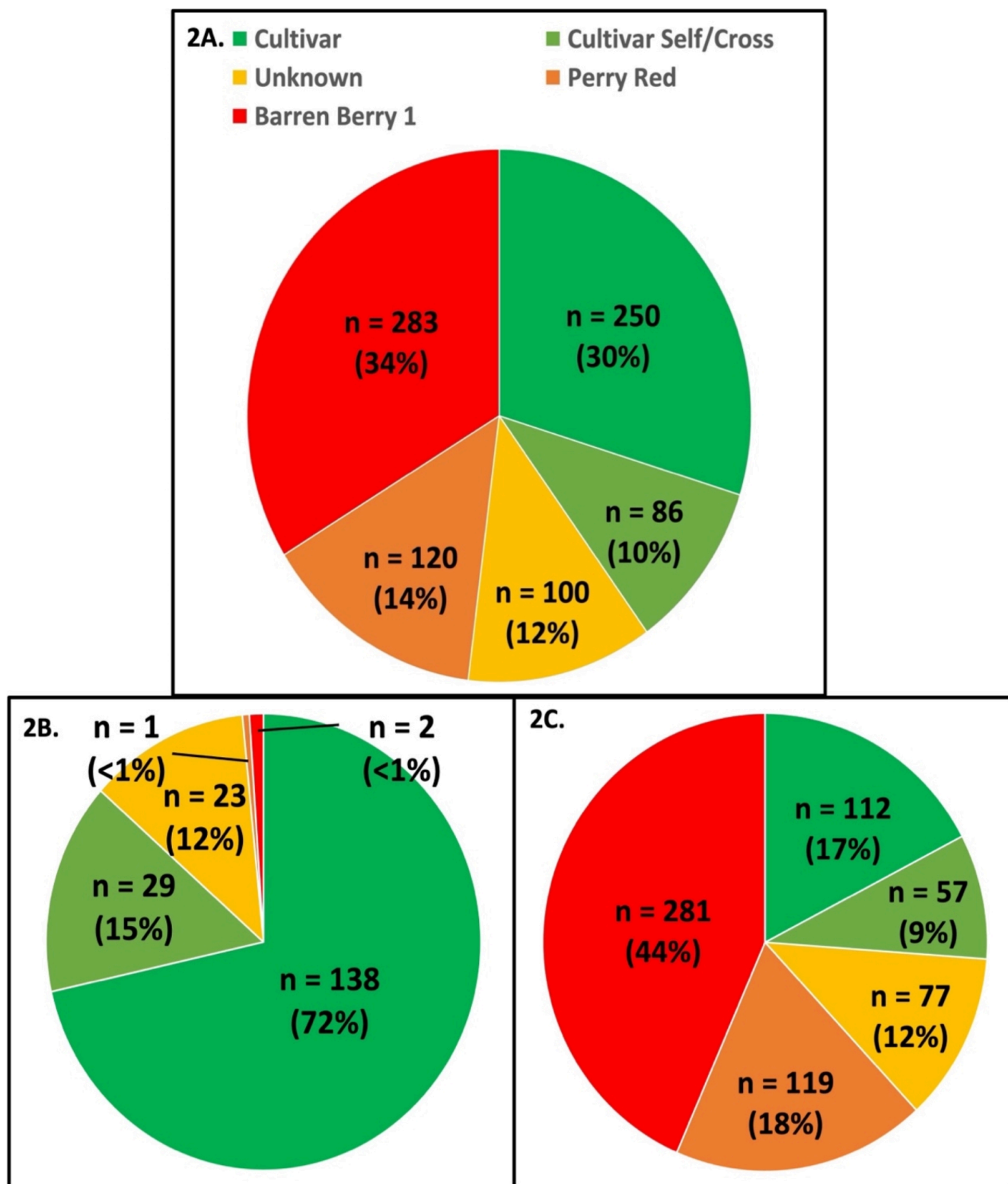


Figure 2

Genetic contamination identified using nine microsatellite markers among 839 cranberry samples collected at 14 grower sites in central Wisconsin (Table 1). (A) Barren Berry 1 (n=283; 34%), Perry Red (n=120; 14%), unknown genotypes (n=100; 12%), and pure cultivars (n=250; 30%), cultivar selfs and crosses (n=86; 10%). (B) Partitioning of samples collected as perceived pure cultivar (n=193), actual pure cultivars (n=138; 72%), cultivar self and crosses (n=29; 15%), unknown genotypes (n=23; 12%), Perry Red (n=1; <1%), and Barren Berry 1 (n=2; <1%). (C) Partitioning of

samples collected as perceived genetically contaminated (n=646), Barren Berry 1 (n=281; 44%), Perry Red (n=119; 18%), unknown genotypes (n=77; 12%), cultivar crosses or selves (n=57; 9%), pure cultivars (n=112; 17%).

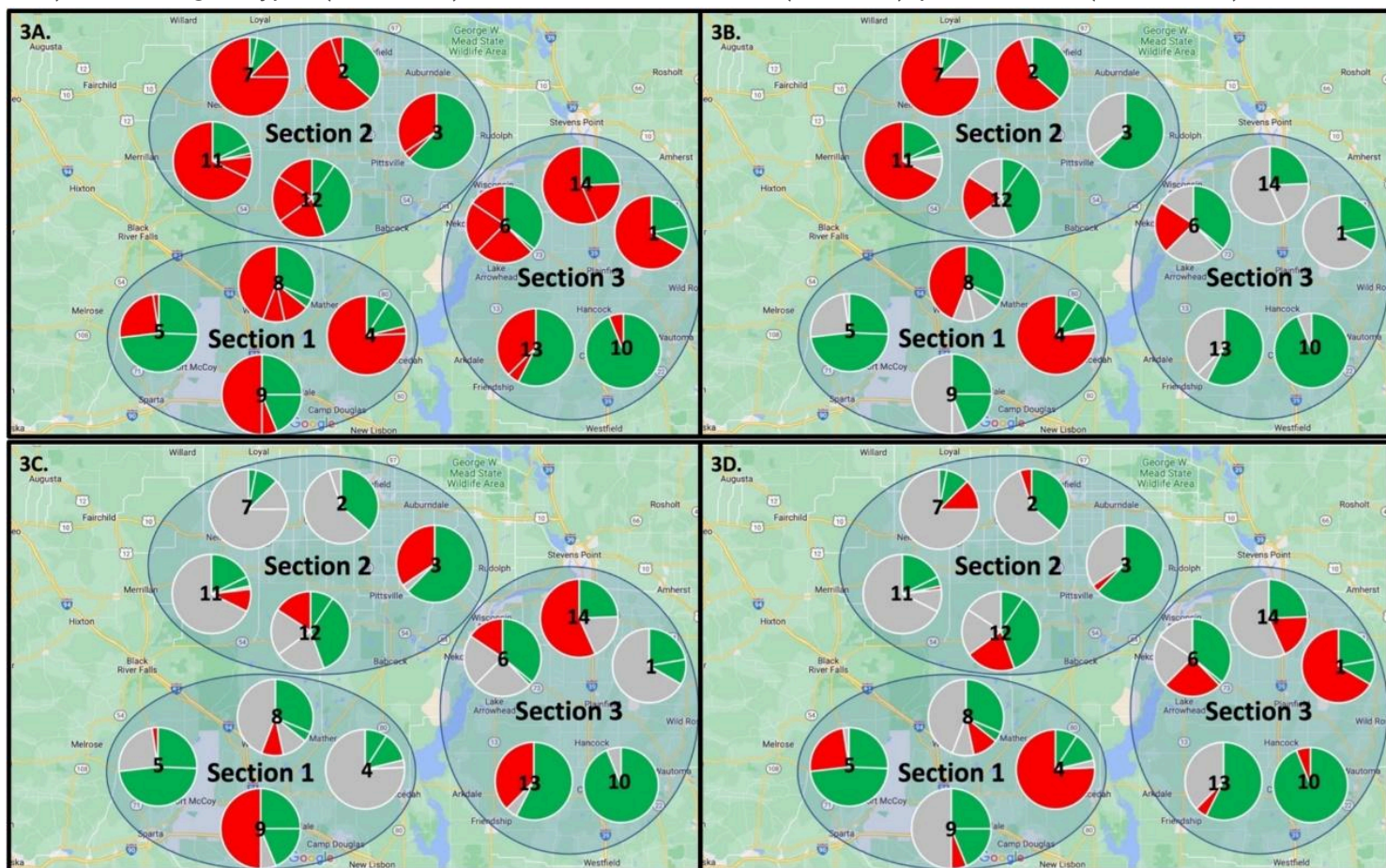


Figure 3

Geographical distribution of participating cranberry growers and genetic analysis results based on nine microsatellite markers (Table 1). 3A. Number of samples per grower representing unproductive genotypes (Barren Berry 1, Perry Red, and unknown; shown in red) and productive genotypes (cultivar, crosses, and self; shown in green). 3B. Number of samples per grower representing Barren Berry 1 detected per grower (shown in red) and productive genotypes (cultivar, crosses, and self; shown in green). 3C. Number of samples per grower representing Perry Red detected per grower (shown in red) and productive genotypes (cultivar, crosses, and self; shown in green). 3D. Number of samples per grower representing unknown genotypes detected per grower (shown in red) and productive genotypes (cultivar, crosses, and self; shown in green). Exact location of each grower has been hidden due to privacy concerns.

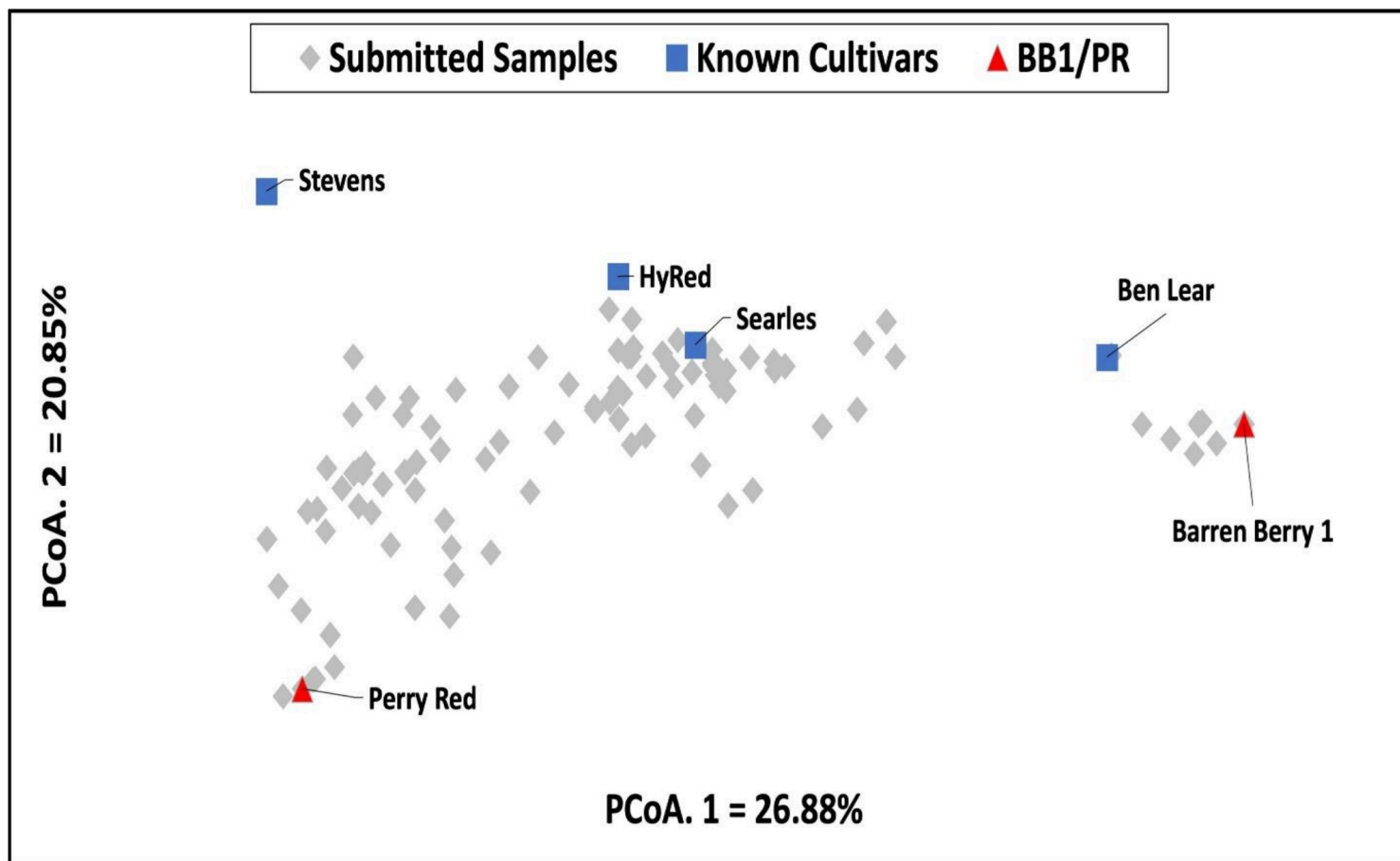


Figure 4

Principal coordinate analysis (PCoA) of 839 cranberry samples submitted as productive or unproductive/barren (Table 1; Fig. 2) based on nine microsatellite markers. Cultivar genotypes of the submitted beds are shown in blue. The genotypes of Barren Berry 1 and Perry Red are shown in Red.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementary.docx](#)