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Cechy biologiczne i użytkowe wybranych hybrydowych odmian winorośli

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Publikacja nr 1 **Kowalczyk B.**, Bieniasz M., Błaszczyk J., Banach P. 2022. The effect of rootstocks on the growth, yield and fruit quality of hybrid grape varieties in cold climate conditions. Horticultural Science. 58/2021. https://doi.org/10.17221/58/2021-HORTSCI

IF_{2022} - 0,833; $MNiSW_{2022}$ - 70 pkt

Publikacja nr 2 Kowalczyk B., Bieniasz M., Kostecka-Gugała. 2022. Flowering Biology of Selected Hybrid Grape Cultivars under Temperate Climate Conditions. Agriculture (Switzerland) 12(5): 1-18 nr 655

IF_{2022} - 2,925; $MNiSW_{2022}$ - 100 pkt

Publikacja nr 3 **Kowalczyk B.**, Bieniasz M., Kostecka-Gugała. 2022. The Content of Selected Bioactive Compounds in Wines Produced from Dehydrated Grapes of the Hybrid Variety 'Hibernal' as a Factor Determining the Method of Producing Straw Wines. Foods 11(7): 1-14

IF_{2022} - 4,350; $MNiSW_{2022}$ - 100 pkt

Sumaryczny IF – 8,108

Sumaryczna punktacja MNiSW – 270 pkt

1.2 Wykaz pozostałych oryginalnych prac

Dziedzic E., Bieniasz M., Kowalczyk B. 2019. Morphological and physiological features of sweet cherry floral organ affecting the potential fruit crop in relation to the rootstock. Scientia Horticulturae 251: 127–135

IF_{2019} - 2,925; $MNiSW_{2019}$ - 140 pkt

Gondek K., Mierzwa-Hersztek M., Kopeć M, Zaleski T., Bogdał S., Bieniasz M., Błaszczyk J., Kaczmarczyk E., Kowalczyk B., Knaga J., Nawrocki J., Pniak M., Jarosz R. 2020. "Mineral composition of fruits and leaves of San Andreas® everbearing strawberry in soilless cultivation" Journal of Elementology" 25(4): 1333-1347

IF_{2020} - 0,781; $MNiSW_{2020}$ - 70 pkt

Sumaryczny IF – 3,706

Sumaryczna punktacja MNiSW – 210 pkt

1.3 Prezentacje na konferencjach naukowych

29.07 - 1.08 2017, Kraków-Wieliczka:
The XLVI Esna Annual Meeting:

- Kruczek M., Bieniasz M., Kaszycki P., Kostecka-Gugała A., **Kowalczyk B**. 2017. Straw wine as a noble Polish sweet wine. Production techniques and their impact on the bioactivity of product.

20.09 – 21.09.2017, Kraków:
Ogólnopolska Ogrodnicza Konferencja Naukowa „Ziemia-Roślina-Człowiek".

- Błaszczyk J., Bieniasz M., **Kowalczyk B.** 2017. Wpływ podkładek na wzrost, plonowanie i jakość owoców kilku odmian winorośli, 87

16.09 -18.09.2019, Warszawa:
V Zjazd PTNO:

- **Kowalczyk B**., Bieniasz M. 2019. Morfologia kwiatów hybrydowych odmian winorośli, 5

07.11.2019, Jachranka:
III Międzynarodowa Konferencja Kamczacka:

- **Kowalczyk B.** Wina i nalewki z jagody kamczackiej. Referat.

20.06 – 21.06.2020 Kraków:
Nowe Trendy w Badaniach Naukowych:

- **Kowalczyk B.** 2020. Sposoby wykorzystania i potencjał prozdrowotny Jagody Kamczackiej (*Lonicera caerulea* L.*)*, nowego gatunku uprawianego w Polsce.

1.4 Staże i wyjazdy naukowe, szkolenia

02-08.03.2018	Staż naukowy - Katedra Chemii Analitycznej Wydziału Chemii Uniwersytetu Mikołaja Kopernika w Toruniu.
2018/2019	Program Erasmus plus – „Wykorzystanie nowoczesnych technologii do wspierania branży enologicznej w Europie": 13-18.04.2018 Czechy 17-24.06.2018 Cypr 22-28.07.2018 Słowenia 01-06.09.2019 Polska.
2020	Międzynarodowy kurs – Sommelier II stopnia – Wine & Spirit Education Trust.

2021	Kurs Pedagogiczny dla doktorantów i asystentów – Politechnika Krakowska.
04.04.2022	Szkolenie „Dachy zielone – aspekty prawne inwestycji”. Abrys.
13.05.2022	Szkolenie „Plac zabaw w pigułce” Abrys.
02.06.2022	Szkolenie „Zieleń Przyuliczna, Technologie, Praktyka, Przyszłość”. Abrys.

2. Streszczenie

Cechy biologiczne i użytkowe wybranych hybrydowych odmian winorośli.

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Odmiany hybrydowe winorośli, ze względu na większą tolerancję niskich temperatur, uprawiane są w strefie klimatu zimnego, do którego zakwalifikowana jest także Polska. W badaniach przeanalizowano trzy etapy związane z cechami biologicznymi i użytkowymi hybrydowych odmian winorośli.

Przeanalizowano wpływ pięciu podkładek na jakość i wielkość plonu trzech odmian hybrydowych winorośli. W wynikach odnotowano brak wpływu podkładek na wielkość plonu, natomiast znacząco poprawiły jego jakość. Zastosowana podkładka może zmienić w zaszczepionej na niej odmianie parametry fizykochemiczne jagód tj. zawartość ekstraktu i kwasowość. Wybór podkładki może także podkreślić lub zniwelować wpływ mikroklimatu na cechy fizykochemiczne uzyskanego plonu.

W kolejnym etapie badano biologię kwitnienia, zapylenia i zawiązania owoców i nasion wybranych jedenastu odmian hybrydowych winorośli. Wykazano różnice w budowie kwiatów odmian hybrydowych w porównaniu do kwiatów winorośli właściwej, w szczególności w odniesieniu do liczby pylników i płatków korony. Stwierdzono również, że liczba pyłku w kwiecie jest zależna od odmiany i sezonu wegetatywnego oraz może podlegać dużym wahaniom.

W trzecim etapie dotyczącym wykorzystania owoców odmian hybrydowych analizowano, jak metoda produkcji win słomkowych wpływa na zawartość wybranych związków bioaktywnych. Wyprodukowane trzema różnymi metodami wina słomkowe, porównano pod względem fizyko-chemicznym. Dzięki wynikom określono optymalną metodę produkcji pod względem związków bioaktywnych tj. metodę produkcji dla win typu *passito* z dodatkową maceracją winogron. Przeprowadzona analiza sensoryczna tych win również potwierdziła, że dzięki tej metodzie wyprodukowane wino, było najbardziej zbalansowane w porównaniu do pozostałych. Badanie to pokazało, że w Polsce produkcja win typu *passito* z hybrydowych odmian winorośli, jest alternatywą dla tradycyjnego procesu produkcji i z powodzeniem może być wykorzystywana w chłodnym klimacie.

Słowa kluczowe: podkładka, kwitnienie, jakość pyłku, liczba pyłku, polifenole, wino słomkowe

3. Summary

Biological and functional traits of selected hybrid grape cultivars

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Hybrid grapevine cultivars, due to their higher tolerance for low temperatures, can be grown in the cold climate zone, which includes Poland. The research concerned the three stages of grapevine growth and fruiting related to the biological and functional features of hybrid grapevine cultivars.

Firstly, the effect of five rootstocks on the quality and yield of three hybrid grapevine cultivars was analyzed. The results showed that rootstocks had no effect on yield, while they significantly improved yield quality. The used rootstock can change the physicochemical parameters of berries i.e. soluble solids content and acidity of the cultivar grafted on it. The choice of rootstock can also enhance or eliminate the influence of microclimate on physicochemical characteristics of the obtained yield.

In the next stage, the biology of flowering, pollination and setting of fruit and seeds of selected eleven hybrid grape cultivars was studied. The differences in the flower structure of hybrid cultivars in comparison to flowers of grapevine proper were demonstrated with respect to the number of anthers and corolla petals, in particular. It was also found that the number of pollen in the flower depends on the cultivar and growing season and can fluctuate widely.

In the third stage of studies concerning the use of fruit of hybrid varieties, it was analyzed how the method of producing straw wines influences the content of selected bioactive compounds. The straw wines were produced by three different methods, and were compared in terms of physicochemical aspects. Based on the results obtained, the optimum production method in terms of bioactive compounds was determined, i.e. the production method for *passito* type wines with additional maceration of grapes. Sensory analysis of these wines also confirmed that thanks to this method the produced wine was the most balanced compared to the others. This study showed that in Poland, the production of *passito*-type wines from hybrid grape varieties is an alternative to the traditional production process and can be successfully used in cold climates.

Keywords: rootstock, flowering, pollen quality, pollen number, polyphenols, straw wine

4. Wstęp

Winorośl właściwa *'Vitis vinifera'* L. jest ważnym gospodarczo gatunkiem uprawianym w wielu krajach na świecie. W ponad 90 krajach powierzchnia uprawy wynosi około 8 mln hektarów, a roczna globalna produkcja wynosi około 67 mln ton owoców, z czego 12 państw reprezentuje 80% całej produkcji na świecie. Winogrona są spożywane na świeżo i suszone, przetwarzane są na wino i inne napoje fermentowane, używane jako niesfermentowany sok i koncentrat. Wino jest jednak najważniejszym zastosowaniem winogron zarówno pod względem tonażu, jak i obszaru produkcji (Reisch et al., 2012).

Udomowienie winorośli sięga czasów prehistorycznych. Produkty powstałe z *'Vitis vinifera'* wpisywały się w życie codzienne, ale także w związki kulturowe czy religijne (Censi et al., 2014).

W drugiej połowie XIX wieku przypadkowo sprowadzona z Ameryki Północnej mszyca filoksera winiec '*Phylloxera vastatrix'*, zdziesiątkowała europejskie winnice, a później także winnice na innych kontynentach. Także inne choroby nękały winnice jakie jak mączniak prawdziwy '*Erysiphe necator'*, mączniak rzekomy '*Plasmospora viticola'* i czarna zgnilizna *Guignaria bidwellii*. Odbudowa upraw winorośli, po innych nieudanych próbach, oparła się na metodzie szczepienia szlachetnych odmian *'Vitis vinifera'* na odpornych na filokserę podkładkach autochtonicznych gatunków północnoamerykańskich. Początkowo były to '*Vitis riparia', 'Vitis rupestris',* później także '*Vitis labrusca'* i '*Vitis berlandieri'* (Mijowska et al., 2017; Ollat et al., 2016; Reisch et al., 2012; Vršič et al., 2015). Zwrócono wówczas też uwagę na inne cechy, możliwe do uzyskania dzięki stosowaniu podkładek, takie jak np. przystosowanie winorośli do warunków glebowych i klimatycznych (Ferlito et al., 2020; Hoover et al., 2004; Walker and Clingeleffer, 2009; Zhang et al., 2016). Badania wykazały także, że oddziaływanie podkładki na roślinę może wpływać na pobieranie składników pokarmowych z gleby, a tym samym na wzrost roślin, długość okresu wegetacji, wielkość plonu. Wszytko to przekłada się na cechy jakościowe owoców i w efekcie końcowym na jakość uzyskanych z nich produktów i co za tym idzie: stwarza to możliwość sterowania rośliną w celu osiągnięcia założonych cech produktu końcowego (da Silva et al., 2019; Walker and Clingeleffer, 2009).

Winogrona *'Vitis vinifera'* zazwyczaj uprawia się w regionach o odpowiednich warunkach dla wegetacji, czyli w zakresie od 30°N do 50°N oraz od około 30°S do 40°S (Fig.1). Oczywiście praktyki uprawy winorośli pozwalają na uprawę poza nią, chociażby przez stosowanie odmian odpornych na niskie temperatury nawet do -35°C lub częściej odmian hybrydowych.

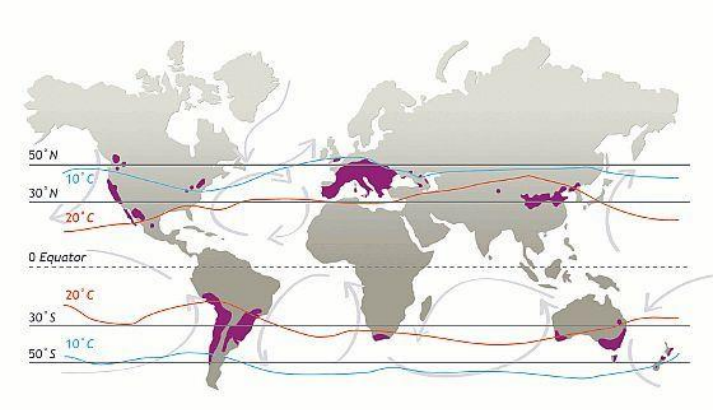

Fig. 1. Obszary uprawy winorośli właściwej 'Vitis vinifera'. (http://viniculture.pl/wino/regiony-winiarskie-na-swiecie/)

'Vitis vinifera' w chłodniejszych krajach ma niewielkie szanse na rozwój i prawidłową plenność, dlatego też, zdecydowano się na krzyżowanie różnych odmian z gatunku winorośli właściwej, czyli *'Vitis vinifera'* z odmianami innych gatunków, jak na przykład amerykańską *Vitis labrusca* i *Vitis rupestris, lub dalekowschodnią Vitis amurensis*. Hybrydyzacja winorośli rozpoczęła się w XIX wieku w Stanach Zjednoczonych (Teissedre, 2018). Później powstało kilka programów realizowanych już na całym świecie, gdzie uzyskano przede wszystkich odporność na choroby i szkodniki, ale także taką cechę jak odporność na niskie temperatury. Hybrydy jednak nadal są źle postrzegane przez winiarzy, ponieważ zbyt dużo niepożądanych cech sensorycznych dziedziczą po gatunkach amerykańskich czy azjatyckich. Są to m.in. zbyt wysoka kwasowość, mała ilość cukrów, tanin, nadmiernie ziołowe lub inne, niepożądane aromaty. Z tego względu, tylko niewielka liczba odmian hybrydowych winorośli jest w krajach europejskich dozwolona do produkcji win (Council of the European Union, 2008). Być może wprowadzane coraz szerzej ograniczenia zużycia pestycydów w ramach ekologicznych programów, które stało się podstawowym społecznym żądaniem (Barnaba et al., 2017; Montaigne et al., 2016; Teissedre, 2018), zwróci uwagę winiarzy w kierunku odporniejszych na choroby hybryd winorośli. Tym bardziej, że w literaturze można znaleźć doniesienia poparte badaniami genetycznymi, które dowodzą, iż obecne odmiany *'Vitis vinifera'* to w większości krzyżówki z dzikimi gatunkami europejskimi *Vitis silvestris* (Migicovsky et al., 2016).

W Polsce winorośl pojawiła się razem z nastaniem chrześcijaństwa i do połowy XVII w. była uprawiana z różnym efektem. Silne ochłodzenie klimatu, rozwijający się eksport win węgierskich oraz liczne wojny, skutecznie zniechęciły polskich ogrodników do kontynuacji wymagającej uprawy *'Vitis vinifera'* (Kapusta et al., 2018; Myśliwiec et al., 2018). W latach 80 XX wieku w okolicach Jasła powstała winnica, która przede wszystkim uwzględniała realizację programu przydatności różnych odmian winorośli do polskich warunków klimatycznych. Dzięki ich doświadczeniu zaczęto powoli odbudowywać w naszym kraju areał winnic. Obecnie w Polsce mamy ponad 400 winnic, na około 500 hektarach. W stosunku do krajów europejskich jest to bardzo mała liczba. Nadal dużą przeszkodą w rozwoju upraw w Polsce i konkurowaniu z innymi krajami winiarskimi, mającymi dużo większe tradycje winiarskie, jest wysoki koszt produkcji win.

W większości, w winnicach polskich królują odmiany hybrydowe winorośli, a jedynie w winnicach położonych w cieplejszych rejonach, winogrodnicy ryzykują nasadzenia

winoroślą właściwą *'Vitis vinifera'*. Największy jednak rozwój upraw, można obserwować w południowo-wschodniej Polsce, który jest efektem zarówno globalnego ocieplenia, zmian kulturowych wynikających z przystąpienia do Unii Europejskiej, jak i dywersyfikacji upraw sadowniczych oraz poszukiwania alternatywnych źródeł dochodów (Kapusta et al., 2018).

Pomimo licznych badań na temat kwitnienia *'Vitis vinifera'* pod wpływem czynników środowiskowych i kulturowych, proces ten pozostaje słabo poznany (Boss et al., 2003; Carmo Vasconcelos et al., 2009; García-Breijo et al., 2020; Heazlewood et al., 2005; Heazlewood and Wilson, 2004; Kimura et al., 1998; Lebon et al., 2008; Meneghetti, 2006; Panchenko et al., 1975; Pratt, 1971; Sabir et al., 2020; Sabir and Kucukbasmaci, 2020). Badania wskazują, że winogrona mają dość złożoną biologię zapylania, brakuje natomiast doniesień na ten temat dla odmian hybrydowych. Kwitnienie winorośli, szczególnie w chłodnym klimacie, wykazuje duże sezonowe zróżnicowanie. Wiąże się to przede wszystkim z interakcją między genotypem rośliny, środowiskiem i praktykami jej uprawy. Na jakoś pyłku składają się takie cechy jak: żywotność pyłku, zdolność kiełkowania oraz wzrost łagiewki pyłkowej. Cechy te wpływają na wielkość i jakość produkcji winogron. Sam proces zapylenia może odbywać się przez samozapylenie lub zapylenie krzyżowe, dla niektórych odmian niezbędne ze względu na budowę kwiatów.

W ostatnich latach zwiększyło się zainteresowanie żywnością funkcjonalną, czyli taką, która oprócz walorów smakowych i odżywczych, ma także działanie prozdrowotne i jest naturalną alternatywą dla sztucznych substytutów. Wykorzystując naszą dotychczasową wiedzę, możemy znaleźć i opracować substancje, które będą wspomagać nasz system antyoksydacyjny organizmu. Kierunkiem najbardziej pożądanym jest pozyskiwanie roślinnych metabolitów (Cybul and Nowak, 2008).

Wino zostało docenione przez współczesne nauki medyczne jako jeden z produktów prozdrowotnych i jedno z najważniejszych źródeł pod względem ilości przeciwutleniaczy, którymi w winie są przede wszystkim związki fenolowe (Martin and Rasmussen, 2011). Oprócz działania przeciwutleniającego, mają także działanie przeciwdrobnoustrojowe oraz kardioprotekcyjne. Wykazano działanie ochronne przeciwutleniaczy biologicznych m.in. w stosunku do raka, chorób serca, miażdżycy, cukrzycy, reumatoidalnego zapalenia stawów, choroby Parkinsona i Alzheimera (Cory et al., 2018; Di Lorenzo et al., 2021). Pozytywne właściwości polifenoli, jak również rosnące obawy, iż syntetyczne polifenole mogą mieć niepożądane skutki uboczne dla naszego zdrowia, spowodowały zwiększenie ich rangi (Hooper and Frazier, 2012; Mazzanti et al., 2015). Całkowita zawartość związków fenolowych w białym winie wynosi od 100 do 400 m/l. W winach różowych od 400 do 800 mg/l, a w winach czerwonych wynoszą one od 1000 do 2000 mg/l (Kennedy, 2008).

Polskie wina gronowe są i będą w najbliższej przyszłości produktem niszowym, dlatego też przede wszystkim winiarze powinni skupiać się na osiągnięciu jak najlepszej jakości win regionalnych. W warunkach klimatycznych Polski, winogrona w pełni dojrzałe osiągają poziom cukru w granicach 17-23%. Efektem tego jest niska zawartość alkoholu w winach i wysoka kwasowość, a także niski poziom związków polifenolowych. W winach produkowanych z winogron uprawianych w południowej Polsce, w badaniach wykazano poziom polifenoli średnio: w winach czerwonych 1210,4mg/L a w winach białych 96,42 mg/L (Kapusta et al., 2018; Lisek, 2008). Wzrost antyoksydantów możemy osiągnąć przez maceracje lub fermentację moszczu winogronowego razem ze skórkami i pestkami.

W przypadku technologii produkcji win czerwonych maceracja jest normą, w przeciwieństwie do win białych, gdzie fermentowany jest czysty moszcz zaraz po tłoczeniu. Równoczesne zwiększenie zawartości cukrów oraz polifenoli można uzyskać poprzez produkcję win technologiami specjalnymi tj. np. metodami *passito*, Tokajską czy win lodowych. Metody te w Polsce są mało znane, natomiast na świecie, szczególnie w Europie południowej, cieszą się dużą popularnością. We Włoszech, wino w stylu *passito* ma 10% udziału w rynku win i jest produkowane w 17 regionach. Korzystając ze sprawdzonych metod produkcji lub dostosowując ją do polskich warunków, można uzyskać zmianę słabych parametrów biochemicznych, w tym zwiększenie zawartości związków fenolowych kilkukrotnie.

Hybrydowe odmiany winorośli zyskują coraz większą popularność w krajach klimatu chłodnego. Agrotechnika i przeznaczenie tych odmian jest analogiczna do odmian *'Vitis vinifera"*, jednakże szczepy hybrydowe cechuje szereg różnic anatomicznych i agrotechnicznych w porównaniu do '*Vitis vinifera'*. W związku z tym podjęto badania dotyczące biologicznych i użytkowych hybrydowych odmian winorośli.

5. Hipoteza badawcza i cel pracy

Po przeprowadzeniu analizy danych literaturowych postawiono trzy hipotezy dotyczące wykorzystania odmian hybrydowych w uprawie winorośli.

1. Zastosowanie różnych podkładek dla odmian winorośli hybrydowych ma wpływ na wzrost roślin, plonowanie i jakość owoców.
2. Odmiany winorośli różnią się od siebie pod względem heterogeniczności liczby pylników, jakości pyłku, liczby i jakości pyłku w kwiecie.
3. Metoda produkcji win z podsuszanych winogron wpływa na wzrost substancji biologicznie aktywnych w winie.

Odmiany hybrydowe, przewyższając w niektórych aspektach winorośl właściwą *'Vitis vinifera'* np. w aspekcie ekonomicznym, powinny być bardziej rozpropagowane, nie tylko w krajach klimatu zimnego. Co więcej, wykorzystując techniki produkcji win metodami specjalnymi, które są stosowane na całym świecie, można będzie tworzyć wina o niezwykłych wartościach prozdrowotnych.

Celem pracy było określenie najważniejszych cech biologicznych wybranych hybrydowych odmian winorośli: wzrost roślin, biologia kwitnienia, plonowanie, jakość owoców, oraz ocena wpływu produkcji win z podsuszanych winogron na zawartość wybranych substancji biologicznie aktywnych.

Szczegółowo:

- w doświadczeniu oceniano trzy odmiany, z których każda była zaszczepiona na pięciu podkładkach,
- przeprowadzono ocenę biometryczną krzewów oraz parametry użytkowe owoców,
- przeprowadzono analizę różnic w budowie kwiatów pomiędzy jedenastoma hybrydowymi odmianami winorośli,

- oceniono zapylenie oraz zawiązywanie owoców i nasion,
- wprowadzono metody produkcji win zwiększające koncentrację moszczu i tym samym związków w nim zawartych,
- określono wybrane cechy użytkowe i wartości prozdrowotne win z podsuszanych winogron,
- przeprowadzono analizę organoleptyczną win z podsuszanych winogron.

Zaplanowane i zrealizowane zadania badawcze przedstawiono w tabeli 1.

Tabela 1. Badane czynniki i cele badawcze przeprowadzonych eksperymentów w publikacjach wchodzących w skład pracy doktorskiej.

		Etapy	Badany czynnik	Pomiary
Publikacja nr	1	Wzrost roślin	Wpływ podkładki na: - morfologię winorośli - jakość owoców	- biometria krzewów - zawiązywanie kwiatostanów - plon winorośli - parametry chemiczne soku z jagód
	2	Kwitnienie	- biologia kwitnienia - wydajność zapylania	- biometria kwiatów - liczbę i jakość ziaren pyłku w kwiatach - zapylanie i przerastanie łagiewki pyłkowej przez szyjkę słupka - jakość owoców
	3	Owocowanie	- cechy chemiczne i enologiczne win z podsuszanych winogron	- cechy fizykochemiczne win - cechy organoleptyczne win

6. Materiały i metody

6.1 Materiał roślinny

Do badań przeprowadzonych w publikacji 1 w 2013 roku, w winnicy Uniwersytetu Rolniczego w Krakowie „Garlicki Lamus" w Garlicy Murowanej, posadzono trzy odmiany hybrydowe winorośli 'Solaris', 'Seyval Blanc' i 'Johanniter' zaszczepionych na pięciu podkładkach 125AA, 5BB, SO4, 5BB i Bőrner. Materiał roślinny pobrano w 2016 i 2018 roku. W roku 2017 wiosenne przymrozki uszkodziły kwiaty, eliminując sezon z badań.

Do badań przeprowadzonych w publikacji 2 wykorzystano kwiaty i owoce winorośli pochodzące z winnicy Uniwersytetu Rolniczego w Krakowie „Garlicki Lamus". Materiał

pobrano w 2018 i 2019 roku z dwunastoletnich krzewów odmian hybrydowych winorośli: ‘Aurora’, ‘Bianca’, ‘Hibernal’, ‘Jutrzenka’, ‘Leon Millot’, ‘Marechal Foch’, ‘Muscat Odesskij’, ‘Regent’, ‘Rondo’, ‘Seyval Blanc’ oraz ‘Solaris’.

Do badań przeprowadzonych w publikacji 3 wykorzystano wina z podsuszanych winogron hybrydowej odmiany ‘Hibernal’, pochodzących z winnicy Uniwersytetu Rolniczego w Krakowie „Garlicki Lamus”. Trzy wina wykonano trzema różnymi metodami opisanymi w publikacji.

6.2 Stosowane metody

W tabeli 2 przedstawiono wykaz stosowanych w badaniach metod. Szczegółowy opis metod stosowanych w badaniach znajduje się w publikacjach wchodzących w skład rozprawy doktorskiej.

Tabela 2. Wykaz stosowanych metod przeprowadzonych eksperymentów w publikacjach wchodzących w skład pracy doktorskiej

		Analizowane parametry	Metoda
Publikacja nr	1	Biometria krzewów	przyrost pola powierzchni przekroju podstawy pnia (ITCSA) [cm^2]
			długość pędów [cm]
			masa gron w 5 stopniowej skali [gr]
		Zawiązywanie kwiatostanów	liczba kwiatostanów [szt.]
		Plon winorośli	masa plonu i średniej masa grona [gr]
		Cechy fizykochemiczne owoców	pH - pehametr Janway 3020
			TA - miareczkowanie kwasowo-zasadowe [g/L]
			TSS – refraktometr [°Brix]
			TSS/TA – wskaźnik dojrzałości zbiorczej
	2	Biometria kwiatów	liczba pylników [szt.]
			liczba zalążków w zalążni- mikroskop steroskopowy Carl Zeiss Discovery [szt.]
			receptywność znamienia – mikroskop steroskopowy Carl Zeiss Discovery 2.0 (Dafni et al., 2005; Dafni and Maués, 1998)
			liczba ziaren w pylniku – mikroskop Carl Zeiss Image M2 AXIO przy dziesięciokrotnym powiększeniu w świetle białym (Dziedzic et al., 2019) [szt.]
		Jakość i liczba ziaren pyłku w kwiatach	wielkość ziaren pyłku - oprogramowanie Axio vs. 40V [µm]

			żywotność ziaren pyłku – metoda barwienia Alexandra (Alexander, 1969) [%]
		Zapylenie znamion i przerastanie łagiewki pyłkowej przez szyjkę słupka	barwienie błękitem anilinowym kalozy zawartej obecnej w ziarnach pyłku i łagiewce pyłkowej, mikroskop fluorescencyjny Carl Zeiss Image M2 Axio (Bieniasz et al., 2017)
		Parametry jakości owoców	biometria jagód – masa, długość i szerokość jagód, liczba nasion i powierzchnia kulistości jagód
	3	Parametry fizykochemiczne win	podstawowe parametry chemiczne - pH - pehametr Janway 3020 - TA - miareczkowanie kwasowo-zasadowe [g/L] - TSS –refraktometr [°Brix]
			zawartość etanolu – metoda referencyjna [%]
			profil kwasowy –izotachoforeza kapilarna (Farkaš and Koval, 1982) [g/L]
			całkowita zawartość fenoli, zdolność przeciwutleniająca (test FRAP i CUPRAC) i zdolność do usuwania wolnych rodników – metoda spektrofotometryczna (Apak et al., 2007; Benzie and Strain, 1996; Brand-Williams et al., 1995; Defouquette et al., 2001; Sánchez-Rangel et al., 2013; Singleton et al., 1999) [g/L]
			analiza polifenoli – metoda wysokosprawnej chromatografii cieczowej (HPLC) [g/L]
		Parametry organoleptyczne win	ocena w oparciu o materiały The Wine & Spirit Education Trust (WSET).

7. Omówienie uzyskanych wyników

7.1 Publikacja 1

Kowalczyk B., Bieniasz M., Błaszczyk J., Banach P. 2022. The effect of rootstocks on the growth, yield and fruit quality of hybrid grape varieties in cold climate conditions. Horticultural Science. 58/2021. https://doi.org/10.17221/58/2021-HORTSCI

Wykorzystanie podkładek u uprawie winorośli stosowane jest na szeroką skalę od XIX wieku. Rozpoczęto ten proces w celu ochrony winorośli przed filokserą, ale znalazło to również zastosowanie w przystosowaniu winorośli do istniejących warunków biotycznych i abiotycznych. W badaniu skupiono się na wpływie pięciu podkładek 125AA, 5BB, SO4, 5BB i Bőrner na wzrost roślin, plonowanie i jakość owoców, trzech odmian hybrydowych winorośli

‘Solaris’, ‘Seyval Blanc’ i ‘Johanniter’ w warunkach klimatu chłodnego. Wykonano pomiary biometryczne winorośli i chemiczne moszczu. Z analiz biometrycznych analizowano przyrost powierzchni przekroju pnia (ITCSA), przyrost pędów, liczbę kwiatostanów na winorośli, plon ogólny, średnią masę kiści oraz parametry chemiczne, takie jak całkowita zawartość rozpuszczalnej substancji stałej (TSS) i kwasowość miareczkowa winogron (TA). Masę gron poszczególnych odmian oceniano również w pięciu kategoriach wagowych (0–700 g).

Dla odmiany Solaris w analizie ITSCA stwierdzono, że najbardziej stymulującą przyrost pola powierzchni przekroju podstawy pnia jest podkładka Bőrner (281.8mm^2). Ta sama podkładka w pierwszym roku obserwacji zmniejszyła (91.0g) a w drugim roku zwiększyła średnią masę grona (127.9g). W pozostałych parametrach biometrycznych różnice nie wystąpiły, bądź były nieznaczne. W analizach chemicznych również podkładka Bőrner miała największy wpływ na poprawę parametrów jakościowych, w obu latach TSS (25.2°B, 23.3°B) i w drugim roku TA (6.8g/L).

Dla odmiany Seyval Blanc podkładka 5BB miała wpływ na największy przyrost ITCSA (226.6mm^2), zmniejszył się średni przyrost pędów (91cm), natomiast zwiększyła się średnia liczba kwiatostanów (31.7szt/win.). Zauważono także pozytywny wpływ tej podkładki na poprawę jakości owoców, w obu latach zwiększyła się zawartość TSS (20.8°B i 19.5°B) jak również obniżyła się kwasowość moszczu (26.6 i 7.5g/L) i tym samym stosunek TSS/TA (3.15 i 2.60).

W odmianie Johanniter, podobnie jak w odmianie Solaris, podkładka Bőrner pozytywnie wpłynęła na parametry biometryczne i na parametry chemiczne. Zwiększył się przyrost ITCSA (140.6mm^2) i zanotowano mniejszy średni przyrost pędów (80.0cm). W stosunku do innych podkładek TSS w drugim roku zwiększył się (20.1g/L), przy wyższej zawartości TA (9.0g/L) co spowodowało równocześnie obniżenie stosunku TSS/TA (2.24). Wyższe wartości TSS/TA zanotowano dla podkładek R101-14M, 5BB i SO4 (2.62, 2.64 i 2.56). W doświadczeniu grona w plonie podzielono na pięć klas wielkości dla każdej z kombinacji. Nie odnotowano wpływu podkładki na zróżnicowanie masy w udziale w klasach, natomiast widoczna jest prawidłowość rozkładu masy gron dla każdej z odmian. Dla odmiany Solaris masa grona była w zakresie od 60 do 200g, w odmianie Johanniter od 100 do 200g, w odmianie Seyval Blanc od 200 do 300 g.

7.2 Publikacja 2

Kowalczyk B., Bieniasz M., Kostecka-Gugała. 2022. Flowering Biology of Selected Hybrid Grape Cultivars under Temperate Climate Conditions. Agriculture (Switzerland) 12(5): 1-18 nr 655

Kwitnienie jest kluczowym procesem, wpływającym na jakość plonu, w uprawie wszystkich gatunków owocowych, w tym winorośli. W doświadczeniu skupiono się na analizie biologii kwitnienia i zapylenia wybranych jedenastu hybrydowych odmian winorośli. Analizowano parametry biometryczne i jakość kwiatów, jakość i liczbę ziaren pyłku w kwiatach, oceniono zapylanie i przerost łagiewki pyłkowej przez szyjkę słupka, oraz oceniono jakość owoców.

W analizie liczby pylników wykazano, że większość odmian hybrydowych, podobnie jak ‘*Vitis vinifera’*, ma 5-krotną strukturę kwiatów (5 pylników i pięć płatków), natomiast odnotowano zróżnicowanie pomiędzy odmianami i sezonami, największe w odmianie ‘Seyval Blanc’, gdzie stwierdzono od 4 do 11 pylników w kwiecie. Największą średnią liczbę pylników w obu latach doświadczenia miała odmiana ‘Solaris’ (6.0, 5.9). Analizując liczbę zalążków w zalążni zaobserwowano od 3 do 7 zalążków, gdzie statystycznie największą liczbę miała odmiana 'Solaris' (6.1), a najmniej 'Jutrzenka' (4.3) i 'Marechal Foch' (4.2). Receptywność znamienia przedstawiono w czterech etapach rozwoju i nie stwierdzono istotnych różnic pomiędzy odmianami. Obliczono liczbę pyłku w pylniku i w kwiecie, które były bardzo zróżnicowane w zależności od odmiany oraz warunków pogodowych podczas mikrosporogenezy.

Najmniejszą średnią z dwóch lat liczbą pyłku w pylniku jak i w kwiecie charakteryzowała się odmiana ‘Hibernal’ (1410.6, 7054.9) a największą odmiana ‘Solaris’ (5979.7, 34950.9). Wielkość pyłku podzielono na dwie główne grupy: poniżej i powyżej 20 µm. W grupie odmian powyżej 20 µm znalazły się tylko cztery odmiany: ‘Aurora’ (21.3, 21.3), ‘Bianka’ (22.2, 22.2), ‘Leon Millot’ (21.6, 21.8) i ‘Muscat Odeskij’ (20.5, 20.5). Następnym badanym parametrem była żywotność pyłku, która różniła się w zależności od roku i odmiany. Odmiana 'Muscat Odesskij' charakteryzowała się niską żywotnością pyłku w 2018 roku (25.5%), natomiast odmiany 'Bianka', 'Aurora', 'Rondo' i 'Solaris' zaliczono do grupy o wysokiej żywotności pyłku (89.5%, 78.0%, 81.5%, 84.0%). W 2019 odmiana 'Seyval Blanc' należała do grupy o niskiej żywotności pyłku (37.0%), a odmiany 'Regent' i 'Aurora' do grupy o bardzo wysokiej żywotności pyłku (91.7%, 93.3%).

W ocenie zapylenia obliczono liczbę kiełkujących ziaren pyłku na znamię oraz liczbę łagiewek pyłkowych u podstawy słupka. Statystycznie najwięcej kiełkujących ziaren pyłku było w odmianach 'Solaris' (43.5%), 'Marechal Foch' (40.5%), 'Aurora' (40.8%), 'Bianca' (40.9%) i 'Muscat Odesskij' (32.2%). Najmniej kiełkujących na znamię ziaren pyłku odnotowano dla odmian 'Hibernal' (0.8%) i 'Leon Millot' (1.7%). W ocenie liczby łagiewek u podstawy słupka najmniej odnotowano dla odmian ‘Hibernal’ (0.1), ‚Seyval Blanc’ (1.3), ‘Leon Millot’ (1.7), ‘Rondo’ (0.3) i ‘Jutrzenka’ (2.1) , a najwięcej zaobserwowano dla odmian 'Aurora' (30.1), 'Bianca' (24.2) i 'Solaris' (21,7).

W doświadczeniu oceniono także jakość owoców. Wielkość jagód i ich kształt w omawianych odmianach zależy od odmiany a masa winogron zazwyczaj jest zależna od liczby nasion. Odmiana ‘Regent’ miała jedną z najniższych zanotowanych mas jagód (1.6g), najmniejszą wielkość (1.8cm^2), najmniejszą liczbę nasion (1.6) i najwyższy procent jagód z jednym nasionem (50%). Odnotowano natomiast odstępstwo dla odmiany ‘Solaris’: statystycznie jagody miały najwyższą średnią liczbę nasion, największy procent jagód z 4 nasionami (45%), natomiast nie spowodowało to zwiększenia masy (1.9g) i wielkości jagód (2.0cm^2). Odmiany o największej powierzchni kuli w niniejszym eksperymencie to 'Seyval Blanc' (2.2 cm^2), ‘Bianca’ (2.2 cm^2) i ‘Marechal Foch’ (2.1 cm^2), a najniższej ‘Regent’ (1.8cm^2) i ‘Leon Millot’ (1.8 cm^2).

7.3 Publikacja 3

Kowalczyk B.A., Bieniasz M., Kostecka-Gugała. 2022. The Content of Selected Bioactive Compounds in Wines Produced from Dehydrated Grapes of the Hybrid Variety ‘Hibernal’ as a Factor Determining the Method of Producing Straw Wines. Foods 11(7): 1-14

W trzeciej publikacji analizowano, jak metoda produkcji wina z podsuszanych winogron z hybrydowej odmiany ‘Hibernal”, wpływa na zawartość związków bioaktywnych w nich zawartych. Wprowadzono metody produkcji win zwiększające koncentrację moszczu. W pierwszym etapie podsuszono dojrzałe winogrona, następnie wykonano trzy wina, trzema różnymi metodami: wino A - metodą *passito,* wino B – zmodyfikowaną przez autorów metodą *passito* i wino C - metodą Tokajską. Następnie dokonano analizy, podstawowych dla win, parametrów chemicznych.

Poziom pH analizowanych win różnił się pomiędzy sobą i nie był ściśle skorelowany z kwasowością miareczkową win. Najniższe pH zanotowano dla wina C (3.1) przy średniej kwasowości miareczkowej TA (8.95g/L) a najwyższe pH dla wina A (3.91) przy najniższym TA (8.18g/L). Zawartość ekstraktu wykazała podobną tendencję do pH wina: wina A i B zakwalifikowano do win bardzo słodkich powyżej 150g/l (182 i 159g/L) z zawartością etanolu 18%, a wino C do win słodkich tj. w zakresie od 60 do 90g/L (69g/L) i 15% alkoholu. Wykonano także analizę zawartości głównych kwasów organicznych metodą izotachoforezy. W doświadczeniu próbka B miała najwyższą zawartość kwasu winowego (4.61g/L), jabłkowego (2.99g/L), bursztynowego (2.40g/L), a także kwasu cytrynowego (0.55g/L). W winie C oznaczono najmniejsze zawartości kwasów winowego (2.27g/L), cytrynowego (0.28g/L), jabłkowego (1.25g/L), bursztynowego (0.57g/L) i octowego (0.19g/L). Kwas octowy nie jest pożądany w winie, ale wysoka zawartość winie A 1.50 g/L mieści się w granicach dla win z odwodnianych winogron.

W winach analizowano także całkowitą zawartość polifenoli, testy określające zdolność przeciwutleniającą win i zdolność do usuwania wolnych rodników metodą spektrofotometryczną. Najwyższe wartości uzyskało wino B, najniższą wino C. Skład polifenolowy próbek wina analizowano za pomocą wysokosprawnej chromatografii cieczowej (HPLC). Tu również najwyższe wartości uzyskało wino B poza stylbenem (trans -resweratrol), którego nie wykryto w próbce, natomiast wystąpił w pozostałych dwóch. Polifenole zidentyfikowano jako flawonole ((+)katechina 64.29mg/L, kwercetyna 0.32g/L), kwasy hydroksybenzoesowe (kwas wanilinowy 2.83mg/L i kwas syryngowy 2.25g/L), kwasy hydroksycynamonowe (kwas p-kumarowy 3.00g/L, kwas kawowy 7.10g/L, kwas chlorogenowy 0.57g/L, kwas ferulowy 2.68g/L, kwas trans-cynamonowy 0.43g/L).

Wykonano także analizę organoleptyczną win przez grupę 30 respondentów, w której oceniono ich wygląd, aromat, smak i jakość. Wina A i B zostały ocenione jako wina o intensywnie bursztynowej barwie, pełnym smaku i długim finiszu. Zarówno pod względem aromatu, jak i smaku dominowały smaki określane jako typowe dla win *passito* (tj. głównie suszone owoce, miód, karmel i orzechy. Wysoka zawartość alkoholu w tych winach (18%) była w pełni wyczuwalna, a także widoczna przez „łzy” na ściankach kieliszka. Spośród dwóch win wyżej oceniono wino B. Wino C, pod względem aromatów, odpowiadało charakterystyce

odmianowej, która przypominała Rieslinga, z lekkim dodatkiem wanilii, miodu i kwiatów lipy. Smak składał się głównie ze smaków zielonych owoców, grejpfruta i cytryny. Wino to zostało dobrze przyjęte i ocenione jako bardzo dobre pod względem jakości.

8. Podsumowanie i wnioski

Przeprowadzone badania zaprezentowane w przedstawionych publikacjach przybliżają cechy biologiczne i użytkowe wybranych hybrydowych odmian winorośli.

Wykorzystanie ich w warunkach klimatu umiarkowanego pozwala na zakładanie winnic, a dzięki możliwości wyboru podkładek, można osiągać plony o dobrych parametrach fizykochemicznych. W przeprowadzonym badaniu wykazano, że podkładki, w zależności od odmiany, nie determinują wielkości plonu, ale znacząco poprawiają jego jakość.

Oceniono także biologię kwitnienia, i wydajność zapylania jedenastu hybrydowych odmian winorośli. Pomimo odmiennej budowy kwiatów od budowy kwiatów Vitis vinifera, badane odmiany wykazały się dużą liczbą pyłku, co świadczy o możliwości zapylenia nawet przy bardzo niskich wskaźnikach żywotności pyłku.

Zawartość związków bioaktywnych w produkowanych winach, można zwiększyć poprzez modyfikację metody produkcji win, wykorzystując koncentracje moszczu. W pracy przeanalizowano trzy wina, wyprodukowane z podsuszanych winogron trzema odmiennymi metodami. Zarówno analizy chemiczne win, jak i ich ocena sensoryczna pokazują, że produkcja win typu *passito* z hybrydowych odmian winorośli, jest skuteczną alternatywą dla tradycyjnego procesu produkcji i z powodzeniem może być wykorzystywana w chłodnym klimacie.

Wnioski szczegółowe - Publikacja 1

Parametry biometryczne

1. Podkładka Bőrner miała pozytywny wpływ na odmiany ‘Solaris’ oraz ‘Johanniter’. Przyczyniła się do zwiększenia parametru ITCSA, zmniejszenia przyrostów oraz zwiększenia średniej masy grona.
2. Podkładki SO4 i 5BB wykazały dobrą kompatybilność z odmianą ‘Seyval Blanc’.
3. Podkładki nie miały wpływu na wielkość plonu.

Jakość owoców

1. Podkładki miały wpływ na jakość owoców i mogą istotnie zmienić parametry fizykochemiczne tj. zawartość ekstraktu i kwasowość.
2. W odmianie ‘Solaris’ podkładka Bőrner przyczyniła się do zwiększenia zawartości ekstraktu oraz zmniejszenia kwasowości. W mniejszym stopniu podobna zależność była dla podkładki 5BB.
3. Podkładki R101-14 M, 5BB i SO4 pozytywnie wpłynęły na zawartość ekstraktu i kwasowości odmian 'Seyval Blanc' i 'Johanniter'.

Wnioski szczegółowe - Publikacja 2

Biologia kwitnienia

1. Odmiany winorośli różnią się od siebie pod względem heterogeniczności liczby pylników, jakości pyłku, liczby i jakości pyłku w kwiecie.
2. Odmiany hybrydowe pod względem budowy kwiatów odbiegają od Vitis Vinifera.
3. Odnotowano anomalie w liczbie pylników w większości analizowanych odmian od 4 do 7, a w przypadku odmiany ‘Seyval Blanc’ od czterech do jedenastu.

Wydajność zapylania

1. Liczba pyłku w kwiecie jest zależna od odmiany i sezonu wegetatywnego oraz może podlegać sezonowym wahaniom.
2. Odmiany ‘Rondo’ i ‘Solaris’ charakteryzowały się najwyższymi wartościami takimi jak: liczba pyłku w kwiatach, żywotność pyłku, liczba zalążków w zalążni, oraz liczbą zawiązywanych nasion.
3. Odmiana ‘Regent’ ma zdolność w niewielkim procencie do zawiązywania owoców partenokarpicznych.
4. Masa jagód nie jest skorelowana z liczbą zawiązanych nasion.

Wnioski szczegółowe - Publikacja 3

Analizy chemiczne

1. Metoda produkcji wina w typie *passito* z siedmiodniową maceracją, akumuluje najwięcej bioaktywnych związków (3 748.5 mg/L GAE), wpływających pozytywnie na zdrowie człowieka. Całkowita zwartość polifenoli, jego zdolność antyrodnikowa i antyoksydacyjna była porównywalna z winami czerwonymi.

Analiza sensoryczna

1. Wino w typie *passito* z siedmiodniową maceracją, pomimo wysokiej kwasowości, było najbardziej zbalansowane.
2. Wino w typie *passito* z siedmiodniową maceracją oraz wino wyprodukowane metodą Tokajską oceniono jako wina o bardzo dobrej jakości.
3. Aromaty w winach w typie *passito,* jak i ich smak określono jako typowe dla win *passito* tj. głównie suszone owoce, miód, karmel i orzechy.
4. Wino wyprodukowanym metodą Tokajską pod względem aromatów, odpowiadało charakterystyce odmianowej z lekkim dodatkiem wanilii, miodu i kwiatów lipy. Smak składał się głównie ze smaków zielonych owoców, grejpfruta i cytryny.

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10. Załączniki

Załącznik 1 – Oświadczenie współautorów publikacji 1

Załącznik 2 – Publikacja 1

Załącznik 3 – Oświadczenie współautorów publikacji 2

Załącznik 4 – Publikacja 2

Załącznik 5 – Oświadczenie współautorów publikacji 3

Załącznik 6 – Publikacja 3

Rozprawa doktorska

Publikacja nr 1

Kowalczyk B., Bieniasz M., Błaszczyk J., Banach P. 2022. The effect of rootstocks on the growth, yield and fruit quality of hybrid grape varieties in cold climate conditions. Horticultural Science. 58/2021

Oświadczenie współautorów

Oświadczam, że w wymienionej publikacji mój wkład polegał na konceptualizacji doświadczenia, opracowaniu metodologii, walidacji, recenzji i redakcji manuskryptu, oraz na pozyskiwaniu finansowania dla publikacji.

Udział – 20%

dr hab. inż. Monika Bieniasz, prof. UR .....

Oświadczam, że w wymienionej publikacji mój wkład polegał na: konceptualizacji doświadczenia, opracowaniu metodologii.

Udział – 15%

dr hab. inż. Jan Błaszczyk .....

Udział – 5%

Oświadczam, że w wymienionej publikacji mój wkład polegał na założeniu i agrotechnice doświadczenia

dr inż. Przemysław Banach .....

Oświadczenie doktorantki

Oświadczam, że w wymienionej publikacji mój wkład polegał na prowadzeniu doświadczenia, zebraniu wyników, przeprowadzeniu analiz statystycznych, opracowaniu wykresów i tabel, przygotowaniu wstępnej wersji manuskryptu, oraz w uczestnictwie w redakcji końcowej wersji manuskryptu.

Udział - 60%

mgr inż. Barbara Kowalczyk .....

https://doi.org/10.17221/58/2021-HORTSCI

The effect of rootstocks on the growth, yield and fruit quality of hybrid grape varieties in cold climate condition

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Abstract: Viniculture in colder countries requires the use of rootstocks adapted to the climatic and soil conditions, which influence the essential characteristics of the vine yield in terms of the physiological and morphological features. The current study was carried out in 2015–2018 in southern Poland to examine the impact of the '5BB', '125AA', '101-14M', 'SO4' and 'Börner' rootstocks on the growth, yield and fruit quality of three grape varieties: 'Seyval Blanc', 'Johanniter' and 'Solaris'. The following biometric parameters were compared: the increment in the trunk cross-sectional area, number of inflorescences on the vine, total yield, mean weight of a cluster and chemical parameters, such as the total soluble solid (TSS) content and grape titratable acidity (TA). The cluster weight of the individual varieties was also assessed in eight categories by weight (0–700 g). The results showed that 'Solaris' and 'Johanniter' grafted onto 'Börner' and 'Seyval Blanc' onto '5BB' had significantly increased trunk diameters. For the 'Solaris' cultivar, the 'Börner' rootstock increased the TSS volume by 8.2%. In the 'Seyval Blanc' cultivar, '125AA' and 'Börner' reduced the TSS content and increased the content of TA in the berries. In the 'Johanniter' cultivar, the 'Börner' rootstock led to an increase in the TSS content with a concomitant increase in the TA.

Keywords: berry quality; grape ripeness; strength of growth; TA; TSS; grape size

The domestication of grapevines goes back to prehistoric times. Products made of *Vitis vinifera* were part of everyday life as well as cultural and religious celebrations (Censi et al. 2014). In Poland, the grapevine appeared with the advent of Christianity (10th century) and was cultivated until the middle of the 16th century with various results. The cooling of the climate effectively discouraged Polish gardeners from continuing the demanding cultivation of *V. vinifera*. In the second half of the nineteenth century, an accidentally imported aphid – Grape phylloxera (*Daktulosphaira vitifoliae)* – from North America, decimated European vineyards, and afterwards vineyards on other continents also, excluding only a few countries. The reconstruction and saving the remaining grapevines, after other unsuccessful attempts, was based on the grafting of noble varieties of *V. vinifera* onto resistant rootstocks of North American species or interspecies hybrids: *V. labrusca, V. riparia, V. rupestris* and *V. berlandieri* (Vršič et al. 2015; Ollat et al. 2016; Mijowska et al. 2017). Grafting is one of the oldest horticultural practices, already in use 9 000 years ago in China and now used in most commercial crops worldwide (Mudge et al. 2009; Gautier et al. 2019). The combination of independent progeny and rootstock traits adapts the grapevine to the existing biotic and abiotic conditions. The selection of appropriate rootstocks has become a protective tool against phylloxera, but the adaptability to climatic and soil conditions can also be taken into account (Gu 2001; Walker, Clingeleffer 2009; Somkuwar et al. 2011; Zhang et al. 2016; Ferlito et al. 2020). Rootstocks should be selected both in terms of the characteristics of a given variety and the

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clone (growth rate, yield, nutrient requirements), as well as the soil conditions (soil moisture, soil fertility, active calcium content, etc.). Rootstocks affect the nutrient uptake from the soil, and thus the plant growth, length of the growing season and yield (Reynolds, Wardle 2001; Mijowska et al. 2017).

Climate change, which has a destabilising effect on wine-growing, is setting new limits for viticulture around the world. Poland, as a zone A country, could become a good region for the production of quality wine, provided that the material is adapted to the environmental conditions (Duchêne 2016). The aim of this study that was conducted in 2015–2018 was to investigate the influence of '5BB', '125AA', '101-14M', 'SO4' and 'Börner' rootstocks on the growth, yield and fruit quality of three grape varieties: 'Seyval Blanc', 'Johanniter' and 'Solaris'. The results will provide information to growers that will help to better select rootstocks for the above varieties, grown under "cool climate" conditions.

MATERIAL AND METHODS

Description of the area. Field research was carried out in a vineyard at the Experimental Station of the University of Agriculture in southern Poland (50°08'29.4"N 19°55'50.7"E). The experiment was established in 2013 and data were collected during three growing seasons. Plants were planted from two-season potted cuttings, one year after inoculation on individual rootstocks. The plants had a well-developed root system.

Characteristics of the varieties. 'Seyval Blanc' is an interspecific hybrid: 43.75% *V. vinifera*, 28.15% *V. rupestris*, 12.5% *V. berlandieri*, 12.5% *V. riparia* and 3.1% *V. licencumi*.

'Solaris', a German variety that is a cross of Merzling × Geisenheim 6493 ('Zarya severa' × 'Muskat Ottonel'), registered as *Vitis vinifera* (73.4% *V. vinifera* + 7% *V. rupestris* + 3.1% *V. berlandieri* + 3.1% *V. riparia* + 0.8% *V. lincecumi*) (Julius Kühn-Institut).

'Johanniter', a cross of Riesling and Freiburg 589-54 [Seyve-Villard 12.481 × ('Ruländer' × 'Gutedel')] (Julius Kühn-Institut).

Rootstocks used. 'Kober 5BB' *V. berlandieri* × *V. riparia*: rootstock often used in Europe due to its very high tolerance to the soil conditions. 'Kober 5BB' transfers a short growing season to the grafted varieties. Frost resistance is highest in the group *V. berlandieri* × *V. riparia*, but with a tendency to negatively affect the effectiveness of the flowering (Mijowska et al. 2017).

'Kober 125AA' *V. berlandieri* × *V. riparia*: (intermediate rootstock between '5BB' and S04). Like '5BB', it tolerates a wide range of soils, except dry soils. It is characterised by medium growth and high resistance to freezing. It has a good effect on both the flowering and fruit setting (Shaffer 2004).

Arnold Bőrner *V. riparia 183 G* × *V. cinerea:* shows high drought tolerance to the contribution from *V. cinerea* in the species cross. It may also shorten the growing period (Zhang et al. 2009).

'Selection Oppenheim No. 4' ('SO4') *V. berlandieri* × *V. riparia*: rootstock developed in Germany. It requires light, fertile soils, and is not suitable for dry soils. It is characterised by a short growing season having high resistance to phylloxera and weak frost resistance. It has a beneficial effect on the flowering and fruit setting (Shaffer 2004).

'R101-14M' *V. riparia* × *V. rupestris*: a widely used rootstock, especially in the northeast US and Ukraine. It shows a high sensitivity to a water deficit in the substrate. It is characterised by the early ripening of fruits and the weakening of the growth of the grafted varieties. It has a very beneficial effect on the yield (Shaffer 2004).

Arrangement of the experiment. The cultivation system used in this study is the most common system used in vineyards in Poland, i.e., permanent turf in inter-row spaces and 80 cm wide herbicide strips. Irrigation was not installed, and point watering was used only in the period immediately after planting. Spraying against fungal diseases was performed according to the Fruit Plant Protection Programme, valid for a given year. During the experiment, no "green harvest" was carried out and the shortening of the shoots was carried out in the veraison phase.

Vines were grown 2.5 m × 1 m apart and were trained in a single Guyot system. The experiment included 'Seyval Blanc', 'Johanniter' and 'Solaris' varieties grafted onto five rootstocks: 'Kober 5BB', 'Kober 125AA', 'Bőrner', 'SO4' and '101-14'.

The measurements of each combination of rootstock and variety were conducted in three replicates, one replicate being comprised of five vines (Figure 1).

Weather data. Meteorological data was collected from the iMetos go IMT meteorological station located next to the vineyard in Garlica Murowana in 2015–2018. The average air temperatures during the growing season in two consecutive years, 2016 and 2017, did not differ from each other, while the

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Solaris	*****	○○○○○	◊◊◊◊◊	□□□□□	●●●●●
Solaris	○○○○○	◊◊◊◊◊	*****	□□□□□	●●●●●
Solaris	□□□□□	*****	○○○○○	◊◊◊◊◊	●●●●●
Johanniter	*****	○○○○○	◊◊◊◊◊	□□□□□	●●●●●
Johanniter	○○○○○	◊◊◊◊◊	*****	●●●●●	□□□□□
Johanniter	◊◊◊◊◊	*****	○○○○○	□□□□□	●●●●●
Seyval Blanc	*****	○○○○○	◊◊◊◊◊	□□□□□	●●●●●
Seyval Blanc	□□□□□	●●●●●	*****	○○○○○	◊◊◊◊◊
Seyval Blanc	○○○○○	◊◊◊◊◊	□□□□□	●●●●●	*****

* rootstock 12AA
○ rootstock 5BB
◊ rootstock SO4
□ rootstock 5BB
● rootstock Bőrner

Figure 1. Combination of the vine plantings in the experiment

temperature in 2018 was 12% higher than in 2016 and 2017 (Figure 2A). The temperatures dropped below 0 °C in April and May in 2016 and 2017 and caused the freezing of the young shoots and flower buds. The highest total precipitation was recorded in 2017 (Figure 2B), which was 50% higher than the average total precipitation for 1951–2010 in Poland and was optimal for viticulture. The lowest rainfall was recorded in 2016 and was 100% lower than the minimum cultivation requirements. The highest air humidity was recorded in 2016, while the lowest was recorded in 2018 (Figure 2C).

Methods. The assessed indices included the increment in the trunk cross-sectional area (ITCSA), number of inflorescences on a vine, total yield, average weight of one cluster, total soluble solid (TSS) content and grape titratable acidity (TA).

Vine measurements and biometric assessment of the ITCSA. The vine trunk diameter at a height of 30 cm from the base was measured in spring (bud break phase) and autumn (leaf fall phase) in 2015 and 2018, using electronic callipers. The cross-sectional area of the trunk (mm^2) was calculated according to the formula:

$$P = \frac{\pi \times d^2}{4} \quad (1)$$

where: d – the trunk diameter.

The following measurements and calculations were performed for the tested combinations: number of inflorescences/plants (pieces), total yield (kg), and average cluster weight (g).

In order to determine the qualitative parameters at harvest maturity, ten clusters were collected for each combination, from which ten completely healthy berries with intact skin (total of 100 berries) were randomly selected.

Cluster evaluation. The grape weight was assessed according to the scale of the individual varieties, and divided into five categories by the cluster weight. The percentage of each class was calculated for all the combinations: 0–59 g, 60–100 g, 101–200 g, 201–300 g, 301–700 g.

Chemical measurements. The total TA was determined by the acid-base titration method using 0.1 mol/L NaOH to pH 8.1. A Jenway 3020 pH meter (Jenway Ltd., Dunmow, UK) was used for this pur-

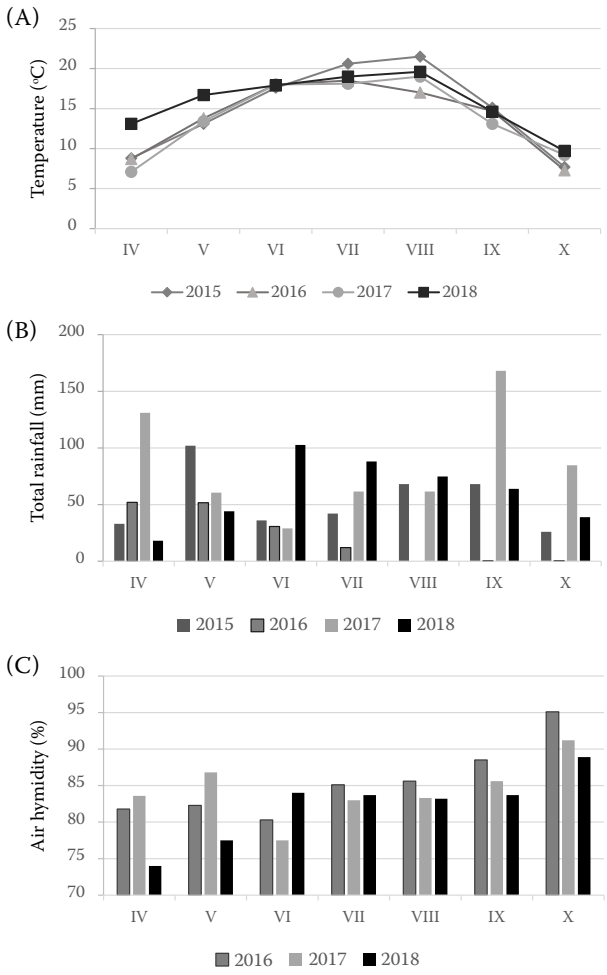

Figure 2. Weather in 2015–2018

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pose. Measurements were performed in three replicates. The results are expressed as tartaric acid (g/L).

The content of the TSS and soluble substances was tested at room temperature using an ATAGO PR-100 digital refractometer (ATAGO, PR-100, Tokyo, Japan) with a measuring range of 0.0–32.0%. Measurements were performed in three replicates and the results were expressed on a scale (°B).

The fruit harvest maturity index was calculated as the ratio of the TSS to the TA and expressed in °B/g/L.

Statistics. All the statistical calculations were performed for each year and for each variety separately. The results were analysed using one-way analysis of variance (ANOVA). Tukey's test was used to assess the significance of the differences between the means at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

This study provides information on the effects of five rootstocks on the quality and yield of three hybrid grape varieties. There are few reports in the literature regarding the influence of rootstocks on the fruit quality and grapevine morphology in zone A conditions. Fruits obtained from grapevines grown in a "cool climate" are characterised by lower sugar levels, and thus a lower alcohol content is obtained from them with a high acidity and polyphenol content (Izajasz-Parchańska et al. 2014).

Vine analysis – biometric assessment. The ITCSA variable (Figure 3) is a direct tool applied in the dendrological evaluation of the growth rate. The measurements were performed in 2015 and 2018. Figure 3 shows the results of the cross-sectional area increments for the three discussed varieties on the five rootstocks. After three growing seasons of the ITSCA experiment, the increment for the varieties 'Solaris' and 'Johanniter' was similar, regardless of the applied rootstocks, and averaged 109.6 mm^2 and 87.6 mm^2, respectively. The highest trunk increment for 'Seyval Blanc' was recorded on the 'SO4' and '5BB' rootstocks, although Whiting (2004) pointed to frequently arising compatibility problems of these two rootstocks with noble varieties. In this study, symptoms of incompatibility were not recorded for the observed varieties, and the rootstocks stimulated the growth of the stem base, similar to the study by Clingeleffer and Emmanuelli (2006) on the variety 'Sunmuscat'. In 2018, the highest increment of the stem base of the varieties 'Solaris' and 'Johanniter' was observed on the 'Börner' rootstock.

It was found, that the rootstocks influenced the shoot length of the grapevine varieties (Tables 1, 2, 3). In 2016, significant differences in the shoot lengths were recorded for the variety 'Solaris' depending on the rootstock (Table 1). The longest shoots were recorded for the grapevine grafted on the 'SO4' rootstock, while the shortest ones were recorded for the grapevine grafted on the '125AA' rootstock. No statistical differences in the length of the shoots were recorded in 2016 for the varieties 'Seyval Blanc' and 'Johanniter' (Tables 1, 2), and in

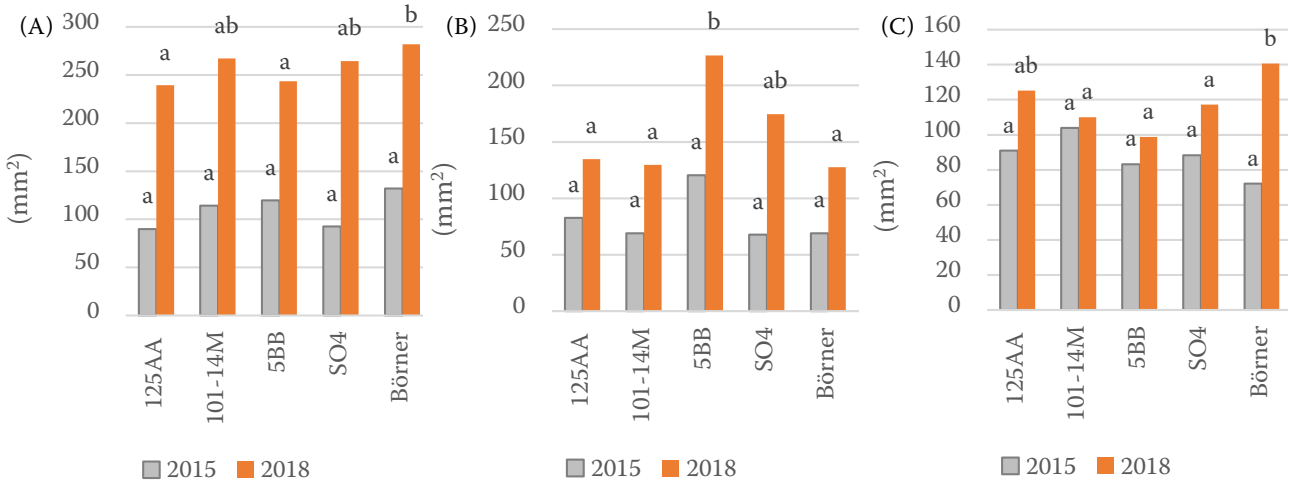

Figure 3. Increment in the trunk base cross-sectional area (mm^2) in the two seasons, 2015 and 2018 for the varieties: (A) 'Solaris', (B) 'Seyval Blanc', (C) 'Johanniter'

All the statistical calculations were performed for each year and for each variety separately. [a,b]Mean values followed by different letters are significantly different ($P < 0.05$)

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Table 1. Biometric characteristics of the vine and quality parameters of the 'Solaris' must depending on the rootstock

Rootstock	Biometric assessment								Quality parameters of the juice							
	Shoot length (cm)		Number of inflorescences (pcs /vine)		Yield (kg/vine)		Average cluster weight (g)		pH		TSS (°B)		TA (g/L)		TSS/TA	
	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018
125AA	106.9^{b}	110.0^{a}	17.0^{a}	24.7^{a}	0.1^{a}	2.8^{a}	108.0^{c}	122.4^{ab}	3.41^{b}	3.16^{b}	22.2^{c}	21.8^{bc}	7.5^{ab}	7.6^{a}	2.96^{c}	2.86^{cd}
R101-14M	128.1^{ab}	126.0^{a}	15.7^{a}	25.5^{a}	0.3^{a}	2.7^{a}	305.0^{a}	122.2^{ab}	3.49^{a}	3.20^{ab}	24.0^{ab}	22.0^{bc}	7.5^{ab}	7.5^{a}	3.20^{b}	2.93^{c}
5BB	137.6^{ab}	138.0^{a}	16.7^{a}	22.9^{a}	0.4^{a}	2.2^{a}	196.0^{b}	114.6^{b}	3.43^{a}	3.21^{ab}	23.8^{bc}	22.8^{ab}	7.9^{a}	7.4^{a}	3.01^{c}	3.08^{b}
SO4	139.0^{a}	136.0^{a}	14.0^{a}	19.0^{a}	0.3^{a}	2.0^{a}	347.0^{a}	119.0^{ab}	3.49^{a}	3.22^{ab}	24.1^{ab}	21.4^{c}	6.6^{b}	7.7^{a}	3.65^{a}	2.78^{d}
Börner	127.6^{ab}	106.0^{a}	17.9^{a}	21.2^{a}	0.1^{a}	2.4^{a}	91.0^{c}	127.9^{a}	3.42^{b}	3.25^{a}	25.2^{a}	23.3^{a}	8.1^{a}	6.8^{a}	3.11^{b}	3.43^{a}

$^{a–d}$Mean values followed by different letters are significantly different ($P < 0.05$)

All the statistical calculations were performed for each year separately; TSS – total soluble solid; TA – titratable acidity

2018 for the variety 'Solaris'. In 2018, differences in the shoot length in relation to the rootstock were noted for the varieties 'Seyval Blanc' and 'Johanniter'. The 'Seyval Blanc' variety produced the longest shoots on the 'SO4' and 'Börner' rootstocks, while for 'Johanniter', the longest shoots were recorded for the '5BB' and 'SO4' rootstocks. Li et al. (2019) investigated eight rootstocks, including 'R101-14M', '5BB' and 'SO4', but they found no effect of the rootstocks on the grapevine Marselan shoot length.

The three grapevine varieties varied depending on the dates of flowering and the number of inflorescences (Tables 1, 2, 3). In terms of the flowering dates, 'Solaris' was the first to bloom, followed by 'Seyval Blanc' and 'Johanniter'. These findings were in agreement with other studies described in Polish climatic conditions (Myśliwiec et al. 2018). In the first year of full fruiting (2016 year), no differences in the number of inflorescences related to the rootstock for any of the varieties were recorded. In the next season (2017), the spring frost damaged 100% of the inflorescences. The first differences in the number of inflorescences were recorded in 2018. For the variety 'Seyval Blanc', a significantly greater number of inflorescences were recorded for the '5BB' and 'Börner' rootstock, while the grapevines on the 'SO4' and 'R101-14M' rootstocks showed a tendency to have a greater number of inflorescences (Table 2). For the variety 'Johanniter', the 'Börner' rootstock also showed a positive effect on the number of inflorescences, and a tendency towards having more inflorescences was noted for the '125AA' and '5BB' rootstocks (Table 3). Generally, it was found that the grapevines grafted on the '5BB' and 'Börner' rootstocks produced up to 28.3% more inflorescences per plant in comparison

Table 2. Biometric characteristics of the vine and quality parameters of the 'Seyval Blanc' must depending on the rootstock

Rootstock	Biometric assessment								Quality parameters of the juice							
	Shoot length (cm)		Number of inflore-cences (pcs /vine)		Yield (kg/vine)		Average cluster weight (g)		pH		TSS (°B)		TA (g/L)		TSS/TA	
	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018
125AA	120.9^{a}	81.0^{b}	13.5^{a}	23.1^{b}	2.9^{a}	4.9^{a}	110.0^{a}	161.4^{a}	3.26^{a}	3.18^{b}	20.1^{bc}	17.0^{b}	7.2^{a}	8.6^{a}	2.80^{a}	1.98^{c}
R101-14M	108.2^{a}	90.0^{b}	9.8^{a}	26.4^{ab}	3.1^{a}	4.8^{a}	162.0^{a}	168.2^{a}	3.29^{a}	3.20^{ab}	19.8^{c}	19.1^{a}	6.7^{a}	7.4^{b}	2.95^{ab}	2.58^{a}
5BB	109.0^{a}	91.0^{b}	10.1^{a}	31.7^{a}	2.8^{a}	5.4^{a}	74.0^{b}	164.6^{a}	3.29^{a}	3.23^{a}	20.8^{a}	19.5^{a}	6.6^{a}	7.5^{b}	3.15^{a}	2.60^{a}
SO4	113.6^{a}	110.0^{a}	10.2^{a}	24.1^{ab}	3.1^{a}	4.7^{a}	73.0^{b}	169.3^{a}	3.28^{a}	3.24^{a}	20.7^{ab}	19.2^{a}	7.0^{a}	7.6^{b}	2.95^{ab}	2.52^{a}
Börner	113.9^{a}	118.0^{a}	12.3^{a}	32.0^{a}	3.8^{a}	5.3^{a}	151.0^{a}	161.0^{a}	3.24^{a}	3.17^{b}	20.2^{ab}	17.7^{b}	8.0^{a}	7.9^{a}	2.52^{b}	2.24^{b}

$^{a–c}$Mean values followed by different letters are significantly different ($P < 0.05$)

All the statistical calculations were performed for each year separately; TSS – total soluble solid; TA – titratable acidity

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Table 3. Biometric characteristics of the vine and quality parameters of the 'Johanniter' must depending on the rootstock

Rootstock	Biometric assessment								Quality parameters of the juice							
	Shoot length (cm)		Number of inflorescences (pcs /vine)		Yield (kg/vine)		Average cluster weight (g)		pH		TSS (°B)		TA (g/L)		TSS/TA	
	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018	2016	2018
125AA	98.7^{a}	90.0^{ab}	15.7^{a}	29.6^{ab}	0.6^{a}	3.2^{a}	232.0^{a}	205.5^{ab}	3.26^{a}	3.32^{a}	18.8^{ab}	19.5^{ab}	7.1^{ab}	9.3^{a}	2.64^{a}	2.10^{c}
R101-14M	99.3^{a}	92.0^{ab}	15.0^{a}	24.8^{b}	0.6^{a}	3.9^{a}	231.0^{a}	214.6^{ab}	3.31^{a}	3.31^{a}	20.0^{a}	19.6^{ab}	7.7^{ab}	7.1^{b}	2.58^{a}	2.62^{a}
5BB	101.9^{a}	101.0^{a}	15.7^{a}	31.5^{ab}	0.4^{a}	3.7^{a}	242.0^{a}	201.9^{b}	3.31^{a}	3.30^{a}	19.8^{a}	18.9^{b}	7.6^{a}	7.2^{b}	2.61^{a}	2.64^{a}
SO4	110.3^{a}	101.0^{a}	17.0^{a}	25.4^{b}	0.4^{a}	3.6^{a}	254.0^{a}	222.6^{a}	3.33^{a}	3.30^{a}	17.8^{b}	19.5^{ab}	7.6^{ab}	7.6^{b}	2.34^{b}	2.56^{a}
Börner	107.7^{a}	80.0^{b}	12.8^{a}	34.6^{a}	1.1^{a}	3.9^{a}	331.0^{b}	199.9^{b}	3.28^{a}	3.33^{a}	17.8^{b}	20.1^{a}	6.7^{b}	9.0^{a}	2.65^{a}	2.24^{b}

^{a,b}Mean values followed by different letters are significantly different ($P < 0.05$)

All the statistical calculations were performed for each year separately; TSS – total soluble solid; TA – titratable acidity

to the other rootstocks. The tendency of having a different number of inflorescences on the shoots depending on the rootstock was also observed in other species, e.g., the kiwi (Wang et al. 1994), apple trees (Tworkoski, Miller 2007), pear trees (Almeida et al. 2020), peach trees (Bussi et al. 1995) and sweet cherry trees (Long et al. 2019; Dziedzic et al. 2019). Keller et al. (2001), studying the effect of the rootstock on the number of flowers in inflorescences, found that the 'SO4' rootstock stimulated a greater number of flowers in the inflorescence in the variety Müller-Thurgau. Overall, there are very few studies on the effect of the rootstock on the number of inflorescences in grapevines.

The yield of the grapevine fruit was obtained in 2016 and 2018 (Tables 1, 2, 3). In both years of the experiment, no significant differences in the yield related to the applied rootstock were observed. The low yield of the varieties 'Solaris' and 'Johanniter' in 2016 was associated with the spring frost damage to the flowers (Tables 1, 3). No significant differences in the yield between the rootstocks were found in 2018. Stevens et al. (2008), Wooldridge et al. (2010) and Wang et al. (2019) reached similar findings and showed no rootstock effects on the grapevine yield. Pulko et al. (2012) also used three rootstocks ('5BB', 'Börner' and 'SO4') in their experiment with the variety 'Sauvignon Blanc'. The authors noted an increase yield on the 'Börner' rootstock (13% to 35%) in comparison to the other rootstocks analysed. Wooldridge et al. (2010) and Bou Nader et al. (2019) found that the yield was dependent on a clear interaction between the noble variety, rootstock and soil type. Lovisolo et al. (2016) and Romero et al. (2018) demonstrated that rootstocks had an effect on the water uptake from the soil, and thus on the vigour, productivity and berry quality. However, other studies reported no significant effect of the rootstocks on the grapevine yield, probably due to differences in the experimental and edaphoclimatic conditions (Kidman et al. 2014; Zhang et al. 2016). The results of the experiment have suggested that further studies on the effect of the rootstocks on the yield are necessary.

The weight of a single fruit cluster was assessed to determine the yield quality (Tables 1, 2, 3). In 2016, for the variety 'Solaris', the greatest impact on the cluster weight was obtained on the 'SO4' and 'R101-14M' rootstocks. The smallest clusters were observed on the 'Börner' rootstock (Table 1). For the variety 'Seyval Blanc', the largest clusters were recorded on the '125AA', 'R101-14M' and 'Börner' rootstocks (Table 2). No significant differences in the cluster weight were noted for the variety 'Johanniter' (Table 3). In 2018, rootstock did not significantly affect average cluster weight. The rootstocks showed a different effect on the average cluster weight depending on the year. The difference in the average cluster weight for all cultivars in 2016 was very large and ranged from 73 g ('Seyval Blanc') to 347 g ('Solaris'), while more uniform results were only observed in 2018. The 'Solaris' cultivar with the Börner rootstock had a higher average cluster weight compared to the other rootstocks, similarly for the 'Johanniter' cultivar with the 'SO4' rootstock. In the cultivar 'Seyval Blanc' there were no significant differences in the mean cluster weight. The study of Romero et al. (2018) demonstrated a very high variation in the average cluster weight depending on the applied rootstock.

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In the presented experiment, the cluster weights were divided into five weight classes for each combination. Figure 4 shows the percentage of each class in the yield. Many authors have reported that the average cluster weight of *V. vinifera* varieties varied between 100 and 200 g (Gatti et al. 2012; Bogicevic et al. 2015; Sabir et al. 2020). In the discussed experiment, no statistical differences in the average cluster weight were found between the rootstocks, but a regularity in the cluster weight distribution was visible for each cultivar. For the variety 'Solaris', the cluster weight most often ranged from 60 to 200 g (Figure 4A). For the variety 'Johanniter', the clusters weighing from 100 to 200 g represented the highest percentage (Figure 4B). For the variety 'Seyval Blanc', the cluster weight most often ranged from 200 g to 300 g (Figure 4C). This last variety was very heterogeneous with regard to the cluster weight, with a large proportion of small clusters below 60 g, as well as five other weight categories.

Chemical measurements. The total soluble solid (TSS), organic titratable acid (TA) contents and the pH value of the grape must are the most important indicators of the grapevine fruit maturity and harvest date. The values depend primarily on two variables: the climatic conditions between the flowering and harvest and also on the fruit to leaf ratio of the plants (Duchêne 2016; Lampíř, Žaloudek 2018).

To assess the effect of the variety and rootstock on the inner characteristics of the grape must, the grape must quality parameters were assessed (Tables 1, 2, 3). The quantitative measure of the acidity of grape must is the pH level, known as an important factor affecting the must and wine stability (Mpelasoka et al. 2003). Zhang et al. (2015). In the discussed work, the authors noted a comparable pH range of the must for the same variety. For the variety 'Solaris', the pH value ranged from 3.09 to 3.43 (Table 1). The lowest pH parameters were recorded for the '125AA' rootstock in both years and for the 'Börner' rootstock in the first year of the experiment. Pulko et al. (2012) observed the highest pH on the 'Börner' rootstock. In 2018, higher pH was recorded for 'Seyval Blanc' cultivar grafted on '5BB' and 'SO4' rootstocks compared to other rootstocks. Significant differences, depending on the rootstock, were not observed for the cultivar Johannite (Table 3).

In a region with relatively cool conditions for viticulture, such as Poland, the TSS during harvesting is the main parameter determining the later wine quality, conditioning the lack of additional treatments before fermentation (Zhang et al. 2015). The TSS depends on the variety and climatic conditions occurring in the period between the flowering and fruit harvest (Duchêne 2016). In the present experiment, a high difference in the TSS values was observed for three cultivars on five rootstocks (Tables 1, 2, 3). No recurring relationship was observed for the effect of the rootstock on the TSS; however, the TSS for the varieties 'Solaris' and 'Seyval Blanc' was the highest for the 'SO4' and 'Börner' rootstocks in 2016. For the variety 'Seyval Blanc', the '5BB' rootstock was also present in the same significance

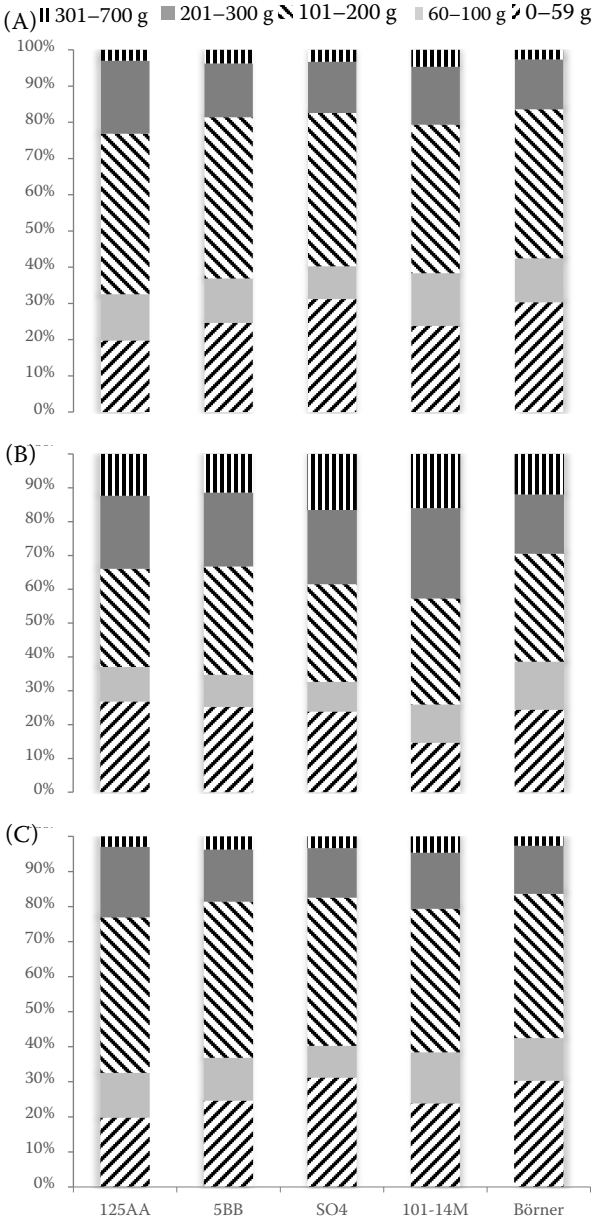

Figure 4. Yield share in the five cluster weight categories: (A) 'Solaris', (B) 'Seyval Blanc', (C) 'Johanniter'

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group. In 2018, these relationships were not recorded again, but the '5BB' and 'Börner' rootstocks contributed to the highest TSS for the variety 'Solaris'. Liu et al. (2015) determined a level of 21.1 °B for the variety 'Solaris' as being adequate for a "cool region", in our study we obtained significantly higher TSS for this variety in both study years, a mean 23.9 °B in 2016 and 22.3 °B in 2018. These results were similar to the values published in the work of Samoticha et al. (2017). Pulko et al. (2012) analysed the effect of the 'Börner' and '5BB' rootstocks on the variety Sauvignon Blanc, and similar our experiment, recorded an increase in the TSS levels on these rootstocks. When analysing the impact of the rootstocks, it can be concluded that the 'Börner' and '5BB' rootstocks increased the TSS on average by 11.7% in 2016 and by 8% in 2018 compared to the other rootstocks. In a study carried out in similar climatic conditions, Izajasz-Parchańska et al. (2014) observed a significantly higher TSS (22 °B) for the cultivar 'Seyval Blanc' than in our experiment, where we obtained similar results to Slegers et al. (2017) (19.7 °B). Jin et al. (2016) analysed the effect of the '101-14M', '5BB' and'SO4' rootstocks on the variety 'Summer Black' and found that the '101-14M' rootstock accumulated a higher TSS content compared to the other two, while we did not observe a similar relationship in our work.

The TA in grapes, similar to the pH, affects the microbiological stability of wine. It is assumed that hybrid cultivars have a higher TA content than *V. vinifera* varieties (Riesterer-Loper et al. 2019; Schrader et al. 2020). The must acidity is an important parameter and determines the later taste of wine (Cioch, Tuszyński 2014). In the discussed experiment, the highest TA content for the variety 'Solaris' was recorded in 2016 on the '5BB' and 'Börner' rootstock, while the lowest was recorded on the 'SO4' rootstock (Table 1). For the variety 'Seyval Blanc', no statistical differences were found in 2016, while in 2018, the '125AA' and 'Börner' rootstocks contributed to an increased TA content (Table 2). A similar relationship was observed for the cultivar 'Johanniter' in 2018 (Table 3). The same variety in 2016, on the '5BB' rootstock, had a statistically higher TA level than the other combinations. Li et al. (2019), for the 'Marselan' variety, recorded the highest acidity on the 'SO4' rootstock and the lowest on the '101-14M' rootstock. In study conducted by Izajasz-Parchańska et al. (2014), the TA for the 'Solaris' variety was 6.5 g/L, while Samoticha et al. (2017) reported a significantly higher TA – at 9.3 g/L. The study of Greyling (2019) on the variety 'Seyval Blanc' showed differences in the TA content in relation to the amount of sunlight in the vineyard. The values obtained by Greyling (2019) and Izajasz-Parchańska (2014) were similar to the values obtained by us in the present study. The study of Slegers at al. (2017) recorded a higher TA (9.7 g/L) for the same cultivar.

The TSS to TA ratio is defined as the grape maturity coefficient. A high or low index may indicate an incorrect harvest date or a disturbance in the veraison stage caused by inappropriate weather conditions (Ju et al. 2016; Shahab et al. 2020). A TSS/TA ratio in a range of 2.1–3.3 is regarded as optimal for white grapes (Yair 2004). During two seasons of the experiment, significant differences were recorded for the TSS/TA ratio between the rootstocks. 'Solaris', as the earliest maturing variety, was in the upper limit of this range, sometimes even exceeding it (Table 1). In 2016, the highest ratio was recorded for the 'SO4' rootstock, i.e., 3.65, which was associated with a low TA level in relation to a high TSS. The phenomenon of an increase in the sugar concentration and a decrease in the acidity associated with a higher pH during grape maturation has been described by many researchers (Ollat et al. 2002; Keller 2010; Téthal et al. 2015). In 2018, the relationship occurred again for the 'Börner' rootstock. In the variety 'Seyval Blanc' grown on the '125AA' rootstock, a low TSS and high TA resulted in a decrease in the coefficient to 1.98 in 2018 only, which was below the range defined as optimal (Table 2). For the remaining rootstocks, the coefficient was within the optimal range, as for the variety 'Johanniter' in both study years (Table 3).

CONCLUSION

A significant factor when selecting varieties for new plantings is the time required by a given variety to produce a crop with good physicochemical parameters. The conducted experiment showed that the rootstocks did not determine the yield size, but significantly improved its quality.

In Polish climatic conditions, we found that the rootstock can significantly change the physicochemical parameters, i.e., TSS content and acidity. This was particularly evident with the 'Börner' rootstock, and '5BB' to a lesser extent, for the 'So-

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laris' strain. The rootstocks 'R101-14M', '5BB' and 'SO4' positively affected the TSS and TA of the 'Seyval Blanc' and 'Johanniter'cultivars.

Further research on the effect of the rootstocks on the physicochemical characteristics of a variety will allow the creation of a physicochemical profile of cultivated hybrid varieties.

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Article

Flowering Biology of Selected Hybrid Grape Cultivars under Temperate Climate Conditions

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Abstract: Climate change is being felt in all vineyards around the world, opening up new perspectives for regions with a growing winemaking industry. In this study, 11 hybrid grapevines grown in cold climates were assessed in terms of flowering biology and pollination efficiency. The flowers were evaluated for the number of anthers and pollen grains in the flower; pollen viability and pollen grain size, the number of ovules in the ovary, and, consequently, the size and the weight of berries and the number of seeds in the berries were also analyzed. The flowers of *Vitis vinifera* L. usually have 5 stamens and 5 petals in their structure; this number for hybrid varieties ranged from 4 to 7, and in the case of the variety 'Seyval Blanc', it was 4 to 11 stamen and petals. Pollen grain size varied and ranged from 17.01 to 22.25 µm, while pollen grain pro-duction in flowers ranged from 5073 to 34,976 grain, which was calculated using a Bürker hemocytometer. The number of ovules in the ovary for the cultivars in question was highly variable, ranging from 3 to 7. One of the most important factors affecting flower pollination is stigma receptivity. Stigma receptivity appeared when the cap starts to fall off and disappeared at the browning of the cap. In connection with climatic changes, grapevine production is expanding to cool-climate countries. The aim of this study was to expand our knowledge about the flower morphology of 11 hybrid grapevine varieties most commonly cultivated in Poland. Knowledge of the flowering process can be important for improving yield and its quality.

Keywords: number grains; pollen viability; pollen size; ovule; pistils; stigma receptivity; seeds; grape cultivars; fruit quality

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1. Introduction

The grapevine (*Vitis vinifera* L.) *Vitaceae* is the most widely cultivated and one of the most economically important fruit crops in the world. In Poland, it was not until the 1980s that the suitability of various grape cultivars for large-scale cultivation in colder climatic conditions began to be tested [1]. Currently, hybrid grape cultivars are mostly grown in Polish vineyards. Interspecific hybrid cultivars selected from the crosses of *V. vinifera* with species such as *V. rupestris*, *V. riparia*, *V. labrusca*, *V. berlandieri*, *V. lincencumii*, or *V. amurensis* are a small fraction of the global grapevine production, but are nonetheless very important locally. Increasing temperatures in Poland and the extension of the vegetation period contribute to the increase in grapevine acreage [2].

The quality of grapes depends on many factors including cultivar, terroir [3,4], management practices [5,6], and changing climate [7–10]. Ideal grapefruit composition, considering the sugar to acidity ratio, is obtained when grapes are produced in a temperate climate [10]. The last three decades have been warmer than any other since 1850 [11]. This forces growers to adapt their vineyards by selecting appropriate plant material and cultivation techniques.

Another method is shifting the cultivation area, which is also associated with a change in the profile of wines produced [11–14].

Acceptance of hybrid grape cultivars and their recognition by world oenologists will allow the cultivation of these grape cultivars to be improved [15]. There is a strong conservatism in viticulture in terms of recognizing only the *V. vinifera* cultivars. Hybrid cultivars possess a combination of characteristics that are superior to those of *V. vinifera* grapevines. Increasingly warmer summers are affecting the quality of white grapes in particular, due to a decrease in fruit acidity associated with malic acid degradation [16]. In colder regions, intense wines with a complex aromatic expression can be obtained using hybrid cultivars. Summer droughts, on the other hand, will favor the production of dark cultivars, for which water shortages at certain stages reduce the size of the berries and increase their polyphenol content [7,11,15,17–19]. The production of grapes with the quality parameters required by winemakers will largely depend on the environment and viticultural practices. There is a large seasonal variability in yields in a climate described as cool.

Fruit and seed production of most crops increases when cross-pollination occurs. Although self-pollination is a widespread biological process in grapevine cultivation, there are still some uncertainties regarding the consequences of cross-pollination on grapevine productivity and quality. The biology of grapevine fertilization becomes an important issue, especially when cover cropping is considered [20]. The yield and quality of grapes are markedly enhanced by cross-pollination although self-pollination ensures some level of berry set. Cross-pollination is also employed to breed a new fruitful genotype to sustain the food to feed the increasing world population in the face of climate change [21]. The flowering and pollination process itself is well understood in *Vitis vinifera*, while the cultivation of hybrid species still lacks comprehensive information on this topic. This knowledge would be of great cognitive importance. *V. vinifera* flowering and flower morphology of the vine have been extensively described by many authors [20–32]. Due to the complexity of inflorescence formation, the authors divided generative development into 22 stages designated as BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) encoded with numbers from 0 to 50 based on the phenological development of the grapevine [25,33]. These stages coincide with the sum of active temperatures of each phase, e.g., in highbush blueberry to the onset of flowering 800 degrees [34] and apple trees to harvest around 2600 degrees [35]. The flowering process is known as anthesis and lasts about a week. The rate at which flowers develop depends primarily on environmental factors such as light and temperature, but also on internal factors such as hormonal changes. Low temperatures, which often occur in cold climates, can damage flower tissues and affect ovule development and pollen tube growth. The response to cold stress varies among genotypes [30,36,37]. The flower clusters of grapevines are quite inconspicuous. They are panicles (loose, irregularly branched flower clusters) with individual flowers, or blossoms, on the end of each branch. The flower consists of 5 sepals, 5 petals, 5 stamens, and a pistil ending in a stigma. The stigma of *Vitis vinifera* is of the solid type. It consists of a layer of the epidermis, parenchymatous tissue, and central conducting tissue. The epidermis consists of a single layer of tannin-rich cells [38]. The receptivity of a stigma is related to its structure. The stigma of *Actinidia deliciosa* flowers has a similar structure to that of *Vitis vinifera*, which has a surface covered with papillae; the stigma is covered with an abundant secretion when the flower is open. Papillae are mostly unicellular and contain numerous phenolic components. During the life of the flower, these papillae gradually lose their turgor, and from the opening of the flower (anthesis), they begin to burst their contents are released into the environment where the stigmas germinate. When the flower opens, the receptivity of the stigma is high and lasts for several days depending on the weather. For *Lonicera* spp., which also has a wet nevus type, it has been observed that receptivity decreases when the papillae rupture and the nevus tissue browns [39,40]. It was observed that hand-pollination *Vitis coignetiae* carried out at 0, 2, 4, and 6 days after anthesis showed that stigmas were most receptive two days after flower opening [32]. Pollen contains many nutrients that

are used by flower pollinating organisms. Bees require 10 amino acids such as arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, and valine for proper development. Among the plant species examined so far in this respect, among the species of fruit plants there is a large variation in the percentage of these substances, and thus in the attractiveness of pollen for pollinating insects [41]. Pollen viability and quantity are determining factors for successful pollination and fruit setting. To evaluate the biological value of pollen, pollen viability, and germination capacity should be taken into consideration. The germination capacity is subject to atmospheric factors including temperature during flowering and bud differentiation [34,40]. There are reports that the application of fungicides during flowering in Pinot Noir cultivar significantly reduced pollen germination [22]. The source of pollen has a significant effect on the percentage of fruit sets and their quality. The phenomenon of metaxenia is also observed, including a more intense berry color. Pollen sources obviously affected the berry detachment and skin rupture forces. The highest values regarding beery detachment and skin rupture forces were detected in 'Michele Palieri' × 'Italia' [21]. The petals are connected to each other by epidermal cells and form a cap (calyptra), whose function is to protect the flower's generative organs from environmental fluctuations during the early stages of bud burst. The cap falls off during blossoming and the individual flowers appear, and a phenomenon called capfall occurs. The released anthers burst and release pollen [25,29]. Recent studies have confirmed that the pollination process relies heavily on self-pollination and occurs before the capfall [29,42]. However, some cultivars of *V. vinifera* need cross-pollination, as protective mechanisms against self-pollination. One of these involves covering the stigma by the calyptra and lowering the anthers relative to the stigma [26,29]. In pollination, an important aspect is pollen fertility, i.e., its viability and germination. These traits will affect yield and are essential for fruit setting [43,44].

In connection with climatic changes, grapevine production is expanding to cool-climate countries. The aim of this study was to expand our knowledge about the flower morphology of 11 hybrid grapevine cultivars most commonly cultivated in Poland. Knowledge of the flowering process can be important for improving yield and its quality.

2. Materials and Methods

2.1. Biological Material

The experiment was set up in the South of Poland (50°08′29.4″ N 19°55′50.7″ E) in a temperate climate zone in 2018–2019. The research material consisted of 12-year-old vines of the following hybrid grapevine cultivars: Aurora, Bianca, Hibernal, Jutrzenka, Leon Millot, Marechal Foch, Muscat Odesskij, Regent, Rondo, Seyval Blanc, and Solaris. Table 1 details the origin of the hybrid grape cultivars discussed.

Vines were grown at 2.5 × 1 m in 24 rows of 100 of each variety spacing as a single Guyot pruning type, with permanent turf in between rows and herbicide strips 80 cm wide under vines. The vineyard lacked installed irrigation, and the average annual precipitation is sufficient in this region for vine growing (multi-year average of about 700 mm) (Figure 1B). Diseases and insects were controlled according to commercial guidelines.

Table 1. Origin of hybrid grape cultivars.

Cultivar	Parents *	*V. Vinifera* (%)	*V. rupestris* (%)	*V. riparia* (%)	*V. labrusca* (%)	*V. berlandieri* (%)	*V. lincecumii* (%)	*V. amurensis* (%)	Flowering Date of Grape Cultivars **
Aurora	Seibel 788 × Seibel 29	68.75	18.75	12.5					medium-early
Bianca	Eger 2 × Bouvier	78.09	14.58		1.56	3.13	2.64		medium-late
Hibernal	Chancellor × Riesling	82.04	14.06	1.95	1.95				late
Jutrzenka	SV 12-375 × Pinot Blanc	78.01	14.58		1.56	3.12	2.64		late
Leon Millot	MGt101 OP (V.rip × V. rup.) × Goldriesling	50	25	25					medium-late
Marechal Foch	MGt101-14 (V.rip × V. rup.) × Goldriesling	50	25	25					medium-late
Muscat Odesskij	Muskat Sinyj Rannij × CV 12-375	78.1	14.58		1.56	3.12			medium-early
Regent	Diana (Ga 30N-8-127) × Chambourcin	80.06	14.32	0.98	1.76	1.56	1.38		medium-late
Rondo	Zarya Severa × Sait Laurent	75						25	medium-early
Seyval Blanc	s. 4995 × s.4986	43.75	28.15	12.5	12.5	3.1			late
Solaris	Merzling × Geisenheim 6493	73.4	7	3.1		3.1	0.8	12.5	medium-early

* Prepared on the basis of data from the Julius Kühn Institut—Federal Research Centre for Cultivated Plants (VIVC). ** According to observations in the vineyard.

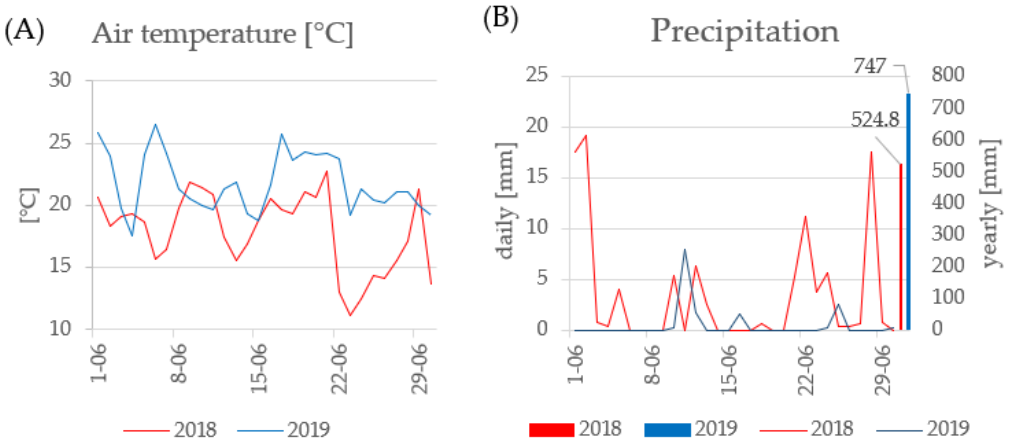

Figure 1. (**A**) Average daily temperatures recorded during the flowering period in 2018–2019, (**B**) Average daily precipitation recorded during the flowering period in 2018–2019.

Weather Data

Meteorological data from the 2018–2019 flowering period was collected from the iMetos go IMT meteorological station located in the vicinity of the vineyard.

2.2. Biometric Measurements and Flower Quality

2.2.1. The Number of Anthers

Flowers of 11 hybrid grapevine cultivars were collected in early June and analyzed for the number of anthers in the flower. A random sample of 100 fully developed flowers, from 50 plants, was taken for analysis (4 replicates of 25 each).

2.2.2. The Number of Ovules in the Ovary

In the next step, the number of ovules in the ovary was calculated. Measurements were carried out in four replicates, each replicate consisting of 25 ovaries. The ovaries were cut with a scalpel and the ovules were extracted using a micro-needle. Calculations were performed using a Carl Zeiss Discovery stereoscopic microscope.

2.2.3. Stigma Receptivity

The receptivity of the grapevine stigma was determined [45,46]. Flowers were taken from each cultivar and classified according to the following developmental stages: stage 1—closed flower (with calyptra); stage 2—flower with calyptra cut off but covered anthers; stage 3—flower without calyptra with anthers exposed; stage 4—flower with brown anthers. In the laboratory, a 3% H_2O_2 solution was applied to the stigma surface to assess receptivity and the stigma was observed under a Carl Zeiss Discovery 2.0 binocular magnifier. A receptive stigma actively releases oxygen in the form of gas bubbles, whereas a non-receptive stigma does not exhibit this ability. The percentage of receptive pistils at each flower stage was calculated (ten flowers were evaluated at each stage).

2.2.4. The Number of Pollen Grains in the Anther

The pollen yield of 11 selected cultivars was evaluated using a Bürker's hemocytometer by calculating the number of pollen grains in anthers and flowers. The sample consisted of 10 anthers randomly collected from 10 flowers of a given cultivar; the calculations were replicated four times. Observations were carried out using a Carl Zeiss Image M2 AXIO microscope at ten-fold magnification under a white light [47].

2.3. Quality and Quantity of Pollen Grains in the Flowers

2.3.1. Pollen Grains Size

Pollen grains size was measured using a light microscope. Pollen was selected from one hundred randomly selected flowers. The pollen mixture was spread onto slides, pollen size measurements were carried out in four replicates, one replicate consisted of one hundred pollen grains. The Axio vs. 40V software was used.

2.3.2. Pollen Grains Viability

Anthers were collected from 50 flowers for each cultivar and placed in Petri dishes and subsequently kept for 24 h at 25 °C for the anthers to open. The viability of pollen grains was assessed using Alexander's staining method [48], i.e., triple staining of pollen grains (malachite green, orange G, and acid fuchsin). Malachite green does not pass through the living cell membrane in viable pollen grains, and then the living pollen protoplast stains carmine, while the membrane of dead pollen is broken down and the pollen cytoplasm stains green.

According to Tello et al. [29], viability was divided into five groups determining pollen viability in percentage:

1. Very high viability > 90%;
2. High viability: 90–75%;
3. Medium viability: 75–50%;
4. Low viability: 50–25%;
5. Very low viability < 25%

2.4. Assessment of Pollination and Overgrowth of the Pollen Tube by the Pistil Neck

The number of germinating grains on the stigma and the number of pollen tubes at the stylopodium were calculated for the eleven cultivars. The experiment was performed in four replicates, each replicate consisting of twenty pistils with ovaries. The pistils were fixed in FAA (formalin:ethyl alcohol:acetic acid, 8:1:1) for 10 to 12 h. The pistils with ovules were subsequently macerated in 30% NaOH solution for two to three hours, and then the tissue was cleared with a 6% H_2O_2 solution. In the next step, after washing with water, the pistils with ovules were stained with aniline blue for three hours. In the final stage, the preparations were closed with glycerol, and observations were conducted using a Carl Zeiss Image M2 Axio fluorescence microscope [49] under ultraviolet light at a wavelength of about 356 nm. Under these conditions, callose fluoresces bright yellow-green and contrasts strongly with the bluish or grayish fluorescence of the stylar tissue. The pollen tubes are outlined by a callose lining and irregularly spaced callose plugs [50].

2.5. Evaluation of Fruit Quality

The fruit clusters were randomly selected for each cultivar and for each of the four replicates. From each cluster, four berries were collected from different levels of the cluster. The sample consisted of forty berries and four replicates. Biometric measurements of fruit such as berry weight, length, and width, number of seeds per berry, and berry spherical area were taken to assess fruit quality. The correlation of berry weight to seed number was calculated.

2.6. Statistical Analysis

The results were statistically analyzed using one-way analysis of variance. Tukey's post hoc test was used to assess the significance of differences between means, at the significance level of $\alpha = 0.05$. In addition, cluster analysis (Ward's method) was performed for biological parameters (number of pollen grains, anthers, ovules, and seeds). Statistical analysis was performed using the STATISTICA 13 software.

3. Results and Discussion

3.1. Biometric Measurements and Flower Quality

3.1.1. The Number of Anthers in Flowers of 11 Hybrid Grape Cultivars

When comparing the average number of anthers between the 11 hybrid grape cultivars and between seasons, it can be seen that the differences were small. The cultivar 'Hibernal' had the lowest number of anthers in both years of the experiment, and the cultivars 'Jutrzenka' and 'Marechal Foch' in 2019 (Figure 2A). The cultivars 'Rondo' and 'Solaris' had the highest number of anthers in the first year of the experiment, while the cultivars 'Solaris', 'Rondo', 'Aurora', 'Bianca', and 'Leon Millot' were in the group with the highest number of anthers in the second year. The average number of anthers (in absolute values) of the evaluated cultivars in both years of the experiment is comparable, only in the case of three cultivars 'Jutrzenka', 'Marechal Foch', and 'Seyval Blanc', it is slightly lower. As the number of anthers in flowers in the analyzed samples varied greatly, Figure 2B shows the percentage of flowers with 4, 5, 6, 7, 9, and 11 anthers. Most of the flowers of the discussed cultivars were characterized by a typical 5-fold flower structure (5 stamens and 5 petals), while exceptions to this rule were noted, including flowers with 4 to 11 anthers and petals, respectively (Figures 2B and 3A).

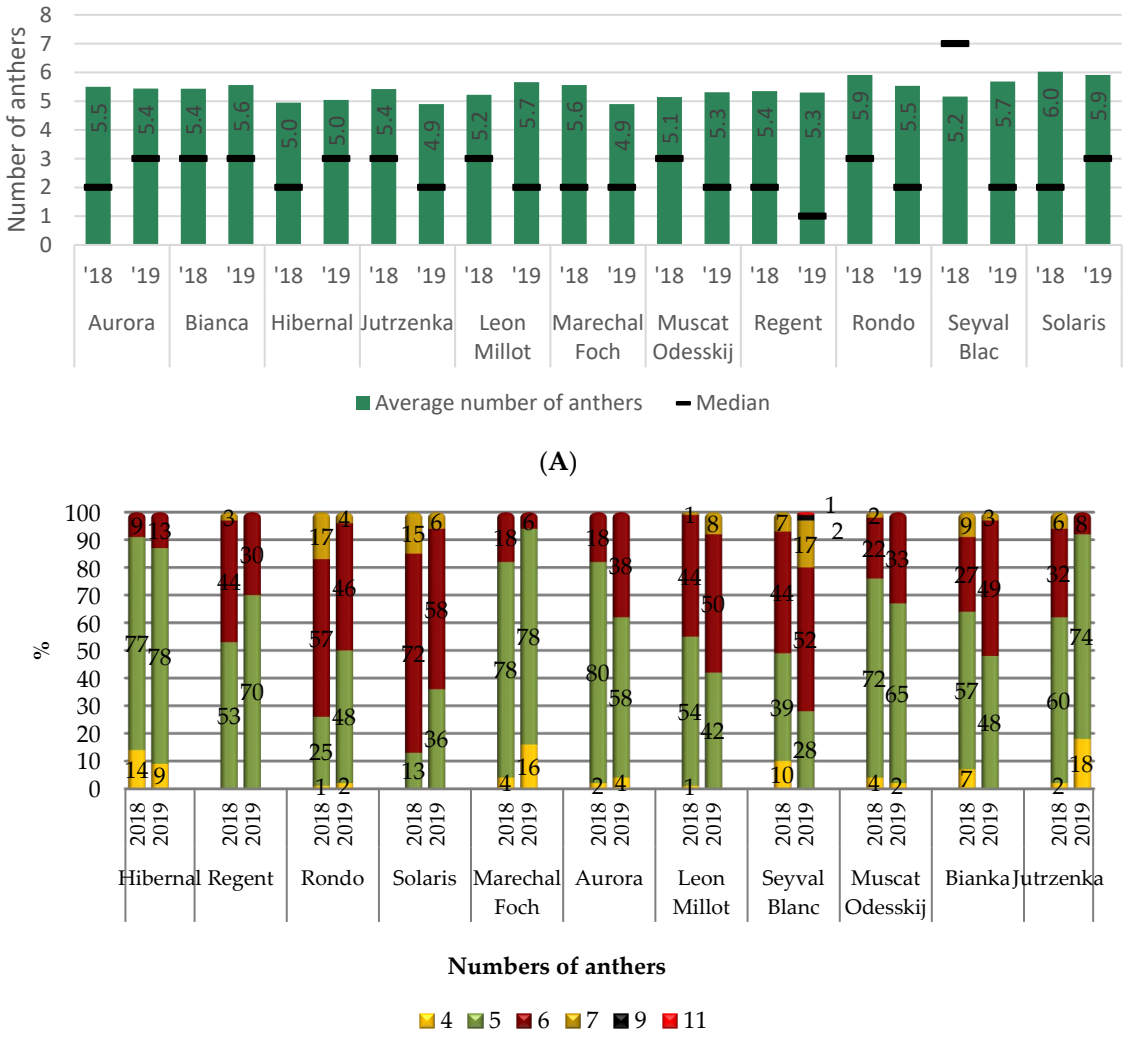

Figure 2. (**A**) Average number of anthers in flower of hybrid grapevine cultivars. (**B**) Variability in anther number in flowers of hybrid grapevine cultivars (%).

The number of anthers was always the same as the number of petals in the present study (Figure 3A) and in studies by other authors [29,51]. The group of cultivars that had the most flowers with 6 anthers included 'Solaris', 'Rondo', 'Leon Millot', and 'Seyval Blanc', recorded flowers containing 7 anthers in the same cultivars. In 'Seyval Blanc', flowers with 9 and 11 anthers were observed (Figure 3B). Kelen and Demirtas [52] analyzed 8 *Vitis vinera* cultivars grown in Turkey and also found differences in the number of anthers depending on the cultivar. The number of anthers for most of the analyzed cultivars was 5.0, only for two cultivars the average number of anthers varied between 5.6 and 6.0. Biasi and Conner [53] analyzed the flower structure of hermaphroditic muscadine cultivars (*Vitis rotundifolia* Michx.). These authors noted between 5 and 10 anthers per flower depending on the cultivar, with 6 to 8 anthers per flower being the most common. Padureanu and Patras [44] studied generative organs of two hybrid grapevines, 'Noah' and 'Otello', and reported that the number of anthers ranged from 4 to 6. Pierozzi and Moura [54] observed from 4 to 7 anthers in two mutants of the cultivar 'Niagara'.

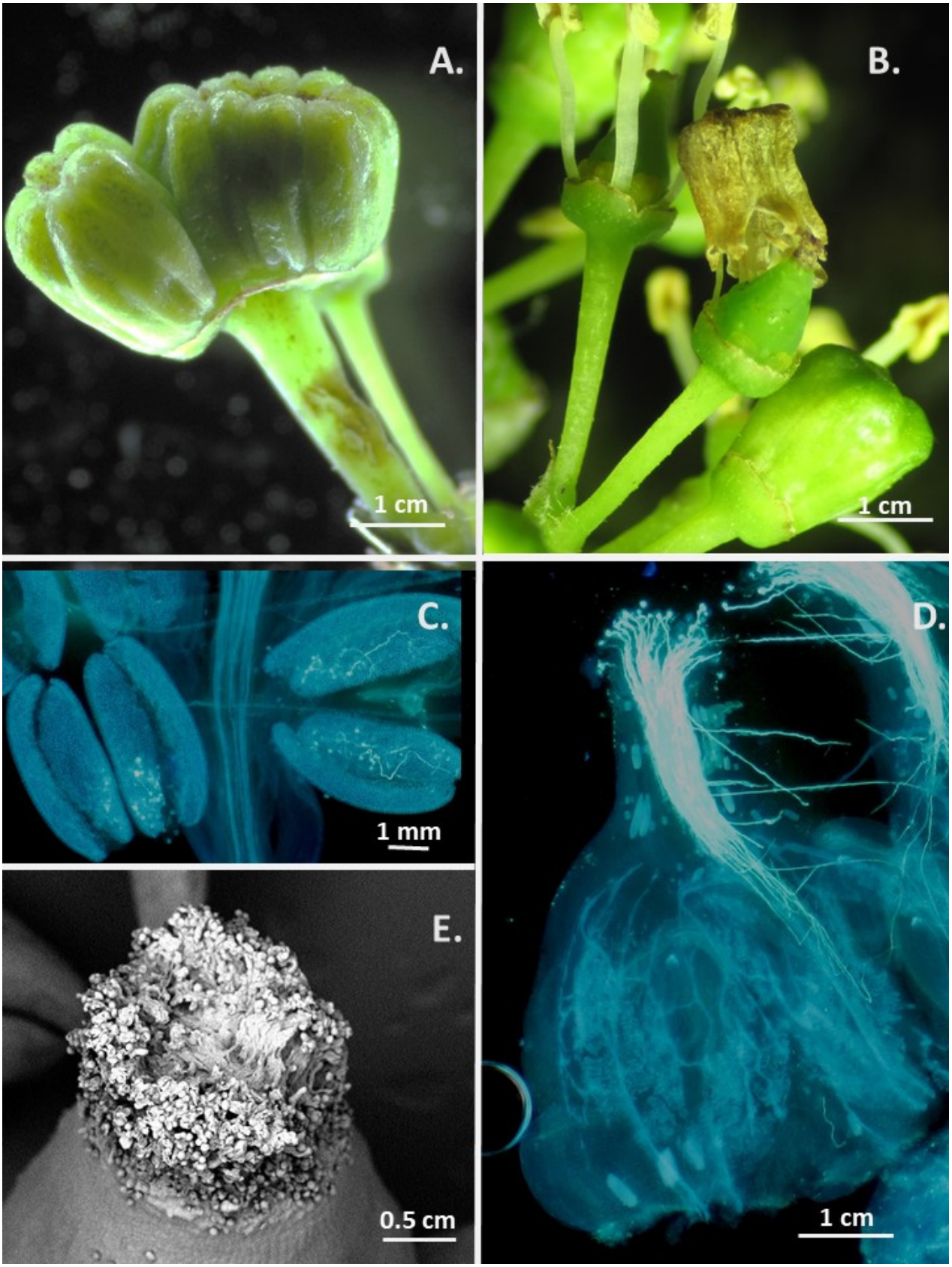

Figure 3. (**A**) Closed flower bud of a 'Seyval Blanc' cultivar with 11 anthers. (**B**) Capfall. (**C**) Pollen grains germinating inside closed anthers. (**D**) Pollen tubes growing through the pistil neck into the ovary. (**E**) Pollinated stigma of the 'Solaris' cultivar of pistil.

3.1.2. The Number of Ovules in the Ovary of 11 Grapevine Cultivars

In this study, the average number of ovules in the flowers of the discussed cultivars was calculated (Table 2). Statistically, the cultivar 'Solaris' had the highest number of seeds, and 'Jutrzenka' and 'Marechal Foch' the lowest. The ovary of the grapevine consists of two chambers, which usually contain two ovules [26]. In the present study, from 3 to 7 ovules were observed.

Table 2. Pollen viability and pollen tube outgrowth through the pistil neck.

Cultivar	Pollen Viability%		Average Number of Germinating Grains	Average Number of Pollen at the Base of the Pistil	Average Number of Ovules	
	2018	2019				Min–Max
Aurora	78.0 ^{cd}	93.3 ^{d}	40.8 ^{c}	30.1 ^{d}	4.6 ^{ab}	4–6
Bianca	89.5 ^{d}	74.0 ^{bcd}	40.9 ^{c}	24.2 ^{d}	4.6 ^{ab}	4–6
Hibernal	47.0 ^{b}	50.7 ^{abc}	0.8 ^{a}	0.1 ^{a}	4.6 ^{ab}	4–6
Jutrzenka	63.5 ^{bc}	78.0 ^{bcd}	12.1 ^{b}	2.1 ^{ab}	4.3 ^{a}	3–6
Leon Millot	59.5 ^{b}	47.7 ^{abc}	1.7 ^{a}	1.7 ^{ab}	5.2 ^{b}	3–6
Marechal Foch	53.5 ^{b}	45.7 ^{ab}	40.5 ^{c}	29.2 ^{d}	4.2 ^{a}	3–7
Muscat Odesskij	25.5 ^{a}	78.0 ^{bcd}	32.2 ^{bc}	9.1 ^{bc}	4.6 ^{ab}	3–6
Regent	60.0 ^{b}	91.7 ^{d}	19.5 ^{b}	6.7 ^{bc}	4.8 ^{ab}	3–6
Rondo	81.5 ^{d}	86.0 ^{d}	9.2 ^{b}	0.3 ^{a}	5.0 ^{ab}	4–7
Seyval Blac	49.0 ^{b}	37.0 ^{a}	17.2 ^{b}	1.3 ^{ab}	5.2 ^{b}	4–7
Solaris	84.0 ^{d}	80.3 ^{cd}	43.5 ^{c}	21.7 ^{cd}	6.1 ^{c}	4–7

The values are given as means, followed by the letters $^{a–d}$ to indicate statistical significance. The values marked with the same letters in one column are not statistically different at $\alpha < 0.05$.

Cluster analysis (Figure 4) distinguished two groups of the studied cultivars that characterized quantitatively the generative organs. The analysis was based on the number of pollen grains in the flower, the number of ovules in the ovary, and the number of seeds in the berry. The group containing 'Rondo' and 'Solaris' cultivars, which were characterized by a high number of ovules, pollen grains per flower, and seeds, was clearly separated. The second group consisted of more combinations and two subgroups could be distinguished within it. The first subgroup included 'Hibernal', 'Jutrzenka', 'Bianca', 'Seyval Blanc', and 'Leon Millot' cultivars, and the second subgroup 'Muscat Odesskij', 'Regent', 'Aurora', and 'Marechal Foch' cultivars. In these groups, we observed similarity in terms of the average number of pollen grains in flowers, ovules, and seeds.

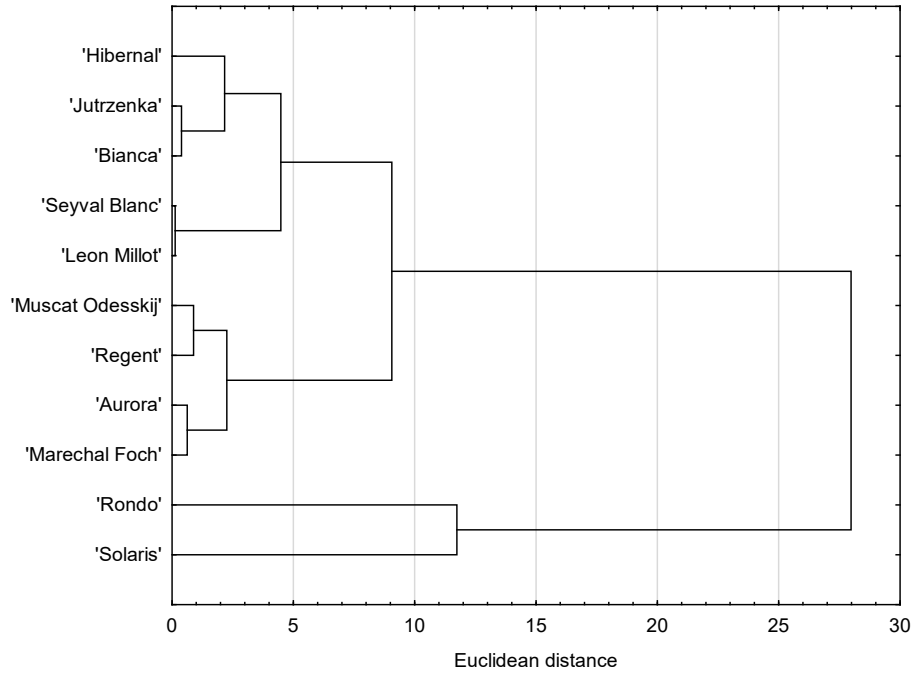

Figure 4. Cluster analysis characterizing the generative organs of the discussed cultivars in terms of the quantity of 11 hybrid grapevine cultivars.

3.1.3. Stigma Receptivity

Stigma receptivity, defined as the ability of the flower to accept pollen, changes during its life. Four stages (Figure 5) of flower development were distinguished for grapevine flowers. Table 3 shows that stigma receptivity varied depending on the stage of flower development. The receptivity of flower stigmas in the analyzed grapevine cultivars appears with the capfall (Figure 3B) and disappears when the stigma turned brown (Table 3, Figure 3E). Baby et al. [55] also found no significant differences in stigma receptivity between the three studied cultivars, i.e., 'Cabernet Sauvignon', 'Merlot', and 'Shiraz'.

Table 3. Mean percent flower pistil receptivity of 11 grapevine cultivars in relation to phenological phases.

Cultivar	Immediately after Capfall	Full Flowering	Dried Anthers	Stigma Discoloured to Brown
Aurora	83.5 ± 0.67	96.2 ± 0.12	35.4 ± 0.12	9.1 ± 0.06
Bianca	84.1 ± 0.06	96.2 ± 0.06	35.9 ± 0.15	8.6 ± 0.09
Hibernal	82.8 ± 0.12	96.4 ± 0.19	38.3 ± 0.09	8.8 ± 0.07
Jutrzenka	82.7 ± 0.12	97.0 ± 0.09	36.5 ± 0.25	8.5 ± 0.15
Leon Millot	82.8 ± 3.94	97.1 ± 0.20	35.4 ± 1.82	8.7 ± 0.17
Marechal Foch	82.0 ± 0.06	96.3 ± 0.27	36.9 ± 0.06	8.5 ± 0.18
Muscat Odesskij	84.5 ± 0.12	97.2 ± 0.49	35.9 ± 0.29	8.6 ± 0.34
Regent	82.4 ± 0.12	97.1 ± 0.06	36.9 ± 0.42	8.4 ± 0.12
Rondo	83.4 ± 0.12	96.7 ± 0.06	37.8 ± 0.09	8.8 ± 0.20
Seyval Blanc	83.4 ± 0.12	96.9 ± 0.12	36.1 ± 0.18	8.7 ± 0.12
Solaris	83.8 ± 4.30	96.5 ± 0.25	35.7 ± 0.21	9.0 ± 0.03

The values are given as means ± standard deviations. Not statistically different at $\alpha < 0.05$.

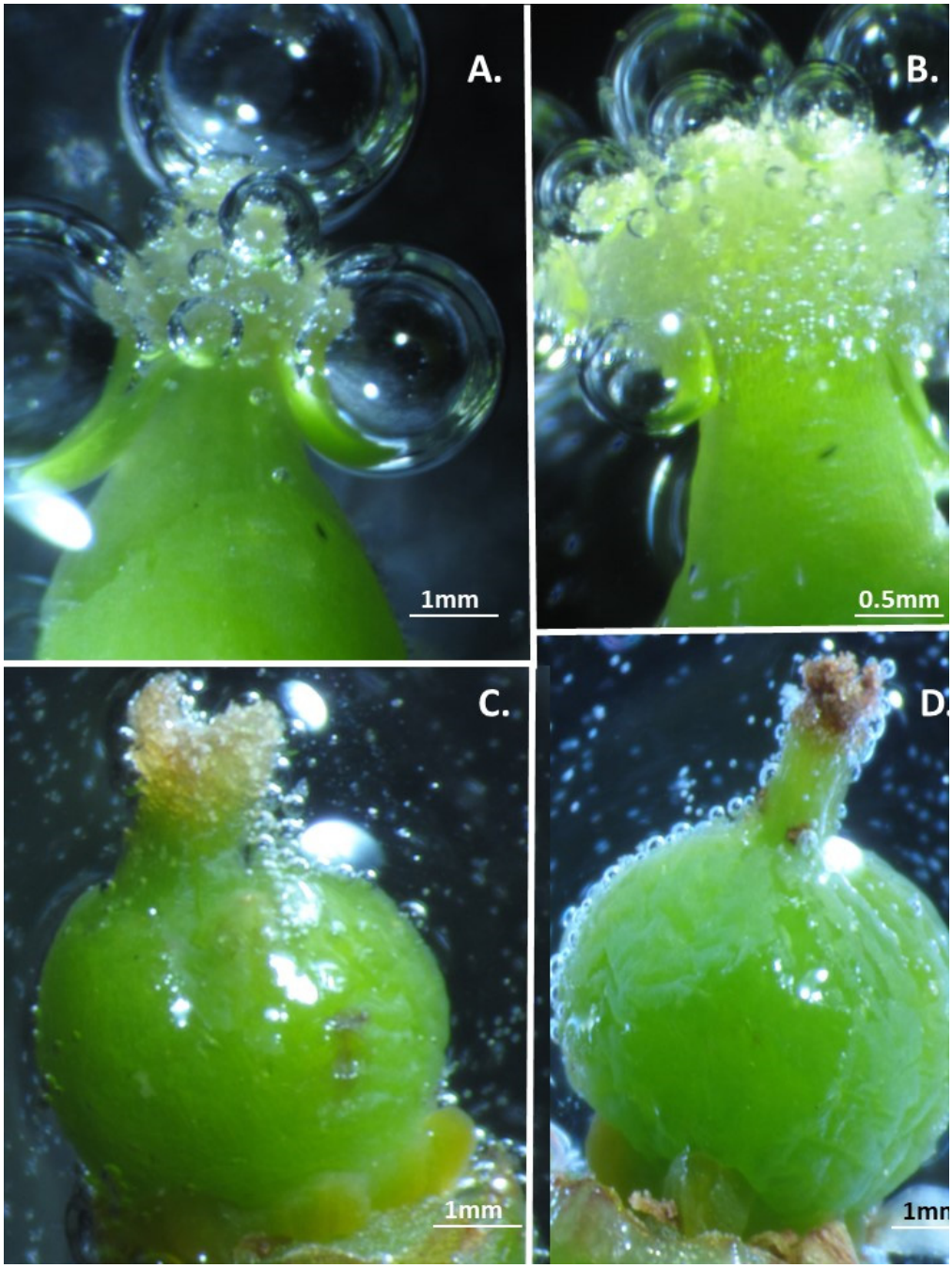

Figure 5. Stigma receptivity. (**A**)—Immediately after capfall, (**B**)—Full flowering, (**C**)—Dried anthers, (**D**)—Stigma discolored to brown.

3.1.4. The Number of Pollen in the Anther

The number of pollens in the anther varies greatly depending on the species and cultivar as well as weather conditions during microsporogenesis [40,47,49]. The group of cultivars with the lowest number of pollen grains in the anther in the first year of the experiment included 'Hibernal', 'Seyval Blanc', 'Leon Millot', and 'Regent' cultivars (1162.5–1981.25), while the lowest number of pollen grains in the second study year was observed for 'Hibernal', 'Dawn', and 'Bianca' cultivars (912.34–1657.81) (Table 4). The cultivar 'Solaris' in both years of the experiment was characterized by the highest number of pollen grains per anther and flower (Table 4). A high number of pollen grains in the anther and in the flower in the first year of the experiment was characterized by the cultivar

'Rondo', while in the second year 'Rondo' and 'Regent'. Kelen and Demirtas [34] recorded from 2906 to 9000 pollen grains per flower. Comparing the results obtained by the authors of the present study with the results of Kelen and Demirtas [52], it could be seen that the values were significantly higher. The number of pollen grains per anther in muscadin cultivars in the study of Biasi and Conner [53] ranged from 2539 to 5776.8, while per flower it was from 16,380 to 39,860. These values were more similar to the authors' results.

Table 4. Biometric measurements of flowers of 11 hybrid grape cultivars.

Cultivar	Average Pollen Size Diameter [µm]		Average Number of Grains per Anther		Average Number of Grains per Flower	
	2018	2019	2018	2019	2018	2019
Aurora	21.3 ^{ef}	21.3 ^{ef}	2618.7 ^{de}	2631.2 ^{d}	14403.1 ^{e}	14314.0 ^{cd}
Bianca	22.2 ^{f}	22.2 ^{f}	2368.7 ^{cde}	912.3 ^{a}	12909.7 ^{cde}	5072.6 ^{a}
Hibernal	19.4 ^{cd}	19.6 ^{cd}	1162.5 ^{a}	1657.8 ^{c}	5754.4 ^{a}	8355.4 ^{a}
Jutrzenka	17.6 ^{ab}	17.8 ^{ab}	2212.5 ^{cd}	1281.2 ^{b}	11991.7 ^{cde}	6278.1 ^{a}
Leon Millot	21.6 ^{f}	21.8 ^{f}	1862.5 ^{bc}	2300.6 ^{d}	9722.2 ^{bc}	13021.5 ^{c}
Marechal Foch	19.5 ^{cd}	19.5 ^{cd}	2337.5 ^{cde}	3025.0 ^{e}	12996.5 ^{de}	14852.7 ^{cd}
Muscat Odesskij	20.5 ^{e}	20.5 ^{de}	2863.5 ^{e}	3296.9 ^{e}	14713.2 ^{e}	17506.4 ^{e}
Regent	19.0 ^{c}	19.2 ^{c}	1981.2 ^{bc}	3914.1 ^{f}	10599.7 ^{bcd}	20744.5 ^{g}
Rondo	18.8 ^{bc}	19.0 ^{c}	4020.3 ^{f}	4153.1 ^{f}	23760.0 ^{f}	22925.2 ^{g}
Seyval Blac	17.0 ^{a}	17.0 ^{a}	1537.5 ^{ab}	2579.7 ^{d}	7675.5 ^{ab}	15271.7 ^{d}
Solaris	18.5 ^{c}	18.6 ^{bc}	5801.6 ^{g}	6157.8 ^{g}	34925.4 ^{g}	34976.4 ^{f}

The values are given as means, followed by the letters $^{a–g}$ to indicate statistical significance. The values marked with the same letters in one column are not statistically different at $\alpha < 0.05$.

3.2. Quality and Quantity of Pollen Grains in the Flowers

3.2.1. Pollen Grains Size

The group of cultivars with pollen size above 20 µm included 'Aurora', 'Bianca', 'Leon Millot', and Muskat Odesskij cultivars. The smallest pollen grains were recorded for the cultivars 'Jutrzenka', 'Seyval Blanc', and 'Solaris', the other cultivars did not differ statistically (Table 4). Vasconcelos et al. [29] reported that grapevine pollen dimensions ranged from 25 to 30 µm in length and 12 to 15 µm in width. The same authors indicated that pollen fertility was determined by its shape, fertile pollen was barrel-shaped, while non-fertile pollen had an oblong shape. Similar observations were made by Baby et al. [55], who described spherical pollen as hydrated, capable of fertilization, while oblong pollen was considered dehydrated, incapable of fertilization. Pierozzi and Moura [54] recorded pollen with mean sizes of 20.67 and 26.43 µm for two mutants of the dessert cultivar 'Niagara'. İşçi [56,57] analyzed pollen of many *V. vinifera* cultivars and its size varied greatly between them (16.26–29.91 µm) [56]. The pollen of *V. vinifera* cultivars described by the author was much smaller compared to pollen sizes in the current experiment.

3.2.2. Pollen Viability

Effective pollination and generation of well-formed seeds largely depend on pollen quality [52,55,58]. In the current experiment, the cultivar 'Muscat Odesskij' was characterized by low pollen viability in 2018, while 'Bianka', 'Aurora', 'Rondo', and 'Solaris' cultivars were included in the group of high pollen viability (Table 3). In 2019, pollen viability of the study cultivars was altered. In that season, the cultivar 'Seyval Blanc' belonged to the group with low pollen viability, while 'Regent' and 'Aurora' cultivars were included in to the group with very high pollen viability. Many authors have reported differences in pollen viability in *Vitis vinifera* [52,55,57,59,60]. Baby et al. [55] analyzed pollen viability in

three *Vitis vinifera* cultivars: 'Merlot', 'Cabernet Sauvignon', and 'Shiraz'. They showed a high proportion of low viability pollen in 'Merlot' and 'Cabernet Sauvignon' cultivars. Defouquette et al. [61] found higher pollen viability in muscadine and hermaphroditic cultivars (*Vitis rotundifolia*) compared to the present study, ranging from 69.8% to 99.8%, which was similar to the results of Biasi and Conner [53].

3.3. Assessment of Pollination and Overgrowth of the Pollen Tube by the Pistil Neck

In the current experiment, the number of germinating pollen grains on the stigma and the number of pollen tubes at the base of the pistil were calculated (Table 2, Figure 3E). Statistically, the highest number was observed for 'Solaris', 'Marechal Foch', 'Aurora', 'Bianca', and 'Muscat Odesskij' cultivars. The lowest number of pollen grains germinating on the stigma was recorded for 'Hibernal' and 'Leon Millot' cultivars. Ebadi et al. [62] also studied the growth of the pollen tube in the cultivars 'Chardonay' and 'Shiraz'. These authors observed that low temperature during flowering weakened pollen germination in the tested cultivars and was a cultivar-specific feature expressed by quantitative differences in the disturbance of pollen functions. In the present study, flowering in 2018 began on June 8, and in 2019 on June 10. It lasted, depending on the cultivar, from 7 to 14 days. In 2018, the lowest average daily temperature was recorded on June 17 (18.7 °C), while in 2019, on June 13 (15.56 °C) (Figure 1A). The rainfall in both years was at a similar level and did not cause any changes in flowering (Figure 1B). According to some authors [29,63], temperatures below 15 °C and rainfall delay flowering.

In our study, the lowest number of pollen tubes at the base of the pistil was recorded for the 'Hibernal', Seyval,' 'Leon Millot', 'Rondo', and 'Jutrzenka' cultivars (Table 2, Figure 3D). The highest number in turn was observed for 'Aurora', 'Bianca', and 'Solaris' (from 21 to 30%). Ebadi et al. [62] reported that 20% of ovules were penetrated by pollen tubes and fertilized in the cultivar 'Chardonay'. Padureanu and Patras [44], investigating the fertility of two hybrid cultivars, found that the cultivar 'Otello', which contained genes from *Vitis vinifera*, was characterized by 2 times higher pollen yield compared to the cultivar 'Noego' derived from *Vitis labrusca* and *Vitis riparia*. In the present study, all the cultivars studied had some share of *Vitis vinifera* (Table 1), and regardless of their genotype, were characterized by the variability in pollen germination and the number of pollen tubes at the stylopodium relative to the cultivar.

The authors observed pollen germination in closed anthers in 10% of flowers of all cultivars (Figure 3C). There are several theories about how grapevines are pollinated, ranging from cleistogamy, i.e., self-pollination, insect pollination, to wind pollination [29]. Staud et al. [64] performed observations of *Vitis vinifera* flower opening in 'Müller-Thurgau' and 'Pinot Noir' cultivars. They noticed that about 25–35% of the pollen showed pollen tube growth as early as in the capfall phase (Figure 3B). Similar observations were made by Defouquette et al. [61]. They reported that self-pollination in *Vitis roundifolia* Michx cultivars was approximately 22%. Maghradze [59] recorded self-pollination in Georgian cultivars at a level from 16.9% to 61.9%, and this trait was cultivar-specific. Depending on the cultivar, the authors indicated that self-pollination could have had a positive or negative effect on berry size, or the number of seeds produced; however, they added that apart from self-pollination, cross-pollination was also necessary for good fruiting. [42,59,61]. Pollination is usually positively correlated with the number of seeds per fruit [42,65] and it was also confirmed in our study.

3.4. Evaluation of Fruit Quality

Parameters such as the average berry weight, length, and width, along with spherical area and average number of seeds were considered in fruit quality evaluation. The correlation between berry weight and seed number in berry was also calculated (Table 5).

Table 5. Fruit quality 11 hybrid grape cultivars.

Cultivar	Average Berry Weight	Average Berry Width	Average Berry Length	Globular Surface (cm^2)	Average Number of Seeds in Berry	Correlation (between Berry Weight and Number of Seeds)
Aurora	1.9 ^{de}	14.0 ^{ab}	14.6 ^{b}	2.0 ^{bcd}	2.6 ^{b}	0.31
Bianca	2.0 ^{de}	14.4 ^{b}	15.3 ^{b}	2.2 ^{e}	2.4 ^{b}	0.86
Hibernal	2.0 ^{de}	13.9 ^{ab}	14.8 ^{b}	2.1 ^{cde}	2.4 ^{b}	0.65
Jutrzenka	1.9 ^{cd}	13.5 ^{ab}	14.3 ^{b}	1.9 ^{bcd}	2.2 ^{ab}	0.47
Leon Millot	1.7 ^{b}	13.0 ^{ab}	14.1 ^{b}	1.8 ^{ab}	2.1 ^{ab}	0.78
Marechal Foch	2.0 ^{e}	13.9 ^{ab}	15.4 ^{b}	2.1 ^{e}	2.4 ^{b}	0.81
Muscat Odesskij	1.6 ^{ab}	13.2 ^{ab}	14.4 ^{b}	1.9 ^{abc}	2.3 ^{b}	0.77
Regent	1.6 ^{a}	12.8 ^{a}	14.1 ^{b}	1.8 ^{ab}	1.6 ^{a}	0.87
Rondo	1.9 ^{d}	13.8 ^{ab}	14.9 ^{b}	2.1 ^{cde}	2.3 ^{b}	0.63
Seyval Blanc	1.8 ^{bc}	13.4 ^{ab}	15.0 ^{b}	2.2 ^{e}	2.1 ^{ab}	0.79
Solaris	1.9 ^{de}	13.9 ^{ab}	14.5 ^{b}	2.0 ^{cd}	3.6 ^{c}	0.91

The values are given as means, followed by the letters $^{a–e}$ to indicate statistical significance. The values marked with the same letters in one column are not statistically different at $\alpha < 0.05$.

The fertilized ovule develops into a seed [66]. The size of the berries and their shape in individual cultivars is genetically controlled; the weight of grape berries also depends on the number of seeds [42], i.e., berries without seeds will be much smaller than berries containing them. The same is true for other species such as apples [67]. This relationship in the current study could is observed particularly for the cultivar 'Regent', as its berries had the lowest weight, the smallest size, the smallest number of seeds (Table 5) and the highest percentage of berries with a single seed (Figure 6). Moreover, parthenocarpic berries were also found in this cultivar (Figure 6). Spena and Rotino [68] have argued that parthenocarpy in grapevines and other species is a way of producing fruit under conditions not conducive to pollination and/or fertilization. In Central European climatic conditions, parthenocarpic fruits very often develop from fruits damaged by spring frosts [67].

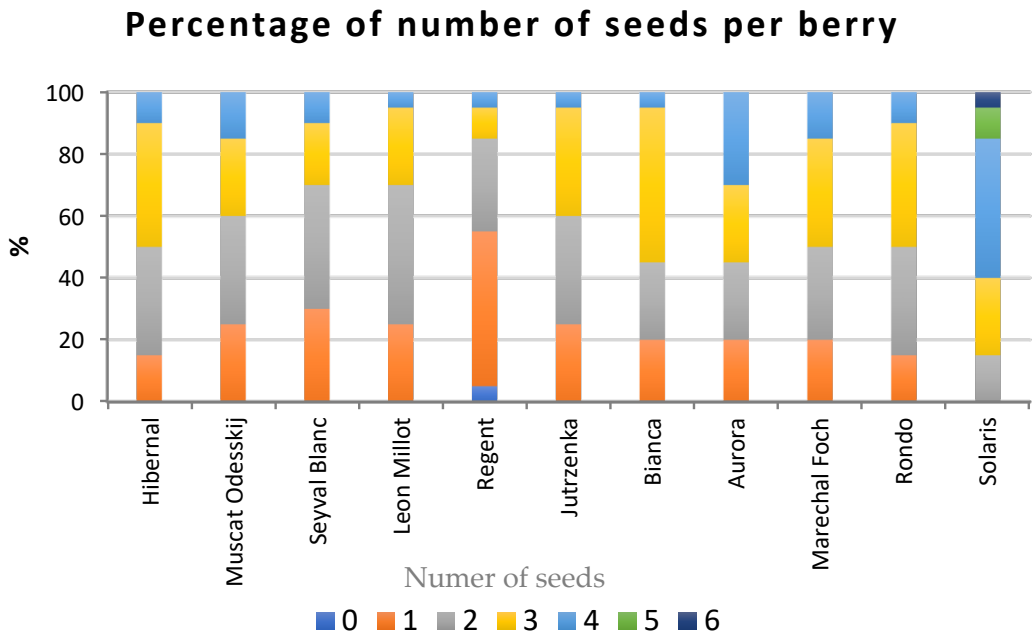

Figure 6. Percentage of number of seeds per berry in 11 hybrid grape cultivars.

The cultivar 'Solaris' was clearly deviating from Sabir's observations [42], as its berries did not increase in weight or size despite a significantly higher number of seeds (Table 5).

This cultivar had the highest percentage of berries with 4 seeds and a small percentage of berries with 5 and 6 seeds (Figure 4). Maghradze [59] found in his work that there was a possibility of producing more seeds in grapevine berries than usually observed (i.e., 0, 1, 2, 3, 4), due to aberrations during micro- and macrosporogenesis, embryogenesis, and genotype variation. The correlation between berry weight and number of seeds was the highest for this cultivar (Table 5). Lebon et al. [25] analyzed inflorescence and flower development of the cultivars 'Gewurztraminer' and 'Pinot Noir' under different conditions. The number of seeds per berry averaged from 1.4 to 1.8. In the present experiment, for most cultivars, the number of seeds was higher than Lebon et al. [25] and ranged from 1.6 to 2.45.

Cultivars with the highest spherical area in the present experiment included 'Seyval Blanc', 'Bianca', and 'Marechal Foch', while 'Regent' and 'Leon Millot' were characterized by the lowest (Table 5).

4. Conclusions

Hybrid cultivars differ from *Vitis vinifera* in terms of flower structure. We recorded anomalies in the number of anthers ranging from 4 to 7 for most of the described cultivars and up to 11 anthers in the cultivar 'Seyval Blanc'. The number of pollen grains in a flower varied greatly and depended on the cultivar and growing season. The cultivars 'Rondo' and 'Solaris' were characterized by the highest values of flower structure and quality (number of pollens in flowers, pollen viability, number of ovules in the ovary, number of set seeds). Stigma receptivity appered with the capfall and disappeared with its browning. The cultivar 'Regent' had the ability to form a small percentage of parthenocarpic fruits. Berry weight is not correlated with the number of seeds set. The existence of *Vitis vinifera* cultivars has been described in many publications, while hybrid cultivars require better knowledge, evaluation of the source of pollen on fruit quality (metaxenia), and checking fruit quality after pollination with their own pollen (kleistogamy). These issues require further research.

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The Content of Selected Bioactive Compounds in Wines Produced from Dehydrated Grapes of the Hybrid Variety 'Hibernal' as a Factor Determining the Method of Producing Straw Wines

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Abstract: Sweet wines are appreciated worldwide; many are produced by fermenting the must of dehydrated (semi-dried) grapes, using methods that vary from region to region. The aim of this study was to evaluate the basic chemical and oenological characteristics of wines obtained by three technologies of production. The wines were made from a hybrid cultivar 'Hibernal', grown under cool climate conditions. 'Hibernal' is a hybrid variety. This 'Hibernal' variety is widely cultivated in central and eastern Europe, where it is of great economic importance. Wines produced from this variety are popular in local markets. In comparison with the production of varieties belonging to *Vitis vinifera*, a very small percentage of the 'Hibernal' variety is cultivated. The methods used in the experiment for the production of wines were: classical method in the Italian *passito* style, modification of the *passito* style with a seven-day maceration of grapes, and a method of production in the Tokaj wine style at five Puttonyos. Basic chemical parameters, acid profile, total phenolic content, antioxidant and antiradical capacities, and quantitative analysis of selected polyphenols was performed. The sensory features and quality of the wines was assessed using a sommelier analysis based on The Wine & Spirit Education Trust guidelines. The results indicated that the seven-day maceration of the dehydrated grapes resulted in the highest polyphenol content, as well as the largest antioxidant and antiradical contents. The oenological evaluation of wines produced by the Tokaj method and Italian *passito* method with seven-day maceration found that the wines were appreciated due to their rich taste, flavor, and overall quality. The present study confirms the promising opportunities to obtain special sweet wine with a valuable composition and oenological characteristics in regions with cooler climates.

Keywords: hybrid cultivars; *passito* wine; straw wine; polyphenols; antioxidant capacity; antiradical capacity

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1. Introduction

Sweet dessert wines are very popular in the world. They are produced using various technologies primarily based on the grape dehydration process. This treatment is aimed at increasing sugar concentration in the must, and can be carried out using several methods, such as cryoconcentration, for German and Canadian Eiswein, *Botrytis cinerea* infection for Tokaj wines, stopping fermentation by distillate addition for Porto or Madeira wines, or by partial grape drying. The latter method is the most common and has been used for centuries. Several countries have a long tradition in producing these types of wines. For instance, Italy's production of Vino Passito and Vin Santo, Austria's production of

Strohwein, France's production of Vin de Paille, and Spain's production of Pedro Ximenez. The old methods were based on spreading the grapes on mats and drying them in the sunshine. At present, modern dryers with a forced warm, dry air convection are mostly used, thus avoiding problems associated with fungal growth and ochratoxin, contamination, or insect problems [1,2]. The winemaking process itself also provides many problems. A high sugar concentration and increased alcohol content create osmotic stress for yeasts, and only some of them are able to carry out the fermentation process. The end products are wines with a very distinctive taste and aroma, which are often more complex than standard red or white wines made from ordinarily ripe grapes [3,4]. Mostly, they are sweet to very sweet, capable of a long life, usually deep golden in colour, with a viscous appearance. In general, the nose and palate is met with a complex, alluring blend of peaches, dried apricots, and marmalade, with flavours of almonds and honey. The intense mouthfeel is balanced by a clean, fresh, and very long finish of dried apricots [3–5].

In Poland, in recent years, there has been an increase in the number of vineyards where mostly hybrid varieties are grown. *Vitis vinifera* strains are cultivated only in the west of the country. The whole country is located in the cool climate zone, and it is rarely possible to produce wines with higher residual sugar contents under natural conditions. Generally, it is only possible to harvest grapes with a relatively low extract (usually 17–23° Brix), although these can give good, fragrant dry, or semi-dry wines with a balancing acidity. [5]. The only way to obtain dessert wines is to use special production methods. The climate that prevails in Poland should favor the production of ice wines, however, there is no guarantee that a frosty winter will happen every year. At the turn of 2020 and 2021, only 500 L of ice wine were produced, which accounted for 0.03% of the total wine production in Poland. Straw wines (i.e., wines made of semi-dried grapes being dehydrated indoors) are a safer solution for producers, as they are independent of weather conditions [1].

A number of studies provide evidence for the beneficial effects of grapes on human health, both in their basic and modified forms. A special role is attributed to polyphenolic compounds contained in grapes, which greatly contribute to the prevention of cardiovascular diseases and cancer [6–8]. Most of them are found in red wines, due to their production technology. Maceration, together with skins and seeds, results in the extraction of their ingredients into the must. For white wines, the process is slightly different, as the must is fermented directly after pressing, reducing valuable compound contents such as polyphenols by up to 10-fold. For winemakers, polyphenols are important, primarily for the colour and taste of wine [9,10]. Phenolic compounds contained in unfermented grapefruits are not fully assimilated by the human body because they occur as large polymeric complexes. Ethanol formed during the fermentation process is a good solvent for phenolic compounds, hence facilitating their extraction into wine [11].

One grape variety (i.e., hybrid variety 'Hibernal') was used in the experiment, but three different production methods were applied. In commercially available wines, one can also find similar examples of different technologies being used during production in order to produce different wine characteristics (e.g., Amarone della Valpolicella and Recioto della Valpolicella, where the maximum sugar content of the former is 12 g/L, and over 100 g/L of the latter) [12]. In this study, three wines with different styles and compositional parameters were obtained. Their variability was mainly due to different production technologies (i.e., classic for straw wines: grape drying (wine *A*), grape drying and maceration (wine *B*) and wine refermentation using dehydrated grapes (wine *C*)).

During the long preliminary procedure (i.e., drying, fermentation, and maturation to the final product), straw wine is enriched with several compounds, similarly to classic wines. In wines made from dried grapes, substances dissolved in water, mainly sugars, but also acids, are concentrated [13–15].

By applying the red wine production method to straw wine making, a product with a higher concentration of polyphenols, that is comparable to red wines, can be obtained. The objective of this study was to evaluate the production methods of straw wines, considering their oenological characteristics, as well as the content and proportion of essential

health-promoting compounds. The results will help improve the quality of straw wines produced under cool climate conditions.

2. Materials and Methods

2.1. Biological Material

The research was carried out for three types of straw wines produced in 2015, from the hybrid 'Hibernal' variety (Figure 1A), derived from the 'Seibel 7053' (Chancellor), and 'Riesling' cultivars.

(**A**) (**B**) (**C**)

Figure 1. (**A**)—'Hibernal' variety; (**B**)—openwork boxes; (**C**)—dehydrated grapes after six weeks.

Straw wines used for the comparison were made of grapes from the vineyard 'Garlicki Lamus', located nearby to Krakow, in the south of Poland (50°08′29.4″ N 19°55′50.7″ E).

The collected fruits were fully ripe, without any damage, infection, mold, or rot. The process of dehydrating the grapes, which lasted six weeks, took place in a dry and well-ventilated room, in openwork boxes (Figure 1B). Two fans were used to force air circulation. The temperature during drying was kept at about 18 °C. During the entire process, the grape clusters were inspected, and any damaged or infected fruit were immediately removed (Figure 1C). The basic chemical parameters of fresh and dried grapes are summarized in Table 1.

Table 1. The basic chemical parameters of the grapes used in the experiment: pH, titrable acidity (TA, in tartaric acid equivalents), and the total extract content (TSS).

	Fresh Grapes	Withered Grapes
pH	3.14 ± 0.01	3.31 ± 0.01
Ta (TAE, g/L,)	8.7 ± 0.15	17.9 ± 0.71
TSS (°Brix)	17.63 ± 0.01	40.10 ± 0.00

The straw wines used for the comparison were made using three methods:

A: The 50 kg semi-dried grapes were pressed, then the must (about 20 L) was fermented (the classical straw wine method);

B: The 50 kg semi-dried grapes were ground and macerated for 7 days at 15 °C. Then, the free-run juice was poured off and the remaining grapes were pressed. The must was combined with the free-run juice and subjected to a further fermentation process—about 20 L (the classical method with fermentative maceration);

C: The 3 kg semi-dried grapes were poured with 20 L of fresh 'Hibernal' wine in a ratio of 1 kg per 7 L and subjected to refermentation. The fresh wine had a residual sugar content of 6.7 g/L and an acidity of 6.05 g/L (the Tokaj method). Fermentation of the young wine was carried out with ZYMAFLORE® VL1 yeast made by Laffort. IOC Bayanus yeast was used for refermentation at 50 g/hl.

Active dried yeast IOC Bayanus produced by Institut Oenologique de Champagne (*Saccharomyces cerevisiae bayanus*) was used to produce and referment the wines used in the research. After the supernatant was decanted, the wine was allowed to clarify. The wines were aged at 10 degrees Celsius and 75% humidity.

2.2. Physicochemical Analyzes

2.2.1. pH, the Total Acidity, the Extract Content

The pH and the total acidity of the wine were determined with a Janway 3020 pH meter. Titration with NaOH was performed to pH = 7.0. The analysis was performed in triplicate. The results were expressed in g $\times$ 100 g^{-1} of tartaric acid equivalents. For determination of the extract content, an electronic ATAGO Digital PR-101a refractometer (Tokyo, Japan) with a range of 0.0–45.0 °Brix was used. The measurement was performed in triplicate and the results were expressed in °Brix.

2.2.2. Ethanol Content

The determination of the ethyl alcohol content of the straw wine samples were tested according to the OIV reference method (OIV-MA-AS312-01A). Briefly, 100 mL of wine with the addition of 10 mL of 2 M $Ca(OH)_2$ was distilled at 20 °C. Calcium hydroxide is necessary to avoid the presence of volatile acids which could affect the ethanol quantification. Distillation was stopped when approximately 75% of the original volume was reached, and after 20 min, pure water was added to the total volume of 100 mL. The ethanol content was measured with an oscillating type densimeter (Handheld DensityMeter, Densito, Mettler-Toledo, Columbus, OH, USA).

2.2.3. Acid Profile

The content of organic acid anions was investigated with an EA 100 capillary isotachoresis (ITP) analyzer (Villa Labecos. r.o. Spisska Nova Ves, Slovakia). The apparatus was equipped with two capillaries: a pre-separation capillary (90 mm $\times$ 0.8 mm ID) and an analytical capillary (90 mm $\times$ 0.3 mm ID). The initial separation was carried out at an AC current of 250 µA, while the separation in the analytical capillary column at 60 µA during the initialization step and at 50 µA during the detection step. The lead electrolyte (LE) was 10 mM HCl with 0.1% HEC (Hydroxyethyl Methyl Cellulose) and 10 mM β-alanine. The final electrolyte was a 0.01 M caproic acid solution [16,17]. All the reagents used for ITP analysis (i.e., β-alanine, caproic acid, tartaric acid, citric acid, malonic acid, succinic acid, lactic acid, and acetic acid, as well as hydrochloric acid) were from Sigma–Aldrich (Burlington, MA, USA).

2.2.4. Total Phenolic Content (TPC)

The number of phenolic compounds in the extracts was determined based on the reaction with the Folin–Ciocalteu reagent. The wine sample (0.25 mL) was mixed with 0.25 mL of 25% Na_2CO_3, 0.125 mL of the Folin–Ciocalteu reagent (Sigma–Aldrich, diluted twice with water prior to the analysis), 2.25 mL of water, and then incubated for 15 min. The absorbance was measured at 760 nm (JASCO V-530 UV–Vis spectrophotometer). The final results were expressed as mg of gallic acid (Sigma–Aldrich) per L (gallic acid equivalents, GAE mg L^{-1}) [17,18].

2.2.5. Antioxidant Capacity—A FRAP Assay

The FRAP (ferric reducing antioxidant power) assay is based on the reduction of the ferric–tripyridyl-*s*-triazine (Fe^{+3}–TPTZ) complex to its ferrous (Fe^{2+}) derivative. The FRAP working solution was freshly prepared by mixing together 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (Sigma–Aldrich) in 96% ethanol, and 20 mM $FeCl_3$ (10:1:1, *v:v:v*). Then, 3 mL of the FRAP working solution were mixed with 0.1 mL of the wine sample and 0.3 mL of water. The absorbance was measured at 595 nm after 5 min. The results were expressed as

μmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma–Aldrich) per L (Trolox equivalents, TE μmol L^{-1}) [19].

2.2.6. Antioxidant Capacity—A CUPRAC Assay

The CUPRAC (cupric ion reducing antioxidant capacity) assay is based on the measurement of the utilization of copper (II)-neocuproine as a chromogenic oxidizing agent. Briefly, 1 mL of 10 mM $CuCl_2$, 1 mL of 7.5 mM neocuproine (Sigma–Aldrich) in 96% ethanol and 1 mL of 1 M NH_4Ac buffer, pH 7.0, were mixed with 0.25 mL of the wine and 0.8 mL of distilled water. The absorbance was measured at 450 nm after 15 and 30 min. The results were expressed as μmol Trolox (Sigma–Aldrich) per L (TE μmol L^{-1}) [20,21].

2.2.7. Radical Scavenging Capacity (RSC)—A DPPH Assay

The radical scavenging capacity of extracts was tested following the reduction of a synthetic, stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The colorimetric method enables the measurement of absorbance changes to DPPH solution at 517 nm as a result of the antioxidant activity of the sample. Briefly, 2.8 mL of 0.1 mM DPPH (Sigma–Aldrich) solution in 96% ethanol was mixed with 0.2 mL of the wine sample. The DPPH absorbance was measured after 10 min. The RSC results were expressed as μmol Trolox (Sigma–Aldrich) per L (TE μmol L^{-1}) [20].

2.2.8. Polyphenol Analysis

High performance liquid chromatography (HPLC) (Shimadzu LC-10AS chromatograph equipped with a C18 RP column and SPD-10AV UV-Vis detector) was used to identify phenolic compounds in wine. Signal detection was set at 265 and 325 nm. Chromatographic separation was performed at 33 ± 1 °C using the following solvents: (A) water (Sigma–Aldrich) with acetic acid (0.1%), (B) methanol (Sigma–Aldrich, ultrapure) with acetic acid (0.1%), and use of a solvent gradient: 90% A, 10% B for 20 min; 75% A, 25% B for 30 min; 65% A, 35% B for 40 min; 55% A, 54% B for 50 min; 50% A, 50% B for 60 min; 30% A, 70% B for 62 min; 100% B for 80 min; 80% A, 10% B up to 85 min. The flow rate was 1 mL/min. Identification of phenols was based on the retention times of vanillic, syringic, *trans*-Cinnamic acids, quercetin (Sigma–Aldrich), and (+)-catechin (LGC Standards), at 265 nm, and chlorogenic, caffeic, *p*-coumaric, ferulic acids, *trans*-resveratrol (Sigma–Aldrich), at 325 nm. Before separation, the samples A and C were diluted twice, and the sample *B* was diluted four times, all with methanol (Sigma–Aldrich, HPLC gradient, ultra pure).

2.3. Organoleptic Analyses

All wines were subjected to organoleptic analysis by a group of 30 respondents in the age group of 22–55. Study participants were free of upper respiratory tract infections. All respondents were amateur wine consumers, and they received basic training in organoleptic evaluation of wines based on The Wine & Spirit Education Trust (WSET) materials. The training was conducted by two persons with WSET Level II sommelier certifications. During the training, 64 aromas from LE Nez du Vin (Jean Lenoir, Provence, France), as well as solutions for taste blindness (flavors: sour, sweet, bitter, salty, and umami) were utilized. Wines were evaluated on "wine evaluation sheets" which were compiled based on the WSET materials. The scores presented on the sheets were intended to illustrate the quality rating of individual wines. The Table 2 includes answers that were marked by at least 60% of the panellists in the survey.

2.4. Statistical Analysis

The collected results were processed using a one-way analysis of variance. Tukey's test was used to assess the significance of differences between the means, with a significance level of $\alpha = 0.05$. All the statistical calculations were performed using Statistica 13 computer software.

Table 2. Organolepic evaluation criteria.

APPEARANCE	
Clarity	clear-hazy (faulty?); intensity: pale, medium, deep
Colour	lemon, green, lemon, gold, amber, brown
Other observations	e.g., legs/tears, deposit, petillance, bubbles
NOSE	
Condition	clean, unclean (faulty?); intensity: light, medium (−), medium, medium (+), pronounced
Aroma characteristics	e.g., fruits, flowers, spices, vegetables, oak aromas, other
Development	youthful, developing, fully developed, tired/past its best
PALATE	
Sweetness	dry, off-dry, medium-dry, medium-sweet, sweet, luscious
Acidity	low, medium (−), medium, medium (+), high
Tannin	low, medium (−), medium, medium (+), high
Alcohol	low, medium (−), medium, medium (+), high
Body	light, medium (−), medium, medium (+), full
Flavour	intensity light, medium (−), medium, medium (+), pronounced
Flavour characteristics	e.g., fruits, flowers, spices, vegetables, oak flavours, other
Length	short, medium (−), medium, medium (+), long
CONCLUSIONS	
Quality level	faulty, poor, acceptable, good, very good, outstanding
Level of readiness for drinking/potential for an ageing	can drink now, drink now, not for drinking, too young but has potential, suitable for ageing, too old for ageing, for ageing or further ageing.

3. Results and Discussion

The physical and chemical parameters of the obtained straw wines were determined in the experiment. The first analyzed parameter was the pH of the juice, which is a factor that influences must and wine stability [22]. The initial juice pH in the grapes after drying increased from 3.14 to 3.31 (Table 1). This process is observed when the juice is concentrated, but also during extended maturation [23]. During fermentation, the pH value increased to 3.91 for sample *A*, and the statistically lowest and most desired pH was achieved for sample *C* (Table 3). Aponte and Blaiotta [24] analyzed the passito wine 'Moscato di Saracena', and recorded a pH range from 3.1 to 3.93, which was similar to the values obtained in the present study. Bondada et al. [23] confirmed that pH was not strictly correlated with acidity (TA), and pH increase did not always show the same trend, which was also reflected in the results of this study.

The main organic acids in wines, such as tartaric acid, citric acid, and malic acid come from fruits; others are fermentation products (i.e., lactic acid, succinic acid or acetic acid), and affect wine sensory characteristics such as colour, and its microbiological stability [25,26]. The titratable acidity of wines in the experiment was in the range of 8.18–10.34 g/L; it was statistically the highest in sample B, which was probably due to the high initial acid content in the macerate which was then fermented (Table 3). Climatic conditions are key factors in determining grape maturity and maturity-related parameters, such as sugar content and total grape acidity. In classic wines, it is assumed that total acidity should be between 5 and 12 g/L [27]. Croce et al. [12] studied 302 samples of Italian wines made from dried grapes. The acidity recorded by these authors ranged

between 3.73 and 11.31 g/L. The values obtained in this experiment were at the upper end of this range.

Table 3. Basic chemical characteristics of wines using semi-dried grapes, and which were obtained using three methods of production.

	Wine *A*	**Wine *B***	**Wine *C***
pH	3.91 a ± 0.18	3.53 b ± 0.01	3.1 c ± 0.01
TA (g/L)	8.18 c ± 0.11	10.34 a ± 0.41	8.95 b ± 0.05
TSS (°Brix)	18.2 a ± 0.00	15.9 b ± 0.06	6.9 c ± 0.00
ethanol (vol %)	18.0 a ± 0.00	18.0 a ± 0.00	15.0 b ± 0.00
tartaric acid (g/L)	2.23 b ± 0.03	4.61 a ± 0.33	2.27 b ± 0.45
citric acid (g/L)	0.50 a ± 0.04	0.55 a ± 0.05	0.28 b ± 0.04
malic acid (g/L)	2.47 b ± 0.10	2.99 a ± 0.19	1.25 c ± 0.10
succinic acid (g/L)	1.60 b ± 0.03	2.40 a ± 0.07	0.57 c ± 0.20
lactic acid (g/L)	0.52 a ± 0.27	0.54 a ± 0.14	0.58 a ± 0.25
acetic acid (g/L)	1.50 a ± 0.01	0.95 b ± 0.01	0.19 c ± 0.01

The values are given as means ± standard deviations, followed by the letters a–c to indicate statistical significance. The values marked with the same letters in one line are not statistically different at $\alpha < 0.05$; TA—titrable acidity, TSS—total extract content.

Wines made from dried grapes can vary in typology, with sugar concentrations reaching even 600 g/L. Wines in the present study could be divided into two groups in terms of sugar content: wines A and B belonged to very sweet wines with a sugar content higher than 150 g/L, and wine C belonged to the group of sweet wines, with sugar in the range of 60 to 150 g/L. Domizio & Lencioni [28] demonstrated a high diversity among *passito* styles. They summarized that Tuscan wines (Vin Santo) had residual sugar content between 10 and 250 g/L. Their observations indicated that recent trends in consumption have prevailed toward slightly sweet and sweet wines. Laureati et al. [29] also described the production of traditional Vin Santo, made from three varieties: Trebiano, Malvasia and Grechetto. The wines were divided into three types: semi-sweet wines (10–50 g/L residual sugar), slightly sweet wines, and sweet wines (up to 100 g/L residual sugar content). The described production technology for Vin Santo was very similar to that used by the authors for sample A; however, a residual sugar in the experiment was determined at 182 g/L, which classified it as a very sweet wine (Table 3). By reducing the degree of dehydration, and thus sugar level in the must, it is possible to match the style of Polish wines to traditional Italian wines. Giordano et al. [30] described obtaining Passito di Pantelleria DOC wines using a base wine and refermentation to sweeten the product, yielding wines containing 200 to 340 g/L residual sugar. In the present study, the authors used the same method for wine *C* but obtained a lower sugar concentration (i.e., 69 g/L). Such a result was due to the low residual sugar content in the base wine and in the dehydrated grapes. No reports were found in the literature on using dried grapes and the maceration method to produce wines, which was applied in sample B. Such a method is sometimes used to produce traditional white wines (very short maceration), and almost always to produce red wines (longer maceration).

The yeast used in the study (i.e., *S. bayanus*), is applied to resume fermentation, refermentation, fermentation of meads, and production of sparkling beverages [31,32]. In this case, the fermentation in two samples (wine *A* and *B*) stopped at the maximum alcohol concentration of 18% vol, whereas the alcohol concentration in sample *C* was 15% vol (Table 3). Croce et al. [12] recorded alcohol content in *passito* wines ranging from 10.18 to even 20.52% vol. Similar results were obtained by Domizio and Lencioni [28], who examined 63 Tuscan Vin Santo wines and determined that the alcohol concentration was in the range of 10–19% vol. They divided Vin Santo into three styles depending on residual sugar and alcohol content: dry style (16–19% vol alcohol and 10–50 g/L sugar), slightly sweet and sweet style (14–16% vol alcohol and up to 100 g/L sugar), and very sweet (14–16% vol alcohol and up to 250 g/L sugar). Relating the results of the present study to the above division, wine *C* can be classified as a slightly sweet style, whereas wines *A* and

B would be classified as very sweet in terms of sugar content, and as having a dry style in relation to alcohol concentration.

The current study analyzed the content of major organic acids by isotachophoresis (ITP) (Table 3). This method is used for electromigration separation and allows the analysis of mixtures of ionic substances [33]. In the experiment, sample *B* had the highest content of tartaric, malic and succinic acids, and together with wine *A*, also citric acid. The lowest contents of tartaric, citric, malic, succinic, and acetic acids were measured in sample *C*. Aponte and Blaiotta [24] obtained similar values as the authors for wine *A*: tartaric acid content—2.56–2.65 g/L, citric acid—0.54–0.60 g/L, malic acid—2.16–2.11 g/L, succinic acid—1.72–1.89 g/L, acetic acid—0.79–1.51 g/L, and in only one case, lactic acid—0.84 g/L. Giordano et al. [30] studied three Italian *passito* wines and acquired similar contents of the aforementioned acids to those obtained in the present study. Wine *C* proved to be the most similar to Passito di Panteleria DOC in terms of organic acid content; the production method of both wines was also very similar. In the experiment, as in a study by Aponte and Blaiotta [24], wine *A* statistically had the highest acetic acid content at 1.5 g/L, and fermentation dynamics and acetic acid production were also evaluated. The maximum concentration of acetic acid formed in wines from dehydrated grapes has been defined in several countries; for example, the maximum value of acetic acid in Canadian Eiswein is 2.1 g/L [4,34]. In Europe, the standard for white and rosé wines is 1.08 g/L, and 1.8 g/L for red wines. An elevated acetic acid content in table wines usually indicates a spoilage process; however, in the case of wines made from dried grapes, it is responsible for retaining a redox balance when responding to osmotic stress induced by high sugar levels [34].

The total polyphenol content of wine is influenced by winemaking techniques, namely maceration, fermentation, and aging [35]. In white wines, it is assumed to range from 100 to 400 mg/L, in rose wines from 400 to 800 mg/L, and in red wines from 1000 to 2000 mg/L [36]. The straw wines used in the present study contained 907.5 to 3748.5 mg of gallic acid equivalents per liter (mg GAE/L); sample *B* (i.e., the one in which the dried grapes were macerated) was the richest in phenolic compounds (Table 4). Many authors have shown that the maceration process leads to an increase in total polyphenols due to their high concentration in the skins and seeds. Their greatest increments occur at the end of maceration, when the produced alcohol destroys the skin lipid layer that protects the seeds [37,38]. For wines *A* and *C*, the concentration of polyphenols was over 70% lower than for sample *B* in the current study. Fhurman et al. [39] studied the effect of skin contact with grapes on the polyphenol content and antioxidant capacity of white wine. During maceration, which lasted from 2 to 18 h, the latter authors noted a gradual increase in the concentration of polyphenols (up to 41%). They also analyzed the effect of fresh grape maceration in alcohols of different concentrations (2–18% vol). The 18% vol alcohol resulted in the highest polyphenol extraction, 60% higher than in the wine without any added alcohol. In the present study, wine *A* had no contact with skins and seeds, whereas polyphenols present in the dried grapes in wine *C* were dissolved in the base wine. A lower alcohol concentration, compared to other samples, may not have been sufficient to fully extract polyphenols from dried grapes. Panceri and Bordignon-Luiz [26] analyzed the effect of grape dehydration, among others, on the chemical composition of young wines from dark Merlot and Cabernet Sauvignon varieties using a five-day maceration after 22 months of maturation. They determined total phenolic content (TPC, in GAE) in the range of 1221.78–1588.50 mg GAE L^{-1} (i.e., they obtained values similar to samples *A* and *C* after 5 years of maturation). Much lower TPC values (an average of 249 mg/L) were obtained by Loizzo [35], who analyzed *passito* from Saracena that was aged between one to four years; however, he expressed the results as (+)-catechin equivalents. The production of this wine was slightly different from the one used in this study. Fresh must was obtained from overripe grapes and dehydrated grapes were added to it. During the wine aging process, the phenolic composition changes quantitatively and depends on the type of wine and its storage conditions [40]. The conducted analysis confirmed the differentiation of the total polyphenol content depending on the production method.

Table 4. The total phenolic content (TPC, mg/L GAE) in straw wines which were obtained by three methods, their antioxidant capacities (FRAP and CuPRAC, both in mmol/L TE), and the radical scavenging capacity (RSC, mmol/L TE).

	Wine *A*	**Wine *B***	**Wine *C***
TPC	1 082.2 b ± 90.76	3 748.5 a ± 354.74	907.5 b ± 33.23
FRAP	2.4 b ± 0.28	10.2 a ± 1.20	3.4 b ± 0.39
CuPRAC	4.9 b ± 0.22	24.6 a ± 1.66	5.6 b ± 0.42
RSC	1.8 c ± 0.05	5.0 a ± 0.74	1.9 b ± 0.04

The values are given as means ± standard deviations, followed by the letters a–c to indicate statistical significance. The values marked with the same letters in one line are not statistically different at $\alpha < 0.05$.

The antioxidant properties of the wines were evaluated by FRAP and CuPRAC assays. FRAP is commonly used in many studies of antioxidative compounds, CuPRAC is less known, but is considered to be more sensitive [41]. The analysis of the obtained values showed that sample *B* revealed the strongest antioxidant properties. The results from FRAP and CuPRAC analyses are well correlated with TPC [42], and as shown above, sample *B* was richest in phenolic compounds. This also confirmed that the phenolic compounds in straw wine *B* were characterised by a higher antioxidant potential.

Studies of of radical scavenging capacity (RSC), defined as the ability to quench the DPPH free radical, showed that sample *B* was 2.7 times more effective as an antiradical agent than sample *A*, and 2.5 times more effective than sample *C*. In red wines, RSC is usually more than 10 times higher than in white wines [43]. Panceri et al. [44] showed the RSC in the range of 2.67–4.67 mM/L (TE). Li et al. (2009) [45] tested 24 Chinese classic wines and reported activity for red wines ranging from 4.19 to 21.30 mM/L (TE), for rosé wines from 1.40 to 3.41 mM/L (TE), and for white wines 0.08 to 1.12 mM/L (TE). Noting the results of the antiradical capacity with the literature data, it could be observed that the values obtained for samples *A* and *C* corresponded to the characteristics of typical white wine, compared with the red wines of sample *B*.

The polyphenol composition of wine samples was analyzed using high-performance liquid chromatography (HPLC) with the use of standards (Table 5). Polyphenols were identified as flavonols ((+)catechin, quercetin), hydroxybenzoic acids (vanillic acid and syringic acid), hydroxycinnamic acids (*p*-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, *trans*-cinnamic acid), and stilbene (*trans*-resveratrol). Two of the phenolic compounds (e.g., syringic acid; (+)-catechin), belonged to the chemical markers of 'Hibernal' white wine [46].

(+)-Catechin content of the wines in the present experiment was in the range of 37.82–64.29 mg/L, with only wine *C* having statistically lower contents than wines *A* and *B* (Table 5). Loizzo [35] recorded 71.7 mg/L of catechins in fresh Passito of Saracena wine. Avizcuri-Inac et al. [47] analyzed the chemical and sensory characteristics of mature sweet wines obtained using different techniques, and determined that catechins in white wines range from nd.–0.12 mg/L, and in red wines from nd.–0.17 mg/L. In twelve white commercial wines, Han et al. recorded catechins in the range of 3,57–16,87 [48]. Rozdrigez-Delgrado et al. [49] analyzed 55 fresh red wines from the Canary Islands and obtained values ranging from 17.70 to 30.77 mg/L. Panceri et al. [44] also analyzed catechin contents in red wines and acquired values ranging from 5.82 to 18.19 g/L. Low catechin content is usually found in older wines and it is related to their polymerization Tannins that contribute to dark colour and astringency of wine mainly belong to condensed tannins (or procyanidins) derived from seeds and skins [35,50]. The main forms are polymers of flavan-3-ols ((+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epicatechin-3-O-gallate) with C4–C6 or C4–C8 linkages and monomeric units [51]. Quercetin in the wines was determined in the range of 0.22–0.32 g/L, with sample *A* having a statistically lower content. Similar results were obtained by Budic-Leto et al. [52] in a study on Croatian red dessert wines, where the determined quercetin levels varied from 0.00 to 12.402. In contrast, Panceri et al. [44] obtained slightly higher values ranging from 2.33 to 15.58 g/L.

The concentration of flavonols in wines largely depends on grape variety, but also on environmental factors and winemaking practices [52].

Table 5. Content of selected phenolic compounds in the straw wine obtained by three methods of production.

Phenolic Compound, mg/L	Wine *A*	Wine *B*	Wine *C*
FLAVONOLS			
(+)-catechin	56.91 a ± 10.12	64.29 a ± 3.65	37.82 b ± 0.30
quercetin	0.22 b ± 0.06	0.32 a ± 0.01	0.32 a ± 0.01
HYDROXYBENZOIC ACIDS			
vanilin acid	1.97 b ± 0.30	2.83 a ± 0.32	1.38 b ± 0.32
syringic acid	1.55 c ± 0.20	2.25 a ±0.19	1.27 b ± 0.20
HYDROXYCINNAMIC ACIDS			
chlorogenic acid	0.2 b ± 0.02	0.57 a ± 0.04	0.51 a ± 0.02
caffeic acid	0.04 c ± 0.01	7.10 a ± 0.71	3.43 b ± 0.03
p-coumaric acid	1.28 b ± 0.04	3.00 a ± 0.25	1.58 b ± 0.03
trans-cinnamic acid	0.28 b ± 0.06	0.43 a ± 0.01	0.26 b ± 0.01
ferulic acid	0.19 c ± 0.03	2.68 a ± 0.13	0.64 b ± 0.04
STILBENES			
trans-resveratrol	0.03 b ± 0.003	nd.	0.18 a ± 0.05

The values are given as means ± standard deviations, followed by the letters a–c to indicate statistical significance. The values marked with the same letters in one line are not statistically different at $\alpha < 0.05$; nd.—not detected.

The examined wines were characterized by a rather high content of vanillic and syringic acids: 1.38–2.83 g/L and 1.27–2.25 g/L, respectively (Table 5). Avizcuri-Inac et al. [47] reported much lower values—nd.–0.15 mg/L and nd.–0.25 mg/l, and in one case, only 1.41 mg/L. The scatter in the values obtained for these acids in red wines from dried grapes was high. According to Rozdrigez-Delgrado et al. [49] these contents were higher—1.71–2.99 g/L and 1.64–2.77 g/L, and similar to the results obtained in this study. On the other hand, the content of these acids in the experiment of Panceri et al. [44] was significantly higher (i.e., 3.53–13.42 g/L and 1.40–8.85 g/L).

The concentrations of hydroxycinnamic acids determined in wine samples *A*, *B*, and *C* were within typical ranges characteristic of white wines [6,48,52] (Table 5). Wine *B* was distinguished by having the highest content, which, taking into account the maceration process, is the expected result.

A very low content of *trans*-resveratrol (0.03–0.18 mg/L) was determined in the examined wines *A* and *C*, and this stilbene was completely absent in sample *B* (Table 5). Vitalini et al. [53] tested, among others, three Italian dessert wines from dried grapes, and only in Santelmo—Vin Santo DOC did they determine a *trans*-resveratrol content of 0.02 mg/L. A much higher concentration was recorded by Loizzo et al. [35] in Passito of Saracena wine—4.6 mg/L. Classic red wines, according to a large study by Stervbo et al. [54] contained between 0 and 14.3 g/L *trans*-resveratrol. Grapes, and products made from them, are considered a very good source of *trans*-resveratrol, which was attributed as being a potent anti-cancerous compound, although recent studies have not confirmed its unique properties. Numerous analyses of the chemical composition of wines have revealed that these products differ greatly in their *trans*-resveratrol content. It is most often found in red wines, less often in rosé wines, and least often in white wines [53]. The method of beverage production is also of great importance, as also demonstrated in this study. Although the skins of 'Hibernal' grapes, despite their golden-pink colour, contain a small amount of *trans*-resveratrol, possibly due to their Seibel 7053 genetic lineage, we would expect the highest content of this compound in sample *B* but due to the use of maceration, it probably degraded during this process for unknown reasons.

A sensory evaluation of the straw wines was carried out, where the respondents assessed their appearance, aroma, flavor, and quality (Table 6). Wines *A* and *B* were rated

as wines with an intense amber colour (Figure 2), full flavor, and a long finish. Both in terms of aroma and taste, flavors defined as typical for *passito* wines prevailed (i.e., mainly dried fruit, honey, caramel and nuts [3,5,6]). The high alcohol content of these wines (18%) was fully palpable and also visible through "tears" on the walls of the glass [55]. Of the two wines, wine *B* was rated higher. Wine *C*, in terms of aromas, corresponded to the varietal characteristics, which was reminiscent of Riesling, with a slight addition of vanilla, honey, and linden flowers. The flavor was mainly green fruits, grapefruit, and lemon. This wine was well received and assessed to be very good in terms of quality.

Table 6. Sensory evaluation of wines obtained by three methods of production according to "Wine evaluation sheets" based on the WSET materials.

APPERANCE	**Wine *A***	**Wine *B***	**Wine *C***
Clarity	Clear	Clear	Clear
Intensity	Medium to deep	Deep	Medium
Colour	Amber	Intese amber	Amber
Other observations	Tears	Tears	Tears
NOSE			
Condition	Clean	Clean	Clean
Intensity	Pronounced	Pronounced	Medium
Aroma characteristics	Almonds, walnuts, golden apples, honey, wood, dried plum	Almonds, walnuts, golden apples, honey, wood, dried plum	Green apples, pear, hay, vanilla, herbs
Development	Developed	Fully developed	Fully developed
PALATE			
Sweetness	Medium sweet to sweet	Medium sweet to sweet	Dry to medium dry
Acidity	Medium	Medium (−)	Medium
Alkohol	High	High	Medium
Body	Full	Full	Medium
Flovour intensity	Pronounced	Pronounced	Medium
Flavour characteristics	Pear, jam, caramel, dried fruit	Apple, jam, caramel, dried fruit	Apple, lemon, grape, pear
Lenght	Long	Long	Medium (+)
CONCLUSIONS			
Quality level	Good	Very good	Very good
Level of readiness for drinking/potential for an ageing	Can drink now, but has potential for ageing	Can drink now, but has potential for ageing	Can drink now, not suitable for ageing

Figure 2. Differences in the colour intensity of wine depending on the production method.

4. Conclusions

This study analyzed three wine types from semi-dried grapes using three different methods—classical straw wine *A*, straw wine with fermentative maceration *B*, and refermentation (or Tokaj) *C* methods. They were compared in terms of chemical characteristics, including acid and phenolic profiles, and potential antioxidant properties.

The conducted analyses determined the chemical parameters of wines and their quantitative ranges, after which it was possible to ascertain which of the production methods accumulated the most bioactive compounds. The results of this study showed that the wine produced using the method with seven days maceration had stronger antioxidant and anti-radical properties compared with others.

Sensory analysis showed that wine *B*, despite its high acidity, was the most balanced. The research group selected wine *B* (modification of the passito style with a seven-day maceration of grapes) and wine *C* (Tokaj wine style five Puttonyos) as wines of very good quality. Both the chemical analyses of the wines and their sensory evaluation have demonstrated that the production of *passito* wines from hybrid grape varieties is an effective alternative to the traditional production process and can be successfully applied in cold climates. Future studies will focus on the chemical and sensory changes occurring in wine produced using various techniques during the aging process, combined with a detailed analysis of polyphenols and volatile compounds.

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