Winery On-site Assessment of Grapevine Bunch Rot: In Pursuit of Sustainable Practices

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Producer wineries are responsible for processing 75 % of South Africa's annual wine grape production. A characteristic of producer wineries is the processing of large volumes of wine grapes, thus incorporating enormous variability in grape quality, which includes the presence of grapevine bunch rots. Grapevine bunch rots are detrimental to grape and wine quality. The "Introduction" provides background information on the industrial working conditions at producer wineries and the economic effect of rot on wine production. The next two sections discuss the principal reasons for bunch rot being an inevitable part of grape production, namely cultivar susceptibility and climatic conditions. The challenges regarding grapevine bunch rot assessment, specifically concerning producer wineries or wineries processing large amounts of grapes, are set out in "Assessing rot intensity". Representative sampling, mechanical harvesting of grapes and visual evaluation are discussed from an industrial perspective. The last section of this review focuses on the quest for sustainable appraisal of grapevine bunch rot. Infrared spectroscopy (IR) could provide a sustainable option for objectively assessing rot on-site at producer wineries. However, even with the availability of plenty of spectroscopic methods and the demonstrated potential of IR spectroscopy for rapid assessment of grapevine bunch rot, these methods are yet to be applied routinely under industrial working conditions.

INTRODUCTION

Industrial scenarios at large producer wineries

Currently, South Africa has 43 producer wineries responsible for processing 75 % or 1 094 000 tonnes of South Africa's annual wine grape production (SAWIS, 2021). A producer winery, also referred to as a producer cellar, is defined as "a role player or entity where grapes are received and processed on behalf of a group of wine grape producers, its members, into wine grape products and the marketing thereof as packaged or bulk" (SAWIS, 2021). Globally, producer wineries account for one-quarter to one-third of global wine production, with well-known winery examples in France, Italy, Spain, Germany, and South Australia (Bezuidenhout, 2014).

A unique characteristic of producer wineries is the scale of production associated with processing large volumes of wine grapes. Thirty-two of South Africa's producer wineries process more than 10 000 tonnes per annum (SAWIS, 2021), but processing could amount to as much as 60 000 tonnes per annum at a single premise. Producer wineries arguably have advantages in the cost-effectiveness of operational costs due to economy of scale and market competitiveness by guaranteed volume supply to retail. However, the production of large volumes of grapes by many grape growers from commercial vineyards spread out over large surface areas creates significant logistical challenges, primarily related to grape quality control and resulting wine quality.

The producer winery's operational structure usually includes the advisory service of viticulturists, aiming to provide input on vineyard management practices at the farm level and thus manage grape quality according to desired wine style. Reviewed by Steel *et al*. (2013), the detrimental effect of grapevine bunch rots on grape berry composition and eventual wine quality is well-researched.

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Thus, rot assessments are imperative to grape quality control at producer wineries. Constraints in terms of time, cost, and trained assessors required for in-field rot assessments justify consideration of alternatively assessing grape quality at the point at which grapes are delivered on-site at the winery, also referred to as grape or winery intake. Assessing individual grape loads at winery intake would also encompass in-vineyard variability, often overlooked by in-field assessments. It is long known that rot can exist as "pockets" in vineyard blocks (Seem, 1984). Grape (load) assessment at winery intake would also be advantageous if grape transportation by road from farm to winery is needed. Extended travel time and exposure to high temperatures during transport could significantly increase the microbial activity in grape loads (Swanepoel, 2006). In the instance where microbial rot pathogens are already present on the grapes in the vineyard, these travelling conditions could further deteriorate grape quality.

Currently, the parameters assessed at winery intake include sugar concentration (ºBrix), pH, total titratable acidity (TA, measured in g/L), temperature (ºC) and visual assessment of grape loads for rot and matter other than grapes (MOG). These parameters are limited since they do not account for the detrimental chemical compositional changes in the grape berries brought about by rot. Furthermore, the reported subjectivity and biased nature of visual rot assessments (Hill *et al*., 2014) often have led to disputes between grape growers and wineries (Longbottom *et al*., 2013). Logistical challenges during harvest require that producer wineries often employ seasonal workers with minimal knowledge of/or training in the visual assessment of rot in grape loads delivered to the winery. Furthermore, assessments must be done fast and not slow down harvest-related operations, which are hectic at large producer wineries.

Considering that this final grape quality evaluation at winery intake will ascertain grape growers' income, wineries are responsible for ensuring that quality assessment is objective and accurate. However, the presence of rot also has an impact on winery profits.

Economic implications of rot on wine quality

The wide variety of grapevine bunch rots was discussed in a large body of recently published literature (Jackson, 2014; Ioriatti *et al*., 2015; Steel *et al*., 2016; Hall *et al*., 2018; Steel *et al*., 2018; Gao *et al*., 2020; Crandall *et al*., 2022; Kellner *et al*., 2022). Regarding the economic impact of rot on wine production, it is especially botrytis rot and sour rot that are of concern (Ky *et al*., 2012; Hall *et al*., 2018; Molitor *et al*., 2018). Thus, this review focuses on the deleterious botrytis rot, also referred to as grey rot and sour rot.

The economic impact of rot can already be observed during the early stages of wine production by the significant decrease in juice yield obtained from rot-affected compared to healthy grapes. Sour rot-affected Cabernet Sauvignon grapes with 30 % severity could lead to as much as a 25 % decrease in the volume of grape juice recovered for fermentation (Barata *et al*., 2011b). Calculating a theoretical scenario: One tonne of healthy grapes provides an average juice yield of 770 L (Price Waterhouse Cooper, 2013), compared to rot-affected grapes, which could decrease the volume of juice recovered per tonne of grapes by as much as 25 %. This would mean that a winery could incur an estimated loss of as much as 40 % in South African rand (ZAR) value for each tonne of rot-affected grapes received compared to healthy grapes (Table 1).

In addition, rot increases wine production costs with specific interventions needed to mitigate the effect of rot on the grape must and wine matrix. For example, the presence of laccase in grape must and wine poses a serious threat to the quality of wine produced. Laccase is an oxidising enzyme produced by *Botrytis cinerea*, the causative fungi responsible for botrytis rot. Uncontrolled oxidative reactions of phenolic compounds, catalysed by laccase, cause browning and premature ageing of wine (Claus *et al*., 2014). Processing strategies to reduce laccase activity include short heat treatment of grape must and application of oenological tannins as early as crushing (AWRI, 2023; Vignault *et al*., 2019, Claus, 2020).

Another processing difficulty with a consequent increase in production costs caused by rot, is the early clogging of filter membranes. Wine filtration removes unwanted particles from the wine and enhances its stability. Decreased filterability is associated with the increased presence of β-glucans in wines (Francioli *et al*., 1999). *Botrytis cinerea* produces β-glucans responsible for botrytis rot in wine grapes (Jackson, 2014). Remedial actions include the addition of enzymes responsible for the hydrolysis of glucans to improve the filterability of wines (Jadhav & Gupta, 2016; Espejo, 2021).

However, the detrimental effect of rot on wine quality is primarily associated with the negative impact on wines' sensory properties. Rot could have a detrimental impact on the aroma of grape must and wine due to the following reactions: The laccase-induced oxidation of phenolic aroma compounds, as earlier discussed, the production of detrimental flavours, and the hydrolysis of aromatic grape components. The latter two complications are discussed in the following paragraphs.

Meneguzzo *et al*. (2008) reported that Gewürztraminer wines made from rot-affected grapes differed significantly from healthy grapes due to the sensory detection of mould flavour and a dominating acetic character in rot-affected wines. Off-flavours regularly associated with rot-affected wines include earthy, mouldy, and fresh mushroom (Steel *et al*., 2013). Sweet-like honey off-flavours of ethyl phenylacetate and phenylacetic acid are associated with wine produced from sour rot-affected grapes (Barata *et al*., 2011a). Volatile markers reported for fungal rots include sesquiterpenes, 1,5-dimethylnaphthalene, 2-(4-hexyl-2,5-dioxo-2,5dihydrofuran-3-yl) acetic acid, m-cresol, γ-nonalactone (Schueuermann *et al*., 2019) and 2-trans-hexenal (Santos *et al*., 2022).

Besides producing unwanted sensory volatiles, rot can also induce wine aroma losses. Volatile organic compounds (VOCs) are the main contributor to wine aroma (Dudareva *et al*., 2013). Botrytis rot berry modifications could include the transformation of monoterpenes, the aromatic compounds responsible for the varietal aromas of Muscat cultivars, into less odorous compounds (Rienth *et al*., 2021). Furthermore, the disappearance of fruity aromas in young wines could be related to the hydrolysis of fatty acids' ethyl esters, which contribute to the fermentation aromas of wine by the esterase activity of *Botrytis cinerea*. Barata *et al*. (2011b) suggested that sour rot significantly affects the wine yeast's secondary metabolism, decreasing the levels of volatiles related to fatty acids and amino acid synthesis. Unwanted sensory properties in wine or the loss of varietal character could warrant wine quality downgrades with consequent economic implications for wineries (Table 1).

Furthermore, rot could also influence the food safety aspects of wine via the production of the mycotoxin, ochratoxin A (OTA) (Rousseaux *et al*., 2014; Welke, 2019; Ubeda *et al*., 2020). Classified by the International Agency for Cancer Research's (IARC) monographs (1993) in Group 2B as "possibly carcinogenic to humans", OTA levels in wine are regulated (European Commission, 2006). Selected winemaking practices could decrease OTA levels in wine, but these practices could be detrimental to the impact of wine's positive volatile compounds (Gambuti *et al*., 2005).

GRAPE CULTIVAR SUSCEPTIBILITY TO ROT

Over time, an extensive body of literature has developed on wine grape cultivars' natural differences in susceptibility to rot. As an example of ranking cultivars' susceptibility to rot, research by Pañitrur-De La Fuente *et al*. (2018) is discussed. Based on visual assessment, the in-field susceptibility of 13 wine grape cultivars naturally infected with botrytis rot was compared and classified in relation to fruit maturity under Chile and France's contrasting climate and vineyard practices. Despite differing climatic conditions and vineyard practices, cultivar susceptibility ranking on both sites was similar. High susceptibility to botrytis rot was associated with the cultivars Sauvignon blanc and Gewürztraminer, followed by Chardonnay and Pinot noir. Petit Verdot, Cabernet Sauvignon, Mourvèdre and Syrah were highly resistant to botrytis rot (Pañitrur-De La Fuente *et al*., 2018).

Cultivar characteristics regularly reported as influencing susceptibility to rot include bunch compactness (Hed *et al*., 2009; Lisek & Lisek, 2021), berry skin traits such as physical berry resistance and cuticle thickness (Herzog *et al*., 2015). Grapevine breeders consider loose bunch architecture as the most important selection criterium to improve cultivars' resistance to botrytis rot (Herzog *et al*., 2022). Bunch compactness is defined as the tightness or the spatial arrangement of berries within a bunch (Tello & Ibáñez, 2018). Figure 1 illustrates an example of the difference in

TABLE 1
Estimated loss of income (%) per tonne of rot-affected grapes.

Cultivar	Juice yield of healthy grapes (L/tonne)	Healthy grapes traded as cultivar wine (ZAR/tonne of grapes)	Juice yield of rot-affected grapes (L/tonne)	Rot-affected grapes traded as dry red/white wine (ZAR/tonne of grapes)	Loss/tonne of rot-affected grapes[4] (%)
Cabernet Sauvignon	770L[1]	ZAR7 253[3]	558L[2]	ZAR4 380[3]	-40%
Chenin blanc	770L[1]	ZAR4 543[3]	558L[2]	ZAR2 784[3]	-39%

[1]Average juice recovery (L)/tonne of healthy grapes at approximately 770L/tonne (Price Waterhouse Cooper, 2013). [2]Average juice recovery (L)/tonne of rot-affected grapes estimated at 558L/tonne (Barata *et al*., 2011b). [3]Wine price (ZAR/L): Cabernet Sauvignon – ZAR9.42/L; Chenin blanc – ZAR5.90/L; Dry Red – ZAR7.85/L; Dry White – ZAR4.99/L (SAWIS, 2022). [4]Decrease in juice yield + wine quality downgrade.

FIGURE 1
Bunch compactness of white wine grape cultivars.
Left: Healthy Colombar bunches with berries in loose contact with each other. Right: Botrytis rot susceptible cultivar Sauvignon blanc with berries not readily movable. Regions where berries are dense are indicated with white circles, and often the growth of *Botrytis cinerea* begins in these dense bunch regions because of berry injury.

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bunch compactness amongst cultivars.

Several factors contribute to dense bunches being more susceptible to rot. After flower dehiscent, compact bunches retain more flower debris between berries than more loose bunches leading to rot, for example, by harbouring *Botrytis* inocula (Hed *et al*., 2009). Insufficient development of berries' epicuticular wax between compact berries reduces resistance to pathogen attack (Marois *et al*., 1986). Berries in dense bunches press against each other as berry size increases towards maturity, which could lead to berry rupturing. This injury to the berry skin provides entry for secondary microorganisms while leaking grape juice supplies nutrients that stimulate microbial growth. Berry injuries are considered the primary source of sour rot (Hall *et al*., 2017; Ioriatti *et al*., 2018). The dense arrangement of berries could prevent fungicide spray from penetrating between berries and faster drying of bunches after rainfall, leaving a conducive environment for developing rot (Hed *et al*., 2009). However, Molitor *et al*. (2018) found that differences in clonal bunch compactness do not primarily explain the natural variation in botrytis rot susceptibility of nine White Riesling clones. The authors suggested that the general link between bunch compactness and rot susceptibility may not hold true within-cultivar, for example, for a group of clones from the same cultivar.

The berry skin is an important physical and mechanical barrier to rot. Thin-skinned cultivars are more prone to injury, providing entry for microbial colonisation and rot development. A recent study by Lisek & Lisek (2021) concluded that the resistance of cultivars to sour rot strongly correlated with increased berry skin thickness. For example, research has provided evidence that the berry skin of Ugni blanc (115 µm) is significantly thinner compared to the skins of Chardonnay (203 µm) and Viognier (243 µm) (Jin *et al*., 2017). Thin-skinned berries are also more predisposed to rot in high temperatures and high visible light vineyard environments. These conditions more easily cause skin injury to thin-skinned berries compared to thicker-skinned cultivars (Steel *et al*., 2016; Lisek & Lisek, 2021).

Berry cuticle thickness and hydrophobic epicuticular waxes on the berry outer skin also influence cultivars' rot susceptibility (Herzog *et al*., 2015). The cuticle covers the outer surface of the berry skin. In-field evaluation of naturally infected grapes showed that a thicker cuticle associated with epicuticular waxes negatively correlates with susceptibility to botrytis rot, especially for compact-bunch cultivars (Herzog *et al*., 2015).

Considering reported differences in cultivar susceptibility to rot, it is conceivable that grape growers should be advised to plant rot-resistant cultivars and clones. However, cultivar choice is a long-term and strategic investment. Over time wineries build a relationship with the retail and clients, which could include the guaranteed volume supply of specific varietal wines. Furthermore, prominent wine regions in the world are characterised by cultivar-specific production; these include Merlot from Bordeaux, (Ky *et al*., 2012), Sauvignon blanc and Sémillon from Sauternes (Kallitsounakis & Catarino, 2020), Chenin blanc from the Loire Valley, France (Carbajal-Ida *et al*., 2016), and the sought-after, unique fruity Sauvignon blanc wines from Marlborough, New Zealand (Benkwitz *et al*., 2012). These cultivars are known to be susceptible to rot (Paňitrur-De La Fuente *et al*., 2018), but due to being an economic driving force of these wine regions, they will continue to be planted for many years. Furthermore, even targeting cultivar and clone selection strategies associated with resilience to rot, climatic conditions still significantly influence the intensity of rot.

CLIMATIC INFLUENCES ON BOTRYTIS AND SOUR ROT OF GRAPEVINE BUNCHES

Botrytis rot can either manifest as unwanted grey rot, also called gray mold or just bunch rot, or as the special form of *Botrytis cinerea* infection, namely noble rot (Jackson, 2014). Noble rot is associated with the highly valued sweet white wine styles of Sauternes (Landrault *et al*., 2002) and Tokaj (Furdiková *et al*., 2019). Under controlled conditions, optimal botrytis rot development is observed at high relative humidity (≥ 90 %) and cool temperatures of 20ºC to 25ºC (Ciliberti *et al*., 2015). Very specific climatic conditions of humid nights, cool and foggy mornings, followed by dry, sunny, and windy afternoons are required for the development of noble rot (Furdíková *et al*., 2019). However, the occurrence of this rare botrytised noble rot grapes is geographically restricted (Kallitsounakis & Catarino, 2020), and under most conditions, botrytis rot is undesirable. Optimum conditions for the development of grey rot include temperatures of approximately 18ºC to 22ºC, high humidity (> 90 %), and prolonged wet conditions (Würz *et al*., 2020; Rienth *et al*., 2021). In contrast to the cool temperatures associated with botrytis rot, sour rot is favoured by hot summers (VanderWeide *et al*., 2020; Lisek & Lisek, 2021). Average monthly temperatures of approximately 18ºC during berry ripening were reported by Lisek & Lisek (2021) for the vintages having severe sour rot. Significant to inducing sour rot is rainfall from veraison to harvest (VanderWeide *et al*., 2020; Lisek & Lisek, 2021). Rainfall during berry ripening could lead to grape berry splitting. Berry injuries are conducive to the development of sour rot by providing grape juice as a suitable substrate for yeast and acetic bacteria proliferation (Ioriatti *et al*., 2018).

Different amounts and the distribution of rainfall, which lead to the increase in relative humidity and berry wetness duration, influence the intensity of rot (Becker & Knoche, 2012; Paňitrur-De La Fuente *et al*., 2018). Botrytis rot's potential infection occurrences under future European climatic conditions (2030 and 2050) were modelled using 15 years of temperature and leaf wetness duration data (Bregaglio *et al*., 2013). Mixed results emerged from the study, ranging from more than a 100 % increase in the occurrence of botrytis rot, maintaining similar levels of pressure compared to the current situation, or decreasing disease occurrences. The rise in botrytis rot relates to more humid conditions, favouring pathogen development. Bregagli *et al*. (2013) concluded that botrytis rot will remain a relevant problem to European wine production in the next 10 to 30 years. Using South African weather data from 1984 to 2015 and modelling plausible future conditions, similar heterogeneous climatic changes and conditions for the future were reported (Southey, 2022).

The published literature mainly focuses on the economic effect of a change in temperature on wine production (Ashenfelter & Storchmann, 2014). However, the effect of climate change relating to increased rainfall events during the summer months, i.e. the berry ripening period from veraison to harvest, is also of economic concern to the wine industry. These rainfall events would significantly impact the intensity of sour rot. An increase in the intensity of sour rot has already been observed in Europe (Hausinger *et al*., 2015; Lisek & Lisek, 2021). In-field observations of an increase in sour rot intensity could explain the series of recent studies on the etiology of sour rot (Ioriatti *et al*., 2015; Madden *et al*., 2017; Hall *et al*., 2018; Hall *et al*., 2019; Entling *et al*., 2019; Pinto *et al*., 2019; Gao *et al*., 2020).

Long-term strategies to lower the intensity of rot related to climatic conditions include site-specific plantings to avoid climatic conditions favourable to the development of rot. However, there is no strong indication in the literature that this strategy has been followed. For example, recent research suggests that new plantings are established under climatic conditions, knowing that botrytis rot will be problematic (Würz *et al*., 2020). Due to the dominant effect of climatic conditions on rot, it can be concluded that rot will inevitably be part of grape production.

Earlier literature reviews focused on the predisposing factors contributing to the development of rot, microorganisms associated with grapevine bunch rots, disease expression such as visual symptoms, the effect of rot on grape composition and wine chemistry, as well as vineyard management practices aimed at the control of rot (Mundy, 2008; Steel *et al*., 2013; Rousseaux *et al*., 2014; Latorre *et al*., 2015; Kallitsounakis & Catarino, 2020; Rienth *et al*., 2021; Crandall *et al*., 2022). However, grapevine bunch rot assessment from an industrial perspective has not been reviewed before. In the following sections, this review provides an industrial perspective on challenges specific to large or producer wineries concerning the assessment of grapevine bunch rots.

ASSESSING ROT INTENSITY

Before discussing the challenges relating to the accurate assessment of rot under industrial working conditions, a summary of relevant terms in the field of plant disease assessment is given. For a comprehensive glossary, the reader is referred to Bock *et al*. (2022a).

Plant disease assessment or phytopathometry concerns the visual estimation or instrument or sensor-based measurement of the amount of plant disease that usually is symptom-based (Bock *et al*., 2022a). Disease assessment focuses on detection, identification, quantification, or a combination of these objectives (Bock *et al*., 2022a). Disease intensity is a general term used to describe the amount of disease present in a population (Nutter, 1991). It can be defined by the terms prevalence, incidence, or severity (Bock *et al*., 2022a). In earlier research, prevalence, incidence, and frequency were often used as synonyms (Seem, 1984). However, incidence is the proportion or number of the whole of disease entities within a sampling unit, and prevalence is the proportion of diseased plots or fields in a defined area (Bock *et al*., 2022b). On the other hand, severity measures the quantity of disease within the sampling unit (Bock *et al*., 2022b).

Compared to prevalence or incidence, severity is more evident in research describing rot intensity on wine grape cultivars. The reason could be that as severity increases, the detrimental effect on wine quality also increases (Zoecklein, *et al*., 2000; Barata *et al*., 2011b; Steel *et al*., 2018). Globally, visual assessment is still the reference method to determine grapevine bunch rot intensity in-field, which could include visually estimating the percentage of berries infected per bunch, the percentage of tissue infected per bunch, or severity estimations according to scales (Hed *et al*., 2009; Hill *et al*., 2013; Molitor *et al*., 2020; Steel *et al*., 2020). Rot intensity assessment of grape loads at winery intake includes visual detection of rot-affected bunches, and/or calculating the severity by percentage of weight of rot in the sample. (Durgun, 2010). Bock *et al*. (2022a) strongly advised clearly defining how a disease assessment term is applied under specific industrial conditions. Wineries often impose a price penalty if rot intensity exceeds a certain threshold (Hill *et al*., 2014). Imposing price penalties could severely reduce grape growers' income; accordingly, rot intensity assessments must follow a standard protocol and ensure repeatability, objectivity, and accuracy. The next section will discuss three challenges concerning rot intensity assessment from an industrial perspective. These challenges include representative sampling, mechanical harvesting of grapes and visual assessment of rot.

Challenges relating to rot intensity assessment under industrial working conditions

Trustworthy assessment stems from representative samples (Wagner & Esbensen, 2015). The reality of variability within a vineyard is well-known (Bramley *et al*., 2011), which also applies to the variability of disease intensity within a single vineyard block. Clumping or pockets of rot within a single vineyard block is not uncommon, and rot intensity could differ between parts in the same vineyard block (Evans *et al*., 2010). Under industrial conditions, the sample size, and the spatial distribution from which the sample is collected, should be representative of the vineyard block if the assessment is executed at vineyard level; or the grape load which is assessed at winery intake. Non-representative sampling procedures may lead to invalid results.

Accuracy, or the lack thereof, in vineyard sampling comes with an increasing cost for both grape growers and wineries. Theoretically calculated, Hill *et al*. (2019) estimated that annual crop losses/ha could be as high as NZ$2 578 (approximately ZAR27 000/ha) due to incorrect estimates of botrytis rot severity in vineyards. The cost concerning the time spent and human resources needed to accurately assess many vineyard blocks in a large producer winery's setup could render in-vineyard assessment unsustainable. Interpolating vineyard blocks from small samples taken within the specific vineyard block was proposed by Hill *et al*. (2019) to reduce the amount of sampling. Assessing rot intensity at winery intake could provide an economical substitute for in-vineyard checks. However, mechanisation of the grape harvesting process confronts winery intake visual assessment with structural integrity loss of bunches

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(Durgun, 2010), which obscures the presence of rot.

When grapes are machine-harvested, mechanical actions are exerted on grapevines to remove the berries from the stalks. Grape bunches and berries lose their structural integrity due to the mechanical forces exerted on them. Grape loads delivered to the winery consist of a shapeless mass of juice, berry skins and pulps called grape mash; see Figure 2 for a visual demonstration of the effect of mechanical harvesting on grape bunches. However, regarding the sampling strategy of grape loads at winery intake, the sample size of machine-harvested grape loads could be smaller compared to hand-harvested loads. Marois *et al*. (1993) found that hand-harvest grape loads had a more aggregated distribution of *Botrytis cinerea* than machine-harvested grape loads, which had a more uniform distribution of *Botrytis* antigen.

In addition to the above two challenges, objective visual assessment proves to be difficult. For a review of the sources of error related to the visual assessment of plant diseases Bock *et al*. (2010) can be consulted. Under industrial working conditions, subjectivity can intensify by using seasonal employees without prior training or experience with visual rot assessments, as is often employed in large wineries. Several studies have demonstrated that experienced assessors estimate severity more accurately (Bock *et al*., 2022b).

Aside from the subjectivity and bias associated with visual assessment, this method does not fully capture the unseen chemical berry composition changes induced by rot. In the event of latent rot, visual symptoms would not yet have manifested, but chemical changes to the berry have already started to occur (Versari *et al*., 2008). It is also possible that an extended rot period increased the concentration of rot-associated grape berry metabolites compared to a shorter infection period. Still, visually berries are assessed with the same intensity (Fischer & Berger, 2007).

Since early investigations, such as Berg's *et al*. (1958) study into a practical grading system for defect detection at winery intake, efforts are still ongoing in the quest to improve the objectivity and speed of rot intensity assessments. The following section critically reviews the industrial application of sustainable rot assessment in the last 15 years, from 2007 to 2022.

THE QUEST FOR SUSTAINABLE ASSESSMENT OF GRAPEVINE ROT IN THE FOURTH INDUSTRIAL REVOLUTION

Sustainability has three core concepts, namely economic, environmental, and social sustainability (Contini & Peruzzini, 2022). Characteristic of the current fourth industrial revolution (Industry 4.0) is digitisation and automation in the manufacturing environment aiming to increase sustainability (Ortt *et al*., 2020; Hassoun *et al*., 2023). The vine and wine sectors also focus on digitisation to increase efficiency, productivity, transparency, value proposition and new business models, and sustainability (OIV, 2021).

Initiatives for sustainable development in the vine sector were launched in recent years. Examples of this inventiveness applied under industrial working conditions include the use of the normalised difference vegetation index (NVDI) that characterises in-vineyard variability (Best *et al*., 2015; Pañitrur-De la Fuente *et al*., 2020), and crop protection modelling (Molitor *et al*., 2020) which use meteorological conditions to predict the risk of rot.

Sustainable development in wine grape production also embraces replacing wet chemistry methods with non-destructive technologies such as infrared (IR) spectroscopy to quantify grape compounds rapidly (Swanepoel *et al*., 2007; Petrovic *et al*., 2020; Ferrer-Gallego *et al*., 2022). Infrared spectroscopy methods contribute to all three of the core sustainable concepts. Concerning the economic concept, accuracy, speed, and simplification of IR analysis methods can provide rapid results for actionable operational insights. Instrumentation and so-called global calibration models for the prediction of grape compounds are also available at a reasonable cost. Environmentally, IR methods reduce the use of harsh chemicals and thus also reduce chemical waste. Socially, since less or no chemicals are used during analysis, operators' health and safety are also considered. It is also possible for non-specialist operators to use spectroscopy instrumentation (Marcinkowska *et al*., 2019) as part of routine applications.

The above sustainable development initiatives indicate that the vine sector may be receptive to decision support using technological interventions. Infrared spectroscopy methods could provide options for sustainable rot assessment, and grape production can notably benefit from rot assessment decision support (Porep *et al*., 2014; Schmidtke *et al*., 2019). However, hurdles need to be cleared to achieve automated rot assessment including ensuring representative sampling, data analytics for actionable insights and winery decisions on sorting rot-affected grapes into different production streams.

Evaluating the progress towards this stance, published studies in the last 15 years, from 2007 to 2022, on the use of IR spectroscopy for grapevine bunch rot assessments in wine grapes are critically reviewed from an industrial perspective (Table 2).

Overview of published studies (2007 to 2022) using infrared (IR) spectroscopy for grapevine bunch rot assessment

Quantifying rot intensity was the focus of earlier IR studies (Versari *et al*., 2008; Durgun, 2010; Hill *et al*., 2014). In these studies, Fourier-transform mid-infrared (FTIR-MIR) showed an ability to predict severity (%) with $R^2 = 0.8$ (Versari *et al*., 2008) and $R^2 \geq 0.9$ (Durgun, 2010; Hill *et al*., 2014). Near infrared (NIR) spectroscopy also demonstrated potential for quantifying rot intensity ($R^2 = 0.9$). However, for the ease of in-field industrial application, Hill *et al*. (2014) recommended the use of digital image analysis instead of IR spectroscopy techniques. Digital image analysis does not require additional equipment for in-vineyard assessments and could save time. Still, emphasising the previously discussed challenges of rot assessment for producer wineries, the time implications of in-vineyard assessments are not sustainable. However, with an RPD of 2.5 and 2.0 for NIR and FTIR-MIR severity (%) predictions, respectively (Hill *et al*., 2014), these methods show potential for screening purposes at winery intake. Hill *et al*. (2014) noted that compared to visual assessments, both the beforementioned IR techniques appear to be more accurate at predicting severity ≥ 50 %, and considerable non-linearity was observed in low severities of ≤ 25 %.

FIGURE 2
Mechanical harvesting of wine grapes.
Left: Grape bunches still intact on the grapevine before mechanical harvesting. Middle: When grapes are machine-harvested, mechanical actions are exerted on grapevines to remove the berries from the stalks. After mechanical harvesting only the stalks of the grape bunches are left on the grapevine. Interestingly, the brown stalks indicated by a white circle, represent the bunches with rot, compared to the green stalk of a healthy grape bunch. Right: Machine-harvested grapes are delivered to the winery as a shapeless mass of juice, berry skins and pulps called grape mash.

Infrared spectroscopy techniques were especially explored for the detection of rot (Table 2). Detection of grapevine bunch rot could be achieved by following either of two strategies, namely detecting an increase in the concentration(s) of rot-associated disease marker(s) (Versari *et al*., 2008; Hausinger *et al*., 2015; Porep *et al*., 2015a; Porep *et al*., 2015b; Gelhken *et al*., 2022), or discriminating between rot-affected and healthy grapes (Beghi *et al*., 2017; Giovenzana *et al*., 2017; Giovenzana *et al*., 2018). The compounds in the beforementioned studies that were targeted to support the rapid detection of rot by increased concentrations include gluconic acid and glycerol (Versari *et al*., 2008; Hausinger *et al*., 2015; Porep *et al*., 2015a), acetic acid (Porep *et al*. 2015a), the fungal sterol, namely ergosterol (Porep *et al*., 2014; Porep *et al*., 2015b), as well as VOC's (Gelhken *et al*., 2022). Interestingly, the classification studies of Giovenzana *et al*. (2018) and Beghi *et al*. (2017) only aimed at discriminating between healthy and rot-affected grapes, which will be downgraded by the winery for high-quality vinification. From an industrial perspective, a two-class grading system could be limiting, especially in a year with extreme weather conditions conducive to rot development. A third group of "classified as infected, but in need of winemaking remedy" is probably a more realistic situation at a producer winery.

The identification of rot pathogens using IR spectroscopy has only recently been explored (Table 2). Although Giovenzana *et al*. (2018) did report visual spectral differences between grape must infected with botrytis compared to sour rot, no classification modelling was performed. Schmidtke *et al*. (2019) were the first to explore the use of IR techniques in combination with classification modelling for identifying rot pathogens in grape must. It should be noted that the latter study was conducted on a small sample set consisting of only 30 samples per pathogen. Furthermore, grape berries were inoculated with pure cultures; thus, identification of these rot pathogens will have to be tested with naturally infected berries (Schmidtke *et al*., 2019). Although the results from this study are promising for identifying rot pathogens, pathogen identification does not provide an answer to the amount of rot present. Knowing the intensity of rot is crucial to guide mitigation practices in wineries.

In addition, some of the reviewed studies listed in Table 2 lack industrial robustness, for example by using only a small sample size per parameter that was modelled. For optimal results with calibration modelling, more than 75 samples might be required (Versari *et al*., 2008). Furthermore, variation in the sample set must encompass different cultivars, vintages, climatic conditions and more than one type of rot. Rot rarely exhibits a single form under natural conditions. An example of lacking robustness for industrial application could be drawn from Durgun (2010) who explored the use of FTIR-MIR and Raman spectroscopy for rot quantification in the severity range of 0 % to 5 %. Even though this rot intensity range is likely to result in the downgrading of grapes at winery intake, it does not represent the observed in-field severities. Thus, this model would be limiting in sorting grapes into different production streams within the winery. Robust models can perform measurements in the various scenarios encountered under industrial conditions (Giovenzana *et al*., 2018).

Near-infrared spectroscopy features strongly in rot assessment studies (Table 2). Earlier studies did explore the potential of transmittance FTIR-MIR spectroscopy for rapid detection of rot (Versari *et al*., 2008; Durgun, 2010). However, the sample preparation requirements for transmission FTIR-MIR could be a drawback for rapid analysis at winery intake. Attenuated total reflectance mid-infrared (ATR-MIR) spectroscopy, which requires minimal or no sample preparation for analysis, could fulfil the requirements for routine application at winery intake. Schmidtke *et al*. (2019) is the only study exploring the potential use of ATR-MIR in rot assessments.

Importantly, spectroscopy methods are coupled with multivariate data analysis (MVDA) techniques, such as partial least square (PLS) regression and partial least square discriminant analysis (PLS-DA) (Table 2). Spectroscopic methods lead to the generation of large volumes and a variety of data, also referred to as big data (Simsek *et al*., 2019). Although information-rich, this spectral data needs to be transformed into decision-support tools relevant for the problem at hand. The process of extracting information from these datasets is called data mining (Provost & Fawcett, 2013). However, the decision-support information needed

TABLE 2

Published studies in the last 15 years (2007 to 2022) that focussed on the use of infrared (IR) spectroscopy in grapevine bunch rot assessment.

References Countries where research was conducted	Cultivars	Sample numbers	Sample matrix	Rot pathogen	Rot assessment objective	IR technique MVDA	Spectral range	Reference method used for rot assessment	Value for industrial decision support
Gelhken *et al*. (2022) Germany	n = 20 Red and white cultivars	n = 725 For individual regression modelling of grape aroma compounds sample numbers ranged from 47 to 725	Naturally infected grape mash collected at winery intake	n/a	Detection	Vis/NIR (reflectance) PLS	400 to 1700 nm	GC-MS	Rapid, easy, and simultaneous quantification of aroma quality of wine grapes which could relate to rot .
Schmidtke *et al*. (2019) Australia	n = 1 White: Chardonnay	n = 120 30 samples for each of four fungal rot pathogens	Grape berry juice from inoculated fresh berries	*Aspergillus niger, Aspergillus carbonarius, Botrytis cinerea, Pencillium expansum*	Identification	ATR-MIR SIMCA, SVR, KNN, RFM	1850 to 375 cm^{-1}	Pure fungal cultures analysed using ATR-MIR	Potential rapid discrimination between *Aspergillus* spp., *Botrytis cinerea*, and *Penicillium expansum.*
Giovenzana *et al*. (2018) Giovenzana *et al*. (2017) Italy	n = 15 Red and white cultivars	n = 159 Healthy = 98; Diseased = 88; of which *Botrytis cinerea* = 23; Powdery mildew = 15; Sour rot = 23.	Naturally infected grape must collected by auger from grape load at winery intake	*Botrytis cinerea*, sour rot	Detection	Vis/NIR (reflectance and transmittance) PLS-DA	400 to 1650 nm	Visual rot assessment of grape bunches: Infection level 0 (healthy) versus infection level 1 (extended colonies of > 2 mm with evident grey-whitish powdery appearance and brown senescent-necrotic lesion)	Potential classification of diseased and healthy samples
Beghi *et al*. (2017) Italy	n = 7 Red and white cultivars	n = 2 559 Healthy = 1 235; Diseased = 1 324; of which *Botrytis cinerea* = 684; Powdery mildew = 395; Sour rot = 245							

TABLE 2 (CONTINUED)

References Countries where research was conducted	Cultivars	Sample numbers	Sample matrix	Rot pathogen	Rot assessment objective	IR technique MVDA	Spectral range	Reference method used for rot assessment	Value for industrial decision support
Hausinger *et al*. (2015) Germany	n = 4 Red: Pinot noir White: Riesling, Müller-Thurgau, Pinot gris	n/a	Naturally infected grape must	*Botrytis cinerea*	Detection	Optical grape sorter	Optical grape sorter with an integrated high-speed red, green and blue camera system and an infrared laser (765 nm)	Enzymatic determination of gluconic acid and glycerol concentrations	Automated optical grape-sorting gave successful separation between healthy and rot-affected berries which could help to ensure and improve wine quality
Porep *et al*. (2015a) Germany	n = 12 Red and white cultivars	n = 1 160 Red = 698; White = 462	Grape mash collected after destemming and crushing at winery	n/a	Detection	Vis/NIR (reflectance) PLS	Vis: 450 to 850 nm NIR: 1050 to 1650nm	Routine wet chemistry methods: enzymatic, automatic titrator, density meter, pH meter	Vis/NIR potential tool for rapid, multifactorial, and holistic assessment of grape chemical composition. PLS calibration models for 10 grape parameters were built
Porep *et al*. (2015b) Germany	n = 5 Red: Dornfelder Pinot Meunier Trollinger Lemberger Schwarzriesling White: Müller-Thurgau	n = 177	Grape mash collected after destemming and crushing at winery	n/a	Detection	Vis/NIR (reflectance) PLS	400 to 1800 nm	HPLC-UV analysis for determination of ergosterol content	Vis/NIR applied as a rapid tool for online determination of ergosterol under industrial working conditions

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TABLE 2 (CONTINUED)

References Countries where research was conducted	Cultivars	Sample numbers	Sample matrix	Rot pathogen	Rot assessment objective	IR technique MVDA	Spectral range	Reference method used for rot assessment	Value for industrial decision support
Porep *et al*. (2014) Germany	n = 6 Red and white cultivars	n = 168	Grape mash from naturally infected grapes, as well as mash from grapes infected with pure mould strains	n/a	Detection	Vis/NIR (reflectance) FT-NIR (reflectance) PLS	400 to 1800 nm 650 to 2500 nm	Reference analysis of: Ergosterol - HPLC; laccase activity - colourimetric assay; glucose, fructose, gluconic acid, malic acid, glycerol, acetic acid, ethanol - enzymatic UV test kits; titratable acidity - automatic titrator; tartaric acid - Rebelein method; pH - pH-meter, density – density meter	Rapid quantification of rot-associated disease markers with prediction results ranging from differentiation between low and high concentrations, rough estimates or semi-quantitative, to quantitative predictions of glycerol and ergosterol
Hill *et al*. (2014) Australia	n = 1 White: Riesling	n = 66	Naturally infected grape bunches and berries	*Botrytis cinerea*	Quantification	NIR (reflectance) MIR (reflectance) Digital image analysis	NIR: 1260 to 1370 nm MIR: 1142 to 1050 cm^{-1} Digital image analysis: Hue distribution information is used to determine hue range attributable to diseased and healthy bunch tissue	Visual rot assessment – estimation of severity (%)	Suitable alternatives for quantification of botrytis rot severity in white wine grapes

TABLE 2 (CONTINUED)

References Countries where research was conducted	Cultivars	Sample numbers	Sample matrix	Rot pathogen	Rot assessment objective	IR technique MVDA	Spectral range	Reference method used for rot assessment	Value for industrial decision support
Durgun (2010) California	n = 2 Red: Zinfandel White: Chardonnay	n = 174 Zinfandel = 66; Chardonnay = 108	Grape must from inoculate berries	*Aspergillus niger, Penicillium italicum, Rhizopus stolonifer*	Quantification	FTIR-MIR (transmittance) Raman PLS	MIR: 3044 to 1887 cm^{-1} and 1555 to 950 cm^{-1} Raman: 3400 to 200 cm^{-1}	Healthy and mouldy homogenates were mixed in weight:weight (%) to obtain severities 0.5%, 1%, 2%, 3%, 4%, 5%	MIR (transmittance) and Raman spectral analysis showed promise for objective quantification of grapes' mould content MIR models contained white and red cultivars
Versari *et al.* (2008) Italy	n = 2 Red: Sangiovese White: Trebbiano	n = 320 Hand-picked in vineyard = 200; Mechanically sampled from grape loads at winery intake = 120	Naturally infected grape must	*Botrytis cinerea*	Detection Quantification	FTIR-MIR (transmittance) PLS	Three spectral regions: 2971 to 2435 cm^{-1}; 2280 to 1717 cm^{-1}; 1543 to 965 cm^{-1}	Visual rot assessment HPLC analysis of gluconic acid and glycerol concentrations	Rapid method for the simultaneous measurement of the rot-associated disease markers gluconic acid and glycerol. Models contained white and red cultivars. MIR (transmittance) suitable for process control

ATR-MIR = attenuated total reflectance mid-infrared. FTIR-MIR = Fourier transform mid-infrared. GC-MS = gas chromatography-mass spectrometry. HPLC = high performance liquid chromatography. HPLC-UV = high performance liquid chromatography-ultra-violet spectroscopy. IR = infrared. KNN = k-nearest neighborhood. MIR = mid-infrared. MVDA = multivariate data analysis. n = total number of cultivars or samples. n/a = not available. NIR = near-infrared. PLS = partial least square. PLS-DA = partial least square discriminant analysis. RFM = random forest model. Vis/NIR = visible/near-infrared. SIMCA = soft independent modelling of class analogies. SVR = support vector regression.

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for industrial implementation will be revealed only if MVDA data are combined with problem-specific interpretation (Esbensen & Julias, 2009). For an introduction to the MVDA methods coupled with spectroscopy in wine and grape analysis, the reader is referred to Cozzolino *et al*. (2009) and Musingarabwi *et al*. (2016).

CONCLUSIONS

Grapevine bunch rots are an inevitable part of grape production. They force attention to the unique challenges of producer wineries where large volumes of grapes need to be assessed for their health status in a relatively short harvest period. The need for rot assessment as part of grape quality evaluation relates to rot's detrimental effect on grape composition and wine quality. Inaccurate rot assessments lead to a loss in income, both for grape growers and wineries. Although grape cultivars naturally differ in susceptibility to rot, climatic conditions have overriding influences on the intensities of encountered infections.

From the published articles on the use of IR methods for grape rot assessments (Table 2), it is evident that the studies predominantly aimed at the detection of rot by rapid quantification of rot-associated disease markers. However, the first step towards the detection of rot at winery intake using rot-associated disease markers would require the identification of disease marker threshold values for rejection.

Infrared spectroscopy may provide sustainable options for rapid and objective rot assessment for routine application at producer wineries. The potential use of ATR-MIR for rapid detection and quantification of rot under industrial working conditions is unexplored. Furthermore, MVDA featuring discriminant analysis for quantifying the severity (%) has not been done before.

Compared to the fruit and vegetable sectors' use of decision support by automated analyses (Giovenzana *et al*., 2015), the vine and wine sector fail to keep pace. Creating an example for practical application and overcoming practical challenges would benefit sustainable practices in the wine industry.

However, the first step for industrial implementation would be based on having a standardised tool to assess rot at winery intake. As discussed by Hill *et al*. (2014), using a standardised alternative method for rot assessment would depend on the goal. The question arises whether the alternative method must predict severity (%) directly comparable to visually estimated severity (%)? Thus, to be used as a threshold level upon which penalties would be implied? Or is the goal to find an alternative assessment method that removes the subjectivity of visual assessment regardless of the variable used? The latter is a more generalised strategy and advantageous for implementation under different industrial working situations. The next step would be for wineries customising the assessment method for example, per cultivar and even per cultivar per quality goal. Following this stage, would be aligning process flows for an increase in the potential value of wine by sorting grapes according to health status into different production streams.

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