Comparison of quantification methods for *Botrytis cinerea* biomass in grape bunches

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Introduction and aims

- *Botrytis cinerea* bunch rot (grey mould) can result in significant crop loss particularly when rain falls close to harvest.
- Wine made from grey mould affected bunches potentially has unwanted mouldy and earthy characters.
- Quantifying *B. cinerea* biomass in grape bunches has been problematic, especially for in-field applications.
- This study aimed to compare accuracy and efficiency of several quantification methods for *B. cinerea* biomass in grape bunches.

Methods and materials

- Red table grape bunches (18.4 °Bx) were inoculated with *B. cinerea*.
- Bunches were incubated at room temperature (~22°C) for 2 to 5 days.
- Different levels of infection were visually categorised.
- Hyperspectral images were taken with a Specim FX10 hyperspectral camera.
- The fungal biomass of the infected bunches was estimated by measuring the fungal sterol, ergosterol.
- Further analysis of the bunches for organic acids and by loop-mediated isothermal amplification (LAMP) and quantitative Polymerase Chain Reaction (qPCR).

Results

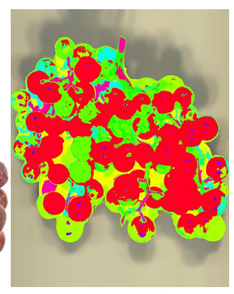
Visual and hyperspectral imaging scoring

Infection percentage for hyperspectral images was calculated as infected area pixel count over the sum of infected area pixel count and healthy area pixel count.

Incubation time (days)	Visual scoring (%)*	Hyperspectral image scoring (%)*
2	0	0.25
3	3	5.19
4	12	7.77
5	10	7.17

Note: * Adjusted results with subtraction of controls

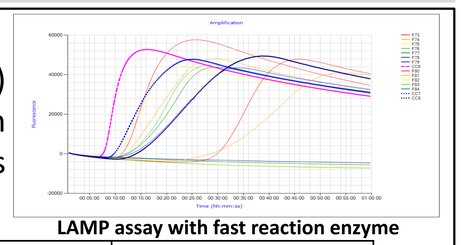
Visual scoring



Hyperspectral image scoring

LAMP and qPCR

Two types of LAMP reaction enzyme (fast and slow) were tested, which results in different detection time and detection sensitivity. Taqman probe was used in the qPCR assay.



LAMP assay with fast reaction enzyme

Incubation time (days)	LAMP slow (T_d[1])	LAMP fast (T_d)	qPCR (CT[2])
2	Not detected	26.12	36.76
3	38.76	20.19	33.70
4	38.60	14.41	31.40
5	43.63	15.15	29.29
Control	Not detected	Not detected	Not detected

Note: 1. T_d=Adjusted time of detection in minutes; 2. CT= Cycle threshold

Ergosterol measurement

Ergosterol was extracted through a liquid-liquid extraction procedure and quantified via reverse-phase HPLC. A conversion factor was used to calculate *B. cinerea* fungal biomass.

Incubation time (days)	Ergosterol (mg/kg)	Biomass* (g/kg)
2	0.12	0
3	0.21	0.04
4	0.50	0.09
5	0.70	0.11

Note: * Adjusted results with subtraction of controls

Organic acid measurement

Gluconic, acetic and malic acid were measured using enzymatic kits through a Konelab Arena 20 selective chemistry analyser.

Incubation time (days)	Gluconic acid (mg/L)	Acetic acid (g/L)	Malic acid (g/L)
2	not detected	BDL	2.209
3	not detected	BDL	2.269
4	BDL*	BDL	2.211
5	BDL	BDL	2.335
Control	BDL	BDL	2.429

Note: * BDL= Below the designed determination limit of the enzymatic kit, Gluconic acid: <50mg/L, Acetic acid: <0.04g/L.

Cross-comparison

	Advantages	Disadvantages
Visual	Very fast.	Subjective. Difficult to maintain consistency. Limited to superficial infections. Semi-discriminative.
Hyperspectral imaging	Fast (takes minutes for each image when an image fragment library can be applied universally).	Difficult to establish universal image fragment library due to colour changes caused by different varieties, maturity and climatic conditions. Difficult to apply on dark colour grape varieties due to similarity in skin and infection colour. Limited to superficial infections. Semi-discriminative.
Ergosterol measurement	Quantitative. Sensitive to low infection.	Very slow (takes days). Non-discriminative. Limited to measuring total fungal biomass.
Organic acid measurement	Fast (takes several minutes for each sample).	Highly restricted by enzymatic kit determination limits especially when infection levels are low. Non-discriminative.
LAMP	Medium fast (takes less than 30 minutes from sample preparation to final result when using the fast reacting enzyme). Highly discriminative. Highly sensitive even to very low infection. Portable instrument available.	Semi-quantitative. Difficulty in separating different infection levels due to the nature of its chemistry. High cost for portable instrument.
qPCR	Quantitative. Highly accurate. Highly discriminative. Highly sensitive even to very low infection.	Takes around 1.5 to 2 hours to perform. Difficult to apply in field due to requirements of sophisticated non-portable instruments and computer programs.

Conclusion

- The qPCR assay is still the gold standard in quantification of fungal contamination in harvest fruit. However, it is still hard to be carried out quickly and in field conditions.
- The LAMP assay can be applied quickly and easily in field conditions to detect *B. cinerea* contamination in harvest fruit. Despite its semi-quantitative nature, its detection sensitivity to different level of contaminations can be carefully adjusted via different combinations of reaction enzymes and reaction conditions.
- The accuracy and efficiency of visual and hyperspectral imaging methods are heavily limited by the natural characteristics of harvest crop.
- Ergosterol measurement provides an indication of the total fungal biomass present on the harvest fruit.

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