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Overall efficacies of combined measures for controlling grape bunch rot can be estimated by multiplicative consideration of individual effects

Research note

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Abstract

Aims: The present analyses aimed at evaluating the performance of two models for estimating the overall effect of combining two or more measures (leaf removal, cluster division, late shoot topping, botryticide application, bioregulator application) for controlling grape bunch rot based on the efficacy of the individual measures.

Methods and results: Field trials with the white *Vitis vinifera* cultivars Pinot gris and Riesling on the efficacy of three bunch rot control measures applied either alone or in combination were analyzed. Bunch rot disease severities prior to harvest were assessed and efficacies were calculated for each treatment. Observed efficacies of single measures were used to estimate the overall efficacies of all possible measure combinations. Calculated efficacies matched observed efficacies more accurately when assuming multiplicative interaction among the individual measures ($R^2 = 0.8574$, $p < 0.0001$; average absolute deviation: 7.9%) than in case of assuming additive effects ($R^2 = 0.8280$; average absolute deviation: 14.7%).

Conclusions: The multiplicative approach assumes that each additional measure is affecting (in case of efficient measures: reducing) the disease severity level as the result of the additional treatments rather than compared to the disease severity level in the untreated control.

Significance and impact of the study: The high goodness of fit as well as the observed low deviations between the estimated and the observed efficacies suggest that the multiplicative approach is appropriate for estimating the efficacy of combined viticultural measures in a complex practical bunch rot control strategy assembled of different modules.

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Introduction

Botrytis bunch rot caused by *Botrytis cinerea* Pers.:Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) is a major fungal disease of grapevine causing severe economic damage worldwide (Kassemeyer and Berkelmann-Löhnertz, 2009; Wilcox *et al.*, 2015). Common bunch rot control strategies were traditionally based on the application of fungicides with known activity against *B. cinerea* (botryticides). Substantial evidence for a loss of fungicide efficacy due to resistance in *B. cinerea* was reported on several occasions [see for instance Leroch *et al.* (2011) or Walker *et al.* (2013)], illustrating the need for better fungicide resistance management and alternative control strategies beyond fungicides. In recent years, the high efficacy of non-chemical crop cultural measures such as pre-flowering or early post-flowering leaf removal in the cluster-zone (Molitor *et al.*, 2011a; Poni *et al.*, 2006; Poni *et al.*, 2008; Sternad-Lemut *et al.*, 2015), cluster division (Molitor *et al.*, 2012), late first shoot topping (Molitor *et al.*, 2015a), artificial shading (Basile *et al.*, 2015), leaf anti-transpirants (Intrieri *et al.*, 2013) and flower debris removal (Jaspers *et al.*, 2016; Molitor *et al.*, 2015b) has been demonstrated in different regions. The success of individual disease control measures is usually measured as first described by Abbott, 1925).

Being aware of the significant impact of annual meteorological conditions on bunch rot epidemics (González-Domínguez *et al.*, 2015; Molitor *et al.*, 2016), an estimation of the overall degree of control efficacy of complex strategies built on a set of modules is of interest for the annual bunch rot control strategy. Furthermore, it is crucial to avoid combining control methods that may act in an antagonistic manner with regard to the overall efficacy.

Ostensibly, a straightforward approach for estimating the cumulative efficacy ($E_{ab\ldots x}$) of combined measures would be the accumulation (additive consideration) of the efficacies (E) of each single measure. This approach might deliver an acceptable estimation of the real efficacy at low efficacy levels and/or low numbers of measures combined. However, there is an obvious limitation to this approach at high efficacy levels and/or in case of high numbers of measures combined: the overall efficacy cannot, by definition, exceed 100%. However, theoretical efficacies above 100% might be reached when accumulating efficacies of several single measures. Furthermore, combining several control measures in other pathosystems indicated multiplicative rather than additive effects of combining control measures (Blandino *et al.*, 2012; Edwards, 2004).

Consequently, we hypothesize that the efficacy of bunch rot control strategies combining two or more measures could be more correctly estimated based on the multiplicative consideration of the efficacies of single measures than based on additive consideration.

To test this hypothesis, three field examinations on the efficacy of three single non-chemical and/or chemical measures to control bunch rot as well as of all possible combinations of these measures were conducted and analyzed in the white *Vitis vinifera* L. cultivars Pinot gris and Riesling in the years 2009 and 2015 in Luxembourg.

Materials and methods

1. Vineyard sites and experimental design

Field investigations were carried out in the years 2009 and 2015 in the Luxembourgish Moselle Valley on the white *Vitis vinifera* L. cultivars Pinot gris and Riesling. Experimental vineyards were described in detail before [vineyard A (Pinot gris, Ahn): Molitor *et al.* (2011b); vineyards B (Pinot gris, Remich) and C (Riesling, Remich): Molitor *et al.* (2015a)].

Fungicides with efficacy against *Plasmopara viticola* and *Erysiphe necator* were applied at 10- to 12-day intervals. No fungicides with known activity against *B. cinerea* were used. Each experiment was conducted as a randomized block design with four replicates of eight to twelve vines per plot.

Treatments, precise dates of applications and the developmental stages of the grapevines recorded according to Lorenz *et al.* (1995) are summarized in Table 1. Treatments consisted of bioregulator application (Regalis®; active ingredient: prohexadione-Ca; application dose: 1500 mL ha^{-1}), botryticide application (Teldor®; active ingredient: fenhexamid; application dose: 1600 g ha^{-1}) or leaf removal in the cluster-zone on the north or east exposed sides of each row. For a precise description of the implementation of the treatments of trial A, see Molitor *et al.* (2011b). Assessment data of trial A have partly been published before (Molitor *et al.*, 2011b).

Field trials B and C were performed specifically for the present analyses. Here, in treatments 3, 4, 7 and 8 two to four leaves were removed (depending on the number of clusters per shoot) in the cluster-zone. Vertical cluster division eliminating the lower part (approximately 50%) of each cluster took place in treatments 2, 4, 6 and 8 (for exact dates see Table 1).

In treatments 1 to 4, the first shoot topping was realized at BBCH 71-73 (P. gris) or 71 (Riesling) on 25/06, while in treatments 5 to 8 late first shoot topping took place 22 days later [17/07; BBCH 79 (P. gris) or 77-79 (Riesling)].

2. Assessment of the final *B. cinerea* disease severity

The final *B. cinerea* disease severities were assessed visually directly prior to harvest according to the EPPO guideline PP1/17 classifying disease severities in seven classes (0%; 1-5%; 6-10%; 11-25%; 26-50%; 51-75%; 76-100%) by examining 100 randomly selected clusters per plot (50 on each side of the row) as described before (Molitor *et al.*, 2015a).

3. Statistical analysis

Data sets consisting of average disease severities per plot were analyzed for the treatment effects by one-way ANOVAs using SPSS Statistics 19 (IBM, Chicago, IL, USA) after testing Gaussian distribution and homogeneity of variances. In case the null-hypothesis was rejected ($p \leq 0.05$), multiple comparisons according to Tukey were performed.

Efficacies were calculated according to equation (1) as defined by Abbott (1925).

$$E = \frac{DS_{control} - DS_{treatment}}{DS_{control}} = 1 - \frac{DS_{control}}{DS_{treatment}} = 1 - R \quad (1)$$

E= efficacy
DS= disease severity
R= disease severity relative to control

Based on the efficacies of single measures [calculated according to equation (1)], expected efficacies for combined measures were computed by:

a. assuming additive effects according to equation (2)

$$E_{1,2...n} = E_1 + E_2 + ... + E_n = (1 - R_1) + (1 - R_2) + ... + (1 - R_n) \quad (2)$$

E= efficacy
R= disease severity relative to control

b. assuming multiplicative effects according to equation (3):

$$E_{1,2...n} = 1 - (R_1 x R_2 x ... x R_n) = 1 - [\ (1 - E_1) x (1 - E_2) x ... x (1 - E_n) \quad .(3)$$

E= efficacy
R= disease severity relative to control

Estimated efficacies were compared with observed efficacies. Deviations (Δ) between the observed ($E_{obs.}$) and estimated efficacies ($E_{est.}$) were calculated for each combination of measures in all three trials and for both approaches.

Absolute deviations ($\Delta_{abs.}$) (representing absolute values of deviations) were determined. Coefficients of determination (R^2) of linear regressions between estimated and observed efficacies were computed. Average values of deviations and absolute deviations were calculated for each trial. In addition, global averages of deviations and absolute deviations (representing averages of the data of all three trials) were computed. In case of multiplicative considerations, the ratio between observed ($E_{obs.}$) and estimated efficacies ($E_{est.}$) was calculated.

Results and discussion

As shown in Table 1 B. *cinerea* disease severities in the untreated control ranged from 14.6 (Pinot gris 2009) to 51.9% (Riesling 2015). Efficacies of single measures ranged from 9.9% (bioregulator; Pinot gris 2009) to 68.4% (leaf removal; P. gris 2015). Combining three crop cultural measures [namely, bioregulator + leaf removal + botryticide (trial A) or cluster division + leaf removal + late first shoot topping (trials B + C)] resulted in the numerically highest efficacies [range from 66.7% (Riesling 2015) to 96.9% (Pinot gris 2015)] (Table 1). Generally, efficacies of the measures in 2015 tended to be higher in Pinot gris than in Riesling. This might be explained by the dense cluster structure of Pinot gris grapes in 2015. Here, control measures might have been more efficient than in Riesling, which showed less compact clusters in 2015.

Assuming additive effects, average deviations per trial between estimated and observed efficacies ranged from -1.0% to -21.3% with average absolute deviations between 6.7% and 21.3%. Here, the global average deviation was -10.6% and global average absolute deviation 14.7% (Table 1). The negative average deviations in all three trials suggest that additive considerations tend to overestimate the overall efficacies. This effect is, as expected, most pronounced in case of combining measures with high efficacies, as this was the case particularly in trial B. Here, assuming additive effects leads to estimated efficacies above 100%, confirming the limitations of this approach.

In case of the multiplicative consideration, the average deviations per trial between estimated and observed efficacies of combined measures ranged from 5.1% to 6.3% with average absolute deviations between 6.4% and 9.0%. Here, the global average

Table 1. Trials, treatments, dates of application and BBCH stages, assessed *B. cinerea* disease severities at the final assessments prior to harvest [assessment dates: 01/10/2009 (A); 30/09/2015 (B, C)], their standard errors (SE) as well as indications of homogenous subsets according to the Tukey post hoc test following one-way ANOVAs.

										Additive consideration			Multiplicative consideration			
	Year	Cultivar	Nr.	Treatment	Date	BBCH	Disease sev. (%)		$E_{obs.}$ (%)	$E_{est.}$ (%)	Δ (%)	$Δ_{abs.}$ (%)	$E_{est.}$ (%)	Δ (%)	$Δ_{abs.}$ (%)	$E_{obs.}/E_{est.}$
A	2009	P. gris	1	untr. control			14.6±4	c	0.0							
			2	bioregulator	16-juin	65	13.1±4	bc	9.9							
			3	leaf removal	18-juin	71	4.6±1	ab	68.4							
			4	bioregulator+ leaf removal	16-juin 18-juin	65 71	2.4±0	a	83.7	78.4	5.4	5.4	71.6	12.2	12.2	1.17
			5	botryticide	10-juil	77	10.2±2	abc	29.9							
			6	bioregulator+ botryticide	16-juin 10-juil	65 77	7.6±2	abc	47.8	39.9	7.9	7.9	36.9	10.9	10.9	1.29
			7	leaf removal+ botryticide	18-juin 10-juil	71 77	4.4±1	ab	70.1	98.3	-28.3	28.3	77.9	-7.8	7.8	0.90
			8	bioregulator+ leaf removal+ botryticide	16-juin 18-juin 10-juil	65 71 77	2.2±1	a	85.2	108.3	-23.1	23.1	80.1	5.2	5.2	1.06
								average			-9.5	16.1		5.1	9.0	1.11
B	2015	P. gris	1	untr. control			40.3±3	d	0.0							
			2	cluster division	21-juil	79	12.9±5	bc	68.0							
			3	leaf removal	18-juin	68	18.5±2	c	54.2							
			4	cluster division+ leaf removal	21-juil 18-juin	79 68	2.6±0	ab	93.6	122.2	-28.6	28.6	85.3	8.2	8.2	1.10
			5	late 1st sh. topp.	17-juil	79	34.1±2	d	15.4							
			6	cluster division+ late 1st sh. topp.	21-juil 17-juil	79 79	11.9±2	abc	70.5	83.4	-13.0	13	72.9	-2.5	2.5	0.97
			7	leaf removal+ late 1st sh. topp.	18-juin 17-juil	68 79	13.4±2	c	66.7	69.6	-2.9	2.9	61.2	5.5	5.5	1.09
			8	cluster division+ leaf removal+ late 1st sh. topp.	21-juil 18-juin 17-juil	79 68 79	1.3±0	a	96.9	137.6	-40.7	40.7	87.6	9.3	9.3	1.11
								average			-21.3	21.3		5.1	6.4	1.06

										Additive consideration			Multiplicative consideration			
	Year	Cultivar	Nr.	Treatment	Date	BBCH	Disease sev. (%)		$E_{obs.}$ (%)	$E_{est.}$ (%)	Δ (%)	$Δ_{abs.}$ (%)	$E_{est.}$ (%)	Δ (%)	$Δ_{abs.}$ (%)	$E_{obs.}$/ $E_{est.}$
C	2015	Riesling	1	untr. control			51.9±6	b	0.0							
			2	cluster division	21-juil	79	33.8±3	ab	34.9							
			3	leaf removal	18-juin	68	41.7±5	b	19.5							
			4	cluster division+ leaf removal	21-juil 18-juin	79 68	17.7±4	a	65.8	54.4	11.4	11.4	47.6	18.2	18.2	1.38
			5	late 1st sh. topp.	17-juil	77-79	44.0±7	b	15.2							
			6	cluster division+ late 1st sh. topp.	21-juil 17-juil	79 77-79	30.5±4	ab	41.2	50.0	-8.9	8.9	44.8	-3.6	3.6	0.92
			7	leaf removal+ late 1st sh. topp.	18-juin 17-juil	68 77-79	35.8±5	ab	31.0	34.7	-3.7	3.7	31.7	-0.8	0.8	0.98
			8	cluster division+ leaf removal+ late 1st sh. topp.	21-juil 18-juin 17-juil	79 68 77-79	17.3±3	a	66.7	69.6	-2.9	2.9	55.5	11.1	11.1	1.20
								average			-1.0	6.7		6.3	7.9	1.12
								global average			-10.6	14.7		5.5	7.9	1.10

Treatments in the same trial marked with the same letter did not differ significantly ($p = 0.05$). Observed ($E_{obs.}$) and estimated (Est.) efficacies (i) according to equation (2) (additive consideration) or (ii) according to equation (3) (multiplicative consideration) as well as deviations (Δ) and absolute deviations ($Δ_{abs.}$) between observed and estimated efficacies are depicted. In case of multiplicative consideration, ratios between observed and predicted efficacies are shown. Generally, global averages represent averages calculated from the data of all three trials.

deviation was 5.5% and global average absolute deviation 7.9% (Table 1).

Treatments in the same trial marked with the same letter did not differ significantly ($p = 0.05$). Observed ($E_{obs.}$) and estimated ($E_{est.}$) efficacies (i) according to equation (2) (additive consideration) or (ii) according to equation (3) (multiplicative consideration) as well as deviations (Δ) and absolute deviations ($Δ_{abs.}$) between observed and estimated efficacies are depicted. In case of multiplicative consideration, ratios between observed and predicted efficacies are shown. Generally, global averages represent averages calculated from the data of all three trials.

Estimated and observed efficacies were in both approaches significantly correlated. Coefficients of determination of linear regressions between estimated and observed efficacies were higher in case of the multiplicative consideration ($R^2 = 0.8574$; $p < 0.0001$) than in case of the additive consideration ($R^2 = 0.8280$; $p < 0.0001$) (Figure 1). The multiplicative approach assumes that each additional measure is affecting (in case of efficient measures: reducing) the disease severity level as the result of the previous/additional treatments rather than compared to the disease severity level in the untreated control.

Generally, the high goodness of fit as well as the observed low deviations between the estimated and the observed efficacies demonstrated the suitability of the approach assuming multiplicative effects to estimate the efficacy of combined viticultural measures. Ratios between observed and estimated efficacies ($E_{obs.}/E_{est.}$) > 1 mean that the overall efficacy of the combination of two or more measures is above the expected efficacy according to equation (3). Such ratios are indicating that besides multiplicative effects slight synergistic effects might exist, while, on the other hand, $E_{obs.}/E_{est.}$ ratios < 1 are indicating slight antagonistic effects. In the present investigations, both slightly synergistic as well as slightly antagonistic effects were observed in the different trials as well as in the different combinations. Generally, the fact that the global average ratio between observed and estimated efficacies of 1.10 was close to 1 demonstrates the usefulness of equation (3) with a slight tendency towards synergistic effects in some combinations: e.g., in treatments that combined leaf removal in the cluster-zone with other measures that lead to a reduction of the cluster compactness (such as cluster division or the application of a bioregulator), slightly synergistic effects were observed in all three trials, while in other combinations (e.g., cluster division + late first shoot topping), slight antagonistic effects

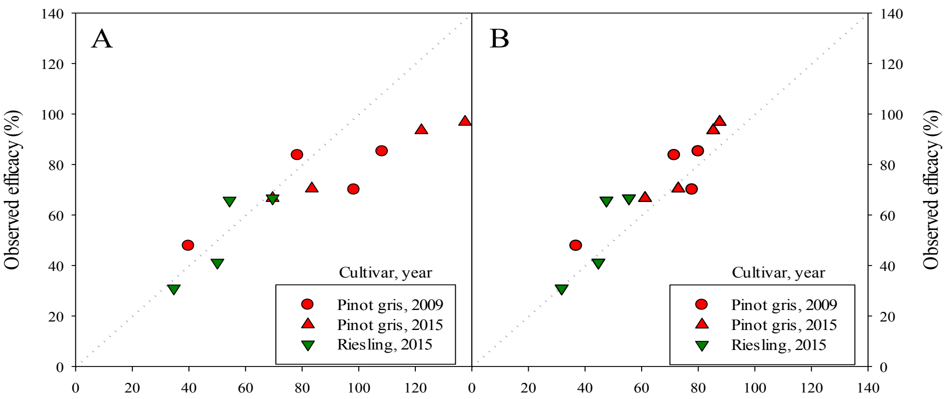

Figure 1 - Estimated efficacies according to equation (2) (additive consideration; A) or according to equation (3) (multiplicative consideration; B) plotted against the observed efficacies of combined viticultural measures to control bunch rot in the three field trials.

($E_{obs.}/E_{est.} < 1$) were recorded (Table 1). In the case of combining leaf removal with a late first shoot topping in 2015, synergistic efficacies were observed in Pinot gris ($E_{obs.}/E_{est.} = 1.09$), while the efficacies in Riesling (0.98) were slightly antagonistic. The question of which combinations of measures under which conditions in which cultivar tend to show (besides generally multiplicative effects) synergistic or antagonistic effects and the underlying principles would merit further investigations based on a broader data set. Potentially, combining different measures affecting the complex pathosystem grapevine/bunch rot at distant loci, in different ways or at distant time points (e.g., bioregulator (effect on cluster compactness) + botryticide (direct effect on pathogen); Table 1) might tend to slightly synergistic effects while the combination of measures inhibiting the pathogen at similar positions (e.g., cluster division (effect on cluster structure) + late first shoot topping (effect on cluster structure); Table 1) might exhibit slight antagonistic effects (efficacy lower than expected based on the multiplicative consideration of single efficacies).

Under practical conditions, a broad spectrum of crop cultural measures is available to optimize the grape health status and hence to enable a prolongation of the maturation period (Molitor *et al.*, 2012). Present (Table 1) as well as previous studies demonstrate that crop cultural measures (non-chemical control) are often of higher efficacy than the application of botryticides (chemical control) (Evers *et al.*, 2010; Molitor *et al.*, 2011b) and might save costs as well as energy (Sternad-Lemut *et al.*, 2015). Consequently, such non-chemical crop cultural measures represent efficient tools for reducing or partially replacing the pesticide input in viticulture as intended in Integrated Pest Management. For instance, a chemical treatment (such as a botryticide application) can be replaced by an efficient crop cultural measure, without jeopardizing the efficacy of the bunch rot control regime. As an efficient risk management strategy, the combination of several measures is recommended in practical bunch rot control programs. According to the present results, the efficacy of the overall control strategy can be estimated based on multiplicative consideration of the expected effects of the single measures. Which and how many viticultural measures are integrated in the strategy is determined by the specific local conditions, the specific varietal degree of bunch rot susceptibility as well as the production target.

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