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Pre-selection in laboratory tests of survival and competition before field screening of antagonistic bacterial strains against *Botrytis* bunch rot of grapes

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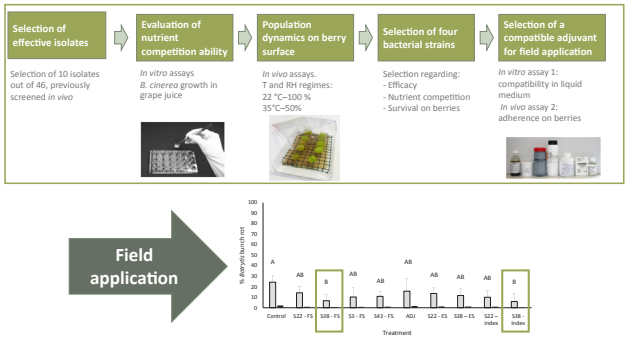
GRAPHICAL ABSTRACT



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ABSTRACT

With only a few biocontrol products currently registered against *Botrytis* bunch rot (BBR) of grapes, there is a crucial need for new antagonistic strains that are able to survive and efficiently suppress *B. cinerea* under vineyard conditions. The aim of this study was to establish and follow a pre-selection process among potential antagonistic bacterial strains, previously identified *in vivo* for efficacy, and to carry out a further field screening assay using a reduced strain number. Ten bacterial strains were pre-selected and tested, *in vitro* and *in vivo*, to characterise their mode of action and population dynamics under simulated climatic regimes. Four candidate strains were then selected and characterised for high efficacy *in vivo*, known mode of action and marked survival ability. Some suitable additives for increasing strain adherence on grape berry surface were tested prior to field applications, indicating one commercial adjuvant for potential improved bacterial persistence in the field. The four strains were applied separately in an experimental Merlot vineyard near Bordeaux (SW France), either at five key phenological stages, or following a specially developed Disease Risk Index (DRI). The *Bacillus ginsengihumi* S38 strain treatments significantly reduced BBR incidence by 72–75% compared to the control, whereas sprays applied according to the DRI decision support system tended to improve disease control. The study validates a laboratory pre-selection process followed by a field screening step, resulting in a candidate *B. ginsengihumi* strain S38 with a high potential for BBR biocontrol and future development in vineyards.

Abbreviations: BBR, *Botrytis* bunch rot; BCA, biological control agent; CFU, colony forming units; DRI, disease risk index; MoA, mode of action; RH, relative Humidity; SDW, sterile distilled water; T, temperature

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1. Introduction

Biological control of fruit pathogens with microbial antagonists is regarded as a disease management strategy alternative to the use of synthetic fungicides, with a high potential to control fruit crop diseases (Nicot et al., 2011; Romanazzi et al., 2016). In this context, many studies have investigated, using *in vitro* or *in vivo* experiments, biological control of *Botrytis cinerea* Pers.:Fr., the causative agent of grey mould affecting economically important crops such as tomatoes, apples, strawberries or grapes (Ballet et al., 2016; Marín et al., 2016; Passera et al., 2017; Qin et al., 2017; Ruiz-Moyano et al., 2016; Sylla et al., 2015; You et al., 2016). In vineyards, the disease, also known as *Botrytis* bunch rot, represents, mostly in temperate climate regions, a major challenge for yield and wine quality (Ky et al., 2012). In the last ten years, several studies have shown new advances in biological control strategies against BBR, by developing either new biological control agents, or new application strategies using yeasts, filamentous fungi and/or bacteria prior to harvest (Calvo Garrido et al., 2017; Haidar et al., 2016c; Parry et al., 2011). In addition, only a few commercial products, based on fungal or bacterial genera, are available in Europe for biological control of BBR (Nicot et al., 2016). However, the control rates performed by these products tend to vary between orchards and from one season to the next. Consumers also increasingly demand zero-residue fruit goods and wines. However, despite more than 30 years research in biological control, reliable commercial solutions to control BBR are still lacking, indicating that greater research effort are still needed to develop new BCAs, adapted to the vineyard environment and effective in those field conditions, with lower variability in disease control. Recent research in INRA Bordeaux-Aquitaine has evaluated the efficacy of up to 46 bacterial strains against *B. cinerea* infection, using *in vitro* and *in vivo* biotests (Haidar et al., 2016a). As these strains were originally isolated from grape berry surface or grapevine wood (Bruez et al., 2015b; Martins et al., 2013), this may be considered an *a priori* advantage, possibly allowing these BCA bacterial strains to better survive and compete in this particular crop ecosystem, following their introduction in vineyards.

The mode of action of a BCA strain is one of the most important features to be investigated and understood in order to i) better analyse efficacy results and those factors which may interfere with efficacy, and ii) determine optimal BCA application strategy in the field, and the possibility to include it in combinational strategies (Haidar et al., 2016c; Spadaro and Droby, 2016). Many studies in biocontrol literature have shown disease reductions under *in vitro* and/or *in vivo* conditions, thereby suggesting new microbial candidates. Due to economic, technical and time constraints, only a few studies have also tested the BCA candidates in the field, with biocontrol efficacy often being markedly reduced, even lost, compared to laboratory experiments (Köhl et al., 2011; Nicot et al., 2011). The major reason for such an unsuccessful outcome comes mainly from the difficulties of BCA populations to adapt to, and survive on, the aerial host organs (phyllosphere or carposphere) under field environment conditions (Köhl et al., 2011; Nicot et al., 2011). Consequently, specific studies evaluating the survival ability of BCA candidates, MoA and their suppressive efficiency under different conditions should be included in preliminary laboratory experimental steps before subsequent field screening. Other strategies may also improve survival ability and population persistence after field application, e.g. the use of adjuvants that may improve cell adherence to fruit surfaces and favour BCA establishment on leaves, flowers and/or berries (Ballet et al., 2016; Calvo-Garrido et al., 2014b; Di Francesco and Mari, 2014; Marín et al., 2016).

A systematic stepwise screening process has been proposed by Köhl et al., (2011) as a general framework procedure for any biological control pathosystem. Following these guidelines may be very useful to achieve a short, efficient and field-oriented selection process of strains that are also adapted to specific pathosystem or biological control conditions. In this context, the present study is aimed at developing such a selection process for the bacterial strain collection at INRA Bordeaux-Aquitaine (Haidar et al., 2016a; Haidar et al., 2016b). Although a single BCA candidate with appropriate characteristics may prove sufficient in a general screening process, including more than one BCA candidate in field trials could also maximise success opportunities. Doing so might include candidate strains with lower laboratory efficacy, but with other potential advantages. Selecting and developing a microbial BCA is a long and costly process in which the likelihood of successful strains becoming commercial products is extremely low (Köhl et al., 2011). Well adapted pre-selection procedures are, therefore, needed to maximise success probabilities and shorten this screening process, providing practical solutions for growers.

The major objectives of this work were, firstly, to characterise some key life traits of ten pre-selected bacterial strains, effective against *B. cinerea*, to assess their potential as BCAs in vineyards. The life traits included nutrient competition ability, antibiosis aptitude based on diffusible compounds, survival under simulated climatic regimes, and compatibility with spray adjuvants. The second objective was to select four of those strains with high biocontrol potential, to be tested and compared in a one-season field screening trial, by assessing their efficacy against BBR and their survival capacity under Bordeaux region climatic conditions.

2. Materials and methods

2.1. B. cinerea strain used in laboratory experiments

The *B. cinerea* pathogenic strain (code = 213), selected from the INRA-UMR 1065 SAVE collection in Bordeaux, belongs to the *transposa* genotype and has been characterised as highly virulent on grapevine berries at different stages (Deytieux-Belleau et al., 2009; Martinez et al., 2005). Routine cultures were maintained on malt agar medium ($15\,g\,L^{-1}$ of malt from Biokar Diagnostics and $20\,g\,L^{-1}$ of Setaxam® agar) at 22 °C. In order to obtain a conidial suspension, *B. cinerea* conidia were collected by adding SDW to sporulating cultures on malt agar medium plates and gently rubbing with a sterile spatula. Concentration of the conidial suspension was determined using a haematocytometer, and then diluting to adjust to the desired final concentration.

2.2. Bacterial strains and pre-selection of ten effective BCA candidate strains

Bacterial isolates were all originally isolated from grapevine tissues (Bruez et al., 2015a; Martins et al., 2013) and were maintained in the collections of either INRA Bordeaux-Aquitaine or “Biological Resources Center for Enology” (University of Bordeaux and Bordeaux Polytechnic Institute). These strains were characterised in previous studies (Haidar et al., 2016a; Haidar et al., 2016c; Haidar et al., 2016d). The strains were maintained on cryogenic storage beads (Viabank MWE, Wiltshire, England) at −20 °C, then subcultured on TSA solid medium (Trypto-casein Soy Agar; Biokar Diagnostics, Beauvais, France) and incubated at 27 °C in the dark, before use. Liquid cultures were obtained by inoculating bacterial colonies from TSA medium in Erlenmeyer flasks containing TSB liquid medium (Trypto-casein Soy Broth; Biokar Diagnostics, Beauvais, France), and then incubated at 27 °C for 48 h using an orbital shaker at 150 rpm. Liquid cultures were then centrifuged in sterile centrifuge flasks at 5000 rpm during 10 min and then re-suspended in phosphate buffer to obtain the liquid suspensions used in laboratory and/or field experiments. The bacterial strain populations in suspension were quantified by fluorochrome staining (500 µL Chemsol B16 buffer + 2,5 µL de fluorochrome Chemchrome V6 fluorescein acetate; Biomérieux, Marcy l’Etoile, France) followed by epifluorescent direct counts using an optical microscope (Model BH2, Olympus France, Rungis, France). A minimum of 300 cells were counted in at least 10 different fields of view, and the average number of dyed cells

Table 1
List of ten pre-selected strains with high overall efficacy in previous *in vivo* tests (Haidar et al., 2016b).

Code	Strain name in original collection	Overall *in vivo* efficacy (%)[a]	Efficacy profile[b]
S3	*Pantoea agglomerans* 3	40.3	Unwounded berries
S6	*Pantoea agglomerans* 6	39.1	Regular
S18	*Paenibacillus* sp. 3	39.3	Leaves
S22	*Enterobacter cowanii* 2	47.8	Regular
S23	*Enterobacter* sp. 1	41.0	Regular
S24	*Enterobacter* sp. 2	20.6	Unwounded berries
S38	*Bacillus ginsengihumi* 1	65.4	Wounded berries
S39	*Bacillus ginsengihumi* 2	38.4	Wounded berries
S43	*Bacillus* sp.	60.2	Regular
S46	*Bacillus* sp.	41.9	Wounded berries

[a] Overall efficacy was calculated as the mean reduction *B. cinerea* rot symptoms in four *in vivo* tests conducted with grapevine leaves (two tests), unwounded berries and wounded berries, respectively (Haidar et al., 2016b).

[b] Efficacy profile was assigned when the strain presented more than ten % points of *B. cinerea* reduction in one of the grapevine substrates used in the *in vivo* tests (leaves. unwounded berries and wounded berries) compared to the other substrates. Regular profile was assigned when *B. cinerea* reduction in all tests not differed more than 10%.

per field was finally expressed as CFU ml^{-1}.

Previous *in vivo* tests at INRA UMR SAVE, Bordeaux, confronted *B. cinerea* with different bacterial strains, isolated from grapevines (Haidar et al., 2016b). By further analysing these results from biotests on grapevine leaves, and on unwounded and wounded grape berries, an overall average efficacy rate was calculated for every strain (Table 1). The 15 strains presenting high overall efficacy were then further characterised by an efficacy profile: either showing a higher efficacy on specific grapevine organs or a similar efficacy, whatever the host organ. Ten strains were finally pre-selected since they showed high efficacy, different profiles, and also represented a variety of genera and species. They were considered as the best BCA candidates for laboratory tests in this study and for further short-listing them for field trials (Table 1).

2.3. Nutrient competition ability of the pre-selected candidate strains

The capability of the bacterial strains to inhibit *B. cinerea* germination by competing for available nutrients in grape juice was tested *in vitro* according to a methodology adapted from Janisiewicz et al. (2000). Grape juice was obtained by crushing mature berries (cv. Sugraone seedless) using a domestic blender. After removing larger solid particles by filtering through a sieve, juice was centrifuged at 5000 rpm for 15 min. The supernatant was filtered through a filter paper (Filtres Prat-Dumas Number 3; Lalinde, France), then pasteurised at 70 °C for 30 min, before being finally cool stored at 4 °C until used.

The test was carried out using 24 well plates with the appropriate PTFE membrane inserts (Millicell -24 plate and Millicell Cell culture inserts, respectively; Merck Millipore Ltd., Cork, Ireland). *B. cinerea* conidia were incubated jointly with the candidate bacterial strain, but separated by the insert membrane (0.4 μm pore size), in the same liquid medium containing either 0, 0.1 or 1.0% grape juice. Adjusted final concentrations were 2×10^5 *B. cinerea* conidia mL^{-1} (added into the inserts) and 1×10^7 CFU mL^{-1} for the bacterial strain (added into the plate well). Three replicate wells were designed per each juice concentration*bacterial strain combination. After 18 h incubation at 22 °C in the dark, the inserts were removed from plate wells and the germination process was stopped by adding a drop of methyl blue onto the membrane. The percentage of germinated conidia was determined under a light microscope by assessing 150 conidia per membrane (by observing three different zones of 50 conidia each). A conidium was defined as germinated when the germ tube length was greater than the conidium length.

2.4. Survival ability of bacterial strains on grape berry surface under simulated climatic conditions

Since survival may be a major factor contributing to field efficacy in biological control, the capacity of the candidate bacterial strains to survive and multiply under various controlled conditions was tested in climatic chambers simulating two contrasted Temperature (T) and Relative Humidity (RH) regimes occurring in the vineyard. Mature table grape berries (cv. Sugraone seedless), which were apparently sound, were placed onto grids and sprayed until runoff with bacterial suspensions of each of the 10 pre-selected strains (1×10^7 CFU ml^{-1}) using a hand pump sprayer (Model F2 plus; Berthoud EXEL GSA, Villefranche, France). Because of technical reasons, the ten strains were evaluated in two different bioassays: the first bioassay included the strains S3, S18, S22, S23, S24 and S46; the second bioassay included the strains S6, S38, S39 and S43. Beforehand, grape berries had been washed for 15 min under continuous tap-water flow to remove particles and/or synthetic fungicide residues. Ten berries on one grid constituted a replicate sample, there were three replicates per bacterial strain. After drying for 2 h at room temperature, treated berries were incubated in controlled climatic chambers (PGR14 model, CONVIRON Ltd, Winnipeg, Canada), at two Temperature (T) and Relative Humidity (RH) regimes: a) 22 °C and 100% RH, simulating favourable conditions for *B. cinerea* infection; b) 35 °C and 50% RH, simulating a hot and dry period during the grapevine growing season. After 0, 24 and 48 h of exposure, bacterial populations were recovered from the berry surface and quantified as follows. The ten berries of each replicate were shaken in an Erlenmeyer flask with phosphate buffer for 20 min at 150 rpm on a rotary shaker, and then sonicated for 10 min in an ultrasonic bath (Branson® 2510, Branson Ultrasonics Corp., Danbury, Connecticut, USA). After serial dilutions of the washing suspension, 100 μL aliquots were plated onto TSA plates. Duplicate plates were incubated in the dark at 27 °C and, after 24 h, colonies were visually recognised, according to morphological characteristics, and then counted. Data, collected as CFU mL^{-1}, was transformed into CFU g^{-1} of tissue sampled. Results of the bacterial populations were finally expressed, at each exposure time (0, 24 and 48 h), as Log (N/N_c), where N = populations in treated sample (CFU g^{-1}) and N_c = Mean value of the population in the three replicates of the Control treatment (CFU g^{-1}).

2.5. Compatibility of bacterial strains with commercial adjuvants and natural products for use as additives in field applications

The use of adjuvants is a key parameter in a biocontrol strategy to improve effective colonisation and persistence of BCA cells on the surface of the host tissues to be protected. We established a list of commercial adjuvants, all of them presenting surfactant and/or adherent properties, which included different active ingredients (Table 2). Moreover, a list of natural products (NPs) with a potential to improve bacterial adherence due to the composition was also evaluated (Table 2). Some of these NPs may also present a potential suppressive effect on *B. cinerea*. The tested doses corresponded to 1.2 times the dose recommended by the manufacturer for field applications, to show more easily a hypothetical toxic effect of the product. The compatibility was tested *in vitro* with four strains (*P. agglomerans* S3, *E. cowanii* S22, *B. gingsengihumi* S38 and *Bacillus* spp S43) by incubating bacterial cells in 50 mL Erlenmeyer flasks containing 20 mL of a solution of the different products in SDW. After preparing the additive solution, the bacterial strain was inoculated by adjusting its concentration to $1x10^6$ CFU mL^{-1} in the flask. Then, for incubation, flasks were placed in rotary shakers at 150 rpm in climatic chambers (PGR14 model, CONVIRON Ltd, Winnipeg, Canada) at 27 °C. Four flasks per strain*additive combination were used as replicate samples. After 0 and 24 h of incubation, 10 μL samples were recovered from replicate flasks, diluted and plated for the evaluation of bacterial populations.

Table 2
Effect of commercial adjuvants and natural products on the multiplication of bacterial strains in distilled water.

Treatment name	Product name	Manufacturer	Recommended dose	Reported antibotrytic effect	Bacterial strain concentration (Log CFU ml^{-1})								
					S3		S22		S38		S43		
Water Control					7.29	a	7.29	a	4.76	c	4.93	b	
Acteon	Acteon	SDP, Pinon, France	0.5% (v/v)	No	6.47	bc*	6.63	cd*	3.94	d*	3.65	bc	
Actirob	Actirob	Bayer Cropscience France, Lyon, France	0.3% (v/v)	No	6.67	ab	7.03	ab	5.34	bc	3.48	bc	
AT	Agrotonic	Agrimer, Plouguemeau, France	0.75% (v/v)	No	5.98	c*	6.85	bc*	7.32	a*	6.96	a*	
Dash	Dash HC	BASF Agro France SAS, Ecully, France	0.3% (v/v)	No	UDL	*	UDL	*	UDL	*	2.61	c*	
Sticman	Sticman	Agridyne-DeSangosse, Pont du Casse, France	0.14% (v/v)	No	6.20	bc*	7.02	ab	4.96	bc	3.89	b	
SW	Silwet L-77	Helena Chemical Company, Collierville, TN, USA	0.15% (v/v)	No	6.25	bc*	6.41	d*	5.70	b*	3.82	b	
Water Control					6.81	a	6.87	a	4.96	cd	3.00	b	
AZ	ArmourZen	BotryZen Ltd., Dunedin, NZ	1% (v/v)	Parry et al. (2011)	2.32	c*	4.76	b*	4.85	d	2.41	b	
FC	Fungicover Base	BioDurcal, Granada, Spain	2.5% (v/v)	Calvo-Garrido et al. (2014a)	7.19	a	7.36	a	7.40	a*	6.05	a*	
HML Cu	HML Copper product	Henry Manufacturing Ltd., Napier, NZ	0.22 g/l	No	6.91	a	7.00	a	4.96	cd	2.94	b	
HML Zn	HML Zinc product	Henry Manufacturing Ltd., Napier, NZ	0.3 g/l	No	7.14	a	6.88	a	5.06	cd	2.43	b	
HML32	HML32	Henry Manufacturing Ltd., Napier, NZ	1.25% (v/v)	http://www.henrymanufacturing.co.nz/products/hml-32/	1.86	c*	3.09	c*	5.06	cd	UDL		
MZ	MidiZen	BotryZen Ltd., Dunedin, NZ	3% (v/v)		7.11	a	7.31	a	6.52	b*	6.96	a*	
PRT	Protector	Henry Manufacturing Ltd., Napier, NZ	2% (v/v)	Calvo-Garrido et al. (2014a)	4.93	b*	2.21	d*	5.28	c	2.19	b	

*Significant difference ($p < 0.05$) compared to water control. Values linked by the same letter are not significantly different according to LSD Student's *t* test.
UDL: Under detection level.

2.6. Cell adherence on surface of grape berries with selected adjuvants

The four selected strains, *P. agglomerans* S3, *E. cowanii* S22, *B. gingsengihumi* S38 and *Bacillus* spp S43, were applied on grape berries alone, or associated with different adjuvants, to test the potential improvement in bacterial cell adherence following application. Out of the products considered as compatible in the previous assay (see above), only the commercial adjuvants were selected. Since these NPs have been reported to possibly directly affect *B. cinerea* development in the bibliography on this phenomenon, or by manufacturers (Table 2), they were not used in the ulterior field experiments, in order not to interact with the results of BBR control by the antagonistic candidate bacterial strains. Bacterial suspensions (1×10^7 CFU mL^{-1}) of S3, S22, S38 and S43 were prepared and mixed with Acteon, Actirob, AT, Sticman or SW products, used at the dose recommended by the manufacturer (Control treatment consisted of suspensions in SDW). Then, treatment suspensions were sprayed until runoff with a hand pump sprayer (Model F2 plus; Berthoud EXEL GSA, Villefranche, France) over ten berries placed onto one grid, i.e. one replicate sample. There were three replicates per bacterial strain*adjuvant combination. The treated samples were then dried for 2 h at room temperature and afterwards populations were subsequently recovered from the berry surface and quantified (CFU g^{-1}), as described above in the experiment under simulated climatic conditions. Because of technical constraints, this test was carried out in three different bioassays, each with the corresponding water control, first evaluating S22 and S43 with Agrotonic, Sticman and Silwet; then S22 and S43 with Actirob and Acteon; and, finally, evaluating S3 and S38 with all the five adjuvants. Results were finally expressed as Log (N/N_c), where N = populations in treated sample (CFU g^{-1}) and N_c = Mean value of the population in the three replicates of the Control treatment (CFU g^{-1}).

2.7. Field application of four selected bacterial strains

The field efficacy assay for testing the four finally selected bacterial isolates was conducted in 2015 in an INRA experimental vineyard (*Vitis vinifera* L.) near Bordeaux ("Grande Ferrade", Villenave d'Ornon, France). The cultivar was Merlot noir grafted onto '101-14' rootstock. The planting density was approximately 5350 vines ha^{-1} with a row and vine spacing of 1.7 m × 1.1 m and a north–south row orientation. The experimental vineyard was not treated with any specific anti-*Botrytis* fungicide. As shown in Table 3, different treatment strategies using the four selected isolates were carried out, consisting of different applications either at key phenological stages or following a new disease risk index that was developed for this study (see next section). Experimental design was based on randomised blocks with four replicate plots per treatment. Each replicate plot consisted of seven consecutive vines in the same row. For each BCA treatment, bacterial suspensions were adjusted to 5×10^7 CFU l^{-1} and applied with the commercial adjuvant Sticman at a dose of 0.14% (v/v), by using a motorised back-sprayer for spraying grape bunches until runoff. At the end of the growing season, two assessments of *Botrytis* bunch rot (BBR) and sour rot (SR) were carried out: 1) at commercial harvest time (09/09/2015), and 2) two weeks later, when grape bunches were over-ripe (23/09/2015). Both disease incidence and severity were assessed on 50 bunches, scored individually, per replicate unit. BBR incidence corresponded to the percentage of bunches with typical *B. cinerea* rot symptoms, and BBR severity was measured visually as the percentage of *B. cinerea* infection in rotten berries per bunch.

2.7.1. Development and use of a Disease Risk Index as a decision support system for field applications

The specific disease risk index (DRI) developed for this field study was based on the first equation, i.e. "equation 1" in Ciliberti et al. (2015), in which the dependent variable "y" is the rescaled infection incidence, a function of air relative humidity "RH" and of air

Table 3
Field treatments with selected bacterial strains during 2015 season.

	Treatment name	A 10% flowering	A+ Fruit set (100% capfall)	B Pre-bunch closure	C 10% Veraison	D 21 days before harvest
Untreated	Control	–	–	–	–	–
Full season strategy	ADJ	Adjuvant	Adjuvant	Adjuvant	Adjuvant	Adjuvant
	S3 – FS	S3[a]	S3	S3	S3	S3
	S22 – FS	S22[b]	S22	S22	S22	S22
	S38 – FS	S38[c]	S38	S38	S38	S38
	S43 – FS	S43[d]	S43	S43	S43	S43
Early season strategy	S22 – ES	S22	S22	S22	–	–
	S38 – ES	S38	S38	S38	–	–
Late season DRI strategy[e]	S22 – Index	–	–	–	S22 – DRI Output	
	S38 – Index	–	–	–	S38 – DRI Output	

[a] *Pantoea agglomerans* S3.
[b] *Enterobacter cowanii* S22.
[c] *Bacillus ginsengihumi* S38.
[d] *Bacillus* sp. S43.
[e] DRI: Disease Risk Index, calculated with Temperature and Relative Humidity hourly data.

temperature "Teq". The corresponding hourly input climatic data were issued from an INRA automatic weather station 15 m close to the experimental vineyard (data checked by INRA and downloaded from the INRA internal web meteorological service CLIMATIK). The DRI was calculated three times a week from veraison onwards. The DRI value used for decision making was the daily DRI value, calculated as the mean of rescaled infection incidences calculated at hourly intervals (Ciliberti et al., 2015). The initiation date of the model was implemented at the beginning of veraison, i.e. on 27 July 2015, corresponding to an average of 10% of berries having a change of colour. This 10% veraison stage was assessed visually in the experimental vineyard. In order to take into account the variations in fruit ontogenic resistance to the pathogen after veraison, i.e. susceptibility increase due to fruit maturation (Deytieux-Belleau et al., 2009), the decision rule was based on two different thresholds, as follows: i) 50% during the first 3 weeks after the initiation date (i.e. until 17 August); ii) 30% from 18 August onwards. The dates of the modelled applications were decided when the daily DRI value was equal or superior to the threshold, leading to the following four application dates in 2015: 12 August, 24 August, 4 September and 11 September. These application dates respected a minimal gap period between two treatments of 10 days.

2.8. Field population dynamics of bacterial strains

The populations of the bacterial strains applied in the field were quantified throughout the growing season, by recovering bacterial cells from treated grapevine tissues after every spray application, as well as just before the next application. At flowering sampling times (10% flowering and 100% flowering, BBCH stages 61 and 69, respectively), BCA populations were recovered from 2 g of floral organs collected randomly from eight inflorescences per replicate unit. Samples were then immersed in 20 mL of phosphate buffer. At pre-bunch closure (BBCH stage 75), 40 pea-sized berries, sampled randomly from 20 bunches per unit plot, were weighed and then immersed in 50 mL of phosphate buffer. After veraison (BBCH stage 83), 20 berries were sampled randomly from 10 bunches, weighed and then immersed in 50 mL of phosphate buffer. After serial dilutions of the washing solution, aliquots of each replicate were plated in duplicate. After 24 to 48 h of incubation at 27 °C, colony counts were carried out, based on morphological recognition of the bacterial strains. Data of CFU ml^{-1} were finally expressed as CFU g^{-1} of sample.

2.9. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using JMP® Pro 12.0.1 (SAS Institute). CFU data were log transformed before ANOVA. When significant differences between treatments were detected ($P < 0.05$), the Tukey test was used to separate treatment means, whereas least significant differences (LSDs) were used in treatment comparisons with an untreated control.

3. Results

3.1. Evaluation of the nutrient competition ability of bacterial strains

In the first experiment (Fig. 1a), comparing five strains, the percentages of *B. cinerea* germination in the Non-Bacteria control samples (NB), were 58.6, 88.0 and 96.6%, for the 0, 0.1 and 1% juice concentrations, respectively. When bacteria and *B. cinerea* conidia were incubated in the absence of nutrient sources (0% juice concentration), only the *E. cowanii* S22 strain significantly reduced germination (42% reduction compared with the NB control). For two strains, germination rates were significantly higher than in the NB control (*P. agglomerans* S6 and *Enterobacter* sp. S24: 76.6% and 70.0%, respectively). In the presence of nutrients from grape juice at 0.1%, three strains significantly reduced *B. cinerea* germination by 30% (*E. cowanii* S22), 21% (*P. agglomerans* S3) and 16% (*Paenibacillus* sp S18), compared with the NB control. For the highest grape juice concentration tested (1%), three strains significantly reduced germination by 16% (S22), 11% (S18) and 7% (S3). However, the other two strains (*Pantoea agglomerans* S6 and *Enterobacter* sp. S24) did not show any significant effect on *B. cinerea* germination.

In the second experiment (Fig. 1b), seven strains were evaluated, by including again the two strains S3 and S18 that had previously significantly inhibited the conidial germination, only, in the presence of nutrients from grape juice. *B. cinerea* germination percentages in the NB controls were 35.2, 37.1 and 65.5% for the 0, 0.1 and 1.0% grape juice concentrations, respectively. With no nutrient source (0% grape juice), two strains significantly reduced *B. cinerea* germination by 67% (*B. ginsengihumi* S38) and 52% (*B. ginsengihumi* S39). All the other strains (*P. agglomerans* S3, *Paenibacillus* sp. S18, *Enterobacter* sp. S23, *Bacillus* sp. S43, *Bacillus* sp. S46) were not significantly different from NB. Similarly, in 0.1% juice, the same two strains significantly lowered the *B. cinerea* germination compared with NB, *i.e.* S38 and S39 showing 72% and 42% reduction, respectively. However, the *P. agglomerans* S3 strain showed a significant inhibition reaching 37% compared with NB. Lastly, in the 1.0% grape juice solution, three strains, S3, S38 and S39 significantly reduced the germination rate by 68, 82 and 49% compared with the NB control, respectively (Fig. 1b).

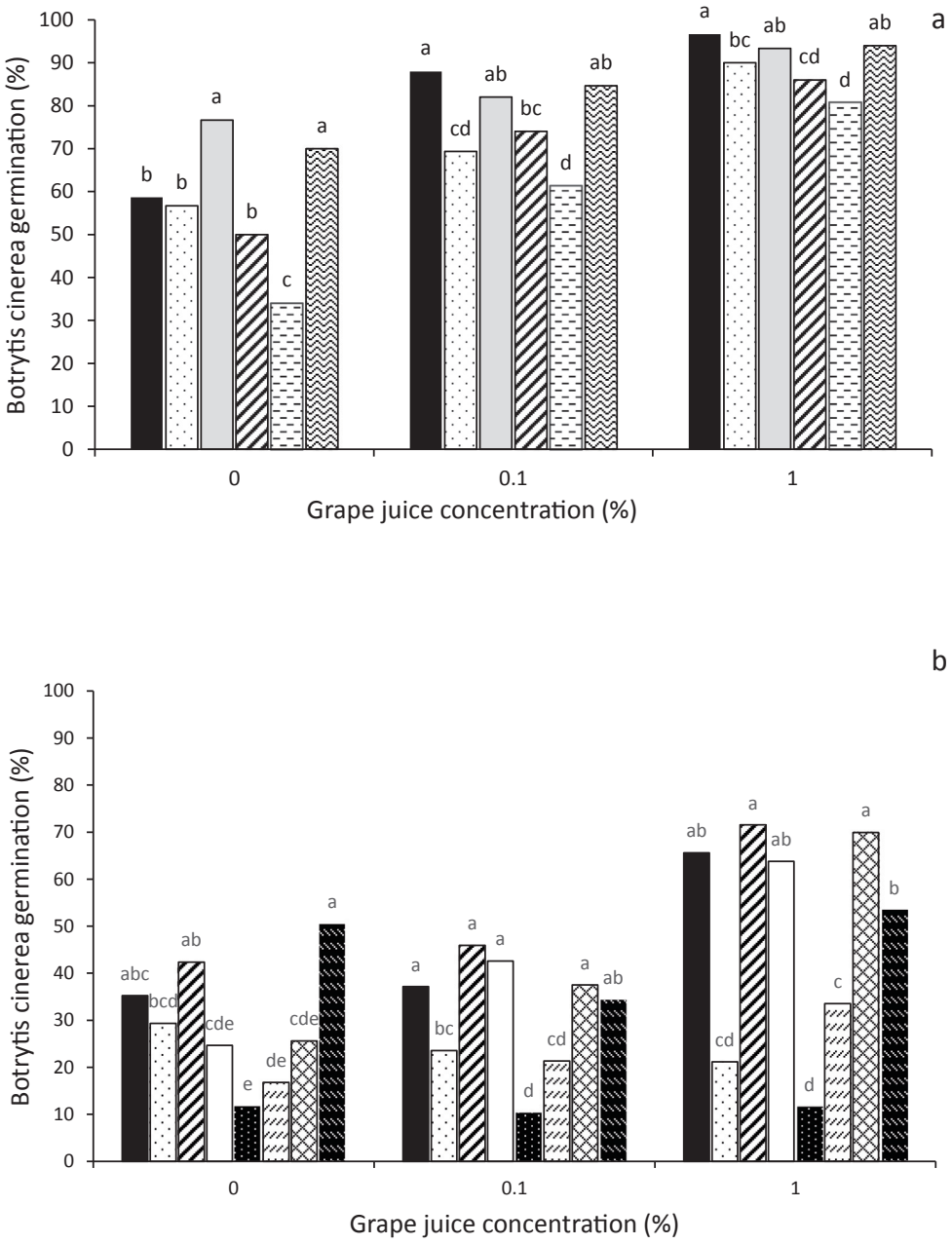


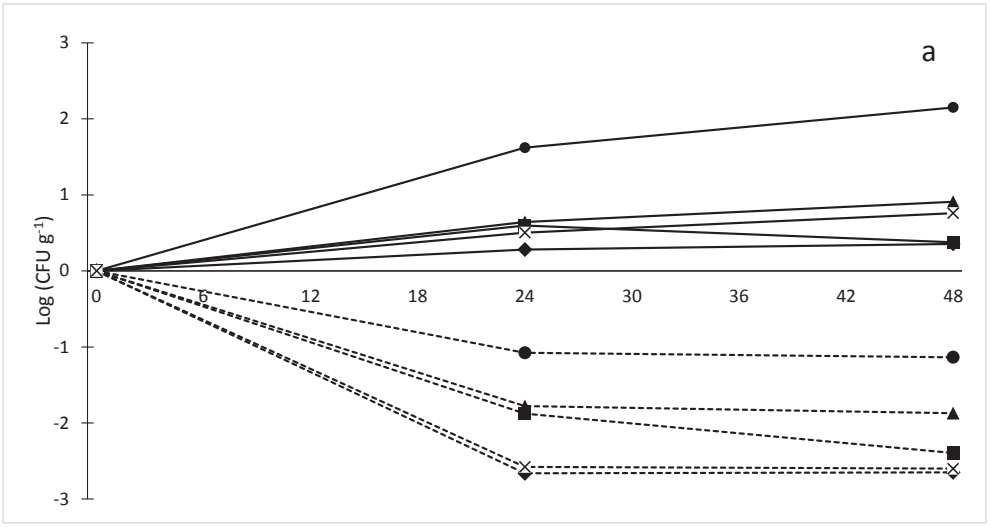
Fig. 1. Germination of *B. cinerea* conidia in diluted grape juice for assessing the effect of nutrient competition by antagonistic bacterial strains Bacterial cells were inoculated in 24 well plates at 1×10^7 CFU ml^{-1} in different grape juice solutions (0%, 0.1% or 1%), whereas conidia were inoculated in membrane inserts placed inside the wells, physically separated from bacteria by the membrane (0.4 μm pore), but allowing liquid flow. After 18 h incubation, germination was assessed under optical microscope based on at least 150 conidia for each of the three replicates. a) First bioassay evaluating five bacterial strains; b) second bioassay evaluating seven bacterial strains. Treatments correspond to different bacterial strains: (■) NB No bacteria; (□) *Pantoea agglomerans* S3; (▨) *Pantoea agglomerans* S6; (◪) *Paenibacillus* sp. S18; (⊟) *Enterobacter cowanii* S22; (▢) *Enterobacter* sp. S23; (▨) *Enterobacter* sp. S24; (▩) *Bacillus ginsengihumi* S38; (◪) *Bacillus ginsengihumi* S39; (▨) *Bacillus* sp. S43; (▩) *Bacillus* sp. S46. Bars characterised by the same letter, within each grape juice concentration, are not significantly different according to LSD Student's *t* test.

3.2. Survival of bacterial strains on grape berries under simulated climatic conditions

At 22 °C and 100% RH, most of the *Pantoea* and *Enterobacter* strains (Fig. 2a) presented a very similar population dynamics during 48 h, showing a progressive increase in population between 0.35 log (S23) and 0.91 log (S3; Fig. 2a). The S6 strain presented similar dynamics, but with higher multiplication rates, increasing up to 2.15 log after 48 h, compared with at 0 h. Only the S22 strain presented slightly lower populations after 48 h rather than after 24 h of incubation. These strains were also similar in terms of the population dynamics pattern at 35 °C and 50% RH, with slightly different quantitative results. All of them showed a remarkable population decrease after 24 h, ranging from −1.08 log (S6) to −2.6 log (S23 and S24). The S22 and S3 strains showed an intermediate decrease of approximately −1.8 log after 24 h, compared with their initial population level. Then, between 24 and 48 h, most of the population levels stayed relatively stable.

As for population dynamics in 48 h of the *Bacillus* and *Paenibacillus* strains (Fig. 2b), at 22 °C and 100% RH, only the populations of one strain (S46) did not significantly increase, presenting only 0.18 log more at 48 h, compared to the 0 h samples (Fig. 2b). On the other hand, the strains S38 and S43 rapidly multiplied up to 1.80 log and 1.43 log after 24 h, respectively, although populations decreased to 1.27 log or stayed at 1.46 log over the initial level after 48 h, respectively. With an intermediate profile, the two other strains, S18 and S39, progressively increased in 48 h, up to 1.02 log for S18 and to 0.78 log for S39 (population level at 48 h was 0.14 lower than at 24 h, but was non-significant at $p = 0.05$).

Under the simulated hot and dry conditions (35 °C and 50% RH), S46 presented the most remarkable decrease (−1.83 log after 48 h), whereas S38 and S18 decreased to −0.90 log and −1.27 log in the same time lapse, respectively (Fig. 2b). These three strains showed a similar pattern, with a considerable decrease in the first 24 h, followed by a more stable population level between 24 and 48 h. In contrast, S39 presented a slight decrease in the first 24 h (−0.33 log), but then populations kept dropping to −0.83 log compared to the 0 h samples. In



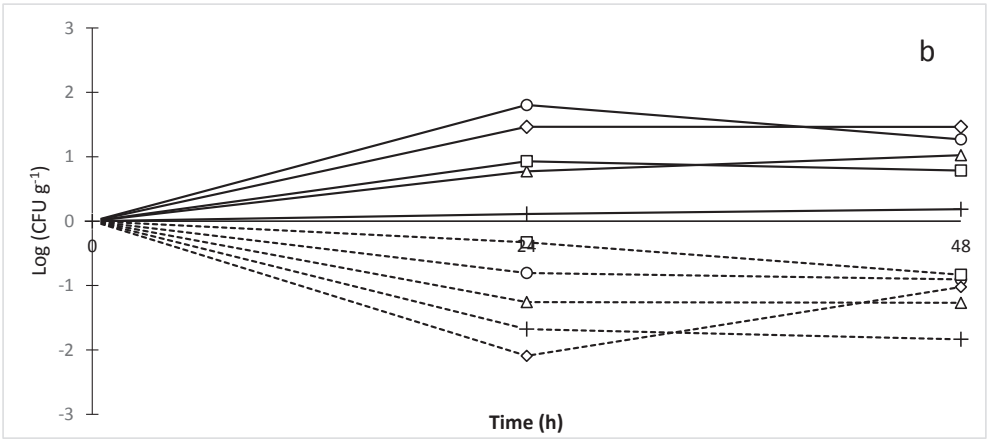


Fig. 2. Population dynamics of bacterial strains on grape berries under simulated climatic conditions a) *Pantoea* spp. and *Enterobacter* spp. strains, b) *Bacillus* spp and *Paenobacillus* spp. strains. Detached grape berries were sprayed with bacterial suspensions at 1×10^7 CFU ml^{-1} of the strains (▲) *Pantoea agglomerans* S3, (●) *Pantoea agglomerans* S6; (△) *Paenibacillus* sp. S18; (■) *Enterobacter cowanii* S22; (♦) *Enterobacter* sp. S23; (x) *Enterobacter* sp. S24; (○) *Bacillus ginsengihumi* S38; (□) *Bacillus ginsengihumi* S39; (◇) *Bacillus* sp. S43; (+) *Bacillus* sp. S46. Treated berries were then incubated in climatic chambers at 22 °C and 100% RH (solid lines) or at 35 °C and 50% RH (dotted lines) during 24 h or 48 h before recovery of surface populations. CFU values are per gram of berry sampled and were log-transformed. Values represent the means of three replicates.

the case of S43, a dramatic decrease was observed after 24 h (−2.09 log), although final levels after 48 h of exposure were similar to the other strains (−1.02 log compared to initial populations).

3.3. *In vitro* compatibility of bacterial strains with commercial adjuvants and natural products

The effect of the commercial adjuvants varied according to the different strains, although some general trends were noticeable (Table 2). One formulation (Dash) was very toxic to all tested strains, depleting S3, S22 and S38 populations under the detection level and reducing S43 by −2.32 log. Similarly, the Acteon treatment significantly reduced the populations of three strains, with differences ranging from −0.66 log (S22) to −0.82 log (S3 and S38), although it did not significantly affect the S43 population level. The two adjuvants, Actirob and Sticman, were not statistically different from the water control, except for the Sticman*S3 combination, resulting in −1.09 log compared to the water control.

In contrast and interestingly, the Agrotonic adjuvant (AT treatment) significantly favoured the *Bacillus* species populations compared with the water control, with a significant increase of 2.56 and 2.03 log for S38 and S43, respectively. However, the same product significantly reduced S3 and S22 populations by −1.31 and −0.44 log, respectively. The SW treatment also significantly increased S38 populations by 0.94 log, but reduced S3 and S22 populations by −1.04 and −0.88 log, respectively.

The compatibility tests with the tested NPs (Table 2) showed that two products, HMLCu and HMLZn, did not show any effect on all the bacterial strains tested. Two other products, FC and MZ, significantly increased the *Bacillus* populations of S38 and S43, presenting between 1.56 and 3.96 log more than the water control (WC). However, these products did not affect S3 and S22 population levels. Lastly, the three other NP formulations significantly reduced the populations of two or three strains. AZ, HML32 and PRT reduced the S3 and S22 populations by −1.88 to −5.01 log, respectively, without affecting S38 or S43 (except for the HML32, which markedly decreased the S43 populations).

3.4. Cell adherence on surface of grape berries with selected adjuvants

The products tested as potential adjuvants, which showed full or partial compatibility *in vitro* (see previous paragraph), were further investigated *in vivo* to assess their effect on adherence of bacterial cells at the grapevine berry surface. Out of three different bioassays, Fig. 3 represents the differences in bacterial populations applied with the adjuvants, compared to a water control (corresponding to the 0 h level in Fig. 3). Considering the means of recovered populations per gram of grape berry, no significant differences in S3 cell adherence were detected between the tested products, and when they were compared to the pertinent assay control (LSD test at $P = 0.05$). As for S22 cells, three products, i.e. Agrotonic, Silwet and Sticman, increased cell adherence by more than 1 log, compared with the corresponding water control, inducing a significantly higher adherence than the other two tested products (Acteon and Actirob). For the S38 strain, the three products

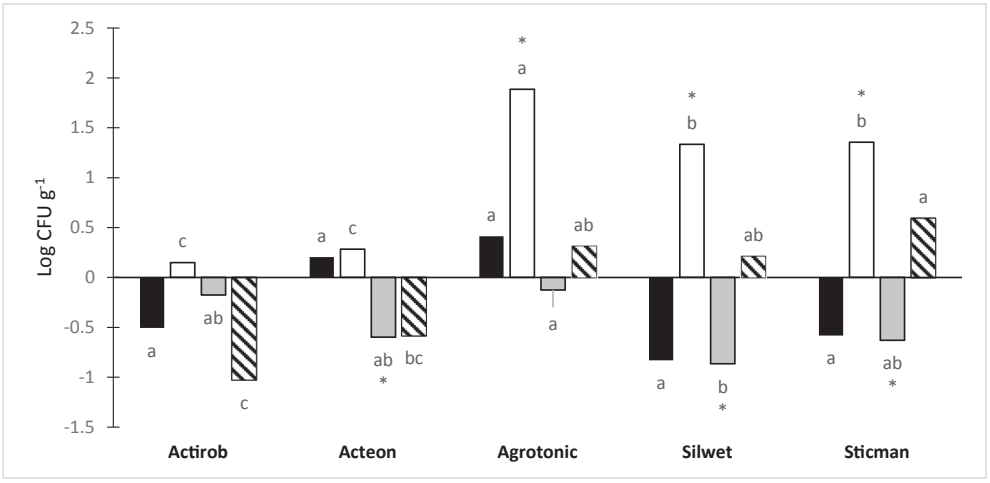


Fig. 3. Effect of commercial adjuvants on the adherence of bacterial cells to grape berry surface Detached grape berries were sprayed with a bacterial suspension of (■) *Pantoea agglomerans* S3, (□) *Enterobacter cowanii* S22, (▣) *Bacillus ginsengihumi* S38, (◪) *Bacillus* sp. S43 in sterile water with the addition of commercial application adjuvants Actirob (0.3% v/v), Acteon 0.5% (v/v), Agrotonic 0.75% (v/v), and Sticman 0.14% (v/v). After drying for 2 h, bacterial populations from the surface were recovered and counted. Values are means of three replicates and represent populations on berries compared to the application of bacterial suspension on water only. *Indicates significant differences with water control. Values linked by the same letter are not significantly different.

Acteon, Silwet and Sticman markedly lowered populations at the berry surface by more than −0.5 log. Moreover, for S38 adherence, Agrotonic (−0.12 log) was significantly better than Silwet (−0.86 log), and the other product effects were intermediate. Lastly, only Sticman significantly improved S43 adherence, compared to water control, and the resulting adherence level was significantly higher than that with Actirob or Acteon.

3.5. Field application of selected bacterial strains

The untreated control presented 24.3% incidence and 2.2% severity at harvest (Fig. 4a). Two treatments with the *B. ginsengihumi* S38 strain significantly reduced BBR incidence by 72% and 75%. The treatments consisted of five applications throughout the season, or four applications after veraison, following the DRI (S38 FS and S38–Index, respectively). The other treatments reduced the incidence levels

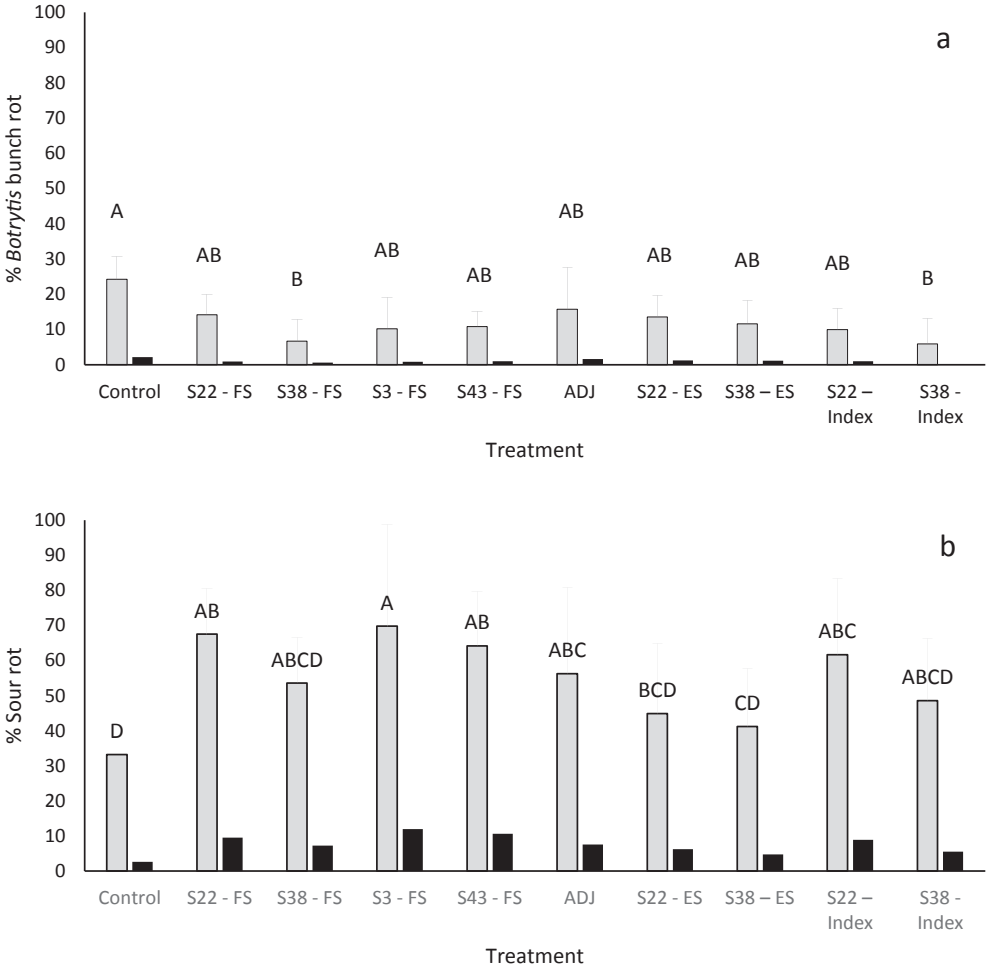


Fig. 4. Incidence and severity of two main bunch rot diseases in a Bordeaux vineyard (cv. Merlot) at 2015 season harvest a) Botrytis bunch rot and b) Sour rot. Incidence (grey bars) and severity (black bars) of the two diseases were assessed at harvest on 50 bunches per replicate plot. Vines were treated until runoff with bacterial suspensions plus a commercial adjuvant (ADJ) at key phenological stages or according to a *Botrytis* Disease Risk Index (DRI). **Control**: Untreated; **ADJ**: Sticman® at 0.14 mL l^{-1}; **S3**: *P. agglomerans* S3 + ADJ; **S22**: *E. cowanii* S22 + ADJ; **S38**: *B. gingsengihumi* S38 + ADJ; **S43:** *Bacillus* spp S43 + ADJ; **FS**: Full season strategy including five applications between 10% flowering and 21 days before harvest; **ES**: Early season strategy including three applications between 10% flowering and pre-bunch closure; **Index**: four post-veraison applications following a DRI. Values are means of four replicates. Values linked by the same letter are not significantly different according to LSD Student's *t* test.

compared with the untreated control, but not significantly. The corresponding efficacies ranged from 35% (ADJ) to 59% (S22-Index) reduction compared to the control. Compared to the regular spray application strategy, either “Early season” or “Full season”, the strategies based on modelling using the DRI after veraison, tended to be more effective for reducing BBR. This was noticeable for both the S22 and S38 strains applied according to this DRI-based strategy (S22-Index, S38-Index) which resulted in 10% or less BBR incidence at harvest. Although no significant differences at $P = 0.05$ were observed in BBR severity, the S38-FS and S38–Index treatments presented the lowest severity levels, with 71% and 89% reductions compared to the untreated control, respectively. Severity reductions in the other treatments ranged from 24% (ADJ) to 61% (S3 – FS).

As for SR intensity at harvest (Fig. 4b), the untreated control presented 33.2% incidence and 2.6% disease severity. All the treatments presented higher SR levels than the control. This difference was significant in SR incidence for the treatments S22-FS, S3-FS, S43-FS, ADJ and S22-Index, but not for treatments using the S38 strain (S38-FS, S38-ES and S38-Index) or the S22-ES treatment. No significant differences were detected in SR severity, although all treatments presented higher disease levels than the control, ranging from 4.7% (S38-ES) to 11.9% (S3-FS).

3.6. Field population dynamics of bacterial strains

Fig. 5a shows the temporal population dynamics of the four bacterial strains applied in the Bordeaux vineyard in the field during the 2015 season. After the first application at 10% flowering, bacterial populations per sample gram ranged from 5.43 log (S22) to 6.33 log (S38). Populations of the S43 strain were not determined, due to sample contamination. Before the second application at 100% flowering, populations of S22, S38 and S43 decreased to 4.60, 4.03 and 2.95 log, respectively, whereas the S3 populations only slightly decreased to 5.77 log. The second application increased populations in flowers by 0.33, 0.4 and 1.14 log in the S3, S43 and S22 samples, respectively, while S38 populations could not be determined, due to sample contamination. The most remarkable population decrease occurred between the end of flowering and the pre-bunch closure stage, when populations ranged from 0.35 to 3.26 log. Interestingly, the application of another spray at pre-bunch closure only increased significantly the populations of the *Bacillus* species S38 and S43, by 1.21 and 2.91 log, respectively, compared to the previous sample. After pre-bunch closure, the S3, S22 and S43 strains did not significantly increase their populations until the end of the season, despite the last two applications at 10% veraison and 21 days before harvest. The S38 strain significantly decreased after pre-bunch closure to approximately 1.50 log at veraison, and maintained this level until the end of the season.

Regarding population dynamics of the *E. cowanii* S22 and *B. ginsengihumi* S38 strains, applied following the DRI after veraison (Fig. 5b), the first spray application left between three and four log units of the two bacterial strains at the berry surface. These populations decreased slightly until the second application, after which populations reached 3 log units, approximately. However, none of these differences were significant. Similarly, populations of both strains decreased slightly between the second and third applications. After the third application, populations of S22 were significantly recharged over 3 log units, whereas S38 populations did not significantly increase. Then, before the fourth spray, the S22 populations decreased significantly and were again recharged over 4 log units. Lastly, the S38 populations remained similar, before and after the last application.

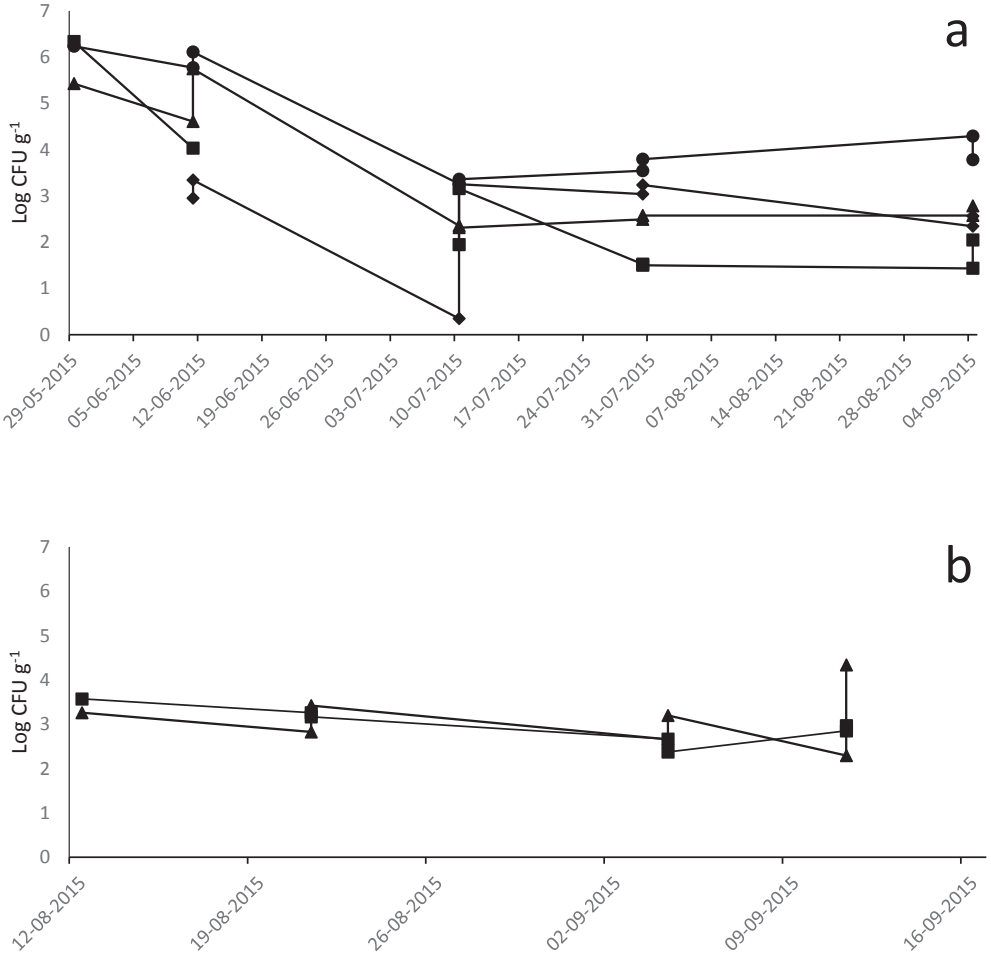


Fig. 5. Population dynamics of four selected bacterial strains on grapevine flowers and berries after spray applications of bacterial suspensions during the 2015 season in a Bordeaux vineyard (cv. Merlot) a) Bacterial populations applied at key phenological stages (five applications between 10% flowering and 21 days before harvest); b) bacterial populations applied according to a post-veraison Disease Risk Index. Vines were treated until runoff with bacterial suspensions at 5×10^7 CFU ml^{-1} plus the commercial adjuvant Sticman® at 0.14 mL l^{-1}. Populations were recovered before and after each spray application. (●) *P. agglomerans* S3; (▲) *E. cowanii* S22; (■) *B. gingsengihumi* S38; (♦) *Bacillus* spp S43. Flower or berry samples were taken after spraying and again just prior to the next spray application. CFU values are per gram of tissue sampled and were log-transformed. Values are the means of four replicates.

4. Discussion

In this paper, we described the application of a field-oriented approach for screening bacterial isolates as BCAs against BBR, after an initial multi-criteria selection step in the laboratory to assess potential BCA efficacy under vineyard conditions. We subsequently evaluated four candidate strains following different application strategies in a same field experiment to maximise the opportunities for finding an effective isolate adapted to the vineyard environment. This approach represents an advance compared to the application of just one single BCA candidate strain based exclusively on laboratory efficacy tests. This selection process is in accordance with a recent body of literature recommending multi-criteria screening for BCAs (Bouaoud et al., 2017; Köhl et al., 2011; You et al., 2016).

First, we pre-selected ten bacterial strains out of 46 and, by performing an *in vitro* test, we singled out the MoAs of five strains. Two strains showed nutrient competition ability, i.e. *P. agglomerans* S3 and *Paenibacillus* spp S18, since they only suppressed *B. cinerea* germination in the presence of grape juice (whatever its concentration). This capacity was confirmed for S3 in a second assay, but not for S18, which showed more variable ability. In addition, three strains (*E. cowanii* S22, *B. ginsengihumi* S38 and *B. ginsengihumi* S39) showed significant *B. cinerea* germination reduction in both SDW and in grape juice, indicating that the MoA of these three strains arose more from metabolite production than from nutrient competition. This MoA is widely described for other *Bacillus* species (Ambrico and Trupo, 2017; Baruzzi et al., 2011; Ongena and Jacques, 2008). Equally, although examples are less common, Enterobacteriaceae members have been also described as producing soluble compounds or enzymes, active against plant pathogenic fungi (Weller, 2007) (Chernin et al., 1996; Fravel, 1988; Wallace et al., 2018). In a previous study (Haidar et al., 2016a), neither the S3 or S18 strains, showed any mycelial antagonism by diffusible compounds, in accordance with the nutrient competition MoA emerging from the present study. Those *in vitro* results showed that a direct antagonistic effect by diffusible compounds characterised the strains *Enterobacter* sp. S23, *Bacillus* sp. S43 and *Bacillus* sp. S46, but this effect was not observed in our experiment with grape juice. This discrepancy might be attributable to the different experimental conditions: i) solid *vs.* liquid medium, ii) available nutrient source: rich dextrose medium *vs.* low concentrated grape juices; and iii) the *B. cinerea* structures targeted for antagonism (mycelium *vs.* conidia). As often highlighted in the literature (Di Francesco et al., 2016; Haidar et al., 2016c; Nicot et al., 2016), a wide range of laboratory tests results is needed to define the sole MoA (or the different MoAs) of one BCA candidate strain. Furthermore, whenever multiple MoAs account for a BCA strain's efficacy, it is difficult to separately demonstrate and quantify each and every MoA (Pertot et al., 2017a). Such a combination of effects could have occurred in our nutrient experiment, where some strains may have reduced germination by direct antifungal activity (S22, S38 and S39), but also, to a certain extent, by competition for the nutrients. This is the case for many antagonists, because not all their potential MoAs and interactions have been studied thoroughly, and detailed information on such complex mechanisms is often incomplete (Teixidó et al., 2011).

Concerning survival ability, the results revealed that the *Pantoea* and *Enterobacter* strains tended to multiply less in optimal conditions for *B. cinerea* growth, and to be more depleted under limiting conditions, when compared to the *Bacillus* and *Paenibacillus* strains. These results may signal the *Bacillus* and *Paenibacillus* strains as being potentially better adapted for biological control in the field. Survival ability of *Bacillus* species in field conditions has been already highlighted in other studies (Demoz and Korsten, 2006; Fan et al., 2017; Gotor-Vila et al., 2017; Leibinger et al., 1997), and may be explained mostly by spore formation and dormancy (Mutlu et al., 2018). However, the population dynamics in our study was different for each particular strain. In this sense, the results helped to identify strains with lower interest in terms of survival ability, notably the two *Enterobacter* sp. S23 and S24, and *Bacillus* sp. S46.

By considering three key features contributing to an effective biological control, *in vivo* efficacy level, MoA and survival ability, we identified a short-list of four candidates for field applications, out of the ten initially pre-selected strains. The final selection took into account the following criteria: 1) high overall efficacy associated with the strain efficacy profile described in previous *in vivo* biotests (Haidar et al., 2016b); 2) presence of several of the potential MoAs considered; 3) high and/or intermediate survival ability under two contrasting simulated climatic conditions. Of the ten pre-selected strains, three presented a higher overall *in vivo* efficacy (*B. ginsengihumi* S38, *Bacillus* sp. S43 and *E. cowanii* S22), and also corresponded to the chosen criteria. Of the strains with lower overall efficacy levels, the *P. agglomerans* S3 strain presented two very interesting features in terms of selection criteria, which complemented the characteristics of the first three strains: nutrient competition as its main MoA (confirmed in two assays), and a different specific efficacy profile (high efficacy in unwounded berries). Accordingly, these four strains were selected for the evaluation of suitable additives and for field applications, as shown in the results section: *P. agglomerans* S3, *E. cowanii* S22, *B. gingsengihumi* S38 and *Bacillus* spp S43.

Our *in vitro* results have provided a list of compatible adjuvants for field applications and NPs for integrated control strategies. These data may be interesting for other research programs dealing with bacterial BCAs, since combining BCAs with additives in pre- or post-harvest applications is regarded as a major mechanism in improving efficacy of biological control strategies (Rhodes, 1993; Tesfagiorgis and Annegarn, 2013). However, to avoid any interaction due to the direct anti-*Botrytis* efficacy of certain NPs, we preferred to use commercial adjuvants in our field trials. Overall, the Agrotonic and Sticman formulations, resulted in significantly higher populations of *E. cowanii* (S22) and *Bacillus* sp. (S43). However, the polysaccharide matrix of Agrotonic, the algae-based product, may, as a sugar nutrient source, favour *B. cinerea* development, as well as other communities in the grape berry microbiota. Since such a product composition may have interfered with the efficacy results of the candidate strains, we considered Sticman as a more appropriate adjuvant.

In the vineyard screening experiment comparison using the four strains (S3, S22, S38 and S43), only the *B. ginsengihumi* S38 strain was able to significantly reduce the incidence of BBR (S38-FS and S38-Index treatments). The same treatments were also successful in reducing severity, thereby confirming the clear BCA effect of this *B. ginsengihumi* strain. Decreased BBR incidence and severity in the DRI-Index-based strategy was also observed with the *E. cowanii* S22 strain, supporting the idea of good performance being attributable to the DRI strategy for positioning BCA. This good DRI performance represents one particularly positive outcome of this study, since it constitutes the first application and use of the infection model recently published by (Ciliberti et al., 2015). Other models for *B. cinerea* epidemics in vineyards have been developed (Broome et al., 1995; González-Domínguez et al., 2015), and there are other examples of BCAs being applied on the basis of a DRI (Shtienberg, 2004). However, to the best of our knowledge, the present study is also the first reported successful outcome resulting from application of a BCA following a DRI-based spray timing in vineyards. These results still have to be validated in following growing seasons and/or regions, and the decision rules may also be partly modified in the future. As for the early season strategy, it did not show any particular effect on BBR at harvest-time, although the stages between flowering and bunch closure are generally considered as key epidemiological stages for BBR development (Calvo-Garrido et al., 2014a; Sanzani et al., 2012). This lack of efficacy could be linked to the unfavourable meteorological conditions for secondary inoculum build-up between the beginning of flowering and veraison during the experimental season (2015) in the Bordeaux region (only approx. 26 mm cumulative rainfall in 64 days; data not shown).

The efficacy data presented came from one growing season and one

location only, under low BBR levels associated with early disease assessment. Furthermore, a second BBR evaluation at maturity (data not shown, assessment of 23/09/2015) also presented disease reductions, but these were not statistically significant. These BBR reductions may be considered as interesting preliminary results, whose efficacy percentages need to be confirmed in repeated seasons and site locations, even if our reduction percentages are similar to those achieved by other *Bacillus* BCAs in developmental stages (Aziz et al., 2016; Ben Maachia et al., 2015). The *in vivo* results of (Haidar et al., 2016b) have been corroborated with the *B. ginsengihumi* S38 strain, which was shown to be the most effective. This finding is also consistent with the literature, since *Bacillus* species, which are of prime importance in biological control of grapevine diseases, are extensively investigated (Di Francesco et al., 2016; Haidar et al., 2016c; Ongena and Jacques, 2008; Sawant et al., 2016). In some currently biocontrol commercialised products against BBR, *B. subtilis* strains, for instance, constitute the major active ingredient (Nicot et al., 2016; Pertot et al., 2017b).

Overall, the selection after one out of four bacterial candidate strains in a vineyard experiment, prioritising key biological features, such as survival capacity and MoA, can be considered as a success, since it validates the selection process employed. Our whole screening programme followed the systematic stepwise process proposed by (Köhl et al., 2011). However, we ended with a short and field-oriented step, in which we integrated only a few parameters from Kohl's step 3 to step 8 (full field testing), i.e. ecological traits of strains and a few formulation issues, and (up to step 5) by complementing previous efficacy bioassays from (Haidar et al., 2016a; Haidar et al., 2016b). This multi-criteria selection process is expensive (Köhl et al., 2011), hence we only applied it to a reduced list of candidate strains. In addition, we introduced another factor into the full field testing (step 8), by testing several candidate strains at the same time, allowing us to compare them and save time. Although not presented in this work, steps 4 and 6 (database mining and preliminary assessment of mass production, respectively) were also carried out in order to better characterise the candidate strains.

The field experiment also led to interesting findings related to SR. Interestingly, the Sticman product favoured SR development, whereas three bacterial strains (S22, S43 and, notably, S3) showed a trend favouring SR, but not significantly. Furthermore, the S38 strain tended to lower SR incidence and severity. These effects may be related to the modification of the natural microbiota at the berry surface and the stimulation of SR-related microorganism species, i.e. bacteria and/or yeasts (Barata et al., 2012; Blancard et al., 2000). The high rate of SR in the *P. agglomerans* S3-treated plots was associated with high S3 populations, but this was not the case with *E. cowanii* S22 and *Bacillus* spp S43, pointing to an unclear relationship between BCA bacterial population levels and SR development. The SR analysis at harvest highlights the importance of studying more than one pathosystem in the same field experiment when dealing with biological control. Some treatments may have undesired effects, according to the disease targeted, as already demonstrated *in vivo* (Haidar et al., 2016b). According to these field results, the Sticman adjuvant should not be used for vineyard application in association with BCAs. The favourable effect on SR development of the *P. agglomerans* S3 strain completely excludes this strain from further BCA research programmes.

In conclusion, the present study has pointed out a potential effective BCA against BBR, *B. ginsengihumi* S38 strain, for future product development associated with its formulation optimisation and adapted application strategies. The selection process used to pass from laboratory experiment to field applications has been validated, since one out of ten candidate strains has been positively selected, in accordance with the systematic stepwise screening process proposed in the literature (Köhl et al., 2011; You et al., 2016). In addition, a first application of a DRI, an application strategy based on a BBR epidemiological model, has produced a positive outcome. However, this needs to be validated in future seasons. Lastly, the results of the SR evaluation at harvest highlighted the complexity of biocontrol interactions in the field, indicating that a multi-pathogen approach is always important and desirable in further biocontrol studies.

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