New multipest damage indicator to assess protection strategies in grapevine cropping systems

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Abstract

Background and Aims: Social, environmental and regulatory evolution encourages grapegrowers to reduce chemical use. Organic production is one possible answer, but controlling pests and diseases by products allowed in organic production is less easy than with chemicals. We integrated disease severity levels (*Botrytis* bunch rot, powdery and downy mildews) and pest incidence (grape berry moths) to develop an indicator of pests and disease damage in grape bunches, named assessment indicator of damage in grape bunches (AIDB), which can be used to assess pest incidence in networks of grower vineyards.

Methods and Results: In 2011 and 2012, we tested AIDB by monitoring damage at key phenological stages in 20 vineyards in the Bordeaux and Languedoc regions in the south of France, under three modes of production: conventional production, organic production and in conversion to organic production. The indicator proved accurate in describing the total damage on grape bunches under all conditions tested.

Conclusions: Irrespective of the mode of production, the AIDB value was negatively correlated with yield parameters and the technical expertise of the grower. This indicator proved to be an innovative tool to estimate the multipest damage on grapevines over a range of conditions.

Significance of the Study: The AIDB enables grapegrowers and advisors to assess the integrated impact of their treatment strategies on grape production, which is necessary when reducing pesticide use.

Keywords: *assessment indicator, organic farming, pest management, pesticide, production system*

Introduction

As for most horticultural crops, pesticide use has long been a key factor in increasing and stabilising yields and fruit composition in grapevines (Ehler 2006). This heavy dependence on pesticides is challenged by the increasing knowledge of the harm caused by pesticides to human health and to the environment (Damalas and Eleftherohorinos 2011). Stricter rules for pesticide registration and natural resources protection have now been adopted, especially in Europe. As a consequence, the list of registered pesticides is regularly and significantly reduced, and systematic use of chemicals, based on persistence period only, has been questioned (Hillocks 2012). The future of grape production for wine will therefore rely on low-input and efficient cropping systems, built on innovative strategies for pest and disease management (Atkinson et al. 2004, Deguine et al. 2008, Thiéry 2011).

Innovative approaches to plant protection, however, remain to be developed (Kogan 1998, Cook and Proctor 2007, Médiène et al. 2011, Thiéry 2011). Among these approaches, damage indicators and decision rules for pesticide application (Léger et al. 2010) are required to reduce efficiently the amount of pesticides used without reduction in yield and wine quality. Reducing the application of chemicals requires operational integrated pest management (IPM) strategies to be implemented (Suckling et al. 1999, Hillocks 2012, Rossi et al. 2012), based on new tools and technologies (e.g. precision viticulture, plant elicitors and new cultivars), more information on vineyard status (e.g. observation networks), new management practices and the development of biological control (Hillocks and Cooper 2012) and modelling for a decision support system (Gill et al. 2011). These new strategies require the integration of functional ecological processes related to pathogens or pests interacting with crop growth and development (Wearing 1997, Caffi et al. 2013). Compared with systematic use of pesticides, this is likely to result in more complex strategies to control pests and diseases that have to be assessed, in an integrated way, for their efficiency in limiting pesticide use without affecting grape yield and wine quality (Trevisan et al. 2009). Thus, there is a crucial need to compare current and alternative strategies and to monitor their performance in vineyards, in order to promote the most effective for a sustainable pesticide reduction.

Disease and pest indicators, and associated decision tools, are needed for such assessments (Bockstaller et al. 2008) in order to evaluate objectively the sanitary status of the crop. Three types of indicators can be defined, depending on their use and users (Wery et al. 2012): (i) 'analysis indicators' are used to describe and understand system processes and properties (Valdés-Gómez et al. 2008); (ii) 'management indicators' guide the implementation of improved management practices (Pellegrino et al. 2006, Barrabé et al. 2007), generally as input variables to a decision rule; and (iii) 'assessment indicators' evaluate performance and environmental impacts. For an efficient input reduction, operational management indicators are needed to inform growers about the dynamic of the system's state including, for example, intensity of water stress (Pellegrino et al. 2006) or nitrogen stress and leaching (Cuny et al. 1998), level of a pest population, risk or potential severity of a disease (Wearing 1997, Carisse et al. 2009, Rossi et al. 2014). This type of information is needed to adjust the spraying

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dose (Davy et al. 2010, Gill et al. 2011), the frequency (Léger and Naud 2009) and the type of applications and/or active ingredients to be used (Penrose et al. 1994). Although these management indicators are essential tools to assist in a sustainable reduction of pesticides, they often fail in the ex post assessment of these strategies to maintain a safe state of the crop with regard to pests and diseases. Examples of more integrative assessment indicators have been proposed in the literature but failed because of the lack of operational use or focus on environmental aspects, forgetting agronomic performance assessment (Trevisan et al. 2009, Zhan and Zhang 2012). Finally, integrative, easy-to-calculate and easy-to-share assessment indicators are required, such as the environment exposure to pesticides index (Wijnands 1997) or the frequency treatment index widely adopted in France (Butault et al. 2010). Integrated assessment indicators, considering several pathogens and pests together, are also essential because they could be shared by various actors in different agricultural contexts, notably with various intensity of dependence on chemicals (Wearing 1997, Fragoulis et al. 2009).

Vineyard systems are highly susceptible to several key pests and diseases. Their control is crucial to maintain the quantitative and qualitative production objectives for this crop. Currently, in France, vineyards consume 20% of all the pesticides used, on only 3% of the total agricultural area, and receive on average 14–15 applications annually (Agreste 2010). A similar trend is also noticeable in Europe, and several actions have been initiated to favour IPM to reduce the use of pesticides in vineyards, for example, through the ENDURE (2014) network or the PURE (2014) research program. Three main diseases and a pest are targeted for their impact on grape bunches in many vineyards worldwide:

- Downy mildew (DM), due to *Plasmopara viticola* (Berk & Curt.), is one of the major diseases of grapevine (Rossi et al. 2013). Between fruitset and veraison, the pathogen infects inflorescences and young berries, affecting seriously the grapevine yield.
- Powdery mildew (PM), due to the ectoparasitic fungus *Erysiphe necator*, grows mostly on the surface of the green aerial organs in *Vitis* spp. (Corio-Costet 2007) and can decrease yield, especially by developing on berries, from flowering to the berry touch stage (Calonnec et al. 2004).
- Botrytis bunch rot or grey mould (GM) is caused by the necrotrophic fungal pathogen *Botrytis cinerea*. This fungus is a species complex that infects mostly inflorescences and ripening berries (Martinez et al. 2005, Deytieux-Belleau et al. 2009, Walker et al. 2011). This pathogen, reported worldwide in tablegrapes and winegrapes, is responsible for important crop loss and qualitative oenological damage (Elmer and Michailides 2007, Ky et al. 2012, Steel et al. 2013).
- The tortricid grape moths (Lepidoptera: Tortricidae) (TM), *Eupoecilia ambiguella* (Hübner) and *Lobesia botrana* (Denis and Schiffermüller) are the most harmful pests in grapevines in the western Palearctic region (Thiéry 2008). In Europe, these polyphagous moths undergo generally two and two-to-four generations, respectively, for the two species. The main direct damage results from the summer (second and next) generations larvae injuring grape berries (Pavan et al. 1987, Fermaud 1998), but they also encourage rot pathogens, such as *B. cinerea* (Fermaud and Le Menn 1992, Cozzi et al. 2006).

The intensity of attack, however, by these key damaging organisms may vary between locations depending on the year (Savary et al. 2009), the cultivar (Thiéry et al. 2014), the soil (Valdés-Gómez et al. 2011), the microclimate/mesoclimate (Pieri and Fermaud 2005, Caffi et al. 2013) and the landscape environment (Veres et al. 2013). In response to environmental concerns, alternative modes of production have emerged and are appreciated by consumers, with organic farming being a prime example. In vineyards, the market and societal pressures have stimulated conversion to organic farming production in recent years. For example, in France, since 2008, the area devoted to organic vineyards (either in conversion or already certified) has increased almost threefold (Agence bio 2011). One of the main differences between organic farming and conventional production (CP) is the type of active ingredients used against pests and diseases. With synthetic chemicals being forbidden, growers use mostly copper and sulfur compounds, which are more easily washed off from the leaves and are less efficient than more recent synthetic and specific pesticides. The efficiency of disease control is therefore likely to depend on the strategy of the organic grower to reduce pest pressure by other means (e.g. reduced plant vigour) or to increase the frequency of fungicide spraying.

In this context, there is a crucial need to develop generic, operational and multipest assessment indicators to evaluate the efficiency of pesticide strategies in a wide variety of vineyard management systems, including conventional, IPM and organic production schemes. In this paper, we propose a new integrative assessment indicator of bunch damage intensity resulting from major grapevine pests and pathogens. By relying solely on assessment of infection and pest population in inflorescences and bunches, the indicator was intended to be used as a vine health indicator, more than an indicator of the impact of these pests and diseases on yield or wine quality. It was necessary to assess total cumulative loss because of the different bunch-damaging organisms and to evaluate the potential of this indicator as an assessment tool, when used in the context of growers' vineyard networks.

Materials and methods

The new indicator was calculated using an equation established on a theoretical basis (see below) and was tested with a database obtained by monitoring a major pest and several pathogens damaging bunches in a network of vineyards in the two major French grapegrowing regions of Bordeaux and Languedoc.

Climatic conditions and natural pest and disease pressure

The Languedoc area near Montpellier and the area near Bordeaux are, respectively, characterised by typical Mediterranean and Oceanic climates. The average annual climatic features (1981–2010) are, respectively, as follows: (i) minimal temperature, 10.4°C and 9.1°C; (ii) maximal temperature, 19.9°C and 18.5°C; (iii) rainfall, about 630 and 940 mm; and (iv) number of rainy days, 58 and 124.

To estimate the natural pest and pathogen pressure in both regions in 2011 and 2012, we used information from surveys in unsprayed vineyards conducted by various experts throughout France and published by the French Agricultural warning services (Grosman et al. 2011, 2012). Such annual reports rely on a large number of experts and numerous vineyards observed in various regions and therefore represent a good characterisation of the overall pest and disease pressure on vineyards. We transformed the textual qualitative information into a class variable according to Zwankhuizen and Zadoks (2002) for each pest or pathogen, corresponding to their frequency of occurrence. The grid scoring proposed was as follows:

- 0, no symptom or not recorded.
- 1, low intensity, sporadic, locally referenced symptoms. Approximately, maximum 25% of the vineyard affected, with less than 10% of diseased bunches or less than one tortricid larva for ten bunches.
- 2, moderate, widely referenced symptoms. Approximately, 50% of the vineyard affected, with about 10% of diseased bunches or one tortricid larva for ten bunches.
- 3, severe but locally referenced symptoms. Approximately, maximum 25% of the vineyard affected, with about half of diseased bunches or one tortricid larva in two bunches.
- 4, severe, important and geographically widespread symptoms. Approximately, more than 50% of the vineyard affected, with more than 25% of diseased bunches or one tortricid larva per bunch.

Vineyard network and modes of production monitored

The study was undertaken on a network of 20 vineyards all planted with the same cultivar (Merlot), in both Languedoc (nine vineyards) and Bordeaux regions (11 vineyards), but covering a wide range of environmental and management conditions. Because of their location in France, these two regions will be hereafter referred to as south-east (SE) and south-west (SW), respectively (Figure 1). The vines were grafted to 140Ru or SO4 in SE, and to 101-14, Gravesac or SO4 in SW. The year of planting ranged from 1984 to 1999 in SE, and from 1972 to 1993 in SW. The pruning system was Cordon de Royat or simple Guyot in SE, and simple or double Guyot in SW. The planting density varied between 3600 and 6300 vines per hectare.

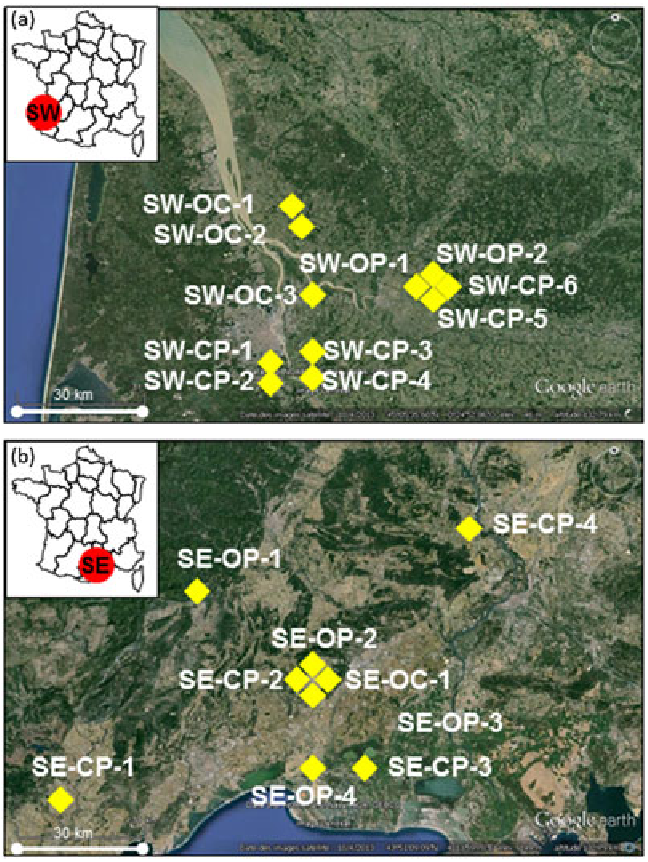

Figure 1. Location of the vineyard network in (a) south-east France (SE) and (b) south-west France (SW). CP, conventional production; OP, organic-labelled production; and OC, production in organic conversion.

The mode of production was classified as follows: (i) CP, that is, using synthetic pesticides with some integration of the IPM principles, ten vineyards, four in SE and six in SW; (ii) organic growing labelled production (OP), six vineyards, four SE and two SW; and (iii) production in organic conversion (OC), that is, undergoing transition from CP to OP, four vineyards, one SE and three SW.

The conventional mode of production includes some variability between growers. The decisions about spraying frequency are sometimes calendar based as proposed by the local pesticide supplier and/or adjusted according to extension services, taking into account vineyard monitoring, extension advice and weather forecasts. In the SE, 95% of the vineyards receive between 2.2 and 16.4 fungicide treatments per year, and half of them receive 7.2 to 10.9 sprays per year; in the SW, 95% of the vineyards receive between 3.7 and 18.3 fungicide treatments per year, and half of them receive 9.4 to 12.9 sprays per year (Agreste 2010).

Technical expertise of growers

We characterised the quality of vineyard management by growers with a qualitative variable, named 'technical expertise' (Tech), characterising the ability of the winegrowers to implement grapevine management practices related to IPM principles, based on their knowledge, know-how and observations. We categorised some technical aspects of cropping practices, farm equipment (machinery) and human resources (Table 1) on a scale of 1 (poor) to 3 (good). For every vineyard, the value remained the same for both years because this variable varies little in the short term.

Characterisation of plant protection treatment strategies

We characterised the vine protection strategies, including the intensity of product use, according to the phytosanitary treatment schedules provided by the growers for every vineyard. We used the treatment frequency index (TFI), which is the equivalent annual number of treatments applied to the crop, calculated on the basis of the amount of product actually applied compared with the corresponding amount of product at the official dose (Butault et al. 2010). This index was calculated every year for each target pest or disease.

Pest and disease sampling

The vineyards were surveyed in 2011 and 2012, and the symptoms due to the following major diseases and pest, DM, PM, GM and TM, were assessed, focusing on grape bunches. Every vineyard was monitored at key stages by visual assessment in order to evaluate the different infection/infestation level in inflorescences and grape bunches by the pathogens and pest when they were most damaging for yield and/or must composition, as follows: (i) at flowering, in inflorescences, for DM (Savary et al. 2009) and GM (Dubos 1999); (ii) at bunch closure, in bunches, for DM and PM (Savary et al. 2009); and (iii) at ripening, near harvest time, for GM and TM (Delbac et al. 2006, Thiéry 2008, Deytieux-Belleau et al. 2009). In the SW region, these three stages corresponded, in 2011, to 10–20 May, 4–8 July and 30 August–8 September; and in 2012, to 31 May–8 June, 23–25 July and 12–17 September. Corresponding dates in the SE region were as follows: 22–24 May, 25–27 July and 17 August–5 September in 2011, and 23–25 May, 16–18 July and 16 August–7 September in 2012.

Table 1. Components of the 'technical expertise' variable.

Expertise level and components	Low level (1)	Intermediate level (2)	High level (3)
Implementation of cultural practices	Badly planned and performed Imprecise choice of cultural practices Poor use of decision tools Lack of knowledge or skills on the biophysical system and cultural practices	Intermediate, irregularity in controlling some of the components	Precise management of cultural practices Detailed and appropriate procedures Integration of information from outside the farm (via private advisers)
Pesticide use	Errors or lack of precision in pesticide application (frequency, conditions, phytotoxicity, persistence, etc.)		Fine decision-making process on doses and application conditions Good knowledge of chemical characteristics and use (e.g. treatments at night to reduce drift by wind)
Production factors	Insufficient ratio of labour to productive area		Satisfactory labour/productive area ratio with sufficient staff training
	Unsuitable or incorrectly adjusted machinery		Sufficient and optimal farm equipment

In each vineyard, we observed five randomly sampled plots, each consisting of at least five contiguous vines. Pest and pathogen populations and/or associated symptoms were counted, at the rate of 30 bunches per plot. We measured disease severity in every sampled bunch as the proportion of external surface area visually attacked and showing typical symptoms (Dubos 1999). The number of TM larvae per bunch was estimated with the 'brine method' (Stockel et al. 1994) by immersing with agitation a batch of five bunches in 3 L of brine (NaCl, ~170 g/L of water) for 60 to 90 min in a bucket and counting the number of larvae at the brine surface. Then, the number of TM larvae per bunch was converted into a severity scale, corresponding to the proportion of berries attacked, as follows. We assumed that a bunch can be completely destroyed by 10–30 larvae at harvest time depending on the cultivar (Thiéry 2008) and that Merlot is a medium class compactness cultivar (Fermaud 1998, Galet 2000). Furthermore, in 1995 in the Medoc region, a 100% loss of Merlot grapes was observed in a commercial vineyard following an attack by 15 third-generation larvae per bunch (Mr Lionel Delbac, pers. comm., 1995). Thus, we considered that the maximal severity of 100%, that is, total destruction, was achieved with 15 larvae per bunch. In the following AIDB formula, the proportion of infestation by TM (YTM) was calculated as follows:

$$\mathrm{YTM}(\%) = (\mathrm{LTM}\times 100)\div 15 \text{ for } 0\leq \mathrm{LTM}\leq 15 \quad (1)$$

$$\mathrm{YTM} = 100 \text{ for } \mathrm{LTM}\geq 15.$$

with LTM corresponding to the mean number of TM larvae per bunch near harvest.

Assessment of grapevine growth—development parameters

Canopy state (CanS). Several cropping operations are targeted for the management of grapevine architecture and seasonal leaf development. We have categorised, using a score of 1–3, different states of the vine canopy by taking into account various components (Table 2). For every vineyard surveyed, the value may change between years according to the choices of the grower in response to the vine growing conditions.

Grapevine vigour. The grapevine vegetative vigour was estimated by using the normalised difference vegetation index (NDVI) obtained from ground-based measurements with a Greenseeker (N-Tech Industries, Ukiah, CA, USA; and Oklahoma State University, Stillwater, OK, USA). The measurement protocol was described previously by Drissi et al. (2009) and resulted in an index varying from 0 to 1. High values correspond to maximum vegetative vigour and leaf density in the canopy. The NDVI was assessed at approximately the bunch closure stage.

Table 2. Ranked multi-component categories classifying different states of the plant canopy.

Canopy states and components	Poor state (1)	Intermediate state (2)	Good state (3)
Ventilation in bunch zone	Bunches not visible, hidden by foliage	Irregularity in the control of certain components, usually two to four uncontrolled	Visible bunches, well exposed to light and accessible to spraying
Branch growth and architecture	Overgrown, with tangled branches, trimming and topping absent or poorly executed		No tangled branches, controlled growth
Base of the vinetrunk	Not clear: suckers present		Clear: suckers eliminated
Impact of chemical applications	Incorrect application of treatments (e.g. phytotoxicity)		Control of the dose and of the application

Yield components and yield estimate

We monitored several yield components in the 20 vineyards. On the same 25 vines per vineyard used for pest and disease sampling, we counted the number of bunches at harvest time (Bn) and estimated the mean bunch mass (BM) by averaging the measured mass of one randomly chosen bunch per vine. We obtained the number of vines per hectare (VD) from the grower and calculated the yield (CY) as follows:

$$\mathrm{CY} = \mathrm{Bn} \times \mathrm{BM} \times \mathrm{VD} \quad (2)$$

Every year, we collected from each grower the yield expected from the monitored vineyard, called the 'target yield' (TY). It represents the quantitative production per vineyard, planned at the beginning of the season, allowing the grower to cover the production costs and to ensure the targeted economic margin. At harvest time, the effective mass of the mechanically or manually harvested crop was called the 'harvested yield' (HY). We calculated, for each year and each vineyard, the rate of achievement of the yield (YAR) initially targeted by the grower, as follows:

$$\mathrm{YAR}\,(\%) = (\mathrm{HY} \div \mathrm{TY}) \times 100 \quad (3)$$

The YAR value is expressed as a proportion but may exceed 100% when the HY exceeds the initial TY.

Calculation of the new assessment indicator of damage in grape bunches

We integrated the severity level of each disease and the number of TM larvae per bunch in a single indicator, named 'assessment indicator of damage in grape bunches' (AIDB), expressed as the proportion of damage visually assessed (Delbac et al. 2012). We did not include in the AIDB formula the known interactions between the pests and diseases studied. Two key periods of possible damage by the pest and diseases were taken into account in the indicator calculation, as follows:

$$\mathrm{AIDB}\,(\%) = 100 - (\mathrm{A} \times \mathrm{B}) \quad (4)$$

where A corresponds to the early attacks of the pest and diseases, from flowering to fruitset, and B to later infections/infestations occurring during berry growth and ripening.

A Early in the season, at flowering, DM (YDMF) and GM (YGMF) may infect parts or the entire inflorescence, which become necrotic and sporulating and may be abscised (Martinez et al. 2005, Rossi et al. 2013). The corresponding reduction factor A is

$$\mathrm{A} = [100 - (\mathrm{YDMF} + \mathrm{YGMF})] \quad (5)$$

B After fruitset, the four diseases and pests studied can affect the remaining fruit.

- Downy mildew infects berries (YDMB) (Jermini et al. 2010a, 2010b, 2010c). It does not generally sporulate, and berries become brown, totally dehydrated and depressed (brown rot) (Lafon and Clerjeau 1998).
- Powdery mildew (YPM) covers the berries with conidia and gives them a grey-green aspect. Their growth is affected, and the skin becomes corky; berry bursts may occur (Calonnec et al. 2004).
- Grey mould may infect the berries (YGMB) and progress through the dissemination of conidia. It results in greyish berries, turning brown and rot under cover of a grey down (Valdés-Gómez et al. 2008). This damage increases with grape ripening (Deytieux-Belleau et al. 2009).
- Tortricid moths (YTM) neonate larvae of the summer generation penetrate berries immediately after hatching; they feed on grape pulp (Thiéry 2008). Often, larvae leave their original gallery and damage neighbouring berries, gathering them together with silk. At the end of larval development, the damage can include two to six berries per larva (Pavan et al. 1987, Fermaud 1998).

The fruit biomass is therefore potentially reduced at this stage by a factor of

$$\mathrm{B} = \{1 - [(\mathrm{YDMB} + \mathrm{YPM} + \mathrm{YGMB} + \mathrm{YTM}) \div 100]\}. \quad (6)$$

Statistical analysis

We analysed the results of our vineyard surveys with R statistical software (R Core Team 2015). Differences between regions and between modes of production were tested using ANOVA or Student's *t*-tests. Because we monitored the same vineyards in 2011 and 2012, we analysed the differences between years using paired *t*-tests (Logan 2010). When a significant difference between more than two groups was found, we performed Tukey's multiple mean comparison tests to distinguish the diverging groups. Last, we used standard correlation (Pearson correlation coefficient) tests to quantify the relationship between two numeric variables (Logan 2010).

The main relationships among all the variables recorded were identified by principal component analysis (PCA), using the STATBOX software (version 6.6; Grimmer Logiciels, Paris, France); 27 variables were submitted to the PCA. As active variables in this analysis, 21 variables characterising the modes of production were used to calculate relative contributions to the axes. The six other variables (AIDB, TFI, Tech, CanS, Year and VD) were considered as supplementary variables, either because they had been elaborated directly using some of the basic variables (AIDB and TFI) or because they were semi-quantitative variables (Tech, CanS, Year and VD) (Table 3).

Results

Regional natural pest pressure and annual field surveys

In 2011 and 2012 in the Bordeaux region (SW), and in the Languedoc region (SE), the regional natural pest and disease pressures are summarised in Figure 2. In the SW, both the DM and TM pressures appeared to be higher in 2012 than in 2011. In the SE, the natural pressures were closer between the 2 years, with PM and TM being present slightly more than the other pests and diseases.

In the data obtained from our field monitoring, the severity of DM, PM, GM and TM larvae infections/infestations in the monitored vineyards is presented in Figure 3, for each region and each year, and depending on the production system.

Downy mildew. In 2011, we found few vineyards showing DM infection on bunches (Figure 3a). This pathogen was observed only once, in a vineyard with high vegetative growth and a dense canopy. In 2012, however, DM damage was observed in eight out of the 20 vineyards of our survey, with a wide range of severity (from 0.6% to 39.4% of the bunch surface infected). Overall, the severity of DM damage appeared significantly higher in 2012 than in 2011 ($t = -2.62$, $P = 0.017$). It was lower in the SE than in the SW (2.2% vs 9.2%), but the wide variability between vineyards did not allow us to conclude whether this was significant.

Table 3. Variables used in monitoring and analysing pest and disease damage to grape bunches.

Variable	Abbrev.	Unit	Value
Farm variables			
Vineyard region	VGR	Category	BA, Bordeaux-Aquitaine; LR, Languedoc-Roussillon
Mode of production	MP	Category	CP, conventional production; OP, organic production for at least 5 years; OC, organic conversion, i.e. in-transition growers, undergoing conversion to OP
Year of survey	Year	Number	2011; 2012
Technical expertise	Tech	Category (1 to 3)	1, Technically outdated or faulty equipment; 2, sometimes efficient sometimes outdated; 3, technically efficient and reactive
Vineyard variables			
Targeted yield	TY	t/ha	Grower's initial yield objective
Vine density per hectare	VD	Number/ha	Obtained from the grower
Number of bunches per plant	Bn	Number/plant	
Mean bunch mass	BM	g	
Calculated yield	CY	t/ha	Calculated yield of the plot: $CY = Bn \times BM \times VD$
Harvested yield	HY	t/ha	Real yield of the plot, measured by the grower
Yield achievement rate	YAR	%	$YAR = 100 \times (HY \div TY)$
Normalised difference vegetation index	NDVI	Numerical value from 0 to 1	Measured by a Greenseeker (N-Tech Industries, Ukiah, CA, USA; and Oklahoma State University, Stillwater, OK, USA)
Canopy state	CanS	Categorical (1 to 3)	1, Poor crop maintenance; 2, sometimes efficient sometimes bad; 3, vegetation well managed during the whole growing season
Pesticides variables			
Total number of treatments with pesticides	T-P	Number	Number of pesticide applications for all pests and diseases considered
Treatments against downy mildew	T-DM	Number	As T-P for downy mildew
Treatments against tortricid moths	T-TM	Number	As T-P for tortricid moths
Treatments against grey mould	T-GM	Number	As T-P for grey mould
Treatments against powdery mildew	T-PM	Number	As T-P for powdery mildew
Treatment frequency index	TFI	Number	Index calculated after Butault et al. (2010) for all pests and diseases considered
Epidemiological variables			
Downy mildew in flowers (severity)	DMF	%	
Downy mildew in bunches (severity)	DMB	%	
Number of tortricid larvae/bunch	LTM	Number	
Tortricid moths in bunches (severity)	TMB	%	$(LTM \div 15) \times 100$
Grey mould in flowers (severity)	GMF	%	
Grey mould in bunches (severity)	GMB	%	
Powdery mildew in bunches (severity)	PMB	%	
Assessment indicator of damage in grape bunches	AIDB	%	Formula: refer to Materials and methods section

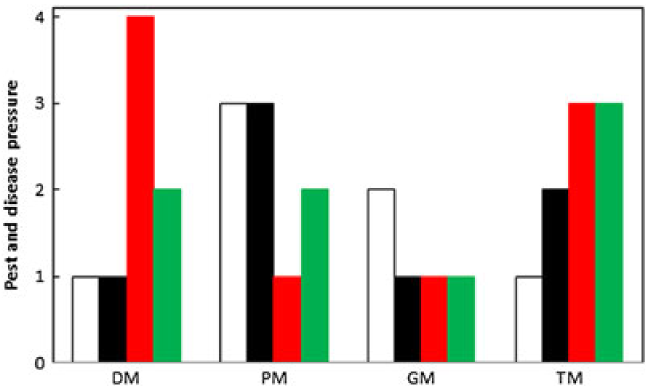

Figure 2. Natural grapevine pest and disease pressure observed by extension services in untreated vineyards in the south-west (□, 🟥) and south-east (■, 🟩) of France in 2011 (□, ■) and 2012 (🟥, 🟩). DM, downy mildew; PM, powdery mildew; GM, grey mould; and TM, tortricid moths.

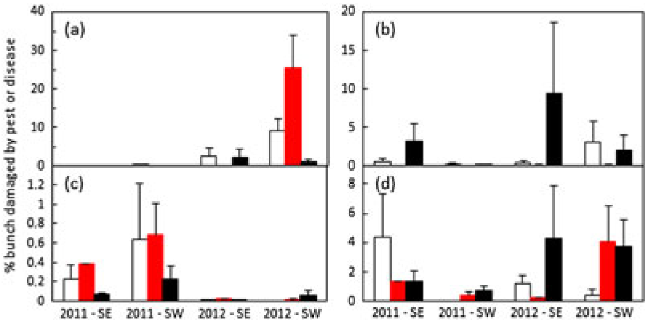

Figure 3. Proportion of (a) downy mildew, (b) powdery mildew, (c) grey mould and (d) tortricid damage in grape bunches from 20 monitored vineyards in south-west (SW) and south-east (SE) of France in 2011 and 2012. The vineyards were managed according to three modes of production – organic farming production (□), conventional production (■) or organic conversion (🟥) – that is, in transition from conventional production towards organic conversion. Error bars indicate standard errors of the mean.

The difference between modes of production was difficult to reveal, because of the wide variability in severity and the small number of vineyards in each combination of region/production system, leading to weakness in the statistical analyses. In 2012, however, we found a significant influence of the production system on DM severity ($F_{2,17}=6.99$ $P=0.006$). In both regions, the vineyards under CP were the least infected, with 1.5% of the bunch surface attacked (2.3% in the SE and 1.0% in the SW), and the OP vineyards were more infected (4.8% overall, 2.6% in the SE and 9.3% in the SW). In 2012 in the SW, the three OC vineyards showed more than 10% DM on grapes, with an average of 25.6%.

Powdery mildew. Powdery mildew occurred in all but one of the vineyards surveyed in both years (Figure 3b). The level of infection was often low: it was more than 1% of the bunch surface area in only three out of 20 vineyards in 2011 and in four out of 20 vineyards in 2012. But in some, the infection was widely spread across the vineyard. As much as 9.5% of the grape surface was infected by PM in 2011 and 39.4% in 2012 in the same vineyard in the SE region, grown under CP. No significant difference could be found between the two regions or between the modes of production.

Grey mould. Grey mould infection on ripe berries at harvest was more frequent in 2011 than in 2012 (Figure 3c). All vineyards but one showed GM symptoms in 2011, whereas only eight out of the 20 vineyards were infected in 2012. The difference between years on the whole vineyard sample was significant ($t=3.52$, $P=0.002$). But no significant difference could be found between the two regions or the different modes of production in any year.

Tortricids. Tortricid larvae were found in 2011 and 2012 in both regions, but with different patterns according to the region (Figure 3d). In the SW, the number of larvae per bunch was significantly different between years: we found few TM in 2011, with an average of 0.5% of the bunches damaged by the larvae, whereas in 2012, the proportion was 3.2% ($t=-2.72$, $P=0.022$). In the SE, the average level of infestation by TM did not differ between years (2011, 2.7%; 2012, 2.4%), with one vineyard showing a higher infestation level than all the others in both years (>10% damage on the bunches).

Assessment indicator of damage in grape bunches (AIDB)

In every vineyard, we calculated the AIDB in order to evaluate the overall proportion of bunches affected by the diseases and pest considered. The AIDB values ranged from less than 0.1 to 60%, indicating various degrees of success in the protection strategies adopted by the growers, as well as a wide variety of pest and pathogen pressures. Despite this high variability, we observed a significant difference in AIDB ($t=-2.56$, $P=0.019$) between 2011 (2.6% on average) and 2012 (11.9% on average). The AIDB value was higher in 2012 than in 2011 in 14 out of the 20 surveyed vineyards. In the SW (Figure 4), the difference between the 2 years was marked with mean AIDB values reaching 1.0% in 2011 and 14.2% in 2012. Moreover, in ten out of the 11 SW vineyards, the AIDB value was higher in 2012 than in 2011, whatever the mode of production. In 2011, only one AIDB value exceeded 10%, in a SE vineyard managed in OP (13.8%). In 2012, AIDB was above 10% in eight out of the 20 vineyards surveyed. In neither year did we find any significant difference in AIDB between the vineyards managed under the three modes of production (2011, $F=0.57$, $P=0.57$; 2012, $F=1.13$, $P=0.34$).

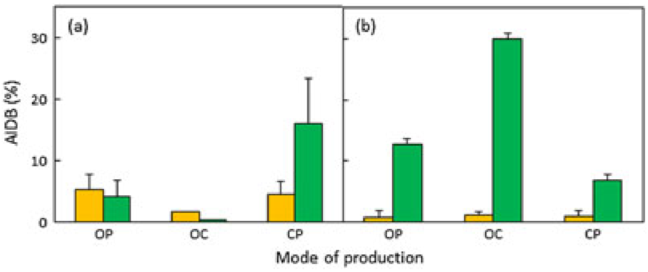

Figure 4. Calculated values of the new assessment indicator of damage in grape bunches (AIDB). Data from 20 vineyards in (a) south-east and (b) south-west of France in 2011 (🟨) and 2012 (🟩). The vineyards were managed according to three modes of production – organic farming production (OP), conventional production (CP) or organic conversion (OC) – that is, in transition from CP towards OP. Error bars indicate standard errors of the mean.

Relations among production system variables and the AIDB indicator

Interrelations were investigated by PCA among various variables characterising the production system and some of the main indices calculated in this study, such as AIDB and TFI (Figure 5). The first two principal components account for 42.0% of the total variance. The first composite axis is mainly representative of the vineyard yield by comprising the following variables (relative contribution to the axis in parenthesis). On one side of the axis are production variables, such as the calculated yield (CY) (10.3%), the harvested yield claimed by the grower (HY) (10.0%) and the targeted yield (TY) (8.2%), as opposed to, on the other side, the DM brown rot intensity (DMB) (6.5%) and the number of treatments against it (T-DM) (6.2%). As expected, the negative correlations observed between variables opposed on the axis suggest that the yield was reduced by a high DM pressure, notably brown rot

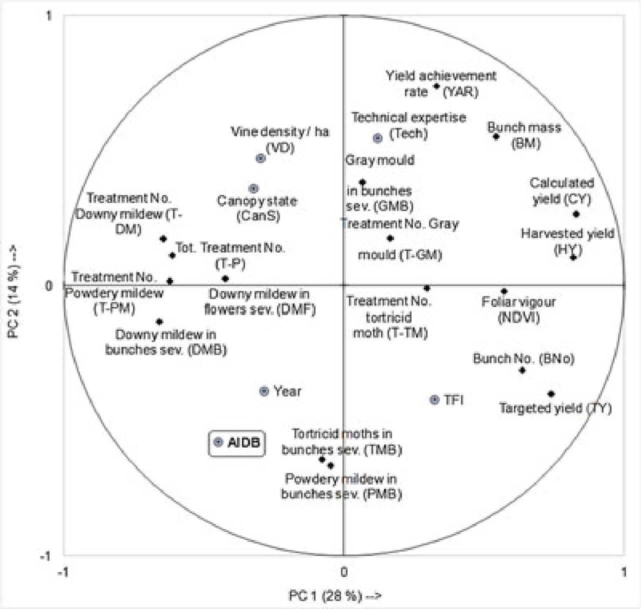

Figure 5. Principal component analysis of active (♦) and supplementary (●) numerical variables monitored in 20 vineyards in two French winegrowing regions in 2011 and 2012. Principal components (PCs) 1 and 2 are built to represent the maximal portion of the overall data variability (28% and 14%, respectively). For variable abbreviations, see Table 3.

symptoms affecting older berries without sporulation. The second main axis represents mostly, on its negative part, the PM severity variable PMB (13.3%) and the intensity of injury because of the third-generation TM larvae (TMB) (12.4%). On its positive part, yield-related variables such as the yield achievement ratio (YAR) and the mean bunch mass (BM) also contribute clearly to this second axis (16.4 and 9.1%, respectively).

The PCA shows that the AIDB indicator (supplementary variable) was, as expected, clearly associated with damage due to the different pest and diseases as shown by its negative coordinates on both axes and by its location close to DMB, PMB and TMB variables (Figure 5). Furthermore, the AIDB indicator was located diametrically opposed to the variables YAR and BM, based on significant negative correlations. It was also opposed noticeably to the technical expertise indicator (Tech), suggesting that the most expert growers were those who were able to minimise the damage caused by pests and diseases, whatever their mode of production.

Relations between the AIDB indicator and yield variables

The target yield (TY) expected by growers in the SW ranged from 6.1 to 11.5 t/ha (mean, 7.6 t/ha) and from 9.4 to 16 t/ha in the SE (mean, 11.8 t/ha). No significant correlation with the calculated AIDB was found ($t = -0.46$, $P = 0.64$). But the difference in TY between regions was highly significant ($t = 7.5$, $P < 0.001$). Because of this difference in objectives, neither the HY nor CY could be compared between regions. The YAR, which overcomes this limitation, was not significantly dependent on the region ($t = -0.914$, $P = 0.36$).

As expected, the resulting yield harvested by the grower (HY) and the value of the yield calculated with the measured yield components (CY) were tightly correlated ($R = 0.73$, $t = 6.58$, $P < 0.001$). In the SW vineyards, the average HY was significantly lower in 2012 than in 2011 ($t = 3.56$, $P = 0.005$), being 8.1 t/ha in 2011 and 6.8 t/ha in 2012. In the SE, the difference in HY was smaller (11.1 t/ha in 2011 and 10.2 t/ha in 2012) and not significant ($t = 1.28$, $P = 0.24$). Interestingly, we found a significant negative correlation between the HY or CY and the AIDB: R (HY, AIDB) $= -0.35$, $t = 2.37$, $P = 0.023$, and R (CY, AIDB) $= -0.39$, $t = -2.59$, $P = 0.013$.

Based on the 20 vineyards surveyed in both seasons 2011 and 2012, Figure 6 also shows a significant negative correlation between the YAR and the level of damage characterised by AIDB ($R = -0.45$, $t = -3.11$, $P = 0.0035$). In 2012, the YAR was generally lower (87.6%) than in 2011, where the objective was even slightly exceeded (100.1%) ($t = 3.24$, $P = 0.004$). This was more acute in SW (YAR = 88.2% in 2012 vs 104.4% in 2011, $t = 3.56$, $P = 0.005$) than in SE (YAR = 86.9% in 2012 vs 94.9% in 2011), where the difference between years was not statistically significant ($t = 1.22$, $P = 0.26$).

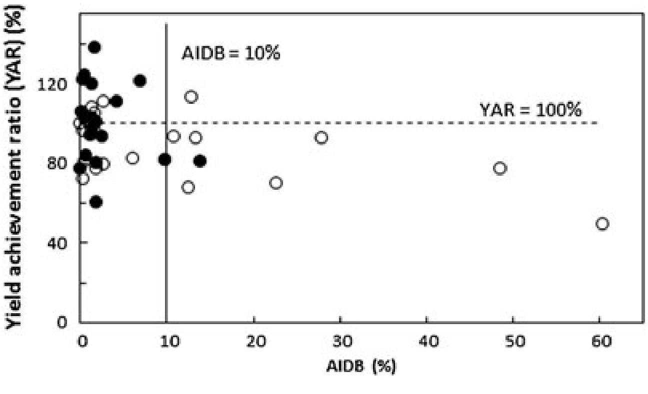

Figure 6. Yield achievement ratio (YAR) observed in 20 vineyards in south-east and south-west of France in 2011 (●) and 2012 (○), as a function of the assessment indicator of damage in grape bunches (AIDB) calculated in the vineyard.

In our vineyard network, AIDB values above 15% occurred four times (Figure 6), all in 2012, and YAR was always less than 100% in these vineyards, meaning that the grower's TY was not reached. And in ten out of the 12 cases where AIDB was above 5%, the YAR was under 93.5%.

Relations between the AIDB indicator, technical expertise and TFI variables

While all levels of technical expertise occurred in both regions, most of the vineyards were managed by growers with high technical expertise (16 out of 20 were rated 3) including all the vineyards managed in OP. The AIDB level was significantly lower in the vineyards where the grower had a higher level of technical expertise ($F = 15.4$, $P = 3.5 \times 10^{-4}$), and this was even more significant in 2012 when the disease pressure was higher ($F = 26.8$, $P < 0.001$).

The total number of pesticide treatments applied in the surveyed vineyards was significantly different between 2011 (average: 13.0 treatments) and 2012 (average: 15.9 treatments) (paired *t*-test: $t = -3.71$, $P = 0.001$). This difference was highly significant in the SW ($t = -4.25$, $P = 0.002$), but not significant in the SE ($t = -1.25$, $P = 0.24$). The majority of the treatments were fungicide sprays (97% on average), mainly against PM and DM. The mode of production also affected the number of treatments: the CP and OP modes led to a similar number of treatments (12.8 vs 12.9), but the OC growers applied an average of 20.8 treatments. These differences were observed in both regions and in both years.

The TFI is linked to the total number of treatments, but it also takes into account the dose of chemical actually applied in each treatment, as compared with the recommended dose. It therefore shows common trends with the number of treatments: TFI was also significantly higher in 2012 (average 9.65) than in 2011 (8.37) ($t = -2.48$, $P = 0.023$). But when considering the mode of production, the TFI was highest in CP vineyards (average: 10.91), intermediate in OC vineyards (7.38) and lowest in OP vineyards (6.94). The overall difference between modes of production was significant ($F = 4.97$, $P = 0.012$) but depended on the region: this effect was significant in SW France ($F = 9.01$, $P = 0.002$) but not in the SE ($F = 0.45$, $P = 0.64$).

The correlation analysis showed no significant link between the number of treatments and the AIDB value ($R = 0.21$, $t = 1.37$, $P = 0.18$). The correlation between the amount of pesticide used, characterised by TFI, and the AIDB value was even lower ($R = -0.05$, $t = -0.31$, $P = 0.76$). This result was also confirmed by considering each year separately.

Discussion

Why do we need a new indicator?

In this work, we proposed an original indicator of multipest damage intensity in the grape bunch (AIDB) to assess the efficiency of the grapevine protection strategy under a large set of conditions. The objectives were to develop and test an assessment indicator, that is, a variable that can be easily accessed in a network of growers' vineyards, to assist in the analysis of the efficiency of growers' practices to control pest and disease. This assessment indicator would therefore allow for the application to vineyards of the regional agronomic diagnosis approach (Doré et al. 1997), which would not be relevant in the absence of a variable to assess the plant protection efficiency. Despite the relatively small number of vineyards surveyed, they provided a range of damage profiles and treatment patterns representative of regional and/or national trends in French vineyards. The range of conditions and grower practices provided by this network was broad enough to test the indicator, which was the main purpose of this work, but the statistical power of such a network is not sufficient to draw firm conclusions on the influence of grower practices. Nevertheless, the results illustrate how indicators such as TFI (assessing intensity of pesticide use) and AIDB (related to efficacy of plant protection) can be used, for example, to question the beliefs that organic farming necessarily leads to lower crop protection efficiency or that farmers in organic farming use less pesticides.

In order to proceed further with this correlative analysis between AIDB and other variables, we assessed with our network whether AIDB has the typical qualities of an indicator (Wery et al. 2012): *sensitivity* to the process aimed to be 'indicated', that is, here pest and disease pressure levels; *robustness* to varied measurement conditions; *consistency* with the underlying processes to be assessed; and *accessibility* for the user (i.e. grower or advisor).

Sensitivity of AIDB

The *sensitivity* test was ensured by the measurement of the indicator over a range of vine production conditions. The experimental network covered different production objectives and types of vineyard with different modes of production (including the organic label). Across the two regions (Oceanic vs Mediterranean) and the 2 years, we explored various climatic patterns, soil types, and pest and diseases pressures. We also took into account different protection strategies, associated or not with the organic production label. Over this range of conditions, AIDB showed a high sensitivity (Figure 4).

Robustness of AIDB

The *robustness* of AIDB is demonstrated by the possibility to calculate it, without additional parameterisation, in such a wide range of conditions. The production conditions we investigated are common in vineyards. Many grapegrowing regions are located in a Mediterranean or Oceanic climate, notably in France, and also in Europe and worldwide. The vines studied were trained traditionally with vertical shoots that are the most common in modern viticulture. Furthermore, the selected cultivar, Merlot, is one of the most widely used in the world (Galet 2000). Thus, even if further investigations are necessary on other cultivars and under other disease/pest, soil, climatic and technical conditions, we consider that AIDB may be applicable to a wide range of vineyard growing conditions.

Knowledge consistency

The knowledge *consistency* of the AIDB indicator can be derived from its method of calculation. The meaning of AIDB is self-explanatory from its method of calculation, which includes the following: (i) the biology of the major grapevine pests and diseases; and (ii) their potential impact on yield determination.

- The AIDB integrates infectious and parasitic processes from different living organisms, that is, insect pests and different fungi. These processes include feeding by the insect pest (Thiéry 2008, Ioriatti et al. 2012) as well as diversion of host assimilates and/or host tissue enzymatic degradation by the pathogens (Savary et al. 2009, Jermini et al. 2010a,b,c).
- The overall damage to the grape berries was quantified according to the main developmental and epidemiological steps: (i) the early phase on floral buds and flowers and (ii) the fruit phase including berry development and ripening. For TM, this corresponds to different antophagous versus carpophagous generations (Thiéry 2008, Ioriatti et al. 2012). For DM and GM, the symptoms observed in these two phases are different and may presumably be caused by different types of inoculum produced through either sexual or asexual reproduction. The first part of the AIDB equation takes into account the level of destruction of inflorescences caused by two of the pathogens considered in this study, that is, *P. viticola* and *B. cinerea*, or DM and GM, respectively. Such early floral attacks may directly affect the number of inflorescences per plant, which is a key yield component. In addition, for the partially affected inflorescences, the mean number of flowers may markedly decrease following these early pathogenic infections. The second part of the AIDB equation indicates the crop loss caused by the attacks and/or infections directly affecting grape berries by all the pathogens and pests studied. These grape bunch symptoms, during berry development and ripening, are considered generally as of prime importance for all three PM, DM and GM pathogens and the last generation of TM larvae (Calonnec et al. 2004, Martinez et al. 2005, Elmer and Michailides 2007, Savary et al. 2009). Therefore, the AIDB method of calculation gives good agreement between the processes generating the pest and disease severity (proportion of damaged berries in the grape bunches) and the indicator value. This is consistent as far as the major hypothesis of our indicator is respected: that is, the four diseases and pest we took into account should cause the majority of the damage in the vineyard.

Furthermore, we found that the AIDB indicator was negatively correlated with both final yield estimates from either the grower's statement (HY) or the calculation based on measured yield components (CY). Similarly, the yield achievement rate (YAR) was correlated negatively with the AIDB indicator. The BM explained 30% the total yield (Guilpart 2014), and the BM depends significantly on attacks by pests and diseases, either during berry growth and ripening or during flowering, by interfering with the number of inflorescences/flowers and/or with the rate of berry setting. Moreover, among the parasitic processes in the grapevine inflorescences and berry bunches accounted for by AIDB, various interactions between the pest(s) and pathogen(s) may occur in the fruit zone, such as the known synergistic and mutualistic relationship between the grape berry moth *L. botrana* and *B. cinerea* (Mondy et al. 1998). Being based on the maximum symptom expression for every pest/disease considered, the AIDB implicitly includes such interactions, even if no interaction term appears in the equation. Nevertheless, it should be outlined that the AIDB indicator may not be relevant in order to analyse grapevine qualitative losses, particularly when considering oenological damage in wines.

Synthetically, a high AIDB value indicates an inefficient protection strategy leading to potentially marked yield losses due to pests and diseases. In contrast, a low value (less than 1–2%) denotes success in the grapevine protection but should also be analysed along with the level of pest and disease pressure of the year/region considered, with the intensity of pesticide use (TFI) and the grower's decision system (technical expertise indicator). Intermediate values should encourage the combined use of the AIDB and YAR assessment indicators, to analyse the efficiency of the crop protection system over several consecutive years within the vineyard context (e.g. quality wine or table wine). Our results suggest that the grapegrower should aim at AIDB values below 10%, which should help in reaching a significant reduction in pesticide use while maintaining quantitative yields close to the target. This preliminary threshold, however, should be confirmed by further studies under various winegrowing conditions, such as cultivar and type of wine, and should be used cautiously when qualitative loss is considered.

Accessibility of AIDB to potential users

The fourth requirement for an operational indicator is its *accessibility* under growers' vineyard conditions. Because of the complexity of processes taken into account in terms of pest and disease dynamics and impacts, AIDB requires variables to be measured in the vineyard at three key phenological stages: flowering, bunch closure and ripening. In total, acquisition of these data requires a maximum of 4 h per vineyard, including both vineyard observations and assessment of the population of TM larvae. No other costs are involved, apart from some materials costing about €10. These observations require some expertise, notably in disease and pest diagnosis. Nevertheless, these are typical observations already widely made within the Biological Monitoring Plan of French vineyards through a network of vineyards across various regions (Grosman 2010). This monitoring focuses on DM, PM, GM and TM, with simplified protocols for evaluating severity on bunches throughout the growing season. The AIDB indicator may benefit from this survey provided the accuracy of these basic measurements is consistent with the AIDB sensitivity. As in the case of the Mildium strategy (Naud et al. 2011), a test of the quality of the indicator with different levels of accuracy in the input variables would be required before connecting the two approaches. Thus, further studies are needed to adjust the sampling grid, taking into account the vineyard diversity and management plans in order for the indicator to efficiently assess the overall protection strategy at the vineyard level.

Combination of AIDB with other indicators

When developing and testing a new indicator, an important point is the consistency between this indicator and other important indicators already in use. In order to assess pesticide strategies, the most popular indicators are the average number of treatments and the TFI. No significant correlation was established between the number of treatments and the AIDB value. Similarly, no significant correlation was detected between the AIDB indicator and the TFI, which represents the amount of pesticide used (Butault et al. 2010). This appears a paradox, because the pesticide treatments are made with certified products, supposed to be efficient in controlling the pests and diseases concerned. But the number of pesticide treatments and TFI do not explicitly take into account the pest and disease pressure: the grower's aversion to risk largely determines the dose and frequency of applications, as a response to natural pressure. Thus, it should be considered that the TFI index does not reflect the real efficiency of the pesticide use and associated strategy, unlike the AIDB indicator. In this context, the grower's technical expertise (Tech), proposed in our study, is a complementary indicator to be analysed in relation to AIDB. Interestingly, we showed a highly significant negative correlation between Tech and AIDB whatever the production system (OC or CP). This suggests that the more expert farmers were, the better they were at controlling the pests and diseases studied and hence in minimising the cumulative damage (AIDB) while trying to limit the amount of pesticides applied. This relationship may result directly from a positive effect of the high level of grower technical expertise on the quality and efficiency of treatments under well-adapted conditions of pesticide use (spraying conditions, choice of the machinery and active ingredient). In addition, a grower with a high value of the Tech indicator may also have more precise canopy management and cultural practices in order to reduce plant vigour and to improve shoot, leaf and bunch spatial arrangement and architecture. Some of these practices, such as cover-cropping, shoot suppression and/or leaf removal in the fruit zone, which are associated with reduced bunch compactness and/or decreased vegetative growth, have been clearly demonstrated to lower the epidemiological risk of all the pests and diseases considered in this study (Fermaud 1998, Valdés-Gómez et al. 2008, 2011).

Conclusions

The TFI, Tech and AIDB could form an indicator framework combining the intensity of pesticide application (TFI), the quality of the vineyard management (Tech) and the efficiency of the control strategy (AIDB). The proposed AIDB was shown to fulfil the four mandatory qualities for an assessment indicator: *sensitivity* to pest and disease pressure, *robustness* to measurement conditions in growers' fields, *consistency* with knowledge and *accessibility* for users (Wery et al. 2012). Thus, AIDB constitutes a helpful assessment indicator to assist the agroecological transition in grapevines, leading to a significant decrease in pesticide application in improved modes of production.

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