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REVIEW - FOCUS ISSUE ON PLANT HEALTH SUSTAINING MEDITERRANEAN ECOSYSTEMS

Fungicide models are key components of multiple modelling approaches for decision-making in crop protection

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Summary. Decision-making for integrated pest management (IPM) in crops requires the assessment of multiple risk factors. Plant disease models have been used to predict disease risk and support decisions about whether and when to protect crops based on environmental conditions. In addition to requiring information about disease risks, correct decision-making also requires answers to several questions. These include: Is the plant susceptible to infection? Is the plant protected by a previous fungicide application? Which fungicide should be used for the specific application? Which dose of the product should be used, and when should it be applied? Obtaining answers to these questions requires a multiple-modelling approach in which models for diseases are combined with those for plant growth and for the effects of fungicides. This review discusses models that predict fungicide efficacy dynamics based on fungicide physical mode of action, fungicide localisation on/within host plants, fungicide effects on the pathogen, and the dynamics of fungicide residues. Empirical and mechanistic models are considered. Empirical models have been mainly developed by fitting equations to field data. Mechanistic models consider the processes that determine fungicide dynamics and effects, and these are generally considered to be superior to empirical models, but parameterisation of mechanistic models is challenging. A new modelling approach is described that combines the main processes of mechanistic models with simple parameterisation based on laboratory experiments, practical knowledge, and technical information. Examples are also provided of systems that calculate fungicide dose and application time. Decision support systems are described as tools that provide farmers with all of the information required for correct decision-making in IPM.

Key words: risk algorithms, physical mode of action, fungicide models, multi-criteria decision making.

Introduction

Plant disease control has been traditionally based on calendar applications of fungicides to keep the plants constantly protected from fungal pathogens. Integrated pest management (IPM) is an important alternative to this traditional approach. Directive 128/2009/EC on the Sustainable Use of Pesticides makes IPM mandatory across Europe, with the goals of reducing the negative effects of pesticides on human health and the environment. Art. 3 of this Directive states that integrated pest management means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms. Furthermore, the Directive keeps the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. Integrated pest management emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms. Annex III of the Directive 128/2009 defines eight general principles for IPM.

These eight principles have been integrated into a logical framework that emphasizes four types of decisions (Rossi *et al.*, 2012). Type I decisions concern the

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selection of measures for the prevention and/or suppression of harmful organisms. Type II decisions concern whether and when plant protection actions are required, based on crop monitoring, mathematical models, and action thresholds. Type III decisions, which are made after growers or managers have decided that crop protection is needed, concern the control measures to be used, and non-chemical alternatives should be considered in addition to chemical pesticides. When pesticides are used, they must be selected based on their effects both on target and non-target organisms; they must also be applied at the lowest levels needed to achieve pest control; and they must be selected and applied to prevent the development of pesticide resistance in the target population. Type IV decisions concern the implementation of the management actions including adjustment of spray volume, sprayer speed, and spray timing during the day.

The decision to apply a fungicide to control a plant disease is based on analysis of multiple risk factors, i.e., the variables associated with an increased risk of disease or infection. The identification and quantification of disease risk factors is fundamental to decision-making for crop protection (Hughes and Burnett, 2015).

Identifying disease risk factors is facilitated by reference to the generic disease triangle, including the three components necessary for disease development: a susceptible host, a virulent pathogen, and a favourable environment. Risk factors are the particular host, pathogen, and/or environmental factors that increase disease risk in a given pathosystem.

For the quantification of disease risk factors, Madden *et al.* (2007) defined a risk algorithm as 'any calculation that uses observations of identified risk factors from the host crop, the pathogen population and the environment to make an assessment of the need for crop protection measures'. Models can be then regarded as risk algorithms in that they predict the risk factors.

Plant disease models

As risk algorithms, plant disease models are relevant parts of IPM because they provide useful information for deciding whether and when crop protection action must be implemented, i.e., for the type II decisions of Rossi *et al.* (2012).

The first plant disease models were developed in the middle of the 20th century and were empirical, i.e., they consisted of simple equations that described, in a non-mechanistic manner, the relationships between particular stages of respective pathogens and weather conditions. The so called "3–10 rule" for predicting the first seasonal infection of grape downy mildew, caused by the oomycete *Plasmopara viticola*, is an example of this empirical approach for understanding relationships between pathogens, plants, and the environment. Empirical models are developed by identifying mathematical or statistical relationships between field-collected data, but these relationships do not necessarily have cause-and-effect meanings (Rossi *et al.*, 2010). Lack of knowledge, accuracy, and especially robustness are the main weaknesses of these models, which require accurate validation and usually adaptation when used in agricultural contexts different from where they have been developed or under changing climate. Advanced methods for data analysis, like neural networks, can facilitate the identification of the mathematical or statistical relationships that are central to such models, but they do not overcome the above-mentioned weaknesses.

Although the number and complexity of disease models have increased with improvements in weather monitoring and automatic data processing, empiricism predominated for a long time. In recent years, however, new research approaches have increased the ability to obtain a more mechanistic understanding of the complex relationships between plants, pathogens, and their environments, and this new understanding has been integrated into mechanistic models (Rossi *et al.*, 2010). Mechanistic models are based on knowledge of the biological and epidemiological behaviour of the system under study. Mechanistic models are dynamic, because they analyse the changes in the components of an epidemic over time, due to the external, influencing variables. Dynamic modelling is based on the assumption that the state of a pathosystem at every moment can be quantitatively characterised and that changes in the system can be described by mathematical equations. These models overcome most of the weaknesses of empirical models. Accuracy and robustness are greater for mechanistic models than for empirical models. Compared to the 3–10 rule, a mechanistic model for grape downy mildew increased the overall accuracy of the predictions by 60 to 90% (Caffi *et al.*, 2007).

Although plant disease models are important tools in IPM, correct decision-making requires additional information, including answers to the following questions: Is the plant susceptible to infection? Is the plant already protected by a previous fungicide spray? and Which fungicide should be used? The first two questions address type II decisions and the third addresses type III decisions of Rossi *et al.* (2012).

Answering these questions requires a multiple modelling approach that includes models for plant disease, for plant growth and development (with consideration of disease resistance), and for the effects of fungicides.

Whereas plant disease and plant growth models have been extensively reviewed (Rossi *et al.*, 2010; Chuine *et al.*, 2013; Thimme Gowda *et al.*, 2013), fungicide models have not; hence, they are considered in the next section.

Fungicide models

Fungicides are specific types of pesticides that control diseases by specifically inhibiting or killing the pathogenic fungus or oomycete causing the disease (McGrath, 2004).

Fundamental components of fungicide modelling

The following are important components in fungicide models: i) the physical mode of action (PMoA) of fungicides; ii) fungicide activity in relation to fungicide localisation on or within the plant; iii) the fungicide effect; and iv) the dynamics of the fungicide residue after application onto plant surfaces.

Physical mode of action of fungicides

The term PMoA refers to the effects of fungicides with respect to the time of their placement in relation to the host-pathogen interactions, so that the following fungicide activities can be distinguished: i) pre-infection, ii) post-infection, iii) pre-symptom expression, and iv) post-symptom activity (Pfender, 2006). Fungicide activities influence different steps of the "infection chain" and epidemic development (Figure 1). PMoA also considers the duration and degree of the fungicide's activity (Pfender, 2006) as well as fungicide movement on or within the plant and the temporal dynamics of fungicide residues on or in plant tissues (Szkolnik, 1981; Koller, 1994). Fungicide movement refers to the movement of the fungicide to non-sprayed parts of the plant caused by rain and/or vapour activity, as well as to systemic movement (Szkolnik 1981; Wong and Wilcox, 2001).

Pre-infection activity concerns fungicides that are applied before or during an infection period. An infection period (also termed an "infection window") is the time between spore germination on host surfaces and the production of infection structures such as appressoria. Pre-infection fungicide activity may reduce pathogen spore germination, germ tube growth, and infection efficiency (i.e., the proportion of spores that establish an infection) (Figure 1). Because pre-infection fungicides are usually on the plant before infection is initiated, their activity is also called "protective", "preventive" (or "preventative"), or "prophylactic".

Post-infection fungicide activity refers to the capability of a fungicide applied hours or days after infection has occurred to stop the infection or to inhibit fungal development on or within plant tissues and to prevent the establishment of a lesion; in epidemiological terms, post-infection activity also reduces infection efficiency (Figure 1). This activity is also called "curative", "therapeutic", "kickback", or "suppressive".

Pre-symptom fungicide activity is an extension of post-infection activity. When applied beyond its limit of post-infection activity, a fungicide with pre-symptom activity has no or little effects on the lesion number (i.e., it does not reduce infection efficiency), but can increase the incubation period (the period between infection and symptom onset) and can result in small and atypical lesions that produce few or no spores (Figure 1).

Post-symptom activity refers to the capability of fungicides applied to visible and sporulating lesions to reduce the production of new spores from those lesions or to reduce spore viability. In epidemiological terms, post-symptom activity lengthens latency (i.e., the period between infection and the production of new spores on lesions) and shortens infectiousness (i.e., the period when a lesion continues to produce spores and contribute to epidemic development) (Figure 1). Like pre-symptom activity, post-symptom activity reduces the further spread of the of the pathogen and disease. "Eradicative" and "eradicant" activity have been used as alternative terms.

Because several categories of host tissues (i.e., healthy, with latent infection, with visible lesions, and with visible and sporulating lesions) and different processes (infection, sporulation, and dispersal) are present at the same time during an epidemic, a single fungicide application can have multiple effects on the epidemic. For example, post-symptom fungicides may reduce spore viability and spore production, but

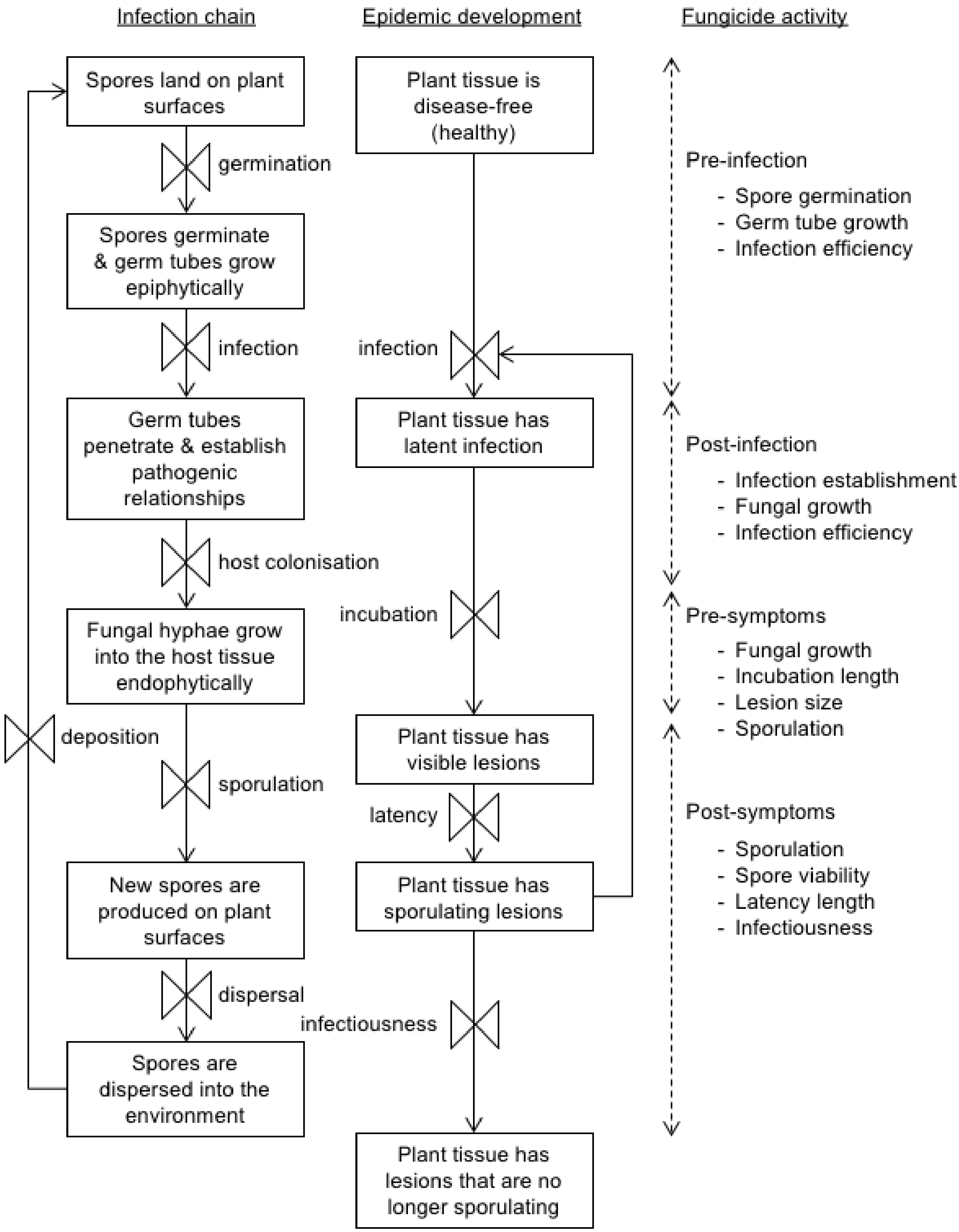

Figure 1. Relationships between steps of the infection chain of plant pathogenic fungi, epidemic development, and fungicide activity. The figure uses systems analysis symbols (Leffelaar and Ferrari, 1989): boxes are state variables; arrows show flux and direction of states; and valves in a flux are rates.

may also provide protection for noninfected host tissues (Buck *et al.*, 2011).

Fungicide activity vs. fungicide localisation on/in the plant

Once fungicides have been deposited on plant surfaces, they may remain there or penetrate the plant tissues, i.e., fungicides can be divided into non-penetrant, protectant fungicides and penetrant, systemic fungicides.

Protectant fungicides are contact materials that remain on the plant surface and kill fungal spores and hyphae upon contact. Systemic fungicides are absorbed by the plant cuticle and underlying tissues and can kill spores and hyphae on the surface as well as incipient infections beneath the surface. Among systemic fungicides, some are only locally systemic (they have limited uptake around the site of application but they lack long distance transport), and others are fully systemic. Some fungicides show xylem mobility: they are translocated over long distance only in the direction of the xylem stream; movement is upwards to the growing point of the plant (i.e., acropetal or apoplastic). Other fungicides show phloem mobility: they are translocated over long distances in direction of the phloem stream; movement is downwards from the shoots to the roots (i.e., basipetal or simplastic). The term "translaminar" is also frequently used; it refers to the local movement of a fungicide from one side of the leaf (upper vs. lower) to the other, resulting in disease control on both sides of the leaf.

For pathogens that infect endophytically (e.g., downy mildews, rusts, etc.), both protectant and systemic fungicides are effective if applied before infection occurs (i.e., they have pre-infection activity), but only systemic fungicides are effective after the fungus has penetrated the plant, i.e., they have post-infection activity (but only for a limited time, e.g., 24 to 72 h, depending on the fungicide, dosage, and disease) and sometimes pre-symptoms activity (also depending on the fungicide, dosage, and disease). Products that are more systemic tend to have longer post-infection activity because they penetrate deeper into the plant tissues and are able to interact with more advanced infections. In the latter case, the higher the dosage, the better the post-infection activity.

For pathogens that infect ectophytically (e.g., powdery mildews), both protectant and systemic fungicides show pre- and post-infection activity as well as pre- and post-symptoms activity.

Fungicide effects

The term fungicide effect refers to the duration and degree of activity. Both the duration and degree of activity are influenced by the characteristics of the fungicide (its active ingredient and formulation), its dosage, the uniformity of its distribution, the weather conditions after application, plant growth after application, and the multiple interactions among these factors.

Temporal dynamics of fungicide residues

After a protectant fungicide is applied, its residues progressively decrease over time due to chemical deterioration (vaporization, photolysis, and hydrolysis), microbial activity on the plant surfaces, weathering including wash-off by rainfall, and redistribution over the plant surfaces as vapour or through effects of rainfall, dew, or irrigation water (Lukens, 1971). Rainfall is widely recognised as one of the main factors reducing the persistence of fungicides on plant surfaces. Growing plant tissues will also "dilute" the fungicide because expanding plant surfaces create unprotected areas in the protective coating of a fungicide and may also dislodge residues already present (Lukens, 1971).

The concentration of systemic fungicides is reduced, mainly due to redistribution and dilution by growing plant tissues, as well as by possible breakdown by plant metabolism. Because systemic fungicides are absorbed by plant tissues and are redistributed, they are less susceptible to wash-off by rainfall than protectant fungicides, which remain on plant surfaces.

Under commercial cropping conditions, performance failures of fungicide sprays often result from poor application, i.e., from insufficient or non-uniform coverage of plant surfaces. Locally systemic fungicides can redistribute within the host waxy layers or epidermal cells by lateral diffusion, and this makes them less dependent on uniformity of spray coverage (Evans, 1977). Thus, systemic fungicides can provide better control than protectants under poor coverage conditions. Because of their mobility, systemic fungicides may also be more effective than protectant fungicides on rapidly expanding plant surfaces, such as leaves and young shoots.

In summary, the following factors are known to affect the rates at which the concentration of fungicide deposits decline: i) the nature of the treated plant and especially of its surface; ii) the dilution of fungicides by plant growth; iii) loss due to weathering by rain and other weather variables; iv) fungicide formulation; and v) the susceptibility of the fungicide to volatilization and to chemical, photochemical or microbial degradation.

The relevant processes involved in fungicide deposits decline are: i) retention (or adhesion), ii) drying and rainfastness, iii) redistribution, iv) vapour movement, v) tenacity, vii) uptake by the plant and movement for penetrant fungicides.

When a spray droplet lands on a leaf (or another plant part), it may be retained on the leaf surface or bounce off the surface and ultimately land on some other plant part or the soil. Retention is a consequence of dynamic interactions among the chemical components of the droplet during flight and impact, the physical properties of the droplet (size and velocity), leaf surface morphology, and leaf orientation (Forster *et al.*, 2001). The degree to which plant surfaces can be wetted by fungicides differs greatly among plants and plant parts (Gaskin *et al.*, 2005).

Protectant and penetrant fungicides both require the drying of plant surfaces after application. Failure of a protectant fungicide to dry on the plant surface will often reduce efficacy; penetrant fungicides are less sensitive to drying than protectant fungicides, but still require a minimum number of pre-rainfall drying hours to be fully absorbed by plants. “Rainfastness” refers to the time required between application and rain for the product to perform effectively. Rainfastness is a characteristic of the fungicide product; it depends on the active ingredients and adjuvants, which can be increased by the addition of sticking agents to the respective formulations. Depending on the product, proper drying and absorption may require less than 0.5 or up to 8.0 h (Kudsk *et al.*, 1991; Bardsley and Thompson, 1995; Lindner *et al.*, 1995; Andersen *et al.*, 2014).

Redistribution refers to how fungicides are redistributed within plant canopies by rain (Brent and Atkin, 1987). With light rain, some of the fungicide applied to upper canopy layers may be moved to lower layers, such that both layers are protected (Hislop, 1966). With heavy rainfall, however, the fungicide may be washed-off (Pereira *et al.*, 1973).

Vapour movement, or “episystemicity”, refers to movement (redistribution) of fungicide vapours through the waxy layers of leaf surfaces. If the vapour pressure of the active ingredient is high, movement can begin from the time of deposition on the plant surface, via vaporization and gas-phase transportation (Oliver and Hewitt, 2014).

Fungicide tenacity is the ability of the fungicide to resist being washed-off by rainfall (Cohen and Steinmetz, 1986). Factors influencing wash-off include the properties of both the product (its active ingredient and adjuvants) (Taylor and Matthews, 1986; Thacker and Young, 1999; Cabras *et al.*, 2001; Vicent *et al.*, 2007; Gaskin and Steele, 2009; Hunsche *et al.*, 2006 and 2011) and the rainfall (rain intensity, raindrop size, impact energy, and the cumulative number of impacts on the plant surface) (Pérez-Rodríguez *et al.*, 2015). The time elapsed between fungicide application and rainfall may also influence the amount washed-off by rain. However, there is no clear relationship between drying time and wash-off, because wash-off was greatly decreased by short drying times in some studies (Bruhn and Fry, 1982; Bryson, 1987; Mashaya, 1993; Willis *et al.*, 1994; Reddy and Locke, 1996; Schepers, 1996) but was unaffected by drying time in other studies (Schepers, 1996; Ditzer, 2002; dos Santos *et al.*, 2002). The degree of wash-off can also be affected by environmental conditions before application (Stevens *et al.*, 1988) and time of day (night/day) of fungicide application (Augusto *et al.*, 2010).

Uptake is the process by which the fungicide crosses the cuticle and is absorbed by the plant (Kirkwood, 1999). The absorbance of a fungicide involves a complex interplay of factors, as recently reviewed by Klittich (2014).

Models for fungicide effects

In disease models, the duration and degree of pre-infection activity, post-infection activity (i.e., preventive or curative), or eradicant activity will influence a fungicide’s effect on the weather-related infection events. The availability of quantitative information on these influences for commonly used fungicides enables researchers to construct disease and management models that realistically incorporate the effects of fungicide on the dynamics of plant disease epidemics (Pfender, 2006). These models may help clarify the appropriate role and optimal use for fungicides in disease management (Lalancette and Hickey, 1986; Schoeny and Lucas, 1999; Albrigo *et al.*, 2005; de Kraker *et al.*, 2005; Pfender and Eynard, 2009).

Empirical models

As for disease models, empirical models for fungicide dynamics over time have been developed by fitting results obtained in field or laboratory experiments to mathematical equations. First-, second-, or third-order polynomial models have been frequently used to describe the duration of fungicide control of dollar spot on creeping bentgrass (*Agrostis stolonifera*) (Latin, 2006), peanut leaf spot (Nokes and Young, 1992), stem rust on perennial ryegrass (*Lolium perenne*) (Pfender, 2006), hop downy mildew (Gent *et al.*, 2015), and melanose, scab and Alternaria brown spot on citrus (Mondal *et al.*, 2007; Vicent *et al.*, 2007). To describe the duration of fungicide control of dollar spot of creeping bentgrass, researchers used a first-order decay model (negative exponential) in the form y = a × exp(– r × t), where y is the residual efficacy proportion, a is the initial efficacy proportion (i.e. at the time of application), and r is the rate of decay over time (t) after fungicide application (Latin, 2006). In the case of brown patch disease on creeping bentgrass, researchers used a two-parameter Weibull distribution function in the form y = 1/[1+exp(a+b×t)], where y describes the decline in residual efficacy over time (t), a is the scale parameter indicating the point at which protection is compromised, and b is the shape parameter of fungicide efficacy decay (Daniels and Latin, 2013).

In general, the data reported for fungicide tests under field conditions are not useful for understanding fungicide dynamics over time, because natural (and often repeated) infection periods make it impossible to separate pre-infection activity from post-infection and/or eradicant activity (Mathiassen *et al.*, 1997).

Process-based models

Arneson *et al.* (2002) developed a model for fungicide effects on apple scab, for teaching purposes. This fungicide effect model, which can be parameterised for any fungicide and can be applied to other diseases, includes two compartments. The first (the fungicide concentration compartment) predicts the temporal dynamics of fungicide residue after application, with a time step of 1 d, as influenced by fungicide characteristics, weather conditions, and plant growth. The second compartment (the fungicide efficacy compartment) predicts the effect of the fungicide residue.

The fungicide concentration compartment

The model of Arneson *et al.* (2002) assumes uniform coverage of host tissue by the fungicide at the time of spraying. The initial concentration of the fungicide (C_0) is set at 1 by assuming that each fungicide is applied at the label-recommended dose. This model compartment reduces the fungicide residue on the plant tissue on a daily basis throughout the growing season. The reduction is predicted by combining three equations that account for temporal dynamics of fungicide residues. Because of the complexity of the interactions among these factors in the field, prediction of their individual effects on fungicide residue dynamics is difficult, and little information is available in the literature. For this reason, all factors, except rainfall wash-off (C2) and dilution due to plant growth (C3), are combined into a single reduction factor (C1). The product of these three factors indicates the fungicide residue at any time after application.

For the reduction factor C1, disappearance of canopy-applied fungicides from plant tissues has been found to follow first-order kinetics (Courshee, 1967). This is, however, only an approximation of a bilinear or trilinear loss relationship (Van Dyk, 1974): the first part of the bilinear relationship indicates rapid loss due to weathering; the second part shows a loss primarily due to pesticide degradation and volatilization. In the model of Arneson *et al.* (2002), the fungicide reduction equation is therefore the following first-order approximation of the bilinear relationship:

$$C1_t = C_0 \times \exp(- K \times t) \quad [1]$$

where t = time (in d after fungicide application), C_0 = fungicide concentration at the time of application on d 0 (with $C_0 = 1$), $C1_t$ = fungicide residue at any d t after application, and K = the reduction rate constant.

Although the rate constant should be separately determined for each commercial fungicide, plausible default values for pre-infection activity are 0.10 for protectant fungicides, 0.07 for locally systemic fungicides, and 0.06 to 0.05 for systemic fungicides. These default values determine that half of the initial fungicide concentration will remain after approx. 7, 10, 12, and 14 d, respectively, for the four rate constants (Figure 2A). Default values for post-infection activity are, respectively, 0.7, 0.4, and 0.25 for the three fungicide categories. These default values determine that half of the initial fungicide concentration will remain, respectively, after approx. 1, 2, and 3 d from after application for the three fungicide types (Figure 2B).

When rainfall occurs, the fungicide residue on the plant surface is reduced based on the precipitation amount for that day, and this wash-off by rain is accounted for by C2. Wash-off by rain has been estimated with a bilinear loss relationship (Burchfield

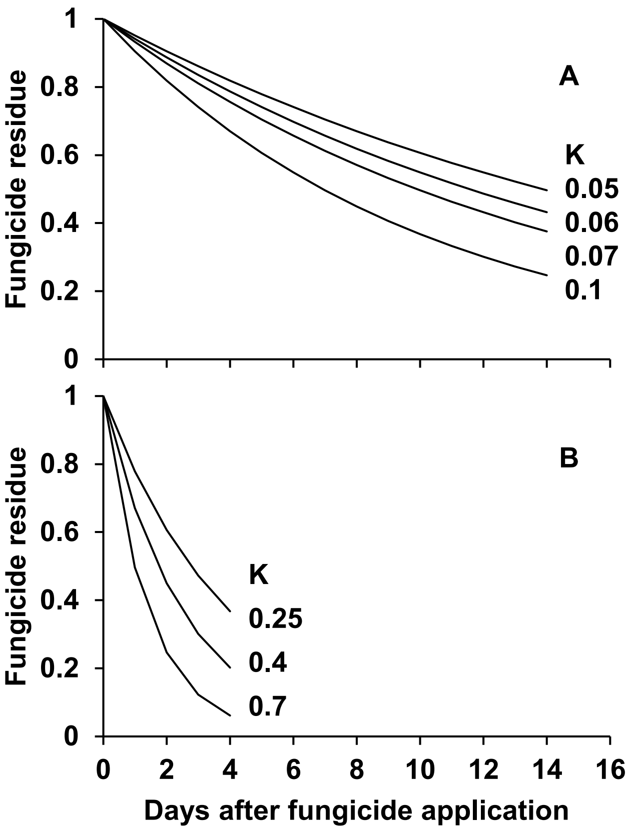

Figure 2. Changes over time in the proportion of fungicide residue remaining as calculated by equation $C1_t = C_0 \times \exp(-K \times t)$ [1], for (A) pre-infection activity and (B) post-infection activity. C_0 is the fungicide concentration at the time of application, day 0, and is set at 1. K is the attenuation rate constant, which will differ among fungicides (K values in the figure are examples).

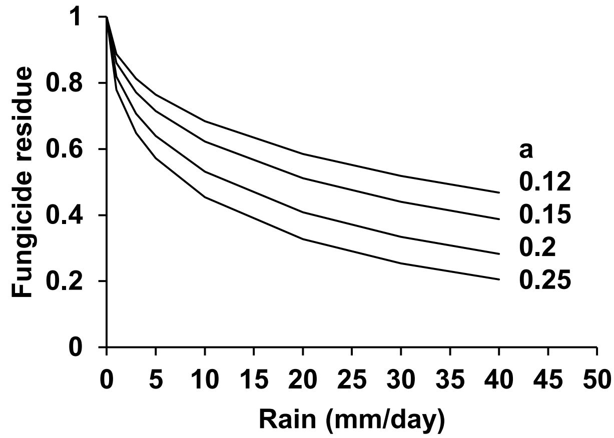

Figure 3. Changes in the proportion of fungicide residue remaining as affected by rainfall, and as calculated by equation $C2_t = \exp(-a \times c_t \times \sqrt{R_t})$ [2]. "a" is the tenacity factor, which will differ among fungicides (the values in the figure are examples).

and Goenaga, 1957). Because fungicides are known to vary in their susceptibility to wash-off by rainfall, the model of Arneson *et al.* (2002) uses the following tenacity function to estimate C2:

$$C2_t = \exp(-a \times c_t \times \sqrt{R_t}) \quad [2]$$

where a = tenacity factor, c_t = concentration factor, and R = rainfall (in mm d^{-1}). The fungicide concentration factor modifies the fungicide loss so that removal becomes increasingly difficult as fungicide is removed by rainfall. The concentration factor is calculated as follows:

$$c_t = \exp[(C_{t-1} - 1) \times 3] \quad [3]$$

The tenacity factor, a, must be calculated for each commercial fungicide. Default values determining the quantity of rainfall that reduces the initial fungicide concentration by 50% range from 0.25 with 10 mm of rainfall to 0.12 with 40 mm of rainfall (Figure 3).

The equation for rainfall wash-off applies to protectant (i.e., non-penetrant) fungicides only. Penetrant fungicides are absorbed by plant surfaces after deposition; once this occurs, they are no longer subject to wash-off by rain. Although poorly understood, the absorption dynamics of the penetrant fungicides are likely to be affected by commercial product formulation characteristics, plant surface features, and temperature (Bouma, 2007).

The absorption dynamics should be determined for each fungicide and host plant. The model of Arneson *et al.* (2002) assumed that most of the product is absorbed in the first hours after application, and that part of the remaining residue is absorbed each day thereafter (Solel and Edgington, 1973). The model divides fungicide residues into external and internal residues, with part of the external residues becoming internal at each time step (i.e., 1 d); only the external residues are subject to rainfall wash-off. Default values for fungicide absorbance are 70% in the first 24 h after application and 15% on the second and third days; the possible range for absorption in the first 24 h is 50 to 90%.

Dilution by plant growth (C3) is calculated separately for protectant and penetrating fungicides based on the daily increase in plant biomass as follows:

$$\text{if } B_t \geq B_0;\ C3_t = 1 \ / \ (B_t \ / \ B_0) \qquad [4]$$
$$\text{if } B_t < B_0;\ C3_t = 1$$

where B_0 = plant biomass at the time of fungicide application (i.e., day 0), and B_t = plant biomass at day t after fungicide application.

The fungicide efficacy compartment

In the model of Arneson *et al.* (2002), fungicide efficacy is predicted by the following logistic function:

$$EF_t = EF_0 \ / \ [1 + \exp\ (a - b \times C_t)] \qquad [5]$$

where EF_t = fungicide efficacy on day t, EF_0 = fungicide efficacy on day 0, C_t = the fungicide residue on day t as calculated in the fungicide concentration model compartment, and a and b are shape parameters of the relationship.

Values of EF_0, b, and c must be determined for each commercial fungicide, for each target organism, and for pre-infection activity, post-infection activity, or both pre- and post-infection activity. If reliable quantitative information on the effects of various fungicides on the various diseases is lacking, reasonable default values can be used. For protectant, broad-spectrum inorganic and organic fungicides (e.g., copper and sulphur-based fungicides, and dithiocarbamates), pre-infection (or preventive) EF_0 default values range from 0.8 to 0.9. For penetrant (locally systemic and systemic) fungicides (e.g., strobilurins and sterol biosynthesis inhibitors), pre-infection (or preventive) EF_0 default values range from 0.9 to 1.0, and post-infection (curative) values range from 0.7 to 0.9. For eradicant fungicides, EF_0 default value range from 0.6 to 0.8. Default values for parameter a range from 3.5 to 4.5, and default values for parameter b range from 8 to 9 (Figure 4).

Other models

Rossi *et al.* (2012, unpublished) developed a different modelling approach that incorporates the main processes accounted for by the model of Arneson *et al.* (2002), but enables parameterization based on laboratory experiments, practical knowledge, and technical information. The model is based on the following equation:

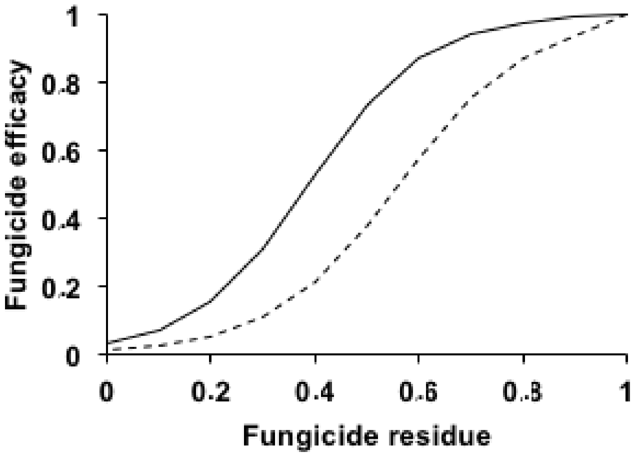

Figure 4. Relationship between fungicide residue remaining and efficacy as calculated by equation $EF_t = EF_0 / [1 + \exp(a - b \times C_t)]$ [5]. The following equation parameters were used in this example: EF_0 (fungicide efficacy at the time of application, day 0) = 1, a = 3.5, b = 9 (solid line); EF_0 = 1, a = 4.5, b = 8 (dotted line).

$$EF_t = EF_0 \ / \ (1 + \alpha \times \exp\ (\beta \times (t + (\sum Rain \times P \ / \ R) + (B_t - B_0) \ / \ B))) \qquad [6]$$

where EF_t = fungicide efficacy on day t after fungicide application, EF_0 = fungicide efficacy at the time of application (i.e., day 0), α = tenacity factor, β = fungicide efficacy reduction rate, $\Sigma Rain$ = summation of rainfall from day 0 to day t (mm), P = fungicide persistence with no rain (in d); R = rain amount causing complete wash-off of the fungicide (in mm), B_0 = plant biomass on day 0, B_t = plant biomass on day t, and B = biomass increase resulting in complete lack of efficacy.

Experimental data are necessary for precise estimation of model parameters (i.e., for calibration). For overcoming the problems of models based on field data and for overcoming parameterization problems in particular, relating to physical modes of fungicide action, laboratory experiments may be required (Figure 5). These experiments should satisfy the following criteria: i) increasing dosages of the fungicide should be applied to the plants or plant parts; ii) plants or plant parts should be artificially inoculated with the pathogen at different times before or after fungicide application (to study, respectively, pre- and post-infection activity), or before or after sporulation (to study, respectively, pre- and post-symptom activity); iii) efficacy should be determined for each com-

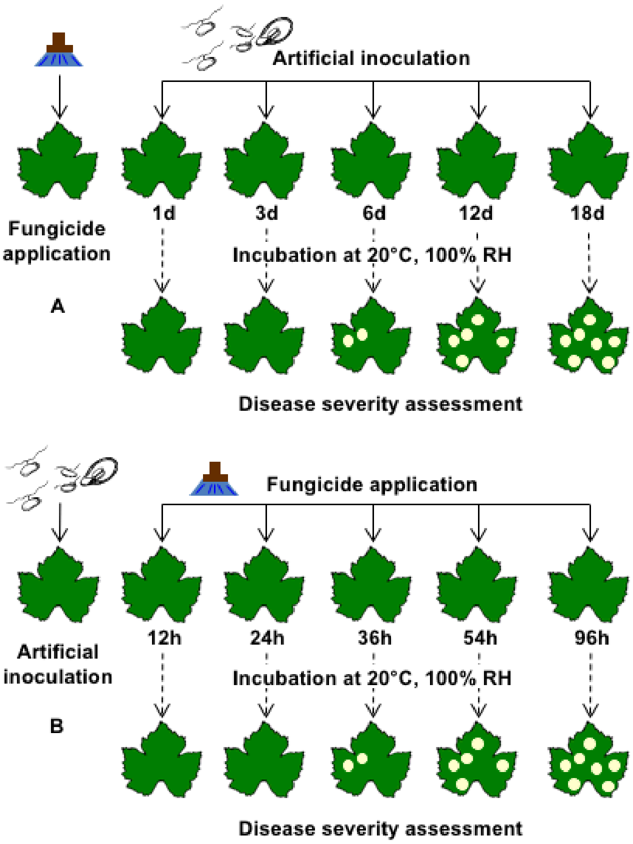

Figure 5. Examples of experiments used for the parameterisation of equation $EF_t = EF_0 / [1 + \exp(a - b \times C_t)]$ [5], for *Plasmopara viticola*, the causal agent of grape downy mildew. In (A) (pre-inoculation activity), the fungicide is applied to the leaves, and leaves are inoculated with a *P. viticola* sporangial suspension after 1, 3, 6, 12, or 18 d. the leaves are incubated at 20°C and 100% relative humidity until disease develops and disease severity is assessed. In (B) (post-inoculation activity), leaves are artificially inoculated with a sporangial suspension, and the fungicide is applied 12, 24, 36, 54, or 96 h later. The leaves are then incubated as above before disease severity is assessed.

bination of dosage and inoculation time in terms of number of lesions, disease severity and sporulation severity; and iv) dosage- and time-response relationships should be developed.

Using this modelling approach, Caffi *et al.* (2016) evaluated the post-inoculation activity of two copper-based fungicides. The fungicides were applied at 1, 3, 6, 12, or 24 h post-inoculation (hpi), and at 2, 3, or 4 g L^{-1} to potted grape plants (cv. Barbera); distilled water was applied as a control treatment. For each combination of product × dosage × hpi, eight leaf discs were inoculated with a suspension of *Plasmopara viticola* sporangia and were incubated under optimal conditions for the pathogen. At 13 d post-inoculation, the percentage of leaf disc area covered by downy mildew lesions (i.e., disease severity or SEV) was assessed. For each combination, the efficacy was then calculated as $(SEVu-SEVt)/SEVu$, where the sub-script *u* refers to the untreated leaf discs and *t* refers to the treated ones. Because fungicide identity and the interaction between dosage and time of application (hpi) were not significant, the data were pooled, and the following parameters were estimated for equation [6] using a non-linear regression analysis: $EF_0 = 1$, $\alpha = 0.005$, and $\beta = -0.994$ ($R^2 = 0.965$).

In a similar experiment with the same copper-based fungicides (Caffi *et al.*, unpublished), the pre-inoculation activity was tested at 12, 9, 6, 3, or 1 d before artificial inoculation, and the following parameter estimates were obtained: $EF_0 = 1$, $\alpha = 0.00005$, and $\beta = -0.984$ ($R^2 = 0.986$). For both pre- and post-inoculation activity, the other parameters of equation [6] were set as follows: P = 7 d (based on commonly accepted technical knowledge); R = 10, 30, or 40 mm, respectively, if dosages of 30, 50, and 70 g of Cu^{2+} hL^{-1} of copper were used (Kuflik *et al.*, 2009); and B = three leaves (Kuflik *et al.*, 2009). Figure 6 shows some output of equation [6] for pre-infection activity; dots in the figure represent model validation. Validation was performed by spraying the fungicide in a commercial cv. Barbera vineyard at three times in 2016, corresponding to the following growth stages: ten leaves unfolded (Figure 6A), pea-sized berries (Figure 6B), and veraison (Figure 6C). The vineyard was located in Castell'Arquato (North Italy), was 20 years old, and was trained with a Guyot system. At 1, 3, 6, 9, or 12 d after each spray, 30 random leaves were collected, placed in a cooler, and transported to the laboratory, where the leaves were artificially inoculated with *P. viticola* as described by Caffi *et al.* (2016) and then assessed for fungicide efficacy.

Multi-criteria decision-making

According to Rossi *et al.* (2012), type II decisions regarding whether and when it is necessary to protect the crop depend on the following risk factors: i) the risk of disease or infection (as indicated by plant disease models); ii) plant susceptibility to the disease (as indicated by plant growth models); and iii) the residual efficacy of previous fungicide sprays (as indi-

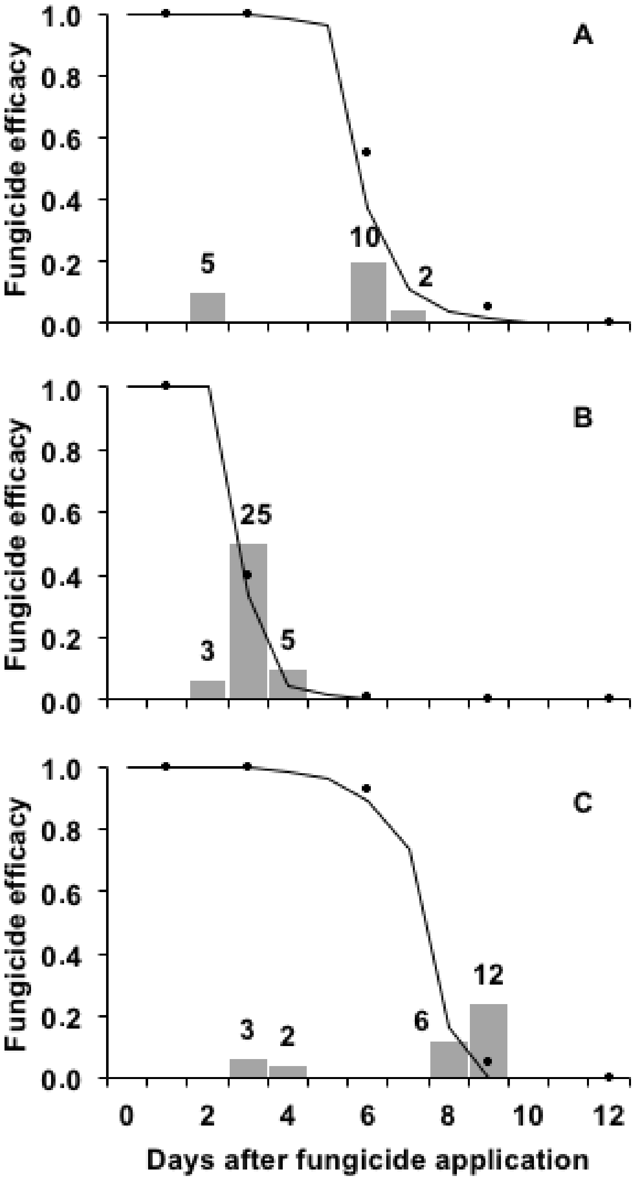

Figure 6. Loss of fungicide efficacy over time in a commercial vineyard in 2016, as predicted by equation $EF_t = EF_0 / (1 + \alpha \times \exp(\beta \times (t + (\sum Rain \times P / R) + (B_t - B_0) / B)))$ [6], with the following parameter estimates: $EF_0 = 1$; $\alpha = 0.00005$; $\beta = -0.984$; P = 7 d; R = 40 mm rain; and B = three leaves. Bars indicate the rain (mm) that fell in the vineyard, which was located at Castell'Arquato (North Italy). Treatments of a copper-based fungicide were applied in the vineyard at the following stages: ten leaves unfolded (A), pea-sized berries (B), and veraison (C). At 1, 3, 6, 9, or 12 d after each spray, leaves were collected, artificially inoculated with *Plasmopara viticola* sporangia in the laboratory, incubated for 7 d at 20°C and 100% relative humidity, and assessed for fungicide efficacy, as indicated by dots (real values) and by curves connecting the dots.

cated by fungicide models). A method of integrating these risk factors is needed, and one such method is the use of a fuzzy control system (FCS). González-Dominguez *et al.* (2016) developed a FCS to determine whether a fungicide application is needed to control *P. viticola* in a vineyard. The FCS uses the following information provided by mathematical models: i) grapevine phenology (Cola *et al.*, 2014); ii) risk of primary infection (Rossi *et al.* 2008); iii) abundance of secondary sporangia; iv) risk of secondary infection (Caffi *et al.*, 2013); and v) residual protection provided by the last fungicide application. All possible combinations of these risk algorithms are expressed as IF–THEN rules; the fuzzification interface, inference engine, and defuzzification interface provide the FCS output as 'treatment' or 'no-treatment'.

The FCS was tested in 18 organic vineyards in Italy by comparing the scheduling of copper fungicides against *P. viticola*, as determined by a panel of five experts *vs.* the FCS. The FCS was able to reproduce the expert reasoning with an overall accuracy of 0.992 (with 1 indicating perfect agreement). The probability that the FCS recommended a treatment given that the expert panel did was 0.878, and the probability that the FCS did not recommend a treatment given that the expert panel did not was 1. From the total of 2,754 d evaluated, the FCS recommended a treatment when the experts did not on only 21 days. The reasons why the experts did not recommend a treatment on these days were as follows: i) the level of fungicide protection was 60–75% and a treatment had been applied 1 or 2 d before; and ii) the harvest time was near. Therefore, using the information provided by multiple models, the FCS was able to reproduce the expert reasoning regarding the decision to apply a fungicide for controlling grape downy mildew.

Completing the decision-making process

In the IPM decision framework of Rossi *et al.* (2012), once the timing is decided and the plant protection product is selected, the following additional questions require answers: At what dose should the selected product be applied? When should the selected product be applied? Systems for defining the product dose and when the environmental conditions are suitable for application are therefore needed to complete the decision-making process.

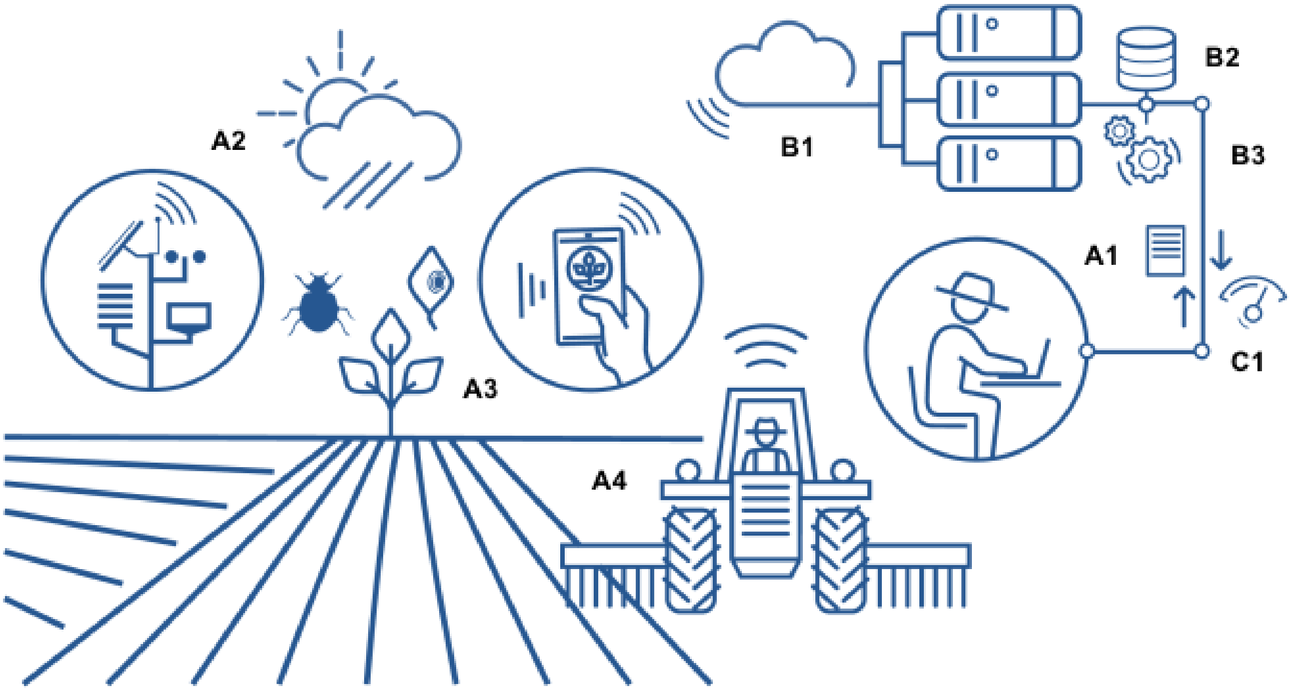

Figure 7. Scheme of a modern decision support system (DSS) for plant disease management. Information about crop-specific characteristics (A1), environmental conditions (A2), crop and plant status (A3), and agricultural operations (A4) flows asynchronously from the crop to a remote server (B1), and is stored in databases (B2). This information is then used as input for running mathematical models and decision algorithms (B3), which generate decision supports and alerts that help each grower decide whether, when and how to apply a fungicide.

Crop-adapted fungicide application

Grapevines have no leaves at the start of each growing season and abundant leaves at the end. Consequently, the leaf area to be treated increases considerably during the growing season, from nearly zero at bud burst to over 23,000 m^2 ha^{-1} at fruit set (Siegfried *et al.*, 2007). It follows that the application of a fungicide at a fixed rate per hectare will over-treat early season foliage but under-treat late season foliage. Fungicide dosages must therefore be adjusted according to the leaf area at the time of application.

Crop-adapted spray application makes it possible to obtain constant quantities of active ingredient per unit of leaf area throughout the growing season. The vine row volume (VRV), the leaf wall area (PWA), and the unit canopy growth (UCR) have all been used in the last decade to determine the optimal volume for spraying in vineyards based on achieving the optimal coverage (impacts cm^{-2}) according to the characteristics of the crop canopy (Gil *et al.*, 2007; Siegfried *et al.*, 2007; Barani *et al.*, 2008).

Researchers have also developed methods to modify the volume of spray applied based on the type of sprayer used, nozzle types and sizes, operational parameters, and weather conditions (Walklate and Cross, 2010; Gil and Escolà, 2009). Crop-adapted spraying reduces the quantity of fungicide applied while achieving disease control equivalent to traditional spraying (Gil *et al.*, 2011).

Application time of pesticides

Weather conditions before, during, and after the application of plant protection products greatly affect their efficacy. Bouma (2003) developed a model that evaluates the relationships between meteorological conditions, the timing of pesticide application, and pesticide efficacy. Knowledge of the behaviour of products in relation to weather conditions can increase their efficacy and even serve as a tool for reducing the doses. Formulation and the build-up of the wax layers on upper leaf surfaces affect fungicide adhesion and

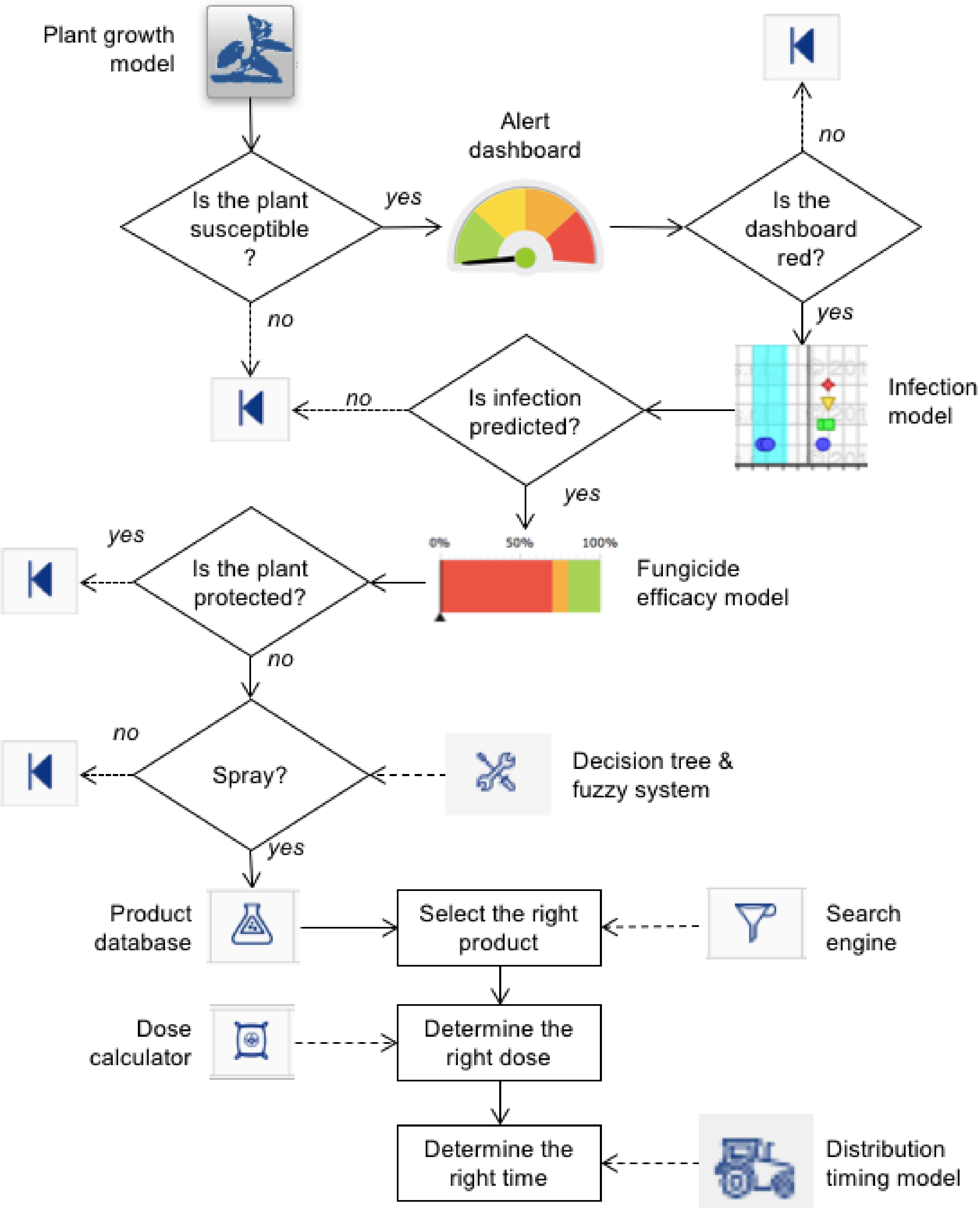

Figure 8. Scheme of how a farmer navigates within the DSS vite.net®, in order to decide whether a fungicide spray is required, and when it should be applied if it is required, based on plant growth, infection risk, and the protection provided by any previous fungicide application. If the user decides to apply fungicide, the DSS helps the user select the best product (based on multiple selection criteria including active ingredient, mode of action and activity) and, given that product, the correct application dosage and application time during the day.

the rate and method of uptake of active substances into leaf tissues. Farmers require tools to account for all of these processes. The decision support tool GEWIS, for example, combines and integrates knowledge of relationships between plant protection products and meteorological conditions, and helps farmers select the best moment of the day to apply a product.

Decision support systems for multi-criteria decision-making

The decision-making processes discussed in this review require expertise and access to updated information on multiple risk factors. Advances in information and communication technologies have made it possible to incorporate models into decision support systems (DSSs) and to deliver these systems to farmers. Although the acceptance by users of early DSSs was low because of the so-called "implementation problem" (Rossi *et al.*, 2014a), modern DSSs are useful tools for supporting informed decision-making in plant protection (Magarey *et al.*, 2002) (Figure 7).

One example of a modern DSS is vite.net® (Rossi *et al.* 2014a). This is intended for vineyard managers (the people making decisions about vineyard management or who suggest the proper actions to grape growers). The DSS has two main parts: i) an integrated system for real-time monitoring of vineyard components (air, soil, plants, pests, and diseases); and ii) a web-based tool that analyses these data, using advanced modelling techniques, and then provides up-to-date information for vineyard management as alerts and decision supports; the decision about fungicide application remains with the users. The information is tailored to particular vineyards, part of a vineyard, or a number of vineyards that are uniformly managed throughout the season. In the design and development of vite.net®, the implementation problem was solved, in part by involving potential users during development and testing of the DSS (Rossi *et al.*, 2014b).

The DSS vite.net® incorporates all of the models and tools described in this paper. Figure 8 shows how a user can navigate within the DSS to obtain a recommendation at any time during the grape-growing season, to not spray or to spray with the appropriate product at the right dose and at the right moment.

When expert viticulturists used vite.net®, the number of copper treatments against downy mildew in organic vineyards was reduced by 24%, and the total amount of copper applied was reduced by 37%, compared to a calendar scheduling of copper application that provided the same level of protection (Rossi *et al.* 2014a). From 2012 to 2014, vite.net® was used in different grape-growing conditions within the PURE project (www.pure-ipm.eu): the results confirmed its accuracy in predicting disease risk and confirmed its ability to provide useful information to growers. The use of vite.net® allowed growers to calibrate the amount of fungicides applied with any treatment, and this resulted in an average reduction in fungicide usage of 33% in IPM farming and of 44% in organic farming, with a consequent reduction in disease management costs of about 200 € per ha per season (Pertot *et al.*, 2017).

The DSS vite.net® has been commercially available since January 2013, and is now used on a large-scale by grape growers (Rossi *et al.*, 2014b). Approximately 400 growers or managers used the DSS in 2017 for management of more than 15,000 ha of vineyards throughout Italy. The DSS is also used in Spain, Portugal, Greece, Romania, and the United Kingdom. The use of the DSS has made it possible to maintain yields and grape quality while reducing pesticide usage by as much as 50%. This has increased farmer profits (Pertot *et al.*, 2017), and enhanced environmental and social sustainability of the grape production system (Metral *et al.*, 2013).

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