Alternatives to synthetic fungicides using small molecules of natural origin

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Alternatives to synthetic fungicides using small molecules of natural origin

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Abstract

Several small molecules of natural origin have been reported to act as alternatives to synthetic fungicides. These are reviewed in this chapter, and some ideas of new development are given.

The list, which is not exhaustive, is the following: acetaldehyde, acetic acid, aldehydes (other than acetaldehyde), aminobutyric acids, ascorbic acid, ethanol, ethylene, jasmonic acid and methyl jasmonate, saicylic acid and methyl salicylate, salts (e.g. sodium bicarbonate, calcium chloride, copper sulphate), sorbic acid and sulphur. There is also a paragraph about the potential of combinations (e.g. additive or synergistic effects).

Introduction

There are many grape diseases, among which downy and powdery mildew and gray mold, causing important pre- and post-harvest losses. Most commercial grapevines are susceptible to such fungi. To counteract this disease development a wide panel of synthetic fungicides is in use nowadays. However, solutions to limit pre-harvest treatments with synthetic fungicides are of particular interest as chemical residues are limiting access to many markets, and there is a diminishing number of antifungal compounds that are still registered (Nigro et al., 2006). Moreover, some pathogen strains may develop resistance to some pesticide, thus alternative strategies are required. One of the most sustainable alternative strategies would be to develop grape cultivars that are naturally resistant to such fungi, and there are available genotypes for this trait (Dry et al., 2010). But it is a long term development and most food industries using grapes are relying on specific cultivars, around which all marketing efforts have been made for decades. So finding alternative treatments with existing cultivars is still of interest for the grape industry.

The purpose of this review is to list various compounds of natural origin that have been tested with or without success in the past. They were ranged by alphabetical order.

Acetaldehyde

As this compound has such a low boiling point (20°C), it is very volatile at ambient temperature, so most trials were performed for postharvest applications using it in the vapour phase (Pesis, 2005). As most aldehydes, it has a strong bactericidal and fungicidal potential, as aldehydes are very oxidative compounds. One must attract attention to users that they are very toxic compounds to manipulators and quite oxidative to many parts of the equipment.

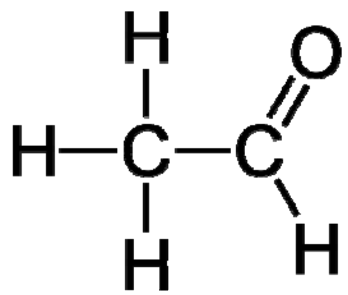


Figure 1 : Acetaldehyde (MW: 44g; BP: 20.2°C, 68°F)

Utama et al. (2002) showed that aldehydes were more effective that alcohols in blocking fungus growth *in vitro*. They stated that acetaldehyde was quite effective against various fungi: *Rhizopus stolonifer, Penicillium digitatum, Colletotrichum musae, Erwinia carotovora,* and *Pseudomonas aeruginosa,* showing germicidal effects at concentrations below 1 mmole/per dish, as the assays were run in Petri dishes; but they did not report trials *in vivo*. These germicidal concentrations corresponded approximately to 60 µmoles acetaldehyde/ litre of air, in the vapour phase, after one hour of application, and they went down to 20 µmoles/litre of air in 5 days at 25°C. When applied on 'Sultanina' and 'Perlette' grape berries grapes with low sugar content and high acidity, the acetaldehyde was found to increase total soluble solids, to decrease acidity, and to enhance sensory preference (Pesis and Frenkel, 1989), but these authors did not report effects on fungus. These observations were reported later by Avissar and Pesis (1991) who showed that acetaldehyde was controlling the decay of table grapes in a postharvest trial.

Acetaldehyde is probably present in most plant and fruit, but at very low concentration around a few nmoles.g_{FW}^{-1}(Chervin et al., 1999), so whether it is effective or not at this natural concentration is not known. However there was an interesting report by Miyake and Shibamoto (1995) showing that acetaldehyde can be produced in aerobic and relatively mild conditions by oxidation of L-ascorbic acid. Thus during the oxidative stress following a fungus infection, there may be some acetaldehyde produced, and it may be part of the natural defence; this has been shown in the case of resistance of potato plants to *Phytophthora infestans* (Tadege et al., 1998).

Acetic acid

The use of acetic acid fumigation for postharvest control of fungi has been reviewed by Tripathi and Dubey (2004). These authors referenced the fact that acetic acid is a natural metabolite occurring in several fruit, and that fumigation with acetic acid onto grapes has been proven an effective treatment to control gray mold.

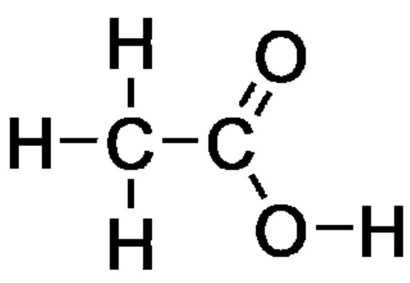


Figure 2 : Acetic acid (MW: 60g; BP:118.1°C, 245°F)

A recent paper by Camili et al. (2010) showed that acetic acid might stimulate natural defences in Italia grapes, as the best control of Botrytis was obtained when the fruit was treated with acetic acid vapours 48 hours prior inoculation with Botrytis spores. To my knowledge, there no or very scarce data about a potential control of grape disease by spraying acetic acid in vineyards, but the trials may be worth it.

Interestingly, sprays of indole-acetic acid on tomato seedlings were shown to reduce the symptoms caused by a phytopathogen, *Pythium ultimum*. (Gravel et al., 2007). The indole-acetic acid, one of the auxins, is obviously quite different to acetic acid, however no application has been tested onto grapevines to check if such an effect would be observed with grape specific pathogens.

In a more comprehensive approach, Martin and Maris (2004) tested the antifungal and antibacterial efficacy of seventeen organic and mineral acids against several strains of bacteria and fungi, known as food contaminants. They found interesting inhibitory effects by formic, mandelic and lactic acids.

Aldehydes (other than acetaldehyde)

The remarks regarding their toxicity, outlined in the acetaldehyde paragraph, are valid for the following compounds too.

The cinnamaldehyde is part of the cinnamon aroma. It can be used as a food additive, and has anti-microbial properties at quite low concentration around 10 mM (Smid et al., 1996). There no report of exogenous treatment of cinnamaldehyde on grapes, but a recent report shows an additional potential of such treatments with this aldehyde or other compounds listed in this chapter: Viazis et al. (2011) show that cinnamaldehyde limit the viability of an enterohemorrhagic Escherichia coli strain on leafy vegetables, when sprayed at 0.5% (v/v).

The hexanal and hexenal are two natural compounds, oxidation products of lipids. They have been shown to harbour anti-fungal properties against

Botrytis sp., *Alternaria sp.*, and *Penicillium sp.* among others (Tripathi and Dubey, 2004 and refs herein). This has been confirmed recently by Song et al. (2007), as hexanal vapours gave a excellent control of *Monilinia fructicola* on peaches and a very good control of *Botrytis cinerea* on raspberries at doses around 900 µl.l^{-1}.

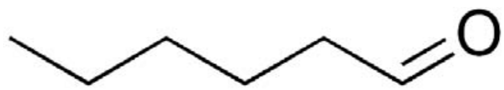


Figure 3 : Hexanal (MW: 100g; BP: 120°C, 248°F)

In planta experiments have been published recently showing that Arabidopsis over-producing C6 aldehydes (e.g. hexanal and hexenal) were more resistant to *Botrytis* infection, mainly through a direct effect of the aldehydes on the fungus growth rather than through an elicitor role that the aldehydes might have had (Kishimoto et al., 2008). This study also leads to think that the spraying of such aldehydes on grapevines may reduce the development of various fungi, however the cost and the hazard of manipulating such molecules have to be considered.

Utama et al. (2002) tested in vitro the bactericidal and fungicidal properties of several aldehydes listed above and also benzaldehyde, which was shown to be slightly more efficient than acetaldehyde.

Aminobutyric acids

These compounds are involved in plant induced resistance to fungi, and most recent studies report the effects of β-aminobutyrate (Dubreuil-Morizi et al., 2010; Walz and Simon, 2009; Slaugther et al., 2008). Its chemical structure is close to the γ-aminobutyrate which is a well known neurotransmitter. For in-field applications to various crops, the toxicity to humans has to be considered.

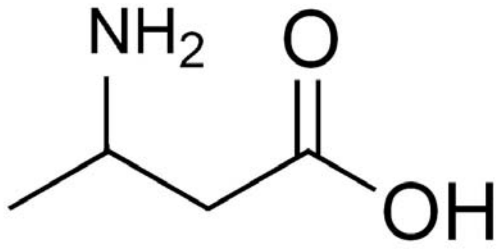


Figure 4 : β-aminobutyric acid (MW: 103g, solid at ambient temperature)

The β-aminobutyrate was shown to induce resistance to downy mildew in sensitive and resistant grape cultivars, and this was related to increased stilbene synthesis and accumulation (Slaugther et al., 2008). These enhanced protection effect against various fungi have been observed in other plants, like cucumber (Walz and Simon, 2009), and these effects were associated with callose accumulation and rapid cell death around the infected area. Recently, a report stated that this enhanced resistance to mildew due to β-aminobutyrate application could be associated with reactive oxygen species produced by a NADPH oxidase (Dubreuil-Morizi et al., 2010).

Ascorbic acid

It is a natural compound which accumulates in many fruit, but in grapes it does not reach high levels as in citrus, as it is a precursor of tartaric and oxalic acids (DeBolt et al. 2004).

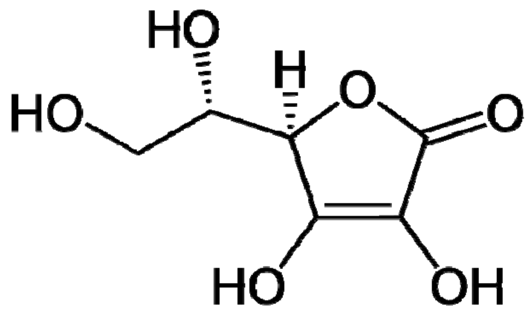


Figure 5: Ascorbic acid (MW: 176g, solid at ambient temperature)

It accumulates at low levels in berries, some µmoles per gram of fresh weight (Cruz-Ruz et al., 2010). Whether it shows some antifungal activity at this level is unknown. Authors have observed antimicrobial effects of ascorbic acid at higher concentration such as 2.5% (Van der Wolf et al., 2008), however no test has been recorded on grapevines. However the role of ascorbic acid on phytopathogens is probably complex, indeed Barth et al. (2004) have shown that Arabidopsis mutants deficient in ascorbic acid are more resistant to bacteria and fungi, may be through an increase in salicylic acid accumulation, thus leading to activation of plant natural defences.

Ethanol

This compound is also naturally present in plant and fruit tissues, and can be found under normal aerobic conditions when the inside of the cells become too acidic or under hypoxic conditions (Sweetman et al., 2009, and refs herein).

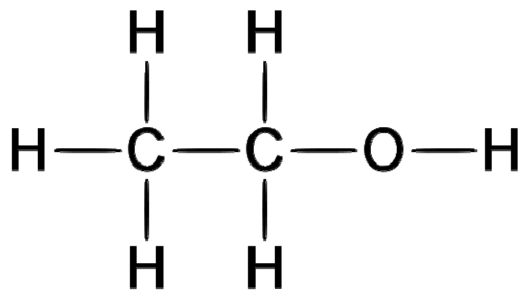


Figure 6 : Ethanol (MW: 46g; BP: 78°C, 172°F)

Our work regarding applications of ethanol has been initiated by reading an article by Beaulieu and Saltveit (1997). They observed that exogenous ethanol was stimulating ethylene production and tomato fruit ripening, so we tested it on grapes in order to modulate ripening and anthocyanin accumulation (El Kereamy et al., 2002) and wine colour (Chervin et., 2001) using hand-held sprayers directed towards the bunches. Later we found that ethanol sprays with commercial sprayers increases mostly the berry diameter (Chervin et al., 2005a).

And then we tested ethanol for its efficacy to limit fungus growth. The first application was post-harvest, we will present pre-harvest applications later. The idea came from a paper by Lichter et al. (2002) showing that dipping grapes at harvest in ethanol solutions was decreasing the *Botrytis cinerea* growth. The ethanol dip has two drawbacks which are the need to promptly dry the grapes after treatment to prevent berry cracking (Karabulut et al., 2004), and the possible cross-contamination with fungus spores from a previously infected grape crate, when working with low ethanol concentrations. We adapted these ethanol treatments to commercial practices using ethanol in the vapour phase (Chervin et al., 2005b). Indeed the industry is already using fumigation with SO_2 in adapted chambers, or in crates with SO_2 pads releasing the SO_2 in contact with air humidity, so if ethanol was going to be efficient and accepted by the industry, a simple change of the active ingredient was possible. The application of ethanol vapours was optimised over two seasons for 'Chasselas' table grapes and at a dose rate of 2 ml . kg^{-1} of grapes, the ethanol vapour was as effective as sulphur dioxide pads to prevent rot development, caused by *Botrytis cinerea*, and stem browning. Further tests with consumer panels showed no significant difference in sensory perception between controls and treated grapes. The application of evenly distributed ethanol vapours is critical, as higher concentrations of ethanol may enhance stem browning. Materials releasing ethanol are already on the market, such as the "ethanol powder" (Suzuki et al., 2004). Postharvest applications of ethanol may also present potentials to reduce berry shatter (Chervin et al., 2005c) but these need further development.

Then we tested pre-harvest applications of exogenous ethanol in the vineyard, to prevent fungus development ahead; the results that are detailed below have been reported recently (Chervin et al., 2009). The idea came from the reading of an article by Karabulut et al. (2003). These authors found that spraying 1 litre per 5 vines of a solution at 50% ethanol, 24 hours prior harvest, was effective in reducing the rots over the postharvest period. We then adapted this to commercial practices, reducing the amount of solution to 150 litres per hectare (using a mist blower) and no treatment in the last two weeks prior havest. The treatments were performed every two weeks from veraison, we tested a late harvest, scheduled two months and a half after veraison, was chosen for these trials, so the conditions were optimised for high *Botrytis* development. The fungus development was assessed at harvest and after 4 to 6 weeks cold storage. We always found a higher impact of the treatments after cold storage when Botrytis development had occurred at higher rates.

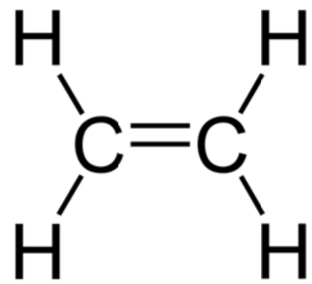
In preliminary trials, we found that even a very low concentration of 2% ethanol was reducing the Botrytis growth. It is not likely that ethanol would have had a direct effect on fungus growth at this low percentage, as Lichter et al. (2003) showed that at least 30% EtOH is necessary to prevent *Botrytis cinerea* spore germination. Thus the 2% EtOH dose is more likely to induce plant defence.

The optimal dose of ethanol to reduce *Botrytis* growth by pre-harvest spraying was found to be around 16%. However to match the industry demand, we had to combine it with calcium chloride in order to further reduce the gray mold growth. This was done after reading an article by Nigro et al. (2006) in which the authors reported the efficacy of various salts to reduce the *Botrytis* development. This will be detailed in a paragraph below in this chapter. Thus we reported that preharvest applications of a 16% ethanol solution, containing 1% $CaCl_2$, reduced gray mold development. At harvest the losses due to rotten clusters dropped from 15% in controls to 5% in grapes treated with ethanol & $CaCl_2$. Over 6 weeks of cold storage, the losses due to gray mold were reduced by 50% in bunches treated with ethanol & $CaCl_2$, compared to untreated controls. These treatments did not induce significant changes in fruit quality assessed by sensory analysis of healthy berries.

The ethanol has a much higher boiling point that acetaldehyde and is not oxidative, which renders it safer to use; it is already used by industry as a wetting agent or for its solvent properties. As it is flammable, precautions are necessary. Ethanol is rather cheap to produce, and its worldwide production is increasing, mainly due to its use as ethanol fuel, so its cost will decrease.

Ethylene

Its a gas at ambient temperature and it plays important roles as phytohormone in most plants (Lin et al., 2009). These authors review all aspects of ethylene metabolisms in plant biology, including production and perception.



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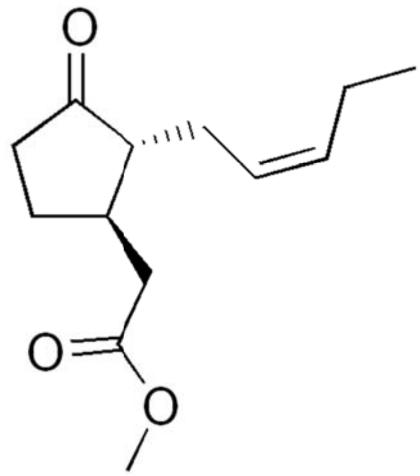
Regarding the ethylene role in grape defence, there has been a series of recent works by J.M. Merillon's team showing that 2-chloroethylphosphonic acid, an ethylene precursor also called ethephon, led to a decrease of fungus growth when sprayed onto grapevines, through elicitation of natural defences (Belhadj et al., 2008a). More details will be developed in J.M. Merillon's chapter in this book.

Additionally a recent series of microarray analyses, using mRNAs extracted from berry tissues after exogenous application of ethylene on grape clusters, has been partly published by Chervin et al. (2008): the expression of some genes involved in plant defence was shown to be modulated by such a phytohormone.

Jasmonic acid and methyl jasmonate

Jasmonic acid is present naturally in grape berries, particularly in seeds (Kondo and Fukuda, 2001) up to 50 pmoles/seed, and methyl jasmonate id also present to a lesser extent (about 10 times less).

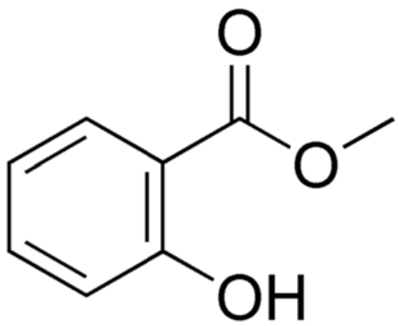
Data are lacking to check the variability over various ciltivars.



Methyl jasmonate has been shown to promote stilbene accumulation (Krisa et al., 1999) and grape natural defenses. There will be more details developed in J.M. Merillon's chapter, in this book.

Salicylic acid and methyl salicylate

The salicylic acid is well-known to be part of the SAR: Systemic Acquired Resistance (Durrant and Dong, 2004). Park et al. (2007) showed that both conversions of salicylic acid to methyl salicylate and methyl salicylate to salicylic acid were essential for SAR in tobacco, and they concluded that methyl salicylate is a SAR signal in this plant.



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However there is still some controversy about the direct involvement of salicylic acid and methyl salicylate in SAR (Durrant and Dong, 2004; Attarana et al., 2009).

Nevertheless, there are a few papers reporting application of salicylic acid on grapes (e.g. Wen et al., 2005), showing that this compound can induce metabolisms known to be involved in plant defence mechanisms, but no report with grapes showing induced resistance. On strawberries, Babalar et al. (2007) showed that pre-harvest sprays of salicylic acid had potential to limit fungal decay. The concentration that gave a rather good control was 2 mM, and several sprays were necessary: at the vegetative growth stage, then the fruit development stage and postharvest. This gives good ideas to set-up an experiment with grapes. Salicylic acid may also interest companies and growers as it seems to be available at low cost (Dr Liliana Martinez, Mendoza, pers. comm.).

In addition to controlling the fungal diseases, salicylic acid and derivatives might have other potentials for grape growers. Indeed methyl salicylate was also used to “recruit” beneficial insects in vineyards (James and Price, 2004). These authors showed that use of controlled-release methyl salicylate in a crop could increase recruitment and residency of populations of certain beneficial insects. This strategy may have the potential to enhance the efficacy and reliability of conservation biological control in crop pest management.

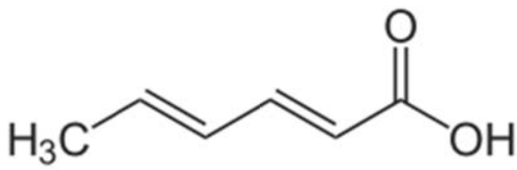
Lastly, salicylic acid was shown to delay ripening in particular conditions (Kraeva et al., 1998). Thus the fruit quality is obviously a parameter to take into account in any experiment dealing with applications of such a compound, and all treatments described in this chapter.

Salts (e.g. sodium bicarbonate, calcium chloride, copper sulfate)

Salts are cheap, accepted by consumers, with minor environmental impact at the effective concentrations, which are non-toxic, and they are already used by the food industry. One of the most recent and most comprehensive study about their use as antifungal agents in vineyards has been published by Nigro et al., 2006). These authors tested 19 differet salts, from sodium phosphate dibasic, the most efficiant against Botrytis, to potassium carbonate the les efficient in a small trail using artificially infeste berries. Then they ran larger field trials in field in which the most efficient salts against bunch rots were calcium chloride, sodium bicarbonate and sodium carbonate at concentrations around 1% (w/v). the spray timing was variable, some treatments preformed 90 and 30 days prior harvest in the last trial, but also 20 and 5 days prior harvest in earlier trials. The salts were as efficient as the classical chemical treatments in some field trials.

Another wellknown salt used in viticulture is the copper sulfate pentahydrate, which has a blue colour, and its natural form is called chalcanthite. It is one of most common antifugal when it is mixed with lime, particularly against downy mildew, and it is named Bordeaux mixture. It is one of the rare antifungal treatments, with sulfphur, allowed in organic vineyards. However its intensive use is known to ‘pollute’ soils and to render some cultures difficult after several years of vine growing, as other crops are not so tolerent to high Cu concentrations in soils.

Sorbic acid



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It is a known food preservative, it can be associated with sodium or potassium, among others. Karabulut et al. (2005) showed that potassium sorbate when applied on 'Thompson Seedless' grapes after harvest, at concentrations around 1%, reduced the incidence of gray mold over storage. No data was found regarding pre-harvest sprays of sorbate salts to control various fungal or bacterial diseases on grapes, but there is some potential.

Sulphur

Sulphur remains a very efficient and simple alternative to synthetic fungicides against powdery mildew. Recent reports by Crisp et al. (2006) confirmed this fact. They compared the efficacy of several compounds, such as milk, whey, canola oils and potassium bicarbonate to sulphur, and this latter was most often the best blocker of powdery mildew. Whey showed some potential, however in field trials the acceptable yield (i.e. bunches with less than 5% powdery mildew infections) was lower than when treated with sulphur. The whey compounds are mainly lactose and lactoglobulin. Why these compounds or the whey pH might have an inhibitory effect on the fungus is not discussed in the article.

Combinations

These are always interesting as they can generate additive or synergistic effects. One example has been detailed above when combining ethanol with calcium chloride during field sprays gave a better control of gray mold at harvest, and after storage (Chervin et al., 2009). Another paper is reporting such effects with ethanol and potassium sorbate (Karabulut et al., 2005). Belhadj et al. (2008b) have tested with success the combination of methyl jasmonate and sucrose to induce accumulation of polyphenolics in grape cell cultures.

Many combinations have been tested on different fruit, giving ideas for grape future treatments. For example Spadaro et al. (2004) have tested with some success combinations of hot water, backing soda and ethanol against *Penicillium expansum* and *Botrytis cinerea* over apple storage, but the potential may be extended to grapes and field trials. Wang et al. (2010) showed that the combined treatment with methyl jasmonate and ethanol resulted in a greater control of green mold due to *Penicillium citrinum* and also improved antioxidant capacities of bayberries. These authors reported a clear synergistic effect of the combination of methyl jasmonate and ethanol leading to approximately 10% decay in bayberries when each treatment led to 40 % decay and the controls showing 80% decay. Interestingly the combined treatments led to a better sensory appreciation of the fruit by a sensory panel than for all other treatment.

There is an infinite number of combinations, (i) as the number of potential individual treatments is great, (ii) as the combination order may vary (e.g. treatment A before treatment B, or B before A), and (iii) as the number of repeated applications over the pre- and post-harvest periods (e.g. one treatment every week over the ripening period or one treatment every fortnight). Combinatorial optimisation has an obvious interest in such approaches.

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