INHIBITION OF THE APPLE SCAB PATHOGEN VENTURIA INAEQUALIS AND THE GRAPEWINE DOWNY MILDEW PATHOGEN PLASMOPARA VITICOLA BY EXTRACTS OF GREEN WASTE COMPOST

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SUMMARY

Extracts of green waste compost have been shown to inhibit plant diseases. In this study, the factors influencing the mechanism of inhibition of apple scab (*Venturia inaequalis*) and grapevine downy mildew (*Plasmopara viticola*) were studied. Extracts were prepared from samples of 30 composts from commercial composting plants. Composts were extracted with 1:2 or 1:5 water for 2 or 7 days. Extracts were applied to seedling of apple and grapevine. The seedlings were artificially inoculated with *V. inaequalis* or *P. viticola*, respectively and incubated under controlled conditions. After inoculation, severity of diseases and lesion diameter were measured. The incubation time and the compost/water ratio did not influence the capacity of the extracts to protect the apple plants. All treatments with compost extracts reduced disease severity in both host-pathogen systems, and there was no difference in efficacy between autoclaved, sterile filtrated (0.2 µm) and untreated extracts. From this, we conclude that the inhibition by compost extracts is not linked to their microbial activity. Rinsing apple seedling leaves 1 and 48 hours after application of the compost extracts did not diminish the protective effect against *V. inaequalis*. On the other hand, the severity of *P. viticola* increased, when the seedlings were rinsed after the application of compost extracts, and was similar to the untreated control. Compost extracts enhanced in vitro germination of conidia of *V. inaequalis* and showed no fungicidal effect. Thus, inhibition apparently acts indirectly in this host-pathogen system. On the other hand, the activity of zoospores of *P. viticola* was inhibited by 70 % compared to the control. The salt content of the extracts and their effect on the zoospores were positively correlated. For this host-pathogen system, there is thus evidence for a direct inhibition by compost extracts. We conclude that the active principle against *V. inaequalis* and *P. viticola* must be a water soluble, heat-stable metabolite produced in the compost before its extraction. The mechanism of inhibition in both plant-pathogen systems is different.

1 Introduction

Over the last years, organic agriculture has looked for alternatives to substitute the utilisation of copper fungicides against some plant fungal diseases such as apple scab and grapevine downy mildew. Compost extracts could represent a potentially attractive alternative to fungicides.

Indeed, compost extracts have been shown to improve plant health (Scheuerell and Mahaffee, 2002; Weltzien, 1992). The efficacy of compost extracts varies with the type of compost and the host-pathogen system, but the reasons for this variability are unknown so far. Moreover, there is no final conclusion about the mechanisms of inhibition of plant diseases by compost extracts. Most observations about the mechanisms of inhibition of plant diseases were carried out with composts based on manure (Cronin et al., 1996; McQuilken et al., 1994; Weltzien, 1992). Likewise, the production mode for compost extracts varies widely in relation to the extraction duration and compost to water ratio (Weltzien, 1990).

In this study, the disease suppressiveness of a range of different composts representative for the Swiss composting plants was investigated. The aims of our study were (i) to determine the optimal extraction duration and compost/water ratio (ii) to determine the mechanisms of inhibition of the apple scab pathogen *Venturia inaequalis* and the grapevine downy mildew pathogen *Plasmopara viticola*

2 Materials and methods

2.1 Compost samples

The composts were sampled from various Swiss professional composting plants. The feedstock, ages of composts and the composting system are shown in *Table 1*.

TABLE 1 Description of the composts used for the production of extracts

Sample name	Age at sampling (weeks)	Compost feedstocks	Composting system
MeS08-1	08	33 % green waste, 67 % manure composts	Mesophyl sludge issue of mechanization
TaW08-1	08	45 % floating wood, 20 % sewage sludge, 35 % green waste, feathers and vegetables	Open tabular windrow (2.5 to 4 m in height)
TaW10-1	10	15 % green waste, 25 % horticulture waste, 20 % municipal green waste, 20 % industrial waste, addition of 10 % fresh compost and 10 % mature compost	
TaW30-2	30	50 % green waste (without wood), 25 % horticulture waste, 25 % of the screening rejects	
TaW40-1	40	50 % green waste (without woods), 25 % horticulture waste, 25 % of the screening rejects	
TrW08-1	8	Mixture between green waste, feathers, vegetables and papers fibres	Open triangular windrow (1.5 to 2 m in height)
TrW08-2	8	Mixture between green waste, feathers, vegetables and papers fibres	
TrW09-1	9	25 % green waste, 45 % horticulture waste, 30 % industrial waste	
TrW09-2	9	30 % green waste, 40 % horticulture waste, 20 % municipal waste, 10 % of land	
TrW09-3	9	40 % green waste, 30 % horticulture waste, 15 % municipal green waste, 10 % industrial waste +enzymes	
TrW10-1	10	Mixture between green waste, feathers, vegetables and papers fibres	
TrW12-2	12	25 % woods, 25 % green waste, 30 % dead leaves, 10 % clayey land and 10 % paper fibres	
TrWt08-1	8	50 % green waste, 40 % horticulture waste, 10 % municipal green waste	Open triangular windrow (2 to 3.5 m in height)

2.2 Production of compost extracts

Eight to 40-weeks-old composts were used for preparing compost extracts. The compost samples were added to demineralised water (1:2 or 1:5, v/v, compost/water) in open bottles. The mixtures were manually stirred for 2 min and incubated at room temperature for 2, 3, 4 or 7 days without stirring and aeration. Following each incubation period, mixtures were screened through four layers of cheesecloth and the filtrate was used immediately. Batches of composts were autoclaved before their extraction at 121 °C for 1h. Where indicated, the compost extracts were also autoclaved at 121 °C for 1h or filter-sterilized through a 0.2 µm-pore membrane.

2.3 Preparation of inoculum

Two pathogens were used in the seedlings bioassays: *Venturia inaequalis*, the apple scab pathogen, and *Plasmopara viticola*, the agent causing downy mildew in grapevines.

Conidia of *V. inaequalis* were harvested from inoculated, dried apple leaves maintained in a glass box at 4 °C. The inoculum was obtained by soaking the infected leaves in demineralised, sterile water, and then stirring for 15 min and filtering through nylon cloth (stitch diameter 0.25 mm). The concentration of the conidia suspension was determined with a Bright-line Hemacytometer and then adjusted to $5 \cdot 10^4$ conidia/ml.

Sporangia of the obligate biotroph *P. viticola* were obtained by washing infected seedling grapevines with cold, demineralised water (4 °C). The concentration of the sporangia suspensions was determined with Bright-line Hemacytometer and then adjusted to 10^5 sporangia/ml.

2.4 Seedling bioassays

Seedlings of apple (*Malus domestica* L) cv. "Mc Instosh" (stage of 6 to 8 leaves) were sprayed with different compost extracts by means of a hand-sprayer till near run-off and then kept in the growth room (70 % relative humidity (RH), 14 h light, 20 °C during day, 18 °C during night) for 2 days. After that, the apple seedlings leaves were sprayed with conidia suspensions of *V. inaequalis* (50'000 conidia / ml) by means of a hand-sprayer till near run off. Inoculated apple seedlings were incubated in the humidity chamber (100 % RH, 14 h light, 5 kLux) for 24 h at 20 °C. After incubation, the apple seedlings were transferred back to growth room. Disease severity (% of leaf area infected) of apple seedlings was assessed 10–12 days after inoculation.

Seedlings of grapevine (*Vitis vinifera*) cv. "Chasselas" (stage of 6 to 8 leaves) were also sprayed with various compost extracts by means of a hand-sprayer till near off and then kept in the growth room (70 % relative humidity (RH), 14 h light, 20 °C during day, 18 °C during night) for 3 days. The grapevine seedlings were drop inoculated with *P. viticola*. Two drops of 10 µl of sporangia suspension were applied to the underside of the leaves. Five leaves per plants were inoculated. After inoculation, the plants were incubated in the humidity chamber (100 % RH, 14 h light, 5 kLux) for 24 h at 20 °C. Grapevine plants were transferred to the growth room and brought back to the humidity chamber the evening before scoring in order to initiate sporangia production.

Disease severity (% of leaf area infected) of grapevine leaves was assessed after 7 days.

The efficacy of the composts extracts was calculated according to (Abbott, 1925), as follows:

$$\text{Efficary (\%)} = 100\left(1 - \frac{a}{b}\right) \quad (1)$$

with

a disease severity (or lesion diameter) of treatment

b disease severity (or lesion diameter) of control.

2.5 In vitro bioassays

In order to evaluate the influence of compost extracts on the conidial germination of *V. inaequalis*, the method of Cronin et al. (1996) was slightly modified. One gram of saccharose was added to 500 ml conidia suspension of *V. inaequalis* (50'000 conidia/ml). The growth stages of conidia were evaluated according to the following scale:

0: no germination

1: The length of the germ-tubes lower than the conidia diameter

2: The length of the germ-tubes equal to the conidia diameter

3: The length of the germ-tubes greater than the conidia diameter

To determine the influence of the compost extracts on the zoospore activity of *P. viticola*; 0.1 ml of the sporangia suspension of *P. viticola* (100'000 sporangia/ml) were inoculated into the centre of slides of grapevine leaves (10 mm diameter) previously treated with demineralised water or compost extracts and placed on water agar in Petri dishes. After incubation in the growth room (70 % RH, light) for 24 h at 18 °C, the number of colonized stomata was counted under the microscope. Approximately 50 zoospores or sporangia per slide were evaluated.

3 Results

3.1 Effect of production mode on the efficacy of compost extracts

The compost/water ratio did not influence the efficacy of compost extracts to protect apple plants against *V. inaequalis*. No significant difference was observed for ratios between 1:2 and 1:5. Variation in extraction duration between 2 and 7 days had no effect on the efficacy of compost extracts against *V. inaequalis*.

In the water controls, disease severity was 32 % (average for three experiments). All compost/water ratios and all extraction durations protected apple seedlings by up to 40 % compared to the control (*Table 2*).

TABLE 2 Effect of compost/ water ratio and extraction duration on compost extracts efficacy against severity of V. inaequalis

	Efficacy (%)*			
	TaW08-1	TaW40-1	TrW10-1	SEM
Compost/water ratio				
1:2	50.5a	44.5a	47.3a	2.49
1:5	42.5a	41.7a	46.2a	2.46
Extraction duration (days)				
2	43.7a	44.5ab	47.2a	3.02
3	44.4a	34.6b	42.0a	3.82
4	46.1a	37.0ab	36.4a	2.97
7	50.7a	56.4a	61.3a	2.27

*see text for experimental details

Table shows means of three experiments with 8 plants per replicate, except for TrW10-1 (two experiments).

SEM is the standard error of the mean

Different letters indicate statistically significant differences between means (Tukey-B test; $P < 0.05$).

3.2 Effect of autoclaving and filtration on extract efficacy

Sterilization of the compost or the extract did not affect the inhibition of *V. inaequalis* (*Table 3*, *Figure 1*). Moreover, there was no difference in efficacy between autoclaved, sterile filtrated (0.2 µm) and untreated extracts in apple seedlings bioassay (*Figure 1*).

TABLE 3 Effect of heat sterilisation of diverse compost extracts on disease severity (%) of V. inaequalis

	Disease severity (%)					
	Control	TaW30-2	TrW09-1	TrW09-3	TrW12-2	MeS08-1
CnEn	20.8a	7.9b	10.7b	11.8b	9.1b	4.50b
CaEa	20.8a	8.7b	9.1b	7.7b	7.0b	5.0b
SEM	2.02	1.28	1.53	1.31	1.49	0.69

The compost extraction was performed with compost/water ratio (1:2 ; v:v). The extraction duration was 3 days.

Control: treated with water.

CnEn: compost and extract not autoclaved (crude extract).

CaEa: compost and extract autoclaved.

The table shows means of disease severity in one experiment (n = 8 or n = 10). Experiment was repeated with similar results (data not shown).

SEM is the standard error of the mean.

Different letters indicate statistically significant differences between means (Tukey-B test; $P < 0.05$).

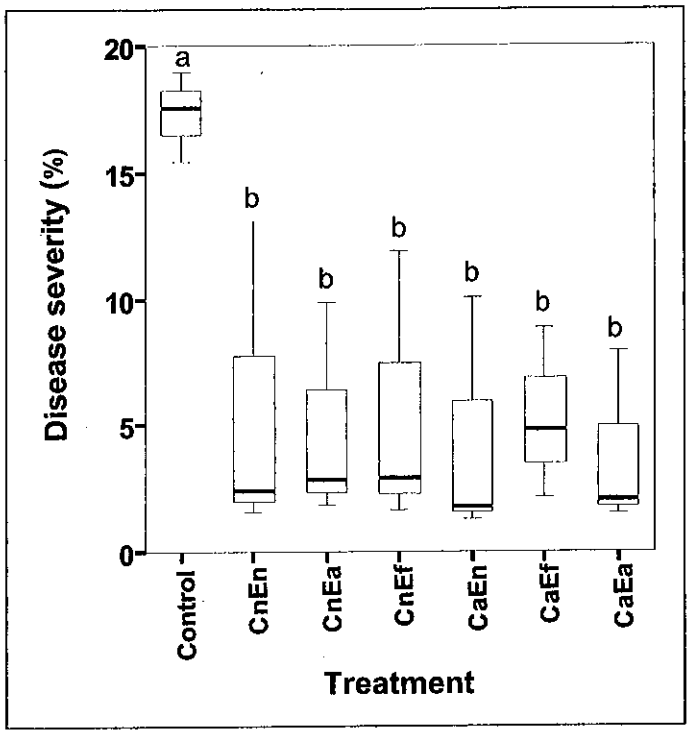

FIGURE 1 Effect of different sterilisation modes of compost and compost extracts against V. inaequalis

The compost extraction was performed with a compost/water ratio of 1:2. The extraction duration was 3 days.

Control: treated with water
CnEn: compost and extract not autoclaved (crude extract)
CnEf: compost not autoclaved before extraction, extract filter-sterilized through a 0.2 µm-pore membrane
CnEa: compost not autoclaved before extraction, extract autoclaved (121 °C for 1h)
CaEn: compost autoclaved, extract not autoclaved
CaEf: compost autoclaved, extract filter-sterilized through a 0.2 µm-pore membrane
CaEa: compost and extract autoclaved

The figure shows medians from three experiments as well as the 50 and 95 % percentile (n = 8).

The box plots with different letters indicate statistically significant differences (Tukey-B test; $P < 0.05$).

Likewise, sterilization did not significantly affect the inhibition of *P. viticola*. In many cases, however, there was a tendency that sterilization decreased lesion diameter of *P. viticola* (TrW09-1 and TrW12-2; *Table 4*). The highest efficacy against *V. inaequalis* was reached by the extract of MeS08-1, which equalled more than 75 % in both treatments (CnEn and CaEa) (*Table 3*). The maximum efficacy of the same extract against *P. viticola* was greater than 80 % in both treatments (CnEn and CaEa) respectively (*Table 4*).

TABLE 4 Effect of heat sterilisation of diverse compost extracts on lesion diameter (mm) of P. voticola

	Lesion diameter (mm)					
	Control	TaW30-2	TrW09-1	TrW09-3	TrW12-2	MeS08-1
CnEn	11.3a	4.5cd	8.2bc	2.9d	7.1b	2.2d
CaEa	11.3a	3.6cd	4.9d	2.6d	2.7d	1.8d
SEM	0.88	0.67	0.67	0.46	0.93	0.51

For details see *Table 3*.

The table shows means of lesion diameter in one experiment (n = 8 or n = 10). Experiment was repeated with similar results (data not shown).

3.3 Effect of rinsing leaves

In order to demonstrate whether the mechanism of inhibition of compost extracts is due to a direct inhibitory effect or to induction of resistance, the leaves of apple and grapevine seedlings were rinsed after they had been treated with compost extracts.

In the case of apple scab, rinsing the leaves 1 or 72 hours after their treatment did not diminish the disease severity of *V. inaequalis* (*Figure 2*A). In contrast, infection by *P. viticola* (lesion diameter) increased when plants were rinsed off and was similar to the untreated control (*Figure 2*B). In both host-pathogen systems, efficacy of compost extracts was higher when leaves were not rinsed.

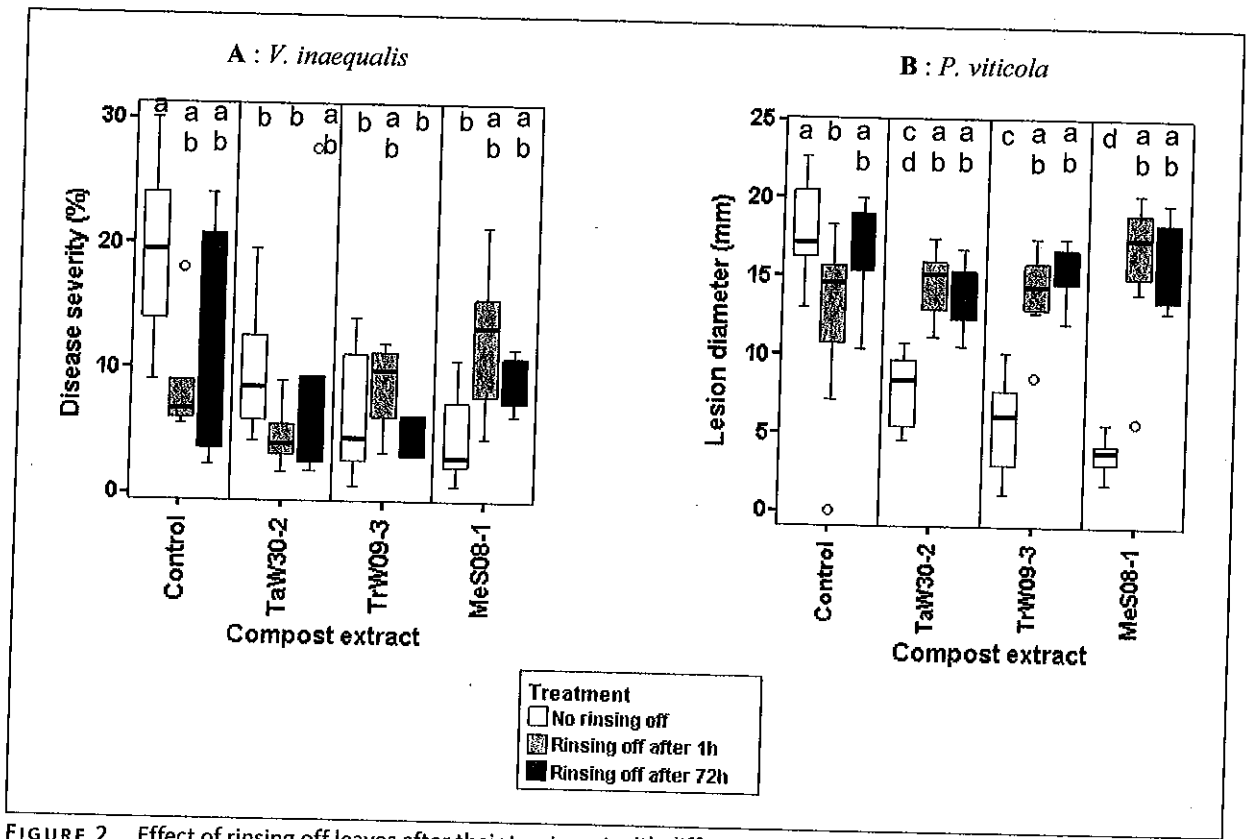

FIGURE 2 Effect of rinsing off leaves after their treatment with different compost extracts on disease severity of V. inaequalis (A) and lesion diameter of P. viticola (B)

The figure shows medians of disease severity (A) and lesion diameter (B) as well as the 50 and 95 % percentile in one experiment (n = 8). Experiment was repeated with similar results (data not shown). For details see *Figure 1*.

3.4 Effect of compost extracts on in vitro conidial germination of V. inaequalis and zoospores activity of P. viticola

Compost extracts were tested *in vitro* for a fungistatic effect on conidial germination of *V. inaequalis* and zoospore activity of *P. viticola*. The conidial germination of *V. inaequalis* was higher than 90 % in all compost extracts tested, and less than 80 % in demineralised and sterile water (*Figure 3*). The percentage of germs-tubes which were longer than conidia diameter (germination scale 3) was higher in compost extracts than in demineralised water.

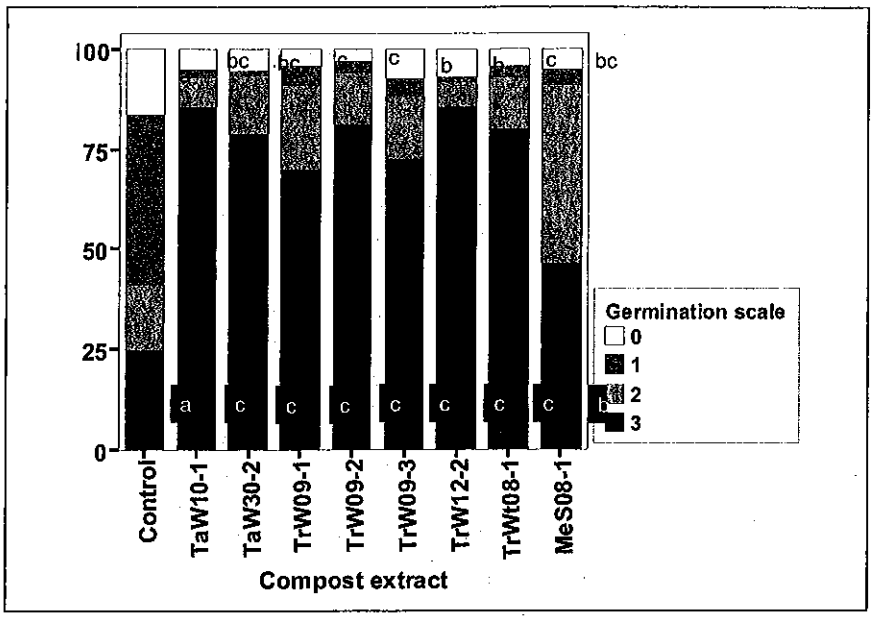

FIGURE 3 Effect of different compost extracts on in vitro conidial germination of V. inaequalis

The figure shows means of three experiments with 6 replicates per experiment (n = 100). For details see *Figure 1*.

In grapevines, compost extracts or copper greatly reduced the percentage of stomata occupied by zoospores (*Figure 4*). The activity of zoospores of *P. viticola* was inhibited by up to 70 % compared to the control. 100 % inhibition of zoospores was observed with MeS08-1 (*Figure 4*). The efficacy of compost extracts *in vitro* disappeared when leaves were rinsed 72 hours after application (*Figure 4*).

Solutions of sodium chloride (NaCl) at concentrations corresponding to those of the compost extracts 2.5 mS/cm and 5 mS/cm were also tested. These significantly reduced the number of zoospores fixed over the stomata of grapevine leaves. Inhibition of zoospores was proportional to the salt concentration. Solutions of 5 mS/cm of NaCl corresponded to copper treatment (0.4 g/l) (*Figure 4*).

A positive correlation between salt content in the composts extracts and their zoospore inhibition was observed: TrW09-3, with salinity of 2.6 mS/cm, inhibited zoospore fixation by 48 %, TaW30-2 extract with salinity of 2.92 mS/cm by 59 %, MeS08-1 extract, with salinity of 4.2 mS/cm by 100 %.

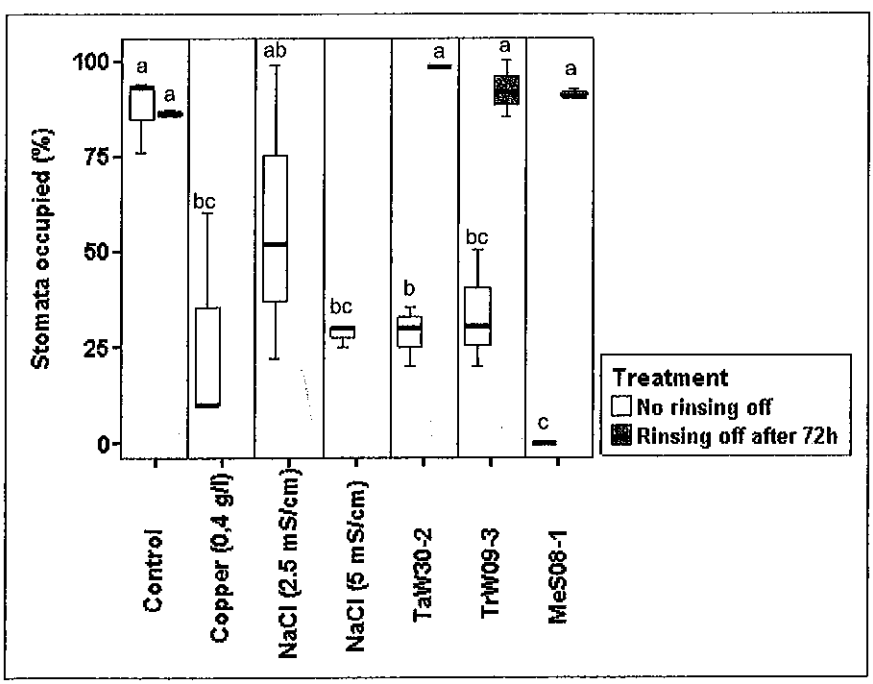

FIGURE 4 Effect in vitro of rinsing grapevine leaves after their treatment with different compost extract on zoospores activity of P. viticola

The figure shows medians of occupied stomata as well as the 50 and 95 % percentile in one experiment (n = 50). Copper and NaCl (2.5 and 5 mS/cm) treatments were not rinsed. For details see *Figure 1*.

4 Discussion

In this study, we have demonstrated that green waste compost extracts protect apple plants against scab (*V. inaequalis*) and grapevines against downy mildew (*P. viticola*) under controlled conditions. These results confirm the findings for *V. inaequalis* (Yohalem et al., 1994 and Cronin et al., 1996) and *P. viticola* (Ketterer and Weltzien, 1987; Weltzien et al., 1987).

The efficacy of compost extract against *V. inaequalis* was not affected by the compost/water ratio and extraction duration. In many cases, different compost/water ratios have reduced the incidence or disease severity of pathogens. This confirms the work of Weltzien (1990) who had not observed differences in compost extracts between 1:3 and 1:10. With regard to extraction duration, several studies indicate that disease suppression varies widely in relation extraction duration. Our findings corroborate with the studies of (Achimu and Schlosser, 1991; Tsror and Bieche, 1998) who did not find any influence of extraction on the extracts efficacy. By contrast, several studies have shown that the extracts' efficacy increases with increasing extraction duration (Ketterer, 1990; McQuilken et al., 1994; Weltzien, 1990). However, the composts we used were different from those used in the above studies. The effect of extraction duration was mostly related to the micro-organisms in the extracts, in particular bacteria (McQuilken et al., 1994).

Scheuerell and Mahaffee, (2002) have proposed that the ideal extraction duration may need to be determined for each host-pathogen-compost system.

Sterilisation of composts before extraction and of the extracts (by autoclaving and sterile filtration at 0.2 µm) did not influence efficacy against *V. inaequalis* and even increased inhibition of *P. viticola*. These observations confirm those of (Achimu and Schlosser, 1991; Cronin et al., 1996; Elad and Shtienberg, 1994; Zhang et al., 1997), but contradict (McQuilken et al., 1994; Stindt, 1990; Weltzien, 1992), who demonstrated involvement of micro-organisms in the suppression of various pathogens. In our studies, there is strong evidence that the protective effect of compost extracts is not directly due to their microbial activity. It may be caused by a substance present in extract and probably produced during the composting process, which is water soluble and thermo-stable.

Washing the leaves after application of extracts did not reduce the efficacy against *V. inaequalis*, but reduced the efficacy against downy mildew of grapevines. Thus, it seems that compost extracts have a direct inhibitory effect on the growth of *P. viticola* but not on *V. inaequalis*.

Moreover, *in vitro* bioassays showed that compost extracts enhanced germination of conidia of *V. inaequalis* and revealed no fungicidal effect. Cronin et al. (1996) and Yohalem et al. (1994) observed inhibition *in vitro* on conidial germination of *V. inaequalis* by up to 98 % by spent mushroom substrate (SMS) extracts. This contrast with the findings of those authors may well be explained by the nature of feedstock compost and physical and chemical properties of SMS extracts such as salinity and pH. Generally, SMS extracts contain higher levels of salts. The mechanism of conidial germination inhibition for *V. inaequalis* by SMS extracts may well be different from our compost based on heterogeneous input mixture.

On the other hand, the activity of zoospores of *P. viticola* was inhibited by up to 70 %. Thus, *in vitro* results confirm in vivo observations made before and emphasize a direct inhibitory effect by compost extracts on zoospore germination of *P. viticola*. This effect may be due to either a direct inhibition of sporangia of *P. viticola* or to the metabolism or mobility of evicted zoospores. Weltzien and Ketterer (1987) reported direct inhibition of sporangia of *P. viticola* by horse manure extracts. The present work has demonstrated a positive correlation between salt content in compost extracts and their inhibitory effect on zoospores. However, salinity alone cannot explain all of the fungicidal effect of extracts, but it seems to play an important role in the fixation inhibition of zoospores. In fact, the osmotic pressure due to salinity could have an effect on the zoospores' membrane and provoke their lysis, or prevent their motility.

In conclusion, we infer that the mechanism implicated in the inhibition of *V. inaequalis* and *P. viticola* is different. The inhibition of compost extract is not linked to their microbial activity. The active principle against both pathogens studied, has to be a water soluble, heat-stable metabolite produced in compost before its extraction. Considering that compost extracts had no direct fungicidal effect against *V. inaequalis*, the mechanism must be either through the plant (perhaps induction of resistance) or through influence on the phyllosphere. Better knowledge of the processes taking place in the phyllosphere might be useful. With respect to protection of grapevine against *P. viticola*, a direct effect on the pathogen seems to be important.

The green waste compost extracts have shown an interesting effect against foliar plant diseases under controlled conditions. Further trails under practical conditions would be worthwhile to optimize the use of extracts and to determine the limitations of this technique

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