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Suppression of grapevine diseases with compost tea in the greenhouse.

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Introduction and Objective: There is considerable interest in developing alternative approaches/materials to manage grapevine diseases due to concern over synthetic pesticide residues on fruit and the development of pathogen resistance to pesticides. Within the last 15 years, several studies have demonstrated the control of grapevine diseases with compost teas (Urban and Trankner 1993, Elad and Shtienberg 1994, Weltzien 1991). Our focus was to explore the potential of compost teas, made from locally available composts, in the suppression of grapevine powdery mildew and *Botrytis*. Greenhouse and laboratory assays were conducted to determine the effects of compost type, brewing method, and nutrient additives on the microbial composition and disease control efficacy of compost teas. Assays were conducted at the Fruit Research and Extension Center in Biglerville, PA (FREC) and at the Lake Erie Grape Research and Extension Center in North East, PA. (LERGREC).

Methods and Materials: Compost and compost teas. Two fresh composts were used for making teas. The compost from Roth Vineyards, Fairfield, PA, consisted of 52% hay, 17% mushroom aggregate, 18% fresh wood chips, 12% raw, litter-free chicken manure and 1% calcium sulfate. The second compost, a fresh manure based vermicompost, was obtained from Orner Farms, Rockton, PA.

Compost teas were made via two methods; Non-aerated and aerated. Non-aerated compost teas (NCTs) were made by mixing compost with water at a 1:5 ratio (v:v) and allowing for passive growth of microorganisms for 3 to10 days in open bucket containers with no active aeration (fermentation). During fermentation, NCTs were stirred every other day for 20 minutes and temperatures ranged from about 18-26 C. After fermentation, NCTs were applied with and without nutrients, and with and without a wetting agent (Nu-Film P or milk). Nutrients were added to NCTs *after* fermentation was complete to avoid excessive oxygen depletion and odor. Non-aerated compost teas and nutrients were strained through a 40 mesh screen before application to avoid clogging of spray equipment.

Aerated compost teas (ACTs) were prepared by adding 3 lb of compost to 22 gal of water for a 24-36 h brewing period. A nutrient mixture was added to the water at the *start* of the brewing cycle. Preliminary tests showed that levels of culturable organisms could be increased 1000 fold by adding nutrients at the beginning of the aerated brewing cycle. The brewer has a 30 gallon capacity with Mazzei injectors to supply aeration, and a Shertech closed coupled centrifugal pump for agitation. Compost is loaded into a 20 mesh screened bucket that is immersed in the water during brewing. All ACTs were applied with a wetting agent (Nu-Film P). All teas were applied full strength. In some treatments, additional nutrients were added at the time of application. NCTs were analyzed for bacterial numbers by culturing 0.1 ml aliquots of various dilutions of the teas on petri plates of agar media. Total numbers of culturable bacteria were determined on nutrient agar and fluorescent Pseudomonads on King's B media. Spore forming bacteria were determined by passing dilutions through a 70C water bath for 30 minutes before application to nutrient agar. Samples of NCTs and ACTs were sent to the Soil Foodweb New York Inc. to determine active and total bacterial and fungal biomass.

Results: In general, the Roth teas had superior bacterial and fungal profiles when compared to the vermicompost teas, and NCTs (3 and 6 day fermentations) were superior to ACTs. *Active* and *total* bacteria of vermicompost and Roth NCT increased with age. With vermicompost, ACT had higher *active* bacterial biomass than NCT, but lower *total* bacterial biomass than NCT. However, *active* and *total* bacteria were in good to excellent range in all vermicompost teas and were well suited for foliar applications. Vermicompost NCTs had higher *active* and *total* fungal biomass than ACTs. *Total* fungal biomass of vermicompost NCT was within acceptable range, but *active* fungal biomass was still below minimum standards in the 3 and 6 day NCTs. With Roth compost, NCT was almost ten times higher in *active* bacteria than ACT whereas *total* bacteria were roughly equal. *Active* and *total* bacteria were rated good and excellent in Roth NCTs and low and excellent in ACTs, respectively. With respect to *active* and *total* fungal biomass, Roth NCTs were in the low and good range and ACTs were rated low and low. Roth NCTs were higher in *active* and *total* bacteria and fungi than vermicompost NCTs. Roth ACTs were lower in *active* bacteria and higher in *total* bacteria than vermicompost ACT and roughly equal to vermicompost teas in *active* and *total* fungi. In NCTs, levels of

Pseudomonad bacteria decreased and spore forming bacteria increased, as fermentation time increased. These groups of bacteria are important because many Pseudomonad and spore forming bacteria are biocontrol agents. The addition of Nu-Film P did, in some tests, have a slight negative effect on bacterial numbers but results were not consistent.

Powdery mildew assays: Methods and Materials. Assays were conducted as randomized complete blocks with 5 replications. Each replication consisted of one potted grapevine cutting. Powdery mildew isolates used in the experiment were from unsprayed Chancellor vines at the LERGREC and one isolate supplied by investigators at Cornell University.

LERGREC: Leaves of potted cuttings of *Vitis vinifera* 'Chardonnay' were inoculated with various NCT combinations (teas from two different composts, 3 and 6 day fermentation, with and without nutrients) in the greenhouse.

Trial 1: The nutrient mixture included Hydra-Hume-AN (Helena Chemical Co., Biglerville, PA, 4.75 ml/gal), SP-85, a formulation of humic acid (Organic Approach, Lancaster, PA, 1.6 g/gal), granular molasses (4.5 g/gal), and fish Hydrolysate (Neptune Harvest, Agway, 0.2 ml/gal). Nu-Film P (0.047 %) was added to all tea treatments and the nutrient check just before application.
Trial 2: One percent whole milk served as nutrient and wetting agent. Therefore, treatments without nutrient were applied without a wetting agent.

Trial 1: Vines were sprayed with NCTs to runoff and left in the greenhouse overnight to dry. Cuttings were inoculated the following day with a spore suspension of powdery mildew conidia from the Cornell isolate. To prepare the inoculum, spores were washed from infected leaves with distilled water and 0.05 % tween 20, adjusted to 10^5 spores/ml and sprayed out within 45 minutes, onto the three newest fully expanded leaves on each plant. A one second burst of inoculum was applied to each leaf. Vines were placed on a bench inside the lab at room temperature and indirect natural light, with a fan to hasten drying. After drying, individual vines were enclosed in clear plastic cylinders with a breathable top for isolation. The same inoculation procedure, for tea and powdery mildew (7×10^4 spores/ml), was repeated 3 weeks later on the next three youngest leaves with spores from the PA isolates. Three weeks later, disease was rated on the six leaves/vine inoculated with powdery mildew (table 1).

Trial 2: To improve the establishment of tea organisms on leaves, vines were sprayed twice with NCTs, 8 days and 1 day before inoculation with powdery mildew (as above). After inoculation, Vines were placed on a bench inside the lab at room temperature and indirect natural light with a fan to hasten drying. Vines were left uncovered for the duration of the experiment. Three weeks later, disease was rated on the six youngest leaves/vine inoculated with powdery mildew (table 1).

FREC: Leaves of potted *Vitis* interspecific hybrid 'Vidal' were inoculated with various ACT combinations (teas from two different composts, with and without nutrients (same nutrients as above)) in the greenhouse. Nu-Film P (0.03 %) was added to teas and nutrient check just before application and vines were sprayed to runoff and left in the greenhouse overnight to dry. The following day, cuttings were inoculated with powdery mildew by spraying the three newest fully expanded leaves/vine with a spore suspension (10^5 spores/ml) of the Cornell isolate (as above). After inoculation, cuttings were left in the greenhouse and fans were used to hasten drying. Vines were left uncovered for the duration of the experiment and continuous air circulation was provided to enhance natural re-inoculation with the pathogen. In trials 2 and 3, all treatment applications were repeated every 7-11 days in response to subsequent natural infection (table 2).

Results: Control of powdery mildew with non-aerated compost teas.
Trial 1*:* Compost type, fermentation time (3 days and 6 days), and presence/absence of added nutrients did not have a significant effect on efficacy. Although none of the NCTs provided significant control over the water check, the addition of nutrients numerically improved the efficacy of NCTs made with Roth compost, regardless of fermentation time.

Trial 2*:* The addition of 1 % whole milk to teas just before application appeared to consistently provide complete control. Milk alone (at much higher rates) has been shown to have efficacy on powdery mildew and microbial populations in the tea likely benefited from nutrients in the milk. These differences may also reflect the beneficial wetting/spreading/sticking effect that was observed on the leaves with the addition of milk. However, trial 2 results should be interpreted cautiously, as disease development was very weak and there is some doubt as to how thoroughly the treatments were tested. As in trial 1, there was enough variability in disease control among vines of the same treatment to render any treatment differences statistically insignificant.

Table 1. Incidence and severity of powdery mildew on leaves of potted 'Chardonnay' (NCTs at LERGREC).

Treatment[z]	Trial 1[y] % infected	Trial 1 % Control	Trial 1 % area infected[wv]	Trial 1 % Control	Trial 2[x] % infected	Trial 2 % Control
VC (3days)………………………	43.2 ab[u]	44	11.6 ab[u]	0	8.4	58
VC (3days) + nutrient……………	46.6 ab	39	8.8 ab	8	0	100
VC (6 days)……………………..	36.8 ab	52	8.5 ab	11	10.2	50
VC (6 days) + nutrient…………..	56.0 ab	27	17.2 b	0	0	100
RC (3days)……………………….	40.0 ab	48	16.4 ab	0	10.2	50
RC (3days) + nutrient……………	40.0 ab	48	5.5 ab	43	0	100
RC (6 days)………………………	56.0 ab	27	13.2 ab	0	6.6	67
RC (6 days) + nutrient…………..	50.0 ab	35	5.9 ab	39	0	100
Nutrient…………………………..	70.2 b	9	8.6 ab	10	13.6	33
Sulfur: 4 lbs actual S/100 gal……	0.0 a	100	0.0 a	100		
Serenade: 4 lbs/100 gal………………………………………………………………………					3.4	83
Water check……………………....	76.8 b		9.6 ab		20.2	

[z] VC = Orner Farms Vermicompost; RC = Roth chicken cattle manure compost
[y] Nu-Film P was added to all tea and nutrient treatments at 0.047 %. Nutrient mixture is detailed in Methods and Materials.
[x] 1 % whole milk was added as both nutrient and wetting agent (Nu-Film P was not used in trial 2).
[w] Severity was rated using the Barratt-Horsfall scale and was converted to % area infected using Elanco conversion tables.
[v] Actual data are shown. Data were transformed by arcsinsqrt transformation before statistical analysis
[u] Means followed by the same letters within columns are not significantly different according to Tukey-Kramer ($P \leq 0.05$).

Control of powdery mildew with aerated compost teas.

Compost type, or presence/absence of added nutrients did not have a consistent or significant effect on efficacy in any of the trials. In trial 1, where only one tea treatment application was made, only Roth ACT with nutrients provided significant reduction in disease when rated 3 weeks later. In trials 2 and 3, additional applications were made every 6-12 days to refresh microbe populations and combat subsequent natural infection. Control with teas was noticeably improved in trial 2; the VC, RC and RC with extra nutrients significantly reduced disease. None of the treatments in trial 3 gave statistically significant control. No phytotoxicity was associated with any of the compost teas (NCT or ACT) or nutrients used in these experiments.

Table 2. Severity of powdery mildew on leaves of potted 'Vidal' (ACTs at FREC).

treatment[z]	Trial 1[y x]	% Control	Trial 2[y x]	% Control	Trial 3[y x]	% Control
VC………………………………	40.3ab[w]	21	2.1a[w]	82	12.3	25
VC + nutrient…………………..	38.5ab	24	11.5bc	2	18.8	0
RC……………………………....	43.2ab	15	2.1a	82	11.1	32
RC + nutrient…………………....	30.9a	39	3.3ab	72	12.5	24
Nutrient…………………………	43.9abc	14				
Sulfur: 2 lbs actual S/100 gal…...	31.5a	38	9.4abc	20	4.9	70
Nu-Film P (alone)………………	60.8c					
Water check…………………….	50.8bc		11.7c		16.4	

[z] VC = Orner Farms Vermicompost; RC = Roth chicken cattle manure compost; Nu-Film P added to all tea and nutrient treatments at 0.03 %.
[y] Severity was rated using the Barratt-Horsfall scale and was converted to % area infected using Elanco conversion tables.
[x] Trial 1: tea spray 20 May; inoculated 21 May; rated 10 June. Trial 2: tea sprays 19, 26 June, 8 July; inoculated 20 June, rated 10 July. Trial 3: tea spray 30 July, 5 August; inoculated 31 July; rated 12 August.
[w] Means followed by the same letters within columns are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

***Botrytis* assays: Methods and Materials.** The *Botrytis* assays were conducted as randomized complete blocks with 4 replications. Each replication consisted of a clear sealed plastic container with five berries of each treatment. The *Botrytis* isolate used to inoculate berries was from a naturally infected Vignoles berry at LERGREC. In assays at LERGREC, berries (with pedicels) were simultaneously inoculated with teas and the pathogen, by submersion in a mixture of 50 % NCT (Roth and vermicompost, with and without nutrients, 4, 7, and 10 day fermentation) and 50 % spore suspension of *Botrytis* (final concentration of 3×10^4 spores/ml). Appropriate controls were included. Nutrients consisted of 1 % proteose peptone and 1 % yeast extract (Urban and Trankner, 1993). Milk was not added to any tea treatments as testing at PSFREC had indicated a clear association between milk application to berries and elevated *Botrytis* rot. Nufilm P at 0.03 % was added to each treatment. Berries were rated 6 and 10 days after inoculation. At PSFREC, berries were dipped into various ACTs (with Nu-Film P at 0.03 %) and inoculated with the pathogen 24 hours later by misting berries with a spore suspension of *Botrytis* (10^5 spores/ml). After inoculation, berries (in all assays) were incubated inside sealed clear plastic containers, suspended on a wire screen above a layer of water to maintain high relative humidity. Berries were rated 5 days after inoculation.

Results: None of the NCTs or ACTs, with the exception of the Roth 7 day tea without nutrients (10 days after inoculation), significantly reduced *Botrytis* on grape berries. Aerated teas generally provided higher levels of control of incidence than non-aerated teas although a direct comparison cannot be made.

Table 3. Incidence and severity of *Botrytis* on grape berries (Red seedless) treated with non-aerated compost teas (LERGREC). Nufilm P at 0.031 % was added to all treatments except the non-inoculated control and Elevate.

	6 days after inoculation			10 days after inoculation		
treatment[z]	% infected	area infected[y]	% control	% infected	area infected[y]	% control
VC (4 day)……………………...	93 b[x]	3.1 b[x]	10	100 c[x]	4.9 c[x]	0
VC (4 day) + nutrient…………..	100 b	3.1 b	8	100 c	4.9 c	0
RC (4 day)……………………...	100 b	3.2 b	6	100 c	4.9 c	0
RC (4 day) + nutrient…………..	93 b	2.9 b	16	100 c	4.5 bc	4
VC (7 day)……………………...	90 b	2.9 b	15	100 c	4.2 bc	11
VC (7 day) + nutrient…………..	100 b	4.3 b	0	100 c	5.0 c	0
RC (7 day)……………………...	70 b	3.1 b	9	100 c	3.8 b	20
RC (7 day) + nutrient…………..	100 b	3.6 b	0	100 c	5.0 c	0
Non-inoculated water control…..	5 a	0.1 a	-	5 a	0.1 a	-
Elevate (1 lb/100 gal)…………..	10 a	0.1 a	97	25 b	0.6 a	87
Nutrient only…….……………..	100 b	3.5 b	0	100 c	5.0 c	0
Inoculated water control………..	93 b	3.4 b		100 c	4.7 c	
VC (10 day)……………………	90 b	2.4 bc	0	100 c	4.5 bc	0
VC (10 day) + nutrient………..	100 b	2.4 bc	0	100 c	4.7 bc	0
RC (10 day)……………………	90 b	1.8 b	20	100 c	3.8 b	13
RC (10 day) + nutrient………..	100 b	2.9 bc	0	100 c	4.8 bc	0
non-inoculated water control…...	5 a	0.1 a	-	5 a	0.2 a	-
Elevate (1 lb/100 gal)…………..	10 a	0.2 a	91	35 b	0.6 a	87
Nutrient only……………………	100 b	3.5 c	0	100 c	4.9 c	0
inoculated water control………..	95 b	2.3 b		100 c	4.4 bc	

[z] VC = Orner Farms Vermicompost; RC = Roth chicken cattle manure compost. 4, 7, 10 day = fermentation time; Nufilm P at 0.031 % was added to all treatments except non-inoculated water control and Elevate.
[y] Disease severity was rated using 1 = 1-5 %, 2 = 5-25 %, 3 = 25-50 %, 4 = 50-75 %, 5 = 75-100 %
[x] Means followed by the same letters within columns are not significantly different according to Tukey-Kramer ($P \leq 0.05$).

Table 4. Percent berries infected with *Botrytis* 5 days after inoculation (FREC).

treatment	White Seedless	% Control	Cayuga	% Control
VC……………………….……………….	10.0[y]	50	10.0[y]	67
VC + nutrients……………………………...	0.0	100	10.0	67
RC………………………………………….	5.0	75	5.0	83
RC + nutrients………………………………	5.0	75	15.0	50
Sovran (0.04g/500 ml)……………………..	5.0	75	10.0	67
Inoculated water control……………………	20.0		30.0	

[z] VC = Orner Farms Vermicompost; RC = Roth chicken cattle manure compost
[y] There were no significant differences according to Duncan's New Multiple Range Test ($P \leq 0.05$).

Discussion: Despite high levels of bacterial activity and biomass in most teas, and numbers of total and spore forming bacteria similar to studies in which good disease control was achieved by others (Urban and Trankner 1993), NCTs and ACTs from these fresh, manure based composts, provided rather modest control of grape powdery mildew and *Botrytis*. Washings from field collected berries sprayed with NCTs and ACTs from these composts, generally had higher bacterial levels than water sprayed berries, one week after application. This suggests that tea applications were capable of elevating bacterial populations on vine surfaces for at least a week. However, fungal biomass and activity were generally low in these teas and the compost source may have lacked the specific organisms needed to control these pathogens. The low fungal component may have been a result of inadequate aeration (although 'a highly aerobic mix is not required', Brinton and Trankner 1996), improper combination of nutrients (not the right fungal foods), or limited extraction of fungi from parent material (improper agitation of teas). To boost the fungal component of teas, additional fungal food in the form of humates was added, but had no effect on biomass or activity.

Although bacterial and fungal profiles of NCTs were generally superior to ACTs, ACTs were generally more efficacious than NCTs of the same compost. However, brewing methods were not directly compared in the assays. In assays at FREC, powdery mildew control was improved in some ACTs when reapplied every 6-12 days after inoculation, indicating some potential for powdery mildew management when applied early and frequently, under low disease pressure. Despite the rather modest disease control efficacy of these teas, the results of these assays have provided a basis for further exploration with compost teas in integrated control programs.

Literature Cited:

Brinton, W.F. , A Trankner, and M. Droffner. Investigations into liquid compost extracts. Biocycle. November 1996.

Elad, Y. and D. Shtienberg. 1994 Effect of compost water extracts on gray mould (Botrytis cinerea). Crop Protection. 13(2):109-114.

Urban, J. and A. Trankner. 1993 Control of gray mould (Botrytis cinerea) with fermented compost/water extracts. In: N.J. Fokkema, J. Kohl and Y. Elad (eds.) Biological control of foliar and post-harvest diseases. Proceedings of a workshop. West Palearctic Region Section 16:8-11.

Weltzien, H. 1991. Biocontrol of foliar fungal diseases with compost extracts. In: J.H. Andrews and S. Hirano (eds.) Microbial ecology of leaves. New York, NY, pp. 430-450.