Author/s:
Moslemi, A;Ades, PK;Groom, T;Nicolas, ME;Taylor, PWJ

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Influence of waterlogging on growth of pyrethrum plants infected by the crown and root rot pathogens, *Fusarium oxysporum, Fusarium avenaceum* and *Paraphoma vinacea*

Azin Moslemi[1], Peter K. Ades[2], Tim Groom[3], Marc E. Nicolas[1], Paul W.J. Taylor[1]

[1] Faculty of Veterinary and Agricultural Sciences, University of Melbourne, VIC, 3010, Australia

[2] School of Ecosystem and Forest Sciences, University of Melbourne, VIC, 3010, Australia

[3] Botanical Resources Australia Pty Ltd, Ulverstone, Tasmania, 7315, Australia

Corresponding author: Paul W.J. Taylor, E-mail: paulwjt@unimelb.edu.au

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Abstract Yield decline in pyrethrum fields of northern Tasmania was thought to be due to an interaction between soil-borne pathogens and abiotic stresses. Glasshouse trials were conducted to assess the influence of a 4-day waterlogging period on growth of pyrethrum plants already infected with the crown and root rot pathogens, *Fusarium oxysporum*, *F. avenaceum* and *Paraphoma vinacea.* In plants grown at optimum soil water capacity, *F. oxysporum* and *P. vinacea* significantly reduced the below-ground and total biomass of plants before waterlogging (0 bw = 2 months after inoculation, 2 mai), at 2 months after waterlogging (2 maw = 4 months after inoculation, 4 mai) and 6 months after waterlogging (6 maw = 8 months after inoculation, 8 mai) but had little effect on above-ground biomass. Although *F. avenaceum* was pathogenic it only had a significant effect on below-ground biomass at 4 and 8 mai. At 7 days after waterlogging treatment, plants infected with *P. vinacea* had more severe wilting, necrosis and chlorosis of the basal leaves and petioles than plants infected with the other two pathogens or non-infected plants. Significant interaction between pathogen treatments and waterlogging occurred at 2 maw, whereas at 6 maw plants had recovered and no significant interaction was observed between the pathogen treatments and waterlogging. The effect of waterlogging on below-ground dry weight of the plants infected with *F. oxysporum* and *P. vinacea* at 2 maw was more severe than those infected with *F. avenaceum*. There was no significant interaction between waterlogging and pathogens on photosynthesis 2 maw however, plants infected by each of the pathogens had the lowest photosynthesis rate in both waterlogged and the non-waterlogged treatments. At 6 maw the number of flowers, flower stems, petioles and leaves were significantly reduced by waterlogging however, there was no significant effect by pathogens nor an interaction between pathogens and waterlogging on these growth parameters. Overall, waterlogging exacerbated the effect of *F. oxysporum, F. avenaceum* and *P. vinacea* on below-ground dry weight and total biomass of the root-dip inoculated pyrethrum plants 2 maw and affected the flower and flower stem production.

Introduction

Pyrethrum (*Tanacetum cinerariifolium* (Trev.) Schultz. Bip.) is a perennial plant of the *Asteraceae* family from which natural pyrethrins are obtained (Ambrizic et al. 2007). Natural pyrethrins are the active ingredients in a number of commercial insecticides used for a range of agricultural, veterinary and domestic applications (Pethybridge et al. 2008). The majority of world's natural pyrethrin production occurs in northern Tasmania and near Ballarat, Victoria, Australia. However, East Africa (Kenya, Rwanda and Tanzania); China and Papua New Guinea also commercially cultivate pyrethrum (Pethybridge et al. 2003).

Pyrethrum fields have been expected to yield for at least three consecutive years under ideal environmental conditions (Hay et al. 2015). However, Pyrethrum yield decline was recently identified where plants failed to regrow after first harvest or yield reduction occurred after the second harvesting season (Moslemi et al. 2017b). Poor persistence or yield decline has no obvious single cause and has been attributed to interaction between various crown and root rot pathogens and abiotic stresses (Moslemi et al. 2017b).

Plants affected by yield decline exhibited severe discolouration of crown tissues, reduced root growth and stunting (Moslemi et al. 2017b). The fungal pathogens *Fusarium oxysporum*, *Fusarium avenaceum* and *Paraphoma vinacea* were found to be associated with these affected plants and affect plant growth (Moslemi et al. 2016; Moslemi et al. 2017b). Foliar pathogens such as *Stagonosporopsis tanaceti* and *Didymella tanaceti* (Pearce et al. 2015; Vaghefi et al. 2015) have also been observed in the yield decline affected plants. Recently, Bhuiyan et al. (2016) showed that infection by *S. tanaceti* reduced plant vigour causing poor regrowth after harvest, which suggested that *S. tanaceti* may be one of the causes of yield decline. *Alternaria infectoria* and *Stemphylium herbarum* were also isolated from the bases of dead flower stems in yield decline affected fields in northern Tasmania and may also contribute to the complex pathology of yield decline syndrome (Moslemi et al. 2017a).

According to Pethybridge et al. (2010), various abiotic factors such as soil compaction and cutting height also contributed to poor regrowth of pyrethrum plants. Previous studies attempting to improve production of pyrethrum have focused on soil fertility (Salardini et al. 1994a, b), weed competition (Rawnsley et al. 2006; Rawnsley et al. 2007) and waterlogging (Javid et al. 2013), as well as plant pathogens (Jones et al. 2007; Pethybridge et al. 2008). Identification of pathogens to which the crops are susceptible and developing pathogen management strategies should reduce the sensitivity of crops to environmental stresses.

Waterlogging is an important abiotic factor having a significant role in pyrethrum yield reduction and a negative effect on flower and pyrethrin production. Developing molecular strategies to produce waterlogging-resistant genotypes (Dennis et al. 2000), planting crops on graded surfaces to reduce water accumulation and avoiding prolonged irrigations after a waterlogging period (Setter and Waters 2003) can help reduce the exposure of the plants to prolonged waterlogging in field conditions.

Javid et al. (2013) subjected pyrethrum plants to a 6-day waterlogging period followed by inoculation with *S. tanaceti* and found that the combination of stresses affected root expansion and dry weight. The number of stems per plant in the combined treatment decreased within 3 months. This synergistic effect may have been due to the abiotic stress predisposing the plants to more severe infection. Bradford (1983) showed that waterlogged tomato plants were more susceptible to a range of fungal diseases as waterlogging changed the nutritional balance and increased temperature and humidity of the canopy of the stressed tomato plants.

Javid et al. (2013) reported that waterlogging affected cytokinin production, a phytohormone vital for root expansion, in root tips and shoots of the waterlogged pyrethrum plants. Suraweera et al. (2015) showed that a water deficit treatment significantly reduced flower production and pyrethrin content of the flowers.

Since pyrethrum crops are subjected to regular waterlogging, and crown and root rot pathogens are prevalent in field plants, further studies should focus on the interaction between biotic and abiotic stresses and the effect on plant growth and development. Hence, the aim of this study was to assess the effect of a 4-day period of waterlogging on growth and flower production of pyrethrum plants infected with the crown and root rot pathogens *F. oxysporum*, *F. avenaceum* and *P. vinacea*.

Materials and methods

Ten-week-old seedlings of pyrethrum cultivar Pyrate were grown from steam sterilised seeds and raised in seedling mix in Tasmania and sent to the University of Melbourne, then 250 seedlings were individually transferred to 20 cm-diam (2.8 L) pots with potting mix (Debco, Victoria, Australia) and fertilised with 5 g of Osmocote Plus (Scotts Australia Pty. Ltd.) per pot. Seedlings were maintained in a glasshouse for 2 months at 25–27 °C under natural light and Osmocote Plus was applied once a month.

Two separate glasshouse experiments were conducted to assess the effect of waterlogging on infected pyrethrum plants. The first experiment involved seedlings being inoculated with *F. oxysporum* (BRIP 64449) and *F. avenaceum* (BRIP 64445) as these were shown to be pathogenic to pyrethrum causing root and crown rot (Moslemi et al. 2017b). The second experiment involved inoculation with *P. vinacea* (BRIP 63684) causing severe crown infection (Moslemi et al. 2016). Detailed information of the reference isolates can be found in Moslemi et al. (2016; 2017b). Drip irrigation was used to water the plants to minimize cross infection by water splash.

Fifty plants per treatment were inoculated by immersing the roots in 10^5 spore/mL spore suspension of each pathogen as described in Moslemi et al. (2017b). Controls were identically treated but with sterilised water. Plants were maintained in the glasshouse for two months before a waterlogging treatment was applied. To assess the effect of the pathogens, waterlogging and their interaction plants were sampled at three time intervals: before waterlogging (0 bw) when plants were 4-months-old; 2 maw when plants were at the rosette stage; and 6 maw when plants were at the flowering stage. At each sampling time, dry weight was measured for individual plants inoculated with each pathogen treatment, after drying in an oven at 71 °C for 3 days.

Two mai, pre-waterlogging (0 bw): incidence of infection and effect of each pathogen *on growth of infected pyrethrum plants*

Two months after root-dip inoculation 10 plants were randomly sampled from each treatment as described by Moslemi et al. (2017b). At least 10 pieces of each tissue per plant including basal petiole, crown and upper root tissues were cultured on water agar (WA) and potato dextrose agar (PDA) to assess infection.. Plates were incubated under a light regime of 12h light/12h dark for 3-4 days for plants inoculated with *Fusarium* spp. (Burgess and Summerell 1992), and in the dark for those inoculated with *P. vinacea* (Boerema et al. 2004) at 23-25 °C.

Waterlogging treatment

Of the 40 root-dip inoculated plants remaining after undertaking the first harvest in each treatment, 20 were subjected to a 4-day-waterlogging treatment and the other 20 were non-waterlogged. Pots to be waterlogged were placed individually in 16 L plastic buckets. Buckets were then filled with tap water until the water level reached the surface of the soil. Buckets were then left on the bench in the glasshouse for four days. During the waterlogging period O_2 concentration was measured for each pot daily to assess the depletion of O_2 using a dissolved oxygen meter (model HI 9147, Hanna® instruments, USA). This was done by immersing the O_2 electrode in water inside the 16 L buckets and waiting until the O_2 levels stabilized. O_2 concentration dropped gradually but significantly from day 0 to 3 (Fig. 1).

Symptoms caused by waterlogging treatment were also checked daily. Waterlogging treatment was stopped after 4 days at the onset of wilting of the new petioles and leaves, leaf chlorosis and necrosis. Pots were removed from the buckets and allowed to drain, then all plants were maintained in the glasshouse as described before. A stress severity class of 0 to 4 was attributed to each plant 7 days after waterlogging by visual assessment of the degree of necrosis and chlorosis of the lower leaves and petioles (Table 1).

Two maw: effect of waterlogging alone and combined with each pathogen's infection on disease incidence, growth and photosynthesis of pyrethrum plants

The effect of waterlogging on growth alone and combined with prior infection by each of *F. oxysporum, F. avenaceum* and *P. vinacea* was assessed 2 maw. By this time waterlogged plants had partly recovered and had new shoots and young leaves emerging from the crown. Twenty plants, 10 waterlogged and 10 non-waterlogged from each inoculation treatment were randomly sampled.

Roots were washed gently under tap water and tissues from the upper roots, crowns, basal petiole and leaves were cultured on WA and then hyphae were subcultured onto PDA as described.

The effect of waterlogging and *F. oxysporum, F. avenaceum* and *P. vinacea* on photosynthesis rate was assessed on these plants before they were destructively sampled.Photosynthesis rate was measured using a LI-COR® portable photosynthesis system (LI-6400, *LI-COR*, Lincoln, Nebraska, USA) with the CO_2 uptake measurement method. This was carried out using an Infra-Red Gas Analyzer, which compared the CO_2 concentration in gas entering and leaving a chamber in which the first fully grown leaf was enclosed. Leaf areas were measured by capturing images using a Nikon Coolpix A100 compact camera (Digidirect, Australia) and analysing with MATLAB (MathWorks® R2016a) image analysis software and photosynthesis rate was calculated per cm^2 leaf area.

Six maw: effect of waterlogging alone and combined with each pathogen's infection on disease incidence, growth of pyrethrum plants and on flowering

Two maw (i.e. 4 mai) the remaining 20 plants per infection treatment (10 waterlogged and 10 non-waterlogged) were moved outside the glasshouse for vernalisation to induce flowering (June 2016) and maintained there for 4 months. At 6 maw each plant was assessed for the number of flowers and flower stems, petioles, green and yellow leaves, then destructively sampled to assess disease incidence and dry weights as described before. Leaf tissues were not cultured but flower stem bases were sampled as no leaf infection had been found in the previous studies (Moslemi et al. 2016; 2017b).

Data analysis

In each experiment above-ground, below-ground and total biomass were measured and results were analysed using statistical analysis system (SASV.9.4). A completely randomised design was used in each experiment. Dry weight data were analysed at 0 bw, 2 maw and 6 maw. Flowering parameters were analysed 6 maw when plants had produced flowers. The GLM procedure was used in a 2-way ANOVA for analysis of the experiments using pathogen and waterlogging treatments as independent variables in different time periods. Significant differences between waterlogging and non-waterlogging treatments for each pathogen were calculated using Tukey's HSD (honest significant difference) test at α=0.05.

Data from plants that were inoculated but could not be confirmed to have been infected successfully were eliminated from the analyses therefore, ANOVA with unbalanced number of replicates was performed. Data were log transformed to improve residuals distributions.

Results

Disease incidence

Disease incidence was compared in infected plants at 0 bw (pre-waterlogging) and at 2 and 6 maw. At 0 bw (2 mai), in 100% of sampled plants inoculated with *F. oxysporum* the upper root and crown tissues were infected, while in 80% the basal petiole tissues were infected. Of the 10 plants inoculated with *F. avenaceum* 70% were infected with four plants having upper root infection, four crown infection, and two basal petiole infection. Of the 10 plants inoculated with *P. vinacea* eight were infected with severe infection in the upper roots, three in the crowns and one infected in the basal petioles. None of the three pathogens was isolated from any of the non-inoculated controls plants (Table 2 and 3).

Two maw the disease incidence of the plants inoculated with *F. oxysporum* and *P. vinacea* was similar in both waterlogged and non-waterlogged plants with 100% of plants infected by *F. oxysporum* and 90% infected by *P. vinacea*. 80% of plants inoculated with *F. avenaceum* and waterlogged were infected, while only 50% of non-waterlogged plants were infected by *F. avenaceum* (Table 2).

All plants infected by *F. oxysporum* had crown tissue infection in both waterlogged and non-waterlogged treatments 2 maw, whereas 70% and 60% of plants had crown tissue infected by *P. vinacea* in the waterlogged and non-waterlogged treatments, respectively (Table 3). For *F. avenaceum* infected plants only 50% and 20% of plants had crown tissue infection in the waterlogged and non-waterlogged treatments, respectively. Again, no infection was observed in any of the control plants in both waterlogged and non-waterlogged treatments.

At 6 maw disease incidence remained similar to the pre-waterlogging and 2 maw stages for both *Fusarium* and *Paraphoma* trials. More plants had the basal flower stems infected by *F. oxysporum* than by *P. vinacea* or *F. avenaceum*. There was a higher incidence of crown infection in plants inoculated by *F. avenaceum* at 6 maw than at 2 maw (Table 2 and 3). *Stress severity classes of waterlogged plants*

The waterlogging treatment was terminated on day 4 at the onset of chlorosis and necrosis of the young petioles and leaves (Fig. 2). A shift in severity class, in comparison with the controls, was observed 7 days after waterlogging when 75% and 70% of plants infected with *F. oxysporum* and *F. avenaceum* respectively had severity class 1, and 40% of the plants infected with *P. vinacea* had severity class 2 (Table 4). The stress severity in plants inoculated with *P. vinacea* was higher than in plants inoculated with *F. oxysporum* and *F. avenaceum*. Only 10% of the waterlogged plants showed no symptoms while 40% had severe wilt and necrosis of the lower leaves and petioles, 10% had severe wilt and 5% died. Stress severity in both *F. oxysporum* and *F. avenaceum* treatments was mostly similar with *F. oxysporum* causing slightly higher degrees of necrosis and chlorosis of the lower leaves and petioles and wilt than *F. avenaceum*. Non-infected control plants in both *Fusarium* and *Paraphoma* trials showed no severe symptoms and most had moderate necrosis and chlorosis of the lower leaves and petioles (Table 4).

After 6 months, waterlogging caused a significant ($p<0.05$) reduction in the number of flowers, flower stems, petioles and yellow leaves in the waterlogged plants in both *Fusarium* and *Paraphoma* trials. There was no significant difference in green leaves in the *Fusarium* trial. However, none of the pathogens had a significant effect on these traits in the waterlogged and non-waterlogged plants (Table 5).

Dry weight analyses

At each sampling time there was a significant ($p<0.05$) difference in mean dry weight between inoculated and non-inoculated plants for each pathogen treatment, and between waterlogged and non-waterlogged treatments. The interactions between sampling time and waterlogging treatment, and sampling time and pathogen treatments for all the three pathogens were highly significant (Fig. 3). In plants grown at optimum soil water capacity *F. oxysporum* and *P. vinacea* significantly reduced the below-ground and total biomass of plants at pre-waterlogging (0 bw), and 2 and 6 maw (Table 6). Although *F. avenaceum* was pathogenic it had a significant effect only on below-ground biomass at 2 and 6 maw (Table 6).

Waterlogging generally caused a significant reduction in above-ground, below-ground and total biomass of the pyrethrum plants inoculated with *F. oxysporum, F. avenaceum* and *P. vinacea* as well as in the non-infected controls (Fig. 3). At 2 maw, significant reduction of dry weight occurred in plants infected with each of three pathogens, whereas, at 6 maw only *F. oxysporum* and *P. vinacea* significantly affected plant growth in the waterlogged treatment. The waterlogging main effect was significant for below-ground and total dry weight at both 2 and 6 maw (Table 6).

By 6 maw plants had begun to recover from the waterlogging treatment by producing new leaves and increased growth. However, there was a highly significant difference in dry weights of the plants between the waterlogged and non-waterlogged groups

(Table 6). Plants infected with *F. oxysporum* had significantly reduced above-ground, below-ground and total biomass in both waterlogged and non-waterlogged treatments. Plants infected with *F. avenaceum* had no significant difference in above-ground and total biomass in both groups. Plants infected with *P. vinacea* had no significant difference in above-ground dry weights.

Photosynthesis at 2 maw

At 2 maw there was a significant ($p<0.05$) interaction between inoculated and non-inoculated plants on photosynthesis rate, and a significant interaction between waterlogging and no waterlogging treatments however, there was no significant interaction between inoculation and waterlogging treatments. Plants infected by each of the pathogens had the lowest photosynthesis rate in both waterlogged and the non-waterlogged treatments (Table 7). Photosynthesis rate measurements showed that leaf photosynthesis in the waterlogged plants was significantly lower than in those which had not been waterlogged in both trials (Table 7).

Discussion

Waterlogging caused wilt, necrosis and chlorosis of young leaves and petioles of the infected pyrethrum plants. All three pathogens had a significant effect on dry weight of the infected plants from 0bw to 6 maw. The interactions between sampling time and pathogens, and sampling time and waterlogging treatment were highly significant. Significant differences in stress severity occurred when plants were exposed to a 4-day waterlogging stress. A 4-day waterlogging treatment significantly affected the growth of pyrethrum plants infected by *F. oxysporum, F. avenaceum* and *P. vinacea*. At 2 maw there were significant differences in above-ground, below-ground and total dry weights of plants infected by these pathogens, whereas, at 6 maw *F. oxysporum* and *P. vinacea* had larger effect than *F. avenaceum* causing significant reduction in total dry weight.

Although *F. avenaceum* did not significantly reduce above-ground or total dry weights at 6 maw, the below-ground biomass was significantly reduced. These results confirmed previous studies by Moslemi et al. (2017b) that *F. oxysporum* and *P. vinacea* were important pathogens of pyrethrum but that *F. avenaceum* was a minor pathogen although it retained its pathogenicity 6 maw and significantly reduced the dry weight.

Above-ground dry weight of plants was less affected by infection caused by *F. avenaceum* and *P. vinacea* than by *F. oxysporum,* which is indicative of the severity of pathogenicity of *F. oxysporum*. At 7 days after waterlogging, plants infected by *P. vinacea* showed more severe necrosis and chlorosis of leaves and severe wilting of the shoots and petioles than the controls and plants inoculated with the two *Fusarium* spp. This may have indicated that these plants had more severely weakened root systems due to infection by *P. vinacea,* which resulted in more severe symptoms of stress when subjected to the waterlogging stress. This was confirmed in a previous study when Moslemi et al. (2016) showed that pyrethrum plants inoculated with spore suspension of *P. vinacea* had significant reduction of below-ground and total biomass of root-dip inoculated plants in two separate glasshouse experiments.

Significant reduction of O_2 during the 4-day waterlogging period would have caused a reduction in the amount of oxygen in roots (hypoxia). Hypoxia decreases adenosine triphosphate (ATP) production that is vital for respiration and photosynthesis and has direct effect on root and shoot growth of waterlogged plants (Davies et al. 2000). The reduction in O_2 may not have affected growth of the fungal pathogens but enhanced infection and colonisation of affected roots weakened by hypoxia.

Waterlogging did not appear to enhance disease incidence for any of the pathogens perhaps because incidence of infection by all three pathogens was high before the waterlogging treatment. At 6 maw treatment plants infected with *F. oxysporum* had high incidence of infection of flower stem bases and crown tissues, whereas *P. vinacea* was isolated from a high proportion of crown and root tissues. Waterlogging treatment caused significant reduction of the number of flowers, flower stems, petioles, green leaves in the *Paraphoma* trial and yellow leaves in the *Fusarium* trial. Similar results were reported by Javid et al. (2013) where a 6-day waterlogging treatment followed by inoculation with the foliar pathogen *S. tanaceti* reduced plant growth. Javid et al. (2013) suggested that poor plant growth occurred as a result of the reduction of ATP synthesis in the roots of the waterlogged plants, which affected the ability of the plants to uptake nutrients from the soil.

Photosynthesis rate was significantly lower at 2 maw in all the plants including the controls, although plants recovered at 6 maw. However, pyrethrum has been known as sensitive to waterlogging (Javid et al. 2013). McDonald and Dean (1996) showed that waterlogging increased the concentration of ethylene in shoots, which resulted in plants becoming more susceptible to diseases by causing stomatal closure and photosynthesis reduction.

This study will enable a better understanding of the synergistic effect between waterlogging and important fungal pathogens that infect pyrethrum. This expands the pyrethrum industry's knowledge of the range of diseases that can affect pyrethrum growth and production. Assessment of the interaction between these pathogens and abiotic stresses such as waterlogging is a step forward to a better understanding of pyrethrum reduction in the fields of northern Tasmania and Ballarat, Victoria, Australia.

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Table 1. Post-waterlogging stress severity class of plants inoculated with *Fusarium oxsyporum, Fusarium avenaceum,* and *Paraphoma vinacea*, 7 days after waterlogging.

Stress class	Description
0	Healthy looking plants showing no wilt, chlorosis or necrosis
1	Plants begin showing necrosis and chlorosis of the lower leaves and petioles, moderate wilting
2	Wilting of the newly emerged petioles and leaves, chlorosis and necrosis of the lower leaves and petioles
3	Severe wilt, chlorosis and necrosis of the young and senesced leaves, shoots and petioles
4	Plants wilted and dead

Table 2. Number of infected plants two months after inoculation (mai), with *Fusarium oxysporum, Fusarium avencaeum* and *Paraphoma vinacea* per 10 replicates. 0 bw= before waterlogging, 2 maw= 2 months after waterlogging, 6 maw= 6 months after waterlogging. W= waterlogged, NW=non-waterlogged.

		W	**NW**	**W**	**NW**
Treatment	**0 bw**	**2 maw**		**6 maw**	
Control	0	0	0	0	0
F. oxysporum	10	10	10	10	10
F. avenaceum	7	8	5	7	6
Control	0	0	0	0	0
P. vinacea	8	9	9	9	9

Table 3. Disease incidence 2 months after inoculation (mai) at pre-waterlogging (0 bw), 2 months after waterlogging (2 maw) and 6 months after waterlogging (6 maw) for plants inoculated with *Fusarium oxysporum*, *Fusarium avenaceum* and *Paraphoma vinacea*. Each cell shows the number of infected plants out of 10 replicates referring specifically to the type of tissue infected. W= waterlogged, NW=non-waterlogged.

		0 bw				**2 maw**								**6 maw**							
						W				**NW**				**W**				**NW**			
	Treatment	Leaf	Basal petiole	Crown	Root	Leaf	Basal petiole	Crown	Root	Leaf	Basal petiole	Crown	Root	Basal flower stem	Basal petiole	Crown	Root	Basal flower stem	Basal petiole	Crown	Root
Fusarium trial	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F. oxysporum	0	8	10	10	0	8	10	9	0	7	10	8	7	3	10	3	4	3	10	2
	F. avenaceum	0	2	4	4	0	1	5	3	0	0	2	3	1	0	7	2	0	0	5	1
Paraphoma trial	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P. vinacea	0	1	3	8	0	1	7	7	0	2	6	6	1	1	5	5	3	1	3	5

Table 4. The number of plants in each stress severity class and relative stress severity scores of plants inoculated with *Fusarium oxysporum, Fusarium*

avenaceum, and *Paraphoma vinacea*, 7 days after waterlogging. Twenty plants per treatment were considered. Controls represent non-inoculated and waterlogged plants.

Severity class	**Control *Fusarium***	***F. oxysporum***	***F. avenaceum***	**Control *Paraphoma***	***P. vinacea***
0	9 (45%)	4 (20%)	5 (25%)	6 (30%)	2 (10%)
1	11 (55%)	15 (75%)	14 (70%)	13 (65%)	7 (35%)
2	0	1 (5%)	1 (5%)	1 (5%)	8 (40%)
3	0	0	0	0	2 (10%)
4	0	0	0	0	1 (5%)

Table 5. Effect of waterlogging on the number of pyrethrum flowers, flower stems, basal petioles and leaves (green and yellow), 6 months after

waterlogging (maw). Means for each pathogen treatment are shown in each column. Significant differences between waterlogging and non-waterlogging treatments for each parameter infected with individual pathogen treatments in *Fusarium* and *Paraphoma* trials are shown as HSD in rows using a Tukey's HSD (honest significant difference) test at α=0.05. * Significant t-value; ns= not significant t-value. W=waterlogged, NW=non-waterlogged.

	Flowers			**Flower stems**			**Basal petioles**			**Green leaves**			**Yellow leaves**		
Treatment	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**
Control	7.6	3.2		5	1.7		68.5	41.1		6.4	3.5		9.6 b	11.2	
F. oxysporum	6.5	2.5	2.28*	4.2	2.1	1.2*	63.1	40.3	9.53*	4.7	2.4	2.13 ns	22.8	13	3.93*
F. avenaceum	7.6	1.7		4.9	1		67.3	37.2		4.7	3.9		20.9	12.5	
	NW	**W**	**HSD**	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**	**NW**	**W**	**HSD**
Control	10	2.7	2.93*	5.7	1.2	1.6*	72.6	34.7	15.46*	6.6	2.6	1.85*	6.6 a	6.1	1.91 ns
P. vinacea	5.9	1.6		3.3	1		56.5	35.4		3.9	2.1		4.6 a	4.5	

Table 6. The effect of waterlogging on dry weights of pyrethrum plants infected with *Fusarium oxysporum*, *Fusarium avencaeum* and *Paraphoma vinacea*, pre-waterlogging (0 bw), 2 months after waterlogging (2 maw) and 6 months after waterlogging (6 maw). Means with different letters in each column for each pathogen treatment were calculated using Tukey's HSD (honest significant difference) test at α=0.05 and are significantly different. Significant differences between waterlogging and non-waterlogging treatments for each dry weight category are shown as P-values in rows. ns= not significant, ai= after inoculation, maw= months after waterlogging. W=waterlogged, NW=non-waterlogged.

			Means						
			0 bw	2 maw[y]			6 maw[y]		
	Dry weight	Treatment		NW	W	P-value	NW	W	P-value
Fusarium trial	Above-ground	Control	4.17 a	8.28 a	8.01 a	ns	15.66 a	10.57 a	P<.0001
		F. oxysporum	2.35 b	6.38 b	5.65 b		12.65 b	7.17 b	
		F. avenaceum	3.13 ab	7.99 ab	5.88 b		13.95 a	9.62 a	
	Below-ground	Control	3.38 a	13.38 a	6.78 a	<.0001	18.50 a	15.08 a	<.0082
		F. oxysporum	2.07 b	6.02 b	2.1 b		13.99 b	11.03 b	
		F. avenaceum	2.58 ab	7.99 b	3.70 b		15.24 b	12.32 b	
	Total	Control	7.55 a	21.38 a	14.80 a	<.0005	34.17 a	25.65 a	P<.0001
		F. oxysporum	4.42 b	12.40 b	7.98 b		26.65 b	26.20 b	
		F. avenaceum	5.71 ab	16.27 b	9.35 b		29.19 ab	21.94 ab	
		Treatment	Mean	NW	W	P-value	NW	W	P-value
Paraphoma trial	Above-ground	Control	4.87 a	8.22 a	7.20 a	ns	15.42 a	6.28 a	P<.0001
		P. vinacea	2.63 b	7.40 a	4.58 a		12.62 a	7.97 a	

Below-ground	Control	2.95 a	8.34 a	5.21 a	<.0005	16.75 a	10.79 a	P<.0001
	P. vinacea	2.12 b	4.89 b	1.72 b		12.55 b	5.99 b	
Total	Control	7.83 a	16.56 a	12.41 a	<.0070	32.17 a	17.07 a	<.0001
	P. vinacea	4.76 b	12.29 b	6.31 b		25.17 b	13.78 b	

[y] Equivalent to 4 and 8 months after inoculation.

Table 7. The effect of waterlogging on photosynthesis of the plants inoculated with *Fusarium oxysporum, Fusarium avenaceum* and *Paraphoma vinacea*, 2 months after waterlogging. Means with different letters in each column for each pathogen treatment were calculated using Tukey's HSD (honest significant difference) test at α=0.05 and are significantly different. Significant differences between waterlogging and non-waterlogging treatments for each pathogen trial are shown as P-values. W=waterlogged, NW=non-waterlogged.

		Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})		
Trial	**Treatment**	**W**	**NW**	**P-value**
Fusarium trial	Control	0.91 a	1.64 a	<.0001
	F. oxysporum	0.26 b	1.37 b	
	F. avenaceum	0.44 b	1.60 b	
Paraphoma trial	Control	0.91 a	2.73 a	<.0001
	P. vinacea	0.31 b	1.24 b	

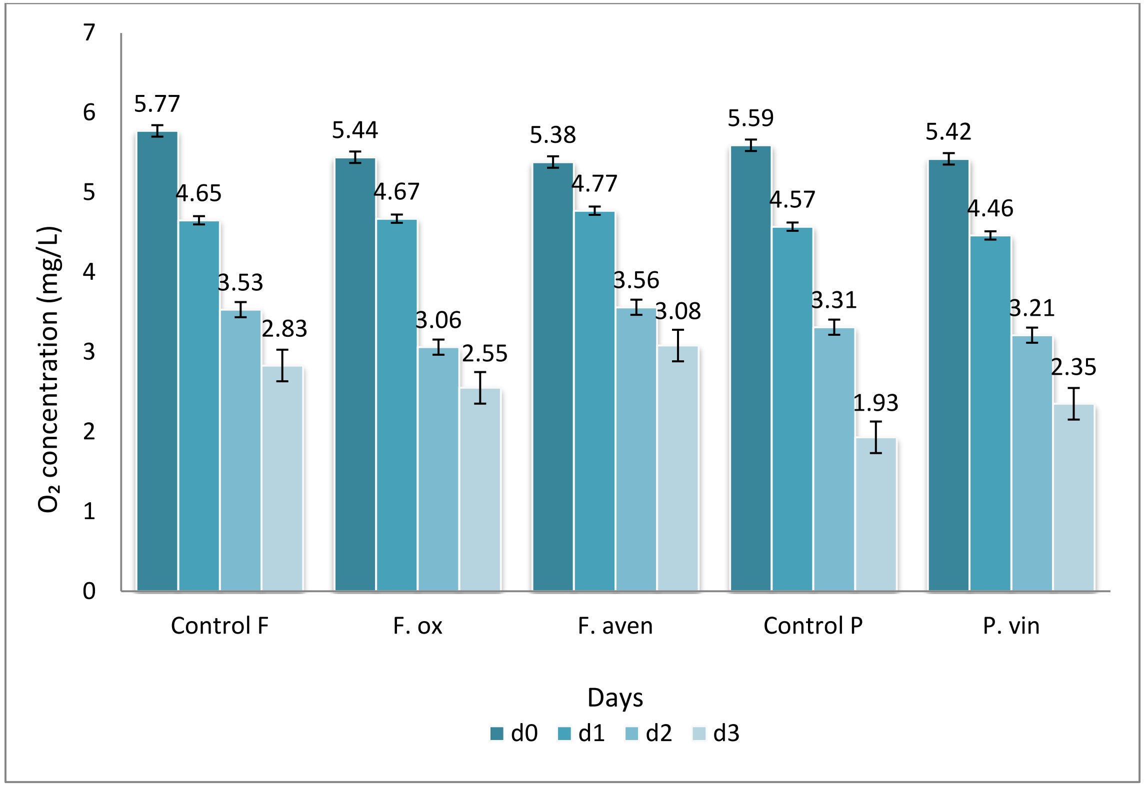

Fig. 1. O_2 concentration (mg/L) measured for 20 plants per treatment from day 0 to 3; means were calculated for 20 plants/day. F= *Fusarium*, P= *Paraphoma*, F. ox= *F. oxysporum*, F. aven= *F. avenaceum*, P. vin= *P. vinacea*. Capped lines show +/- standard error of the mean (n=20).

Fig.2. Effect of waterlogging on pyrethrum plants; **(a)** wilting of the new shoots and young leaves 7 days after the waterlogging treatment. **(b)** chlorosis and necrosis of lower leaves and petioles, wilting of the young petioles and leaves, 7 days after waterlogging.

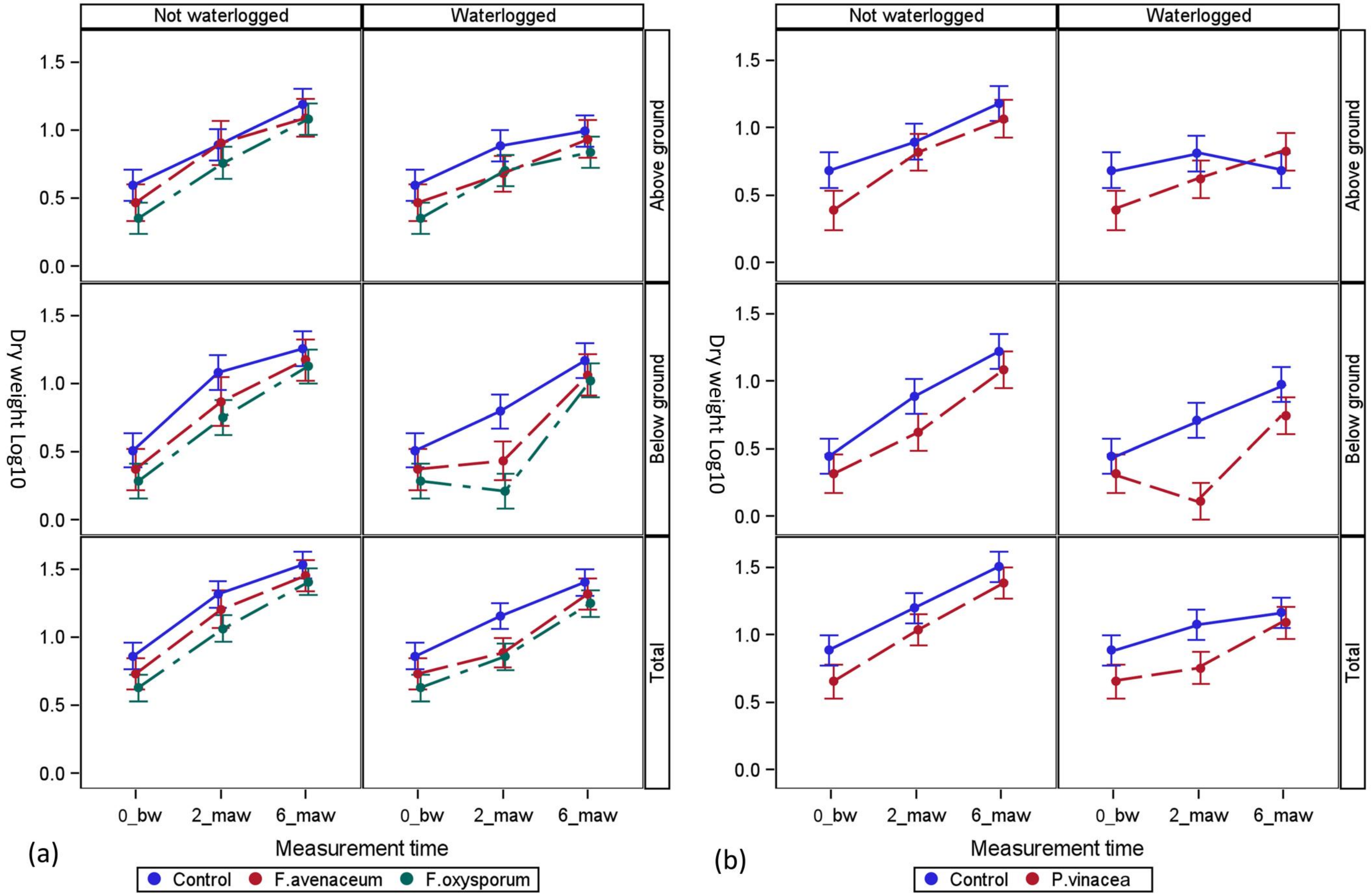
Not waterlogged
Waterlogged
Above ground
Below ground
Total
Dry weight Log10
1.5
1.0
0.5
0.0
0_bw
2_maw
6_maw
Measurement time
(a)
Control
F.avenaceum
F.oxysporum
(b)
Control
P.vinacea

Fig.3. Interaction plots between waterlogging and each pathogen treatment, pre-waterlogging (0 bw), 2 months after waterlogging (2 maw) and 6 months after waterlogging (6 maw) in the waterlogged and non-waterlogged plots. **(a)** plants infected with *Fusarium oxysporum* and *Fusarium avenaceum* **(b)** plants infected with *Paraphoma vinacea*. Y axis refers to mean biomass of the plants (log transformed). X axis refers to the sampling time in which the dry weight of the inoculated plants was measured. 0 bw (before waterlogging) has been used in the analyses of both waterlogged and non-waterlogged treatments for consistency. Error bars indicate 95% confidence intervals for the means.