Biocontrol of Athel Pine
Glasshouse Studies 2010

Research conducted by Christine Crossley and Vic Galea – School of Agriculture and Food Sciences, The University of Queensland, Gatton Campus.

Experiment 1. Adding fungal cultures (isolated from diseased Athel Pine stems) grown on millet into the stem and on the surface of roots of Athel Pine cuttings growing in a glasshouse.

Aim: To determine if dieback disease will result from inoculating fungal cultures (isolated from diseased Athel Pine stems) grown on millet into the stem and root of Athel Pine cuttings.

Materials and Methods:

Source of Plants Cuttings of *Tamarisk aphylla* growing in 50:50 mix of sand and potting mix were placed in the glasshouse with a watering regime of 3 minutes irrigation every 8 hours one week prior to inoculation. Saucers were placed under pots to ensure the media was moist at all times.

Inoculum White French millet seeds were rinsed twice and soaked in deionised water in a 500ml flask for 24 hrs at room temperature. The excess water was drained and the seeds rinsed again with deionised water. Six grams of moist millet seed was placed into each McCartney bottle, then autoclaved at 121°C for 25 mins. The following day, this autoclaving was repeated. Once cool, the McCartney bottles were inoculated with culture. Two squares of culture on ½ PDA were added to the McCartney bottles with 200µl of sterile deionised water. The McCartney bottles were placed in a 25°C incubator and grown for 2 weeks.

Cultures Control (millet with water but no culture)
NT211
NT213
NT214
NT215 (*Nigrospora* sp.)

Experimental design Four cultures plus 1 control, totalling 5 treatments for the experiment. Four plants (replicates) for each treatment were used. Plants of a similar size were arranged into replicates and placed together in the glasshouse and then each treatment randomly

assigned within the replicate. This gave 20 plants for stem inoculating and 20 plants for root inoculating. For the stem inoculated plants the plant heights at the time of inoculation varied from 62cm to 125cm, (mean = 100.0cm, SE = 4.5cm) . The stem width at the height of inoculation varied from 8.8mm to 20.6mm (mean = 12.9mm, SE = 0.8mm). For the root inoculated plants the plant height at the time of inoculation varied from 45cm to 120cm, (mean = 82.6cm, SE = 4.0cm)

Inoculation The Athel Pine plants were inoculated on 10 August 2010. For the stem inoculation, a 4.5mm drill bit was used to drill approx 5mm into the stem. Four grains of millet inoculum was placed into the hole with sterile forceps, and the hole was then sealed with silicone sealant.

Placing millet grains with fungal isolates into stem hole

For the root inoculations, 3 holes were gently dug around the pot with a pencil to gently wound the roots, 10 grains of millet inoculum was placed into each hole. An additional treatment of using all the cultures was added to the design, 5 grains of each of the 4 cultures were placed into 3 holes dug in the same way.

Millet grains containing fungal isolates being placed into media near roots

Millet grains from the inocula were placed onto ½ PDA to determine viability and to check that the cultures were pure. Each culture was found to be viable and was the correct culture.

After inoculation the plants were monitored with a disease ranking scale used to determine severity of disease.

Disease ranking scale	0	completely green healthy plants
	1	foliage yellowing / discoloration (1-19%)
	2	foliage yellowing / discoloration (20-39%)
	3	foliage yellowing / discoloration (40-59%)
	4	foliage yellowing / discoloration (60-79%)
	5	foliage yellowing / discoloration (80-99%)
	6	completely dead plants (100%)

Plants in replicates 1 & 2, for all treatments were water stressed by removing drippers and upturning saucers in weeks 12, 15 & 16 until wilting commenced and then normal irrigation was reinstated.

Results:

Figure 1., shows the results for the stem inoculated plants. A mean of 1.5 ranking was the highest observed. Dieback symptoms were not observed in these plants, with no significant difference between treatments.

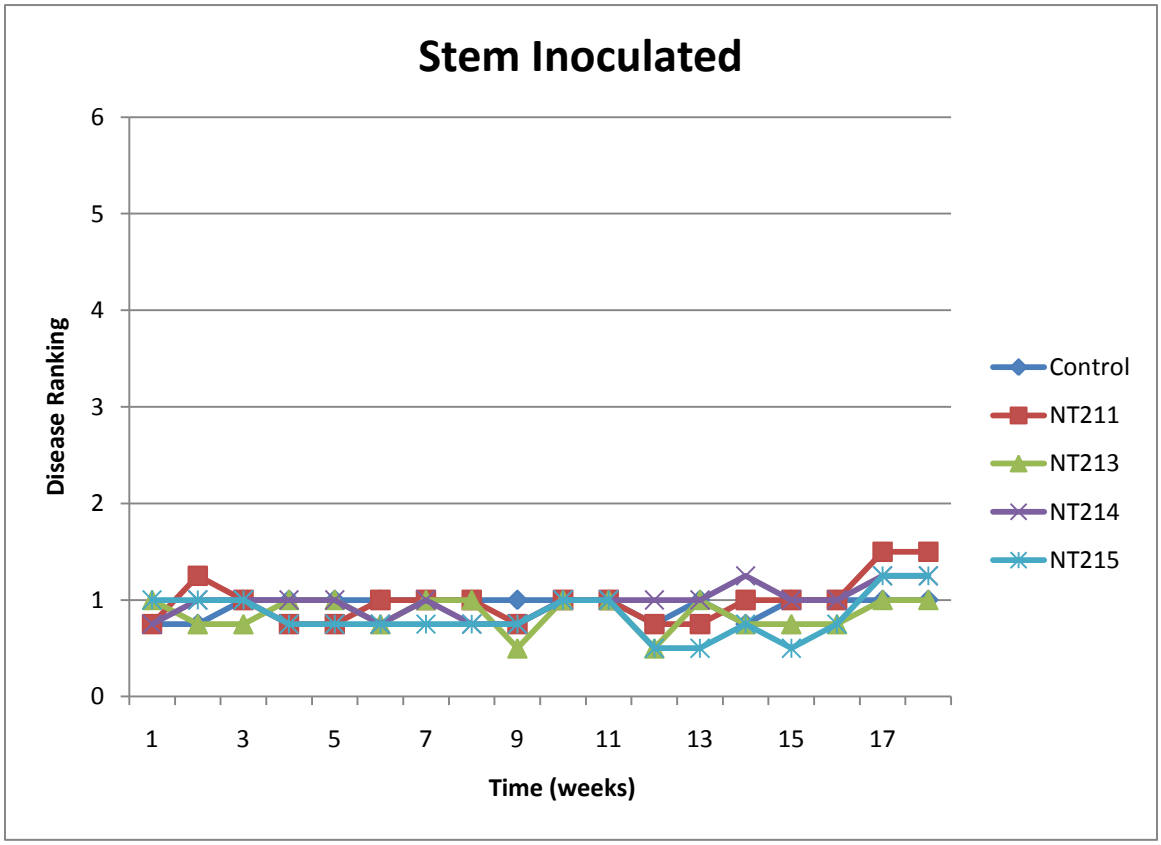


Fig. 1. Means of disease ranking for plants that were stem inoculated.

Figure 2., shows the results for the root inoculated plants. Dieback symptoms were also not observed in these plants although the disease ranking was slightly higher than for the stem inoculated plants. A mean of 2.5 was the highest ranking recorded. There was also no significant difference between treatments.

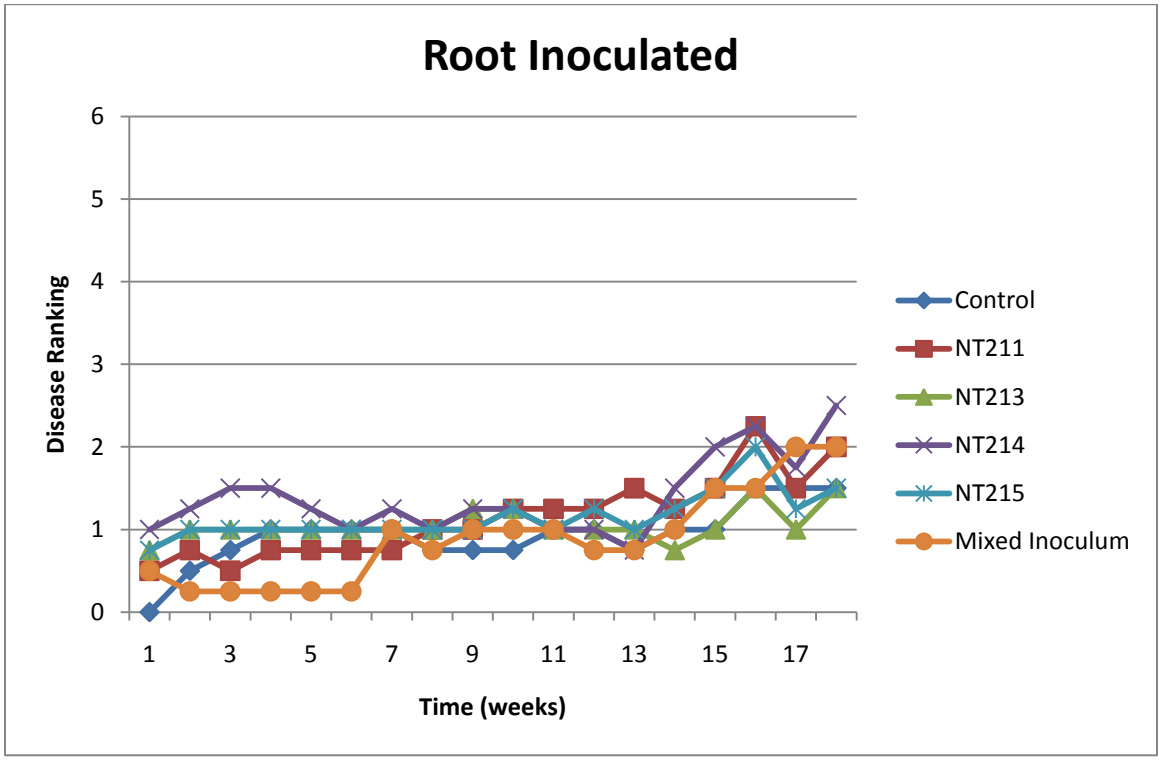


Fig. 2. Means of disease ranking for plants inoculated with root wounding.

Discussion: Dieback symptoms were not able to be produced in this experiment with either stem inoculating or root inoculating. Cycles of water stressing in replicates 1 & 2 was also tried to see if this would cause onset of disease. This also failed to produce dieback symptoms. When the experiment ceased, stem and root samples were kept from all plants. Laboratory work will be undertaken to establish the presence or absence of the inocula in the samples. If colonisation has occurred this will show that the organisms are part of the natural ecology of *Tamarisk* sp. but is not the causal agent of dieback disease.

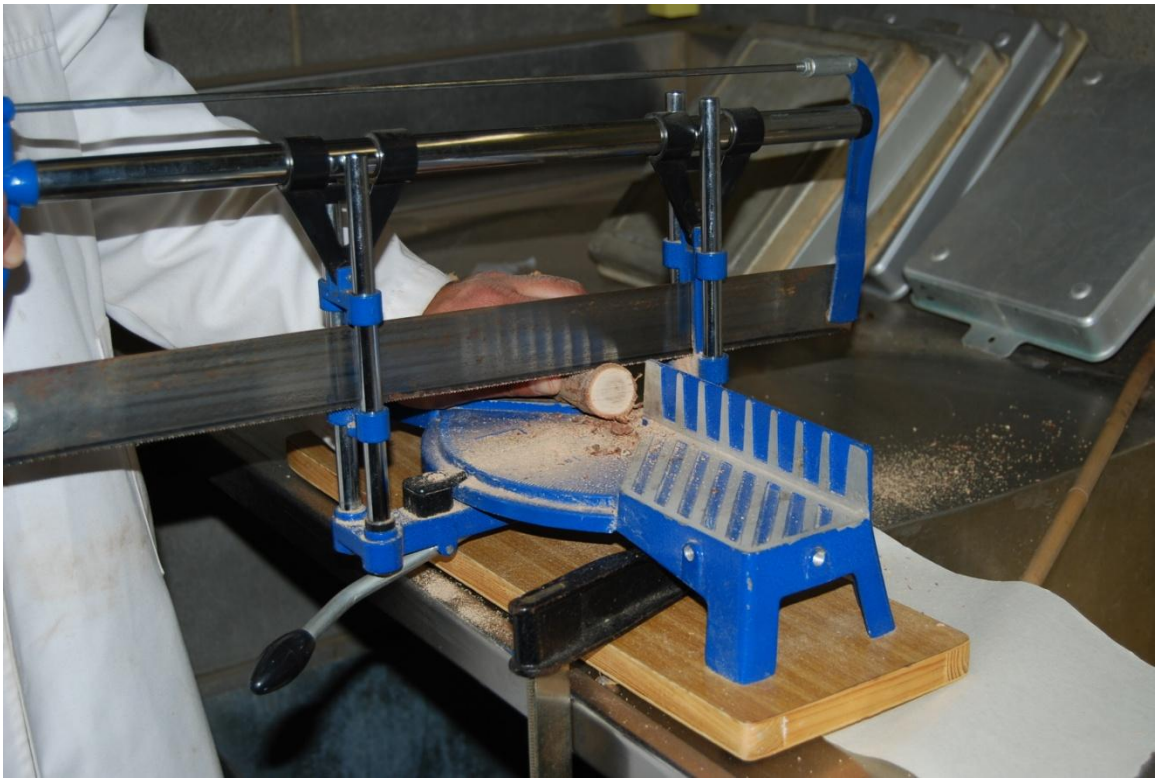
Experiment 2. Adding diseased discs cut from Athel Pine affected with dieback from the Finke River to pots of Athel Pine cuttings growing in a glasshouse.

Aim: To determine if dieback disease will result from adding diseased discs into the growing media of Athel Pine cuttings in pots.

Materials and Methods:

Source of Plants Cuttings of *Tamarisk aphylla* growing in 50:50 mix of sand and potting mix were placed in the glasshouse with a watering regime of 3 min irrigation every 8 hours one week prior to inoculation. Saucers were placed under pots to ensure the media was moist at all times.

Discs Three diseased stems (A, B, C) and one healthy stem from dieback affected Athel Pine were collected from the Finke River (November 2009). A hand saw was surfaced sterilized and used to cut thin segments. The thickness of the discs ranged from 3.0 to 5.8 mm (mean = 3.9mm, SE = 0.3mm).



Cutting stem discs with a mitre saw

Experimental design Three diseased stems and 1 healthy (4 treatments). Four plants (replicates) were used for each treatment. Plants of a similar size were arranged into replicates and placed together in the glasshouse and then each treatment randomly assigned within the replicate. A total of 16 plants were used. Plant heights at the time of inoculation varied from 57cm to 90cm (mean = 76.9cm, SE = 2.0cm).

Inoculation The Athel Pine plants were inoculated on 11 August 2010. A scalpel was used to cut slots in the potting media. Two slots were made on opposite sides of the pot and right angles to the stem, discs were then inserted with the top edge being 25mm below the surface.

Stem disc being inserted into root zone of a potted athel pine plant

After inoculation the plants were monitored with a disease ranking scale used to determine severity of disease.

Disease ranking scale	0	completely green healthy plants
	1	foliage yellowing / discoloration (1-19%)
	2	foliage yellowing / discoloration (20-39%)
	3	foliage yellowing / discoloration (40-59%)
	4	foliage yellowing / discoloration (60-79%)
	5	foliage yellowing / discoloration (80-99%)
	6	completely dead plants (100%)

Results:

Figure 3. shows the results for the plants where discs were inserted into the pots. Dieback symptoms were also not observed in this experiment. There was no significant difference between treatments.

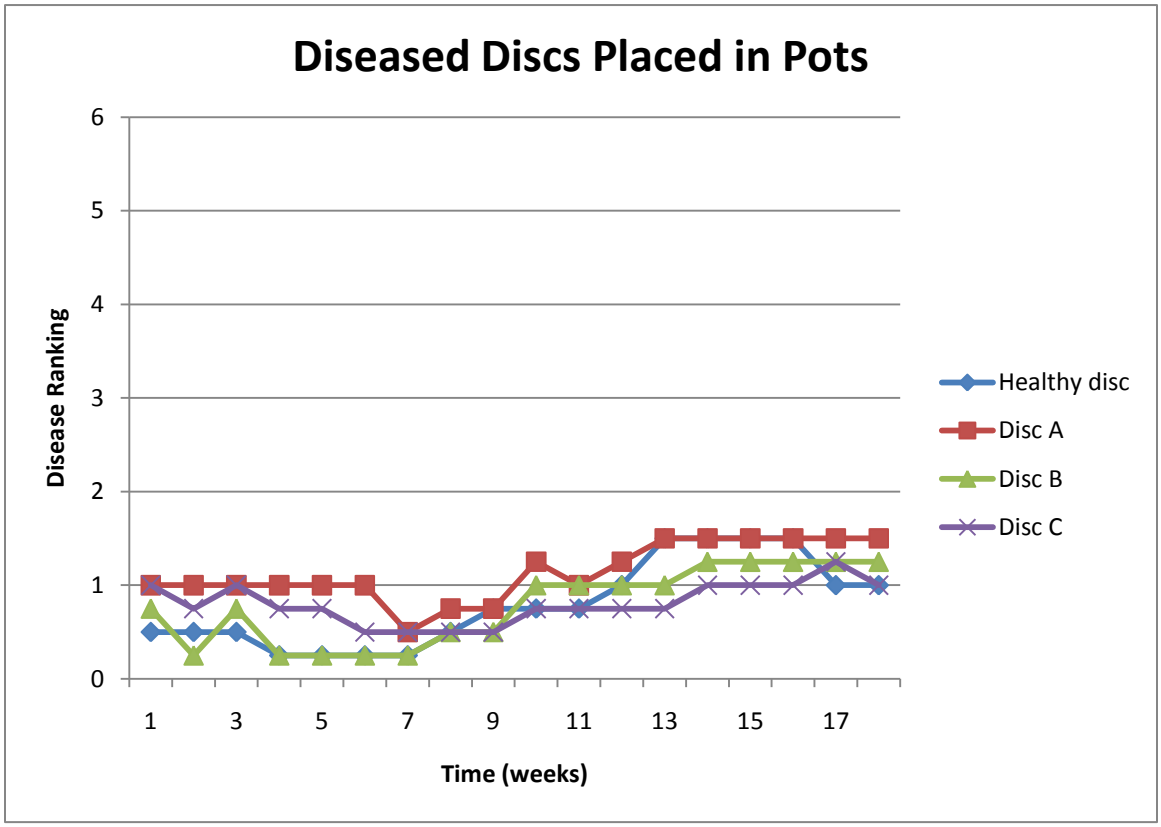


Fig. 3. Means of disease ranking for plants where discs were placed in pots.

Discussion: Dieback symptoms were not able to be produced in this experiment. Cycles of water stressing in replicates 1 & 2 was also tried to see if this would cause onset of disease. This also failed to produce dieback symptoms. When the experiment ceased, stem and root samples were kept from all plants. Laboratory work will be undertaken to establish the presence or absence of fungal cultures in the samples.

Conclusions: These trials represented the third series of experiments in which attempts were made to inoculate potted Athel pine plants with fungal isolates taken from the stems of dieback affected athel pine plants from a dieback site in the Finke river (Horseshoe Bend station – NT). In all three experiments, we have been unable to re-create the stem dieback symptoms which are apparent in field observed cases. This could lead to three possible conclusions:

1. That the isolates taken from the stems of dieback affected athel pine are not primary pathogens, but secondary organisms, and therefore not the causative agents of dieback.
2. That the conditions in the glasshouse, and the inoculation techniques used are not appropriate for disease development
3. That the principle cause of dieback may be located elsewhere in the plant – most likely in the root system, and not in the stems.

Further work to take isolates from the roots of dieback affected athel pine plants will soon be underway. Samples of root material were collected for two sites at Horseshoe Bend station in November 2010. These will be worked upon in the laboratory, and isolates taken will be compared to those previously used. New isolates will be assessed in the glasshouse using methods as described earlier in this document (Experiments 1 & 2).

Report prepared by Christine Crossley and Vic Galea.

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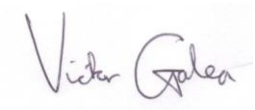

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