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<u>Analysis of the effect of CUTEX on root growth – final report</u>

1. Introduction

We were asked by GeoFabrics to assess whether the copper containing fabric 'CuTex' was effective in the inhibition of root growth, particularly with respect to Japanese knotweed. If so, we were asked to investigate the mechanism by which CuTex affects root growth.

2. Does CuTex inhibit root growth?

To test whether CuTex inhibits root growth, we have used a range of approaches and species.

<u>a) Sterile culture</u>
We initially focussed on experiments using 'sterile culture'. This allowed us to directly visualise the effects of CuTex on root growth. We used the model plant species *Arabidopsis thaliana* for these experiments. As Figure 1 shows, a strip of CuTex embedded in an agar plate creates a 'zone of inhibition' around it. Roots grow toward this zone, but then cease at a distance of approximately 1 to 1.5 cm away from the CuTex.

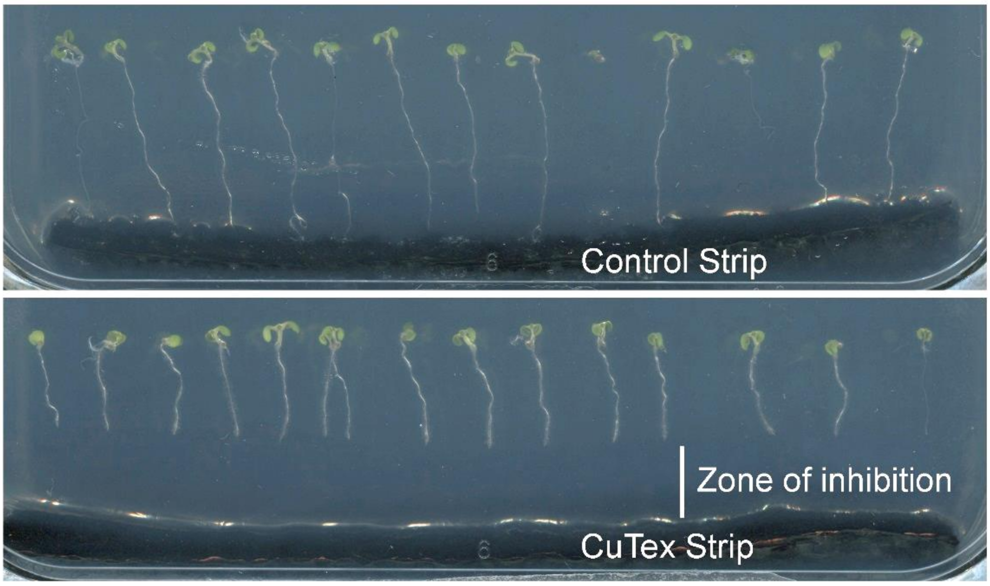

Figure 1: Comparison of root growth of *Arabidopsis thaliana* seedlings in the presence of a control or CuTex strip. Seeds were planted 20mm away from the bottom of the plate, and allowed to grow for 7 days

We grew seedlings at different distances away from CuTex, but irrespective of their starting point, all roots stopped at the zone of inhibition (exemplified in Figure 2, graphs in Figure 3). Roots grown on plates without CuTex continue growing until they hit the bottom of the plate. These results show that CuTex appears to be highly effective at locally inhibiting the growth of roots.

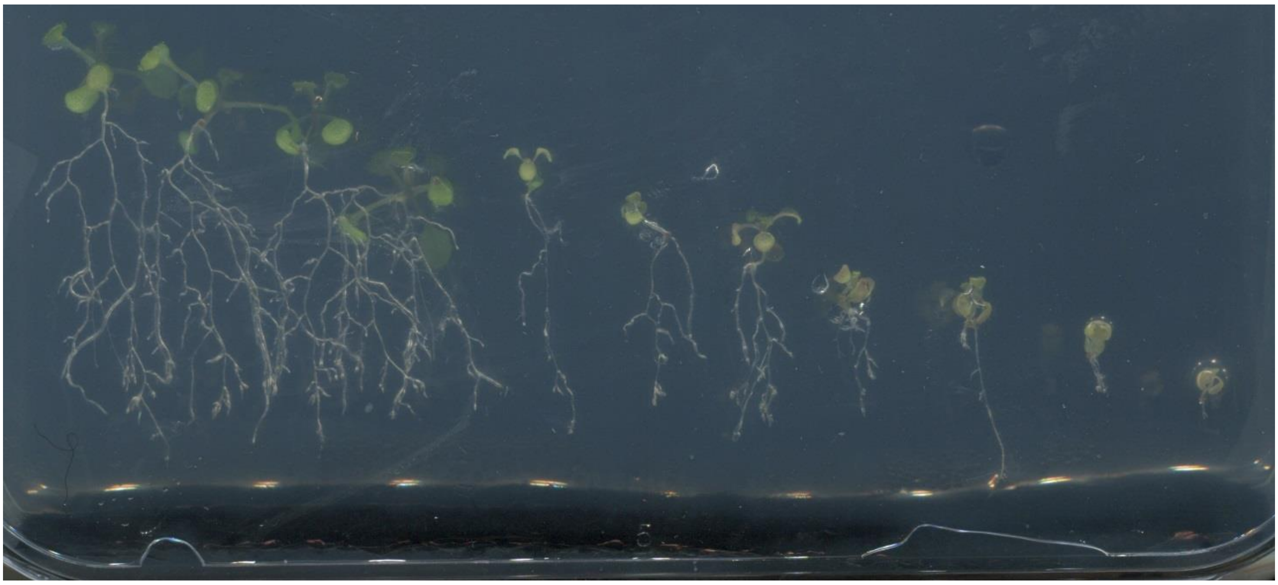

Figure 2: Comparison of root growth in *Arabidopsis thaliana* seedlings planted at distances progressively closer to a CuTex strip (from 40mm away to 10mm away) at 14 days. Seedlings planted closer the CuTex are stunted and yellow.

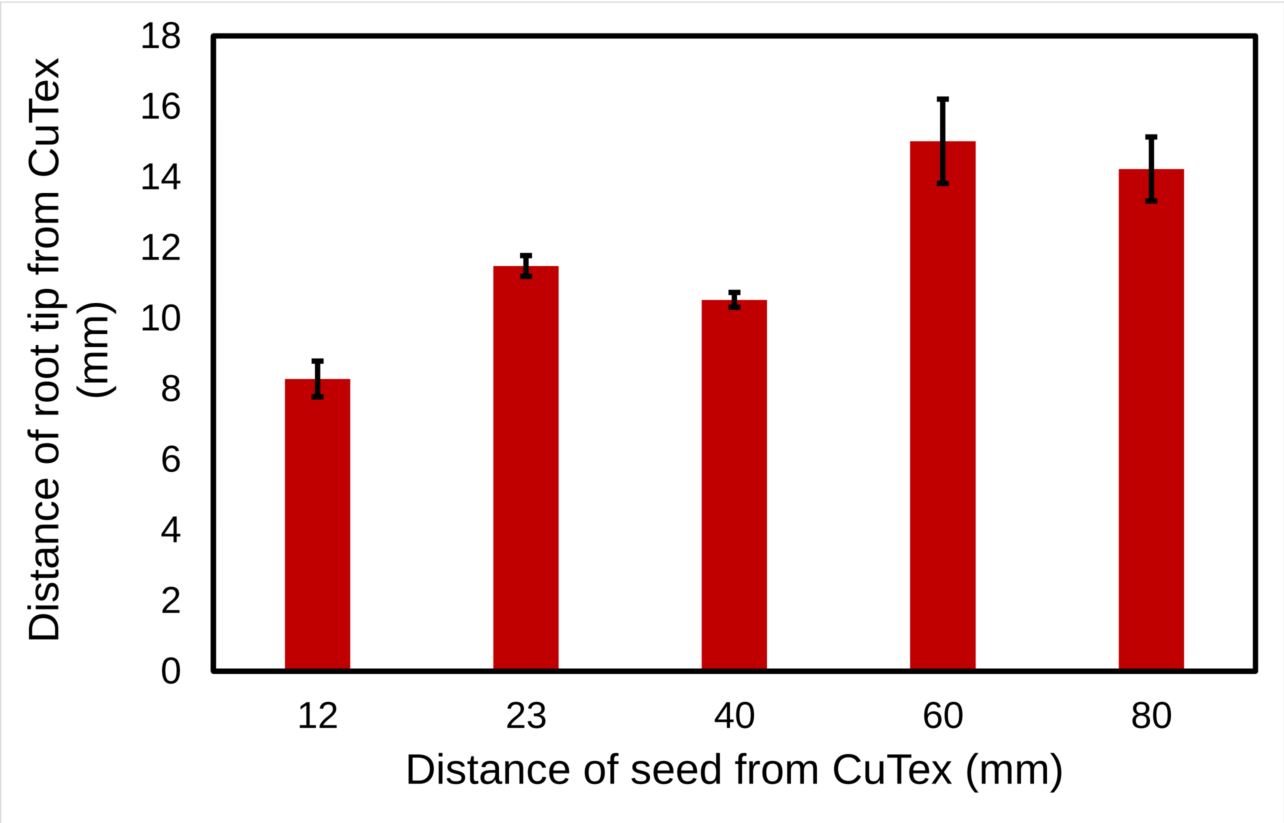

Figure 3: Graph showing the 'stopping distance' in *Arabidopsis thaliana* roots planted at varying distance from the CuTex (x-axis). Measurements of the gap between the root tip and the CuTex layer were made after 14 days. Data are the mean distance (n=16-24 seedlings), error bars show the standard error of the mean. The zone of inhibition is thus generally 10-15mm deep, except for seedlings planted very close to the CuTex.

As can be seen in Figure 2, seedlings which were planted close to the CuTex strip are yellow and fail to grow properly. Seedlings planted further away do not suffer these symptoms. This suggests CuTex may have strong local toxicity to plants.

On a side note, we removed the copper sheet from a sample of CuTex, and used this in isolation in our sterile culture system. This causes the same growth arrest symptoms as intact CuTex (Figure 4). Although there was little doubt that the copper was the active component in CuTex, this nicely confirms it.

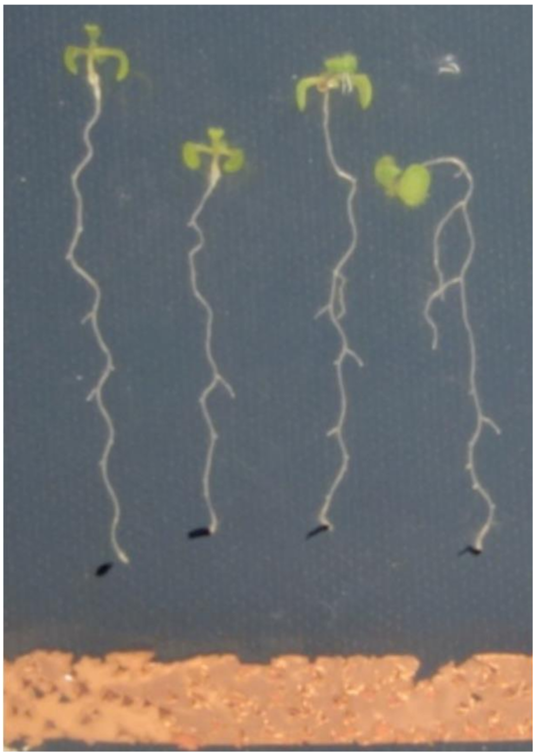

Figure 4: The copper component of CuTex is sufficient to inhibit root growth in *Arabidopsis thaliana* seedlings.

<u>b) Growth in soil - Arabidopsis</u>

We also grew Arabidopsis roots in soil, surrounded by pieces of CuTex. In these experiments no roots grew through the CuTex, but there seemed to be little evidence of root growth inhibition in the vicinity of the CuTex. Indeed, roots grew into the fabric layer of the CuTex quite happily (Figure 5). This suggests that the zone of inhibition is much smaller in soil than in agar.

Figure 5: In soil, roots of *Arabidopsis thaliana* can grow in close proximity to CuTex without obvious inhibition.

c) Growth in soil - tomato

We next trialled tomato roots in soil. We used pots in which the central core of soil was surrounded on all sides by pieces of CuTex, or by control fabric. In these experiments no roots grew through the CuTex, but very few roots grew through the control fabric either. In general, the roots preferred to grow through the gaps between the CuTex/control fabric, rather than through the fabric itself. We then trialled pots with horizontal discs of CuTex placed halfway down the pot, separating the soil into upper and lower sections. We found no roots grew into the lower section when we used CuTex, but equally, we found very few roots in the lower section with the control fabric. In general, the tomato roots were reluctant to grow through either fabric when they had no incentive to do so.

d) Growth in vermiculite/soil - wheat

We therefore modified our system, using a CuTex or control disc to separate an upper vermiculite layer from a lower soil layer. The vermiculite layer has no nutrients, and thus the long-term growth of a plant in these pots depends on growing through the fabric to access the soil. This set up thus gives the plants every incentive to grow through the CuTex if they are able to.

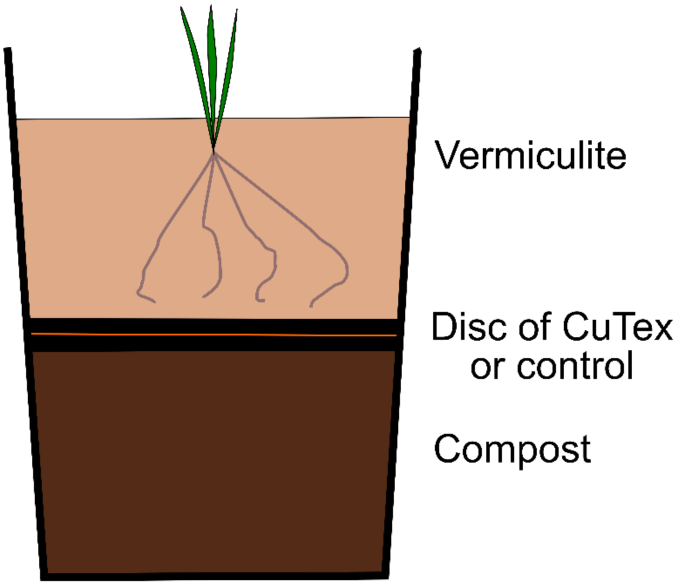

Figure 6: Set-up for wheat experiments.

When then trialled how wheat grew in this system. While some roots grew through the control fabric, no roots grew through the CuTex. Again, roots seemed to be able to grow into the fabric component of the CuTex, so there did not seem to be much long-range inhibition of root growth, unlike in the sterile culture system.

e) Growth in vermiculite/soil – Japanese knotweed

Japanese knotweed is an invasive species that reproduces exclusively through its root system. It has much more vigorous root growth than the previously described species. As with wheat, we grew the plants in nutrient-free vermiculite, with a disc of control/CuTex separating them from nutrient-replete compost (Figure 4). The discs were taped into the pots to prevent roots from growing around the sides of the discs.

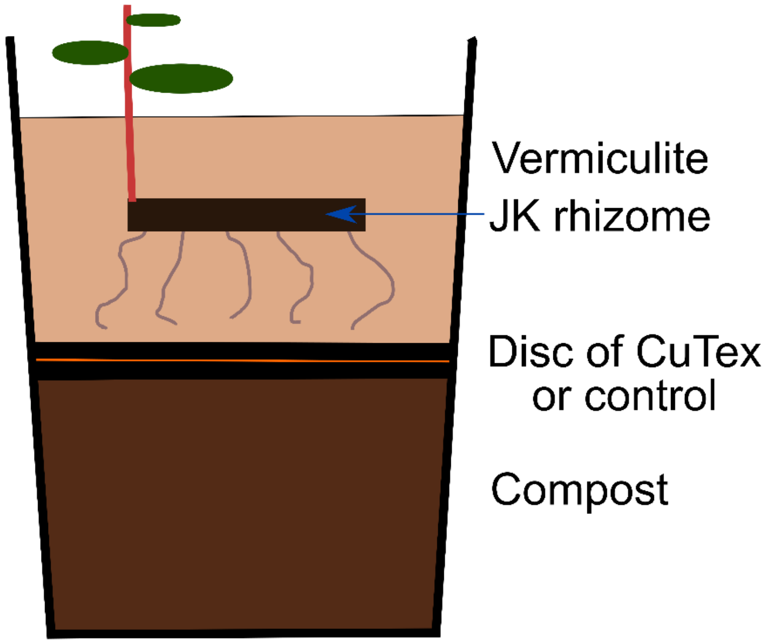

Figure 7: Set-up for Japanese Knotweed experiments.

- In both kinds of pot, there was vigorous shoot system growth (Figure 8), and strong root system growth within the vermiculite layer, which created a solid ‘root ball’ in the vermiculite (Figure 9).
- In control pots, there was clear growth of roots through the fabric disc (Figure 11), leading to a dense mass of roots in the compost layer. These roots bound the compost together into a tight ‘root ball’ (Figure 12), and grew strongly out of the bottom of the pot (Figure 13). It was clear that some of the roots that grew through the disc became major roots (Figure 14). There was also quite a lot of root density in the lower section from roots that had grown around the edge of the CuTex disc — the taping of the disc to the pot was evidently not completely root-proof (Figure 9).
- By contrast, in the CuTex pots, there was generally very little root growth in the compost layer, which was not bound into a root ball (Figure 12), and few roots emerged from the bottom of the pot (Figure 13). Almost all the roots present in the compost layer originated from roots that had grown around the edge of the CuTex disc. In pots where no roots had grown around the CuTex, there was essentially no root mass at all in compost layer (Figure 10). Nevertheless, it is clear that some small roots had grown through the disc in the majority of pots (Figure 11). However, these roots did not grow strongly or to contribute to overall root mass in the compost layer – they may have been ‘mortally wounded’ by growing through the CuTex.

Figure 8: Vigorous shoot growth from rhizomes in these experimental conditions.

Figure 9: Sample removed intact from pot. In this control sample, roots bind the vermiculite and compost layers into separate solid 'root balls', and bind the fabric disc to both the vermiculite and compost layers. Despite precautions, some roots have grown around the edge of the fabric disc (green arrow).

Figure 10: Japanese knotweed sample with no root growth either through or around the CuTex layer. Vigorous root growth can be seen in the vermiculite layer.

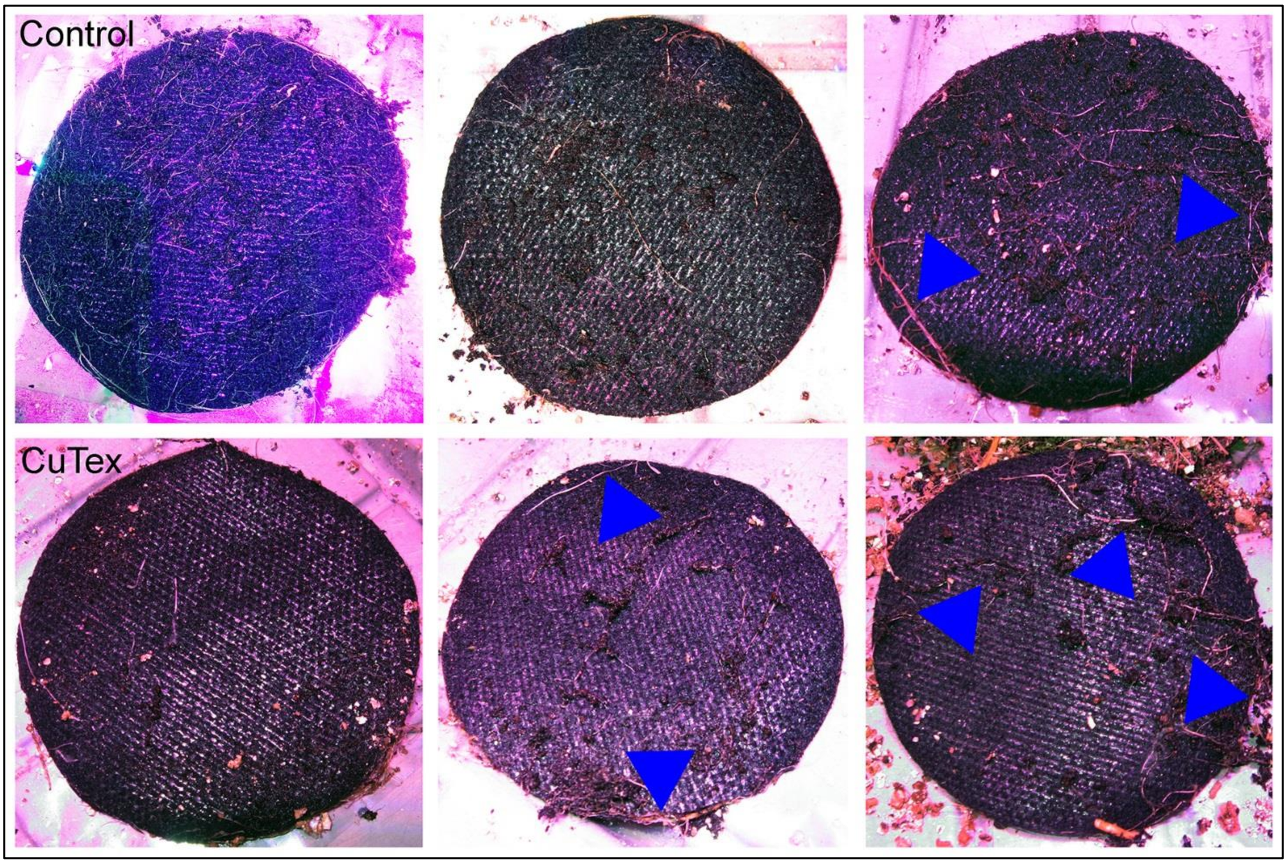

Figure 11: Underside of control and CuTex discs. Roots have grown through both, but far more roots are present in the control samples. Roots that have grown around the edge of the disc are indicated with blue arrow heads.

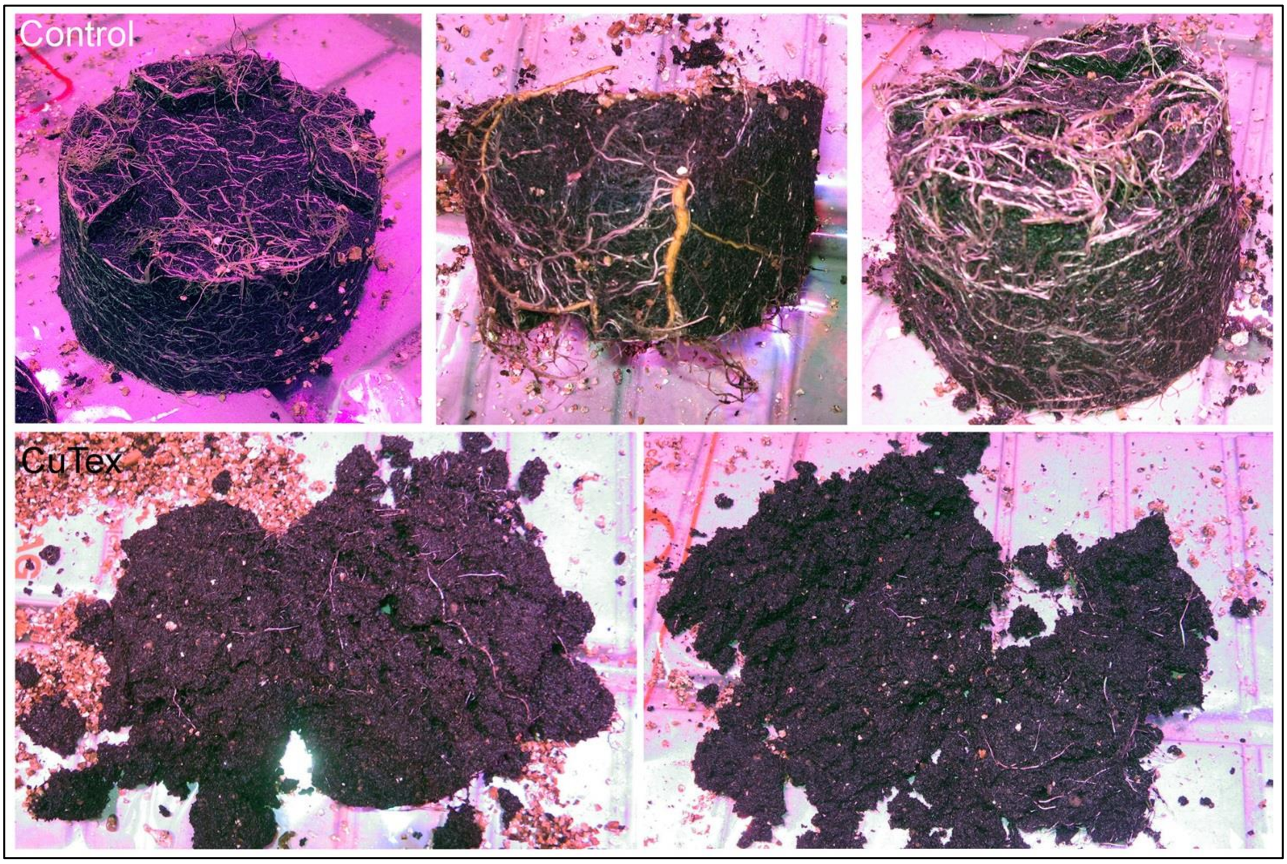

Figure 12: Compost layers from control and CuTex pots. The control compost layers are tightly bound together by dense roots. The CuTex layers by comparison fall apart when taken out of the pot. Although some roots are present, the density is very low.

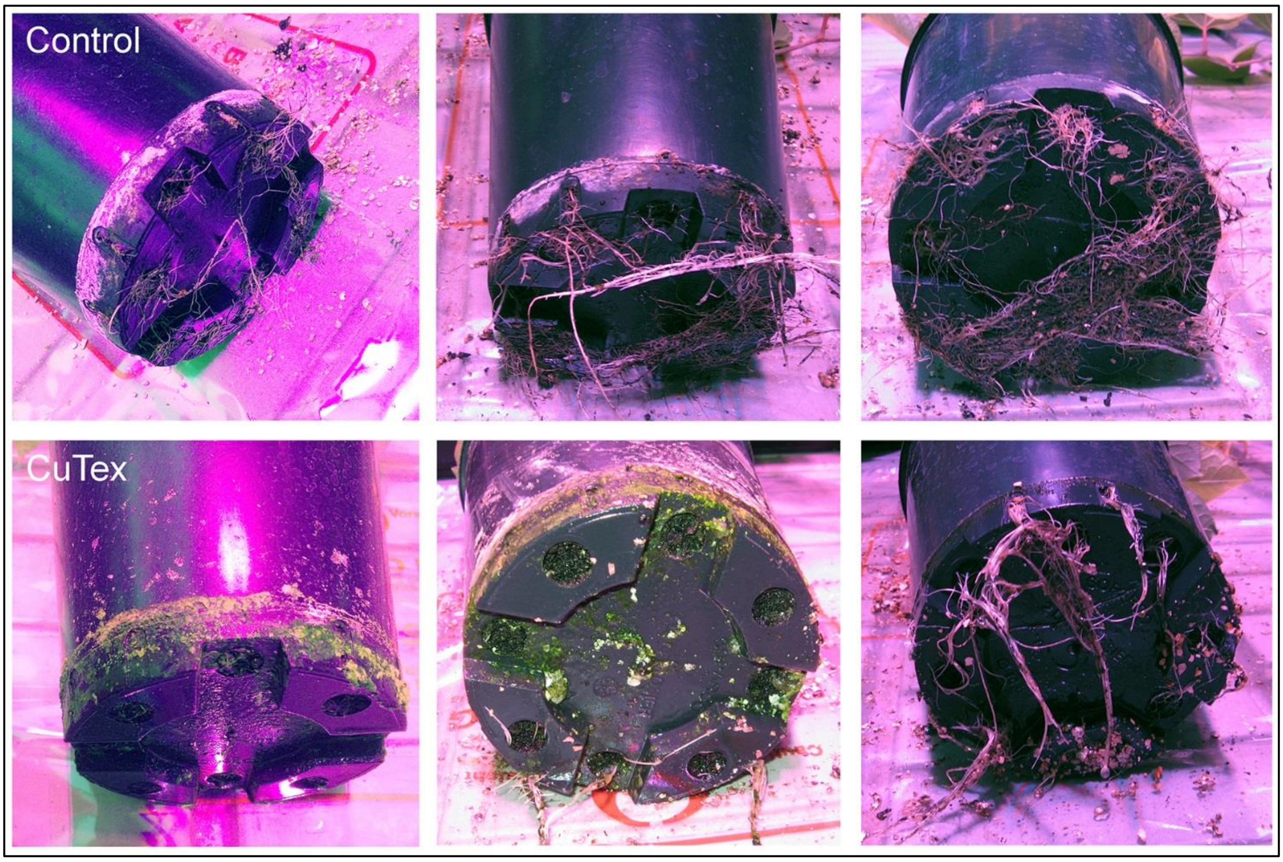

Figure 13: Control pots have abundant growth of roots out of the bottom of the pot. There is much less or no root growth from the bottom of CuTex pots.

Figure 14: Major roots can grow through control fabric (green arrow).

f) Conclusion

From this combination of experiments, we conclude that CuTex is indeed an effective barrier against root growth, even from strongly-rooting species such as Japanese knotweed.

In the sterile culture system, CuTex is effective 'at a distance', suggesting that diffusion of Cu^{2+} ions from the material creates a chemical barrier, and 'zone of inhibition'. However, it is clear that CuTex is much less effective 'at a distance' in soil. This suggests that the chemical barrier is much slower to form in soil. Freshly installed CuTex in soil is therefore probably only really effective as pseudo-physical barrier — only when roots are directly in contact with the copper layer are they inhibited by it.

3. Does the effectiveness of CuTex increase with time?

Given the results in the sterile culture system, we hypothesised that CuTex in soil might eventually form an effective chemical barrier, given enough time, as Cu^{2+} ions diffused into the soil.

We tested this idea in our sterile culture system, by pre-incubating CuTex or control strips in the agar media for 4 weeks. We then sowed out Arabidopsis seeds onto the plates. The effect is striking – the seedling growth is almost completely abolished on the CuTex plates, indicating a much larger and stronger zone of inhibition (Figure 15). These results clearly demonstrate the CuTex can form a chemical barrier, but that even in an agar based system, the diffusion of the Cu^{2+} ions is relatively slow.

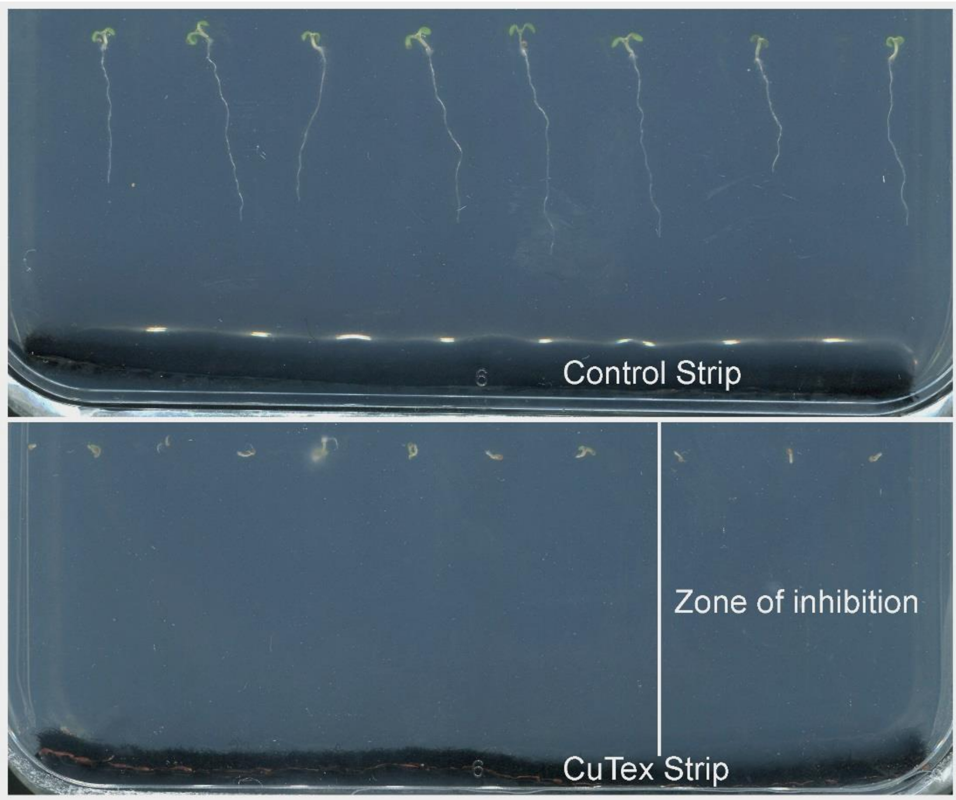

Figure 15: Comparison of root growth of *Arabidopsis thaliana* seedlings grown on plates pre-incubated for 4 weeks with a control or CuTex strip. Seeds were planted 40mm away from the bottom of the plate, and allowed to grow for 7 days.

4. Is CuTex safe?

Since CuTex probably works by releasing Cu2+ ions into the soil, we have pre-emptively tested the bio-safety of CuTex by assessing how it affects overall plant health and growth. So far, we have found no evidence that the presence of CuTex is detrimental to overall plant growthin soil. The biomass of *Arabidopsis thaliana* plants grown in soil surrounded by CuTex is no different to the biomass of plants grown without CuTex (Figure 16). The obvious caveat here is that these experiments are only 8 weeks in duration. It is possible that longer term exposure to CuTex may have some deleterious effects on plant growth (although we believe this is unlikely).

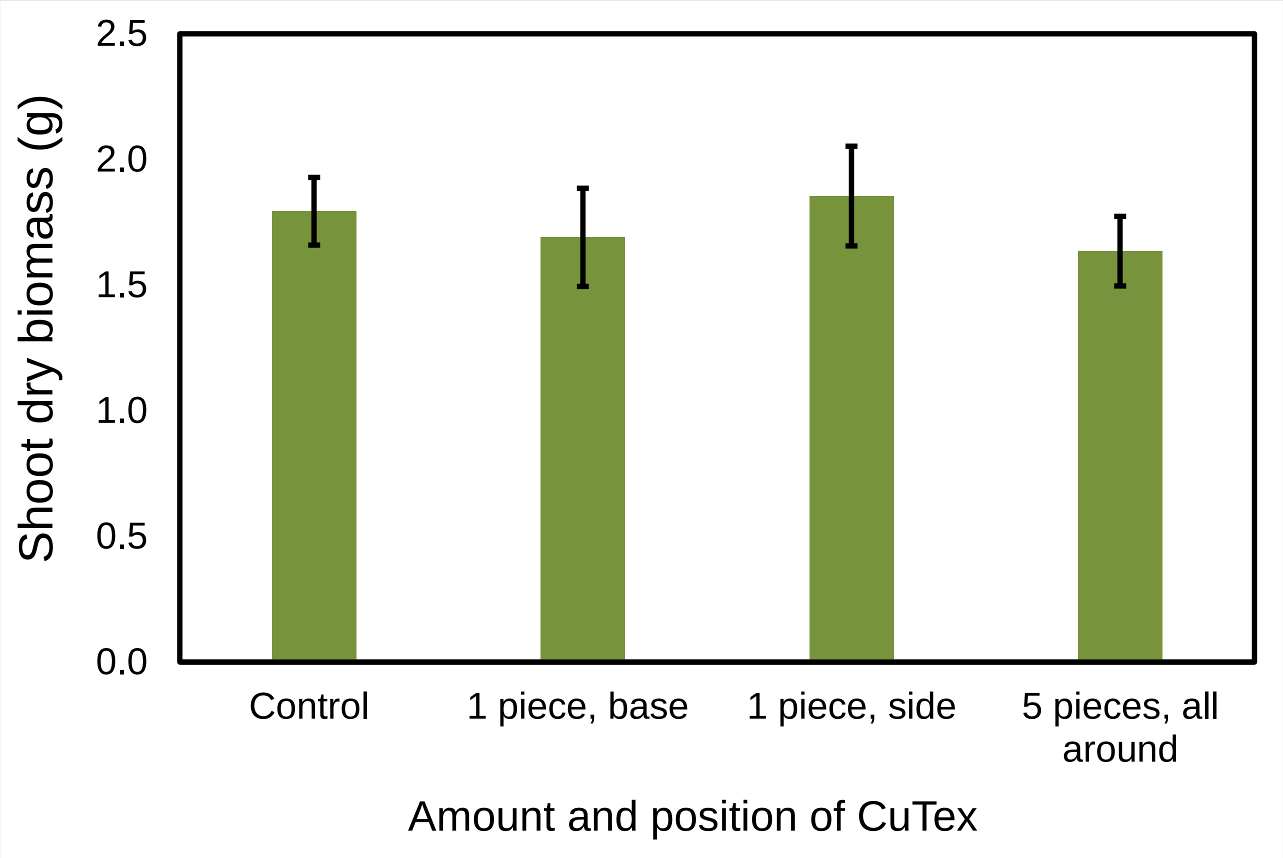

Figure 16: Graph showing average dry shoot biomass of *Arabidopsis thaliana* plants grown on soil containing varying amounts of CuTex. N=8 per treatment, bars indicate standard error of the mean.

We performed similar experiments with tomato, and again saw no effect of the presence of CuTex on the plant's growth or biomass (Figure 17).

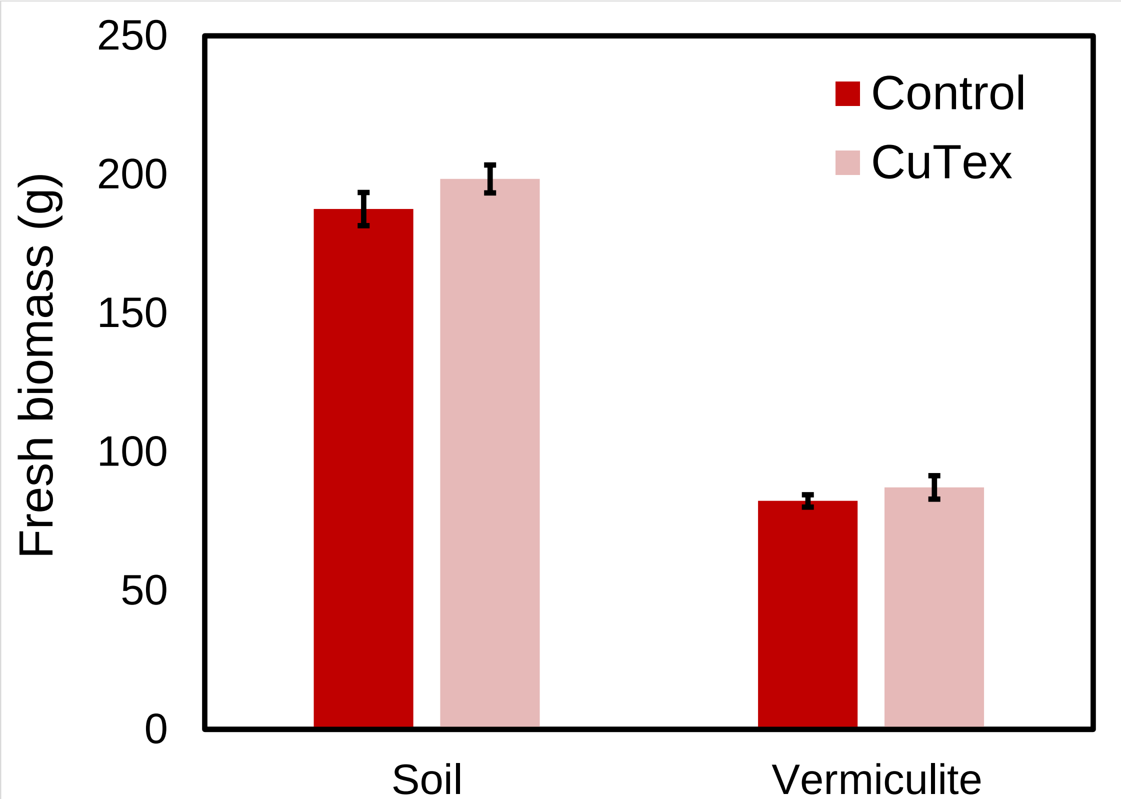

Figure 17: Graph showing average fresh shoot biomass of tomato plants grown on soil or vermiculite in the presence of either a CuTex or control disk. N=6-8 per treatment, bars indicate standard error of the mean.

5. How does CuTex inhibit root growth?

It is fairly clear that CuTex acts by releasing Cu^{2+} ions into solution. But what is the effect of this on the roots themselves? We initially questioned whether CuTex might cause deflection of roots (i.e. altering their growth direction) or cause inhibition of root growth *per se*. The results exemplified in Figure 1 strongly suggested that CuTex inhibits root growth. To gain deeper insight into this process, we used confocal laser-scanning microscopy (CLSM) or differential contrast interference microscopy (DCIM). We analysed the morphology of root tips of plants grown in the presence and absence of CuTex. The results clearly demonstrate that when the root tips approach the zone of inhibition, they undergo a progressive 'collapse'. The dividing cells at the very tip of the root (the 'meristem') die off, and the cells above the meristem differentiate (Figure 18). All indications so far suggest that copper toxicity is the main mode of action of CuTex on plant roots. However, this copper toxicity is clearly a very local effect within the root system – only the part of the root exposed to the 'zone of inhibition' seems to be affected, and overall there is little effect on the health of the plant.

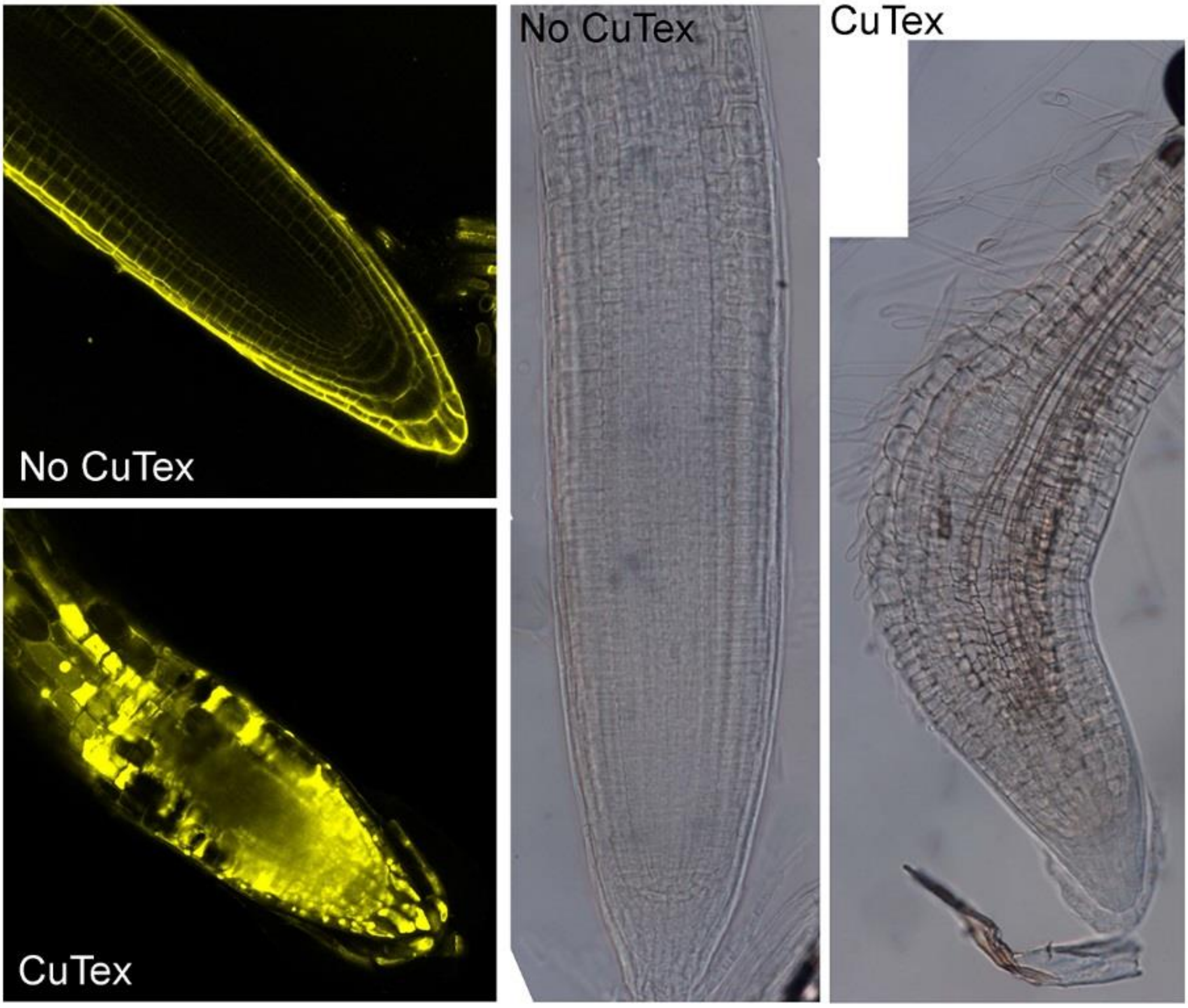

Figure 18: Left hand side: CLSM images of *Arabidopsis thaliana* root tips grown in the presence or absence of CuTex. The roots have been stained with the fluorescent dye propidium iodide (yellow), which labels the outside of intact, live cells. In the absence of CuTex, the root tips have their characteristic morphology. In the presence of CuTex, the propidium iodide has entered into the cells, indicating that they are dead or dying.
Right hand side: DCIM images of *Arabidopsis thaliana* root tips grown in the presence or absence of CuTex. Again, in the absence of CuTex, the root tips have their characteristic morphology. In the presence of CuTex, cells are dead or differentiated, with root hairs visible – something which does not normally occur.

6. Overall conclusions

From the data we have collected, CuTex is clearly an effective and safe material for blocking root growth. We believe that freshly installed CuTex in soil largely acts as a pseudo-physical barrier, in the sense that the roots have to more-or-less come into contact with the copper to be inhibited by it. Our results clearly indicate that CuTex can form a chemical barrier, but the process is slow even in conducive conditions. It seems highly likely that the effectiveness of CuTex in soil will therefore increase over time.