Chapter 2

Allelopathic influence of residues of the agronomic weeds *Cyperus esculentus*, *Bidens pilosa* and *Conyza albida* on the early growth and development of *Pinus patula*

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1. Introduction

The failure of *P. patula* to become established on previously cultivated lands (oldlands) in South Africa has resulted in considerable financial loss. Differences in pine stands on oldlands and afforested soil were evident on the Giant's Castle Estate in Mooi River, KwaZulu-Natal. Due to a history of crop production in certain areas, various annual weeds infest the pine plantations. Of these weeds, *Cyperus esculentus* (yellow nutsedge) is the dominant species during spring and early summer. It occurs in early spring with tubers sprouting after the first rain, and lasts through the winter as a residue on the soil surface. In January, when nutsedge matures, *Bidens pilosa* (common blackjack) and *Conyza albida* (tall fleabane) are growing actively.

According to Radosevich & Holt (1984), numerous naturally occurring chemicals are present in plant material, and when residues are left on the soil surface after harvest, or plowed in, the chemicals can be released by rain or by micro-organisms during decomposition. Subsequent mortality or growth suppression do not have to be related directly to the release of a toxic organic substance from the plant material. The modification in micro-environments could account for the phytotoxic response. Another source of allelochemicals is the production and release by growing plants of toxins that ultimately inhibit development of adjacent plants. Wardle, Nicholson & Rahman (1993) found that residual matter of roots and shoots of *Carduus nutans* (musk thistle) and the stems of dead plants were especially inhibitory to shoot and root biomass of certain grasses and legumes. Ahmed & Wardle (1994) used tissue decomposition bioassays and aqueous extracts of *Senecio jacobaea* (ragwort) to demonstrate strong allelopathic effects on pasture species.

Mycorrhizal fungi are obligate symbionts which derive nutritional benefits from plants and contribute to plant nutrition (Tinker, 1984). According to Agrios (1988), they do not prevent disease, but the absence of mycorrhizae in certain fields results in plant stunting and poor growth. Marx (1969) concluded that in instances where plants have difficulty in obtaining nutrients, mycorrhizae will increase the efficiency of uptake, resulting in enhanced plant growth. Parke, Linderman & Black (1983) stated that mycorrhizae can also increase water uptake and/or alter the plant's physiology to reduce stress response to drought. The work of Marais (1974) confirmed that mycorrhizal infection is essential for the growth and survival of *P. patula*. He reported enhanced phosphate status of the macrobiont and that the microbionts act as biological deterrents to root pathogens such as *Phytophthora cinnamoni*.

2. Aims

1. To evaluate the effect of different weed residues on the growth of *P. patula* seedlings.
2. To determine whether the residues could influence the mycorrhizal colonization on *P. patula* roots.

3. Materials and Methods

Experiment 1: Influence of *C. esculentus* residues on growth of *P. patula* seedlings and mycorrhizal colonization

Site and growth medium description

In January 1996, *C. esculentus* tubers collected at the Giant's Castle Estate of Mondi Forests at Mooi River, KwaZulu-Natal (29°59'S; 29°12'E; altitude 1400m), were planted in quartz sand in Mitscherlich pots (250 mm diameter x 250 mm deep). The pots were kept in a temperature-controlled glasshouse (25°C ± 3 max. and 15°C ± 3 min.) at the University of Pretoria's experimental farm (25 °45'S; 28°15' E; altitude 1320 m). Every second day the pots received 250 ml of a complete nutrient solution (Nitch, 1972).

Foliage of flowering *C. esculentus* growing on the Mondi Forests estate was collected by cutting the plants at their base. The material was left in the sun to dry and then milled to a coarse texture (particles about 2 mm long) with a WileyArthut H. Thom Asco mill. Mitscherlich pots that drained freely were filled with 6 kg of a 50:50 soil mixture consisting of oldland soil (clay loam) and pure quartz sand. Oldland soil was collected on the Estate at a site where, for several years, *P. patula* seedlings had failed to grow properly. The soil properties were as follows: pH (H_2O) 4.87; Bray I P 7.93 and exchangeable Ca, Mg, K and Na of 77.90; 111.40; 151.40 and 20.10 mg kg^{-1} respectively. "Dilution" of the natural soil with quartz sand was done so that allelopathic effects were less likely to be masked by adsorption of allelochemicals on soil colloids such as clay minerals and organic matter. The milled *C. esculentus* foliage was incorporated on a 2 % m/m basis into the soil mixture. This weed residue concentration was estimated to simulate reasonably accurately, on the basis of amount of weed material per $1m^2$, the natural

C. esculentus infestation of mature plants at the study site.

Experimental design

The experiment consisted of three treatments:

- Control, with no weed material added;
- *P. patula* seedlings growing in the soil mixture containing incorporated foliage material of *C. esculentus*;
- Leachate, collected from the root zone of immature *C. esculentus* plants in the glasshouse, applied to *P. patula* seedlings.

P. patula seedlings, about 50-60 mm in height, were obtained from the Mondi Forest nursery in Pietermaritzburg. A day after pots were filled with the soil mixture, two *P. patula* seedlings were planted about 50 mm apart in the pots. Special care was taken not to disturb the root systems unduly. After transplanting, pots received water to about 80% of field capacity. Afterwards, depending on the treatment, seedlings received 250 ml of the nutrient solution or 250 ml of the root leachate. The latter was obtained by pouring the nutrient solution over *C. esculentus* plants which were grown separately in the Mitscherlich pots.

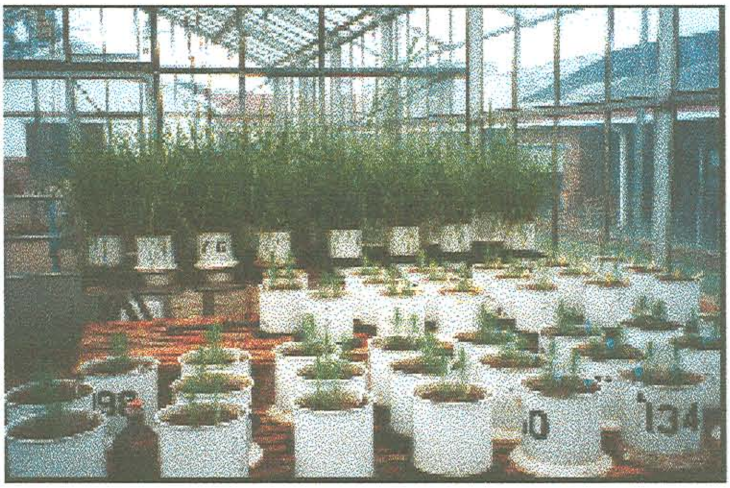

Figure 1 View of the pot experiment. Root leachate was collected from the Mitscherlich pots in the background

Two weeks after transplanting, height and stem diameter measurements were taken. Thereafter, growth measurements were made fortnightly. The growth parameters of height and stem diameter are frequently used to define growth of tree seedlings (Knowe, Nelson, Gjerstad, Zutter, Glover, Minogue & Dukes Jr., 1985; Bacon & Zedaker, 1987). Height was measured from a constant point, 20 mm from the soil surface, to the apical growth point, while the stem diameter was taken from a fixed point at the base of the stem. Seedlings were harvested nine months after they were transplanted to the pots and foliage dry mass was determined after the foliage had been dried in an oven at 70°C for three days.

After nine months' growth, the colonization of ectomycorrhizae on the roots of seedlings was measured. Ectomycorrhizal development on the root system of the pine seedlings originated from natural inoculum present in the pine bark medium used for growing seedlings in the nursery. Fifty randomly selected root pieces per treatment were analysed. Cleaned root pieces were placed in a petri dish with water and scanned under a stereoscope at 10X magnification. The number of ectomycorrhizal morphological types cm^{-1} of root was recorded. Rating numbers 0.5, 1.0, 2.5, and 6.0 were multiplied by the corresponding number of ectomycorrhizal morphological types, *viz.* monopodial, bifurcated, coralloid (3-5 tips) and coralloid (>5 tips), respectively. This rating system was used to determine more accurately the total number of ectomycorrhizal tips cm^{-1} of root.

Statistical analysis

Each treatment was replicated ten times. A completely randomized design was used. Analysis of variance was carried out to determine the effect of the different *C. esculentus* treatments on the percentage height and stem diameter increase of the seedlings. Percentage growth increase was calculated as the final height or stem diameter minus the initial height or stem diameter divided by the initial height or stem diameter multiplied by 100. Differences between treatment means were identified using the Least Significant Difference Test at P=0.05.

Experiment 2: Influence of incorporated residues of *B. pilosa* and *C. albida* on the growth of *P. patula* seedlings and mycorrhizal root colonization on two types of soil

Site and growth medium description

Above-ground parts of actively growing *B. pilosa* and *C. albida* were collected at the Hatfield experimental farm of the University of Pretoria. The same procedure for weed material preparation was used as that in Exp. 1. Two types of soil, afforested and oldland soil, were collected at Mondi's Giant's Castle Estate (KwaZulu-Natal). The two collection sites were about 30 m apart. Soil properties are given in Table 1.

Table 1 Soil properties of afforested and oldland soil collected at the Giant's Castle Estate of Mondi Forests at Mooi River

	pH (H_2O)	Bray I mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Na mg kg^{-1}
Afforested Soil	4.48	5.12	99.70	102.70	109.40	14.40
Oldland Soil	4.87	7.93	77.90	111.40	151.40	20.10

The soil was left in the sun to dry for three days and then sifted through a 2 mm sieve. Plastic pots, 160 mm in height and 195 mm in diameter, with holes in the base for drainage, were filled with 2.2 kg of each type of soil. The milled weed materials were incorporated into the soil on a 1% (m/m) basis, and again 1.5 months into the experiment. Therefore the total weed material added was 2% (m/m). No weed material was present in the control. The weed residue concentration of 2% was estimated to represent a field infestation of approximately 90 mature plants m^{-2}.

Experimental design

The experiment consisted of six treatments:

- Control, with no weed material added;
- Incorporated *B. pilosa* material into both types of soil, and
- Incorporated *C. albida* material into both types of soil.

The same batch of *P. patula* seedlings as in Exp.1 was used. A single *P. patula* seedling was transplanted to each pot early in February 1996, a day after the pots were filled with soil. The seedlings received 250 ml of the same complete nutrient solution every second day. Growth parameters were the same as in Exp. 1. The seedlings were harvested and the foliage dry mass determined, twelve weeks after seedling transplant.

Ectomycorrhizal colonization was measured, as described for Exp. 1.

Statistical analysis

Each treatment was replicated ten times in a factorial design with factors treatment and soil type. Beginning height and stem diameter data of seedlings growing on afforested soil required transformation to squared values and analysis of variance was conducted on the transformed data.

4. Results and Discussion

Experiment 1: Influence of *C. esculentus* residues on growth of *P. patula* seedlings and mycorrhizal colonization

1. Height

Data for the percentage increase in seedling height are not presented as there were no significant effects (ANOVA appears in Table 1 in Appendix A).

2. Stem diameter

The control seedlings had a significantly higher growth rate than seedlings watered with *C. esculentus* root leachate (Table 2). The differences in percentage stem diameter increase between the two weed treatments were not significant. Seedlings treated with root leachate of *C. esculentus* had a significantly greater stem diameter than the other treatments at the onset of the experiment. Despite this, the distinct significant growth difference between plants exposed to the root leachate of *C. esculentus* and the control plants strongly suggests the presence of inhibitory compounds that were released from the weed material into the soil.

Table 2 Stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments (ANOVA in Table 2, 3 and 4 in Appendix A)

Treatment	Final Diameter (mm)	Initial Diameter (mm)	Growth Increase (%)
Control	11.50 a	1.38 b	740.20 a
Applied leachate	11.50 a	1.63 a	611.76 b
Incorporated material	10.54 b	1.41 b	670.25 ab
Standard error	0.189	0.048	30.94
CV (%)	6.55	12.68	17.95

Means followed by the same letter are not significantly different at P=0.05.

Allelochemicals were probably continuously applied to the pine seedlings through the leachate, while with the incorporated material, once it was degraded, it was not applied again. Also different types of allelochemicals and/or varying concentrations of these compounds could have been involved. This could explain the difference in growth inhibition between the weed treatments.

Information on the allelopathic abilities of *C. esculentus* and *C. rotundus* (purple nutsedge) are available. Tames, Getso & Vieitez (1973) and Drost & Doll (1980) concluded that compounds in *C. esculentus* extracts were inhibitory to seed germination and growth of certain crop species. Meissner, Nel & Smit (1979) grew *C. rotundus* in sterilised, well-fertilized soil and showed that the growth of *H. vulgaris* (barley), *Cucumis sativus* (cucumber) and *Lycopersicum esculentum* (tomato) were considerably reduced after the weed was removed. *C. esculentus* was near maturity when collected for incorporation into the soil, while the leachate was periodically obtained from plants growing actively. Growth stage can be vital for the release of growth-inhibiting allelochemicals. Yakle & Cruse (1984) stated that large quantities of crop residues are left on the soil surface due to non-tillage, but that reductions in maize root and shoot mass were more pronounced when fresh residues were incorporated.

3. Dry mass

Data for the dry mass of seedlings are not presented as differences between the means were not significant (ANOVA in Table 5 in Appendix A).

4. Ectomycorrhizal growth

The presence of *C. esculentus* residues and the application of root leachate from live plants significantly inhibited colonization of ectomycorrhizae on roots of *P. patula* seedlings (Table 3). The treatment effect was significant in the case of each EM morphological type.

Table 3 Ectomycorrhizal colonization of *Pinus patula* roots in soil containing root leachate or leaf material (2% m/m) of *Cyperus esculentus*

	EM morphological types cm^{-1} root				
Treatment	**Monopodial**	**Bifurcated**	**Coralloid (3-5 tips)**	**Coralloid (>5 tips)**	**Rated Total ***
Control	2.44 ab	1.72 b	0.88 a	2.68 a	20.98 b
Leachate	1.80 b	3.00 b	0.96 a	0.52 b	9.33 a
Leaf tissue	3.52 a	4.60 a	0.28 b	0.20 b	8.28 a

Means sharing a common letter are not significantly different at P=0.05

* EM rating: Monopodial = 0.5 x value; Bifurcated = 1.0 x value; Coralloid 3-5= 2.5 x value
> 5= 6.0 x value

According to Perry & Choquette (1987), allelopathic effects on ectomycorrhizae vary not only with allelochemical concentration, but are highly specific to fungal species and the nature of the allelochemical. They found that different water soluble leachates from various types of plant litter and at different concentrations significantly inhibited ectomycorrhizae colonization. Several fulvic acids and soil extracts stimulated growth of *Pisolithus tinctorius*, a fungus that was also inhibited by leachates from different plant litters. Chu-Chou (1978) found that water extracts of old *P. radiata* roots completely inhibited the growth of a mycorrhizal fungus associated with the pine species.

The significant inhibition of the mycorrhizal growth on the roots of the *P. patula* seedlings in the present study can probably be attributed to the presence of allelochemicals in the incorporated *C. esculentus* material and those contained in the root leachate. The inhibition of *P. patula* seedling growth on oldlands could therefore be indirectly due to insufficient colonization of mycorrhizae and thus be ascribed to the loss of benefits provided by the symbiont to the tree species.

<u>Experiment 2</u>: Influence of incorporated residues of *B .pilosa* and *C. albida* on the growth of *P. patula* seedlings and mycorrhizal root colonization on two types of soil

1. Height

The interaction Treatment x Soil type was not significant for *P. patula* height increases (ANOVA appears in Table 6 in Appendix A). The percentage height increase of *P. patula* seedlings was significantly influenced by the incorporation of *B. pilosa* and *C. albida* on both types of soil (Tables 4 and 5).

Table 4 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage height increase of *Pinus patula* seedlings on afforested soil (ANOVA for each parameter presented appears in Table 7, 8 and 9 of Appendix A, respectively)

	Final Height (mm)	Initial Height (mm)	Growth Increase %
Control	20.20 b	9.81 b	102.40 b
Bidens pilosa	26.65 a	12.90 a	106.29 b
Conyza albida	19.34 b	7.80 c	150.13 a
Standard Error	1.55	0.43	14.90
CV (%)	22.29	24.81	36.39

Means followed by the same letter are not significantly different at P=0.05.

Table 5 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage height increase of *Pinus patula* seedlings on oldland soil (ANOVA for each parameter presented appears in Table 10, 11 and 12 in Appendix A, respectively)

	Final Height (mm)	Initial Height (mm)	Growth Increase %
Control	15.89 b	10.43 b	53.34 b
Bidens pilosa	20.30 a	11.65 a	74.18 ab
Conyza albida	17.94 ab	9.55 c	87.01 a
Standard Error	0.92	0.22	7.61
CV (%)	15.28	6.62	32.38

Means followed by the same letter are not significantly different at P=0.05.

Incorporated *C. albida* material significantly increased the height growth of *P. patula* seedlings on both soil types (Table 4 and 5). Although not significant, *B. pilosa* also showed a tendency to increase growth. According to Rice (1984), allelopathy encompasses both stimulatory and inhibitory effects, depending on the concentration of allelochemicals present. If the concentration is low, growth increase may occur, while at a higher concentration the same allelochemicals can cause inhibition of growth. Based on current data the foregoing explanation seems the most plausible for the effects shown in Tables 4 and 5, although it is realized that only much more fundamental work could provide concrete evidence of actual growth stimulatory allelopathic effects.

2. Stem diameter

The interaction Treatment X Soil type was not significant (ANOVA appears in Table 13 in Appendix A). As shown in Table 6 and 7, the incorporated weed material significantly increased the stem diameter of the pine seedlings.

Table 6 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage stem diameter increase of *Pinus patula* seedlings on afforested soil (ANOVA for each parameter presented appears in Table 14, 15 and 16 in Appendix A, respectively)

	Final Diameter (mm)	Initial Diameter (mm)	Growth Increase %
Control	8.43 b	1.43 a	489.83 b
Bidens pilosa	9.81 a	1.15 b	539.55 b
Conyza albida	9.00 b	1.55 a	694.66 a
Standard Error	0.22	0.04	22.49
CV (%)	7.68	12.41	12.41

Means followed by the same letter are not significantly different at P=0.05.

Table 7 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage stem diameter increase of *Pinus patula* seedlings on oldland soil (ANOVA for each parameter presented appears in Table 17, 18 and 19 in Appendix A, respectively)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase %
Control	6.86 b	1.42	394.08 b
Bidens pilosa	8.83 a	1.48	514.41 a
Conyza albida	9.28 a	1.35	554.02 a
Standard Error	0.24	ns	26.72
CV (%)	8.71	13.70	16.56

Means followed by the same letter are not significantly different at P=0.05.

The incorporated *C. albida* and *B. pilosa* material caused significant seedling stem diameter increases compared to the control. Meissner, Nel & Beyers (1986) reported the inhibitory effect of *B. pilosa* on certain crop species. The significance of the increase in growth is that it indicates that the inhibitory compounds were either absent or present at concentrations too low to retard growth of seedlings.

Small differences in the nutrient status of the two types of soil (Table 8), which were measured at the end of the trial, are unlikely to have been responsible for the difference in growth between the different treatments (Table 4, 5 ,6 and 7).

Table 8 Soil analysis for the two different types of soil after seedlings grew for a period of twelve weeks

Soil	pH (H_2O)	Bray I P mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Na mg kg^{-1}
Afforested					
Control	4.57	24.23	299.5	930.6	128.4
Bidens pilosa	4.72	28.96	141.5	926.6	126.9
Conyza albida	4.62	28.63	302.5	459.6	121.3
Oldland					
Control	4.42	16.62	296.5	1004.6	124.1
Bidens pilosa	4.62	18.58	318.5	521.6	124.2
Conyza albida	4.66	29.21	357.5	884.6	113.2

3. Dry mass

Only main effects were significant (ANOVA appears in Table 20 in Appendix A). A significant greater dry mass was obtained for pine seedlings with incorporated weed material on both soils (Table 9). This is in accordance with height and diameter data obtained.

Table 9 Dry mass for *Pinus patula* seedlings growing on two types of soil with incorporated *Bidens pilosa* and *Conyza albida* foliage material (ANOVA in Table 21 and 22 in Appendix A)

	Foliage Dry Mass	
	Afforested Soil	**Oldland Soil**
Control	15.72 b	10.15 b
Bidens pilosa	23.64 a	18.89 a
Conyza albida	19.51a	17.18 a
Standard Error	1.125	0.588
CV (%)	18.12	12.22

Means sharing a common letter are not significantly different at P=0.05

4. Ectomycorrhizal growth

Only main effects were significant. The effects the weed treatments had on mycorrhizal growth are shown in Table 10.

Table 10 Ectomycorrhizal colonization of *Pinus patula* roots on both afforested and oldland soil containing *Bidens pilosa* and *Conyza albida* residues (ANOVA appears in Tables 23 and 24, Appendix A)

	EM morphological types cm^{-1} root	
	Afforested Soil	**Oldland Soil**
Control	15.3 a	11.2
Bidens pilosa	8.9 b	8.9
Conyza albida	7.7 b	9.7
Standard Error	3.95	NS
CV (%)	37.2	

Means sharing a common letter are not significantly different at P=0.05

The incorporated weed foliage had a significant inhibiting effect on mycorrhizal growth on both types of soil. Due to optimal growth conditions, the inhibition of mycorrhizae probably did not manifest in poor pine growth. As mentioned earlier, mycorrhizae help the host acquire nutrients and water, and it is thus conceivable that, with optimal supply of both growth factors, the benefits of mycorrhizae would have been masked in the present study. It is evident that both weed treatments suppressed the EM colonization on pine roots.

5. Conclusions

Incorporation of residues of different weed species had different effects on the growth of pine seedlings. *C. esculentus* was deleterious towards pine growth, while *B. pilosa* and *C. albida* were not. It is therefore likely that inhibition of seedling growth can be expected in new plantations where *C. esculentus* has grown for some time. However, it is also possible that, with time, *B. pilosa* and *C. albida* could also inhibit pine growth, given situations where weed infestations have become well established over several years. Inhibitory effects may be more pronounced in the field situation where, with every growth season, residues build up. Because all the weed treatments significantly inhibited EM colonization on pine roots, it is suggested that pine seedling growth will be indirectly impeded as a result, particularly under field conditions where the bionts' vitality is closely linked. It is concluded that compounds released from the foliage of *C. esculentus* were responsible for inhibition of both seedling growth and mycorrhizal colonization on seedling roots, but the possible stimulatory effect of *B. pilosa* and *C. albida* warrants further investigation.

6. References

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Appendix A

Cyperus esculentus

1.Height

Table 1 Analysis of variance of the percentage height increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	1867.696	933.848	0.13	0.8817
Error	42	310591.773	7395.042		
Total	44	312459.569			

R^2.100 = coefficient of determination

R^2 = 0.0600

2.Diameter

Table 2 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	124055.868	62027.934	4.24	0.0211
Error	42	614928.461	14641.154		
Total	44	738984.330			

R^2 = 0.1679

Table 3 Analysis of variance of the initial stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	0.556	0.278	7.97	0.0012
Error	42	1.467	0.035		
Total	44	2.023			

R^2 = 0.2750

Table 4 Analysis of variance of the final stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	9.152	4.576	8.53	0.0008
Error	42	22.539	0.537		
Total	44	31.691			

R^2 = 0.2888

3. Mass

Table 5 Analysis of variance of seedling dry mass due to different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	20.725	10.363	0.67	0.5189
Error	42	653.075	15.549		
Total	44	673.800			

R^2 = 0.0308

Bidens pilosa and Conyza albida

1. Height

Table 6 Analysis of variance of *Pinus patula* seedling height increase on both types of soil growing containing incorporated *Bidens pilosa* and *Conyza albida* material

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	22191.278	11095.134	9.23	0.0004
Time	1	28833.274	28833.274	23.98	0.0001
Treatment X Soil	2	2520.806	1260.403	1.05	0.3579
Error	52	62532.349	1202.545		
Total	57	116076.708			

R^2 = 0.4613

Table 7 Analysis of variance of the initial height of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	58333.231	29166.615	39.47	0.0001
Error	27	19952.223	738.971		
Total	29	78285.454			

R^2 = 0.7451

Table 8 Analysis of variance of the final height of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	319.261	159.630	6.60	0.0046
Error	27	653.269	24.195		
Total	29	972.530			

R^2 = 0.3283

Table 9 Analysis of variance of the percentage height increase of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	14048.999	7024.499	3.16	0.0583
Error	27	59946.898	2220.255		
Total	29	73995.897			

R^2 = 0.1899

Table 10 Analysis of variance of the initial height of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	22.254	11.127	22.88	0.0001
Error	27	13.131	0.486		
Total	29	25.285			

R^2 = 0.6289

Table 11 Analysis of variance of the final height of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	92.130	46.065	6.11	0.0069
Error	25	188.521	7.541		
Total	27	280.651			

$R^2 = 0.3283$

Table 12 Analysis of variance of the percentage height increase of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	5516.121	2758.060	5.29	0.0121
Error	25	13035.331	521.413		
Total	27	1855.452			

$R^2 = 0.2973$

2. Diameter

Table 13 Analysis of variance of *Pinus patula* seedling stem diameter increase on both types of soil growing containing incorporated *Bidens pilosa* and *Conyza albida* material

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	337696.614	168848.307	29.54	0.0001
Time	1	110392.796	110392.796	19.31	0.0001
TreatmentxSoil	2	32137.941	16068.970	2.81	0.0693
Error	52	297214.201	5715.658		
Total	57	777441.551			

$R^2 = 0.6178$

Table 14 Analysis of variance of the initial stem diameter of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	214801.190	107400.595	21.23	0.0001
Error	27	136605.156	5059.450		
Total	29	351406.346			

R^2 = 0.6113

Table 15 Analysis of variance of the final stem diameter of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	9.618	4.809	9.89	0.0006
Error	27	13.130	0.486		
Total	29	22.748			

R^2 = 0.4229

Table 16 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	214801.190	107400.595	21.23	0.0001
Error	27	136605.156	5059.450		
Total	29	351406.346			

R^2 = 0.6113

Table 17 Analysis of variance of the initial stem diameter of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	0.0847	0.042	1.12	0.3397
Error	27	1.017	0.038		
Total	29	1.102			

R^2 = 0.0769

Table 18 Analysis of variance of the final stem diameter of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	31.878	15.939	30.70	0.0001
Error	25	12.980	0.519		
Total	27	44.858			

$R^2 = 0.7106$

Table 19 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	133289.497	66644.748	10.37	0.0005
Error	25	160677.163	6427.087		
Total	27	293966.670			

$R^2 = 0.4534$

3. Mass

Table 20 Analysis of variance of *Pinus patula* seedling dry mass on both types of soil containing incorporated *Bidens pilosa* and *Conyza albida* material

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	712.605	356.303	43.30	0.001
Soil	1	267.267	267.267	32.48	0.001
TreatmentxSoil	2	28.477	14.238	1.73	0.187
Error	52	427.860	8.228		
Total	57	1434.195			

$R^2 = 0.701$

Table 21 Analysis of variance of seedling dry mass pine seedlings growing on oldland soil containing incorporated *Bidens pilosa* and *Conyza albida*

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	413.198	206.599	59.70	0.0001
Error	25	86.511	3.460		
Total	27	499.710			

R^2 = 0.8268

Table 22 Analysis of variance of seedling dry mass pine seedlings growing on afforested soil containing incorporated *Bidens pilosa* and *Conyza albida* foliage

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	313.825	156.912	12.41	0.0002
Error	27	341.509	12.648		
Total	29	655.334			

R^2 = 0.4789

Table 23 Analysis of variance of ectomycorrhizal colonization of *Pinus patula* roots in afforested soil containing *Bidens pilosa* and *Conyza albida* residues

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	168.11	84.06	5.40	0.021
Error	12	186.93	15.58		
Total	14	355.04			

R^2 = 0.569

Table 24 Analysis of variance of ectomycorrhizal colonization of *Pinus patula* roots in oldland soil containing *Bidens pilosa* and *Conyza albida* residues

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	13.63	6.82	0.28	0.757
Error	12	287.32	23.94		
Total	14	300.95			

R^2 = 0.661