Chapter 3

Growth stage of *Cyperus esculentus* influences its allelopathic effects on ectomycorrhizal growth and higher plant species

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1. Introduction

The genus *Cyperus* comprises over 600 species, which occur mainly in tropical and temperate regions of the world, causing large reductions in crop yields (Holm, *et al.*, 1977; Gifford & Bayer, 1995). *Cyperus esculentus* (yellow nutsedge) is a herbaceous perennial weed that is characterized by prolific vegetative activity which produces a complex underground system of rhizomes and tubers (Gifford & Bayer, 1995). It can spread asexually by the formation of rhizomes that end in the production of underground tubers (Wills, Hoagland & Paul, 1980; Stoller & Sweet, 1987; Gifford & Bayer, 1995). Tubers are recognized as the primary dispersal unit. Flowering is variable and many populations do not flower after a cropping season's growth. Sometimes mature, viable seed will develop, but seedlings from seed apparently lack the vigour required for survival (Stoller & Sweet, 1987).

Stoller, Wax & Slife (1979) investigated the competition effect of *C. esculentus* with *Zea mays* (maize). An 8% yield reduction was achieved for every 100 shoots m^{-2}. Yield reduction of 41% occurred when no weed control was done on a field initially infested with 1200 shoots m^{-2}.

C. esculentus and *C. rotundus* (purple nutsedge) are also known for their allelopathic abilities. Drost & Doll (1980) concluded that extracts and residues of *C. esculentus* have an inhibitory effect on growth of *Glycine max* (soyabeans) and *Z. mays*

(maize). Tames, Getso & Vieitez (1973) found compounds in *C. esculentus* tubers that were inhibitory to *Avena fatua* (oat) coleoptiles and seed germination of other crops. Horowitz & Friedman (1971) dried *C. esculentus* tubers and mixed them with soil. The root and top growth of *Hordeum vulgaris* (barley) planted in the soil were significantly reduced. Meissner, Nel & Smith (1979) grew *C. rotundus* in sterilized, 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 and the crops established.

According to Graham (1988), mycorrhizal colonization begins after seedling germination, when the radicle is growing rapidly. Several researchers have reported that mycorrhizae help plants to acquire mineral nutrients from the soil, especially immobile elements such as phosphorous (P), zinc (Zn) and copper (Cu) (Marais, 1974; Schenck, 1981; Tinker, 1984; Graham, 1988). Marx & Bryan (1971), concluded that mycorrhizae can further reduce transplant injury and help plants to withstand high temperatures. It is also clear from research done by Marx (1973) that ectomycorrhizae protect trees from root pathogen infection. Robinson (1972) demonstrated that run-off from roots of living and raw humus of *Calluna vulgaris* (heather) contained a factor toxic to several mycorrhizal fungi. The data also showed that the inhibitor may have prevented infection of *Calluna* by certain pathogenic fungi. Aqueous extracts of *Populus temula* leaves inhibited the growth of several species of *Boletus*, a mycorrhizal fungus. They also have a weaker inhibitory effect on litter-decomposing species of *Marasmius*. The inhibitors was identified as catechol and benzoic acid.

According to Rice (1995), phenols, benzoic acid and derivates have a mixed origin. Some compounds originate directly from dehydoshikimic acid, others from acetate, but apparently most are derived from cinnamic acid. These compounds have been the most commonly identified allelopathic chemicals produced by higher plants. *p*-Hydroxybenzoic acid and vanillic acid are the most commonly identified benzoic acid derivates involved in allelopathy.

2. Aims

1. To verify that *C. esculentus* is inhibitory towards ectomycorrhizal growth.
2. To investigate the effects of aqueous extracts of *C. esculentus* of varying age on seed germination and early seedling growth of certain crop species.
3. To evaluate the effect of compounds identified in *C. esculentus* on seed germination and early seedling development.

3. Materials and Methods

Experiment 1: Effect of aqueous extracts of *C. esculentus* tubers and parts of varying age on ectomycorrhizal growth

Preparation of extracts

In May 1998, *C. esculentus* foliage and tubers were collected at the Giant's Castle Estate of Mondi Forests (Pty) Ltd. KwaZulu-Natal province (29°59'S; 29°12'E; altitude 1400m). Foliage from immature and mature plants were kept fresh by cooling it and cutting it separately into 20 mm lengths. Fifty grammes of foliage material and tubers were mixed with 1000 ml distilled water and macerated in Waring Commercial blender for 30 seconds. The suspension were filtered through Whatman no. 1 filter paper and the supernatant was used for testing allelopathic potential.

Ectomycorrhizal growth

A modified Melin-Norkrans (MMN) agar medium (Marx, 1969) was prepared for mycorrhizal growth. After autoclaving the mixture for 20 minutes at 121 °C, the pH of the medium was adjusted to between 5.5 and 5.7. Sterilized petri dishes (90 mm in diameter) were filled with 30 ml of MMN medium. One method involved placing the ectomycorrhiza (EM) inoculum (*Boletus maxaria*) in the middle and applying 1 ml of the different *C. esculentus* extracts, filtered through a 0.2 μm micropore filter and a sterilized syringe, around it in four holes in the agar medium. In the second method, 1 ml extract was spread evenly over the agar surface and the inoculum placed in the middle. For the control, 1 ml of distilled water was applied. After three weeks the colony diameter was calculated, diagonally and across.

Statistical analysis

A factorial design (2 X 4) design was used with two application methods and four *C. esculentus* growth stages. Each treatment was replicated five times. Analysis of variance was done to determine the effect of the different *C. esculentus* extracts on the growth of the EM. Mean separation was done by the Least Significant Difference (LSD) test at P=0.05.

Experiment 2: Effect of aqueous extracts of *C. esculentus* tubers and plants of varying age on the germination of *L. sativa*

Preparation of extracts

The same weed material, extraction method and treatments were used for this experiment as described above. Two extract concentrations (2% and 5% (m/v)) were used.

Seed germination test

Thirty *L. sativa* seeds were randomly placed on two pieces of Whatman no. 1 filter paper in a covered petri dish, 90 mm in diameter. Treatments were kept moist by applying 5 ml of the appropriate extract once, while the control only received 5 ml of distilled water. All treatments were incubated in a growth chamber at the University of Pretoria's phytotron at a day/night temperature regime of 30/20°C with a 12/12h day/night interval. Each day, for a week, the number of seeds that had germinated in each treatment was recorded and expressed as a percentage of the total number of seeds sown. Germination was considered to be when the radicle had extended at least 2 mm.

Statistical analysis

A factorial design (2 X 4) design was used with two extract concentrations and four *C. esculentus* growth stages. A completely randomized design was used with five replicates. Analysis of variance was done on data expressed as percentage seed that had germinated. Germination data for the 2% extract concentration required transformation to logarithm values and analysis of variance was conducted on transformed data. Mean separation was done by the Least Significant Difference (LSD) test at P=0.05.

Experiment 3: Effect of extracts of *C. esculentus* tubers and plants of varying age on the growth of *L. sativa*

Preparation of extracts

The same extract procedure and treatments were used as described in Exp. 2. Only the 5% extract concentration was tested.

Growing seedlings

L. sativa var. Great Lakes was used as test species. Water was used as growth

medium to eliminate the potential confounding influence of soil factors. *P. patula* seedlings were not used because they are not suited for use in hydroponics. Plastic pots (125 mm diameter) were lined with plastic bags and filled with 800 ml of a complete nutrient solution (Nitch, 1972).

The pots were kept in a temperature-controlled glasshouse (25/15°C) at the University of Pretoria's phytotron. *L. sativa* seedlings, approximately two weeks old, were carefully removed from the seed trays so as not to disturb the root system and washed in water to remove the growth medium. Roots of *L. sativa* seedlings were then suspended in the nutrient solution through a polystyrene lid fitting on the pot. The combination of seedling, lid and nutrient solution was weighed to ascertain the initial mass of the test system. After a week of growth, the whole system was weighed every second day. A maximum of 50 ml of the different extracts was applied to replace the water lost through evaporation/transpiration, and the balance was made up with nutrient solution. At the end of the growth period (14 days), the plants' fresh and dry foliage mass were determined. The seedlings were dried in an oven at 70°C for 72 h and their individual mass recorded.

Statistical analysis

Each treatment was replicated ten times. A completely randomized design was used. Analysis of variance was done to determine the effect of the different extracts on the fresh and dry mass of the test species. Mean separation was done by the Least Significant Difference test at (P=0.05).

Experiment 4: Effect of *C. esculentus* on the emergence rate of *Z. mays* on soil

Growth of seedlings

In October 1998, pots (195 mm diameter x 200 mm deep), with holes in the base

for drainage, were filled with 2.5 kg soil collected at the University of Pretoria's Hatfield experimental farm. The soil was classified as a sandy loam. The first and second treatment involved sowing *C. esculentus* tubers and leaving it for 28 days to grow (day minus 28). In treatment one, the tubers were removed when the maize seed was planted (day 0). In the second treatment, the *C. esculentus* plants were left undisturbed when the maize seed was planted. The *C. esculentus* tubers and maize seed were planted at the same day (day 0) and left for 28 days to grow in treatment four, before the *C. esculentus* plants were removed. In the fourth treatment the maize and *C. esculentus* tubers were also planted on the same day (day 0), but the weed was allowed to grow undisturbed. No *C. esculentus* plants or material were present in the soil of the control treatment at any stage. All pots received 250 ml of a complete nutrient solution (Nitch, 1972), on alternate days, for the duration of the trail. Crop emergence in each treatment was measured by counting the number of coleoptiles that had emerged from the soil. Measurements commenced 8 days after seeding of the crop, *i.e.* on the day the first coleoptiles emerged from the soil, and were made on each of the next three days. Data were expressed as the percentage seedlings that emerged out of a total number of seeds sown in each pot.

Statistical analysis

Each treatment was replicated 10 times in a completely randomized design. Data for the emergence of seeds sown were subjected to analysis of variance. Mean separation was done by the Least Significant Difference (LSD) test at P=0.05.

Experiment 5: Influence of an allelochemical identified in *C. esculentus* growth media on the germination of *L. sativa* and *Z. mays*

Preparation of extracts

The aim was to identify natural compounds in *C. esculentus* which could be biologically active against *P. patula* or its associated mycorrhizae. Four soil samples were studied. They were respectively termed:

A = Quartz sand in which *C. esculentus* was grown;

B = Oldland soil kept weed-free;

C = Oldland soil with no weed control;

D = Oldland soil with only *C. esculentus* present.

Each sample was separated into four parts and extracted with water and ethanol. Subsequently, biochemical analysis and biological tests were done.

I. Extractions:

1.1 Water extraction

A sample of 100 g dry soil or quartz sand was stirred at 100 rpm for 2 min. in 400 ml of demineralized water, and then incubated (dark, 15°C) for 14 hours. After filtration, a part of the extract was used for chemical analysis. It was mixed with 150 ml of ether and the supernatant collected. This operation was performed three times. The etheric fraction was then evaporated at room temperature during the night and compounds were solubilized in 5 ml acetaldehyde for analysis.

1.2 Ethanol extraction

Twenty grammes of dry soil and 30 g quartz sand were incubated in 50:50 (v/v) methanol:water solution at 35 °C for 20 min. After filtration, the extract was passed through a rotavator in order to remove water and alcohol. Compounds were then solubilized in 5 ml acetaldehyde for analysis.

II. Biochemical analysis

1. Total phenols

Seven ml of the water extract were added to 1 ml of Folin-Ciocalteu and 2 ml Na_2CO_3 and incubated for 20 min at 40°C. Absorbency was then read at 760 nm. Quantities were obtained with standard gallic acid.

2. H.P.L.C.

This method gave the identity and quantity of phenolic compounds. The compounds identified in the water extract are presented in Table 1. The data for the ethanolic extracts are not presented, as the same compounds were identified.

Table 1 Phenolic compounds identified in water extracts of artificial and natural growth media for *C. esculentus*

	Quartz sand µg/g	Oldland weed-free µg/g	Oldland with weeds µg/g	Oldland soil with only *C. esculentus* µg/g
***P*-OHbenzoic acid**	0.012	0.003	0.004	0.008
***P*-OHbenzaldehyde**	0.005	0.001	0.001	0.001
Vanillic acid	0.141	0.056	0.071	0.183
Syringic acid	0.000	0.000	0.015	0.000
Vanillin	0.000	0.014	0.015	0.003
***P*-coumaric**	0.000	0.000	0.000	0.000
Ferulic acid	0.000	0.000	0.000	0.000

Vanillic acid was identified in *C. esculentus* growth media in the highest concentrations and therefore, it was decided to work with this compound. It was obtained from Merck laboratories and a concentration range was prepared with

distilled water, to give: 0, 0.04, 0.08, 0.12, 0.16, 0.2 and 0.24 mg l^{-1}.

Seed germination test

Ten ml of vanillic acid were added to lettuce seeds while 6-ml, 8-ml or 10-ml volumes were applied to the maize seeds. Fifteen lettuce or ten maize seeds were placed on Whatman no. 3 filter paper, in sterilized petri dishes. The maize seeds were surface sterilized with 1.5% sodium hypochlorite for 1 min. All these steps were performed in a laminar flow cabinet. The pH (H_20) of the solutions was between 4.93 and 5.03. All treatments were incubated at 25°C throughout the trail in the dark at the University of Pretoria's phytotron. Seeds were considered germinated when radicles were at least 2 mm in length and also healthy in appearance.

Statistical analysis

Treatments were replicated ten times in a completely randomized design. Data for the number of seeds germinated were subjected to analysis of variance, as described in Experiment 1.

4. Results and Discussion

Experiment 1: Effect of aqueous extracts of *C. esculentus* tubers and parts of varying age on ectomycorrhizal growth

The first method, where *C. esculentus* extracts were applied to holes in the growth medium, was unreliable because the EM grew over the growth medium cavity. Data obtained using the second method appears in Table 2. All the extract treatments caused significant inhibition of EM growth. The inhibition caused by the mature *C. esculentus* plant extracts was significantly greater than that effected by the tuber extracts (Table 2). The influence of the tuber and immature plant extract was not significantly different. This indicates that all the extracts contained

growth inhibitory substance(s).

Table 2 Effect of the different *Cyperus esculentus* extracts on ectomycorrhizal (*Boletus maxaria*) growth on artificial medium (ANOVA appears in Table 1, Appendix B)

Extract	Ectomycorrhizal diameter growth (mm)
Control	7.20 a
Tubers	6.33 b
Immature plants	6.00 bc
Mature plants	5.83 c
Standard Error	0.141
CV (%)	9.92

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

The inhibition of growth of *B. maxaria* in the present study was less pronounced than that reported by Reinhardt, Khalil, Labuschagne, Claassens & Bezuidenhout (1996) for unidentified mycorrhizal types found on the roots of *P. patula* seedlings, which were exposed to *C. esculentus* and two other agronomic weeds. They concluded that the inhibition of mycorrhizae, which are essential for successful pine establishment (Marais, 1974), represents an indirect allelopathic effect on the pine seedlings. This may be crucial in determining their resistance to other stress factors.

Bioassays using microorganisms have been employed to evaluate allelochemical effects. Rice (1984) describes such a method, whereby saturated paper disks with material elaborated by marine blue-green algae were used. It was placed on agar

impregnated with yeast and their action, according to the extent of an inhibition zone, was evaluated. Nilson, Hogberg, Zackrisson & Wang (1993) found that aqueous extracts of the shrub *Empetrum hermaphroditum* impaired the growth of the mycorrhiza, *Paxillus involutus*, associated with *Pinus sylvestris* (scots pine). The extract also impaired the mycorrhizal growth in culture.

Experiment 2: Effect of aqueous extracts of *C. esculentus* tubers and plants of varying age on the germination of *L. sativa*

Germination rates for seeds exposed to the different aqueous extracts of *C. esculentus* are shown in Figures 1 and 2.

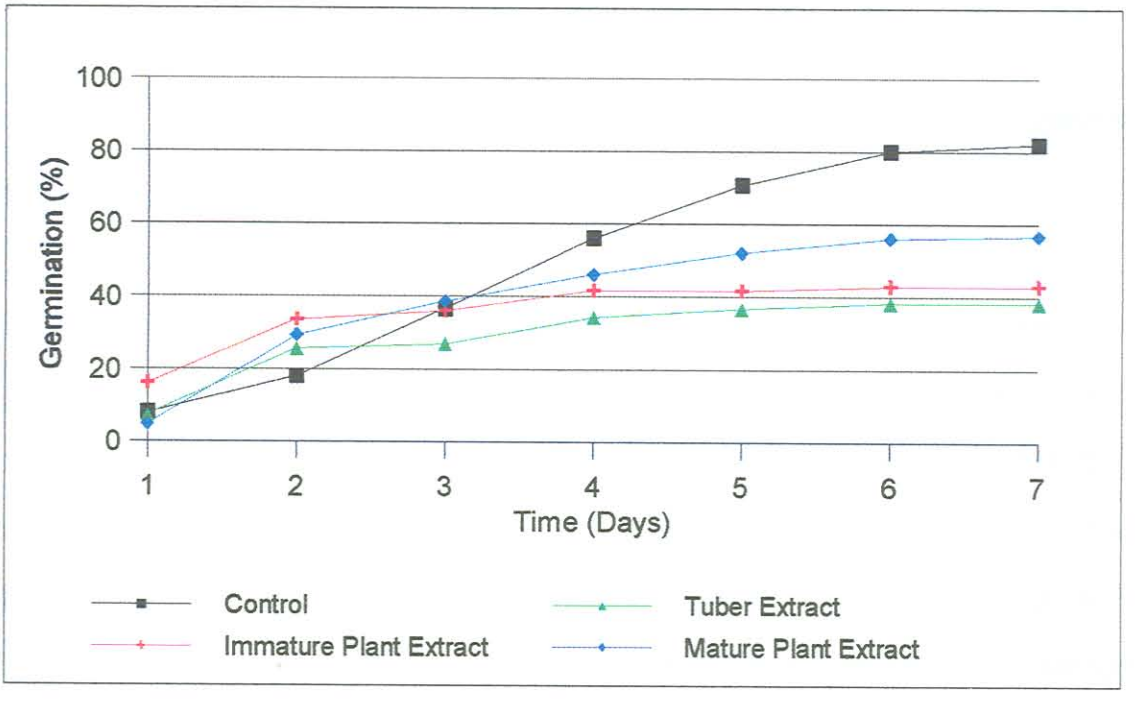

Figure 1 Germination of *Lactuca sativa* seed exposed to different *Cyperus esculentus* extracts at the 2% concentration level

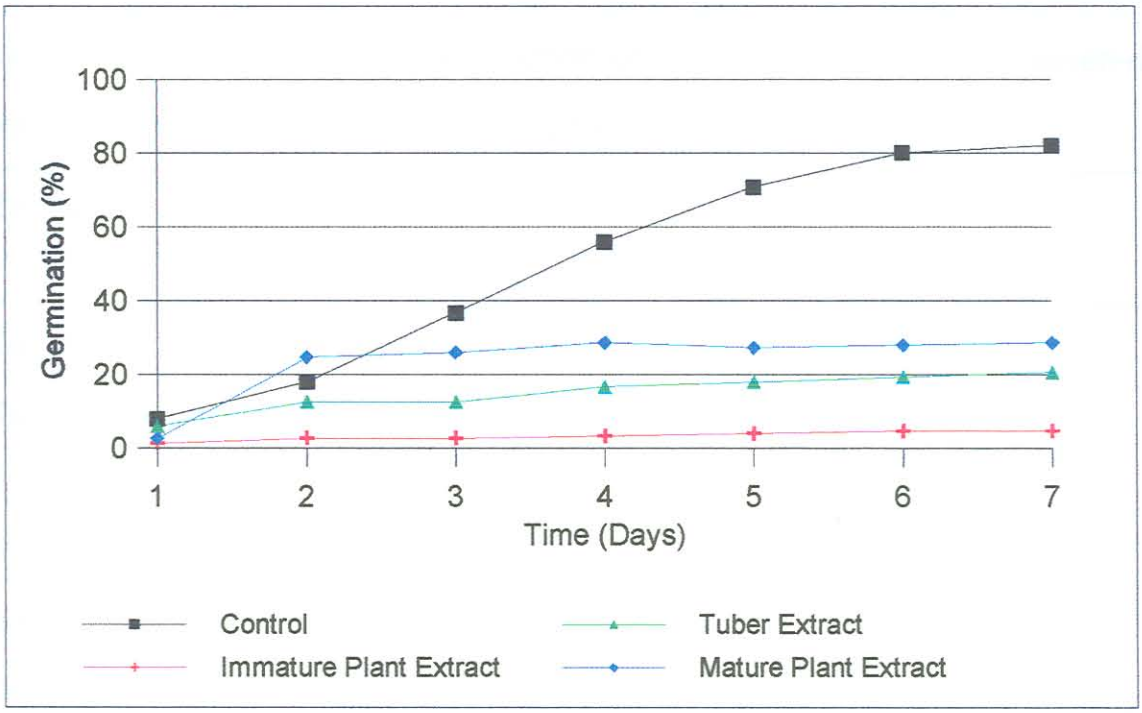

Figure 2 Germination of *Lactuca sativa* seed exposed to different *Cyperus esculentus* extracts at the 5% concentration level

The interaction Concentration X Treatment was not significant. After seven days of exposure to the extracts, at the 2% extract concentration, only the mature plant extract did not significantly inhibit seed germination, while all the 5% extracts significantly inhibited seed germination, compared to the control (Table 3). These observations correspond with the results of Exp. 1. Although not significantly different from the other treatments, the immature *C. esculentus* extract had the most inhibitory effect on seed germination. This shows that the inhibitory compounds were either different or, the same compounds more concentrated in the immature plant material than in the tubers and mature plants.

Table 3 Effect of different concentrations of aqueous *Cyperus esculentus* extracts on the percentage germination of *Lactuca sativa* after a seven-day exposure period (ANOVA appears in Tables 2 and 3, Appendix B)

Extracts	Germination	
	(2%)	(5%)
Control	82.00 a	82.00 a
Tubers	38.00 b	20.67 b
Immature plants	42.67 b	4.67 c
Mature plants	60.67 ab	28.67 b
Standard error	0.225	5.48
CV (%)	18.91	36.06

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

Experiment 3: Effect of extracts of *C. esculentus* tubers and plants of varying age on the growth of *L. sativa*

The effects of the different *C. esculentus* extracts on the fresh and dry mass of *L. sativa* seedlings are presented in Table 4. There were no significant differences among the different *C. esculentus* treatments. All extracts significantly reduced the fresh and dry matter yield of the *L. sativa* seedlings relative to the control. In contrast to the germination results, there were no significant differences in the effect of the immature plant extract compared with the other plant part extracts, although it caused the lowest fresh and dry mass yield of the test species. This could be because the enzymatic processes involved in germination are more sensitive to the extracts than seedling development (Putnam, 1985). Brandsæter & Haugland (1999) concluded that the volume of extracts and distilled water used in bioassays influenced the results considerably and hence the conclusion of studies

where bioassays are used.

Table 4 Effects of different aqueous *Cyperus esculentus* extracts on the fresh and dry mass of *Lactuca sativa* seedlings (ANOVA appears in Table 4, Appendix B)

Extracts	Fresh Mass (g)	Dry Mass (g)
Control	93.36 a	8.26 a
Tubers	40.53 b	4.31 b
Immature plants	34.02 b	3.43 b
Mature plants	47.65 b	4.64 b
Standard error	5.92	0.56
CV (%)	34.72	34.54

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

Experiment 4: Effect of *C. esculentus* on the emergence rate of *Z. mays* on soil

Z. mays emergence was significantly retarded when *C. esculentus* tubers were established 28 days prior to crop seeding and then either totally removed or left (Table 5). This suggests that allelochemicals, which were released into the soil by actively growing *C. esculentus* plants, reduced the rate of emergence and probably the rate of germination as well. The finding demonstrates the need to recognize the risk of poor crop establishment on fields with current or recent infestations of the weed. Weed tubers planted on the day of crop sowing did not affect the rate of crop emergence. This is in accordance with the findings of Meissner, Nel & Smith (1979). They found that the growth of *H. vulgaris* (barley), *Cucumis sativus* (cucumber) and *Lycopersicum esculentum* (tomato) were considerably reduced when, after *C. rotundus* grew in sterilized, well-fertilized soil, the weed was removed.

Burgos & Talbert (1996) reported an emergence reduction of 43-63% of *Zea mays* var. *Rugosa* when the crop was planted into residues of allelopathic rye and wheat. According to Tollenaar, Mihajlovic & Vyn (1993) removal of the above rye and wheat phytomass before maize planting, generally did not influence the delay in development and reduction in yield of the subsequent maize crop.

Table 5 Percent *Zea mays* seedlings that emerged from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop (Day=0) (ANOVA for each day presented appears in Table 6, 7, 8 and 9 of Appendix B, respectively)

Timing of *Cyperus esculentus* tuber planting relative to *Zea mays* seeding	*Cyperus esculentus* presence	Days after seeding			
		7	8	9	10
Control	Absent	90.00 a	95.00 a	95.00 a	95.00 a
Day 0 minus 28*	Undisturbed growth	12.50 b	37.50 b	70.00 b	92.50 a
Day 0 minus 28	Removed on day 0	17.50 b	35.00 b	75.00 b	92.50 a
Day 0 plus 28**	Undisturbed growth	95.00 a	97.50 a	97.50 a	97.50 a
Day 0 plus 28	Removed on day 28	82.50 a	97.50 a	97.50 a	97.50 a
Standard Error		6.739	8.019	7.169	NS
CV (%)		35.82	34.98	26.06	

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

* Day minus 28: Planting of weed tubers, 28 days prior to crop seeding.

** Day 0: Sowing of maize seed and planting of weed tubers.

Experiment 5: Influence of an allelochemical identified in *C. esculentus* growth media on the germination of *L. sativa* and *Z. mays*

Although not significant, there was a tendency for germination inhibition when concentrations of vanillic acid were increased (Table 6). However, some anomalies were observed. Only from 0.16 mg L^{-1} onwards, germination percentages was reduced. Therefore, if the concentration range had included higher concentrations, significant differences could have been possible. According to Willamson & Weidenhamer (1990), it is likely that the toxicity of allelochemicals is a function of both concentration (static availability at a given point in time) and flux rates (dynamic availability based on the total amount of chemical moving in and out of a system over a period of time).

Table 6 Percentage *L. sativa* germination with added vanillic acid (ANOVA appears in Table 10, Appendix B)

Concentration (mg l^{-1})	Germination (%)
0	62.23 ab
0.04	65.56 a
0.08	65.56 a
0.12	60.37 ab
0.16	62.44 ab
0.2	59.24 ab
0.24	53.96 b
Standard error	2.3
CV (%)	20.55

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

The effects of the different volumes and concentrations on the germination of maize are presented in Table 7. Anomalies were apparent at the 0.04 and 0.16 mg l^{-1} concentrations. At the control treatment, the high volume (10 ml) reduced germination significantly compared to the lowest volume (6 ml). It is concluded that the volume of solution the seeds were exposed to had a greater effect on germination than the concentrations of vanillic acid tested.

Table 7 Effect of different volumes of the concentration range of vanillic acid on the percentage germination of *Zea mays* after three days (ANOVA appears in table 11, Appendix B)

	Volume of solution added		
Concentration	**6ml**	**8ml**	**10ml**
0	84 a	75 ab	54 c
0.04	72ab	74 ab	74 ab
0.08	76 ab	79 ab	60 c
0.12	69 b	81 a	50 c
0.16	79 ab	78 ab	71 ab
0.2	78 a	68 b	49 c
0.24	81 a	83 a	53 c
Standard Error		21.47	
CV (%)		4.81	

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

5. Conclusions

Results of Exp. 1 suggest that inhibitory allelopathic effects from *C. esculentus* on mycorrhizae could be pivotal in interactions between the weed and higher plant

species associated with the symbiont. The growth inhibition on the mycorrhizae associated with *P. patula* could at least partly explain the establishment problems of new pine seedlings on former crop fields infested with *C. esculentus*.

The finding that mature weed extract had a greater inhibitory effect on the mycorrhizal growth than the other extracts was not confirmed in Exp. 2 where lettuce was used as indicator species. This apparent anomaly may, in fact, indicate that differential test species' responses should be considered in allelopathy research. With the elimination of soil as factor in the hydroponics experiment (Exp. 3), it is evident that compounds contained in *C. esculentus* material have an inhibitory effect on the growth of *L. sativa*. Different *C. esculentus* plant parts at different development stages have differential allelopathic effect on mycorrhizae and crop species. It is therefore important that the growth stage of weeds be considered in assessments of their allelopathic potential.

It is impossible to extrapolate results from the maize emergence experiment (Exp. 4 & 5) to field conditions, but it is conceivable that, under conditions favouring the production and release of allelochemicals in high concentrations by *C. esculentus*, maize seedlings would be placed under chemical stress that might weaken their resistance to other environmental stress factors.

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Appendix B

1. Mycorrhizal Growth

Table 1 Analysis of variance of ectomycorrhizae growth on agar treated with different *Cyperus esculentus* extracts

Source	Df	Sum of Square	Mean Squares	F	Pr > F
Model	3	21.307	7.102	17.83	0.0001
Error	72	28.688	0.398		
Total	75	49.995			

R^2.100=coefficient of determination

R^2 = 0.4262

2. Germination test

Table 2 Analysis of variance of *Lactuca sativa* germination with a 2% *Cyperus esculentus* extract concentration applied

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	3.061	1.020	4.03	0.0258
Error	16	4.046	0.253		
Total	19	7.107			

R^2=0.4307

Table 3 Analysis of variance of *Lactuca sativa* germination with a 5% *Cyperus esculentus* extract concentration applied

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	16853.333	5617.778	37.38	0.0001
Error	16	2404.444	150.278		
Total	19	19257.777			

R^2 =0.8751

3. Mass

Table 4 Analysis of variance of the effects of different *Cyperus esculentus* extracts on the fresh mass of *Lactuca sativa* seedlings

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	21702.745	7234.248	20.67	0.0001
Error	36	12599.455	349.984		
Total	39	34302.00			

$R^2 = 0.6327$

Table 5 Analysis of variance of the effects of different *Cyperus esculentus* extracts on the dry mass of *Lactuca sativa* seedlings

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	136.042	45.347	14.28	0.0001
Error	36	114.321	3.176		
Total	39	250.363			

$R^2 = 0.5434$

Table 6 Analysis of variance of the percent *Zea mays* seedlings that emerged seven days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	66925	16731.25	36.84	0.0001
Error	45	20437.5	454.16		
Total	49	87362.5			

$R^2 = 0.823$

Table 7 Analysis of variance of the percent *Zea mays* seedlings that emerged eight days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	43875	10968.75	17.83	0.0001
Error	45	27687.5	615.28		
Total	49	71562.5			

$R^2 = 0.797$

Table 8 Analysis of variance of the percent *Zea mays* seedlings that emerged nine days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	8175	2043.75	4.41	0.006
Error	45	20875	463.89		
Total	49	29050			

$R^2 = 0.775$

Table 9 Analysis of variance of the percent *Zea mays* seedlings that emerged ten days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	250	62.5	0.59	0.956
Error	45	4750	105.56		
Total	49	5000			

$R^2 = 0.767$

Table 10 Analysis of variance of the percentage *L. sativa* germination with added vanillic acid

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Concentration	6	2924.501	487.416	3.07	0.0069
Time	2	78789.191	39394.596	247.88	0.0001
Concentration X Time	12	708.226	59.019	0.37	0.9721
Error	189	30036.626	158.924		
Total	209	112458.545			

$R^2 = 0.732$

Table 11 Analysis of variance of the effect of different volumes of the concentration range of vanillic acid on the germination of *Zea mays* after three days

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Concentration	6	2619.048	436.508	1.89	0.0851
Volume	2	15482.857	7741.429	33.45	0.0001
Concentration X Volume	12	6603.810	550.317	2.38	0.0071
Error	189	43740.000	231.429		
Total	209	68445.714			

$R^2 = 0.361$