BIOLOGICAL AND BIOCHEMICAL INTERACTIONS BETWEEN UNICORN-PLANT (*PROBOSCIDEA LOUISIANICA*) AND COTTON (*GOSSYPIUM HIRSUTUM*)

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INTRODUCTION

Each part of this thesis is a separate manuscript to be submitted for journal publication. Parts I and II are to be submitted to Weed Science, a journal of the Weed Science Society of America. Part III is to be submitted to Weed Technology, a journal of the Weed Science Society of America. Part IV is to be submitted to Plant and Soil, the international journal on plant-soil relationships. Part V is to be submitted to the Journal of Chemical Ecology, the journal of the international society of chemical ecology. Articles in each of these journals are peer reviewed and must report experiments repeated over time and/or space. Because of the latter requirement, some preliminary data previously collected by W. Eugene Thilsted were included in Part I of this thesis.

PART I

GERMINATION AND SEED PRODUCTION OF UNICORN-PLANT (PROBOSCIDEA LOUISIANICA)

Germination and Seed Production of Unicorn-plant (*Proboscidea louisianica*)

Abstract. Field and laboratory experiments were conducted with unicorn-plant to determine conditions required for germination and to measure seed production. Field grown plants produced an average of 122 pods/plant with an average of 71 seed/pod. The highest percent germination from seed harvested in 1980 occurred following a 2 week prechill treatment of 4 C. Seed harvested in 1979 and stored at 4 C had greater germination than seed stored at room temperature of approximately 23 C. Germination increase was greater by removing the seed coat and the membrane enclosing the embryo than by removing the seed coat alone. Germination of seed from all pod compartments were similar. Aqueous extracts of unicorn-plant testa, leaf, stem, root, and exocarp were inhibitory in petri dish bioassays to cotton radicle growth. Extracts of stem, root and exocarp were inhibitory to wheat radicle growth, and extracts of endocarp, leaf, and exocarp were inhibitory to unicorn-plant radicle growth. Seed buried in the field 10 cm deep for 1 to 8 months showed increased germination over time. Germination was lower when seed were stored at 4 C for 1 to 8 months in a soil having 25% (v/v) water. Nomenclature: Unicorn-plant,

Proboscidea louisianica (Mill.) Thell. #[1] PROLO; cotton, Gossypium hirsutum L.; wheat, Triticum aestivum L.

Additional index words. Germination, seed production, germination inhibitors, weed biology, devilsclaw, PROLO

INTRODUCTION

Unicorn-plant, also known as devilsclaw and Ram's horn, is a member of the Martyniaceae. Unicorn-plant is generally tolerant to herbicides applied preplant to cotton[2]. Hand-hoeing or spot treatment with labeled herbicides are usually used to remove plants from cotton fields.

Unicorn-plant is a very efficient competitor with cotton (5). A unicorn-plant density of 1 plant/6 m of row caused a lint yield reduction of 8.4% while 4 plants/6 m of row caused a yield reduction of 33.6%[3]. Higher densities of 32 weeds/10 m of row can cause lint yield reductions up to

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[1]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

[2]Smith, D. T., R. C. Berner, and A. W. Cooley. 1973. Devilsclaw herbicidal control. Texas Agric. Exp. Sta. Prog. Rep. 3202.

[3]Bridges, D. C. and J. M. Chandler. 1984. Devilsclaw and wild okra competition with cotton. Proc. South. Weed Sci. Soc. 37:312.

73% (5). The distance-of-influence of unicorn-plant on cotton lint yields has been reported by some scientists to be at least 1 m^3 while others reported that this distance can extend up to 1.5 m^4

The fruit of the unicorn-plant is a large, crested, long-beaked, drupaceous, three compartment capsule up to 10 cm long (3). As the fruit matures the exocarp sloughs off and the endocarp splits from the apex to the base forming a two-horned claw. The seed are dull black with a corky-tuberculate seed coat (7).

Phillippi and Tyrl (6) reported an average seed production of 42 to 62 seed per fruit, and germination values of freshly harvested seed ranged from 6 to 57 percent. The populations of plants used in their studies occurred in overgrazed pastures and the edges of abandoned corn fields. In the dryland and irrigated cropping areas of West Texas, seed production was in excess of 1000 and 2300 seed/plant, respectively[5].

Although native to the southwestern United States, unicorn-plant now occurs from Florida to California and as

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[4]Mercer, K. L., D. S. Murray, and L. M. Verhalen. 1985. Distance of influence of unicorn-plant (Proboscidea louisianica) on the production of cotton. Proc. South. Weed Sci. Soc. 38:361.

[5]Cooley, A. W., D. T. Smith, and L. E. Clark. 1973. Devilsclaw germination, growth, and competition. Texas Agric. Exp. Sta. Prog. Rep. 3201.

far north as Minnesota. Unicorn-plant appears to be spreading into new areas of the Oklahoma and the severity of existing infestations is increasing. Only limited information is available regarding the seed production and germination of unicorn-plant which each contribute to the spread of this species. Therefore, the objectives of this research were to determine the potential seed production, the conditions required for germination, and evaluate the roll of germination inhibitors to unicorn-plant seed germination.

MATERIALS AND METHODS

Seed and pod production. Seed from a locally collected source were germinated in a greenhouse on May 10, 1980 and transplanted that day in a field on an area 40 m by 50 m with each seedling spaced 1 m apart in a grid system. An overhead irrigation system was used three times to supply water during the summer months to minimize plant water stress. Phosphorus and potassium contents of the soil were tested adequate, but 60 kg/ha N as ammonium nitrate was supplied at planting. The experimental area was kept free of unwanted plants by hoeing and hand pulling. At maturity in late September, 15 of these plants were selected at random to determine the average number pods/plant and seed/pod. Seed from the center compartment and the combined two outer compartments of individual pods were collected and saved separately. These seed were counted and used for

subsequent germination experiments.

Germination. A series of germination experiments were conducted to investigate the effects of age, chemical and mechanical scarification, and prechill exposures. Seed were germinated on two layers of moist absorbent paper held in clear plastic boxes (7 by 7 cm wide and 3 cm deep). Day and night temperatures were 30 and 20 C, respectively, with a 16 h photoperiod with fluorescent light (375 $\mu E \cdot m^{-2} \cdot s^{-1}$). The experimental design was a factorial arrangement of treatments in a randomized complete block design. One box containing 25 seed of each treatment per tray level was a replication and there were four replications. A seed was recorded as germinated when the radicle reached a length of at least 2 mm. Germination data in each experiment were collected at 7 day intervals for 28 days. All experiments were repeated and the results pooled following appropriate statistical tests.

Seed used in the germination studies were hand-collected near Stillwater in 1979 and stored at 4 C and 40% humidity until additional seed could be collected again in 1980. Seed collected in 1980 were stored similarly, but were never stored for more than 16 weeks prior to being used in germination studies. Heavy, well-filled seed were separated from the light or immature seed with a Model B, South Dakota seed blower[6]. Only those seed that were not

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[6]Seedburo Equip. Co., 1022 W. Jackson Blvd., Chicago, IL 60607.

removed by air-flow were utilized. This insured a uniform seed size (by pod compartment).

Scarification. The effect of chemical scarification on seed germination was determined by soaking the seed in concentrated H_2SO_4 (98% pure) for 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32, 64, and 128 min. Following each soaking duration, the seed were washed with tap-water, rinsed in a 50% $NaHCO_3$ solution, rinsed again with tap water, and air dried prior to placement in germination boxes.

Four different methods of manual scarification were evaluated. First, a Forsburg[7] seed scarifier using 50 grit paper was used for intervals of 0, 5, 10, and 20 seconds on lots of 25 seed each. The second method involved a manual scarification with emery cloth. Small lots of unicorn-plant seed were placed on a flat emery cloth surface and gently rubbed with another flat surface faced with emery cloth. The third method involved the removal of the entire outer seed coat by hand removing the seed coat to expose the embryo. These removed embryos were inspected under 5x magnification to assure that they were not injured prior to germination tests. While observing and discarding the injured embryos, it was noticed that the embryos were also enclosed in a thin transparent inner membrane. The fourth scarification method involved the removal of the seed coat (as in method three) and the removal of the thin transparent membrane surrounding the embryo. The membrane was removed

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[7]Forsberg Inc., Thief River Falls, MN 56701.

by soaking the extracted embryo in water for 1 h, puncturing the membrane with a sharp needle, and removing it with tweezers.

Prechill. The 1-year old 1979 seed and freshly collected seed in 1980 were used to determine the effects of prechill on germination. Seed were stored on moistened absorbent paper in the 4 C seed storage room for 0, 2, and 4 weeks in germination boxes prior to being placed in the germinator previously described.

Seedcoat extract. Amounts of 10 g of intact seed which had been stored at 4 C for 2 years were shaken in 110 ml of distilled water for 2 h. The solution was filtered through Whatman No. 1 filter paper and lyophilized. The residue was weighed and redissolved in distilled water to concentrations of 1.0 and 5.0 mg/ml. Ten cotton seed were placed between two layers of Whatman No. 1 filter paper in glass petri dishes and 3 ml of the redissolved extract were added to each dish. The petri dishes containing seed were placed in the dark at 27 C for 72 h. The cotton radicle was measured after 72 h. The experimental design was completely randomized with four replications. The experiment was conducted twice and the data pooled.

Tissue extract. Mature plants (beginning to senesce) were harvested on September 26, 1985 by removing all above ground foliage and carefully digging the roots (primarily the tap root). This plant material was separated into seven plant parts; root, stem, leaf, exocarp, endocarp,

testa, and embryo, air dried for 7 days at 30 C, and ground separately in a Model 4 Wiley Mill[8] to pass a 20 mesh screen. Each ground tissue separate was shaken in 2.5 gm amounts in 97.5 ml of distilled water for 2 h, filtered through Whatman No. 1 filter paper, and centrifuged at 15,900 G for 30 minutes. The pH of the supernatant from each extract were as follows: exocarp, 6.9; endocarp, 6.1; embryo, 6.8; testa, 7.1; leaf, 4.5; stem, 6.4; root, 6.1. Each extract was adjusted to pH 7.0 with 0.2 N NaOH or 0.2 N HCl. Ten seed of either cotton, wheat, or unicorn-plant where placed between two layers of Whatman No. 1 filter paper in petri dishes, and moistened with 3 ml of the filtered tissue extracts. The germination temperatures were 27 C for cotton, 20 C for wheat, and 29 C for unicorn-plant. These temperatures are optimum germination temperatures for each species. Germination and radicle measurements were made 72 h after initiation of the experiments. The experimental design was completely randomized with five replications. A second 2.5 g sample of each tissue separate was extracted as previously described and used as a second run of the experiment. The data were not different between runs and the data were pooled.

Seed burial. Seed harvested on October 15, 1985 were sized with the seed blower previously described and placed into 15 by 10 cm nylon mesh screen bags (1 mm openings, 7 strands of nylon/cm). The screen bags, each containing 25 seed, were

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[8]Authur H. Thomas Company, Philidelphia, PA 19105.

buried 10 cm deep in loamy soil at two locations on October 15, 1985. The soil at the Stillwater location was a Norge loam (Fine-silty, mixed, thermic Udic Paleustoll) and the soil at Chickasha was a Reinach silt loam (Course-silty, mixed, thermic Pachic Haplustoll). In addition, seed were stored at 4 C in 500 ml sealed glass jars containing a loamy sand soil with 25% (v/v) water. On the 15th of every month (beginning in November and ending in June) seed were removed from six jars, and six bags were exhumed from each field location for emergence determinations. To determine emergence, seed were planted into a loamy sand potting soil (80% sand, 15% silt, and 5% clay) to a depth of 1.3 cm and greenhouse temperature of 30 C. Percent emergence was measured after 14 days. The experimental design was a randomized complete block with six replications. At the termination of the experiments in June and following the emergence tests, the seed which did not emerge were separated from the soil by wet sieving. The seed coats of the intact seed were removed as previously described and naked embryos placed in petri dishes between moistened filter paper as previously described. After incubation at 27 C for 72 h, germination measurements of the seed were taken.

All data were subjected to analyses of variance. If there were no significant differences between experiments, the data were pooled. Treatment means were separated based on the least significant difference with a 5% probability

level. Data from the burial study were subjected to regression analysis. The two burial locations, Perkins and Chickasha, were not significantly different, and the data were pooled. Regression analyses were based on mean measurements and linear and quadratic equations were tested for goodness of fit.

RESULTS AND DISCUSSION

Seed and pod production. Unicorn-plants growing without interspecific competition were prolific seed producers. Each plant produced an average of 122 pods/plant with each pod containing an average of 71 seed/pod (data not shown). The center compartment contained an average of 28 seed and the two outer compartments contained 43 seed. Thus, unicorn-plants produced over 8660 seed/plant. The seed production in the present study was higher than those reported by Cooley et al.[7] and Phillipi and Tyrl (6). Greater seed production in these experiments may be partially explained by the irrigations, fertilization, and lack of interspecific competition.

Seed germination. No germination occurred when seed were chemically scarified with sulfuric acid for 0.5 to 128 min (data not shown). Since the unicorn-plant testa is somewhat spongy-rough with ridges and tubercles, acid may have penetrated the seed coat and destroyed the embryo. Germination for unscarified seed from 1979 was 22%. Germination for seed scarified using the Forsburg seed

scarifier for 5, 10, 20 seconds was 13, 11, and 9%, respectively. Mechanical scarification for longer than 20 seconds caused extensive damage to the embryo (cracked or chipped) which resulted in no germination.

There was no difference in the germination due to the compartment in which the unicorn-plant seed were produced. Germination was increased by rubbing the seed against emery cloth and by removing the seed coat and inner membrane (Table 1). Before removing the seed coats only 16% of the seed germinated; however, following seed coat removal, over 60% of the seed germinated. Germination percentage was highest when both the seed coat and the thin, transparent membrane enclosing the embryo were removed or when the seed coat was rubbed against emery cloth. This experiment would indicate that not only is the outer seed coat hindering germination, but also the thin, transparent membrane enclosing the embryo is inhibiting germination.

Prechill. The 2 week prechill treatment gave the highest germination percent when compared to the 4 week prechill or no prechill treatments (Table 2). Freshly collected and year old seed from 1980 exposed to prechill germinated similarly. These findings would indicate that exposing freshly collected unicorn-plant seed to a prechill environment may enhance germination, so that their germination is comparable to 1-year old seed. Again there were no differences in germination between seed from the center or outer capsule compartments with seed collected in

1980. However, seed located in the two outer compartments are tightly enclosed and protected by the tough outer pod covering. This may prevent all seed from a single pod from germinating the same year. Since the pod splits at maturity, seed in the center compartment easily disperse during the fall and winter while the seed in the outer compartments remain intact within the outer pod and thus would not be able to germinate until the compartment is degraded or physically broken.

Seedcoat extract. Cotton radicle length was inhibited by aqueous extracts of unicorn-plant seedcoat (data not shown). A radicle length of 38 mm was obtained with the control while the 1 and 5 mg/ml extract rates resulted in a radicle length of 30 and 23 mm, respectively. Although not identified, a water soluble material is contained in the unicorn-plant seed coat which affects cotton radicle growth.

Tissue extract. Aqueous extracts of various unicorn-plant tissues were more inhibitory to cotton and wheat than to unicorn-plant when compared to a distilled water control (Table 3). Extracts of unicorn-plant testa, leaf, stem, root, and exocarp were inhibitory to cotton radicle growth. Extracts of the stem, root, and exocarp were inhibitory to the radicle growth of wheat while the testa extract was actually stimulatory to wheat radicle growth. The reason for this stimulation is not known. Extracts of the leaf, endocarp, and exocarp were inhibitory to unicorn-plant.

Exocarp extracts were very inhibitory to unicorn-plant radicle growth suggesting that a mechanism exists for inhibiting seed germination until the exocarp dries and falls away. The endocarp, which is the woody pod material enclosing the seed, appears to contain chemicals which are inhibitory to unicorn-plant, suggesting that the pod material enclosing the outer compartments must first be physically destroyed or broken down by decay with microorganisms to release the seed. This is likely a major mechanism by which the seed can remain protected and viable in the soil.

Seed burial. Seed buried in October 1985 at Chickasha and Perkins, OK had very low germination after 1 month of burial (Figure 1). Germination increased at each successive month to approximately 45% after being buried for 8 months. Seed kept in moist soil in cold storage during this same period had low germination at the beginning of the experiment but steadily increased to about 15% germination after 8 months. Balyan and Bhan (1), working with horse purslane, Trianthema portulacastrum L., showed higher levels of germination when seed were buried in the field compared to seed being held at a constant storage temperature. Baskin and Baskin (2) working with witchgrass, Panicum capillare L., showed that seed were dormant at maturity and dormancy was broken during the oncoming winter months when buried in the field. In Oklahoma, a large part of the annual rainfall occurs in the fall and spring months.

These unicorn-plant seed were buried during this high rainfall period. Perhaps this moisture moving through the soil profile and through the mesh bags containing the weed seed was responsible for leaching some inhibitors from the seed coat leading to higher germination. Also, the fluctuating soil temperatures, which help loosen the seed coat from the embryo may increase germination.

Percent emergence of 25 seed/bag exhumed in June from the Stillwater, Chickasha, and from glass jars averaged 48, 40, and 16 %, respectively (Table 4). An average of 13 seed from Stillwater, 15 seed from Chickasha, and 21 seed from cold storage did not emerge. Following removal from the soil and seed coat removal, 60 to 77% of the unemerged seed germinated. When the experiments were terminated and the emergence and subsequent germination results were combined, only 13, 21, and 25% of the seed from Stillwater, Chickasha, or cold storage, respectively, did not emerge or germinate.

In well fertilized cropping areas, unicorn-plant is capable of producing a large number of seed that will germinate for an extended period of time. The thick leathery seed coat contributes greatly to the variable germination and emergence. Apparently, the seed coat inhibits germination because it presents a physical barrier, and it also contains water soluble inhibitors which could delay germination. The endocarp which enclosed nearly 60% of the total seed in a unicorn-plant capsule also contains inhibitory materials which would assist the

survival of the seed. After 8 months of burial, under 50% of the seed germinated with most remaining seed having the potential to germinate. A seed bank of viable unicorn-plant seed in the soil can present persistent problems for several years.

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Table 1. Germination of unicorn-plant following seed coat or embryo treatment.

Seed treatment	Germination by compartment[a]	
	Center	Outer
	--------%---------	
Intact seed	16	16
Rubbed against emery cloth	78	75
Seed coat removed	61	63
Seed coat and membrane removed	78	75
LSD (0.05)	4	4

[a]Means between center and outer compartments were not significantly different at the 5% probability level using LSD.

Table 2. Effect of prechill on germination of 1979 and 1980 unicorn-plant seed.

	Germination[a]		
	1979	1980	
Prechill	Combined	Center	Outer
(Weeks)	----------%	----------	
0	17	0	0
2	46	44	54
4	31	24	28
LSD	5	5	5

[a]Means between center and outer compartments or year were not significantly different at the 5% probability level using LSD.

Table 3. Effects of aqueous extracts of unicorn-plant tissue on plant radicle growth.

Plant part extracted	Radicle length		
	Cotton	Wheat	Unicorn-plant
	----------(mm)----------		
Distilled water	25	29	21
Embryo	23	30	19
Testa	20	35	18
Endocarp	22	28	15
Leaf	19	27	15
Stem	16	21	16
Root	18	23	22
Exocarp	17	10	9
LSD (0.05)	5	4	6

Table 4. Emergence of unicorn-plant seed following burial at Stillwater and Chickasha or kept in constant 4 C storage for 8 months and germination of the unemerged seed following seed coat removal.

	Emerged seedlings		Unemerged seed	Germination of unemerged seed following seed coat removal		Total seed emerged and germinated	
	(no.)	(%)	(no.)	(no.)	(%)	(no.)	(%)
Stillwater	12	48	13	10	77	22	87
Chickasha	10	40	15	9	60	19	79
Cold storage	4	16	21	15	71	19	75
LSD (0.05)	4	11	5	4	10	5	12

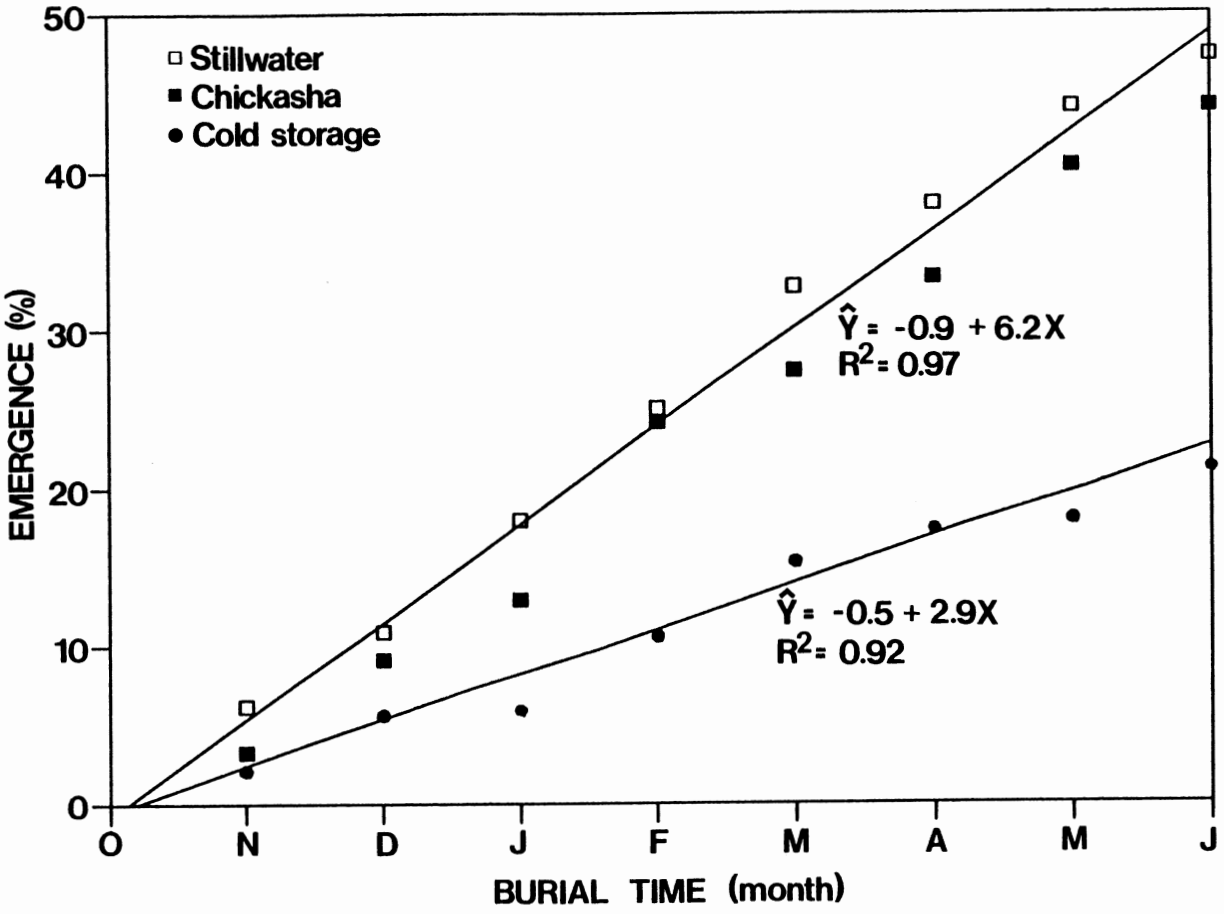

Figure 1. Percent emergence of unicorn-plant as effected by length of soil burial at 10 cm depth and constant cold storage at 4 C. Data from Stillwater and Chickasha were pooled.

PART II

SOIL WATER RELATIONS OF UNICORN-PLANT (*PROBOSCIDEA LOUISIANICA*) WITH COTTON (*GOSSYPIUM HIRSUTUM*)

Soil Water Relations of Unicorn-plant (*Proboscidea louisianica*) with Cotton (*Gossypium hirsutum*)

Abstract. A neutron probe was used throughout the growing season to measure soil water beneath plots containing cotton alone, unicorn-plant alone, cotton growing with unicorn-plant, and bare soil. Volumetric water content between treatments was unchanged throughout a profile depth of 180 cm, prior to the 5th and 6th weeks after cotton emergence in 1986 and 1987, respectively. The greatest amount of water depletion in plots containing only unicorn-plant occurred during the last week of July and the first 2 weeks of August; this corresponded to a period of rapid unicorn-plant growth. In plots containing only cotton, the largest reduction in water content occurred during the last 2 weeks of August and the first week of September; this corresponded to peak bloom and early boll formation. There were no differences in total water depletion between plots containing cotton or unicorn-plant alone during the time from the last week of July to the first week of September. Soil water remained unchanged at profile depths greater than 105 cm. In plots where interference between cotton and unicorn-plant was measured, cotton lint yield was reduced 94% in 1986 and 45% in 1987 when compared to cotton growing alone. Soil water in the soil profile was depleted nearly

equally by cotton and unicorn-plant. The higher rainfall amounts received in 1987 compared to 1986, could partially explain the yield differences between years. Nomenclature: Unicorn-plant, Proboscidea louisianica (Mill.) Thell. #[1] PROLO; cotton, Gossypium hirsutum L. 'Paymaster 145'.

Additional index words. Neutron probe, volumetric water content, soil water depletion, competition, interference, devilsclaw, PROLO.

INTRODUCTION

Unicorn-plant, also known as devilsclaw and rams horn, is a member of the Martyniaceae family, and is native to the southwestern United States. It now can be found from Florida to California and as far north as Minnesota. Unicorn-plant is a course viscid-pubescent spreading annual herb with prostrate branches up to 1 m long and a well developed tap root (8). The leaves are opposite or subalternate and broadly ovate. The fruit body is stout, up to 100 mm long, and is somewhat fleshy (3). Unicorn-plant is sometimes cultivated for its young pods which are pickled, and for mature pods which are used as ornaments and in basketweaving (11). At maturity the exocarp of the fruit sloughs off to reveal a woody hard endocarp and the incurved

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[1]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Supplement 2. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

dehiscent beak splits apart into two hook-like appendages that are one and one-half to three times longer than the body (5).

Natural infestations of unicorn-plant have been reported in the cotton growing areas of Oklahoma and West Texas (4). Those authors reported an 83% cotton lint yield reduction when unicorn-plant was seeded in the cotton row at 90 cm intervals and they attributed the yield loss to a rapidly forming leaf canopy over cotton plants in addition to removing soil water. Bridges and Chandler (2) found that a unicorn-plant density of 1 plant/6 m of row caused a cotton lint yield reduction of 34%. Mercer et al. (10) reported a 21% lint yield reduction for a unicorn-plant density as low as 1 weed/10 m of row. Higher densities of 16 weeds/10 m of row caused lint yield reductions up to 61%. The distance of influence of unicorn-plant, or the distance from the plant that cotton lint yield is affected is at least 1 m (9) and can extend up to 1.5 m (2).

There is a close relationship between the amount and availability of soil water and the competitiveness of weeds. Pavlychenko and Harrington (12) report that under arid conditions, competition is intensified as the available water is limited. They further reported that competition under the soil surface begins when plant root systems overlap during their search for water and nutrients. Examples of this phenomenon have been demonstrated. Common cocklebur, Xanthium strumarium L.;

competes well with soybean, Glycine max L., for available soil water (6). The roots of common cocklebur extended into a greater soil volume than did soybean roots. This would give the weed a competitive advantage over soybeans during dry periods. A similar situation occurs for common lambsquarters, Chenopodium album L., in competition with wheat, Triticum aestivum L. (13). The roots of common lambsquarters uniformly remove soil water through a depth of 90 cm. In this way it removed much more water and nutrients than did wheat which resulted in a grain yield loss.

Several researchers have studied the soil water relations of crops and weeds and related part of the competitiveness of the weeds to the ability of weeds to extract water from the soil. Wiese and Vandiver (14) found that barnyardgrass, Echinochloa crus-galli (L.)Beauv; common cocklebur, and large crabgrass, Digitaria sanguinalis (L.)Scop., grow vigorously and compete well in humid or irrigated farming areas. With dryland farming or in semi-arid or arid areas, kochia, Kochia scoparia (L.)Schrad.; Russian thistle, Solsola iberica Sennen & Pau; buffalobur, Solanum rostratum Dun., and tumblegrass, Schedonnardus paniculatus (Nutt.)Trel., compete well and become major problems.

Unicorn-plant is an efficient competitor and can cause substantial yield losses when growing with cotton. When unicorn-plant is present, cotton appears to wilt more rapidly than when unicorn-plant is absent. Past research

has shown that other weeds affect soil water, but soil water relations with unicorn-plant has not yet been studied. Therefore, the objectives of this research were to measure soil water content in plots containing unicorn-plant alone, cotton alone, cotton growing with unicorn-plant, and bare soil, and to relate this to cotton yield changes. The relationship of soil water and plant growth stage were also determined.

MATERIALS AND METHODS

Experiments were conducted on a Teller fine sandy loam (Udic Argiustoll) near Perkins in north central Oklahoma in 1986 and 1987. Soil fertility levels were amended each year according to state extension soil test recommendations for cotton. Soil pH was 6.9. A stormproof stripper type cotton cultivar, 'Paymaster 145', was planted with a conventional four row planter into an area heavily infested with unicorn-plant. Cotton planting dates were June 24, 1986 and June 6, 1987. The final cotton stand each year averaged 15 plants/m of row.

Plots were 6 rows wide and 7 m long with the rows spaced 91 cm apart. The experiment was arranged in a randomized complete block with four treatments and four replications. The four treatments consisted of cotton alone, unicorn-plant alone, cotton growing with unicorn-plant, and bare soil. Treatments were established 1 week after emergence by hoeing out either unicorn-plant, cotton,

neither, or both. Alachlor, 2-chloro-N-(2,6-diethyl-phenyl)-N-(methoxymethyl)acetamide, was applied preemergence broadcast at 2.1 kg ai/ha to the experiments both years. All plots were kept free of unwanted weeds by hand hoeing or pulling once a week. Supplemental water was applied in 2.5 cm amounts with a side-roll sprinkler system on July 18 and August 3, 1986, and July 22 and August 4, 1987. Rainfall and irrigation amounts from April to September are shown for 1986 and 1987 in Figure 1.

Soil water data and phenological development of unicorn-plant and cotton were recorded weekly in 1986 beginning at cotton emergence on June 30 and ending 11 weeks later on September 9, except no reading was made on September 2, 1986. These same data were recorded weekly in 1987 beginning 4 weeks after cotton emergence on July 13 and ending 12 weeks later on September 8, except no reading was made on August 10, 1987. All data were collected in relation to date of emergence, regardless of year. Measurements were discontinued in early September each year at the time of the onset of weed senescence.

Measurements of soil water content were taken with a Troxler Model 3333 neutron probe[2] with an Am:Be source. Each plot contained one 195 cm-long neutron probe access

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[2]Troxler Electronic Laboratories, Inc., P.O. Box 12057, Research Triangle Park, NC 27709.

tube (Nominal 3.8 cm EMT thin-walled steel tubing[3]) driven into the soil in the center of the 6-row plot to a depth of 180 cm, and readings were taken at 15 cm increments to a depth of 180 cm. Neutron probe readings at 15 cm were interpreted from a calibration curve specifically made for that shallow depth. All readings taken from deeper depths used a separate calibration curve. The neutron probe was assumed to give an average reading of soil water content from a spheroid bounded 7.5 cm above and 7.5 cm below the point at which the neutron source is positioned. Neutron probe readings were converted to volumetric water content (θ) in cm^3 of H_2O/cm^3 of soil. The data were used to explain the soil water relationships in three ways: volumetric water content vs. depth, total water in the profile, and soil water depletion.

Volumetric water content vs. depth. Graphs of θ vs. soil profile depth were made for the four treatments for each of the 10 reading dates in 1986 and the 8 reading dates in 1987. All graphs of θ vs. depth were compared visually to note apparent volumetric water content changes and differences caused by each treatment in a manner similar to Green et al. (7). Following visual observations of the plotted data and statistical analysis it was decided that all graphs of θ vs. depth would be plotted to a depth of 135 cm. There were no significant differences between any

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[3]Emsco Electric Supply Co., Inc., Oklahoma City, OK 73113.

treatment at depths below 105 cm; however, water contents at the 120 and 135 cm depths are shown for illustration purposes only.

Total water in the profile. In order to analyze total water in the profile over time, θ was converted to cm of water at each depth increment to a depth of 105 cm by multiplying θ for each depth by 15 cm. The water contents at each depth were then added for each treatment to obtain the total cm of water for the profile. Total water content by treatment was then plotted against time for both years.

Soil water depletion. Soil water depletion from the top 105 cm of soil was compared to the phenological growth stages of cotton and unicorn-plant. After viewing and analyzing the data the most logical way to present the results was to divide the season into two periods. The first period corresponded to the period of rapid growth for unicorn-plant, and the second period corresponded to the period of peak bloom for cotton. The first period was from July 22 to August 12, 1986 and July 20 to August 18, 1987. The second period was from August 12 to September 9, 1986 and August 18 to September 8, 1987. Water depletion was calculated by subtracting the total water at the end of a period from the total water at the beginning of a period for each plot. Total water use for both periods combined was also calculated.

Weed interference. In addition to measuring soil water in this research, unicorn-plant interference with cotton was

measured by comparing the cotton yield, when in competition with unicorn-plant, to that of weed-free cotton. Measurements were also made to determine cotton interference with unicorn-plant by comparing the dry weight of unicorn-plant growing alone with the weed weight when growing with cotton. During weed senescence, on the last week of September each year, unicorn-plant was counted and harvested from a 19 m^2 area in the center of the plot by cutting the plants at the soil surface and taking fresh weights. A subsample from each plot was weighed and dried at 49 C for 10 days. Percent moisture was calculated from the subsample and used to convert all unicorn-plant fresh weights to a dry weight basis. Cotton was harvested on December 3, 1986, and December 22, 1987. Killing freezes occurred on November 11, 1986 and October 12, 1987. At cotton harvest, 1 mature boll was collected from 15 randomly selected cotton plants in each plot. These bolls were hand ginned to determine lint percent of the snapped cotton. The four center rows were then hand harvested and weighed. Snapped cotton weight was converted to lint weight by multiplying by the lint percentage estimated by the 15 boll samples.

Statistical analysis. All soil water and interference data were subjected to an analysis of variance. Comparisons between treatment means were made using a 5% LSD. The total water content data were analyzed as a split-unit design with cotton, unicorn-plant, cotton plus unicorn-plant, or bare soil as the whole-unit treatment and the dates of reading as

the sub-unit treatment. Soil water data collected in June and the first 3 weeks of July for each year, were not significantly different and were therefore omitted from any further statistical analysis. Total water measurements used for statistical analysis included measurements made from July 29 to September 9, 1986 and from July 27 to September 8, 1987. Each year was analyzed separately and a 5% LSD was calculated for each year.

RESULTS AND DISCUSSION

Volumetric water content vs. depth. Volumetric water content was constant throughout the profile for the first 4 weeks of neutron reading in 1986 (data not shown). During this time, both cotton and unicorn-plant were emerging and establishing themselves with the benefit of adequate soil water and frequent rainfall. Accordingly, they did not cause an appreciable change in the soil water content. Differences in the volumetric water content between the four treatments began to occur in the top 45 cm of the soil profile during the 5th week in 1986; however, most of these differences were between only the bare soil and the plots containing both cotton and unicorn-plant (Figure 2A). At the time of the 5th week measurement, cotton was between 25 and 35 cm tall while unicorn-plant was variable and had between 5 and 30 leaves and was 15 to 70 cm in diameter. Water content in plots containing unicorn-plant and cotton growing with unicorn-plant were not significantly different

during the 5th week, and this continued through the remainder of the season. By the 6th and 7th weeks the plots containing unicorn-plant alone showed a significantly lower water content to a depth of 45 cm than plots containing only cotton (Figure 2B and 2C). Cotton is a perennial, grown as an annual, and it does not establish itself as rapidly as an annual such as unicorn-plant. During the 8th, 9th and 11th weeks the volumetric water content in the soil profile in all plant-containing plots was similar; however, there were significant differences between the plots containing plants and bare soil (Figure 2D, 2E and 2F). During the course of this experiment it was obvious that rooting depths and changes in volumetric water content were occurring at successively deeper depths as the season progressed (Figure 2A through 2F). Significant differences in the volumetric water contents showed a progression to deeper depths and by the 11th week differences were apparent at a depth of 105 cm.

Soil water content was unchanged prior to 6 weeks after cotton emergence in 1987 (data not shown). The third reading date (July 27) where changes in water content began to occur was approximately the same time after emergence of the first water content differences in 1986. Soil water content in plots containing cotton alone and plots containing unicorn-plant alone were different to a depth of 30 cm during the 6th and 7th week after plant emergence in 1987 (Figure 3A and 3B). More frequent and certainly higher

amounts of rainfall occurred previous to and during this period in 1987 (Figure 1). Plots containing unicorn-plant alone and cotton growing with unicorn-plant were not significantly different during these dates with the exception of the 30 cm depth where water content in plots of unicorn-plant alone was less than that of cotton growing with unicorn-plant. Water content in plots of these two treatments were similar the remainder of the season. The volumetric water content changed dramatically by the 9th week (Figure 3C). Statistically different water contents were apparent to depths of 90 cm. In relation to time of plant growth this agreed with the differences in the volumetric water contents at this depth and time (weeks) in 1986. It was also apparent that plots containing unicorn-plant, whether alone or with cotton showed lower volumetric water contents than bare soil or plots containing cotton only. During the 10th, 11th, and 12th weeks the plant-containing plots shows significantly less volumetric water content than bare soil, but there were no differences between any of the plant-containing plots (Figure 3D, 3E, and 3F). This was the same as shown in 1986.

Another similarity between 1986 and 1987 was the steady change in the volumetric water content to deeper depths as the season progressed until by the end of each season the maximum depth at which significant differences could be shown was 105 cm. It was not surprising that the water content was unchanged below a depth of 105 cm. The

rooting depth of cotton can often reach 2 m, but usually more than 90% of the total root dry matter is found in the upper 30 cm of soil (8). Unicorn-plant has a rooting pattern similar to cotton in that it has a large taproot. Thus with these two plant species, competition for water was most evident in the upper 1 m of soil. During both years, there was a time, in early and mid-August, when plots containing unicorn-plant alone had significantly less water than plots containing cotton alone. This period occurred just before the beginning of cotton bloom both years. But soil water depletion in plots containing cotton was such that by the end of August the water content for plant-containing plots was not significantly different.

Total water in the profile. Treatment differences were evident in the top 105 cm of soil during the 5th week after cotton emergence in 1986 and the 6th week in 1987 (Figure 4A and 4B). During the 6th and 7th week in 1986, plots containing unicorn-plant showed significantly less profile water than plots containing cotton (Figure 4A). During this time, unicorn-plant was developing rapidly whereas cotton was smaller and not developing as quickly. During the 6th and 7th week after cotton emergence, a stage of rapid weed growth and development occurred. This was a time of rapid leaf and flower production when the plant was very succulent. The hollow stems of unicorn-plant are often filled with water during this stage. By the 9th week, profile water in plots containing cotton was not

significantly different from that in plots containing unicorn-plant. In 1987, from the 7th to the 11th week, profile water content was significantly less in plots containing unicorn-plant than in plots containing cotton (Figure 4B). There were no significant differences in profile water in plant-containing plots during the 12th week.

In both 1986 and 1987, the soil profile to a depth of 105 cm showed approximately the same total water content and the progression of reduced total water content was similar for both years (Figure 4A and 4B). By the end of both seasons the profile contained similar total water contents; however, the profile with cotton only in 1987 did show slightly more total water than in 1986.

Soil water depletion. Water depletion in plots containing unicorn-plant was significantly more than plots containing cotton during the first period (4 to 7 weeks) in 1986 (Table 1). However, during the second period (7 to 11 weeks) water depletion in plots containing cotton alone was significantly greater than plots containing unicorn-plant alone or cotton growing with unicorn-plant. Water depletion in plots containing cotton during the second period accounted for 73% of the water depletion for both periods combined. Water depletion in plots containing unicorn-plant was approximately the same during each period. The accumulative water depletion (4 to 11 weeks) between plant-containing plots was not significantly different; however,

the bare soil treatment showed significantly less water depletion than all other treatments.

Water depletion during the first period in 1987 (5 to 9 weeks) from plots containing unicorn-plant was significantly greater than from plots containing cotton alone (Table 1). During the second period (9 to 12 weeks), cotton alone plots showed the same level of water depletion as unicorn-plant alone or cotton growing with unicorn-plant. After the slow, initial establishment period cotton develops rapidly and depletes soil water from the profile. Water depletion in plots containing cotton during the second period accounted for 75% of the water depletion for both periods combined. There were no significant differences in water depletion among treatments with cotton and/or unicorn-plant present when the two periods in 1987 were combined. It was evident that with the plant densities present in this experiment, both cotton and unicorn-plant deplete the soil water at nearly equal amounts, however, unicorn-plant depletes water much earlier in the growing season than cotton.

The amount of water depletion caused by unicorn-plant which occurred during the first period of 1986 (4 to 7 weeks) and 1987 (5 to 9) is explained by the phenological development of the plant. During the early season of both years, unicorn-plant increased in growth from approximately 70 cm to 170 cm in diameter (data not shown). The increased water depletion by cotton during the second period of both

years corresponds to peak bloom for cotton. According to Jordan (8), there is a rapid increase in water extraction as the first blooms occur and the cotton plant reaches its peak water use during early to mid-bloom. The cotton plant continues to use large amounts of water throughout the boll development period.

Weed interference. Cotton lint yield reductions of 94 and 45% occurred when compared to weed-free cotton in 1986 and 1987, respectively. In 1986, the weeds were dense and covered 100% of the ground (data not shown). Unicorn-plant dry weights and stand counts were not reduced by the presence of cotton. In 1987, cotton did not appear to be adversely effected as much by the presence of unicorn-plant compared to 1986. Weed-free cotton, in 1987, yielded higher than in 1986, but the yield reduction caused by the presence of unicorn-plant remained significant. Unicorn-plant weights and stand numbers were less than reported in 1986. In 1987, the cotton plants were capable of competing with and reducing the weed weight and stand number; therefore, cotton was more capable of producing fiber and hence only a 45% cotton lint yield reduction occurred. The varied yield reductions of 94% in 1986 to 45% in 1987 may also be partly explained by Figures 1 and 4. There was more rainfall in 1987 than in 1986 (Figure 1) and this was at least in part shown by the slightly higher water contents shown for cotton alone in 1987 compared to cotton alone in 1986 (Figure 4). More rainfall or soil moisture coupled with fewer weeds

would likely account for less competition and improved crop yields.

Soil water relations play a significant part in unicorn-plant interference with cotton. Water depletion by unicorn-plant early in the season depleted the soil of much of its available water. Cotton plants extracted significant amounts of water in its reproductive stage only after unicorn-plant was fully developed. Cotton lint yield reductions are influenced by water extraction by the weed early in the growing season (Table 1). This is evident because if water replenishment is low as in 1986 (Figure 1), unicorn-plant depletes the soil of water before any major water use by cotton, therefore cotton suffers drastic yield losses (Table 2). However if water is replenished as unicorn-plant depletes water early in the season as in 1987, enough water is left for cotton growth. Cotton is then competitive and reduces the growth of unicorn-plant.

The results of the present study are similar to those of Banks et al. (1). Water use early in the season by sicklepod depleted the soil of much of its available water. The pod-filling stage of soybean growth occurs later in the season when less water was available. The same is true for common lambsquarters interference with wheat (13). Common lambsquarters depletes water from the soil at an earlier stage and before grain filling. This appears to be a common occurrence with weed/crop competition when the reproductive parts of the crop are the harvested item. Reproduction and

fruit maturation occur later in the season after most of the available soil water has been depleted by weeds. Another factor is the shading of cotton that occurs during mid and late season. It is likely that these two factors together play a significant role in reducing cotton yield.

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Table 1. Soil water depletion under cotton, unicorn-plant, cotton grown with unicorn-plant, and bare soil.

	Soil water depletion from top 105 cm of soil					
	Weeks after emergence - 1986			Weeks after emergence - 1987		
Treatments	4 to 7	7 to 11	4 to 11	5 to 9	9 to 12	5 to 12
	(cm of H_2O)					
Bare soil	0.2 c	1.3 c	1.5 b	<0.1 d	0.7 b	0.7 b
Cotton	2.0 b	5.6 a	7.6 a	1.4 c	4.4 a	5.8 a
Unicorn-plant	3.6 a	3.2 b	6.8 a	3.5 a	3.4 a	6.9 a
Unicorn-plant + cotton	3.2 a	3.9 b	7.1 a	2.9 b	4.5 a	7.3 a

[a]Means within a column followed by the same letter are not significantly different at the 5% level using LSD.

Table 2. Cotton lint yield and unicorn-plant dry weight harvested from plots in the water depletion study.

Treatment	Year[a]					
	1986			1987		
	Cotton lint yield	Unicorn-plant dry weight	Weed number	Cotton lint yield	Unicorn-plant dry weight	Weed number
	(kg/ha)		(#/ha)	(kg/ha)		(#/ha)
Bare soil	----	----	-----	----	----	----
Cotton	583 a	----	-----	757 a	----	----
Unicorn-plant	----	1623 a	12400 a	----	1068 a	9100 a
Unicorn-plant + cotton	26 b	1361 a	11000 a	411 b	454 b	4200 b

[a]Means within a column followed by the same letter are not significantly different at the 5% level using LSD.

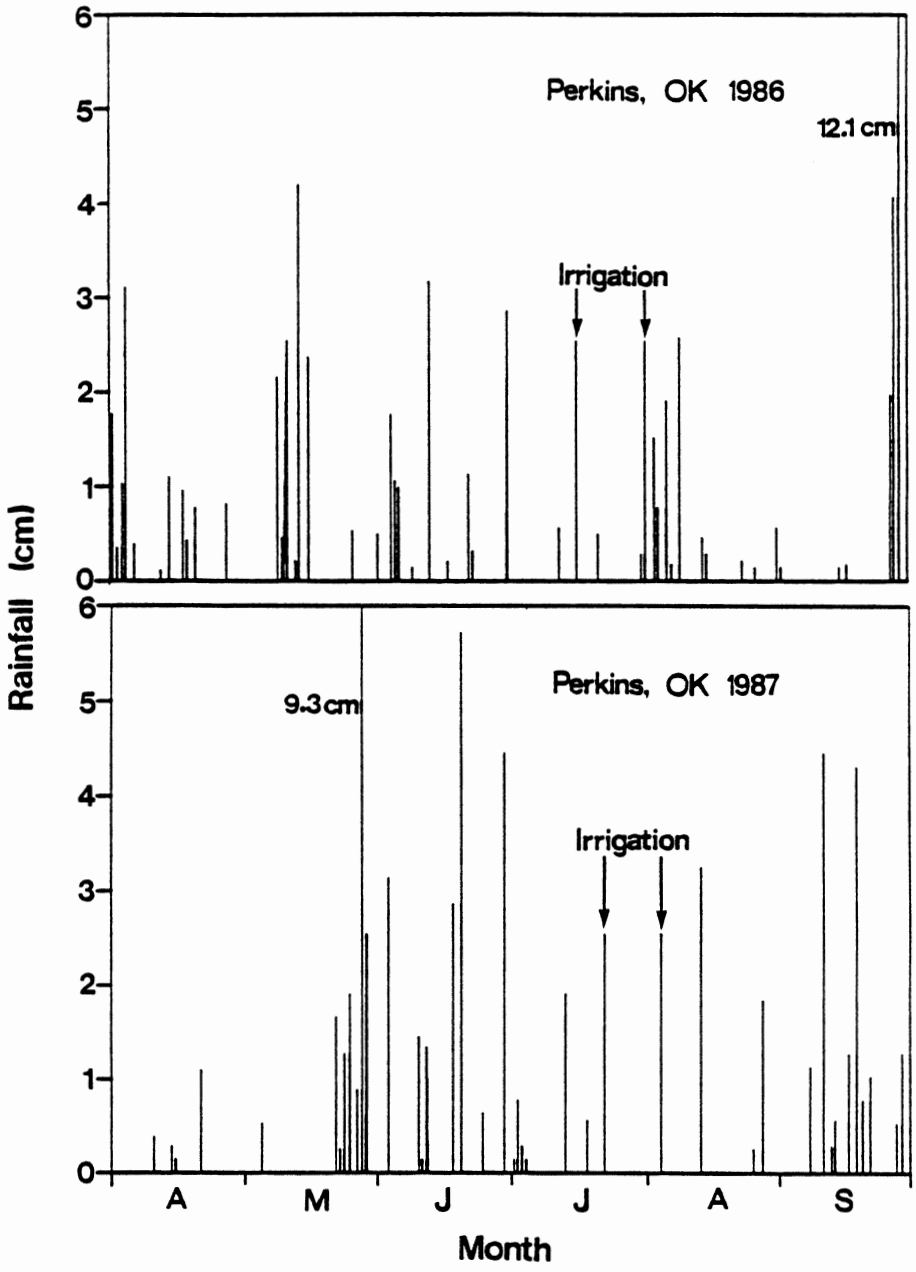

Figure 1. Rainfall and irrigation amounts and frequencies from April to September at Perkins, OK during 1986 and 1987.

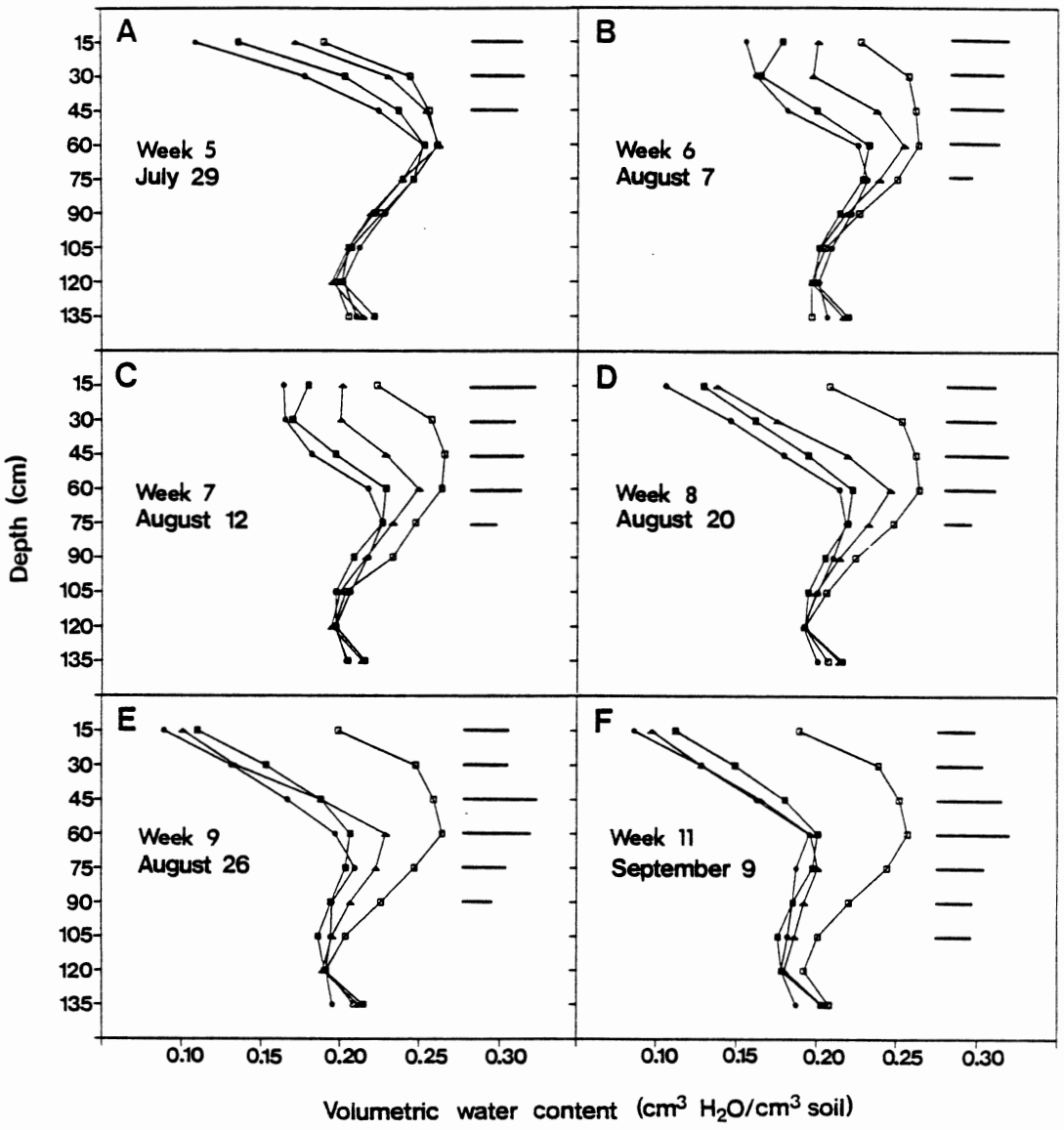

Figure 2. Volumetric soil water content by depth from 5 to 11 weeks after cotton emergence in 1986. Treatments consisted of cotton alone (▲), unicorn-plant alone (■), cotton grown with unicorn-plant (●), and bare soil (□). Horizontal lines represent the LSD at the 5% level only in cases where significant treatment differences occur.

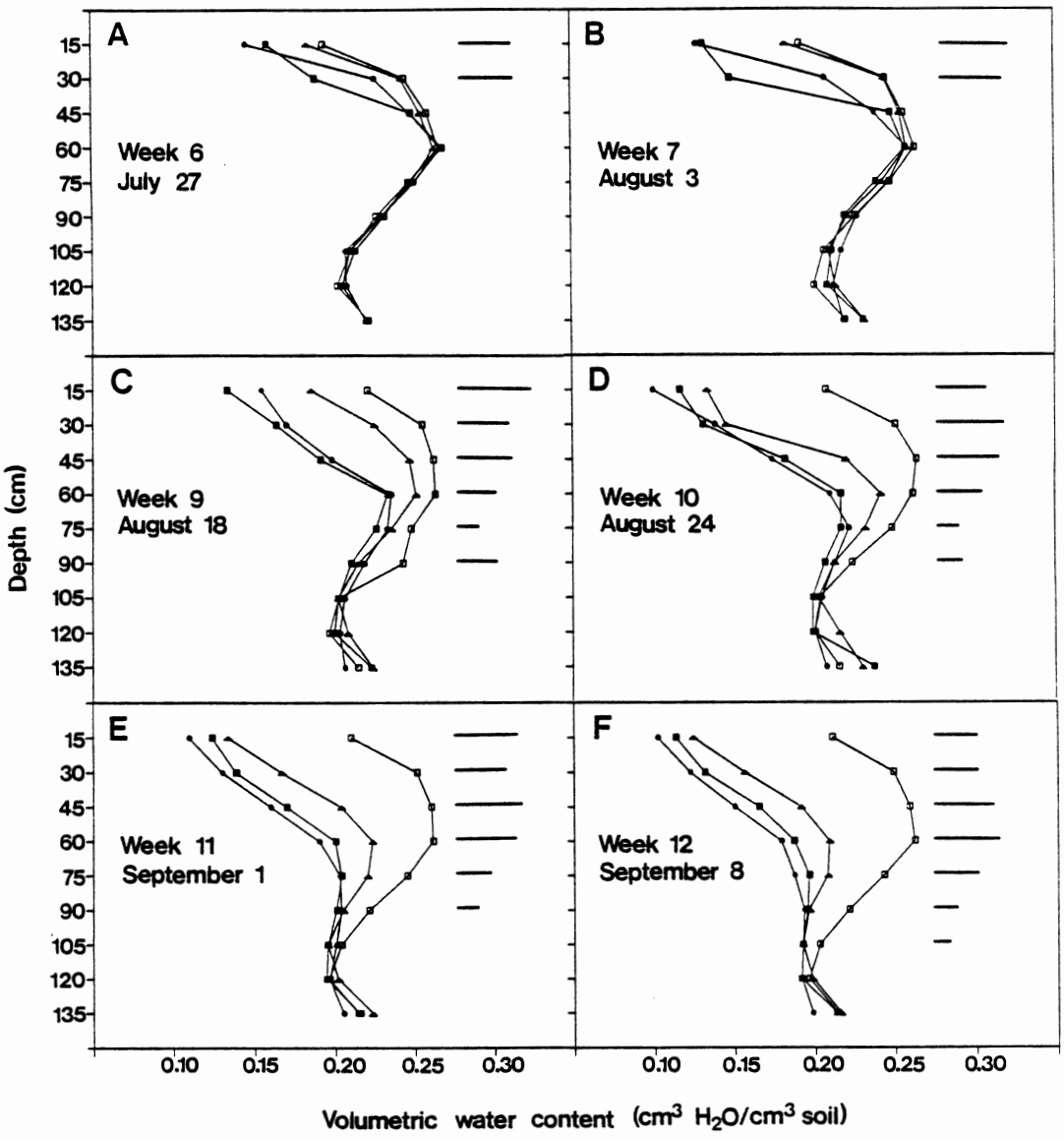

Figure 3. Volumetric soil water content by depth from 6 to 12 weeks after cotton emergence in 1987. Treatments consisted of cotton alone (▲), unicorn-plant alone (■), cotton grown with unicorn-plant (●), and bare soil (□). Horizontal lines represent the LSD at the 5% level only in cases where significant treatment differences occur.

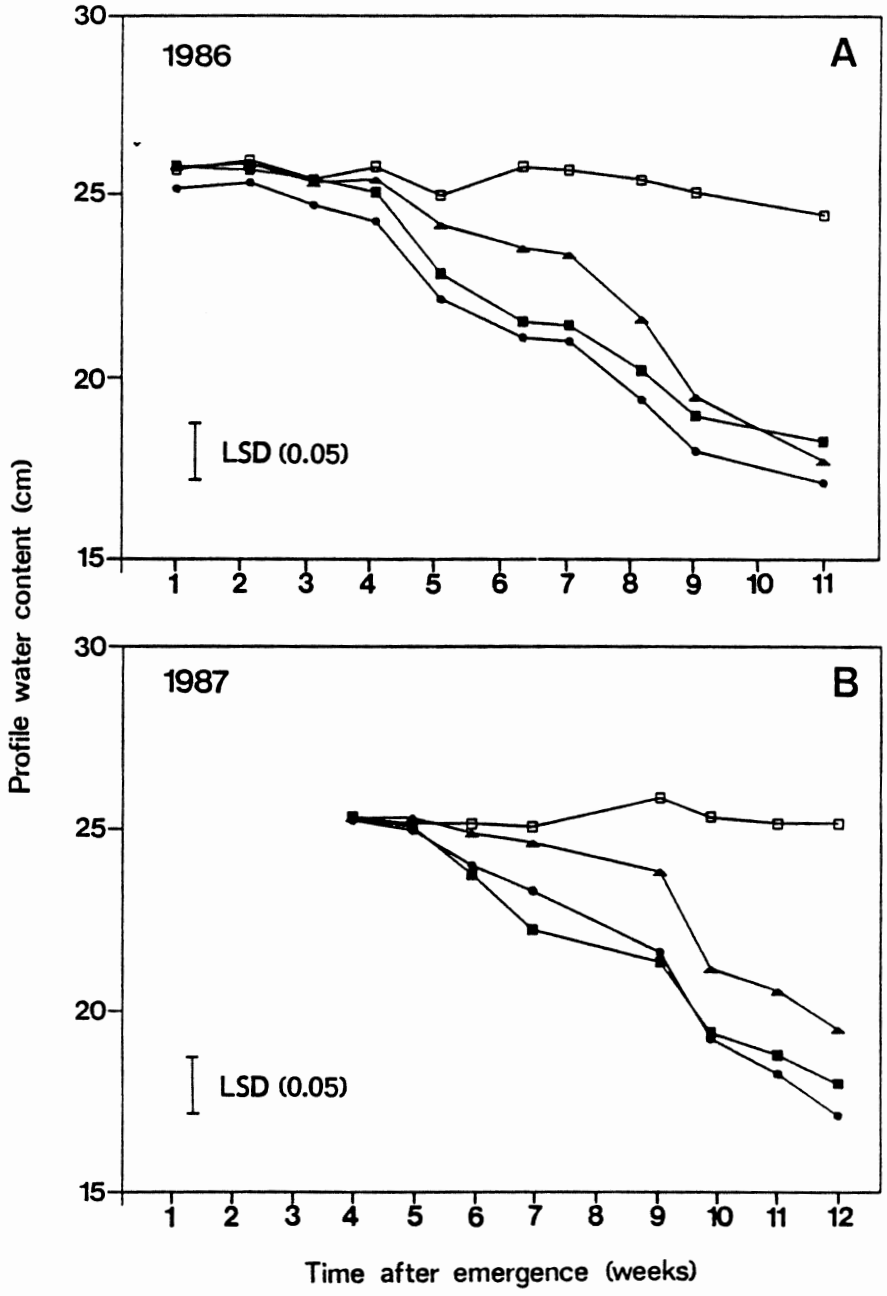

Figure 4. Total soil water content in the profile to a depth of 105 cm. Treatments consisted of cotton alone (▲), unicorn-plant alone (■), cotton grown with unicorn-plant (●), and bare soil (□).

PART III

DURATION AND INTENSITY OF UNICORN-PLANT (*PROBOSCIDEA LOUISIANICA*) INTERFERENCE ON LINT YIELD OF COTTON (*GOSSYPIUM HIRSUTUM*)

Duration and Intensity of Unicorn-Plant (Proboscidea louisianica) Interference on Lint Yield of Cotton (Gossypium hirsutum)

Abstract. The duration and density of unicorn-plant interference on lint yield of cotton was evaluated in three field experiments in 1986. Two experiments contained random, but very high, weed population densities averaging 5.5 ± 1.1 unicorn-plants/m^2 while a third experiment contained densities of 0, 4, 8, and 12 weeds/10 m crop row. In the two experiments with random weed densities, yield reductions of 41 kg/ha or 4.9% occurred for each week that the weeds were present. In the third experiment with fixed weed densities, 4, 8, and 12 weeds/10 m row decreased yield each week by 22, 49, and 56 kg/ha, respectively. Each kg/ha of unicorn-plant dry weight caused a corresponding lint yield reduction of 0.26 kg/ha. A simple linear regression based on weed dry weight was highly related to cotton lint yield and could be used to predict yield changes regardless of duration of weed interference or intensity. In the third experiment, unicorn-plant dry weight was at a maximum level by 8 weeks after emergence for the density of 4 weeds/10 m row and by 10 weeks after emergence for the densities of 8 and 12 weeds/10 m row. Intraspecific competition occurred at the higher weed density. Nomenclature: Unicorn-plant,

Proboscidea louisianica (Mill.) Thellung #[1] PROLO; cotton, Gossypium hirsutum L., 'Paymaster 145'.
Additional index words. Competition, time of weed removal, devilsclaw, PROLO.

INTRODUCTION

Unicorn-plant, also known as "devilsclaw" and "ram's horn", is a member of the family Martyniaceae and is native to the southwestern United States. Natural infestations of unicorn-plant have been reported in the cotton-growing areas of Oklahoma and West Texas, and the weed can cause yield losses up to 83% when growing in the presence of cotton[2].

Competition of annual weeds with cotton has been reported from both a population density (3, 5, 7, 8, 10, 12, 13) and a duration (2, 4, 6, 11, 14) standpoint. Density-oriented research is usually based on season-long weed interference. The density at which cotton lint yields began to decline when growing in full-season competition with

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[1]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street., Champaign, IL 61820.

[2]Cooley, A. W., D. T. Smith, and L. E. Clark. 1973. Devils claw - germination, growth and competition. Pages 14-17 in Weed and Herbicide Research in West Texas 1971-73. Texas Agric. Exp. Stn. Prog. Rep. PR-3201.

buffalobur, Solanum rostratum Dun. (12), and tumble pigweed, Amaranthus albus L. (13), was as low as 2 and 4 plants/10 m crop row, respectively. Green et al. (8) used linear regression to predict cotton lint yield losses of 1.54% caused by full-season interference from each silverleaf nightshade, Solanum elaeagnifolium Cav., plant/10 m cotton row. High densities of common cocklebur, Xanthium strumarium L., and redroot pigweed, Amaranthus retroflexus L., when left for the entire season reduced cotton lint yield by 80 and 90%, respectively (3). Tall morningglory, Ipomoea purpurea (L.) Roth, was reported as the most competitive to cotton among four Ipomoea species. Lint yield reductions up to 88% occurred following full-season interference from 32 weeds/15 m row (7).

Duration of weed interference has been evaluated by numerous scientists (1, 2, 4, 9, 14, 15). Buchanan et al. (4) reported a delay in crop maturity when prickly sida, Sida spinosa L., was allowed to compete with cotton for more than 8 weeks. Reductions in cotton plant height and stem diameter have been reported when weeds competed with cotton for 4 and 6 weeks, respectively (2). Cotton can withstand only 2 to 4 weeks of competition from cocklebur without a reduction in yield (14, 15). Six weeks of seedling johnsongrass, Sorghum halepense (L.) Pers., competition was required to reduce yield; however, a mixed stand of seedling and rhizome johnsongrass caused cotton yield losses after only 3 weeks of interference (1). In an extreme case,

a dense stand of coffee senna, Cassia occidentalis L., caused cotton yield reductions of 118 kg/ha each week (9).

Full-season unicorn-plant interference with cotton was studied in detail by Mercer et al. (10). They reported a 21% lint yield reduction for a unicorn-plant density as low as 1 weed/10 m row. Densities of 16 weeds/10 m row caused reductions in plant height up to 40% and in lint yield up to 61%. Within the range of 1 to 32 weeds/10 m row, as unicorn-plant densities doubled, lint yield reductions ranged from 84 to 146 kg/ha. Unicorn-plant is an efficient competitor with cotton and can cause substantial yield losses when growing in the crop. Full-season competition of unicorn-plant with cotton can result in greatly reduced yields of up to 74% in Oklahoma (10), but little is known about the relationship of unicorn-plant density and duration of interference with cotton. The objective of this research was to determine the critical time of removal of unicorn-plant to prevent lint yield losses in cotton.

MATERIALS AND METHODS

Experiments were conducted on a Tipton silt loam (Pachic Argiustoll) near Tipton in southwest Oklahoma and on a Teller fine sandy loam (Udic Argiustoll) at two sites (designated as Perkins I and Perkins II) near Perkins in north central Oklahoma during 1986. Soil fertility levels were amended each year according to state extension soil test recommendations for cotton. Soil pH was 7.8 at

Tipton, 6.5 at Perkins I, and 6.1 at Perkins II. A stripper-type cotton cultivar, 'Paymaster 145', was planted with a conventional four-row planter into areas heavily infested with unicorn-plant on May 11 at Tipton and on June 9 at Perkins I. At Perkins II, cotton was planted into an area free of unicorn-plant on June 11. Following cotton planting, 10 unicorn-plant seed/hill were hand planted in the Perkins II experiment approximately 8 cm to the south side of three cotton rows. Seedlings were thinned one week after emergence to uniform densities of 0, 4, 8, and 12 weeds/10 m row in a manner similar to that described by Mercer et al. (10). At Tipton and Perkins I, the unicorn-plant density was 5.5 ± 1.1 weeds/m^2. Individual plots were four rows wide and 10 m long with the rows spaced 101 cm apart at Tipton and 91 cm apart in both Perkins experiments. The outside rows of each plot served as border rows between adjacent plots. The final cotton stand at all three locations averaged 15 plants/m of row. Treatments were arranged in a randomized complete block design with four replications. At Tipton, unicorn-plant was allowed to compete with cotton for 0, 4, 6, 8, 10, 12, and 14 weeks after cotton emergence. At Perkins I and II, unicorn-plant was allowed to compete with cotton for 0, 4, 6, 8, 10, and 12 weeks after crop emergence.

Alachlor, 2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide, was applied preemergence broadcast at 2.1 kg ai/ha to all experiments. Malathion,

O,O-dimethylphosphorodithioate of diethylmercapto-succinate, plus cupric hydroxide, a fungicide, was applied in late July and August at 1-week intervals to control feeding insects and bacterial blight, caused by Xanthomonas campestris pv. malvacearum (Smith) Dye, in the unicorn-plant stand. All experiments were hand-hoed to keep plots free of unwanted weeds throughout the growing season.

Cotton lint yield data were collected from all three experiments, and weed dry-weight data were collected at Perkins II. In the Tipton and Perkins I experiments, weeds were removed from the entire plot at the specified time by cutting them at the soil surface. In the Perkins II experiment, weeds adjacent to the two center cotton rows in each plot were cut at the soil surface, dried in a forage drier at 49 C for 14 days, and weighed.

Immediately prior to cotton harvest (December 3, 1986, for Perkins and February 12, 1987, for Tipton), one mature boll was sampled from 15 randomly selected plants in each replication to calculate lint percent. Seed cotton from the two center rows was machine harvested with a one-row brush-type mechanical stripper. Lint yield in kg/ha was calculated for each plot using the estimates of lint percentage from the 15-boll samples.

Data from all three experiments were initially subjected to analyses of variance. Simple linear regression equations were then plotted for cotton lint yield vs. duration of weed interference for all three

experiments in addition to cotton lint yield vs. weed dry weight for Perkins II. The slopes of the regression equations from Tipton and Perkins I were not significantly different; therefore, a common slope was calculated and used for both locations. After conversion to percent yield no significant location by week interactions were detected; and all data could by pooled and regressed. At Perkins II, regression analysis was used to relate weed dry weight to cotton lint yield.

RESULTS AND DISCUSSION

Cotton lint yield reduction was linear through the 14-week removal period for Tipton and through the 12-weed period for Perkins I in 1986 (Figure 1). A high density of 5.5 ± 1.1 unicorn-plants/m^2 was present in each of these experiments, and it is not surprising that yield reductions were evident when this density was allowed to compete for only 4 weeks after emergence. Yield level at Tipton was significantly higher than at Perkins I; therefore, the data were plotted separately. However, the rate of yield reduction/week was the same for both locations. According to the equations for each location, for every week that the unicorn-plant remained in competition with cotton, a lint yield reduction was noted of 41 kg/ha. In an effort to eliminate the significant location by week interaction, yield data from each experiment were converted to a percentage of the weed-free control in a procedure described

by Green et al. (8). After statistical analysis, a significant location by week interaction was no longer present; and percent yield data from the Tipton and Perkins I locations could by pooled. According to this equation, for each week that a density of 5.5 ± 1.1 unicorn-plants/m^2 was present, a cotton lint yield reduction of 4.9% occurred (Figure 2). This prediction equation is appropriate for the approximate weed density present in this experiment, but not for other densities of unicorn-plant.

Cotton lint yield reduction was linear over the 12-week removal period for all three densities of unicorn-plant at Perkins II (Figure 3). Lint yield losses are expected to be 22, 49, and 56 kg/ha for each week of interference at unicorn-plant densities of 4, 8, and 12 weeds/10 m row, respectively. Lint yield reduction more than doubled when the density was doubled from 4 to 8 weeds/10 m row. When weed density was further increased to 12 weeds/10 m row, only a slightly greater reduction was noted. This would suggest some degree of intraspecific interference in populations of unicorn-plant, when the density was increased above 8 weeds/10 m row. Unicorn-plant densities of 12 weeds/10 m row resulted in 100% ground cover causing the weeds to overlap one another (data not shown). These results are similar to those of Mercer et al. (10) who found that weight/weed tended to decrease as weed density increased up to 32 weeds/10 m row.

Analysis of variance for unicorn-plant dry weight

showed no duration by density interaction; therefore, data from all densities and durations were pooled, and a common slope and intercept were calculated. Cotton lint yield reduction was linear when compared to unicorn-plant dry weight (Figure 4). The regression equation estimates, regardless of density or time of removal, a 0.26 kg/ha cotton lint yield reduction for every kg/ha of unicorn-plant dry weight. The slope is listed with two significant digits due to large rounding error over the range of data were only one to be used. Thus, high unicorn-plant densities permitted to interfere with cotton for only a short time at the beginning of the season will have a relatively small dry weight; and therefore, a relatively small lint yield reduction will occur. However, low densities of weeds allowed to interfere for an extended time can cause substantial lint yield losses. This model is useful because it is independent of density and length of interference time. However, calculation of weed dry weight is inconvenient and requires some time and effort. If unicorn-plant dry weight is calculated in kg/ha, it is possible to predict cotton lint yield reduction.

Unicorn-plant dry weight increased rapidly until the 8-week removal time for all weed densities in the Perkins II experiment (Table 1). A very rapid growth stage occured for unicorn-plant, especially from 4 to 8 weeks after emergence; consequently, unicorn-plant at the 4 plants/10m row density had reached its maximum growth by 8 weeks after emergence.

The 8 and 12 weeds/10m row densities had significant increases between the 8- and 10-week removal periods, but the rates of those increases had declined. Plant dry weight increased about three fold from 4 to 6 weeks after emergence and nearly doubled from 6 to 8 weeks for all densities. Unicorn-plant dry weight actually declined at the 12-week removal date for the two higher densities when compared to 10-weeks. At this last harvest date, the weeds were beginning to senesce.

There were no significant differences in weed biomass among densities of unicorn-plant until the 6-week removal date when all three densities were significantly different (Table 1). From 6 weeks through 12, weed biomass was significantly different among the three densities. At the 8-, 10-, and 12-week removal dates, unicorn-plant dry weight essentially doubled from the 4 to the 8 weeds/10 m densities, but the difference between 8 and 12 weeds/10 m densities averaged only a 25.5% increase. This data suggests that intraspecific competition was occurring late in the season in the 8 and 12 weeds/10 m density.

Experiments studying duration and intensity of weed interference are important in the decision-making process concerning weed control. When present, it is helpful to know when weeds will affect crop yield during the growing season . The removal of weeds on a timely basis can minimize crop losses. It is already known how full-season interference of the unicorn-plant with cotton affects lint

yield (10). The findings of this research supplement the earlier work by shedding light on the timing and extent of unicorn-plant influence on cotton lint yield during the growing season. Because the unicorn-plant grows very rapidly from 4 to 8 weeks after emergence it probably should be removed prior to that time interval.

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Table 1. Unicorn-plant dry weight at three densities and five durations of weed interference for the Perkins II experiment[a,b].

Unicorn-plant density		Duration of interference				
Row basis	Area basis	4	6	8	10	12
(plants/10 m)	(plants/ha)	(kg/ha)				
4	4400	123 aC	410 cB	725 cA	797 cA	737 cA
8	8800	154 aD	702 bC	1500 bB	1696 bA	1451 bB
12	13200	220 aD	950 aC	1824 aB	2158 aA	1855 aB

[a]Means within a column followed by the same small letter are not significantly different at the 0.05 probability level using the protected LSD.

[b]Means within a row followed by the same capital letter are not significantly different at the 0.05 probability level using the protected LSD.

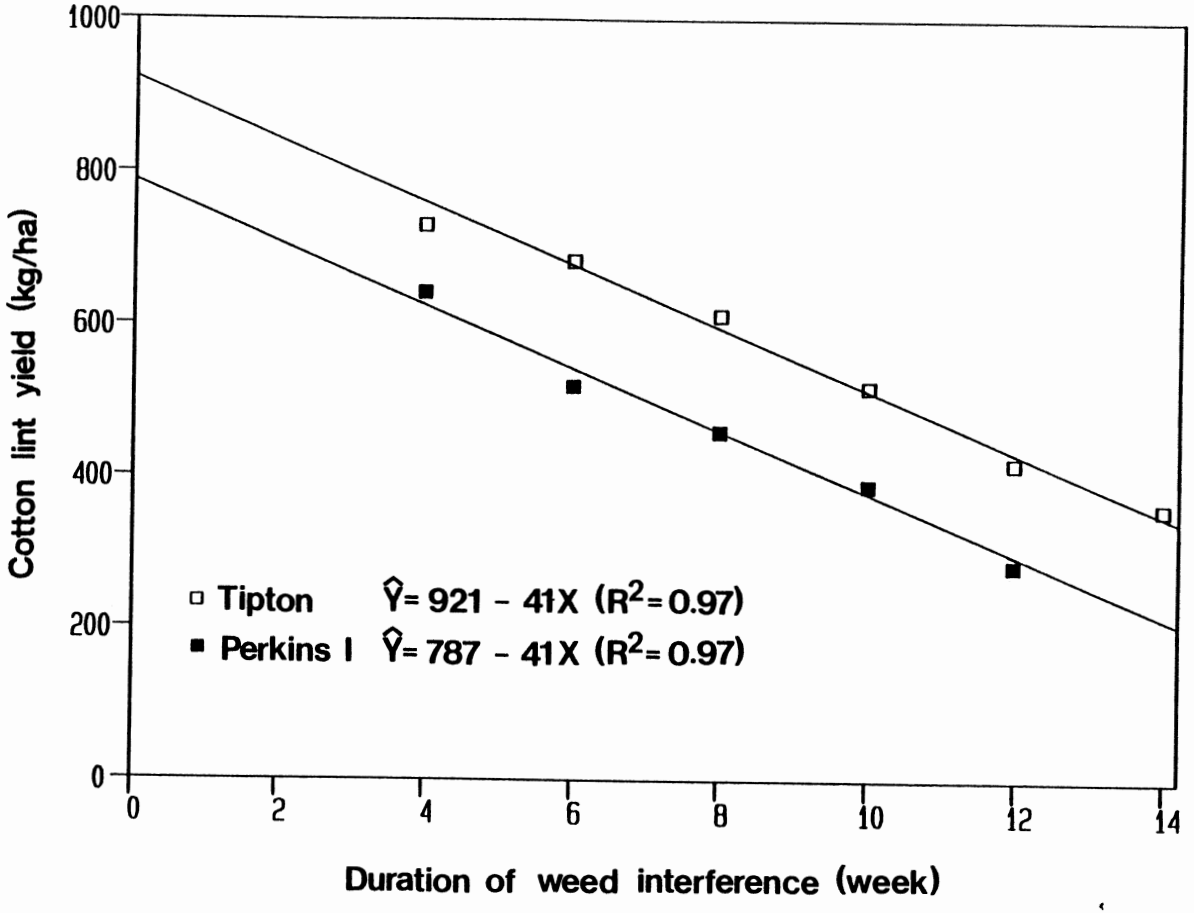


Figure 1. Effect of unicorn-plant interference duration (calculated from cotton emergence) on cotton lint yield at Tipton and Perkins I.

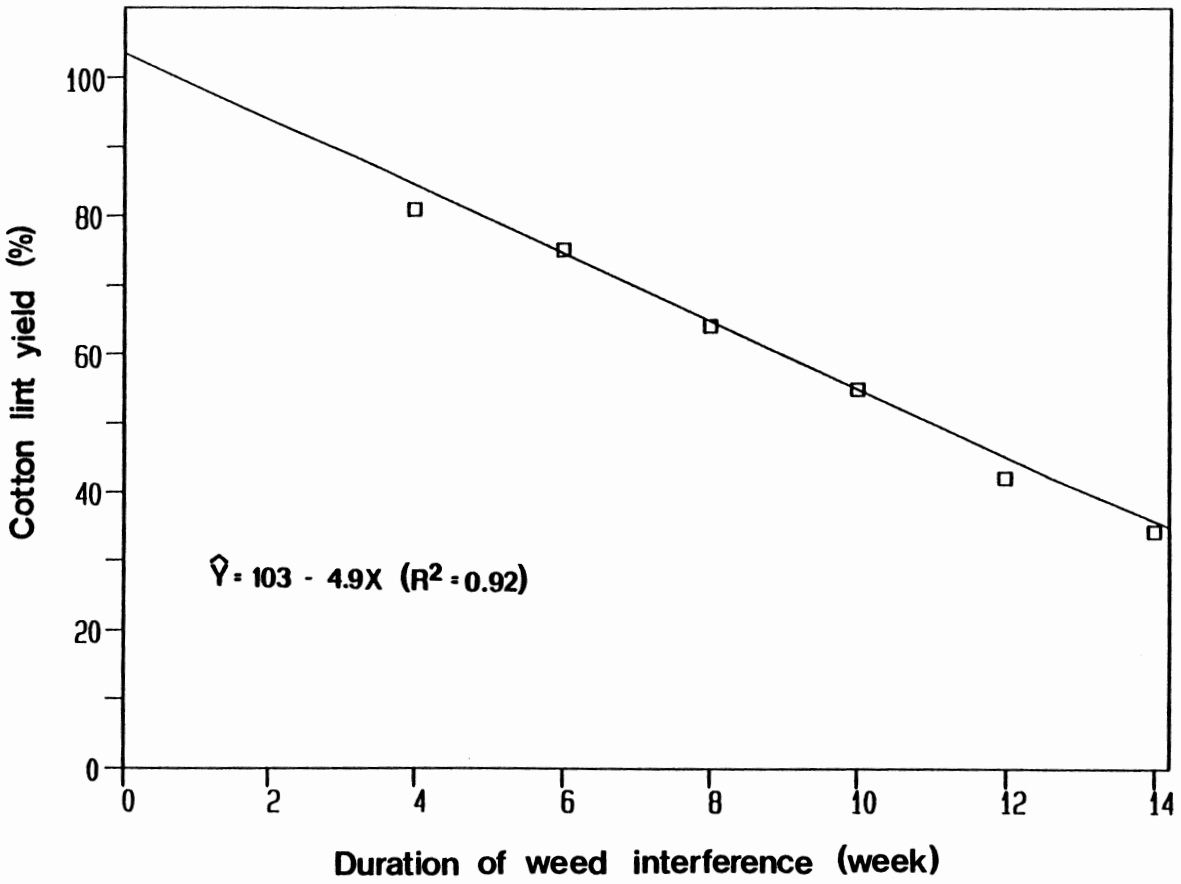


Figure 2. Percent cotton lint yield as influenced by unicorn-plant interference duration (calculated from cotton emergence). Data from Tipton and Perkins I were pooled.

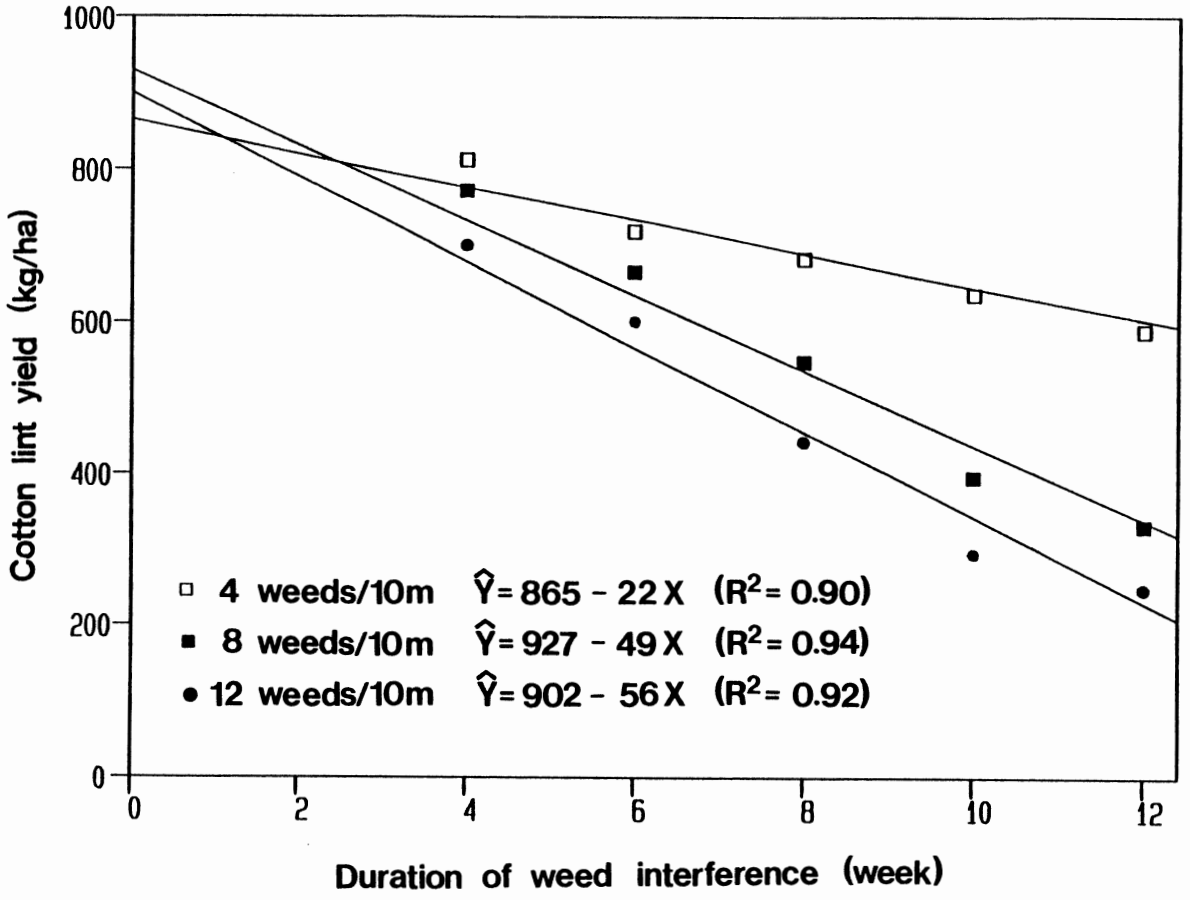


Figure 3. Effect of unicorn-plant interference duration (calculated from cotton emergence) on cotton lint yield with densities of 4, 8, and 12 weeds/10 m row at Perkins II.

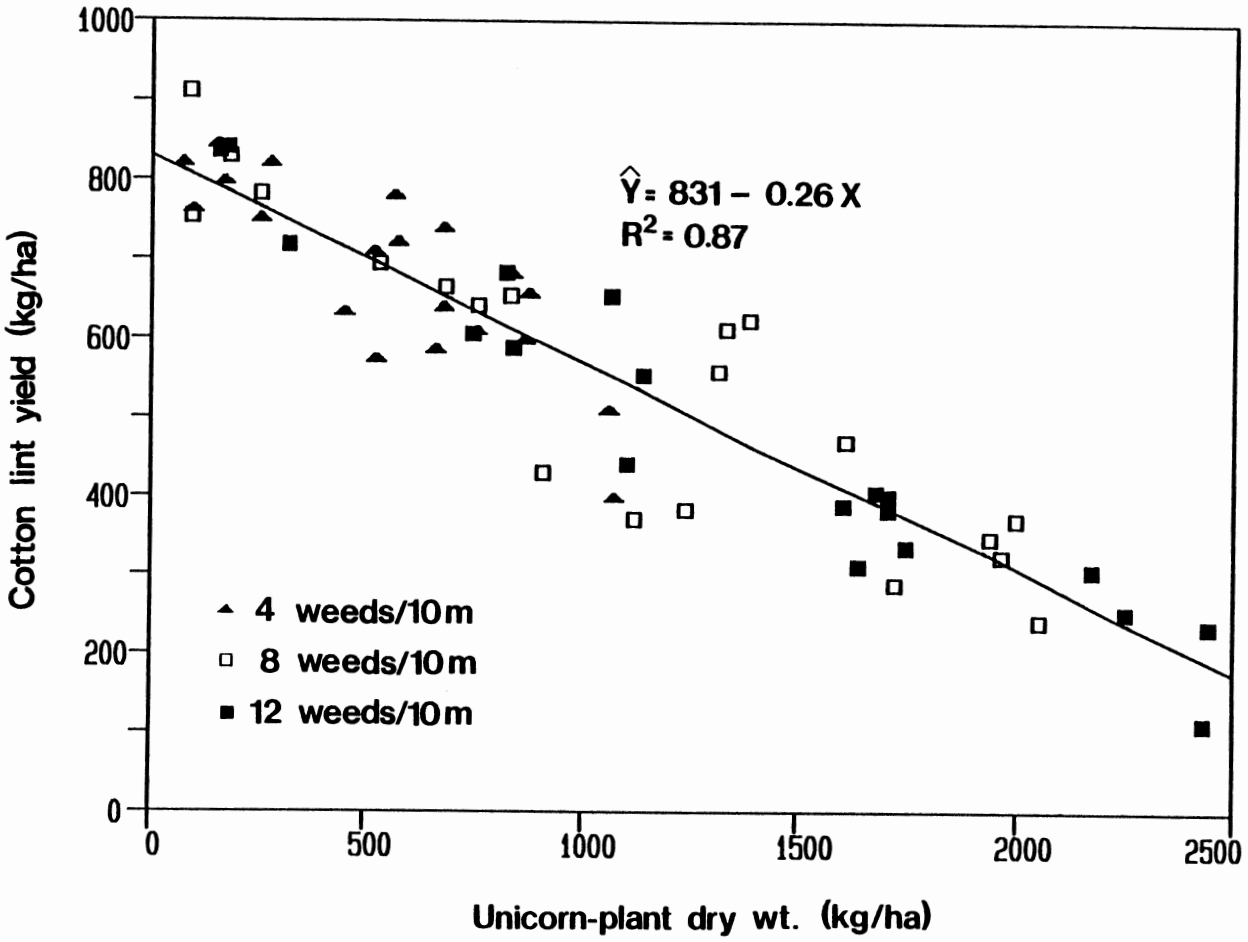


Figure 4. Effect of unicorn-plant dry weight accumulation over weed densities and interference durations on cotton lint yield at Perkins II.

PART IV

COMPOSITION OF ESSENTIAL OIL FROM *PROBOSCIDEA LOUISIANICA* (MARTYNIACEAE)

Composition of Essential Oil from Proboscidea louisianica (Martyniaceae)

Summary The essential oil was collected from mature plants of Proboscidea louisianica by neutral or acidic steam distillation and analyzed by CGC/MS/DS. The MS-50 profile of the essential oils required approximately 140 min and 3,500 spectra for each sample and between 150-220 compounds were detected. From this mixture the following peaks were identified from the normal essential oil of the roots: vanillin, perillyl acetate, δ-cadinene, α-bisabolol, traxolide, 2-methyl-1,4-naphthoquinone, 9,10-anthracenedione-(1-hydroxy-2(or 3)-hydroxymethyl), hexadecanoic acid, with small amounts of 6-methyl-5-hepten-2-one and piperitenone with the remaining compounds being mostly terpenes, terpenoids, and hydrocarbons. In the HCl-treated steam distillate of the pods the identities confirmed were vanillin, phenylethyl alcohol, p-cymen-9-ol, trimethylcyclohexanone, dodecanoic and hexadecanoic acids, and tentatively 2-ethylbenzimidazole.

Keywords 9,10-anthracenedione-(1-hydroxy-2(or 3)-hydroxymethyl) α-bisabolol δ-cadinene p-cymen-9-ol devilsclaw essential oil hexadecanoic acid 6-methyl-5-hepten-2-one 2-methyl-1,4-naphthoquinone perillyl acetate

phenolics phenylethyl alcohol piperitenone Proboscidea louisianica steam distillation traxolide trimethylcyclohexanone unicorn-plant vanillin

INTRODUCTION

Unicorn-plant [Proboscidea louisianica (Mill.) Thell.], a member of the family Martyniaceae, is sometimes referred to as devilsclaw or rams horn. It is a spreading annual with stems up to 80 cm long and large, entire, opposite leaves up to 30 cm wide. The entire plant is covered with trichomes and each is tipped by a droplet of oil which makes the plant oily to the touch and odoriferous[5]. The fruit is a drupaceous dehiscent capsule with a stout fruit body up to 100 mm long. The fruit body is terminated by an incurved beak that is longer in length; at maturity the outer exocarp dries and falls away and the endocarp beak splits to form a 2-horned claw[10].

A white seeded devilsclaw is sometimes cultivated in the Western U. S. and the young fruit may be pickled for food or the mature fruit may be used as ornaments or as a basketry fiber[15]. Unicorn-plant is native to the southwestern U. S. and northern Mexico, but now is the most widely distributed member of its family ranging from Florida to California north to Minnesota and south to Mexico[17].

There are only limited data on the biochemistry of unicorn-plant. Ghosh and Beal[8] conducted experiments on the seed lipid constituents. Linoleic acid ($C_{18:2}$) was found to

be the major component of the oil comprising about 60% of the total fatty acids with oleic acid ($C_{18:1}$) making about 30%. Palmitic acid ($C_{16:0}$) was the major saturated acid and made up about 6% of the oil. Traces of eight other fatty acids made up the rest of the oil. The fatty acid with the longest chain length was behenic acid ($C_{22:0}$). The sterol composition of the oils was mostly β-sitosterol (80%) and campesterol (15%) with four other sterols present in minor quantities. The tocopherol content in the seeds was comprised of τ-tocopherol (50 - 60%), α-tocopherol (15%), and δ-tocopherol (30%). The oil content of the seed totaled about 40%, and the oil composition of the seeds closely resemble that of soybean oil.

The objectives of this research were to isolate and identify the constituents of unicorn-plant essential oil. A preliminary account of this work has been presented[20].

MATERIALS AND METHODS

Steam distillation Nine mature unicorn-plants were collected on September 10, 1986 and separated into roots, stems and leaves, and pods. These were cut into 3 to 6 cm parts, loaded separately into a 6 L round-bottom flask, and steam distilled for 5 h. The steam distillation apparatus was an all glass assembly with teflon stopcocks and sleeves. The condensate (approximately 3 L) was saturated with 1.1 kg NaCl, and extracted three times with 1 L of ethyl-ether, dried over anhydrous Na_2SO_4 and evaporated to dryness under

nitrogen. The residue left after distillation was acidified with 2 N HCl to a pH of 0.8 and redistilled for 5 h. The condensate was processed in the same way as the normal distillation. After the ether was driven off of the ether extract, a viscous, dark yellow to light brown oil with a very acrid odor remained. The weight of the essentials oils recovered was 130 mg for the normal distillation of the roots, and 210 mg for the acidic distillation of the pods.

Capillary gas chromatography The initial capillary gas chromatographic run was carried out on a Hewlett Packard Model 5880 gas chromatograph containing a flame ionization detector and an OV-1 fused silica column 50 m x 0.32 mm. The samples were taken up in ether and analyzed using a 1.5 μl injection with a splitter ratio of 25:1, the oven at 50°C, programmed at 2°C/min to 225°C and held for 60 min using a He flow of 0.5 ml/min.

Capillary gas chromatography/mass spectrometry/data system Two of the samples, the normal distillation of the roots and the acidic distillation of the pods were subjected to gas chromatography and mass spectrometry. The Kratos MS-50 mass spectrometer was equipped with a Varian model 3700 gas chromatograph containing an OV-1 fused silica column 50 m x 0.32 mm and the samples were analyzed using a 1.0 μl injection with the splitter turned off, the oven at 50°C, which was programmed at 2°C/min to 225°C and held for 60 min using a He flow of 0.5 ml/min. The data were acquired and analyzed using a modified Kratos DS-55 data system[4].

Identifications were based on the comparison of known with unknown spectra and visual interpretation of the fragmentation patterns.

RESULTS

Capillary gas chromatography/mass spectrometry/data system The MS-50 profile of the essential oils required approximately 140 min and 3,500 spectra for each sample and the results indicated that between 150-220 compounds were detected. The compounds that were identified are listed in Table 1. The complete reconstituted total ion current chromatograms from each sample are shown for comparison purposes in Figures 1 and 3. The sensitivity was increased for the total ion current monitors to show more detail in Figures 2 and 4. The following peaks were identified from the normal essential oil from the roots (Figure 1). From scan 500 to 1000 (Figure 2A), peaks identified were p-vinyl-phenol (Figure 5), piperitenone (Figure 6), and vanillin (Figure 7). From scan 1000 to 1500 (Figure 2B), peaks identified were 2-methyl-1,4-naphthoquinone, ionol, 1,3,5-tritertbutyl-benzene, and α-bisabolol (Figure 8). Ionol may be a natural compound but it is also a preservative present in the ether used for extraction of the essential oils. From scan 1500 to 2000 (Figure 2C), hexadecanoic acid, δ-cadinene (Figure 9), and traxolide (Figure 10) were identified. From scan 2000 to 2500 (Figure 2D), 9,10-anthracenedione-(1-hydroxy-2(or 3)-hydroxymethyl) was

identified. Trace amounts of 6-methyl-5-hepten-2-one was found from scan 0 to 500 (data not shown). The remaining compounds were mostly terpenes, terpenoids, and hydrocarbons; but they were not further identified.

The following peaks were identified in the HCl-treated steam distillate from the pods (Figure 3). From scan 500 to 1000 (Figure 4A), peaks identified were phenyl-ethyl alcohol, trimethyl-cyclohexanone, p-cymen-9-ol (Figure 11), and vanillin. A peak at scan 665 has a molecular weight of 142 but remains unidentified. From scan 1000 to 1500 (Figure 4B), peaks identified were 2-ethylbenzimidazole (tentatively) and dodecanoic acid. An isomer of a C_{12} acid is present at scan 1265. From scan 1500 to 2000 (Figure 4C), hexadecanoic acid was identified. A peak at scan 1650 has a molecular weight of 232 but remains unidentified. A very large peak of an unknown, high molecular weight hydrocarbon is present at scan 1880. The remaining peaks were mostly sesquiterpenes, and hydrocarbons.

The mass spectra shown are with mass spectral standards from the NBS/EPA/NIH Mass Spectral Data Base[19] except the p-vinyl phenol which was run in this laboratory[13] and traxolide which is available only in the laboratory of International Flavors and Fragrances. International Flavors and Fragrances has their own standards which represent compounds that are in some cases more pure than the NBS/EPA/NIH library compounds. Therefore, the mass spectra in some cases do not necessarily agree with the

NBS/EPA/NIH library, but the interpretations and identifications were made with almost 100% accuracy according to International Flavors and Fragrances.

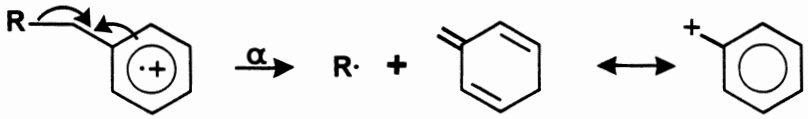
The molecular weight of p-vinyl-phenol is 120 (Figure 5). The loss of a hydroxyl group gives rise to the ion at m/z 103. An ion at m/z 94 represents the loss of a ethyl group. Piperitenone has a molecular weight of 150 which is also its base peak (Figure 6). The ion at m/z 135 represents the loss of a methyl group from the parent compound and the ion at m/z 107 represents the loss of a isopropyl group.

The molecular weight of vanillin is 152 (Figure 7). The loss of a hydrogen results in a prominent m/z 151 base peak. The ion at m/z 137 represents the loss of a methyl group. The loss of an aldehyde (CHO) gives rise to an ion at m/z 123. The molecular weight of α-bisabolol is 220 but the loss of H_2O occurs simultaneously and this is shown in the mass spectrum (Figure 8) which gives rise to m/z 204, the pseudo molecular ion. The loss of a methyl group results in the formation of an ion at m/z 189. The ion at m/z 161 is characteristic of the loss of an isopropyl group.

The molecular weight of δ-cadinene is 204 (Figure 9). The loss of either methyl group results in the ion at m/z 189. The loss of the isopropyl group results in a very prominent ion at m/z 161. The molecular weight of traxolide is 272 (Figure 10) and the loss of a methyl group gives rise to an ion at m/z 257. The m/z 229 ion indicates

the loss of an isopropyl group. The molecular weight of p-cymen-9-ol is 150 (Figure 11). The ion at m/z 119 is the base peak and represents the loss of the CH_2OH groups.

The m/z 91 species represents the tropylium ion and is a characteristic ion present in large or small amounts in the following spectra: p-vinyl-phenol, piperitenone, α-bisabolol, δ-cadinene, traxolide, and p-cymen-9-ol. The tropylium ion is shown below:



DISCUSSION

Unicorn-plant is densely covered with glandular hairs, each tipped by a droplet of oil. This gives the plant a very oily appearance and a strong acrid odor. Since the entire plant is covered with these hairs, large quantities of oil are formed. Unicorn-plant essential oil volatilizes from the plant when growing in the field and gives a distinct acrid odor to the air around these fields. This release of volatiles is similar to many other plants that release volatile chemicals from their essential oil. These volatiles were captured by vacuum on activated charcoal and eluted with methanol, and the same distinct odor was present in the eluate, but these were not analyzed due to the very small amount of material.

The essential oil of Siparuna guianensis leaves

contains δ-cadinene, a common volatile sesquiterpene[1]. It is also found in the volatile components released from wheat leaves[6], and in the essential oils of Sideritis spp.[11], clove (Eugenia caryophyllus)[14], and Rhus typhina[3]. It is a major constituent of unicorn-plant essential oil.

The volatile constituents of kumquat (Fortunella margarita) essential oil contain p-cymene[9]. Kumquat essential oil is similar to unicorn-plant essential oil in that it contains many mono- and sesquiterpenes and hydrocarbons. The essential oil of Sideritis spp. contains p-cymene and p-cymeme-8-ol[11] and the latter compound is very similar to the p-cymene-9-ol found in the HCl treated unicorn-plant essential oil.

Monoterpene aldehydes and alcohols, and monoterpene hydrocarbons make up much of the floral fragrance of Platanthera stricta[16]. Many terpenoid compounds can act as insect attractants[2,6] including δ-cadinene, which is present in the volatile components of wheat leaves and unicorn-plant.

Piperitenone is present in minute quantities in Sideritis spp. essential oil[11]. It is present in relatively small amounts in unicorn-plant essential oil. Bisabolene is also not very common in essential oils but is found in small amounts in the constituents of the rhizome of calumus (Acorus calumus)[12]. An alcohol of bisabolene, α-bisabolol is present in unicorn-plant essential oil.

The volatile constituents of Amaranthus palmeri

seedheads were rich in 2-heptenone, and vapors at a concentration of 1 ppm of these compounds strongly inhibited the germination of onion (Allium cepa) and carrot (Daucus carota) and almost completely suppressed the germination of tomato (Lycopersicon esculentum)[7]. Clove essential oil contained 6-methyl-5-heptene-2-one, a compound identical to that in unicorn-plant essential oil[14].

Phenolic compounds such as vanillin are commonly found in the soil. They are released as root exudates or from decomposing plant litter[22]. Vanillin is unbiquitous in the soil due to the fact that it is a degradation product of lignin[18]. These compounds can act as plant growth inhibitors when present in the soil[21]. Vanillin is a major constituent of unicorn-plant essential oil and is most likely released in large amounts by decomposing plants late in the summer when the plants senesce and die.

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Table 1. Compounds present and relative amounts in *Proboscidea louisianica* essential oil.

Coumpound	Plant part	Relative amount
9,10-anthracenedione-(1-hydroxy-2(or 3)-hydroxymethyl)	root	high
α-bisabolol	root	moderate
δ-cadinene	root	moderate
p-cymen-9-ol	pod	low
hexadecanoic acid	root, pod	high
6-methyl-5-hepten-2-one	root	low
2-methyl-1,4-naphthoquinone	root	moderate
perillyl acetate	root	low
phenylethyl alcohol	pod	low
piperitenone	root	low
traxolide	root	moderate
trimethylcyclohexanone	pod	high
vanillin	root, pod	high

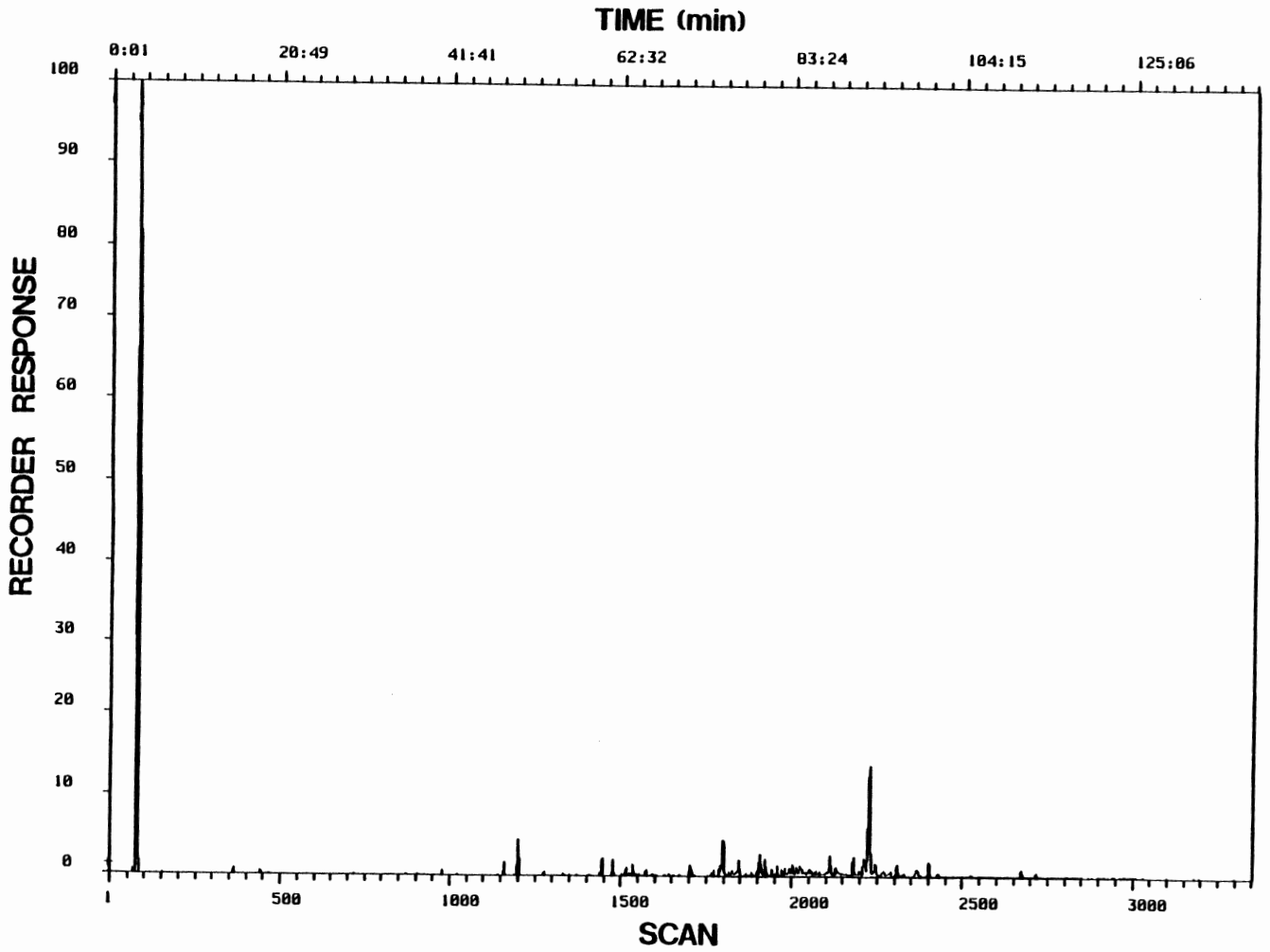

Figure 1. Complete reconstituted total ion current chromatogram of normal essential oil of Proboscidea louisianica.

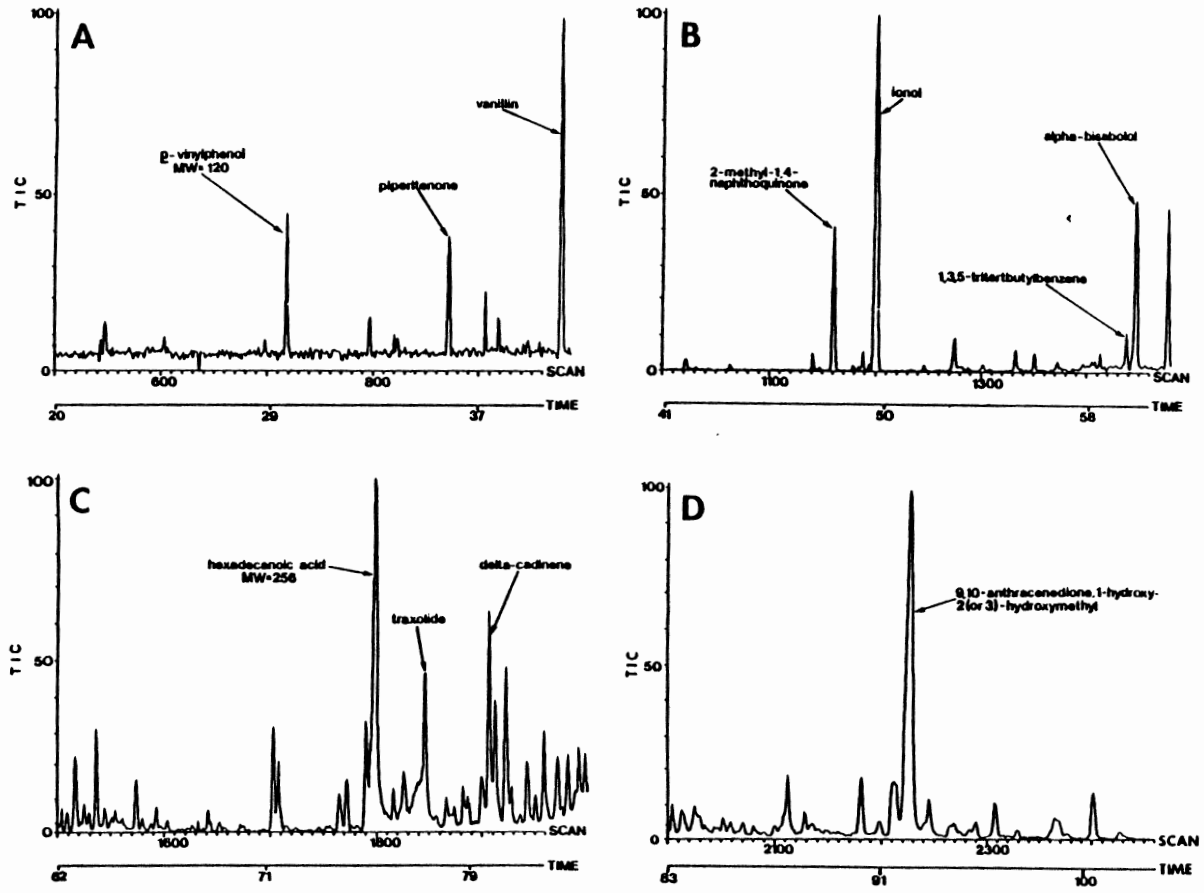

Figure 2. Partial reconstituted total ion current (TIC) chromatograms of normal essential oil of Proboscidea louisianica. These represent a higher sensitivity than those shown in Figure 2. A) Scan No. 500-1000, B) Scan No. 1000-1500, C) Scan No. 1500-2000, D) Scan No. 2500-3000.

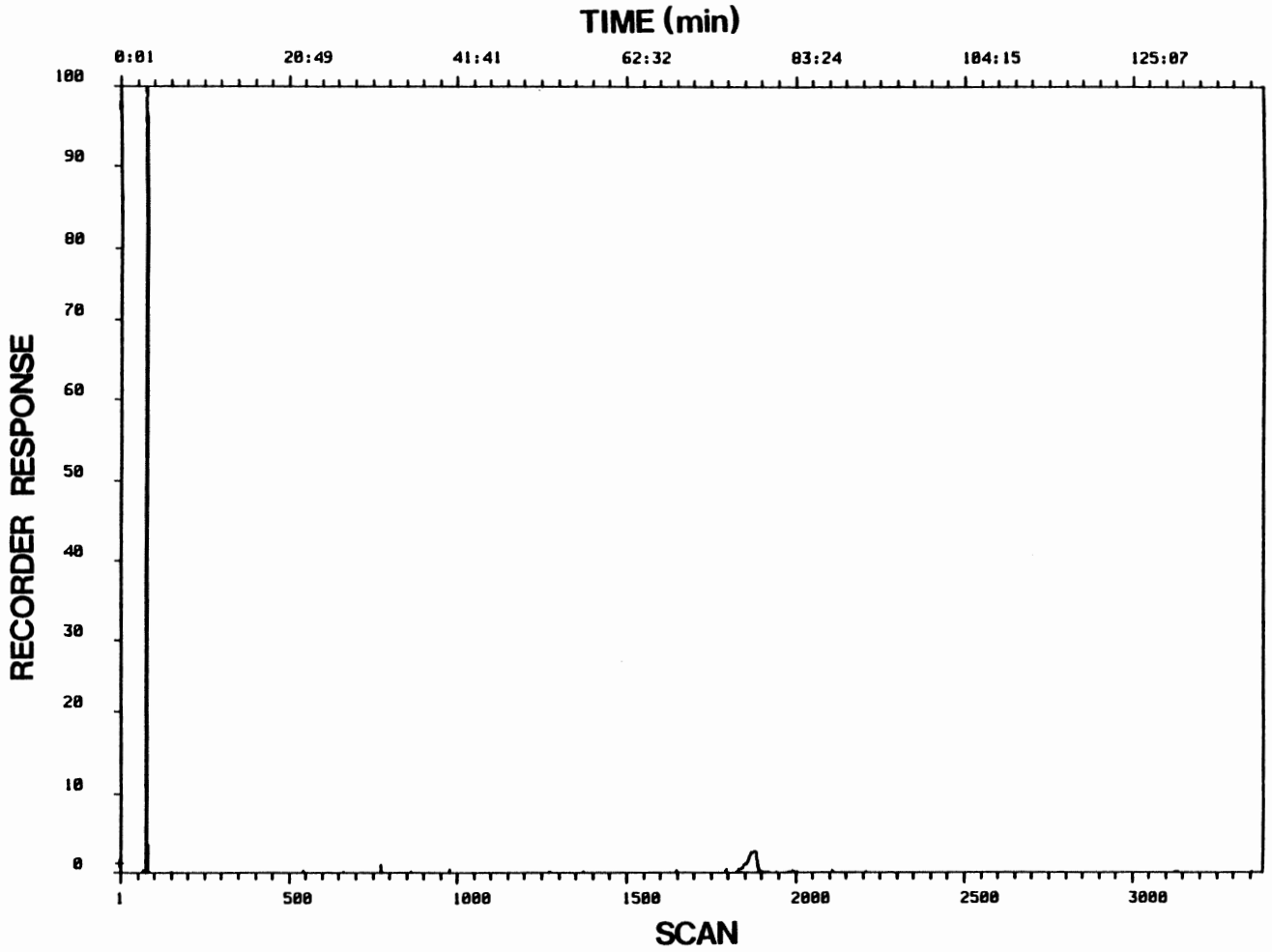

Figure 3. Complete reconstituted total ion current chromatogram of HCl treated essential oil of Proboscidea louisianica.

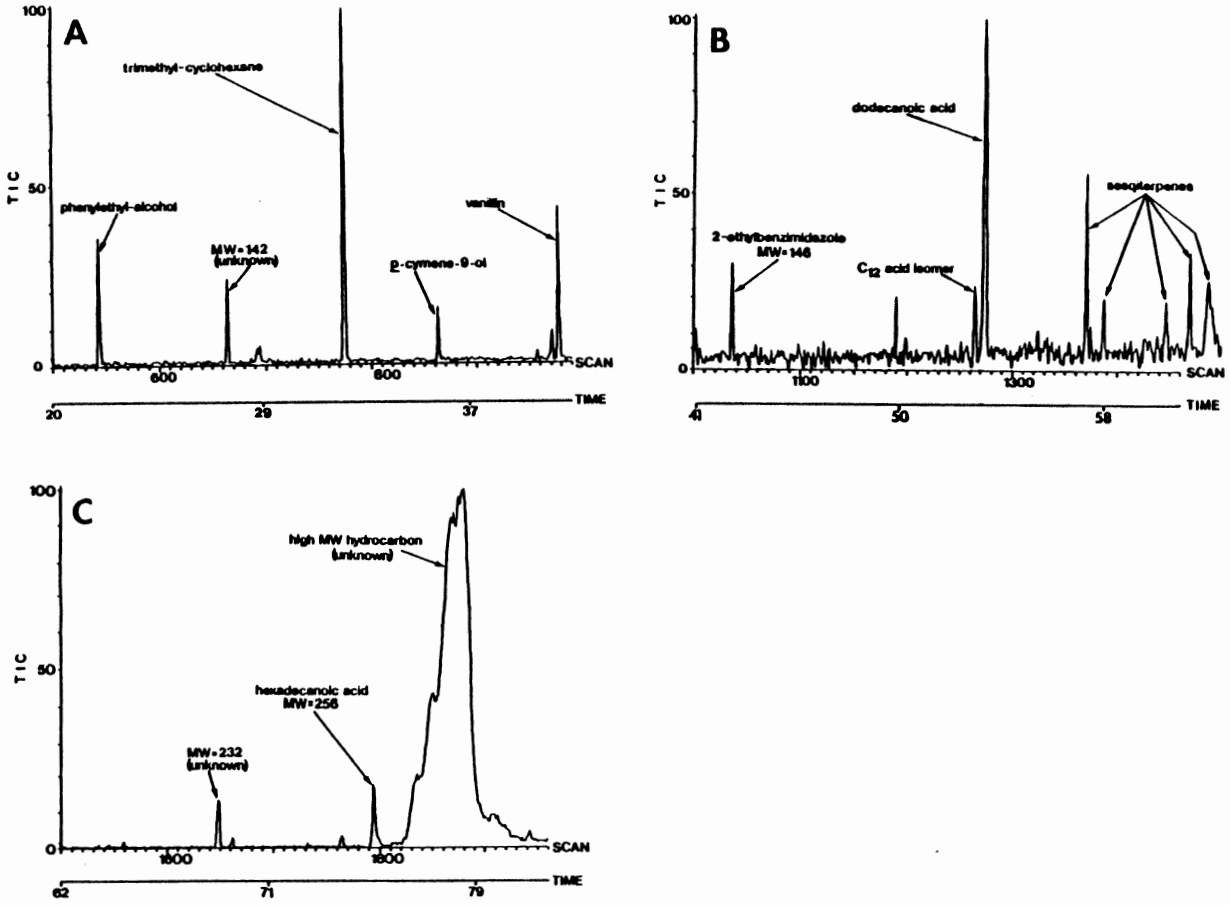

Figure 4. Partial reconstituted total ion current (TIC) chromatograms of HCl treated essential oil of Proboscidea louisianica. These represent a higher sensitivity than those shown in Figure 2. A) Scan No. 500-1000, B) Scan No. 1000-1500, C) Scan No. 1500-2000.

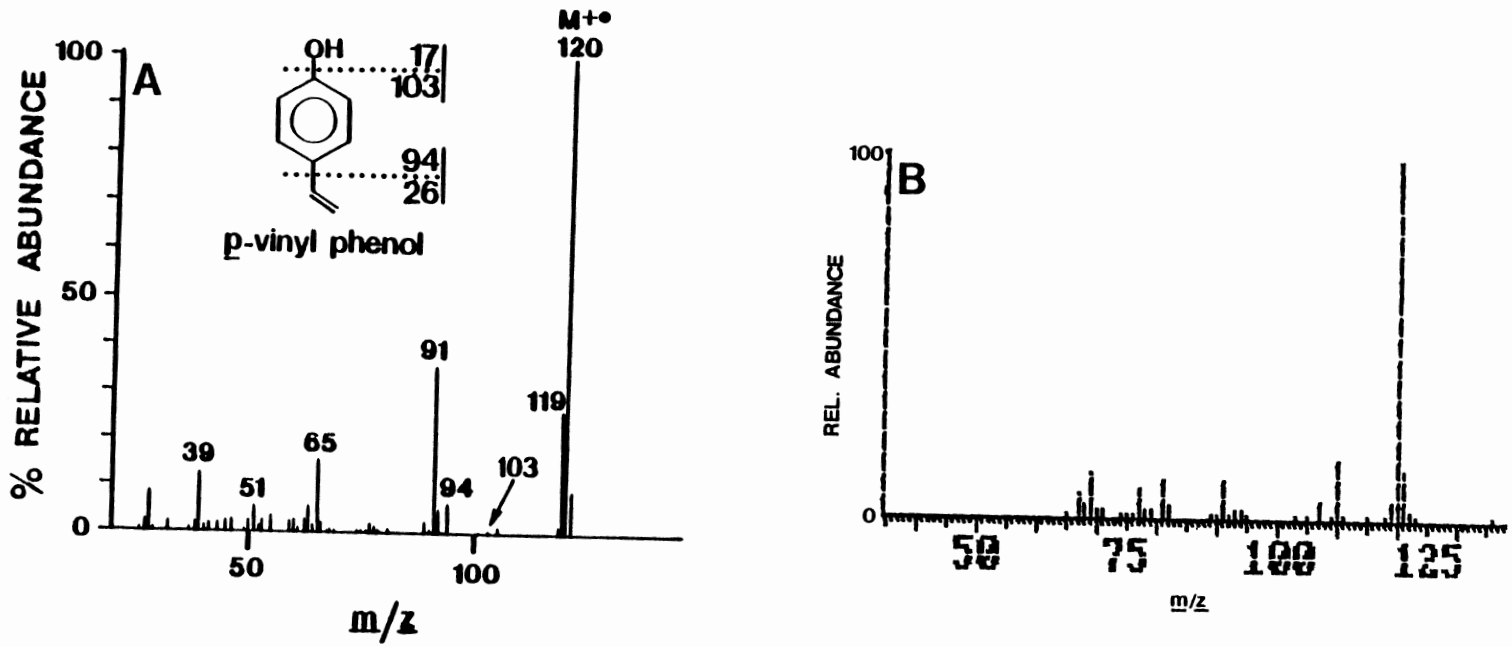

Figure 5. A) Mass spectrum of p-vinyl-phenol from Proboscidea louisianica essential oil, B) Standard mass spectrum of p-vinyl-phenol from LKB-2091 CGC/MS/DS.

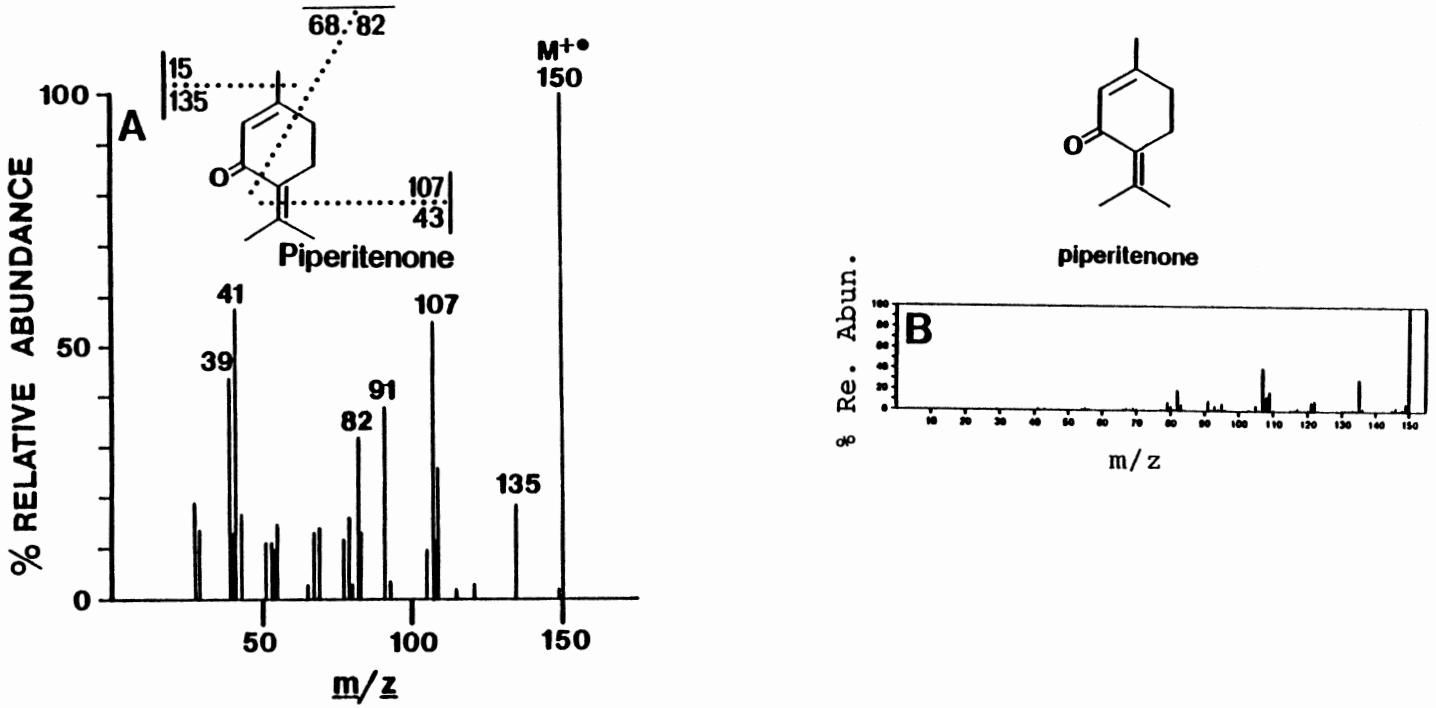

Figure 6. A) Mass spectrum of piperitenone from Proboscidea louisianica essential oil, B) Standard mass spectrum of piperitenone (Source: NBS/EPA/NIH Mass Spectral Data Base. p. 459, Chem. Abstract No. 491-09-8).

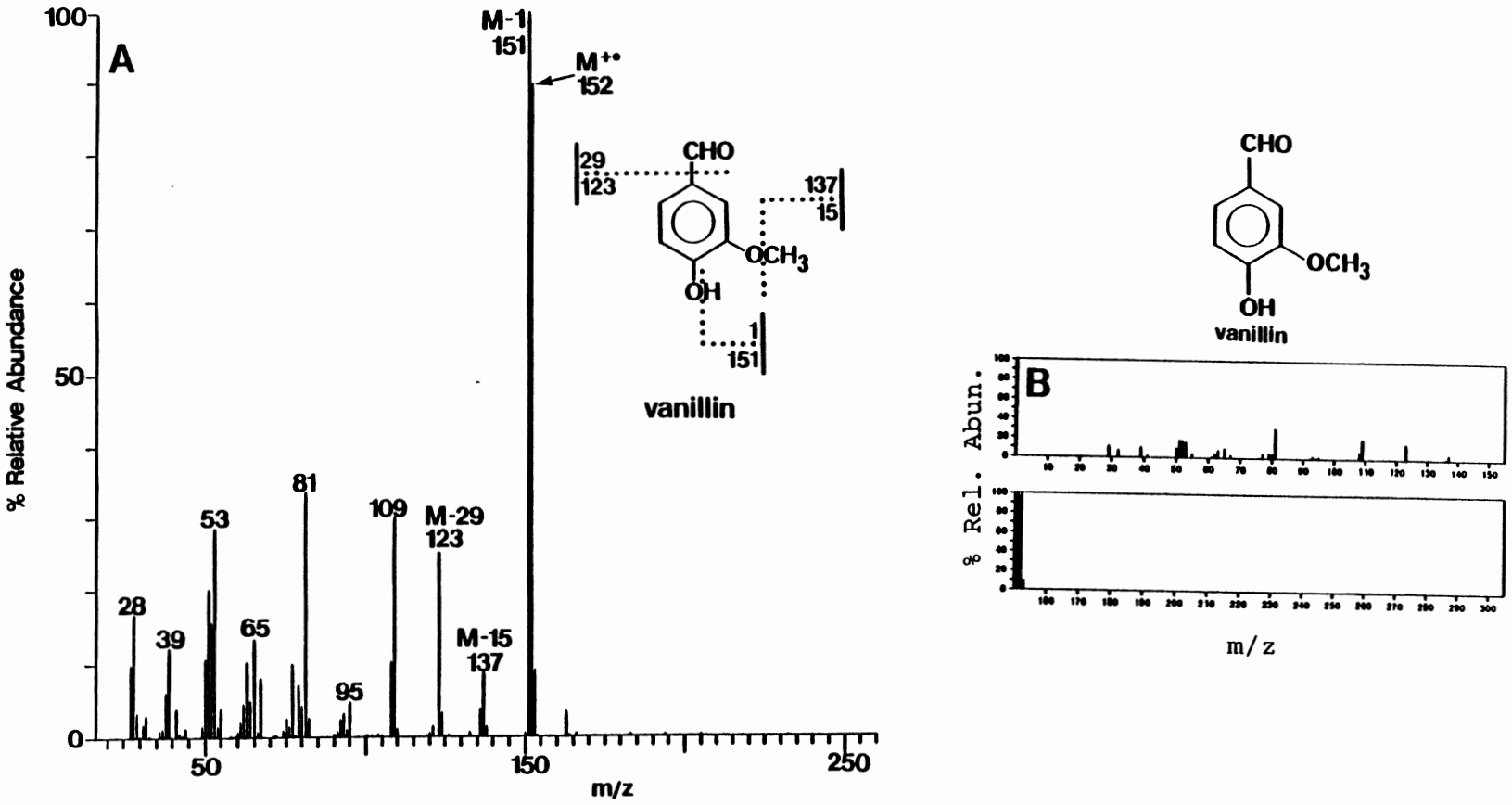

Figure 7. A) Mass spectrum of vanillin from Proboscidea louisianica essential oil, B) Standard mass spectrum of vanillin (Source: NBS/EPA/NIH Mass Spectral Data Base. p. 152, Chem. Abstract No. 121-33-5).

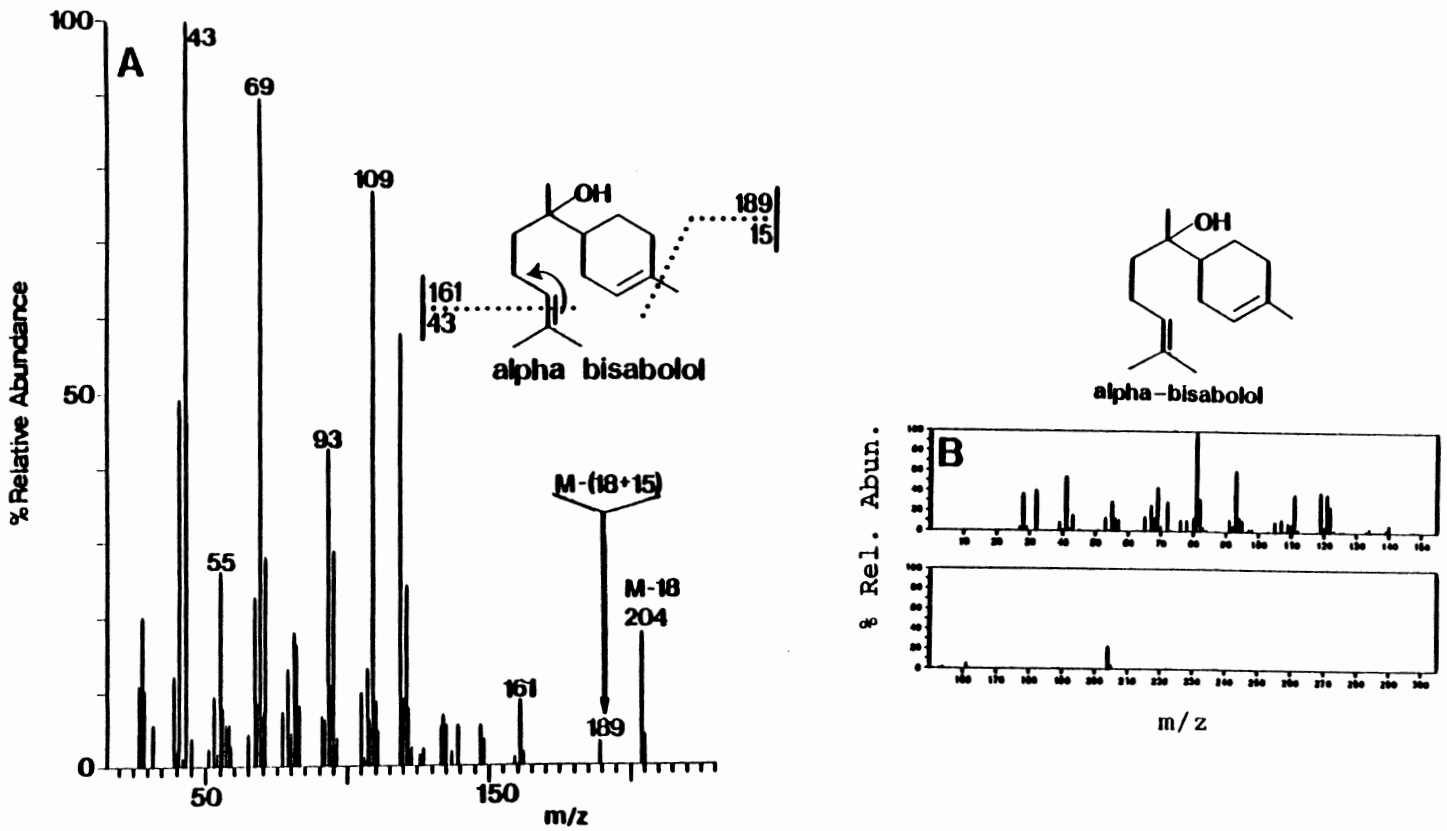

Figure 8. A) Mass spectrum of α-bisabolol from Proboscidea louisianica essential oil, B) Standard mass spectrum of α-bisabolol (Source: NBS/EPA/NIH Mass Spectral Data Base. p. 4526, Chem. Abstract No. 15352-77-9).

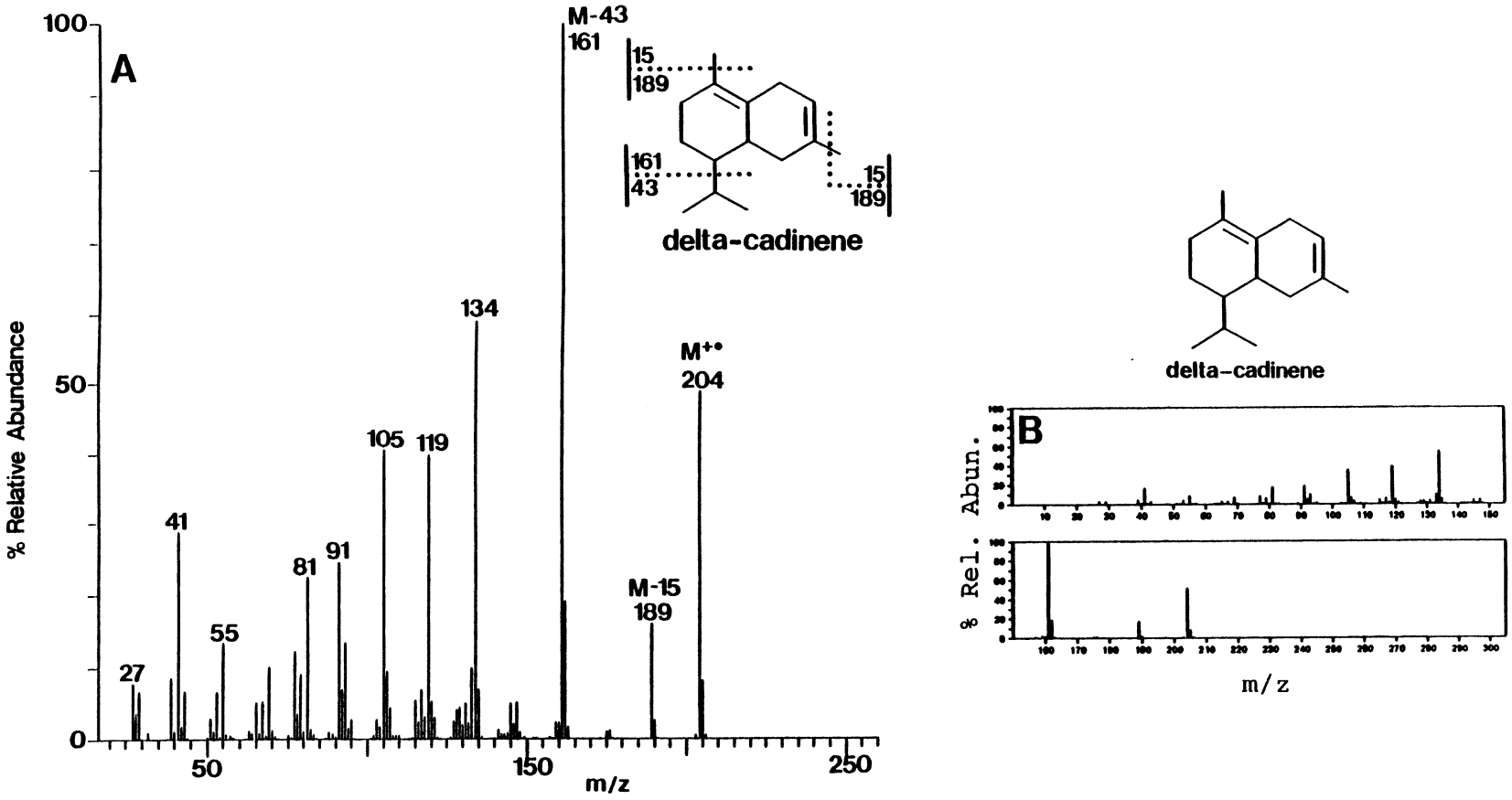

Figure 9. A) Mass spectrum of δ-cadinene from Proboscidea louisianica essential oil, B) Standard mass spectrum of δ-cadinene (Source: NBS/EPA/NIH Mass Spectral Data Base. p. 4441, Chem. Abstract No. 483-76-1).

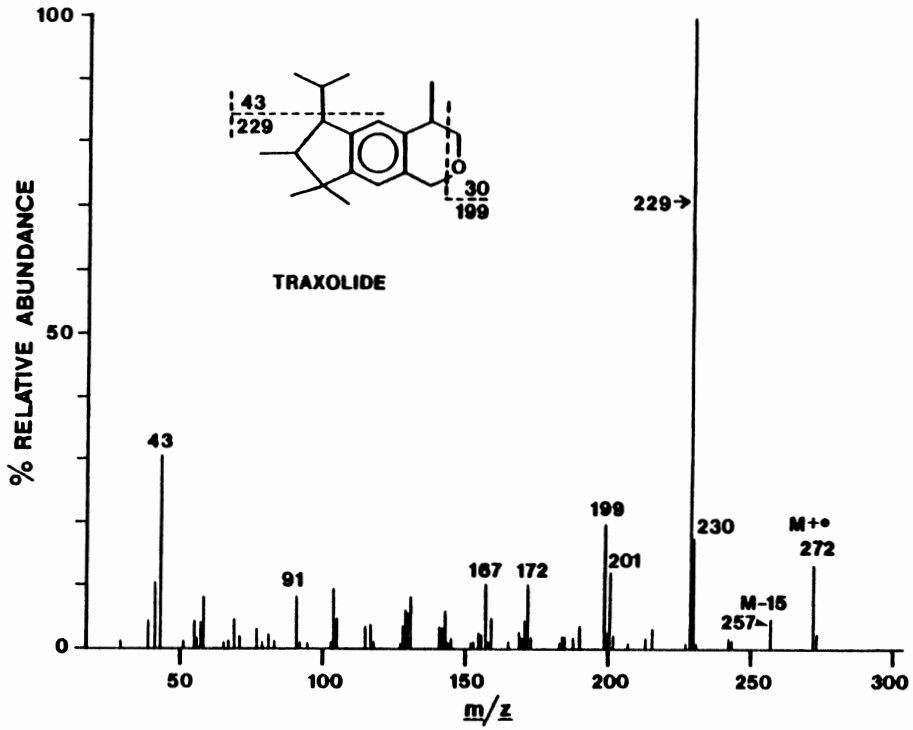

Figure 10. Mass spectrum of traxolide from Proboscidea louisianica essential oil.

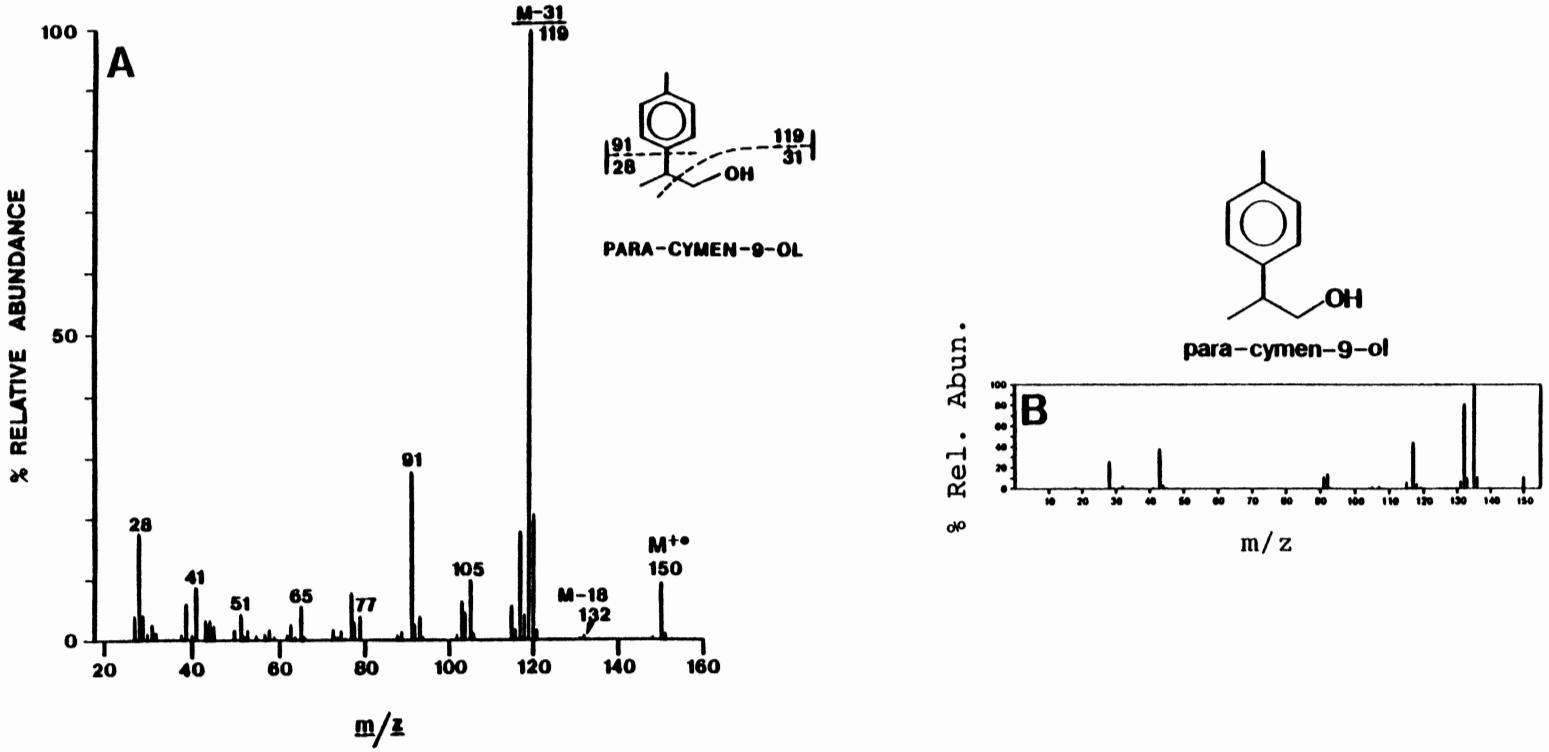

Figure 11. A) Mass spectrum of p-cymen-9-ol from Proboscidea louisianica essential oil, B) Standard mass spectrum of p-cymen-9-ol (Source: NBS/EPA/NIH Mass Spectral Data Base. p. 461, Chem. Abstract No. 4371-50-0).

PART V

ESSENTIAL OIL OF THE UNICORN-PLANT (*PROBOSCIDEA LOUISIANICA*), AND SIX OF ITS COMPONENTS, EVALUATED AS ALLELOCHEMICAL AGENTS ON COTTON AND WHEAT

Essential Oil of the Unicorn-Plant (Proboscidea louisianica), and Six of its Components, Evaluated as Allelochemical Agents on Cotton and Wheat

Abstract-The allelopathic activity of unicorn-plant [Proboscidea louisianica (Mill.)Thell.] essential oil and some of its compounds on the growth of cotton and wheat radicles was studied using a petri dish bioassay. Essential oil was collected by steam distillation using an all-glass and teflon assembly. The results indicate that ether extracts of the steam distillates from fresh unicorn-plant were inhibitory to cotton and wheat radicle growth. Six components of unicorn-plant essential oil identified by CGC/MS/DS were inhibitory to cotton and/or wheat at a concentration of 1 mM. These include vanillin, piperitenone, δ-cadinene, p-cymen-9-ol, α-bisabolol, and phenethyl alcohol.

Keywords-Allelopathy, allelochemical, bioassay, α-bisabolol, δ-cadinene, p-cymen-9-ol, essential oil, germination, Gossypium hirsutum, phenethyl alcohol, piperitenone, Proboscidea louisianica, steam distillation, Triticum aestivum, vanillin, volatiles.

INTRODUCTION

Unicorn-plant is a member of the Martyniaceae family and is native to the southwestern United States and northern Mexico (Martin and Hutchins, 1980). It is sometimes cultivated for its young pods which are pickled, and for mature pods which are used as ornaments and in basketweaving (Nabhan et al., 1981). Unicorn-plant is a spreading annual with prostrate branches spanning up to 1 m, and is densely covered with clammy, articulate, glandular hairs which gives the plant a very oily appearance, and a strong, musty odor (Brook and Weedon, 1986).

Seed germination of unicorn-plant is erratic and the seeds often show extreme dormancy after being freshly harvested (Heit, 1971). The best germination occurred when both the outer black leathery seed coat and the inner, papery white membrane were removed (Heit, 1971; Cooley et al., 1973). Gibberellic acid (CGA_3) greatly increased germination, and was inhibitory to subsequent growth (Anderson, 1968).

Many weedy pests in the cotton growing areas of Oklahoma and West Texas are responsible for yield reductions (Rushing et al., 1985; Rushing et al., 1985; Mercer et al., 1987). Early research of unicorn-plant interference with cotton showed that cotton lint yield is reduced 83% in weed infested areas (Cooley et al., 1973). Bridges and Chandler (1984) reported a cotton lint yield reduction of 34% when the weed population density was 4 plants/6 m of row. Mercer

et al. (1987) reported cotton lint reductions of 20% when the weed density was only 1 plant/10 m of row. Cotton plant height was reduced 43% and lint yield was reduced up to 74% when the weed density was 32 weeds/10 m of row.

Much of the research in allelopathy has centered on the crop-weed association. Results of this research have identified many specific cases of biochemical interactions between crop and weed. Tuber extracts and residues of yellow nutsedge (Cyperus esculentum) reduced the growth of corn (Zea mays) and soybean (Glycine max) (Drost and Doll, 1980). Plant residue and ethanolic extracts of Canada thistle (Cirsium arvense) were inhibitory to barley (Hordeum vulgare) and cucumber (Cucumis sativis) radicle growth (Stachon and Zimdahl, 1980). A glycoside of molecular weight 460 was isolated from the rhizomes of quackgrass (Agropyron repens) by methanol/water extraction, purified, and was inhibitory to the seedling root growth of corn, oat (Avena sativa), cucumber, and alfalfa (Medicago sativa) (Gabor and Veatch, 1981). Much of the allelopathic research has concentrated on compounds that are leached from plant litter or released by plant decomposition.

Volatile allelochemicals can be released from plants into the air and soil due to their low molecular weight and high vapor pressure. Research has been conducted on the inhibitory nature of the volatile chemicals that emanate from the leaves of Salvia leucophylla (Muller et al., 1964; Muller and del Moral, 1966). It was found that the

volatiles released from this shrub contained two terpenes, cineol and camphor, and these compounds were highly inhibitory to root and hypocotyl growth in germinating herb seeds (Muller et al., 1968). Recently, it was found that allelopathic volatiles are associated with the weed Palmer amaranth (Amaranthus palmeri). One of the compounds identified was 2-heptanone and its vapors were strongly inhibitory to the germination of onion (Allium cepa) and carrot (Daucus carota) at concentrations of 1 ppm (Connick et al., 1987).

When growing in the presence of cotton, unicorn-plant can cause substantial cotton lint yield losses (Bridges and Chandler, 1984; Mercer et al., 1985; Mercer et al., 1987). Since unicorn-plant is oily and odoriferous when growing in the field, it was decided that this weed would be a good candidate for research on the allelopathy of plant produced volatiles. Therefore, the objectives of this research were to isolate the essential oil of unicorn-plant and test them for allelochemical activity. A preliminary account of this research has been presented (Waller et al., 1987).

METHODS AND MATERIALS

Plant materials. Unicorn-plant was collected on August 27 and September 10, 1986 at Perkins, OK and separated into roots, stems and leaves, and pods. The plants collected in September were in the early stages of senescence. Fresh weight of the plant tissues collected on August 27 were 1.7

kg of stems and leaves, 3.7 kg of pods, and 0.2 kg of roots. Fresh weight of the plant tissues collected on September 10, were 1.9 kg of stems and leaves, 4.3 kg of pods, and 0.2 kg of roots. In 1987, unicorn-plant was collected in an active growth stage on the 14th, 22nd, and 27th of August. Approximately 5 kg of plant material were collected on each date by cutting the stems at ground level. Plant material was not separated in 1987. Both years, all plant material was collected and immediately taken to the laboratory for steam distillation.

Steam distillation. Steam distillation was used to isolate the unicorn-plant essential oils. The steam distillation apparatus was an all glass assembly using teflon stopcocks and sleeves. Normal and acidic distillations were made in 1986 by loading the plant parts separately into a 6 L round-bottom flask and steam distilling for 5 h. The condensate collected (approximately 3 L) was saturated with 1.1 kg NaCl, and extracted three times with 1 L of ethyl-ether, dried over anhydrous Na_2SO_4 and evaporated to dryness under nitrogen. The residue left after distillation was acidified with 1 L of 2 N HCl to a pH of 0.8 and redistilled for 5 h. The condensate was processed in the same way as the normal distillation. In 1987, only normal distillations were made of the whole plant (minus the roots) in 5 kg amounts without separating them into the various parts as in 1986. Also in 1987, because of the odor noticed in the laboratory during

distillation, a dry-ice/acetone trap was added to the steam distillation apparatus in an attempt to capture the very volatile constituents of the essential oil. After distillation was completed, the ice that formed on the dry ice/acetone trap was quickly thawed, collected in a 15 ml test tube, and stored at -18 C.

CGC/MS/DS analysis. The normal and HCl treated essential oils from the roots and pods, respectively, were analyzed by capillary gas chromotography/mass spectrometry/data system (CGC/MS/DS) using a Kratos MS-50 mass spectrometer with a resolution of 2000. The MS-50 was equipped with a Varian model 3700 gas chromatograph containing an OV-1 fused silica column 50 m x 0.32 mm and the samples were analyzed using a 1.0 μl injection with the splitter turned off, the oven at 50°C, which was programmed at 2°C/min to 225°C and held for 60 min using a He flow of 0.5 ml/min. The data were acquired and analyzed using a modified Kratos DS-55 data system (Bondarovich et al., 1987). Identifications were based on the comparison of known with unknown spectra and visual interpretation of the fragmentation patterns.

Bioassays. In 1986, 18 ml of methanol was added to each of the normal and acidic steam distillates. These solutions were added in 2 ml amounts to 9.5 cm petri dishes containing two layers of 9 cm Whatman No. 1 filter paper. The methanol was allowed to evaporate for approximately 2 h. The length of time of methanol evaporation was short to keep

to a minimum time the loss of volatiles. Ten cotton or wheat seed were then placed between the filter paper layers, 3 ml of distilled water was added, and the covered dish placed in a sealed polyethylene bag to prevent the further loss of volatiles. In 1987, the essential oil collected from unicorn-plant during August, and selected reference compounds[1] were dissolved in methanol to a 1 mM concentration. Reference compounds that are 100% pure were not available; therefore, those obtained represent compounds that are greater than 90% pure. To make a 1 mM concentration of unicorn-plant essential oil in methanol it was assumed that the average molecular weight would be 200. The bioassays were conducted in the same way as those in 1986, with the exceptions of the 1987 essential oil bioassay where 4 ml of the 1 mM solutions were added to the petri dish, in addition to wheat being added as a bioassay species. All experiments were designed as a randomized complete block (Montgomery, 1984) with each of four replications comprising a tray level in the germinator. The germination temperature was 27 C for cotton and 20 C for wheat. The seeds were allowed 72 h for germination and growth and then the measurements of radicle length were made.

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[1]Reference compounds were obtained from International Flavors and Fragrances, 800 Rose Lane, Union Beach, New Jersey 07735 and Firmenich Inc., P.O. Box 5880, Princeton, New Jersey 08543.

Statistical analysis. All bioassays consisted of two runs with four replications except for the bioassay with the ice that formed in the dry-ice/acetone trap. Enough ice was recovered for one run of four replications. Before data analysis, the variance of all treatments were checked using the VARCOMP procedure in SAS[2]. Generally, if the treatment inhibited radicle growth, the variance for that treatment was smaller than uninhibited treatments. Therefore the variances were checked and weighted before analysis. After analysis, a run by treatment interaction was not detected for any of the bioassays, so the runs were pooled and analyzed. The treatment means were separated using the LSD at the 0.05 level of probability.

RESULTS AND DISCUSSION

Steam distillation. The ether extracts of the steam distillates were a viscous, dark yellow to light brown oil with a pungent, musty odor. Recovery was generally higher for the August 27 distillation (Table 1). The unicorn-plant was in an active growth stage at this time, and was green and succulent. Recovery for the September 10 distillation was less due to the fact that unicorn-plant was in a stage of early senescence. The older leaves were beginning to die and fall away and were less oily than in August. In 1987, steam distillations of the above ground portion of unicorn-

[2]Statistical Analysis Systems, SAS Institute Inc., Box 8000, Cary, NC 27511.

plants were made at three dates during August when the plants were in an active growth stage. Because of the active growth stage recovery was equal to or more than in 1986. The weight of the steam distillates were 61 mg for August 18, 549 mg for August 22, and 76 mg for August 27 (0.0012%, 0.0110%, and 0.0015% of fresh weight respectively). The ice that collected in the dry-ice/acetone trap was approximately 5 ml for each distillation. The water that collected on the trap had a very strong sulfide odor, indicating the presence of CHS compounds which were not identified.

CGC/MS/DS analysis. The MS-50 profile of the essential oils required approximately 140 min and 3,500 spectra for each sample and the results indicated that between 150-220 compounds were detected. From this mixture the following peaks were identified from the normal steam distillate of the roots in order of appearance from the CGC column: p-vinyl-phenol, piperitenone, vanillin, 2-methyl-1,4-naphthoquinone, ionol, 1,3,5-tritertbutyl-benzene, α-bisabolol, hexadecanoic acid, traxolide, δ-cadinene, and 9,10-anthracenedione-(1-hydroxy-2(or 3)-hydroxymethyl). Trace amounts of 6-methyl-5-hepten-2-one was found. The remaining compounds were mostly terpenes, terpenoids, and hydrocarbons and were not identified. The following peaks were identified in the HCl-treated steam distillate from the pods: phenylethyl alcohol, trimethyl-cyclohexanone, p-cymen-9-ol, vanillin, tentatively 2-ethylbenzimidazole, dodecanoic

and hexadecanoic acids. The remaining peaks were mostly sesquiterpenes, and hydrocarbons and were not identified.

Bioassays. In 1986, the normal essential oils collected on August 27 of the leaves and stems, and the pods and the HCl-treated essential oil of the pods were inhibitory (27, 37, and 15%, respectively) to cotton radicle elongation (Table 2). In this bioassay, the essential oil recovered was brought up in methanol to 18 ml, so the concentration was different between extracts. On August 27, a high concentration of the normal essential oil of the leaves and stems, and the pods were present in the petri dish and these treatments were highly inhibitory. However, inhibition was not entirely dependent on concentration. The HCl-treated essential oil of the leaves and stems was not as inhibitory as the HCl-treated essential oil of the pods in which the latter was present in much less concentration (2.60 vs. 0.62 mg/dish). The essential oil collected from pods was the most inhibitory within the normal and acidic steam distillations. Essential oil from the roots, whether normal or acidic, did not significantly inhibit cotton radicle growth when compared to the control. Of the steam distillations conducted on September 10, only the HCl-treated essential oil of the leaves was inhibitory to cotton radicle elongation with 33% inhibition (Table 2). Since the plants were in a state of senescence, a buildup of degradation products could have been responsible for the inhibition, but the oil was not analyzed. The normal

essential oils were less concentrated in this bioassay and were not significantly inhibitory.

After analysis of the essential oil in 1987, several compounds present in the essential oil were obtained. A 1 mM concentration of these compounds made in methanol and tested on cotton and wheat shows that of the 13 compounds tested, six were inhibitory to cotton and five were inhibitory to wheat (Table 3, Figure 1). Of the six compounds, three were identified in the root, two in the pods, and one (vanillin) was found in both the root and the pod. Of the six inhibitory compounds, four are terpenoid in nature. Piperitenone and p-cymen-9-ol (Figure 1a, 1b) are monoterpenes, and α-bisabolol and δ-cadinene (Figure 1d and 1f) are sesquiterpenes. Monoterpenes are widely known to be inhibitory (Asplund, 1968; Asplund, 1969; Muller, 1968; Muller et al., 1968; Fischer, 1986). Asplund (1969) reported that the monoterpenes camphor, pulegone, and borneol were extremely toxic to radish (Raphanus sativus) and wheat and that monoterpenes are among the most allelopathic compounds produced by plants. Sesquiterpenes, such as β-bisabolene, isolated from common ragweed (Ambrosia artemisiifolia) caused strong germination inhibition to onion, oats, and ryegrass (Lolium multiflorum) (Fisher, 1986).

Phenolic compounds, such as vanillin (Figure 1c), have been implicated as being allelopathic agents released as root exudates and from decomposing plant litter (Wang et

al., 1967). In the mitochondria, phenolic compounds such as vanillin act as electron transport inhibitors (Moreland and Novitzky, 1987). Inhibition appears to be the result of alterations produced in the inner membrane by the allelochemical.

The concentration of the compounds tested for inhibitory activity against cotton and wheat are listed in μg/dish, corresponding to 2 ml of a 1 mM solution being added to the dish (Table 4). The column of data for cotton shows that the two monoterpenes, p-cymen-9-ol and piperitenone were 16 and 13% inhibitory, respectively. The sesquiterpene alcohol, α-bisabolol, and vanillin were 9 and 11% inhibitory, respectively. All the compounds listed, except vanillin were inhibitory to wheat. The concentration of compounds used in this bioassay is relatively low and is equal or below the concentrations used by many scientists (Colton and Einhellig, 1980; Patterson, 1981; Williams and Hoagland, 1982). Piperitenone, a monoterpene with a ketone group, was the most inhibitory compound tested. Asplund (1968) reported that monoterpenes with a ketone functional group such as camphor and pulegone were the most inhibitory of all monoterpenes tested, and were an order of magnitude greater in toxicity to radish seeds than HCN.

The essential oils of unicorn-plant extracted from the upper plant parts on August 22 and 27 were inhibitory to cotton radicle growth (Table 5). Piperitenone and α-bisabolol were included for comparison purposes. In this

bioassay, 4 ml of the 1 mM essential oil solution was added to each dish and this is shown as 800 μg/dish of essential oil. The essential oil was inhibitory to cotton to the same magnitude as the compounds piperitenone and α-bisabolol. Only the essential oil from August 14 was inhibitory to wheat, while piperitenone and α-bisabolol were extremely inhibitory to wheat.

It should be noted that the essential oil contains up to 220 compounds, so that each compound is acting in only minute quantities, possibly synergistically. Asplund (1969) suggests that the phytotoxic monoterpenes exhibit a marked synergistic action when used in combination and that the phytotoxic concentrations are enhanced up to 100 times by using two compounds simultaneously.

The ice collected on the dry-ice/acetone trap was not inhibitory to cotton radicle growth (data not shown). This material contained the very volatile compounds from distillation because they were not trapped in the distillate. The concentration of the solution was not known but it had a very strong sulfite odor.

Unicorn-plant, when growing with cotton can cause substantial yield reductions (Mercer et al., 1987). The essential oils collected from the upper portions of unicorn-plant in late August were inhibitory to cotton and this is a very sensitive growth stage for cotton in Oklahoma because of the initiation of flowers and bolls. Essential oils from the roots were not inhibitory. The fact that unicorn-plant

leaves and pods release volatile chemicals while growing in the presence of cotton suggests that cotton could be effected with subsequent yield reductions due to the chemicals toxic action. The essential oil would be volatilizing from the upper portion of unicorn-plant at all times and thus be available to penetrate cotton leaves and cause inhibition.

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Table 1. Amount of essential oil recovered from steam distillation of the unicorn-plant in 1986.

	August 27		September 10	
Distillation	Essential oil recovered	Percent of fresh weight	Essential oil recovered	Percent of fresh weight
	(mg)	(%)	(mg)	(%)
Normal distillation				
leaves and stems	57.2	0.0034	4.0	0.0002
pods	40.0	0.0011	16.2	0.0004
roots	9.4	0.0047	14.0	0.0070
Acidic distillation				
leaves and stems	46.8	0.0028	23.1	0.0012
pods	11.2	0.0003	13.0	0.0003
roots	13.7	0.0069	15.1	0.0076

Table 2. Effects of steam distillates of unicorn-plant, collected on August 27 and September 10, on cotton radical length.

	Date of distillation[a]			
	August 27		September 10	
Distillation	Concentration	Inhibition	Concentration	Inhibition
	(mg/dish)	(%)	(mg/dish)	(%)
Control (methanol)	---	0 a	---	0 a
Normal distillation				
leaves and stems	3.18	27 b	0.22	2 a
pods	2.22	37 b	0.90	10 a
roots	0.52	8 a	0.78	0 a
Acidic distillation				
leaves and stems	2.60	7 a	1.28	33 b
pods	0.62	15 b	0.72	11 a
roots	0.76	8 a	0.84	12 a

[a]Means followed by the same letter are not significantly different at the 5% level using LSD.

Table 3. Compounds tested at a 1mM concentration for allelochemical activity on cotton or wheat[a].

Compound	Plant part	Cotton	Wheat
α-bisabolol	root	+	+
δ-cadinene	root	+	+
p-cymen-9-ol	pod	+	+
methyl-heptanone	root	-	NT
1,4-naphthoquinone	root	-	-
perillyl acetate	root	-	NT
phenethyl alcohol	pod	+	+
piperitenone	root	+	+
2,4,4-trimethyl-cyclohexanone	pod	-	NT
vanillin	root, pod	+	-
p-vinylphenol	root	-	NT

[a]+ indicates inhibition, - indicates no inhibition, NT = not tested.

Table 4. Effects of 1 mM concentration of volatile compounds on cotton and wheat radicle growth

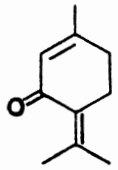
Compound	Plant part	Concentration	Inhibition[a]	
			Cotton	Wheat
		(μg/dish)	-----(%)-----	
control		0	0 a	0 a
α-bisabolol	root	440	9 bcd	39 c
δ-cadinene	root	410	2 ab	20 b
p-cymen-9-ol	pod	300	13 cd	34 bc
phenethyl alcohol	pod	240	6 abc	28 bc
piperitenone	root	300	16 d	43 c
vanillin	root, pod	300	11 cd	2 a

[a]Means followed by the same letter are not significantly different at the 5% level using LSD.

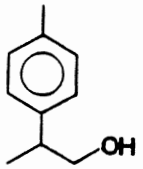
Table 5. Effects of a 1 mM concentration of unicorn-plant essential oil from 1987 (based on average molecular weight of 200) and selected compounds on cotton and wheat radicle growth.

Sample	Concentration	Inhibition[a]	
		cotton	wheat
	(μg/dish)	-------(%)-------	
control	0	0 a	0 a
essential oil - 8/14	800	7 ab	9 b
essential oil - 8/22	800	15 bc	6 ab
essential oil - 8/27	800	12 bc	4 ab
piperitenone (root)	600	17 c	75 d
α-bisabolol (root)	890	12 bc	42 c

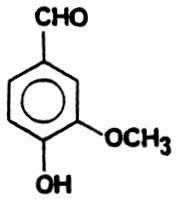
[a]Means followed by the same letter are not significantly different at the 5% level using LSD.



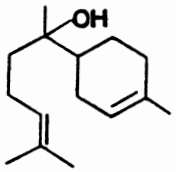
a. piperitenone (root)



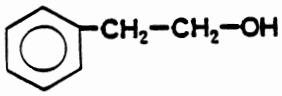
b. p-cymen-9-ol (pod)



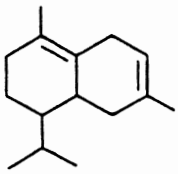
c. vanillin (root, pod)



d. α-bisabolol (root)



e. phenethyl alcohol (pod)



f. δ-cadinene (root)

Figure 1. Componants of unicorn-plant essential oil that were inhibitory to cotton and/or wheat at a 1 mM concentration. Compounds are listed from most inhibitory to least inhibitory.

VITA

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