CHAPTER 6

ALLELOPATHIC EFFECT OF *PASPALUM PANICULATUM* AND *PASPALUM URVILLEI* ON GROWTH OF SUGAR CANE

6.1 Introduction

Sugar cane is a very important crop in Mauritius and occupies approximately 80% of the arable land. This perennial plant of the grass family is grown over a period of 12 to 18 months during the first year, followed by a 12-month ratoon crop for another six to eight years. As the growing period is relatively long, taking between 4 to 8 months for complete canopy closure, weeds need to be controlled efficiently (Rochecouste, 1967). The traditional practice has been to target 100% control of all weeds from sugar cane fields irrespective of the amount and species of the weeds and stage of growth of the cane, by use of large amounts of pre- and post-emergence herbicides. The average amount of herbicides used annually has varied between 8 to 10 kg a.i. ha^{-1} during the last three decades (MSIRI, 2004). The costs for weed control have increased significantly during the last ten years; the average cost for herbicides exceeds MUR 4 000 ha^{-1} (120 US \$ ha^{-1}) annually (see Chapter 1).

Increasing pressure on farmers to optimise their use of pesticides to reduce environmental effects and to minimize costs has led to the development of strategies for integrated weed management (IWM) and use of alternative methods to herbicides for weed control. IWM has also become the basis of all FAO plant protection activities because it contributes directly towards the achievement of sustainable agriculture in developing countries (Labrada & Parker, 1994). Development of such strategies in the Mauritian sugar industry became even more urgent with the announcement and implementation of a price reduction of 37% by 2009 by the EU, the main importer of Mauritian sugar. Several projects have been initiated since 1998 to develop weed management strategies in sugar cane. Firstly, trials studying critical periods of weed competition under the worst agroclimatic conditions of the island have revealed that weed competition started 12 WAH and ended 26 WAH in ratoon cane, and control measures may need to be maintained up to 29 WAP to keep yield losses below 5% in plant cane (Chapter 2; Seeruttun & Lutman, 2004). These studies to compare relative competitiveness of various weed species commonly present in sugar cane fields have revealed sugar cane as a stronger competitor than most of the weeds tested; the time of emergence and rate of development of the weed species influencing the effect. The mechanism of the aboveground competition in sugar cane has been studied by comparing the competitive ability of two *Paspalum* species with different morphological traits; *P. urvillei* being a tussocky mostly erect perennial reaching 150-200 cm in height and leaves 12-50 cm long while *P. paniculatum* reaches a maximum height of 100-150 cm with lanceolate leaves 20-40 cm long and 1.0-2.5 cm broad, with a more planophile arrangement (Mc Intyre, 1991). *Paspalum paniculatum* has been found to be relatively more competitive than *P. urvillei* despite the latter growing taller and having higher relative leaf areas. This difference led to investigations on mechanisms of competition occurring between sugar cane and the two *Paspalum* species (Chapter 5). Shoot versus root competition trials showed that root (underground) competition was important in sugar cane. However, the trials were not able to elucidate the cause of the difference in competitiveness between the two *Paspalum* species.

Weed interference is a term used to express competition by both indirect interaction (e.g. crop and weeds competing for limited resources such as light, mineral nutrients, water, or volume of space) and direct interactions/interference (e.g. suppression of growth of one individual by the other releasing phytotoxic chemicals). Allelopathy is a phenomenon observed in many plants that release chemicals into their near environment either from their aerial or underground parts in the form of root exudates (Rice, 1984). The chemical compounds released into the environment act on the other organisms, such as weeds, plants, animals and microorganisms, by inhibitory or excitatory ways. These chemicals accumulate and persist for a considerable time, thereby imparting significant interference on the growth and development of neighbouring weeds and plants (Putman & Duke, 1974). Literature reviews by Putnam (1988) and Williamson (1990) have described allelopathy caused by substances from a number of cultivated plants and weeds. Allelopathic potential of many gramineous weeds have been reported including that of extracts of *Paspalum notatum* Flueggé (bahiagrass) and other warm-season grasses on alfalfa and Italian ryegrass (Martin & Smith, 1994), interference between bermudagrass [*Cynodon dactylon* (L.) Pers] or johnsongrass [*Sorghum halepense* (L.) Pers] and cotton or corn (Vasilakoglou *et al*., 2005) and nutgrass (*Cyperus rotundus*) on rice seedlings (Quayyum *et al*., 2000). Ishmine *et al.* (1987) studied the potential of some dominant weeds of sugar cane on the Ryukyu Islands and reported that exudates of *P. urvillei* caused an adverse effect on growth of *Phaseolus vulgaris* in greenhouse trials. Root exudates of *P. notatum* have also been reported to reduce soybean and okra (*Hibiscus esculentus*) height increments (Pope *et al*., 1984). Mc Intyre (1998) reported an allelopathic effect of *C. rotundus* on sugar cane.

Considerable current research on allelopathy is focused on its use in weed management strategies, either by identifying allelochemicals for production of bioherbicides or to serve as leads for synthetic herbicides. Much research effort is also spent on identification of crop cultivars having allelopathic properties which can suppress weeds. One means of exploiting allelopathy for weed control is through the use of decaying crop residues, for example, the release of allelochemicals from rice straw (Fujii, 1992; Chou, 1999; Ahn & Chung, 2000). In sugar cane, evidence of allelochemical substances continually being leached from trash that suppressed weeds has been reported by Lorenzi *et al.* (1988). The leachates from sugar cane trash have also been reported to cause autotoxicity; Viator *et al.* (2006) contended that benzoic acid in leachates from trash blanket impairs cane ratooning and growth.

One concern often voiced by researchers of allelopathic interactions is that many laboratory bioassays do not adequately predict the responses observed in field situations. Inderjit and Weston (2000) concluded that a laboratory bioassay could not demonstrate that allelopathy is operational in natural settings. Current research is addressing this issue and many new methodologies and techniques for identification, assessment, etc. are being developed. Recent examples include a ‘sandwich method’ for elucidating allelopathic effect of leaf litter leachates under laboratory conditions (Fujii *et al*., 2004) and use of dose-response curves with known standard allelochemicals in bioassay based on hydroponic culture to screen cultivars for allelopathic traits (Belz & Hurle, 2004).

Benzoxazolin-2(3*H*)-one (BOA) or hydroxamic acids are commonly occurring secondary metabolites in cultivated and wild Gramineae (Zuniga *et al*., 1983; Niemeyer, 1988 and Friebe *et al*., 1998) and have been shown to have an effect on radicle growth and elongation (Aiupova *et al*., 1979) or causing abnormal growth (Wolf *et al*., 1985). BOA has been reported by Barnes and Putnam (1987), and Belz (2004) as the responsible agent for the inhibitory activity of rye residues.

In the present study root exudates (leachates) from *P. urvillei* and *P. paniculatum* have been tested for allelopathic properties in four glasshouse experiments between December 2005 and July 2007. The main objectives of the trials were (i) to determine if root exudates from the two *Paspalum* species exert allelopathic effects on sugar cane and, if yes, (ii) would there be different varietal responses to such chemicals, and (iii) to compare the two *Paspalum* species with respect to their allelopathic properties.

6.2 Materials and methods

Methodology for collection and application of leachates

The methodology used in classical allelopathy trials, i.e. laboratory bioassays with extracts applied on seeds in Petri dishes or other techniques such as the “sandwich method” for leaf litter, could not be used with sugar cane as the plant is vegetatively propagated using cuttings from the stem and the growth period is relatively long. Furthermore, the collection of leachates from the donor plant was more difficult and the approach for continuous trapping of chemicals from an undisturbed root system as developed by Tang and Young (1982) was not possible for practical reasons. The methodology used by Mc Intyre (1998) for transferring leachates from *Cyperus rotundus* to young sugar cane shoots was also physically limiting, as the *Paspalum* species would grow much taller than *C. rotundus*.

For this study, the methodology consisted of applying leachates collected from the donor plant grown in a relatively ‘inert’ medium to young pre-germinated cane setts of four sugar cane varieties grown in a similar medium.

Trial site and plant material

The experiments were carried out in an unheated glasshouse with no supplementary lighting at Réduit (MSIRI) experiment station. Seedlings or young plants of the donor plants, i.e. *P. paniculatum* and *P. urvillei*, were uprooted/collected from sugar cane fields or abandoned lands in the Belle Rive area where it is more humid and these two *Paspalum* species are common weeds. The recipient plant in the four experiments consisted of young sugar cane plants of four widely grown varieties namely M 3035/66, R 570, R 579 and M 695/69. They were selected on the basis of the total area cultivated with them and their tolerance to post-emergence herbicide treatments (MSIRI, 2003). M 3035/66, cultivated on approximately 5% of the area cultivated by Miller-Planters (growers possessing a mill and owning approximately 45% of total land under sugar cane) in 2005, is classified as a tolerant variety (MSIRI, 2006). R 570, occupying more than 23% of the area grown by that group of growers, is very susceptible to herbicide treatments. R 579 and M 695/69, respectively covering 10% and 8% of the acreage by Miller-Planters, are classified as moderately susceptible varieties.

Sugar cane was planted using two-eyed cuttings (cane setts with two buds each) obtained by cutting cane stalks 9 to 12 months old (plant cane) from fields on the station or nearby nursery. They were allowed to germinate in filter mud before transplanting in the buckets.

Containers and growing medium

Plastic containers with a diameter of 20 cm and 15 cm deep (10 litres capacity) were used for the weeds. These were perforated at the bottom to allow excess irrigation water (leachates) to collect in plastic bowls/trays placed 10 cm below each container. The clearance between the growing container and the collecting device was assured by placing the container on wooden frames (Fig. 6.1). When the pre-germinated sugar cane setts were at the 2-leaf stage they were uprooted from the filter mud medium, cleaned to remove most of the filling medium before being transplanted in larger plastic containers (buckets) of 20 L capacity. These buckets also had perforations at the bottom but were placed directly on the collecting bowls to enable excess water to be absorbed back into the medium though capillarity movement.

Fig. 6.1 *Paspalum paniculatum* (left) and *P. urvillei* (right) transplanted in trays filled with mixture of rocksand and filter mud (left), and containers and collecting bowls arrangement for leachates collection from weeds (right)

The growing medium used for both cane and the weeds was a mixture of ‘rocksand’ and filter mud at a ratio of 2:1. The ‘rocksand’ consists of small size (max. 4 mm) particles obtained by crushing basaltic rocks (volcanic origin); this material is usually used in construction. The inert property of the rocksand was assured by washing it with clean water prior to mixing with filter mud. The latter is a cake which is produced after filtration of the precipitated cane juice and also contains much of the colloidal organic matter anions that precipitate during clarification. The filter mud consists mainly of moisture (>60%) and has approximately 1% by weight of phosphate (P_2O_5) (Paturau, 1989).

The medium used was analysed by the Agricultural Chemistry department of MSIRI for pH, CEC, total N, P & K, and dry matter content. Pre-experimentation analysis of the filling medium in

Trial IV had revealed the presence of total N at 1.17%, total P at 0.83% and total K at 0.11%; the soil pH was 6.7 with a CEC of 19.3 cmol kg^{-1}. At the end of the experiment, analysis showed the presence of total N at 0.75%, total P at 0.46% and total K at 0.07%, with a pH of 7.0 and a CEC of 14.0 cmol kg^{-1}.

Planting weeds and transplanting of sugar cane

The collected weeds were transplanted at a density of four stools per container after their leaves were pruned to reduce transpiration. In all trials, 15 containers were planted with each weed species for leachate collection while 10 others were kept unplanted to act as a control.

The two-eyed cane setts for each variety were treated (cold dip) against 'pineapple' disease (caused by *Ceratocystis paradoxa*) with a solution of benomyl at 0.3 g per litre before being planted in large trays filled with rocksand and filter mud (50:50) for germination. Once the setts had germinated, they were uprooted and transplanted in the buckets – one pre-germinated sett per bucket (Fig. 6.2). This step was done to guarantee homogeneity of having two well-developing primary shoots per bucket. For Trial III, due to a poor and erratic germination, the two-eyed cuttings were cut into planting material with only one primary shoot before transplanting into the buckets.

Fig 6.2 Pre-germinated two-eyed cuttings planted in buckets to receive leachates from *P. paniculatum* and *P. urvillei*.

Leachate collection and application to recipient plant

Distilled water was used to irrigate all weeded containers on a daily basis as from establishment; the containers without weeds also received the same amount of water. All excess water percolating through the containers were collected from the bowls every morning and were bulked together into three treatments: leachate from *P. paniculatum*, leachate from *P. urvillei* and leachate from unplanted containers. The containers with the collected leachates were covered and stored under the bench to avoid direct sunlight.

Cane setts were irrigated with distilled water for one or two weeks after transplanting before treatments commenced. Once treatment started the cane received only leachates collected from the donor containers or control. The onset of treatments varied across trials as the establishment of the weeds differed (Table 6.1). The volume of water used to irrigate the weeds varied between 300 and 750 ml depending on the stage of growth and rate of evapotranspiration. This was monitored closely and adjustments were made according to volume of water left in collecting bowls and physiological state of the weeds – water-stress conditions or the presence of too much (diluted) leachates were avoided. All cane buckets received the same volume of leachates from the treatments; the volume applied again varied with water requirements of cane plant with respect to evapotranspiration and its stage of growth. In Trial I, distilled water was applied directly in the control buckets whereas in the other experiments the control received water collected through the similar containers without weeds.

Table 6.1 Treatment dates in trials assessing allelopathic potential of two *Paspalum* species on sugar cane

	Dates			
Trial	Weeds transplanted	Cane transplanted	Start irrigating with leachates	End of trial
Trial I	14 December 2005	28 December 2005	12 January 2006	23 March 2006
Trial II	14 April 2006	2 May 2006	15 May 2006	7 October 2006
Trial III	20 October 2006	4 November 2006	11 November 2006	12 February 2007
Trial IV	3 February 2007	23 February 2007	5 March 2007	7 July 2007

Experimental layout and data collection

The buckets with cane plants were placed on a bench (1 m above floor) on one side of the glasshouse while the weeds were placed in a similar manner on the opposite side. The temperature in the glasshouse was slightly higher than the outside temperatures during the day; all window/opening were left open with a fine mesh wire gauze screen to prevent any insects, etc. Natural day-light was used and the main advantage of placing the trays indoors were to control water regimes by preventing the effect of rainfall.

Data collection consisted of measuring dewlap heights of the primary shoots in each bucket at regular intervals. For Trial I, the first measurement of cane shoot height (dewlap height) was made on 11 January 2006 and was followed by a second one on 6 February 2006. On 23 February 2006 (12 weeks after transplanting), all cane shoots were cut and measurements were taken for stalk height. The harvested material was sorted into primary tillers and new tillers from each bucket and weighed. Sub-samples from the harvested material were weighed before and after being oven-dried at 105°C for 48 hours. The buckets were emptied on 25 February 2006 for dry weight analysis of root biomass.

For Trial II, cane measurements started on 15 May 2006 and subsequently were taken on 2 June 2006, 19 June 2006, 3 July 2006, 17 July 2006, 1 August 2006, 14 August 2006, 29 August 2006, 13 September 2006 and 28 September 2006. On 6 October 2006, all shoots were measured for the last time before being cut at ground level and the roots excavated. All harvested samples were weighed before and after oven-drying at 105°C for 48 hours.

For Trial III, dewlap height was measured for each primary shoot on 24 November 2006, 4 December 2006, 14 December 2006, 26 December 2006, 10 January 2007, 19 January 2007 and 29 January 2007. Aboveground and root biomass (dry weights) of cane were measured for each treatment at the end of the trial, as described above.

Cane measurements in Trial IV started on 6 March 2007 and were also carried out on 21 March 2007, 6 April 2007, 26 April 2007, 10 May 2007, 25 May 2007, 20 June 2007 and 5 July 2007. All cane shoots were chopped and roots excavated on 5 July 2007. They were weighed before and after being oven-dried at 105 °C for 48 hours; dry weight of cane stalks, cane leaves and biomass of cane root per bucket were recorded.

For determining root biomass, the buckets were emptied and all roots separated from the filling material before being washed to remove all filter mud. The roots were separated from cane setts and oven-dried for 48 hours before being weighed.

Statistical design and analysis

Genstat (Discovery Edition 2) was the statistical package used for all the statistical analyses. All data recorded from cane measurements (dewlap heights), aboveground and root biomasses were subjected to analysis of variance (ANOVA) by using a split-plot design, and main effects and interactions were tested for significance. The four cane varieties were the main-plots and the three sub-plot treatments consisted of leachates from *P. paniculatum*, *P. urvillei* and control; each treatment was replicated three times. Treatment means obtained by ANOVA were compared using LSD procedures at $P = 0.05$ level of significance.

Chemical analysis of leachates from P. paniculatum and P. urvillei

Leachates from the two grass weeds were collected from Trial IV and brought to the Agricultural Chemistry department of MSIRI for analysis for the presence (and quantification if present) of 2(3H)-benzoxazalinone, commonly called BOA, and for identification of other allelopathic substances present using gas chromatography-mass spectrometry (GC-MS).

Test for BOA

Analysis of BOA in the leachates was conducted using an HPLC equipped with a DAD detector (HP 1050). A polar C-18 reversed phase column was used, and eluted with a gradient of 5% acetonitrile and 95% Na_2HPO_4–buffer (1 mM, pH 2.4, 10% acetonitrile) at 0.35 ml min^{-1} flow rate. Quantitative analysis was done by the external calibration method using certified BOA (2-Benzoxazolinone) standards (Sigma-Aldrich, Germany; CAS 59-49-4). Identification of BOA peaks was based on retention time window of pure standards; the retention time was 6.5 ± 0.05 minutes.

Identification of allelopathic substances by GC-MSD

Leachate aliquots of 100 ml from both *Paspalum* species plus samples collected from bowls without weeds and irrigated with distilled water were extracted twice with dichloromethane and once with hexane. The combined organic extract was rotary evaporated to 1-2 ml, followed by reconstitution into 7-8 ml hexane. The hexane extracts were evaporated under a gentle N_2 stream, followed by reconstitution in 1 ml hexane (US EPA, 1996). An aliquot of 1 ml was injected (splitless) into the GCMSD (GC HP 6890, MSD 5973). The chromatographic data were obtained on an HP 5mS column (30 m x 0.25 mm I.D., 0.25 µm film thickness) and were screened for allelochemicals using the NIST 2002 Mass Spectral library, inbuilt in the software.

6.3 Results

6.3.1 Trial I

6.3.1.1 Effect of leachates on shoot elongation and cane growth

Pre-treatment cane measurement

Cane measurement made on 11 January 2006 showed a difference in mean stalk height among the main factors (varieties) and no difference between leachate treatments and control (distilled water), thus confirming that all shoot heights were similar before irrigation with leachates started (Table 6.2).

Table 6.2 Mean dewlap height at start of experimentation (before irrigating with leachates) in Trial I

Cane variety	Mean dewlap height (cm shoot^{-1})			
	Distilled water	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	8.8	12.0	12.2	*11.0*
R 570	8.5	8.2	8.7	*8.4*
R 579	10.3	8.0	9.3	*9.2*
M 695/69	11.3	13.5	12.8	*12.6*
Mean (leachates)	*9.8*	*10.4*	*10.8*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for variety (d.f.=6) = 1.10 and s.e.d. of means for leachate treatments (d.f. = 16) = 0.92. S.e.d. for comparing between individual varieties x leachate treatments = 1.85 (d.f. = 16).

Second cane measurement

Dewlap height of cane stalks in the all buckets was again measured on 6 February 2006 (3.5 weeks after start of irrigation with leachates). A few new shoots (tillering) were observed in some of the buckets. Statistical analysis carried out separately on the mean height of primary shoots alone or the latter together with the new tillers revealed no significant difference between the leachate treatments (Table 6.3). Irrespective of the effect of the leachate treatments, variety M 695/69 produced taller cane shoots than the other three varieties.

Table 6.3 Effect of leachates from *P. paniculatum* and *P. urvillei* on mean dewlap height of four cane varieties 3 weeks after start of treatments

Variety	Mean dewlap height (cm shoot^{-1})			
	Distilled water	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	23.7	26.3	24.8	*24.4*
R 570	26.5	22.5	23.5	*24.2*
R 579	22.7	23.0	23.2	*22.9*
M 695/69	32.7	32.5	31.3	*32.2*
Mean (leachates)	*26.4*	*26.1*	*25.7*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f. = 6) = 1.82 and s.e.d. of means for subplot treatments (d.f. = 16) = 1.47. S.e.d. for comparing between individual varieties x leachate treatments = 2.95 (d.f. = 16).

Final cane measurement

The experiment was stopped 10 weeks after the start of irrigation with leachates (on 23 March 2006). There were significant differences ($P< 0.01$) in total dewlap heights between the cane varieties (main plots). For the leachate treatments, a significant difference in the dewlap height of all shoots (primary + tillers) for variety M 695/69 was noted with leachates of *P. paniculatum*. This difference was also observed in the means of all four varieties (Table 6.4). *Paspalum urvillei* did not cause a significant decrease in shoot height.

Table 6.4 Effect of leachates on total dewlap height (primary shoots + tillers) 10 weeks after start of leachate application in Trial I

Variety	Mean dewlap height (cm bucket^{-1})			
	Distilled water	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	139.7 a	115.0 a	113.3 a	*122.7*
R 570	129.0 a	94.7 a	113.0 a	*112.2*
R 579	177.3 a	118.7 a	155.3 a	*150.4*
M 695/69	340.3 a	227.0 b	270.7 ab	*279.3*
Mean (leachates)	*196.6 a*	*138.8 b*	*163.1 ab*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for variety (d.f.=6) = 19.44 and s.e.d. of means for leachate (d.f. = 16) = 21.33. S.e.d. for comparing between individual varieties x leachate treatments= 42.67 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

However, measurements of the individual primary shoot (two per bucket) showed a highly significant ($P < 0.01$) difference between both the leachate treatments and the control (Table 6.5). The decrease in mean dewlap height with leachates from *P. paniculatum* was highly significant ($P < 0.01$) while that from *P. urvillei* was significant at $P < 0.05$. Irrespective of the data set analysed, the difference in dewlap heights between the four varieties was highly significant, and no interaction between the main-plot factors (variety) and the sub-plot treatments (leachates) was recorded. However, the response of the leachates was mainly due to that observed on cane variety M 695/69.

Table 6.5 Effect of leachates on mean shoot dewlap height of primary shoots 10 weeks after start of leachate application in Trial I

Variety	Mean dewlap height (cm shoot^{-1})			
	Distilled water	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	55.2 a	39.8 a	44.2 a	*46.4*
R 570	64.5 a	41.0 a	47.7 a	*51.1*
R 579	65.2 a	52.2 a	49.7 a	*55.7*
M 695/69	125.0 a	73.2 b	89.2 b	*95.8*
Mean (leachates)	*77.5 a*	*51.5 b***	*57.7 b**	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 5.23 and s.e.d of means for subplot treatments – leachate (d.f. = 16) = 6.0. S.e.d. for comparing between individual varieties x leachate treatments= 12.0 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test). Treatment significant at * $P < 0.05$ and ** $P < 0.01$.

6.3.1.2 Effect of leachates on cane biomass

Total aboveground biomass

The dry weights of the ‘aboveground’ biomass of each treatment are shown in Table 6.6. Irrespective of cane variety, leachates from both weed species caused a reduction in aboveground biomass compared to cane shoots receiving distilled water; the decrease was more pronounced with leachates from *P. paniculatum*. Cane variety M 695/69 did not show any sensitivity to leachates from the *P. urvillei*.

Table 6.6 Effect of leachates on total aboveground biomass (dry wt) in Trial I

Variety	Total biomass (g)			
	Distilled water	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	99.2 a	67.9 b	73.3 b	*80.1*
R 570	102.5 a	64.1 c	80.3 b	*82.3*
R 579	105.5 a	65.3 b	76.1 b	*82.3*
M 695/69	104.3 a	77.7 b	96.2 a	*92.7*
Mean (leachates)	*102.9 a*	*68.8 c*	*81.4 b*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 6.48 and s.e.d. of means for subplot treatments - leachate (d.f. = 16) = 3.81. S.e.d. for comparing between individual varieties x leachate treatments= 7.63 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

6.3.1.3 Effect of leachates on root development

Root biomass

The root biomass was easily removed and washed from the filling mixture used. The effect of the leachates on development of cane roots was visible, particularly for those being receiving leachates from *P. paniculatum* (Fig. 6.3).

Dry weight of roots

The dry weight analysis of root biomass showed that leachates from *P. paniculatum* had an adverse effect on root formation of sugar cane (main-plot - mean of four varieties) (Table 6.7). Among the four varieties, leachates applied to M 3036/66 and M 695/69 caused a significant reduction. Irrespective of leachates/distilled water treatment, R 579 had a higher biomass of roots compared to the other three varieties.

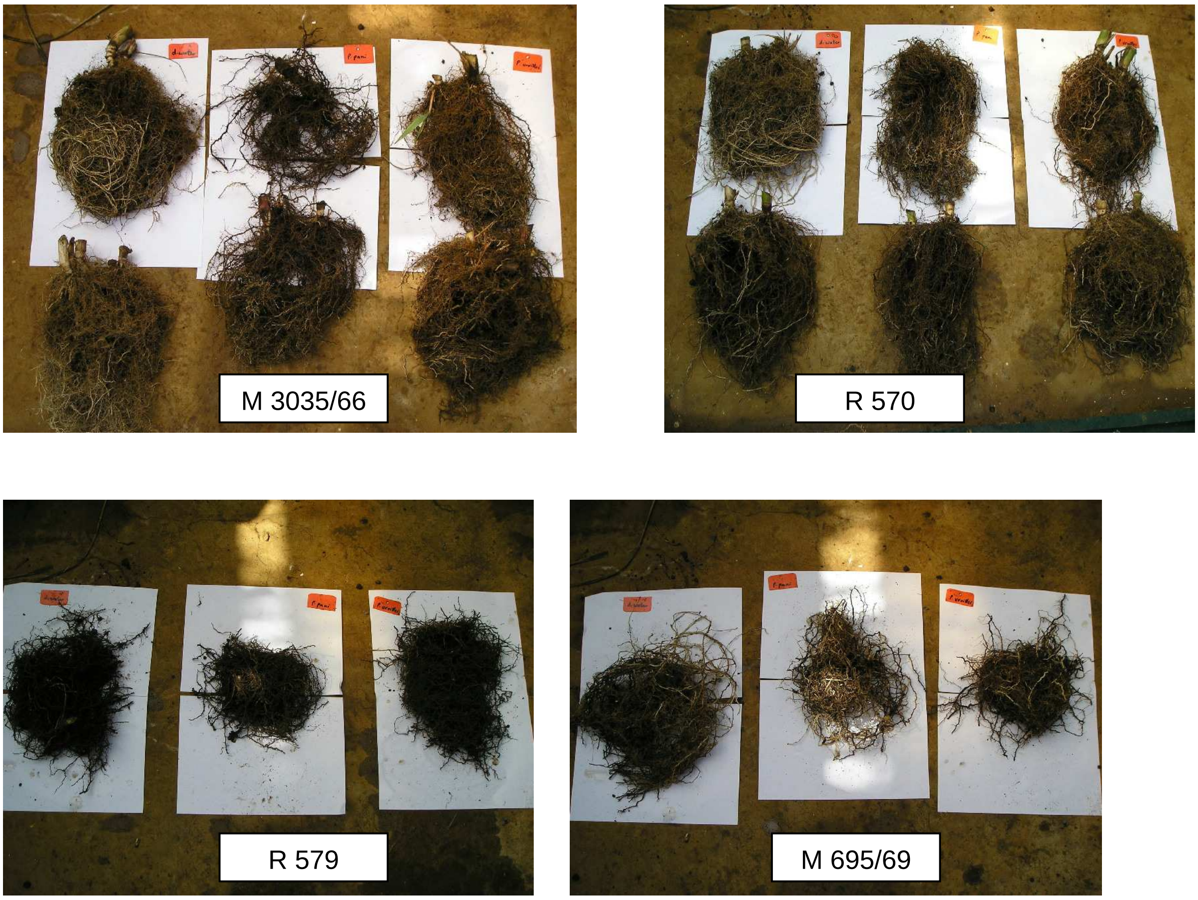

Fig. 6.3 Effect of leachates from *Paspalum species* on root biomass of sugar cane. For each variety, roots on left are from distilled water, in centre for *P. paniculatum* and right for *P. urvillei.* (For M 3035/66 and R 570, roots from two repetitions (top & bottom) are shown)

Table 6.7 Effect of leachates from *P. paniculatum* and *P. urvillei* on root biomass (dry wt) of sugar cane in Trial I

Variety	Total root biomass (g bucket^{-1})			
	Distilled water	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	12.8 a	6.8 b	11.2 ab	*10.3*
R 570	12.1 a	8.6 a	10.4 a	*10.3*
R 579	14.8 a	12.9 a	15.3 a	*14.3*
M 695/69	12.3 a	7.2 b	11.7 a	*10.4*
Mean (leachates)	*13.0 a*	*8.9 b*	*12.2 a*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 1.57 and s.e.d. of means for subplot treatments - leachate (d.f. = 16) = 1.05. S.e.d. for comparing between individual varieties x leachate treatments= 2.09 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

6.3.2 Trial II

6.3.2.1 Effect of leachates on shoot elongation and cane growth

Pre-treatment cane measurement

The first cane measurement made on 5 May 2006 showed a slightly lower germination and initial development of variety R 579 compared to the others but showed no difference between treatments (leachates v/s control) for the same level of variety (Table 6.8). The latter confirmed that all shoot heights were similar before start of irrigation with leachates.

Table 6.8 Mean dewlap height at start of experimentation (before irrigating with leachates) in Trial II

Cane variety	Mean dewlap height (cm shoot^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	13.2	11.2	15.0	*13.1*
R 570	11.7	13.2	11.7	*12.2*
R 579	8.3	7.7	8.7	*8.2*
M 695/69	11.5	11.3	10.3	*11.1*
Mean (leachates)	*11.2*	*10.8*	*11.4*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot - variety (d.f.= 6) = 0.78 and s.e.d. of means for subplot treatments - leachate (d.f.= 16) = 0.72. S.e.d. for comparing between individual varieties x leachate treatments= 1.44 (d.f.= 16).

Cane elongation

Dewlap height measurements over a 20 weeks period showed that stalk elongation varied for each variety, but were in general relatively slow, particularly as from end of June. Variety M 3035/66 grew 5 cm during the first month but slowed down almost completely later on (Fig 6.4) and no difference between the respective treatments was observed.

The elongation rate for variety R 570 was relatively higher during the first six weeks after start where a 15 cm increase was recorded (Fig. 6.4). The rate of growth slowed down later and no difference between the various treatments was recorded.

The early growth of variety R 579 was similar to R 570 but had a slightly higher rate of growth as from the end of August for the ‘control’ and *P. urvillei* treatments (Fig. 6.4). Cane shoots irrigated with water collected from the *P. paniculatum* containers seemed to reduce stalk elongation.

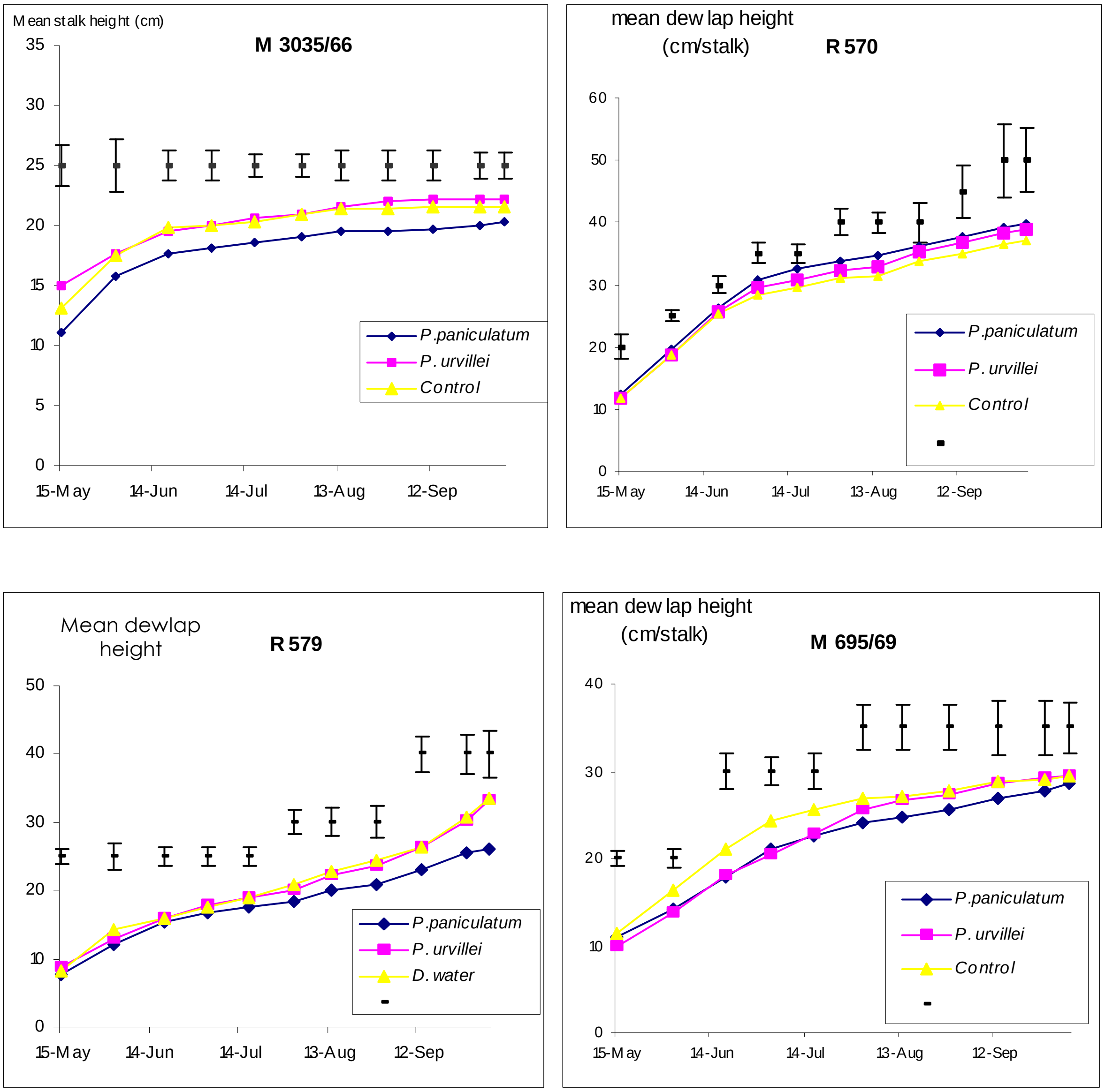

Fig 6.4 Effect of leachates from *P. paniculatum* and *P. urvillei* on stalk elongation of variety M 3035/66 (top left), R 570 (top right), R 579 (bottom left) and M 695/69 (bottom right) in Trial II. The vertical error bars indicate 2 x s.e.d. at each observation date.

The stalk elongation for variety M 695/69 was also slowed down as from the month of August and no difference between the three treatments was observed for each date of measurement (Fig 6. 4).

Final cane measurement

The experiment was stopped 20 weeks after start of irrigation with leachates (on 6 October 2006). Cane measurements showed a mean increase in dewlap height of shoots of 10 cm, 26 cm, 18 cm and 18 cm for varieties M 3035/66, R 570, R 579 and M 695/69 respectively. The final dewlap height for variety R 570 was significantly higher than R 579 and M 695/69, which were themselves higher than

M 3035/66. The final dewlap measurement also revealed that there was no significant difference among the treatments (means of four varieties). However, a decrease in the dewlap height was confirmed for variety R 579, the mean dewlap height of shoots receiving leachates from *P. paniculatum* was significantly reduced (Table 6.9).

Table 6.9 Effect of leachates on final mean dewlap height (primary shoots) 20 weeks after start of leachates application in Trial II

Variety	Mean dewlap height (cm shoot^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	21.5 a	20.3 a	22.2 a	*21.3*
R 570	37.2 a	39.8 a	38.8 a	*38.6*
R 579	33.3 a	26.2 b	33.3 a	*30.9*
M 695/69	29.5 a	28.5 a	29.5 a	*29.2*
Mean (leachates)	*30.4 a*	*28.7 a*	*31.0 a*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 2.06 and s.e.d. of means for subplot treatments (d.f. = 16) = 1.65. S.e.d. for comparing between individual varieties x leachate treatments= 3.31 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

6.3.2.2 Effect of leachates on shoot biomass

Dry weight of stalks and leaves

The dry weight of cane stalks for variety R 570 were found to be higher than for R 579 and M 3035/66. No difference in weight of stalks was found between treatments for the same variety (Table 6.10). The higher dewlap heights for R 579 with the control and leachates from *P. urvillei* did not result in higher biomass of stalk compared to those receiving leachates from *P. paniculatum* though the difference approached significance.

For each variety, the total aboveground biomass (stalk + leaves) was also found to be similar for all treatments (Table 6.10).

Table 6.10 Effect of leachates on aboveground biomass (dry weight) 20 weeks after start of application in Trial II

Variety	Mean dry weight (g bucket^{-1})							
	Control		*P. paniculatum*		*P. urvillei*		*Mean*	
	Stalk	stk+lvs	Stalk	stk+lvs	Stalk	stk+lvs	*Stalk*	*stk+lvs*
M 3035/66	5.5	17.1	9.5	19.1	3.7	11.5	*6.2*	*15.9*
R 570	29.6	70.7	27.2	71.2	29.9	68.6	*28.9*	*70.2*
R 579	12.3	30.6	8.8	28.3	13.7	35.6	*11.6*	*70.2*
M 695/69	12.8	28.6	14.1	28.6	12.2	26.7	*13.1*	*28.0*
Mean (leachates)	*15.1*	*36.7*	*14.9*	*36.8*	*14.9*	*35.6*		

Stk+lvs = stalk + leaves. Values are means of three replications. For stalk dry weight, standard error of difference (s.e.d.) of means for main plot – variety (d.f.= 6) = 6.54; s.e.d. of means for subplot treatments (d.f.=16) = 1.60 and s.e.d. for comparing between individual varieties x leachate treatments= 3.21 (d.f.=16). For total aboveground biomass, s.e.d. of means for main plot – variety (d.f.= 6) = 12.88, s.e.d. of means for subplot treatments (d.f.= 16) = 2.54 and s.e.d. for comparing between individual varieties x leachate treatments= 5.08 (d.f.=16).

6.3.2.3 Effect of leachates on root development

The dry weight analysis showed no difference in root biomass between the various treatments; i.e. leachates from the two weed species had no effect of root biomass (Table 6.11). The difference between the main-plot factor (variety) was significant; variety R 570 which produced higher aboveground biomass also had more roots.

Table 6.11 Effect of leachates from *P. paniculatum* and *P. urvillei* on root biomass (dry wt) of sugar cane in Trial II

Variety	Mean dry weight (g bucket^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	4.3	3.7	2.3	*3.4*
R 570	38.8	38.0	47.4	*41.4*
R 579	15.9	12.4	15.1	*14.5*
M 695/69	5.1	3.5	6.0	*4.9*
Mean (leachates)	*16.1*	*14.4*	*17.7*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 1.94 and s.e.d. of means for subplot treatments (d.f. = 16) = 2.49. S.e.d. for comparing between individual varieties x leachate treatments= 4.97 (d.f.=16).

6.3.3 Trial III

6.3.3.1 Effect of leachates on shoot elongation and cane growth

Pre-treatment cane measurement

The first cane measurement made on 24 November 2006 showed a slightly lower germination and initial development with varieties R 579 and M 695/69 compared to the others but no difference between treatments (leachates v/s control) was obtained for the same level of variety (Table 6.12). The data confirmed that all shoot heights were similar before start of irrigation with leachates.

Table 6.12 Mean dewlap height at start of experimentation (before applying leachates) in Trial III

Cane variety	Mean dewlap height (cm shoot^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	14.7	9.7	16.7	*13.7*
R 570	10.3	9.7	10.3	*10.1*
R 579	5.7	7.7	7.3	*6.9*
M 695/69	8.3	7.7	7.2	*7.7*
Mean (leachates)	*9.8*	*8.7*	*10.4*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 0.96 and s.e.d. of means for subplot treatments (d.f. = 16) = 1.19. S.e.d. for comparing between individual varieties x leachate treatments= 2.38 (d.f.=16).

Cane elongation

Dewlap height measurements made over a 12 weeks period showed that stalk elongation was quite satisfactory; each stalk gained an average of 70 cm over that period. The elongation was quite similar for all varieties, irrespective of the treatments, for the first four to six weeks before some differences started to occur. For variety M 3035/66, stalk elongation for the three treatments were similar till the first weeks of January 2007 when a slowing down in the *P. urvillei* treatment was observed (Fig. 6.5). Similarly, a more rapid growth was recorded in the control treatment compared to *P. paniculatum*; the difference was, however, not significant.

Stalk elongation of variety R 570 was similar for the three treatments for the first seven weeks (Fig. 6.5). After that, the cane shoots receiving leachate from containers with no *Paspalum* plants (control) elongated at a higher rate than the two treatments receiving leachates from the weeds.

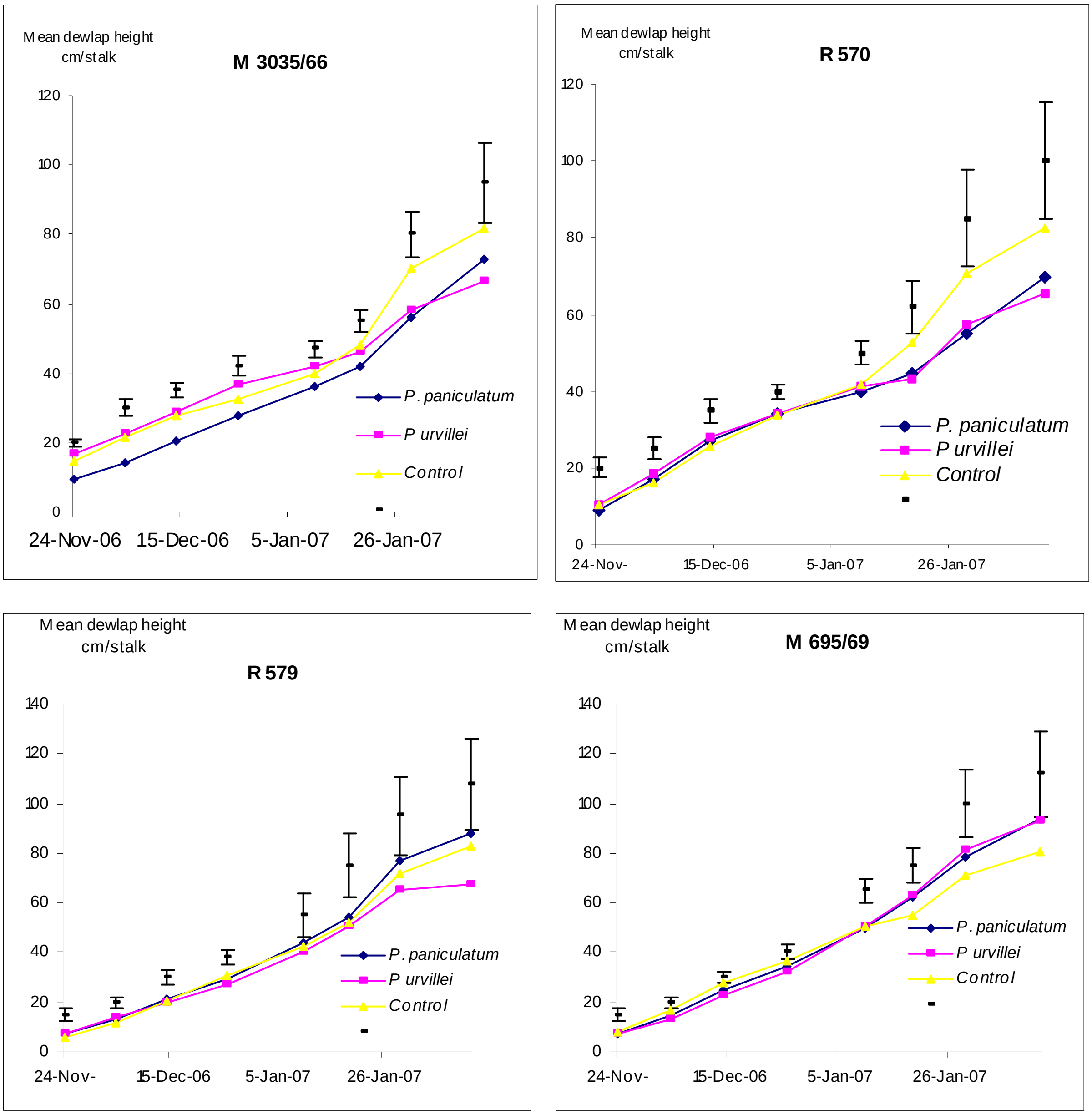

Fig 6.5 Effect of leachates from *P. paniculatum* and *P. urvillei* on stalk elongation of variety M 3035/66 (top left), R 570 (top right), R 579 (bottom left) and M 695/69 (bottom right) in Trial III. The vertical error bars indicate 2 x s.e.d. at each observation date.

Cane shoots in the three subplot treatments with variety R 579 elongated at the same rate for the initial five weeks (Fig. 6.5). It seemed that leachates from *P. urvillei* caused a reduction in the elongation rate of R 579 as from the third week of January 2007. No significant difference was, however, noted.

Variety M 695/69 did not seem to be affected by the leachates treatments (Fig. 6.5).

Final cane measurement

As some of the cane shoots had reached more than 80 cm in dewlap height, the experiment was stopped 12 weeks after start of leachates application. Cane measurements showed the mean dewlap height of M 695/69 to be slightly higher than the other varieties, the difference, however, was not significant. Compared to the control, a tendency for *P. urvillei* causing a reduction in dewlap heights of all the varieties was observed; the differences were, however, not significant.

6.3.3.2 Effect of leachates on shoot biomass

Dry weight of stalks and leaves

The aboveground biomass (dry weight) of cane shoots was found to vary with variety; R 570 producing higher biomass and M 695/69 the least (Table 6.13). Irrespective of cane variety, the effect of leachates on mean (main-plot means) aboveground biomass was significant, *P. urvillei* caused a reduction in shoot development. *Paspalum urvillei* adversely affected shoot development of varieties M 3035/66 and R 570. Leachates from *P. paniculatum* caused no adverse effect on weight of aboveground biomass, thus confirming effect on dewlap height.

Table 6.13 Effect of leachates on aboveground biomass (dry weight) 12 weeks after start of application in Trial III

Variety	Mean dry weight (g bucket^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	83.0 a	71.4 ab	52.8 b	*69.1*
R 570	100.4 a	101.6 a	81.1 b	*94.4*
R 579	83.1 a	83.2 a	67.2 a	*77.8*
M 695/69	51.3 a	66.7 a	49.4 a	*55.8*
Mean (leachates)	*79.5 a*	*80.7 a*	*62.6 b*	

Values are means of three replications. For stalk dry weight, standard error of difference (s.e.d.) of means for main plot – variety (d.f.= 6)= 3.35 and s.e.d. of means for subplot treatments (d.f.= 16)= 4.54. S.e.d. for comparing between individual varieties x leachate treatments= 9.08 (d.f.= 16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

6.3.3.3 Effect of leachates on root development

A significant reduction in root biomass between the control and the two leachates treatments was obtained for the main-plot treatments (four varieties). Significant effects on individual cultivars were less evident, though the data showed similar trends for all cultivars. Leachates from *P. urvillei* were found to cause a significant reduction only in variety M 695/69 (Table 6.14).

Table 6.14 Effect of leachates on root biomass (dry weight) 12 weeks after start in Trial III

Variety	Mean dry weight (g bucket^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	17.1 a	14.8 a	14.5 a	*15.4*
R 570	18.8 a	18.3 a	20.9 a	*19.4*
R 579	15.7 a	13.1 a	13.5 a	*14.1*
M 695/69	12.9 a	10.6 a	8.2 b	*10.5*
Mean (leachates)	*16.1 a*	*14.2 b*	*14.3 b*	

Values are means of three replications. For stalk dry weight, standard error of difference (s.e.d.) of means for main plot – variety (d.f.= 6)= 0.48 and s.e.d. of means for subplot treatments (d.f.= 16)= 0.83. S.e.d. for comparing between individual varieties x leachate treatments= 1.67 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

6.3.4 Trial IV

6.3.4.1 Effect of leachates on shoot elongation and cane growth

Pre-treatment cane measurement

The first cane measurement made on 6 March 2007 showed a lower germination and initial development with variety R 579 (Table 6.15). No difference between treatments (leachates v/s control) was obtained for the same level of variety, thus confirming that all shoot heights were similar before start of irrigation with leachates.

Table 6.15 Mean dewlap height at start of experimentation (before applying leachates) in Trial IV

Cane variety	Mean dewlap height (cm shoot^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	11.8	11.3	10.8	*11.3*
R 570	11.3	10.7	12.0	*11.3*
R 579	8.7	8.0	8.2	*8.3*
M 695/69	11.8	11.0	10.7	*11.2*
Mean (leachates)	*10.9*	*10.3*	*10.4*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 0.73 and s.e.d. of means for subplot treatments (d.f. = 16) = 0.80. S.e.d. for comparing between individual varieties x leachate treatments= 1.60 (d.f.=16).

Cane elongation

Dewlap height measurements made over a 17 weeks period showed that stalk elongation was quite steady; stalk elongation gained between 55 and 70 cm over that period. Variety R 579 had the highest gain in dewlap and M 3035/66 the least. The elongation was quite similar for all varieties, irrespective of the treatments, during the first six weeks before some differences started to occur as from the end of April 2007.

For variety M 3035/66, stalk elongation for the three treatments were similar till the end of April 2007. As from early May, the rate of elongation recorded in the *P. urvillei* treatment was slower than the other two treatments, the gap increasing with time (Fig. 6.6). No difference in rate of elongation was noted between the *P. paniculatum* treatment and the control.

For R 570, a similar tendency as for M 3035/66 was observed but this time, the rate of elongation of both leachates treatments was lower than the control (Fig 6.6). The effect of the leachates seemed to increase with time.

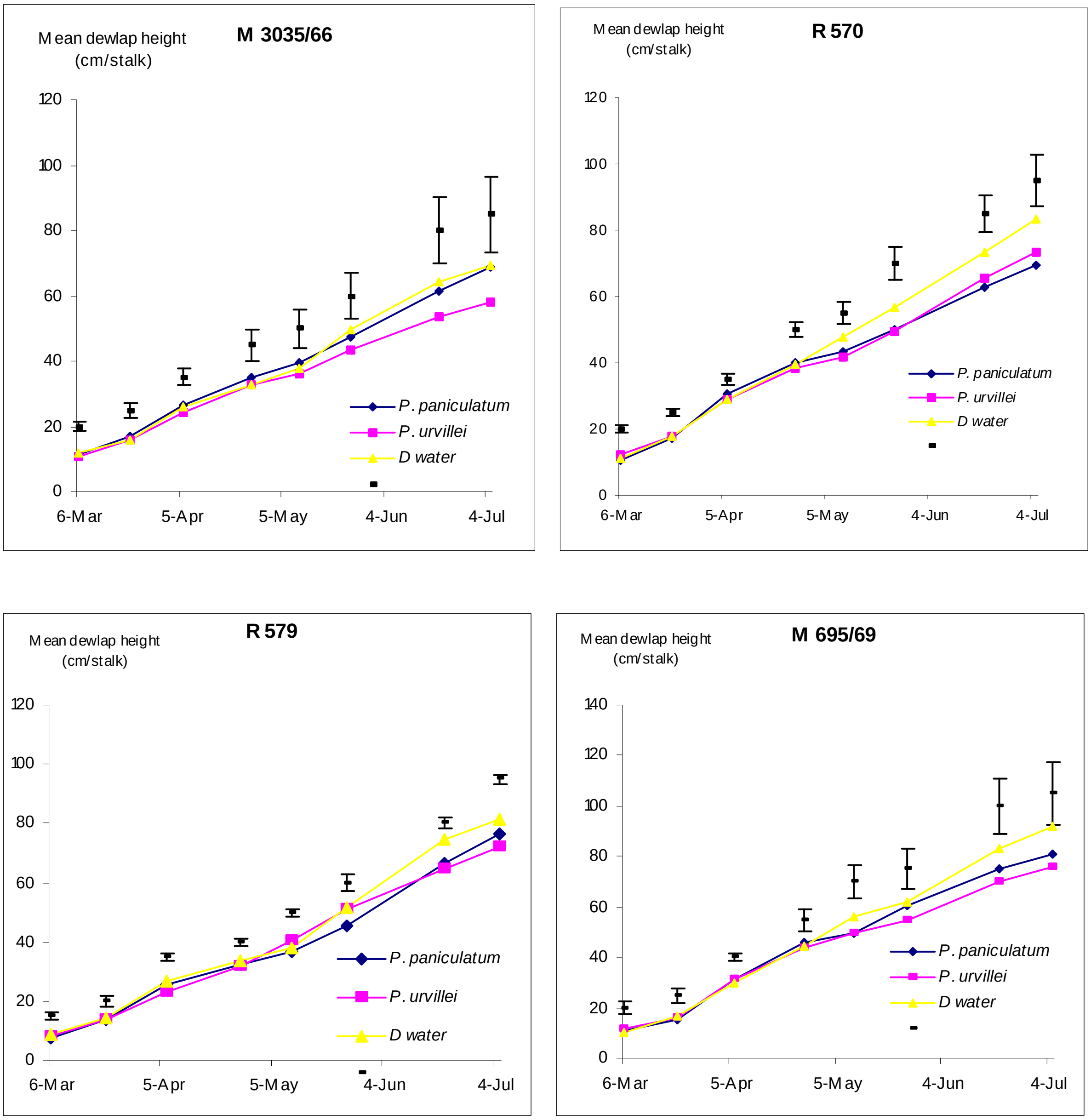

Fig 6.6 Effect of leachates from *P. paniculatum* and *P. urvillei* on stalk elongation of cane variety M 3035/66 (top left), R 570 (top right), R 579 (bottom left) and M 695/69 (bottom right) in Trial IV. The vertical error bars indicate 2 x s.e.d. at each observation date.

The difference between the control and the *P. urvillei* treatment was observed later in variety R 579 (Fig. 6.6); the gap was more visible during the last two measurements. *Paspalum paniculatum* did not seem to reduce elongation rate of this variety.

Variety M 695/69 behaved similarly to R 570 and the mean dewlap height with the control seemed to increase faster than those treated with leachates from the two grasses at the later observation dates (Fig. 6.6).

Final cane measurement

The experiment was stopped 17 weeks after start of leachates application as cane was relatively tall in the buckets. Cane measurements showed the mean dewlap height of M 695/69 to be significantly higher than M 3035/66 but they were not different to R 570 and R 579. Leachates from *P. urvillei* caused a reduction in the mean dewlap height of cane shoots of main-plot treatments (varieties) though no significant effects were recorded for the individual varieties (Table 6.16). *Paspalum paniculatum* also appeared to cause a reduction compared to the control but the difference was not significant.

Table 6.16 Effect of leachates on final mean dewlap height (primary shoots) 17 weeks after start of application of treatments in Trial IV

Variety	Mean dewlap height (cm shoot^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	69.5	68.5	56.5	*64.8*
R 570	83.3	68.0	73.2	*74.8*
R 579	80.5	75.8	72.2	*76.2*
M 695/69	91.2	83.2	79.7	*84.7*
Mean (leachates)	*81.1 a*	*73.9 a*	*70.4 b*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 5.21 and s.e.d. of means for subplot treatments (d.f. = 16) = 4.61. S.e.d. for comparing between individual varieties x leachate treatments = 9.22 (d.f.=16). Mean values in the same row not sharing the same lower-case letter are significantly different at $P < 0.05$ (LSD test).

6.3.4.2 Effect of leachates on shoot biomass

Aboveground biomass

The total biomass of leaves and stalks were reduced by the *P. urvillei* treatment (mean of main-plot treatments) (Table 6.17). For individual varieties, the response was again not significant as for the total dewlap and weight of leaves.

Table 6.17 Effect of leachates on aboveground biomass (dry weight) 17 weeks after start of application in Trial IV

Variety	Mean dry weight (g bucket^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	137.6	124.5	109.7	*123.9*
R 570	139.7	129.0	128.9	*132.6*
R 579	124.2	131.0	117.0	*124.0*
M 695/69	114.9	90.9	91.4	*99.0*
Mean (leachates)	*129.1 a*	*118.8 a*	*111.7 b*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot – variety (d.f.=6) = 6.20 and s.e.d. of means for subplot treatments (d.f. = 16) = 7.49. S.e.d. for comparing between individual varieties x leachate treatments = 14.99 (d.f.=16). Mean values of four varieties not sharing the same lower-case letter are significantly different at $P <$ 0.05 (LSD test).

6.3.4.3 Effect of leachates on root development

As seen in earlier trials, M 695/69 produced less roots irrespective of treatments. For the main-plot treatments, a reduction in root biomass was observed between the control and treatments consisting of leachates from *P. paniculatum*; the latter was not different from *P. urvillei* (Table 6.18).

Table 6.18 Effect of leachates on root biomass (dry weight) 17 weeks after start in Trial IV

Variety	Mean dry weight (g bucket^{-1})			
	Control	*P. paniculatum*	*P. urvillei*	*Mean (varieties)*
M 3035/66	33.4	29.4	33.2	*32.0*
R 570	50.6	41.4	40.5	*44.1*
R 579	28.7	17.6	20.9	*22.4*
M 695/69	19.3	12.3	17.8	*16.5*
Mean (leachates)	*33.0 a*	*25.2 b*	*28.1 ab*	

Values are means of three replications. Standard error of difference (s.e.d.) of means for main plot (variety) = 3.53 (d.f.= 6) and s.e.d. of means for subplot treatments= 2.89 (d.f.= 16). S.e.d. for comparing between individual varieties x leachate treatments= 5.78 (d.f.=16). For mean of four varieties, values not sharing the same lower-case letter are significantly different at $P <$ 0.05 (LSD test).

6.3.5 Chemical analysis of leachates from *P. paniculatum* and *P. urvillei*

6.3.5.1 Presence of BOA (2-benzoxazolinone)

The samples analysed did not show any presence of BOA in the leachate samples collected. Although BOA often exists or is converted to other derivatives such as DIBOA, MBOA, etc, any trace of BOA should have been detected by the analysis. These preliminary analyses therefore excluded detectable levels of BOA in leachates from the two *Paspalum* species.

6.3.5.2 Chemical composition of leachates from *P. paniculatum* and *P. urvillei*

The GC-MSD revealed the presence of 2-Propenoic acid, 3-(4-methoxyphenyl)- (CAS number: 5466-77-3) in leachates from both weeds but not from the control treatment. The retention time was 26.94/26.95 minutes. 2-Propenoic acids form part of the family commonly known as cinnamic acids which include cinnamic acid (2-propenoic acid, 3-phenyl), ferulic acid (2-propenoic acid, 3-(4-hydrxy-3-methoxyphenyl)-), p-coumaric acid (2-propenoic acid, 3-(4-hydroxyphenyl)-), isoferulic acid (2-propenoic acid, 3-(3-hydroxy-4-methoxyphenyl)-) and caffeic acid (2-propenoic acid, 3-(3,4-dihydroxyphenyl)-). All these compounds are known to have allelopathic properties (Fernandez *et al*., 2006). P-Coumaric acid, in particular, has been proven to cause a significant effect on the growth of roots and aboveground organs of *Linum usitatissimum* (Ray & Hastings, 1992).

The chromatograph area (%) covered by the 2-propenoic acid, 3-(4-methoxyphenyl) was three to four times higher in the *P. paniculatum* samples than in leachates from *P. urvillei* (Appendix 1). This may suggest that *P. paniculatum* produced more of that allelopathic chemical, but this needs to be studied further as the amount of chemicals released from the roots would vary with time and several other factors. However, the presence of this chemical in the two *Paspalums* confirms the potential interference from allelopathic substances released by weeds over and above the other mechanisms of competition between sugar cane and weeds.

6.4 Discussion and conclusions

Stalk elongation and cane growth

This study has shown that leachates from both *Paspalum* species can cause an adverse effect on cane growth. Irrespective of varieties (mean of main-plot treatments), leachates from *P. urvillei* caused a significant reduction in mean dewlap of primary shoots in three trials (Trial I, III and IV). A significant reduction was obtained by leachates of *P. paniculatum* in Trial I where the effect was more pronounced to that of *P. urvillei*. No difference between the three treatments was observed in Trial II where cane growth was much slower than the other trials; this may be attributed to the lower mean temperatures which prevailed during the respective trials (Table 6.19).

Table 6.19 Effect of temperature on rate of cane stalk elongation

Trial	Mean daily temperatures (°C)		Rate of stalk elongation
	Max.	Min.	cm/week
Trial I	28.4	21.0	6.7
Trial II	23.9	15.3	1.0
Trial III	28.7	20.6	6.0
Trial IV	26.0	18.2	4.2

The lower cane growth in Trial II resulted in maximum dewlap heights not exceeding 35 cm per shoot except for variety R 570. A reduction in stalk elongation with *P. paniculatum* leachates on R 579 was also recorded in this trial.

Cane growth and initiation of allelopathic effect

Cane measurements showed that the difference between the control and the ‘leachate’ treatments was not apparent during the early weeks after start of experimentation. The difference was in general visible after the cane shoots had reached a mean dewlap height of 40 cm or more. This may also explain why no difference was noted in Trial II. In general, the differences between the control and the leachate treatments increased with time; it is possible that more significant differences would have been observed if the trials were prolonged for a few weeks more. Increasing growth-inhibiting or phytotoxic effects from the weeds on sugar cane with time could have been due to increased allelochemical release from weed roots as the plants matured.

Variety response to leachate treatments

There was no interaction between variety and treatments; all varieties showed susceptibility to the leachates. The order of susceptibility of the cane varieties to leachates differed from their known relative tolerances towards herbicides or herbicide mixtures. M 3035/66 is known to be a more resistant variety towards herbicides than M 695/69 and R 579, with R 570 classified as a susceptible variety. As the leachates were applied as irrigation water underneath the leaves (not applied on cane leaves), it means that water and allelochemical uptake were solely by the roots. Variability in the tolerance of sugar cane varieties to herbicides is mostly associated with foliar-applied herbicides.

Effect of leachates on root biomass

The leachates from both weed species were found to have a growth-inhibiting effect on root development in all the trials except in Trial II where a slight (non-significant) reduction was caused by *P. paniculatum* leachate on roots of varieties M 3035/66 and M 695/69. The difference in root biomass observed in Trials I and III seems to explain the difference caused by the leachates; a correlation between reduction in root biomass and effect on dewlap height indicated that the primary effect of the allelochemicals was on root development. An adverse effect on root development also impacted negatively on aboveground biomass development, although M 695/69 with the least root biomass produced the tallest stalks (dewlap height).

P. paniculatum vs *P. urvillei*

On basis of results from the four trials, *P. urvillei* was found to cause more allelopathic (phytotoxic) effects than *P. paniculatum*, although the reverse occurred in Trial I. Although both weeds were transplanted at the same initial density, growth of *P. urvillei* was more vigorous and it produced more leaves and biomass; suggesting that more root exudates may have been released. Both weeds had a quick and similar development in Trial I and this may have influenced the allelochemical production of *P. paniculatum* to the extent that the latter species seemed to cause more reduction in root biomass than *P. urvillei* in that trial. The implication of this finding is that, on a unit mass basis, *P. paniculatum* may be more allelopathic than *P. urvillei*.

The effect on root growth may have been due to the presence of 2-propenoic acid, 3-(4-methoxyphenyl) found in root exudates from both weeds. Cinnamic acids are known for their allelopathic properties, in particular for impairing root development (Rice, 1984; Fernandez *et al*., 2006). The presence of a higher concentration of 2-propenoic acid in *P. paniculatum* leachates may partly explain the greater reduction in root biomass of sugar cane that was observed at this treatment. These results confirm the allelopathic potential of weeds on sugar cane; the effect of leachates from *Cyperus rotundus* has been reported by Mc Intyre (1998). However, the results presented in this study are only preliminary ones as there may be other allelochemicals involved and the exact effects of cinnamic acids need to be confirmed by simulating effects using pure chemicals. With the same approach, dose-response curves may be used to estimate the minimum concentrations required for any effect on cane. Allelopathic effects also need to be verified under natural conditions.

Although this study proved some interference due to allelopathic effects from the two *Paspalums* on sugar cane, the results cannot completely explain the higher interference (competitiveness) reported earlier for *P. paniculatum*, as both weeds seemed to cause similar allelopathic effects. Their relative rates of development and competitiveness under field conditions need to be studied more closely together with the identification and quantification of the major allelochemicals involved as well as their effects on sugar cane. Further studies are also required to ascertain whether allelochemical production in the live weeds and their release from live plants or from decomposing plant material is governed by growth stage, plant part (leaves or roots), or by stage of decomposition of residual plant material.

CHAPTER 7

A NEW HERBICIDE TANK-MIX OF TRIFLOXYSULFURON + AMETRYN AND AMICARBAZONE TO PROVIDE A COST-EFFECTIVE BROAD-SPECTRUM PRE- AND POST-EMERGENCE TREATMENT FOR MANAGING WEEDS IN SUGAR CANE

7.1 Introduction

Traditionally, weed control in sugar cane in Mauritius was geared towards eradication of all weeds from planting or harvest up to complete canopy closure. In the humid and superhumid areas, canopy closure may take between 20 to 30 weeks; consequently, two or three herbicide applications had to be made, often complemented by manual weeding (MSIRI, 2004). The work presented in earlier Chapters have shown that it is possible to reduce costs of weed control by developing, new weed management strategies based on critical periods of weed competition. The research presented in Chapter 2 showed that critical periods varied between 6 and 27 weeks after planting or 12 and 26 weeks after harvest in the humid areas where cane growth is slower and weed infestations are higher (Chapter 2; Seeruttun & Lutman, 2004). The new strategies proposed included delaying of the first herbicide application to coincide with onset of the critical periods of weed competition. The success of such an approach would rely on the efficacy of the herbicide treatment in knocking down all emerged weeds present on the day of spraying and providing a relatively long residual activity against a broad spectrum of weeds.

A mixture of trifloxysulfuron 1.85% + ametryn 73.15% (Krismat® - WDG 75), developed by Syngenta Crop Protection AG has been tested in Brazil where all the key sugar cane weed species including the most economically important grass species such as *Brachiaria* spp., had been controlled (Howard *et al*., 2001). The efficacy of this mixture on many grass species including *Rottboellia cochinchinensis* (Lour.) Claiton and some broad-leaved weeds such as *Euphorbia heterophylla* L. has also been reported in Cuba (Diaz *et al*., 2004). At rates of 1.5 kg a.i. ha^{-1}, the new herbicide was well tolerated by sugar cane. Amicarbazone (triazolinone) (Dinamic® WDG 70), from Arysta LifeScience has also been reported to provide excellent control of many major annual dicotyledonous weeds and grasses in sugarcane (Philbrook *et al*., 1999).

The current standard herbicide treatments available in Mauritius have limited effectiveness on some grasses and sedges and are not fully effective if control is delayed until after early weed emergence. A tank-mixture of trifloxysulfuron + ametryn and amicarbazone appeared from research elsewhere to have the potential to provide broad-spectrum pre- and post-emergence control. The new management strategies proposed would imply the application of early post-emergence treatments at timings which differ from the traditional approach where the selectivity of the herbicides was achieved by applying either pre-emergence of the cane, or when the latter had reached at least a growth stage of 12 to 14 weeks after planting. At 12 or 14 weeks, the crop better tolerates some of the herbicide treatments. According to the new strategies, herbicide treatments would be applied post-emergence of the crop (and weeds) and most probably at a stage of growth between four to eight weeks after planting when risks of herbicide phytotoxicity would be higher. Consequently, this set of experiments was done to assess the performance of these two new products and to investigate the feasibility of developing new weed control approaches based on the critical period research. The objectives of the trials were to:

1. Evaluate the pre-emergence potential of the two products and their tank-mixes against the weeds present in sugar cane in Mauritius, and to compare the length of residual activities obtained to that of other currently available herbicides.
2. Assess the potential and spectrum of control of the new herbicides and their tank-mixes applied post-emergence to weeds in both plant and ratoon cane.
3. Determine any phytotoxicity of the new products or tank-mixes on the crop when applied both pre- and post-emergence of cane.

7.2 Materials and methods

Trial characteristics and treatments

Eleven trials were conducted in plant and ratoon cane between March and December 2005. Details and characteristics of the trial sites are given in Table 7.1. In the first four trials, treatments were applied pre-emergence of plant cane and weeds. Amicarbazone at 0.7, 0.875, 1.05 and 1.4 kg a.i. ha^{-1}, trifloxysulfuron+ametryn at 0.0263 + 1.097 and 0.0315 + 1.317 kg a.i. ha^{-1}, and amicarbazone at 0.875 and 1.05 kg a.i. ha^{-1} tank-mixed with trifloxysulfuron + ametryn at 0.0263 + 1.097 kg a.i. ha^{-1} were compared to two standards, namely, oxyfluorfen + diuron (0.5 + 2.0 kg a.i. ha^{-1}) and tebuthiuron + atrazine (1.6 + 2.0 kg a.i. ha^{-1}). An untreated control was also included.

In the second series of four trials (Trials V - VIII), treatments were applied post-emergence at the same corresponding sites between 10 and 12 weeks after planting. Treatments comprised of amicarbazone at 0.875, 1.05, 1.25 and 1.4 kg a.i. ha^{-1}, trifloxysulfuron + ametryn at 0.0263 + 1.097 and 0.0315 + 1.317 and amicarbazone at 0.875 and 1.05 kg a.i. ha^{-1} tank-mixed with trifloxysulfuron + ametryn at 0.0263 + 1.097 kg a.i. ha^{-1}. A standard treatment consisting of the tank-mix tebuthiuron + atrazine + 2,4-D amine salt (1.3 + 2.0 + 2.0 kg a.i. ha^{-1}) and an untreated control were also included.

The last three trials (Trials IX, X and XI) were conducted in ratoon cane and post-emergence of the weeds. The rates of amicarbazone, trifloxysulfuron + ametryn and amicarbazone + trifloxysulfuron+ametryn were similar to those used post-emergence of plant cane, except that amicarbazone alone at 1.25 kg a.i. ha^{-1} was excluded. A tank-mix of hexazinone + atrazine + 2,4-D amine salt (0.6 + 2.0 + 2.0 kg a.i. ha^{-1}) was included as an additional standard.

Experimental layout and treatment application

In all post-emergence trials, a non-ionic surfactant at 0.025% v/v was added to all treatments. At all sites, the experimental design was a randomized complete block with three replicates and a plot size of 64 m^2 (4 rows of 10 m length at a spacing of 1.6 m). Treatments were applied with hand-operated knapsack sprayers with double hollow cone jet nozzles delivering 350 L ha^{-1} of spray mixture at a working pressure of 300 kPa.

Table 7.1. Characteristics and details of trial sites

Trial no.	Site	Soil group *	Mean annual rainfall (mm)	Altitude (m)	Date of planting	Cane variety	Date of spraying
I	Sans Souci	Humic Ferruginous Latosol	3800	290	28.02.05	M 1400/86	02.03.05
II	Deux Bras	Latosolic Brown Forest	2350	140	10.03.05	M 1394/86	16.03.05
III	Belle Mare	Lithosol	1500	40	12.04.05	M 2024/88	15.04.05
IV	Valetta	Latosolic Brown Forest	3200	430	07.04.05	M 52/78	13.04.05
V	Sans Souci	Humic Ferruginous Latosol	3800	290	28.02.05	M 1400/86	05.05.05
VI	Deux Bras	Latosolic Brown Forest	2350	140	10.03.05	M 1394/86	20.05.05
VII	Belle Mare	Lithosol	1500	40	12.04.05	M 2024/88	11.07.05
VIII	Valetta	Latosolic Brown Forest	3200	430	07.04.05	M 52/78	27.07.05
IX	Gros-Bois	Latosolic Brown Forest	2950	245	04.07.05	R 575	18.08.05
X	Combo	Humic Ferruginous Latosol	3300	410	13.07.05	M 52/78	02.09.05
XI	Côte D’Or	Humic Ferruginous Latosol	2800	450	19.07.05	M 52/78	22.09.05

* According to Parish & Feillafé (1965). Soil groups are described in Chapter 1.

Data collection and statistical analysis

For pre-emergence trials in plant cane, data collection comprised regular observations on weed infestation and cane growth. Visual observations were made at 4 and 8 weeks after spraying (WAS), whereas weed surveys were carried out twice between 12 and 19 WAS using the ‘Frequency Abundance Method’ (Rochecouste, 1967). The latter method consists of, firstly, a listing of all weeds present in the treatment plots, and then assigning their relative presence/cover on a scale varying between 0 and 8. Stalk height was measured from ground level to the first visible dewlap at 12 WAS.

For the post-emergence trials in plant cane, a weed survey was carried out prior to spraying in each individual plot to identify and quantify all weeds present. The first post-treatment weed survey was conducted between 4 and 6 weeks after spraying to assess the post-emergence potential of each treatment. Results were expressed in % weed kill for each plot by dividing the difference in weed infestation (Frequency Abundance Method) between the two surveys by the initial infestation. The second survey carried out between 10 and 13 WAS was mainly geared towards assessing the residual activity following early post-emergence application.

For the post-emergence trials in ratoon cane, a weed survey was conducted to record weed species and infestation levels in all plots a few days prior to spraying of treatments. Two formal weed surveys were carried out between 6 and 12 WAS using the ‘Frequency Abundance Method’ to calculate the % weed kill. Regular visual observations were made to assess any phytotoxicity on the different cane varieties.

Data for weed control (expressed as % of the untreated control) and % weed kill were transformed using the arcsine square root before statistical analysis was performed. Likewise, the % increase in stalk height (x) for effect of the treatments on cane elongation was transformed using $(x + 0.5)^{0.5}$ (Steel *et al*., 1997).

7.3 Results and discussion

7.3.1 Potential of amicarbazone and trifloxysulfuron + ametryn for pre-emergence weed control

Efficacy on weeds

Both trifloxysulfuron + ametryn and amicarbazone provided good pre-emergence control compared to the two standards. The efficacy of amicarbazone improved with increasing rates as opposed to trifloxysulfuron + ametryn where the two rates tested provided a similar level of control (Table 7.2). In general, trifloxysulfuron + ametryn was superior to amicarbazone as the former proved more effective on sedges (*C. rotundus* and *Kyllinga* spp.) and some grasses (Table 7.3). Amicarbazone showed a higher efficacy on broad-leaved weeds which explains its better efficacy in Trial III. Amicarbazone also provided good control of *Digitaria horizontalis* which was poorly controlled by trifloxysulfuron + ametryn (Table 7.3). Tank-mixing amicarbazone with trifloxysulfuron + ametryn improved the level and spectrum of control (Table 7.2). The residual activity of the tank-mix trifloxysulfuron + ametryn + amicarbazone was comparable to the two standards. Weed surveys at 16 or 19 WAS showed a satisfactory level of control in Trials I, III and IV. Cane growth was faster at Deux Bras (Trial II) and the cane canopy had almost closed before 16 WAS.

Observations made during the first eight weeks showed that all the treatments were safe towards the four cane varieties tested. These observations were confirmed when cane measurements taken between 12 and 16 WAS revealed no significant differences in stalk height and number of shoots. The tank-mix trifloxysulfuron + ametryn and amicarbazone showed no adverse effect on the mean dewlap height (Fig. 7.1) compared to the standard treatment. There were very few weeds left uncontrolled in the plots treated with either the standard herbicides or the new tank-mixes, so these could not have caused any additional adverse effect on the cane due to weed competition.

Table 7.2. Pre-emergence control of weeds presented as % of weed infestation on the untreated treatment (detransformed arcsine data) by trifloxysulfuron+ametryn and amicarbazone in plant cane. Values in parentheses represent transformed (arcsine) data

Treatments	kg a.i. ha^{-1}	Weed control (expressed as % of untreated control *)						
		Trial I		Trial II	Trial III		Trial IV	
		12 WAS	16 WAS	12 WAS	12 WAS	19 WAS	12 WAS	16 WAS
Amicarbazone	0.7	78 (1.08)	81 (1.11)	64 (0.92)	24 (0.51)	44 (0.72)	57 (0.86)	91 (1.26)
Amicarbazone	0.875	63 (0.912)	73 (1.02)	63 (0.92)	24 (0.51)	35 (0.64)	51 (0.80)	83 (1.15)
Amicarbazone	1.05	71 (1.00)	71 (1.00)	58 (0.87)	11 (0.34)	27 (0.54)	33 (0.61)	73 (1.02)
Amicarbazone	1.4	66 (0.95)	70 (0.99)	51 (0.79)	9 (0.30)	20 (0.46)	25 (0.52)	67 (0.96)
Trifloxysulfuron+ametryn	0.0263+1.097	46 (0.75)	59 (0.88)	44 (0.73)	16 (0.42)	34 (0.62)	44 (0.72)	68 (0.97)
Trifloxysulfuron+ametryn	0.0315+1.317	55 (0.84)	60 (0.89)	33 (0.61)	19 (0.45)	37 (0.67)	49 (0.78)	68 (0.97)
Amicarbazone + trifloxysulfuron+ametryn	0.875 + 0.0263+1.097	33 (0.61)	47 (0.76)	54 (0.83)	12 (0.36)	42 (0.70)	32 (0.60)	69 (0.98)
Amicarbazone + trifloxysulfuron+ametryn	1.05 + 0.0263+1.097	45 (0.74)	57 (0.85)	49 (0.77)	13 (0.37)	32 (0.60)	30 (0.58)	73 (1.03)
Oxyfluorfen + diuron	0.5 + 2.0	48 (0.76)	67 (0.96)	45 (0.74)	23 (0.37)	37 (0.66)	28 (0.56)	66 (0.94)
Tebuthiuron + atrazine	1.6 + 2.0	52 (0.80)	56 (0.84)	42 (0.71)	9 (0.31)	18 (0.44)	26 (0.53)	59 (0.87)
Standard error of transformed data		*0.20*	*0.19*	*0.12*	*0.18*	*0.25*	*0.13*	*0.12*

WAS = weeks after spraying
* values represent detransformed (arcsine) data

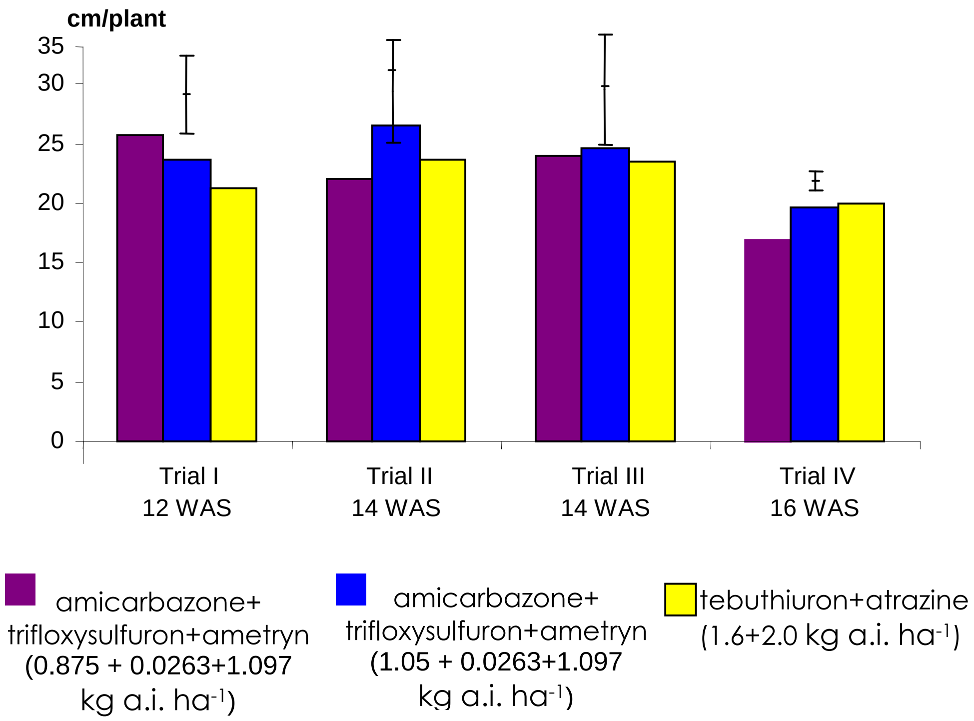

Fig. 7.1 Effect of trifloxysulfuron+ametryn and amicarbazone on cane growth. Error bars represent 2 x s.e.d.

Table 7.3 Relative efficacy of amicarbazone, trifloxysulfuron + ametryn and the tank-mix amicarbazone + trifloxysulfuron+ametryn for the pre-emergence control of some common weeds in sugar cane

	Herbicide treatments (kg a.i. ha^{-1})				
	Amic (1.4)	trif+amet (0.0315+1.317)	trif+amet+amic (1.05+0.0263+1.097)	oxyf+diur (0.5+2.0)	teb+atraz (1.6+2.0)
Ageratum conyzoides	+++	++	++++	+++	+++
Amaranthus dubuis	++++	+++	++++	++++	++++
Cyperus rotundus	+	++	++	+	+
Digitaria horizontalis	+++	+	++++	++++	++++
Digitaria timorensis	+++	+	++++	++++	++++
Drymaria cordata	+++	+++	++++	++++	++++
Kyllinga bulbosa	+	+++	+++	+++	+++
Oxalis corniculata	+++	++	+++	+++	+++
Paspalum paniculatum	+	+++	+++	+++	+++
Phyllanthus sp.	+++	+++	+++	+	+++

\+ Poor ++ Fair +++ Good ++++ very good
amic = amicarbazone, trif+amet = trifloxysulfuron+ametryn, oxyf+diuron= oxyfluorfen + diuron, teb+atraz= tebuthiuron + atrazine

7.3.2 Potential of amicarbazone and trifloxysulfuron + ametryn for post-emergence control

Efficacy on weeds

With the exception of Trial V, the efficacy of the two new herbicides applied alone was comparable or superior to the standard (tebuthiuron + atrazine + 2,4-D amine salt). In general, both herbicides effectively controlled most broad-leaved weeds. Trifloxysulfuron + ametryn provided good knockdown of *C. rotundus*, *Kyllinga* spp. and *Paspalum* spp. that were poorly controlled by amicarbazone (Table 7.4). Likewise, amicarbazone was effective on *D. horizontalis*, which was not controlled by trifloxysulfuron + ametryn (Table 7.4). Tank-mixing the two products improved significantly their level of control (Table 7.5); the combination controlled *Digitaria timorensis* (Kunth.) Balans, which was found to be weakly controlled by both products applied alone (Table 7.4). For post-emergence control, increasing the rate of amicarbazone within the new tank-mix did not improve the level of efficacy (Table 7.5).

Table 7.4 Level of post-emergence control on weed species by amicarbazone, trifloxusulfuron + ametryn and the tank-mix amicarbazone + trifloxusulfuron + ametryn

Weeds	Herbicide treatments		
	amicarbazone	trifloxysulfuron+ametryn	amicarbazone + trifloxysulfuron+ametryn
Ageratum conyzoides	+	+	+
Cyperus rotundus	-	+	+
Digitaria horizontalis	+	-	+
D. timorensis	-	-	+
Eleusine indica	-	+	+
Kyllinga sp.	-	+	+
Oxalis debilis	-	+	+
Paspalum paniculatum	-	+	+
P. urvillei	-	+	+
Setaria barbata	-	+	+
Youngia japonica	+	-	+

+ Good control - Poor control

Table 7.5 Post-emergence control by trifloxysulfuron+ametryn and amicarbazone in plant cane expressed as % kill (detransformed arcsine data) by trifloxysulfuron+ametryn and amicarbazone in plant cane. Values in parentheses represent transformed (arcsine) data

Treatments	kg a.i. ha^{-1}	Weed control (% kill*)			
		Trial V	Trial VI	Trial VII	Trial VIII
		5 WAS	5 WAS	6 WAS	6 WAS
Amicarbazone	0.875	55 (0.83)	52 (0.80)	83 (1.14)	74 (1.04)
Amicarbazone	1.05	69 (0.98)	76 (1.05)	79 (1.09)	76 (1.05)
Amicarbazone	1.25	73 (1.02)	72 (1.01)	83 (1.14)	78 (1.08)
Amicarbazone	1.4	68 (0.97)	69 (0.98)	75 (1.05)	87 (1.21)
Trifloxysulfuron+ametryn	0.0263+1.097	73 (1.03)	63 (0.91)	66 (0.95)	77 (1.07)
Trifloxysulfuron+ametryn	0.0315+1.317	74 (1.03)	62 (0.91)	81 (1.12)	78 (1.08)
Amicarbazone + trifloxysulfuron+ametryn	0.875+ 0.0263+1.097	89 (1.23)	82(1.14)	84 (1.16)	96 (1.38)
Amicarbazone + trifloxysulfuron+ametryn	1.05+ 0.0236+1.097	96 (1.36)	77 (1.07)	86 (1.19)	98 (1.42)
Tebuthiuron + atrazine + 2,4-D	1.3 + 2.0 + 2.0	73 (1.02)	57 (0.85)	87 (1.21)	73 (1.03)
Standard error of transformed data		*0.092*	*0.133*	*0.105*	*0.086*

* values represent detransformed (arcsine) data

Effect on cane growth

Cane measurements made prior to spraying and 6 weeks later revealed that neither of the two new herbicides nor their tank-mixes caused a reduction in tillers or lower cane dewlap heights when compared to the standard (tebuthiuron + atrazine + 2,4 D amine salts). As the latter is known to be safe for post-emergence application in sugar cane, the new tank-mix should therefore be relatively safe for such application. As the level of post-emergence weed control by the new tank-mixes was superior to that obtained in the standard plots, the few weeds left uncontrolled in the latter plots may suggest some weed competition which would mask the effect of crop damage by the new herbicides. The possibility of the latter occurring was minimised by also comparing the cane growth parameters with the measurements recorded in the plots from the pre-emergence trials, which were initiated in the same field at each locality (same variety and planting dates).

7.3.3 Potential of amicarbazone and trifloxysulfuron+ametryn for early post-emergence weed control in ratoon cane

Post-emergence control of weeds

The three trials conducted in ratoon cane were sprayed 6 to 8 weeks after harvest to assess trifloxysulfuron+ametryn and amicarbazone for use within the newly developed weed management strategy. The two new herbicides, applied alone, were again found to be as effective as the two standards for their knockdown effect. Higher rates of amicarbazone resulted in increased efficacy (Table 7.6). The tank-mix of trifloxysulfuron+ametryn + amicarbazone once more tended to show higher level of control than the two standards. Thus, superiority was achieved as a result of a more effective control of species such as *D. horizontalis*, *P. paniculatum*, *P. urvillei*, *S. barbata*, *Kyllinga* spp. and *C. rotundus* (see Table 7.4).

Table 7.6 Post-emergence control and residual activity following application of trifloxysulfuron+ametryn and amicarbazone in ratoon cane expressed as % kill (detransformed arcsine data) and % of untreated control (detransformed data) respectively. Values in parentheses represent transformed (arcsine) data.

Treatments	kg a.i. ha^{-1}	Trial IX		Trial X		Trial XI
		% kill[a]	% of untreated control[b]	% kill	% of untreated control	% kill
		7 WAS	12 WAS	7 WAS	11 WAS	6 WAS
Amicarbazone	0.875	64 (0.92)	27	54 (0.82)	53 (0.81)	57 (0.86)
Amicarbazone	1.05	57 (0.86)	30	59 (0.87)	42 (0.71)	68 (0.97)
Amicarbazone	1.4	79 (1.10)	27	66 (0.66)	38 (0.66)	83 (1.155)
Trifloxysulfuron+ametryn	0.0263+1.097	68 (0.97)	20	67 (0.96)	16 (0.42)	84 (1.15)
Trifloxysulfuron+ametryn	0.0315+1.317	61 (0.90)	24	58 (0.86)	22 (0.49)	85 (1.17)
Amicarbazone+ trifloxysulfuron+ametryn	0.875+ 0.0263+1.097	87 (1.21)	20	82 (1.13)	16 (0.41)	90 (1.25)
Amicarbazone+ trifloxysulfuron+ametryn	1.05+ 0.0263+1.097	65 (0.94)	10	80 (1.11)	19 (0.45)	87 (1.20)
Tebuthiuron+atrazine+2,4-D	1.6+2.0+2.0	57 (0.86)	23	56 (0.84)	39 (0.68)	73 (1.03)
Hexazinone+atrazine+2,4-D	0.6+2.0+2.0	49 (0.77)	49	63 (0.91)	43 (0.71)	71 (1.00)
Standard error of transformed data		*(0.192)*	*n/a+*	*(0.071)*	*(0.092)*	*(0.037)*

[a] – post-emergence control; [b] – residual activity= recovery of weeds + new emergence
\+ data from only one rep – no statistics

Residual herbicide activity on weeds

The residual activity of the new tank-mix following the knockdown of weeds was significantly superior to the two standards (Table 7.6), particularly to the one containing tebuthiuron which is known to provide fairly long pre-emergence control (approx. 14 WAS). It seemed that the higher rate of amicarbazone within the tank-mix extended the residual activity.

Visual observations made throughout the duration of the trials did not show any phytotoxic effects of the tank-mix on the different cane varieties.

7.4 Discussion and conclusions

The good potential of herbicides trifloxysulfuron + ametryn and amicarbazone as both pre- and post-emergence treatments was demonstrated in plant and ratoon cane. Applied pre-emergence of weeds, both herbicides were effective on most broad-leaved weeds and some annual grasses. Trifloxysulfuron + ametryn was less effective on *Digitaria horizontalis* and *D. timorensis*, and amicarbazone did not control *Cyperus rotundus*, *Paspalum* spp. and *Kyllinga* spp (Table 7.3). Tank-mix at lower rates of both herbicides overcame their individual weaknesses while maintaining a residual activity of over 14 to 16 weeks. When applied early post-emergence of weeds, both trifloxysulfuron + ametryn and amicarbazone were effective on most broad-leaved weeds and some grasses. The efficacy of trifloxysulfuron + ametryn on *Paspalum* spp., *C. rotundus* and other sedges, and that of amicarbazone on *Digitaria horizontalis* compensated for their individual inefficacies when they were tank-mixed (Table 7.4). As far as could be ascertained from the trials, which were not set up to specifically assess crop tolerance, the tank-mixes trifloxysulfuron + ametryn + amicarbazone were well tolerated by both young plant and ratoon cane.

The efficacy (pre- and post-emergence) of the new tank-mix offers a new perspective for managing weeds in sugarcane by delaying of the first herbicide application which will result in savings of at least one herbicide treatment per season. The tank-mix trifloxysulfuron + ametryn + amicarbazone (0.0263 + 1.097 + 0.875-1.05 kg a.i. ha^{-1}) has been registered and recommended for use in Mauritius; the higher rate of amicarbazone would be useful where a relatively longer residual activity is required. At these rates, the cost of the new tank-mix is comparable to the conventional treatments, but the possibility of saving one treatment per season renders the new tank-mix more cost-effective.

CHAPTER 8

GENERAL DISCUSSIONS & CONCLUSIONS

8.1 Weed competition in sugar cane

Competition between sugar cane and the major weeds

This study has shown that sugar cane is affected by competition from weeds just like other crops but the effect is often relatively small. Under the worst scenarios assessing the critical period of weed competition in sugar cane, the maximum reduction in cane yield was recorded in plant cane and was 53% of the weed-free treatments after weeds were left in competition with sugar cane for nearly 30 weeks. This reduction is lower than that reported by Suwanarak (1990) who found cane yields to be lowered by more than 70% after no weeding during the first four months after planting in the wet season in Thailand. In ratoon cane, the maximum losses in cane yields varied between 20% and 30%. Similarly, in the trials evaluating competition from individual species (Chapters 3 & 4), competition on the total dewlap height or biomass from very high weed densities rarely exceeded 50%. In other crops, some yield losses due to weed competition have been reported by Naylor (2002); a summary of 51 experiments carried out in UK and involving wild oats densities ranging 8 to 662 plants m^{-2} caused yields of spring barley to decrease by 0 to 72% while canary grass (*Phalaris minor*) and black grass (*Alopecurus myosuroides*) reduced yields of winter wheat by 26% and 45% at densities of 300 and 500 plants m^{-2} respectively.

Relative competitiveness ‘q’ values of eight weed species commonly found in sugar fields, determined by model developed by Kropff and Spitters (1991), showed that sugar cane was a stronger competitor than most of the weeds tested. Although use of this model, based on the relative leaf areas of the weed and crop, showed similar trends when the same weeds were compared, their q values were found to vary across trials. However, the variations in q values found for weeds in sugar cane are smaller than those reported for competition between *Sinapis alba* L. (white mustard) and sugar beet (*Beta vulgaris* L.) or spring wheat (*Triticum aestivum* L.) (Lotz *et al.*, 1996). The varying q values may limit the use of this model for predicting yield losses in sugar cane and comparisons between various species would only be possible if all the weeds were tested under a range of similar conditions. Despite these limitations, it was, however, possible to identify some of the weeds as being more competitive, i.e. *A. conyzoides, P. paniculatum, D. horizontalis* and *S. barbata*, compared to a lesser competitive group including *B. pilosa, P. urvillei, P. conjugatum* and *P. commersonii*. The latter information conflicts with the perception of many growers that grasses are more competitive than broad-leaved weeds. The difficulty of achieving control of all grasses with selective herbicides in sugar cane may have created this belief.

Timing of competition

The critical periods of weed competition determined in Chapter 2 revealed that the adverse effect of weed competition in sugar cane was not experienced before several weeks following cane and weed emergence. This was also confirmed in the different trials, both under glasshouse and field conditions, assessing competition from one weed species at a time; the adverse effects on cane growth were measurable only 10-12 weeks after imposing weed infestations. In some of the trials with the broad-leaved weeds, some treatments at higher densities showed the adverse effects earlier due to the quicker rate of growth of the weeds. This lag period between weed emergence and competition explains why the onset of the critical periods of weed competition is several weeks later in ratoon cane. Competition started earlier (6 WAP) in the critical period trial carried out in plant cane and this may be explained by the presence of more broad-leaved weeds at that site, the period of the year and the relatively slower cane growth.

The relative competitiveness based on ‘q’ values of both *P. paniculatum* and *P. urvillei* was found to remain unchanged with time within the first nine weeks after establishment of weed infestations. A reduction in their competitiveness was recorded after 13 WAT (in Trial III, chapter 4), mainly explained by the distribution of the leaves within the canopy though they had similar relative leaf areas (L_w).

The timing of weed emergence on the final cane yield was illustrated in Chapter 4 (Trials 1 & III). Both trials revealed that the second transplanting of weeds tested caused no significant difference on cane yield. The physiological difference between the two dates of transplanting included both mean height of shoots and the stage of tillering. The results indicate that weed infestations, occurring when the cane approaches peak tiller density for that variety and when shoot heights are more than 40 cm, would be less prone to weed competition.

Measurements of the total cane dewlap height at the different observation dates had shown some significant reduction although the same treatments did not show any difference at harvest. It is believed that due to its long growing period after the cane leaves are less exposed to the competition for light till harvest, sugar cane has an ability to recover and compensate for earlier losses. Apparent effects of weed competition observed before canopy closure do not necessarily translate into yield losses.

Effect of weed density on weed competition

Although it was difficult to maintain the 'original' densities as at transplanting, increasing weed density was found to influence weed competition and result in earlier weed competition. However, there was often little difference between the higher weed densities, as a result of high level of intra-specific competition between the weeds. Broad-leaved weeds such as *A. conyzoides* and *B. pilosa* have a more prostrate growth and hence were subjected to more intra-specific competition as compared to the grasses with a more upright growth of the leaves. This may explain the lack of a major difference between the two infestation levels studied in some of the critical period trials; the 50% infestation level was most probably not so different to the natural infestations.

Mechanisms of weed competition

Weed competition impaired both tillering (shoot density) and stalk elongation (dewlap height of stalks). In most of the trials, early weed competition resulted more in a reduction of the number of shoots or stalks. Stalk elongation was reduced when competition occurred after the peak of the tillering phase or stalks had reached a mean dewlap height of 25 cm or more. The effect of competition on stalk elongation was also demonstrated in the split-box and allelopathy trials where the effect of competition was observed only after the stalks had reached a dewlap height of 35 to 40 cm.

One of outcomes of this study has been the identification of the involvement of other mechanisms of weed competition as well as that for light. In the critical period trials, competition was still observed with weeds that emerged towards the end of the CPWC or when the cane stalks were higher than the weeds. This was confirmed with the comparison of *P. paniculatum* and *P. urvillei* where the former proved to be more competitive in some treatments although the latter produced more leaf area (for similar densities) and grew taller to intercept more light within the canopy. The vertical distribution of leaf area of cane and weeds (Chapter 4) showed that *P. paniculatum* was as or more competitive even though most of the cane leaf area was found higher in the canopy than the weeds. This indicated that other mechanisms might be involved and that competition for light was more important during the earlier growth stages where tillering was mostly affected.

Root competition was shown to be as important as shoot competition or more in Chapter 5. Root development of sugar cane was impaired by both root and shoot competition and the fact that they were not resulting in a more severe competition when both occurred simultaneously suggested that they were not affecting root development in the same manner. The effects of root competition were observed several weeks after imposing competition when the cane stalks reached more then 35 cm in dewlap height suggesting that root competition was more important than competition for light after the post-tillering phase.

Although root competition seemed to cause more reduction in root biomass of *P. urvillei* compared to *P. paniculatum*, the higher competitiveness of the latter was still not completely explained. Collection of leachates (root exudates) from the two grasses applied daily to sugar cane confirmed an effect from allelopathic compounds resulting in a reduction of root biomass of sugar cane. In one trial (Trial I), *P. paniculatum* developed vigorously and the effect of its leachates on cane growth was more pronounced than those from *P. urvillei*. In the other trials, where an adverse effect from the allelochemicals was observed, *P. urvillei* was more competitive; *P. paniculatum* had not developed so vigorously as in the first trial. One chemical identified from the leachates that may be responsible for the allelopathic effects was 2-Propenoic acid, 3-(4-methoxyphenyl), from the known (for their allelopathic properties) family of cinnamic acids. The presence of higher concentration of this chemical in the leachates (samples taken in Trial IV) from *P. paniculatum* suggests a link with the greater reduction in root biomass observed between this treatment and the control (distilled water).

In conclusion, although weeds appear to impact on the growth of sugar cane by competing for light, there are also effects arising from below ground competition. This may be linked to competition for water and nutrients but may also involve allelochemicals. The allelopathic potential of the other weeds, particularly grasses such as *D. horizontalis* and *Panicum species*, occurring in sugar cane fields need to be assessed. The allelopathic properties of *C. rotundus* on sugar cane had been demonstrated by Mc Intyre (1998). Further research is needed in the mechanism of below ground competition and its importance for other weed species apart from the two *Paspalum* spp.

The mechanisms of weed competition may be summarized by competition for light at the earlier stages of growth (germination/tillering) and root competition, with or without allelopathic exudates from the weeds, later within the tillering/elongation phase.

8.2 Applications and recommendations for the Mauritian sugar industry arising from this research study

The main application of the above findings for the Mauritian sugar industry would be a change in the timing of application of herbicide treatments. The critical periods study shows that the ‘traditional’ approach of applying a pre-emergence treatment immediately after planting or within a few days after harvest to prevent any weed emergence is not totally justified. Although the trials to determine the CPWC were established under the most severe agro-climatic conditions, the results can be extrapolated on the basis of the GDDs to other areas and cane varieties (early v/s late maturing). Similarly, the CPWC would imply an earlier end of weed control compared to the current approach where fields are maintained almost weedfree until the complete closure of the crop canopy. Application of the CPWC will, in general, result in the reduction of at least one herbicide application per season. This is possible by delaying the first herbicide treatment until onset of the first flush of weeds and applying an effective herbicide treatment to kill all weeds present and provide a fairly long residual activity to keep field weed-free until the end of the CPWC (Fig. 8.1).

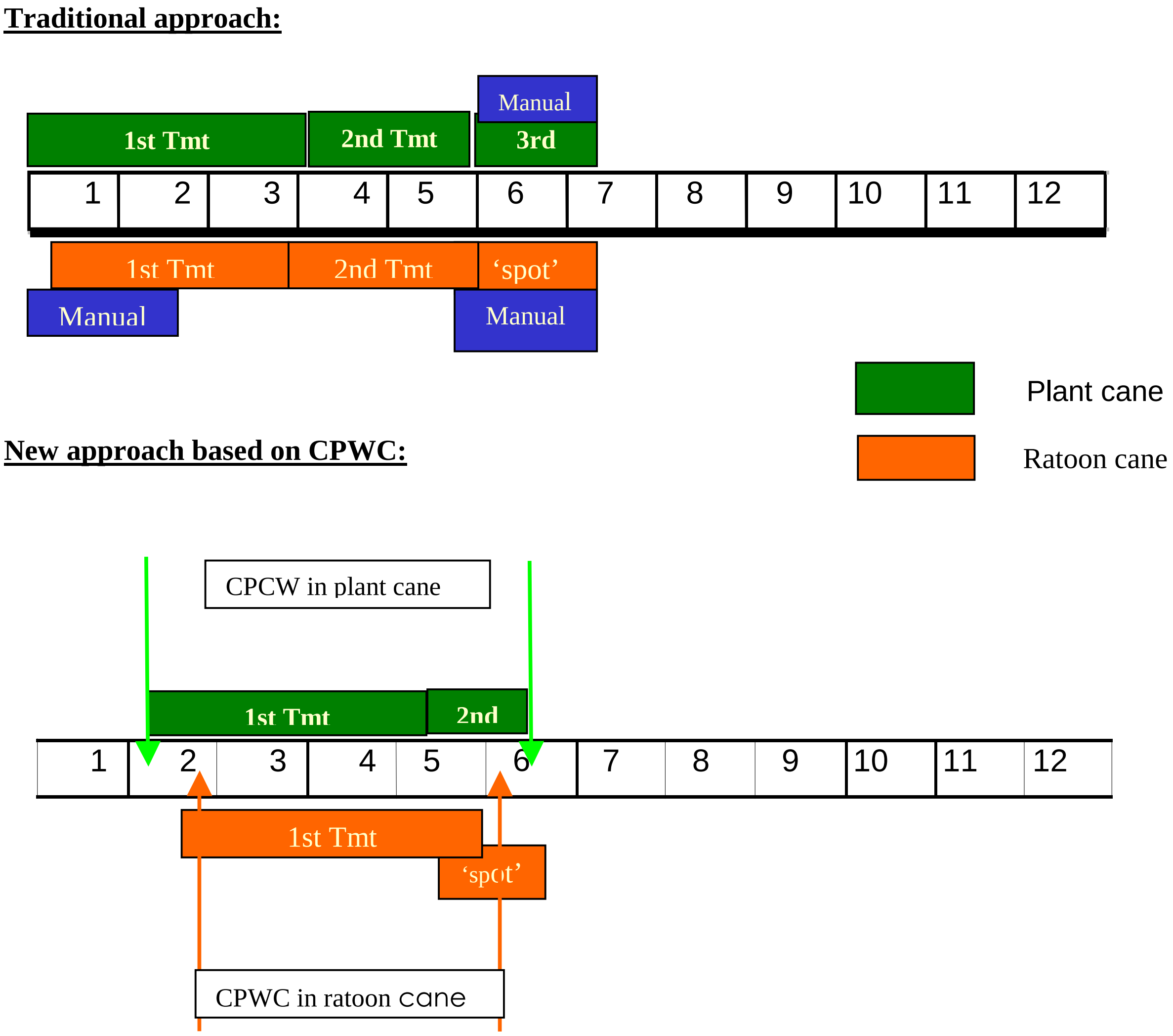

Fig. 8.1 Timing of herbicide applications in sugar cane based on CPWC (arrows showing start and end of control period) (bottom) compared to the conventional method (top). Figures in boxes represent months after planting or harvest. Treatments for plant cane are represented in green boxes and ratoon cane in orange boxes.

Row spacing influences the critical timing for weed removal (Knezevic *et al*., 2003). Planting cane at higher density by changing the row spacing would reduce further the period of control based on the CPWC. Dual row planting, consisting of pairs of cane rows 0.5 m apart with 1.8 m between their centres, has been tested successfully and recommended to the producers in 2006 (MSIRI, 2006; Ismael *et al*., 2007). The new row spacing also has the potential of increasing cane yield with the same amount of planting material and with no increase in fertilizer used compared to conventional planting (1.62 m spacing). It also reduces costs of production by improving weed management and the efficiency of chopper-harvesters. This improvement in weed management results from earlier canopy closure and consequently the end of the CPWC is reached four to eight weeks earlier (Ismael *et al*., 2007).

The success of such a weed management strategy as above would only be possible if the herbicide treatments are able to kill all the weeds present at the time of application and provide effective residual control of most of the weeds present for the duration of the CPWC. Traditional herbicide treatments did not have that potential and the evaluation and the recommendation of the new tank-mix amicarbazone + trifloxysulfuron+ametryn (Chapter 7) has satisfied this requirement. The new tank-mix consisting of trifloxysulfuron+ametryn (0.0263+1.097 kg a.i. ha^{-1}) and amicarbazone (0.875 to 1.05 kg a.i./ha) has a residual activity varying between 14 to 16 weeks and, has post-emergence activity. It is able to control almost all weeds found in sugar cane in Mauritius including *D. horizontalis*, *D. timorensis*, *C. rotundus*, *Paspalum* spp. and *Kyllinga* spp. Moreover, trifloxysulfuron+ametryn has the potential of controlling partly *C. rotundus* pre-emergence. The tank-mix, amicarbazone + trifloxysulfuron+ametryn (0.875-1.05 + 0.0263+1.097 kg a.i. ha^{-1}) did not cause crop injury in young plant or ratoon cane. The efficacy (pre- and post-emergence) of this new tank-mix has offered a new opportunity for managing weeds in sugar cane, as delaying of the first herbicide application will result in savings of at least one herbicide treatment per season.

New weed management strategies based on the CPWC include the exploitation of control methods other than use of herbicides. The use of mechanical weeding during the first two or three months after planting has also been tested successfully (MSIRI, 2006). Two or three passes of duck's foot cultivators have proved to be sufficient to control weeds up to the end of the critical periods. This method of weed control has been recommended in plant cane and where fields are either in rock-free soils or have been derocked for mechanized harvest; this approach would be possible on some 50% of the replanted area every year.

The concept of limiting weed control during the CPWC period, particularly that of leaving weeds uncontrolled after the end of the CPWC, has been discussed by many growers in the past. They were concerned about the production of seeds from the 'residual' weeds and its consequences on the seedbank in the mid- or long-term. Trials (not reported in this study) initiated in parallel to the above development have shown that there was no significant increase in the seedbank between the same plots where weed control had been stopped 16 weeks after harvest for three consecutive years and plots which were kept weed-free. This study is being pursued but as the new weed management strategies are geared towards weed control until 20 to 26 WAH, the risks of increasing the seedbank is minimised. Riemens *et al.* (2007) has shown that appropriate weed management practices in organic farming resulted in no increase in the weed seedbank after seven years. Weed control strategies based on density thresholds were found more cost-effective than spraying every year after modelling seed production of *Alopecurus myosuroides* and *Poa annua* (Munier-Jolain *et al.*, 2002). Similarly, Smith *et al.* (1999) reported a reduction in the population of *Anisantha sterilis* in winter wheat through changes in patterns of management. In sugar cane, Witharama *et al.* (1997) reported that the similarity between species in the seed bank and emerged seedling population in the field was low. This may imply that all the seeds produced do not necessarily pose a threat of more competition later on.

Green cane trash blanketing (GCTB) is practised on approximately 25% of the area harvested and is expected to increase as more fields are harvested mechanically in the near future. The trash blanket controls the weeds effectively until it decays; in humid areas this may happen before end of the CPWC and a herbicide treatment may be required. Similarly under some agro-climatic conditions, especially in plant cane, a second treatment, over and above the new tank-mix applied before the onset of the CPWC, may be justified. Under these conditions, the use of models to predict the weed competition expected from the different infestation levels and weed species present would be beneficial and would suggest further savings of herbicides. However, the findings of this study have revealed varying relative competitiveness (q) values across trials and standardization of the results needs more work. Furthermore, the use of such parameters in sugar cane would be more difficult due to the length of the growing season; the q values changes with time of weed emergence and assessment date.

The allelopathic potential of the other weeds needs to be determined before making any decision on leaving such weeds in the fields after the end of the CPWC. As root competition seems to be important and sugar cane roots do not exploit the cane interrows entirely, weed management could be envisaged that was focused in the vicinity of the stubble or cane roots. This is supported by work carried out by Witharama *et al.* (2007) who found that more weeds emerged in the cane furrows than on the ridges and the difference was influenced by the soil moisture. The latter may imply a herbicide treatment on a localised band nearer to the cane stools in situations where a second post-emergence treatment would be required to reach the end of the CPWC. As the soil moisture varies within the three agroclimatic zones of Mauritius, such approaches would require more research and development.

8.3 Suggestions for future research

Relative competitiveness (q value) for more weeds

This study has indicated two groups of weeds according to their relative competitiveness. More trials should be conducted to evaluate the relative competitiveness (q values) of more weed species occurring in sugar cane fields; the data would be useful for prediction of yield losses for management purposes or Decision Support Systems. The q values could be used to regroup weeds into two or three categories. The results of this study would assist in redefining the various densities for estimating q values. New technologies using Multi Spectral Reflectance (MSR) or radiospectrometry are being successfully tested and calibrated in sugar cane. The use of such technology would give quicker leaf areas estimations.

Threshold for sugar cane and testing of herbicides

The variability within the weed infestations and cane measurements or leaf area estimates observed in this study may restrict the use of threshold infestation levels in sugar cane under the Mauritian conditions. However, with a reliable estimation of leaf areas with the new or forthcoming technologies, prediction of yield losses near the end of the critical periods may assist in the necessity of a second or 'spot' application.

The q values of the different weed species will certainly be useful in the choice of the herbicide treatments. DSS using the relative competitiveness (or any other index) together with information on the level of infestation of each species (e.g. frequency abundance method) will certainly enable more precise selection of herbicide treatments and their rates for cost-effective management of weeds.

The current methods for evaluating herbicides for sugar cane do not provide information on the interaction between weed infestation level or size of weeds and rates of treatments. The efficacy of lower rates on weaker weed infestation levels or smaller weeds or less competitive ones would permit further savings of herbicides.

Allelopathic potentials of more weeds

The screening of more allelopathic compounds from the weeds occurring in sugar cane fields will not only enable a better understanding of the mechanisms of weed competition, but could be used to identify some potential bio-herbicides, for use in other crops.

Future work would be necessary to identify, using dose-response curves with known amounts of the chemicals, the minimum dose of the allelochemicals (e.g. cinnamic acids) required to cause adverse effects on sugar cane. The release of the various chemicals with time and the amounts released will also enable a more complete understanding of the mechanism of weed competition in sugar cane.