

RESUMPTION AND GROWTH OF *Cynodon dactylon* RHIZOME FRAGMENTS

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ABSTRACT

Cynodon dactylon is considered as noxious perennial weed in agricultural or horticultural practices in Tunisia. This weed is difficult to control due to its biological features. Its high degree of competition results not only from the competition growth factors but also the emission of toxins from vegetative and underground decaying biomass. Series of experiments were conducted over three years at the High Institute of Agronomy in Chott-Mariem, Tunisia to understand the biological behavior of rhizome fragments. A new plant will develop from pieces of *C. dactylon* rhizomes only when the pieces have an uninjured and completely developed bud. *Cynodon dactylon* showed a rhizomatous system which had the major masse of rhizomes located in the upper layer. The resumption and the emergence of rhizome fragments depended on the size, the temperature and the planting depth. Short and deep rhizome fragments were unable to emerge. As the temperature went out, the growth increased. Inverse relationship could be established between length of rhizome fragments and bud resumption. Pieces of rhizomes with 1 cm long containing developed bud produced a high percentage of new plants. Also, an inverse relationship was established between planting depth and emergence. The addition of nitrogen to the culture media decreased the inhibiting effect of apical bud and improved the resumption of lateral buds. To elaborate an efficient management strategy, we should take into account these biological parameters.

Key words: Bermudagrass, *Cynodon dactylon*, interference, rhizome, vegetative growth.

INTRODUCTION

Cynodon dactylon (L.) Pers., a herbaceous perennial grass, is a widely distributed species that has been reported as one among the 10 most harmful species in the world (Holm *et al.*, 1977). It likes warm climates and dry situations. In Tunisia, the species is common everywhere (Chabrolin, 1934; Labbé, 1950). It grows on all types of soil, but it still has a preference for sandy soils to sandy loam, dry, well-lit (Labbe, 1950). It grows mainly in patches in gardens,

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roadsides, trampled areas, waste lands, localities with high levels of nitrogen, and often found in wet sites along rivers (Nabli, 1989).

Cynodon dactylon is very aggressive and has been very harmful to the crops in Tunisia posing greater monetary losses to the farmers in the country (Omezine, 1991). In the non-irrigated crops the bermudagrass is very competitive, turning water to its own advantage and giving a huge ground biomass (rhizomes) and aerial (stolons and stems with leaves). This grass is even stronger and highly drought resistant, suspected to issue its roots in the fresh, toxic substances and leave an abundance of organic matter decomposition which is itself a source toxins (Horowitz, 1972; Omezine, 1990). In addition, the bermudagrass is the host of various pests (Datnoff *et al.*, 1997). However, it plays an important role in conserving water and soil. It provides good grazing for the animals during the summer and when there is little greenery. Locally, it is used for indigestion, the treatment of wounds and against constipation (LeFloch, 1983).

Cynodon dactylon is spread mainly by rhizomes and stolons. Seedlings are rare but possible to be obtained in particular conditions (Holm *et al.*, 1977; Montegut, 1990). In Tunisia, in the spring and summer crops under irrigation pipes, the seedling is important, but the survival of these seedlings is short because of the work floor made in due course. The invasion of the plots is mainly done by *C. dactylon* rhizomes (Omezine, 1990). This spread of bermudagrass is favored by tillage tools, especially the use of disc harrows (Omezine, 1991) that break up and disseminate the rhizomes. Recovery and removal of these fragments depend on several factors including the size, position in the soil and the presence of the terminal bud of the fragment and the period of tillage. Rhizomes are the most important organs to be studied to understand the behavior of this species.

The objective of this work is to understand the resumption of growth of *C. dactylon*, and to evaluate its regeneration potential in order to develop effective control processes. These basic studies have been undertaken in a glasshouse and or the field at the High Institute of Agronomy (ISA) of Chott-Marien Sousse, Tunisia during the years 2006, 2007 and 2008.

MATERIALS AND METHODS

Plant material, culture medium and maintenance

Samples of rhizomes were collected from ISA fields. These samples were stored in plastic bags in a refrigerator for later use and without exceeding four days period. All samples used had visible buds. The culture medium used in this work were made up of 1/3 peat, 1/3 sand and 1/3 perlite, this substrate is called standard horticultural culture medium. The plots were irrigated every two days to avoid

water stress (deficit or excess). All experiments are installed in a glasshouse at 25 ± 2 °C.

Spatial distribution of rhizomes

Five holes were dug in ISA pomegranate orchard. The holes were 20 cm by 20 cm to a depth of 70 cm. Each 10 cm horizon was collected. The samples were transported immediately to the laboratory to extract the rhizomes. The extraction was performed as follows: the samples were placed in a tray filled with water to separate the rhizome fragments from soil particles. The separated rhizome fragments were immediately weighed and then planted in 16 cm diameter pots filled with horticultural culture medium to test their viability.

Effect of terminal bud on the resumption of lateral buds from the rhizomes of six buds

To determine the effect of the growth of a bud on another bud, rhizomes of one year's age were collected. These rhizomes were planted in growth chambers filled with sterilized sand. Half of these growth chambers were fertilized and the other half of growth chambers was not fertilized (without fertilization). Fertilization was made with ammonium nitrate at a rate of 2 g per 2000 g of sand. After emergence, the number of buds resumed and not resumed was counted. The experimental design was factorial having two factors with 20 repetitions.

Effect of fragmentation of the rhizome into three either real or virtual equal zones

This experiment concerns the effect of the terminal bud on the lateral buds. Rhizomes with 15 lateral buds either or not were cut into three virtual equal zones (apical, middle and distal) i.e. each rhizome zone contained five lateral buds except the apical zone that contained the apex and four other lateral buds. These fragments were planted in pots filled with horticultural standard potting soil. All the pots were placed in a glasshouse at 25 ± 2 °C under mist-system for 2 months.

Effect of the length of the rhizomes of *C. dactylon* on the resumption of growth

To determine the smallest length of viable rhizome fragments, rhizomes collected from the pomegranate orchard were cut into 1, 3, 6, 9 and 12 cm long. These rhizome fragments were planted in pots filled with horticultural standard potting soil and placed in the glasshouse at 25 ± 2 °C. The pots were irrigated every day to avoid water stress. The experimental design was completely random (CRD) with 20 repetitions.

Effect of burial depth on the resumption of growth

The planting depths were zero, 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70 cm. The rhizomes were placed in 10 cm diameter plastic tubes and buried at indicated depths. The plastic tubes were filled with horticultural potting soil at a level of 3 cm below the top edge. The tubes were plunged into the ground with the top tubes at 5 cm from

the soil surface. The potting soil in the tubes was not disrupted throughout the experiment. The experiment was irrigated every day to avoid water stress. The experimental design was completely random with 20 repetitions.

Effect of time of planting rhizomes on the resumption and growth of rhizomes

To determine the effect of temperature on the growth of the rhizomes, 20 rhizomes with 5 cm length were planted in 16 cm diameter pots which were filled with horticultural potting soil in winter and spring. The pots were irrigated every day. The experiment was installed in a greenhouse in winter (8 °C) and in spring (20 °C) which was without control of temperature and ventilation. The experimental design was completely random with 20 repetitions.

Statistical analysis

The original intent was to analyze growth by using appropriate ANOVA for each experiment. However due to the frequency of failure recovery, it was decided to analyze the proportions of rhizomes resumed combining replicates and calculating the percentages of 20 repetitions (Little and Hills, 1978). The analysis of variance and least significant difference were used.

RESULTS AND DISCUSSION

Spatial distribution of rhizomes in the soil

Rhizomes are the primary over-wintering structure (Holm *et al.*, 1977). *Cynodon dactylon's* success as a weed is thought to be a result of the adaptive rhizome characteristics. In this study, the rhizome system is superficial as well as deep, which may account for the ability of this species to infest both arable and waste lands in a variety of conditions. The excavation of *C. dactylon* rhizomatous system shows that the majority of rhizomes are in the top soil. About 85% of rhizomes are located within 20 cm of soil and few rhizomes are able to reach a depth exceeding 30 cm (Fig. 1). The observation reveals that these rhizomes have different colors from white to black, indicating that they have different ages. Topsoil is a densely populated area by the young rhizomes and deep layers are populated by rhizomes a little older. Horowitz (1972) found approximately 70% of the rhizome weight of two and a half year old plants in the upper 20 cm of the soil and no rhizomes below 40 cm (Horowitz, 1972). Other investigators reported the existence of rhizomes 1 m deep (Holm *et al.*, 1977).

Rhizomes are organs of propagation and survival for *C. dactylon* (Holm *et al.*, 1977). The success of the *C. dactylon* as a weed results from characteristics of adaptation and biological behavior of rhizomes buds. Rhizomatous system of *C. dactylon* is not only superficial but also deep; the distribution of rhizomes into the soil allows the infestation of

cultivated land and uncultivated land under various conditions (Fernandez, 2003). The excavation of rhizomatous system of *C. dactylon* showed that the rhizomes are concentrated near the soil surface. Thus, almost 80% of the mass of rhizomatous are in the top 20 cm of soil. However few rhizomes can drop to below 60 cm. At this depth, the rhizomes allow the plant to resist and survive the severe constraints. These rhizomes are able to form rhizome stock in order to restore new individuals. The position of rhizome in the ground can modify the new shoot growth. The deep rhizomes give weak new shoots and the surface rhizomes give strong ones. Similarly, the fragments of rhizomes which are buried after the removal grow slowly. The cultivation destroys the rhizomatous system into different rhizome fragment lengths and disturbs these fragments into the soil. Thus, this cultivation perturbs the dynamics of the resumption and emergence of new shoots. This disturbance affects the regenerative capacity of rhizome fragments and hence the degree of control with systemic herbicides (Omezine, 1991).

Effect of terminal bud on the resumption of lateral buds of *C. dactylon*

The effect of the presence of the terminal bud on the resumption of lateral buds in soil culture rich or poor in nitrogen is shown in Fig. 2. The terminal bud has reduced the resumption and the growth of lateral buds no matter whether the soil culture is nitrogen rich or poor. However, if the soil nitrogen level is high, the effect of the terminal bud is slightly attenuated and the addition of nitrogen to the soil has improved the resumption of lateral buds. If the apex is decapitated, the resumption of lateral buds is improved, but this improvement is better when the soil contains more nitrogen (Fig. 2). However, once the terminal bud is suppressed, the lateral bud which is well developed on the rhizome dominated the other lateral buds (Fig. 3). This bud has played the same role as the terminal bud. In this case, the bud which has the position 1 dominated the other ones (Fig. 3) and inhibited the resumption of bud 2 and 5, while reducing the growth of other shoots from the buds 4 and 6, respectively.

The evaluation of the regeneration potential of severed portions of rhizomes showed that a portion of rhizome with buds has the potential to reconstruct whole plants. This resumption of portions of rhizomes is proportional to the energy reserves in the rhizomes. The resumption of a bud is also dependent on the actions of other buds on the same piece of rhizome (McIntyre, 1972). Interaction between buds is modulated by several factors. Based on observations made on the resumption of lateral buds on rhizomes not decapitated, the growth of lateral buds showed a basipetal gradient of decreasing activity and was strongly inhibited at the basal nodes. Basal buds are inhibited by the development of the apex. Once the rhizome is beheaded the

resumption of lateral buds was better. The terminal bud inhibited to varying degrees the development of lateral buds (Table-1). Most lateral buds are not able to resume during the vegetative period since the terminal bud is present (Cline, 1991).

Effect of fragmentation of the rhizome into three equal zones

The results of rhizomes fragmentation with or without terminal buds in three virtual equal segments in length and number of buds (apical, middle and distal zone) are presented in Table-1. For non-decapitated rhizomes (with terminal bud), the apical region has a high recovery rate. However, the distal region has the lowest rate. The buds in the apical area have dominated the buds in the other two zones. This dominance is high in the distal zone. However, if the apex is decapitated, the recovery is improved for both middle and distal zones with a sharp reduction in the resumption of buds in the apical region (Table-1). The real fragmentation of rhizomes in three equal segments has improved the resumption of buds (Table-2). Thus the rhizome of 15 buds non-fragmented into three zones allowed the recovery of 15.2% of the buds. However, the fragmentation of rhizomes into three zones has increased the recovery of not only the apical but also the resumption of buds in the other two zones.

Removing the terminal bud produces a correlative inhibition between lateral buds. The lateral bud, which was the best placed and the well fed started first, and once started, it dominated the other lateral buds. Thus, this first bud reacted as an apical bud, i.e. dominance of shoot. Indeed, the first shoot bud which resumed inhibited some buds and limited the growth of other shoots from other buds (Fig. 3). The terminal bud and the equivalent bud acted in the same way on the resumption of buds. Probably, the growth of rhizome buds is largely dependent on how the buds on the same rhizome interact. The conventional explanations invoked in these cases of correlative inhibitions blamed hormones/nutrients. Since its resumption, the apex or lateral bud if the apex is decapitated controls the delivery of food, this bud developed preferentially by diverting to its growth elements located in the rhizomes and synthesizes a growth inhibitor and transmit it to other buds. This gives an initial advantage in the competition for the compounds for growth (Kramer, 1980; Cline, 1991). This is a complex phenomenon that despite much research is still not fully understood at present (Cline, 1991). Another explanation attributed the higher level of nitrogen and humidity in the first bud formed comparatively with the other buds (Beasley, 1970; Cline, 1991). The addition of nitrogen in the culture medium at 2 gr in 10 kg of soil increased further the rate of resumption of lateral buds (Fig. 2), either the terminal bud was decapitated or not (Table-1). The high nitrogen content transformed the apical or lateral bud into aerial shoot instead of

the continuation of rhizome growth. This transition of the rhizome (differentiation) to the aerial shoot is induced by the increase of nitrogen.

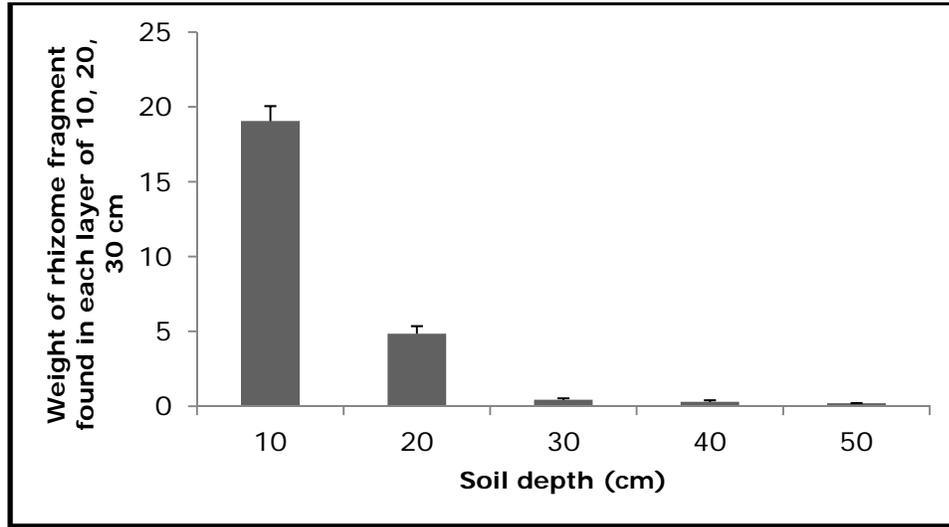


Figure 1. Spatial distribution of *C. dactylon* rhizomes.

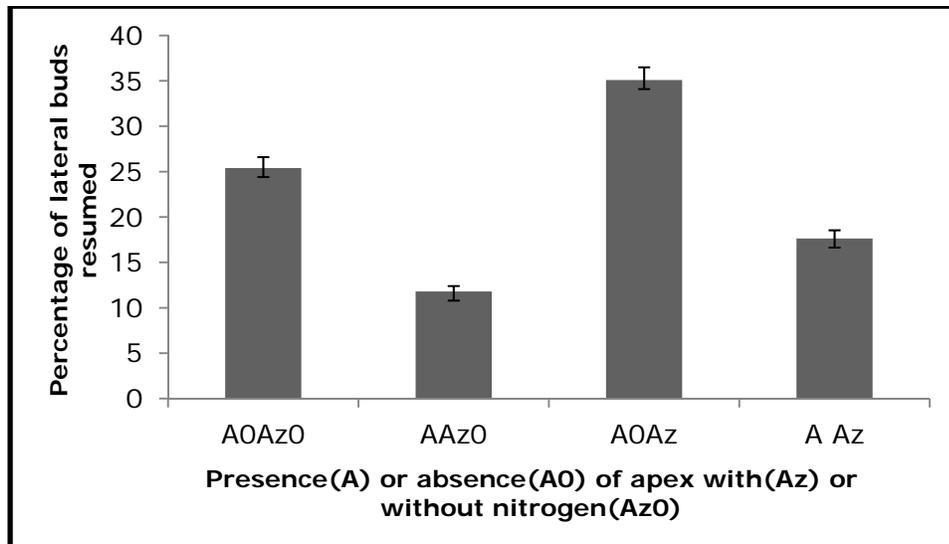


Figure 2. Effect of the presence of the terminal bud on the resumption of the lateral buds in an environment rich or poor in nitrogen (AOAz0 = without apex and without nitrogen, AOAz = without apex and with nitrogen, AAz = with apex and with nitrogen, AAz0 = with apex and without nitrogen).

Nitrogen seems the major factor affecting apical dominance by reducing the inhibitory effect (Fig. 2) probably due to the gradient of nitrogen directed toward the apex (McIntyre, 1965 and 1969). In addition it is well known that nitrogen promotes the use and mobilization of carbohydrates, so when the terminal bud starts to grow, food stocks are moving to it to be an aerial shoot, this migration of reserves is affected by growth substances (Cline, 1991).

Effect of the length of the rhizomes of *C. dactylon* on the resumption of growth in the presence of the apex, and nitrogen

All fragments of rhizomes, regardless of their length resumed, but the number of buds which resumed varies with the length of the rhizome (Fig. 4). Thus, the absolute number of fragment buds increases with the length.

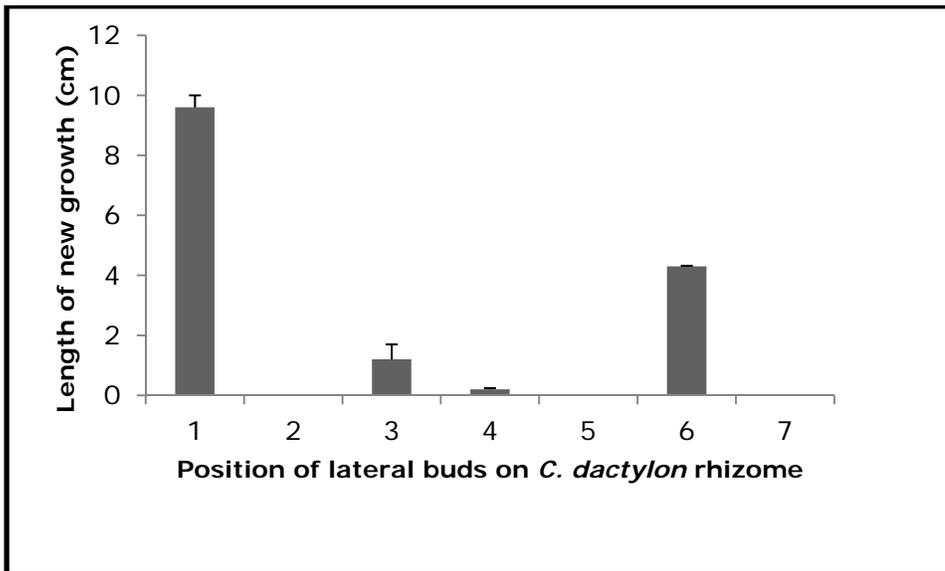


Figure 3. Effect of terminal bud on the new growth of shoots from lateral buds.

Table-1. Effect of the apex on the resumption of lateral buds on rhizomes fragmented contained 12 buds (the results are in percentage of buds that have resumed).

Rhizomes	Position of buds on rhizome		
	Apical zone	Middle zone	Distal zone
Without apex	42.0 ± 1.2 a	36.2 ± 2.3 b	21.8 ± 1.6 c
With apex	75.2 ± 2.3 d	15.6 ± 1.5 e	9.2 ± 0.9 f

Means followed by the same letter are not significantly different at $P < 0.05$ level.

Table-2. Apex effect on lateral buds of non-fragmented rhizomes (each fragment contains 4 buds).

Position of buds on rhizome			
Complete rhizome	Apical zone	Middle zone	Distale zone
15.2 ± 3.1 a	22.1 ± 3.5 b	23.5 ± 1.8 b	24.2 ± 3.5 b

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

However, the relative number of fragment buds decreases with the length of the rhizome fragments (Fig. 5). The addition of nitrogen as ammonium nitrate in the soil culture has improved the resumption of buds either the apex is present or absent. These results showed a direct relationship between the number of buds and length and indicated an inverse relationship between the percentage of buds resumed and length of rhizomes either with in the presence of the apex and fertilization or in their absence. The effects of correlative inhibition of buds and the effects of fragment length on resumption are sometimes confused. The rhizomes having a large number of buds produce few shoots compared to a smaller number of buds. The absolute number of buds increased with the length of rhizomes (i.e. number of buds), but relatively, an inverse relation between the length of the fragment and the resumption of buds (Fig. 3). For 1 cm length fragments with a single bud, all buds had given a new growth; for the rhizomes of 3 cm long, 80% of buds produced shoots. This percentage dropped to 40% of buds on the rhizomes of 12 cm length. The explanation of the effect obtained blames the length of rhizomes, when the number of rhizome buds decreases, the relationship of dominance is reduced.

Effect of burial depth on emergence of rhizomes of *C. dactylon*

The position of the rhizomes in the soil mass affects the emergence and shoot growth. The results show that the emergence period of shoot is longer for deep planting than the shallower planting. The first aerial shoot emerged 7 days after planting at the 5 cm deep. Similarly, the aerial shoot emerged 11 days after planting the rhizomes at 10 cm depth. For rhizomes buried at a depth of 20 cm, shoots emerging after one month, the rhizomes buried deeper, beyond 30 cm, did not emerge. The excavation of buried rhizomes showed that some buds had resumed but they are not able to reach the surface, they are rotten and dead halfway.

The biomass of shoots from the rhizomes was measured (Fig. 6). The rhizomes deeply burial significantly reduced shoot growth when compared to a lowly burial. The rhizomes less deeply buried give more biomass than the rhizomes more deeply buried and the rhizomes are able to produce more perennial organs. This difference results in

the length of the growing season, the rhizomes which emerged first formed many leaves, while the rhizomes deeply burial were slow to from leaves. The more leaves are the more the growth is.

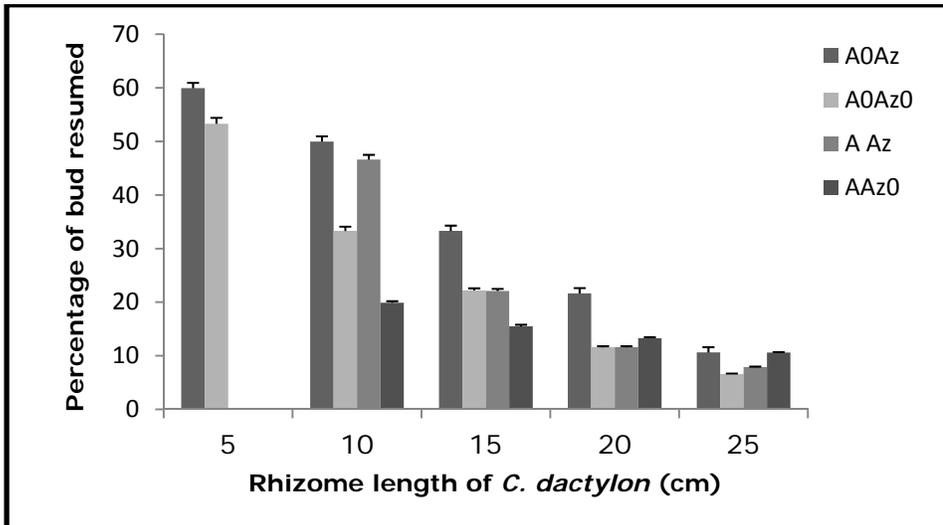


Figure 4. Effect of rhizome length (cm) of *C. dactylon*, nitrogen and the apex on the resumption of lateral buds.

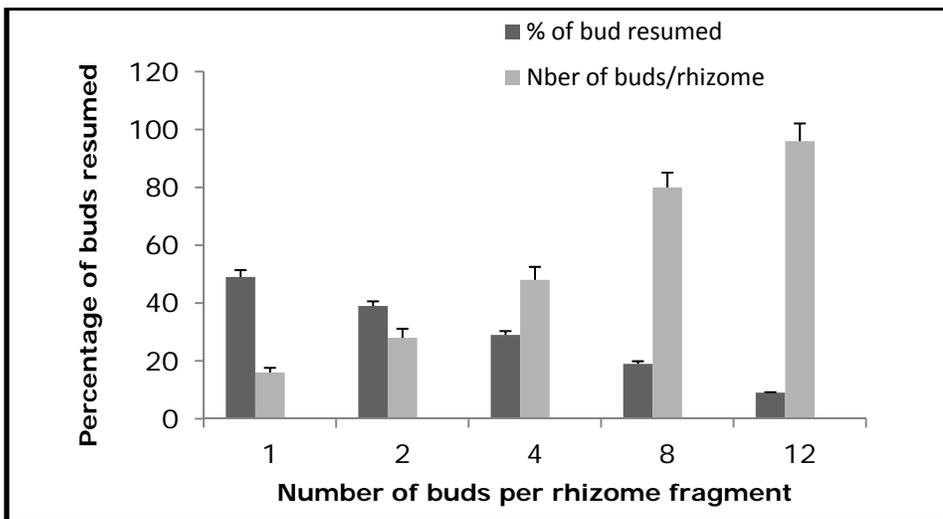


Figure 5. Effect of the length and the apex of rhizomes on the resumption of *C. dactylon* lateral buds (% resumed bud/rhizome = % of bud resumed/rhizome buds).

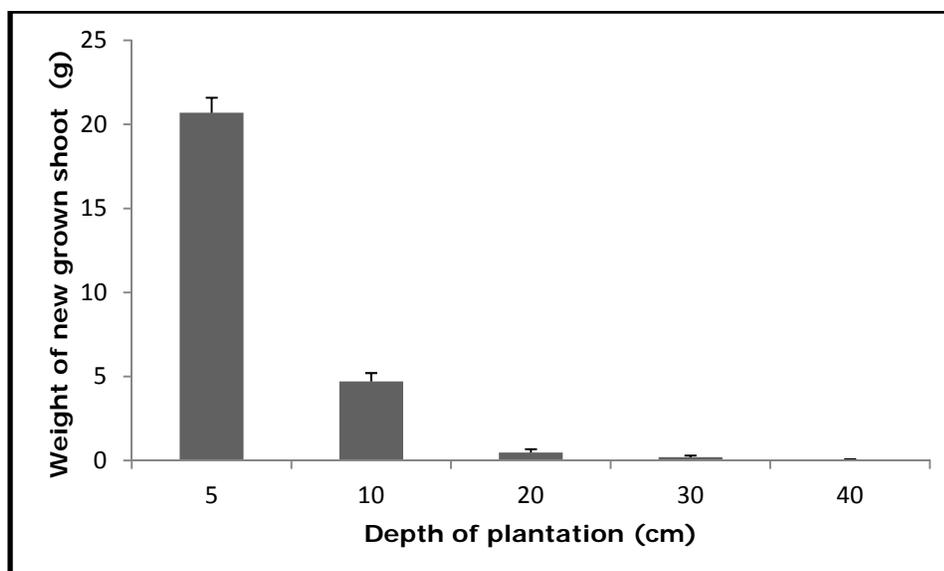


Figure 6. Effect of burial depth on new growth of shoots.

Tillage reduces annual weeds and increases perennial weeds such as *C. dactylon*. However, this operation of soil cultivation may improve partially the control of *C. dactylon* by deep positioning of short fragments of rhizomes into the soil. Burial fragments of rhizomes in the soil allowed to modulate the emergence new growth. Buds which were deep placed did not emerge because the ecological conditions were not conducive to growth or, even if conditions were favorable, small fragments will fail to reach the surface, they die halfway through lack carbohydrate content in the fragment (Chicouène, 2001). By tilling the soil, tillage tools cut the underground system into fragments. These fragments of rhizomes can be short or long. The survival of these pieces of rhizomes is closely related to the length, the short fragments and deeply buried are not able to emerge. The period of cultivation is of vital importance in the emergence and success of the species. Tests on the resumption of rhizomes in the winter and spring indicate that there was a significant difference between the two tests (Table-3). In the spring, the resumption of lateral buds is higher than in winter. The increase of temperature in spring resulted in the formation of stems, and not in the formation of rhizomes. This increase in temperature had favored the growth of the aerial part, but the number of dormant buds has increased. The choice of tool and timing of tillage management are two factors of this species monitoring. The length of the rhizomes is very significant in the

management and control of this species because the type of tillage and the number of tillage may affect the length of the rhizomes.

Effect of time of planting rhizomes (season) on the resumption and growth of rhizomes

The results for the effect of planting season on the resumption of vegetative growth and rhizomes of *C. dactylon* were presented in Table 3. In the spring plantation, the resumption of growth exceeded that obtained in winter planting; it was 30.4% for the winter planting and to 95.2% in spring planting. The emergence period is much longer for winter planting compared to spring planting, it took one week and three weeks for spring planting and winter planting, respectively. Probably, this is due to the effect of thermo-dormancy on buds. Similarly, the growth of stems in the spring planting gave more shoots than the winter planting. The number of shoots obtained was 40 and 15 shoots for spring planting and winter planting, respectively.

Finally, the soil cultivation before winter or autumn is more effective in reducing the number of rhizomes into the soil than the cultivation in the spring. The best resumption of buds takes place at the end of winter when the temperature rises. This renewed growth is governed by the terminal bud, which dominates the resumption of other buds. The agro-ecological conditions imposed by the farmer change this dominance but do not eliminate it and modulate resumption. The ability of rhizomes to produce new growth increases with the reserves of carbohydrates. For a better management strategy of *C. dactylon* must take into account these obtained biological parameters.

Table-3. Effect of planting season on the resumption and growth of rhizomes in the absence of the apex.

Spring planting		Winter planting	
Percentage of rhizomes resumed	Number of shoots obtained	Percentage of rhizomes resumed	Number of shoots obtained
95,2 % a	40 a	30,4% b	15 b

Means followed by the same latter are not significantly different at $P < 0.05$ level.

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