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Using Growth Analysis to Interpret Competition between *Cynodon dactylon* (L.) Beauv. (Bermudagrass) and Newly Planted *Olea europea* L. (Olive Seedlings)

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ABSTRACT

A greenhouse experiment was conducted to determine the effects of bermudagrass [*Cynodon dactylon* (L.) Beauv.] on the growth of newly planted olive trees (*Olea europea* L. var. *Chemlali*) over a one-year period. *C. dactylon* and olive seedlings were grown individually and in competition with each other. Soil water content and growth period were evaluated weekly and nutrient content in the leaves was analyzed at the end of the experiment. Detailed growth analysis together with information on seedling growth was used to interpret competition between *C. dactylon* and olive seedlings. Competition reduced the growth of olive seedlings for different reasons. The decrease in the leaf area ratio was associated with an increase in the root/shoot ratio implying that olive seedlings were limited by competition for below-ground resources. Olive tree seedling leaf area was the most sensitive growth parameter measured in response to bermudagrass. Leaf area was reduced by 79% and specific leaf weight, a relative measure of leaf thickness, was reduced by 34.4%. Moreover, bermudagrass reduced olive tree seedling dry weight by 54%. Olive tree seedling trunk diameter relative growth rate and shoot length were reduced by 55 and 68%, respectively. In addition to visual symptoms of nutrient status, olive tree seedling nutrient status was reduced by bermudagrass. The negative effects of bermudagrass increased over time. This reduction in olive tree seedling growth cannot be explained entirely by competition for essential nutrients and water. However, a competitive interaction through allelopathy might be induced by *C. dactylon*.

Keywords: anatomical index, interference, leaf area, leaf thickness, shot dry weight, soil water, water flow

INTRODUCTION

The olive tree (*Olea europea* L.) is one of the most important plants in the Mediterranean basin. Over 850 million olive trees are cultivated worldwide, 95% of which are in the Mediterranean region; olive tree is an important cash crop accounting for about 3% of the world's oil needs (Luchetti 2002). Tunisia is one of the world's largest olive oil producers; 60 million olive trees occupy 1.5 million ha representing 30% of Tunisia's cultivated soil and 16% of world olive tree acreage. The olive sector plays an important part in Tunisia's economy as it employs some 300,000 farmers who are fully or partially involved in it. This sector is a source of income for around one million Tunisians. Figures recently released show that 70% of the country's olive oil is exported (INS 2009).

Cynodon dactylon (L.) Beauv. is native to Africa, but occurs throughout the world; it is very common in Mediterranean countries and America. The weed probably came from the Middle East; it prefers warmer regions but it is becoming established in cooler regions as well. *C. dactylon* occurs on almost all soil types, especially in fertile soil (Holm *et al.* 1977). This is a vigorous, warm season perennial that expands rapidly with an extensive system of rhizomes and stolons. Since it is a creeping perennial grass, it can establish easily according to mechanical machinery (Holm *et al.* 1977). Seeds also help *C. dactylon* expand to new locations. *C. dactylon* is difficult to control because of its extensive rhizome system and the sprouting ability of fragmented rhizomes. In Tunisia, the weed is widely distributed from deserts to garden lawns through to roadsides, overgrazed, trampled areas, uncultivated lands, localities with high levels of nitrogen, and moist sites along rivers (Cuénod 1952; Carène 1990). The infestation of *C. dactylon* in olive plantation varies with locations from high to low infestation (**Fig. 1**); in severe infestations, *C. dactylon* biomass has been shown to range between 400 and 800 g/m^2 on a dry weight (DW) basis (Omezine, pers. obs.). *C. dactylon* is a very competitive weed; it prevents the normal development of the tree and reduces the total production. Competition is most severe during the first 5 years of the tree's life or where root growth is limited (Combrement 1978); thus, *C. dactylon* is posing a serious threat to olive production. *C. dactylon* around the tree trunk not only competes directly with tree growth, but also provides a good habitat for field mice or voles, which can girdle and kill young trees. Earlier experiments showed that *C. dactylon* competes with olive trees for water and nutrients (Pastor 1989; Saavedra *et al.* 1989). However, the mechanism of *C. dactylon* interference with olive trees is not known and no work has been done in this respect, at least in Tunisia.

Plant growth analysis is an explanatory, holistic and integrative approach to interpreting plant form and function. It uses simple primary data in the form of weights, areas, volumes and contents of plant components to investigate processes within and involving the whole plant (Evans 1972; Hunt 1990). Plant growth analysis is now a widely used tool in such different fields as plant breeding (Wilson and Cooper 1970; Spitters and Kramer 1986), plant physiology (Clarkson *et al.* 1986; Rodgers and Barneix 1988) and plant ecology (Grime and Hunt 1975; Tilman 1988). So, why not in weed science to explain weed competition?

The objective of this study was to determine the nature of interference in olive tree seedlings under greenhouse

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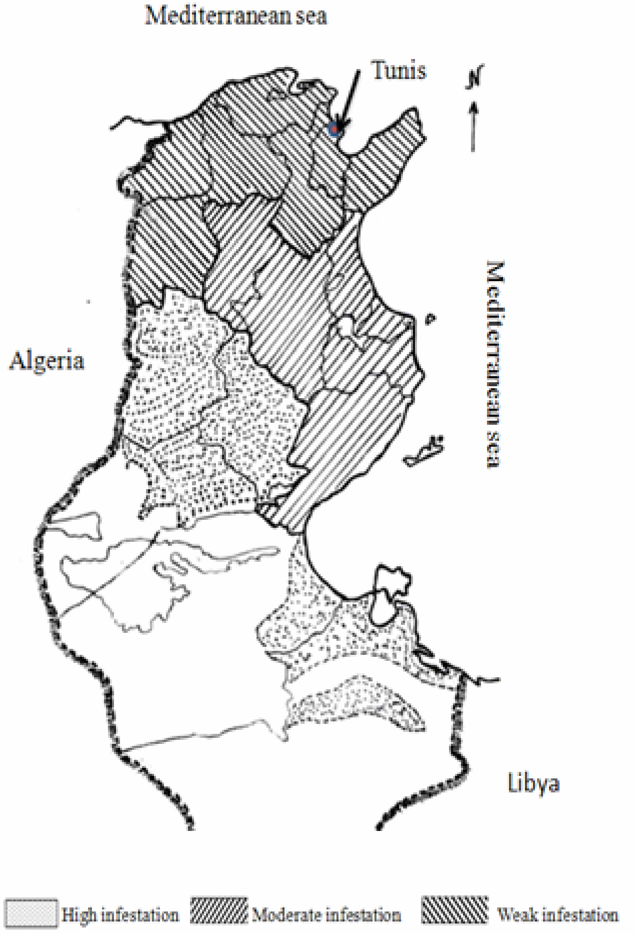

Fig. 1 ***Cynodon dactylon*** **infestation in Tunisia.**

conditions and to ascertain the extent of this competition of olive tree seedling growth by *C. dactylon* using growth analysis.

MATERIALS AND METHODS

Experiments were started in December, 2008 in the greenhouse at the High Institute of Agronomy Station, Chott-Marien (Sousse), Tunisia. The temperature of the greenhouse was maintained between 20 and 25°C with a cooling and heating system. No additional lighting was used in the greenhouse. The pots were planted on December 15, 2008 with one-year-old, ungrafted olive tree seedlings, about 16 cm tall and 4 mm in diameter that had been selected in the nursery for uniformity. The experiments were carried out in plastic pots measuring 32 cm by 20 cm in height, with draining holes, filled with an artificial medium (mechanical composition: clay, 35%; sand, 40%; silt, 10% and organic matter, 3.5%). Each competition unit consisted of one pot which was planted, each with an olive tree seedling and four 4-cm rhizomes of *C. dactylon*.

As soon as *C. dactylon* emerged, moisture content was measured for several weeks in containers where olive seedlings were growing alone or with *C. dactylon*, by means of tensiometers placed 15 cm deep and 5 cm near the olive tree seedlings.

Olive tree seedling length was measured weekly over the whole season and was used to calculate the relative growth rate (RGR) for each tree seedling. All olive tree seedlings were harvested and sampled for leaf area, shoot and root fresh weight (FW) at the end of the growing season. The DW was recorded following oven drying at 60°C for at least 2 days. From these direct simply primary measurements of growth parameters, several variables were calculated (Evans 1972; Hunt 1990): the proportion by DW of the individual parts (leaf-, stem-, and root- weight ratios), the ratio of leaf area to total plant weight (leaf-area ratio = LAR). A leaf area index (LAI = the ratio of the total surface area of a plant's leaves to the ground area) for each tree seedling was determined by passing all leaves through a LI-3100 Leaf Area Meter. Specific leaf area (SLA, leaf area per unit DW) and leaf dry matter content (LDMC, the ratio of leaf dry mass to fresh mass) were evaluated. Leaf area/shoot diameter, leaf area/shoot length, leaf fresh and dry weight/root fresh and dry weight, leaf DW/leaf area, and root/shoot ratios were evaluated. The (SLA × LDMC)$^{-1}$ product was used to estimate leaf thickness, where LDMC is the leaf DW content (leaf DW/FW) (Vile *et al*. 2005).

The evaluation of leaf thickness through the evaluation of leaf water content is of great important for many scientists; it provides valuable information in irrigation management and helps avoid plant drought stress (Jördens *et al.* 2009).

All olive trees' leaves were collected separately from each treatment at the end of the experiment. Three composite leaf samples were taken from each treatment to determine leaf nutrient content. The analysis was done for nitrogen, phosphorus and potassium in the soil chemistry laboratory at the High Institute of Agronomy (Chott-Mariem).

Fifteen elementary units per treatment either with *C.dactylon* or without *C. dactylon* were planted within a completely randomized design. Growth and nutrient data were analyzed using analysis of variance and *F*-tests. Least significant difference was used to compare treatments means (Little and Hill 1978).

RESULTS AND DISCUSSION

Weeds constitute a major problem in crop production in Tunisia. Weeds compete with crops for limited growth factors reducing its availability to crops. Growth factor stresses reduce the photosynthetic capacity of a plant by reducing the rate of area production and consequently the total amount of photosynthetic assimilatory surface present (Fischer 1965; Cabrera-Bosquet *et al.* 2009). When *C. dactylon* olive seedlings were grown together in the same pot, the effect of *C. dactylon* on olive seedlings was significant. The development of olive seedlings grown in separate containers was markedly greater than that in the same containers, due to the utilization of additional growth factors. The negative effect of *C. dactylon* was severe and increased with time during the growing season of competition. The cumulative influence of *C. dactylon* on olive seedlings for 27 weeks was well illustrated by the weight of the above-ground tree parts. The above-ground weight of olive tree seedlings treated with *C. dactylon* was 30% of that of corresponding control. It appears as if the rate of growth with and without *C. dactylon* was approximately constant and was highly greater in the absence of *C. dactylon*. However, relative growth rate derived from these data did not show any significant differences for both treatments, only over the first few weeks. The differences subsequently increased later (**Fig. 2**). The height of the plant can be characterized by the length of the stem. The height of the plant is one of the most responsive parameters to growth factors. The elongation of olive stem seedlings during the period of competition is detailed graphically (**Fig. 2**); their height above the ground was 0.41 m vs 0.83 m for the control. Significant

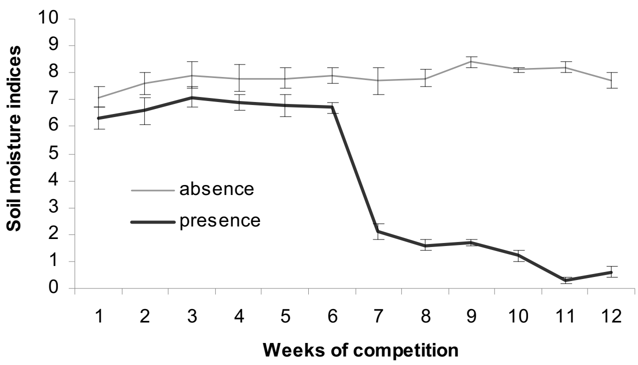

Fig. 2 Repeated observations of olive seedling height (cm) in presence and absence of *Cynodon dactylon*, Values represent means of 15 units± Standard Error (SE).

Table 1 Growth parameters of olive seedlings.*

	Without *Cynodon*	With *Cynodon*
Leaf area (cm^2)	429.3 ± 0.3 a	90.1 ± 0.4 b
Leaf fresh weight (g)	2.1 ± 1.2 a	2.5 ± 0.6 b
Leaf dry weight (g)	4.9 ± 0.5 a	1.4 ± 0.3 b
Shoot fresh weight (g)	2.9 ± 0.8 a	4.0 ± 0.9 b
Shoot dry weight (g)	5.4 ± 0.6 a	2.2 ± 0.7 b
Root fresh weight (g)	8.8 ± 1.1 a	4.4 ± 0.9 b
Root dry weight (g)	3.1 ± 0.5 a	2.6 ± 0.2 b
Diameter increase (mm)	2.9 ± 0.5 a	0.9 ± 0.6 b
Length increase (cm)	66.8 ± 0.8 a	24.9 ± 1.2 b
Whole seedling dry weight (g)	3.4 ± 0.9 a	6.2 ± 0.8 b

*Values within the same row followed by the same letter are not significantly different (P=0.05) according to the LSD test

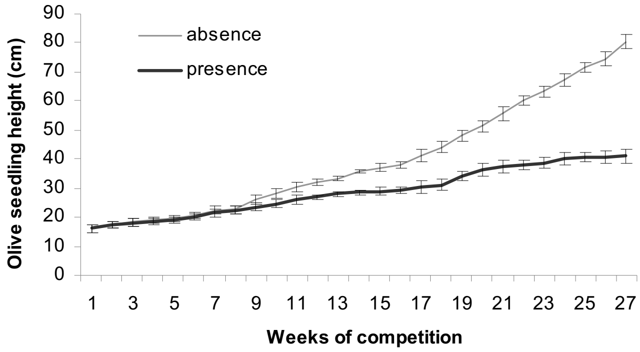

Fig. 3 Repeated observations of soil moisture in the presence and absence of *Cynodon dactylon*. Values represent means of 15 units ± Standard Error (SE).

Table 3 Leaf analysis.*

	Nitrogen[#]	Phosphorus[#]	Potassium[#]
Without *Cynodon*	1.82 ± 0.21 a	1.92 ± 0.28 a	1.60 ± 0.21 a
With *Cynodon*	1.42 ± 0.14 b	0.17 ± 0.25 b	0.21 ± 0.31 b
Healthy olive leaves[β]	1.27	1.88	1.59

*means of 3 composite leaf samples, # Values within the same cologne followed by the same letter are not significantly different (P=0.05) [β] data From FAO (1977).

differences in plant height between weedy and control plants were recorded after only 10 weeks of competition. Olive seedlings in the presence of *C. dactylon* showed an even larger reduction in height, about 50% less than the control. The enlargement of olive seedling stem diameter growth and other growth parameters with and without *C. dactylon* during the growth season of competition are presented in **Table 1**; their base stem diameter was about 4.9 mm versus 6.9 mm for the control. Olive seedlings in the presence of *C. dactylon* also showed a reduction in stem diameter which was about 50% less than the control. Also, the total leaf surface area was consistently reduced as a consequence of the presence of *C. dactylon*: it was about 105 cm^2 in stressed olive seedlings vs about 140 cm^2 in control leaves, i.e. leaves from stressed olive seedlings were 25% smaller than leaves from non-stressed olive seedlings. The response of the various growth parameters varied in intensity but showed generally similar trends. However, they did not have the same distribution of DW between leaves, stem and root (**Table 2**). Olive seedlings accumulated more DW in their roots so that more water and nutrients would be taken up to overcome the presence of *C. dactylon*.

This overall growth reduction pattern of olive seedlings was due probably to water and nutrient deficiencies. Plant growth is controlled directly by water supply and nutrient availability (Sun *et al.* 2009). A water deficit may reduce the rate of growth of plant organs (Basiouny 1977). In fact, the repeated observations of soil water content were represented and water stress was shown to occur after 10 weeks (**Fig. 3**). In the presence of *C. dactylon*, the soil water content decreased with time; however, in its absence the soil water content was likely constant. Both a deficit in water and nutrients likely caused a reduction in growth of olive seedlings. The leaf analysis showed deficiencies in the levels of nitrogen, phosphorus and potassium associated with the presence of *C. dactylon* (**Table 3**). These nutrients were lower in the leaves of seedlings grown with *C. dactylon* than those grown without *C. dactylon*. In the presence of *C. dactylon*, the olive seedling leaves were considerably smaller than those without *C. dactylon*. Also these nutrient deficiencies resulted in necrotic areas at the tip and along the lateral margin and leaves were light green in colour rather than deep green. Combrement (1978) indicated that these symptoms resulted from water and nutrient deficiencies. The same author showed that young olive trees grown with *C. dactylon* usually are stunted, have chlorotic and small leaves, delayed tree fruit maturity and reduced yield compared to those grown in its absence (Combrement 1978). Water and nutrient deficiencies are not the only limiting environmental factors. As soon as these deficiencies occur, many others deficiencies may occur such as light and residues from *C. dactylon*. Paul and Peterson (1980) reported that shading typically decreases leaf thickness. Moreover, competitive interactions between olive seedlings and *C. dactylon* through allelopathy may occur (Omezine 1999). Water and nutrient stresses are one of the most critical environmental stresses that plants are exposed to; they affect both the evolutionary and the economic performance of a plant by directly reducing its survival in the natural environment and its productivity in agriculture (Torres *et al.* 2007).

The main morphological and anatomical effects of water and nutrient stresses caused by *C. dactylon* are summarized in **Table 4** and **5**, respectively. The morphological and anatomical studies demonstrated the existence of a series of effects upon the root system and the arial system which dependent upon the presence of *C. dactylon*. The olive seedlings grown with *C. dactylon* had changes in their morphological and anatomical structures (Shao *et al.* 2008; Jaleel *et al.* 2009). Water and nutrient deficiencies promote greater relative allocation of photosynthetates to root growth, ultimately resulting in plants that have higher root-shoot ratios and a greater capacity to absorb water and minerals relative to the shoots that must be supported (Molas 1997; Kozlowski and Pallardy 2002). A reduction of leaf blade area and the growth of specific leaf area were noticed in plants treated with *C. dactylon* (Trubat *et al.* 2006). *C. dactylon* also reduced transpiration rate (Nagarajah 1980; Otoo *et al.* 1989). The LAR of the control plants increased more than those with *C. dactylon*. The higher leaf DW/area (specific leaf area: SLA) of the stressed plants probably came in part from an increase in leaf thickness. The resulting leaf thickness indices are recorded together with leaf DW/area. The thickness of leaves, a sensitive indicator of plant water status, increased on average by 28.5% and leaf DW/area by 26.6% in the presence of *C. dactylon*. Assuming that thicker leaves would have proportionately more DW per unit area, the difference between these two percentages is presumably due to accumulated assimilates in olive leaves in the presence of *C. dactylon*. The change in leaf thickness was mainly due to changes in the volumes of special water-storage tissues (Jördens *et al.* 2009) or leaf water potential (Syvertsen and Levy 1982). Búrquez (1987) and Seeling *et al.* (2008) found the same result that is a strong correlation between leaf thickness and relative water content.

The root/shoot ratio increased from 0.5 to 1.5 for olive seedlings grown without and with *C. dactylon*, respectively. This increase of root/shoot ratio of olive seedling associated without *C. dactylon* came from a higher increase in both shoot fresh or dry weight of olive seedlings with *C. dactylon*. Harris (1992) indicated that a decrease in soil fertility was associated with an increase in the root shoot ratio; that is, root growth increased more in weight than shoot growth. This increased proportion of roots relative to the shoot would be caused by water and nutrient deficiencies (Kuchenbuch *et al.* 1988; Bonifas *et al.* 2005). In dry sites species tend to have higher root/shoot ratios than those in messic

Table 4 Growth analysis of olive seedlings.*

	Without Cynodon	With *Cynodon*
LAR	32.000 ± 1.200 a	14.500 ± 1.600 b
LAI	2.100 ± 0.200 a	0.400 ± 0.250 b
SLA (LA/LDW)	87.600 ± 2.400 a	64.300 ± 3.400 b
LDMC (LDW/LFW)	0.400 ± 0.100 a	0.560 ± 0.090 b
LWR	0.300 ± 0.010 a	0.200 ± 0.011 b
SWR	0.400 ± 0.050 a	0.300 ± 0.056 b
RWR	0.200 ± 0.100 a	0.400 ± 0.980 b
Root/shoot ratio	0.500 ± 0.100 a	1.500 ± 0.950 b
Leaf area/shoot diameter	148.000 ± 3.400 a	100.100 ± 3.240 b
Leaf area/shoot length	6.500 ± 0.800 a	3.000 ± 0.790 b
Leaf weight/root weight	1.500 ± 0.200 a	1.500 ± 0.250 a
Leaf thickness (LA/FW)	0.400 ± 0.060 a	0.560 ± 0.075 b
Unit leaf area	0.011 ± 0.001 a	0.015 ± 0.002 b

*Values within the same row followed by the same letter are not significantly different (P=0.05) according to the LSD test.

Table 5 Anatomical indices.* (Relative Water Content)

	Without *Cynodon*	With *Cynodon*
Leaf (FW/DW)	2.4 ± 0.2 a	1.7 ± 0.3 b
Shoot	2.3 ± 0.1 a	1.8 ± 0.2 b
Root	2.8 ± 0.2 a	1.6 ± 0.3 b
Whole plant	2.5 ± 0.1 a	1.7 ± 0.4 b

*Values within the same row followed by the same letter are not significantly different (P=0.05) according to the LSD test.

Table 6 Relative transpiration and water flow rates.*

	Without *Cynodon*	With *Cynodon*
Leaf	0.58 ± 0.11 a	0.44 ± 0.10 b
shoot	0.58 ± 0.13 a	0.45 ± 0.12 b
Root	0.63 ± 0.12 a	0.40 ± 0.11 b
Whole	0.60 ± 0.10 a	0.43 ± 0.12 b
Water flow rates	1.50 ± 0.12 a	0.60 ± 0.10 b

*Values within the same row followed by the same letter are not significantly different (P=0.05) according to the LSD test.

and hydric areas (Monk 1966; Koocheki *et al*. 2008).

The increase in root/shoot ratio increased the transpiration rate per unit leaf area. This ratio is one of the most important factors contributing to plants with a larger root system that will explore a larger volume of soil and therefore will have removed more total water when the wilting coefficient is reached (Parker 1956; Ashraf *et al*. 2009). That drought increases the ratio of root to top has long been known (Maximov 1929; Syvertsen and Hanlon 2008). It has found that if this relative increase in root system is taken into account, the total DW of plants grown under drought conditions may actually exceeded that of similar ones grown with full moisture, though the above-ground part is considerably reduced in size. Consequently, more water can be delivered per unit leaf area, leading to a higher transpiration rate (**Table 6**). Evidence of the relation between photosynthesis and transpiration is the parallelism between growth and transpiration rate (Robelin 1967; Omami 2005). This relative rate, in fact provides a useful growth index. The marked reduction of the relative rate of water flow indicated that the seedlings with *C. dactylon* showed a difficulty in supplying water to their upper parts. The major significance of reduction in leaf size is the reduced functional resistance to flow of water (Xu *et al*. 2008). This follows from the modification of the anatomical structure of olive seedlings (**Table 5**). The vacuolated nature of most plant cells makes the ratio of FW a valuable anatomical index. Tissue water content may vary widely depending on the environment in which trees olive seedlings grow. The presence of *C. dactylon* influenced olive tree seedling tissue moisture, since the anatomical indices were significantly different (**Table 5**). Since the tissue water content was reduced by the presence of *C. dactylon*, it probably modified olive tree seedling cell structure by making the cell vacuoles smaller (Brian *et al.* 1999). The competition of *C. dactylon* changed the olive tree seedling's morphogenesis during water stress (Dimitrina *et al*. 2002). Studies have reported the effects of competition on plant growth and development leading to changes in their architecture (Varlet *et al.* 1993; Fourcaud *et al*. 2008). The use of growth analysis could particularly useful to explain the interference of a weed with crops.

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