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Effect of Low Temperature on Purple Nutsedge (*Cyperus rotundus*) Reproductive Biology

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Purple nutsedge is considered to be the worst weed in the tropical and subtropical regions of the world. Although the plant is a low grower it has very strong competitive abilities. The influence of initial tuber size and cold treatment on tuber sprouting, accumulation of plant biomass and new tubers formation was studied. Tubers sprouted continuously over 30 to 50 d with significantly lower sprouting ability of small tubers (0.1 to 0.2 g). Short cold treatment (4 C for 4 d) significantly stimulated sprouting process. The early sprouting of cold treated tubers led to increased number of shoots and inflorescences and therefore more intensive biomass accumulation, as well as more intensive formation of new tubers. The increase in total biomass accumulation raises the reproductive and spreading potential of the weed.

Nomenclature: Purple Nutsedge, *Cyperus rotundus* L.

Key words: Sprouting, vegetative reproduction, biomass accumulation, tuber formation, cold treatment.

Purple nutsedge is considered to be the most damaging weed in tropical and subtropical regions of the world (Bryson et al. 1994; Gupta et al. 2002; Horowitz 1972). Worldwide, purple nutsedge is a serious weed of rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.), cotton (*Gossypium* spp.), corn (*Zea mays* L.) and vegetables. In the continental United States, purple nutsedge is the major weed of peanut (*Arachis hypogaea* L.), soybean [*Glycine max* (L.) Merr.], cotton, sugarcane, turfgrass, vegetables and strawberry (Kadir and Charudattan 2000). This weed reduces yield and quality and interferes with pesticide applications and harvest operations (Grichar and Sestak 2000; Inglis et al. 2001; Wilcut et al. 1993). Although the weed is a low grower, it has strong competitive abilities (Bryson et al. 1994; Horowitz 1972; Inglis et al. 2001; Williams 1982). Purple nutsedge is tolerant of wet soil and high temperature, and its ability to convert CO_2 into carbohydrate via both C_4 and C_3 pathways is extremely efficient (Bendixen and Nandihalli 1987). The high propagation potential and competitive capacity of its vegetative reproduction by rhizomes and tubers explains its aggressiveness and worldwide distribution, and impedes its control (Bendixen and Nandihalli 1987; Wills and Briscoe 1970). Purple nutsedge has become a major weed problem worldwide as a result of the use of selective herbicides that do not control it, use of monoculture that reduces considerably the number of selective herbicides that may be used, lack of crop rotations, and reduced use of hand weeding (Glaze 1987; Kadir and Charudattan 2000).

Purple nutsedge produces numerous seeds but only few survive in the soil; of those, only 5% germinate, and the seedlings have inadequate vigor (Justice and Whitehead 1946; Okoli et al. 1996; Stoller 1973). In most cases, purple nutsedge infestation starts with tubers, which are the driving force for the weed's propagation and dissemination (Horowitz 1965; Wills 1987; Wills and Briscoe 1970). In a pot experiment, one tuber produced 550 tubers and 356 shoots after 4 months and in a field experiment one tuber produced about 1200 tubers within 3 months of planting, with a longevity of about 17 months (Gilreath and Santos 2004). Moreover, the presence of shoots and tubers affects crop yields through the production of allelopathic substances and via competition for moisture, nutrients and space (Kadir and Charudattan 2000). The weed's rapid growth and development confer high survival ability under the stressful conditions prevailing in agricultural fields (Williams 1982). Previous studies have indicated that purple nutsedge can grow actively under continuous foliage removing (Santos et al. 1997a). Tubers accumulate reserves for further sprouting, therefore tuber weight may define regrowth potential for new shoots.

Although light is not a requirement for sprouting, it does promote tuber sprouting (Nishimoto 2001). Light deficiency may be one of the limiting factors for purple nutsedge which has higher light compensation point than yellow nutsedge (Santos et al. 1997b). Biomass partitioning to purple nutsedge tubers was decreased under high shade conditions without increases in partitioning to the shoots. But in tropical areas, where purple nutsedge is generally distributed, light intensity is extremely high and light could not be taken into consideration as a limiting factor. Also oxygen and moisture levels in the soil may be limiting factors for sprouting, but the main factor regulating the process is temperature (Lati et al. 2011; Nishimoto 2001). Nishimoto (2001) indicated that no sprouting occurs below 10 C or above 45 C, and maximal sprouting is achieved under constant temperatures between 25 and 35 C. A daily short duration of high temperature in his experiments was found to increase sprouting to nearly 100% of the tubers. However, most buds did not elongate if the tuber remained at 20 C. Daily fluctuations in soil temperature are probably a major signal for purple nutsedge emergence (Horowitz 1972; Kamabata and Nishimoto 2003; Sun and Nishimoto 1999; Webster 2003), and its distribution is limited by its sensitivity to cold temperature (Glaze 1987; Okoli et al. 1996). Purple nutsedge tubers exposed to 2 C for 3 months lost their ability to germinate (Stoller 1973). However, information on the positive influence of exposing the tubers to a short period of low temperature on sprouting and development is lacking. Data on the influence of tuber size on their resprouting potential and propagation, as well as their reaction to short cold treatment, will shed light on the biology of the weed and in the long run may aid in the development of strategies for its management.

Materials and Methods

Plant Material. Fresh purple nutsedge tubers were collected from a field at the Newe Ya'ar Research Center located in the

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Jezreel Valley in the northern part of Israel (32°42′2″N, 35°10′47″E).

Experimental Design. Tubers were divided into 19 biomass groups from 0.1 to 0.2 g up to 1.9 to 2.0 g in 0.1 g increments with 40 tubers in each group. Twenty tubers from each group were kept for 4 d at 4 C while the other 20 tubers were left at room temperature. Immediately after the cold treatments, each tuber was planted in a pot (15 cm diam, 1.5 L) containing Newe Ya'ar soil (medium-heavy clay–loam soil containing, on a dry weight basis, 55% clay, 23% silt, 20% sand, 2% organic matter, pH 7.1). The pots were placed in a nethouse with a temperature range of 15 C to 30 C and natural photoperiod (12 to 14 h d /12 to 10 h night) for the 15 wk of the experiment. Shoots and inflorescences were counted on a daily basis. At the end of the experiment, the foliage was cut at the soil surface and its dry weight was determined. The soil from the pots was washed under tap water onto a metal net (3 by 3 mm) and the tubers and roots on the net were washed gently, and root dry weight and number and weight of newly formed tubers were evaluated.

Statistical Analysis. The results were subjected to ANOVA by means of JMP Software, version 5.0 (SAS Institute Inc., Cary, NC, USA). Data were compared by least-significant differences (LSD), on the basis of Tukey–Kramer Honestly Significant Difference test ($\alpha = 0.05$), except data on number and weight of new formed tubers, which were compared by LS Means Contrast test ($\alpha = 0.05$). To meet the assumption on ANOVA, percentage data were arcsine-transformed before analysis. On the graphs, back-transformed means are presented. Data of tuber sprouting of various tuber weight groups were compared by Fisher's *t*-test. Groups with no differences were combined together.

The experiment was conducted twice with 20 replicates in each experiment. Comparison of the two experiments was performed using Fisher's *t*-test in order to combine data with homogenous variances. Data of the two experiments on shoot and root biomass, number and weight of new formed tubers were combined. Data of the two experiments on accumulated tuber sprouting and accumulated number of shoots and inflorescences were not combined because of heterogeneity of variances.

Results and Discussion

Tuber Sprouting. Data of the two experiments were not combined because of heterogeneity of variances. In experiment 1, sprouting of nontreated tubers lasted 45 d (Figure 1a). First shoots appeared above soil level after 4 d, but tuber sprouting reached its maximum level after 30 to 45 d. Sprouting of the smallest tubers (0.1 to 0.2 g) was significantly lower than in the other weight categories. By the end of the experiment, only about 65% of the smallest tubers had sprouted, whereas sprouting rate in the other weight groups varied between 85% (0.3 to 0.5 g) and 98 to 99% (0.5 to 2.0 g). The biggest tubers, weighting 1.7 g and more, sprouted more slowly than the others. In experiment 2, tubers sprouted a little bit slower, probably because of the difference in the temperature. First shoots in this experiment appeared above soil level after 6 d, and tuber sprouting reached its maximum level after 35 to 50 d. Except for this difference, all other data on the influence of tuber size on sprouting were similar to those in experiment 1 (data not shown).

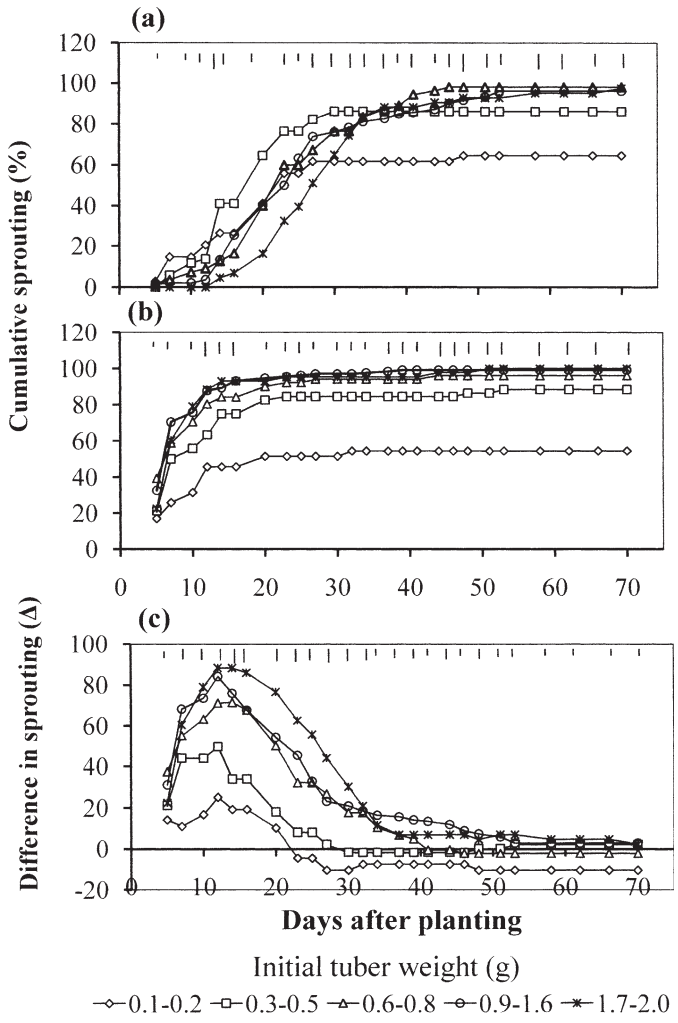

Figure 1. Effect of cold treatment on sprouting of purple nutsedge tubers with different initial biomass. (a) Sprouting of nontreated tubers. (b) Sprouting of cold-treated tubers. (c) Difference (Δ) in sprouting rate between cold-treated and nontreated tubers. Vertical lines indicate least-significant differences (LSD) for specific dates at $\alpha = 0.05$.

Cold treatment did not significantly influence the percentage of sprouted tubers. Sprouting levels of all tuber weight groups were similar to those of their nontreated counterparts. However, the cold-treated tubers sprouted more rapidly, reaching maximum sprouting levels after 14 to 20 d in experiment 1 (Figure 1b) and after 16 to 24 d in experiment 2 (data not shown). Sprouting of the cold-treated tubers was positively correlated with their biomass. In experiment 1, the difference in sprouting level between cold-treated and nontreated tubers 2 wk after planting reached 85% for the big tubers (0.9 to 1.6 g and 1.7 to 2 g), while for the small tubers (0.1 to 0.2 g and 0.3 to 0.5 g), the difference did not exceed 25 and 45%, respectively (Figure 1c). Similar data were obtained in experiment 2 (data not shown).

Early sprouting led to increased shoot number for big (1.7 to 2.0 g) and intermediate (0.6 to 1.6 g) cold-treated tubers relative to nontreated tubers (Figure 2a). In experiment 1, tubers with a weight of 1.7 to 2.0 g produced two additional shoots compared to their nontreated counterparts, and tubers weighing 0.9 to 1.6 g and 0.6 to 0.8 g had 1.5 and 1.0

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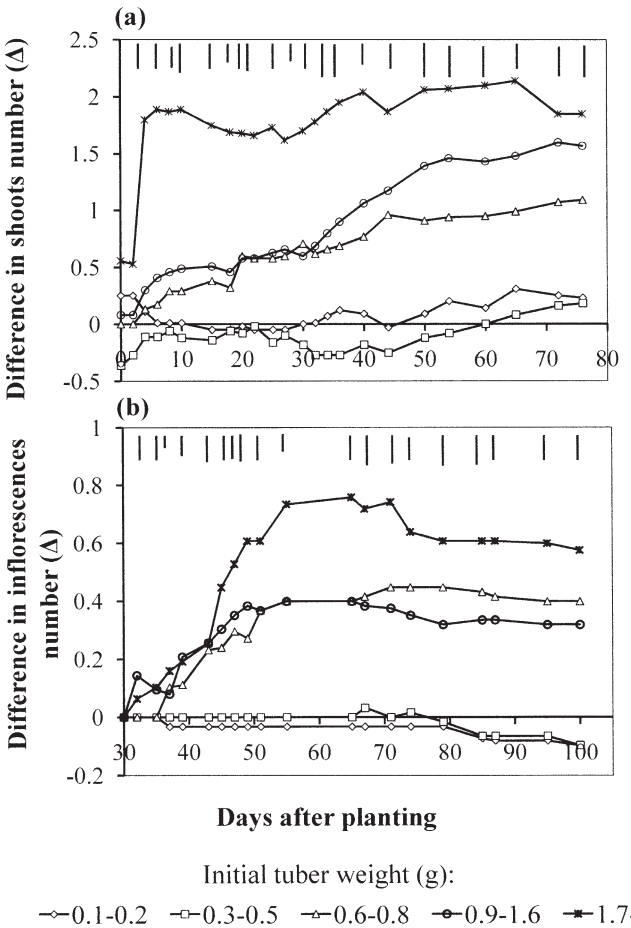

Figure 2. Effect of cold treatment of purple nutsedge tubers with different initial biomass on formation of shoots and inflorescences. The difference (Δ) between the numbers of (a) shoots of cold-treated and nontreated tubers, (b) inflorescences of cold-treated and nontreated tubers. Vertical lines indicate least-significant differences (LSD) at $\alpha = 0.05$.

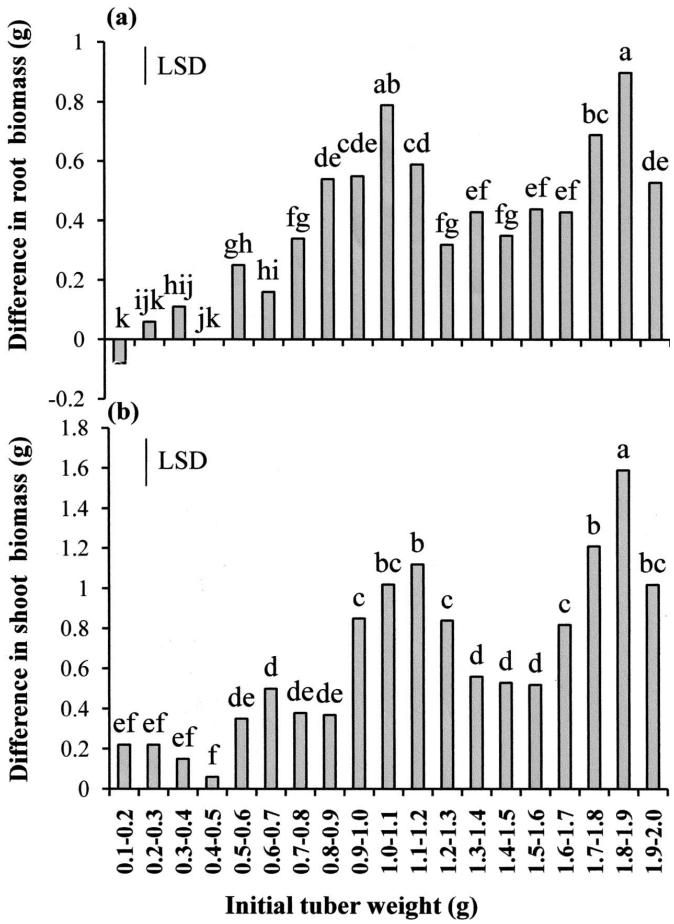

Figure 3. Effect of cold treatment of purple nutsedge tubers with different initial biomass on root and shoot biomass. Different letters indicate significant differences according to Tukey-Kramer Honestly Significant Difference test at $\alpha = 0.05$. Vertical lines indicate LSD.

additional shoots, respectively. Small cold-treated tubers weighting less than 0.5 g did not show any increase in the number of shoots. In experiment 2, the number of shoots after cold treatment increased by 2.5 for tubers with a weight of 1.7 to 2.0 g, and by 1.3 and 0.8 shoots for tubers with a weight of 0.9 to 1.6 and 0.6 to 0.8, respectively. As in experiment 1, small tubers (less than 0.5 g) did not show any increase in the number of shoots.

Similar results were obtained for inflorescences (Figure 2b). In both experiments intermediate-sized (0.6 to 0.8 g and 0.9–1.6 g) cold-treated tubers formed 0.5 more flowering shoots, and big tubers (1.7–2.0 g) formed 0.8 more flowering shoots than untreated tubers in the corresponding weight groups. Flowering of plants from small tubers was not affected by the cold treatment.

Dorado et al. (2009) monitored seedling emergence over two years in corn fields. Purple nutsedge emergence started early and continued throughout the corn life-cycle. The results obtained in his experiments revealed a high correlation between sprouting rate and accumulated temperature (GDD). In our experiments, a short cold treatment significantly stimulated the sprouting process. This phenomenon might be explained by the activation of starch breakdown and accumulation of sucrose and fructose, factors that are known to break tuber dormancy (Zhang et al. 2011). Travlos et al. (2009) reported that sprouting rate and total tuber sprouting increased significantly after a shift of daily temperature fluctuation from 0 to 12 C and was correlated to the depth from which the tubers were dug up. A significantly higher sprouting level of tubers from the 5 upper cm of the soil layer was observed as compared with tubers obtained from the 5- to 15-cm layer, for all temperature treatments. Pena-Fronteras et al. (2009) explained this phenomenon by the differences in tuber size in the shallower and deeper soil layers.

Shoot and Root Biomass. Unexpectedly, shoot and root biomass were not directly correlated with initial tuber weight (Figure 3a,b). Tuber weight groups 0.5 to 1.2 g and 1.7 to 2.0 g produced high levels of shoot biomass. No correlation between initial tuber weight and root biomass was found.

Cold treatment significantly increased shoots and root biomass compared to their nontreated counterparts. This difference was higher for tubers with a biomass of 0.5 g and above.

Tuber size was also a dominant factor in Santos et al. (1997a) study which reported that tubers in the two heaviest weight categories (0.75 and 1.0 g) had greater sprouting rate than the two smallest categories (0.25 and 0.5 g) as shown by their larger shoot biomass after single foliage removal and the absence of tuber depletion after multiple (up to seven) removals. Our experiments indicated that most of the very

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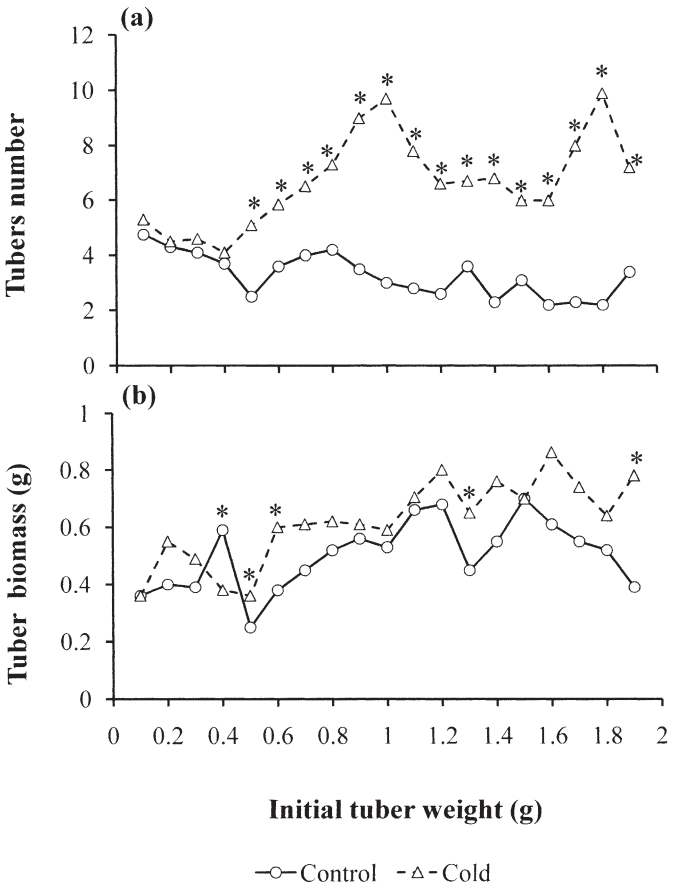

Figure 4. Effect of cold treatment of purple nutsedge tubers with different initial biomass on number (a) and biomass (b) of newly formed tubers. * Indicates significant differences between control and cold treatment according to MS means contrast test at $\alpha = 0.05$.

small tubers, with biomass ranging from 0.1 g to 0.2 g, were able to sprout, to a level of about 65%. Tubers with a biomass of 0.2 g and higher had sprouting levels of 85 to 100%. Geographical origin, depth in the soil, duration of the period between the actual start of the experiment and the date the tubers are dug up from the soil, preservation conditions and the conditions prevailing during the experiment might partially explain the different results obtained in these studies.

Formation of New Tubers. About 2.5 to 4.5 new tubers were produced by each plant regardless of the initial weight of the mother tuber (Figure 4a). Cold treatment had no influence on the number of newly formed tubers by plants originating from tubers with biomass less than 0.5 g; however, it significantly increased (up to fivefold) the number of new tubers formed by plants originating from tubers with larger biomass. Cold treatment did not influence the average biomass of newly formed tubers (Figure 4b). However, as a consequence of the higher number of tubers produced by cold-treated tubers, the total tuber biomass per plant was significantly higher.

The higher sprouting rate of cold-treated tubers led to the formation of an increased number of shoots and inflorescences, resulting in greater biomass and new tuber production. This increased number of newly formed tubers increases the reproductive, dissemination and infestation potential of the plant. Understanding this potential for enhanced reproductive ability may contribute to the development of purple nutsedge management systems. For example, Dodet et al. (2008) examined the influence of emergence date on the growth and development of yellow nutsedge. They have found that total shoot production by tubers sprouting in July compared to tubers sprouting in May, was not compensated during 2 years. Therefore for agricultural practice it was recommended to avoid periods without crops between April and October, that delay weed emergence.

This study contributed a more thorough understanding of the biology of purple nutsedge and the factors governing its development and reproductive cycle. Knowledge of this aspect (cold treatment) may serve as a tool and a trigger for studies aiming to differentiate between the aggressiveness of purple nutsedge in the tropics where temperatures never drop below 18 C as opposed to the subtropics where winter temperatures may frequently get to 6 C and lower. In the long run, it may provide means to improve integrated management systems. As with many noxious weeds for which no single effective control method exists, an integrated approach is preferred.

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