FIELD PERFORMANCE OF _FRAXINUS ORNUS_ BAREROOT PLANTS TO DROUGHT STRESS

Rahman M.S., Tsitsoni T., Tsakaldimi M*. , Ganatsas P.

*Corresponding author: marian@for.auth.gr


Abstract: In this study an experiment was conducted to find out the influence of drought on _Fraxinus ornus_ plant morphology, biomass allocation, architecture of first-order lateral roots (FOLR) and field survival at the early stages of outplanting. Totally 120 undercut bareroot plants were transplanted to field conditions in a Mediterranean area. After establishment, half of the seedlings were kept well-watered (control) while the rest received no other irrigation except natural rainfall (water stress). In the field, measurements were taken after 6 months (before summer), after 8 months (midsummer) and after 10 months (after summer). The results showed that the root biomass, the seedling quality index and the number of first order lateral roots were significantly greater in watered seedlings. The mean length of FOLR increased over time in watered and stressed seedlings as well. However, after the summer period the stressed seedlings had also significantly lower length of FOLR than watered ones. Similarly, the leaf RWC was highly affected by the treatments applied and was found significantly greater in water seedlings during the summer. The first summer period after outplanting was crucial for stressed and watered seedlings as well. Eight months after outplanting the survival rate of stressed seedlings was 45% while that of well-watered seedlings was 75%. There is much evidence for the positive relationship between root biomass, seedling quality index and number and length of FOLR with seedlings survival.

**Keywords:** biomass, _Fraxinus ornus_, Mediterranean, root architecture, survival, water stress.

INTRODUCTION

Seedling establishment in the field is very important because in this stage, rate of failure is high in many species (Tsakaldimi et al. 2013). Seedlings of many species die due to various causes such as high or low temperature, transplanting shock, low soil moisture, competition, allelopathy, etc. Especially, in Mediterranean areas, the planting stock is very drought sensitive and the establishment process is often limited to irregular rainfall (Holmgren and Scheffer 2001; Valdecantos et al. 2006). Thus, it is important to assess drought responses of whole seedling i.e. survival and growth under water stress compared
to well-watered individuals. The quick root growth and the survival of a seedling mainly depend on how its root system is at the time of outplanting. To use bareroot seedlings, sufficient root systems along with large first order lateral roots (FOLR) that grow from the central root should be ensured in order to achieve high field performance (Thompson and Schultz 1995). After field planting, seedlings are generally exposed to extreme temperature, wind, drought, low humidity and other adverse conditions. Under such conditions, transpiration of the seedlings occurs very rapidly but the seedlings do not get enough soil moisture. As a result, seedlings get transplanting shock and their establishment and survival are encountered serious problem (Zaady and Perevolotsky 1995; Ffolliott et al. 2003).

In Mediterranean climates the severe drought period lasts from June to September and during these months the temperature is very high. This post-planting period negatively affects the success of seedling field establishment (Ganatsas et al. 2012). However, the degree of tolerance to drought varies from species to species and this variation may be helpful to choose species for planting in the drought prone areas (Gindaba et al. 2004). The establishment of seedlings in the field depends on their morphological and physiological characteristics, and the environmental conditions of the planting site (Puttonen 1996, Tsakaldimi et al. 2013). There are various morphological and physiological methods to test the seedling quality before and after field plantation (Mattsson 1997). Among them, measurements of shoot and root morphological growth parameters are easily and commonly used to determine the seedling quality (Rose et al. 1990, Puttonen 1996, Tsakaldimi et al. 2013). The study of root morphology is necessary to analyze the response of the seedling in the water stress condition. Also, leaf relative water content has been used to measure the plant water condition in terms of cellular hydration under the possible effect of leaf water potential and osmotic adjustment (Kramer and Boyer 1995, Gindaba et al. 2004). Though many studies were conducted in the field condition, those were mainly on above ground morphology or physiology (e.g. Roy et al. 2001, Jacobs et al. 2005, Wilson et al. 2007). Few studies were reported on morphology and architecture of the whole seedlings including root systems in relation to field performance (e.g. Dey and Parker 1997, Martínez-Sánchez et al. 2003, Tsakaldimi et al. 2009, Tsakaldimi et al. 2013) and even less in relation to water stress (Padilla and Pugnaire 2007, Zida et al. 2008). Such studies are helpful to find out promising species for reforestation as well as restoration programs in Mediterranean areas where water deficit is common.

The present study was undertaken to find out the influence of drought on Fraxinus ornus L. (Manna Ash) plant morphology, biomass allocation, architecture of first-order lateral roots (FOLR), and field survival at the early stages of outplanting. Moreover, seasonal variation of soil available moisture was also evaluated.

MATERIALS AND METHODS

Fraxinus ornus L. (Oleaceae family) is a plant species native to southern Europe and southwestern Asia, from Spain and Italy north to Austria, Poland and the Czech Republic, and east through the Balkans, Turkey, and western Syria to the Lebanon. This
species selected as it is known that it has not been affected much from climate changes and it possess tolerance against drought (Yücedag and Sen 2008). Three years old bareroot seedlings of Fraxinus ornus were lifted from an open-air nursery of the Forest Service Department of Thessaloniki, northern Greece. After lifting, the root systems of the seedlings were undercut. Thirty undercut seedlings were taken randomly (Zida et al. 2008) for initial morphology and biomass measurements. The field experiment was conducted at the Arboretum of the Lab. of Silviculture, AUTH, Greece (longitude 23°08′13.23″ E, latitude 40°30′50″ N, altitude 123 m) on November 2011. The climate of the area is typical Mediterranean with hot dry summers and cool winters. In 2011 and 2012, mean annual precipitation was 484 mm and 498 mm respectively, while the days of rainfall were totally 91 and 96 respectively. There was no rain in July 2011 and there was only one day of rainfall in June 2012. The climatic data were derived from the weather station (Forest Research Institute, Vasilika, northern Greece) near the experimental field.

The experiment was laid out as a randomized complete block design with two treatments and three replications; 20 seedlings of same age and similar size x 3 replications x 2 treatments (120 seedlings totally) were outplanted in pits (0.3 m x 0.3 m). After establishment, half of the seedlings were kept well-watered (control) where the soil was maintained at field capacity, by regular irrigation which was applied daily, while the rest received no other irrigation except natural rainfall (water stress). Planting distance was 2m x 2m. Buffer zones of 3m were created to separate the experimental units. All seedlings were hand-planted with planting shovel and spade and no fertilization was applied. To hold rain water, a boundary of soil was made around the seedling of a pit. The spot weeding was done around the seedlings when needed. Field measurements were taken 6 months after planting (before summer), 8 months (midsummer) and 10 months (after summer). Leaf relative water content (RWC) was determined on 10 discs (4 mm size) per treatment from the leaves similar in age and orientation. All leaf disk samples were taken at the noon on uppermost fully expanded leaves from three seedlings (one from each replication) of each treatment. Then they put into polythene bags and they were transferred to the laboratory inside a mini refrigeractor. The leaf RWC was calculated according to Iannucci et al. (2000). For each harvest period, 12 seedlings (4 seedlings x 3 replications) per treatment were randomly chosen and excavated for the root morphology by the total excavation method (Martínez-Sánchez et al. 2003, Tsakaldimi et al. 2009), shoot morphology and biomass measurements. Before excavation, sufficient water was poured around the seedling to loose and wet the soil. In the laboratory, roots and soil were repeatedly submerged in water. A sieve was used to collect any root fragments detached from the system. Then seedlings with their whole root system were kept in polythene bags and stored in the fridge for further measurements. Seedling parameters measured were: height, root collar diameter, sturdiness index (H/D), length of central root, number of living branches, shoot and root dry weight and seedling quality index (QI) calculated using the equation (Dickson et al. 1960): QI=total seedling dry weight (g)/[height (cm)/diameter (mm)+shoot dry weight (g)/root dry weight (g)]. Also, the first-order lateral roots (FOLR, d ≥ 1 mm) were counted and their length and diameter were measured (Dey and Parker 1997). Then, each FOLR was categorized into five different diameter classes D1 (1-2 mm), D2 (2-3 mm), D3 (3-4 mm), D4 (4-5 mm) and D5 (>5 mm).
Survival rate of seedlings was recorded during 17 months after transplantation. After 10 months in the field, the plants of both treatments were left in same natural environment without irrigation. In addition, from June to September 2012, soil samples were collected from 3 points of equal distance from the centre of each replication and from three different soil depths viz. 0-20 cm, 20-40 cm, and 40-60 cm, in order to measure the available soil moisture in each treatment. Soil moisture was calculated as follows: soil available moisture % = (B1-B2)/B2 x 100, where B1: the initial soil weight and B2: oven-dried soil weight at 105° C. All statistics were calculated by SPSS software. Distribution was tested for normality by Kolmogorov–Smirnov criterion and the homogeneity of variances was tested by Levene’s test. Two samples Independent t-test was conducted to compare the means of seedlings parameters.

RESULTS AND DISCUSSION

Plant morphological characteristics

Shoot height of *F. ornus* decreased over time and it did not vary significantly among survived seedlings of control and water stress treatment in the field (Table 1). This happened due to the shoot top drying which was observed during the summer months, while the stressed seedlings showed more shoot drying than watered ones. Probably, the survived seedlings started to be adapted in the field by reducing their above ground transpiration area. Planting seedlings with great height and transpiration area may be a disadvantage for dry sites that leads to poor field growth or death (Haase 2007). Some shoots of seedlings completely died back and new coppice shoots grew from the soil level. Dey and Parker (1997) also reported partial shoot dieback of bareroot red oak species, followed by less height and formation of lateral shoots after planting in dry sites. Similarly, Schultz and Thompson (1997) studied undercut bareroot seedlings and found that height and diameter growth was hampered by stem dieback on the largest seedlings. Root collar diameter of seedlings was also not affected by drought. Similarly, the sturdiness ratio (H/D) did not vary significantly between the two water regimes even though it was found slightly lower in stressed seedlings. Roller (1977) found that black spruce seedlings with high sturdiness quotients were more susceptible to damage from wind, drought, and frost exposure.

The number of branches did not increase during six months after outplanting. New branches appeared after 8 months in both water regimes and they significantly varied due to water stress treatment. The central root, six months after outplanting, was 5 cm longer than the initial central root but this difference was not statistically significant. After applying the water stress treatment, the central root length of stressed seedlings was significantly shorter than that of control ones. However, two months later, alive stressed seedlings increased their central root more (104.9%) to become statistically similar with those of control ones. Gazal et al. (2004) reported longer tap roots of *Pterocarpus indicus* seedlings at lower moisture levels (75% and 50% of field capacity) instead of 100% field capacity.
Table 1. Morphological characteristics of shoot and root of *Fraxinus ornus* seedlings at two water regimes during 10 months in the field.

<table>
<thead>
<tr>
<th>Months after outplanting</th>
<th>Treatment</th>
<th>Shoot height: H (cm)</th>
<th>Root collar diameter: D (mm)</th>
<th>Number of branches</th>
<th>Sturdiness ratio (H/D)</th>
<th>Length of central root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>177.1±5.0&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>11.1±0.2&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.4±0.3&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>16.2±0.5&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>18.6±0.9&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 -</td>
<td></td>
<td>174.4±4.6&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>11.5±0.2&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.5±0.3&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>15.3±0.3&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>23.8±4.0&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 -</td>
<td></td>
<td>162.7±8.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>12.4±0.6&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.4±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.0±0.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>36.0±3.3&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>8 Control</td>
<td></td>
<td>143.8±11.1&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>12.0±0.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>3.4±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.4±0.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>20.6±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 Control</td>
<td></td>
<td>161.9±8.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>12.2±0.6&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.9±0.8&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>13.7±0.6&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>43.3±6.1&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 Stressed</td>
<td></td>
<td>142.4±14&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>10.9±0.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>3.4±0.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>13.1±0.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>42.1±4.1&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± S.E. Different lower case letters (a, b) show significant differences (P < 0.05) between 0 and 6 months or between the two treatments within the same time and column. ns: non significant.

**Biomass allocation**

The shoot biomass of seedlings was not affected by drought as a result of the shoot die back and the new leaves development in the lower part of shoot, which were common in seedlings of both treatments (Table 2). On the contrary, the stressed seedlings presented significantly lower root biomass than control ones. However, the shoot/root ratios that show the balance between the transpiration area and the water absorbing area of the seedlings (Hermann 1964, Haase 2007) were found statistically similar between watered and stressed seedlings. This may be resulted from the fact that while the roots of stressed seedlings dried and became inactive, on the other hand the shoot’s dieback and leaves’ damage due to excessive sunlight, in both treatments, increased. According to the values of Dickson quality index, the drought significantly affected the quality of survived bare-rooted seedlings. After applying the water stress treatment (8 and 10 months after outplanting), the stressed seedlings showed significantly lower quality than watered ones. It is evident that the lower root dry weight has contributed to this reduction.

Table 2. Biomass allocation and seedling quality index of *F. ornus* seedlings in different times and water regimes in the field.

<table>
<thead>
<tr>
<th>Months after outplanting</th>
<th>Treatment</th>
<th>Shoot dry weight (g)</th>
<th>Root dry weight (g)</th>
<th>Total biomass (g)</th>
<th>Shoot/root ratio</th>
<th>Quality Index (QI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17.3±0.8b</td>
<td>11.8±0.8b</td>
<td>29.1±1.4b</td>
<td>1.6±0.0b</td>
<td>2.6±0.2b</td>
</tr>
<tr>
<td>0 -</td>
<td></td>
<td>88.7±31.0a</td>
<td>31.7±10.2a</td>
<td>120.4±40.8a</td>
<td>2.7±0.3a</td>
<td>8.2±3.2a</td>
</tr>
<tr>
<td>6 -</td>
<td>Control</td>
<td>113.4±16.1ns</td>
<td>51.6±7.4a</td>
<td>165.0±23.1a</td>
<td>2.3±0.1ns</td>
<td>11.8±1.4a</td>
</tr>
<tr>
<td>8 Stressed</td>
<td></td>
<td>71.1±13.7ns</td>
<td>28.2±4.8b</td>
<td>99.3±17.5b</td>
<td>2.6±0.3ns</td>
<td>7.0±1.2b</td>
</tr>
<tr>
<td>10 Control</td>
<td></td>
<td>109.3±20.3ns</td>
<td>49.1±8.1a</td>
<td>158.3±27.9ns</td>
<td>2.3±0.2ns</td>
<td>10.0±1.8a</td>
</tr>
<tr>
<td>10 Stressed</td>
<td></td>
<td>69.6±14.3ns</td>
<td>29.2±5.6b</td>
<td>98.8±19.7ns</td>
<td>2.4±0.2ns</td>
<td>6.6±1.4b</td>
</tr>
</tbody>
</table>

Values are means ± S.E. Different lower case letters (a, b) show significant differences (P < 0.05) between 0 and 6 months or between the two treatments within the same time and column. ns: non significant.
Architecture of FOLR

Many authors argue that FOLR could be one of the best field predictors of field response and competitive capacity of planted seedlings (e.g. Teclaw and Isebrands 1993, Kormanik et al. 1995, Jacobs et al. 2005, Grossnickle 2005, Tsakaldimi et al. 2013). These roots provide the structural framework of the root system and are active in water and nutrient uptake. When irrigation was withdrawn and air temperature was increasing, mean number of FOLR increased significantly in control seedlings at 8 and 10 months after transplantation compared to stressed seedlings, although the latter showed a tendency to increase their number of FOLR (Figure 1). Diameter class-wise analysis showed that the number of FOLR was greater at the lower diameter classes than at the higher diameter classes (Figure 2) and this trend was observed both in watered and stressed seedlings. This may be attributed to the increase of the newly grown (thinner) roots. Mean length of first order lateral roots increased over time (Figure 3A). Although watered and stressed seedlings showed statistically similar mean length of FOLR after 8 months, 2 months later watered seedlings had significantly longer FOLR than stressed ones. Interestingly the mean diameter of FOLR decreased from 8 months to 10 months period in both water regimes (Figure 3B). This probably happened because the old roots gradually dried and the new ones (thinner) started to grow. The increase of the length of FOLR in expense of their diameters especially for the stressed seedlings may be one the adaptive mechanisms of these seedlings in dry Mediterranean site.

Figure 1. Total number of FOLR (diameter ≥1mm) per plant in two water regimes and at different time period after field transplanting.

Figure 2. Number of FOLR per plant according to five diameter classes in two water regimes after 8 months (A) and 10 months (B).
Leaf Relative water content (RWC)

The leaf RWC of plants was significantly affected due to the drought treatment during the summer period (June and July 2012). Thus, control (watered) seedlings had significantly higher RWC than stressed ones in all studied dates (Figure 4). However, even within the treatment, the RWC varied with the time because of the soil moisture and temperature fluctuation. In stressed plants, the lack of water caused a marked dehydration of the leaves. Similar results were reported by Rahman et al. (2013) for Cercis siliquastrum bareroot seedlings. Medrano et al. (1992) also reported low values of water parameters in plants subjected to drought. The maintenance of high leaf RWC has often been suggested as an indicator of drought resistance mechanism in seedlings under dry soils (Iannucci et al. 2000). Also, the ability of plant to recover rapidly after stress condition can be related to its physiological tolerance to drought (Ludlow and Muchow 1990).

Soil moisture fluctuation

The applied treatments greatly affected soil moisture; thus, the soil moisture content of control treatment was higher in all dates compared to the stressed treatment,
due to additional water given to control (Table 3). As a consequence the soil moisture content in stressed plots was found low (almost less than 8%) for the whole summer period. The lowest value was observed on 30\textsuperscript{th} July 2012 (5.56% in a depth of 0-20 cm), attributed to high temperatures and prolonged dry period. The soil moisture content in the control plots ranged between 19-25% for the whole summer period, while the lowest value (18.8%) was found on 1\textsuperscript{st} July 2012. Soil moisture content of the deeper layers (20-40 cm and 40-60 cm) was slightly higher than of the top soil in all cases.

<table>
<thead>
<tr>
<th>Date/Treatment</th>
<th>0–20 cm depth</th>
<th>20–40 cm depth</th>
<th>40–60 cm depth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stressed</td>
<td>Control</td>
</tr>
<tr>
<td>15/6/2012</td>
<td>19.45±1.91</td>
<td>8.10±0.00</td>
<td>22.09±1.03</td>
</tr>
<tr>
<td>1/7/2012</td>
<td>18.81±0.44</td>
<td>7.65±0.15</td>
<td>20.40±0.09</td>
</tr>
<tr>
<td>15/7/2012</td>
<td>19.46±0.80</td>
<td>6.69±0.02</td>
<td>21.56±0.09</td>
</tr>
<tr>
<td>30/7/2012</td>
<td>21.31±1.43</td>
<td>5.56±0.08</td>
<td>22.54±0.76</td>
</tr>
<tr>
<td>15/8/2012</td>
<td>19.46±2.89</td>
<td>7.64±0.17</td>
<td>23.26±0.23</td>
</tr>
<tr>
<td>30/8/2012</td>
<td>25.53±0.74</td>
<td>7.24±0.34</td>
<td>26.26±0.63</td>
</tr>
<tr>
<td>15/9/2012</td>
<td>21.03±0.03</td>
<td>9.07±0.29</td>
<td>26.29±0.01</td>
</tr>
<tr>
<td>4/10/2012</td>
<td>25.50±0.23</td>
<td>11.07±0.14</td>
<td>27.57±0.21</td>
</tr>
</tbody>
</table>

Survival rate

The first summer drought was proved crucial for bare-rooted seedling survival in this Mediterranean area where the extreme high temperature and very low rainfall play a significant role (Gindaba et al. 2005). Similar results were reported for bareroot seedlings of *Cercis siliquastrum* (Rahman et al. 2013). Eight months after outplanting, the survival rate of the stressed seedling sharply decreased to 45% (Figure 5); however, the survival rate of watered seedlings was also decreased to 75%. The survival rate of all seedlings after 10 and 17 months was same as it was after 8 months growth. During the June and July 2012, the total rainfall was only 4 mm and the highest temperature in July was 39.9 °C. On the other hand, the average minimum air temperatures in December 2011, January and February 2012 were 1.8°C, -2.4°C and 0.4°C respectively. Internal water stress of plants occurs either from excessive transpiration or slow water absorption from soil or a combination of both, that adversely affects their survival and growth. Newly planted seedlings can not cope with extreme cold weather as well. A part of the initial mortality of the studied bareroot seedlings may be also attributed to the disturbance and desiccation of their root system during lifting, transportation and planting. Once roots have dried, rewetting has been shown to be ineffective in preventing growth reductions, even when shoot water potential recovers (Hasse 2007). Another possible explanation for the slow root growth and mortality of *F. ornus*, during the summer months, could be the age (3 years old) of the bareroot seedlings. Younger seedlings are established faster than older ones due to less root damages (Packer and Clay 2003, Navarro et al. 2006). Nevertheless, the watered seedlings presented significantly greater field survival than drought stressed
ones during the whole studied period (17 months after outplanting). So, a positive relationship between root biomass, seedling quality index and number and length of FOLRs with the plant’s field survival is evident. The watered plants which had significantly greater values of these parameters presented better field survival in contrast to water stressed ones. Similar positive relationship was found by Thompson and Schultz (1995), Davis and Jacobs (2005), Tsakaldimi et al. (2005), Tsakaldimi et al. (2013), Bayala et al. (2009), Manas et al. (2009), Rahman et al. (2013).

**Figure 5.** Course of mean survival rate of *F. ornus* seedlings in two water regimes in the field.

**CONCLUSIONS**

The shoot dieback which was observed in both watered and stressed bareroot seedlings of *F. ornus* decreased their height. The reduction of the initial height and the leaf dieback followed by new coppice shoot, leaf and branch growth in the lower part of stems, seems to be an adaptive mechanism of seedlings to minimize excessive transpiration and to establish in the field. Although the water stress treatment did not affect the shoot morphology of the seedlings, however, it negatively affected the root parameters and the seedling quality index. The first summer months after transplanting reduced the survival of *F. ornus* seedlings of both treatments. However, the withdrawal of irrigation in the plants during the dry summer hot months was crucial in water-stressed plants resulting in very high mortality rate. The higher survival rate of watered seedlings during the dry summer months was positively related to the moisture availability and their greater root biomass, quality index and number and length of FOLR in relation to stressed plants. Therefore, to reduce the mortality rate and for a successful establishment of bare-rooted undercut seedlings, first year irrigation is highly recommended during summer months.

**REFERENCES**


