# Influence of Biostimulants and Water-Retaining Polymer Root Dips on Survival and Growth of Newly Transplanted Bare-Rooted Silver Birch and Rowan[1]

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## Abstract

The influence of five commercially available biostimulant products (Trade names; Generate, Resistim, Fulcrum CRV, Bioplex, Maxicrop) in combination with a water-retaining polymer applied to the root system of silver birch (*Betula pendula* Roth.) and rowan (*Sorbus aucuparia* L.) during the winter period under field conditions was investigated. The short and long-term efficacy of biostimulants on growth was quantified by assessing root and shoot vigor and survival rates at week 8 and 20 post bud break. Improvements in tree vitality were also assessed by measurement of leaf photosynthetic rates, chlorophyll fluorescence emissions and chlorophyll content. Significant effects of species, biostimulant and concentration were found on the majority of growth and tree vitality parameters measured. Only two of the biostimulants tested induced significant growth responses in both tree species. Regardless of species, applications of a water retaining polymer alone had no significant effect on tree survival rates or tree vitality. However, growth of birch was significantly reduced compared to controls indicating a detrimental effect of polymer application alone on this species. Results conclude that use of commercially available biostimulant product in combination with a water retaining polymer can be of use to reduce transplant losses and improve tree vitality and growth over a growing season in silver birch and rowan. Selection of an appropriate biostimulant(s), however, is important as effects on growth and vitality varied widely between species and concentration of biostimulant applied.

**Index words:** Seaweed extracts, zinc complex, molasses, natural plant extracts, betaine, transplant shock, tree vitality, growth, transplant stress.

**Species used in this study:** rowan (*Sorbus aucuparia* L.); silver birch (*Betula pendula* Roth.).



### Significance to the Nursery Industry

Poor root vigor following planting out of bare rooted ornamental trees into amenity landscapes is associated with high mortality rates during the initial years of establishment; a concept known as transplant stress. Biostimulants have been recommended to reduce transplant stress while synthetic water retaining polymers are commonly used to prevent root desiccation after lifting and during transit to the planting site. The influence of a biostimulant and synthetic water retaining polymer combination on reducing transplant stress of bare rooted trees has not been investigated. Results of this investigation show that use of a appropriate commercially available biostimulant in combination with a synthetic water retaining polymer gel applied at the time of planting under field conditions can be of benefit in reducing transplant stress and enhancing growth over the following growing season in silver birch and rowan. Selection of an appropriate biostimulant, however, is important as effects on growth and vitality varied between species.

### Introduction

Trees planted into United Kingdom amenity landscapes provide important benefits to urban populations. These include absorption of pollutants, reduction of traffic noise, windbreaks, shelter and reduction of radiation and solar heat gain through shading and evapotranspiration. Trees also provide shape, scale, form and seasonal changes to a landscape. There are an estimated over 100 million trees in urban areas in Britain, planted on publicly and on privately owned land (1) with an estimated £300 ($450) million spent annually within the UK on amenity tree plantings (2). Creation of amenity landscapes are predominantly established using bare-rooted deciduous ornamental trees and shrubs (3). Poor growth and survival following transplanting is common during the first few years of establishment (4) with mortality rates ranging from 30 to 70% (5). These losses can prove to be a heavy financial burden to those involved with urban tree care. Improving survival and growth of bare-rooted trees will have large financial consequences (2, 3). Poor growth and death of newly transplanted trees has been attributed to water stress symptoms due to internal water deficits within the tree (3, 6). Physical loss of roots during lifting from the nursery bed has been postulated as a major cause of internal water deficits since as little as 5% of a tree's root system may be moved with a tree, even when accepted nursery practices (root pruning, wrenching or undercutting in the nursery) are followed (3, 7, 8). Following leafing out, the capacity of roots to supply the leaves with water can be severely restricted. This post-transplant water stress can be manifest by reduced shoot growth, branch die-back, and ultimately death, a concept frequently referred to as transplant stress. Further damage to root systems can also occur during handling and storage of trees due to desiccation by exposure to air (9).

Products sold as biostimulants differ from traditional nitrogen, phosphorous and potassium (NPK) fertilizers in that their active ingredient consists of a range of organic compounds such as plant hormones, humic acids, marine algae extracts, sea kelp, vitamins and other chemicals that vary according to the manufacturer (10). Recent investigations into the efficacy of biostimulants in enhancing root vigor of urban trees have been conflicting. Evaluation of a range of biostimulant products on root growth of three transplant sensitive tree species demonstrated a significant increase in root vigor (11). Humic acids, an integral component of many

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biostimulant products have been credited with increasing root growth and water uptake of red oak and olive (12) while sea kelp extracts contain high levels of cytokinins which may be beneficial under water stress conditions (13). Contrary to this, a range of biostimulants and humate-based products marketed as aids to plant establishment in balled and burlapped red maple (*Acer rubrum* L. 'Franksred') had little beneficial effects on root growth (14). Further work investigating the interaction between fertilization, irrigation and biostimulants on red maples and washington hawthorns (*Crataegus phaenopyrum* (Blume) Hare) again found little effect on root growth (15). A potential problem regarding the use of biostimulants is their breakdown in soils (16). Planting of bare-rooted trees is generally performed during dormancy (November–January) in the United Kingdom thus simultaneous application of these compounds may have little post-transplant effect when trees break dormancy three to five months later in spring (2). Application of biostimulants after bud break would increase labor costs since repeat visits for spray applications or soil injections would be necessary and could act as a commercial deterrent.

Water-retaining synthetic polymers are soil additives used to improve plant establishment and growth in arid conditions and aid in post transplant survival (17). When irrigated, the polymer gel swells to form particles with a water holding capacity several hundreds times their own dry weight; 95% of which is released to plants (18). Several studies have shown that these polymers can be beneficial to plants grown under water stress and saline conditions (19, 20). Consequently these polymers are recommended as a means of reducing root desiccation and post planting mortalities (17, 18).

The use of a water-retaining synthetic polymer in combination with a biostimulant at the time of planting has not been investigated. Such a combination may provide a means of increasing the longevity of biostimulants in soil so they are available to the tree following dormancy break in spring. Aims of this investigation were to determine the influence of five commercially available biostimulants incorporated into a synthetic water-retaining polymer on growth (stem diameter, height, root growth potential, root length, leaf, shoot, root and total plant dry weight and the root:shoot ratio), tree vitality (chlorophyll fluorescence, leaf chlorophyll content, photosynthetic rates), and survival of silver birch (*Betula pendula* Roth.) and rowan (*Sorbus aucuparia* L.), transplant-sensitive and intermediate tree species respectively, under field conditions.

## Materials and Methods

Bare rooted silver birch (*Betula pendula* Roth.) and rowan (*Sorbus aucuparia* L.) were obtained from a commercial supplier (Oakover Plants, Ashford, Kent, United Kingdom). The physical characteristics of twenty trees selected at random were destructively analyzed to provide an estimation of stock uniformity for experimental purposes. Birch: height 80.9 ± 4.40 cm, stem diameter 2.7 ± 0.10 cm, height:stem diameter ratio 30.0 ± 0.93, shoot dry weight 17.9 ± 1.08 g, root dry weight 12.7 ± 0.54 g, root:shoot ratio 0.70 ± 0.02, root area 357.2 ± 21.89 $cm^2$. Rowan: height 90.2 ± 6.33 cm, stem diameter 1.6 ± 0.06 cm, height:stem diameter ratio 56.4 ± 2.12, shoot dry weight 12.9 ± 0.82 g, root dry weight 61.6 ± 5.22 g, root:shoot ratio 4.78 ± 0.31, root area 968.5 ± 58.30 $cm^2$. All remaining experimental trees were then further root pruned by removal of about 55% (silver birch) and 93% (rowan) of total root volume to produce a root:shoot ratio of 0:33; a ratio associated with transplant shock in trees (21). Trees were then sorted into four bundles of sixty trees, sealed in plastic bags, placed inside larger paper bags, and stored at 6C ± 0.5 (43 ± 0.33F) in darkness. Trees were removed from cold storage (January 26, 2004) and all treatments were applied on the same day as removal.

All biostimulant products (Table 1) were diluted with water at 10 and 30 ml biostimulant per liter of water. Ten ml per liter of water was a general recommended rate for the majority of biostimulants applied. The water-retaining synthetic polymer Aquastore F was hydrated with the biostimulant solution at 5 g polymer per liter of solution. Following hydration of each polymer, the root systems of ten trees were dipped for 30 sec and gently agitated throughout the polymer to ensure maximal contact with the root system. The influence of the water-retaining synthetic polymer alone (no biostimulant treatment) on tree growth and vitality was also investigated and bare-rooted stock dipped for 30 sec in water only (no biostimulant treatment or polymer) acted as controls. Following dipping, trees were immediately planted out into field trial plots at the University of Reading, Shinfield Experimental Station, Reading (51º43' N, –1º08' W) at 1.0 m spacing. A randomized complete design was used. There were twelve treatments (5 biostimulant × 2 concentrations, 1 water-retaining polymer and 1 control and ten trees per treatment. The soil was a sandy loam containing 4–6% organic matter with a pH of 6.2. Weeds were controlled chemically using glyphosate (Roundup) prior to planting, and by hand during the trial. During the experimental period the mean minimum and maximum air temperatures were 2.1C (36F) and 32.6C (91F) respectively, mean daily relative humidity, sunshine hours and rainfall were 72.3%, 8.10 h and 1.44 cm (0.58 in) respectively, the mean soil surface temperature was 3.2C (38F) and soil temperatures at 20 cm (8 in) depth averaged 7.6C (45F); (Reading University Meterological Dept, Whiteknights, Reading, United Kingdom). No irrigation was required and no fertilizer was applied to trees during the ex-

**Table 1. Selected biostimulants applied in combination with a water retaining synthetic polymer on growth and tree vitality of birch (*Betula pendula* Roth.) and rowan (*Sorbus aucuparia* L.)**

| Product | Active ingredient | Supplier |
|---|---|---|
| Water-retaining synthetic polymer | polyalcrylamide gel (WHP) – Aquastore F | Green-Tech, Sweethills Park, Nun Monkton, York, UK |
| Maxicrop Original + WHP | seaweed extract | Maxicrop (UK) Ltd, P.O. Box 6027, Corby, UK |
| Generate + WHP | zinc ammonium acetate complex (2.7%) plus nitrogen (16%) | Loveland Industries, Swaffham Bulbeck, Cambridge, UK |
| Resistim + WHP | betaine | Mandops UK Ltd, Eastleigh, Hampshire, UK |
| Bioplex + WHP | seaweed + humic acid extract | United Agri Products Ltd, Alconbury Weston, UK |
| Fulcrum CRV + WHP | molasses | Banks Cargill Agriculture Ltd, St Hughs, Lincoln, UK |

periment. The effects of biostimulants on growth and vitality measurements were taken at weeks 8 and 20 after bud break with bud break occurring in early April 2004.

Five leaves randomly selected throughout the crown per tree were used for chlorophyll fluorescence and chlorophyll content measurements. Leaves were then tagged to ensure that only the same leaf was measured throughout. Leaves were adapted to darkness for 30 min by attaching light-exclusion clips to the leaf surface, and chlorophyll fluorescence was measured using a HandyPEA portable fluorescence spectrometer (Hansatech Instruments Ltd, King's Lynn, UK). Measurements were recorded up to 1 sec with a data-acquisition rate of 10 ms for the first 2 ms and of 1 ms thereafter. The fluorescence responses were induced by a red (peak at 650 nm) light of 1500 mmol/$m^2$/s photosynthetically active radiation (PAR) intensity provided by an array of six light-emitting diodes. The ratio of variable (Fv = Fm – Fo) to maximal (Fm) fluorescence, i.e. Fv/Fm where Fo = minimal fluorescence, of dark-adapted leaves was used to quantify any effects on leaf tissue. Fv/Fm is considered a quantitative measure of the maximal or potential photochemical efficiency or optimal quantum yield of photosystem II (22). Likewise Fv/Fm values are the most popular index used as a measure of plant vitality and early diagnostic of stress (23).

Leaf chlorophyll content was measured at the mid point of the leaf next to the main leaf vein by using a hand held optical Minolta chlorophyll meter SPAD-502 (Spectrum Technologies, Inc. Plainfield, Illinois, USA). Calibration was obtained by measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam, Portsmouth, UK) after extraction with 80% v/v aqueous acetone (regr. eq. $y = 5.80 + 0.057x$; $r^2$ adj = 0.82, $P \leq 0.01$) (24).

The light-induced $CO_2$ fixation (Pn) was measured in pre-darkened (20 min), fully expanded leaves from near the top of the canopy (generally about 4 nodes down from the apex) by using an Infra Red Gas Analyser (LCA-2 ADC BioScientific Ltd Hoddesdon, Herts, United Kingdom). The irradiance on the leaves was 700 to 800 (mol/$m^2$ photosynthetically active radiation saturating with respect to Pn; the velocity of the airflow was 1 ml/s/$cm^2$ of leaf area. Calculation of the photosynthetic rates was carried out according to Von Caemmerer and Farquhar (25). Two leaves per tree were selected for measurements.

Trees were destructively harvested, and leaf, shoot, and root dry weight recorded after oven drying at 85C (185F) for 48 h. Stem diameter was quantified using Manta blue precision calipers (Langsele, Haglof AB, Sweden) at 60 cm above ground level. Height was recorded by measuring the distance from the tip of the leading apical shoot to the soil surface. Soil was gently removed from the root system by gently shaking the root system after lifting using a garden fork and then washing with water through a 4 mm screen to collect any roots accidentally removed during the shaking and washing process. Once the soil was removed the root system was easily distinguishable. The number of new white roots >1 cm (0.4 in) was counted as a measure of the root growth potential (RGP) and the root length (the straight line distance from the trunk to the furthest root tip) was measured.

Effects of biostimulants application on chlorophyll fluorescence, photosynthetic rates, chlorophyll concentrations and growth were determined by both two and one way analyses of variance (ANOVA) as checks for normality and equal variance distributions were met using an Anderson-Darling test. Differences between treatment means were separated by the Least Significance Difference (LSD) at the 95% confidence level ($P > 0.05$) using the Genstat for Windows 2000 program. Dunn's or Dunnett's tests (respectively, for unequal and equal sample sizes) were then used to compare biostimulant treatments to the control values.

## Results and Discussion

In the case of rowan, none of the treated or control trees died following transplanting. Mortality rates for silver birch (control) were 20%. All biostimulant and polymer treated birch trees survived. Irrespective of biostimulant and concentration, no symptoms of phytotoxicity were observed in either test species. Marked differences in growth (stem diameter, height, root growth potential, root length, leaf, shoot, root and total plant dry weight and the root:shoot ratio) and tree vitality (chlorophyll fluorescence, leaf chlorophyll content, photosynthetic rates) between treatments and species were recorded (Tables 3–6). There was a significant ($P < 0.05$) effect and interaction of species, biostimulant and concentration for the majority of growth and tree vitality parameters. Week twenty data are shown which reflects data obtained at week 8 (Table 2). Regardless of species, applications of a water-retaining polymer alone had no significant effect on virtually all growth and tree vitality measurements (Tables 3–6). In the case of silver birch, significantly reduced ($P < 0.05$) RGP and shoot and total tree dry weight at week 20 post bud break than water dipped controls (Table 4) indicate a potential detrimental effect of polymer application alone on this species. Application of the biostimulants Maxicrop and Bioplex had few significant effects on growth and tree vitality of rowan at week 8 and 20



**Table 2. P values[z] for growth and tree vitality of birch (*Betula pendula*) and rowan (*Sorbus aucuparia* L.) at week 20 following biostimulant treatments.**

| Factor | Stem diameter | Height | RGP | Root length | Leaf DW | Shoot DW | Root DW | Total plant DW | Root: Shoot ratio | Chloro-phyll content | Fv/Fm | Pn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species (S) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Concentration (C) | 0.012 | <0.001 | 0.684 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.036 | <0.001 | <0.001 | <0.001 |
| Biostimulant (B) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.048 |
| S × C | 0.897 | <0.001 | 0.628 | <0.001 | 0.603 | 0.011 | 0.080 | 0.446 | 0.338 | 0.091 | 0.080 | 0.245 |
| S × B | <0.001 | <0.001 | 0.064 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| C × B | 0.001 | 0.089 | 0.007 | 0.002 | 0.019 | 0.003 | 0.295 | 0.668 | 0.415 | 0.002 | 0.018 | <0.001 |
| S × C × B | 0.002 | 0.821 | 0.015 | 0.247 | 0.008 | 0.002 | 0.293 | 0.160 | 0.331 | 0.225 | 0.364 | 0.333 |

[z]$P < 0.05$ are considered significant based on Dunnetts test.

RGP = root growth potential; DW = dry weight (g), Fv/Fm = chlorophyll fluorescence, Pn = light-induced $CO_2$ fixation

**Table 3. Influence of biostimulants in combination with a water-retaining polymer on tree vitality and growth of birch (*Betula pendula* Roth.) at week 8 after bud break under field conditions[z].**

| Treatment[y] | Stem diameter (cm) | Height (cm) | RGP | Root length (cm) | Leaf DW (g) | Shoot DW (g) | Root DW (g) | Total plant DW (g) | Root: Shoot ratio | Chloro-phyll content | Fv/Fm | Pn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 2.9 | 102.9 | 9.7 | 32.5 | 4.62 | 29.1 | 15.8 | 49.5 | 0.47 | 13.5 | 0.635 | 3.80 |
| WRP | 3.0$^{ns}$ | 113.1* | 10.4$^{ns}$ | 23.1$^{ns}$ | 6.22$^{ns}$ | 27.1$^{ns}$ | 15.3$^{ns}$ | 48.6$^{ns}$ | 0.48$^{ns}$ | 12.8$^{ns}$ | 0.615$^{ns}$ | 3.67$^{ns}$ |
| Maxicrop 10 ml + WRP | 2.9$^{ns}$ | 114.6* | 12.8$^{ns}$ | 40.2$^{ns}$ | 6.10$^{ns}$ | 26.9$^{ns}$ | 23.2* | 56.5$^{ns}$ | 0.70$^{ns}$ | 13.1$^{ns}$ | 0.650$^{ns}$ | 3.69$^{ns}$ |
| Maxicrop 30 ml + WRP | 2.9$^{ns}$ | 113.7* | 12.6$^{ns}$ | 43.6* | 6.32$^{ns}$ | 31.4$^{ns}$ | 29.9* | 67.5* | 0.80* | 14.5$^{ns}$ | 0.673$^{ns}$ | 4.02$^{ns}$ |
| Generate 10 ml + WRP | 3.4$^{ns}$ | 107.7$^{ns}$ | 15.4$^{ns}$ | 33.7$^{ns}$ | 8.73* | 29.7$^{ns}$ | 20.7$^{ns}$ | 59.1* | 0.54$^{ns}$ | 13.8$^{ns}$ | 0.679$^{ns}$ | 4.17$^{ns}$ |
| Generate 30 ml + WRP | 2.4$^{ns}$ | 103.4$^{ns}$ | 11.8$^{ns}$ | 40.5$^{ns}$ | 8.43* | 39.1* | 20.6$^{ns}$ | 68.1* | 0.43$^{ns}$ | 15.9* | 0.744$^{ns}$ | 4.40$^{ns}$ |
| Resistim 10 ml + WRP | 3.2$^{ns}$ | 113.6* | 22.4* | 39.1$^{ns}$ | 7.73* | 37.9* | 28.8* | 74.4* | 0.65$^{ns}$ | 15.8* | 0.698$^{ns}$ | 4.34$^{ns}$ |
| Resistim 30 ml + WRP | 3.1$^{ns}$ | 110.9* | 17.6* | 26.8$^{ns}$ | 7.64* | 33.2$^{ns}$ | 27.1* | 68.0* | 0.66$^{ns}$ | 14.6$^{ns}$ | 0.685$^{ns}$ | 4.18$^{ns}$ |
| Bioplex 10 ml + WRP | 3.3$^{ns}$ | 102.3$^{ns}$ | 10.0$^{ns}$ | 34.8$^{ns}$ | 7.21* | 30.0$^{ns}$ | 29.1* | 66.3* | 0.78* | 16.7* | 0.611$^{ns}$ | 3.31$^{ns}$ |
| Bioplex 30 ml + WRP | 3.3$^{ns}$ | 110.7* | 9.8$^{ns}$ | 44.1* | 9.17* | 46.2* | 39.5* | 94.8* | 0.75* | 15.8* | 0.637$^{ns}$ | 3.29$^{ns}$ |
| Fulcrum CRV 10 ml + WRP | 2.6$^{ns}$ | 89.6* | 10.8$^{ns}$ | 28.9$^{ns}$ | 3.50$^{ns}$ | 23.0$^{ns}$ | 16.8$^{ns}$ | 43.3$^{ns}$ | 0.66$^{ns}$ | 11.0$^{ns}$ | 0.640$^{ns}$ | 3.59$^{ns}$ |
| Fulcrum CRV 30 ml + WRP | 3.0$^{ns}$ | 108.3$^{ns}$ | 16.0* | 33.3$^{ns}$ | 3.38$^{ns}$ | 31.4$^{ns}$ | 32.6* | 67.4* | 0.99* | 17.1* | 0.707$^{ns}$ | 4.90$^{ns}$ |
| SED[x] | 0.31 | 3.62 | 2.94 | 5.11 | 0.918 | 3.48 | 3.69 | 4.71 | 0.133 | 1.13 | 0.0675 | 0.741 |
| df[x] | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 |

[z]All values mean of 5 trees except control (n = 4).

[y]WRP = Water-retaining polymer, (Trade name Aquastore F); Maxicrop = a seaweed based biostimulant; Generate = a zinc complex based biostimulant; Resistim = a Betaine based biostimulant; Bioplex = seaweed + humic acid extract; Fulcrum CRV = a molasses based biostimulant.

[x]df = degree of freedom, SED = standard error of the difference between means; ns = not significant from control, * = $P \leq 0.05$ using Dunnett's tests.

RGP = root growth potential; DW = dry weight (g), Fv/Fm = chlorophyll fluorescence, Pn = Light-induced $CO_2$ fixation.

after bud break irrespective of concentration applied, indicating little or no tree growth or vitality enhancing properties of these biostimulants for this species (Tables 5–6). Similar results were recorded for silver birch following application of Maxicrop at 10 ml per liter of water (Tables 3–4). However, applications at 30 ml per liter of water generally increased ($P < 0.05$) silver birch height, root dry weight, total plant dry weight at both week 8 and 20 (Tables 3–4). In addition this treatment generally ($P < 0.05$) increased the root:shoot ratio at week 8. Applications of Bioplex at both 10 and 30 ml per liter of water significantly ($P < 0.05$) increased silver birch leaf, root and total dry weight, the root:shoot ratio and leaf chlorophyll content of silver birch at week 8 post bud break (Table 3). By week 20, however, these effects were only significantly higher ($P < 0.05$) than controls at a concentration of 30 ml Bioplex per liter of water (Table 4). Irrespective of concentration (10 or 30 ml) applications of the biostimulants Resistim and Generate induced positive increases in both growth and tree vitality of rowan compared to water dipped controls (Tables 5–6). For example, at week 8 a significant ($P < 0.05$) increase in height, stem diameter, root and total plant dry weight and chlorophyll content was recorded compared to controls (Table 5). By week 20 a significant ($P < 0.05$) increase in total plant dry weight and leaf chlorophyll content was recorded

**Table 4. Influence of biostimulants in combination with a water-retaining polymer on tree vitality and growth of birch (*Betula pendula* Roth.) at week 20 after bud break under field conditions[z].**

| Treatment[y] | Stem diameter (cm) | Height (cm) | RGP | Root length (cm) | Leaf DW (g) | Shoot DW (g) | Root DW (g) | Total plant DW (g) | Root: Shoot ratio | Chloro-phyll content | Fv/Fm | Pn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 3.8 | 104.7 | 11.8 | 32.4 | 7.89 | 39.4 | 26.2 | 73.4 | 0.56 | 14.5 | 0.630 | 4.01 |
| WRP | 3.5$^{ns}$ | 112.0$^{ns}$ | 7.6* | 26.0$^{ns}$ | 5.65$^{ns}$ | 30.0* | 16.4$^{ns}$ | 52.1* | 0.47$^{ns}$ | 13.1$^{ns}$ | 0.622$^{ns}$ | 3.93$^{ns}$ |
| Maxicrop 10 ml + WRP | 3.8$^{ns}$ | 111.7$^{ns}$ | 14.4$^{ns}$ | 47.7* | 7.20$^{ns}$ | 32.8$^{ns}$ | 31.7$^{ns}$ | 71.8$^{ns}$ | 0.79$^{ns}$ | 13.9$^{ns}$ | 0.643$^{ns}$ | 4.24$^{ns}$ |
| Maxicrop 30 ml + WRP | 3.4$^{ns}$ | 115.8* | 11.4$^{ns}$ | 34.6$^{ns}$ | 7.09$^{ns}$ | 43.7$^{ns}$ | 40.0* | 90.8* | 0.78$^{ns}$ | 15.5$^{ns}$ | 0.698$^{ns}$ | 4.22$^{ns}$ |
| Generate 10 ml + WRP | 4.0n$^{s}$ | 112.2$^{ns}$ | 13.4$^{ns}$ | 37.1$^{ns}$ | 7.30$^{ns}$ | 36.4$^{ns}$ | 30.8$^{ns}$ | 74.6$^{ns}$ | 0.71$^{ns}$ | 13.8$^{ns}$ | 0.585$^{ns}$ | 3.19$^{ns}$ |
| Generate 30 ml + WRP | 3.7$^{ns}$ | 103.1$^{ns}$ | 18.2* | 36.3$^{ns}$ | 9.66$^{ns}$ | 36.1$^{ns}$ | 57.5* | 103.4* | 1.25* | 18.9* | 0.801* | 5.23* |
| Resistim 10 ml + WRP | 4.4* | 113.4* | 15.2 * | 33.7$^{ns}$ | 8.64$^{ns}$ | 36.7$^{ns}$ | 35.1$^{ns}$ | 80.5$^{ns}$ | 0.79$^{ns}$ | 14.7$^{ns}$ | 0.698$^{ns}$ | 4.71$^{ns}$ |
| Resistim 30 ml + WRP | 4.1$^{ns}$ | 115.5* | 10.6$^{ns}$ | 39.1$^{ns}$ | 9.24$^{ns}$ | 40.7$^{ns}$ | 39.34* | 89.3* | 0.72$^{ns}$ | 14.6$^{ns}$ | 0.624$^{ns}$ | 4.10$^{ns}$ |
| Bioplex 10 ml + WRP | 4.1$^{ns}$ | 101.6$^{ns}$ | 11.8$^{ns}$ | 45.6* | 9.89$^{ns}$ | 36.4$^{ns}$ | 37.0$^{ns}$ | 83.2$^{ns}$ | 0.80$^{ns}$ | 16.9$^{ns}$ | 0.657$^{ns}$ | 4.55$^{ns}$ |
| Bioplex 30 ml + WRP | 3.8$^{ns}$ | 109.5$^{ns}$ | 14.8* | 40.5$^{ns}$ | 8.25$^{ns}$ | 42.4$^{ns}$ | 53.9* | 104.5* | 1.07* | 18.0* | 0.775* | 5.30* |
| Fulcrum CRV 10 ml + WRP | 4.0$^{ns}$ | 101.2$^{ns}$ | 14.0$^{ns}$ | 45.8* | 9.46$^{ns}$ | 37.1$^{ns}$ | 23.0$^{ns}$ | 69.4$^{ns}$ | 0.50$^{ns}$ | 12.3$^{ns}$ | 0.667$^{ns}$ | 3.88$^{ns}$ |
| Fulcrum CRV 30 ml + WRP | 4.6* | 122.9* | 11.6$^{ns}$ | 42.0$^{ns}$ | 9.88$^{ns}$ | 44.7$^{ns}$ | 46.0* | 100.6* | 0.90* | 17.9* | 0.748* | 5.00* |
| SED[x] | 0.30 | 3.84 | 1.46 | 4.97 | 1.596 | 3.62 | 5.38 | 6.31 | 0.153 | 1.28 | 0.0447 | 0.400 |
| df[x] | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 |

[z]All values mean of 6 trees except control (n = 4).

[y]WRP = Water-retaining polymer, (Trade name Aquastore F); Maxicrop = a seaweed based biostimulant; Generate = a zinc complex based biostimulant; Resistim = a Betaine based biostimulant; Bioplex = seaweed + humic acid extract; Fulcrum CRV = a molasses based biostimulant

[x]df = degree of freedom, SED = standard error of the difference between means; ns = not significant from control, * = $P \leq 0.05$ using Dunnett's tests.

RGP = root growth potential; DW = dry weight (g), Fv/Fm = chlorophyll fluorescence, Pn = light-induced $CO_2$ fixation.

**Table 5. Influence of biostimulants in combination with a water-retaining polymer on tree vitality and growth of rowan (*Sorbus aucuparia* L.) at week 8 after bud break under field conditions[z].**

| Treatment[y] | Stem diameter (cm) | Height (cm) | RGP | Root length (cm) | Leaf DW (g) | Shoot DW (g) | Root DW (g) | Total plant DW (g) | Root: Shoot ratio | Chloro-phyll content | Fv/Fm | Pn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1.8 | 94.0 | 7.0 | 24.0 | 12.2 | 14.9 | 72.8 | 100.0 | 2.70 | 16.0 | 0.576 | 2.98 |
| WRP | 1.9$^{ns}$ | 101.8$^{ns}$ | 5.8$^{ns}$ | 17.7* | 11.9$^{ns}$ | 13.7$^{ns}$ | 67.2$^{ns}$ | 92.8$^{ns}$ | 2.66$^{ns}$ | 15.5$^{ns}$ | 0.580$^{ns}$ | 3.11$^{ns}$ |
| Maxicrop 10 ml + WRP | 1.8$^{ns}$ | 92.3$^{ns}$ | 5.6$^{ns}$ | 17.2* | 11.2$^{ns}$ | 14.1$^{ns}$ | 61.6$^{ns}$ | 86.9$^{ns}$ | 2.44$^{ns}$ | 17.2$^{ns}$ | 0.600$^{ns}$ | 3.24$^{ns}$ |
| Maxicrop 30 ml + WRP | 1.9$^{ns}$ | 92.7$^{ns}$ | 6.0$^{ns}$ | 21.7$^{ns}$ | 12.7$^{ns}$ | 14.4$^{ns}$ | 70.2$^{ns}$ | 97.3$^{ns}$ | 2.61$^{ns}$ | 17.3$^{ns}$ | 0.561$^{ns}$ | 2.76$^{ns}$ |
| Generate 10 ml + WRP | 2.0* | 103.7* | 9.7* | 25.8$^{ns}$ | 14.6$^{ns}$ | 16.0$^{ns}$ | 96.4* | 127.1* | 3.17* | 20.2* | 0.644* | 3.44$^{ns}$ |
| Generate 30 ml + WRP | 2.0* | 107.2* | 11.0* | 21.6$^{ns}$ | 13.7$^{ns}$ | 15.2$^{ns}$ | 89.4* | 118.3* | 3.10$^{ns}$ | 18.9* | 0.598$^{ns}$ | 3.50$^{ns}$ |
| Resistim 10 ml + WRP | 2.2* | 123.0* | 8.6$^{ns}$ | 24.8$^{ns}$ | 15.5* | 17.8* | 90.9* | 124.2* | 2.75$^{ns}$ | 19.5* | 0.676* | 3.51$^{ns}$ |
| Resistim 30 ml + WRP | 2.1* | 121.4* | 9.8* | 24.5$^{ns}$ | 16.2* | 18.8* | 91.7* | 126.7* | 2.66$^{ns}$ | 22.3* | 0.655* | 3.49$^{ns}$ |
| Bioplex 10 ml + WRP | 1.9$^{ns}$ | 96.9$^{ns}$ | 5.2$^{ns}$ | 18.3* | 11.9$^{ns}$ | 15.1$^{ns}$ | 65.4$^{ns}$ | 92.4$^{ns}$ | 2.43$^{ns}$ | 14.9$^{ns}$ | 0.544$^{ns}$ | 2.84$^{ns}$ |
| Bioplex 30 ml + WRP | 1.9$^{ns}$ | 98.2$^{ns}$ | 5.2$^{ns}$ | 16.8* | 11.9$^{ns}$ | 14.4$^{ns}$ | 64.4$^{ns}$ | 90.7$^{ns}$ | 2.45$^{ns}$ | 16.7$^{ns}$ | 0.562$^{ns}$ | 2.51$^{ns}$ |
| Fulcrum CRV 10 ml + WRP | 1.8$^{ns}$ | 96.9$^{ns}$ | 5.8$^{ns}$ | 16.6* | 11.0$^{ns}$ | 14.3$^{ns}$ | 62.3$^{ns}$ | 88.5$^{ns}$ | 2.49$^{ns}$ | 14.9$^{ns}$ | 0.583$^{ns}$ | 2.39$^{ns}$ |
| Fulcrum CRV 30 ml + WRP | 2.0* | 103.9* | 11.2* | 22.7$^{ns}$ | 14.7* | 16.3$^{ns}$ | 101.3* | 132.3* | 3.29* | 23.8* | 0.693* | 3.74* |
| SED[x] | 0.097 | 4.67 | 1.32 | 2.31 | 1.38 | 1.25 | 6.01 | 7.48 | 0.205 | 1.39 | 0.0332 | 0.291 |
| df[x] | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 |

[z]All values mean of 5 trees.

[y]WRP = Water-retaining polymer, (Trade name Aquastore F); Maxicrop = a seaweed based biostimulant; Generate = a zinc complex based biostimulant; Resistim = a Betaine based biostimulant; Bioplex = seaweed + humic acid extract; Fulcrum CRV = a molasses based biostimulant.

[x]df = degree of freedom, SED = standard error of the difference between means; ns = not significant from control, * = $P \leq 0.05$ using Dunnett's tests.

RGP = root growth potential; DW = dry weight (g), Fv/Fm = chlorophyll fluorescence, Pn = Light-induced $CO_2$ fixation.

(Table 6). For all remaining growth (stem diameter, height, root growth potential, root length, leaf, shoot, root dry weight, root:shoot ratio) and tree vitality measurements (chlorophyll fluorescence, photosynthetic rates), values were consistently higher if not significantly so than controls (Table 6). Although not as pronounced, similar growth and vitality stimulatory effects were manifest in silver birch following applications of Resistim and Generate at both week 8 and 20 (Tables 3–4). For example, at week 20 post bud break total plant dry weight was consistently higher than controls. Likewise in the majority of cases leaf chlorophyll content, chlorophyll fluorescence and photosynthetic rates were in general 20–40% higher than control values (Tables 3–4). For both rowan and silver birch few significant effects on growth and vitality were recorded following application of the biostimulant Fulcrum CRV at 10 ml per liter of water indicating no beneficial effects of this compound when applied at this concentration (Tables 3–6). Applications of 30 ml per liter of water, however, induced positive growth and tree vitality responses in both species (Tables 3–6). By week 8 after bud break, for example, significant ($P < 0.05$) increases in RGP, root dry weight, root:shoot ratio and leaf chlorophyll content were recorded compared to controls (Tables 3, 5). At week 20 significant ($P < 0.05$) increases in stem diameter, height, root and total plant dry weight,

**Table 6. Influence of biostimulants in combination with a water-retaining polymer on tree vitality and growth of rowan (*Sorbus aucuparia* L.) at week 20 after bud break under field conditions[z].**

| Treatment[y] | Stem diameter (cm) | Height (cm) | RGP | Root length (cm) | Leaf DW (g) | Shoot DW (g) | Root DW (g) | Total plant DW (g) | Root: Shoot ratio | Chloro-phyll content | Fv/Fm | Pn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 2.2 | 117.2 | 6.2 | 36.2 | 13.6 | 18.5 | 87.9 | 119.9 | 2.72 | 19.3 | 0.608 | 3.23 |
| WRP | 2.1$^{ns}$ | 117.1$^{ns}$ | 5.4$^{ns}$ | 34.3$^{ns}$ | 14.1$^{ns}$ | 17.9$^{ns}$ | 82.9$^{ns}$ | 114.8$^{ns}$ | 2.62$^{ns}$ | 20.0$^{ns}$ | 0.604$^{ns}$ | 3.18$^{ns}$ |
| Maxicrop 10 ml + WRP | 2.1$^{ns}$ | 115.7$^{ns}$ | 6.6$^{ns}$ | 38.0$^{ns}$ | 14.6$^{ns}$ | 18.2$^{ns}$ | 77.4$^{ns}$ | 110.2$^{ns}$ | 2.35$^{ns}$ | 18.2$^{ns}$ | 0.625$^{ns}$ | 3.46$^{ns}$ |
| Maxicrop 30 ml + WRP | 2.1$^{ns}$ | 113.6$^{ns}$ | 6.6$^{ns}$ | 38.8$^{ns}$ | 12.3$^{ns}$ | 17.3$^{ns}$ | 79.5$^{ns}$ | 108.0$^{ns}$ | 2.67$^{ns}$ | 17.6$^{ns}$ | 0.611$^{ns}$ | 3.04$^{ns}$ |
| Generate 10 ml + WRP | 2.3$^{ns}$ | 125.1$^{ns}$ | 8.6$^{ns}$ | 43.4* | 15.0$^{ns}$ | 19.3$^{ns}$ | 108.7$^{ns}$ | 143.0* | 3.17$^{ns}$ | 25.2* | 0.709* | 4.02* |
| Generate 30 ml + WRP | 2.3$^{ns}$ | 126.8* | 10.5* | 39.1$^{ns}$ | 16.0$^{ns}$ | 19.2$^{ns}$ | 107.3$^{ns}$ | 142.6* | 3.06$^{ns}$ | 23.4* | 0.699* | 3.87* |
| Resistim 10 ml + WRP | 2.4* | 136.8* | 9.0* | 49.9* | 19.8* | 20.2$^{ns}$ | 120.4* | 160.5* | 3.01$^{ns}$ | 22.8* | 0.666$^{ns}$ | 4.11* |
| Resistim 30 ml + WRP | 2.3$^{ns}$ | 139.8* | 10.4* | 48.3* | 20.7* | 20.6$^{ns}$ | 121.2* | 162.5* | 2.94$^{ns}$ | 27.1* | 0.729* | 4.11* |
| Bioplex 10 ml + WRP | 2.0* | 115.7$^{ns}$ | 6.4$^{ns}$ | 38.1$^{ns}$ | 14.0$^{ns}$ | 17.6$^{ns}$ | 82.3$^{ns}$ | 114.0$^{ns}$ | 2.59$^{ns}$ | 16.8$^{ns}$ | 0.579$^{ns}$ | 3.10$^{ns}$ |
| Bioplex 30 ml + WRP | 2.2$^{ns}$ | 115.6$^{ns}$ | 5.0$^{ns}$ | 33.8$^{ns}$ | 13.7$^{ns}$ | 17.4$^{ns}$ | 78.1$^{ns}$ | 109.1$^{ns}$ | 2.51$^{ns}$ | 19.0$^{ns}$ | 0.632$^{ns}$ | 3.28$^{ns}$ |
| Fulcrum CRV 10 ml + WRP | 2.2$^{ns}$ | 120.5$^{ns}$ | 6.3$^{ns}$ | 37.5$^{ns}$ | 13.3$^{ns}$ | 17.3$^{ns}$ | 77.0$^{ns}$ | 107.6$^{ns}$ | 2.49$^{ns}$ | 17.5$^{ns}$ | 0.620$^{ns}$ | 3.11$^{ns}$ |
| Fulcrum CRV 30 ml + WRP | 2.4* | 127.3* | 9.4* | 40.1$^{ns}$ | 15.2$^{ns}$ | 19.7$^{ns}$ | 110.8* | 145.7* | 3.18$^{ns}$ | 22.8* | 0.707* | 4.25* |
| SED[x] | 0.080 | 4.66 | 1.34 | 3.30 | 1.46 | 1.19 | 11.07 | 11.09 | 0.255 | 1.50 | 0.0440 | 0.323 |
| df[x] | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 |

[z]All values mean of 5 trees.

[x]df = degree of freedom, SED = standard error of the difference between means; ns = not significant from control, * = $P \leq 0.05$ using Dunnett's tests.

[y]WRP = Water-retaining polymer, (Trade name Aquastore F); Maxicrop = a seaweed based biostimulant; Generate = a zinc complex based biostimulant; Resistim = a Betaine based biostimulant; Bioplex = seaweed + humic acid extract; Fulcrum CRV = a molasses based biostimulant.

RGP = root growth potential; DW = dry weight (g), Fv/Fm = chlorophyll fluorescence, Pn = Light-induced $CO_2$ fixation.

leaf chlorophyll content, chlorophyll fluorescence photosynthetic rates were recorded in both species (Tables 4, 6).

Promotional literature distributed by manufacturers suggests biostimulants can play an important role in increasing transplant survival. To date, however, most published studies on trees indicate little or no effect of these products (10, 12, 14, 15, 26). In agreement with these findings, the biostimulants Maxicrop and Bioplex applied at 10 and 30 ml in combination with a synthetic water retaining polymer was shown to have few positive effects on growth and vitality of rowan at week 8 and 20 post bud break. Results of this investigation and those elsewhere (11, 27, 28) however, highlight specific constraints which must be taken into account when using biostimulants as a means of reducing transplant losses.

First, the effects of biostimulants on plant growth can markedly differ as a result of differing active ingredients (auxins, cytokinins, vitamins, humates, salicylic acid, etc.) contained within a product. For example application of the biostimulants Generate (active ingredient (a.i.) zinc complex) and Resistim (a.i. betaine) were beneficial to both species increasing total plant dry weight and leaf chlorophyll content at the cessation of the experiment (20 weeks after bud break). Research has shown root drenches and foliar sprays of the biostimulant Generate to three transplant-sensitive tree species; red oak (*Quercus rubra* L.), silver birch (*Betula pendula* Roth.) and beech (*Fagus sylvatica* L.) were effective in reducing transplant losses in all three species (11). Likewise, the active ingredient present in Generate (zinc ammonium acetate) has been shown to induce positive growth responses in other plants. Zinc ammonium acetate, an organic zinc chelate, provides available zinc to plants without any soil fixation problems that occur when zinc is applied alone (29). Zinc is one of the major nutrients required in the synthesis of auxins (30). Supplementing plants with zinc ammonium acetate provides plants with an easily absorbed form of zinc that is rapidly translocated to the leaf meristematic tissue enhancing the production of the root promoting hormone auxin, in turn stimulating root and then shoot vigor (31). Applications of Maxicrop (a.i. seaweed extract), Fulcrum CRV (a.i. molasses) and Bioplex (a.i. seaweed and humic acid extract) either failed to induce any growth promontory responses or where positive effects were recorded these were not, in general, as great as Generate or Resistim.

Second, even when the active ingredient is the same, effects on tree growth can differ from marked increases to no significant effects depending on species. For example, Maxicrop and Bioplex application significantly increased total tree dry weights at the cessation of the experiment in silver birch yet failed to have any significant effect on rowan. This species specific response to a biostimulant has been demonstrated elsewhere. Percival and Gerritsen, (6) using individual and combinations of indole-butryic, indole-acetic and napthyl-acetic acid showed improved growth of red alder (*Alnus rubra* Bong.) rowan (*Sorbus aucuparia* L.), and linden (*Tilia* x *europea* L.) but little effect on English oak (*Quercus robur* L.). Similarly, applications of the plant growth regulator indole-butryic acid increased root initiation, root elongation, shoot growth and leaf area of beech (*Fagus sylvatica* L.) but had no promontory effects on growth and vitality of English oak (*Quercus robur* L.) (3). Similar species specific responses to hormonal based products have been shown using scarlet oak (*Quercus coccinea* Muenchh.), palm, pistachio (*P. vera* L.), silver birch (*Betula pendula* Roth.) and beech (*Fagus sylvatica* L.) as test models (32, 33). Differing growth responses between tree species to an active ingredient present in a biostimulant would be disadvantageous to the tree care industry where products with universal applicability for a wide range of species are required.

Third, application of the biostimulant Fulcrum CRV at 10 ml per liter of water had no significant effects on tree growth; however, application at 30 ml per liter of polymer significantly enhanced stem diameter, height, root and total plant dry weight, leaf chlorophyll content and chlorophyll fluorescence at week 20 of both rowan and silver birch. This result demonstrates a significant dose response effect. Consequently, where biostimulants are used, prior experimentation maybe needed to determine the correct application for trees in a garden or landscape setting (28). In many cases where biostimulants have been trialed unsuccessfully as a means of reducing transplant mortalities, few dose response treatments were tested (10, 12, 14, 15, 26). Evidence elsewhere has shown that the active ingredient in Fulcrum CRV i.e. molasses, a sugar, can induce significant root growth in plants and provide a concomitant increase in stress resistance. Supplementing plant root systems with sugars significantly increased lateral root branching and root formation of a range of monocotyledonous plant species while application of sucrose enhanced root vigor of containerized and field planted tree stock (34, 35, 36, 37, 38). Similarly, soil/root injections with sucrose were found to significantly enhance root dry weight of established mature horse chestnut (*Aesculus hippocastanum* L.), silver birch (*Betula pendula* Roth.), cherry (*Prunus avium* L.), and English oak (*Quercus robur* L) (39). Recent work also reported a positive significant influence of sucrose on root development of seedling material with a twenty fold increase in root length recorded in the presence of sugars compared to controls (40).

Irrespective of species, applications of a water-retaining polymer alone had no significant effect on virtually all growth and tree vitality measurements. In the case of silver birch, significantly reduced root growth potential and total tree dry weight at week 20 post bud break indicates a potential detrimental effect of polymer application alone on this species. In support of this, Percival and Barnes, (41) demonstrated a detrimental influence on growth of silver birch following polymer application when applied as a post transplant treatment. Earlier work by Davies (42) also concluded that, despite marked improvements in the available water capacity of treated soils, most of the commercially available water-retaining polymers trialed were of little or no use in sustaining the growth of newly planted trees. In contrast others (17, 18) concluded that the use of synthetic water retaining polymers were beneficial in the establishment of woody plants by increasing plant water and nutrient holding capacity and facilitating a favourable root:shoot development under low soil moisture conditions. Further research is suggested before the use of synthetic water-retaining polymers alone should be used as a viable option to reduce transplant losses of bare-rooted woody plants. In this investigation biostimulants were not applied without a synthetic water-retaining polymer. Application of a synthetic water-retaining polymer had no positive effect, indicating the polymer may not be needed at all. However, due to the experimental design, a polymer × biostimulant interpretation could not be provided in this instance.

In conclusion, results of this investigation indicate that use of an appropriate biostimulant and synthetic water retaining polymer combination can potentially provide a means of reducing transplant losses and improve the growth and vitality of silver birch and rowan. Selection of an appropriate biostimulant is, however, critical as effects on growth can vary widely between tree species possibly as a result of i) the differing active ingredient used in the formulation of a product and ii) the concentration applied. With the influx of biostimulants released into the amenity market, evaluating all of them independently is a time consuming and labor intensive process. Consequently where independent scientific data are not available to support the claims of the manufacturer, then using an unevaluated biostimulant is not recommended.

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