RECOVERY RESPONSES OF SIMULATED HAIL-DAMAGED *Pelargonium graveolens* L'Hér. (cv. 'Bourbon') TO DIFFERENT MIXTURES OF BIOSTIMULANTS

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Abstract. The aim of this study was to determine the potential ability of biostimulant-mixtures to recover the herbage and essential oil yield, as well improve the essential oil quality, of simulated hail-damaged rose geranium (*Pelargonium graveolens* L'Her.). The experiment was carried out in a 3 × 4 factorial treatment design and laid out in a randomised complete block design in a temperature-controlled greenhouse during 2019 season. Treatments consisted of three hail damage simulation levels and three biostimulant-mixtures and a control. Plants grew taller when biostimulant-mixtures were applied from levels 2 up to 3. Level 3 treatment increased the essential oil yield of plants which sustained up to 50% foliage loss, and intact plants. Citronellol and geraniol content, as well the citronellol to geraniol ratio improved with the level 2 treatment in plants that sustained 100% foliage loss. It can be concluded that the application of biostimulants-mixture should be applied from level 3 concentration to improve the herbage yield and the essential oil quality of rose geranium plants with more than 50% foliage loss. Plants with less than 50% foliage loss can be treated with a biostimulant-mixture from level 2 to improve essential oil yield and the densities of brevicollate trichomes.
Keywords: *'Bourbon-type', brassinosteroids, cytokinin, essential oil, gibberellic acid, hail damage simulation, trichome density*

Introduction

Prolonged droughts, increased floods, and frequent extreme weather events are evidence of climate change, as a consequence of global warming (Tack et al., 2015). Agriculture is adapting to the variability in global climatic conditions, with farmers continually developing strategies to respond to changing weather patterns (Burke et al., 2015). Aromatic and medicinal plants are among those plants which are negatively affected by climate change (Cavaliere, 2009). In addition, there is a concern over climate change affecting the secondary metabolites of many medicinal and aromatic plants (Das et al., 2016; Kundu and Gantait, 2017). This has been demonstrated with muña (*Minthostachys mollis* [Kunth] Griseb.), where leaf puncturing resulted in reduced menthone levels, while the pulegone concentration increased during the first 48 hours of the experiment (Banchio et al., 2007).

Loss of plant yield, associated with hail damage, is often variable and dependent on the plant species, the timing of injury, relative growth rate, and the prevailing conditions following the damage (Conley et al., 2008; Atkinson et al., 2014). Hailstones >H2 (15.2 to 20.3 mm) can cause extensive damage to growing crops through defoliation, leaf shredding, and stand losses (Changnon, 1999; Conley et al., 2008). Hailstones may cause

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significant damage to aromatic and medicinal plants, such as rose geranium (*Pelargonium graveolens* L'Hér.): post-hail damage strategies are therefore required to recover the lost herbage material. Studies have shown that some species recover slowly from hail damage due to delayed growth patterns, i.e. canola (*Brassica napus* L.), Alaska pea (*Pisum sativum* L.), guar (*Cyamopsis tetragonoloba* [L.] Taub.), and lentil (*Lens culinaris* L.) (Miller and Muehlbauer, 1984; McGregor, 1987; Sij et al., 2005; Bueckert, 2011).

Following hail damage, the tissue around the damaged site initiates a cascade of biochemical or physiological processes to repair the damaged tissue: this further depletes energy stores to rebuild photosynthetic material and regenerate lost plant tissue (Suttle et al., 2013; Atkinson et al., 2014). These physiological activities are primarily driven by the upregulation of endogenous plant growth regulators (Ikeuchi et al., 2017; Nanda and Melnyk, 2018). Extensive studies have demonstrated that exogenous applications of agricultural biostimulants on plants may effectively stimulate vegetative growth, improve nutrient acquisition, and increase the antioxidant capacity of the plant tissue (Torres et al., 2018; Parađiković et al., 2019).

The effects of biostimulant are based on the synergism to improve the growth and development, as well as the recovery, resistance and survival of stressed plants (Prins et al., 2010; Parađiković et al., 2019). Therefore, the objective of this study was to determine the ability of biostimulants-mixtures to recover the herbage and essential oil yield attributes, and as well improve the essential oil quality of simulated hail-damaged rose geranium plants. It is hypothesized that high concentrations of biostimulants-mixtures will improve the essential oil yield and quality of simulated hail-damaged rose geranium plants.

Materials and methods

Experimental site description, plant material and agronomic practices

This experiment was conducted in a 72 m^2 temperature-controlled, plastic-covered greenhouse for 122 days using a drain-to-waste hydroponic system, on the campus of the Central University of Technology, Free State (29°07'S 26°12'E) in Republic of South Africa (RSA). The temperature in the greenhouse was maintained by a fan, which was triggered at ca. 26° C. Rooted rose geranium cuttings 'Bourbon-type' (ca. 10 cm tall) were sourced and obtained from a reputable commercial grower (Siyakholwa Development Foundation, RSA). These rooted cuttings were transplanted into 5 L potting bags filled with a sterile silica-sand root-medium, with a standard average grain size diameter of 2 mm. A single rooted cutting was transplanted into each potting container.

Water analysis was conducted during the growing season and taken into account during the formulation of the nutrient solution. Plants were fertigated three times per day (8:00 am, 12:00 pm, and 4:00 pm) using a 'drain to waste' drip irrigation system, where each dripper supplied 2 L/h of water and nutrient solution (Khetsha et al., 2020). The electric conductivity (EC) and pH of the nutrient solutions were maintained at 1.6 mS/cm and 5.5, respectively. Using a pH and EC meter (Hanna HI 98129 Digital meter), the desired pH and EC levels were achieved by using nitric acid and adjusting the nutrient solution concentration to reach the desired EC (Combrink, 2019). No phytophagous pests were documented during the experiment, however, Malasol (an organophosphate) was preventatively sprayed at 1.75 ml/L throughout the cropping seasons. These applications were repeated for three to six days, at four-week intervals.

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Treatments

A 3 x 4 factorial design, arranged in a randomized complete block design was used, and had three replications. Treatments consisted of three hail damage simulation levels (0% [non damaged plants], 50%, and 100% leaf defoliation). The levels of biostimulants evaluated for are illustrated in *Table 1*.

Table 1. *Treatment levels used as biostimulants-mixtures in this study*

Component	Biostimulants-mixtures treatments (mg/kg)			
	Level 1	**Level 2**	**Level 3**	**Level 4**
Gibberellic acid	Zero	1.26	2.55	3.83
Brassinosteroids	Zero	0.51	1.02	1.53
Cytokinin (traces)	Zero	0.025	0.05	0.075

Hail damage was simulated 61 days after transplanting days using manually-operated garden secateurs, following the procedures described by Khetsha et al. (2021) at ca. 14:00. The 50% hail damage simulation level was achieved by defoliating the top half (average plant height per experimental unit) of each experimental plant (Obeso, 2002). The 100% hail damage simulation treatment was achieved by the total removal of all leaves (Changnon, 1999; Obeso, 2002). This was followed by the removal of stem terminal buds and random bruising of the main stem and lateral branches (using a pair of hand secateurs) on the same day. Plants were subsequently sprayed with a fine water mist, using a nozzle calibrated precision sprayer (0.3 MPa pressure), to simulate humid summer afternoon conditions (Irigoyen et al., 2010; Bal et al., 2014). Subsequently, biostimulants application treatments followed same on the day, an hour after simulated hail damage. A full cover spray of approximately 50 mL of biostimulants treatment solution was applied to each plant. Treatments were subsequently repeated every 14 days between 13:00 and 14:00, and this continued until harvesting that occurred on 31 December 2019.

Parameters

Plant height was determined for six plants in each treatment unit, a day before harvesting using the procedures described by Wood and Roger (2000). The number of branches for each plant was determined during harvesting by counting the number of shoots developing from the main stem, continuing to the last top node (both old and new shoots), following the procedure of Pérez-Harguindeguy et al. (2013). The B:H ratio was used as an indication of bushiness, by dividing the number of branches by plant height (Sedibe, 2012). The plant stand loss, which is the difference between the initial plant stand and the stand after recovery, was also determined (Sij et al., 2005).

Two plants were randomly selected from each treatment to determine the leaf area: all leaves were removed, and the leaf area for each plant was measured using a LI3000 leaf area meter (LI-COR Inc., USA). LI3000 leaf area meter follows a destructive methods to analyse the leaf area, therefore, only two plants where used from six plants to compensate for other destructive sampling for other parameters. Before harvesting, the chlorophyll content was determined using a portable non-destructive chlorophyll meter (Opti-sciences CCM 200, USA), according to the procedure described by Chen and Black (1992). Readings were taken randomly from the upper-six fully-developed leaves of the crop.

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Data for the external leaf morphology was collected from the new fourth leaf from the apical bud, seventy-five days following the recovery of simulated hail-damaged rose geranium plants. Data was collected on a cloudless day, between 10:00 am and 11:00 am. Because the younger leaves have a denser indumentum than the older ones, only the uppermost young fully expanded leaves were selected (Oosthuizen and Coetzee, 1983; Tozin et al., 2015). A sample (ca. 1 cm^2) was cut from the middle part of the leaf blade with a surgical blade and prepared for scanning electron microscopy (SEM - Shimadzu SSX-550; Kyoto, Japan) following the procedures by Aly et al. (2021). The morphology and density of trichome groups were determined from digital photos obtained from a computer connected to the SEM. Photos were analysed with Photoshop 7 Savvy (Sybex San Francisco, USA), then two-dimensional selections were made using the SEM scales printed on the photos.

Plant foliar fresh mass (FFM) was determined following the procedures of Wood and Roger (2000) by weighing foliar fresh plant material with a PGL 2002 Adam scale (USA). Rose geranium essential oil was extracted from three plants using a custom-built steam distillation unit (Sedibe, 2012). Approximately 2 kg - 5 kg fresh plant material was distilled at ca. 98°C for one hour. Oil mass (yield) was determined by weighing the oil volume using a PGL 2002 Adam scale (USA) immediately following extraction, as described by Swamy and Rao (2009).

The extracted essential oil was analysed using gas chromatography (GC) (Agilent 7890B), equipped with a 30 mm x 0.25 mm x 0.25 μm column (Agilent 19091S 433 UI, HP5-MS UI) and a mass selective detector (Agilent 5977A) as described by Sedibe (2012). The compounds were identified using the NIST11 mass spectral library (https://www.nist.gov/system/files/documents/srd/Ver20Man.pdf). The ISO standard (ISO 4731 2012) was used to characterise rose geranium ('Bourbon-type' cv.) essential oil quality parameters for the perfumery industry.

The relative growth rate (RGR) of the main stem and the free proline parameters are described in detail. The RGR was measured every week, measurement only commenced after the first application of biostimulants (Pérez-Harguindeguy et al., 2013). Weekly plant height data were used to determine the RGR (cm cm^{-1}/day) by means the following formula:

$$RGR = In\,(height2) - In\,(height1)/\,time2 - time1 \qquad \text{(Eq.1)}$$

where: *in (height1)* = height at the start of the interval; *in (height2)* = height at the end of the interval; *time1* = time at the start of the interval (in days); *time2* = time end of the interval (Meyer, 1998).

Free proline was extracted and analysed using a ninhydrin-based method (Gibon et al., 2000; Carillo and Gibon, 2011):

$$Proline = (Abs\,extract - blank)/slope * Vol\,extract/Vol\,aliquot * 1/FW \qquad \text{(Eq.2)}$$

where: *Abs extract* is the absorbance of the extract, the absorbance of the *blank*, and the *slope* (expressed as absorbance/nmol) is determined by linear regression, *Vol extract* is the total volume of the extract, *Vol aliquot* is the volume used in the assay and FW (expressed in mg) is the amount of plant material extracted.

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Statistical analysis

All parameters were statistically analysed and compared using PROC GLIMMIX, SAS version 9.4 (PROC GLIMMIX, SAS Institute 2013). Significantly different means among the treatments were separated using Tukey's least significant difference *ad hoc* mean comparison tests, at the 0.05 level of significance (Steel and Tourie, 1980). The data was then subjected to an appropriate analysis of variance (ANOVA) to determine the effects of the tested factors and their interactions. The Shapiro-Wilks test was performed on standardised residuals to test for any deviations from normality (Shapiro and Wilk, 1965).

Results

Plant parameters, leaf trichomes and essential oil yield attributes

The number of branches, branches to height ratio (B:H ratio), leaf area, and chlorophyll content were not affected by interactions between simulated hail damage and the subsequent application of biostimulants-mixture (*Table 2*). Only the plant height was affected by interactions between simulated hail damage and the subsequent application of biostimulants ($P<0.001$) (*Table 2*). Intact plants tended to grow taller when biostimulant-mixtures (all levels) were applied, compared to the control: only level 2 was not statistically different from the control. In the 50% defoliated plants, plant height was similar with all biostimulant treatments, including the control. In 100% defoliated plants, the results were inconsistent between the levels: a marginal increase in growth was recorded when the biostimulant-mixture was applied at level 3 compared to the control; however, this application was not statistically different to levels 2 and 4.

The number of branches per plant declined by 37.9% when the simulated hail damage was intensified up to 100% defoliation compared to 0% defoliation (intact plants) (*Table 2*). The significant decline in number of branches per plant significantly affected the FFM, which declined by 56.1% when the simulated hail damage was intensified up to 100% defoliation compared to 0% defoliation (*Table 2*). Intact plants continued with normal straight elongation of the terminal shoots (*Figure 1*); however, this was not the case in 50% and 100% simulated hail-damaged plants, in which the number of branches were affected. Branches of the 50% and 100% defoliated plants tended to be plagiotropic, with the terminal bud tips drying and not showing any signs of recovery (*Figure 1*). Plants that suffered 100% simulated hail damage were less bushy, with significantly smaller leaves compared to the intact plants and those that suffered 50% simulated hail damage. This is shown by the lower B:H ratio and smaller leaf area in *Table 2*.

The application of biostimulant-mixtures at levels 3 and 4 increased the FFM for all hail damage levels (*Table 2*): this could also be ascribed to the proline content (*Figure 2*). A significantly lower proline content was recorded when levels 3 and 4 were applied, compared to the control and the level 2 treatment ($P<0.02$).

The stem RGR was affected by the interaction between hail damage simulation and time (week factor) (*Table 3, Figure 3*). During the first week, the rate of stem development did not differ between the simulated hail damage intensities. The RGR tended to vary significantly from the second week until the sixth week for all hail damage levels; however, this trend remained the same throughout the recovery period. A consistent RGR was recorded on intact plants; however, a relatively constant slow RGR was recorded on plants that endured simulated hail damage.

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Table 2. *The effects of hail-simulated damage and the application of biostimulants-mixtures on selected plant growth parameters of rose geranium*

Treatments	Plant height	№ of branches	B:H ratio	Leaf area	Chlor. (%)	FFM
Simulated hail damage						
0% defoliation	59.10^{a}	32.51^{a}	55.003^{a}	44.61^{a}	14.65^{a}	692.60^{a}
50% defoliation	47.37^{b}	27.53^{b}	58.11^{a}	42.33^{a}	14.66^{a}	556.04^{b}
100% defoliation	46.45^{b}	20.18^{c}	44.44^{b}	27.82^{b}	13.33^{a}	304.08^{c}
P-value	*0.001	*0.002	*0.03	*0.001	0.18^{ns}	*0.01
Biostimulant (Bio.)						
Level 1	44.93^{b}	23.37^{a}	52.00^{a}	35.51^{a}	13.63^{a}	401.80^{b}
Level 2	51.83^{a}	25.21^{a}	48.64^{a}	38.15^{a}	14.63^{a}	505.55^{a,b}
Level 3	55.16^{a}	27.82^{a}	50.54^{a}	39.92^{a}	14.57^{a}	554.58^{a}
Level 4	51.80^{a}	30.50^{a}	60.88^{a}	39.44^{a}	14.03^{a}	608.35^{a}
P-value	*0.001	0.08^{ns}	0.22^{ns}	0.29^{ns}	0.66^{ns}	*0.001
HD x Bio. levels						
0% x Level 1	51.75^{b,c}	29.36^{a}	56.72^{a}	42.74^{a}	11.86^{a}	565.41^{a}
0% x Level 2	57.41^{a,b}	32.25^{a}	56.17^{a}	43.75^{a}	17.15^{a}	717.50^{a}
0% x Level 3	64.08^{a}	35.75^{a}	55.78^{a}	46.61^{a}	14.58^{a}	759.16^{a}
0% x Level 4	63.16^{a}	32.69^{a}	51.73^{a}	45.36^{a}	15.21^{a}	728.33^{a}
50% x Level 1	43.58^{c,d}	24.50^{a}	56.21^{a}	38.63^{a}	14.16^{a}	446.25^{a}
50% x Level 2	51.50^{b,c}	25.64^{a}	49.78^{a}	42.13^{a}	14.88^{a}	546.66^{a}
50% x Level 3	50.08^{b,c,d}	26.77^{a}	53.45^{a}	43.51^{a}	17.06^{a}	582.91^{a}
50% x Level 4	44.33^{c,d}	33.22^{a}	74.93^{a}	45.04^{a}	12.54^{a}	648.33^{a}
100% x Level 1	39.83^{d}	16.25^{a}	40.79^{a}	25.17^{a}	12.08^{a}	193.75^{a}
100% x Level 2	46.58^{c,d}	17.75^{a}	38.10^{a}	28.56^{a}	11.86^{a}	252.50^{a}
100% x Level 3	51.33^{b,c}	21.14^{a}	41.18^{a}	29.63^{a}	14.35^{a}	321.66^{a}
100% x Level 4	47.91^{b,c,d}	25.61 ±	53.45^{a}	27.94^{a}	15.05^{a}	448.41^{a}
P-value	*0.001	0.46^{ns}	0.54^{ns}	0.99^{ns}	0.22^{ns}	0.39^{ns}

Means followed by the same letter in the same column are statistically non-significant (P<0.05); ns = not significant; * = F-ratio probability of P<0.05. Plant height (cm/plant); Leaf area (cm^2/plant); Chlor. = Chlorophyl; FFM = Foliar fresh mass (g/plant)

Two groups of trichomes were identified: glandular (peltate and capitate) and non-glandular trichomes (attenuate) (*Table 4*). The attenuate trichome type is insignificant to essential oil biosynthesis; therefore, they are also not discussed any further. The peltate type: this type is characterised by a short neck with bigger, rounded tips, and is regarded as a brevicollate trichome, while the capitate type: is characterised by a short, segmented capitate, with a columnar hatchet-shaped tip that has a slightly bent apical cell pointing towards at the leaf apex (Sedibe et al., 2013; Khetsha et al., 2021).

An interaction was observed between simulated hail damage and the subsequent application of biostimulant-mixtures for the asciiform trichome densities on both leaf surfaces of the rose geranium plant (*Table 4*). On intact plants, the densities of the asciiform trichome were significantly (P<0.01) high when biostimulant-mixtures were applied at level 2 on the adaxial leaf surface compared with the control; however, this treatment was not significantly different to level 3. The density of the asciiform trichome started to decline when the biostimulant-mixture was applied from level 3 to level 4.

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When plants sustained 50% simulated hail damage, biostimulant-mixture application produced inconsistent results: a marginally higher density of the asciiform trichomes were recorded when biostimulants-mixture were applied at levels 3 and 4, however, these applications were not significantly different to the control. On the 100% defoliated plants, the asciiform trichome densities declined by 60.6% when biostimulants were applied at level 2 compared with the control.

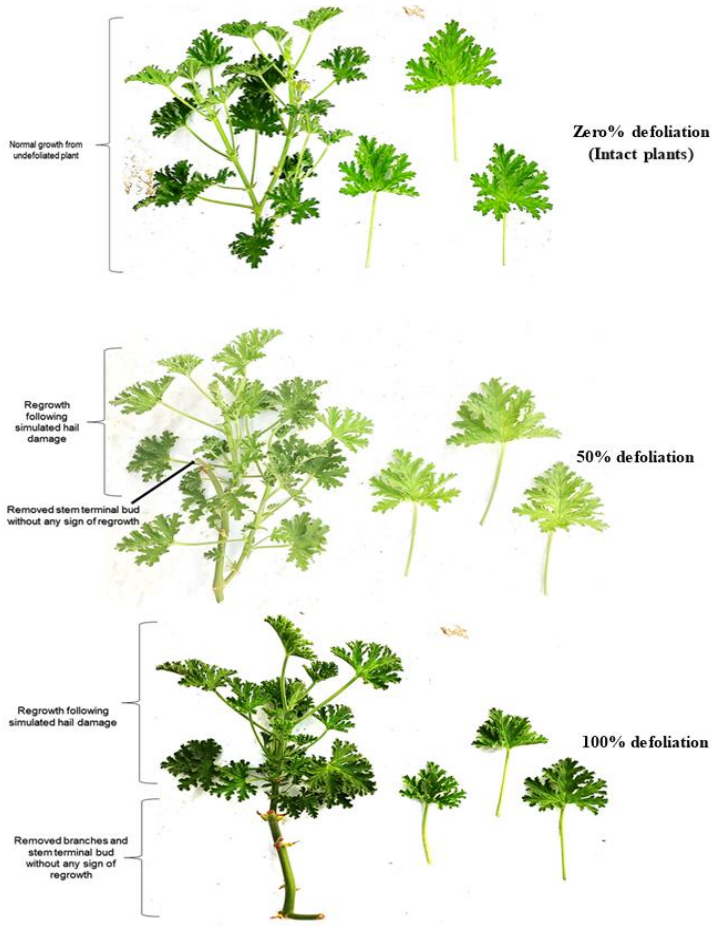

Figure 1. *Terminal buds from simulated hail-damaged rose geranium plant*

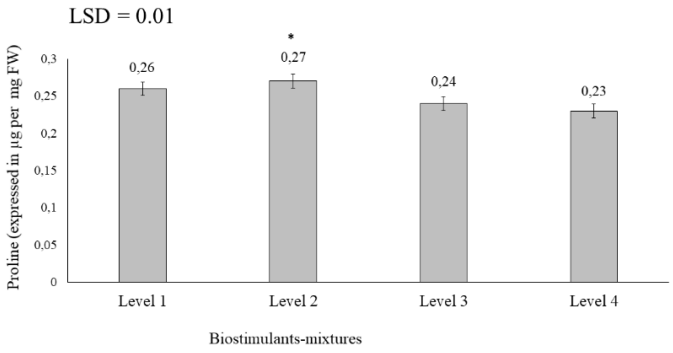

Figure 2. *The effects of biostimulants-mixtures on free proline of rose geranium. Means followed by the same letter in the same column are statistically non-significant (P<0.05); ns = not significant; * = F-ratio probability of P<0.05*

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Table 3. *Summary of ANOVA for the relative growth of the stem (RGR) as affected by hail-simulated damage and the application of biostimulants-mixtures in six weeks on rose geranium plants*

Treatments	Df	*F*-value	*P*-value
Week	5	0.48	0.78
Simulated hail damage	2	5.83	***0.001**
Biostimulants	3	5.01	***0.002**
Week x Simulated hail damage	10	1.96	***0.04**
Week x Biostimulants	15	0.59	0.88
Simulated hail damage x Biostimulant	6	2.06	0.06
Week x Simulated hail damage x Biostimulant	30	0.64	0.92

Means followed by the same letter in the same column are statistically non-significant (P<0.05); ns = not significant; * = F-ratio probability of P<0.05

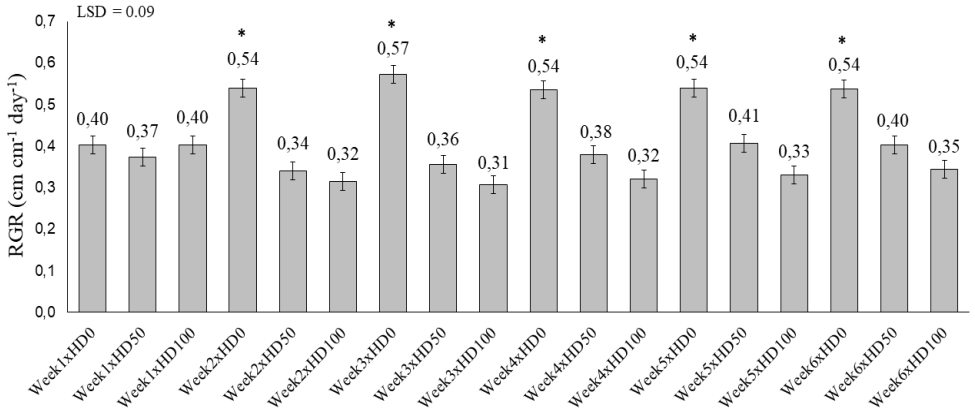

Figure 3. *The effects of simulated hail damage between six weeks on the relative growth rate of rose geranium stems. Abbreviations: HD0 = zero% defoliation; HD50 = 50% defoliation; HD100 = 100% defoliation. Means followed by the same letter in the same column are statistically non-significant (P<0.05); ns = not significant; * = F-ratio probability of P<0.05*

On the abaxial leaf surface, the opposite effect was recorded on refoliating plants that had lost up to 50% defoliation (*Table 4*), with the asciiform trichome densities increasing by 39% when the biostimulants-mixture was applied at level 2. Asciiform trichome densities gradually declined when the biostimulants-mixture was increased from level 2 up to level 4 (P<0.001). The intact plants, and those that sustained 100% simulated hail damage, had similar asciiform trichome densities following all biostimulants treatments, including the control. The asciiform trichome densities were increased by 32.1% on the 50% simulated hail-damaged plants compared to 100% defoliated plants when biostimulants-mixture was applied at level 2.

Brevicollate trichome densities were significantly (P<0.001) higher when biostimulants were applied at level 3 on the adaxial leaf surface (*Table 4*). However, this was not significantly different from level 4. On the abaxial leaf surface, the densities of the brevicollate trichome were affected by the interation between simulated hail damage and the subsequent application of biostimulants-mixtures (*Table 4*). On the intact plants, the densities of brevicollate trichome were higher when the biostimulant-mixtures were applied compared with the control, from level 3 to level 4 (P<0.005). On the 50%

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defoliated plants, the biostimulants-mixture applied at level 4 resulted in marginally higher densities of brevicollate trichomes, compared to the control (P<0.005). With the 100% defoliated plants, all biostimulants treatments were similar to the control.

Table 4. *The effects of hail-simulated damage and the application of biostimulants-mixtures on the leaf surfaces of rose geranium leaves*

Treatments	Adaxial leaf surface		Abaxial leaf surface	
	Asciiform	Brevicollate	Asciiform	Brevicollate
Simulated hail damage				
0% defoliation	27.55^{a}	21.11^{a}	41.33^{a}	47.33^{a}
50% defoliation	24.66^{a}	29.66^{a}	33.77^{b}	44.99^{a}
100% defoliation	15.99^{b}	27.77^{a}	$37.55^{a,b}$	47.22^{a}
P-value	*0.001	0.69^{ns}	*0.001	0.69^{ns}
Biostimulant (Bio.)				
Level 1	22.44^{a}	21.55^{c}	39.55^{a}	30.88^{b}
Level 2	20.33^{a}	$25.33^{b,c}$	39.00^{a}	47.88^{a}
Level 3	25.11^{a}	30.11^{a}	37.66^{a}	55.00^{a}
Level 4	23.00^{a}	$27.55^{a,b}$	33.00^{a}	52.22^{a}
P-value	0.14^{ns}	*0.001	0.80^{ns}	*0.001
HD x Bio. levels				
0% x Level 1	$22.00^{b,c}$	18.33^{a}	48.66^{a}	$27.33^{c,d}$
0% x Level 2	37.66^{a}	21.33^{a}	$35.66^{a,b,c}$	$46.66^{a,b,c,d}$
0% x Level 3	$27.33^{a,b,c}$	24.66^{a}	$41.66^{a,b}$	63.66^{a}
0% x Level 4	$23.33^{b,c}$	20.33^{a}	$38.50^{a,b,c}$	$51.66^{a,b}$
50% x Level 1	$25.00^{b,c}$	24.33^{a}	$29.33^{b,c}$	25.66^{d}
50% x Level 2	$15.33^{c,d}$	30.66^{a}	48.33^{a}	$48.00^{a,b,c,d}$
50% x Level 3	$32.00^{a,b}$	37.00^{a}	$35.00^{a,b,c}$	$47.00^{a,b,c,d}$
50% x Level 4	$26.33^{a,b,c}$	25.00^{a}	22.33^{c}	68.33^{a}
100% x Level 1	$20.33^{c,d,e}$	22.00^{a}	$40.66^{a,b}$	$39.66^{b,c,d}$
100% x Level 2	8.00^{f}	24.00^{a}	$33.00^{b,c}$	$49.00^{a,b,c}$
100% x Level 3	$16.00^{c,d}$	28.66^{a}	$36.33^{a,b,c}$	$54.33^{a,b}$
100% x Level 4	$19.33^{c,d}$	36.33^{a}	$40.00^{a,b}$	$36.66^{b,c,d}$
P-value	*0.001	0.08^{ns}	*0.001	*0.005

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = *F*-ratio probability of $P<0.05$

Essential oil mass and essential oil content were significantly affected by the interaction between reduced simulated hail damage levels and the subsequent application of biostimulants (*Table 5*). In the intact plants, the highest essential oil mass was recorded when biostimulants-mixtures were applied at level 3 (P<0.006); however, the essential oil content was similar in all biostimulants-mixtures levels. In 50% defoliated plants, the essential oil mass was marginally increased (P<0.01) when the biostimulants-mixtures were applied at level 3 compared with the control; however, this was not statistically different from levels 2 and 4. A similar trend was recorded for the essential oil content on the 50% defoliated plants: there were no statistical differences between all biostimulants treatments for both essential oil yield attributes when plants sustained 100% simulated hail damage.

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Table 5. *The effects of hail-simulated damage and the application of biostimulants-mixtures on essential oil mass yield of rose geranium*

Treatments	Oil mass (g/plant)	Oil content (%)
Simulated hail damage		
0% defoliation	3.06^{a}	0.44^{a}
50% defoliation	2.24^{b}	0.39^{a}
100% defoliation	0.55^{c}	0.17^{b}
P-value	*0.001	*0.001
Biostimulant (Bio.)		
Level 1	1.29^{c}	0.27^{b}
Level 2	$1.78^{b,c}$	$0.32^{a,b}$
Level 3	2.45^{a}	0.38^{a}
Level 4	$2.29^{a,b}$	$0.36^{a,b}$
P-value	*0.01	*0.02
HD x Bio. levels		
0% x Level 1	2.44^{b}	$0.43^{a,b,c}$
0% x Level 2	2.51^{b}	$0.35^{a,b,c,d}$
0% x Level 3	3.98^{a}	0.53^{a}
0% x Level 4	$3.31^{a,b}$	$0.45^{a,b}$
50% x Level 1	$1.18^{c,d}$	$0.25^{b,c,d,e}$
50% x Level 2	$2.39^{b,c}$	$0.43^{a,b}$
50% x Level 3	$3.02^{a,b}$	0.52^{a}
50% x Level 4	$2.38^{b,c}$	$0.37^{a,b,c}$
100% x Level 1	0.25^{d}	$0.12^{d,e}$
100% x Level 2	0.44^{d}	$0.19^{c,d,e}$
100% x Level 3	0.35^{d}	0.10^{e}
100% x Level 4	$1.17^{c,d}$	$0.26^{b,c,d,e}$
P-value	*0.01	*0.01

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; * = *F*-ratio probability of $P<0.05$

Essential oil quality

The levels of *cis*-Rose oxide and *trans*-Rose oxide declined by 75% and 80%, respectively, with increasing defoliation intensity, while the linalool content increased in plants that had lost 100% foliage (*Table 6*). In this study, the geraniol esters and the citronellyl formate content were significantly higher in plants that sustained 100% simulated hail damage. The citronellyl formate content accumulated ($P<0.007$) up to 20.2% when biostimulants were applied at level 4; however, this was not significantly different compared to the control. Interactions were observed between simulated hail damage and the subsequent application of different levels of biostimulants for citronellol, geraniol, and the citronellol to geraniol (C:G) ratio (*Table 6*). The highest ($P<0.006$) citronellol content was recorded when biostimulants were applied at level 4 on the intact plants compared with the control; however, this biostimulant level was not significantly different from level 3. On 50% and 100% defoliated plants, the citronellol content was similar in all biostimulant treatments. Although citronellol and geraniol share a similar biosynthetic pathway, the geraniol results were not the same: the geraniol content of the

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intact plants, and those that suffered 50% simulated hail damage were not significantly affected by the subsequent application biostimulants, at any treatment level (*Table 6*). In the 100% defoliated plants, a higher (P<0.02) geraniol content was recorded when the level 2 treatment was applied compared with the control: however, this treatment level was not significantly different to level 4.

Table 6. *The effects of hail-simulated damage and the application of biostimulants-mixtures on essential oil compounds and quality of rose geranium*

Treatments	*cis*-RO	*trans*-RO	Isom.	Lin.	Geranyl formate	Geranyl butyrate	Geranyl tiglate	Citronellyl formate	Guaia-6,9-diene	Citronellol	Geraniol	C:G ratio
Simulated hail damage												
0% defoliation	0.20^{a}	0.10^{a}	1.75^{a}	0.41^{b}	5.40^{b}	1.01^{b}	1.93^{b}	18.71^{b}	11.35^{a}	23.23^{a}	8.37^{a}	2.77^{a}
50% defoliation	0.13^{b}	0.06^{b}	1.83^{a}	0.63^{a}	5.85^{b}	0.93^{b}	1.98^{b}	18.65^{b}	11.92^{a}	24.26^{a}	8.98^{a}	2.70^{a}
100% defoliation	0.05^{c}	0.02^{c}	1.07^{a}	0.76^{a}	8.37^{a}	1.30^{a}	2.74^{a}	19.87^{a}	12.23^{a}	19.97^{b}	9.11^{a}	2.19^{b}
P-value	*0.001	*0.01	0.10^{ns}	*0.009	*0.001	*0.002	*0.001	*0.001	0.20^{ns}	*0.003	0.13^{ns}	*0.001
Biostimulant (Bio.)												
Level 1	0.11^{a}	0.05^{a}	1.36^{a}	0.61^{a}	6.46^{a}	1.16^{a}	2.32^{a}	19.39^{a}	11.86^{a}	20.26^{b}	8.14^{c}	2.48^{a}
Level 2	0.15^{a}	0.08^{a}	1.91^{a}	0.67^{a}	6.40^{a}	0.99^{a}	1.97^{a}	18.23^{b}	11.59^{a}	23.50^{a}	9.76^{a}	2.40^{a}
Level 3	0.13^{a}	0.06^{a}	1.32^{a}	0.54^{a}	6.61^{a}	1.15^{a}	2.42^{a}	18.52^{b}	12.16^{a}	23.31^{a}	8.30^{a,c}	2.81^{a}
Level 4	0.12^{a}	0.05^{a}	1.71^{a}	0.61^{a}	6.72^{a}	0.99^{a}	2.04^{a}	20.15^{a}	11.67^{a}	23.28^{a}	9.24^{a,b}	2.51^{a}
P-value	0.60^{ns}	0.34^{ns}	0.58^{ns}	0.56^{ns}	0.95^{ns}	0.18^{ns}	0.14^{ns}	*0.007	0.12^{ns}	*0.02	*0.03	0.09^{ns}
HD x Bio. levels												
0% x Level 1	0.17^{a}	0.08^{a}	2.00^{a}	0.80^{a}	5.84^{a}	1.23^{a}	2.33^{a}	18.32^{a}	11.29^{a}	17.36^{d}	9.15^{a,b}	1.89^{f}
0% x Level 2	0.22^{a}	0.11^{a}	2.23^{a}	0.73^{a}	5.62^{a}	0.97^{a}	1.79^{a}	18.33^{a}	11.26^{a}	24.24^{b}	8.83^{b}	2.75^{b,c,d}
0% x Level 3	0.20^{a}	0.10^{a}	0.99^{a}	0.69^{a}	5.31^{a}	1.01^{a}	1.91^{a}	18.72^{a}	11.47^{a}	24.99^{a,b}	7.84^{b}	3.18^{a,b}
0% x Level 4	0.22^{a}	0.10^{a}	1.78^{a}	0.88^{a}	4.56^{a}	0.78^{a}	1.57^{a}	19.87^{a}	11.38^{a}	27.87^{a}	7.30^{b}	3.81^{a}
50% x Level 1	0.13^{a}	0.06^{a}	1.34^{a}	0.68^{a}	5.60^{a}	1.02^{a}	2.03^{a}	19.44^{a}	11.89^{a}	24.26^{b}	7.65^{b}	3.17^{b,c}
50% x Level 2	0.13^{a}	0.06^{a}	1.65^{a}	0.71^{a}	6.48^{a}	0.94^{a}	1.96^{a}	17.93^{a}	11.90^{a}	23.85^{b}	9.37^{a,b}	2.54^{c,d,e}
50% x Level 3	0.15^{a}	0.07^{a}	2.11^{a}	0.56^{a}	5.53^{a}	0.89^{a}	1.92^{a}	17.81^{a}	12.09^{a}	25.09^{a,b}	8.78^{b}	2.85^{b,c,d}
50% x Level 4	0.11^{a}	0.05^{a}	2.24^{a}	0.60^{a}	5.77^{a}	0.89^{a}	2.03^{a}	19.41^{a}	11.88^{a}	23.86^{b}	10.11^{a,b}	2.36^{d,e,f}
100% x Level 1	0.04^{a}	0.01^{a}	0.73^{a}	0.35^{a}	7.93^{a}	1.27^{a}	2.74^{a}	20.43^{a}	12.65^{a}	19.17^{d,c}	7.61^{b}	2.51^{b,c,d,e}
100% x Level 2	0.08^{a}	0.05 ^{a}	1.83^{a}	0.53^{a}	7.46^{a}	1.10^{a}	2.27^{a}	18.53^{a}	11.61^{a}	21.86^{b,c}	11.74^{a}	1.86^{f}
100% x Level 3	0.03^{a}	0.01^{a}	0.85^{a}	0.36^{a}	8.99^{a}	1.55^{a}	3.42^{a}	19.02^{a}	12.92^{a}	19.85^{d,c}	8.29^{b}	2.39^{d,e,f}
100% x Level 4	0.06^{a}	0.03^{a}	1.14^{a}	0.46^{a}	8.79^{a}	1.27^{a}	2.37^{a}	21.07^{a}	11.66^{a}	19.65^{d,c}	9.68^{a,b}	2.02^{e,f}
P-value	0.71^{ns}	0.53^{ns}	0.55^{ns}	0.76^{ns}	0.07^{ns}	0.18^{ns}	0.14^{ns}	0.25^{ns}	0.30^{ns}	*0.006	*0.02	*0.01

Means followed by the same letter in the same column are statistically non-significant ($P<0.05$); ns = not significant; RO = Rose oxide; Iso. = Isomenthone; Lin. = Linalool; * = *F*-ratio probability of $P<0.05$

As expected, changes in citronellol and geraniol content affected the C:G ratio (*Table 6*). The perfumery industry prefers a C:G ratio of <3:1 (Saxena et al., 2008). In the intact plants, the C:G ratio was higher than 3:1 when biostimulants-mixtures were applied at levels 3 and 4 (P<0.01). In the 50% defoliated plants, the lowest C:G ratio was recorded when biostimulants were applied at level 4, compared to the control. On plants that suffered 100% simulated hail damage, the C:G ratio was lower when the level 2 treatment was applied, compared to the control (P<0.01). A significantly lower C:G ratio (<3:1) was recorded on all defoliated plants (50 & 100%) compared to the intact plants (P<0.01).

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Discussion

Taller plants in this study after addition of biostimulants could be ascribed to increased plasticity of the cell wall, regulated by the action of GA and BRs (Yamaguchi et al., 2010): Matusmoto et al. (2016) indicated that GA and BRs also regulate stem elongation. Therefore, the accumulation of BRs following the biostimulant application could have facilitated GA to promote stem elongation. According to Sun (2010), an increase in cell elongation and cell division occurs during stem growth because GA induces the transcription of genes involved in growth processes. Dayan et al. (2012) found that endogenous concentrations of bioactive GA in young internodes of defoliated tobacco plants (*Nicotiana tabacum* L.) were reduced by 60% compared to those of intact plants, however, 0.8 mg/L GA restored the cambium and xylem fibre differentiation. In the 100% defoliated plants, taller plants could be attributed to the action of BRs, since this also regulates abiotic stress (Yamaguchi et al., 2010). The stem terminal buds were removed following defoliation to simulate hail bruising in this study, therefore, the accumulation of BRs could have been slower in the stem as the plant recovered from defoliation.

Defoliation and the removal of the shoot apex through hail damage simulations may affect the apical dominance, and stimulate the growth of branches from auxiliary buds (Prins and Verkaar, 1992). Therefore, the alteration of branching patterns following apex removal is not uncommon, as was the case with the simulated hail-damaged plants (50% and 100%) in this study. This could be due to delayed plant growth, as auxiliary branches must first form new leaves before the photosynthetic rate can reach the same levels as pre-defoliation (Prins and Verkaar, 1992).

As expected, the B:H ratio decreased in plants that sustained 100% defoliation. The removed stem terminal buds showed no signs of regrowth (*Figure 1*). This could be attributed to the nutrients and carbohydrates requirements between the auxiliary buds and the shoot apex, since plants need to recuperate following defoliation (Prins and Verkaar, 1992). In this study, the bushiness of rose geranium plants did not recover following the application of biostimulants.

Plant leaf development follows a standard basic sequence that is flexible and adjusted according to species, developmental stage, and environmental conditions (Bar and Ori, 2014). Meyer (1998) also reported that the plant response to extreme defoliation is affected by its capacity to draw upon sufficient resources to maintain growth recovery, which directly affects the leaf maturation process. In a study with perennial ryegrass (*Lolium perenne* L.), decreased leaf area could be ascribed to decreased cell production and expansion. In this study, the intact and 50% simulated hail-damaged plants had a significantly higher number of mature leaves at harvest, compared to plants that endured 100% simulated hail damage (*Figure 1*). Therefore, the reduction in the leaf area in the 100% simulated hail-damaged plants could be because of reduced photosynthetic capacity following complete defoliation (Bar and Ori, 2014). Contrary results were reported for goldenrod (*Solidago altissima* L.), where refoliating plants had bigger leaves compared to the control plants (Meyer, 1998).

According to Pérez-Harguindeguy et al. (2013), agronomic parameters such as plant height, the number of branches, leaf area, and FFM are essential parameters in determining the morphological characteristics and yield components of indeterminate crops, such as rose geranium. Reduced FFM following simulated hail is due to fewer branches per plant, a reduced number of branches per meter, and a smaller leaf area. This confirms the hypothesis of resultant decrease in biomass and related attributes where rose geranium plants endure extreme simulated hail damage intensity (Swamy et al., 1960;

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Dermane and van der Walt, 1989; Weiss, 1997). Similar findings were recorded in a study by Bueckert (2011) on lentils (*Lens culinaris* L.), which is also an indeterminate crop species.

Plants accumulate proline as an adaptive mechanism for stress, but it is also beneficial under normal plant growth conditions (Zhang and Becker, 2015). Besides its proteogenic function, proline also plays a role in energy utilization (Hare and Cress, 1997), protein unfolding (Liang et al., 2013), as well as cell reprogramming and development (D'Aniello et al., 2015). Moreover, recent studies have also shown that proline plays a vital role in plant growth and differentiation at different growth stages (Kishor et al., 2015). In this study, the application of biostimulants could have increased energy utilization, and improved cell development, subsequently increasing the FFM (Hare and Cress, 1997). Therefore, results on the increased FFM and declined proline content could be ascribed to the biostimulants and proline in the chloroplast and cytoplasm. In addition, Li et al. (2012) reported that a BRs and GA interact at the signalling level to promote plant growth and development. This occurs through the regulation of BRs, which directly affects plant growth by modulating GA levels. Amiri et al. (2014) reported that FFM was significantly increased by the application of GA (100 ppm) on German chamomile (*Matricaria recutita* L.).

Growth rate, as an indicator of life history and ecological strategy, may be crucial to post-defoliation recovery strategies. According to Atkinson et al. (2014), plants deploy stored resources to rebuild photosynthetic material following defoliation. Meyer (1998) reported similar results on goldenrod, where less damaged plants recovered more rapidly than completely defoliated plants. In this study, fortnightly applications of biostimulants did not affect the rate of relative stem growth (*Table 3*).

In this study, the densities of asciiform trichomes on both leaf surfaces differed between the simulated hail damage levels. Generally, the intact plants, and those that suffered 50% foliage loss on both leaf surfaces recorded higher asciiform trichome densities than those that suffered 100% simulated hail damage. Prins et al. (2010) also demonstrated that the development of trichomes, and the biosynthesis of essential oil could be influenced by exogenous applications of biostimulants. GA positively contributes to the regulation of trichome development (Zhou et al., 2013). According to Traw and Bergelson (2003), GA modifies the epidermal cell surface area, and also the cell number per unit area. However, in this study, the activities associated with asciiform trichome development on refoliated plants could be ascribed to *TEM*s (Jiao, 2016). *TEM*s regulate the signalling of CK at the later developmental stage compared to GA, signalling in earler developmental stage. CK is a plant growth regulator that also promotes trichome formation under normal growth conditions. Moreover, since BRs and jasmonic acid are directly involved in the formation and variation of trichome density (Campos et al., 2009): the *dpy* mutant (BR-deficient) is the one which enhances pubescence, while the jasmonic acid *jai1-1* mutant produces the opposite phenotypic effect (Campos et al., 2009; Fambrini and Pugliesi, 2019). The Arabidopsis *bls1* mutant, which is impaired in the BR response, developed fewer trichomes on both the abaxial and adaxial leaf surfaces, indicating the possible involvement of BR in trichome development (Pattanaik et al., 2014). There are no studies on refoliating plants treated with biostimulant. Therefore, the changes in asciiform trichome densities is attributed to the traces of CKs.

The increased density of brevicollate trichomes on the intact plants and those suffering 50% simulated hail damage could also be ascribed to the GA and CK in the biostimulants-mixture. Zhou et al. (2011, 2013) reported similar findings in Arabidopsis (*Arabidopsis thaliana* [L.]) Heynh.), where GA and CK, at concentrations as low as 100 µM, increased

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the density of glandular trichomes. According to the author, this was ascribed to GA and CK molecules, which regulated the development of glandular trichome through the combined action of *ZFP5* and *ZFP6* transcription factors (Zhou et al., 2013). As for the 50% defoliated rose geranium, plants deploy stored resources to rebuild photosynthetic material and regenerate new organs or tissues following defoliation and wounding (Stobbe et al., 2002; Nanda and Melnyk, 2018). During the refoliation, it is possible that the endogenous CK content in 50% defoliated plants was already too high; then GA accumulation occurred at the later stage to regulate growth. GA and CK could have combined during these periods via antagonistic crosstalk effects following the fortnightly applications of biostimulants. According to Barnes (2013), the amalgamation of CK and GA following hail damage may cause alterations in morphological features, such as increased trichome density. Other than GA and CK, BRs and jasmonic acid directly affect trichome formation through the accumulation of *zgb* and *PI-I* transcripts, indicating the importance of BRs in leaf recovery following defoliation or wounding (Campos et al., 2009). This was not the case for the 100% defoliated plants, as most energy was focused on recovering plant leaf material.

Asciiform trichome type is characterised by a short segmented capitate with a columnar hatchet-shaped tip, that has a slightly bent apical cell pointing at the leaf apex (Payne, 1978). On the other hand, breviocollate trichomes are characterised by a short neck with bigger round tips (Payne, 1978). Eiasu et al. (2009) reported that the capitate group (asciiform trichome) lack stored essential oil in their sub-cuticular areas compared to the brevicollate trichome. Thus, in this study, biostimulants-mixture should be applied at a rate of level 3 on rose geranium plants that have lost up to 50% foliage to increase the densities of the brevicollate trichomes, with the aim of improving the essential oil yield.

Biostimulants stimulate plant growth and terpene biosynthesis in a large number of aromatic plant species, which result in beneficial changes in terpene accumulation (Farooqi and Shukla, 1990). There are no reports in the literature where a combination of GA, BRs, and CK affected essential oil yield attributes of refoliating plants, however, Poyh and Ono (2006) recorded higher essential oil content for sage (*Salvia officinalis* L.) treated with 100 mg/L GA. Fraternale et al. (2003) reported a higher essential oil yield of Spanish marjoram (*Thimus mastichina* L.) using a medium culture with CK as low as 0.1 mg/L. In the leaves of Spanish marjoram treated with CK, there were a greater number of glandular trichomes recorded at the later leaf developmental stage, which could be ascribed to increased essential oil yield. Foliar application of a plant growth regulator-28-homobrassinolide (10^{-6} M) also enhanced the essential oil yield of mint (*Mentha arvensis* L.) (Naeem et al., 2014). In this study, it is evident that only the 50% hail-damaged plants yield higher essential oil, following treatment with biostimulants at level 3.

Croteau et al. (2000) reported that molecules could change before undergoing enzymatic oxidation, with a vast array of compounds, of remarkably different structures formed under various environmental conditions. *cis*-Rose oxide and *trans*-Rose oxide can be synthesised from the conversion of citronellol, through citronellyl diphosphate (phosphorylated citronellol) in rose geranium (Wüst et al., 1999). This may also occur because of a photo-oxygenation mechanism, including singlet oxygen as the oxidising agent (Yamaguchi, 1981). In this study, the enzymatic oxidation of citronellol could have gradually declined as a result of defoliation, directly affecting the cyclization of the diol to *cis*-Rose oxide and *trans*-Rose oxide (Wüst et al., 1999). Takana et al. (2010) reported

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that when plant tissues undergo physio-morphological changes, such as wounding or defoliation, some chemical compounds are converted to secondary polyphenols by enzymatic and non-enzymatic reactions. This could have been the case in this study; however, there are no similar cases in the literature to support this hypothesis.

Linalool content tends to increase with an increase in the intensity levels of defoliation and wounding, and this could be due to the ontogeny of the leaf factor (Maia et al., 2007). Severely simulated hail-damaged plants could contain younger leaves at the time of recovery, compared to intact and those plants with at least 50% leaf loss. With the ontogeny of the leaf factor, this metabolic process affect the primary and secondary metabolic processes during refoliation. This was demonstrated on balsam fir (*Abies balsamea* L.), where linalool content was higher in defoliated plants compared to intact plants (Deslauriers et al., 2015). In sweet basil (*Ocimum basilicum* L.), younger leaves had higher linalool content compared to mature leaves (Fischer and Hammerschmidt, 2011).

Citronellol shares a similar biosynthesis pathway with geraniol and its esters (Bergman et al., 2019). Chacón et al. (2019) reported that the biotransformation of geraniol occurs through endogenous enzymatic changes. On the intact plants in this study, increased citronellol content could be ascribed to the exogenous application of agricultural biostimulants (Suga and Shishibori, 1973). The results on increased geraniol in this study could be ascribed to leaf ontogeny as well as the effects of GA in the biostimulants-mixture. According to Prins et al. (2010), plants produce essential oils in response to physiological stress, pathogen attack, and other ecological factors. Therefore, simulated hail damage in this study could be responsible for increased geraniol content. Geraniol content fluctuates significantly during leaf development, which subsequently affects its esters and citronellol biosynthesis (Chacón et al., 2019). In this study, 100% simulated hail-damaged plants had younger leaves compared to intact plants at the time of harvest. It is also possible that as plants refoliated, the fortnightly-applied biostimulants could have also affected the biosynthesis of the geraniol. According to Burlat et al. (2004), geraniol and GA share a similar biosynthetic route: the mevalonic acid pathway. This pathway could have been affected at a lower concentration of biostimulants treatment (level 2), and improved the accumulation of geraniol and citronellol as plants refoliated. However, an increase in the biostimulants-mixture up to level 3 & 4 could have led to the toxicity of the GA, causing the decline of the geraniol content.

The C:G ratio is used to classify rose geranium essential oil quality, and is a requirement of the perfumery industry and ISO standards (Saxena et al., 2008). Biosynthesis of citronellol and geraniol affected the C:G ratio in this study. The higher citronellol and low geraniol contents observed in this study after the application of biostimulants could be related to the metabolic pathway of geraniol biosynthesis, GA concentration, and the leaf ontogeny (Suga and Shishibori, 1973; Burlat et al., 2004; Motsa et al., 2006). In this study, it may be beneficial to apply mixtures of biostimulants at level 2 only when plants have suffered complete defoliation, resulting in a lower and favourable C:G ratio <3. This may improve the essential oil quality.

Geranyl formate, geranyl butyrate, and geranyl tiglate are geraniol esters (Liu et al., 2016). The equal accumulation of these esters could be attributed to the biosynthesis of geraniol, and leaf development (Motsa et al., 2006). Plant defoliation affects leaf ontogeny and biosynthesis of secondary metabolites (Burlat et al., 2004; Motsa et al., 2006). Therefore, the conversion rate of geraniol into geraniol esters could have differed between leaf developmental stages in this study. Ganjewala and Luthra (2009) reported

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similar findings, with geraniol esters declining from 59% to 3% during the leaf growth period from day 10 to day 50 after transplanting. In addition, Motsa et al. (2006) also demonstrated that geraniol content declines as rose geranium plants age. Therefore, it may be concluded that the variations between the geraniol esters changed with leaf age factor, especially following the defoliation treatment.

Citronellyl formate is an ester to citronellol. Both constituents, along with geraniol and its esters, share the same chemical structure (citronellyl moiety) and biosynthetic pathway (Lis-Balchin, 2002; Sedibe, 2012; Bergman et al., 2019). In this study, citronellol, as well as geraniol and its esters, were improved by leaf ontogeny factor. Interestingly, this was also the case with citronellyl formate: Malatova et al. (2011) reported a similar situation for rose geranium, where a change in citronellol content affected the citronellyl formate content. Therefore, refoliating plants could have accumulated high content of these compounds (citronellol and geraniol esters), including citronellyl formate.

Conclusion

In this study, simulated hail damage affected the recovery and growth of the measured rose geranium foliage parameters. However, it can be concluded that the application of a mixture of biostimulants should be applied at 2.55 mg/kg (GA), 1.02 mg/kg (BRs), and 0.05 mg/kg (CKs) (level 3 in this study) in order to improve the foliage yield and essential oil quality (to <3, as per industry standards) for plants suffering more than 50% foliage loss. Plants with less than 50% foliage loss can be treated with a lower level of biostimulants: level 2 to level 3 in this study, to improve the essential oil yield and brevicollate trichome density.

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