

The Effects of Paclobutrazol on the Vegetative and Reproductive Traits of *Pycnanthemum*¹

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Abstract

Pycnanthemum is a relatively new market introduction. Due to a lack of existing production procedures, growers may hesitate to work with the genus. Determining optimal plant growth regulator concentrations could make the plant more accessible. In the study, *P. virginianum* were drenched with 0, 0.5, 1, 2, 4, 8, and 16 mg a.i./pot of paclobutrazol (Bonzi®), while *P. flexuosum* were drenched with 0, 6, and 8 mg a.i./pot. The height, growth index, shoot dry weight, density, and inflorescence count were measured to compare growth between treatments. The height was controlled in all treatments except *P. virginianum* treated with 0.5 mg a.i./pot. The inflorescence count and growth index of *P. virginianum* increased at lower concentrations, while the shoot dry weight decreased at higher concentrations. *Pycnanthemum virginianum* plants treated with 8 mg a.i./pot had increased density (mg·cm⁻³). The height, inflorescence count, and growth index of *P. flexuosum* decreased when treated with paclobutrazol. Treating *P. flexuosum* with 6 mg a.i./pot decreased shoot dry weight compared to nontreated plants, although the shoot dry weight was numerically higher when treated with 8 mg a.i./pot compared to 6 mg a.i./pot. Plant introductions can be daunting for growers if plants have no previous protocols for care. Providing information about *Pycnanthemum* production will enable more efficient production and faster distribution.

Species used in this study: Virginia mountain mint, *Pycnanthemum virginianum* (L.); Appalachian mountain mint, *Pycnanthemum flexuosum* (Walt.).

Chemicals used in this study: paclobutrazol (Bonzi®).

Index words: plant growth regulator, *Pycnanthemum*, mountain mint, production protocols, paclobutrazol.

Significance to the Horticultural Industry

Plant growth regulators (PGR) are compounds that influence plant growth and development through means other than supplying nutrients (Rademacher 2015). Plant growth, such as flowering and height, can be altered by manipulating temperature and light (Moe and Heins 1990). However, less specialized equipment is required when applying a PGR compared to controlling environmental factors. Additionally, PGR applications demand less labor than physical methods of controlling plant growth, such as pruning. Production efficiency in the greenhouse and field is integral to the success of new plants. New plant introductions are exciting to consumers but can be daunting to growers if no information exists regarding cultivation. Introducing production information and best practices for growing plants makes new genera accessible for cultivation by growers. Using PGR to improve commercial production has been achieved in various ornamental plants. However, little production information is available for *Pycnanthemum*. Determining optimal PGR treatments for the new genus removes a major limit to production and encourages the introduction of the new genus in the ornamental market.

Introduction

Plant growth regulators (PGR) are compounds, other than nutrients, that affect plant growth and development (Rademacher 2015). Physiological processes in plants, such as flower development and vegetative growth, can be influenced by temperature and light (Moe and Heins 1990). However, chemical applications require less specialized equipment than other methods. Production is an integral step in new plant introductions. Therefore, improving the efficiency of plant production encourages the success of the new plants. Ornamental plants, such as *Osteospermum*, *Dianthus*, and *Helianthus* (Bañón et al. 2002, Barrios and Ruter 2019, Olsen and Andersen 1995) have production protocols. Growers often use a PGR to limit growth, which allows growers to space plants closer together and thus increase product quantity (Whipker and McCall 2000). Plant growth regulators can also extend the saleable period of plants by increasing their shelf life (Kurniawati et al. 2023). Plant growth may also be regulated to meet consumer demands for compact and floriferous plants. Previous studies have also effectively reduced the height of *Zinnia* and *Pelargonium* and increased *Iris* flowering following PGR treatments (Al-Khassawneh et al. 2006, Cox and Keever 1988).

Paclobutrazol ((2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol) is the active ingredient in certain PGRs, such as Bonzi® (Syngenta Crop Protection, LLC, Greensboro, NC). Paclobutrazol limits plant growth by inhibiting gibberellin biosynthesis through a mechanism that prevents kaurene oxidation and the production of kaurenoic acid (Nagar et al. 2021). Gibberellin is responsible for cell elongation rather than cell division, which indicates that disrupting gibberellin biosynthesis

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restricts plant size (Jungklang et al. 2017). Paclobutrazol can decrease plant height and diameter (Dasoju et al. 1998) and influence the number of leaves, chlorophyll content, and root development (Chandra and Roychoudhury 2020). The chlorophyll content of holy basil (*Ocimum sanctum* L.) increased when plants were treated with paclobutrazol, which promotes photosynthetic processes and plant growth (Divya Nair et al. 2009). The ability of paclobutrazol to confer smaller size while encouraging growth characteristics produces more compact plants. Paclobutrazol also affects the reproductive stage of plant development. For example, Singh et al. (2008) described an increase in flower abundance observed in clary sage (*Salvia sclarea* L.) following treatment with paclobutrazol.

Drought tolerance improves after paclobutrazol treatments. Plants treated with paclobutrazol require less water than control plants, as seen in okra (*Abelmoschus esculentus* (L.) Moench) and common sage (*Salvia officinalis* L.) (Bañón et al. 2023, Iqbal et al. 2020). Paclobutrazol induces stress tolerance through various mechanisms such as maintaining turgidity, enhancing antioxidative enzyme activity, and increasing the relative water content of plants (Jungklang et al. 2017). Gopi et al. (2007) found that proline levels increased with increasing paclobutrazol concentrations in wild carrot (*Daucus carota* L.), which helps stabilize cells and macromolecules through osmotic regulation. The activity of reactive oxygen species was reduced in false indigo (*Amorpha fruticosa* L.) and common sage following paclobutrazol treatment (Bañón et al. 2023, Fan et al. 2020). Fan et al. (2020) reported an increase in peroxidase activity (an enzyme responsible for protecting plants against reactive oxygen species [ROS]) as the concentration of paclobutrazol increased. Similarly, Bañón et al. (2023) observed a decrease in non-photochemical quenching (a process that reduces ROS produced from excess excitation energy) in plants treated with paclobutrazol, indicating ROS levels were lower in plants treated with this PGR. Absciscic acid (ABA) levels may also increase in paclobutrazol-treated plants, stimulating stomatal closure and reducing water lost from evapotranspiration (Desta and Amare 2021). Paclobutrazol serves as a stress protectant by enhancing the activity of enzymes and hormone levels while maintaining cell stability. The ability of paclobutrazol to provide drought tolerance could help expand the distribution of plants to consumers in more arid climates.

The method of application, concentration, and species affect how a plant responds to a PGR. Plant growth regulators are often applied as foliar sprays or substrate drenches. Bañón et al. (2002) compared the reduction in plant growth between carnations (*Dianthus caryophyllus* L.) sprayed and drenched with a range of paclobutrazol concentrations. The study determined that drenches of lower concentrations decreased plant height more than higher concentrations of foliar sprays; therefore, drenches were more efficient for carnations (Bañón et al. 2002). One reason for the efficiency of drenches could be that paclobutrazol is transported through the xylem, is immobile in the phloem, and has low solubility in water (Desta and Amare 2021, Ribeiro et al. 2011). Unlike drench applications, foliar

spray applications do not take advantage of the movement of paclobutrazol through the xylem. Therefore, foliar sprays limit the interaction of paclobutrazol beyond the nearest tissue, producing more localized effects. Quinlan and Richardson (1986) reported that ^{14}C -paclobutrazol movement in apples (*Malus domestica* (Borkh.)) was more prevalent in younger tissue (closer to shoot tips) than older tissue following spray applications, demonstrating the limited translocation of paclobutrazol after foliar sprays. Paclobutrazol also moved toward shoot tips rather than roots, consistent with the theory that this PGR is translocated through the xylem (Quinlan and Richardson 1986). Drench applications allow for an even distribution of paclobutrazol throughout the plant, creating more uniform results. Applying paclobutrazol with drenches may also reduce labor costs due to the efficiency of singular applications compared to foliar sprays.

The concentration of a PGR and plant species also affects the treatment results. If the PGR concentration is too high, the treatment can produce plants with less consumer appeal. Al-Khassawneh et al. (2006) explored the effects of various concentrations of paclobutrazol on black iris (*Iris nigricans* Dinsm.). The study found that paclobutrazol concentrations higher than the optimal concentration reduced height to the point that plants were undesirable (Al-Khassawneh et al. 2006). Plants treated with concentrations above the optimal treatments also experienced a reduction in leaf number and delayed flowering (Al-Khassawneh et al. 2006). Dasoju et al. (1998) found a reduction in the inflorescence diameter of sunflower (*Helianthus annuus* L.) plants after treatment with a concentration higher than the optimum, which resulted in a less appealing product. Therefore, the concentration of a PGR applied to plants influences the intensity of the plant response.

Additionally, species or variety can affect how plants react to a PGR, even when applied at the same concentration. Cavins et al. (2002) compared two coleus (*Solenostemon scutellarioides* (L.) Codd) cultivars after spraying plants at two different time points with uniconazole (another triazole-based PGR). The study determined that *Solenostemon scutellarioides* 'Burgundy Sun' required two sprays of uniconazole to control height. Alternatively, the height of *Solenostemon scutellarioides* 'Solar Storm' was efficiently reduced with one spray of the same concentration and severely reduced by the second spray (Cavins et al. 2002). Treatments must improve the growth habits of plants without reducing factors related to the marketability of the plants. For example, consumers prefer compact plants produced from PGR application; however, the flower presence must also be maintained to ensure plants are appealing. Applying PGR concentrations higher than the optimal concentration for a species may reduce the plant's marketability. Concentrations higher than the optimum are also economically inefficient because more of the PGR is used, and fewer plants are likely to be sold. Determining the rates that significantly improve growth habit will ensure resources are used efficiently. *Pycnanthemum* is a relatively unexplored genus; therefore, identifying the optimal PGR concentration for *Pycnanthemum* will make the genus more accessible to growers and appealing to consumers.

Virginia mountain mint (*Pycnanthemum virginianum* (L.)) and Appalachian mountain mint (*Pycnanthemum flexuosum* (Walt.)) are herbaceous perennials in the Lamiaceae with no commercially listed PGR. The genus *Pycnanthemum* is a vigorous North American native (Hummer et al. 2020) characterized by a mint or thyme-like fragrance. The inflorescences are arranged in a cyme of small tubular white or purple flowers speckled with purple dots. Virginia mountain mint grows to a height of 0.6–1.1 m (2.0–3.6 ft) and has 0.8–1.5 cm (0.3–0.6 in) -wide flowers (Chambers 1961). The species grows from north Georgia to North Dakota (Weakley 2020). Appalachian mountain mint grows between 0.5–1.2 m (1.6–3.9 ft) tall, with flowers that are 2–4 cm (0.8–1.6 in) wide (Chambers 1961), and is endemic to the southeastern United States, from Virginia to Mississippi (Weakley 2020). The genus attracts pollinators (Cadotte et al. 2017) and has the potential to appeal to the public, providing the opportunity to supplement pollinator habitats in urban landscapes.

Despite the potential of the genus, *Pycnanthemum* is rarely seen in cultivation and lacks production information. Plant introductions can be daunting for growers if plants have no previous protocols for care. Providing information about *Pycnanthemum* production will enable more efficient production and faster distribution. Additionally, introducing production procedures for *Pycnanthemum* will make the genus more accessible to growers and breeding programs.

Materials and Methods

Plants. Virginia mountain mint and Appalachian mountain mint from the USDA-ARS Western Regional Plant Introduction Station (Pullman, WA) were grown from seed and transplanted into 6.65 cm (2.6 in) (280 mL; 17.1 in³) Deep Press Fit Pots (The HC Companies, Twinsburg, GA) filled with Pro-line C/B Growing Mix (Shady Dale, GA; composed of Canadian sphagnum peat, coarse perlite, medium vermiculite, and processed pine bark fines) and used as stock plants for the cuttings taken to propagate the plants in the study. Once the propagules were established, the plants were transplanted into C300 2.8 L (0.1 ft³) pots (Nursery Supplies Inc., Kissimmee, FL) with an outdoor mix composed of 20% peat moss, 28% 0.95 cm (0.4 in) aged pine bark, 42% 1.59 cm (0.6 in) aged pine bark, and 10% sand (Old Castle, Shady Dale, GA), and spaced 30 cm (11.8 in) apart on a container pad. One plant was transplanted per pot. The experiment in 2022 received 16 g (0.6 oz) of Osmocote Plus 15-9-12, 8-9-month (15-4-10 N-P-K) (ICL Specialty Fertilizers, Summerville, SC) once they were placed on the container pad, while the experiment in 2023 was fertilized with 24 g (0.8 oz) of Osmocote Plus 15-9-12, 8-9-month (15-4-10 N-P-K). Both experiments were watered twice daily by a sprinkler system, culminating in 1.27 cm (0.50 in) of irrigation per day. Once plants were established in the container, each plant was cut down to 4 cm (1.6 in) before applying treatments. Plants were cut 48 hours before treatment to give plants a chance to acclimate and were treated on July 18, 2022 and June 9, 2023.

Treatments in 2022. A preliminary study compared Appalachian mountain mint plants treated with 0 (control treatment), 0.25, 0.5, 1, and 2 mg a.i. (0, 8.82e⁻⁶, 1.76e⁻⁵, 3.5e⁻⁵, and 7.05e⁻⁵ oz) of paclobutrazol (Bonzi®)/pot, a commonly used plant growth regulator. Nine replications of each treatment were arranged in rows of increasing concentration. On the last day of collection (September 23, 2022), plants treated with 1 and 2 mg a.i. of paclobutrazol/pot were smaller than the controls; however, plants treated with 1 and 2 mg a.i./pot were not significantly different from treatments with lower concentrations. Initial results informed additional treatments of Appalachian mountain mint plants in 2023.

Treatments in 2023. The experiment in 2023 examined Virginia mountain mint and Appalachian mountain mint plants. The study in 2022 informed treatments of Appalachian mountain mint, while Virginia mountain mint was treated with a broad range of concentrations due to a lack of preliminary information. Plants were separated by species and arranged in randomized complete blocks with three replicates per block. Plants were drenched with 118 ml of a paclobutrazol and water mixture. Three blocks (nine samples per treatment) of Virginia mountain mint were treated with 0 (control treatment), 0.5, 1, 2, 4, 8, and 16 mg a.i. (0, 1.76e⁻⁵, 3.5e⁻⁵, and 7.05e⁻⁵, 1.41e⁻⁴, 2.82e⁻⁴, and 5.64e⁻⁴ oz) of paclobutrazol/pot. Two blocks (six samples per treatment) of Appalachian mountain mint were treated with 0 (control treatment), 6, and 8 mg a.i./pot (0, 2.12e-4, and 2.82e-4 fl oz).

Data collection. Biweekly measurements were recorded August 11, 2022 to September 23, 2022 and June 26, 2023 to August 7, 2023. The measurements were used to determine if treatments affected plant growth, including width 1, width 2 (perpendicular to width 1), height, and average internode length. The height was measured from the substrate surface to the tip of the highest branch. The width of plants was calculated by averaging width 1 and width 2. The volume was calculated by dividing the elliptical volume by 2 to represent a hemisphere ($V = (\frac{1}{2}) [(4/3) * (\text{height} * (1/2 \text{ width } 1) * (1/2 \text{ width } 2))]$). The growth index was calculated by averaging height, width 1, and width 2. The average internode length was calculated by measuring the length between the third and fourth internode on three branches to provide an average internode length per plant. The inflorescence heads were counted, and the vegetative growth was cut on the last day of data collection (August 21, 2023) to quantify flowering and shoot dry weight. Plants were cut flush with the upper rim of the pots, placed in bags, and allowed to dry for three weeks in an empty greenhouse set to 25 C (77 F) (daytime temperatures often exceeded 40 C (104 F)) before being weighed. The density of plants was determined by dividing the shoot dry weight by the volume of plants on the final day of data collection.

Statistical analysis. A repeated measures ANOVA (RStudio, PBC, Version 1.4.1725, Boston, Massachusetts) was used to analyze the data for significant treatment effects over the collection period. An ANOVA analysis

(RStudio) was used to determine if the concentration of paclobutrazol affected vegetative traits taken on the last day of data collection. The model for the height, volume, and shoot dry weight of the Virginia mountain mint plants was log-transformed to improve the model fit and ensure that models met normality and homoscedasticity assumptions (Hopper et al. 1994, Sanz-Pérez and Castro-Díez 2010). The height and growth index of the Appalachian mountain mint plants were squared, while the density of the Appalachian mountain mint plants was log-transformed to improve model fit and meet normality and homoscedasticity assumptions (Hopper et al. 1994, Sanz-Pérez and Castro-Díez 2010). A Poisson regression (a generalized linear model for discrete and positive response variables, such as count data) was used to compare flowering between Appalachian mountain mint treatments (O'Hara and Kotze 2010). Virginia mountain mint data was analyzed using a non-binary regression to meet the equal dispersion assumption. Outliers were identified using RStudio, characterized by values less than or greater than 1.5 times the standard deviation, and then winsorized. Winsorization replaces outliers with the value closest to the outlier, normalizing the data without creating unequal sample sizes between treatments (Kwak and Kim 2017). A Tukey HSD post-hoc test (RStudio) was used to compare the effects of treatments on Virginia mountain mint. However, a Bonferroni correction (RStudio) was more fitting for Appalachian mountain mint due to the comparison of fewer treatments during the experiment. Significance was reported as $P \leq 0.05$.

Results and Discussion

The concentration of paclobutrazol significantly affected the height and growth index of Virginia mountain mint ($P < 0.001$) and Appalachian mountain mint ($P < 0.05$) throughout the study. The growth of plants over time and the polynomial equations are displayed in Figures 1–4.

Virginia mountain mint was shorter than the control plants in all treatments besides 0.5 mg a.i./pot ($P < 0.05$). Paclobutrazol treatments greater than 0.5 mg a.i./pot reduced height by 33.7–47.6% compared to the nontreated control (Table 1, Fig. 5). Virginia mountain mint drenched with 0.5, 1, 2, and 4 mg a.i./pot had more inflorescence than plants treated with 0, 8, or 16 mg a.i./pot ($P < 0.05$). The inflorescence abundance of plants treated with 0.5, 1, 2, and 4 mg a.i./pot increased by 84.0–141.2% compared to nontreated plants (Table 1), while higher concentrations were not different from the control. The shoot dry weight of Virginia mountain mint drenched with 16 mg a.i./pot were 34.7% lower than the control (Table 1) ($P < 0.001$). Other treatments did not produce shoot dry weights different from the control (Table 1) ($P > 0.05$). No difference in growth index was present between treatments and the control ($P > 0.05$); however, the growth index did differ between paclobutrazol treatments ($P < 0.01$) (Table 1). Lower treatments increased the growth index, while higher concentrations decreased the growth index. The growth index of plants treated with 0.5, 1, 2, and 4 mg a.i./pot were numerically 6.6–10.1% larger than the control, while the 8 and 16 mg a.i./pot treatments had 14 and 11% smaller growth indexes compared to the control, respectively

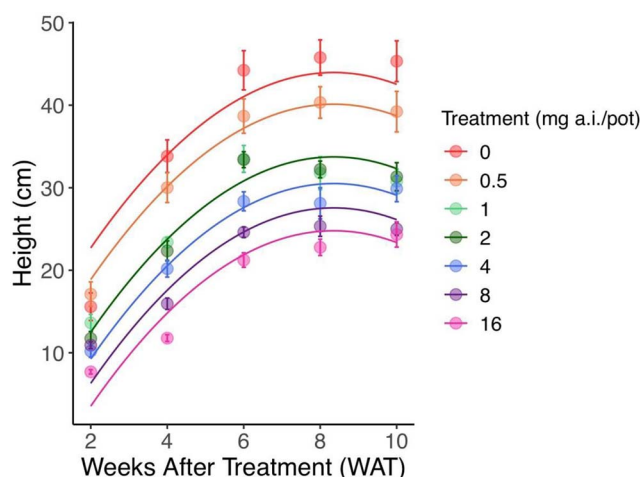


Fig. 1. The height of Virginia mountain mint (*Pycnanthemum virginianum*) after paclobutrazol treatments. The quadratic equation can be used to predict the effects of additional paclobutrazol concentrations: 0 mg a.i./pot ($y = -3.3025x^2 + 26.961x - 7.6053$; $R^2 = 0.78$), 0.5 mg a.i./pot ($y = -2.503x^2 + 20.473x - 0.8169$; $R^2 = 0.71$), 1 mg a.i./pot ($y = -2.3833x^2 + 18.568x - 2.8711$; $R^2 = 0.74$), 2 mg a.i./pot ($y = -2.4786x^2 + 19.726x - 5.82$; $R^2 = 0.84$), 4 mg a.i./pot ($y = -1.7738x^2 + 15.373x - 3.2578$; $R^2 = 0.78$), 8 mg a.i./pot ($y = -1.1787x^2 + 10.896x + 0.3764$; $R^2 = 0.85$), and 16 mg a.i./pot ($y = -0.9952x^2 + 10.44x - 2.7956$; $R^2 = 0.84$).

(Table 1). The density of plants treated with 8 mg a.i./pot increased by at least 41.7% compared to lower treatments ($P < 0.05$), although the density of plants treated with 16 mg a.i./pot were not different than the control ($P > 0.05$) (Table 1).

The heights of Appalachian mountain mint plants treated with 6 and 8 mg a.i./pot were 41.9 and 43% lower than the control ($P < 0.05$), respectively (Table 1, Fig. 6). Plant height did not differ between plants drenched with 6 and 8 mg a.i./pot ($P > 0.05$) (Table 1). Nontreated Appalachian mountain mint had more inflorescences than the plants treated with 6 and 8 mg a.i./pot ($P < 0.05$), which had 89.4 and 100% fewer inflorescences, respectively. Appalachian mountain mint controls had a higher shoot dry weight than plants treated with 6 mg a.i./pot ($P < 0.01$), which were 52.2% lighter than the control. The shoot dry weight of plants treated with 8 mg a.i./pot did not differ from the control or plants treated with 6 mg a.i./pot ($P > 0.05$) (Table 1). Drenching Appalachian mountain mint plants with 6 and 8 mg a.i./pot decreased the growth index by 37.1 and 31.8% compared to the control ($P < 0.01$). The plants treated with 6 and 8 mg a.i./pot also increased the vegetation density by 127.4 and 96.3% ($P < 0.05$).

Paclobutrazol is a triazole that affects plant hormone levels. The triazole is commonly known for inhibiting gibberellin biosynthesis, the plant hormone responsible for stem elongation (Kurniawati et al. 2023). Stem elongation contributes to plant height; therefore, paclobutrazol limits plant size. Appalachian mountain mint and Virginia mountain mint exhibited a decrease in plant height when compared to treated plants and control plants. Other members of the *Lamiaceae*, including peppermint (*Mentha piperita* L.) and basil (*Ocimum basilicum* L.) (Firdausa and

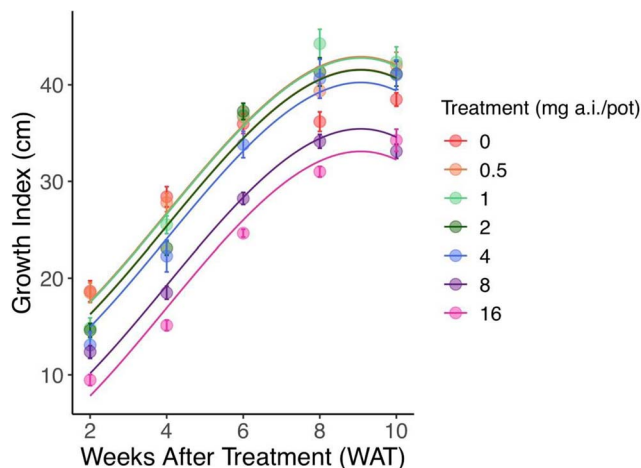


Fig. 2. The growth index (average of the height, width 1, and width 2) of Virginia mountain mint (*Pycnanthemum virginianum*) after paclobutrazol treatments. The cubic equation can be used to predict the effects of additional paclobutrazol concentrations: 0 mg a.i./pot ($y = 0.2727x^3 - 4.0083x^2 + 20.552x + 1.6006$; $R^2 = 0.88$), 0.5 mg a.i./pot ($y = 0.036x^3 - 1.7181x^2 + 15.077x + 4.9351$; $R^2 = 0.89$), 1 mg a.i./pot ($y = -0.7321x^3 + 4.4746x^2 + 2.7326x + 8.4015$; $R^2 = 0.91$), 2 mg a.i./pot ($y = -0.8275x^3 + 5.4896x^2 - 0.6534x + 10.302$; $R^2 = 0.92$), 4 mg a.i./pot ($y = -0.7275x^3 + 4.8634x^2 + 0.3612x + 8.4504$; $R^2 = 0.86$), 8 mg a.i./pot ($y = -0.8402x^3 + 6.5638x^2 - 8.0738x + 14.725$; $R^2 = 0.90$), and 16 mg a.i./pot ($y = -0.5799x^3 + 4.6512x^2 - 3.7281x + 9.0119$; $R^2 = 0.96$).

Kurniawati 2022, Santos Filho et al. 2022), have exhibited a similar reduction in plant height. The effect of paclobutrazol on the internodes of plants could be responsible for the decrease in height. Inhibiting gibberellin biosynthesis allows paclobutrazol to influence internode formation. Paclobutrazol may reduce the length or number of internodes (Hamid and Williams 1997), which reduces plant height. Kasim et al. (2018) noted that the internode length of scarlet sage (*Salvia splendens* Sellow ex Roem. et Schult) decreased as the concentration of paclobutrazol increased. Kurniawati et al. (2023) demonstrated the role

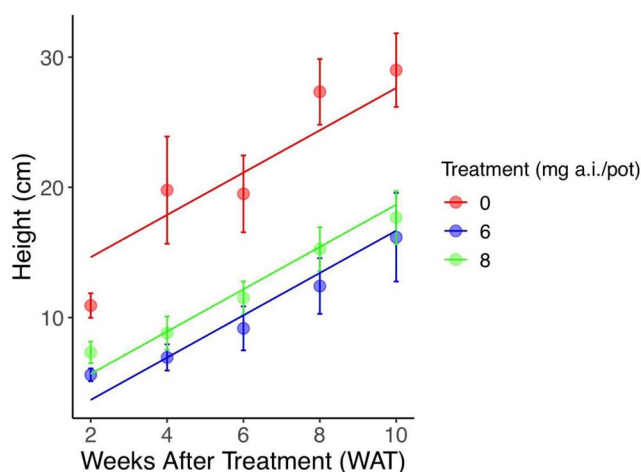


Fig. 3. The height of Appalachian mountain mint (*Pycnanthemum flexuosum*) after paclobutrazol treatments. The linear equation can be used to predict the effects of additional paclobutrazol concentrations: 0 mg a.i./pot ($y = 4.7717x + 7.3917$; $R^2 = 0.55$), 6 mg a.i./pot ($y = 2.76x + 2.2033$; $R^2 = 0.48$), and 8 mg a.i./pot ($y = 2.715x + 3.9617$; $R^2 = 0.57$).

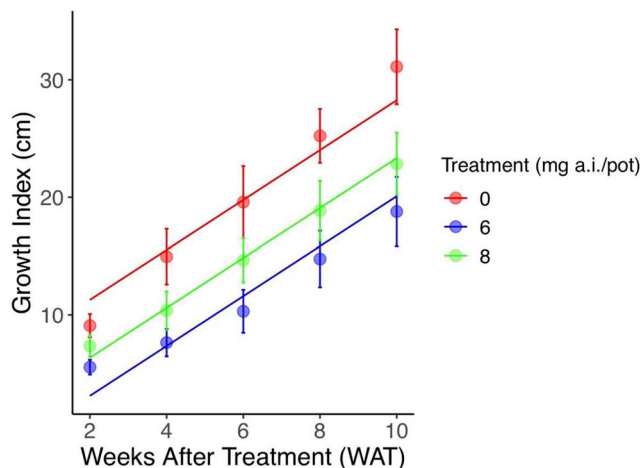


Fig. 4. The growth index (average of the height, width 1, and width 2) of Appalachian mountain mint (*Pycnanthemum flexuosum*) after paclobutrazol treatments. The linear equation can be used to predict the effects of additional paclobutrazol concentrations: 0 mg a.i./pot ($y = 5.5661x + 3.425$; $R^2 = 0.69$), 6 mg a.i./pot ($y = 3.5339x + 1.2339$; $R^2 = 0.62$), and 8 mg a.i./pot ($y = 3.945x + 2.9761$; $R^2 = 0.60$).

of internode count on plant height in basil when describing the reduction in basil height and the number of internodes as paclobutrazol concentrations increase. The internode length of Appalachian mountain mint plants and most Virginia mountain mint did not vary from controls despite differences in plant height ($P > 0.05$) (Table 2). The observed decrease in height without a similar trend in internode lengths suggests that the number of internodes decreases rather than the length. Therefore, a decline in the number of internodes could explain the reduced height of treated Appalachian mountain mint and Virginia mountain mint.

Paclobutrazol increased the flowering of Virginia mountain mint at low concentrations and decreased the flowering at high concentrations (Table 1). However, all concentrations of paclobutrazol decreased the flowering of Appalachian mountain mint plants. Previous studies have observed an increase or a decrease in flowering following paclobutrazol treatment. For example, ornamental plants such as African daisy (*Osteospermum ecklonis* DC. Norl.) experienced more flowering after paclobutrazol treatment (Olsen and Andersen 1995), while flower abundance in carnations and holy basil decreased (Bañón et al. 2002, Divya Nair et al. 2009). The observed increase in flowering could be due to the ability of paclobutrazol to increase chlorophyll content. Applications of paclobutrazol increased chlorophyll content in scarlet sage and habenero-type pepper (*Capsicum chinense* Jacq.) (França et al. 2017, Kasim et al. 2018), which could improve light capture and potential plant growth. On the other hand, the flowering of Virginia mountain mint and Appalachian mountain mint plants decreased at the highest concentrations (8 and 16 mg a.i./pot and 6 and 8 mg a.i./pot, respectively) (Table 1). Common selfheal (*Prunella vulgaris* L.) treated with gibberellin 3 exhibited an increase in flowering (Li et al. 2022). Similarly, chamomile (*Matricaria chamomilla* L.) flowering increased due to applications of gibberellic acid. Gibberellin plays a role in promoting flowering; therefore, inhibiting gibberellin could explain the reduction of flowering. However, the inhibition of

Table 1. The average of *Pycnanthemum* growth and reproductive characteristics following paclobutrazol treatments. Data shown is from the last day of data collection (August 21, 2023). An ANOVA analysis (RStudio) was used to determine if the concentrations of paclobutrazol had a significantly different effect on vegetative traits. A Poisson regression was used to compare flowering between Appalachian mountain mint (*Pycnanthemum flexuosum*) treatments. Virginia mountain mint (*Pycnanthemum virginianum*) inflorescence count was analyzed using a non-binary regression.

	Treatment (mg a.i./pot)	Height (cm)	Growth Index ^z (cm)	Number of Inflorescences ^y	Shoot Dry Weight (g)	Density (mg·cm ⁻³)
Genus						
<i>Pycnanthemum</i>						
<i>P. virginianum</i>	0	46.4 a	38.5 ab	21.6 a	32.7 ab	1.2 a
	0.5	39.2 ab	42.0 a	39.7 b	37.0 a	1.0 a
	1	30.8 bc	42.4 a	45.1 bc	39.3 a	1.1 a
	2	31.3 bc	41.1 a	49.8 c	35.2 ab	1.0 a
	4	29.1 bcd	41.0 a	52.0 c	34.7 ab	1.1 a
	8	25.0 cd	33.1 b	26.4 a	28.4 b	1.7 b
	16	24.3 d	34.3 b	26.7 a	21.3 c	1.4 ab
<i>P. flexuosum</i>	0	31.0 a	33.5 a	119.8 a	26.5 a	1.3 a
	6	18.0 b	21.1 b	12.7 b	12.7 b	3.0 b
	8	17.7 b	22.8 b	0.0 b	16.3 ab	2.6 b

^zThe average of the height, width 1, and width 2.

^yThe average number of inflorescence heads.

gibberellin may not occur at the same magnitude when applying low concentrations of paclobutrazol, which could explain the increase in flowering compared to higher concentrations.

The shoot dry weight of Virginia mountain mint decreased as paclobutrazol concentrations increased. Appalachian mountain mint plants exhibited a similar decrease at 6 mg a.i./pot, although the shoot dry weight numerically increased slightly at 8 mg a.i./pot. Paclobutrazol treatments could reduce shoot dry weight by restricting the number of leaves or leaf area. Singh et al. (2008) described a decrease in clary sage leaf number and suggested that the reduction in leaf number resulted in a decrease in the shoot dry weight. Divya Nair et al. (2009) reported a smaller leaf area in holy basil following paclobutrazol drenches. The slight increase in the shoot dry weight of Appalachian mountain mint plants treated with 8 mg a.i./pot could be due to the complete inhibition of flowering, allowing more resources to be directed to vegetative growth rather than reproductive growth.

The density of Virginia mountain mint was highest for plants drenched with 8 mg a.i./pot (Table 1). The volume of plants treated with 8 mg a.i./pot was smaller than the control plant, while shoot dry weight did not differ, resulting in greater density (Table 2). Applying paclobutrazol reduces the elongation of cells and decreases plant volume by inhibiting gibberellin synthesis. The volume of Virginia mountain mint plants treated with 16 mg a.i./pot was not different from 8 mg a.i./pot treatments or controls; however, the shoot dry weight of plants treated with 16 mg a.i./

pot was lower than control plants (Table 1). Therefore, the plants drenched in 16 mg a.i./pot had a lower density than those treated with 8 mg a.i./pot due to less shoot dry weight (Table 1). The difference in shoot dry weight could be due to smaller or fewer leaves on plants treated with 16 mg a.i./pot, caused by paclobutrazol inhibiting vegetative growth. Paclobutrazol reduced leaf area and number following treatments of common sage, which reduced overall shoot dry weight (Bañón et al. 2023). Additionally, after paclobutrazol treatments, Virginia mountain mint may redirect resources from aboveground into belowground tissue (Jaleel et al. 2008), which reduces the shoot dry weight. An increase in belowground growth redirects plant resources to roots, limiting shoot growth and reducing the shoot dry weight and density of plants.

Despite a decrease in shoot dry weight, the density of treated Appalachian mountain mint increased (Table 1). Teto et al. (2016) found a reduction in shoot dry weight and plant volume in paclobutrazol-treated lion's tail (*Leonotis leonurus* (L.) R.Br.), resulting in a higher vegetative density after treatment. Plant volume declined after paclobutrazol application due to the inhibition of cell elongation. Increasing plant density requires that plant volume decreases by a larger margin than shoot dry weight. The volume of treated Appalachian mountain mint was also smaller than control plants (Table 1). Therefore, the reduction in plant volume contributed to the increased density of Appalachian mountain mint.



Fig. 5. Virginia mountain mint (*Pycnanthemum virginianum*) 10 weeks after paclobutrazol drench. Plants were treated with 0, 0.5, 1, 2, 4, 8, and 16 mg a.i./pot of Bonzi® (left to right).



Fig. 6. Appalachian mountain mint (*Pycnanthemum flexuosum*) 10 weeks after paclobutrazol drench. Plants were treated with 0, 6, and 8 mg a.i./pot of Bonzi® (left to right).

The growth index of Virginia mountain mint increased and then decreased, as evidenced by the decrease when comparing low and high concentrations (Table 1). Paclobutrazol can encourage environmental stress tolerance (Berova et al. 2002) and increase chlorophyll content (Gopi et al. 2007). Under abiotic stress conditions, paclobutrazol is able to protect plants from stress while enhancing the photosynthetic potential, resulting in larger plants than the control; however, paclobutrazol also limits cell elongation. At high concentrations, the PGR limits growth despite environmental conditions, explaining the decrease in the growth index when Virginia mountain mint and Appalachian mountain mint were treated with higher concentrations of the PGR.

Paclobutrazol is a commonly used PGR for ornamental plants, capable of producing dense, compact plantst that appeal to consumers and assist growers. The density of plants is especially important for producing tight canopies, which are more desirable as final products and stock plants in vegetative production. Paclobutrazol is expected to reduce plant size by decreasing internode length or number (Hamid and Williams 1997). The reduction of the plant height and growth index would make the plant more compact and improve vegetative density. The use of PGR can positively or negatively impact flowering (Bañón et al. 2002, Divya Nair et al. 2009). Previous studies have shown a decrease in flowering, potentially due to an inhibition of

gibberellin (França et al. 2017, Kasim et al. 2018), while others exhibited an increase in flowering, which could be related to an increase in the chlorophyll content of treated plants. The effects of paclobutrazol on *Pycnanthemum* are influenced by the dose of the PGR and the species of *Pycnanthemum*, as demonstrated by the differing results of certain concentrations between the species.

Determining the optimal concentrations of paclobutrazol will improve the accessibility of *Pycnanthemum*. Appalachian mountain mint and Virginia mountain mint species exhibited the desired decrease in height; however, both also experienced a decrease in flowering at higher concentrations. Concentrations below 6 mg a.i./pot should be examined for Appalachian mountain mint, while Virginia mountain mint may benefit from paclobutrazol concentrations between 4 and 8 mg a.i./pot to find a balance between increasing flowering and improving density. Examining a range of PGR concentrations informs growers' strategies, ensuring plants are desirable to consumers and efficient for production. Concentrations too low produce leggy plants prone to lodging, while applying high concentrations can reduce flowering and waste resources. Exploring the effects of paclobutrazol at different concentrations allows growers to optimize PGR applications for transport, production, and consumer appeal.

Table 2. The average of *Pycnanthemum* internode lengths and volumes following paclobutrazol treatments. Data shown is from the last day of data collection (August 21, 2023). An ANOVA analysis (RStudio) was used to determine if the concentrations of paclobutrazol had a significantly different effect on vegetative traits.

	Treatment (mg a.i./pot)	Internode length (cm)	Volume (m ³)
Genus			
<i>Pycnanthemum</i>			
<i>P. virginianum</i>	0	0.9 a	0.029 ab
	0.5	1.1 ab	0.039 a
	1	1.0 a	0.038 a
	2	1.4 b	0.034 a
	4	1.3 ab	0.035 a
	8	1.4 ab	0.018 c
	16	1.2 ab	0.020 bc
<i>P. flexuosum</i>	0	2.33 a	0.018 a
	6	1.91 a	0.004 b
	8	2.22 a	0.007 b

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