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Growth regulation of ornamental and vegetable plants under greenhouse conditions by air stream-based mechanical stimulation

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Summary

Plant growth regulation is an integral part within the production chain of ornamentals and vegetable seedlings. This allows plant producers to increase area and volume use efficiency. Furthermore, smaller and compact plants are often characterized by an increased robustness against potential damage during shipping and transport and consumers usually also prefer a compact plant stature with shortened internodes and dark green leaves.

In protected ornamental horticulture, chemical-synthetic plant growth regulators (PGR) are used to reduce plant size. In vegetable production, the use of these substances is prohibited by law in most counties, which is why non-chemical growth regulation methods, such as temperature and light management, must be applied. However, these methods require a high degree of crop-specific expertise and are considered only moderately efficient for growth control by horticulturists. Furthermore, the political objective to strongly reduce the application of pesticides and the scepticism of consumers towards the use of chemical adjuvants suggests that the use of PGR will be more strictly regulated in ornamental horticulture in the future.

In this respect, a production method for non-chemical growth control of ornamentals and vegetable seedlings under greenhouse conditions has been developed that is based on the application of air streams, inducing thigmomorphogenesis, the morphological and structural shaping of a plant organism during its development phase as influenced by touch-like stimuli. Numerous research studies have investigated the underlying cellular signaling events within the thigmomorphogenetic signaling cascade that ultimately reduces plant stem and shoot elongation. Mechanical stimuli act on the cytoskeleton-plasma membrane-cell wall interface, causing ion channels embedded in the cell membrane to open, allowing calcium influx in particular into the cytoplasm. Subsequent cellular reactions and in particular the change in the phytohormonal balance ultimately lead to a gradual morphological adaptation of the plant.

In own experiments jointly performed at the State Horticulture College and Research Station in Heidelberg, Germany, the application of a regularly applied air stimuli significantly reduced plant height by 24% in bellflower (*Campanula* 'Merrybell') compared to the control. In a subsequent practical trial at a local horticulture company (Fleischle GbR, Vaihingen Ensingen, Germany) plant height of creeping inchplant (*Callisia repens*) was significantly reduced by 20% on average compared to the control. In both experiments, a compressor generated the air stream which was then guided through hose lines and ultimately applied to the plant stand through custom-built stainless-steel nozzles (air pressure module). In tomato (*Solanum lycopersicum* 'Romello'), air streams applied by the 'air knife' module, the '360° rotor' module, or the 'air pressure' module resulted in a reduction in plant height of 26%, 33%, and 36% compared to the control, respectively. The air stream of the air knife module and the 360° rotor modules was not generated by a compressor but with a centrifugal fan and guided via a Y-switch, and two downstream connected flexible hoses into two air inlet holes at the backside

of either the air knife module or the 360° rotor module. The air stream guided into the air knife module was applied by an aperture slot, which could be adjusted between 1 and 5 mm, while the air stream guided into the 360° rotor module was applied via two 360° rotating PVC tubes that were inserted on the bottom of a rectangular aluminium box. It turned out, that the air outlet velocity along the aperture slot of the air knife module was highly variable. Consequently, the stimulus intensity perceived by individual experimental plants was unequal. A multiple regression analysis clearly showed that the maximum air velocity explained the variability in plant height reduction by air streams generated with the air knife module best, while the stimulus duration and the cumulative air velocity were less relevant. Plant height reduction by air stream generated with the 360° rotor module was most homogenous compared to the other prototypes.

Therefore, a subsequent series of experiments at the University of Hohenheim, Stuttgart, Germany, was carried out with the most promising prototype, the 360° rotor. The focus here was on addressing questions with a high degree of practical relevance, such as the effect of different treatment frequencies and different air stream velocities on the degree of plant height reduction.

Tomato plants showed a reduction in plant height of about 31% compared to the untreated control. Interestingly, no systematic dose-response relationship related to increasing application frequencies of 8, 24, 40, 56, 72, and 80 d^{-1} was found, confirming previous findings that the plants do not integrate the mechanical stimulus over time. In contrast, plant height reduction was significantly influenced by the air stream velocity. A sigmoidal dose-response relationship was fitted to the data and showed negligible effects on tomato plant height reduction between 0.7 m s^{-1} and 2.0 m s^{-1}, followed by a steep increase in the reduction effect up to 4.7 m s^{-1} and a fading of the effect at 36 % reduction for air velocities beyond that.

With the optimised settings for daily application frequency and air velocity, another experiment was conducted focusing on the effect of air stream application on phenotypic and physiological responses in tomato. Air stream application resulted in a gradual reduction of total leaf area by 14% on day 14 after treatment start, and radial growth was promoted relative to internode elongation compared to the untreated control, resulting in a more compact and stable plant phenotype. Air stream-treated plants translocated proportionally more assimilates to leaves and stems, at the expense of dry matter accumulation to petioles. The reduction in total leaf area was compensated by an increased leaf density, accompanied by a higher leaf green intensity and consequently by an average 8% increase in net CO_2 assimilation rates compared to the control. Thus, air stream-treated plants partially sustained total biomass accumulation at the same level as compared to the control.

Summary

The broad implementation of air-based mechanical stimulation as a method for growth control in ornamental and vegetable horticulture will be in line with the political objective of biologically sustainable and environmentally friendly plant production.

Zusammenfassung (German summary)

Die Regulierung des Pflanzenwachstums ist ein wesentlicher Bestandteil in der Produktions-kette von Zierpflanzen und Gemüsesetzlingen. Dadurch können Pflanzenproduzenten die Flächen- und Volumennutzungseffizienz erhöhen. Darüber hinaus zeichnen sich kleinere und kompaktere Pflanzen häufig durch eine größere Robustheit gegenüber möglichen Schäden bei Versand und Transport aus, und auch die Verbraucher bevorzugen in der Regel einen kompakten Pflanzenwuchs mit verkürzten Internodien und dunkelgrünen Blättern.

Im geschützten Zierpflanzenbau werden chemisch-synthetische Pflanzenwachstumsregula-toren (engl. Plant Growth Regulators - PGR) eingesetzt, um die Pflanzengröße zu reduzieren. Im Gemüsebau ist der Einsatz von PGR in den meisten Ländern gesetzlich verboten, weshalb nicht-chemische Methoden der Wachstumsregulierung, wie Temperatur- und Licht-management, angewandt werden müssen. Diese Methoden erfordern jedoch ein hohes Maß an kulturspezifischem Fachwissen und werden von Gartenbauern als nur mäßig effizient für die Wachstumskontrolle angesehen. Darüber hinaus lassen die politische Zielsetzung, den Einsatz von Pflanzenschutzmitteln stark zu reduzieren, und die Skepsis der Verbraucher gegenüber dem Einsatz chemischer Hilfsstoffe vermuten, dass der Einsatz von PGR im Zierpflanzenbau in Zukunft strenger geregelt sein wird.

In diesem Zusammenhang wurde eine Produktionsmethode für die nicht-chemische Wachs-tumskontrolle von Zierpflanzen und Gemüsesetzlingen unter Gewächshausbedingungen entwickelt, die auf der Anwendung von Luftströmen basiert, die die Bewegung der Pflanzen und folglich die Thigmomorphogenese, die morphologische und strukturelle Formung eines Pflanzenorganismus während seiner Entwicklungsphase unter dem Einfluss von berüh-rungsähnlichen Reizen, induzieren.

In zahlreichen Forschungsstudien wurden die zugrundeliegenden zellulären Signalereignisse innerhalb der thigmomorphogenetischen Signalkaskade untersucht, welche die Streckung des Pflanzensprosses reduziert. Mechanische Reize wirken auf die Grenzfläche zwischen Zytoskelett, Plasmamembran und Zellwand (engl. cytoskeleton-plasma membrane-cell wall interface – CPMCW), wodurch sich in der Zellmembran eingebettete Ionenkanäle öffnen und einen Kalziumeinstrom in das Zytoplasma ermöglichen. Die anschließenden zellulären Reak-tionen und die Veränderung des phytohormonellen Gleichgewichts führen zu einer allmähli-chen morphologischen Anpassung der Pflanze.

In eigenen Versuchen an der Staatlichen Lehr- und Versuchsanstalt für Gartenbau in Heidel-berg, Deutschland, führte die Anwendung eines regelmäßigen Luftreizes bei der Glocken-blume (*Campanula* 'Merrybell') zu einer signifikanten Verringerung der Pflanzenhöhe um 24 % im Vergleich zur Kontrolle. In einem Praxisversuch bei einem örtlichen Gartenbaubetrieb (Fleischle GbR, Vaihingen Ensingen, Deutschland) wurde die Pflanzenhöhe des Kriechenden Schönpolsters (*Callisia repens*) im Vergleich zur Kontrolle um durchschnittlich 20 % deutlich

reduziert. In beiden Versuchen erzeugte ein Kompressor den Luftstrom, der dann durch Schlauchleitungen geleitet und schließlich durch speziell angefertigte Edelstahldüsen (Luftdruckmodul) auf den Pflanzenbestand verabreicht wurde.

Bei der Tomate (*Solanum lycopersicum* 'Romello') führten die Luftströme des „Air knife" Moduls, des „360° Rotor" Moduls oder des Luftdruckmoduls zu einer Verringerung der Pflanzenhöhe um 26 %, 33 % bzw. 36 % im Vergleich zur Kontrolle. Der Luftstrom des "Air knife" und des "360° Rotor" Moduls wurde nicht durch einen Kompressor, sondern durch ein Zentrifugalgebläse erzeugt und über eine Y-Weiche und zwei nachgeschaltete flexible Schläuche in zwei Lufteinlassöffnungen auf der Rückseite des jeweiligen Moduls geleitet. Der in das „Air knife" Modul geleitete Luftstrom wurde über einen zwischen 1 und 5 mm einstellbaren Öffnungsschlitz an die Pflanzen verabreicht, während der in das „360°-Rotor" Modul geleitete Luftstrom über zwei um 360° drehbare PVC-Rohre verabreicht wurde, die auf der Unterseite eines hohlen, rechteckigen Aluminiumkastens angebracht waren. Es stellte sich heraus, dass die Luftaustrittsgeschwindigkeit entlang des Öffnungsschlitzes des „Air knife" Moduls sehr variabel war. Folglich war die von den einzelnen Versuchspflanzen erfahrene Reizintensität ungleich. Tatsächlich zeigte eine multiple Regressionsanalyse eindeutig, dass die maximale Luftgeschwindigkeit die Variabilität in der Reduktion der Pflanzenhöhe durch die mit dem „Air knife" erzeugten Luftströme am besten erklärte, während die Reizdauer und die kumulative Luftgeschwindigkeit weniger relevant waren. Die Verringerung der Pflanzenhöhe durch den mit dem „360° Rotor" Modul erzeugten Luftstrom war im Vergleich zu den anderen Prototypen am homogensten.

Daher wurden die nachfolgenden Versuche an der Universität Hohenheim, Stuttgart, Deutschland, mit dem „360° Rotor" Modul durchgeführt.

Es wurde keine systematische Dosis-Wirkungs-Beziehung bei zunehmender Anwendungshäufigkeit von 8, 24, 40, 56, 72 und 80 Überfahrten pro Tag gefunden, was frühere Erkenntnisse bestätigt, dass die Pflanzen den mechanischen Reiz nicht über die Zeit integrieren. Hierbei zeigte die Tomatenpflanzen eine Verringerung der Pflanzenhöhe von durchschnittlich 31 % im Vergleich zur unbehandelten Kontrolle. Im Gegensatz dazu wurde die Verringerung der Pflanzenhöhe signifikant von der Geschwindigkeit des Luftstroms beeinflusst. Eine sigmoidale Dosis-Wirkungs-Beziehung zeigte vernachlässigbare Auswirkungen auf die Reduktion der Tomatenpflanzenhöhe zwischen 0,7 m s^{-1} und 2,0 m s^{-1}, gefolgt von einem steilen Anstieg der Reduktionswirkung bis zu 4,7 m s^{-1} und einem Abklingen der Wirkung bei 36 % Reduktion für Luftgeschwindigkeiten darüber hinaus.

Mit den optimierten Einstellungen für die tägliche Anwendungshäufigkeit und die Luftgeschwindigkeit wurde ein zweiter Versuch durchgeführt, der sich auf die Auswirkungen der Luftstromanwendung auf phänotypische und physiologische Reaktionen der Tomatenpflanze konzentrierte. Die Anwendung des Luftstroms führte zu einer allmählichen Verringerung der

Gesamtblattfläche um 14 % an Tag 14 nach Beginn der Behandlung, und das Radialwachstum wurde im Vergleich zur unbehandelten Kontrolle relativ zur Internodienstreckung gefördert, was zu einem kompakteren und stabileren Pflanzenphänotyp führte. Die mit dem Luftstrom behandelte Pflanzen verlagerten proportional mehr Assimilate in die Blätter und den Stamm, was auf Kosten der Trockenmasseakkumulation in die Blattstiele ging. Die Verringerung der Gesamtblattfläche wurde durch eine höhere Blattdichte kompensiert, die mit einer höheren Grünintensität der Blätter einherging und folglich zu einem Anstieg der Netto-CO_2-Assimilationsrate um durchschnittlich 8 % im Vergleich zur Kontrollgruppe führte. Somit konnten die luftstrombehandelten Pflanzen die Gesamtbiomasseakkumulation teilweise auf demselben Niveau halten wie die unbehandelten Kontrollpflanzen.

Die breite Anwendung der mechanischen Reizung durch Luft als Methode zur Wachstums-steuerung im Zier- und Gemüsebau steht im Einklang mit dem politischen Ziel einer biologisch nachhaltigen und umweltfreundlichen Pflanzenproduktion.

1. General introduction

1.1. Horticulture in Germany

In 2012, horticulture accounted for about EUR 6.3 billion, a production value of 11% of total agriculture in Germany (Federal Ministry of Food and Agriculture, 2014). Horticulture is divided into production horticulture and service horticulture. In production horticulture, a basic distinction is made between 5 sectors: fruit growing, vegetable growing, tree nurseries, flower and ornamental plant growing, and others. Vegetable and ornamental horticulture generated the largest production value with EUR 2.26 and EUR 2.29 billion, respectively, corresponding to a share of around 36% each, while the production value of tree nurseries and fruit growing accounted for EUR 1.02 (16%) and EUR 0.69 billion (11%), respectively (Federal Ministry of Food and Agriculture, 2014). In 2016, according to the Federal Ministry of Food and Agriculture, a total of 15,543 horticulture companies managed an area of 182,334 ha (Federal Ministry of Food and Agriculture, 2016). Of these, 14,305 companies managed an area of 179,517 ha and were characterized as production companies, as they derive at least 50% of their income from the production of plants and related products. The remaining 1,238 companies are classified as service horticulture, such as gardening and landscaping or cemetery nurseries. The largest percentage share of companies is accounted for fruit growers (31.6%), followed by others (26.6%), vegetable growers (17.1%), ornamental growers (16.7), and tree nurseries (8.0%). The largest percentage share of the total cultivated area is held by vegetable growers (42.9%), followed by fruit growers (25.5%), others (19.7%), tree nurseries (9.2%), and ornamentals plant growers (2.7%) (Fig. 1.1). This shows that the average cultivated area per company is largest for vegetable growers (≈31 ha) and smallest for ornamental growers (≈2 ha), which is probably related to the fact that ornamental plant production is mainly carried out under protected greenhouses (2,185 companies) while vegetable production predominantly under open field conditions (2,300 companies) (Federal Ministry of Food and Agriculture, 2016).

The main objective of production horticulture is to produce high-quality plants for food, landscaping, and private use. Different phases are distinguished within the production process. First, existing, and new plant varieties are multiplied and bred in propagation nurseries. Once the desired quality trait is achieved, they are marketed and delivered to young plant producers or seedling producers who specialise in plant growth management in the juvenile developmental phase of potted plants. The finished end product is then distributed directly to retailers or large grower organisations (Havardi-Burger et al., 2020). During the different stages of production, one objective is growth management, such as maintaining dense and compact plant growth by various methods, one of which is the use of chemical additives, such as Plant growth regulators (PGR).
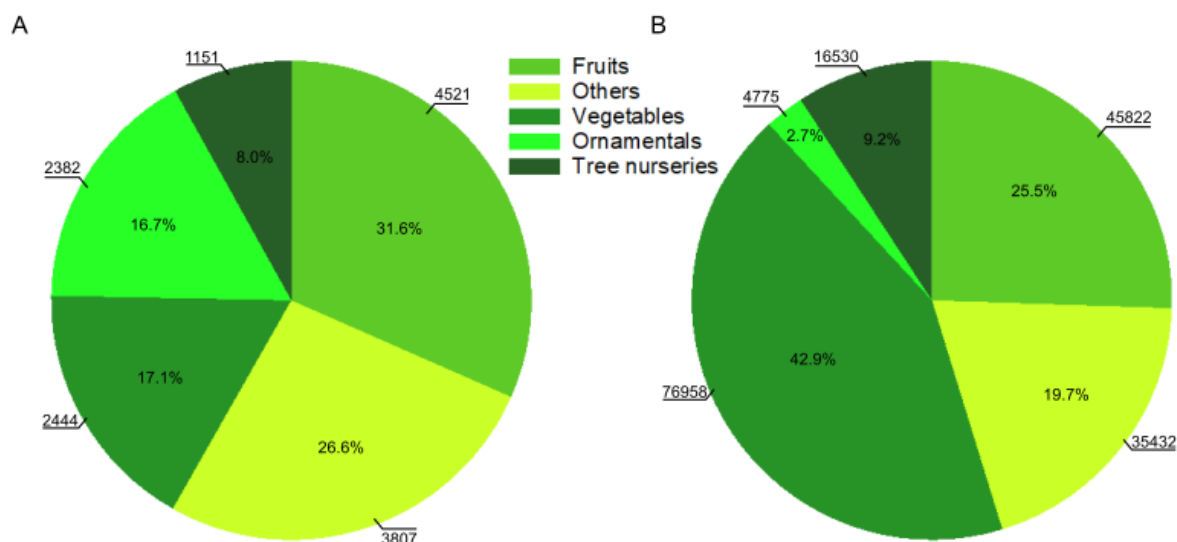
Fig. 1.1. Absolute number of companies and percentage share (A) and total area (ha) cultivated and percentage share (B) of different horticulture sectors with at least 50% focus on production in the Federal Republic of Germany. Data were reproduced from the Federal Ministry of Food and Agriculture 'Der Gartenbau in Deutschland, Auswertung des Gartenbaumoduls der Agrarstrukturerhebung' 2016.

In conventional crop production systems, the use of plant protection products is an essential component. In 2020, the sales volume of plant protection products in Germany was around 100,000 t (inert gases excluded), with herbicides accounting for the largest share of 42,998 t (42.9%), followed by 24,693 t (24.7%) insecticides, 24,624 t fungicides, bactericides, and viricides (24.6%), 5,266 t growth regulators and sprout inhibitors (5.3%), and 2,472 others (2.5%) (Federal Office of Consumer Protection and Food Safety) (Fig. 1.2).

A considerable amount of PGR and sprout inhibitors are likely to be used in agriculture and grassland systems as 53 PGR are officially approved in Germany in 2020 (Federal Office of Consumer Protection and Food Safety, 2020). Apart from that, PGR are typically used in ornamental production for growth control during the production process. Throughout the entire production chain of vegetables, PGR are not permitted by law in most countries, which is why alternative growth control measures have to be taken.

In recent years, there has been a shift from conventional to organic production, which means the abandonment of synthetically produced plant protection products, including PGR, in accordance with government objectives justified by the concerns of potentially hazardous effects on the environment, animals and humans from the application of PGR (de Castro et al., 2004; Sørensen et al., 2006).

Following the global trend towards more sustainable crop production and the ambitious goals to reduce the input of synthetically produced plant protection products, alternative methods of growth control in the different horticulture sectors need to be found to maintain the production levels and the product quality standards.
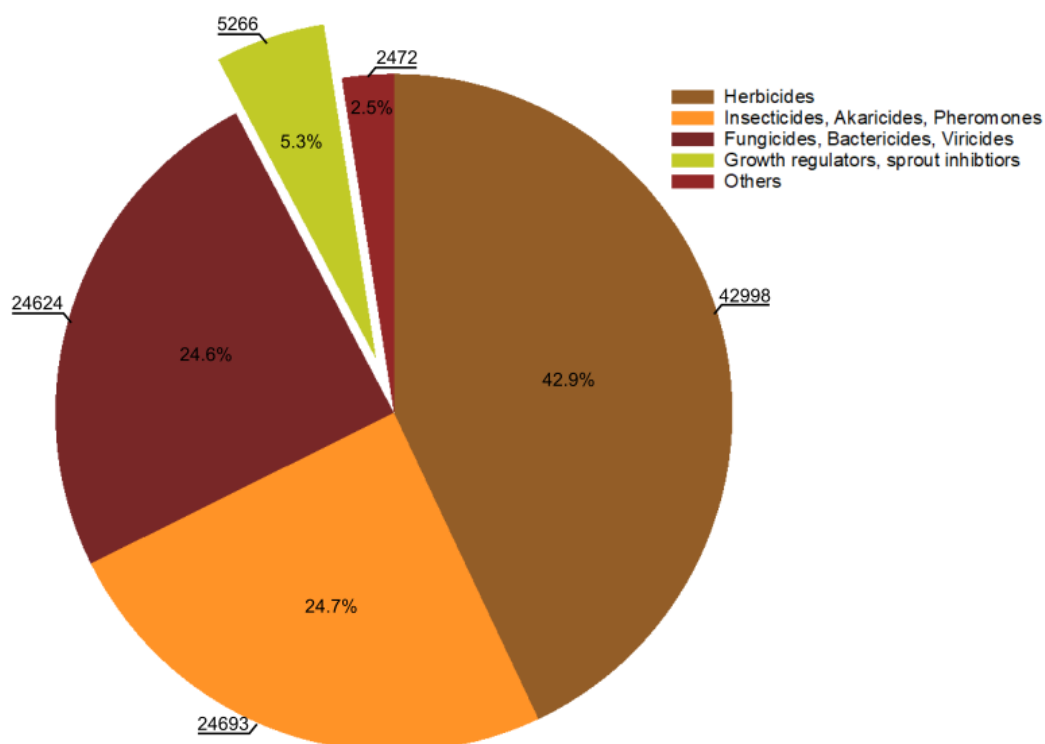
Fig. 1.2. Absolute amount (t) and percentage share of pesticide sales volume in the Federal Republic of Germany in 2020. Data were reproduced from the Federal Office of Consumer Protection and Food Safety, 2020.

1.2.   Plant growth control

An important criterion for the quality of the product is the external appearance and the size of the plant. For example, a dark green canopy colour is preferred, and the canopy size should be compact and dense (Bergstrand, 2017). A reduced growth, e.g., a reduction in stem and shoot length of potted ornamentals but also vegetable seedlings, is a major goal during the production process and is highlighted as a necessary prerequisite for the introduction of new ornamental plants (Andersen and Andersen, 2000). Consequently, there is a commercial need for plant growth control which also applies to the production of vegetable seedlings.

The reasons for this are manifold. Young plant producers must ensure a resource-efficient production to remain internationally competitive, e.g., limiting factors such as production area and transport volume must be used to the maximum. From an economical point of view, plants that are reduced in size and are characterised by general compactness are therefore advantageous compared to plants with long, elongated shoots because the area use efficiency and the volume use efficiency can be increased (Bergstrand, 2017). In addition, compact plants are usually less susceptible to potential damage during handling and transportation as they are tougher and more robust, which consequently reduces the amount of low-quality products. From a consumer point of view, a compact plant with short internodes and dark green leaves is usually preferred compared to plants with a less vital and loosened appearance.

1.3. Growth control methods

1.3.1. Breeding

Artificial mutation induction and subsequent selection for easily determined traits, such as compactness, are proposed to offer great potential for use instead of classical breeding methods which are usually more time consuming (Schum, 2003). Molecular techniques such as genetic modification with biotechnological tools are also very promising and have already been used successfully. For example, Islam et al., (2013) genetically modified poinsettia (*Euphorbia pulcherrima*) with the *Arabidopsis thaliana SHORT INTERNODE* (*AtSHI*) gene, resulting in a maximum of 52% regulation of plant height. Similarly, Lütken et al., (2010) produced several compacted *Kalanchoe* cultivars with agrobacterium *tumefaciens* as vector for the *AtSHI* gene.

1.3.2. Chemical plant growth regulators

PGR can be defined as natural compounds or synthetic analogues that influence plant metabolic processes and consequently plant growth and development of higher plants (Rademacher, 2016). Their range of application is diverse and includes different functionalities in agriculture, horticulture, and viticulture (Rademacher, 2015). For example, PGR are used to reduce logging in small-grain cereals such as wheat or rice, or to induce flowering, fruit set or fruit drop in fruit-bearing trees, to name but a few (Rademacher, 2015). Their physiological mode of action is based on influencing the plant's hormonal balance. In general, PGR have in common that they inhibit different enzymatic steps along the gibberellins (GAs) biosynthesis pathway, depending on the chemical compounds contained in the growth inhibitory product. For example, chlormequat chloride blocks the enzyme copalyl diphosphate in the early stages of the gibberellin metabolism, resulting in a persisting inhibition of all subsequent gibberellin intermediates (Rademacher, 2000), resulting in stem elongation inhibition.

1.3.3. Temperature

The feasibility to control stem extension of greenhouse-grown ornamentals and vegetable crops by temperature adjustment was demonstrated frequently (Jensen et al., 1996; Moe, 1990; Patil and Moe, 2009; Xiong et al., 2002). In general, the difference between day and night temperature highly influences the extent of stem extension in so far, that negative DIF (higher night temperature than day temperature) inhibits stem elongation, whereas positive DIF (lower night temperature than day temperature) promotes stem elongation. For example, a negative DIF of 6°C (day temperature 15°C and night temperature 21°C) reduced stem elongation in bellflower (*Campanula isophylla*) by 25% compared to a constant temperature regime of 18°C during day and night (Jensen et al., 1996).

Short temperature drops are also effective for stem extension control. For example, temperature drops from 19°C to 13° for 2 h after the beginning of the photoperiod effectively reduced stem extension by 25% in Poinsettia (*Euphorbia pulcherrima*), by 12-18% in 3 *Begonia*

x hiemalis cultivars, and by 7% - 29% in bellflower, depending on the duration of the temperature drop (Moe et al., 1995).

1.3.4. Light

The control of light quantity and quality is a useful method to regulate stem extension. The simplest approach is to manipulate the duration of the photoperiod. For example, shortening the photoperiod to 8 h d^{-1} successfully inhibited stem extension in *Calibrachoa*, *Petunia*, *Pelargonium*, and *Scaevola* cultivars by 14% to 27%, depending on when the short-day treatment was applied during the growing cycle and depending on the species (Schüssler and Bergstrand, 2012). Rather than manipulating the duration of the photoperiod, increasing the ratio of red: far red using spectral filters reduced stem elongation in Poinsettia by about 20% (Clifford et al., 2004), similar to the results found in bellflower (*Campanula carpatica)*, bean (*Pisum sativum*), and pansy (*Viola ✗ wittrockiana*) (Runkle and Heins, 2001). Application of red light for 30 minutes at the end of the photoperiod also resulted in a significant 13% reduction in shoot length of poinsettia (Islam et al., 2012). Lykas et al. (2008) increased the ratio of blue: red light and successfully reduced the length of the main shoot of *Gardenia* by about 69%. Also, Cummings et al. (2008) reduced the total plant height of bean by 11% using blue shade cloth material.

1.3.5. Mechanical stimulation

The response of plants to mechanical stimulation (MS) is referred to as thigmomorphogenesis (Jaffe, 1973). The practical implementation of MS to control plant growth and stem elongation of ornamentals and vegetable crops under greenhouse conditions has been frequently demonstrated (Autio et al., 1994; Koch et al., 2011; Latimer and Thomas, 1991; Regnant et al., 2009; Schnelle et al., 1994). For example, plant height of aster (*Callistephus chinensis*), dusty miller (*Senecio bicolor*), and petunia (*Petunia*) seedlings was reduced by 22%-25%, 18%, 44%, respectively by brushing with burlap. Also tomato plants were reduced by 37% in height when exposed to regularly applied mechanical stimuli with a suspended PVC bar (Latimer and Thomas, 1991). Koch et al. (2011) effectively reduced plant height in various herbs by bending the shoot tips with fleece material.

1.3.6. Others

A very simple but efficient approach for growth control is to restrict the space for root growth. A meta-analysis by Poorter et al. (2012) clearly showed that the available volume for root growth, which is determined by the pot size, has a great influence on biomass production. Their analysis showed that biomass production was reduced by 43% on average when the pot size was halved. Álvarez et al. (2009) showed that a controlled water deficit reduced plant height of carnation (*Dianthus caryophyllus*) by 27%. Also, Sánchez-Blanco et al. (2009) exposed geranium to drought stress and reduced plant height by 27%.

2. Research justification and study outline

2.1.   Research justification

In recent years, there has been a strong global trend towards more sustainable crop production systems, particularly related to concerns about negative environmental impacts from the introduction of synthetic substances into ecosystems and consequently into the global food chain.

Thus, with the decision on the Green Deal, the European Commission published the 'Farm to Fork Strategy' in 2020 and adopted ambitious targets to make food production systems more environmentally friendly. For example, the European Commission aims to reduce the amount of chemical plant protection products by 50% by 2030. This is also related to the goal of reducing the decline in biodiversity caused by the incorporation of chemical substances into ecosystems, while at the same time aiming to achieve a 25% share of organic crop production by 2030. Furthermore, societal concern and increasing consumer awareness of production conditions, as well as the scepticism about the introduction of chemically synthesised compounds into the food production chain, are major drivers for this paradigm shift.

So far, PGR are still the most used method to control plant growth in ornamentals. In vegetable production their use is already banned in most counties. Therefore, it can be assumed that in the future, restrictions on registration may also become stricter in ornamental horticulture, since, for example, paclobutrazol and chlormequat, two main chemical components of various commercially available PGR, have been critically considered regarding potentially harmful effects on animals (de Castro et al., 2004; Sørensen et al., 2006). Furthermore, human exposure to PGR may also be associated with health risks (Hjollund et al., 2004). However, growth control is a fundamental requirement for meeting the basic quality criterion of plant compactness and small size to improve area and volume use efficiency during seedling production and transportation to enable an economically viable production system and maintain international competitiveness. Alternative, non-chemical methods of growth control are therefore needed in the future.

MS has frequently been shown to control undesirable, excessive stem elongation in greenhouse-grown ornamentals, vegetables, and herbs (Coutand and Mitchell, 2016; Garner and Langton, 1997; Koch et al., 2011; Latimer, 1998). The underlying response is termed thigmomorphogenesis (Jaffe, 1973), and the cellular signaling events have already been described in great detail (Braam, 2005; Hamilton et al., 2015; Monshausen and Gilroy, 2009; Telewski, 2006).

From a technical point of view, most attempts to use MS for growth control were based on the use of solid materials such as metal (Schnelle et al., 1994), or PVC pipes (Baden and Latimer, 1992), but also soft materials such as fleece (Koch et al., 2011). Although these methods were effective in controlling stem elongation, damage to plant tissue was frequently reported (Garner

and Langton, 1997b; Koch et al., 2011; Latimer, 1994), resulting in severe quality loss. Furthermore, in large greenhouse compartments where different plant species are grown commonly, a continuous adjustment of the suspended material to the different plant heights would be necessary, which is practically not feasible. Therefore, the practical implementation was not pursued further and to date, no standardized and reliable procedure for mechanical growth control is commercially available.

In addition, the quantitative relationship between the application frequency of the air stream i.e., the duration of the stimulus, as well as the quantitative relationship between the stimulation intensity and the resulting degree of stem elongation inhibition still needs to be systematically investigated in order to make application recommendations for potential users. The results to date on this quantitative relationship are contradictory (Coutand and Moulia, 2000; Garner and Langton, 1997a, b; Garner and Björkman, 1996; Jaffe et al., 1980; Jędrzejuk et al., 2020; Telewski and Pruyn, 1998). Apart from this, there is little information on the maximum extent of stem elongation inhibiting by MS, the phenotypic and physiological acclimation response of plants and the associated consequences for plant productivity. A commercially available system for non-chemical growth control, which does not rely on direct touch- based MS, is urgently needed to respond to likely stringent changes in registration restrictions of PGR in the near future.

2.2.   Objectives and Hypothesis

The main objective of this study was the investigate whether air stream-based MS is suitable as a method for non-chemical growth control in greenhouse-grown ornamentals and vegetable seedlings. Following on from this, a further aim was to test various prototype systems for their practicability in terms of (i) easy integration into commonly available greenhouse infrastructure, such as boom irrigation systems, (ii) uniform stimulus application to the plant stand and (iii) comparable effectiveness to other, non-chemical growth control methods.

Furthermore, a fundamental understanding of the dose-response relationship between air stream stimulation frequency and intensity and the resulting plant growth responses should be elaborated to be able to make economically and ecologically justifiable application recommendation for potential users. Based on this, the associated effects of air stream- based MS on the plant phenotype and plant productivity should be investigated.

The following hypothesis were tested:

(i)   Air stream-based mechanical stimulation results in a reduced plant height.

(ii)   The extent of stem elongation inhibition is primarily influenced by the intensity of the air stimulus, while the duration of the stimulus is of less importance because the stimulus does not accumulate and is therefore not integrated over time.

(iii)   The extent of stem elongation inhibition is dose-dependent with defined values for a stimulus threshold and stimulus saturation threshold with an intervening linear dependence.

(iv)   As a result of the adaptive stress response a new equilibrium state is reached that allows the plant to maintain its original productivity through phenotypic plasticity and physiological adaptation.

2.3.   Study outline

This dissertation has been submitted as a cumulative thesis. The main body comprises 4 scientific articles of which 2 have been published and 2 are under review.

The PhD project comprised 4 work packages (WP). The limitations and knowledge gaps were identified by an extensive literature review (WP1), followed by the identification of the most suitable prototype for air stream-based MS for growth control of greenhouse-grown crops (WP2), the investigation of the effectiveness of air stream-based MS with the selected prototype (WP3), and the morphological and physiological plant responses to air stream-based MS (WP4).

One of the prototypes investigated addressed in WP2 was evaluated at the State Horticulture College and Research Station (LVG) in Heidelberg. The evaluation of the other prototypes was carried out at the University of Hohenheim. After the prototype selection, the effectiveness and the plant acclimation response were investigated in a greenhouse chamber at the University of Hohenheim.
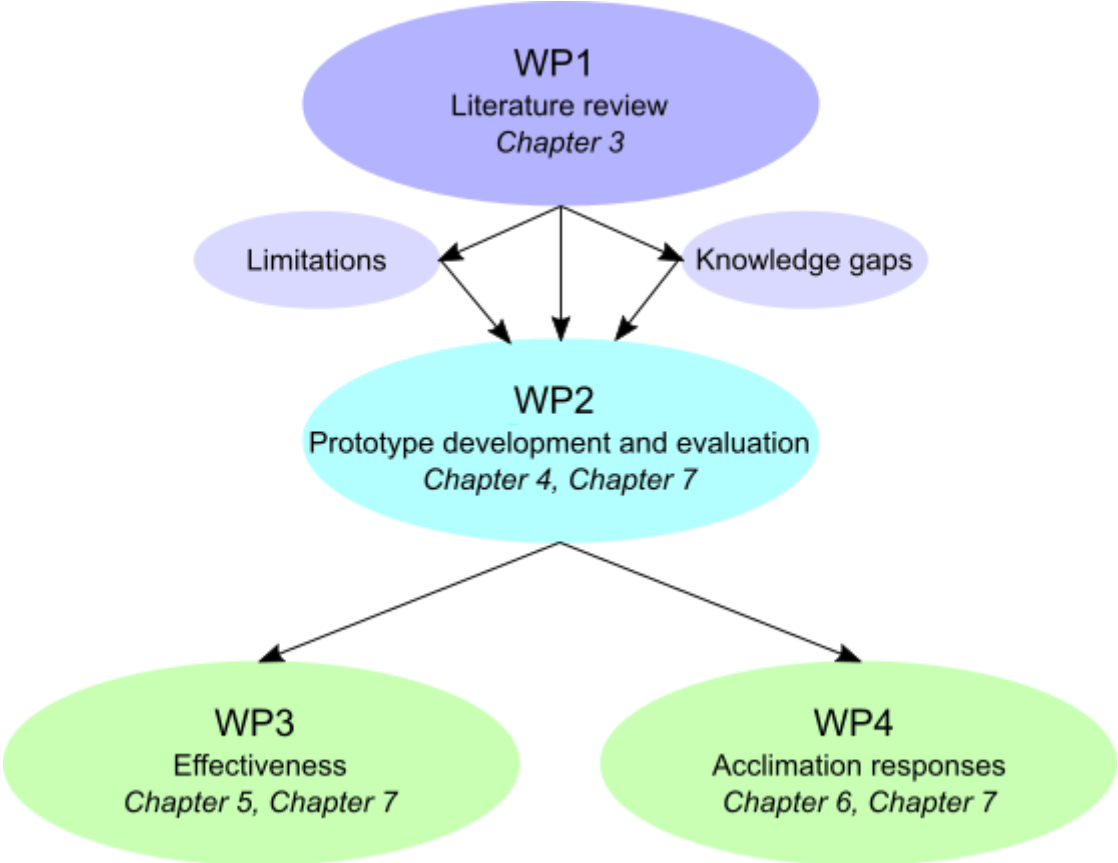
Fig. 2.1. Work packages (WP 1-4) of the PhD study.

2.4.   Funding

3. Mechanosensing of plants

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3.1.   Abstract

Plants possess a remarkable acclimatization capacity at (sub)cellular level that enables them to tolerate unfavourable growing conditions to a greater extent than that possible without such changes. These specialized plant cells can sense mechanical forces or deformation, brought about by environmental cues or crop management practices, and convert these physical impacts into specific response mechanisms. This review describes some possible physiological and molecular mechanisms that are responsible for sensing and transducing mechanical signals by living cells. Mechanical stimuli are perceived at the cytoskeleton-plasma membrane-cell wall interface that subsequently trigger ion channel activity, ion- (e.g., Ca^{2+}) mediated signaling responses, followed by downstream signaling events such as gene expression and protein and metabolite (e.g., plant hormones) adjustment. In turn, a progressive acclimation and morphological change at the tissue and whole plant level takes place in response to mechanoperception. The potential of mechanical stimulation as a technique for horticultural applications under greenhouse conditions is discussed.

3.2.   Introduction

Plants do not possess the ability to escape their surroundings. Once the signal to germinate is received and transferred, young seedlings must cope with the environmental conditions they come upon. During their lifetime, plants in natural habitats are frequently exposed to changing and often highly variable environments, evoked by climatic factors or crop management practices. Plants, including horticultural crops, possess tremendous phenotypic plasticity, allowing them to respond sensitively to a range of environmental conditions in a time-dependent manner through their ability to change certain traits and to adjust developmental processes. Such acclimatization capacity (adaptivity) is associated with specific physiological and biochemical mechanisms such as CO_2 assimilation, mineral uptake, synthesis of hormones and proteins or expression of regulatory genes. The complexity of the underlying ecophysiological mechanisms, however, still poses many questions: which environmental signals control specific plant processes? How are those signals perceived and transduced to bring about the developmental changes associated with, for example, flowering, fruit set, fruit abscission and fruit growth? Answers to these questions will provide the basis for the development of effective and reliable practical tools for crop manipulation, ensuring that genetically intrinsic plant/fruit properties can be expressed to their full potential.

To improve their chances of survival, plants have evolved tolerances to abiotic and biotic stressors, which refer to the plant's fitness to sense and respond sensitively to unfavourable environments. Phototropism is an example of whole plant alignment either towards (positive) or away from (negative) a light source in order to maximize light interception and consequently the acquisition of photosynthetic energy for plant growth and developmental processes or to avoid stress from this stimulus. Furthermore, the internal gravimetric sensing system enables plants to orient their roots towards gravitational pull for resource acquisition, whereas shoots grow in the opposite direction for light exposure.

An excellent example of mechanical stress sensing in the plant kingdom is the carnivorous Venus flytrap (*Dionaea muscipula*), which demonstrates one of the most rapid and intensively studied plant movements. Its finely differentiated antennae system enables the plant to close its modified trap-leaves within a fraction of a second to capture arthropod prey (Braam, 2005; Markin and Volkov, 2012). A further plant species, which shows a quick nastic reaction by leaf folding, is the mechanosensitive plant *Mimosa pudica*. The double compound leaves are folded rapidly after a touch stimulus is perceived, most probably as a defense reaction to reduce the attack surface and visibility to natural enemies (Jaffe et al., 2002, Braam, 2005). However, vascular plants, which do not possess this specialized and rapid morphing behaviour, change also their structural and architectural composition in response to mechanical force perception, even though a visible reaction occurs gradually on a longer time scale. Plant responses to MS was named thigmomorphogenesis by Jaffe (1973). It followed a series of experiments which

investigated the effects of artificial mechanical stimulations on plant growth. Over the last few decades, the research area on thigmomorphogenesis has expanded with an attempt to formulate a unified hypothesis about mechanoperception in plants (Telewski, 2006; Monshausen and Gilroy, 2009). Ecophysiological plant responses and mechanisms include ion gating through mechanosensitive channels (Haswell et al., 2011; Peyronnet et al., 2014), cell-to-cell communication through propagating calcium (Ca^{2+}) and reactive oxygen species (Kurusu et al., 2012a; Sewelam et al., 2016), touch inducible-gene expression (Braam, 2005) and phytohormonal changes (Chehab et al., 2009).
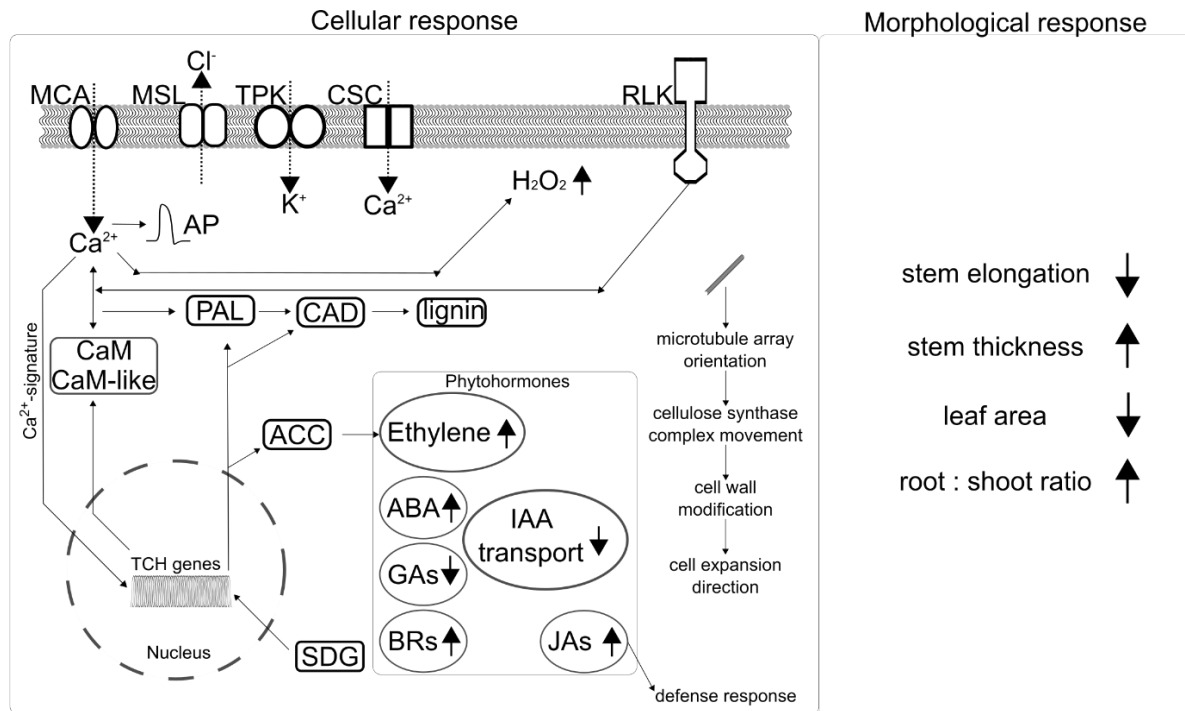
Fig. 3.1. Cellular and morphological plant responses to mechanical stimulation. Mid1-complementing activity (MCA) channels, mechanosensitive channels of small conductance-like (MSL) and two-pore potassium (TPK) channels facilitate ion gating across the cell plasma membrane. Calcium-permeable stress-gated cation channels (CSC) are also suggested to be involved during mechanical force perception. Ion fluxes might trigger action potentials (AP) and Ca^{2+} influx produces oxidative bursts (H_2O_2). Cell wall monitoring membrane proteins of the receptor like-kinases (RLK) family influence the Ca^{2+} - signature. The stimulus-specific Ca^{2+} signatures trigger the expression of touch-inducible genes (TCH) encoding Ca^{2+} signalling proteins like Calmodulin (CaM) and Calmodulin-like (CaM-like), which activate downstream signalling events such as enzyme activation of phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) within the phenylpropanoid pathway to promote lignin biosynthesis. 1-aminocyclopropanecarboxylic acid synthase (ACC) is activated and increases ethylene biosynthesis. Biosynthesis of abscisic acid (ABA), brassinosteroids (BRs) and jasmonic acids (JAs) is increased. Jasmonic acids synthesis enhances plant defence against pests and diseases. Gibberellic acids (GAs) are broken down and polar indole-1-acetic acid (IAA) transport is reduced. Cell wall properties also become modified by the arrangement of microtubules, which correlate with the movement of cellulose complexes. Changes at cellular level lead to morphological alterations such as reduced stem elongation and leaf area or increased stem thickening and root:shoot ratio.

The objective of this review is to describe morphological and physiological plant responses to mechanoperception and to review the underlying key mechanisms at plant organ and cellular scale, respectively (Figure 3.1). The potential of MS as a technique for controlling plant growth under greenhouse conditions without applications of plant PGR is discussed.

3.3.   Thigmomorphogenesis

The term thigmomorphogenesis is composed of three words which originate from the Greek language: *thígma* meaning touch; *morphê* meaning shape and *genesis* referring to creation. Accordingly, thigmomorphogenesis is a term, which describes the morphological and structural shaping of a plant organism during its developmental phase as influenced by touch-like stimuli (Jaffe, 1973).

Over the last five decades, the term thigmomorphogenesis has been used to refer to plant responses associated with the perception of MS by abiotic stressors, pest attacks or specific crop management factors such as pruning and training practices. In general, mechanical stimuli generate pressure, which is perceived by the cytoskeleton-plasma membrane-cell wall interface (CPMCW) that is then integrated into stimulus-specific molecular signaling signatures (e.g., Ca^{2+}), followed by signal transduction, including gene expression, protein synthesis/degradation, plant metabolic adjustment and a progressive acclimation at the tissue and whole plant level (Jaffe et al., 2002). Hence, mechanical force perception by plants triggers cell physiological processes, which in turn result in a gradual morphogenetic plant acclimation. A visual change in plant shape and structure of most vascular plants to mechanical stimuli usually occurs on a longer time scale. In natural environments, climatic factors such as sunlight, wind, rain, or snow are major drivers that exert energy transmissions on the plant body, inducing mechanical strains. Under controlled experimental conditions, mechanical force loads are induced by brushing, stem bending, local touching, and shaking or vibration devices to control and study plant growth as well as to mimic thigmomorphogenesis effects in natural environments.

Distinct forms of MS (e.g., seismomorphism, as a plant reaction to sound waves or vibration) have been categorized by Mitchell and Myers (1995). Although the number of physical stimuli that are perceived by plants is diverse, two main thigmo-related plant responses can be distinguished according to growth and development patterns. Thigmotropism refers to those responses which are aligned towards the direction of the stimulus and are frequently accompanied by a unilateral growth inhibition of the growing structure (Telewski, 2012). For example, a thigmotropic plant movement is the coiling of bean (*Phaseolus vulgaris*) tendrils when an obstacle is sensed by direct physical contact. Negative thigmotropic plant responses are found in tree species, which grow away from the direction of the environmental stimulus, as observed by the asymmetrical canopy formation of trees where growth occurs away from the prevailing wind direction (Telewski, 2012). In contrast, thigmonasty is a specialized reaction

Tab 3.1. Thigmo-related plant responses

Term	Definition	Reference
Thigmotropism	Growth movements related to the direction of the stimulus	(Telewski, 2012)
Thigmonasty	Non-growth movements; not related to the direction of the stimulus	(Telewski, 2012)
Thigmomorphogenesis	Long term growth and developmental changes induced by mechanical force perception	(Jaffe, 1973)

of a plant to a mechanical stimulus, but proceeds independently of the stimulus direction (Monshausen et al., 2008) and includes highly specialized plant organs (Table 3.1). For instance, the Venus flytrap has developed a unique mechanoperception system, which enables the plant to close its trap-leaf within 0.3 s (Markin and Volkov, 2012).

3.4.   Natural and artificial induction of thigmo responses

3.4.1. Natural induction

In natural environments, plants experience MS by kinetic energy of, for example, wind, rain, snow or by physically impairing one another and thus inducing the formation of specific stabilising tissue (e.g., flexure wood) (Gardiner et al., 2016). The gravity force itself is also continuously sensed by plants and plays a major role in mechanical force perception. It influences the plant's architectural structure, inducing cellular stretch and compaction forces at the CPMCW (Telewski, 2006).

Among all of the natural mechanical stimuli that plants may be exposed to, wind forces are probably the most dominant and persistent. Studies on tree structure formation under wind forces exist for pine (*Pinus*) (Gillies et al., 2002; Mayhead, 1973; Vollsinger et al., 2005), Douglas fir (*Pseudotsuga menziesii*) (Mayhead, 1973; Spatz et al., 2007), spruce (*Picea*) (Mayhead, 1973) and walnut (*Juglans*) (Lopez et al., 2011) species and were compiled in a review by Gardiner et al. (2016). Trees need to cope with fluctuating wind conditions, as they grow at the same location for several years or up to decades. Trees which are exposed to weak wind velocities and multidirectional wind directions, maintain a more symmetrical branching alignment, whereas tress that experience moderate to strong winds have a typically asymmetrical branching formation away from the prevailing wind direction. Windswept tree structures have often been recorded both in temperate and in tropical wind-exposed locations like coastal regions, ridges, mountains, flat plains, or at the border line of whole forest systems (Jefferson 1904, Smith 1972, Holtmeier 1981, Telewski and Jaffe 1986a, Noguchi 1997). Tree shape, crown architecture, and branch positioning have often been used as indicators to describe local wind conditions as, for example, the velocity and direction of wind currents (Wade and Hewson, 1979). Wind is considered as the major ecological component (Metzger, 1893) affecting "dosage"-dependent tree form and thus thigmotropic plant reactions (Telewski and Pruyn, 1998; Telewski, 2012). Several biomechanical modelling approaches, describing

the mechanical forces acting on trees, have been undertaken based on experiments in wind tunnels (Mayhead, 1973; Vollsinger et al., 2005) and under natural conditions (Spatz et al., 2007). New terms like "oscillation damping" to describe the up-and-down movement of branches (Spatz and Theckes, 2013) or reconfiguration (Harder et al., 2004; Lopez et al., 2011) and streamlining (Harder et al., 2004) to characterize the direction of branch establishment and leaf orientation have been introduced. From these studies, it can be concluded that the plant structure depends on the wind speed, the air density, the drag force (Speck, 2003; de Langre et al., 2012) and the frontal area density of the respective plant part (Gardiner et al., 2016). Moreover, the tension, torsion and compression forces of wind along the plant system are not evenly distributed and change over time (Telewski, 2012) and thus frequently cause secondary stresses like changes in leaf microclimate and possibly water status (Onoda and Anten, 2011). In general, the acclimation of a plant to continuous wind exposure is, therefore, a strategy to prevent negative impacts of excessive force loads and involves thigmo-responses that have independent or additive effects on endogenous plant signalling pathways (Mitchell, 1996; Smith and Ennos, 2003; Marler and Clemente, 2006; Anten et al., 2010).

3.4.2. Artificial Induction

Research on thigmomorphogenesis was investigated using barley (*Hordeum vulgare*), bean, red bryony (*Bryonia dioica*), cucumber (*Cucumis sativas*), sensitive plant (*Mimosa pudica*) and castor oil plant (*Ricinus communis*) in the early 1970s (Jaffe and Galston, 1966, Jaffe 1970, 1973, 1976). MS was originally applied by internode rubbing between thumb and forefinger. Most of the species showed an inhibition in stem elongation rates. In the 1990s, studies with practical implications reported on MS using suspended PVC pipes (Latimer and Thomas, 1991) or cardboard sheets (Baden and Latimer, 1992) that were manually moved across a plant stand, thereby rubbing or bending the shoot tips. These approaches were further refined by inducing more controlled thigmo effects with different mechanical devices (Gartner, 1994; Osler et al., 1996; Patterson, 1992) or fleece material (Koch et al., 2011; Regnant et al., 2009). However, to better understand the causal relationship between MS intensity (e.g., degree of shoot bending) and the corresponding growth response (e.g., degree of internode reduction), standardized experimental procedures were required using quantifiable force loads with so called thigmostimulators. For example, Coutand et al. (2000) investigated temporal growth retardation dynamics immediately after the application of a stem bending treatment in tomato (*Solanum lycopersicum*) by recording the internode extension growth response with a linear voltage differential transducer.

The application of PGR is still heavily used for inhibiting shoot growth and development and thereby inducing stunted plants in a range of applications in horticulture (Rademacher, 2015). For example, Biddington and Dearman (1987) demonstrated that ethephon, by triggering ethylene biosynthesis, and chlormequat, by inhibiting gibberellin biosynthesis in plant tissue,

induced shoot growth inhibiting effects that were mostly comparable with MS treatments in lettuce (*Lactuca sativa*), cauliflower (*Brassica oleracea*) and bean.

3.5.   Morphological plant responses

Plants exposed to wind not only respond to mechanical strain but also to the consequential change in microclimate (Onoda and Anten, 2011), whereas plants treated with brushing or bending devices mainly respond to the mechanical stimulus alone. Consequently, thigmomorphogenetic plant responses, induced by the perception of mechanical force and environmental stresses, are difficult to separate. Hence, it is problematic to extrapolate from responses to bending or brushing to those due to wind, as has been shown in broadleaf plantain (*Plantago major*) (Anten et al., 2010), papaya (*Carica papaya*) seedlings (Marler and Clemente, 2006) and sunflower (*Helianthus annuus*) (Smith and Ennos, 2003).

Plants respond to MS with specific morphological changes at organ level (Figure 3.1).

3.5.1. Plant Organs

1.   Leaf. Brushing young shoots of cauliflower, lettuce or celery (*Apium*) with bond paper for approximately 1.5 min at each of 40 times per day resulted in a visibly more compact growth habit compared to unbrushed plants (Biddington and Dearman, 1985). The compact plant appearance was attributed to a reduction in petiole length as well as an increase in leaf thickness in celery and cauliflower, and to a reduction in leaf length in lettuce. Other studies have confirmed these morphological leaf alterations in vegetable plants under mechanical stress treatments (Latimer, 1991), specifically in several tomato cultivars (Gartner, 1994; Latimer and Thomas, 1991; Piszczek and Jerzy, 1987) but also in cucumber (Latimer et al., 1991), soybean (*Glycine max*) (Nakaseko, 1988), garden pansy (*Viola wittrockiana*) (Garner and Langton, 1997a), petunia (*Petunia*) (Garner and Langton, 1997a) and aster (*Aster*) (Autio et al., 1994). For example, brushing back and forth the shoot apex of tomato twice daily for 18 continuous days resulted in plants with reduced leaf size, leaf dry weight, and leaf number compared to the controls (Johjima et al., 1992). Moreover, perennial coniferous (Telewski and Jaffe 1986a, 1986b) and deciduous tree species (Flückiger et al., 1978; Marler and Zozor, 1992; Niklas, 1996; Telewski and Pruyn, 1998; Wu et al., 2016) showed a reduction in needle length and leaf size when exposed to MS by wind. Leaves of sugar maple (*Acer saccharum*) sampled from wind-exposed sites showed a significant reduction in leaf petiole length, average petiole diameter and leaf surface area compared to those from wind- protected sites (Niklas 1996). Attempts to establish dose response curves between mechanical stimulus intensity and the degree of leaf area reduction have been made (Flückiger et al. 1978, Marler and Zozor 1992, Telewski and Pruyn. 1998). Flückiger et al. (1978) showed that wind speeds between 0 and 4 m s^{-1} did not have marked effects on leaf area reduction in young aspen (*Populus tremula*) trees, whereas wind speeds between 4 and 6 m s^{-1} induced a decline of about 50%, but this effect became less strong towards 12 m s^{-1}. In contrast, carambola (*Averrhoa*

carambola) seedlings responded more sensitively with an approximate 50% reduction in leaf area at 2 m s^{-1} wind speed. Recently, Wu et al. (2016), evaluating leaf responses of eight oak (*Quercus*) species following an artificial wind treatment, concluded that due to differences in leaf shape, lanceolate and lobed leaves were less susceptible than broad leaves to wind-induced morphological changes. Telewski and Pruyn (1998) found that mean leaf area of American elm (*Ulmus americana*) seedlings was significantly reduced when they were bent at least 40 times per day.

In summary, it can be suggested that leaf size reduction is a general plant acclimation response to MS, including wind, in order to impose less drag on the plant structure and to prevent breakage and uprooting (Gardiner et al., 2016).

2.   Stem. Stem or shoot responses of plants that have perceived mechanical stimuli have a reduction in internode elongation and, frequently, an increase in radial growth (Biddington, 1986; Braam, 2005; Gardiner et al., 2016; Hamant, 2013). Thus, over the cycle of development, plants subjected to such stimuli become shorter and often thicker and therefore more robust to withstand physical stress impacts (Monshausen and Gilroy, 2009). The compacted plant habit induced by thigmomorphogenesis has been shown in tree species exposed to wind in natural environments (Telewski and Jaffe, 1986a, b; Jaffe and Forbes, 1993; Watt et al., 2005) but also in plant species cultivated under greenhouse conditions treated with various mechanical stimuli (Biddington and Dearman, 1987, 1985; Jaffe and Forbes, 1993; Latimer, 1991). Biro et al. (1980) demonstrated that the stunted stem growth of bean seedlings induced by internode rubbing was related to reduced cell elongation rates in epidermal and cortical cells. It was further shown that the wind-induced radial growth extension in bean was attributable to an increase of secondary xylem cell numbers and an increase in cambial activity (Hunt and Jaffe, 1980). However, in other herbaceous plant species with often limited secondary growth, stem girth was not affected after mechanical perturbation, such as in cauliflower (Biddington, 1986), sunflower (Smith and Ennos, 2003) and *Arabidopsis* (Paul-Victor and Rowe, 2011). In contrast, in wind exposed woody tree species, like loblolly pine (*Pinus taeda*) (Telewski and Jaffe, 1986a) and Fraser fir (*Abies fraseri*) (Telewski and Jaffe 1986b), stem thickening occurred due to more rapid cell division rates of the vascular cambium and the formation of specialized tissue structures like flexure wood or reaction wood was enhanced (Gardiner et al. 2016). Consequently, stem thickening seems to be a less conservative and a polygenetic dependent response to MS compared to stem shortening.

Other stem properties may also be affected by mechanical impact: tissue exposed to MS is often more flexible, less rigid and therefore less susceptible to breakage. For example, vibration stress applied to shepherd's purse (*Capsella bursa-pastoris*) plants significantly increased shoot flexibility compared to untreated plants (Niklas, 1998). A decrease in stem rigidity and stiffness, thus an increase in stem flexibility, was also found in sweetvetch

(*Hedysarum coronarium*) (Wang et al., 2008), tobacco (*Nicotiana tabacum*) (Anten et al., 2005) and, more recently, in *Arabidopsis thaliana* stems (Paul-Victor and Rowe, 2011) when exposed to vibration, bending or brushing treatments, respectively. Interestingly, Smith and Ennos (2003) indicated that stem rigidity and strength of sunflower was increased in response to manual stem bending but decreased by air flow treatment. In contrast, Telewski and Jaffe (1986a) demonstrated that mechanically perturbed Fraser fir produced a thick and less elastic, rigid stem. The reasons for the different responses of woody and herbaceous plants to mechanical stresses are not clear; however, differences in tissue specific secondary growth characteristics and in metabolites may be important attributes in this respect.

Experiments to evaluate the response between the intensity/frequency of the mechanical stimulus and the resulting inhibitory effect on stem elongation were undertaken in bean (Biro et al., 1980; Jaffe et al., 1980), elm seedlings (Telewski and Pruyn, 1998), and in tomato (Coutand and Moulia, 2000). In bean, inhibition of stem elongation was observed at very low forces of 0.3 N, and maximal growth reductions were seen at 6 N. In elm seedling stems the relationship between the stimulus frequency (0, 5, 10, 20, 40, 80 flexures per day) and the inhibition of tree height was best fitted with a negative exponential function, thereby confirming the results by Jaffe et al. (1980). However, the force required to induce a thigmomorphogenetic response was considerably greater than that found in bean and is probably related to differences in stem tissue composition between herbaceous and woody plants. Coutand and Moulia (2000), investigating the growth response of tomato at the whole plant level after a defined stem bending treatment, found that neither maximal force nor the maximal bending point of the stem but the sum of the longitudinal strains along the stem during bending explained best the variability of the growth response. Moreover, their study showed that endogenous signal transport processes are required to translocate the signal from the source of the mechanical stimulus to those tissues that are sensitive to stress perception. (Biro and Jaffe 1984) found that the degree of growth inhibition due to mechanical stress was correlated to internode length in bean: shorter internodes (10 mm) became more inhibited than longer internodes (20 mm), indicating that the younger the tissue and the less the terminal internode length is attained, the greater the potential to inhibit growth extension under mechanical stress perception. Consequently, tissue age and tissue sensitivity are important features in the response mechanism to mechanical stimuli.

3.   Root. MS of above-ground plant organs may also have consequences for the development of the below-ground root system. However, the mechanically induced root growth response may also be affected by the complexity of the soil environment which, in turn, will determine to what extent the soil volume can be explored to improve plant anchorage. For example, it was shown that brushed vegetables (Biddington and Dearman, 1985; Johjima et al., 1992; Gartner, 1994), wind-exposed trees (Jaffe and Forbes, 1993; Gardiner et al., 2016;

Wu et al., 2016), manually bent tree stems (Coutand et al., 2008) and plants treated with vibration stress (Niklas, 1998) generally partition proportionally more biomass towards below-ground plant tissues, likely due to the formation of anchorage structures to avoid breakage or uprooting (Stokes et al., 1995; Mickovski and Ennos, 2003). In contrast, Biddington and Dearman (1985) reported that brushed cauliflower, lettuce and celery seedlings all had shorter and fewer roots than occurred on control plants and that the root:shoot dry weight was only increased in lettuce.

Several studies have investigated the effect of unidirectional mechanical stress of plant stems on root morphology and architecture. For example, root formation of wind exposed young Sitka spruce (*Picea sitchensis*) and larch (*Larix decidua*) trees tended to occur towards the windward site compared to the leeward site, indicating that the direction of the prevailing wind can influence the growth and alignment of roots in the soil structure (Stokes et al., 1995). Similarly, unidirectional stem flexing of pine (*Pinus sylvestris*) resulted in greater root cross-sectional area and enhanced root mass at the plane of the flexure direction compared to control plants (Mickovski and Ennos, 2003). When the deciduous woody species robinia (*Robinia pseudoacacia*) and oak (*Quercus robur*) were repeatedly stem flexed into one direction, the formation of first-order deep roots was increased; however, second-order roots were fewer in *Quercus* but more abundant in *Robinia*, suggesting a species-specific biomass allocation (Reubens et al., 2009). Moreover, maize (*Zea mays*) and sunflower plants subjected to regular unidirectional stem flexure increased the number and thickness of first-order lateral roots, yet there were also considerable differences in the growth responses and mechanical properties of lateral roots between the two species which were presumably related to their contrasting anchorage mechanics (Goodman et al., 1998). Bending of *Arabidopsis* roots for 20 s induced the formation of lateral roots on the convex side of the resultant bend (Richter et al., 2009).

4.   Reproductive Organs. Changes in the developmental processes of generative organs have been reported in association with MS. For example, a moderate daily shaking of American sweetgum (*Liquidambar styraciflua*) trees resulted in terminal bud formation within 3 weeks, which did not occur in unshaken plants (Neel and Harris, 1971). In soybean, MS of the upper canopy markedly increased the formation of later branches and, despite the delay of pod development, seed productivity was increased by 38% (Nakaseko, 1988). Moreover, Niklas (1998) demonstrated that MS of shepherd's purse reduced the dry weight of reproductive structures, delayed the formation of the first mature flower and fruit by 5 and 3 days, respectively, accelerated the onset of plant senescence by 8 days, and reduced seed production by 43% when compared to unstimulated plants. The shift in biomass allocation from generative towards vegetative growth upon mechanical stress exposure might be interpreted as an adaptation mechanism when more resources are partitioned into plant structure for plant survival and ensuring the long-term success of reproductive development.

3.5.2. Cytoskeleton-plasma Membrane-cell Wall Continuum

1.   Cell Wall and Membrane. Plant cells continuously sense and respond to mechanical deformations or cell wall perturbations which subsequently affects the physicochemical and cellular processes involved in cell growth and development (Wolf et al., 2012). The cell wall represents a physical barrier between the surrounding environment and the intracellular space, is part of the cytoskeleton-plasma membrane-cell wall interface and thus plays an important role in the perception of mechanical forces (Telewski, 2006). Jaffe et al. (2002) affirmed the importance of the cell wall in transducing thigmo events, as it is the primary acceptor of mechanical stimuli. A force perception proposal by Jaffe et al. (2002) postulates that linkage structures (e.g., integrin-like receptors), which are connected to the cell wall, may be required to further integrate the mechanical signal into the cell via the plasma membrane. There is accumulated support for the involvement of an integrin-binding tripeptide Arg-Gly-Asp (RGD)-like recognition system in physically connecting the plasma membrane to the cell wall and sensing mechanical stress (Monshausen and Gilroy 2009). Indeed, it was shown, that plasma membranes of cultured cells of *Arabidopsis* possess binding sites for heptapeptides containing the Arg-Gly-Asp sequence, suggesting a role of integrin-like receptors in the process of signal transfer between the cell wall and the plasma membrane (Canut et al., 1998). However, such a RGD-like recognition system for integrin strands has not yet been identified in horticultural plants.

Compositional changes of cell wall constituents were shown in young bean plants when "rubbing" led to the formation and deposition of callose in the cells and phloem tissue of internodes within 1.5 to 6 h, but the polysaccharide was fully metabolized after three days (Jaffe et al., 1985). De Jaegher et al. (1985) showed an activation of enzymes within the lignification biosynthesis pathway following internode rubbing in bean. Both studies suggest that cell wall lignification and concomitantly cell wall rigidification is part of the thigmomorphogenetic response. More recently, cross-sectional stem tissue organization of mechanically perturbed *Arabidopsis* wildtype plants was investigated and it was shown that cell walls of lignified tissue were thickened; however, the relative contribution of cortex tissue to cross-sectional area was significantly increased, whereas pith and lignified interfascicular tissue was significantly reduced (Paul-Victor and Rowe, 2011). The authors suggested that this growth pattern indicates an acceleration of plant developmental rates.

Woody plants respond to MS by also changing cell wall composition and structure (Gardiner et al., 2016). This response is a strategic acclimation and has a hardening function to prevent damage by, for example, strong winds in natural environments. Trees exposed to moderate to strong wind loads respond with the formation of differential wood structures: reaction wood forms during permanent positional changes (leaning) of branches and stems on a longer time scale, for example, as a response to continuous gravity impact. In contrast, flexure wood forms

in response to oscillating and swaying movements due to wind forces and occurs on a shorter time scale.

2.   Microtubules. Microtubules (MTs) are composed of longitudinally arranged heterodimers of α- and β-tubulins, which commonly consist of 13 protofilaments that are attached to each other to form a hollow circular shape with a diameter of approximately 20-30 nm (Hashimoto, 2015). These cylindrical tubes are key elements of the cytoskeleton in eukaryotic cells, forming a network that mechanically stabilizes the cell walls and presets the direction of cell expansion in response to environmental signals, including touch (Lloyd, 2009). When mechanical cell wall properties become modified by, for example, the rearrangement of MTs, spatial cell wall stiffness changes, leading to a directed cell expansion vector (Landrein and Hamant, 2013).

It is now well established that the orientation of the cortical MTs array in the plasma membrane correlates with the orientation of the cellulose microfibrils that are deposited in the cell wall, giving the wall its mechanical integrity and properties (Corson et al., 2009; Jacques et al., 2013; Landrein and Hamant, 2013). For example, visualization of cellulose deposition using transgenic *Arabidopsis* plants, expressing a yellow fluorescent protein fused to cellulose synthase, revealed coincidence between the movement of cellulose synthase complexes in the plasma membrane and the alignment of the cortical MTs (Paredez et al., 2006). Moreover, the requirement of MTs to allow an anisotropic, directed growth was shown in *Arabidopsis thaliana* roots when the application of microtubule inhibitors (oryzalin and taxol) resulted in radial swelling and a loss in directed cell growth and expansion (Baskin et al., 1994). The application of oryzalin, a herbicide of the dinitroaniline class that acts through the disruption (depolymerization) of MTs, onto the apical shoot meristem of *Arabidopsis thaliana* seedlings resulted in tissue geometry changing into a globular shape, indicating that growth anisotropy was lost (Corson et al., 2009). These observations suggest an important role of cortical MTs array orientation to guarantee a pattern of anisotropic cell expansion and growth. Experimental data have shown that an external physical force or a mechanical stress substantially influence cortical MTs orientation (Hush and Overall, 1991; Cleary and Hardham, 1993; Hamant et al., 2008; Jacques et al., 2013).

In pea (*Pisum sativum*) roots, the application of a mechanical field directly influenced the orientation array of cortical MTs, aligning them perpendicular to the stimulus direction (Hush and Overall, 1991). In ryegrass (*Lolium rigidum*) epidermal leaf cells, cortical MTs arrays reoriented their direction from transverse to longitudinal when a non-directional hydrostatic pressure was applied (Cleary and Hardham, 1993). However, Hamant et al. (2008) demonstrated, through experimental and modelling approaches at the shoot apex of *Arabidopsis*, that cortical MTs align parallel to the direction of maximal stress. Changes in MTs array alignment as a response to globally applied pressure (Uyttewaal et al., 2012) or to touch-

like stimuli via a microneedle (Hardham et al., 2008) permit the assumption of a functional role of MTs in the responsivity to mechanical stimuli.

3.6.   Physiological plant responses – cellular signaling

3.6.1. Calcium

Ca^{2+} plays a substantial role in many plant physiological processes and is involved in numerous signal transduction pathways in plant cells, including plant responses to mechanical perturbations (Figure 3.1). Several studies have shown that mechanical forces, either by wind or localized touch-stimulation, on plant cells elicit a cytoplasmic Ca^{2+} increase ($[Ca^{2+}]_{cyt}$) (Jones and Mitchel 1989l; Knight et al., 1991, 1992; Thonat et al., 1993; Haley et al., 1995). Knight et al. (1991) monitored *in planta* and non-invasive $[Ca^{2+}]_{cyt}$ changes under MS by transforming plants with the Ca^{2+}-sensitive luminescent protein aequorin. Mechanostimulation, in combination with the application of channel blocker substances and Ca^{2+} chelated forms, to inhibit the influx from extracellular Ca^{2+} sources through the plasma membrane, revealed a substantial role of Ca^{2+} membrane transport mechanisms immediately following cellular mechanoperception in soybean (Jones and Mitchell, 1989). It has also been shown that Ca^{2+} and pH influence the probability of a channel being open (Gobert et al., 2007) and both of these parameters may change upon MS.

Further evidence that Ca^{2+} transport processes at the plasma membrane are involved, was visualized using fluorescent red bryony internode probes (Thonat et al., 1993). Knight et al. (1992) demonstrated that plant movements induced by wind force were accompanied by $[Ca^{2+}]_{cyt}$ peaks, which originated from intracellular organelle sources since, when adding ruthenium red (a potent inhibitor of intracellular Ca^{2+} release) the Ca^{2+} signal was impaired.

Cell membranes are part of the direct interface between a plant cell and its surrounding environment. Therefore, it seems reasonable that plasma membranes must possess putative sensory systems to stay in contact with their surroundings. It is generally accepted that mechanosensitive ion channels are embedded in the plasma membrane and become stretch-activated under MS to promote ion transport across the membrane (Haswell et al., 2011; Peyronnet et al., 2014; Hamilton et al., 2015; Basu and Haswell, 2017). While Ca^{2+} acts as an universal intracellular messenger in many abiotic and biotic stress-related plant reactions, like wind and cold-shock (Knight et al., 1992, 1991) and herbivore attack (Kiep et al., 2015), it is still unclear what makes a touch-induced $[Ca^{2+}]_{cyt}$ signalling pattern specific, leading ultimately to a specific, graded physiological response. This question refers to the so-called Ca^{2+} signature or Ca^{2+} footprint, which was firstly addressed by McAinsh and Hetherington (1998). The Ca^{2+} signature is defined by its spatio-temporal signal distribution inside the cell. The intensity of the stimulating force determines the magnitude of $[Ca^{2+}]_{cyt}$ and, more importantly, the intracellular signalling hot spots and the response competence of single plant cells. Under great stimulus intensities, intracellular Ca^{2+} organelle processes become essentially involved

by releasing Ca^{2+} storage pools to trigger the appropriate downstream signalling cascade by, for example, activating/deactivating Ca^{2+} sensitive sensor proteins (Hashimoto and Kudla, 2011; Monshausen, 2012). In addition, the ability of single plant cells to respond plays a crucial role. The response competence depends on the developmental history of the cell, concomitant with its exposure to different local abiotic conditions and gene-encoding messages, which in turn determine the availability of signal receptors to convert the Ca^{2+} signature into a meaningful message. Additionally, Ca^{2+} signalling frequency and amplitude, meaning $[Ca^{2+}]_{cyt}$ oscillation and spikes, may play key roles for the specificity of the plant response (McAinsh and Hetherington, 1998). It remains unclear if Ca^{2+} signatures contribute to a systemic information transmission at the whole plant level because these specific cellular responses are predominantly considered in the context of local stimuli application (Gilroy et al., 2016, 2014). However, long distance signalling in response to mechanical perturbation is likely because growth inhibition occurs at regions which were not directly stimulated.

3.6.2. Reactive Oxygen Species

Reactive oxygen species (ROS) were originally considered as hazardous by-products arising from aerobic metabolic processes and their occurrence was related to classical plant defence responses. Because of their toxic characteristics in the cell, their abundance is highly regulated by both non-enzymatic and enzymatic reactions. The major ROS forms in plants are singlet oxygen (O_2), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO) (Waszczak et al., 2018).

The key role of ROS molecules in rapid plant signalling responses to environmental stimuli has been established (Gilroy et al., 2014, 2017; Perez and Brown, 2014; Mittler and Blumwald, 2015; Sewelam et al., 2016; Choi et al., 2017; Choudhury et al., 2017). Like the signal transduction of the Ca^{2+} profile, which is characterized by its occurrence spatially within the cell and its temporal dynamics, the information transmitted by ROS depends on the balance between their degree of scavenging, regional production and compartmentalization (Bailey-Serres and Mittler, 2006). Their presence results in stress-specific downstream activation of proteins. ROS are also involved in the plants mechanosensory function. Bradley et al. (1992) have shown that bean and soybean cells extracted from node tissue preferentially have greater H_2O_2-mediated protein cross-linking. They related that to mechanical strains particularly acting on the tissue at the shoot-petiole junction during leaf development, indicating H_2O_2-mediated formation of cell-wall stabilizing structures at bearing positions. Furthermore, Legendre et al. (1993) reported that in suspension-cultured soybean cells, mechanical stirring for the purpose of maintaining cell vigour induced a continuous oxidative H_2O_2 burst (Figure 3.1). They postulated that this H_2O_2 burst was not related to cells stimulated with a purified polygalacturonic acid elicitor, but was rather related to thigmotropism at sites where rapidly growing cells inside the media interacted physically. A targeted and more calibrated

investigation to illuminate the relation between H_2O_2 burst and mechanical stress was undertaken by Yahraus et al. (1995). They showed that both osmotic changes within the cell media and direct physical pressure applied to soybean cells led to similar oxidative burst profiles. They suggested that plant cells can detect mechanical disturbances by initiating a classical defence response reaction. Additionally, Gus-Mayer et al. (1998) demonstrated that continuous non-damaging stimuli of single parsley (*Petroselinum crispum*) cells with a tungsten needle resulted in the generation of reactive oxygen intermediates. The observed oxidative burst was essentially the same as that in response to locally defined mechanical stimuli and to the penetration by a fungal hypha. Although the involvement of ROS as messenger molecules in response to environmental changes at the subcellular, cellular and whole plant level is substantiated, ROS signalling seems to play a primary role in the response to biotic and abiotic stresses and is not solely related to mechanoperception. Moreover, Ca^{2+} signalling is considered as a prerequisite to initiate downstream regulated ROS signalling (Monshausen et al., 2009).

3.6.3. Electrical Signals

Recent literature in the field of electrophysiology underlines the importance of electrical signals in plants as a mechanism to rapidly react to environmental stimuli, to allow intercellular signal translocation (Gallé et al., 2015) and to acquire a systemic signalling response to biotic and abiotic stress factors (Choi et al., 2016, 2017; Gilroy et al., 2016). Many electrophysiological studies have been conducted preferentially on characean algae due to their larger cell size which has strongly facilitated practical research work (Tazawa, 1984; Wayne, 1994; Iwabuchi et al., 2005). Additionally, the carnivorous plant (*Dionaea muscipula*) (Volkov et al. 2007, Markin and Volkov, 2012) and the sensitive plant (*Mimosa pudica)* (Jaffe et al., 2002; Braam, 2005) have been shown to be suitable model organisms for studying electrophysiological responses in plants in conjunction with mechanical stimulus perception. However, many plants if not all perceive and transduce mechanical stimuli by similar means.

It has been suggested that plant electrical communication occurs via the phloem over long distances and via plasmodesmata over short distances from cell to cell (Gallé et al., 2015; Gilroy et al., 2016). Long-distance electrical signalling can be studied by using aphids as bioelectrodes (Hedrich et al., 2016). Further approaches to capture electrical signals can be achieved by extracellular or intracellular electrode arrangement techniques (Fromm and Lautner, 2007; Yan et al., 2009). Extracellular measurements are conducted with surface contact electrodes (calomel [Hg_2Cl_2] or a silver metal electrode), whereas localised intracellular measurements usually require the insertion of a glass microelectrode into the cell, using a micromanipulator. Recently, Volkov et al. (2017) have shown that electrode arrangement (distance of electrodes from each other and intra- vs. extracellular electrode attachment) influences the characteristics (e.g., amplitude and duration) of the electrical signal

and has, therefore, to be considered when interpreting results.

In general, electrical signals are evoked by alterations of the membrane voltage potential as a result of ion movements across the cell plasma membrane (Figure 3.1) when biotic and abiotic environmental stimuli are perceived. The nature of electrical signal evolution is related to changes in permeability for ion (Ca^{2+}, Cl^-, K^+) gating processes across the plasma membrane (Vodeneev et al., 2016). Non-damaging stimuli (e.g., cold, MS) trigger electrical signals which are assigned to be action potentials (APs) (Gilroy et al., 2016; Vodeneev et al., 2016). The prevailing characteristic of APs is to follow the all-or-nothing principle (Fromm and Lautner, 2007); when a certain degree of stimulus threshold is exceeded, Ca^{2+} channels in the plasma membrane open that lead to a cytoplasmic influx of Ca^{2+} ions, followed by a voltage dependent Cl^- efflux across the plasma membrane (depolarization of the plasma membrane). The voltage-shift opens K^+ channels and K^+ cations are relocated out of the cell in order to re-establish an electrochemical equilibrium (repolarization phase) (Fromm and Lautner, 2007). These mechanisms have been elucidated by studying the flow of ions across the cell membrane (patch-clamp) and experiments involving channel blocker substances in *Chara* cells (Okihara et al., 1991; Homann and Thiel, 1994; Wayne, 1994). Specifically, Kaneko et al. (2005, 2009)) demonstrated the involvement of mechanosensitive Ca^{2+} channels in mechanoperception in *Chara*.

Immediate electrical signalling events in plants are clearly one of the first responses to mechanical stimuli. As mentioned above, non-invasive stimuli profiles, such as mechanical stimuli, mainly evoke the propagation of APs throughout the plant body. Whether the electrical signal characteristics correlate to the quality of the mechanical stimulus still needs to be verified in future research. Studies to date have shown that the type of stimulus (temperature, mechanical wounding, chemical application) seems to correlate with the AP profile, i.e., with the AP amplitude and propagation velocity (Volkov and Haack, 1995). However, Pyatygin et al. (2008) reasonably argued that APs only serve as a bioelectric pulse-modulated alarm signal, whereby no information about the quality of the external stimulus can be transmitted to resting, non-affected tissue. They argued that plants lack a clear mechanism to decode the signal, like the central nervous system can in animals. Moreover, the all-or-nothing principle beyond the stimulus threshold makes it less probable to identify a dependency between the intensity of the mechanical stimulus and the manifestation of the generated AP.

3.6.4. Protein Complexes

1.   Mechanosensitive Ion Channels. Considerable research has been conducted on the identification and characterization of mechanosensitive ion channels (MCs) (Telewski, 2006; Monshausen et al., 2008; Monshausen and Gilroy, 2009; Haswell et al., 2011; Peyronnet et al., 2014; Hamilton et al., 2015; Basu and Haswell, 2017). Mechanosensitive ion channels are protein complexes, which facilitate the gating of ions down an electrochemical gradient across

a cell membrane, as a response to mechanical force impacts elicited by touch, gravity, osmotic pressure and sound vibration. In plants, MCs play a crucial role in mediating biological processes, including shape development of plastids (Haswell and Meyerowitz, 2006), osmosensing (Maathuis, 2011) and expansion growth (Gobert et al., 2007). Their characterization commonly requires experiments with pharmacological substances to either inhibit or activate protein channels and to characterize channel properties by electrophysiological and genetic approaches (Hamilton et al., 2015).

When mechanical stimuli are perceived, the channel protein in the cell membranes undergoes a conformational change which leads to either opening or closing its channel pore to control ion transport. There are currently two models of this gating process that explain how membrane-activated ion channels open or close. The so-called stretch-activated opening of MCs relates to the tension in the lipid bilayer, caused by, for example, mechanical force, whereby the cell membrane is laterally stretched and thinned (Kung, 2005; Monshausen et al., 2008). In contrast, a spring-like tether in either the cytoskeleton or the extracellular matrix is attached directly to the MC and acts like the spring mechanisms of a shutter upon MS (Kung, 2005; Monshausen et al., 2008).

Three putative MCs protein families have been identified in plants (Figure 3.1; Hamilton et al., 2015). The first family are homologs of bacterial mechanosensitive channels with small conductance (MscS-like), which are essentially nonselective ion channels (Hamant and Haswell, 2017; Hamilton et al., 2015). In *Arabidopsis* ten MscS-like proteins have been characterized and they are involved in ion transport in the inner membrane of chloroplasts (Haswell and Meyerowitz, 2006) and mitochondria (Haswell, 2007) and in the cell plasma membrane of root cells (Haswell et al., 2008). They become activated upon changes in membrane tension but also upon changes in temperature and turgor pressure. Their major function has been ascribed to the regulation of solute concentration under conditions such as hyper-osmotic shock (Haswell, 2007). The second family are Mid1-complementing activity (MCA) proteins. This family putatively promotes Ca^{2+} fluxes at the cell plasma membrane and has been described in *Arabidopsis* (Nakagawa et al., 2007; Yamanaka et al., 2010), tobacco (Kurusu et al., 2012c), and rice (*Oryza sativa*) (Kurusu et al., 2012b). Overexpression of MCA1 in *Arabidopsis* enhanced $[Ca^{2+}]_{cyt}$ levels under hypo-osmotic stress conditions and also elevated expression levels of touch-inducible genes (TCH3) (Kurusu et al., 2012c). The third family includes the two-pore potassium (TPK) protein channels and, as their name indicates, they are selective for K^+ cations. (Maathuis, 2011), using patch-clamp approaches, revealed that membrane stretch activates TPK channels, indicating sensitivity towards MS.

Recently, two more protein families that become activated by MS have been suggested by Hamant and Haswell (2017). These belong to the (OSCA)/Ca^{2+}-permeable stress-gated cation channel (CSC) family (Figure 3.1) and the mechanosensitive Piezo channel family. However,

Piezo homologues have not yet been characterized in plants.

2.   Receptor-Like Kinases. Receptor-like kinases (RLK) are membrane-localized proteins, which generally consist of three domains: a cytosolic kinase domain, a transmembrane domain and an extracellular domain (Monshausen and Haswell, 2013). The RLKs as potential RGD-binding proteins, connect the plasma membrane to the cell wall and have been described to play an important role in recognizing the mechanical status of the cell wall by forming a cell-wall surveillance system (Monshausen and Gilroy, 2009). Moreover, they are suggested to be involved in mediating and directing cell growth and development in response to diverse environmental signals, including mechanical stresses (Humphrey et al., 2007; Cheung and Wu, 2011).

A protein subfamily of RLKs, the cell wall-associated receptor-like kinases (WAKs), was shown to be required for normal cell elongation and lateral root development, suggesting a crucial role for WAKs in plant developmental processes (Lally et al., 2001). Another subfamily of RLKs is the *Catharanthus roseus* receptor-like kinases (CrRLKs) (Monshausen and Haswell, 2013). Recently, in *Arabidopsis* mutants lacking FERONIA, a member of this subfamily, Ca^{2+} signalling was completely absent or exhibited qualitatively different signatures when stimulated locally by touch or bending (Shih et al. 2014). Moreover, the upregulation of the touch-responsive *TCH* gene was decreased in *fer* loss-of-function mutants after MS and the plant phenotype exhibited typical growth developmental defects related to thigmomorphogenesis. The authors concluded that the CrRLK FERONIA appeared to play an important role in early events of the Ca^{2+} mediated signalling response. This is the first evidence of a likely relationship between an RLK subfamily member and its role in the process of mechanotransduction.

3.   Calcium Sensor Proteins. MS results in elevated expression levels of specific genes (TCH1, TCH2 and TCH3) which encode calmodulin (CaM) and CaM-like proteins (Figure 3.1; Braam and Davis, 1990). In conjunction with Ca^{2+}, these proteins undergo a conformational change and subsequently alter their physiological effectiveness with consequences for downstream signalling events (e.g., enzyme activation/deactivation, ion membrane transport, Ca^{2+} homeostasis) within the thigmomorphogenetic signalling response (Chehab et al., 2009; Monshausen, 2012). Studies on Arabidopsis have shown that mechanical stimuli trigger the synthesis of Ca^{2+}-binding proteins (Braam, 1992; Sistrunk, 1994; Antosiewicz et al., 1995). When calmodulin antagonists (calmidazolium, chlorpromazine) were applied, the stunted growth through MS could be partially negated, indicating a substantial role for Ca^{2+}-binding proteins in mediating thigmomorphogenesis (Jones and Mitchell, 1989). A study investigating a novel Ca^{2+}-binding protein (TCH3) revealed a similar structural identity to previously known Arabidopsis calmodulins. However, a major difference was that instead of having four Ca^{2+}-binding sites, this novel calmodulin-like protein had six (Sistrunk, 1994). In addition, tissue

specific accumulation of the TCH3 protein during plant development was shown. Tissues which had undergone cell expansion, such as the apical meristem of developing roots and shoots, were associated with the accumulation of the TCH3 protein. Furthermore, MS by wind revealed an upregulation of the TCH3 protein, which predominately accumulated at branch points of leaf primordia, at the central zone of the apical meristem, and inside the vascular cambium (Antosiewicz et al., 1995). Therefore, cell modifying properties of the calmodulin-like protein, induced by mechanical stimuli, are suggested and a potential interaction with auxin which, for example, directs cell expansion and growth in young tissues, might be plausible (Chehab et al., 2009).

4. Enzymes. MS activates enzymatic steps inside the phenylpropanoid pathway (Figure 3.1; De Jaegher et al., 1985; Cipollini, 1997; Saidi et al., 2009). The upregulation of phenylalanine ammonia-lyase (PAL) in red bryony (de Jaegher et al., 1985) and peroxidase activity in primary bean leaves (Cipollini, 1997) and in tomato internodes (Saidi et al., 2009) in response to MS was significantly increased in comparison with control plants. Moreover, cinnamyl alcohol dehydrogenase (CAD) activity in tomato (Saidi et al., 2009) and in bean (Cipollini, 1997) was also significantly increased by MS. PAL is the primary enzymatic step for the synthesis of polyphenolic compounds and CAD the last required enzymatic step to complete lignin biosynthesis. In relation to that, it has been shown that the proportion of lignified tissue in mechanically stimulated plants was higher compared to that in control plants, giving evidence that tissue lignification, which can be considered as an aging process, is part of the thigmomorphogenetic response. However, the upregulation of these enzymes is not directly involved in the mechanosensory system but rather becomes activated as a general and non-specific plant defence response. Also, the key enzyme in the ethylene biosynthesis pathway, 1-aminocyclopropanecarboxylic acid (ACC) synthase, was shown to increase in activity after MS (de Jaegher et al. 1987). Cazzonelli et al. (2014) recently identified a chromatin modifying enzyme, SDG8, which promotes the TCH gene in Arabidopsis in response to MS, revealing new molecular insight into thigmomorphogenesis as the loss-of-function mutant sdg8 did not reach maximum TCH expression levels.

3.6.5. Phytohormones

1. Ethylene. Ethylene is a gaseous plant hormone. This volatile molecule plays a ubiquitous role as a signalling compound in multiple plant developmental processes including seed germination, fruit ripening and defence reactions to pathogens (Johnson and Ecker, 1998). In addition, ethylene has long been considered to be the major constituent in mediating thigmomorphogenetic plant responses under the influence of mechanical stress (Figure 3.1; Braam, 2005).

First evidence of the involvement of ethylene release from intact plant tissue under spatially limiting growth conditions was shown in pea epicotyls when glass beads exposed young plants

to a mechanical barrier (Goeschl et al., 1966). They showed that physical stress triggered ethylene production in the non-injured plant tissue. Further studies confirmed that ethylene production occurs under diverse physical stimuli, including stem bending of apple (*Malus domestica)* trees (Robitaille and Leopold, 1974), hand rubbing of bean (Biro and Jaffe, 1984; Takahashi and Jaffe, 1984) and red bryony (de Jaegher et al., 1987) stems, and electric vibration of peach (Prunus persica) trees (Onguso et al., 2006). Also, the direct exposure of plants to external ethylene concentrations without mechanical stimuli resulted in thigmomorphogenetic-like plant responses (Goeschl et al., 1966; Robitaille and Leopold, 1974; Erner and Jaffe, 1982). Ethylene evolution under mechanical stress was often reported to be closely related to increased activity of 1-aminocyclopropane-1-carboxylic acid synthase, which is an important enzymatic step in the biosynthesis of and a precursor for ethylene (Biro and Jaffe, 1984; de Jaegher et al., 1987; Botella et al., 1995; Onguso et al., 2006). However, after (Johnson et al. 1998) showed that ethylene-insensitive Arabidopsis mutants responded similarly to MS compared to the wild type, it was essentially proven that ethylene was not directly required to manifest the morphological developmental changes associated with thigmomorphogenesis. Nevertheless, as ethylene is involved in "cross-talking" with other phytohormones which are directly involved in thigmomorphogenesis (e.g., auxin), it seems reasonable that ethylene at least contributes to some extent to thigmomorphic events (Chehab et al., 2009).

2.   Auxin. The general key role of auxin-mediated plant stature alignment and growth redirection, that are induced by environmental cues (e.g., gravity or light direction), has been clearly established (Morita and Tasaka, 2004; Esmon et al., 2006). The actively regulated polar transport of auxin along plant cells, and the subsequent gradual distribution along tissues and the whole plant, essentially determines plant structure and development (Friml, 2003). Auxin distribution is achieved by a transporter-like membrane protein (PIN), which functions as an efflux carrier, thereby enabling cell-to-cell auxin transport (Benjamins, 2003).

Auxin is considered to play a major role in thigmomorphogenesis (Figure 3.1, Chehab et al., 2009), being involved in the gradual metabolic acclimation processes involved in the responses to mechanical stress treatments (Mitchell, 1977; Erner and Jaffe, 1982; Sanyal and Bangerth, 1998). Mitchell (1977) rubbed hooks of young pea seedlings between thumb and forefinger and excised the first two 5 mm internode sections directly below the apical hook. The time of excision after the MS essentially influenced the subsequent *in vitro* growth response of the respective internode section. The *in vitro* growth of the first sections was stimulated when cut within the first 8 h of stress application, but growth was inhibited when cut after 8 h. In contrast, growth of the second section was progressively inhibited with increased time between treatment and cutting, indicating a time-dependent inhibition of the relocation of growth promoting substances from the shoot apex towards the stem section located below the

apical hook. When using labelled ^{14}C-IAA and the donor-receiver agar cylinder technique as described by Beyer and Morgan (1969), they found that the polar auxin transport in the second sections was inhibited by 52% compared to those of non-stimulated seedlings.

Similar results have been shown by Erner and Jaffe (1982) where mechanical perturbation of bean internodes induced an accumulation of auxin but significantly retarded polar auxin transport rates, which contributed to a retarded elongation of the internodes. Sanyal and Bangerth (1998) demonstrated that down-bending of vertical shoots from mature apple trees significantly reduced polar auxin transport rates. Both, Erner and Jaffe (1982) and Sanyal and Bangerth (1998) suggested that polar auxin transport inhibition is due to increased ethylene evolution in mechanically stressed tissue, proposing cross-talk between auxin and ethylene. Additionally, the apple shoot bending experiment by Sanyal and Bangerth (1998) showed that mechanical stress can be used as a tool to manipulate the endogenous hormonal balance to positively affect flower bud induction.

Interestingly, Benjamins (2003) found that a PINOID protein (serine/threonine kinase), which is essential to mediate auxin signalling, interacts with a Ca^{2+}-binding protein of the touch-inducible gene TCH3, suggesting that the interplay of the two proteins might also be associated with thigmomorphogenetic plant responses.

3.   Jasmonic Acid. Considerable attention has been paid to jasmonates (JAs) as a plant hormone affecting plant growth and development, but in particular the conjugated forms like methyl-jasmonate, serving as a natural endogenous plant growth regulator (Per et al., 2018). Jasmonates are ubiquitous in the plant kingdom and a cross-talk function (Per et al. 2018) with other plant hormones to modulate physiological, biochemical and molecular processes, and also in mediating thigmomorphogenesis, has been established (Figure 3.1; Chehab et al., 2009).

Methyl-jasmonate is synthesized from α-linolenic acid through various enzymatic and oxidative steps within the octadecanoid pathways. One major important intermediate product and precursor of methyl-jasmonate within this biosynthetic pathway is 12-oxophytodienoic acid. Through HPLC and GC-MS analysis, (Stelmach et al. 1998) showed that the content of this intermediate substance is essentially increased during the coiling movement of red bryony tendrils and that it is also accumulated after internode rubbing between thumb and forefinger, suggesting a substantial role of this jasmonate intermediate in directed plant movements and plant responses to MS. Chehab et al. (2012), using *Arabidopsis* mutant plants with an inability to synthesise JA due to a functional loss in allene oxide synthase (aos), verified that touch-induced morphogenesis is JA-dependent. In contrast, a transgenic *Arabidopsis* line which constitutively overexpressed oxophytodienoate reductase, accumulated approx. 30% more JA than the wild type and consequently greatly resembled phenotypic thigmomorphogenetic-like characteristics of the touched wild type (a decreased rosette radius, shorter inflorescences and

delayed flowering). Notably, touched plants with subsequently elevated JA levels were also primed against *Botrytis cinerea* infection and cabbage looper infestation. The observed increased pest and disease resistance following MS is consistent with previous reports (Cipollini and Redman, 1999).

4.   Other Phytohormones. In addition to ethylene, auxin and jasmonates, abscisic acid (ABA), brassinosteroids (BRs) and gibberellins (GAs) are also plant hormones that regulate plant developmental processes and are therefore potential candidates in mediating thigmomorphogenesis (Figure 3.1; Chehab et al., 2009).

ABA accumulation under MS has been reported in young bean plants (Erner and Jaffe, 1982) and in rice (Jeong and Ota, 1980). Also, exogenous applications of ABA resulted in growth retardation similar to that observed under MS (Erner and Jaffe, 1982).

Brassinosteroids are metabolic compounds whose physiological activity depends on the functional group attached to their cholesterol skeleton (Haubrick and Assmann, 2006). Xu (1995) applied a highly potent brassinosteroid (24-epibrassinolide) and observed a subsequent rapid upregulation of the TCH4 gene encoding cell wall modifying enzymes that belong to the touch-inducible gene family. When BR-insensitive mutants were touch stimulated, stable TCH4 gene expression levels were found, indicating that BRs signalling was not directly involved in the thigmomorphogenetic signal transduction (Iliev et al., 2002). In contrast, when brassinolide was applied in conjunction with IAA, a drastic increase in ethylene production, which was greater of that under IAA application alone, was shown in Arabidopsis tissues, indicating a BR - IAA interaction that may be involved in growth retardation under MS (Arteca and Arteca, 2008).

GAs have also been shown to be involved in growth retardation induced by MS (Suge, 1978; Lange and Lange, 2015;). A daily stroking by hand of the top shoot apex of bean plants greatly reduced the concentration of specific GAs (Suge, 1978). Recently, Lange and Lange (2015) found that loss-of-function Arabidopsis mutants (ga2ox7), which are deficient in GAs signalling, did not show morphological alteration when touched. Moreover, touched wild type plants showed a significant reduction in GAs (GA_{12}, GA_{15}, GA_4, and GA_{34}) and the stunted growth could be reversed by application of a bioactive form of gibberellin, suggesting a key role of GAs in mediating thigmomorphogenesis (Lange and Lange, 2015).

3.7.   Molecular aspects

Within the past three decades, research groups have revealed insights into plant responses to mechanical stimuli at the molecular level. The discovery of touch-inducible (*TCH*) genes by Braam and Davis (1990) and the genome-wide analysis of touch- and darkness-regulated genes by Lee et al. (2005) in *Arabidopsis thaliana* are considered as major breakthroughs.

When *Arabidopsis* plants were treated by touch, rain, wind, irrigation, water sprays or even darkness, the expression of *TCH* genes increased by 10 – 100-fold (Braam and Davis 1990).

Subsequently, fundamental knowledge of cause and effect relationships in thigmomorphogenetic plant responses was gained on a molecular level. The mRNA expression levels of *TCH* genes peaked 30 min after the mechanical stimulus and fell back to initial levels after approx. 2 h (Braam and Davis 1990). When a subsequent stimulus 2 or 3 h after the first stimulus was applied, mRNA accumulation was less pronounced, suggesting a decrease in response sensitivity over time (Braam, 1992). Nevertheless, when subsequent stimuli were applied rapidly after one another (2, 5, or 10 back and forth movements of rosette leaves), *TCH* mRNA accumulation increased, revealing a dose-dependent expression behaviour. Sistrunk (1994) demonstrated a tissue-specific expression of the *TCH3* gene, using the β-glucuronidase (GUS) reporter gene technique. Plant tissues that predominantly expressed the *TCH3* gene were the growing regions of roots, the vascular tissue, the root/shoot junction, and the branching points of the shoot, siliques and flowers. Direct wounding or darkness revealed a comparable *TCH* mRNA expression pattern to touch-like stimuli, particularly for the *TCH3* gene, so that the response specificity of the *TCH* genes to stimuli with mechanical properties was put into question. Lee et al. (2005) conducted a genome-wide analysis of touch- and darkness-regulated *Arabidopsis thaliana* genes using microarray analysis and quantitative real-time RT-PCR. They focused particularly on calmodulin (Ling et al., 1991; Perera and Zielinski, 1992; Gawienowski et al., 1993;), calmodulin-like (CML) and xyloglucan endotransglucosylase/hydrolase (XTH) genes and sought to gain insight into both the cellular functions that may be altered by mechanostimulation and the shared regulatory properties of these genes. Moreover, they aimed to find out if genes exist that uniquely respond only to a touch treatment. Overall, 2.5% of the whole *Arabidopsis* genome was touch-inducible which is remarkable and shows the significance of mechanical force perception by plants on a molecular basis. The majority of the touch-related genes in the *Arabidopsis* genome were putatively associated with Ca^{2+} and kinase signalling, wall modification, disease resistance and downstream transcriptional responses. Furthermore, about half of the 589 genes that were up-regulated in response to touch were also up-regulated by darkness, indicating a common or at least partially overlapping signal transduction pathway. This might be due to darkness inducing mechanical perturbation forces by, for example, changes in cell turgidity, which in turn are then genetically encoded. More recently, studies by Ghosh et al. (2016, 2017) identified the overlapping existence of gene transcripts induced either by sound vibration or by touch stimuli in *Arabidopsis thaliana*, suggesting comparable stimuli identities of touch and sound vibrations.

CaM-related cDNAs were also isolated in mung bean (*Vigna radiata*) (Botella and Arteca, 1994) and in rapeseed (*Brassica napus)* (Oh et al. 1996) and expression studies revealed in both cases a transient dramatic increase of the transcript level after touch stimulation. In a subsequent study with *Vigna radiata*, Botella et al. (1996) demonstrated that a Ca^{2+}-dependent

protein kinase gene (*VrCDPK-1*) was inducible by mechanical strain.

3.8.   Application strategies in horticulture

The initiative to use mechanical stimuli as a method for growth regulation under controlled greenhouse conditions was based on the need for alternative solutions to the standard practice of using synthetic PGR and the exclusion of daminozide from use in all vegetable crops in the United States in 1989. Toxicological effects of active ingredients in PGR remain a serious concern, leading to a demand for more sustainable crop management strategies. Available alternatives to the application of PGR are crop management strategies involving, for example, the adjustment of water and nutrient availability. However, these methods may have negative effects on crop productivity and quality. Therefore, mechanical conditioning, that is the application of mechanical stress, was proposed as an appropriate method for controlling plant growth under greenhouse conditions by natural means (Latimer, 1991).

Mechanical conditioning was shown to be effective on various vegetable species and ornamental plants growing under a range of possible commercial systems (Latimer, 1990; Latimer and Thomas, 1991; Autio et al., 1994). For example, stem length of the tomato cultivar 'Sunny' could be reduced by 37% compared to untreated control plants (Latimer and Thomas, 1991). Approaches to develop an electrically driven, fully automated application device equipped with either PVC tubes (Morel et al., 2012) or fleece material (Regnant et al., 2009) proved successful for controlling plant growth. However, observations of plant damage on pepper (Latimer, 1994) and also on petals of ornamental plants (Koch et al., 2011) limited further development and commercialisation. Moreover, simultaneous treatment of different plant species, cultivars or plants of different age within the same greenhouse area were impracticable because of the need to permanently adjust the application device. Laser based or optical scanner tools might be implemented to automatically adjust the device to the required height. However, economic feasibility studies are required. A promising solution might be MS procedures by efficient and less invasive air blast systems.

Besides being an efficient method for growth control, mechanical conditioning was also shown to enhance field establishment of transplants (Latimer and Beverly, 1993) with the additional benefit of increasing resistance towards pests (Latimer et al., 1994; Benikhlef et al., 2013; Pillai and Patlavath, 2015; Tomas-Grau et al., 2018). Latimer et al. (1994) showed that the number of feeding scars per plant and the percentage of damaged leaf area of various vegetables was lowered after brushing when plants were inoculated with western flower thrips and green peach aphid compared to unbrushed plants. In *Arabidopsis*, soft MS triggered Ca^{2+} fluxes accompanied by ROS production and *TCH* gene expression and subsequently decreased vulnerability to the virulent fungus *Botrytis cinerea* (Benikhlef et al., 2013). Similarly, Tomas-Grau et al. (2018) found that soft touch-like stimulation of strawberry plants induced biochemical and molecular changes and also resulted in an increased resistance to *Botrytis*

cinerea. Altogether, therefore, several reports on enhanced plant stress resistance are available, providing a reasonable assumption that thigmomorphogenesis may be useful in increasing plant resistance against pests and diseases. However, the defence response activated by mechanical stimuli would need to be of a general nature and not specific against individual pathogen strains or pest species if it was to be effective.

Yield performance by MS has been inconsistent. Field transplanted broccoli, which had previously been mechanically stimulated did not show any differences in yield performance compared to unstimulated control plants (Latimer, 1990). Moreover, greenhouse grown cucumber cultivars were mechanically treated before being transplanted in beds and three out of four cultivars did not show significant yield differences to the controls (Latimer et al., 1991). However, the duration of the treatment period needs to be well chosen because yield was negatively affected under long-term mechanical treatment periods (28 days) in four tomato lines (Johjima et al., 1992). Nevertheless, it has been suggested that if the application is done during the transplant production stage, no or only minor negative effects on yields of vegetables are expected (Latimer and Beverly, 1993).

Temperature and light intensity tend to influence the efficacy of mechanical conditioning, indicating seasonal variation. Moderate temperatures and low light intensities had a positive effect on the extent of growth depression during MS (Latimer, 1991). Investigating the growth performance of pansy transplants (Garner and Langton, 1997a) and tomato seedlings (Garner and Langton, 1997b) under different mechanical treatment application schemes indicated that growing conditions during summer mitigated MS efficacy, whereas the number of stimuli required to achieve maximum growth depression was lower in spring. The time of the day (9 am vs. 4 pm) when MS was applied had no effect on the magnitude of growth retardations. Garner and Langton (1997a, b) concluded that brushing was a flexible management technique that could be used to control excessive stem elongation under greenhouse conditions and has the potential to reduce the application of PGR. Identifying characteristic plant responses to mechanical stimuli and applying plant specific treatments using electrophysiological signatures might be a future field of application in horticulture plant production. Through such approaches, the next step towards digitalization and fully automated system operation, as recently demonstrated by Pereira et al. (2018), could be realized in horticulture.

3.9.   Conclusions

Mechanosensing is an important and widespread biological phenomenon that allows both multicellular eukaryotes (plants, animals) and prokaryotes (bacteria) to perceive and respond in a well-coordinated manner to mechanical stimuli. The temporal pattern of the thigmomorphogenetic growth response after mechanical stimuli perception is highly variable among plant species.

Although many details of mechanically induced plant responses have emerged over past

decades, the exact physiological and molecular mechanisms of mechanosensing and mechanotransduction remain elusive. Further clarification of the underlying mechanisms of surface-sensing and downstream signalling pathways will provide valuable insights into understanding plant growth responses to mechanical force perturbation. In particular, combining molecular and mechanical experimental perturbations with theoretical multiscale modelling, will provide information on how exactly forces are sensed and transmitted and how cytoskeletal proteins interact in response to those forces.

Understanding the plant processes involved in mechanoperception and identifying physiological and/or molecular markers for early recognition of mechanically induced plant stress symptoms would help to apply mechanical stimuli more precisely in horticultural situations. Thigmo-technologies should indeed be further explored in mechanical perturbation experiments to develop practical and reliable horticultural crop management tools for modulating plant growth and development, particularly in greenhouse conditions.

4. Air-based mechanical stimulation controls plant height of ornamental plants and vegetable crops under greenhouse conditions

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4.1.   Abstract

Mechanical stimulation to produce stable and high quality crops under greenhouse conditions is a promising alternative to chemical growth retardants. However, plant tissue damage and the lack of full automatization have been major constraints for large scale application in the past. We demonstrate the potential of automated touch-less directed air stream application systems to control plant height and appearance of bellflower (*Campanula* 'Merrybell'), creeping inchplant (*Callisia repens)* and tomato (*Solanum lycopersicum* 'Romello'). Plants were cultivated under greenhouse conditions and exposed to regulated air stream stimuli by three different prototype systems. Air stream stimuli of all three prototype systems significantly reduced plant height in all three plant species. Bellflower plants showed a reduction of 24% and tomato plants of 26% to 36%, compared to the respective control plants. The degree of height growth inhibition in tomato was shown to be predominantly influenced by the stimulus intensity. An air pressure prototype system was successfully implemented in a horticultural company and height of creeping inchplant could be sufficiently reduced by 20% on average throughout 1 year of experiment compared to untreated controls. Overall, no plant tissue damages of air stream treated plants were visibly apparent and no difference in number of flowers between air stream treated bellflower plants and untreated controls was observed.

Keywords: automated stimulus application, innovation, mechanosensing, organic crop production, technology, thigmomorphogenesis

4.2.   Introduction

Morphogenetic plant responses to MS have been a subject of several research projects over the past decades. The altered growth behavior induced by MS was first described by Jaffe (Jaffe, 1973) as thigmomorphogenesis. The growth-inhibiting effect caused by a direct physical impact by touching, rubbing or brushing is mainly used to improve plant quality criteria such as stability and appearance, thereby increasing the market value of greenhouse-raised plants (Latimer and Thomas, 1991; Latimer and Beverly, 1993; Jaffe and Forbes, 1993; Garner and Björkman, 1996; Latimer, 1998). Moreover, through the application of mechanical treatments, the obtained compact and stable plants, which are tougher and sturdier because of their increased shear strength and increased modulus of rupture (Heuchert and Mitchell, 1983), are better able to withstand potential impacts during handling, transplanting, transport, and shipping (Latimer and Beverly, 1993; Samimy, 1993; Latimer, 1998). Underlying complex mechanosensing mechanisms are responsible for specific endogenous plant processes such as GA biosynthesis inhibition or Ca^{2+} signaling responses (Chehab et al., 2009; Sparke and Wünsche, 2020), which frequently result in anatomical and morphological plant adaptation. Considering the worldwide growing and competitive horticultural market, producers of high-quality plants are realizing the necessity to establish an efficient and economic production regime to 1) maximize productivity per unit area, 2) effectively use transportation capacity, 3) minimize losses attributable to damage by transportation, and 4) fulfill the market requirements. To meet these numerous challenges, horticulturists have to implement production processes that require a high level of skillful crop management. During greenhouse cultivation, for example, excessive stem elongation can be controlled by regulating the water and nutrient supplies and controlling temperature conditions (Garner and Björkman, 1996). Nevertheless, during ornamental plant production, the application of PGR is still the most commonly used tool for achieving an effective reduction in plant height (Latimer, 1992; Morel et al., 2012). However, increased public concern and governmental restrictions have limited the use of chemicals in plant production. Additionally, the legally defined use of PGR often leads to some constraints in the production regime of the company because, in many cases, re-entry into the greenhouse section is not allowed for at least 24 h after PGR application, and, if necessary, re-entry is only allowed with protective clothing. Consequently, the growing demand for sustainable production systems requires the development and the practical implementation of alternative crop management strategies, such as organic cultivation methods, that might have the potential to increase profitability (Raviv, 2010). In this context, the use of thigmomorphogenetic effects can be a promising application technology in large-scale horticultural greenhouse production as an alternative to PGR. Furthermore, it was previously demonstrated that various mechanical treatments reduced plant height to a similar

extent as PGR applications (Adler and Wilcox, 1987; Biddington and Dearman, 1987; Latimer, 1991).

Beyl and Mitchell (1977) invented an automated system that shakes and touches plants simultaneously to control plant growth, but their device was unsuitable for large-scale crop production. Further technical approaches to control plant shoot elongation involved brushing plants manually with cardboard (Latimer, 1990) or paper sheets (Biddington and Dearman, 1985). These mechanical treatments could only be applied at irregular intensities to a limited number of plants and the high humidity conditions inside greenhouses led to upwelling and wear-out of the brushing material. Latimer and Thomas (1991) used a moving polyvinylchloride (PVC) pipe for successfully reducing the average stem length and leaf area of tomato (*Solanum lycopersicum*) greenhouse plants. However, the friction of the PVC on the plant surface caused undesirable damage to the leaf tissue, particularly when plants were turgid. Alternatively, a smooth bar made of wood or steel, which was vertically adjustable to account for time-dependent plant height differences, reduced the growth of several plant species (Latimer et al., 1991; Baden and Latimer, 1992). Koch et al. (2011) further improved the brushing application by using a fleece material and demonstrated a significant reduction in internode length for several organically cultivated herbs. However, plant quality was frequently diminished through the repeated direct physical impact, resulting in damage to the shoot tips, leaves, and petals (Latimer, 1991; Johjima et al., 1992; Latimer and Beverly, 1993; Garner and Björkman, 1997; Garner and Langton, 1997b; Koch et al., 2011). The extent of the reported plant damage appears to be dependent on plant species, cultivar, cultivation conditions, and growing season (Latimer and Beverly, 1993; Garner and Björkman, 1997; Garner and Langton, 1997b); these factors render the development of a suitable application system difficult. To our knowledge, an efficient and reliable mechanical application system that inhibits plant growth and concomitantly maintains plant quality is not yet available for horticultural crop cultivation under greenhouse conditions. In response to this production constraint, the objectives of this study were to evaluate three air stream application modules that apply a controlled and directed air stimulus to plants sustainably cultivated under greenhouse conditions. During this study, the height growth of 'Merrybell' bellflower (*Campanula*), a favored ornamental plant, 'Romello' tomato, which has frequently been investigated for reactions to MS, and creeping inchplant (*Callisia repens*), a main crop of a horticulture company used for pet feed production, were studied under experimental and commercial greenhouse conditions.

4.3.   Material and Methods

4.3.1. Construction of the air pressure module

The single technical components of the air pressure module are presented in a schematic illustration (Fig. 4.1).
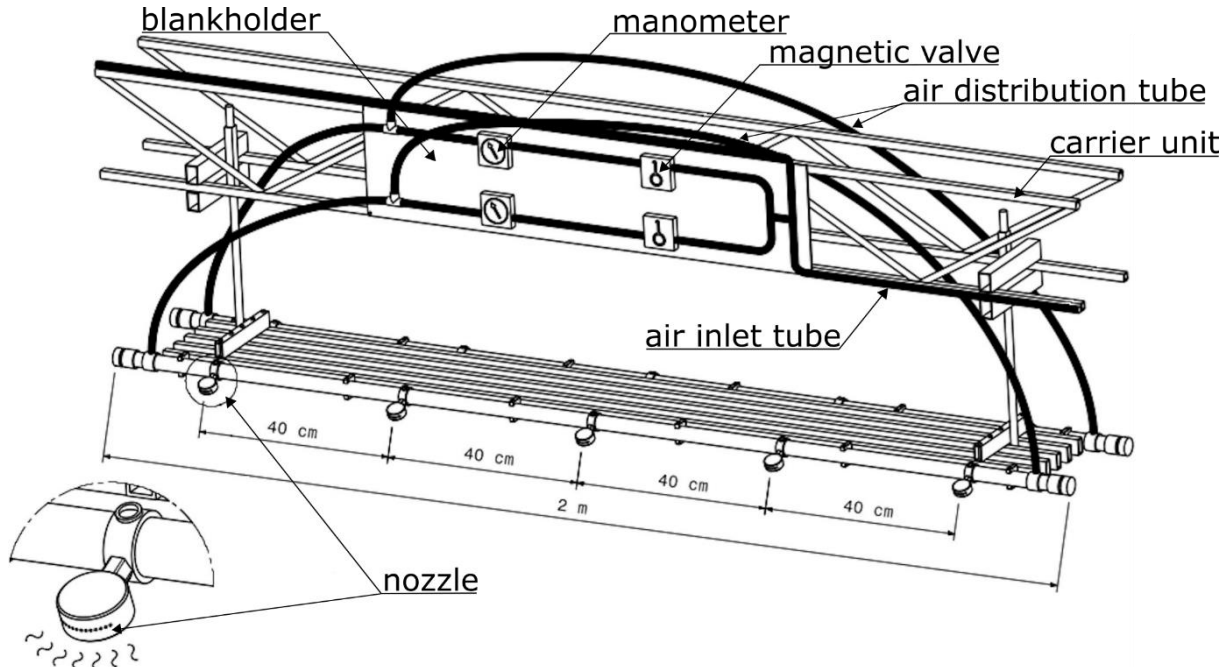

Fig. 4.1. Three-dimensional representation of the air pressure system installed in the greenhouse at the State Horticulture College and Research Institute (Heidelberg, Germany) and a close-up of a single nozzle. A moveable metal frame serves as carrier unit for the entire construction. The air stream is conveyed from the air inlet tube to two polyethylene pipes that are attached to a blankholder. Each pipe is equipped with a magnetic valve and a manometer, respectively. The air stream is further distributed via air distribution tubes to pipes clipped onto the front and rear side of a substructure. 1 cm = 0.3937 inch.

A metal frame, originally conceptualized to support an irrigation system for plant production under greenhouse conditions, served as the carrier unit for all technical components of the air pressure module. A blank holder was mounted onto the front side of the metal frame and supported two individual polyethylene pipes, which separated the incoming air stream of the air inlet tube. Both pipes were equipped with a magnetic valve and a manometer. The magnetic valves facilitated either the opening or closure of the respective pipe, and the manometers allowed for monitoring of the air pressure. Two individual air distribution tubes were attached to each polyethylene pipe to further distribute the air stream to a pipe either at the front or the rear side of a substructure. The substructure was mounted underneath the metal frame by vertically fixed steel struts to both sides. The struts could manually be moved upward and downward along the vertical axis to adjust the height from 0 cm (minimum linkage extension) to 65 cm (maximum linkage extension). The entire length of the substructure was 2 m, and it comprised five consecutively arranged hollow aluminum bars. Thereby, low weight, stability

and reduced vibrations and oscillation during system movement could be achieved. The pipes, which were clipped at the front and rear side of the substructure, were each equipped with five stainless steel nozzles (Lechler; Metzingen, Germany; Knecht, Metzingen, Germany), spaced at 0.4 m apart that served as air stream outlets. Each nozzle had 13 drill holes with different diameters. The diameter of the two outer holes to either side was 0.9 cm. The adjacent three holes on both sides had a diameter of 0.6 cm, and the three center holes were 0.4 cm wide. The angle of incidence of the air stream could be adjusted manually from horizontal to vertical (0° - 90°) by turning the respective pipe at the front or rear side of the substructure.

4.3.2.   Functional principle

The air stream that is conveyed to the air pressure module and subsequently applied to plants was generated by a stationary compressor with a downstream connected pressure regulator. The entire system was fully automated by an electrical control system (IB Ebner, Konstanz, Germany). The metal frame was suspended on a longitudinal guide rail that used an electric motor to facilitate its forward and backward movements across the entire greenhouse complex. Start and reversal positions of the frame were determined by magnets, which were placed at the appropriate position on the guide rail. As soon as a magnet was reached, an electric signal initiated either a reverse movement or a stop impulse of the frame. However, the entire system could be configured with additional options.

The air stream was applied while the frame was driving toward the reversal position, and then back to its start position or only in one of the two directions. Magnetic switches placed at determined positions along the guide rail induced an electric signal, which triggered the activation or deactivation of the air stream; therefore, the air stream could be adjusted for treating specific application scheme-dependent greenhouse areas. The driving speed of the frame ranged from 1.5 to 18 m·min^{-1} and the number of transits could be adjusted from 0.5 to 10 after a start pulse at preset time intervals.

4.3.3.   Construction and function of the centrifugal fan system

Two additional prototype systems were tested in a greenhouse facility at the University of Hohenheim, Stuttgart, Germany.

The air stream was generated by a 0.37-kW centrifugal fan (VBL8; Air Control Industries, Axminster, England) mounted to a vertical aluminum bar as the carrier unit (Fig. 4.2 left). The bar was connected to a wheel drive powered by an electric motor (IB Ebner) and ran across a horizontally positioned guide rail (Fig. 4.2 right). The flow rate of the air stream could be adjusted by throttle valve set at an angle from 0° (fully open) to 90° (fully closed). The air stream was then split by a Y-connector and passed through two arms of 7.6 cm-diameter flexible ducting, which were connected either to the air knife or the 360° rotor module. A horizontal aluminum bar that served as carrier unit for the respective module was fixed with hinges to the vertical bar and rope tensions were used to stabilize the entire system. Vertically
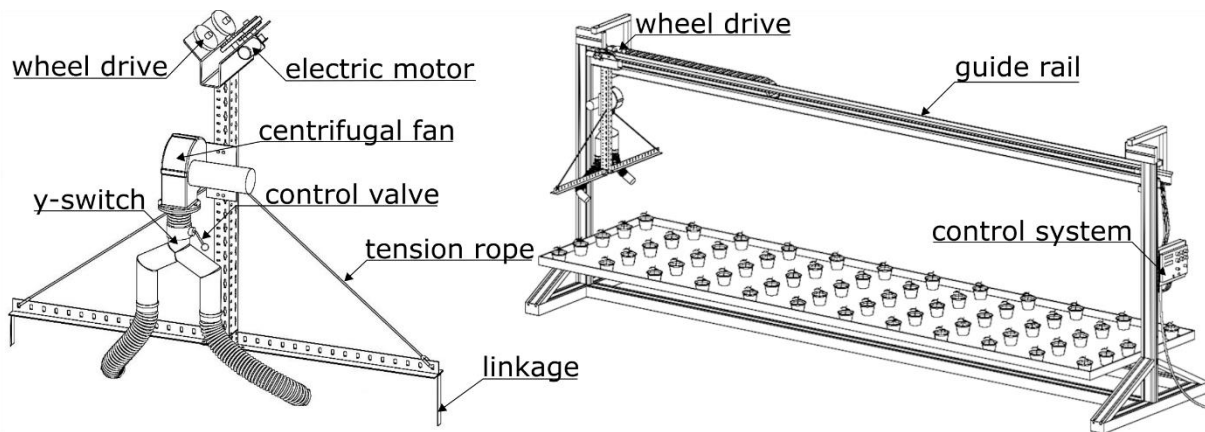
Fig. 4.2. Centrifugal fan system installed in the greenhouse at the University of Hohenheim (Stuttgart, Germany) (left side). A wheel drive is powered by an electric motor that facilitates the movement of the fan system across a longitudinal guide rail above the greenhouse table (right side). A vertical aluminium bar serves as carrier unit for the centrifugal fan. The air stream is passed through flexible ducting and regulated mechanically by a control valve unit.

attached linkages on either side of the horizontal bar permitted the fixation of the respective module with threaded rods on both sides.

4.3.4. Air knife module

The air knife module (Air Control Industries) was constructed of two identical casings of anodized aluminum, which were screwed together to form a hollow space (Fig. 4.3).
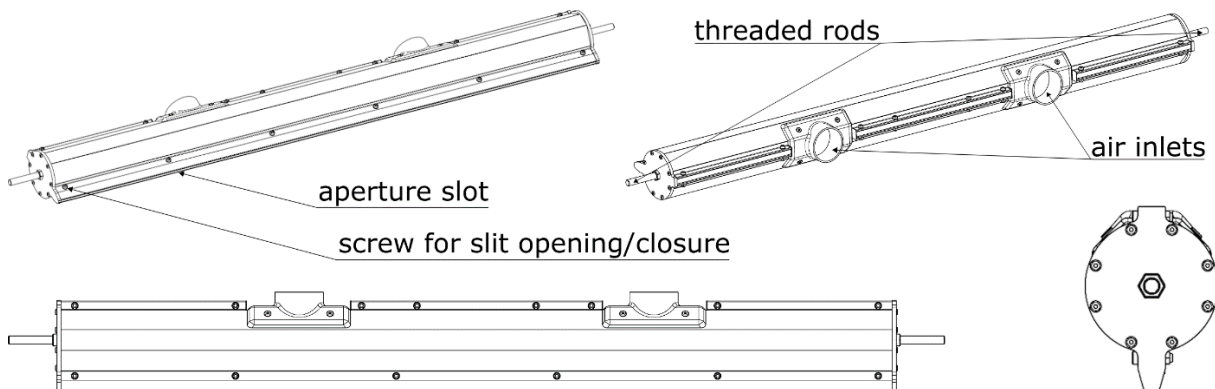

Fig. 4.3. Three-dimensional representation of the air knife module front- and backside (top) installed in the greenhouse at the University of Hohenheim (Stuttgart, Germany) and a top view and a cross-section representation (bottom). Two air inlet holes are inserted on the backside and the air outlet is an aperture slot along the front side. The aperture slot can be adjusted by loosening or tightening screws. Threaded rods are attached on both sides to mount the module onto the carrier unit.

The inner profile of the air knife produced a continuous laminar air stream. Two air inlet holes (diameter, 76 mm) were positioned at the back side of the air knife. The air outlet was an aperture slot that could be adjusted from 1 to 5 mm by loosening (opening) or tightening (closing) screws, thereby extending small springs inside the casing. The effective length of the air knife was 1.2 m and the angle of incidence of the air stream could be manually adjusted by turning the manifold from 0° (vertical) to 90° (horizontal) along its vertical axis. Threaded rods on each side of the module facilitated the insertion of the module into the carrier system.

4.3.5. 360° rotor module

The 360° rotor module (Raith Tec, Ditzingen, Germany) was a hollow, rectangular, aluminum box with a volume of ≈ 1500 cm^3 and an overall length of 1 m (Fig. 4.4). Two air inlet holes (76 mm diameter) at the back delivered the air stream to two rotating PVC tubes inserted on the bottom at 20 cm away from both lateral surfaces of the module. The air flow escaped the air outlet tubes as a turbulent free air stream at a fixed 20° angle below to the horizontal line. The 360° rotation of the tubes was facilitated by a chain drive with gear wheels (60 mm diameter) mounted on the top and powered by a 12-W electric motor (Mädler, Stuttgart, Germany) placed on the front. Threaded rods on each side of the module allowed its fixation on the carrier system. The electrical control system (IB Ebner) was identical to that described for the air pressure module.
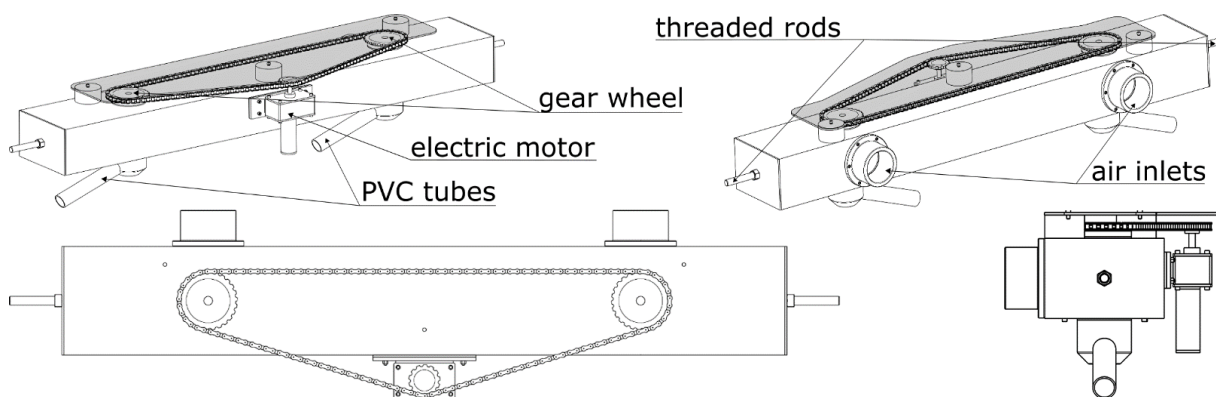
Fig. 4.4. Three-dimensional representation of the rotor module front- and backside (top) installed in the greenhouse at the University of Hohenheim (Stuttgart, Germany) and a top view, and cross-section representation (bottom). Two 360° rotating polyvinylchloride (PVC) tubes are inserted on the bottom side of the aluminium corpus. The rotation of the tubes is caused by chain driven gear wheels that are powered by an electric motor. Threaded rots are attached on both sides to mount the module onto the carrier unit.

4.3.6. Air velocity measurements

A pocket weather meter (Kestrel 4000; PCE Deutschland, Meschede, Germany) placed on a tripod was used to determine the air velocity of the respective module. Air velocity measurements were obtained from 0 to 60 cm away from the air outlet at center positions of individual nozzles of the air pressure module and the PVC tubes of the 360° rotor module, respectively, as well as at five positions equally spaced along the aperture slot of the air knife module corresponding to the plant rows on the greenhouse table (five rows). Moreover, various air stream flow rates were set by adjusting the aperture slot opening width from 1 – 5 mm on the air knife and the throttle from 11.75° (nearly closed) to 90° (fully open) on the 360° rotor module. The throttle on the air knife was set to 90° (fully open). Measurements were recorded as soon as the fluctuations were minimal (±0.5 m·s^{-1}).

4.3.7. Plant material and experimental design at the State Horticultural College and Research Institute

The effect of directed air stimuli applied by the air pressure system on the height growth of bellflower and tomato plants was investigated under greenhouse conditions at the State Horticultural College and Research Institute, Heidelberg, Germany. Three bellflower 'Merrybell' scions were transplanted to one plastic pot (0.5 L volume) each filled with substrate [12N-6.1P-19.9K (RHP 15, Klasmann-Deilmann, Geeste, Germany)]. Bellflower plants were grown for 9 weeks during winter season (Table 1) under natural light conditions with an average photosynthetic active radiation (*PAR*) of ≈ 350 µmol·m^{-2}·s^{-1} and day/night air temperatures of 20/16 °C. During week 10, flowering was induced by long days (14 h/ 10 h day/ night) with high-pressure sodium bulbs (SONT Agro 400 W; Philips, Amsterdam, the Netherlands). Irrigation was performed by table flooding with 1 g·L-1 fertilizer [18N-4.8P-14.9K (Universol blue; ICL Specialty Fertilizers, Tel Aviv, Israel)]. Tomato seeds were sown in 104-cell trays and grown for three weeks during spring season (Table 1). Thereafter, seedlings were transplanted to plastic pots (volume, 2 L) filled with substrate [12N-6.1P-19.9K (Seedlingsubstrat, Klasmann-Deilmann)]. Irrigation was performed by table flooding with 5 mL·L^{-1} liquid fertilizer [7N-0.9P-2.5K (Organic Plant Feed; Plant Health Cure, Oisterwijk, the Netherlands)]. Tomato plants were grown under natural light conditions with an average *PAR* of 850 µmol·m^{-2}·s^{-1}. Relative humidity (RH) was 60% and day/night air temperatures were 20/16 °C.

The bellflower experiment was set up as a one-factorial (air stream) design with two blocks, each with two greenhouse tables that were longitudinally separated in one air stream and one control area, respectively. Air stimuli were applied by one air pressure module in each block. Eight hundred bellflower plants were uniformly arranged across the four tables with plants on two table halves regularly treated with a directed air stream of a defined air velocity for 10 weeks; the plants on the other two table halves served as untreated controls. The planting density accounted for 20 plants/m^2. The experiment with tomato was conducted with only one air pressure module and comprised 76 plants for each table half with a planting density of 10 plants/m^2. Throughout the 2-week tomato experiment, the distance between the air outlet of individual nozzles and plant tips was maintained at ≈ 15 cm distance.

Bellflower and tomato were evaluated for plant height. The bellflower plant height was defined as the length of the longest upright standing shoot, and tomato plant height was determined by measuring the height from the pot rim to the tip of the main shoot apex. On each table half, 12 bellflower plants that were 10 weeks old and four tomato plants that were 5 weeks old were randomly selected for plant height measurements, respectively.

Throughout the experiment, only the nozzles at the front of the modules were activated, and air pressure was generated by two scroll compressors (SF-4 FF; Atlas Copro, Essen, Germany). Details regarding relevant dates related to the plant cultivation procedure are

shown in Table 4.1, and additional functional settings of the experiments with the air pressure module are shown in Table 4.2.

4.3.8. Plant material and experimental design at University of Hohenheim

The effect of directed air stimuli applied by the air knife and the 360° rotor module on the height growth of tomato plants was investigated under greenhouse conditions at the University of Hohenheim. Tomato seeds were sown in 104-cell trays with one seed per plug and grown for 3 weeks during winter season (Table 4.1). Thereafter, seedlings were transplanted to plastic pots (volume, 0.5 L) filled with substrate [12N-6.1K-19.9 (Substrat 5; Klasmann-Deilmann)]. Throughout both experimental runs, plants were grown under natural light conditions with an average *PAR* of 250 µmol·m^{-2}·s^{-1}. Plants were watered manually every second day with 80 mL tap water and with 2.34 g·L^{-1} water-soluble fertilizer [16N-2.6P-21.6K (FERTY 2 MEGA; Hauert, Grossaffoltern, Switzerland)]. The day/night air temperatures were set to 20/16 ºC and relative humidity ranged from 50% to 70%. The carrier unit (Fig. 4.2, right) was placed above the greenhouse table (3 x 1.2 m) that was transversely separated as a control half and air stream-treated half. Each experiment was repeated twice with both treatments randomly placed on the greenhouse table halves. The passage of the modules commenced first at the table end of the control and then at the air stream area. Thirty tomato seedlings were evenly distributed on each table area with a planting density of 16 plants/m^2. When the respective air stream module reached the magnet placed at the center position on the longitudinal guiderail, the air stream was initiated or stopped. A polystyrene board was placed between the control and air stream areas to avoid any air stream stimulation of control plants. Plant height was determined for all 60 plants on days 11 and 14 after treatment initiation in the air knife experiment and on day 14 during the 360° rotor module experiment.

The throttle valve for adjusting the air stream flow rate was at 60°, and it was fully opened for the 360° rotor module. The aperture slot on the air knife module was set to 5 mm. Because the angle of the air jet incidence was set to 10° from the vertical position, there was a gradually increased stimulus duration along the air stream axis on the greenhouse table. This effect was not observed in the rotor module because of the 360° rotation of the air outlet tubes. At the beginning of the experiments, the distance between air outlet and plant tip was ≈ 60 cm. As the plants grew taller over the experimental period of 2 weeks and the distance between air outlet and plant tip was not adjusted, increasingly stronger stimulation intensities were perceived by the plants. Details regarding relevant dates related to the plant cultivation procedure are shown in Table 4.1. Additional functional settings used during the experiments with both modules are shown in Table 4.2.

To evaluate the effect of air velocity on plant height, plants were replaced with the pocket meter at various positions on the table on day 11 after treatment initiation with the air knife module. Measurements were obtained at 2-s intervals using the data logging function of the gadget,

thereby allowing a record of the maximum air velocity, the stimulus duration, and the cumulative air velocity at each single plant position. The cumulative air velocity was calculated from the sum of the air velocity measurements during one transit. In other words, during system movement, the air stream gradually approached plants positioned in backrows. Those plants were exposed to longer air stimulation and cumulative air velocity compared with plants that were positioned closer to the starting position of the air knife module.

4.3.9. Commercial application

The effect of directed air stimuli applied by the air pressure system on the height growth of creeping inchplant was also tested under commercial conditions at a local horticulture company (Fleischle, Vaihingen Ensingen, Germany). Plant management was entirely managed performed by the company owner. Three scions were transplanted in plastic pots (volume, 0.5 L) filled with substrate (14N-P-14.9K; CL T, Einheitserdewerke Patzer, Sinntal-Altengronau, Germany). The air pressure module, which was adjusted to the greenhouse dimension of the company, was attached to the crossbeam of the irrigation system as described previously. The module was 13 m long and equipped with 18 air outlet nozzles that were arranged at uniform intervals on the front of the PVC tube. The air pressure was generated by a piston compressor (SAZ 1011, Mahle, Stuttgart, Germany) and a screw compressor (RSC 40, Renner, Güglingen, Germany); both were fueled by a 250-L diesel tank outside the greenhouse complex. Five consecutive experimental sets (1 – 5) were conducted over the course of 1 year, and plant height was evaluated as described for the bellflower experiment. One greenhouse table was separated longitudinally into halves, with one half assigned to air stream area and the other half assigned to the control area. In each experimental set, 10 plants of the air stream area and the control area were randomly selected for measurements of plant height. Details of the air pressure settings are provided in Table 4.2.

4.3.10. Data analysis

F-tests were performed with the PROC MIXED statement (SAS version 9.4, SAS Institute, Cary, NC) for all experiments, with significance level set to $P \le 0.05$. For the tomato experiment with the air knife module, records of air velocity and plant height on day 11 after treatment initiation were analyzed using the following multiple linear regression model:

$$y = B_0 + B_1 x_1 + B_2 x_2 + B_3 x_3$$

where y is the dependent variable (plant height); B_0 is the intercept; x_1 is the air velocity sum; x_2 is the air velocity duration; x_3 is the maximum air velocity. B_1, B_2 and B_3 are the estimated parameters for the x-variables. Variables that were found to insignificantly contribute to the response variable were removed.

Tab 4.1. Relevant dates of the experiments involving air-based mechanical stimulation control of plant height conducted at the State Horticultural College and Research Institute (Heidelberg, Germany) and the University of Hohenheim (Stuttgart, Germany).

	State Horticultural College and Research Institute			University of Hohenheim		
Parameter	Bellflower	Tomato	Tomato	Tomato	Tomato	Tomato
System type	Air pressure	Air pressure	Air knife	Air knife	360° rotor	360°rotor
Year	2015	2017	2017	2018	2018	2018
Experimental run	1	1	1	2	1	2
Sowing date	-	11 Apr.	16 Oct.	6 Nov.	29 Jan.	26 Feb.
Transplanting date	21 Sept.	27 Apr.	2 Nov.	23 Nov.	16 Feb.	16 Mar.
Treatment starting date	5 Oct.	3 May	6 Nov.	27 Nov.	19 Feb.	12 Mar.
Treatment ending date	16 Dez.	17 May	20 Nov.	11 Nov.	5 Mar.	26 Mar.
Date of height measurement	16 Dez.	17 May	17 Nov.; 20 Nov.	8 Dez.; 11 Dez	5 Mar.	26 Mar.

Tab 4.2. Air supply settings of various mechanical stimulation experiments on ornamental and vegetable plants under greenhouse conditions at the State Horticultural College and Research Institute (Heidelberg, Germany), the University of Hohenheim (Stuttgart, Germany) and a commercial horticulture company (Vaihingen Ensingen, Germany).

Parameter	State Horticultural College and Research Institute		University of Hohenheim		Commercial company
	Bellflower	Tomato	Tomato	Tomato	Creeping inchplant
System type	Air pressure	Air pressure	Air knife	360° rotor	Air pressure
Driving velocity (m·min^{-1})^z	1.5	1.5	1.5	1.5	10
First start impulse (HR)	8000	7000	8000	8000	7000
Start impulses/ day (no./d)	6	4	8	8	8
Transits/ start impulse (no.)	10^y	10^y	10^x	10^x	10^y
Air stimuli/ day (no./d.)	120	80	80	80	160
Time between start impulses (min)	45	45	45	45	15
Angle of incidence^w	60°	60°	10°	70°	0°

^{z}1 m = 3.2808 ft.
^{y}Air stream application during forth and back movement.
^{x}Air stream application only during forth movement.
^{w}From the vertical line.

4.4.   Results

4.4.1. Air velocity measurements

During experiments performed at the State Horticultural College and Research Institute, the average maximal air velocity at the nozzle outlets of the air pressure module after pressure equalization was obtained was ≈ 13 m·s^{-1} (Fig. 4.5A). The air velocity of the turbulent air jet followed an exponential decay curve with increasing measurement distance from the air outlet, decreasing to 3 m·s^{-1} at 30 cm and to ≈ 1 m·s^{-1} at 60 cm. The air velocity perceived at the plant apical meristem was ≈ 4 m·s^{-1}, which was assured by a daily adjustment of the distance to 15 cm between the air outlet and plant tip.

During experiments at the University of Hohenheim, the maximal air outlet velocity at the aperture slot of the air knife module was ≈ 12 m·s^{-1} for the opening widths of 5, 4 and 3 mm; however, the maximal air outlet velocities at the aperture slot of the air knife module were ≈ 10 m·s^{-1} and ≈ 7 m·s^{-1} for the opening widths of 2 mm and 1 mm, respectively (Fig. 4.5B). Air velocity of the laminar air flow decreased exponentially with measurement distance from the air outlet, reaching ≈ 3 m·s^{-1} at a distance of 60 cm, regardless of the slot opening width. The exponential decay of air velocity with distance from the air outlet was less pronounced when the opening width was reduced. The air outlet velocity of the 360° rotor module strongly depended on the air flow that was regulated by the throttle (11.75° - 90°). When the throttle was fully open (90°), the maximal air velocity was 17 m·s^{-1}, and it gradually decreased to 5 m
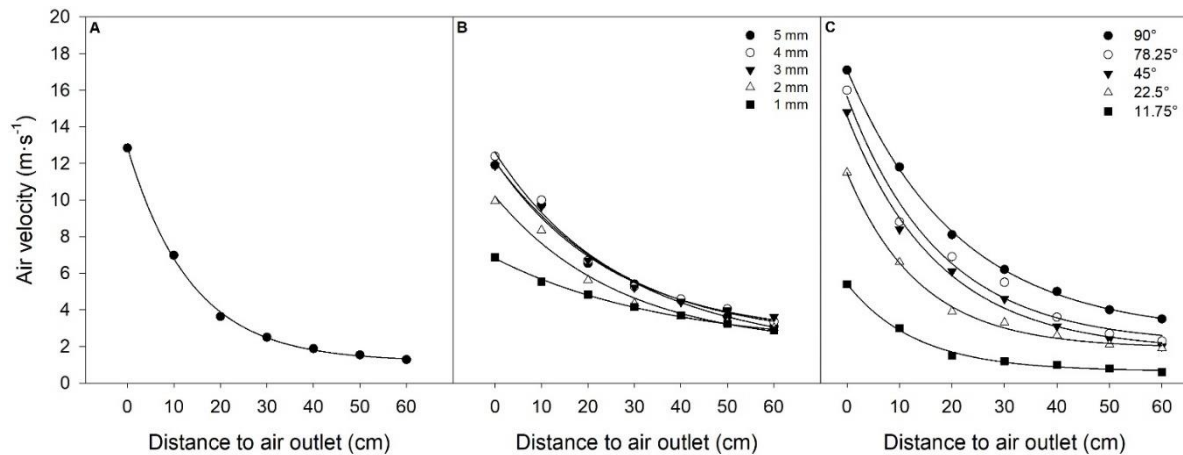

Fig. 4.5. Air velocities of the air pressure module implemented in the greenhouse at the State Horticulture College and Research Institute (A), the air knife module with opening width of the aperture slot from 1 – 5 mm (B), the 360° rotor module with throttle adjustment from 11.75 – 90° implemented in the greenhouse at the University of Hohenheim (Stuttgart, Germany) (C) at measurement distance of 0 to 60 cm from the air outlet. The relationship between the measurement distance from the air outlet and air velocity was significant (P < 0.0001) for all air systems and various slot opening widths of the air knife module and throttle opening angles for the experiment with the 360° rotor module. The R^2 values of the regression line between the measurement distance and air velocity for the air pressure module was 0.99, and it ranged between 0.98 and 0.99 for the air knife and the 360° rotor module at various slot opening widths and throttle opening angles. 1 cm = 0.3937 inch; 1 mm = 0.0394 inch. 1 m·s^{-1} = 2.2369 mph.

s^{-1} when it was almost closed (11.75°) (Fig. 4.5C). The air velocity decreased exponentially for all throttle opening angles, reaching ≈ 3.5 $m \cdot s^{-1}$ when fully open and 0.5 $m \cdot s^{-1}$ at the lowest angle.

After system activation of the air pressure module during experiments at the State Horticultural College and Research Institute, the time required to obtain a uniform air velocity was ≈ 250 s (Fig. 4.6A and B). The third nozzle of the air pressure module 1 (Fig. 4.6A) reached an air velocity of ≈ 16 $m \cdot s^{-1}$ compared with 13 $m \cdot s^{-1}$ reached with the other four nozzles. In contrast, the sixth nozzle of air pressure module 2 (Fig. 4.6B) reached an air velocity of ≈ 7.5 $m \cdot s^{-1}$ compared with ≈ 13 $m \cdot s^{-1}$ reached with the other four nozzles.
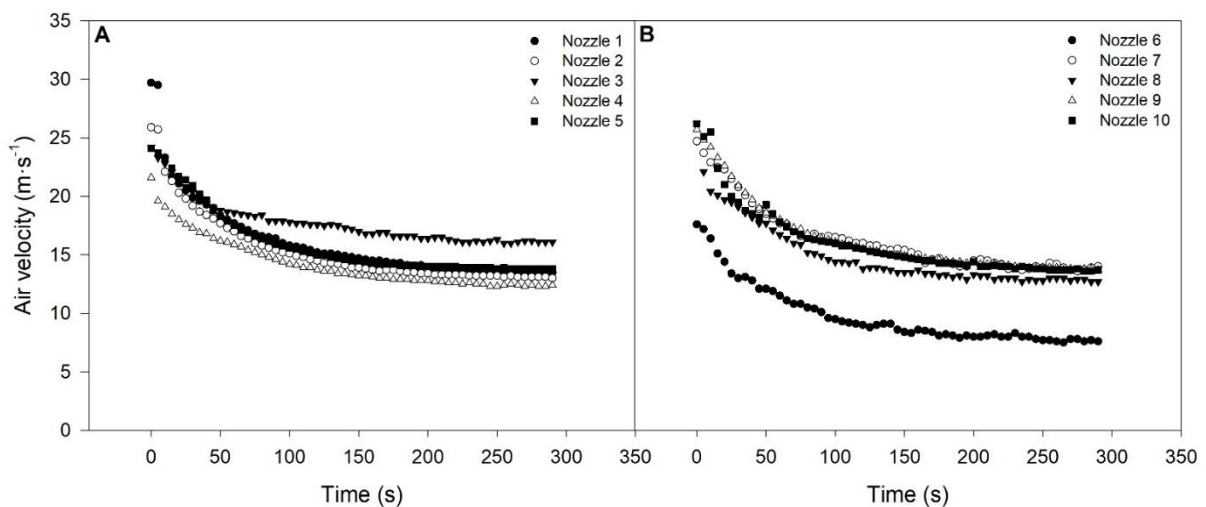

Fig. 4.6. Air velocity at the centre position directly at the air outlet of the individual nozzles (1-10) of air pressure module 1 (A) and air pressure module 2 (B) for a time period of 300 s implemented in the greenhouse at the State Horticultural College and Research Institute (Heidelberg, Germany). 1 m = 3.2808 ft. 1 $m \cdot s^{-1}$ = 2.2369 mph.

Air velocity measurements at five equally spaced positions along the air knife aperture slot with an opening width of 5 mm revealed considerable fluctuation (Fig. 4.7A). Directly at the air outlet, the maximum air velocity was measured at position 4 as 15 $m \cdot s^{-1}$, and the minimum air velocity was measured at position 5 as 9 $m\ s^{-1}$. The fluctuation of the air velocity among the five measurement positions was gradually reduced with increased distance. The air velocity at position 3 was consistently the highest, ranging between 10 $m \cdot s^{-1}$ (distance, 10 cm) and 4 $m\ s^{-1}$ (distance, 60 cm).When the aperture slot was reduced to 1 mm (Fig. 4.7B), less air velocity fluctuation among the measurement positions was observed. Again, there tended to be a gradual decrease in the air velocity at all positions with increased measurement distance from the air outlet. The highest air velocities were measured at distances of 0 cm and 10 cm from the air outlet, with maximum values of 8 and 6 $m \cdot s^{-1}$, respectively, observed at position 1.
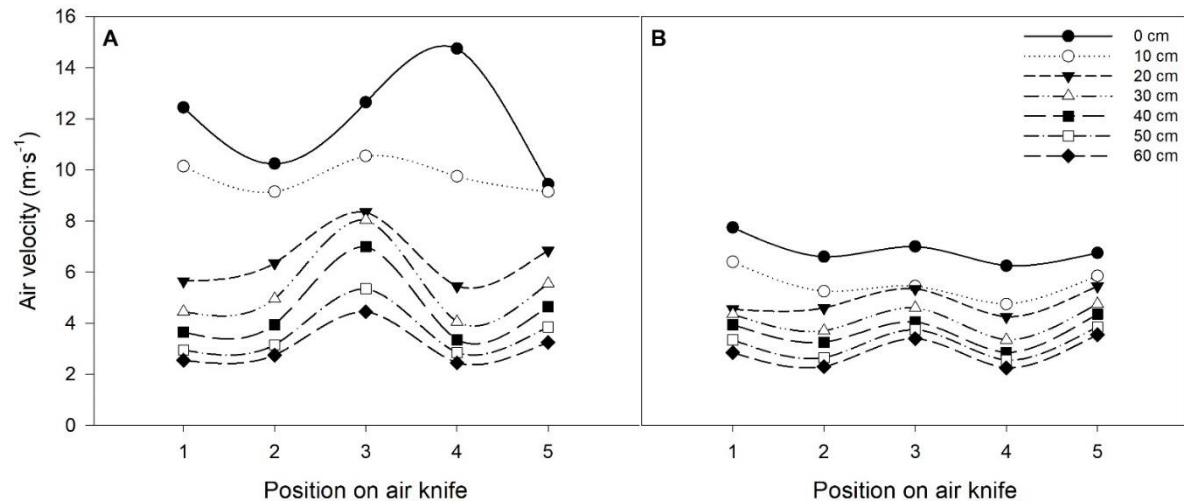
Fig. 4.7. Air velocity with the fully open throttle valve (90°) at five positions along the length of the air knife module implemented in the greenhouse at the University of Hohenheim (Stuttgart, Germany) with measurement distances of 0 to 60 cm from the aperture slot with an opening width of 5 mm (A) and 1 mm (B). 1 cm = 0.3937 inch; 1 mm = 0.0394 inch. 1 m·s^{-1} = 2.2369 mph.

4.4.2. Plant growth responses of bellflower

Before treatment initiation, bellflower plants had an average plant height of 3.9 cm. Air stimuli applied by the air pressure module at 80 transits per day throughout a growth period of 10 weeks induced a significant 24% reduction ($P < 0.0001$) in bellflower plant height compared with untreated control plants (Fig. 4.8). Consequently, air stream-treated bellflower plants showed a denser growth type with less elongated side shoots compared with untreated control plants (Fig. 4.9B). Moreover, no difference in the number of flowers was observed for air stream- treated and control plants.
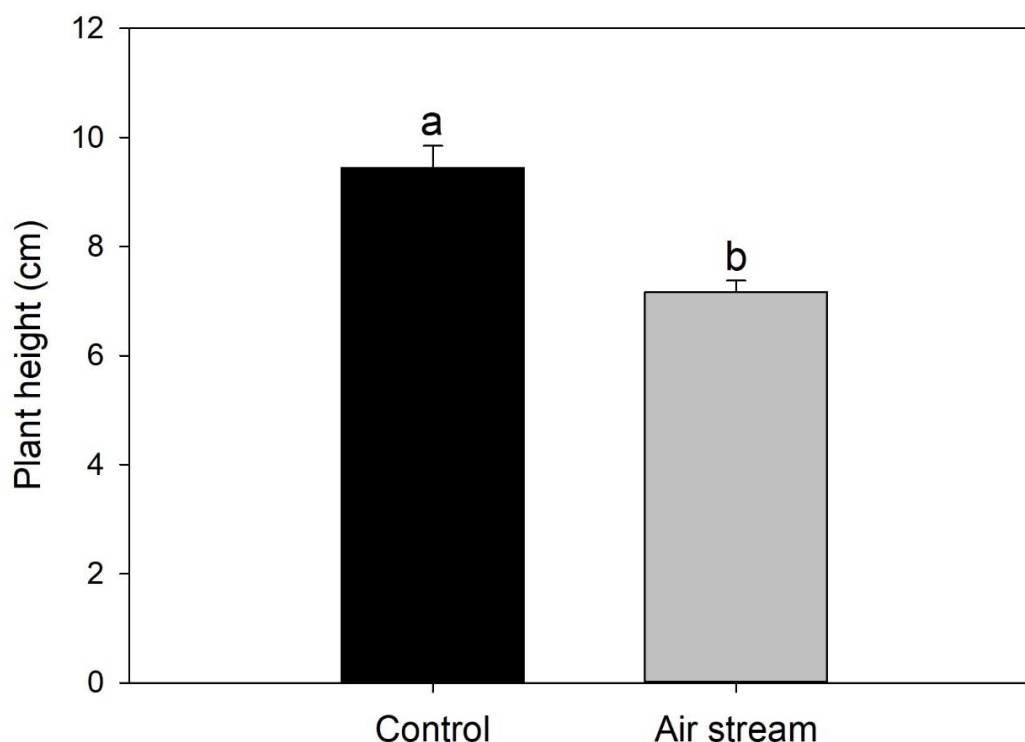
Fig. 4.8. Mean plant height of bellflower control plants and air stream-treated plants of the air pressure module at the State Horticultural College and Research Institute (Heidelberg, Germany). Vertical bars indicate SE (n=24). Bars with different letters indicate significant difference at $P \leq 0.05$. 1 cm = 0.3937 inch.

4.4.3. Plant growth response of tomato

A comparison of the three modules regarding their efficacy for reducing tomato plant height with 80 transits per day over the course of a growth period of 2 weeks is shown in Table 4.3. Before treatment initiation, tomato plants had an average plant height of 2.2cm. Air stimuli applied by the air pressure system significantly reduced plant height ($P < 0.0001$) by 36%

Tab 4.3. Mean plant height of tomato after 14 d of air stream treatment with 80 transits per day using the various air supply modules at the State Horticultural College and Research Institute (Heidelberg, Germany) or the University of Hohenheim (Stuttgart, Germany); n = 8 for air pressure module; n = 60 for air knife and 360° rotor module.

	Plant ht [mean ± SD (cm)^z]		
	Control	Air stream stimulated	Reduction (%)
Air pressure	22.5 ± 1.5 a^y	14.3 ± 1.8 b	36
Air knife	22 ± 2.8 a	16.2 ± 2.9 b	26
360° rotor	21.8 ± 1.9 a	15.4 ± 1.5 b	33

^{z}1 cm = 0.3937 inch.
^{y}Means followed by different letters indicate significant difference between control and air stream-stimulated plants at $P \leq 0.05$.
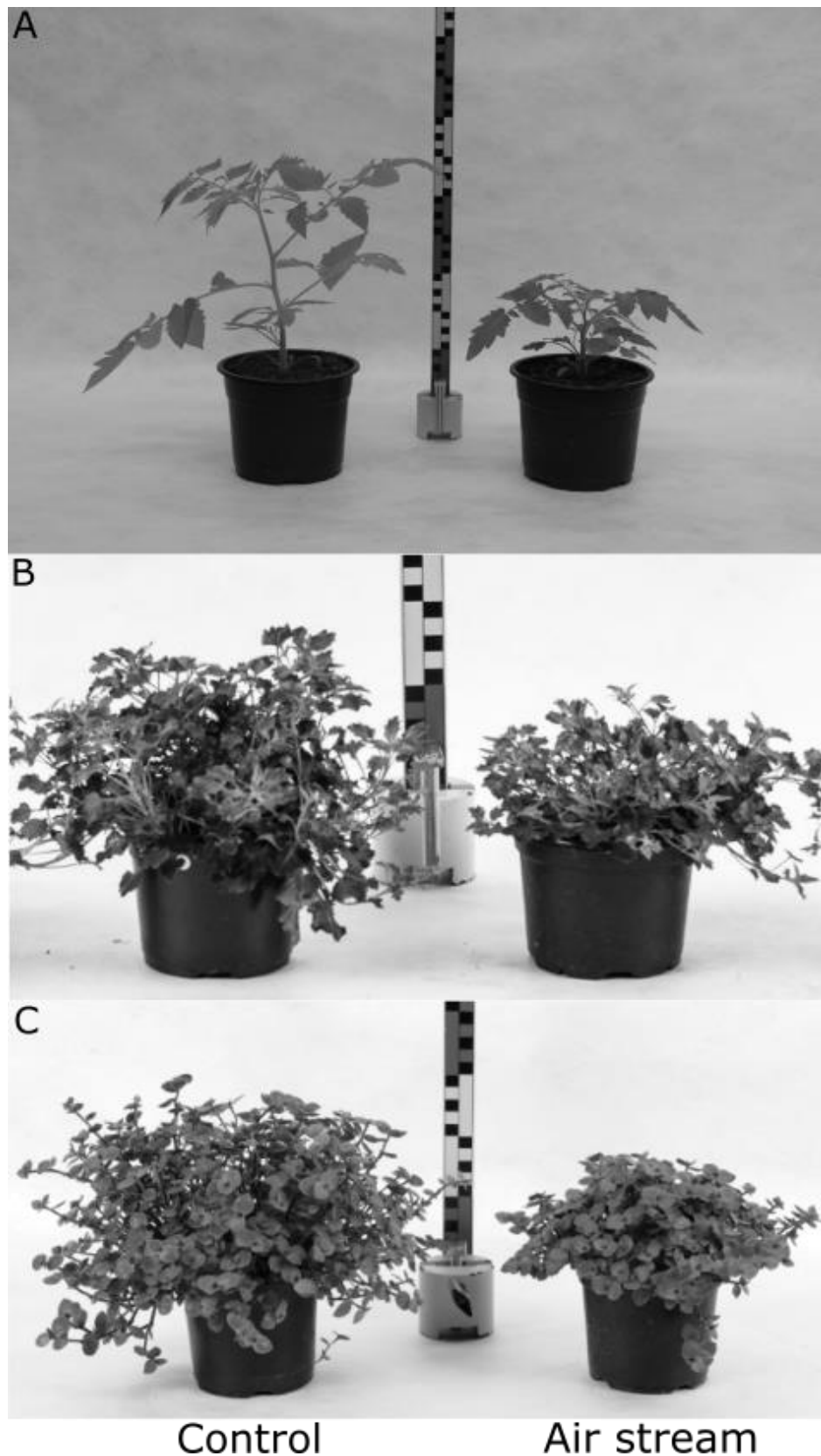
Fig. 4.9. Plant appearance of tomato (A) and bellflower (B) at 2 weeks and 10 weeks after air stream initiation with the air pressure module and untreated control plants at the State Horticulture College and Research Institute (Heidelberg, Germany). Plant appearance of regularly air stream-treated creeping inchplant by the air pressure module and untreated control plants (C) of experimental run three at a local horticulture company (Vaihingen Ensingen, Germany).

compared with untreated control plants (Fig. 4.9A). In contrast, air stimuli applied by the air knife resulted in a significant reduction ($P < 0.0001$) in plant height (26%) compared with control plants. Air stimuli applied by the 360° rotor system also induced a significant reduction ($P < 0.0001$) in plant height (33%).

The height of air knife-treated tomato plants was highly variable (8 – 16 cm). Because of the 10° inclination of the air knife, tomato plants were exposed to increasingly higher stimulus durations ranging from 10 s to ≈40 s per transit along the air stream axis. The maximum air velocity ranged from 1 to 4 m·s^{-1}; consequently, the cumulative air velocity range was between 5 and 30 m·s^{-1} per transit. The multiple regression analysis revealed the following significant ($P < 0.0001$) regression model:

$$y = 16.62 - 0.01x_1 + 0.01x_2 - 2.1x_3$$

Maximum air velocity was the variable that explained best the variability in plant height (Fig. 4.10C); no significant effects of stimulus duration (Fig. 4.10A) and cumulative air velocity (Fig. 4.10B) on plant height were observed. The removal of insignificant variables resulted in the following linear equation model:

$$y = -2.16x + 16.8$$

where y is the response variable (plant height) and x the maximum air velocity.
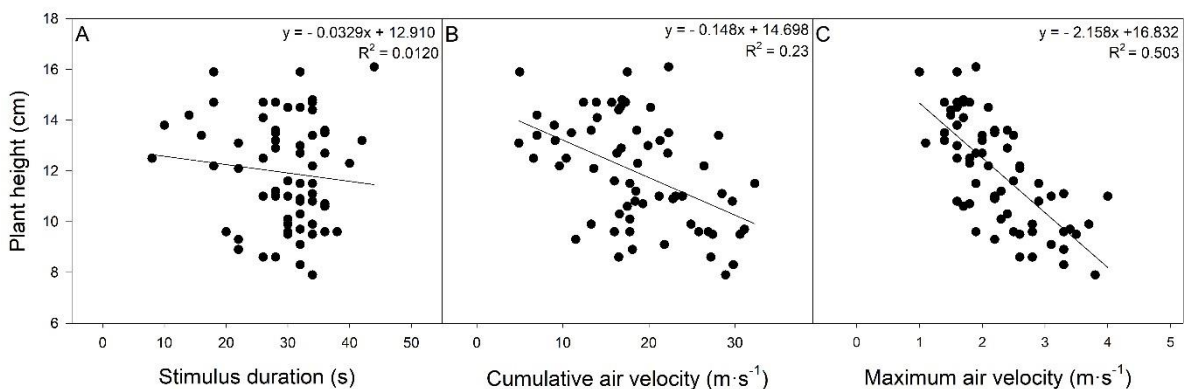
Fig. 4.10. Relationships between stimulus duration (s) (A), cumulative air velocity (m·s^{-1}) (B) and maximum air velocity (m·s^{-1}) (C) and tomato plant height (cm) 11 d after air stream initiation with the air knife module with the throttle valve set at 60° at the University of Hohenheim. P = 0.7812, 0.84, 0.0003 for the effect of cumulative air velocity, stimulus duration and maximum air velocity on plant height at $P \leq 0.05$, respectively. 1 cm = 0.3937 inch. 1 m·s^{-1} = 2.2369 mph.

4.4.4. Creeping inchplant experiment

The evaluation of the air pressure system in a commercial setting showed that the applied air stream stimuli significantly reduced plant height of creeping inchplant (Fig. 4.11). Plant appearance was markedly improved, as indicated by a reduction in plant height with fewer elongated shoots (Fig. 4.9C). The efficiency of air stream stimuli to regulate plant height growth of creeping inchplant was dependent on the season; the experiments performed during fall and winter (set 4 and 5) induced a stronger reduction in height growth (22% to 32%) than those during spring and summer (set 1, 2 and 3), with an average reduction in plant height of 15% compared with untreated controls.
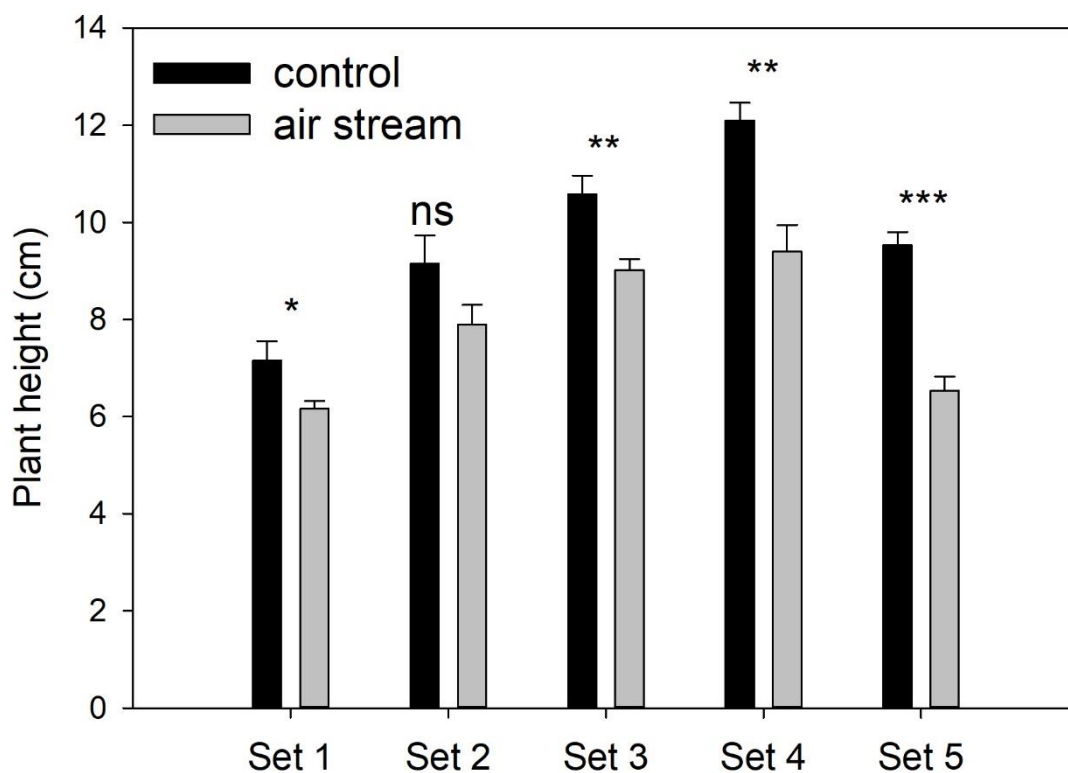

Fig. 4.11. Mean plant height of the control and air stream-treated creeping inchplants of five consecutive experimental sets within 1 year at a local horticulture company (Vaihingen Ensingen, Germany). Vertical bars indicate SE (n=10). ns, $*$, $**$ and $***$ represents nonsignificance or significance at $P \leq 0.05$, $P \leq 0.01$ or $P \leq 0.001$, respectively. 1 cm = 0.3937 inch.

4.5. Discussion

4.5.1. Air module development

One of the first automated brushing modules for mechanical plant growth regulation was implemented at the State Horticultural College and Research Institute in 2009 (Regnant et al., 2008) and 2010 (Koch et al., 2011). Previously proposed system configurations were created by using the boom of the watering systems as the carrier unit for the brushing module (Latimer and Thomas, 1991). Additionally, the reported problem of plant damage caused by direct physical contact between plant tissue and fleece material was addressed, and a system equipped with plastic spray nozzles (Type LU-120-03, Lechler) was used for air stream application. This earlier version was continually modified and resulted in the current air pressure system (Fig. 4.1).

Plastic spray nozzles were replaced by custom-built stainless-steel nozzles (Lechler and Knecht), with each containing 13 outlet holes with increased diameter from the inner toward the outer drill holes, resulting in a more uniform air distribution across the plant stand. However, at ≈ 20 cm distance from the center position of one nozzle, the air streams of the two adjacent nozzles were likely to interfere, thereby causing omni-directed air currents and subsequently resulting in variable air velocities across the plant stand.

Because of these findings, an additional prototype system was developed and implemented on a small scale for greenhouse applications at the University of Hohenheim. The air knife module was implemented to create a more uniform air curtain across the plant stand to induce consistent height growth reduction. However, the air velocity was highly variable along the 1.2-m aperture slot and strongly dependent on the opening width (Fig. 4.7). Consequently, a third prototype module was developed that generated a cone-shaped air jet through two 360° rotating PVC tubes, thus permitting multiple plant stimuli during one transit. Moreover, when the throttle was fully opened, the 360° rotor module produced higher air velocities than the air knife module. Moreover, it could cover a greater greenhouse table area and fulfill the basic requirement for homogenous air distribution across the plant stand. For all these reasons, the rotating air application module seemed to be best for air stimulus applications. Air-based stimulation for inducing thigmomorphogenetic effects does not cause plant and product quality-related damage such as that reported previously for direct touch stimulus systems (Latimer and Beverly, 1993; Latimer, 1994; Koch et al, 2011).

4.5.2. Air velocity performance

Three nozzle types were tested for air velocity along their air stream axis (Fig. 4.5). Custom-built flat spray nozzles, circular PVC nozzles, and continuous slit nozzles showed a gradual decrease in air velocity with increased measurement distance from the air outlet, thereby creating an inverse proportional dependency. The gradual reduction in air velocity is related to friction losses occurring between the air stream and the surrounding air mass and to an

increase in stream mass transport as the range occupied by the air jet increases with distance (Zawadzki et al., 2010). The flat spray nozzles and the circular PVC tube nozzles produced turbulent free air streams comparable to air jets of circular axial-symmetric nozzles (Zawadzki et al., 2010), and the pattern of air velocity loss based on the distance from the air outlet was similar. The greater air outlet velocities of the circular PVC nozzles with fully opened throttles were lost by a stronger decay in air velocity. The profile inside the plenum chamber of the air knife corpus generates a laminar air jet that enters the surrounding media (Trancossi, 2011) and maintains greater, less decaying air velocities along the air stream axis compared with air jets produced by circular nozzle types.

Individual nozzles on the air pressure module 2 tended to have slightly greater fluctuations in air velocity until a stable air outlet velocity was reached compared with the nozzles of module 1 (Fig. 4.6). This was presumably because of module 2 receiving the incoming air stream from the generator. Moreover, the air velocities of individual nozzles were not uniform, likely because of small air leakages in the plastic air tubes.

4.5.3. Plant growth response of bellflower

It was shown that the air pressure module was suitable for controlling plant height of bellflower when applied 120 times per day (Fig. 4.8). Experiments that tested growth responses by MS using burlap material showed a maximum reduction in plant height of ornamentals of 25% for aster (*Callistephus chinensis)*, 19% for dusty miller (*Senecio bicolor)* and 34% for petunia (*Petunia)* (Autio et al., 1994). Garner and Langton (1997) used polythene material or plastic netting to apply touch stimuli but could not regulate the plant growth of geranium (*Geranium*) and impatiens (*Impatiens holstii*) without causing significant plant damage; however the brushing effects induced 17% to 30% reductions in the petiole length in petunia and pansy (*Viola tricolor*) (Garner and Langton, 1997b). The results are of the same magnitude as the data shown in this study, indicating that touchless air stream stimulation has effects similar to those of treatments based on direct physical contact to induce plant movement and, subsequently, thigmomorphogenesis. Furthermore, Autio et al. (1994) found significant effects of daily brushing duration on the degree of plant height reduction of dusty miller and petunia, which is a thigmomorphogenetic response that was not seen for aster. There was no effect of various air stimulus durations on the degree of height reduction for tomato (Fig. 4.10A); however, this relationship for bellflower remains unclear. Bellflower plants located in rows between two adjacent nozzles showed a less stunted height growth compared with those in rows directly underneath a nozzle, indicating that stimulation intensity might have had a role.

4.5.4. Plant growth response of tomato

It was shown that defined and directed air jets applied as either a laminar air stream or turbulent free air streams by three distinct system configurations were suitable to induce (depending on the applied stimulus intensity and the used module) 26% to 36% reductions in tomato plant

height compared with untreated control plants (Table 4.3). The data are in agreement with results of growth regulation experiments using other stimulation techniques and are of the same order of magnitude compared with one PGR treatment (Latimer and Beverly, 1993). For example, Schnelle et al. (1994) applied mechanical stimuli to tomato using a four-bar apparatus and reduced height growth by 26% to 35% compared to untreated control plants. Johjima et al. (1992) achieved growth reductions of ≈30% in six tomato lines compared with controls using a suspended steel bar for 1.5 min twice daily. The lower degree of growth inhibition during the air knife experiment (Table 4.3) might be related to the throttle valve being closed to 60°, resulting in a marked reduction of air velocities that ranged between $1 - 4$ m$\cdot$s^{-1} (Fig. 4.9C) compared with air velocities that were achieved with a 90° throttle opening (Figs. 4.5B and 4.7). However, even with the fully opened throttle, the distance from the air outlet would need to be reduced to 50 cm to obtain an air velocity similar to that at 60 cm from the 360° rotor module with the same throttle opening (Fig. 4.5B and C). Nevertheless, the variability in air velocities along the aperture slot is a major drawback (Fig. 4.7).

The predominant factor that determined the degree of growth inhibition in tomato was the stimulus intensity, resulting in a negative linear relationship between the maximum air velocities perceived ($1 - 4$ m$\cdot$s^{-1}) and plant height (Fig. 4.10C). Studies that have investigated the effects of various stimulation intensity are scarce. Jaffe et al. (1980) applied defined forces on tomato stems and showed that a log linear function best described the relationship between the amount of force applied and the magnitude on stem elongation. More specifically, Hunt and Jaffe (1980) found a curvilinear growth pattern for bean (*Phaseolus vulgaris*) with a tendency toward response saturation with 4.4 m$\cdot$s^{-1} air velocity. Consequently, it is interesting to investigate whether air velocities greater than 4 m$\cdot$s^{-1} of the tested modules will lead to a greater reduction in height than found during this study.

Even with increasingly greater air velocities more than 4 m$\cdot$s^{-1} of the rotor module because there was no adjustment of the distance between the air outlet and shoot tip (Fig. 4.5C), the growth reduction throughout the 2-week experimental period was not greater than that achieved with a constant air velocity of 4 m$\cdot$s^{-1} with the air pressure module because of the constant distance between the air outlet and plant height (Table 4.3). This plant response indicates that air velocities more than 4 m$\cdot$s^{-1} do not induce greater height growth inhibition in tomato, thereby confirming the findings of Hunt and Jaffe (1980).

Garner and Björkman (1996) investigated the effect on tomato stem elongation with variable doses (10 to 40 strokes per day), stroke intervals (0.01 to 10 min) and time of day when the treatment was applied and found no graded plant responses. This was in accordance with the results of this study, which showed that the stimulation frequency/ duration is of minor importance (Fig. 4.10A) for an effective height control, as already suggested by Latimer (1991). Further evidence is provided by the fact that multiple air stimuli per transit were applied (≈10)

with the 360° rotation of the PVC tubes attached to the rotor module but the degree of height growth inhibition was not different to that found in the air pressure and air knife module experiments, during which only one air stimulus per transit was applied. It remains to be investigated to what extend the daily treatment frequency can be reduced while maintaining the maximum degree of height control. We also found that the bending stress applied by air streams does not accumulate (Fig. 4.10B), indicating that plants do not integrate the mechanical stimulus over time, which has also been shown by Garner and Björkman (1996). We postulate that the response to MS follows an all-or-nothing principle with a plant sensitivity response range between the stimulus intensity threshold and the stimulus intensity saturation point.

4.5.5. Commercial application

When creeping inchplants were treated 160 times per day with air streams of the air pressure module, plant height was reduced by 14% to 32%, depending on the season (Fig. 4.11). Seasonal effects were also reported for petunia and dusty miller, which responded to a greater extend during fall compared with spring (Autio et al., 1994). Similarly, Heuchert and Mitchell (1983) found that plant response in tomato to MS was greater in winter than in summer, and that lower light intensities exhibited stronger plant responses compared with higher light levels. Generally, moderate temperatures and low light levels seem to enhance plant responsiveness to MS (Latimer, 1991), similarly to the results found during this study because no additional lighting was applied during winter and the lower room temperatures compared to those during summer. The local horticulturist was able to regulate the height of creeping inchplants throughout 1 year for five consecutive experimental sets, clearly demonstrating that this product can be organically produced at high market value without PGR applications.

4.6.   Conclusions

Plant height reduction of greenhouse crops has been frequently achieved by changing temperature conditions or regulating water and nutrient supplies. PGR are still the most commonly used method of avoiding excessive stem elongation in ornamentals, but there is growing concern regarding the use of chemicals during plant production. Stimuli applied by directed air streams were shown to be a suitable alternative to PGR and showed efficiency comparable to that of previous techniques relying on touch or brush stimulation. The degree of responsiveness to MS in tomato was shown to predominantly depend on stimulus intensity. The full automation of the presented systems allows flexible and user-friendly large scale application for horticultural companies. In the future, the dose-response relationships will help identify economically suitable application regimes and increase resource efficiency of greenhouse plant production.

4.7.   Acknowledgements

5. Growth regulation by air stream-based mechanical stimulation in tomato (*Solanum lycopersicum* L.) – Part I: optimization of application frequency and intensity

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5.1   Abstract

Stem elongation control is a fundamental requirement for the production of high-quality seedlings in terms of plant compactness and stability. It is known that the stem elongation of seedlings can be inhibited with mechanical stimulation. In this study, a custom-built air stream applicator was used to apply intermittent stimuli to tomato (*Solanum lycopersicum* cv. 'Romello'). Tomato plants were cultivated under greenhouse conditions for 21 days and then exposed to intermittent air stimuli at different air stream application frequencies (8, 24, 40, 56, 72 and 80 d^{-1}) and different air stream velocities (0.7 – 6.0 m s^{-1}) for 14 days. Tomato plants responded with an inhibition of stem elongation of approximately 31% compared to the untreated control, without a systematic dose-response relationship related to application frequency. In contrast, stem elongation inhibition was significantly affected by air velocity, with a sigmoid dose-response relationship with negligible effects up to 2.0 m s^{-1}, followed by a steep increase in the reduction effect up to 4.7 m s^{-1} and a fading of the effect at 36 % reduction for air velocities beyond that. Dry mass of leaves, stems, and petioles was reduced by approximately 10%, 41%, and 19%, respectively, after 14 days of treatment at a gradually increasing air velocity from 3.5 m s^{-1} at day 0 to 6.1 m s^{-1} at day 14 and an application frequency of 8 d^{-1}. Root dry mass was less affected by the air stream application, but showed a slight tendency to decrease compared to control plants.

Keywords: air flow, dose-response, mechanical stimulation, thigmomorphogenesis, stimulus threshold

5.2.   Introduction

Ornamental plant and vegetable seedling production under greenhouse conditions requires well-coordinated crop management strategies to obtain a product that meets quality criteria such as compactness and small plant size. Thus, the basic requirements of the market and consumer are met. In addition, downsizing plants allows crop producers to maximize profitability by increasing the production per m^2 and transport capacity (Bergstrand, 2017; Börnke and Rocksch, 2018). Moreover, plants that are reduced in size are more stable and therefore less susceptible to potential damage during handling and transportation (Börnke and Rocksch, 2018; Latimer, 1998). From a consumer perspective, a downsized plant with short internodes is more aesthetically pleasing and therefore more attractive.

In ornamental plant production, control of plant size and organ growth is usually achieved by the use of synthetic PGR. Their mode of action is based on the inhibition of the biosynthesis of GA, which, among other relevant plant hormones, are responsible for internode elongation (Rademacher, 2015). In vegetable seedling production, the use of PGR is not allowed by law in many countries, yet growth control is required. In recent decades, several alternative management strategies to control plant size of greenhouse grown crops have evolved and are now common practice. For example, temperature and light control, as well as regulating water and nutrient supply, allow for the regulation of excessive, undesirable stem elongation (Bergstrand, 2017). However, these techniques require a high degree of management and are difficult to apply uniformly to the entire crop. Therefore, new production techniques that regulate plant size and organ growth are needed. This could also be crucial in the near future in view of tightened regulatory requirements regarding the excessive use of PGR in ornamental plant production, which will most likely become stricter as part of the global trend towards more sustainable crop production.

Plant responses to MS have been the subject of numerous studies in recent decades (Sparke and Wünsche, 2020). When plants perceive touch-like stimuli, molecular signalling events generally lead to a gradual morphological adaptation of the plant, often characterized by stunted growth and consequently a reduction in plant size (Börnke and Rocksch, 2018). This phenomenon is referred to as thigmomorphogenesis (Jaffe, 1973).

Many attempts have been undertaken to put MS into practice for plant size control. Manual brushing of shoot tips with cardboard material (Latimer, 1990) use of polyvinylchloride (PVC) rods (Latimer and Thomas, 1991), or fleece material (Koch et al., 2011), which is dragged through the greenhouse section, or bending the shoot tips while moving, reduced plant size successfully. However, plant damage was frequently reported (Koch et al., 2011; Latimer, 1994), which hindered further development.

A previous study described the development process of different prototype systems applying a defined air-based mechanical stimulus to greenhouse plants, demonstrating that the size of

bellflower (*Campanula* 'Merrybell'), creeping inchplant (*Callisia repens*) and tomato (*Solanum lycopersicum* 'Romello') could be effectively controlled (Sparke et al., 2021). Thereby, frequently reported plant damage from the direct touch-like stimulus could be reduced, providing a promising alternative to PGR.

Several authors (Coutand et al., 2000; Garner and Langton, 1997a,b; Garner and Björkman, 1996; Jędrzejuk et al., 2020; Sparke et al., 2021; Telewski and Pruyn, 1998) examined the growth response to different treatment frequencies but could not find consistent results. Furthermore, to our knowledge, there is little literature on growth regulation experiments with variable treatment intensities, although this appears to be the predominant factor influencing the extent of stem elongation inhibition (Hunt and Jaffe, 1980; Latimer and Beverly, 1993; Sparke et al., 2021).

Therefore, the objective of this study was to develop a fundamental understanding of the dose-response relationship between stimulation frequency and intensity and the resulting plant growth response.

5.3. Material and Methods

5.3.1. Plant material and cultural practice

Seeds of tomato (*Solanum lycopersicum* L.) cv. 'Romello' (Graines Voltz, Colmar, France) were sown in 104-cell trays with one seed per plug and grown under greenhouse conditions at the University of Hohenheim, Stuttgart, Germany. Individual tomato seedlings were transplanted into round plastic pots (0.5 L volume) 20 days after sowing, when the third true tip leaf unfolded. The pots were filled with a soil substrate of raised bog peat and coconut fibre (12N-6.1P-19.9K; Substrat 5, Klasmann-Deilmann, Geeste, Germany) and arranged evenly on a greenhouse table at a density of 16 plants per m^2. The transplanted seedlings were manually irrigated every other day with 80 mL of tap water and 2.34 $g·L^{-1}$ water-soluble fertilizer (16N-2.6P-21.6K; Ferty 2 Mega, Hauert, Grossaffoltern, Switzerland). The day/night air temperature was set to 20/16 °C and relative humidity ranged between 50% and 70% throughout the experiment. Artificial light was provided by high-pressure sodium lamps (SONT Agro 400W; Philips, Amsterdam, Netherlands) with a photoperiod of 14 h. Photosynthetic active radiation ranged from 210 to 270 $µmol·m^{-2}·s^{-1}$ and was uniformly distributed across the plant stand on the greenhouse table.

5.3.2. Air stream application

To apply an air-based mechanical stimulus to the plants, an electric motor facilitated the movement of an air stream applicator along the guiderail mounted longitudinally above the greenhouse table. The air stream was generated by a 0.37-kW centrifugal fan (VBL; Air Control Industries, Axminster, England) and directed through a mechanical throttle, a downstream Y-branch, and two flexible hoses inserted into the back of a rectangular aluminium box. The air stream was directed through two slowly rotating PVC tubes (Ø 28 mm) inserted vertically at an

angle of 70°. The rotation of the tubes at 24 rpm was facilitated by a rotor module consisting of a chain drive with gears driven by an electric motor (Sparke et al., 2021). The system was fully automated with an electronic control unit. Thereby, the starting time, the number of passes per activation signal, and the driving velocity could be set. The driving velocity along the guide rail was set to 1.5 m·min^{-1} in all experiments. The combination of travel speed and rotation speed of the air outlet tubes provided approximately 10 air stimuli per plant each time the table was traversed. The vertical distance D_t of the outlet tubes from the greenhouse table was kept constant at 42 cm in all test runs. When the applicator passed magnetic switches attached to the guide rail, the centrifugal fan and the rotor module were switched on and off, respectively. The applicator was deactivated and retracted after reaching the end of the guide rail, i.e., the air flow was only applied during the forward movement.

The greenhouse table (3.0 x 1.2 m) was divided into two sections (1.5 x 1.2m, each). Plants on one half of the table were left untreated as control. Those plants on the second half of the table were treated daily for 14 days with a specific air stream application scheme with set passing frequencies and air stream intensities, labelled with the day of treatment (DOT 0 to DOT 14). The two sections were separated by a polystyrene foam board, to keep the air stream away from the control group.

Fig. 5.1. The air stream applicator installed at the University of Hohenheim, Stuttgart, Germany.

Tab 5.1. Starting dates of individual experimental runs with the corresponding application frequency and air stream intensity setting of Experiment 1 and 2, conducted with the air stream applicator under greenhouse conditions at the University of Hohenheim, Stuttgart, Germany.

		1	2	3	4	5		6	7	8	9	10	11	12
	Test run	1	2	3	4	5		6	7	8	9	10	11	12
	Year			2018							2019			
Experiment		19	12	5	19	3		14	28	11	25	27	29	17
1	Starting date	Feb	Mar	Nov	Nov	Dez		Jan	Jan	Feb	Feb	Mar	Apr	May
	Application frequency AF (d^{-1})	80	80	24	56	72		40	8	40	8	72	56	24
	Air outlet velocity v_0 (m s^{-1})	17.1	17.1	17.1	17.1	17.1		17.1	17.1	17.1	17.1	17.1	17.1	17.1
	Test run	1	2	3	4	5		6	7	8	9	10		
	Year			2019						2020				
Experiment		6	28	25	11	2		20	3	17	2	16		
2	Starting date	Jan	Jan	Feb	Nov	Dez		Jan	Feb	Feb	Mar	Apr		
	Application frequency AF (d^{-1})	8	8	8	8	8		8	8	8	8	8		
	Air outlet velocity v_0 (m s^{-1})	5.4	17.1	17.1	14.8	11.5		16	5.4	14.8	11.5	16		

5.3.3. Application schedule of Experiment 1

Experiment 1 was designed to investigate the influence of the air stream application frequency (AF). The experiment consisted of twelve test runs starting on certain days in 2018 and 2019 (Table 5.1) with freshly potted plants for each test run. Individual test runs lasted 14 days and comprised eight application cycles per day with starting times at 08:00, 08:45, 10:00, 10:45, 12:00, 12:45, 14:00 and 14:45. The application cycles comprised 1, 3, 5, 7, 9 or 10 table passes, resulting in a daily application frequency of 8, 24, 40, 56, 72 or 80 times. Each application frequency was applied twice at random order. The same air velocity v_0 of 17.1 m s^{-1} was set for all test runs, where v_0 denotes the air velocity at the centre of the air outlet tube.

5.3.4. Application schedule of Experiment 2

Experiment 2 was designed to investigate the influence of the air stream intensity. The experiment consisted of ten test runs that started on certain days in 2019 and 2020 (Table 5.1) with newly potted plants for each test run. Individual test runs lasted 14 days and comprised eight application cycles with the same starting times as in Experiment 1. One table pass per application cycle was performed, resulting in a daily application frequency of 8 table passes for all test runs. The air outlet velocity v_0 was set to 5.4, 11.5, 14.8, 16.0, and 17.1 m s^{-1}, by regulating the volume flow with the mechanical throttle valve using a manually inserted angular scale. Each air outlet velocity was applied twice in random order.

5.3.5. Stem height measurement

Thirty untreated control plants and thirty air stream-treated plants were repeatedly evaluated for stem height (SH, cm) at DOT 0, 2, 4, 7, 9, 11 and 14. SH was measured with a ruler and defined as the height from the edge of the pot rim to the main stem apex.

Relative stem height (RSH) was calculated by dividing the mean SH of air stream-treated plants by the mean SH of untreated control plants at DOT 0, 2, 4, 7, 9, 11, and 14, respectively, with the following equation:

$$RSH = \frac{\overline{SH}_{Air\ stream}}{\overline{SH}_{Control}} \tag{1}$$

5.3.6. Trigonometric model application

As the plants grew during the 14-day test runs, the vertical distance between the tube outlets and the main stem apex continuously decreased. Since the velocity of the airflow decreases with distance from the outlet tube, the velocity of the air stream reaching the plant (v_{plant}) is affected by its height growth. Based on Zawadzki et al. (2010) v_{plant} was estimated as:

$$v_{plant} = \frac{\frac{v_0 \cdot 0.96 \cdot r_0}{a}}{(l_0 + D_a)} \tag{2}$$

Where v_0 is the air outlet velocity of the nozzle, r_0 the radius of the circular air outlet 0.014 m, a is the experimental coefficient 0.137, l_0 the theoretical distance of the pole from the nozzle outlet 0.044, and D_a a function of SH:

$$D_a = \frac{D_t - SH}{cos70°} \qquad (3)$$

where D_t is total height difference between the pot rim and the centre position of the air outlet (Fig. 5.2).
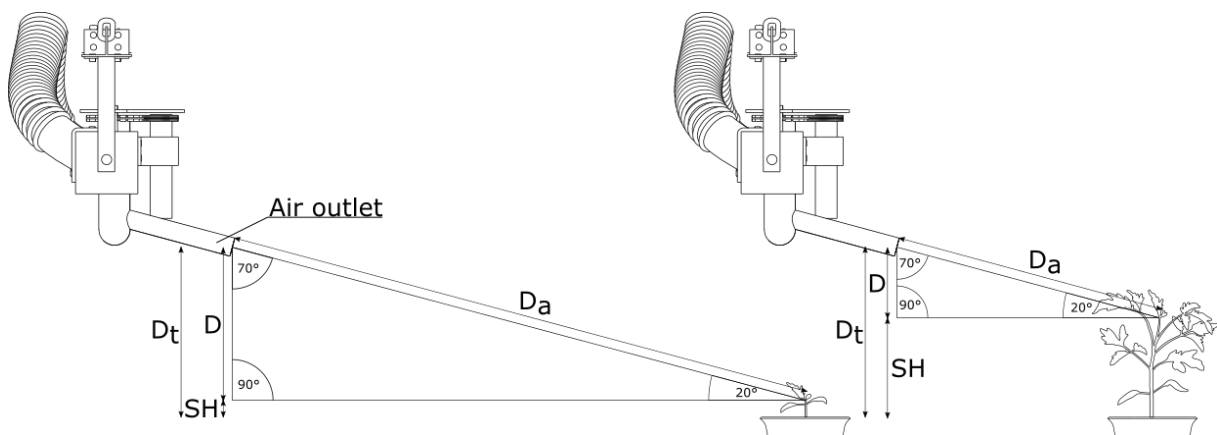

Fig. 5.2. Distance D_a between air tube outlet and the main stem apex at the beginning of the test runs (left) and after growing of the plants (right) with vertical distance of the air tube outlet D_t and stem height SH.

5.3.7. Biomass measurement

At DOT 14, 10 randomly selected control and 10 air stream-treated plants were harvested and separated into leaves, petioles, stem, and roots. After oven drying at 60°C for 4 days, leaf dry mass (LDM, g), petiole dry mass (PDM, g), stem dry mass (SDM, g) and root dry mass (RDM, g) were determined using a microscale (Sartorius ME215S; Sartorius AG, Göttingen, Germany). Dry mass was determined for AF of 0, 8, 40, and 80 d^{-1} in Experiment 1 and for air outlet velocity v_0 of 5.4, 11.5, 14.8, 16.0, and 17.1 m s^{-1} in Experiment 2.

5.3.8. Statistical analysis

SH and biomass measurements were analysed with SAS software 9.4 (SAS Institute, Cary NC) using proc glimmix. Least squares means of SH were calculated for each combination of treatment level and DOT to compare between control and treatment and between treatment levels at each DOT. The significance level was set to P ≤ 0.05.

5.4.   Results

5.4.1.   Influence of application frequency (Experiment 1)

5.4.1.1.   Effect of the application frequency on tomato stem elongation

No significant differences in SH were observed between control plants and the different treatment levels at DOT 0 and 2, but a gradually increasing significant reduction in SH was evident at DOT 4, 7, 9, 11, and 14 (Fig. 5.3).
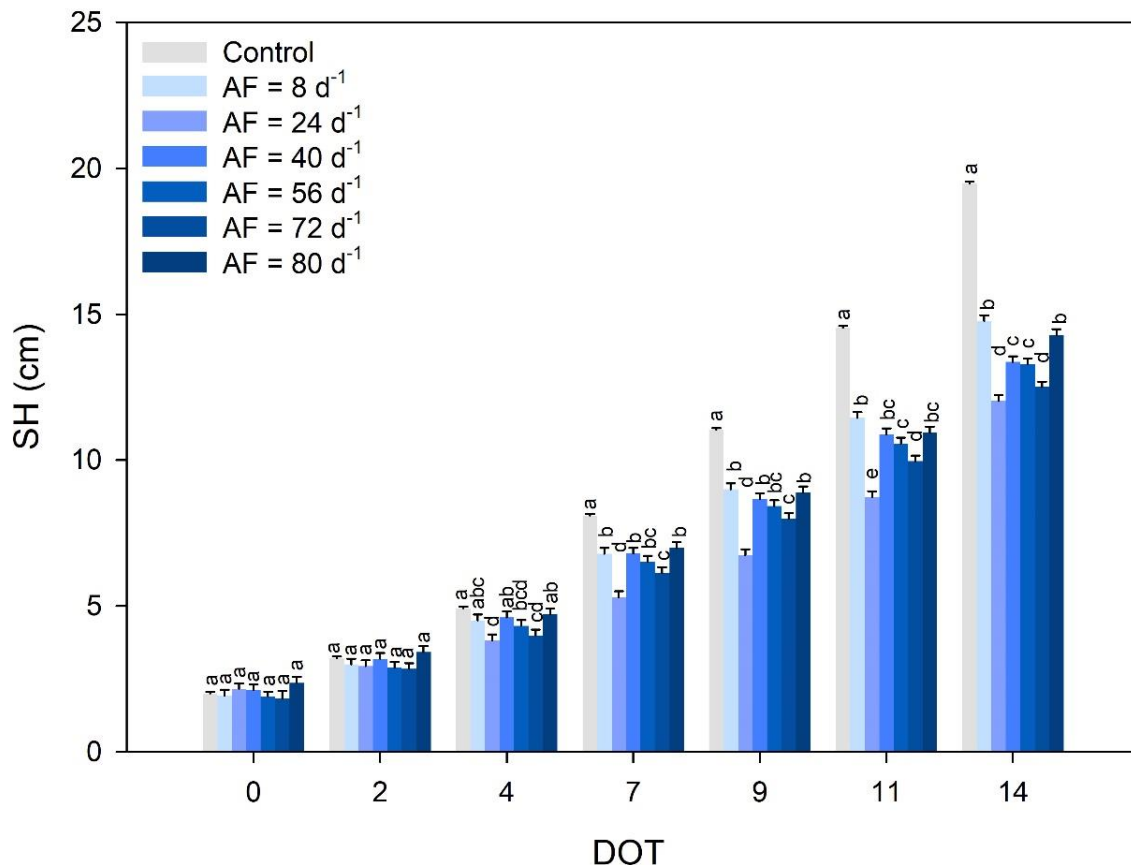

Fig. 5.3. Least squares means (± standard error) for tomato stem height (SH) at different days of treatment (DOT) for various application frequencies (AF) applied by the air stream applicator. Different letters indicate significant differences at P ≤ 0.05.

At DOT 4, the reduction in SH, averaged over all treatment levels, accounted for 12% compared to the untreated control, and gradually increased to 21%, 25%, 28%, and 31% at DOT 7, 9, 11, and 14, respectively. The average RSH ranged between 0.7 and 0.84 and did not show significant differences between AF (Fig. 5.4). As indicated by the weak correlation between RSH and AF with $R^2 = 0.10$, no systematic dose-response relationship for AF in the range between 8 and 80 d^{-1} could be found.
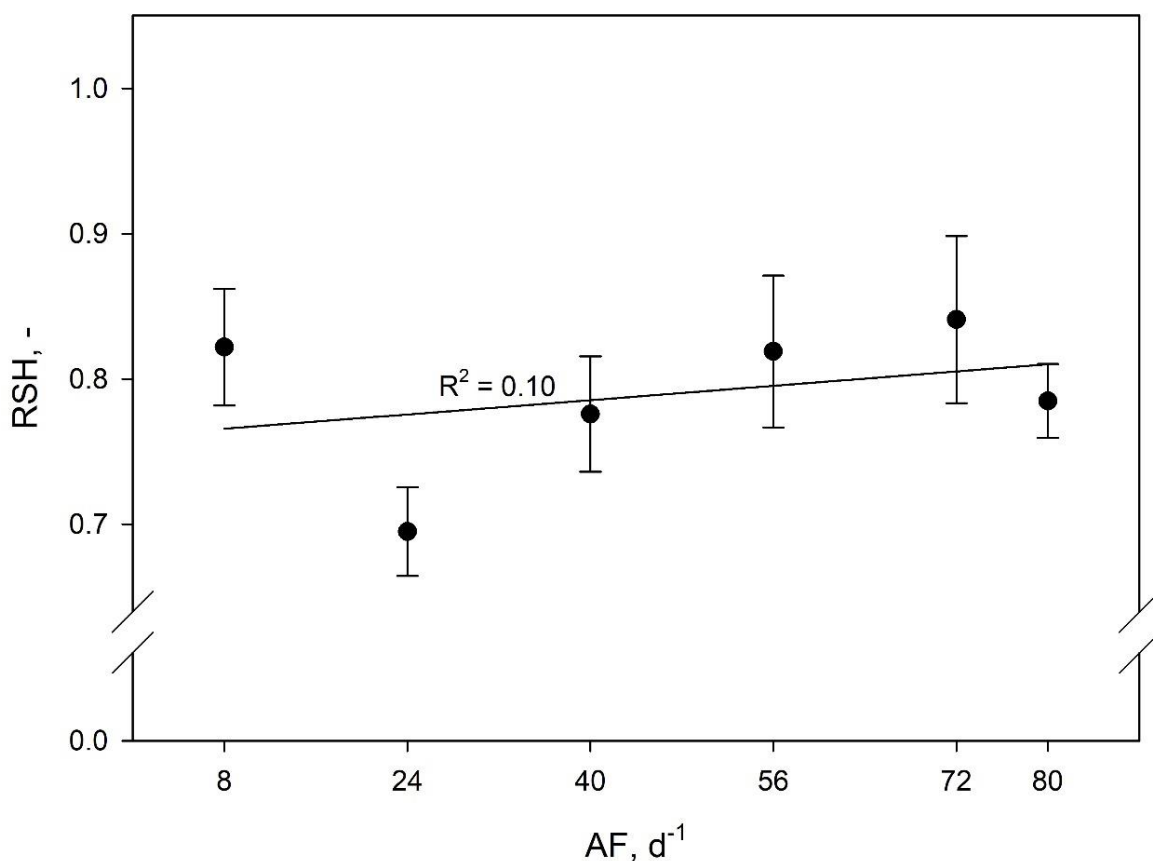
Fig. 5.4. Relative stem height (RSH) (mean ± standard error) of tomato plants as a response to different air stream application frequencies (AF).

5.4.1.2.   Effect of the application frequency on biomass development

LDM of air stream-treated plants was significantly reduced by about 14% compared to the untreated control regardless of the AF (Table 5.2). Stronger effects were found in the reduction of SDW, which accounted for roughly 41% at AF of 80 d^{-1} compared to the untreated control. This reduction was less evident when the AF was reduced from 80 d^{-1} to 8 d^{-1}. PDM was significantly reduced by approximately 26% when treated with AF of 80 d^{-1} compared to the control. Similar to the reduction in SDM, this effect was less pronounced when AF was reduced to 40 d^{-1} and 8 d^{-1}. RDW tended to be slightly reduced by 9% at AF of 80 d^{-1}, and a significant difference was found at AF of 40 d^{-1} compared to the control with a 15% reduction.

Tab 5.2. Leaf dry mass (LDM), stem dry mass (SDM), petiole dry mass (PDM), and root dry mass (RDM) of tomato plants untreated (control) and treated by the air stream applicator for 14 days at various application frequencies (AF). Different letters indicate significant differences at $P \leq 0.05$.

AF (d^{-1})	v_0 (m s^{-1})	LDM (g)	SDM (g)	PDM (g)	RDM (g)
0	0	0.433 ± 0.008^a	0.187 ± 0.004^a	0.192 ± 0.004^a	0.090 ± 0.002^a
8	17.1	0.387 ± 0.018^b	0.147 ± 0.009^b	0.169 ± 0.008^b	0.088 ± 0.005^{ab}
40	17.1	0.371 ± 0.018^b	0.124 ± 0.009^b	0.161 ± 0.008^b	0.077 ± 0.005^b
80	17.1	0.377 ± 0.018^b	0.111 ± 0.009^b	0.137 ± 0.008^c	0.083 ± 0.005^{ab}

5.4.2. Influence of air stream velocity (Experiment 2)

5.4.2.1. Effect of the air stream velocity on tomato stem elongation

At DOT 0, 2 and 4, no significant differences in SH were observed between the control and the different treatment levels or between different treatment levels (Fig. 5.5). At DOT 7, 9, 11 and 14, SH reduced gradually and strongly depended on v_0. At DOT 7, plants treated with v_0 of 17.1 m s^{-1} showed a significant reduction in SH of about 33% compared with the untreated control. Subsequently, the difference in SH continued to increase in air stream-treated plants, resulting in 36%, 26%, and 24% reductions at v_o of 17.1, 16, and 14.8 m s^{-1}, respectively, at DOT 14.
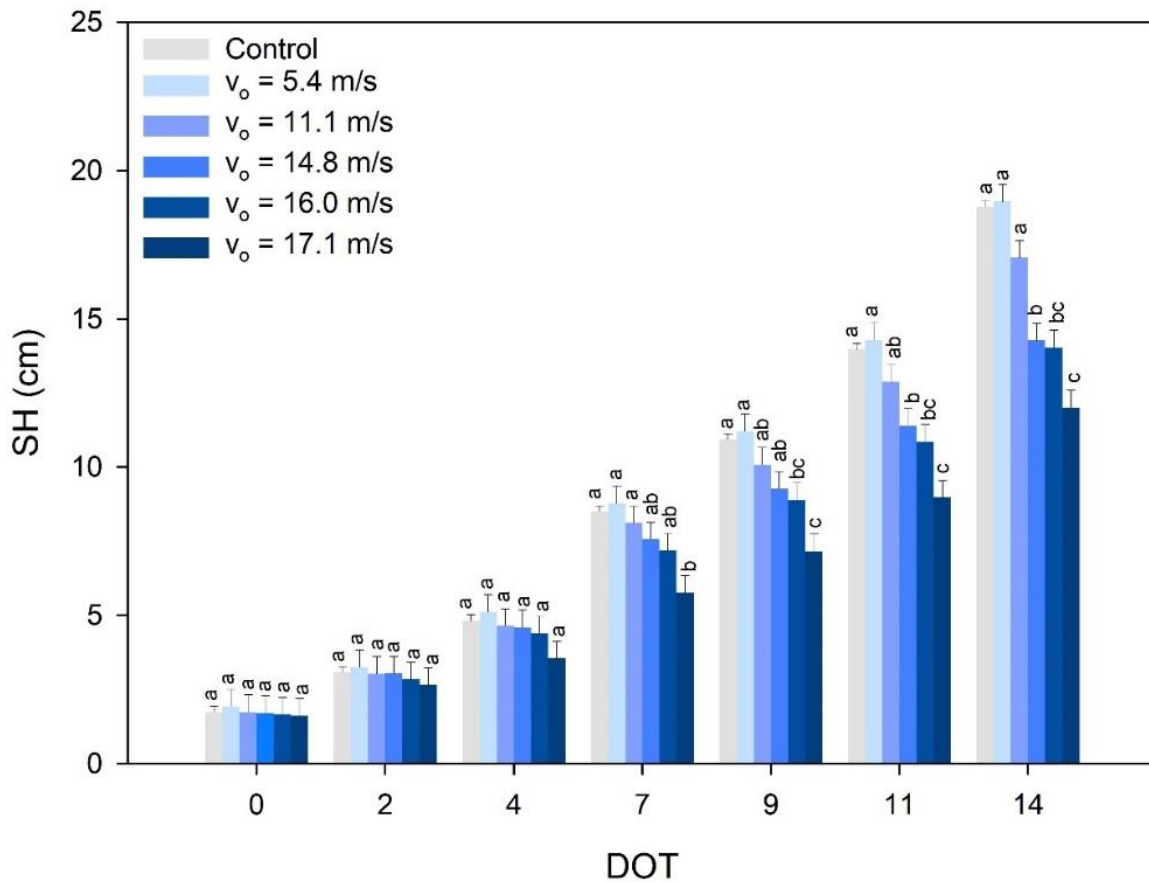
Fig. 5.5. Least squares means (± standard error) for tomato stem height (SH) at different days of treatment (DOT), for various air outlet velocities (v_0) applied by the air stream applicator. Different letters indicate significant differences at P ≤ 0.05.

To quantify the effect of the air stream application, it is necessary to consider the actual air stream velocity arriving at the plant (v_{plant}) as estimated in Eq. (2). At DOT 0, the distance between the apical stem tip and the air outlet (D_a) measured approximately 60 cm and was gradually reduced by plant growth to 33 cm at v_0 of 17.1 m s^{-1} and to 13 cm at v_0 of 5.4 m s^{-1} at DOT 14 (Fig. 5.6A).

Plants treated with v_0 of 5.4 m s^{-1} perceived the lowest v_{plant} of approximately 0.7 m s^{-1} at DOT 0 (Fig. 5.6B). Accordingly, v_0 of 11.5, 14.8, 16.0, and 17.1 m s^{-1} resulted in v_{plant} of about 1.9, 2.4, 2.6, and 3.5 m s^{-1} at DOT 0. As the experiment progressed and the plants were growing, v_{plant} increased exponentially with a maximum of 6.1 m s^{-1} at v_0 of 17.1 m s^{-1} at DOT 14. Although air velocities at DOT 0 differed significantly among all treatment levels, exposure to air velocities at DOT 14 was similar for all treatment levels, except for 5.4 m s^{-1}, due to the different effect on stem growth.
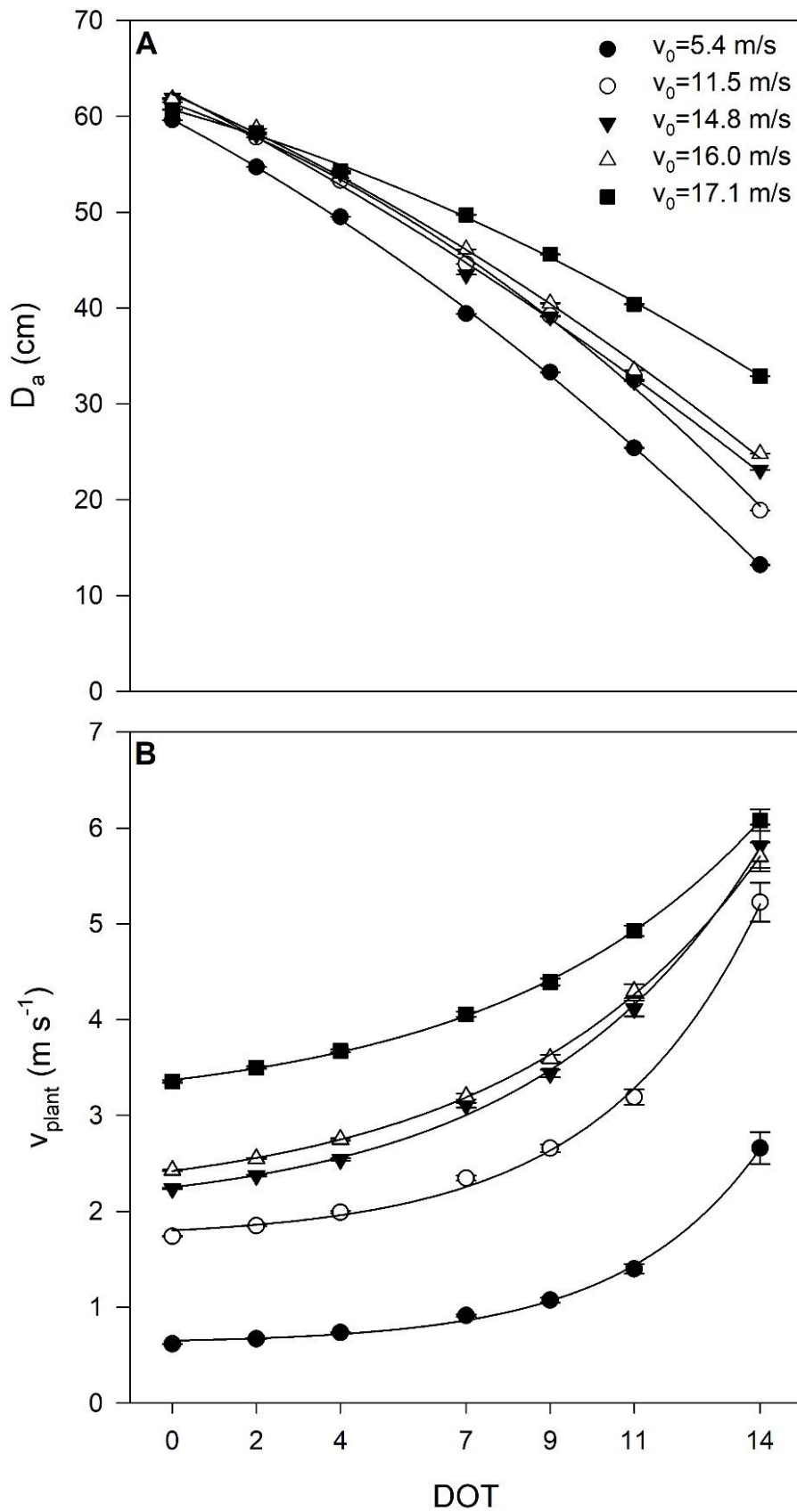
Fig. 5.6. Distance (D_a) between air outlet tube and plant apical stem tip (A) and estimated air stream velocity (v_{plant}) at the apical stem tip (B) on different days of treatment (DOT) for various air stream velocities v_0 applied by the air stream applicator (mean ± standard error).

The plant response as indicated by RSH could be fitted for all values of v_{plant} by a sigmoidal model (Fig. 5.7):

$$RSH = 0.71 + \frac{0.29}{1 + e^{-\frac{v_{plant}-3.38}{-0.49}}}$$

To define stimulus threshold and stimulus saturation, a three-part linear function was fitted to the data. Minimal RSME of 0.007 was found for the following segmentation:

$$RSH = \begin{cases} 1; \, v_{plant} \leq 2.0 \\ -0.1066 \cdot v_{plant}; \, 2.0 < v_{plant} < 4.7 \\ 0.71; \, v_{plant} \geq 4.7 \end{cases}$$

Hence, a v_{plant} of 2.0 m s^{-1} can be considered as stimulus threshold. When v_{plant} exceeds this threshold, RSH is reduced by 0.1 per 1 m s^{-1} until reaching the stimulus saturation at v_{plant} of 4.7 m s^{-1}, where RSH is 0.71. This means that stem length can be reduced by a maximum of 29% through air stream application when the air velocity reaches 4.7 m s^{-1} on the plant.
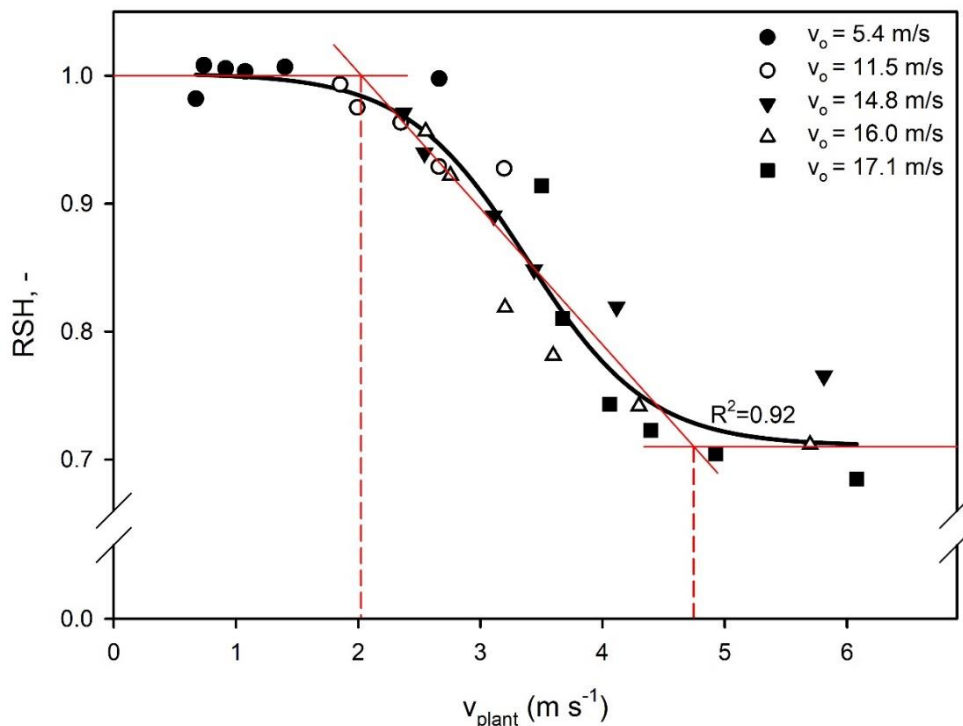

Fig. 5.7. Relative stem height (RSH) of tomato plants as a response to the estimated air velocity at the plant apical stem tip (v_{plant}) for various air stream outlet velocities (v_0) applied by the air stream applicator.

5.4.2.2.   Effect of the air stream velocity on biomass development

LDM of plants treated with v_0 of 16 and 17.1 m s^{-1} was significantly reduced by 10% compared to the control, while no significant differences were found between control and plants treated with v_0 of 5.4, 11.5, and 14.8 m s^{-1} (Table 5.3). SDM was comparatively more affected, with a significant 45% reduction between control and plants treated with v_0 of 17.1 m s^{-1}. The reduction in SDM decreased at lower v_0. Plants treated with v_0 of 17.1 m s^{-1} showed a significant reduction in PDM by 19% compared to the control and the lower v_0 resulted in a lower decrease in PDM. Only plants treated with an air outlet velocity of 17.1 m s^{-1} showed a significant 25% reduction in RDM compared to the control.

Tab 5.3. Least squares means (±standard error) of Leaf dry mass (LDM), stem dry mass (SDM), petiole dry mass (PDM), and root dry mass (RDM) of tomato plants untreated (control) and treated by the air stream applicator for 14 days at various air outlet velocities (v_0). Different letters indicate significant differences at P ≤ 0.05.

AF (d^{-1})	v_0 (m s^{-1})	LDM (g)	SDM (g)	PDM (g)	RDM (g)
0	0	0.392±0.006^a	0.178±0.003^a	0.159±0.002^a	0.080±0.002^a
8	5.4	0.375±0.019^{ab}	0.165±0.009^a	0.143±0.008^{ab}	0.075±0.005^a
8	11.5	0.395±0.019^{ab}	0.179±0.009^a	0.154±0.008^{ab}	0.082±0.005^a
8	14.8	0.385±0.019^{ab}	0.125±0.009^{bc}	0.148±0.008^{ab}	0.084±0.005^a
8	16.0	0.346±0.019^c	0.137±0.009^b	0.136±0.008^b	0.078±0.005^a
8	17.1	0.345±0.019^c	0.104±0.009^c	0.131±0.008^b	0.057±0.005^b

5.5.   Discussion

5.5.1. Effect of the daily air stream application frequency on tomato stem elongation and biomass accumulation

Air stream-based MS with different AF d^{-1} at a constant v_0 of 17.1 m s^{-1} reduced SH by 31% on average (Fig. 5.3), confirming previous results (Sparke et al., 2021) and was in the same range as the results of studies (26% - 35%) investigating the effect of mechanical growth regulation in tomato by non-air-based MS techniques (Johjima et al., 1992; Schnelle et al., 1994). However, no systematic inhibition of height growth was found with increasing AF (Fig. 5.4), suggesting stimulus saturation at AF of 8 d^{-1}, possibly related to the saturation of [Ca^{2+}]$_{cyt}$ influx after repeated air stream application, indicating tissue refractoriness leading to a decreasing response sensitivity to successive stimuli (Knight et al., 1992). Autio et al. (1994) brushed Aster (*Callistephus chinensis*), dusty miller (*Senecio bicolor*), and petunia (*Petunia*) seedlings with burlap 60, 120, and 180 min d^{-1} and found that stem length of aster and petunia was significantly reduced at 60 min d^{-1}, whereas the reduction was not significantly more pronounced at 180 min d^{-1}. Garner and Björkman (1996) used polystyrene foam and brushed

tomato plants 10, 20, 30, or 40 times d^{-1} and found a rapid saturation in stem elongation inhibition with increasing stimulation dosages d^{-1}. Similarly, bending the stems of young elms (*Ulmus americana*) showed no graded response in reducing stem elongation to daily manual bending of 5, 10, 20, 40, and 80 times d^{-1} (Telewski and Pruyn, 1998). In garden roses, even five stimuli per day were sufficient to significantly inhibit first-order axis length (Morel et al., 2012). Interestingly, Jędrzejuk et al. (2020) recently found that petunia (*Petunia x atkinsiana*) cultivars mechanically stimulated with a brushing apparatus showed the greatest reduction in stem length when plants were exposed to 80 stimulations d^{-1} compared to 60 or 40 stimulations. It should be noted that most studies indicate that the daily treatment frequency or total daily treatment duration is not the predominant factor affecting the extent of longitudinal stem growth inhibition, and if it was, this was more likely to be cultivar-specific (Jędrzejuk et al., 2020) or seasonally dependent (Autio et al., 1994; Sparke et al., 2021). Our results confirm that the stimulus duration or the cumulative effect is of less importance, which is supported by previous findings (Sparke et al., 2021). In fact, it would be necessary to also investigate AF below 8 d^{-1} to determine the threshold for the inhibitory effect on stem elongation observed in this study. Once the stimulus threshold is reached, the effect can last markedly long (~ 550 min.), as shown by the results of Coutand et al. (2000). However, using the lowest AF while maintaining maximum stem growth inhibition needs further investigation. Nevertheless, we argue that the main advantage of the air stream-based stimulation technique presented in this study is that multiple air stimuli are applied due to the rotation of the PVC tubes while passing over the table, making potential effects due to variable AF on the degree of growth inhibition, if any, negligible.

LDM, SDM, PDM, were significantly reduced at AF of 8, 40, and 80 d^{-1}, respectively, indicating that aboveground biomass accumulation is negatively affected, while belowground appeared to be maintained, as RDM was less affected (Table 5.2). A reduction in aboveground dry mass in response to MS treatments has been frequently reported (Biddington and Dearman, 1985; Johjima et al., 1992; Latimer, 1991; Piszczek and Jerzy, 1987; Schymanski and Or, 2016). Like the reduction in SH, the biomass reduction was not increased by further increasing AF above 8 d^{-1}.

5.5.2. Effect of the air stream velocity on tomato stem elongation and biomass accumulation

Air stream-based MS was found to gradually reduce SH in tomato by 0-36% with increasing v_{plant} (0.7 – 6.1 m s^{-1}) compared to the control (Fig. 5.5), clearly indicating a plant sensitivity response consistent with the results of previous studies (Hunt and Jaffe, 1980; Jaffe et al., 1980; Latimer and Beverly, 1993; Sparke et al., 2021). The graded response to increasing air velocities, which inevitably results in higher stimulus intensity and thus a longer period of plant oscillation, must be a result of the different cellular signalling events in response to the

perception of mechanical forces (Sparke and Wünsche, 2020), and could be related in particular to the amount of $[Ca^{2+}]_{cyt}$ influx, which correlates positively with the wind force applied (Knight et al., 1992), and is mediated by the opening of mechanosensitive ion channels. Hunt and Jaffe (1980) found that stem elongation in beans (*Phaseolus vulgaris*) decreased linearly between air velocities of 1 m s^{-1} and 3.3 m s^{-1}, with a stimulus saturation toward 4.4 m s^{-1}. Similarly, Sparke et al. (2021) found in a previous study that stem elongation inhibition in tomato increased linearly from 1 m s^{-1} to 4 m s^{-1}. The sigmoidal curve trend between the estimated maximum air velocity (v_{plant}) to which plants were exposed and the effect on stem elongation reduction (Fig. 5.7), revealed a linear trend between 2.0 m s^{-1} and 4.7 m s^{-1} and only marginal effects on stem elongation inhibition between 0.7 m s^{-1} and 2.0 m s^{-1}. A saturation pattern started at about 4.7 m s^{-1} with a curve saturation toward RSH of 0.71. We think that a sigmoidal quantitative relationship between stimulus intensity (v_{plant}) and stem elongation with defined values for a stimulus threshold and stimulus saturation threshold is more appropriate from a biological point of view compared to a pure linear regression (Hunt and Jaffe, 1980; Sparke et al., 2021). Therefore, we propose to build on previous findings (Hunt and Jaffe, 1980; Sparke et al., 2021) by adding to the previous dose-response relationships a stimulus threshold at about 2.0 m s^{-1} and a stimulus saturation threshold at about 4.7 m s^{-1} with an intervening linear dependence, as shown in the dose-response curve (Fig. 5.7). However, it is very likely that the dose-response relationship presented does not hold for different plants species and therefore must be repeatedly elaborated, since, for example, greater forces are required to cause stem elongation inhibition in woody plant stems than in herbaceous ones (Telewski and Pruyn, 1998).

Like the reduction of SH, dry mass was predominantly reduced at v_0 of 16 and 17.1 m s^{-1} in LDM, SDM, PDM and RDM, whereas v_0 between 5.4 and 14.8 m s^{-1} showed less pronounced effects on LDM, PDM, and RDM (Table 5.3). The reduction in LDM is probably due to a reduction in the total leaf area of the plant, and possibly a reduction in the total number of leaves, as previously reported for brushed tomato (Johjima et al., 1992; Piszczek and Jerzy, 1987) and cucumber (Latimer et al., 1991). SDM was more reduced, consistent with previous findings (Johjima et al., 1992) and corresponding to a reduction in stem length. Accordingly, the reduction in PDM was related to a reduced petiole length (data not shown).

5.6.   Conclusions

This study showed that plant growth of tomato seedlings can be reduced by MS based on the application of defined intermittent air streams. The air velocity is the predominant factor determining the extent of stem elongation inhibition, while the daily application frequency is of secondary importance as long as the stimulus saturation point is reached. The graded response to increasing air velocities makes air stream-based MS a flexible method for growth control with great potential for practical implementation in vegetable seedling production, and

at the same time can promote a more sustainable ornamental production, where synthetic PGR are still mainly used to regulate excessive stem elongation.

5.7.   Acknowledgements

6. Growth regulation by air stream-based mechanical stimulation in tomato (*Solanum lycopersicum* L.) – Part II: phenotypic and physiological responses

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6.1.   Abstract

Plant responses to mechanical stimulation has great potential for growth control of ornamentals plants and vegetable seedlings and is a major requirement to ensure plant compactness and stability. 21 days old tomato (*Solanum lycopersicum* cv. 'Romello') plants were exposed to regularly applied mechanical stimuli for 14 days by the employment of a defined air stream through a custom-built air stream applicator. Air stream application gradually reduced total plant leaf area by 14% and radial growth was promoted relative to internode length compared to the untreated control, resulting in a more compact and stable plant phenotype, which was also related to an increased stem dry matter content of air stream-treated plants. The reduction in total plant leaf area was compensated by the translocation of proportionally more assimilates to the light- harvesting tissue and to stems at the expense of dry mass accumulation to petioles. Total stem, leaf and root dry mass of air stream-treated plants was unaffected. Specific leaf area of air stream treated plants was reduced compared to the control and resulted in an increased relative leaf greenness and consequently in higher net carbon assimilation rates by 8% on average compared to the control. Thereby, air stream-treated plants were able to maintain overall biomass accumulation at the same level of the control. Leaf transpiration rate of air stream treated plants was not markedly affected on long-term. The technique presented should be easily transferable to other plants, such as ornamentals where the application of chemical plant growth regulators is still the most common technique for plant growth control.

Keywords: acclimation, air flow, mechanical stimulation, phenotypic plasticity, thigmomorphogenesis

6.2.   Introduction

The production of high quality and marketable ornamentals and young vegetable seedlings is a basic requirement in the horticulture production and supply chain to remain competitive on a growing international horticulture market. Therefore, plant products need to meet external sensory quality parameters (Heuvelink et al., 2004) such as a stunted plant architecture, overall compactness and plants with dark green leaves (Börnke and Rocksch, 2018).

Commonly, in ornamentals the product quality criteria compactness and reduced plant size is met by the application of synthetic PGR (Rademacher, 2015). However, global developments towards more sustainable plant production systems imply the reduction of chemical inputs. Furthermore, PGR are legally not allowed in vegetable seedling production in many countries, but growth regulation required, which is why alternative measures must be undertaken. Usually, growth regulation in vegetable seedling production relies on the control of climate conditions, such as temperature or light management which both are common techniques to reduce excessive stem elongation (Bergstrand et al., 2016; Jensen et al., 1996; Moe, 1990; Myster et al., 1997; Patil and Moe, 2009; Schüssler and Bergstrand, 2012). However, if not managed well, artificial temperature control is considered problematic due to potentially higher energy consumption (Bergstrand, 2017) while changes in photoperiod and light quality require a high level of experience and management.

The induction of thigmomorphogenesis, described as the plant response to MS (Jaffe, 1973), is considered to be a promising alternative methodology to regulate plant growth under greenhouse conditions. By means of MS, similarly to the application of PGR, touch-like stimuli result in the degradation or biosynthesis inhibition of GA and ultimately reduce stem elongation, the most noticeable plant response to MS (Sparke and Wünsche, 2020). Furthermore, plants treated by MS consistently show a reduction in leaf area (Autio et al., 1994; Johjima et al., 1992; Latimer et al., 1991a, Latimer et al., 1991b, Garner and Langton, 1997a) under MS treatment. Although MS reduces leaf area, leaf density was frequently reported to be increased (Biddington and Dearman, 1985; Latimer, 1998; Latimer and Thomas, 1991; Nakaseko, 1988; Wu et al., 2016), which in turn resulted in darker green leaves and consequently in increased leaf chlorophyll concentration (Anten et al., 2010; Johjima et al., 1992; Latimer and Thomas, 1991; Marler and Zozor, 1992; Piszczek and Jerzy, 1987). Accordingly, also leaf nitrogen content and photosynthetic capacity were shown to be increased (Anten et al., 2010).

Other observed effects of MS are the promotion of radial growth to better withstand mechanical force impact (Gardiner et al., 2016) sometimes accompanied by biomass partitioning to roots (Coutand et al., 2008; Gartner, 1994; Goodman et al., 1998).

In a previous study it was shown that the application of defined air stream-based mechanical stimuli successfully reduced plant size of bellflower (*Campanula* 'Merrybell'), creeping

inchplant (*Callisia repens*) and tomato (*Solanum lycopersicum* 'Romello') without causing plant damage (Sparke et al., 2021).

However, the plant physiological acclimation to regularly applied air stimuli with special regard to biomass partitioning and long-term effects on photosynthesis performance need to be described in more detail to evaluate if MS is suitable as an alternative growth regulation methodology.

Therefore, the objective of this study was to investigate the effect of air stream application on plant biometric variables and photosynthesis performance with regard to potential future applications for increasing transportability, where plant size control in order to maximize volume utilization efficiency but also knowledge on its resulting consequences for plant performance is crucial.

6.3. Material and Methods

6.3.1. Plant material and cultural practice

Tomato (*Solanum lycopersicum* L.) seeds cv. 'Romello' (Graines Voltz, Colmar, France) were sown in 104-cell trays with one seed per plug and grown under natural light conditions in a greenhouse of the University of Hohenheim, Stuttgart, Germany. Twenty days after sowing, individual tomato seedlings were transplanted into round plastic pots (0.5 L volume) when the third true tip leaf unfolded. The pots were filled with soil substrate of raised bog peat and coconut fibre (12N-6.1P-19.9K; Substrat 5, Klasmann-Deilmann, Geeste, Germany). The transplanted seedlings were manually watered every other day with 80 mL tap water and 2.34 $g \cdot L^{-1}$ water-soluble fertilizer (16N-2.6P-21.6K; Ferty 2 Mega, Hauert, Grossaffoltern, Switzerland). The day/night air temperature was 20/16 °C and relative humidity ranged between 50% and 70% throughout the experiment.

6.3.2. Experimental design

In each test run, sixty 3-weeks-old tomato seedlings were arranged evenly on a greenhouse table (3 x 1.2 m) at a planting density of 16 plants/m^2. The greenhouse table was divided into halves with a Styrofoam board. On one half of the table, 30 plants were left untreated as control. On the other half of the table 30 plants were regularly treated with a defined air stream by an air stream applicator as shown in Fig. 6.1, and described in detail in Part I. In 2020, four consecutive test runs were conducted, beginning on May 11, May 25, June 8 and June 22, each lasting 14 days. Optimal treatment frequency and intensity as identified in Part I, were chosen. Thus, application frequency AF was 8 d^{-1} by performing eight table passes per day of treatment (DOT), starting at 08:00, 08:45, 10:00, 10:45, 12:00, 12:45, 14:00 and 14:45. Air stream intensity v_0 at the outlet of the applicator tube was set to 17.1 m s^{-1}. The distance between the air outlet and the apical shoot tip was about 60 cm at DOT 0, which resulted in an air velocity perceived by the plants' apical shoot tips v_{plant} of 3.3 m s^{-1}. As the distance between

air outlet and the apical shoot tip gradually reduced throughout the experiment due to shoot elongation, the v_{plant} gradually increased to approx. 5.5 m s^{-1} at DOT 14.

Fig. 6.1. The air stream applicator installed at the University of Hohenheim, Stuttgart, Germany.

6.3.3. Plant growth

6.3.3.1. Biometric measurements

Stem height (SH, cm) was determined for two randomly selected control plants and two air-stream treated plants at DOT 0, 2, 4, 7, 9, 11, and 14, respectively. SH was measured with a ruler and defined as the height from the pot rim to the main stem apex. Internode length (IL, mm) and internode thickness (IT, mm) of internodes 2, 3 and 4 were determined at each DOT with a calliper when the corresponding internode was already present at the respective DOT. IT was measured at the centre of each internode and IL was measured between two consecutive nodes where petioles emerged from the main stem. Total leaf area (TLA, mm^2) was determined destructively by taking scan images with a mobile scanner unit (CanoScan 8400F; Canon, Tokyo, Japan) at each DOT. The digital grayscale photos were imported into ImageJ (Schneider et al., 2012) to calculate leaf area (O'Neal et al., 2002). Specific leaf area (SLA, mm^2 mg$^{-1}_{DM}$) was calculated for each DOT as:

$$SLA = \frac{TLA}{LDM} \qquad\qquad (4)$$

6.3.3.2.   Biomass measurements

The same plants evaluated for SH were harvested and separated into leaves, petioles, stem, and roots. Stem fresh mass (SFM, g) was determined immediately after sampling at each DOT using a micro scale (ME215S; Sartorius AG, Göttingen, Germany). Dry mass (DM, g) was determined by oven drying at 60°C for 4 days with the same microscale and divided into leaves (LDM, g), petioles (PDM, g), stem (SDM, g), and roots (RDM g) at each DOT. Accordingly, mass fractions (MF, %) of leaves (LMF, %), petioles (PMF, %), stem (SMF, %), and roots (RMF, %) were calculated as:

$$XMF = \frac{XDM}{TDM} \cdot 100 \qquad\qquad (5)$$

where X is a placeholder for leaves, petioles, stem, or roots.

Stem dry matter content (SDMC, %) was calculated for each DOT as:

$$SDMC = \frac{SDM}{SFM} \cdot 100 \qquad\qquad (6)$$

6.3.4. Plant physiology

6.3.4.1.   Leaf gas exchange

Leaf transpiration rate (LTR, mmol H_2O m^{-2} s^{-1}) and net carbon assimilation rate (NAR, µmol CO_2 m^{-2} s^{-1}) were determined for the two control plants and air stream-treated plants harvested at each DOT. A portable gas fluorescence system (GFS-3000; Walz GmbH, Effeltrich, Germany) was used to measure LTR and NAR at DOT 2, 4, 7, 9, 11, and 14 on tip leaves 2, 3, and 4 (from bottom to top) when they were already present on the respective DOT. The order of LTR and NAR measurements on individual leaves of one plant was randomized, as well as the order of measurement between control plants and air stream-treated plants at each DOT. The flow rate of the gas fluorescence system was set to 750 µmol s-1 and the light intensity to 1200 µmol m^{-2} s^{-1}. The relative humidity and temperature in the measurement cuvette were set to 50% and 25° C, respectively. Three consecutive measurement points were recorded at 10 s intervals for each leaf approx. 20 min after inclusion of the leaf in the measurement cuvette, once LTR and NAR had stabilized.

6.3.4.2.   Relative leaf greenness

Relative greenness (RG) was determined on individual tip leaves of the main stem of petiole 2, 3 and 4 (from bottom to top) using a chlorophyll meter (SPAD – 502Plus; Konica Minolta, Tokyo, Japan). The chlorophyll meter was positioned at the centre of individual tip leaves and 10 consecutive readings were recorded and then averaged.

6.3.5. Statistical analysis

Data were analysed with SAS software 9.4 (SAS Institute, Cary NC) using proc glimmix with the following model:

$$y = TR + DOT + T + DOT{\times}T$$

Where *y* is the dependent response variable; TR is the number of the test run (1-4), DOT the day of treatment, T the treatment (air stream) and DOT×T the interaction between day of treatment and treatment. The above model was used to calculate least squares means and to compare between control and treated plants. The significance level was set to *P≤0.05*.

6.4.   Results

6.4.1. Biometric measurements

In general, the effect of time for growth (DOT) was significant for all variables except SLA (Table 6.1). The effect of treatment (T) proved significant for SH, TLA, and SLA. The DOT×T interaction had a significant effect on SH.

Tab 6.1. F-test results of the effect day of treatment (DOT), air stream treatment (T), and the interaction between day of treatment and air stream treatment (DOT×T) on SH, TLA, and SLA

Variable		DOT	T	DOT×T
Stem height	SH (mm)	***	**	***
Total leaf area	TLA (mm^2)	***	*	(*)
Specific leaf area	SLA (mm^2 mg$^{-1}_{DM}$)	ns	*	ns

Significant differences are indicated as: (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.001$; *** $p < 0.0001$

The difference in SH gradually increased over the course of the experiment and resulted in a significant reduction of 46 % at DOT 14 between control and treated plants, when SH of the control was about 194 mm and that of air-stream treated plants about 104 mm (Fig. 6.2). The effect in SH reduction of the air stream treatment is illustrated in Figure 6.3.
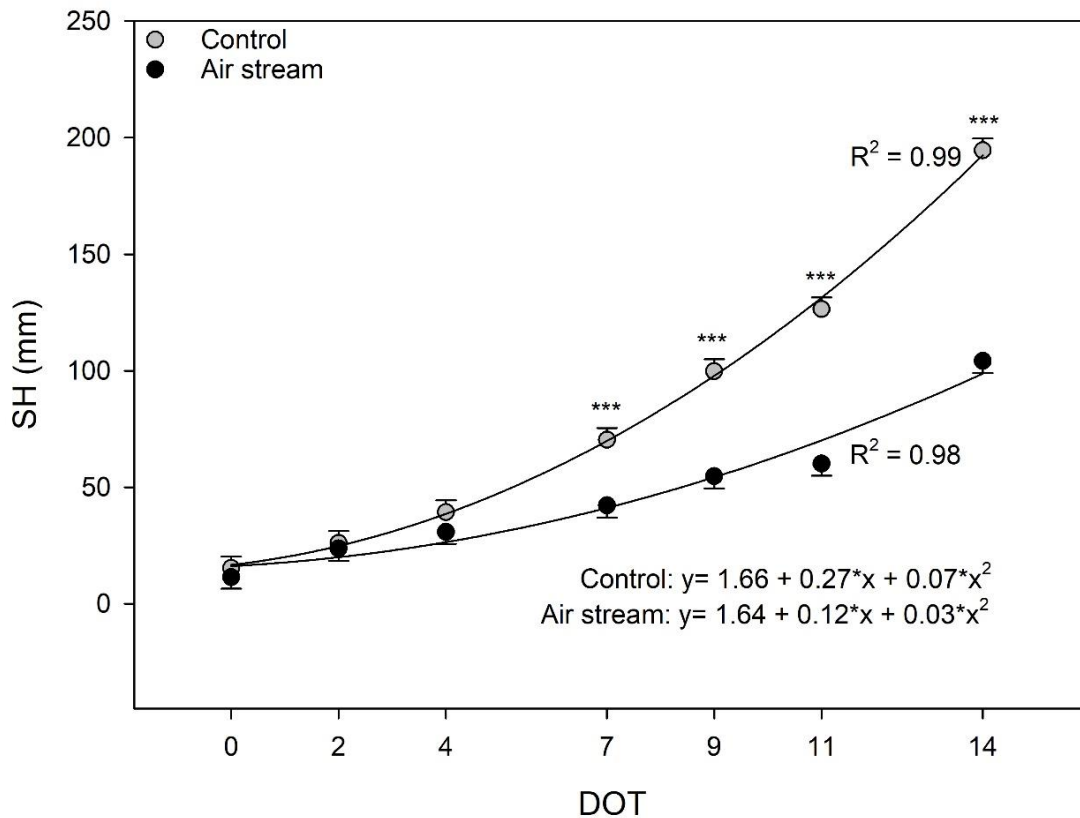
Fig. 6.2. Least squares means (± standard error) for tomato stem height (SH) at different days of treatment (DOT), for control and treated tomato plants. Significant differences are indicated as: (*) = p <0.1; * = p<0.05; ** = p < 0.001; *** = p < 0.0001.
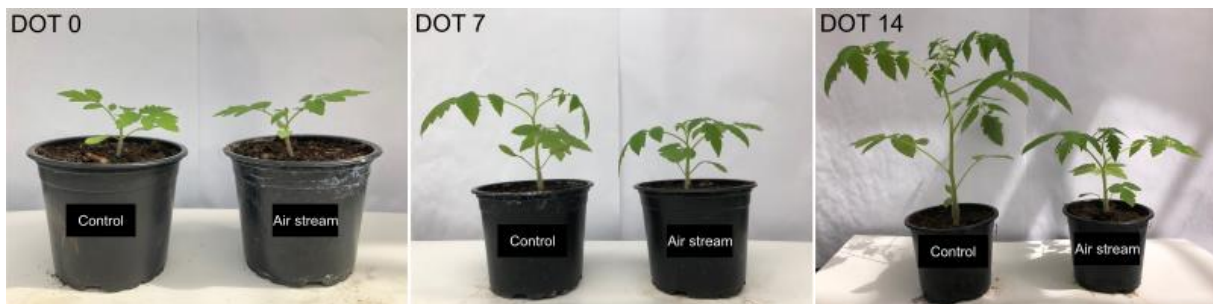
Fig. 6.3. Tomato phenotype of control and treated plant at day of treatment (DOT) 0, 7, and 14.

IL and IT of internode 2, 3, and 4 (Fig. 6.4) were positively correlated. Over the course of the experiment, the treated plants showed a markedly higher IT/IL-ratio than the control, as indicated by steeper slopes of the regression lines. Thus, while air stream application resulted in a reduction of internode length, radial stem growth was promoted compared to internode elongation.
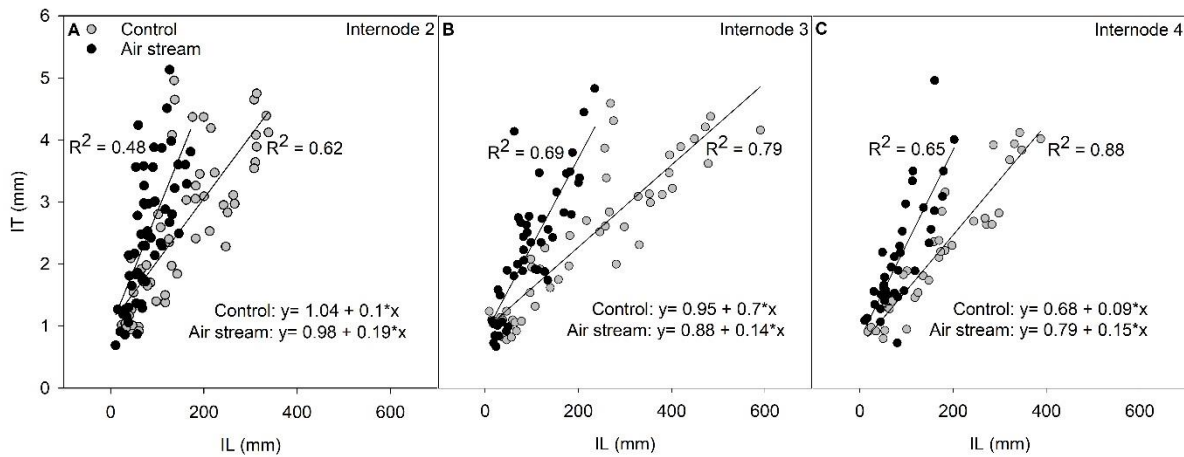
Fig. 6.4. Internode length (IL) and internode thickness (IT) of control and treated tomato plants of internode 2 (A), 3 (B), and 4 (C) of four 14-day test runs.

Figure 6.5 shows the change in TLA and SLA from DOT 0 to DOT 14. The increment in TLA was described by a quadratic regression function for both treatments (Fig. 6.5A). At DOT 9, TLA of air stream-treated plants tended to be reduced compared to the untreated control. This trend intensified at DOT 11 and DOT 14, which resulted in a 12% reduction at DOT 14 of air-stream treated plants compared to the control, when TLA was approx. 2364 mm^2 in control plants and 2084 mm^2 in air stream-treated plants.

SLA of air stream-treated plants followed a similar progression over time as that of the untreated control, but was consistently lower than SLA of the control from DOT 2 to 14, with a 10% reduction at DOT 14 (Fig. 6.5B).

Fig. 6.5. Least squares means (± standard error) of total plant leaf area (TLA) (A), specific leaf area (SLA) (B), of control and treated tomato plants. Significant differences are indicated as: (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.001$; *** $p < 0.0001$.

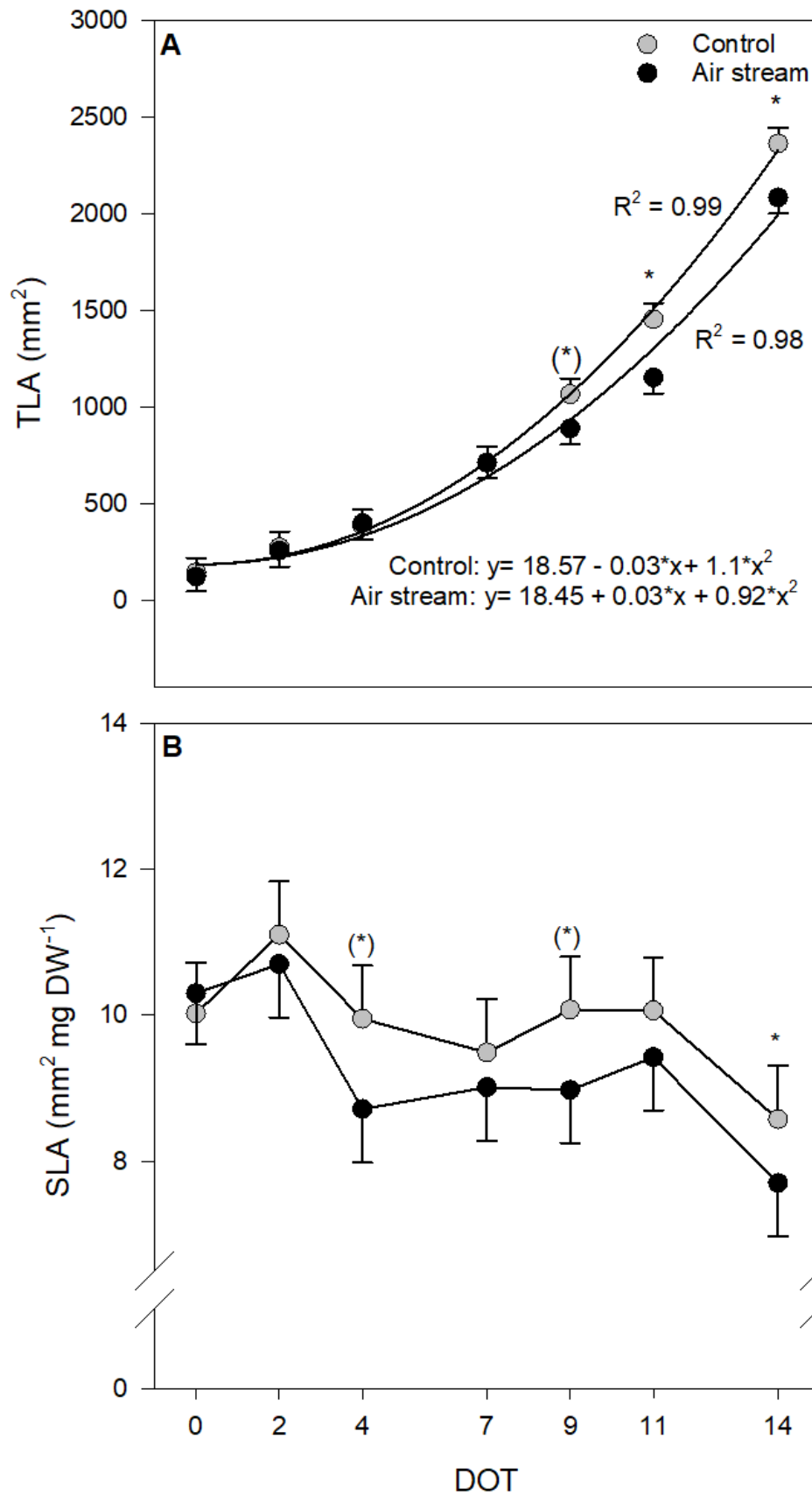### 6.4.2. Biomass measurements

The effect of time for growth (DOT) was significant for all variables (Table 5.2). The effect of treatment (T) proved significant for PDM and SMF. The DOT×T interaction had a significant effect on PDM, LMF, PMF, and SDMC.

Tab 6.2. F-test results of the effect day of treatment (DOT), air stream treatment (T), and the interaction between day of treatment and air stream treatment (DOT×T) on LDM, PDM, SDM, RDM, LMF, PMF, SMF, RMF, and SDMC.

Variable		DOT	T	DOT×T
Leaf dry mass	LDM (g)	***	ns	ns
Petiole dry mass	PDM (g)	***	*	***
Stem dry mass	SDM (g)	***	ns	ns
Root dry mass	RDM (g)	***	ns	ns
Leaf mass fraction	LMF (%)	***	ns	*
Petiole mass fraction	PMF (%)	***	(*)	**
Stem mass fraction	SMF (%)	*	*	ns
Root mass fraction	RMF (%)	***	ns	ns
Stem dry matter content	SDMC (%)	***	*	***

Significant differences are indicated as: (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.001$; *** $p < 0.0001$.

Figure 6.6 shows the increase in dry mass and dry mass fraction of leaves, petioles, stem and roots of the growing plants from DOT 0 to DOT 14. LDM, SDM, and RDM between the control and treated plants did not show significant differences at DOT 14 and accounted for approx. 0.3 g, 0.2 g, and 0.1 respectively (Fig. 6.6A, C, G). PDM of the control and treated plants accounted for 0.1 g and 0.08 g respectively at DOT 14, a significant reduction of 38% compared to the control (Fig. 6.6C).

Air stream-treated plants translocated proportionally more dry matter to leaves (Fig. 6.6B) and stem (Fig. 6.6F), thus resulting in higher LMF and SMF of 7% and 3%, respectively, at DOT 14 compared to the control. PMF increased from approx. 7% at DOT 0 to about 16% at DOT 2 (Fig. 6.6D), but PMF of treated plants gradually declined below that of the control, leading to a significant reduction of approx. 30% at DOT 14 compared to the control.
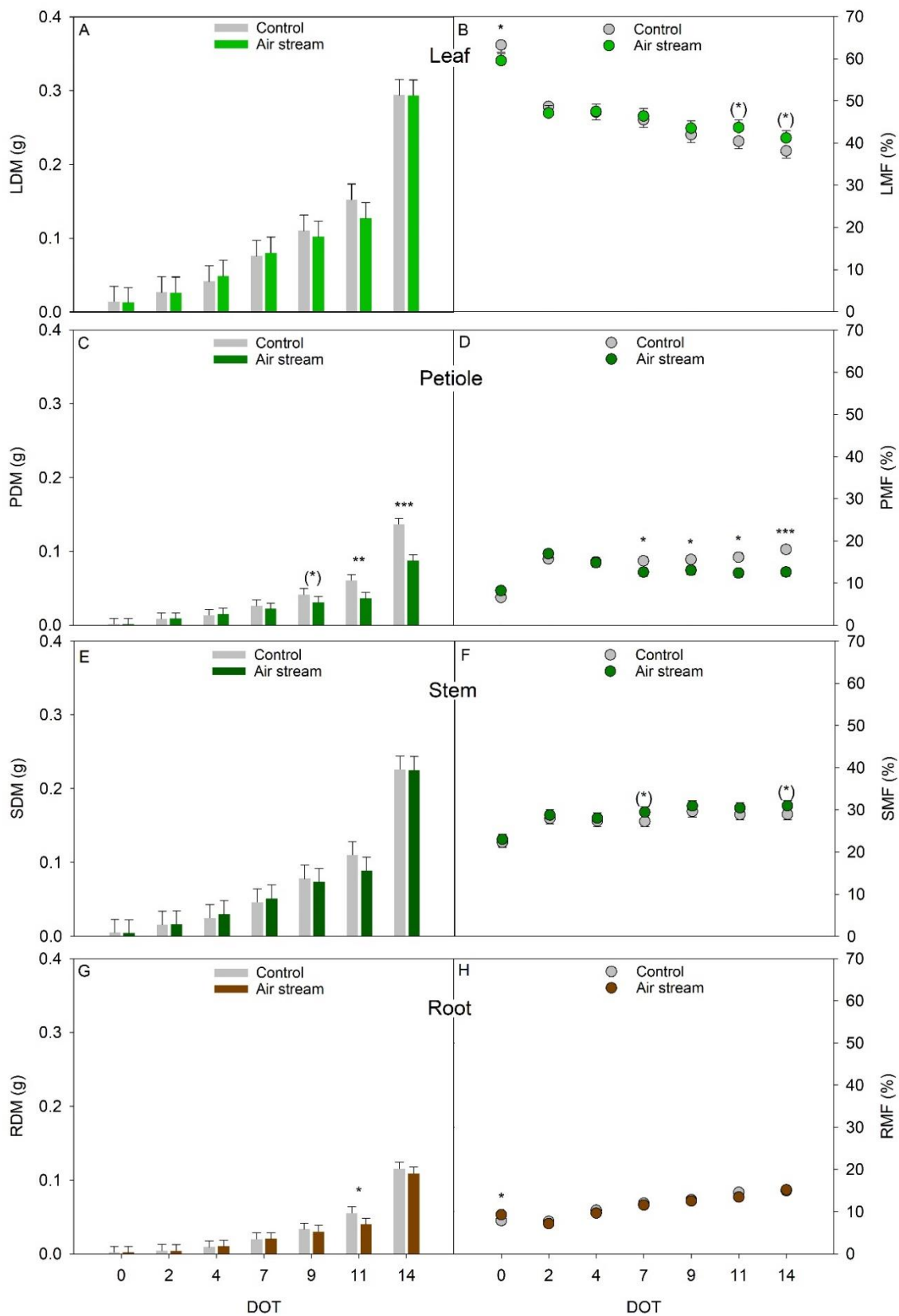
Fig. 6.6. Least squares means (± standard error) of absolute (bars) and percentage (dots) dry mass fraction of leaves (A, LDM; B, LMF), petioles (C, PDM; D, PMF), stem (E, SDM; F, SMF) and H roots (G, RDM; H, RMF) of control and treated tomato plants. Significant differences are indicated as: (*) = p <0.1; * = p<0.05; ** = p < 0.001; *** = p < 0.0001.

RMF increased steadily from approx. 8% at DOT 2 to 15% at DOT 14, while RMF tended to increase slightly in the control compared with the treated plants (Fig. 6.5H).

SDMC of accounted for approx. 4% at DOT 0. As the experiment progressed, the initial increase in SDMC in control plants stagnated at approx. 8%, while SDMC of treated plants increased steadily up to 14% at DOT 14, resulting in a significant 45% increase of treated plants compared to the control (Fig. 6.7).
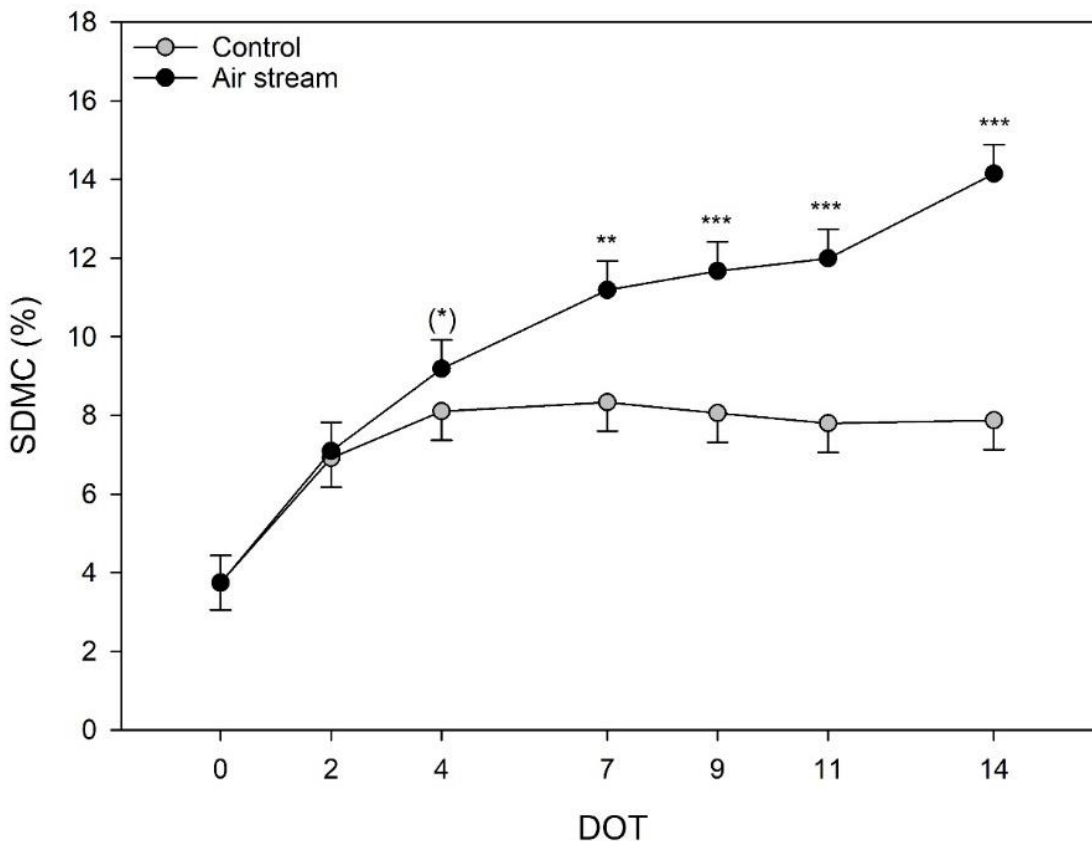
Fig. 6.7. Least squares means (± standard error) of stem dry matter content (SDMC) of control and treated tomato plants. Significant differences are indicated as: (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.001$; *** $p < 0.0001$.

6.4.3. Leaf gas exchange and relative greenness

In general, the effect of time (DOT) and the DOT×T interaction was not significant for leaf gas exchange and relative greenness (Table 6.3). The effect of treatment (T) was not significant for LTR but proved significant for NAR of tip leaf 2 and 3 and for RG of tip leaf 2, 3, and 4.

Tab 6.3. F-test results of the effect of day of treatment (DOT), air stream treatment (T), and the interaction between day of treatment and air stream treatment (DOT×T) on leaf transpiration rate (LTR, mmol m^{-2} s^{-1}), net carbon assimilation rate (NAR, µmol m^{-2} s^{-1}), and relative greenness (RG, -) of tip leaf 2, 3 and 4, respectively.

Variable	DOT	T	DOT×T
LTR (mmol m^{-2} s^{-1})			
Tip leaf 2	ns	ns	ns
Tip leaf 3	ns	ns	ns
Tip leaf 4	ns	ns	ns
NAR (µmol m^{-2} s^{-1})			
Tip leaf 2	(*)	*	ns
Tip leaf 3	ns	*	ns
Tip leaf 4	ns	(*)	ns
RG			
Tip leaf 2	ns	*	(*)
Tip leaf 3	(*)	*	ns
Tip leaf 4	ns	*	ns

Significant differences are indicated as: (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** $p < 0.001$.

Figure 6.8 shows the time course and the respective fitted quadratic regression functions of leaf transpiration, net carbon assimilation, and relative leaf greenness in tip leaf 2, 3, and 4 of the growing plants from DOT 0 to DOT 14. LTR in tip leaf 2 and 3 gradually declined; however, LTR of treated plants tended to be slightly increased in tip leaf 2 and 3 from DOT 9 to DOT 14 compared to the control (Fig. 6.8A, B) Similarly, LTR was consistently slightly higher in tip leaf 4 of plants treated by the air stream at DOT 7, 9, 11 and 14 (Fig. 6.8C). LTR in tip leaf 4 also declined towards DOT 14, similar to the pattern in tip leaf 2 and 3.

NAR of tip leaf 2 and 3 gradually decreased in both treatments during the course of the experiment (Fig. 6.8D, E); however, NAR of air stream-treated plants was on average 10% and 8% higher in tip leaf 2 and 3, respectively, than in the untreated control. NAR of air stream-treated plants in tip leaf 4 was 11% higher at DOT 7 compared to the control but the difference became negligible towards DOT 14 (Fig. 6.8F).

RG of treated plants in tip leaf 2, 3 and 4 was on average 6%, 8%, and 8% higher than the control (Fig. 6.8G, H, I). and followed a similar temporal pattern, respectively.
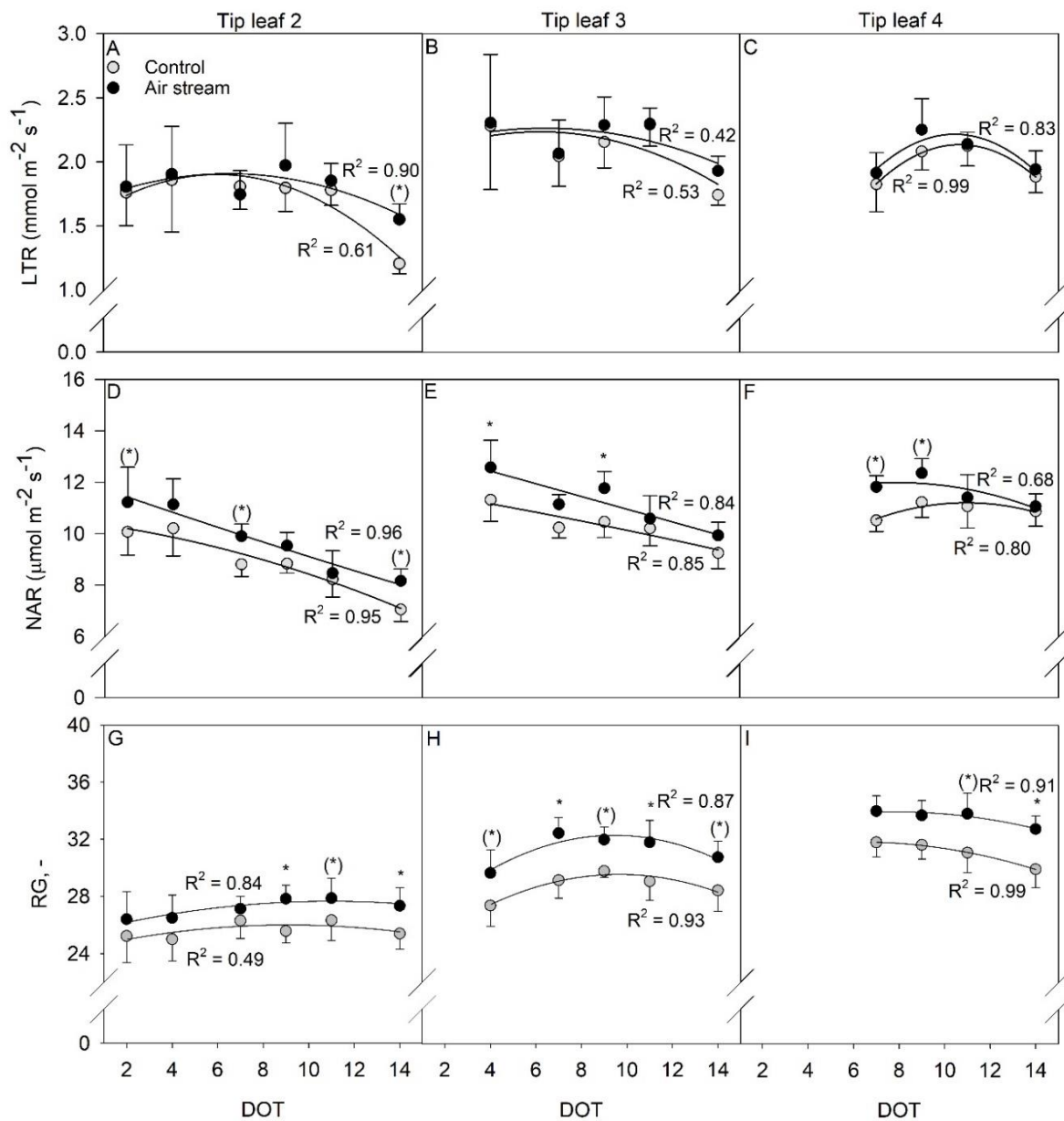
Fig. 6.8. Leaf transpiration rate (LTR), net carbon assimilation rate (NAR), and relative greenness (RG) of tip leaf 2 (A, D, G), tip leaf 3 (B, E, H) and tip leaf 4 (C, F, I) for control and treated plants. Significant differences are indicated as: (*) = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.001$; *** $p < 0.0001$.

6.5.   Discussion

6.5.1. Effect of air stream application on plant biometrics

The air outlet velocity of 17.1 m s^{-1} resulted in the plants being exposed to gradually increasing air velocities between approx. 3.3 and 5.5 m s^{-1} at their apical shoot tip during the course of the experiment, as demonstrated by a computational approach in Part I. As a result, SH was less elongated in the treated plants than in the control during the experimental period (Fig. 6.2). Interestingly, the degree of stem elongation inhibition was even stronger compared to that found in Part I, although the technical set up was the same, which supposes influential

seasonality effects. In general, it is assumed that moderate temperatures and lower light levels enhances the plant responsivity to MS (Autio et al., 1994; Latimer, 1991; Sparke et al., 2021), which contradicts the findings of this study because a stronger inhibition effect occurred in spring/summer compared to the experiment conducted in autumn/ winter (Part I). In fact, a more in-depth analysis to investigate potential seasonality effects, such as different light levels and temperature regimes in combination with MS, will be required.

IL and IT of internode 2, 3, and 4 were each significantly correlated, due to the linear height-diameter allometry (Weiner and Thomas, 2009). A relative shift toward larger stem diameters was observed by air stream application (Fig. 6.4), investing proportionally more into radial growth compared to longitudinal stem elongation. This indicates great phenotypic plasticity of tomato to air stream-based MS by increasing the IT/IL ratio. In any case, the increased IT/IL ratio was not a result of an absolute IT increase of treated plants, but only related to a strong reduction in IL, confirming results found in other woody herbaceous species like sunflower (*Helianthus annus*) (Smith and Ennos, 2003) and other tomato cultivars (Johjima et al., 1992). Consequently, tomato plants treated by air stream-based MS are expected to exhibit similar stability parameters to plants that are touched or flexed, such as increased stem strength and rigidity because of the strong inhibition of IL (Smith and Ennos, 2003). These parameters are important traits for reduced damage during shipping and handling (Latimer and Beverly, 1993), suggesting an improvement in plant quality.

Although, TLA of air stream-treated plants was reduced by approx. 12% at DOT 14 compared to the control (Fig. 6.5A), SLA was consistently reduced (Fig. 5B), indicating higher leaf density of air stream-treated plants. Johjima et al. (1992) and Latimer and Thomas (1991) noted a maximum 31% reduction in leaf area in tomato cultivars when brushed regularly for 5 weeks, similarly to the observations found in four cucumber cultivars (Latimer et al., 1991), and in petunia and pansy (Garner and Langton, 1997a). The reduction in leaf area appears to be a conservative plant response to MS, likely to reduce the attack area and consequently the drag force by wind exposure (Gardiner et al., 2016).

6.5.2. Effect of air stream application on biomass development and partitioning

It was shown that air stream treatment had no significant effect on LDM, SDM, and RDM formation, but PDM was strongly reduced (Fig. 6.4), which was most probably due to a reduction in petiole length (data not shown), indicating plant phenotypic acclimation in conjunction with stem elongation inhibition (Fig. 6.2) to reduce the degree of bending of the entire plant structure under the influence of air stream perception and ultimately avoid breakage. RDM was shown to be reduced in tomato (Johjima et al., 1992) but less in cucumber (Latimer, 1991) when treated with MS by direct contact. The retention of RDM and RMF in this study suggests that assimilate partitioning is maintained belowground under air stream-based MS, indicating potential differences between direct touch-based MS and air- stream based MS,

thus confirming the results of Anten et al., (2010) in great plantain (*Plantago major*) where biomass allocation to roots was stronger reduced under touch-based MS compared to air stream-based treatment. Our results show, that proportionally more assimilates were partitioned to the light-harvesting tissue (Fig. 6B), indicating greater sink strength of leaves most likely to compensate for the reduction in light-harvesting surface area (Fig. 6.5A). Although stem elongation of air stream-treated plants gradually decreased during the experimental period compared to the control, SDM remained unaffected, indicating the formation of more secondary xylem as stabilizing tissue as shown in bean plants when exposed to wind (Hunt and Jaffe, 1980), and confirmed by the increased SDMC of air stream-treated plants (Fig. 6.5B), and greater sink strength of the stem (Fig. 6.6F). In contrast, Latimer (1991) found a significant reduction in SDM when cucumber plants were exposed to MS and also Johjima et al., (1992) showed corresponding results in three tomato parental lines and three hybrid lines. Similarly, in Part I it was demonstrated that SDM of the same tomato cultivar ('Romello') was reduced when treated with air stream-based MS. One possible reason for this discrepancy could be related to seasonal effects. This study was conducted from late spring to early summer, when the natural light intensity was likely higher compared to the studies of Johjima et al. (1992) (March-April) and the own experiment of Part I (November – May). It would be possible that the effect of increased photosynthetic capacity due to MS treatment (Fig. 6.8D, E, F) only occurs under higher light intensity, allowing biomass accumulation to be partially sustained by higher carbon (C) uptake rates.

6.5.3. Effect of air stream application on leaf gas exchange and relative greenness

LTR of tip leaf 2, 3, and 4 were not found to be significantly affected by air stream application (Table 6.3), although marginal differences between control an air stream-treated plants were evident at later stages of the experiment (Fig. 8A, B, C). Changes in LTR in response to seismic stress were determined in soybean (*Glycine max*) (Pappas and Mitchell, 1985), carambola (*Averrhoa carambola*) (Marler and Zozor, 1992), and in great plantain (Anten et al., 2010) treated with either MS, artificial wind, or both simultaneously. Seismic stress in soybean resulted in a 15% to 17% reduction in whole-plant transpiration after a 5-minute shaking event. Seismic stress tended to reduce stomatal conductance in carambola, but stronger effects were seen in the 2.4 m s^{-1} artificial wind treatment, which reduced stomatal conductance by 56% compared to the control. In contrast, brushing had no effect on LTR in great plantain but increased strongly (50-100%) when subjected to a maximum wind load of 2.5 m s^{-1}. Therefore, MS treatments alone are most likely to have differential effects on LTR compared to air stream-based MS as already shown by Anten et al., (2010). The general, expectation is that wind loads have a positive effect on LTR due to the increased evaporative demand, while air streams disrupt the leaf boundary layer and passively remove water from the leaf surface (Schymanski and Or, 2016). In this study, the sampled leaves were exposed to artificial

atmospheric conditions in the measurement cuvette for approximately 20 minutes, so leaf gas exchange was not measured immediately after or during the perception of air streams through the leaves. Therefore, no assumptions about the immediate effect on LTR can be described here, since plants had most likely already recovered from possible short-term effects of air stream perception, altering the leaf microclimate and thus leaf transpiration. It is worth noting, that plants never showed symptoms of drought stress and generally lost the same amount of water after each watering event (data not shown). Our results suggest that although instant effects on LTR are most likely evident, long-term effects from regular air stream application are most likely absent, if any, they could be related to long-term changes such as stomata density (Gokbayarak et al., 2008).

In the study presented, a positive effect of regularly applied air streams on NAR was observed, most likely related to the frequently reported 'greening effect' induced by MS treatments (Anten et al., 2010; Biddington and Dearman, 1985; Latimer, 1991; Marler and Zozor, 1992; Mitchell and Myers, 1995; Piszczek and Jerzy, 1987), similar to the results presented (Fig. 6.8G, H, I). Therefore, it is assumed that specific leaf chlorophyll content per unit leaf area or unit dry weight must have increased, based on the well-established relationship between RG measurements and leaf chlorophyll content (Hay and Porter, 2006). Further evidence for this is the fact that leaf density was higher in air stream-treated plants (Fig. 6.5B), indicating greater dry mass per unit leaf area and, consequently, higher chlorophyll content, which increases light harvesting efficiency per unit leaf area or leaf mass. RG measurements are often used to estimate leaf nitrogen (N) content, which in turn is directly related to Ribulose-1,5 bisphosphate carboxylase-oxygenase (RuBisCO) leaf content, which accounts up to 50% of the total soluble protein in leaves (Evans, 1989), and thus is a major determinant of the C uptake rate. Therefore, it is suggested that the increased RG values may indicate an increased RuBisCO leaf content, which is most likely the main reason for the increased NAR of air stream-treated plants found in his study, building on the results of Anten et al. (2010), who found higher leaf N contents and increased NAR in MS and wind stimulated plants. Although, the short-term effect of MS treatments on photosynthesis is usually described as negative (Jaffe and Forbes, 1993), in this study we have shown that the gradual acclimation to regularly applied air streams leads to higher photosynthesis capacity in the long term. However, as mentioned earlier, this observation might only hold true under growth conditions with higher light intensities, since in this study photosynthesis was measured close to the light saturation point and plants were grown during early summer, enabling higher photosynthesis rates and thus maintaining of C intake at the same range compared with control plants. In fact, it would be interesting to scale these studies at different light intensity to consider the net C gain on whole-plant level to test this hypothesis.

6.6.   Conclusions

Acclimation of tomato to regularly applied air stream-based mechanical stimuli revealed great phenotypic plasticity, allocating proportionally more assimilates to the light-harvesting tissue and stems at the expense of petiole dry mass partitioning while root dry mass fraction is maintained. Thereby, an increased photosynthesis rate, mainly related to a reduced specific leaf area, accompanied by darker green leaves of air stream-treated pants, compensated for the reduction in total leaf area and preserved biomass accumulation, although most likely season-dependent. The increase in internode thickness/length ratio indicates improved plant stability, which is an important characteristic to prevent damage or breakage during transportation. Overall, no major drawbacks of air stream-based MS were found, suggesting the use of defined, artificial air streams as a promising alternative measure to control plant size of ornamental plants and vegetable seedlings under greenhouse conditions.

6.7.   Acknowledgements

7. General discussion

7.1.   Challenges for horticulture in Germany

Production horticulture contributes significantly to the production value of agriculture in Germany, with the ornamental and the vegetable sector having the highest production value compared to the fruit and tree nursery sector (Federal Ministry of Food and Agriculture, 2012). Although, ornamental plant production accounts for by far the smallest share in terms of cultivated area compared to the vegetable, fruit, and tree nursery sector, and others (Fig. 1.1B), the number of companies is comparable to those associated with the vegetable sector (Fig. 1.1A), indicating great economic importance of the ornamental sector. However, the entire production horticulture in Germany underlies a drastic structural change in recent years in that the number of producing companies steadily decreased in all sectors, while the area cultivated remains relatively equal.

The structural change is particularly noticeable in the ornamental sector. The number of production companies reduced by more than 50% from 2005 to 2016, while the cultivated area reduced by 37%, which is the strongest change compared to the other horticulture sectors (Federal Ministry of Food and Agriculture, 2016). A more detailed consideration shows that the number of companies cultivating an area of 20 ha or more increased by 25%, while those cultivating an area below 20 ha strongly decreased, indicating a trend towards company enlargement. However, companies producing on an area of 5 ha or below account for 90% of all companies in the ornamental plant production sector, pointing to the fact that this share particularly presents an integral part of the ornamental horticulture sector. Considering the vegetable sector, a similar trend can be observed; however less strong pronounced compared to the ornamental sector, and the area cultivated even increased from 2005 to 2016 (Federal Ministry of Food and Agriculture, 2016). According to the Zentralverband Gartenbau the main reasons for this change are the increasing international competitiveness and new requirements and regulations which lead to uncertain production conditions. Apart from that, increasingly frequent and prolonged drought periods and increasing labour and energy costs contribute to this production insecurities. As the numbers indicate, smaller production companies that cultivate smaller areas were not able to withstand this pressure economically, while larger companies were able to. Consequently, if this trend continues, the backbone of the German ornamental and vegetable sector might gradually break down in near future. The uncertainties due to changing climate conditions, such as prolonged drought periods, can be partially addressed by shifting the production to protected greenhouse conditions, as observed in the vegetable sector, where there was a 60% increase in production under protected greenhouses between 2005 to 2016 (Federal Ministry of Food and Agriculture, 2016). In ornamental horticulture, the production under protected greenhouse areas is the most widespread of all horticultural sectors in any case.

Against this background, horticulture in Germany is facing major challenges, which will result, among other things, from a very likely increased regulatory policy regarding the use of pesticides, PGR included, in the future. Although PGR accounted for the smallest share of the total sales volume in 2020 (≈5%) (Fig. 1.2), they are the most used method of controlling plant growth in ornamental horticulture compared to other methods such as temperature or light control (Bergstrand, 2017). Therefore, they play a crucial role in the economic success of ornamental producers by enabling to meet quality criteria such as plant height reduction and compactness, while simultaneously maximizing resource use efficiency such as area production capacity and transport capacity, which also applies to vegetable seedling production (Börnke and Rocksch, 2018). Consequently, under the very likely assumption of increased restrictions of the application of PGR in the future, the further development and investigation of other growth control methods will be very crucial. This might ensure that small companies are given opportunities to sustain qualitative product standards to remain nationally and internationally competitive.

7.2.   Evaluation of different growth control methods

The different methods for growth control were described in chapter 1.3 and the objective was that air stream-based MS is comparable in effectiveness to other non-chemical growth methods. To make a general assessment of the different methods various factors such as energy consumption, labor and practicability have to be considered.

Classical or conventional breeding based on sexual reproduction with the aim of developing new ornamental plant varieties characterized by compactness has made little progress in recent decades. This is probably related to the great time investment until new and marketable cultivars emerge. In a fast-moving ornamental plant market with new cultivar releases almost seasonally, this investment is often not feasible economically. It has also been found that breeding for traits such as compactness is often not promising because due to a limited gene pool (Bhattacharya et al., 2010), as dwarfism is naturally eliminated because of the inter- and intraspecific competition for space and resources between plants growing in their natural environment, often resulting in vigorous growth and especially the promotion of stem elongation to guarantee light capture (Busov et al., 2003; Bhattacharya et al., 2010). Nonetheless, the use of biotechnology for genetic modification was shown to offer greater potential to induce compact phenotypes (Lütken et al., 2010; Islam et al., 2013). However, for market release of economically interesting genetically modified varieties a regulatory approval by authorities is required in most countries which usually takes several years and thereby strongly influences the profitability and consequently the development of such plant genotypes (Boutigny et al., 2020). Furthermore, it is questionable whether genetically modified genotypes find broad acceptance at the customer level, although this might be considered less critical in not edible ornamentals.

The effect of greenhouse temperature adjustment to control plant growth was shown to involve the GAs biosynthesis pathway and conversion processes among various GA within this pathway (Jensen et al., 1996; Myster et al., 1997), and consequently has great influence on stem and shoot extension. High day/ low night temperatures (DIF+) promoted stem elongation and increased GAs levels in bellflower, while low day/ high night temperatures (DIF-) had the opposite effect (Jensen et al., 1996). In most cases, continuous temperature regulation is associated with high energy consumption and consequently high costs, as this usually means heating at night and cooling during the day, which is contrary to the natural diurnal rhythm in most regions. Apart from this, the DIF- strategy ultimately has a negative impact on the environmental due to the higher energy requirement (Bergstrand, 2017). Tantau (1998) pointed out, that a deviation of +1 K can cause an increase in energy consumption of about 10%. As energy use efficiency has increasingly become the focus of attention in the last years, temperature adjustment for growth control under greenhouse conditions is reported to lack acceptability at the producer level (Bergstrand, 2017). Therefore, short temperature drops during daybreak, are preferable and similarly effective in reducing undesired stem extension compared to an integral temperature adjustment and thus seem to be more suitable from an economic and ecological point of view (Bergstrand, 2017).

Light is probably the most important factor influencing plant growth and, consequently, stem extension. The effect of a restricted stem extension by manipulating the photoperiod is likely related to an overall growth depression accompanied by a reduced C intake and consequently biomass gain under shorter light periods (Warrington and Norton, 1991). More specifically, Yamaguchi, (2008) reviewed that stem extension in particular must be related to the promotion of GAs biosynthesis under light perception. Although, the shortening of the photoperiod is technically easy to achieve (e.g., black-out screens), it is often accompanied with a delay in flowering (Schüssler and Kosiba, 2006) or a reduction in number of flowers (Schüssler and Bergstrand, 2012). Instead of a reduction in photoperiod length, an inhibition of shoot and stem elongation can also be achieved with filters (e.g., plastic screens) to alter the natural sunlight spectrum by reducing the far red portion (van Haeringen et al., 1998) or increasing the blue:red ratio (Lykas et al., 2008). However, as Mortensen (2014) stated, spectral filters are predominantly suitable at sites with naturally high light intensities, while the loss of light transmission is too high at sites with suboptimal natural light conditions. At higher latitudes, where additional lightning is required during the autumn/winter/spring season, spectral filters are therefore less suitable, because the energy loss from using filters would require additional heating energy to counteract the low outside temperatures, especially given the ongoing trend to replace high-pressure sodium lamps with light-emitting diodes (LEDs), which do not emit long-wave radiation and consequently heat (Van Ieperen, 2012). Nevertheless, the manipulation of light quantity and light quality in combination with an appropriate temperature

strategy offers great potential, especially with the introduction of the energy saving and relatively cheap LED technology.

PGR are an integral component in the production of ornamental pot, bedding plants and cut flowers and are ranked as the number 1 technique when it comes to growth control of ornamentals (Bergstrand, 2017). The cellular mechanisms rely on the inhibition of GAs biosynthesis of various GA intermediates within the GA biosynthesis pathway and ultimately results in reduced stem extension. Their high efficacy combined with great reliability and relatively low prices are probably the main advantages compared to other previously mentioned growth control methods. Moreover, their application is uncomplicated as users do require less skill full crop management compared to e.g., growth control using temperature and light regulation strategies. However, their application requires more labour compared to other electronically automated growth control methods, such as temperature and light regulation, because PGR are usually applied manually. In addition, chemically treated ornamental plants could lose further acceptance among end consumers and policy targets also point to a decrease in the use of PGR in the near future.

Until now, the technical implementation to control plant growth and stem elongation by MS has not been very promising. Previous attempts, such as the use of shaking devices (Beyl and Mitchell, 1977) or a moveable polyvinylchloride pipe (Latimer and Thomas, 1991) to induce plant movement and consequently the thigmomorphogenetic trait, had only limited success, which was due to in particular the lack of flexibility in application and the resulting impracticability. Probably the most promising attempts were demonstrated by Regnant et al., (2009) and Koch et al., (2011). They used an irrigation boom, which is usually standard equipment in most modern greenhouses, to attach the material that bends the plants as it moves over the plant tips. The movement of the irrigation boom was fully automated electronically, resulting in a considerable reduction in labour. However, damage to plant tissue from direct contact hindered further development. Therefore, the main objective of this work was to overcome this problem by using air stream-based MS for control of stem elongation. This objective was shown to be fully achieved as no damage to plant tissue was observed. Furthermore, the results presented in chapter 4 clearly show that the application of air stream -based MS resulted in a 24%, 20%, and 26%-36% reduction in plant height in bellflower (Fig. 4.8), creeping inchplant (Fig. 4.11), and tomato (Table 4.3), respectively. The hypothesis of plant height reduction by air stream-based MS can thus be confirmed.

Similar to this study, Moe (1990) obtained a 25% reduction in stem length of *Campanula isophylla* at a DIF- of 7°C compared to constant day and night temperatures. This was also confirmed by Jensen et al. (1996). A temperature drop of 6°C for 9 h at daybreak resulted in a 29% reduction in plant height in *Campanula isophylla* compared to a treatment without a temperature drop (Moe et al., 1995). Photoperiods with far red deficiency using photoselective

filters had an insignificant effect on plant height in *Campanula carpatica* (Runkle and Heins, 2001). In tomato, 15 min of end-of-day light with red light resulted in a 32% reduction in tomato plant height (Decoteau and Heather, 1991). A blue light treatment resulted in a 43% reduction in plant height in tomato (Mortensen and Stromme, 1987). In tomato plants exposed to MS, a reduction in plant height of 26% to 35% (Schnelle et al., 1994) and about 30% (Johjima et al., 1992) was found compared to untreated control plants. This indicates that air stream-based MS is comparably effective to other growth control methods.

Tab 7.1. Assessment of growth control methodologies in terms of efficacy, energy consumption, labour requirement, user and consumer acceptance, and practicability.

	Breeding	Temperature	Light	PGR	MS
Efficacy	High	High	High	High	High
Energy consumption	Low	High	Medium	Low	Medium
Labour	High	Low	Low	Medium	Low
User acceptance	Medium	Medium	High	High	Medium
Consumer acceptance	Medium	High	High	Medium	High
Practicability	High	Medium	Medium	High	High

7.3.   Air stream-based mechanical stimulation systems

Compared to previous MS techniques (Beyl and Mitchell, 1977; Biddington and Dearman, 1985; Latimer, 1990; Latimer and Thomas, 1991; Regnant et al., 2009), the three different prototypes tested in this study (Fig. 4.1; Fig, 4.3; Fig. 4.4) are the first that use directed air streams to induce plant movement and consequently thigmomorphogenesis. A fundamental prerequisite for their implementation into horticulture greenhouse practice is the availability of a watering boom that functions as the carrier unit. Commonly, watering booms are standard equipment in modern greenhouses nowadays and usually they are fully automatized e.g., driving velocity and starting time through an electronic control unit. Furthermore, watering booms are constructed to cover the dimensions of the entire plant production area. From a practical point of view, watering booms therefore suit ideally as the carrier unit for the respective air stream application module, as no additional constructions are necessary and thus the objective of high practicability is fulfilled by using the given infrastructure.

Considering the requirement of a compressor for the air pressure module and the installation of flexible hose lines for air stream guidance from the compressor to the air outlet nozzles, additional constructions are required. For example, the flexible hose lines may need to be embedded in an additional guide rail or directly in the chain guide cage, which runs parallel to the longitudinal guide rail on which the entire watering boom is hooked. Given these circumstances, the use of the air pressure system is limited to a greenhouse area covered by a single watering boom. Alternating the use of a compressed air module in different areas of

the greenhouse is therefore unlikely, as this would require the installation of additional hose lines and possibly even the purchase of additional compressors. Apart from other factors, such as the 250 s requirement to obtain a uniform air outlet velocity and the variability in air velocity among individual nozzles (Fig. 4.6A, B), the inflexible use and the requirement of a compressor are considered to be strong limiting factors for a broad application in horticulture greenhouse practice. Furthermore, the inconsistent air distribution across the underneath plant stand resulted in variable growth inhibition patterns. Therefore, the air pressure system does not fulfil the objective of a uniform stimulus application.

Subsequently, the air knife module and the 360° rotor module were implemented and evaluated. The main advantage of these two prototypes was that no additional hose lines were required along the guide rail for air stream guidance, because the air stream was generated by a centrifugal fan mounted directly on the watering boom, replacing the requirement of a compressor.

The principal idea of the air knife module was to produce an air curtain that has a uniform air outlet velocity along the entire aperture slot opening, as guaranteed by the manufacturer. Thus, the air knife module should provide a uniform stimulus application to the plant stand. Surprisingly, air velocity measurements at 5 positions equally spaced along the aperture slot of the air knife module revealed a large variability (Fig. 4.7A) which could not be completely solved by adjusting the opening width of the aperture slot to the minimum opening width of 1 mm (Fig. 4.7B). Intriguingly, these circumstances allowed to investigate the effect of different maximum air velocities reaching the plant, giving first insights into the dose-response relationship (Fig.4.10C). Additional experiments with the air knife module conducted at the State Horticulture College and Research Station in Heidelberg, Germany (Fig. 7.1), confirmed the variability in air outlet velocities along the slit opening (data not shown) and showed that the air outlet velocities achieved under these technical settings (more powerful centrifugal fan and longer effective length of the air knife module) were insufficient for an optimal effect on stem elongation inhibition. Based on these findings an implementation of the air knife module into horticulture praxis is not recommendable, again due to the inconsistency in air distribution similar to the major drawback described for the air pressure module. In addition, the use of the air knife module is likely to be rather impractical, given that the air stream should cover and area width of at least 10 m to correspond to the usual dimensions of a watering boom. Apart from that, similar to the main disadvantage of the air pressure module, the fixed installation of the centrifugal fan for air stream generation limits the versatility in use in different greenhouse areas.
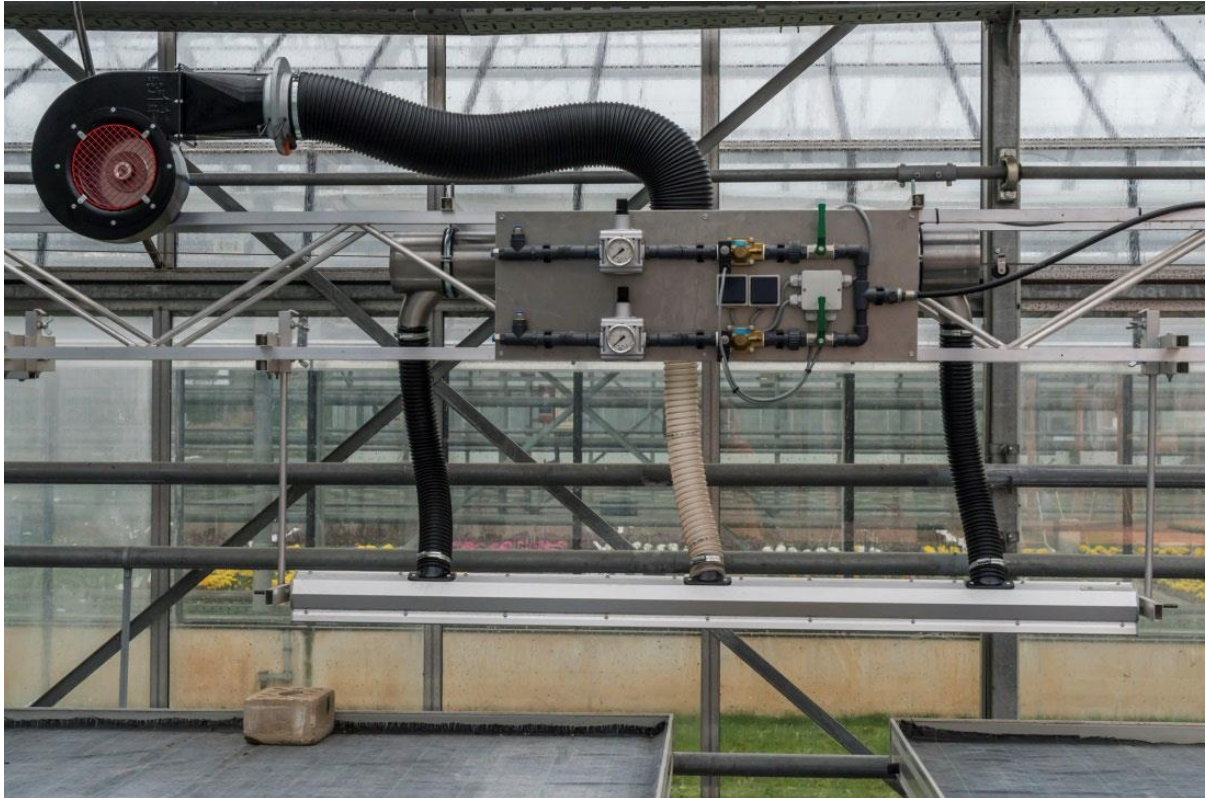
Fig. 7.1. The air knife module was installed in a greenhouse compartment at the State Horticulture College and Research Institute in Heidelberg, Germany.

The 360° rotor module showed the most promising results in terms of stimulus uniformity which is why all following experiments were conducted with this prototype. However, also the 360° rotor requires a centrifugal fan as the central unit for air stream generation, again limiting versatility in use. The most practical approach would therefore be to install individual air stream application modules directly on the crossbar of the watering system. In this case, the air stream must be generated in a decentralised manner by each module itself. A major advantage here would be that no hose lines are required for air stream guidance and the individual modules can be used individually in different areas of the greenhouse, provided that a watering boom is available and the attachment of individual modules to the watering boom is simple to carry out.

This idea was pursued and implemented by the local horticulture company (Fleischle GbR, Vaihingen Ensingen, Germany). Two axial fans (Heylo PowerVent 1500; Heylo GmbH, Achim, Germany) were mounted centrally on the left and right cantilevers of the cross beam of a watering system (Fig. 7.2A). A base plate on which each fan was mounted served as a support for the fan. The base plate and consequently the fan was inclined at an angle of approx. 40° to the vertical. The plate rotated together with the fan at a rotation angle of 120°, driven by a rotary motor. This was to ensure air stream application along the entire surface of the greenhouse table. However, as the fans rotated to the right and left, the distance between the fan's air outlet and the plant, i.e., the theoretical length of the air stream profile
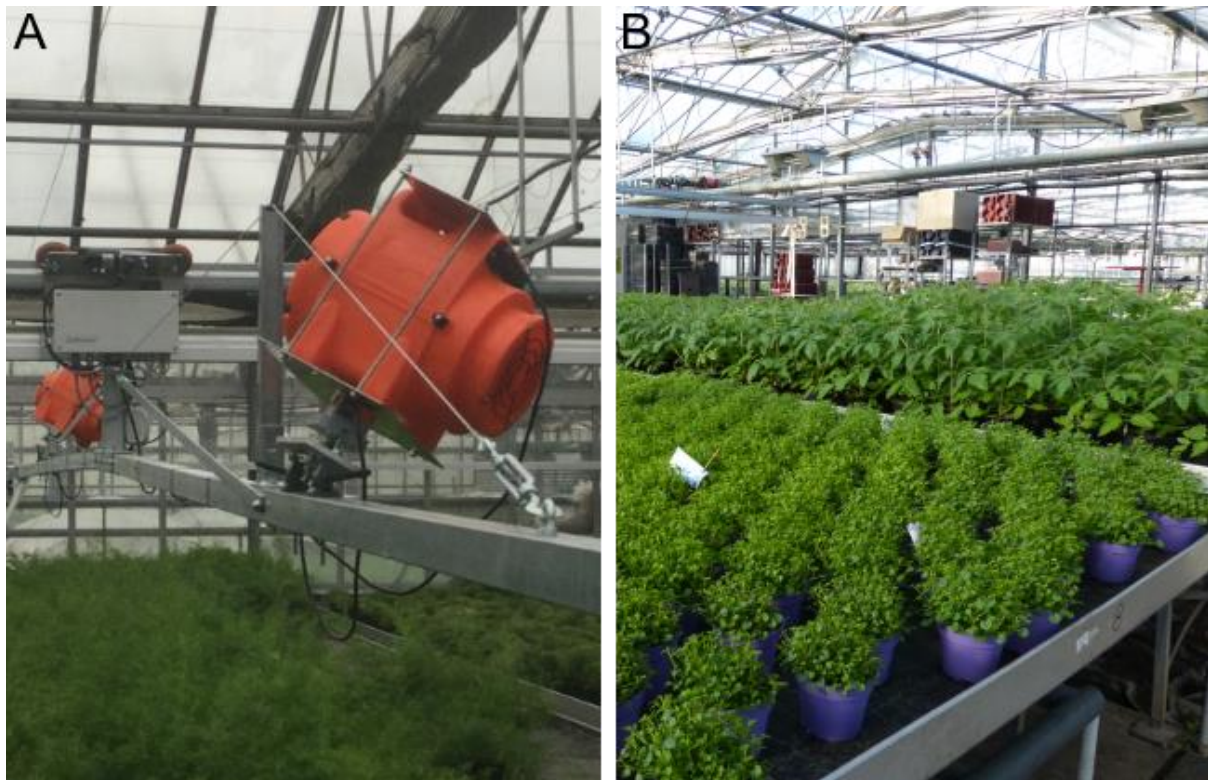
Fig. 7.2. Two axial fans (Heylo GmbH, Achim, Germany) were installed on a crossbeam at Fleischle GbR, Vaihingen Ensingen, Germany for growth control by air stream-based mechanical stimulation (A). Variable stem elongation inhibition in tomato when treated with the Heylo axial fans (B).

to reach the plant, increased simultaneously. This led to a gradually decreasing stimulation intensity for tomato plants that were located closer to the table edges and the centre. As a result, the less strong stimulated tomato plants showed a less strong reduction in plant height (Fig. 7.2B). Although the installation of the axial fans was likely the most practical approach to date, the uneven air stream distribution along the table was still a major constraint to widespread adoption in practice, similar to the major constraints previously described for the air pressure module and the air knife module.

In a subsequent project in cooperation with a mechanical engineering company (Raith Tec, Ditzingen, Germany) a combination of the 360° rotor module and the axial fan system was developed. The promising approach of a decentralised air stream generation was pursued further by integrating a high-performance axial fan into individual modules. The generated air stream by the axial fan is centrally separated into two air outlet tubes mounted opposite each other below and an electric motor empowers a belt drive that enables a 360° rotation of the air outlet tubes. The angle of incidence of the air stream is set to 70° vertically. The new prototype was implemented at the State Horticulture College and Research Institute, Heidelberg (Fig. 7.3). First experiments with the new prototype revealed a high efficacy in growth regulation of poinsettia and basil (data not shown) and according to the company, the energy consumption could be reduced by 80% compared to previous air stream application modules.

Fig. 7.3. The new prototype (Raith Tec, Ditzingen, Germany) was installed at the State Horticulture College and Research Institute, Heidelberg, Germany for growth control by air stream-based mechanical stimulation of poinsettia (A) and basil (B).

The new prototype module can be easily installed provided that a watering boom and a 240 V socket is available. Great flexibility in terms of use in different greenhouse compartments and different plant crops is given as the air stream is generated by each module itself. Furthermore, due to the 360° rotation of the air outlet tubes the air stream is uniformly applied to the plant stand below, thus ensuring a homogeneous plant height reduction. Also, the new prototype was conceptualized in consultation with Raith Tec in such a way that the findings on the dose-response relationship (Fig. 4.7C) were used to achieve a sufficiently high air velocity of at least 5 m s^{-1} at 60 cm from the air outlet. Thus, according to the results presented (Fig. 4.7C) it is likely that the stimulus saturation point is met and thus the maximum inhibitory effect is achieved.

7.4. The effect of air stream-based parameters on stem elongation

Another objective of this study was to establish a fundamental understanding of the dose-response relationships between different air stream parameters and their effects on the extent of stem elongation inhibition. This is of particular interest to potential users of the air stream-based MS technique for height control because knowledge of how often and how strong the air stimulus needs to be applied is of great importance in order to achieve a specific production goal. Thereby, recommendations for an economically and ecologically suitable application schedule can be given which ultimately determines the successful integration of this technique into vegetable seedling and ornamental plant production.

In the prototype systems presented (Fig. 4.1; Fig. 4.3; Fig. 4.4; Fig. 7.2; Fig. 7.3), the duration and the intensity of the air stream stimulus to which the plants were exposed were the primary influencing parameters to be investigated. In addition, it was to be investigated whether a short-lasting but stronger stimulus would have the same effect on the extent of stem elongation inhibition as a long-lasting but milder stimulus.

Numerous observations during different experimental phases of this study, such as the obvious large variability in tomato plant height reduction observed when the air stream was applied unevenly along the greenhouse table with the Heylo fans (Fig. 7.2B), indicate an apparent relationship between the air stream velocity, i.e., the stimulus intensity, and the extent of tomato stem height reduction. The first data that describes this relationship quantitatively were shown in chapter 4 for the experiment conducted with the air knife module. It was found that neither the stimulus duration (Fig. 4.7A) nor the cumulative air velocity, i.e., the cumulative stimulation intensity (Fig. 4.7B), had a significant effect on the extent of stem elongation inhibition in tomato. In contrast, the maximum air velocity reaching the plant tip was shown to significantly correlate with the degree of plant height reduction (Fig. 4.7C). This was further substantiated by the experiments that were specifically designed to investigate the influence of gradually increasing air stream AF and air stream velocities shown in chapter 5 with the 360° rotor module, respectively. It was evident that increasing air stream AF from 8 to 80 did not lead to an increased inhibition of stem elongation (Fig. 5.4), while increasing air stream velocities from 0.7 to 6.0 m s^{-1} (Fig. 5.7) correlated strongly with the inhibitory effect on plant height, confirming previous findings that the stimulus duration appears to be negligible, while the stimulus intensity is crucial.

The results presented (Fig. 5.4) are in great accordance with earlier studies that investigated the dose-response relationship between different MS treatment frequencies and the inhibitory effect on stem elongation (Jaffe et al., 1980; Autio et al., 1994; Garner and Björkman, 1996; Telewski and Pruyn, 1998). Bean internode elongation was consistently inhibited similarly regardless of the number of rubes applied (Jaffe et al., 1980). In tomato, 10 brush strokes day^{-1} significantly reduced stem length with negligible additional effects when the frequency was increased up to 40 strokes d^{-1} (Garner and Björkman, 1996). Similarly, Telewski and Pruyn, (1998) found a rapid saturation in stem elongation inhibition in American elm when exposed to 5 manually stem flexure treatments d^{-1}. In aster, and petunia the maximum inhibition in stem elongation was likewise quickly reached when brushed with burlap 60 min d^{-1}. These previous findings and the results shown in this study (Fig. 5.4) are unequivocal evidence that, provided that the stimulus saturation point is met, the threshold for AF under air stream-based MS is rapidly reached. Consequently, the hypothesis that the stimulation intensity primarily influences the extent of stem elongation inhibition, while the duration of the stimulus is of less importance as the stimulus does not accumulate over time can be confirmed. However, it is

not yet possible to make a concrete statement about the minimum threshold value for the air stream AF, as this would require a systematic experiment with a gradual reduction of the air stream AF below 8. Thus, the question of an optimal treatment scheme within a certain period of time (e.g., 24 h) remains open. Based on the expectation that the resumption of stem elongation after MS is between 30 and 60 min (Telewski, 2006, 2021), it could be assumed that the application of successive stimuli should be at least every hour to be able to use the air stream-based MS technique to its full extent i.e., obtaining the maximum extent of stem elongation inhibition. However, there is no information on whether a subsequent stimulus has the same effect as the previous stimulus in the time period mentioned above. Garner and Björkman (1996) found no graded response in tomato plants when the time interval between 2 successive stimuli was varied between 0.01 and 10 minutes. The time interval chosen in the experiments of this study ranged approx. from 45 to 75 min, and as mentioned previously, no significant difference was found between AF 8 and 80. An analysis on the tissue refractoriness could provide valuable insights for the development of appropriate treatment regimes. High-resolution sensor-based measurements of stem elongation dynamics under repetitive air stream application, for example with linear variable differential transformers (LVDT), could be a promising approach to accurately investigate the actual duration of stem elongation inhibition and clarify the question of when the plant regains its full responsiveness or if it gradually loses its sensitivity to reappearing stimuli.

With the knowledge gained that the air stream AF does not significantly influence the extent of stem elongation, the main focus was shifted on the effect of different stimulation intensities. The originally postulated linear relationship (Fig. 4.7C) between air velocity and stem elongation inhibition was refined by a sigmoidal dose-response relationship in tomato (Fig. 5.7) with defined points for stimulus threshold and stimulus saturation in a subsequent experiment. This quantitative relationship between stimulation intensity and plant height reduction is consistent and of the same range as the results found in bean plants when exposed to increasing wind velocities (Hunt and Jaffe, 1980). The shown data (Fig. 4.7C; Fig. 5.7) and the consistency with the information in the literature allow the confirmation of the hypothesis of a stimulus intensity-dependent inhibition of stem elongation with defined values for stimulus threshold and stimulus saturation.

However, it is worth mentioning that increasing plant height over the 2 week experimental period due to plant growth in the underlying experiment inevitably resulted in a systematic increase in air velocity because the distance between the air outlet and the main stem apex was not continuously adjusted. Consequently, the dose-response relationship shown (Fig. 5.7) contains a temporally dynamic component. Thus, it remains to be investigated whether air velocities at or beyond the stimulus saturation point at the beginning of the experiment, could have ultimately further increased the extent of plant height reduction.

The question remains open as to which physical factors that are related to the induced plant movement cause the gradual response in stem elongation inhibition. Some conceivable physical parameters that include a time component, such as the stimulus duration and the associated oscillation time, i.e., the cumulative stimulation intensity of the entire plant after air stream perception, have already been excluded based on the results presented in chapters 4 and 5. From a physical point of view, it therefore seems very plausible that the stronger maximum deflection of the plant stem due to increasing air velocities is responsible for the graded plant response. Jaffe et al., (1980) used a thigmostimulator to investigate the relationship between the amount of mechanical force applied to bean stems, i.e., bending degree, and the resulting internode elongation inhibition. Similar, to this study a clear linear relationship was found. Coutand et al., (2000) used LVDT to precisely measure the effect on tomato stem elongation after a transient localized stem bending event. Surprisingly, the force intensity did not relate to the internode elongation response in their experiment. However, a major difference between these two experiments was that the stimulus was applied locally to a fully elongated internode i.e., to old tissue, whereas in the experiment by Jaffe et al. (1980) the stimulus was applied to a young, still actively elongating internode. In air stream-based MS the entire plant structure is set into motion, including the leaves, petioles, and young growing shoot tips. Therefore, it is relatively certain that similar to the results presented by Jaffe et al. (1980), the higher bending degrees induced by higher air velocities are responsible for the graded plant response. This is also coherent to the air velocity-dependent $[Ca^{2+}]_{cyt}$ influx (Knight et al., 1992) that is likely to be one driving cellular signalling event for decoding the stimulation intensity.

Although the technical set up was the same in both experiments (chapters 5 and 6), stem elongation was found to be stronger inhibited in spring/summer than in autumn/winter, which adds to the complexity of understanding the growth response to air stream-based MS. Other abiotic factors, such as temperature regime or light intensity are therefore likely to influence the extent of stem elongation inhibition under MS. Furthermore, due to the diurnal pattern of stem elongation (Coutand et al., 2000; Garner and Björkman, 1996), the question arises whether the stimuli should be applied during the night or at daybreak, when elongation peaks. Investigations on this could be crucial for the practical implementation of air stream-based MS.

7.5.   The effect of air stream-based mechanical stimulation on plant growth

Another objective of this study was to examine the effect of air stream application on plant productivity. There is strong evidence that MS leads to an overall reduction in growth i.e., biomass formation (Biddington, 1986; Biddington and Dearman, 1985; Johjima et al., 1992; Latimer et al., 1991; Piszczek and Jerzy, 1987). In cauliflower, lettuce, and celery, manual brushing of young seedlings for 1.5 min d^{-1} reduced SDM in all species (Biddington and Dearman, 1985). Similarly, LDM, PDM, and SDM were significantly reduced in different tomato

cultivars when they were exposed to 2 brushing treatments with a suspended steel bar for 1.5 min d^{-1} (Johjima et al., 1992), confirming the results of SDM reduction found by Piszczek and Jerzy (1987) when tomato plants were subjected to a vibration treatment. In addition, it is considered well established that the TLA is reduced under MS treatments such as manual stem flexing (Telewski and Pruyn, 1998) brushing (Garner and Langton, 1997a; Johjima et al., 1992), and wind (Niklas, 1996). At the same time, SLA was frequently reported to be increased under MS (Latimer, 1991).

The results presented (chapters 5 and 6) are in great accordance with these earlier findings, as also air stream-based MS negatively affected LDM, SDM, and PDM (Table 5.2; Table 5.3), as well as TLA (Fig. 6.5A), and increased leaf density (Fig. 6.5B). Furthermore, similar to the gradual response in stem elongation inhibition to increasing air velocities, there was a graded response in the reduction of the various aboveground plant parts to increasing air velocities (Table 5.3). Taking into account the general concept of plant stress responses to abiotic and biotic stressors (Lichtenthaler, 1996), these observations indicate that air stream-based MS leads to a negative deviation from the original physiological standard in a dose-dependent manner, most likely related to a disturbance of the primary plant metabolism such as photosynthesis. Surprisingly, in contrast to these consistent observations found in the experiments during autumn/winter, the reduction in biomass formation was less pronounced in the experiment conducted in spring/summer (Table 6.2; Fig. 6.6), although the technical set up of the air stream applicator was the same. Therefore, it seems very likely that other seasonal factors such as temperature and/or light intensity must have played a role that enabled the plant to counteract these consistently observed, stress-induced reductions in biomass accumulation as a result of MS. This leads to the assumption of a positive interaction between environmental factors and the phenotypic and physiological acclimation responses to air stream-based MS presented in this study (Fig. 6.6; Fig. 6.8). In more detail, it was shown that the new physiological standard attained by the plant after continuous perception of air streams was characterized by a pronounced biomass allocation to leaves and stems (Fig. 6.6B, D). Ultimately, this led to the maintenance of LDM and SDM (Fig. 6.6A, E), although TLA (Fig. 6.5A) and SH (Fig. 6.2) were significantly reduced by air stream-based MS compared to the control. A possible explanation for this discrepancy between the two technically identical experiments is related to the results shown in Figure 6.8D, E, F, where the NAR of air stream-treated plants was significantly increased compared to the control. These findings are most likely related to the reduced SLA of air stream-treated plants (Fig. 6.5B), indicating an increased leaf density, which in turn suggests an increased N content and ultimately an increased RuBisCO content (Evans, 1989).

In view of this, it can be assumed that the NAR of air stream-treated plants must have been at a similar range compared to that of the control on the whole plant level. To follow up on this

assumption a simple extrapolation of the data presented (Figure 6.8D, E, F) on the whole leaf level by using individual leaf areas of tip leaf 2, 3, and 4 (data not shown) allows to calculate the potential NAR of the examined leaves using the following equation:

$$NAR_{Leaf} = NAR \times LA \qquad\qquad\qquad (7)$$

Where NAR_{Leaf} is the estimated net assimilation rate of the entire leaf 2, 3, and 4, NAR is the observed net assimilation rate, and LA is the leaf area of the respective tip leaf. This allows an estimation of the extent to which the increased NAR of air stream-treated plants compensated for the reduction in light harvesting surface area to ensure biomass maintenance of leaves and stems compared to the control. In fact, this computational approach indicates that tip leaf 2, 3, and 4 of the air stream-treated plants had a higher NAR_{leaf} from DOT 2 to 7 compared to the control (except for tip leaf 3 at DOT 7), whereas this trend reversed from DOT 9 to 14 (except for tip leaf 2 at DOT 14) (Table 7.2). From this, the above-mentioned assumption can be partially confirmed that higher NAR_{leaf} could temporarily increase and ultimately partially maintain C assimilation on the whole plant level within the 14 days experiment.

However, it is worth mentioning that in this study NAR was measured at the light saturation point of 1200 µmol m^{-2} s^{-1} which represents the maximum rate of net C assimilation. Under lower light intensities this effect was shown to become gradually neglectable (Gregory et al., 1981) which could be the reason why tomato plants were not able to maintain biomass accumulation during the autumn/winter experiments. Although this adaptive stress response to air stream-based MS leads to a new physiological standard, the hypothesis of complete maintenance of the original productivity can therefore not be confirmed, as other abiotic factors are most likely decisive for this.

Tab 7.2. Potential net assimilation rate of tip leaf 2, 3, and 4 (NAR$_{leaf}$) of control and air stream-treated plants and the relative change (%) of NAR$_{leaf}$ from DOT 2 to DOT 14. N.A. indicates not available.

DOT	Tip leaf	NAR$_{Leaf}$ (μmol s^{-1})		Relative change (%)
		Control	Air stream	
	2	0.0044	0.0058	↑ 25.1
2	3	N.A.	N.A.	N.A.
	4	N.A.	N.A.	N.A.
	2	0.0046	0.0067	↑ 32.2
4	3	0.0048	0.0063	↑ 24.0
	4	N.A.	N.A.	N.A.
	2	0.0061	0.0068	↑ 10.4
7	3	0.0094	0.0087	↓ 7.9
	4	0.0055	0.0068	↑ 19.0
	2	0.0070	0.0059	↓ 18.0
9	3	0.0121	0.0105	↓ 15.5
	4	0.0089	0.0088	↓ 0.9
	2	0.0060	0.0054	↓ 10.8
11	3	0.0129	0.0108	↓ 19.5
	4	0.0107	0.0103	↓ 4.6
	2	0.0054	0.0055	↑ 2.7
14	3	0.0138	0.0121	↓ 13.6
	4	0.0139	0.0123	↓ 12.6

The reasons for this dynamic change in NAR$_{leaf}$ (Table 7.2) cannot be clarified exactly and require further investigation. However, it can be assumed that if the air stream is applied regularly for a short period of time (e.g., 14 days), the reduction in aboveground biomass formation can be expected to be limited, provided that other factors influencing photosynthesis performance, in particular light intensity, are close to the optimum. It is worth mentioning that under these conditions the series of experiments carried out during spring/summer, the two weeks air stream application had no significant effect on tomato yield (data not shown). By contrast, based on the trend shown (Table 7.2), it can be assumed that air stream-based MS beyond the experimental period chosen here will lead to a gradual reduction in aboveground biomass formation and potentially yield reduction.

8. Conclusions

The use of PGR is still the most common method to achieve the desired quality criteria of compact plant growth and reduced plant height in ornamental plant production. However, in view of the global political objective to strongly reduce the use of chemical-synthetic substances, including PGR, it is necessary to develop suitable alternative production methods for plant growth control and to establish them in horticulture greenhouse practice. The same applies to the production of young vegetable seedlings where the application of PGR is already prohibited by law, but growth regulation is required.

Numerous studies have investigated the effect of MS on plants on a (sub)cellular level and outlined its potential as a technical production method for growth control. In general, the response to MS can be considered as a widespread biological adaptation mechanism in most plant species, which falls under the thigmo-related plant responses and plays a crucial role to withstand harsh environments particularly characterized by the exertion of strong mechanical forces primarily induced by wind loads. Thus, the plant responses to MS are of great ecological significance. Similarly, to the reaction to other abiotic or biotic stressors, the cellular signaling events involve the universal secondary messenger cation Ca^{2+} and a well-coordinated signal transduction which ultimately leads to an upregulation of the plants' defence system in a general manner which, inter alia, also includes the synthesis of defence substances such as JAs. On a morphological scale, changes as a response to MS are the reduction in stem elongation and leaf area, simultaneously to the frequently observed promotion of radial stem extension and root growth. Due to these characteristics, MS could be suitable as a priming treatment to increase the robustness in general against potential abiotic and biotic damage while simultaneously achieving the goal of plant size reduction in ornamental plant and vegetable seedling greenhouse production.

The results of this study and the subsequent contextualisation with the observations described in the literature have clearly shown that the application of air stream-based MS can have a comparable efficacy to direct touch-based MS techniques with regard to the effect of stem elongation inhibition in tomato and bellflower. However, it must be explicitly emphasised that the repeatedly reported plant damage was absent under the application of air stream-based MS, representing a significant advantage compared to previous touch-based MS techniques. Through a constantly ongoing development process, which was composed of various technically refined prototypes for air stream-based growth regulation, it was possible to develop a market-ready end product. The achievement of a uniform stimulus distribution and a practical, flexible application, provided that a system is now available which is comparably effective to non-chemical growth control methods. Factors, such as energy consumption, user acceptance and practicability, which can be evaluated positively in comparison to other non-chemical growth regulating techniques, will determine whether the herein presented technique

will establish itself on the market. However, as long as the use of PGR will not be prohibited by law or at least stronger restricted, it is likely that PGR will remain the first choice for ornamental plant growth regulation due to their established evidence of reliable efficacy, high degree of practicability and barely comparable cost efficiency.

With regard to the quantitative relationship between different relevant influencing variables of the air stimulus, such as the duration of the stimulation frequency, this study has clearly shown that the decisive influencing variable is the stimulation intensity, i.e., the air velocity reaching the plants, which determines the extent of stem elongation inhibition. This is a significant milestone in demonstrating the efficacy and suitability of the air stream-based MS technique for stem elongation control. The graded response in stem elongation inhibition to increasing air velocities demonstrates that air stream-based MS is a flexible method for growth control, in that potential users are able to operate between the stimulus threshold and the stimulus saturation threshold, thus achieving a specific target in plant height reduction. It must be emphasised, however, that in this context it was almost consistently found that biomass formation was negatively affected, with a similar gradual effect with increasing stimulation intensities compared to the reduction in stem elongation. In response to air stream-based MS, the plant initiates a comprehensive adaptation mechanism that essentially involves a redistribution of assimilates towards the light-harvesting tissue and stems, with a simultaneous reduction in total leaf area and petiole length. From an ecophysiological point of view these phenotypic adaptation mechanisms, indicate (i) an accelerated or enhanced lignification process, i.e., the formation of stabilizing stem tissue, in order to better withstand mechanical forces (wind) and (ii) to reduce the maximum stem bending degree by reducing the surface attack area and consequently the drag force, thus avoiding total breakage or uprooting. In this way, the plant increases its chances of survival. Along with this adaptation process, the plant reaches a new physiological standard characterized by an increased maximum photosynthetic capacity, mainly related to an increased leaf density. Thus, the frequently described loss in biomass formation by MS could be partially compensated, provided that the light intensity is sufficiently high to take advantage of this increased photosynthetic capacity.

Air stream-based MS is a new and promising technique for growth regulation of ornamental and vegetable seedling production, but still in its early stages. The established quantitative relationships between air stimulus-related parameters and growth inhibition presented in this study are not necessarily transferable to other plant species. In order to use this new production method on a broad scale, especially in the production of ornamentals, it would be necessary to systematically compile a species-specific catalogue of effects in order to be able to give producers concrete and reliable application recommendations.

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Department of Crop Science
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- Project management
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- Analysis and quantification of plant stress responses e.g. Leaf gas exchange measurements; chlorophyll fluorescence
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08/2015 - 02/2016
Visiting Researcher
International Center for tropical Agriculture (CIAT)
- Tissue sampling for *in vivo* enzyme activity analysis and leaf nutrient concentration
- Measuring greenhouse gas emissions on field scale
- Soil sampling
- Bioluminescence assay with modified Nitrosomonas bacteria on root extracts

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Student Apprentice
German Society for International Cooperation (GIZ)
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04/2010 – 07/2013
Student Research Assistant
Department of Crop Science
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- Mass spectrometry
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- Chemical soil and plant mineral nutrient analysis

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Queen Sirikit Research Station
- Electrical resistance tomography (ERT) for soil water quantification
- Root distribution patterns along the crop-soil-hedge-interface

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M. Sc. Crop Sciences | Crop Physiology and Nutrition
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SKILLS & COMPETENCES

Job-related skills
Good level of scientific reading and writing | Profound knowledge of statistical data analysis softwares (SAS, R, Sigmaplot) | Profound knowledge in office computer applications (Word, Excel, Power Point) Deadline success working | Conscientious working paradigm

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English	spoken	●●●●○
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